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DOTTORATO DI RICERCA TOSCANO IN NEUROSCIENZE

CICLO XXXII

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Adenosine A_{2B} receptors and Carbonic Anhydrase: New therapeutic targets for cerebral ischemia and demyelination

Settore Scientifico Disciplinare BIO/14

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1. Introduction.

The Adenosinergic system

The nucleoside adenosine (Figure 1) is formed by a molecule of the purinergic nucleobase adenine bound to a ribose. Adenosine is a neuromodulator/neurotransmitter in the CNS, usually with a cytoprotective function (Fredholm et al., 2007).

This nucleoside is central in the regulation of several physiological functions in different cell type (neurons, platelets, mast cells, smooth muscle cells, myocytes), due to its role as modulator of neurotransmission, conduction, vasodilation, proliferation and cell death.

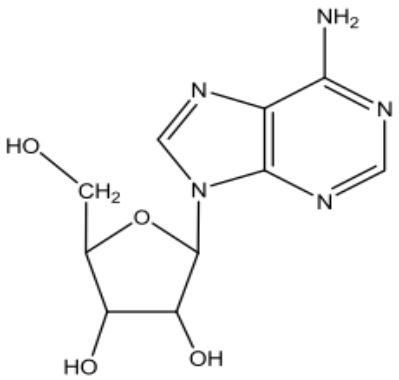


Figure 1. Adenosine molecular structure.

Many of the actions mediated by adenosine, are responsible of tissue protection during situations of stress, such as ischemia or hypoxia. Protective effects and tissue repair are exerted by adenosine by increasing the supply of oxygen, by triggering anti-inflammatory responses and by promoting angiogenic processes (Linden, 2005).

In the Nervous System, adenosine acts both at central and peripheral level. In 1929, Drury and Szent-Györgyi, proved that systemic administration of adenosine in anesthetized animals induced transient bradycardia, vasodilation, decrease of blood pressure and intestinal motility. In the years 1970s Sattin and Rall (Sattin et al., 1970) showed a specific role of adenosine in the central nervous system (CNS), in the increase of cAMP formation in mammalian brain slices. This effect was inhibited by the methylxanthines: caffeine and theophylline.

Later on, a depressant role of adenosine on the firing of cortical (Phillis et al., 1979) and cerebellar neurons (Kostopoulos et al., 1975) and on excitatory synaptic potential amplitude in cortical and hippocampal slices (Kuroda et al., 1976; Dunwiddie et al., 1980; Okasa et al., 1980) was demonstrated. Moreover, it was shown the inhibitory role of adenosine on the acetylcholine release in different brain areas (Harms et al., 1978) and on the release of dopamine (Michaelis et al., 1979), noradrenaline (Harms et al., 1978), excitatory amino acids (Corradetti et al., 1984)

and serotonin (Harms et al., 1979). All these effects were inhibited by methylxanthines, increased by inhibitors of the adenosine reuptake and correlated with changes in cAMP levels.

1.1 Adenosine formation

In the CNS, adenosine originates from neurons and from non-neuronal cells such as glial cells (Halassa et al., 2009). Its formation occurs by dephosphorylation of AMP operated by 5'-nucleotidases. These enzymes are present both intracellularly, (5'-endonucleotidase), and extracellularly, (5'-ectonucleotidase) (Zimmermann, 1992 a). Intracellularly adenosine is generated from AMP and extracellularly by enzymatic conversion of released nucleotides through coupled ectonucleotidases: CD39 that converts ATP/ADP to AMP and CD73 that hydrolyses AMP to adenosine. A further enzyme able to generate adenosine by sequential hydrolysis of ATP, is the alkaline phosphatase (Zimmermann, 2000).

The intracellular adenosine was considered as the most important source of extracellular adenosine. Over the past decade however, it was increasingly recognized that the rapid (1 ms) extracellular conversion of locally released ATP by ectonucleotidases represents another important source of extracellular adenosine in tissue (Fredholm et al., 2007).

Another possible source of extracellular adenosine is represented by released cAMP. In the extracellular space, cAMP can be converted to 5'-AMP by ecto-phosphodiesterase, and then to adenosine by 5'-ectonucleotidase. Alternatively, cAMP can be converted into 5'-AMP within the cell and then released in the extracellular space, where it represents a further source of adenosine. This suggests that many neurotransmitters that act on metabotropic receptors whose signaling is linked to adenylate cyclase, by favouring the accumulation of cAMP, may regulate the adenosine levels and thus the inhibitory effects in the CNS (Latini et al., 2001 a).

Adenosine can also derive from S-adenosylhomocysteine (SAH) hydrolysis by the enzyme SAH-hydrolase (Figure 2). This reaction occurs mainly at cardiac level, where SAH represents an important source of intracellular adenosine but only in physiological and not under hypoxic conditions (Latini et al., 1996 a). At neuronal level, this synthesis pathway does not significantly contribute to the adenosine production either in normoxic or in hypoxic conditions (Pak et al., 1994; Latini et al., 1996 b).

1.1.1 5'Endonucleotidases

Six 5'-endonucleotidases with different subcellular localization have been cloned in mammals (Table 1):

5'-Nucleotidase (abbreviation)		**Aliases**	**Natural substrates**
cytosolic 5'-nucleotidase I (cN-I)	cN-IA	AMP-specific;	AMP, dTMP, dCMP, dGMP, dAMP, dIMP, (probable substrate = dUMP)
	cN-IB	cN-IA homologue; AIRP	AMP
cytosolic 5'-nucleotidase II (cN-II)		high *Km* 5'-NT; purine 5'-NT; GMP, IMP-specific 5'-NT	Nucleotidase reaction: IMP, dIMP, GMP, dGMP, XMP. Phosphotransfer reaction: inosine and Deoxyinosine
cytosolic 5'-nucleotidase III (cN-III)		PN-I; P5'N-1; UMPH	Nucleotidase reaction: CMP, UMP, dUMP, dCMP, dTMP. Phosphotransfer reaction: Uridine, Cytidine, Deoxycytidine
cytosolic 5'(3')-nucleotidase (cdN)		dNT-1; PN-II	3'-dUMP, 3'-dTMP, 3'-UMP, 2'-UMP, 5'-dIMP, 5'-dUMP, 5'-dCMP,5'-dTMP, 5'-dAMP
mitochondrial 5'(3')-nucleotidase (mdN)		dNT-2	5'-dUMP, 5'-dTMP, 3'-dTMP, 5'-, 3'-, 2'-dUMP

Table 1. Classification of 5'-endonucleotidases. Modified from (Bianchi et al., 2003).

Three different cytosolic 5'-nucleotidases cN-I, cN-II, and cN-III have been described; among them, the AMP-selective 5'-nucleotidase (cN-I) mostly accounts for adenosine during enhanced oxygen demand or metabolic load. Two different isoforms of cN-I enzyme exist: cN-IA and cN-IB. cN-IA is able to hydrolyse several substrates but has a preference for AMP. It has been purified from a variety of different tissues including the brain. cN-IB is less characterized and has the lowest expression in the brain (Sala-Newby et al., 2001).

Adenosine can be formed intracellularly from AMP not under basal physiological normoxic conditions but only under different stress conditions (i.e. depolarization, ischemia/hypoxia, seizures) when AMP concentrations increases because of ATP breakdown (Sala-Newby et al., 2000). Only in these conditions, AMP concentration reaches a value in the range of cN-I affinity. In fact the Km of cN-I for AMP (1.2–8.3 mM) is higher than the intracellular concentrations of AMP that have been evaluated under physiological conditions (in rat brain 30–40 μM) (Traut, 1994). Therefore, a massive production of adenosine can occur only in response to a condition of energy imbalance when ATP consumption exceeds the capacity of its own re-synthesis, with a consequent production of adenosine (Dunwiddie et al., 1997).

Typically, in pathological conditions such as ischemia when the energy imbalance is massive, the resulting massive AMP production is the key mechanism that accounts for adenosine formation (Melani et al., 2006 a). In this condition, adenosine cannot be efficiently re-phosphorylated to ATP due to the lack of oxygen and glucose.
In addition, since ATP and ADP inhibit the 5'-endonucleotidase activity, a reduction in the intracellular concentration of ATP and ADP leads to the enzyme activation (Meghji, 1993).

1.1.2 5'Ectonucleotidases

Ecto-5'-nucleotidase (CD73 or 5'-NT) is the enzyme that catalyses the terminal dephosphorylation of 5'–AMP. 5'-ectonucleotidases allow the transformation of the nucleotides released outside the cell into adenosine. These enzymes are homodimers anchored to the plasma membrane through a molecule of glycosyl–phosphatidylinositol. Their catalytic site faces the extracellular space where 5'-ectonucleotidases exert their enzymatic action. CD73 hydrolyses several nucleosides 5'–monophosphate such as: CMP, UMP, GMP, IMP and AMP. On the basis of the Vmax/Km ratio, AMP is the most efficient (Naito et al., 1981) and physiological substrate (Zimmermann, 1992 a), with adenosine being the major nucleoside produced by CD73 (Hunsucker et al., 2005). In fact, CD73 is the major enzyme responsible for the formation of extracellular adenosine from released adenine nucleotides (Zimmermann, 2000).
CD73 is ubiquitously expressed both in central and peripheral nervous system. In CNS it is expressed poorly in proximity of neurons in comparison to glial cells: astrocytes, oligodendrocytes and microglia (Kreutzberg et al., 1978; Zimmermann, 1992 b; Schoen et al., 1993; Resta et al., 1998; Spychala, 2000) . Pathological conditions such as ischemia and hypoxia are major inducers of CD73 expression (Kitakaze et al., 1996; Braun et al., 1997; Ledoux et al., 2003). Moreover, the modulation of the adenosine formation is central since it impacts on neurotransmission processes and CD73 has been reported as the major regulator of the adenosinergic signaling in the mouse brain (Kulesskaya et al., 2013).
CD73 action is limited by ATP and ADP, that act as inhibitors with a *Ki* in the low micromolar range (Zimmermann, 1992 b). In fact, in striatal cholinergic synapses it has been shown that ATP, released in response to a high frequency stimulation, can be degraded to adenosine only at the end of the stimulation, when the concentrations of ATP and ADP decrease under the threshold of inhibition of the enzyme (James et al., 1993).

1.2 Adenosine release

The mode of adenosine release may differ depending on experimental conditions. After electrical stimulation *in vitro*, adenosine seems to originate directly from cells, while in ischemic experimental models *in vitro*, adenosine might have also an extracellular origin due to the degradation of the adenosine nucleotides released following changes in membrane permeability (Pedata et al., 1993).

Adenosine concentrations are regulated by a bidirectional flow mediated by transporters. These transporters are divided into two categories: those capable of a bidirectional transport across the plasma membrane of both purines and pyrimidines, according their concentration gradient (equilibrative transporters); and transporters that mediate the nucleotide influx thank to the coupling with sodium transporters (concentrative). Both transporters have been cloned and classified as: ENT1 and ENT2, equilibrative transporters; CNT1 and CNT2, concentrative transporters (Williams et al., 1991; Anderson et al., 1996; Baldwin et al., 1999). The equilibrative transporters work bidirectionally in order to maintain the intracellular and extracellular concentrations of adenosine in a range of 30-300 nM (Dunwiddie et al., 1997). Inhibitors of adenosine equilibrative transporters such as dypiridamole might increase or decrease the efflux of adenosine differently according to the physiopathological conditions. Under *in vivo* physiological conditions dypiridamole increases adenosine efflux from the striatum indicating that the equilibrative carrier transports adenosine into cells (Pazzagli et al., 1993; Melani et al., 2012). Conversely, under conditions of energetic imbalance, dypiridamole decreases extracellular adenosine indicating that the equilibrative carrier transports adenosine out of the cells (Latini et al., 2001 a).

It has been observed that tetrodotoxin (TTX) completely blocks adenosine release in brain slices electrically stimulated and that a reduction of extracellular Ca^{2+} concentration can cause a remarkable reduction of adenosine release (Pedata et al., 1990). Most recently it was demonstrated that adenosine efflux occurring *in vivo* from the striatum under normoxic physiological conditions does not arises from extracellular degradation of nucleotides and is not inhibited by dypiridamole (Melani et al., 2012 a). Under these conditions the efflux of adenosine is Ca^{2+}sensitive (Melani et al., 2012 a) and is inhibited by TTX (Pazzagli et al., 1993; Dobolyi et al., 2000). On the all observations *in vitro* and *in vivo* led to speculate that adenosine efflux under normoxic physiological conditions is consequent to electrical activity propagated along the typical modality of nervous cells that involves activity of voltage-gated Na^{+} channels. In fact, the TTX sensitivity and the Ca^{2+} dependency of adenosine release indicate that

adenosine release occurs by an excitation-secretion modality which is typical of neurotransmitters. Observation that the presence of adenosine is associated with intracellular vesicles support the notion that adenosine is stored in vesicles and released by exocytosis in an excitation-secretion modality typical of neurotransmitters (Corti et al., 2013).

1.3 Adenosine catabolism

Adenosine metabolism is regulated by two enzymes: adenosine deaminase (ADA) and adenosine kinase (AK) (Figure 2). ADA degrades adenosine to inosine having a Km in the range of 17–45 μM (Phillips et al., 1979) and it is present both at intracellular and at extracellular sites where is anchored to the plasma membrane (Franco et al., 1986).

AK is an enzyme that converts adenosine to AMP, it is characterized by high specificity and a Km for adenosine of 2 μM (Phillips et al., 1979).

Since the basal endogenous levels of adenosine are in the nanomolar range, it is likely that under physiological conditions the main degradation pathway is the phosphorylation operated by AK, while the action of extracellular ADA is important only when significant increases in adenosine concentration occur, such as during ischemia (Latini et al., 2001).

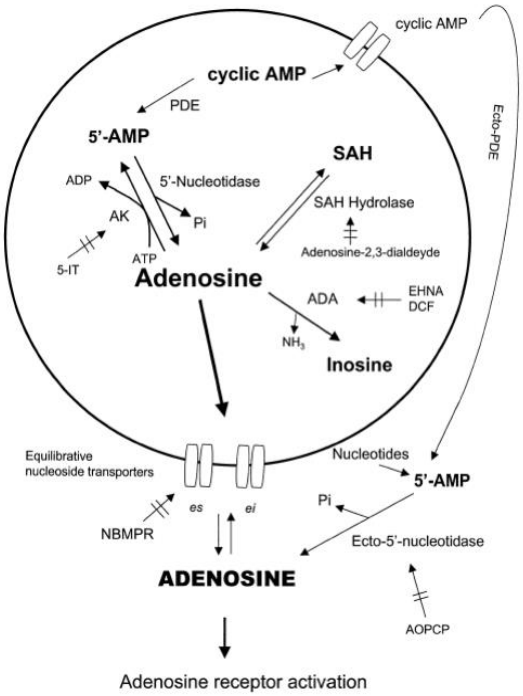


Figure 2. Adenosine synthesis, catabolism and transport. Adenosine is produced intracellularly by AMP dephosphorylation or by SAH hydrolysis and then released from the cell through specific membrane transporters, or extracellularly by ATP hydrolysis. Adenosine is deaminated by ADA and converted to inosine. ADA, adenosine deaminase; AK, adenosine kinase; AOPCP, α, β-methylene ADP; DCF,

deoxyicoformicin; EHNA, erythro-9-(2-hydroxy-3-nonyl) adenosine; es, sensitive transporter ei, insensitive transporter; 5-IT, 5-iodotubercidin; NBMPR, nitrobenzylthioinosine; PDE, cAMP-phosphodiesterase; SAH, S-adenosyl-homocysteine. From (Latini et al., 2001).

1.4 Adenosine receptors

Adenosine receptors were initially distinguished into two receptor classes: A_1 and A_2 on the basis of their excitatory or inhibitory actions on adenylyl cyclase (van Calker et al., 1979). Subsequently, molecular cloning and pharmacological studies have identified four subtypes of adenosine receptors: A_1, A_{2A}, A_{2B} and A_3 receptors (Fredholm et al., 2001).

All adenosine receptors have been cloned from rat, mouse and human tissues (Fredholm et al., 2001). Key features for their classification are their amino acid sequence, the coupled G-protein and the affinity for the endogenous ligand.

All receptors are metabotropic heteromeric G-protein coupled receptor (GPCR). Typically, their structure is formed by a polypeptide chain characterized by 7 transmembrane hydrophobic domains with α helix structure (7TM, helices 1-7) of approximately 25 residues followed by one short membrane-associated helix (helix 8). TM domains are closely associated by three extra-cellular loops (ECL1-3) and three intracellular loops (ICL1-3) (Cristalli et al., 2008). Biochemical experiments and computational approaches have revealed the importance of the TM3 and TM7 in binding the endogenous ligand and agonist molecules (Rivkees et al., 1995). All adenosine receptors present an extra-cellular amino terminus (N-terminus) and a cytosolic carboxy terminus (C-terminus), (Cristalli et al., 2008) (Figure 3). The extracellular N-terminus contains one or more glycosylation sites, while the intracellular C-terminus provides sites for phosphorylation and palmitoylation, thereby playing a role in receptor desensitization and internalization mechanisms (Borea et al., 2018). Recently, details of the structures of human A_1 and A_{2A} receptors have been provided by X-ray crystallography (Jaakola et al., 2008; Lebon et al., 2011; Carpenter et al., 2016; Glukhova et al., 2017) and by electron cryo-microscopy (cryo-EM) studies (Draper-joyce et al., 2018; García-Nafría et al., 2018), which are useful to obtain new GPCR structural information and in the structure-based drug design of A_1 and A_{2A} receptors ligands (Glukhova et al., 2017; Sun et al., 2017).

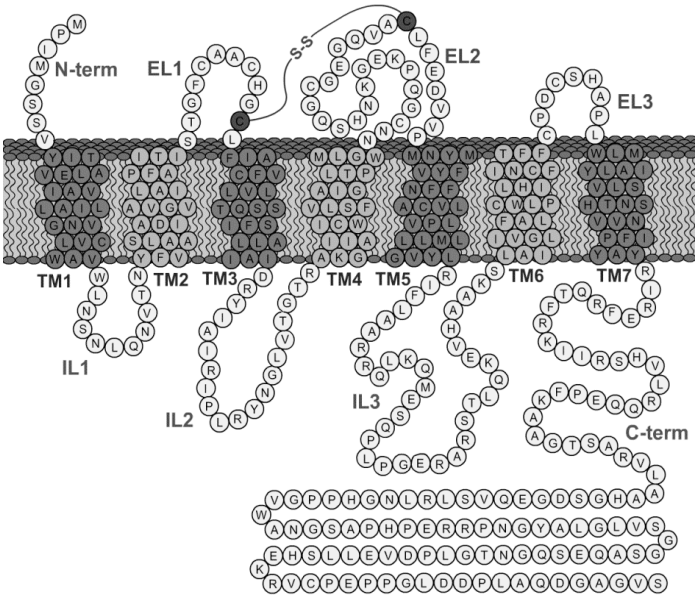


Figure 3. Schematic representation of the molecular structure of the A_{2A} receptor. From (Cristalli et al., 2008).

A_1, A_{2A}, A_{2B} amino acid sequence is well conserved among mammalian species, except for A_3 receptors (Burnstock et al., 2011). This subtype is the latest cloned and pharmacologically characterized (Linden et al., 1993), and presents a considerable structural variability among different species. For instance, almost 30% difference in the amino acid sequence is found between humans and rat (Linden, 1994).

Adenosine receptors transduce signals via G proteins. Hence, A_1 and A_3 subtypes are associated with Gi activation, adenylyl cyclase inhibition and decrease of intracellular cAMP levels, while A_{2A} and A_{2B} receptors are linked to Gs proteins that activate the same enzyme increasing cAMP concentration in the cytosol. However, adenosine receptors have also been reported to couple to other G-proteins, modulating different second messenger systems. For instance, in addition to their effects on adenylate cyclase (and contrary to adenosine A_{2A} receptors) adenosine A_1, A_{2B} and A_3 receptors are also characterized by their stimulatory effect on PLC (Abbracchio et al., 1995; Feoktistov et al., 1997) while A_1 and A_3 receptors can also activate PLD (Fredholm et al., 2001).

In addition, by a direct G protein-channel interaction or by second messenger systems adenosine receptor stimulation also modulates several types of Ca^{2+} and K^+ channels (Figure 4).

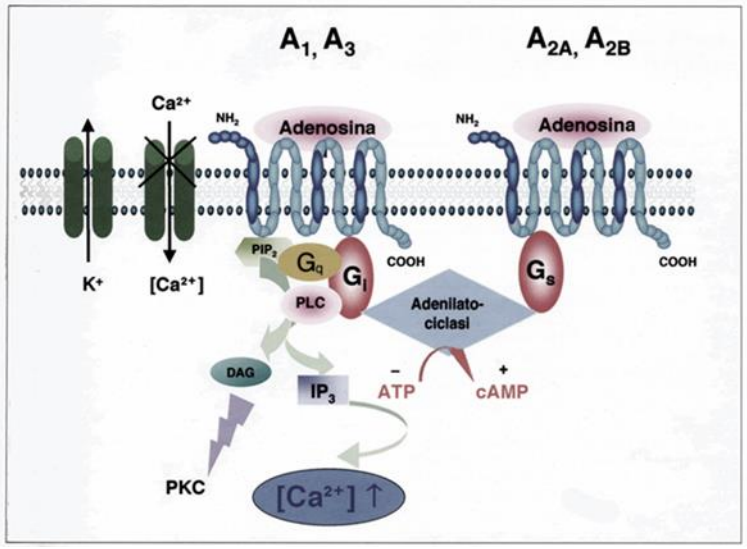


Figure 4. Adenosine receptors and signal transduction mechanisms. Adenosine receptors are G protein-coupled receptors. The A_1 and A_3 subtypes inhibit adenylate cyclase via Gi protein, while the A_{2A} and A_{2B} receptors mediate stimulation of the enzyme via Gs protein. In addition, A_1, A_3 and A_{2B} receptors can modulate, by Go protein or Gq, phospholipase activity, inducing the production of diacylglycerol (DAG) and inositol-triphosphate (IP3) and an increase in Ca^{2+} release from intracellular deposits.

Another key feature for adenosine receptor subtypes classification is the affinity for their endogenous ligand. A_1, A_{2A} and A_3 receptors have high affinity for the endogenous ligand, being activated by nanomolar concentrations of adenosine (Fredholm et al., 2011). On the contrary, the affinity values of A_{2B} receptors for adenosine evaluated in binding and functional experiments are higher than 1 µM (Fredholm et al., 2011). Under physiological conditions, extracellular adenosine concentrations are estimated in the range of 30 to 200 nM (Latini et al., 2001). These levels are sufficient to activate A_1, A_{2A} and A_3 receptors subtypes, but not A_{2B}, which require higher concentrations (micromolar range) of adenosine to be activated. Such higher adenosine concentrations are only reached under pathological conditions, such as during hypoxia or ischemia *in vivo* (Pedata et al., 2001) and *in vitro* (Latini et al., 1999).

1.4.1 A_1 Receptor: molecular structure, distribution and function

The A_1 receptor is a monomeric glycoprotein of 35-36 kDa formed by 326 amino acids. It is highly conserved with an 87%-92% homology between different species. It is coupled with the $G_{i/0}$ protein at the TM3 loop. The different effects of the stimulation of $G_{i/0}$ are mediated by both α subunit and βγ complex (Birnbaumer, 1992). A_1 receptor mediates: inhibition of adenylate cyclase, activation of several types of K^+ channels (probably via β,γ-subunits), inactivation of N, P and Q-type Ca^{2+} channels, stimulation of phospholipase C (PLC)

(Burnstock et al., 2011), and stimulation of phospholipase D (PLD) (Fredholm et al., 2001) (Figure 5).

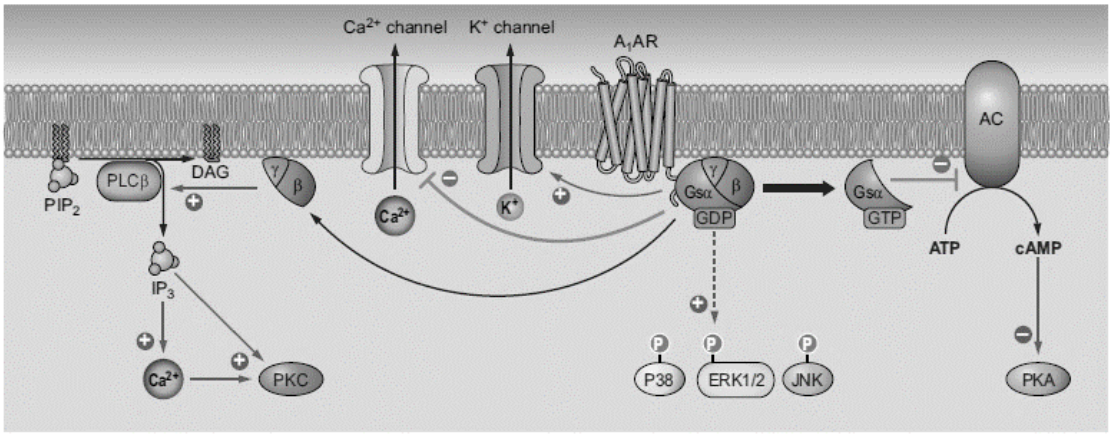


Figure 5. Overview of adenosine A_1 receptor (A_1AR) intracellular signaling pathways. A_1AR stimulation decreases adenylate cyclase (AC) activity and cAMP production, thus inhibiting protein kinase A (PKA), while activated phospholipase C (PLC)-β, and Ca^{2+}. K^+ and Ca^{2+} channels are opened and closed, respectively, by A_1AR enrollement. Mitogen-activated protein kinases p38, ERK1/2, and JNK1/2 phosphorylation are induced by A_1AR activation. Modified from (Borea et al., 2018).

The highest levels of A_1 receptor expression are found in the CNS, suggesting a particularly important role of adenosine in brain functions. In rat the highest expression of A_1 receptors has been found in the cortex, hippocampus, cerebellum and dorsal horn of the spinal cord; intermediate levels in basal ganglia structures including the striatum (Dixon et al., 1996). mRNA encoding A_1 receptors is present in large striatal cholinergic interneurons (Dixon et al., 1996) and in neurons of the globus pallidus, subthalamic nucleus and thalamus. An abundant expression of adenosine A_1 receptor also occurs in the trigeminal ganglia, supporting a role of this receptor in pain regulation (Schindler et al., 2001). Neuronal A_1 receptors are localized both pre- and post-synaptically (Deckert et al., 1988). In the hippocampus subcellular analysis of nerve terminals revealed that A_1 receptor immunoreactivity is strategically located in the active zone of presynaptic terminals, as expected on the basis of the ability of A_1 receptor agonists to depress neurotransmitter release. It has also been demonstrated that A_1 receptor immunoreactivity is evident at postsynaptic sites together with NMDA receptor subunits NR1, NR2A and NR2B and with N-and P/Q-type Ca^{2+} channel immunoreactivity, emphasizing the importance of A_1 receptors in the control of dendritic integration A_1 receptors can be found also extrasynaptically on dendrites (Rivkees et al., 1995) and on the axonal fibres of the hippocampus (Swanson et al., 1995). Activation of A_1 receptors along the axon may be a powerful extra synaptic mechanism by which adenosine alters axonal electric transmission to inhibit neurotransmitter release (Swanson et al., 1995).

Under physiological conditions A_1 receptors mediate sedative-hypnotic, anti-epileptic and anti-nociceptive effects (Dunwiddie, 1985) by exerting a tonic inhibition of synaptic transmission both *in vitro* and *in vivo*. This has been demonstrated in several brain regions containing a high concentration of A_1 receptors such as the hippocampus, but also in striatum and olfactory cortex (Von Lubitz, 1999; Latini et al., 2001). The inhibitory effect of adenosine A_1 receptor stimulation has a pre- and post-synaptic component. At pre-synaptic level, the activation of A_1 receptor reduces Ca^{2+} influx through the preferential inhibition of N-type and, probably, Q-type channels (Yawo et al., 1993; Wu et al., 1994), with a consequent decrease in transmitters release (Prince et al., 1992). In fact, adenosine, by stimulation of A_1 receptor, has been found to inhibit the release of all classic neurotransmitters: glutamate, acetylcholine, dopamine, noradrenaline and serotonin (Fredholm et al., 1988). In particular, a powerful suppression of glutamate release from presynaptic terminals has been described in the hippocampus (Corradetti et al., 1984; Burke et al., 1988).

At post-synaptic level, A_1 receptors mediate a direct hyperpolarization of neurones via activation of G-protein-coupled inwardly rectifying potassium (GIRK) channels (K_{ir} 3.2 and 3.4 channels) (Takigawa et al., 1999, 2002) and by a direct increase of Cl^- conductance (Greene et al., 1991) thus, stabilizing the membrane potential.

Out from CNS, high levels of A_1 receptor expression are found in adrenal glands, eye and atria. Intermediate levels are found in skeletal muscles, liver, kidney, adipose tissue, gastrointestinal smooth muscles and bronchi. Lung and pancreas present low level of A_1 receptor expression (Fredholm et al., 2001).

Pharmacology

Selective agonists for the A_1 receptor have been synthesized from adenosine molecule by modifications on its purine ring or its ribose. By substitution of adenosine in N6 position CPA (N6-cyclopentyladenosine), a compound with greater affinity for A_1 receptor, has been obtained. Its derivative CCPA (2-chloro-N6-cyclopentyladenosine) presents a slightly higher affinity for A_1 receptor in human, rats and mouse tissues and is active at nanomolar concentrations. Further agonist for A_1 receptor analogues of adenosine are CADO (2-chloroadenosine), L-PIA ((R)-N-(1-methyl-2-phenylethyl) adenosine), CHA (N(6)-cyclohexyladenosine) and R-PIA (N6-R-phenylisopropyladenosine) (Jacobson et al., 1992). Allosteric enhancers for this receptor subtype are also available such as PD81,723 (2-amino-3-benzoylthiophene) and analogues. The discovery and development of pyridine-based non-nucleoside agonists of the adenosine receptors started from the publication of patents from

Bayer describing pyridine derivatives endowed with agonist activity at the adenosine receptors. One of these compounds is the adenosine A_1 receptor agonist 2-amino-6-[[2-(4-chlorophenyl)-1,3-thiazol-4-yl] methylsulfanyl]-4-[4-(2-hydroxyethoxy) phenyl] pyridine3,5-dicarbonitrile, also named BAY68-4986 or Capadenoson. Capadenoson, currently classified as an adenosine A_1 receptor partial agonist, has undergone two phase II clinical trials, initially in patients with atrial fibrillation and subsequently in patients with stable angina (Tendera et al., 2012). Structural modifications at the exocyclic amine group of Capadenoson led to the development of the adenosine A_1 receptor agonist 2-(((2-(4-chlorophenyl)-1,3-thiazol-4-ylmethyl) sulfanyl)-4-(4-(2-hydroxyethoxy) phenyl)-6-(pyrrolidin-l-yl) pyridine-3,5-dicarbonitrile, also named Neladenoson. Like Capadenoson, Neladenoson is a selective partial agonist at the A_1 receptor (Meibom et al., 2017; Voors et al., 2017). Neladenoson showed in rat preclinical models cardioprotective effects analogous to Capadenoson, although with lower central effects. Methylxanthines are non-selective antagonists of adenosine receptors (Sattin et al., 1970). Antagonists with high affinity have been synthesized from theophylline, by substitution in position 8 with a cycloalkyl group (e.g. compounds 8-CPT, 8-cyclopentyltheophilline and 8-PT, 8-phenyltheophilline). By substitution of the methyl groups in 8-CPT with propyl groups DPCPX (8-Cyclopentyl-1,3-dipropylxanthine), a selective antagonist for A_1 receptor with high affinity, has been synthetized (Bruns et al., 1987). The substitution of the propyl group in position 3 of DPCPX with a more complex group led to the synthesis of BW-A884U (3-(4-Amino)phenethyl-1-propyl-8-cyclopentylxanthine) (Patel et al., 1988), a compound more selective than DPCPX. Other derivatives as KFM 19 (8-(3-oxocyiclopentane)-1,3-dipropylxanthine), KW-3902 (Rolofylline; 8-(Hexahydro-2,5-methanopentalen-3a(1*H*)-yl)-3,7-dihydro-1,3-dipropyl-1*H*-purine-2,6-dione), KF 15372 (8-dicyclopropylmethyl-1,3-dipropylxanthine) and ENBX (1,3-dipropyl-8-endonorbornyl-xanthine), poorly soluble in water, were obtained from theophylline performing substitutions in position 8 (Jacobson et al., 1992).

1.4.2 A_{2A} Receptor: molecular structure, distribution and function

A_{2A} receptor is a glycoprotein of approximately 45 kDa with an amino acid sequence highly conserved with a homology of 90% between different species.

Its intracellular section is particularly extended (412 amino acids in 4 loops) and it seems to be involved in several responses A_{2A}-mediated (Cristalli et al., 2008). The receptor is associated to a Gs protein at TM2 and TM3 loops (Moreau et al., 1999), it stimulates adenylate cyclase

and increases intracellular cAMP concentrations (Moreau et al., 1999) and mobilizes the intracellular Ca^{2+} (Mirabet et al., 1997) (Figure 6).

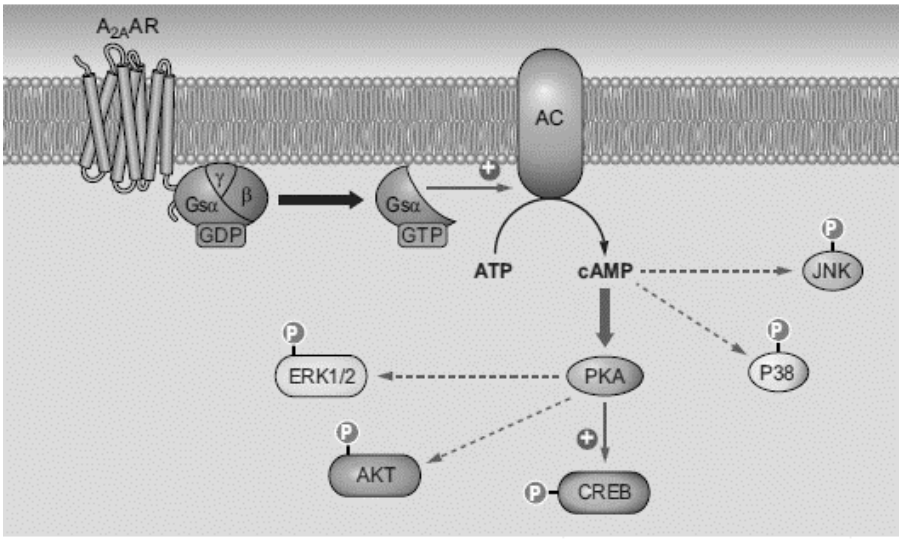


Figure 6. Overview of adenosine A_{2A} receptor ($A_{2A}AR$) intracellular signaling pathways. $A_{2A}AR$ stimulation increases adenylate cyclase (AC) activity, cAMP production, protein kinase A (PKA), and cAMP-responsive element-binding protein (CREB) phosphorylation. AKT and mitogen- activated protein kinases p38, ERK1/ 2 and JNK1/2 are activated following by $A_{2A}AR$ recruitment. Modified from (Borea et al., 2018).

A_{2A} receptors are expressed throughout all the CNS (Dixon et al., 1996). They are principally located in the basal ganglia: in the caudate-putamen, nucleus accumbens and olfactory tubercle (Jarvis et al., 1989; Rosin et al., 1998). In particular, this receptor subtype is expressed on striatopallidal GABAergic-enkephalin neurones (where it co-localises with dopamine D_2 receptors, but not on GABAergic-dynorphin striatal neurones (Fredholm et al., 2003 b). High levels of adenosine A_{2A} receptor mRNA have been found in striatum (Schiffmann et al., 1991; Svenningsson et al., 1997), while lower levels have been found in hippocampus and cortex (Dixon et al., 1996; Svenningsson et al., 1997). Besides post-synaptically, A_{2A} receptors are also located pre-synaptically on different GABAergic, cholinergic, glutamatergic neuron types (Hettinger et al., 2001; Rosin et al., 2003). In the CNS they are also expressed on astrocytes (Biber et al., 1999; Lee et al., 2003), microglia (Pedata et al., 2014), oligodendrocytes (Stevens et al., 2002), blood cells and vasculature (Phillis, 2004).

In recent years, particular interest has been dedicated to study receptor dimerization, either in homomeric and heteromeric structures, since this phenomenon seems to frequently occur in numerous cell types and can modify the pharmacological profile of receptors and their functional role. Various lines of evidence indicate that such an interaction occurs post-

synaptically in the striatum between A_{2A} adenosine and D_2 dopamine receptors, this heterodimerization inhibits D_2 receptor functions (Ferre et al., 1991). While A_1 receptor and A_{2A} receptor heteromers are located pre-synaptically in glutamatergic terminals of the striatum (Ciruela et al., 2006), exerting opposite effects on the modulation of glutamate release through a "concentration-dependent switch" mechanism by which low adenosine concentrations inhibit, while high concentrations stimulate, glutamate release (Ciruela et al., 2006).

In the nervous system, A_{2A} receptor activation mediates excitatory actions, contrary to the A_1 receptor that exerts synaptic inhibition (Pedata et al., 1984; Spignoli et al., 1984; Latini et al., 1996 c; Sebastião et al., 1996). Electrophysiological investigations about the role of A_{2A} receptors under physiological conditions have shown that they increase synaptic neurotransmission. In fact, the A_{2A} receptor G_s-mediated signalling increases cAMP level, a second messenger that activates protein kinase A (PKA) (Gubitz et al., 1996). PKA phosphorylates the Ca^{2+} channels of P type and thus, directly modulates the mechanisms of vesicle release. Vesicle exocytosis can also be regulated by βγ subunits of the Gs protein, which can promote the phosphorylation, mediated by PKC, of Ca^{2+} channels of N-type (Gubitz et al., 1996; Gonçalves et al., 1997). In fact, in the hippocampus *in vitro*, A_{2A} receptor stimulation results in a Ca^{2+}-dependent release of acetylcholine (Spignoli et al., 1984; Cunha et al., 1995). Moreover, the selective stimulation of adenosine A_{2A} receptors augments the amount of glutamate released in hippocampus and striatum of young rats (Popoli et al., 1995; Corsi et al., 1999, 2000), supporting the theory about that A_{2A} receptors increase excitatory amino acid release.

A_{2A} receptor also impacts in long-term potentiation (LTP), a form of synaptic plasticity associated with memory, central for learning process, that is increased by A_{2A} receptor activation (Almeida et al., 2003). In fact, a decreased LTP in the nucleus accumbens was found in knock-out (KO) mice for the A_{2A} receptor (d'Alcantara et al., 2001).

It is worth noticing that the role of A_{2A} receptors in the striatum is recently gaining interest in light of their heterodimerization with D_2 dopamine receptors. The association between A_{2A} and D_2 receptors results in an antagonistic interaction which provided a rationale for evaluating A_{2A}-selective antagonists in Parkinson's disease. It was suggested that A_{2A} antagonists not only provide symptomatic relief but also decelerate dopaminergic neuron degeneration in patients (Xu et al., 2005). On July 2019, A_{2A} antagonists have been approved in the United States by FDA (Food and Drug Administration) in the therapy of Parkinson's disease (*fda.gov*) (Takahashi et al., 2018).

A_{2A} receptors are also highly present in spleen, thymus, immune cells both on cells of innate (macrophages, mast cells, monocytes, dendritic cells, and granulocytes) and on cells of adaptive (lymphocytes) immunity (Haskó et al., 2008; Antonioli et al., 2014). Lower levels are also found in the heart, lung and blood vessels (Fredholm et al., 2001).

Pharmacology

Most of A_{2A} receptors agonists have been obtained from the adenosine molecule by substitution of the purine ring in the position 2 or by different types of substitutions at the ribose ring. By the replacement of an alchilamide group in position 5' of the ribose ring the agonists NECA (5'-N-ethylcarboxamidoadenosine) and CGS21680 have been synthetized. NECA was long considered to be a selective A_2 agonist but it has been largely demonstrated that it is an unselective agonist to all P1 receptors, with a slightly preference for A_{2A} subtype. However, based on evidence that 2-substitution of NECA molecule increased selectivity, CGS21680 was developed as an A_{2A} selective agonist. This compound is less potent and selective in humans than in rats, but it has been replaced by another recently developed A_{2A} agonist, ATL-146e (4-(3-[6-amino-9-(5-ethylcarbamoyl-3,4-dihydroxy-tetrahydro-furan-2-yl)-9H-purin-2-yl]-prop-2-ynyl)-cyclohexanecarboxylic acid methyl ester), which is 50 fold more potent than CGS21680 for the human A_{2A} receptor by substitution in position N6, the compound DPMA has been obtained (Bridges et al., 1988).

Many of A_{2A} receptor antagonists have been developed from the caffeine (1,3,7-trimethylxanthine) molecule. DPMX (1,3-dipropyl-7-methyl-xanthine) and 8-cicloesilcaffeine are low selective A_{2A} receptor antagonists (Williams et al., 1991). CSC (Jacobson et al., 1993) and KF17837 ((E)-8-(3,4-dimethoxystyryl)-1,3-dipropyl-7-methylxanthine) (Nonaka et al., 1994) are more selective but less easy to use since undergo to photoisomerization. Among the numerous A_{2A} antagonists, the most selective so far are SCH58261 (7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolol[1,5-c]pyrimidine) and SCH442416 (2-(2-Furanyl)-7-[3-(4-methoxyphenyl)propyl]-7*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5-amine) and the structurally related ZM241385 (4-(-2-[7-amino-2-{2-furyl}{1,2,4}triazolo{2,3-a}{1,3,5}triazin-5-yl amino]ethyl)phenol). SCH58261 does not derive from xanthine, as well as CP66713 (8-chloro-1-phenyl- [1,2,4] triazolo[4,3-a] quinoxalin-4-amine). However, also CP66713 presents a good selectivity for the A_{2A} receptor (Sarges et al., 1990).

1.4.3 A_{2B} Receptor: molecular structure, distribution and function

The A_{2B} receptor is a glycoprotein of approximately 36-37 kDa, coupled to Gs protein, that stimulates adenylate cyclase, intracellular Ca^{2+} mobilization (Mirabet et al., 1997) and activates PKC and PLC (Abbracchio et al., 1995; Feoktistov et al., 1997).

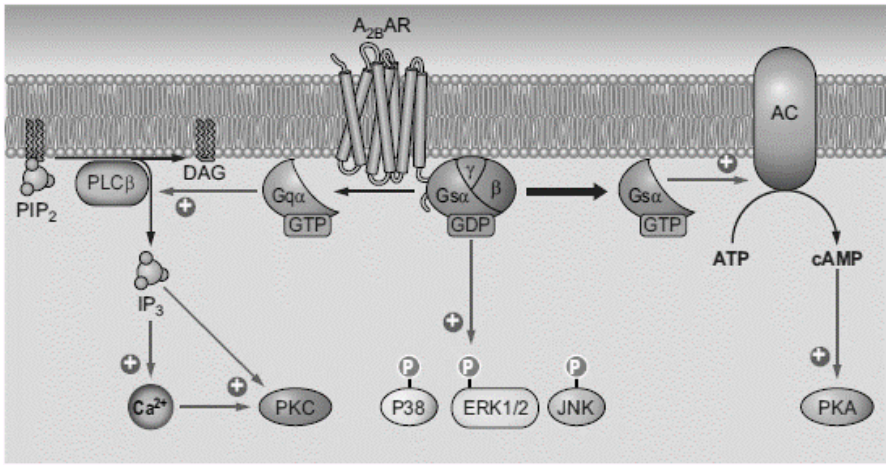


Figure 7. Overview of adenosine A_{2B} receptor (A_{2B}AR) intracellular signaling pathways. A_{2B}AR stimulation increases adenylate cyclase (AC) activity, cAMP production, and protein kinase A (PKA) phosphorylation. A_{2B}AR enrollment activates phospholipase C (PLC)-β and increases Ca^{2+}. Mitogen-activated protein kinases p38, ERK1/2, and JNK1/2 phosphorylation are induced by A_{2B}AR activation. Modified from (Borea et al., 2018).

A_{2B} receptors are expressed both in the CNS and in the peripheral nervous system (PNS) (Dixon et al., 1996). A_{2B} receptors are shown to be ubiquitously distributed in the brain and their mRNA has been detected in all rat cerebral areas studied with a prevalent presence on hippocampal neurons (Mogul et al., 1993; Kessey et al., 1997) and on glial cells (Peakman et al., 1994; Fiebich et al., 1996).

At peripheral level, A_{2B} subtype is particularly abundant in the gastrointestinal tract, mainly in caecum, colon, urinary bladder, lung, blood vessels and adipose tissue (Fredholm et al., 2001). Increasing evidences indicate a role for this receptor in the modulation of inflammation and immune responses in selected pathologies like cancer, diabetes, as well renal, lung and vascular diseases (Borea et al., 2018).

A_{2B} receptors appear to induce the release of excitatory amino acids and acetylcholine, while they reduce the release of γ-amino butyric acid (GABA) in rat cortex (Phillis et al., 1993). In addition, at hippocampal level, they modulate the LTP process (Kessey et al., 1997). The signaling pathways generated by A_{2B} receptors stimulation are strongly influenced by the signaling of other receptors that affect the PLC-Ca^{2+}-PKC pathway. In brain slices, cAMP

accumulation due to the activation of the A_{2B} receptor, is markedly increased by drugs that stimulate PKC (Fredholm et al., 1987; Hollingsworth et al., 1985).

Pharmacology

A class of non-adenosine A_{2B} agonists with affinity values in the low nanomolar range has been synthesised by Beukers and colleagues as pyridine derivatives (Beukers et al., 2004). Among them the most important are LUF5834 (2-amino-4-(4-hydroxyphenyl)-6-(1H-imidazol-2-ylmethylsulfanyl)pyridine-3,5-dicarbonitrile) (Beukers et al., 2004) and BAY60-6583 (2-[[6-Amino-3,5-dicyano-4-[4-(cyclopropylmethoxy)phenyl]-2-pyridinyl]thio]-acetamide) (Kuno et al., 2007; Eckle et al., 2008 a) that are highly selective. LUF5835 (2-amino-4-(4-hydroxyphenyl)-6-(1H-imidazol-2-ylmethylsulfanyl) pyridine-3,5-dicarbonitrile,17) is a full agonist with an EC_{50} of 10 nM for human A_{2B} receptor expressed in CHO cells.

Several potent and relatively selective antagonists have been found among anilide derivatives of xanthines with K_i values in the low nanomolar range, such as MRS 1754 (*N*-(4-Cyanophenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1*H*-purin-8-yl)phenoxy]-acetamide) (Ji et al., 2001), that is over 200-fold selective for A_{2B} versus all other adenosine receptors (Kim et al., 2000) and the selective A_{2B} antagonist 8-[4-[4-(4-Chlopehenzyl) piperazide-1-sulfonyl) phenyl]]-1-propylxanthine (PSB603) (Fusco et al., 2018).

Further antagonists xanthine-derived are MRE 2029-F20 (N-(2H-1,3-benzodioxol-5-yl)-2-{[5-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1H-purin-8-yl)-1-methyl-1H-pyrazol-3yl] oxy}acetamide) and OSIP339391 (N-[2-[[2-phenyl-6-[4-(3-phenylpropyl) piperazine-1-carbonyl]-7H-pyrrolo[3,2-e]pyrimidin-4-yl]amino]ethyl]acetamide) (Kim et al., 2001; Stewart et al., 2004; Gessi et al., 2005).

1.4.4 A_3 receptor: molecular structure, distribution and function

A_3 receptor is a protein of approximately 39 kDa, coupled to G_i protein. It evokes the same effects mentioned for A_1 receptor (Englert et al., 2002), induces Ca^{2+} mobilization (Englert et al., 2002; Shneyvays et al., 2004, 2005) and interacts with MAPKs (Schulte et al., 2003) (Figure 8).

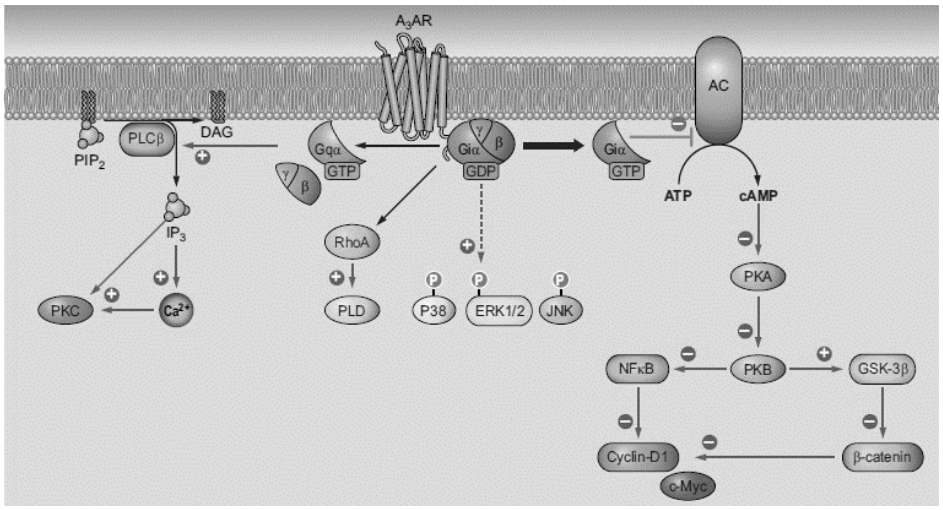


Figure 8. Overview of adenosine A_3 receptor (A_3AR) intracellular signaling pathways. A_3AR stimulation triggers decrease of adenylate cyclase (AC) activity and cAMP production, activation of glycogen synthase kinase-3β (GSK-3β), and consequent decrease of β -catenin, cyclin D1, and c-Myc. Increase induced by A3AR activation of phospholipase C (PLC)- β and Ca2+, as well as of RhoA and phospholipase D (PLD) is shown. Mitogen-activated protein kinases p38, ERK1/2, and JNK1/2 phosphorylation are induced by A_3AR activation. Modified from (Borea et al., 2018).

The expression of A_3 adenosine receptor in the brain is generally lower than that of the other subtypes (Ji et al., 1994) and is highly species-dependent (Fredholm et al., 2000, 2001). By the sensitive technique of real time PCR, A_3 receptors are found in both neuronal and non-neuronal elements, i.e. astrocytes, microglia, and vasculature of the cerebral tissue (Zhao et al., 1997) with widespread distribution. In the rat, a significant expression of A_3 receptors is found in cerebellum and hippocampus (Dixon et al., 1996) where they are mainly expressed at the presynaptic level (Lopes et al., 2003).

In literature discrepancies about the role of adenosine A_3 receptors in the CNS are present. An excitatory role of A_3 receptor has been supported by evidence indicating that, in the rat hippocampus, its activation attenuates long-term depression (LTD) and allows induction of LTP elicited by a subliminal weak-burst protocol (Costenla et al., 2001). Additional evidence for an excitatory role of adenosine A_3 receptors came from studies carried out in hippocampal slices (Pugliese et al., 2007). In the same brain area, A_3 receptor activation through a selective agonist has been shown to antagonize the adenosine A_1 receptor-mediated inhibition of excitatory neurotransmission (Dunwiddie et al., 1997). However, further electrophysiological studies refused this hypothesis, since several authors demonstrated that no significant interaction between A_1 and A_3 receptors occurs in the rat cortex and hippocampus (Brand et al., 2001; Lopes et al., 2003) . Conversely, an inhibitory action has been attributed to A_3 receptors by Brand and colleagues, who demonstrated that, in rat cortical neurons, the selective activation

of A_3 receptor is involved in inhibition of excitatory neurotransmission, suggesting a synergic action with the inhibitory effect mediated by A_1 receptor activation.

Despite results obtained by A_3 receptor stimulation, evidence that selective block of A_3 receptors does not affect neurotransmission in the CA1 region of the hippocampus under normoxic conditions, indicates that endogenous adenosine at physiological concentration does not exert tonic activation of A_3 receptors (Dunwiddie et al., 1997; Pugliese et al., 2003).

In the periphery, A_3 subtype is mainly found in rat testis (Meyerhof et al., 1991) and mast cells, in accordance with the fact that for a long time the unique role assigned to this receptor have been mast cell degranulation and histamine release. Intermediate levels are found in the lung, spleen, thyroid and liver. Interestingly, A_3 receptor is overexpressed in several cancer cells and tissues and is therefore likely to have an important antitumoral role (Borea et al., 2014).

Pharmacology

The most widely used A_3 agonist is Cl-IB-MECA (2-Chloro-N^6-(3-iodobenzyl)-adenosine-5′-N-methyluronamide) for its high affinity (low nanomolar range) and selectivity (more than 100-fold versus A_1 and A_{2A} receptors) towards A_3 receptors. For these features Cl-IB-MECA is the most used pharmacological tool to investigate A_3-mediated effects. Cl-IB-MECA affinity does not vary beyond an order of magnitude among mammal species. The new, highly selective, A_3 agonist (1S,2R,3S,4R,5S)-4-(2-((5-chlorothiophen-2-yl)ethynyl)-6-(methylamino)-9H-purin-9-yl)-2,3 dihydroxy-N-methylbicyclo [3.1.0]hexane-1-carboxamide (MRS5980) was synthesized as reported previously (Tosh et al., 2014; Fang et al., 2015).

Particularly potent agonists can be obtained through the 4'-thio modification of adenosine derivatives, such as in LJ 568 (Jeong et al., 2003).

An emblematic feature of the adenosine A_3 receptor is its insensitivity to the antagonistic actions of methylxanthines, such as caffeine and theophylline, the traditional blockers of adenosine receptors. Hence, A_3 antagonists are dihydropyridines, pyridines and flavonoids. Another class of highly selective compounds are isoquinoline and quinazoline derivatives, such as VUF5574 (*N*-(2-methoxyphenyl)-*N*′-[2-(3-pyrindinyl)-4-quinazolinyl]-urea) that presents a K_i value of 4 nM versus human A_3 receptors but not versus the rat isoform. In this regard, it is worth noticing that significant species differences in the affinity of adenosine A_3 receptor antagonists have been noted, as expected from the high structural inter-species variability already mentioned. The affinity values of several A_3 blockers are typically more than 100-fold greater on human than rat receptors, as described for MRS1220 (*N*-[9-Chloro-2-(2-furanyl) [1,2,4]-triazolo[1,5-*c*] quinazolin-5yl] benzene acetamide).

Among the antagonists are also CCPA and DPMA (N(6) -[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)-ethyl]adenosine), whose structures have been used to get the drug MRS1292 which is a selective antagonist of A_3 receptors, both in rat and in humans (Gao et al., 2002; Yang et al., 2005). At least, selective adenosine antagonist is MRS1523 (3-propyl-6-ethyl-5-[(ethylthio) carbonyl]-2 phenyl-4-propyl-3-pyridine carboxylate).

Role of adenosine in oligodendrocyte precursor maturation

1.5 Oligodendrogenesis

Oligodendrocytes are neuroglial cells responsible, within the central nervous system, for myelin sheath formation that provides an electric isolation of axons and accelerates the transmission of electric signals. In order to be able to produce myelin, oligodendroglial cells progress through a series of highly regulated differentiation steps from oligodendrocyte precursor cells (OPC) to mature oligodendrocytes (OLG) (Barateiro et al., 2014).

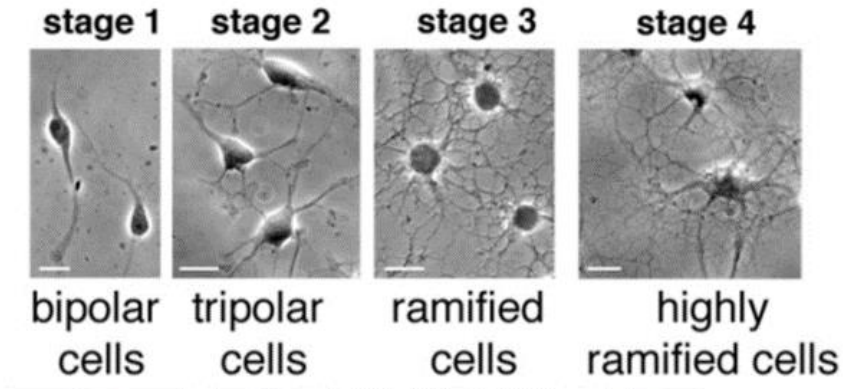
OPC are generated during embryonic development in restricted areas, such as the subventricular zone (SVZ), and present an amazing migration capacity that allow them to spread and populate brain and spinal cord (El Waly et al., 2014). During maturation oligodendroglial cells lose their capacity to migrate and proliferate (Barateiro et al., 2014) and acquire an elaborate morphology with many branched processes, that wrap around axons and form membrane sheaths of myelin, typical of mature OLG (De Castro et al., 2005).

During embryonic development oligodendrocytes are the last cells to be generated after neurons and astrocytes. *In vivo*, differentiation and maturation of oligodendroglial cell are postnatal processes. Rodents (rat and mouse) at postnatal day 2 (P2) present mainly preoligodendrocytes and myelination of the CNS starts only at P7 (Dean et al., 2011).

Oligodendrocyte maturation involves a sequence of cellular transitions: distinct phases of oligodendrocyte maturation can be identified by stage-specific morphological changes and by the expression of stage-specific surface antigens (Raff et al., 1983; Goldman et al., 1986; Levi et al., 1986; Gard et al., 1989; Gallo et al., 1995). It has been observed that the expression of different markers is related to morphological changes of the cell (Fumagalli et al., 2011). This phenomenon has allowed a classification into four stages of differentiation: oligodendrocyte precursor cell (OPC), preoligodendrocyte (or pre-OLG), immature (or pre-myelinating) OLG and mature, myelinating OLG (Barateiro et al., 2014) (Figure 9 A).

The initial stage of maturation (1-2 days in culture), show a bipolar (or tripolar) morphology, typical of OPC (stage 1). OPC are proliferative cells with great migratory capacity and express defined surface antigens, marker of precocious maturation stages, such as platelet-derived growth factor receptor α (PDGFRα), chondroitin sulfate proteoglycan nerve-glial antigen 2 (NG2) and transcription factor Olig2 (Pringle et al., 1992; Nishiyama et al., 1996; Ligon et al., 2006). When OPCs start to differentiate in pre-OLG (stage 2), they are characterized by emerging secondary ramifications and by the expression of intermediate molecular markers such as GPR17 protein (Boda et al., 2011) and O4 antigen (Sommer et al., 1981). At approximately day 6 in culture, they acquire the typical phenotype of immature oligodendrocyte with highly branched morphology (stage 3) (Fumagalli et al., 2011). The expression of the intermediate markers GPR17 and O4 persists while early markers such as NG2 and PDGFRα are downregulated (Yu et al., 1994). Finally, at approximately day 10 in culture, when oligodendrocytes reach the mature myelinating stage (OLG), they acquire immunoreactivity for myelin specific structural proteins such as MAG (myelin associated glycoprotein) and MBP (myelin basic protein) (stage 4) (Fumagalli et al., 2011) (Figure 9 B).

A



B

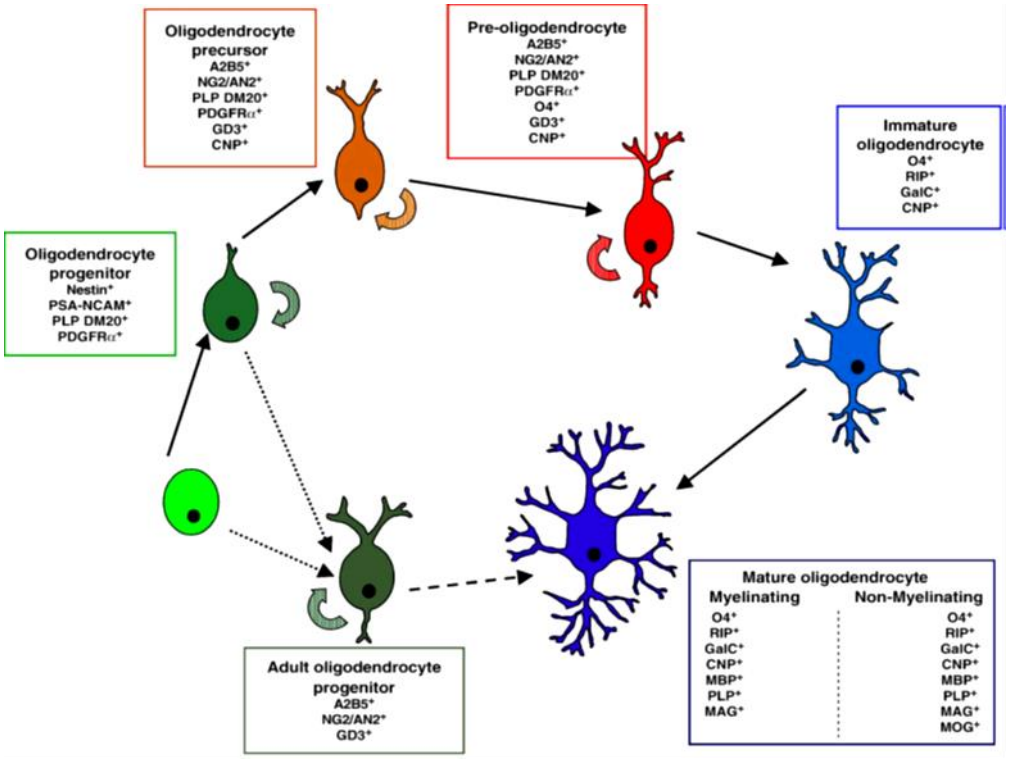


Figure 9. Characterization of the stages of differentiation of oligodendroglial cells. Oligodendrocyte maturation involves a sequences of distinct phases with distinct morphological characteristics (A) From (Fumagalli et al., 2011) and express a defined antigenic pattern (B) From (De Castro et al., 2005).

During their maturation, oligodendrocyte lineage cells display different functional voltage-gated ion channels (Sontheimer et al., 1989) including both inward and outward rectifying K^+ channels (Sontheimer et al., 1988; Williamson et al., 1997), Na^+ channels (Barres et al., 1990), and different subtype of Ca^{2+} channels (Verkhratsky et al., 1990; Berger et al., 1992). Immature OPCs ($NG2^+$) show outward membrane currents whose main component is represented by delayed rectifying K^+ currents (I_K) (Gallo et al., 1996) characterized by low time- and voltage-dependent inactivation and by a threshold of activation at about -40 mV. On the contrary, another K^+ conductance typical of undifferentiated OPCs, the transient outward current (I_A), presents a rapid time-dependent inactivation (approximately 50 ms) and a voltage-dependent inactivation at potentials above -40 mV (Gallo et al., 1996). At this immature stage a subpopulation (about 60%) of OPCs also possess an inward sodium current (I_{Na}) tetrodotoxin-sensitive, characterized by a rapid time-dependent inactivation (less than 1 ms) and a peak of amplitude that is evoked at around -10 mV (Káradóttir et al., 2008), never observed in mature stage (Coppi et al., 2013 a).

During development membrane outward K^+ conductances (both I_K and I_A) undergo to a strong downregulation up to almost completely disappear in mature stages (Sontheimer et al., 1989; Barres et al., 1990; Gallo et al., 1996; Attali et al., 1997; Coppi et al., 2013 a).

In parallel with outward K^+ current downregulation, there is a gradual increase of inward rectifier K^+ currents (K_{ir}), activated at potentials more negative than membrane resting potential (Vm), which are the main conductances represented in mature OLs (Knutson et al., 1997).

Among the mentioned currents, those associated with voltage-gated K^+ channels, such as I_K, I_A and K_{ir} are involved in the regulation of oligodendrocyte differentiation and thus of myelin formation (Sontheimer et al., 1989; Gallo et al., 1996). In addition, the expression of I_K currents is linked to cell cycle regulation and hence to proliferative capacity of OPC (Chittajallu et al., 2005) because of the following: (1) a downregulation of I_K occurs as oligodendrocyte lineage cells mature (Sontheimer et al., 1989; Barres et al., 1990); (2) proliferative OPCs express larger I_K currents than cell cycle-arrested OPCs (Knutson et al., 1997; Chittajallu et al., 2002); (3) pharmacological block of I_K induced by tetra-ethyl-ammonium (TEA) in cells belonging to the oligodendrocyte lineage, is sufficient to delay proliferation and differentiation (Gallo et al., 1996; Chittajallu et al., 2002). Hence, treatments finalized to modulate these currents may affect oligodendrocyte proliferation and differentiation.

It has been demonstrated that a small fraction of OPCs generated during development are maintained in an immature and slowly proliferative or quiescent state in the adult CNS (Dawson et al., 2003) where they are called “adult OPCs”. These adult OPCs are present in all brain structures and represent 2-9% of the CNS cellular population (Dawson et al., 2003), however, they are the largest population of proliferating cells within the CNS (Horner et al., 2000). The adult OPC population persists stable in the adult CNS (Rivers et al., 2008) where they represent an endogenous reservoir of immature cells (De Castro et al., 2005), constantly produced by neural stem cells located in the SVZ (Menn et al., 2006). Current evidences suggest that adult OPCs express the same markers (e.g. NG2 or PDGFRα) and appear morphologically similar to their developmental counterpart (Franklin et al., 2008).

Very little is known about factors that control adult oligodendrogenesis. Upon opportune stimuli both physiological (e.g. voluntary physical exercise; (Simon et al., 2011)) and pathological (e.g. injury, inflammation, demyelination), adult OPCs are able to react with increased proliferation and subsequent differentiation in mature oligodendrocytes (Simon et al., 2011), thus representing the primary source of myelinating cells (Nishiyama et al., 1999; Windrem et al., 2004).

Self-renewal and multipotential features of OPCs have been reported. Thanks to their self-renewal features, adult OPCs continue to proliferate throughout life span (Young et al., 2013) and to differentiate in mature OLG, ensuring myelin integrity. Furthermore, under specific conditions, adult OPCs give rise to neurons, astrocytes (Nishiyama et al., 1999) and Schwann cells (Zawadzka et al., 2010). This potentiality of OPCs is still to be confirmed since remains controversial due to some discrepancy between *in vitro* and *in vivo* data (Crawford et al., 2014). Adult OPCs have shown to contact the axonal membrane (Butt et al., 1999) and the synaptic terminals (Ong et al., 1999). This raises the question of whether adult OPCs may be capable to impact or to react to neuronal activity (Butt et al., 2002; Nishiyama et al., 2002). Concerning this topic a recent study demonstrated the positive impact of neuronal activity on myelination in the adult brain (Gibson et al., 2014).

1.6 Purinergic signaling in oligodendrogenesis

Purinergic signaling has recently emerged as one of the most pervasive mechanism for intercellular communication in the nervous system, affecting communication between many types of neurons and all types of glial cells (Fields et al., 2006).

In the CNS, neurons and multiple types of glia are in close proximity, so that a signal such as ATP released by a neuron could exert effects on many nearby cells (Figure 10).

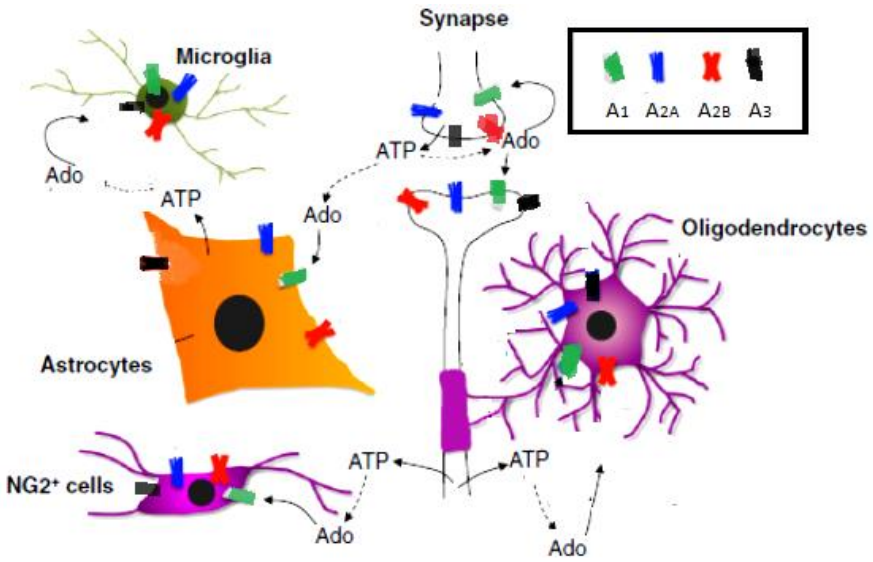


Figure 10. Schematic representation of the role played by extracellular nucleosides in modulating cell-to-cell communication between neurons and glial cells in the central nervous system. ATP is exocytotically released from nerve terminals as a co-transmitter during synaptic transmission. Adenosine is generated by itself or through the activity of ecto-nucleotidases and further contributes to neurotransmission and to the modulation of glial cell functions. Astrocytes and microglia themselves can release ATP, which acts autocrinally or paracrinally on surrounding cells. An axonal non-vesicular

ATP release has been recently identified, which plays a fundamental role in controlling oligodendrocyte myelinating functions, and, possibly, also $NG2^+$ cell activity and maturation. Modified from (Lecca et al., 2012).

Concerning oligodendrocytes, it has been demonstrated that purines exert multiple effects including increased motility, proliferation and differentiation of cultured OPCs (Othman et al., 2003; Agresti et al., 2005).

Adenosine acts as a potent neuron-glial transmitter and by stimulation of its receptors can affect both the process of migration of OPCs and of myelination by mature OLs (Stevens et al., 2000, 2002). Indeed OPCs express all adenosine receptors subtype (A_1, A_{2A}, A_{2B}, A_3); (Stevens et al., 2002; Fredholm et al., 2011).

To date, a functional role on migration of OPCs and on myelination by mature OLs has been attributed to the adenosine A_1, A_{2A} and A_3 receptors (González-Fernández et al., 2014; Coppi et al., 2015).

Adenosine, through the activation of the adenosine A_1 receptors seems to be a primary activity-dependent signal inhibiting the proliferation and promoting differentiation of pre-myelinating progenitors into myelinating oligodendrocytes (Stevens et al., 2002) and stimulating OPC migration (Othman et al., 2003). Coppi et al. (Coppi et al., 2013 b), showed that the selective activation of the adenosine A_{2A} receptors decreases outward rectifying, sustained, I_K currents and inhibits *in vitro* OPC differentiation towards mature, myelinating oligodendrocytes, without affecting cell division. Furthermore, oligodendrocytes apoptosis induced by adenosine A_3 receptor activation was reported by Gonzales-Fernandez (González-Fernández et al., 2014). These adenosine roles may be important during development but also in pathological conditions such as ischemia, when extracellular adenosine concentrations in the brain increase (Latini et al., 2001) and could promote the migration of OPC toward the damaged tissue and the process of neuron remyelination.

Identifying the molecular mechanisms which underlay oligodendrocyte development is not only critical to furthering our knowledge of oligodendrocyte biology, but also has implications for understanding the pathogenesis of demyelinating diseases such as multiple sclerosis (MS). For example, the observation that OPCs are present in MS lesions but fail to differentiate into mature oligodendrocytes (Levine et al., 1999; Chang et al., 2000) suggests that the remyelination process is blocked at a pre-myelinating stage in demyelinating lesions.

Thus, identification of critical regulators that inhibit myelination/remyelination could facilitate the development of therapeutic targets for myelin repair in CNS demyelinating diseases.

To date, no evidence exists concerning the functional role of adenosine A_{2B} receptor in OPC cells.

The bioactive lipid mediator sphingosine 1-phosphate (S1P) has recently gained attention in myelination within CNS since its five receptors ($S1P_{1-5}$) are found in oligodendroglial cells, where they regulate a number of processes, ranging from cell proliferation, and maturation to dendrite elongation (Jaillard et al., 2005; Jung et al., 2007; Miron et al., 2008).
A recent study demonstrate an interaction between the adenosine A_{2B} receptor and S1P pathway in human and mouse erythrocytes *in vitro* (Sun et al., 2015).

1.7 Sphingosine kinase/Sphingosine 1-phoshate pathway

Sphingosine-1-phosphate (S1P) is a sphingolipid signaling molecule that has been implicated in the control of a wide variety of processes, including cell growth, survival, differentiation, apoptosis, and cell migration (Maceyka et al., 2009).
S1P is generated by ATP-dependent phosphorylation of sphingosine by the sphingosine kinase (SphK), an enzyme that exists in two isoforms with different subcellular distribution, $SphK_1$ and $SphK_2$. Cellular levels of S1P are tightly regulated by the balance between its synthesis by SphK and its degradation by either hydrolysis to sphingosine by S1P phosphatases (Mandala et al., 2000; Le Stunff et al., 2002) or cleavage to trans-hexadecenal and ethanolamine phosphate by a S1P lyase (Van Veldhoven et al., 2000) (Figure 11).

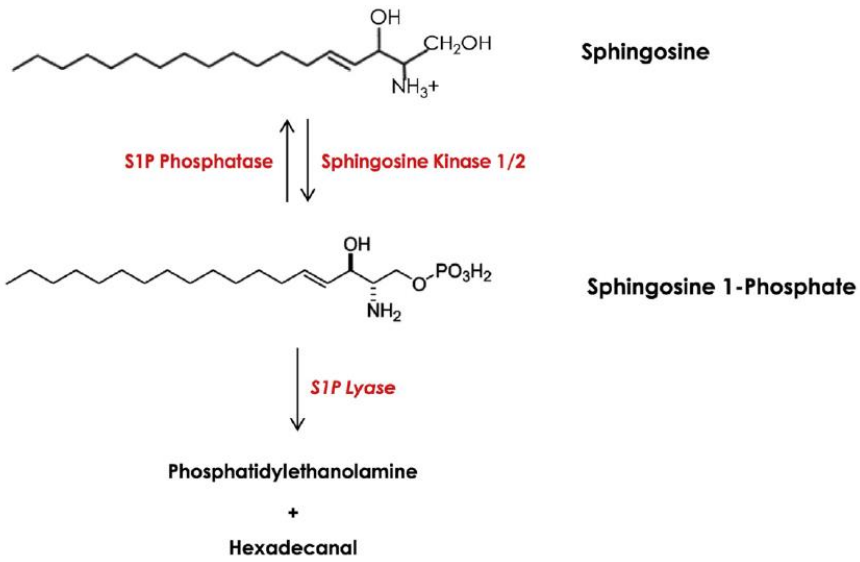


Figure 11. S1P pathway: enzymes involved in the production and degradation of S1P and its derivates. Modified from (O'Sullivan et al., 2017).

After generation, S1P can be exported out of the cell either by the specific transporter Snipster 2 (Snps2) (Nijnik et al., 2012) or by several members of the ABC transporter family (Mitra et al., 2006).

S1P exert extracellular functions through the binding to five S1P-specific G protein-coupled receptors, called $S1P_{1-5}$ receptors (O'Sullivan et al., 2017). S1P receptors are expressed ubiquitously and play important roles in cell survival, growth and differentiation in many cell types, including cells of the immune, cardiovascular and central nervous system (CNS) (Toman et al., 2002; Terada et al., 2004; Kono et al., 2008) (Figure 12).

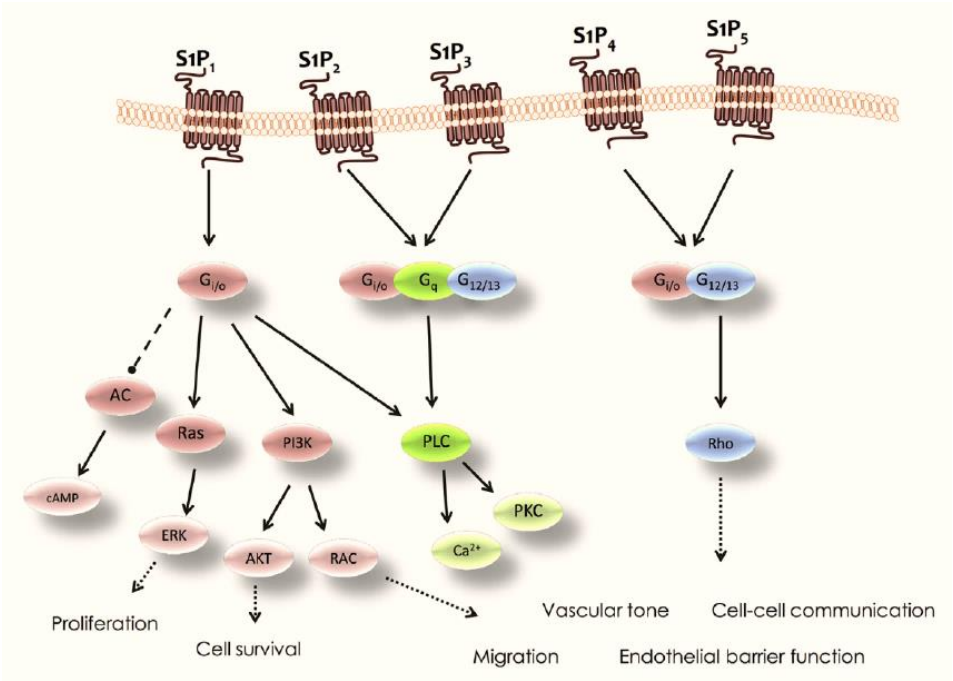


Figure 12. G-protein coupled signaling through S1P receptors. Activation of S1P receptors transduce several well characterized signaling pathways. Modified from (O'Sullivan et al., 2017) .

$S1P_{1-3}$ are widely distributed in the immune and cardiovascular systems and the CNS; $S1P_1$ is highly expressed on T and B lymphocytes (Mandala et al., 2002; Ishii et al., 2004). $S1P_4$ is generally confined to lymphoid and hematopoietic tissues, and $S1P_5$ is predominantly located in CNS white matter (Watterson et al., 2003; Sánchez-Moreno et al., 2004).

S1P receptors modulation has emerged as an effective disease modifying therapeutic strategy in multiple sclerosis (MS). Several oral agents are currently in development, including Fingolimod (FTY720), an immunosuppressant drug which is an analog of sphingosine.

1.8 Fingolimod

Fingolimod, GilenyaR, 2-amino-2[2-(4-octylphenyl)ethyl]-1,3-propanediol) was originally synthesized by the Japanese chemist Tetsuro Fujita from Yoshitomi Pharmaceutical Industries Ltd. (present: Mitsubishi Tanabe Pharma Corporation, Japan) (Adachi et al., 1995) using the natural compound myriocin (ISP-1) as a lead.

Fingolimod (FTY720) is converted *in vivo* by $SphK_2$ to yield its active form, FTY720-phosphate (FTY720-P) which is a structural analogue of sphingosine (Figure 13), a potent ligand of all S1P receptor except $S1P_2$ receptor subtype.

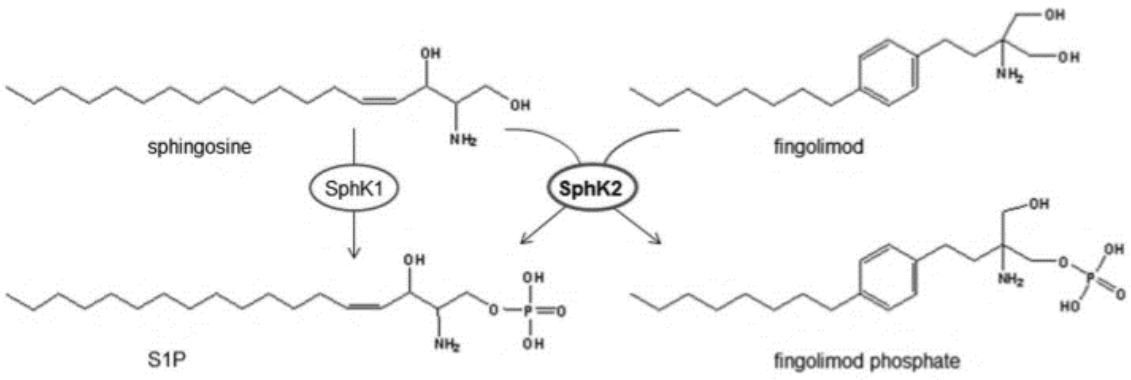


Figure 13. Phosphorylation of fingolimod generates a structural analog to S1P. Fingolimod shows high analogy to sphingosine and is phosphorylated by sphingosine kinases, mainly $SphK_2$, which is the predominant SphK isoform in the brain. Fingolimod is a prodrug of fingolimod phosphate that can signal via S1P receptors and activate intracellular targets of S1P. Modified from (Brunkhorst et al., 2014).

FTY720 has been shown to prolong the survival of allograft in different animal (Chiba et al., 1996) and to induce protective effects in animal of autoimmune diseases (Mizushima et al., 2004; Maki et al., 2005). Administration of FTY720 is highly efficacious in the MS model of experimental autoimmune encephalomyelitis (EAE), both were given at the time of immunization and at the onset of the symptoms (Fujino et al., 2003; Webb et al., 2004).

The therapeutic effect of FTY720 in multiple sclerosis and in its animal model, experimental autoimmune encephalomyelitis (EAE), is attributed to the modulation of $S1P_1$ receptor on lymphocytes. FTY720-P binds with high affinity to $S1P_1$ receptor expressed on lymphocytes and is able to internalize and degrade it. This downregulation renders lymphocytes unresponsive to the normal S1P gradient and thus deprives them of the obligatory signal that would ordinarily allow them to egress from lymphoid tissues and recirculate to the periphery (Brinkmann et al., 2002, 2004; Mandala et al., 2002; Matloubian et al., 2004; Balatoni et al., 2007; Brinkmann, 2007).

Oral fingolimod thus prevents normal egress of lymphocytes, which are retained in lymph organs and away from sites of inflammation (Mandala et al., 2002; Brinkmann et al., 2004; Matloubian et al., 2004).

In addition, there is also evidence supporting the concept of anti-inflammatory, glioprotective and neuroprotective actions of S1P receptors modulators in the CNS (Choi et al., 2011; Kim et al., 2011; Soliven et al., 2011). *In vitro* studies have demonstrated that FTY720-P regulates the survival, differentiation and process dynamics of cultured rodent and human OLG lineage cells (Coelho et al., 2007; Jung et al., 2007; Miron et al., 2008 a). Furthermore, the treatment of mice with FTY720 protects against acute cuprizone-induced OLG injury, demyelination and axonal loss but does not promote remyelination in this model. The protective effect of FTY720 in the cuprizone model may be mediated not only by direct actions on S1P receptors on OLGs, but also by indirect or anti-inflammatory actions on astrocytes and microglia (Kim et al., 2011). FDA approval of fingolimod came in 2010, making this medication the first orally available effective treatment reducing the number of relapses and disease severity in relapsing -remitting multiple sclerosis.

1.9 Effects of S1P signaling in the CNS.

The role of S1P and its receptors in the CNS has been extensively investigated (Choi et al., 2013) (see Table 2).

Cell Type	Receptor	Function
Neurons	S1PRs	Growth cone formation
	$S1P_1$, $S1P_2$	Neurite extension and retraction
	$S1P_2$	Synaptic activity
Oligodendrocytes	$S1P_5$	Mature oligodendrocyte survival
	$S1P_1$	Myelin formation
Astrocytes	$S1P_5$	Proliferation
	$S1P_1$, $S1P_3$	Astrogliosis
Microglia	$S1P_2$ ↑, $S1P_1$& $S1P_3$ ↓	Activated state
	$S1P_1$	↓ Pro-inflammatory cytokine release

Table 2. Functional S1P receptors expression in cells of the CNS. Modified from (O'Sullivan et al., 2017).

Four of the five S1P receptors are found in the CNS ($S1P_1$, $S1P_2$, $S1P_3$ and $S1P_5$), with all four receptors expressed on neurons, astrocytes, microglia and oligodendrocytes (Figure 14).

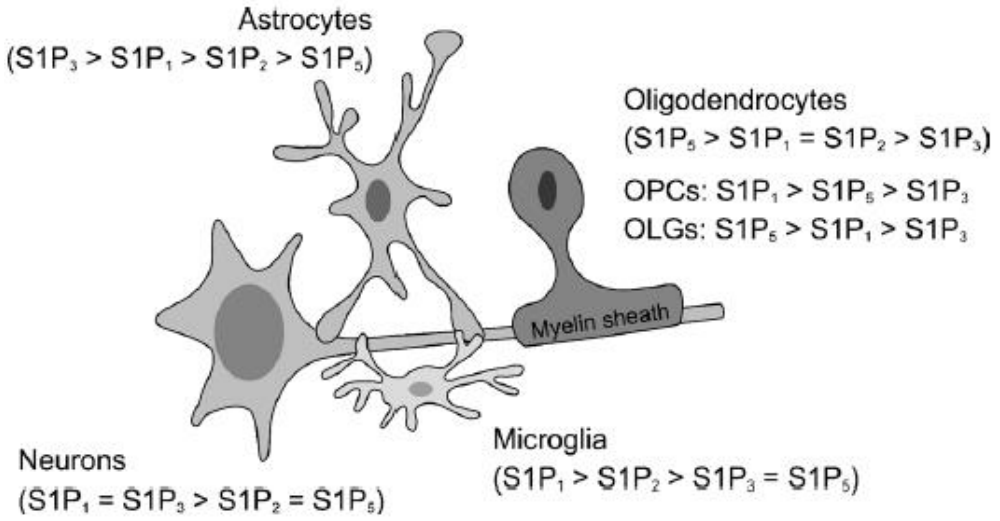


Figure 14. Distribution of S1P receptors subtypes on neural cells. From (Chun, 1999).

In neurons S1P receptors are expressed in brain areas showing active neurogenesis (Chun, 1999; McGiffert et al., 2002; Harada et al., 2004), whereas KO mice with a constitutive genetic deletion of $S1P_1$ showed defective neuronal development (Mizugishi et al., 2005).

S1P has been shown to promote the migration of neural stem/progenitor cells toward areas of damage in the CNS via $S1P_1$, which are abundantly expressed on neural stem/progenitor cells (Kimura et al., 2007). S1P signaling may also be neuroprotective, as suggested from data obtained from several different in vitro systems(Edsall et al., 1997; Shinpo et al., 1999; Chin et al., 2002). These observations suggest that receptor-mediated S1P signaling may be involved in promoting some aspects of neuronal injury repair.

Astrocytes express $S1P_1$, $S1P_2$, $S1P_3$ and $S1P_5$, with $S1P_3$ and $S1P_1$ being expressed at greater levels than the other two receptors subtypes (Rao et al., 2003).

When activated by pathological stimuli, $S1P_1$ and $S1P_3$ receptors are upregulated in activated astrocytes (Van Doorn et al., 2010), indicating that these receptor subtypes may be important in pathological processes.The results of various studies suggest that S1P can influence astrocyte proliferation, migration, and astrogliosis (Rao et al., 2003, 2004; Yamagata et al., 2003; Mullershausen et al., 2007; Miron et al., 2008 a). *In vitro*, S1P induced activation and proliferation of astrocytes, whereas *in vivo*, injection of S1P into the striatum of mouse brains induce astrogliosis (Sorensen et al., 2003). In addition, FTY720-P has been shown to affect S1P receptor-mediated signaling and migration of astrocytes in vitro (Mullershausen et al., 2007; Osinde et al., 2007).

In microglia, the expression of S1P receptors appears to be dependent their activation states. Indeed, in activated microglia, $S1P_1$ and $S1P_3$ receptors are down-regulated but $S1P_2$ receptor

is up-regulated (Choi et al., 2013). The $S1P_1$ deletion in microglia has been shown to reduce activation of these cells in experimental autoimmune encephalomyelitis spinal cords, however the precise function of these receptors on microglia remains to be established (O'Sullivan et al., 2017).

1.9.1 S1P in oligodendrogenesis

Remyelination has been documented to occur in human MS lesions and in animal models of MS. Remyelination is a complex mechanism requiring cellular processes of proliferation, migration, adhesion, process extension/retraction, and differentiation (Miron et al., 2008). S1P signaling has significant effects on these processes in oligodendrocytes (Saini et al., 2005; Novgorodov et al., 2007; Miron et al., 2008). $S1P_5$ and the $S1P_1$ receptors are both expressed on oligodendrocytes, and their relative levels of expression seem to depend on the developmental stages of the cell (McGiffert et al., 2002; Miron et al., 2008).

In particular, mature OLG express $S1P_5$ and at lesser extent $S1P_1$ $S1P_2$ and $S1P_3$, whereas OPC express at a higher level $S1P_1$ than $S1P_2$, $S1P_3$ and $S1P_5$ receptors (Yu et al., 2004; Jung et al., 2007; Novgorodov et al., 2007). *In vitro* studies demonstrated that FTY720-P differently modulates differentiation, proliferation migration and availability of OPCs and/or OLGs relying on the activation of different S1P receptors subtypes whose expression depends upon the developmental stage of OPCS (Jung et al., 2007). Furthermore, it protects from myelin damage in EAE model and chronic progressive CNS inflammation (Kataoka et al., 2005; Rothhammer et al., 2017).

Cerebral ischemia and role of adenosine receptors

1.10 Cerebral ischemia

Stroke is a form of cerebrovascular disease which affects blood supply to the brain.

World Health Organization (WHO) defines stroke as "*rapidly developed signs of focal (or global) disturbance of cerebral function lasting longer than 24 h (unless interrupted by death), with no apparent non vascular cause"* (Aho et al., 1980).

Stroke, being the second leading cause of death (Dariush et al., 2015) and third leading cause of disability (Murray et al., 2012), is considered as major health problem worldwide. Factors

like diabetes, hypertension, hypercholesterolemia, smoking and alcohol abuse have been associated with cardiovascular disease in general and stroke in particular.

The neurological complications and clinical manifestations following a stroke vary considerably as well, depending upon which areas of the brain were affected and to what degree. Deficits after stroke can include depression, partial paralysis, difficulties with memory, thinking, language, and movements.

Stroke can be classified into two basic types: ischemic stroke and hemorrhagic stroke (Figure 15).

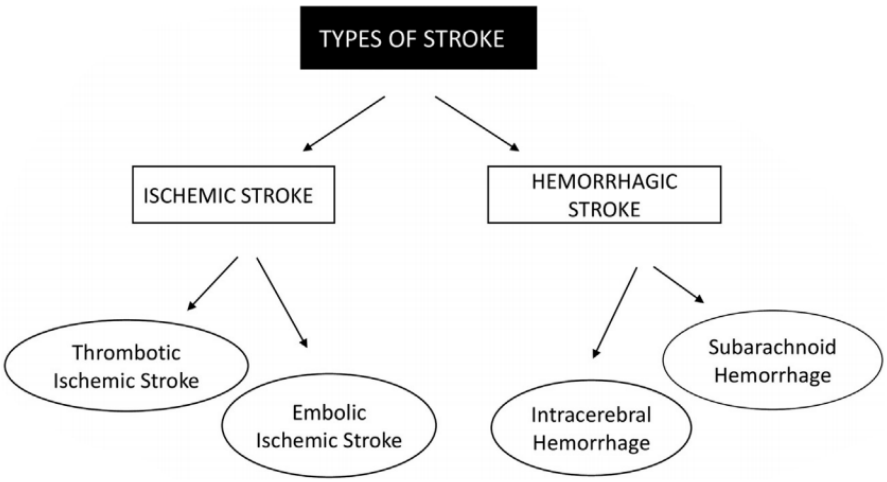


Figure 15. Types of stroke. Modified from (Kumar et al., 2016).

Ischemic stroke accounts for 80–87% of all cases (Sudlow et al., 1997; Dariush et al., 2015) and is caused by occlusion of a major cerebral artery by a thrombus or an embolism, which leads to loss of cerebral blood flow, a condition of hypoxia and glucose deprivation (oxygen, glucose deprivation: OGD) and subsequently tissue damage in the affected region (Gibson, 2013).

Hemorrhagic stroke accounts for about 13-20% of the total stroke cases (Sudlow et al., 1997; Dariush et al., 2015). This type of stroke can be subarachnoid or intracerebral and originate with the rupture of a cerebral blood vessel or an aneurysm within the blood vessel, resulting in an accumulation of blood and compression of brain tissue (Sacco et al., 1989, 1997; Levine, 2004). Ischemic brain injury results from a complex sequence of pathophysiological events that evolve over time and space (Figure 16).

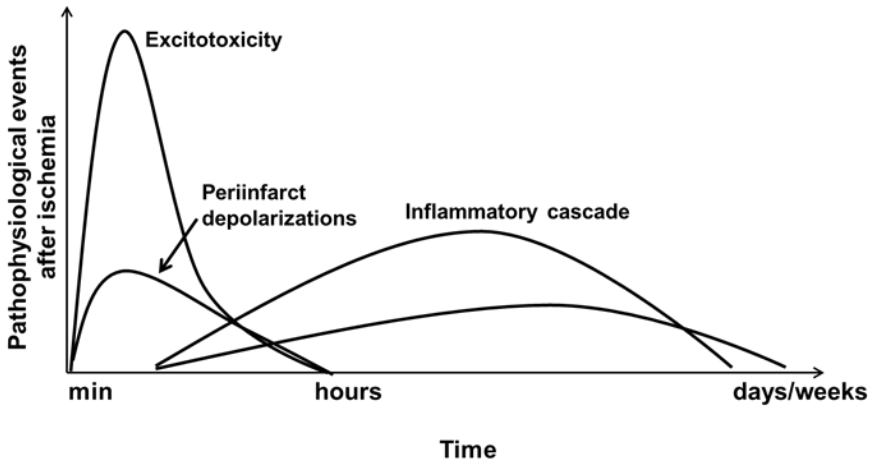


Figure 16. Cascade of pathogenetic mechanisms after ischemia. Primary mechanisms of excitotoxicity lead to acute cell death. Depolarization spreads in the periinfarct areas. In the following several hours, activation of resident immune cells, i.e. microglia and a cascade of production of inflammation mediators occur. Cell death/neurogenetic responses progress along days/weeks after ischemia. Figure modified from (Dirnagl et al., 1999).

The major pathogenic mechanisms of this cascade include: primary acute mechanisms of excitotoxicity and peri infarct depolarizations followed by activation of resident immune cells, i.e microglia, and production or activation of inflammation mediators (Dirnagl et al., 1999). Proinflammatory cytokines, that upregulate cell adhesion molecules, exert an important role in promoting neutrophil infiltration and accumulation in brain parenchyma (Stoll et al., 1998; Huang et al., 2006). Although after ischemia precocious activation of immune cells may be neuroprotective and supportive for regeneration, protracted neuroinflammation is now recognized as the predominant mechanism of secondary brain injury progression.

Following a focal stroke, the ischemic lesion can be divided in two distinct regions (Figure 17): the center of the ischemic area (ischemic *core*) in which the flow reduction is most pronounced and leads to rapid energy failure and cell death (Hossmann, 1994) and the surrounded area, the ischemic *penumbra*, where electrical functions are lost but energy metabolites and ion homeostasis are not significantly altered (Astrup et al., 1981). It is estimated that initially, the area of penumbra corresponds to 50% of the tissue that later will progress into infarct (Dirnagl et al., 1999). The understanding of mechanisms occurring in the penumbra zone is essential to preserve function and promote the survival of nervous cells after reperfusion. The main goal of a neuroprotective pharmacological treatment is to recover the area of ischemic penumbra from an irreversible neurodegeneration.

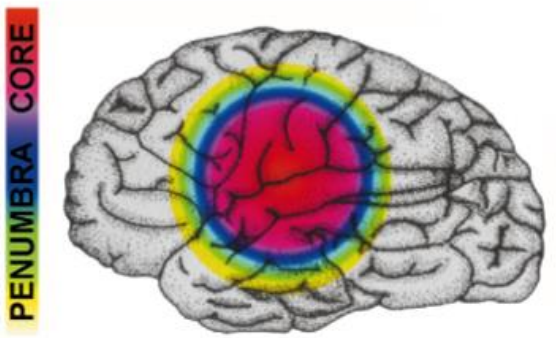


Figure 17. The red area on the figure marks the infarcted *core*, a brain region of low perfusion in which cells have lost their membrane potential. The ischemic *penumbra*, marked by the remaining colors, is potentially salvageable if an appropriate treatment is administered within a specified therapeutic window in which intermediate perfusion prevails and identified as the salvageable brain area. Modified from (Dirnagl et al., 1999).

Despite advances in the understanding of the pathophysiology of cerebral ischemia, therapeutic options for acute ischemic stroke remain very limited (Donnan et al., 2008).

At the moment, the only drug approved to treat ischemia is the tissue plasminogen activator (tPA), a specific enzyme that cleaves proteins which are present in the blood, such as fibrin, an essential protein in blood clotting, and thrombi. The main issue is that in order to perform such a vital function tPA must be necessarily administered in the first 3-4 hours after the manifestation of the symptomatology. Because of its short therapeutic window and by its complication derived essentially from the risk of hemorrhage, the number of patients who might receive treatment and therefore potentially benefit is small (Donnan et al., 2008). Drugs like aspirin, other antiplatelets, and anticoagulants are used as preventive therapy of stroke (Albers et al., 2011; Macrez et al., 2011). Consequently, the successful treatment of acute ischemic stroke remains one of the major challenges in clinical medicine.

1.11 Pathobiology of ischemia

Cerebral ischemia triggers a series of hemodynamic, biochemical and behavioural disorders. Initially, due to the decrease in blood flow, neurological functions are affected. Subsequently, the metabolic activity is suppressed in order to maintain the structural integrity of the cells (Hossmann, 1994). This leads to activation of several signalling pathways that may be neuroprotective or harmful towards the brain. These events that include excitotoxicity, ionic imbalances, oxidative stress, inflammatory reactions and inflammatory cascade, occur simultaneously and depend on the intensity and duration of the ischemic insult.

1.11.1 Changes in cellular homeostasis

It is well known that brain tissue is highly vulnerable to ischemia, such that even transient loss of oxygen and glucose to the cerebral tissue may culminate in cellular death (Woodruff et al., 2011). In contrast to other organs, brain tissue uses glucose as its only substrate for energy metabolism. Brain is unable to store energy, thus it requires a constant supply of oxygenated blood containing an adequate glucose and oxygen concentration to maintain its function and structural integrity (Markus, 2004).

Low blood flow values caused by ischemia can induce electron chain transport failure and an enormous efflux of potassium ions into the extracellular space (Rama et al., 2012). Furthermore, as a consequence of the decrease in oxygen values, mitochondrial oxidative phosphorylation is interrupted, thus resulting in a rapid decline in cellular level of ATP (Katsura et al., 1994; Martin et al., 1994; Rama et al., 2012). Reduction in ATP level further stimulates the glycolytic metabolism of residual glucose and glycogen, which induce protons and lactate accumulation and lead to rapid intracellular acidification and further reduction in cellular ATP production (Martin et al., 1994). Indeed, it has been seen that the lactate content during ischemia rises to ~20mM (normal conditions ~15-20mM), leading to a pronounced acidosis (Katsura et al., 1999). The accumulation of lactic acid in ischemic brain may greatly disturb the normal H^+ homeostasis, and it has been suggested that excessive lactic acidosis facilitates ischemic mechanisms leading to cerebral infarction (Plum, 1983).

pH in the brain is maintained at approximately at 7.2 (Casey et al., 2010) thanks to a strong regulation by active (ion pump transport) and passive (ion channel transport, intracellular buffer solution) mechanisms (Casey et al., 2010). During stroke, extrusion of CO_2 from the cell is limited by poor perfusion thus accumulation of CO_2 in the intracellular space decreases the performance of the buffer solution and contributes to the reduction of pH (Ekholm et al., 1991). In addition, the reduction of glucose and oxygen supply leads to the depletion of glycogen and phosphocreatine (PCr; cellular energy reserves), which increases the production of hydrogen ions. The pH regulates diverse cellular processes (Busa et al., 1984) and modulates the activity of many enzymes and ion channels. There are two main mechanisms of acidosis-induced damage during ischemia: free radical formation and cell calcium metabolism. Free radicals are activated when ischemia is followed by recirculation (Traystman et al., 1991) contribute to tissue damage (Folbergrová et al., 1995) and low pH increases their production (Gebicki et al., 1981; Bralet et al., 1992). Ca^{2+} concentration depends on the level of pH (Kristián et al., 1996). A change in this parameter may trigger apoptosis, which can occur for pH values of approximately 6.5 (Barry et al., 1992). Excessive production of H^+ ions in the cell after stroke

is owing to two factors: consumption of ATP, glucose glycogen and PCr stores, and a high concentration of CO_2 (Roberts, 1999).

Brain pH alteration after ischemic damage have been well documented by Back and co-workers in a model of permanent MCAo, in which areas of acidification and alkalization can be detected in ischemic penumbra surrounding the markedly acid core (Back et al., 2000).

1.11.2 Glutamate-mediate excitotoxicity

Glutamate is the predominant excitatory transmitter in the mammalian CNS and acts at a range of different receptor types. Under normal conditions, neuronal and glial uptake systems maintain the extracellular concentration of glutamate at micromolar levels (Bralet et al., 1992). Under ischemic conditions, glutamate is massively released (initially mediated by vescicular release from nerve terminals, and later by reverse transport from astrocytes), reaching millimolar concentrations in the extracellular space.

These concentrations of glutamate are neurotoxic, and substantial evidence now implicates the toxicity of glutamate (excitotoxicity) in the pathogenesis of neuronal death after ischemia.

Extracellular glutamate accumulating under ischemic conditions overstimulates N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate-type glutamate receptors, promoting an increased permeability to Na^+, K^+ and Ca^{2+} ions which further depolarize the cell membrane and through metabotropic receptors (mGluRs) cause the mobilization of Ca^{2+} from intracellular stores. It is thought that the majority of neuronal degeneration is due to the intense activation of NMDA receptors glutamate-mediated. The increased intracellular concentration of Ca^{2+} leads to the activation of proteases, lipases, phospholipases and endonucleases that determine the rupture of the plasma membrane, of the cytoskeleton and DNA fragmentation, bringing to neuronal degeneration. Moreover, an influx of Ca^{2+} via NMDA receptor, can determine mitochondrial dysfunction, caspase-3 activation through the action of calpain, and production of nitric oxide (NO) and reactive oxygen species (ROS) leading to neuronal death (Dobrek et al., 2011).

Uncontrolled release of glutamate causes one of the early events after ischemia, the peri-infarct depolarization (Figure 16): a series of spontaneous depolarization waves that propagate from the ischemic *core* toward the *penumbra* area. Moreover, the glutamate accumulation leads to an excessive influx of Na^+ and Ca^{2+} together with water, resulting in cytotoxic edema (Furukawa et al., 1997). It has been observed that the infarcted area extends with the number of depolarizations (Mies et al., 1993), thus, drugs able to reduce the number of depolarizations are also able to limit the extension of the infarct (Ohta et al., 2001; Lu et al., 2005).

1.11.3 Oxidative stress

In cerebral ischemia the production of ROS is increased and during reperfusion ROS production is even accelerated due to cytotoxic events such as lipid peroxidation, protein oxidation and fragmentation of DNA (Crack et al., 2005). Furthermore, lipid membrane peroxidation by ROS generates toxic aldehydes that damage a large number of ion channels, transporters, and cytoskeleton proteins.

The nervous tissue is too vulnerable to the action of free radicals because of some peculiarities, such as being rich in lipids and unsaturated fatty acids which may react with ROS to form peroxyl radicals that determine the lipid oxidation of the membrane of neurons (Porter, 1984). Additionally, the brain has low to moderate catalase, and glutathione activity which eliminate the hydrogen peroxidase (H_2O_2) reducing oxidation (Cooper et al., 1997). It has been demonstrated how the application of antioxidant enzymes can be effective in contrasting oxidative stress (Huang et al., 2001). For instance, superoxide dismutase (SOD) plays a protective role in ischemia (Noshita et al., 2001; Sugawara et al., 2002) and SOD1 over-expression attenuates apoptosis (Saito et al., 2004). Also, melatonin is known for its neuroprotective effect in models of ischemia, due to its anti-oxidant properties and capability in eliminating free radicals (Tan et al., 2000; Pei et al., 2002).

The free radical, NO, is synthetized by the enzyme NO synthase (NOS) that is heavily activated during ischemia, as well as during reperfusion. The NO has neuroprotective and neurotoxic properties. The activation of NOS during the ischemia causes the death of neurons by combining with a superoxide yielding peroxynitrite ($ONOO^-$), a potent radical which is an irreversible inhibitor of mitochondrial function in addition to being a pro-oxidant that damages lipids, proteins and DNA and increases glutamate release determining neuronal cell death (Radi et al., 1991). Conversely, the activation of NOS in the endothelial cells is neuroprotective because NO production acts relaxing the adjacent smooth muscle cells leading to vasodilation and increase of blood flow in the affected cerebral region (Bolaños et al., 1999).

1.11.4 Stress signaling in response to cerebral ischemia

Among the pathophysiological events that occur after cerebral ischemia, noteworthy is the activation of mitogen-activated protein kinase (MAPK) cascade (Takagi et al., 2000). MAPKs comprise three subfamilies: the extracellular-signal regulated protein kinases (ERK), c-jun N-terminal kinases (JNK) and p38 (Koistinaho et al., 2002) which are signal transduction pathways that serve several different functions at the cellular level. In particular, following focal

cerebral ischemia in the rat, activation of both ERK and p38 MAPK up to 24 after ischemia was reported (Irving et al., 2000 a).

Most evidence indicates that ERK activation is associated with the regulation of cell proliferation and differentiation (Tibbles et al., 1999) and exerts neuroprotection mediated by neurotrophins and in particular by the brain-derived neurotrophic factor (BDNF) (Han et al., 2000). Differently, JNK and p38 MAPK activation is involved in responses to environmental stress, cell suffering and death (Xia et al., 1995; Kummer et al., 1997; Takeda et al., 2002) and plays a role in neuronal damage and angiogenic response in cerebral ischemia (Lennmyr et al., 2002). Concerning p38 MAPK, data agree on protection by p38 inhibition against focal ischemia induced infarct, neurological deficit and expression of inflammatory cytokines (Barone et al., 1999, 2001 a). Several studies have shown that all MAPKs are activated twenty-four hours after focal brain ischemia in the rat (Irving et al., 2000 a): p38 and ERK in neurons and in microglia (Irving et al., 2000 a; Takagi et al., 2000; Piao et al., 2003; Melani et al., 2009), JNK in neurons and in oligodendrocytes (Melani et al., 2009).

1.11.5 Neuroinflammation

A few hours after the ischemia onset a switch from the ischemic damage to the inflammatory damage occurs. The inflammatory response is characterized by the activation of microglia and the influx of peripheral leukocytes (neutrophils, lymphocytes and monocytes) in the brain parenchyma that promotes alteration of the blood brain barrier (BBB) permeability (Iadecola et al., 2011).

Microglial cells are the resident macrophages of the brain and play a critical role as resident immunocompetent and phagocytic cells in the CNS. Ekdahl and colleagues (Ekdahl et al., 2009) reported an increased number of activated microglial cells up to 16 weeks after two hours middle cerebral artery occlusion (MCAO) in rats. After activation by ischemia, microglia can transform into phagocytes that can release a variety of substances many of which are cytotoxic. There is substantial evidence that activated microglial cells in response to ischemia have the potential of releasing several pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), as well as other potential cytotoxic molecules including NO, ROS and prostanoids (Lucas et al., 2006). Other cytokines related to inflammation in acute stroke are interleukin-10 (IL-10) and transforming growth factor (TGF)-β. While IL-1β and TNF-α, appear to exacerbate cerebral injury, TGF- β and IL-10 may be neuroprotective (Spera et al., 1998; Zhu et al., 2002). Increased production of proinflammatory

and lower levels of the anti-inflammatory IL-10 are related to larger infarctions and poorer clinical outcome.

Astrocytes are known for their ability to express different kinds of inflammatory mediators such as cytokines, chemokines, and the inducible NOS (iNOS) (Dong et al., 2001) Following ischemia astrocytes become activated, they increase the expression of the glial fibrillary acidic protein (GFAP) and participate to the cerebral inflammation by expressing the major histocompatibility complex (MHC) and by stimulating the expression of cytokines such as IL-2 (Dong et al., 2001).

There is increasing evidence that cellular adhesion molecules (CAMs) play an important role in the pathophysiology of acute ischemic stroke (Yilmaz et al., 2008). CAMs are upregulated after stroke and are responsible for the adhesion and migration of the leukocytes. Leukocytes roll on the endothelial surface and then adhere to the endothelial cells. The interaction between leukocytes and the vascular endothelium is mediated by three main groups of CAMs: the selectins, the intracellular adhesion, (ICAM-1,2) and vascular cell adhesion (VCAM-1) molecules and the integrins. Selectins, especially E- and P-selectin are upregulated and mediated leukocytes rolling and recruitment during the early stages of ischemia (Zhang et al., 1998). It has been shown that the blockade of adhesion molecules leads to a reduction of neurological damage (Clark et al., 1995, 1997).

Neutrophils are the first leukocyte subpopulation to be recruited into the ischemic brain and may exacerbate the damage following the release of deleterious substances or of inflammatory mediators (Hallenbeck, 1996). Therefore, the inhibition of neutrophil infiltration can significantly reduce the infarct volume after ischemia (Bowes et al., 1995; Clark et al., 1995; Chopp et al., 1996; Connolly et al., 1996; Yenari et al., 1998; Garau et al., 2005). Following permanent middle cerebral artery occlusion (MCAo) in rats, lymphocytes represent the second most abundant leukocyte subtype, after neutrophils, infiltrated in the ischemic lesion (Stevens et al., 2002) and exert pro-inflammatory actions, harmful for the cerebral tissue (Nadareishvili et al., 2004).

Other molecules upregulated in the brain in response to injury are the matrix metalloproteinases (MMPs) (Montaner et al., 2001).

MMPs are a family of proteolytic enzymes that are responsible for remodeling the extracellular matrix and that can degrade all its constituents. Neurons, astrocytes, microglia, and endothelial cells have all been shown to express MMPs after injury. Stroke is associated with the disruption of the BBB leading to edema and hemorrhage and experimental studies have shown that these events results from the expression and activation of MMPs (Asahi et al., 2001). Among the

MMPs, the MMP-2 and MMP-9 are implicated in cerebral ischemia. Elevated MMP-9 levels were found in brain tissue and in serum from patients with acute ischemic stroke and in animal models of stroke beginning at 12 hours after permanent MCAo (Lakhan et al., 2013).
The described post-ischemic neuroinflammatory changes are summarized in Figure 18.

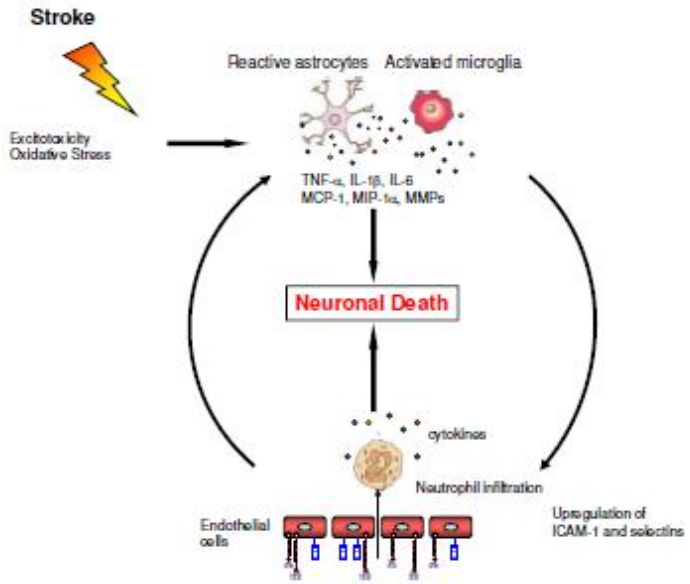


Figure 18. Post-ischemic inflammatory response. Excitotoxicity and oxidative stress caused by the initial ischemic event activate microglia and astrocytes which react by secreting cytokines, chemokines and matrix metalloproteases (MMP). These inflammatory mediators lead to an upregulation of cell adhesion molecules on endothelial cells, allowing blood derived inflammatory cells, mainly neutrophils, to infiltrate the ischemic brain area. Neutrophils themselves also secrete cytokines which cause a further activation of glial cells. These processes all result in neuronal cell death and enhance the damage to the ischemic brain. From Lakhan et al., 2009

1.11.6 Cellular death

After cerebral ischemia, brain cells that are compromised by excessive glutamate-receptor activation, Ca^{2+} overload, oxygen radicals or by mitochondrial and DNA damage can die by necrosis or apoptosis. Necrosis is the predominant mechanism that follow acute, permanent, vascular occlusion, whereas in milder injury, the predominant mechanism is the apoptosis, particularly within the ischemic *penumbra*. Some of the major morphological changes that occur with necrosis include: cell swelling, formation of cytoplasmic vacuoles, distended endoplasmic reticulum, formation of cytoplasmic blebs, condensed, swollen or ruptured mitochondria, disaggregation and detachment of ribosomes, disrupted organelle membranes, swollen and ruptured lysosomes, and eventually disruption of the cell membrane (Trump et al., n.d.; Kerr et al., 1972; Majno et al., 1995). This loss of cell membrane integrity results in the release of the cytoplasmic contents into the surrounding tissue, sending chemotactic signals with eventual recruitment of inflammatory cells (Elmore, 2007).

Apoptosis (or controlled cell death) is induced by many signals. The enzymes involved in apoptosis are the caspases, whose gene is highly expressed during the infarction (Dirnagl et al., 1999). To date, research indicates that there are two main apoptotic pathways: the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway (Figure 19).

Post-ischemic apoptosis occurs via intrinsic (or mitochondrial) activation: caspases become activated when cytochrome C, released from mitochondria, leading to the activation of caspase-9. Caspase-9 activates, among others, the caspase-3 which initiates the cell death by apoptosis (Carbonell et al., 2007).

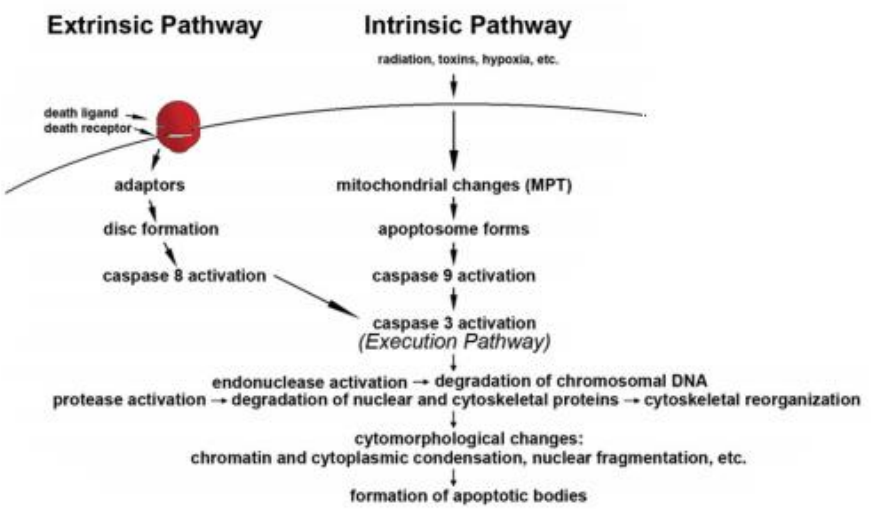


Figure 19. Schematic representation of the two main pathways of apoptosis: extrinsic and intrinsic. Each requires specific triggering signals to begin an energy-dependent cascade of molecular events. Each pathway activates its own initiator caspase (8, 9) which in turn will activate the executioner caspase-3. The execution pathway results in characteristic cytomorphological features including cell shrinkage, chromatin condensation, formation of cytoplasmic blebs and apoptotic bodies and finally phagocytosis of the apoptotic bodies by adjacent parenchymal cells, neoplastic cells or macrophages. Modified from (Elmore, 2007).

1.12 Adenosine in cerebral ischemia

The increase of extracellular adenosine concentration during *in vivo* ischemia (Dux et al., 1990; Hagberg et al., 1987; Matsumoto et al., 1992; Melani et al., 1999; Phillis et al., 1994, 1996; Sciotti et al., 1992) is attributable to different reasons. Early after ischemia, the increase of adenosine is mainly due to extracellular released ATP (Melani et al., 2012) that is hydrolysed by ectonucleotidases (NTPDases 1, 2 and 3 that convert ATP o ADP and AMP) and ecto-5'-nucleotidase that converts AMP to adenosine (Zimmermann, 2000; Fausther et al., 2012). Thereafter adenosine *per se* is mainly released from cells likely by the ENT2 (Melani et al., 2012). Inhibition of adenosine-uptake processes due to down-regulation of CNT2 and 3 and of

the ENT1 also contributes to the extracellular adenosine increase after stroke (Medina-Pulido et al., 2013).

The extracellular adenosine concentrations reached after ischemia allow the stimulation of all four adenosine receptor subtypes (A_1, A_{2A}, A_{2B} and A_3). In particular, their wide distribution on neuronal, glial and inflammatory cells (Fiebich et al., 1996; Brodie et al., 1998; Svenningsson et al., 1999; Hettinger et al., 2001; Yu et al., 2004), suggests that the role of adenosine in ischemia is the consequence of an interplay among different receptor activation, which changes depending on the time-related development of the pathological condition (Pedata et al., 2014).

During ischemia, adenosine has long been identified as a neuroprotectant endogenous agent (Cunha, 2001; Pedata et al., 2007; Fredholm et al., 2003; Ongini et al., 1997; Ribeiro et al., 2002; Schwarzschild et al., 2002). It was demonstrated that adenosine infusion into the ischemic striatum significantly ameliorated neurological outcome and reduced infarct volume after transient focal cerebral ischemia (Kitagawa et al., 2002). All these protective effects are greatly attributed to A_1 receptor activation due to reduced Ca^{2+} influx, thus lowering presynaptic release of excitatory neurotransmitters (Andiné, 1993; Corradetti et al., 1984; Dunwiddie, 1984; Zetterström et al., 1990; Pedata et al., 1993; Kitagawa et al., 2002) and in particular of glutamate which exerts an excitotoxic effect during ischemia mainly by overstimulation of NMDA receptors (Choi, 1990). In addition, by directly increasing the K^+ and Cl^- ion conductances, adenosine stabilises the neuronal membrane potentials, thus reducing neuronal excitability (Choi, 1990). Nevertheless, the use of selective A_1 agonists is hampered by undesirable effects such as sedation, bradycardia, hypotension (White et al., 1996; Fredholm et al., 2005).

Beyond the protective effects of A_1 receptor, important roles in cerebral ischemia of the other receptor subtypes have been outlined in the last years. It has been demonstrated that both antagonist and agonist of adenosine A_{2A} receptor exert protective effects attributable to different mechanism. The neuroprotective properties of adenosine A_{2A} receptor antagonists largely reside in effects mediated by A_{2A} receptors located on brain cells, in particular in control of excitotoxicity as demonstrated by the observation that the A_{2A} receptor selective antagonist, ZM241385, injected peripherally or directly intra hippocampus is protective against excitotoxicity induced by kainate (Jones et al., 1998) and by the combinations of quinolinic acid and IL-1β (Stone et al., 2007). Moreover, A_{2A} receptor antagonists protect from ischemia–induced activation of Mitogen-Activated Protein Kinases (MAPKs) expression. Twenty-four hours after focal ischemia, a definite over expression of A_{2A} receptors (Trincavelli et al., 2008) and an activation of p38 and ERK1/2 MAPKs was found in neurons and in microglia of the

ischemic tissue (Irving et al., 2000 b; Takagi et al., 2000; Piao et al., 2003; Melani et al., 2006 a). Sub-chronic administration of the A_{2A} receptor antagonist, SCH58261, has reduced phospho-p38 in microglia while it did not affect ERK1/2 MAPK activation 24 hours after ischemia (Melani et al., 2006 a). A control of p38 activation by A_{2A} receptor antagonism (Melani et al., 2006) might account for neuroprotection after ischemia as demonstrated in brain slices after OGD (Barone et al., 2001 b).

Apparently in a paradoxical manner, also A_{2A} agonists exert protective effect. Activation of central A_{2A} receptors is known to increase expression and release of neurotrophic factors (Sebastião et al., 1996) such as nerve growth factor (NGF) in microglia (Heese et al., 1997), BDNF in mice hippocampus (Tebano et al., 2008), in rat cortical neurons (Jeon et al., 2011) and in primary cultures of microglia (Gomes et al., 2013); glial cell line-derived neurotrophic factor (GDNF) in striatal neurons (Gomes et al., 2006). The increase in neurotrophic factor expression by adenosine A_{2A} receptor stimulation may contribute to restore neurological functions and cerebral damage after brain ischemia. Besides protective effect attributable to A_{2A} expressed at central level, a bulk of evidences indicate that peripheral effects on A_{2A} receptors located on blood cells greatly account for protective effects of adenosine A_{2A} agonists after ischemia. Adenosine A_{2A} receptors are present, in fact, on peripheral blood marrow derived cells including platelets, monocytes, mast cells, neutrophils and T cells where selective activation of A_{2A} receptors reduces production of adhesion cell factors, reduces platelet aggregation and neutrophil activation exerting therefore an antiplatelet, antioxidant and anti-inflammatory effect. (Haskò et al., 2000; Lappas et al., 2006; Sitkovsky et al., 2004; Varani et al., 1998).

In particular, it has been demonstrated that A_{2A} receptor activation reduced ischemia-induced rolling, adhesion and transmigration of various peripheral inflammatory cells (such as lymphocytes, neutrophils) (Haskó et al., 2008). In agreement, A_{2A} agonists, systemically administered, protected from inflammation in various models of autoimmune disease such as rheumatoid arthritis (Szabó et al., 1998; Mazzon et al., 2011), colitis (Odashima et al., 2005; Di Paola et al., 2010), and hepatitis (Choukèr et al., 2008) and in models of spinal cord trauma (Li et al., 2006; Genovese et al., 2009; Paterniti et al., 2011) and of traumatic brain injury (Dai et al., 2010).

The few studies present in the literature concerning the role of adenosine A_3 receptor in cerebral ischemia are rather contradictory (Borea et al., 2009; Pedata et al., 2010). The effects of adenosine A_3 receptor stimulation appear to depend on drug administration (acute *vs* chronic), dosage and timing of treatment with respect to the onset of the ischemic insult.

1.12.1 Adenosine A_{2B} receptor in cerebral ischemia

Adenosine A_{2B} receptor is expressed at low levels uniformly throughout the CNS (Puffinbarger et al., 1995; Dixon et al., 1996; Fredholm et al., 2000) and has a low affinity for adenosine. Their mRNA and protein expression levels increase on endothelial cells, neurons, and astrocytes to a greater extent than do those of the other three adenosine receptors (A_1, A_{2A}, and A_3) 24 hours after tMCAo in the rat (Li et al., 2017). Thus, during conditions of hypoxia or ischemia when the extracellular adenosine levels rise, A_{2B} receptors might be well activated (Xu et al., 2013). Because of paucity of A_{2B} selective agonists and antagonists (Müller et al., 2011) few studies are till now present on the role of A_{2B} receptors in brain ischemia.

Recently, the role of A_{2B} receptor selective antagonists was investigated under OGD. Fusco et al., (2018) demonstrated that, in the CA1 area of the rat hippocampus, the selective A_{2B} receptor antagonists, N-(4-Cyanophenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1H-purin-8-yl) phenoxy]–acetamide (MRS1754) and 8-[4-[4-(4-Chlorophenzyl) piperazide-1-sulfonyl) phenyl]] -1-propylxanthine) (PSB603) prevented the appearance of AD, a phenomenon strictly related to cell damage and death (Pugliese et al., 2006) and protected from the synaptic activity depression, bringing to a significant recovery of an otherwise disrupted neurotransmission induced by 7 min OGD (Fusco et al., 2018). Adenosine A_{2B} receptor antagonism also counteracted the reduction of neuronal density in CA1 stratum pyramidale, decreased apoptosis at least up to 3 hours after the end of OGD and prevented astrocyte modification (Fusco et al., 2018).

The selective A_{2B} antagonist, N-(4-acetylphenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1H-purin-8-yl) henoxy] acetamide (MRS1706), completely prevented in human astroglial cells elongation of astrocytic processes (a morphological hallmark of *in vivo* reactive astrogliosis) induced by selective stimulation of A_{2B} receptors (Trincavelli et al., 2004). Moreover, a short-term TNF-α treatment induced A_{2B} adenosine receptor desensitization in human astroglial cells (Trincavelli et al., 2004). These results suggest that in the acute phase of brain ischemia characterized by both cytokine and adenosine high release, A_{2B} receptor desensitization on astroglia might represent a cell defense mechanism (Trincavelli et al., 2004). Few studies have investigated the role of A_{2B} receptors in brain ischemia *in vivo*. It was reported that the selective A_{2B} receptor antagonist, MRS1754, reduced the ceramide production in astrocytes and attenuated inflammatory responses and neuronal damage after 30 minutes and 6 hours in a model of global cerebral ischemia induced by four-vessel occlusion in the rat (Gu et al., 2013). This effect was related to an early reduction of p38 MAPK activation. In fact, A_{2B} receptor plays a key role in the rapid activation of p38 and in the subsequent inflammatory

process (Koscsó et al., 2012; Wei et al., 2013). Altogether experiments indicated that antagonism of A_{2B} receptor located on brain cells might be protective from ischemic brain damage.

Besides brain cells, A_{2B} receptors are present on blood immune cells i.e neutrophils, lymphocytes (Gessi et al., 2005; Eckle et al., 2008) where in most cases they are co-expressed with A_{2A} receptors. They are also expressed at low levels on platelets, where they are upregulated following injury and systemic inflammation *in vivo* inducing an inhibition of platelet aggregation (Yang et al., 2010). Moreover, A_{2B} receptors are expressed on the surface of endothelial cells (Feoktistov et al., 2004) where they are up regulated by the hypoxia inducible factor (HIF-1α) (Eltzschig et al., 2004). In agreement, vascular permeability was significantly increased in organs of A_{2B} receptor KO mice subjected to ambient hypoxia. By contrast, hypoxia-induced vascular leak was not accentuated in A_1, A_{2A} or A_3 receptor KO mice, suggesting a specific role of A_{2B} receptor in endothelial cells (Eckle et al., 2007). Moreover, A_{2B} receptor KO mice exposed to hypoxia exhibit increased neutrophil infiltration into hypoxic tissues revealing an inhibitory role for A_{2B} receptors in neutrophil transmigration *in vivo* (Eckle et al., 2007, 2008). Attenuation of hypoxia-associated increases in tissue neutrophil numbers appeared to depend largely on hematopoietic cell A_{2B} receptor signaling (Yang et al., 2006; Eckle et al., 2007).

Recent introduction of new pharmacological tools (Hinz et al., 2014) led to understand a role of A_{2B} receptor in ischemia. Intravenous treatment with the selective A_{2B} receptor agonist BAY60-6583 (1 mg/kg), at the start of reperfusion after brain ischemia induced by tMCAo, reduced lesion volume and attenuated brain swelling and BBB disruption. In the presence of tPA (administered after ischemic stroke to dissolve intravascular clots), BAY60-6583 also mitigated sensorimotor deficits and reduced tPA induced hemorrhages at 24 hours after ischemia (Li et al., 2017). The neurovascular protection afforded by BAY60-6583 appears to derive from stimulation of the tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) production, inhibition of tPA-induced matrix metalloprotease (MMP) activation, and prevention of tight junction protein degradation. In fact, over-activation of MMP leads to increased cerebrovascular permeability after ischemia-reperfusion injury (Mishiro et al., 2012). Thus, this study proposed that A_{2B} receptor agonists might be adjuvant to tPA and could be a promising strategy for decreasing the risk of hemorrhages during treatment for ischemic stroke (Li et al., 2017).

All together these studies point toward a role of central A_{2B} receptors, in synergy with A_{2A} receptors in promoting brain excitotoxicity, while A_{2B} receptors located on vascular endothelial

cells would play a pivotal role in attenuating hypoxia-induced increases in vascular leak. Blood immune cells would be implicated in dampening vascular adhesion signals and hypoxia-induced inflammation (Koeppen et al., 2011).
To date there are no evidences in literature on the protective effects of A_{2B} receptor agonists at more distant times from ischemia when a defined neuroinflammation develops.

Role of Carbonic Anhydrase Inhibitors in cerebral ischemia

The Carbonic anhydrases (CAs; also known as carbonate dehydratases) are a superfamily of metalloenzymes present in prokaryotes and eukaryotes that are encoded by five distinct, evolutionarily unrelated gene families:

- α-CAs: present in vertebrates, plants and algae
- β-CAs: present in bacteria, algae and fungi;
- γ-CAs: present in archaea and bacteria;
- δ-CAs and ζ-CAs: both present in marine diatoms.

Of the five different classes of CA, the α class has perhaps received the most attention because of its role in human pathology. Indeed, a large number of α-CA isoforms have been described in vertebrates: 15 in humans and other primates, and 16 in other mammals, with different catalytic activity, subcellular localization and tissue distribution. There are five cytosolic forms (CA I, CA II, CA III, CA VII and CA XIII), five membrane-bound enzymes (CA IV, CA IX, CA XII, CA XIV and CA XV), two mitochondrial forms CA (Va and CA Vb), and a secreted CA isozymes (CA VI). Three a-catalytic forms are also known, which are denominated CA related proteins (CARP), CARP VIII, CARP X, and CARP XI.

1.13 CA Structure and Mechanism

CA catalyze a simple physiological reaction: the conversion between carbon dioxide and water and the dissociated ions of carbonic acid (i.e. bicarbonate and hydrogen ions) (Figure 20) and are thus involved in crucial physiological processes connected with respiration and transport of CO_2/bicarbonate between metabolizing tissues and lungs, pH and CO_2 homeostasis, electrolyte secretion in a variety of tissues/organs, biosynthetic reactions (such as gluconeogenesis, lipogenesis and ureagenesis), bone resorption, calcification,

tumorigenicity, and many other physiologic or pathologic processes (Hewett-Emmett, 2000; Supuran et al., 2000, 2002).

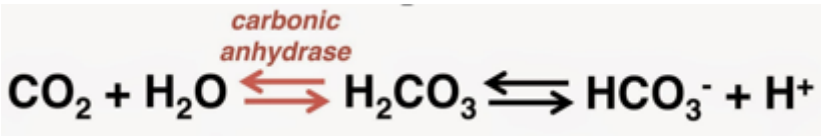


Figure 20. Reaction catalyzed by CA.

The basic catalytic mechanism of CA was established from studies of bovine CA and human CAs I and II (Silverman et al., 1983, 1988).

The active site of CAs contains a zinc ion (Zn^{2+}), which is essential for catalysis. X-ray crystallographic data show that the ion is situated at the bottom of a 15 Å deep active-site cleft, and is coordinated by three histidine residues (His94, His96 and His119) and a water molecule/hydroxide ion (Supuran, 2008) (Figure 21).

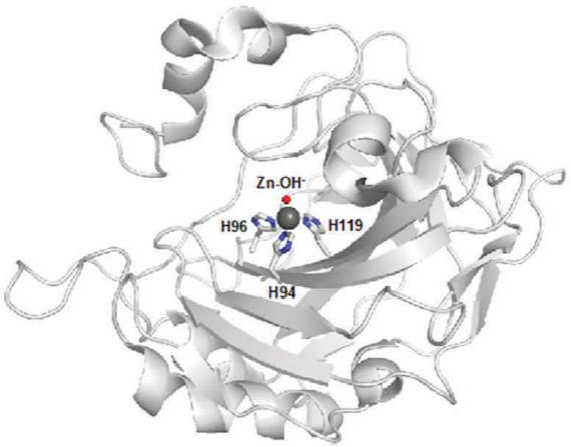


Figure 21. Cartoon structure of CA II; His residues coordinating the catalytic zinc are shown as sticks and labeled. The zinc-bound hydroxide ion/water is represented as a red sphere. Modified from (Lomelino et al., 2016).

The zinc-bound water is also engaged in hydrogen bond interactions with the hydroxyl moiety of Thr 199, which in turn is bridged to the carboxylate moiety of Glu 106; these interactions enhance the nucleophilicity of the zinc-bound water molecule and orient the substrate (CO_2) in a favorable location for the nucleophilic attack (Figure 22).

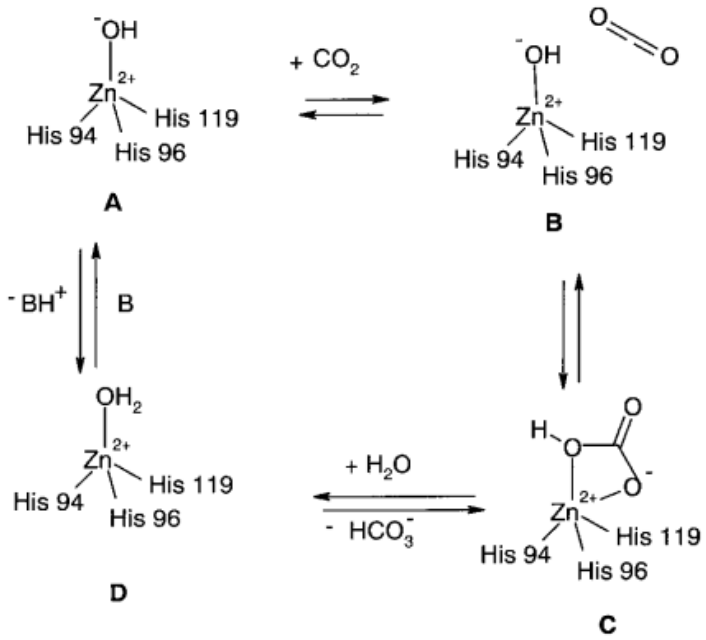


Figure 22. Schematic representation of the catalytic mechanism for the CA. From (Supuran, 2003).

The active form of the enzyme is the basic one, with hydroxide bound to Zn^{2+} (Figure 22 A). This strong nucleophile attacks the CO_2 molecule that is bound in hydrophobic pocket in its neighborhood (Figure 22 B), leading to the formation of bicarbonate coordinated to Zn^{2+} (Figure 22 C). The bicarbonate ion is then displaced by a water molecule and liberated into solution, leading to the acid form of the enzyme, with water coordinated to Zn^{2+} (Figure 22 D), which is catalytically inactive (Supuran, 2008).

1.14 CA Inhibition

Two main classes of carbonic anhydrase inhibitors (CAIs) are known: the metal-complexing anions (thiocyanate, azide etc.) and the unsubstituted sulphonamides and their biososteres. These inhibitors act by binding to the catalytic Zn^{2+} ion in the active site of the enzyme and blocking its function (Neri et al., 2011).

There are over 20 CAIs that have received FDA approval or are in clinical trials for drug therapies (Mincione et al., 2007; Pinard et al., 2013). However, the majority of these compounds are sulfonamide-based and act systemically.

The sulfonamides were recognized to act as potent CAIs already in the 1950, and the first diuretic based on this class of pharmacologic agents, acetazolamide (5-acetamido-1,3,4-thiadiazole-2-sulfonamide), started to be clinically used in 1956, with this drug still being used nowadays (Supuran, 2008).

The CAIs are clinically used as diuretics (Carta et al., 2013), anti-epileptics agents (Thiry et al., 2007; Aggarwal et al., 2013), anti-glaucoma drugs (Carta et al., 2012; Masini et al., 2013 a), and were recently validated for management of neuropathic pain (Carta et al., 2015; Supuran, 2016), cerebral ischemia (Di Cesare Mannelli et al., 2016) and rheumatoid arthritis (Margheri et al., 2016; Bua et al., 2017). A crucial problem in CAIs design is related to the high number of isoforms, their diffuse localization in many tissue and organs (see Table 3) and the lack of isozyme selectivity of the presently available inhibitors.

Isozymes	Subcellular Localization	Tissue/Organ Localization
CA I	Cytosol	Erythrocytes, GI tract
CA II	Cytosol	Erythrocites, eye, GI tract, bone osteoclasts, kidney, lung, testis, brain
CA III	Cytosol	Skeletal muscle, adipocytes
CA IV	Membrane-bound	Kidney, lung, pancreas, brain, capillaries, colon, heart muscle
CA Va	Mitochondria	liver
CA Vb	Mitochondria	Heart and skeletal muscle, pancreas, kidney, spinal cord, GI tract
CA VI	Secreted (saliva, milk)	Salivary and mammary glands
CA VII	Cytosol	CNS
CA VIII	Cytosol	CNS
CA IX	Transmembrane	Tumours, GI mucosa
CA X	Cytosol	CNS
CA XI	Cytosol	CNS
CA XII	Transmembrane	Renal, intestinal, reproductive epithelia, eye, tumours
CA XIII	Cytosol	Kidney, brain, lung, gut, reproductive tract
CA XIV	Transmembrane	Kidney, brain, liver
CA XV	Membrane-bound	Kidney

Table 3. CA distribution. GI= gastro-intestinal. Modified from (Supuran, 2008).

1.15 Role of CAIs in the CNS

The human CNS as well as the choroid plexus are among the tissues/organs having the highest number of CA isoforms, although their functions are still not completely understood.

Given the multitude of CA isoforms in the CNS, their inhibition was exploited therapeutically for anticonvulsant agents already in the '70s, with acetazolamide and methazolamide the most used agents, in some forms of epilepsy (Aggarwal et al., 2013). The mechanisms by which CAIs show antiepileptic action are rather complex and there is not a definitive consensus among researchers on many aspects related to this pharmacological effect (Aggarwal et al., 2013;

Hamidi et al., 2015). Inhibition of brain CAs leads to a diminished formation of bicarbonate and also changes the brain pH, contributing thus to an antiepileptic effect by several diverse pathways, among which the diminished depolarization of principal cells and of interneurons being the crucial factors (Aggarwal et al., 2013; Hamidi et al., 2015). Sulfonamides are scarcely used clinically as antiepileptic drugs, due to their promiscuous inhibition of most CA isoforms and as a consequence a multitude of side effects (Supuran, 2008). More recent, drugs such as topiramate and zonisamide are widely clinically used such agents in many types of epilepsy (Thiry et al., 2007; Aggarwal et al., 2013).

There are several other possible pharmacologic applications of the CAIs related to the inhibition of CNS isoforms, including neuropathic pain (Carta et al., 2015; Supuran, 2016) and cerebral ischemia (Di Cesare Mannelli et al., 2016). Neuropathic pain affects up to 8% of the population with few therapeutic options available for its management (Supuran, 2016). Recent advances in understanding the pathological mechanisms of this syndrome and the biochemical/pharmacological characterization of novel drug targets, evidenced that CA inhibition might be a novel approach for obtaining anti-neuropathic pain agents (Carta et al., 2015; Supuran, 2016). Novel classes of selective sulfonamide CAII/VII inhibitors were designed (Carta et al., 2015), which indeed showed highly improved efficacy in animal model of neuropathic pain, compared to acetazolamide, offering the basis for the development of specific therapies based on selective CA isoforms inhibition.

1.15.1 Role of CAIs in cerebral ischemia

Recently a relationship between hypoxia, a key feature of cerebral ischemia, and several CA isoforms over-expression through the hypoxia-inducible factor (HIF) transcription factor has been highlighted (Neri et al., 2011), suggesting CA IX/XII inhibition as a possible target for this condition (Di Cesare Mannelli et al., 2016; Supuran et al., 2018).

Few studies are present in literature about carbonic anhydrase and its inhibitors in cerebral ischemia. Among them, Regli et al. (Regli et al., 1971) demonstrated that cats underwent middle cerebral artery occlusion and treated with acetazolamide (500 mg via intramuscularly), appeared to have more severe neurological deficits, larger areas of ischemia and infarction and more brain swelling respect to untreated cats.

A recent study (Di Cesare Mannelli et al., 2016) evaluated the pharmacological profile of several coumarin 23 and sulfonamide 24 CAIs in an animal model of cerebral ischemia, rats which underwent a permanent middle cerebral artery occlusion (pMCAO). Vehicle-treated rats showed a clear neurological deficit after pMCAo, and repeated subcutaneous injections, 5 and

20 minutes after pMCAo, of the CAIs 23 and 24 (at 1 mg/kg subcutaneously) were able to significantly reduce the neurological deficit. Sulfonamide 24 also showed the tendency to reduce the infarct volume after pMCAo (Di Cesare Mannelli et al., 2016), whereas the classic CAI, acetazolamide, was ineffective neither to reduce neurological deficit nor to reduce brain damage. The properties of novel CAIs to improve neurological functionalities after cerebral ischemic insult were thus for the first time demonstrated, opening the way to a novel, innovative pharmacological treatment of this condition.

2. Aims of the Research

Aim of Section I:

Role of adenosine A_{2B} receptor on differentiation in cultured oligodendrocyte precursor cell and possible interaction with SphK/S1P signaling pathway.

Oligodendrocyte progenitor cells (OPCs) are a population of cycling cells which persist in the adult central nervous system (CNS) where, under opportune stimuli, differentiate into mature myelinating oligodendrocytes. While the role of adenosine A_1 and A_{2A} receptors during OPCs development was investigated, the role of A_{2B} and A_3 receptors is still unclear.

The bioactive lipid mediator S1P has recently gained attention in myelination within the CNS since its receptors ($S1P_{1-5}$) are found in oligodendroglial cells, where they regulate a number of processes, ranging from cell proliferation and maturation to dendrite elongation.

From these evidences, it appears that both adenosine receptors and SphK/S1P signaling are key regulators of oligodendrocyte development.

The aim of the present study was to investigate the still unknown functional role of adenosine A_{2B} receptors and its possible interaction with SphK/S1P signaling pathway in OPC maturation. We thus investigated the effects of adenosine A_{2B} receptor ligands and SphK/S1P modulators on myelin basic protein (Mbp) and myelin-associated glycoprotein (MAG) expression at different times in culture.

Aim of Section II:

Role of adenosine A_{2B} receptor agonist, BAY60-6583, in a transient model of focal cerebral ischemia in the rat.

Adenosine A_{2B} receptors are expressed at low levels uniformly throughout the CNS and has a low affinity for adenosine. An overexpression of A_{2B} receptors occurs on endothelial cells, neurons, and astrocytes 24 hours after tMCAo in the rat. Thus, during conditions of hypoxia or ischemia when the extracellular adenosine levels rise, A_{2B} receptors might be well activated in the CNS. Besides brain cells, A_{2B} receptors are present on blood immune cells i.e neutrophils, lymphocytes that take part in the inflammatory phenomena that developing hours and days after ischemia, contribute to enlarge the ischemic damage. Thus, A_{2B} receptors might play a crucial role in the inflammatory phenomena.

To date there are no evidences in literature on the effects of A_{2B} receptor ligands after ischemia when a defined neuroinflammation develops. Purpose of the present work was to check the putative neuroprotective effects of the adenosine A_{2B} agonist, BAY60-6583 chronically administered for 7 days after transient (1 hour) focal cerebral ischemia induced by MCAo in the rat.

Aim of Section III:

Role of carbonic anhydrase inhibitors in a rat model of cerebral ischemia

The evidence that hypoxic microenvironments elicit the expression of specific isoform of CA (in particular CA IX and CA XII) through the hypoxia inducible factor allows to hypothesize a possible relevant role of CA in ischemia.

Recently it has been demonstrated that carbonic anhydrase inhibitors (CAIs), sulfonamide and coumarin, were able to improve neurological functionalities after cerebral ischemic insult.

Preliminary data obtained in our laboratory in a model of *in vitro* ischemia (data not yet published) demonstrated that two CAIs, acetazolamide and AN11-740 were able to prevent the appearance of anoxic depolarization (AD), a phenomenon strictly related to cell damage and death, 30 minutes after oxygen and glucose deprivation (OGD) condition in hippocampal slices.

Based on this preliminary result, the aim of this study was to investigate the putative protective effect of two CAIs, acetazolamide and AN11-740 in the *in vivo* model of permanent cerebral ischemia in the rat.

3. Materials and Methods

Section I.

3.1 Cell cultures

Purified cortical OPC cultures were prepared as described elsewhere (Coppi et al., 2013 b). Wistar rat pups (postnatal day 1-2) were killed and cortices removed, mechanically and enzymatically dissociated, suspended in DMEM medium containing 20% fetal bovine serum (FBS), 4 mM L-glutamine, 1 mM Na-pyruvate, 100 U/ml penicillin, 100 U/ml streptomycin (all products are from EuroClone, Milan, Italy), and plated in poly-D-lysin coated T75 flasks (1 flask per animal). After 2-3 days in culture, OPCs growing on top of a confluent monolayer of astrocytes were detached by 5 h of horizontal shaking. Contaminating microglial cells were eliminated by a 1 h pre-shake and by further plating of detached cells on plastic culture dishes for 1 h. OPCs, which do not attach to plastic, were collected by gently washing the dishes and replated onto poly-DL-ornithine-coated (final concentration: 50 μg/ml, Sigma-Aldrich) 13 mm-diameter glass coverslips laid in 24 multi well chambers (l04 cells/well: electrophysiological and immunocytochemical experiments), poly-DL-ornithine-coated 25 mm-diameter glass coverslips laid in 6 multi well chambers (2 x l04 cells/well: Ca2+ imaging experiments) or poly-DL-ornithine-coated 6 cm tissue culture dishes (3.5 x 105 cells/dish: western-blot experiments). OPC cultures were maintained in Neurobasal medium (Thermo Fisher Scientific) containing 2% B27, 4 mM L-glutamine, 1 mM Na-pyruvate, 100 U/ml penicillin, 100 U/ml streptomycin, 10 ng/ml platelet derived growth factor (PDGF-BB) and 10 ng/ml basic fibroblast growth factor (bFGF; both grow factors were from PeproTech EC Ltd, London, UK) to promote cell proliferation (proliferating medium: PM).

3.2 OPC differentiation

OPCs show a bipolar or tripolar morphology, when grown in a proliferating medium (PM); they were allowed to differentiate to mature oligodendrocytes, by switching to a Neurobasal medium lacking growth factors (differentiating medium: DM). The day at which cells were switched from PM to DM is indicated as t0. According to our previous results (Coppi et al., 2013a; Coppi et al., 2013b), cells cultured in DM undergo gradual maturation as demonstrated by the decrease on NG2 expression and the parallel increase in myelin-related proteins such as MAG, Mbp1 and Mbp3 antigens. After 7 days in DM (t7) the expression of myelin-related proteins reaches steady state levels (no further increase at t10 was observed) so the culture is considered to be composed of mature OLs. In order to study the effect of adenosine A_{2B} receptor and/or

SphK/S1P signaling pathway on oligodendroglial maturation, OPCs were grown in DM in the absence or presence of different pharmacological treatments for 7 days (from t0 to t7).

3.3 Real-time PCR

Gene expression analysis was performed by Real-Time PCR, using 2^ (-ΔΔCT) comparative method of quantification (Livak et al. 2001). Briefly, total RNA (500 ng), extracted with GenElute™ Mammalian Total RNA Miniprep (Sigma-Aldrich s.r.l. Milan, Italy at http://www.sigma-aldrich.com) was reverse transcribed using iScript™ Advanced cDNA Synthesis Kit for RT-qPCR (Bio-Rad Laboratories S.r.l., Segrate (MI), Italy, at http://www.bio-rad.com/) according to the manufacturer's instructions. The design of MAG, Mbp and A_{2B} probes was performed employing Primer Express® Software v3.0.1 (Thermo Fisher Scientific INC. Monza (MB), Italy, at https://www.thermofisher.com/) that provides customized application-specific documents for absolute and relative quantitation. Rat oligonucleotide primers employed in gene expression studies are listed in Table 4 The quantification of target gene mRNA levels was performed employing PowerUp™ SYBR™ Green Master Mix (Bio-Rad Laboratories S.r.l). Each measurement was carried out in triplicate, using the automated ABI Prism 7500 Sequence Detector System (Thermo Fisher Scientific, INC) as previously described (Donati *et al.*, 2007), by simultaneous amplification of the target gene together with the housekeeping gene (beta-actin and GAPDH) in order to normalize expression data. Results were analysed by ABI Prism Sequence Detection Systems software, version 1.7 (Applied Biosystems, Foster City, CA). The 2^(−ΔΔCT) method was applied as a comparative method of quantification (2) and data were normalized to beta-actin expression.

Oligo Name	**Sequence 5' to 3' (include modification codes if applicable)**
Mag-FW	TTCCGAATCTCTGGAGCACCTGATAAG
Mag-RV	TCCTCACTTGACTCGGATTTCTGCGT
Actb-FW	GAACACGGCATTGTCACCAACTGGGA
Actb-RV	GCCTGGATGGCTACGTACATGGCT
Mbp1-FW	GCCCTCTGCCTTCTCATGCCC
Mbp1-RV	CCTCGGCCCCCCAGCTAAATCT
ADORA2B-FW	GTGGGAGCCTCGAGTGCTTTACAG
ADORA2B-RV	GCCAAGAGGCTAAAGATGGAGCTCTG

Table 4. Sequences of rat oligonucleotide primers employed in gene expression studies**.**

3.4 Western blot analysis

Primary rat cortical OPC culture were collected after 10 μM BAY60-6583 challenge for 10 min and lysed in a buffer containing 50 mM Tris, pH 7.5, 120 mM NaCl, 1mM EDTA, 6 mM EGTA, 15 mM Na4P2O7, 20 mM NaF, 1% Nonidet and protease inhibitor cocktail for 30 min at 4°C. Then lysates were centrifuged at 10.000xg, 15 min 4°C, supernatant collected for protein quantification and 15 μg of protein from total cell lysates were used to perform a SDS-polyacrylamide gel electrophoresis and Western blot (WB) analysis. The rate of phosphorylation of SphK1 and SphK2 was measured using the Sphingosine kinase activation antibody sampler kit (ECM Biosiences ECM Biosciences, Versailles, KY USA at https://www.ecmbiosciences.com/index.php?content=Contact. PDVF membranes were incubated overnight with the primary antibodies at 4°C and then with specific secondary antibodies for 1 h at room temperature. Binding of the antibodies with the specific proteins has been detected by chemiluminescence, employing Amersham imager 600.

3.5 Adenosine A_{2B} receptor silencing by RNA interference

OPC grown into tissue culture 6-well plates up to 60% confluence, were transfected with siRNA duplexes using Lipofectamine RNAiMAX (Thermo Fisher Scientific INC), according to the manufacturer's instructions as previously described (Bruno et al 2018). Briefly, Lipofectamine RNAiMAX was incubated with siRNA in Neurobasal without supplement and antibiotics for 20 minutes, and afterward the lipid/RNA complexes was added to cells to a final concentration of 50 nM in Neurobasal containing growth factors and supplements and without antibiotics. After 24 hours, OPC was shifted to differentiating medium and then used for the experiments within 72 hours. Successful siRNA repression was confirmed by real-time reverse transcription polymerase chain reaction (RT-PCR).

3.6 Drugs

2-[[6-Amino-3,5-dicyano-4-[4-(cyclopropylmethoxy)phenyl]-2-pyridinyl]thio]-acetamide (BAY60-6583, Figure 23); amidine analogues that inhibits sphingosine kinase, VPC96047 and VPC96091 (Figures 24 and 25), were kindly provided by Prof. K. Lynch (University of Virginia); (2S)-amino-2-[2-(4-octylphenyl)ethyl]-1-(dihydrogen phosphate)-1,3-propanediol. (FTY720 (S)-Phosphate, Figure 26) was provided by Cayman Chemical, (Michigan USAhttps://www.caymanchem.com/); the new, highly selective, adenosine A_{2B} receptor agonist, (2-{[(1H-imidazol-2-yl) methyl] thio}-6-amino-4-[4-(cyclopropylmethoxy)

phenyl]pyridine-3,5-dicarbonitrile), P453 showed in Figure 27 (Betti et al., 2018). 5-[4-Phenyl-5-(trifluoromethyl) thiophen-2-yl]-3-[3-(trifluoromethyl) phenyl]1,2,4-oxadiazole (SEW2871, Figure 28) and [3R-amino-4-[(3-hexylphenyl) amino]-4-oxobutyl]-phosphonic acid (W146, Figure 29) were kindly provided by Prof. F. Cencetti (University of Florence).

3.7 Chemical structure of drugs

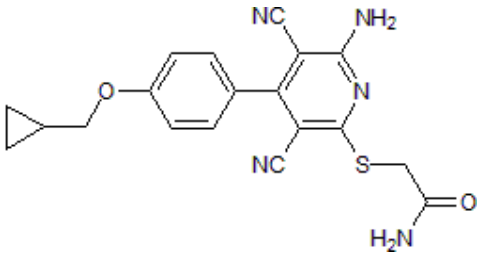


Figure 23. BAY60-6583: adenosine A_{2B} receptor agonist.

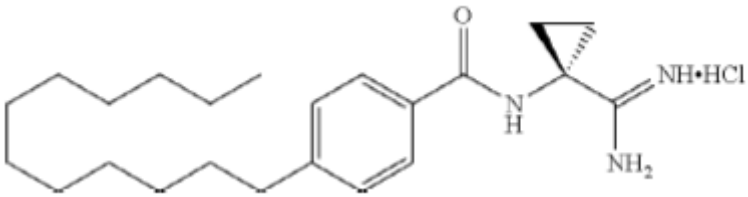


Figure 24. VPC96047: a-specific inhibitor of the two isoforms $SphK_1$ e $SphK_2$.

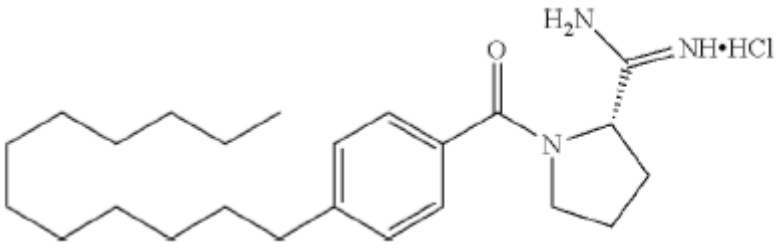


Figure 25. VPC96091: selective inhibitor of $SphK_1$.

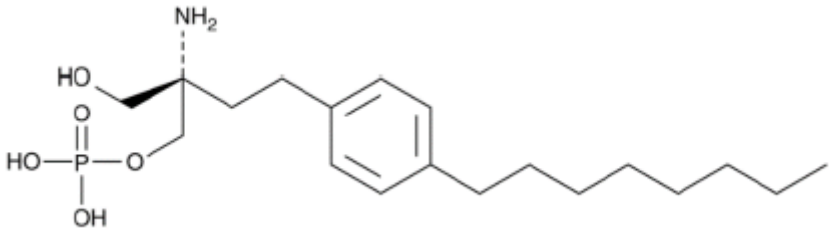


Figure 26. FTY720-P.

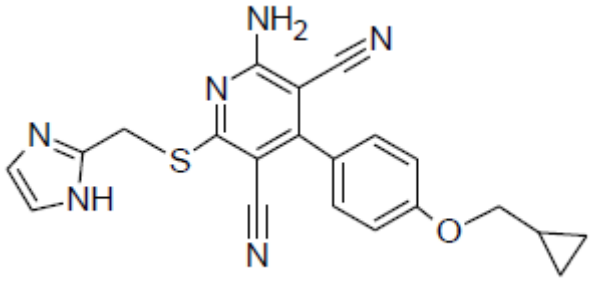


Figure 27. P453: the non-adenosine like adenosine A_{2B} receptor agonist.

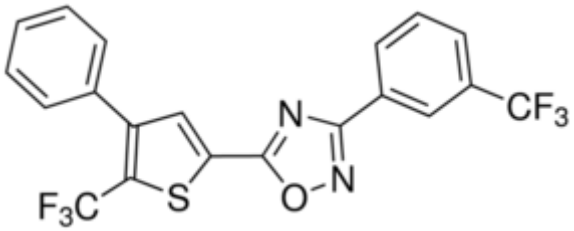


Figure 28. SEW2871: $S1P_1$ agonist.

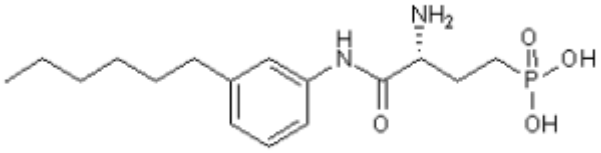


Figure 29. W146: potent and selective $S1P_1$ antagonist.

3.8 Statistical analysis

Data are expressed as mean ± SEM (standard error of the mean). Student's paired or unpaired t-tests or One-way ANOVA followed by Bonferroni's post-hoc test analysis were performed, as appropriate, in order to determine statistical significance (set at $P<0.05$). Data were analyzed using software package Graphpad PRISM (GraphPad Software, San Diego, CA, USA).

Section II and III:

3.9 Animals

Male Wistar rats (Envigo, Italy) weighting 270–290 g were used. Animals were housed in groups of three with free access to food and water and kept under standardized temperature, humidity and light conditions (12 h light/dark cycle) within the animal house facility of the University of Florence. The experimental procedures described were approved by the local Animal Welfare Body (AWB) of the University of Florence and authorized by the Italian Ministry of Health (Authorization n. 118/2016-PR). The ethical policy of the University of Florence complies with to the Directive 2010/63/EU of the European Parliament and to the Italian Regulation DL 26/2014 on the protection of animals used for scientific purposes. According to the law, all efforts were made to fulfill to the principle of 3Rs.

3.10 Surgery

Focal cerebral ischemia was induced by intraluminal middle cerebral artery occlusion (MCAo) in the right hemisphere. The animals were anesthetized with 5.0% isoflurane (Baxter International) and spontaneously inhaled 1.0 to 2.0% isoflurane in air by the use of a mask. Body core temperature was maintained at 37°C with a recirculating pad and K module and was monitored via an intrarectal type T thermocouple (Harvard, Kent, UK). The surgical procedure

to occlude the MCA consisted in insertion of a 4-0 nylon monofilament (Doccol corporation, USA), via the external carotid artery into the internal carotid artery in order to block the origin of the MCA according to the procedure originally described by Longa et al., (1989) and modified by Melani and co-workers (Melani et al., 1999) (Figure 30).

In permanent middle cerebral artery occlusion (pMCAo), the nylon filament was not removed while in the transient (1 hour) model of focal cerebral ischemia (tMCAo), animals were re-anesthetized with isoflurane and re-perfused by withdrawing the filament. The sham operation was conducted by inserting the filament into the internal carotid artery and immediately withdrawing it.

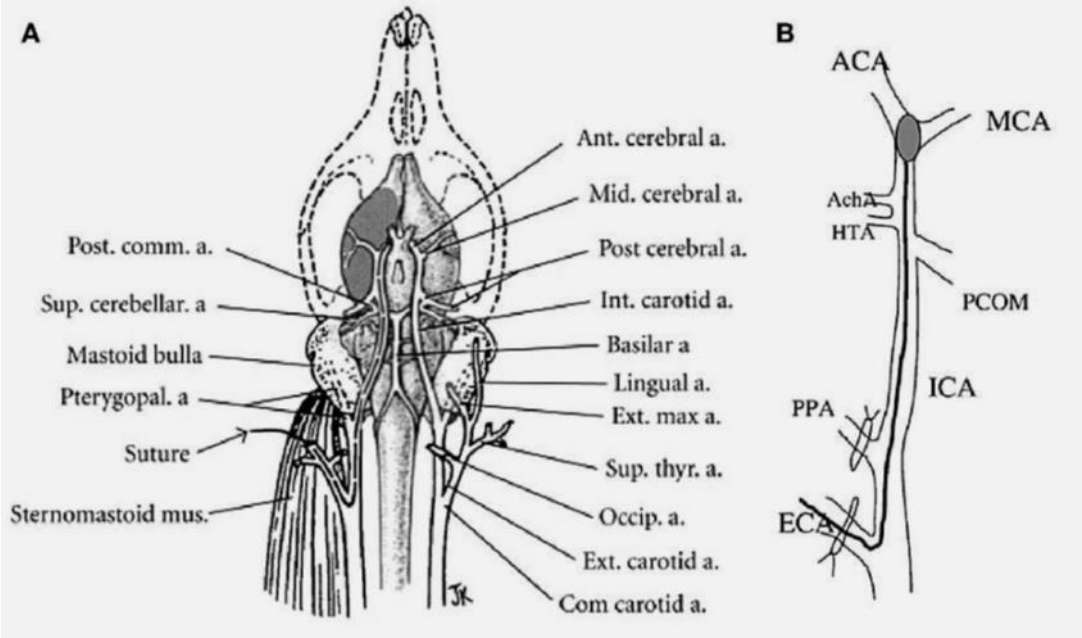


Figure 30. Intraluminal middle cerebral artery occlusion**.** Abbreviations: ACA: anterior cerebral artery; ECA: external carotid artery; ICA: internal carotid artery; MCA: middle cerebral artery; PCOM: posterior communicating artery; PPA: pterygopalatine artery. From (Canazza et al., 2014).

3.11 Chronic treatment with adenosine A_{2B} receptor agonist, BAY60-6583

3.11.1 Drug administration

Adenosine A_{2B} receptor agonist 2-[[6-Amino-3,5-dicyano-4-[4-(cyclopropylmethoxy) phenyl]-2-pyridinyl] thio]-acetamide (BAY60-6583) (Tocris, Bristol, United Kingdom, UK) (Figure 23), was dissolved in DMSO 0.1%. BAY60-6583 was chronically administered at the dose of 0.1 mg/kg, intraperitoneally (i.p.) twice/day for 7 days.

3.11.2 Experimental groups

A group of animals were subjected to transient middle cerebral artery occlusion (tMCAo) and sacrificed 7 days after tMCAo. Rats were randomly allocated in the following groups: (1) sham-operated rats (n=7): did not receive any treatment; (2) tMCAo+vehicle group (n=6): saline with DMSO (0.1%) administered (i.p.) twice/day for 7 days, starting 4 hours after tMCAo; (3) tMCAo+BAY 60-6583 group (n=6): BAY60-6583 administered (i.p.) twice/day for 7 days, starting 4 hours after tMCAo.

A second group of animals were subjected to tMCAo and sacrificed 2 days after tMCAo. Rats were randomly allocated in the following groups: (1) sham-operated rats (n=3): did not receive any treatment; (2) tMCAo+vehicle group (n=3): saline with DMSO (0.1%) administered (i.p.) twice/day for 2 days after tMCAo, starting 4 hours after tMCAo; (3) tMCAo+BAY 60-6583 group (n=4): BAY60-6583 administered twice/day for 2 days after tMCAo, starting 4 hours after tMCAo.

3.12 Sub-chronic treatment with Carbonic Anidrase inhibitors

3.12.1 Drugs administration

Acetazolamide (5-acetamido-1,3,4-thiadiazole-2-sulfonamide), (Figure 31) was dissolved in saline with 0.05% dimethyl sulfoxide (DMSO). Acetazolamide was sub-chronically administered at the dose of 4.4 mg/kg, i.p.

AN11-740 was dissolved in saline with 0.01% DMSO. AN11-740 was sub-chronically administered at the dose of 1 mg/kg, i.p.

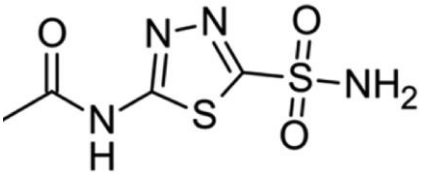


Figure 31. Acetazolamide.

3.12.2 Experimental groups

Animals subject to pMCAo were sacrificed 24 hours after surgery. Rats were randomly allocated in the following groups: (1) sham-operated rats (n=7): did not receive any treatment; (2) pMCAo+vehicle group (n=4): saline+DMSO; (3) pMCAo+AN11-740 group (n=6); (4) pMCAo+AAZ group (n=4). The vehicle or both carbonic anidrase inhibitors were administered i.p. 5 minutes, 6 hours and 16 hours after occlusion.

3.13 Neurological deficit

The neurological deficit was evaluated by modified Neurological Severity Score (mNSS) test described by Chen et al., (2001). All tests were carried out before pMCAo or tMCAo and 1, 5 and 7 days after MCAo. The mNSS test evaluates the sensorimotor deficit: it is composed of motor, sensory, reflex and beam balance tests (Figure 32). The score assigned to each rat at completion of the evaluation equals the sum of all test scores. The test is graded on a scale from 0 (normal score) to 18 (maximal deficit score). In the beam balance test, a score between 0 (normal score) and 6 (maximal deficit score) was assigned to each animal in function of the ability to stay and walk on the beam. Beam balance test score affects 1/3 of the total mNSS score.

TABLE 1. Modified Neurological Severity Score Points

Motor tests	
Raising rat by tail	3
Flexion of forelimb	1
Flexion of hindlimb	1
Head moved >10° to vertical axis within 30 s	1
Placing rat on floor (normal=0; maximum=3)	3
Normal walk	0
Inability to walk straight	1
Circling toward paretic side	2
Falls down to paretic side	3
Sensory tests	2
Placing test (visual and tactile test)	1
Proprioceptive test (deep sensation, pushing paw against table edge to stimulate limb muscles)	1
Beam balance tests (normal=0; maximum=6)	6
Balances with steady posture	0
Grasps side of beam	1
Hugs beam and 1 limb falls down from beam	2
Hugs beam and 2 limbs fall down from beam, or spins on beam (>60 s)	3
Attempts to balance on beam but falls off (>40 s)	4
Attempts to balance on beam but falls off (>20 s)	5
Falls off; no attempt to balance or hang on to beam (<20 s)	6
Reflex absence and abnormal movements	4
Pinna reflex (head shake when auditory meatus is touched)	1
Corneal reflex (eye blink when cornea is lightly touched with cotton)	1
Startle reflex (motor response to a brief noise from snapping a clipboard paper)	1
Seizures, myoclonus, myodystony	1
Maximum points	18

One point is awarded for inability to perform the tasks or for lack of a tested reflex: 13–18, severe injury; 7–12, moderate injury; 1–6, mild injury.

Figure 32. Example of modified Neurological Severity Severity (mNSS) score points scale for sensorimotor deficit evaluation.

3.14 Body weight evaluation

Rats used to induce cerebral ischemia were in the body weight ranging from 270 to 290 g. The body weight was evaluated before pMCAo or tMCAo and after 1, 5, 7 days from occlusion. The weight variation after ischemia of each animal was evaluated with respect to its own pre-ischemia weight.

3.15 Ischemic brain damage

Rats were anesthetized with Zoletil 50/50 (100 mg/kg i.p., Virbac, Carros, Francia) and were perfused transcardially with an ice-cold 4% paraformaldehyde solution (in phosphate buffer, pH 7.4). Brains were post-fixed overnight and cryoprotected in a 18% sucrose solution (in phosphate buffer) for at least 48 hours. Brains were cut with a cryostat and coronal sections (30 µm) were collected at 210 µm intervals at 12 different levels through the striatum (König and Klippel, 1967). One and seven days after pMCAo and tMCAo respectively, brain slices were stained by acetate cresyl violet (1%) or by hematoxylin and eosin (H&E). Histological analysis by cresyl violet staining allows to clearly define the infarct area and volume up to 1 week after ischemia (Rousselet et al., 2012). To evaluate area and volume of ischemic damage, 12 cresyl violet-stained brain sections per animal were placed directly on the scanning screen of a color flatbed scanner (CanoScan LiDE 90; Canon). Following image acquisition, the images were analyzed using ImageJ software. The measurements of infarct area in striatum and cortex were obtained by manually outlining the margins of infarcted area. Ischemic cortical and striatal volumes were calculated by multiplying the infarcted area by the slice thickness and summing the volume of the 12 slices.

After H&E staining, heterochromatic nuclei were counted at Bregma level within an optical field at 40X in ischemic cortex and striatum. Data were then averaged and expressed as mean±SEM of number cells per optical field of "n" animals.

3.16 Neuronal damage, gliosis and blood cell infiltration

Immunohistochemistry was performed with the free-floating method (Giovannini et al., 2002; Lana et al., 2014) on rat brain coronal sections in striatum and cortex.

The following *primary antibodies* were used: for neurons a mouse antineuronal nuclei (NeuN) antibody, dilution 1:400 (Product Code #MAB377, Millipore, Billerica, MA, United States); for astrocytes a mouse anti-GFAP antibody conjugated with the fluorochrome AlexaFluor 488, dilution 1:500 (Product Code #MAB3402X, Millipore, Billerica, MA, United States); for microglia a rabbit anti-ionized calcium binding adaptor molecule 1 (IBA1) antibody, dilution 1:400 (Product Code #016-20001, WAKO, Osaka, Japan); for granulocytes a mouse monoclonal antibody anti-HIS-48, dilution 1:50 (Santa Cruz Biotechnology). The following fluorescent *secondary antibodies* were used: AlexaFluor 555 donkey anti-mouse, dilution 1:400 (Product Code #A31570, Thermo Fisher Scientific); AlexaFluor 635 goat anti-rabbit, dilution 1:400 (Product Code #A31577, Thermo Fisher Scientific). Nuclei were stained using DAPI,

contained in the mounting medium for glass slides, Vectashield (Product Code #H1200, Vector Laboratories, Burlingame, CA, United States). The sections were mounted onto gelatin-coated slides using Vectashield with DAPI (Vector Laboratories). Slices were observed with LEICA TCS SP5 a confocal laser scanning microscope (Leica Microsystems CMS GmbH, Manheim, Germany).

3.16.1 Microscopy Techniques, qualitative and quantitative analysis

The confocal microscopy images were obtained with a LEICA TCS SP5 confocal laser scanning microscope (Leica Microsystems CMS GmbH, Mannheim, Germany). The parameters of acquisition were maintained constant: frame dimension 1024 x 1024 points, frequency of acquisition 200 Hz.

Two experimenters performed all quantitative analyses blind, and data were averaged. All evaluations of cell density were made on z projections of 10 consecutive confocal scans (total thickness 12 µm). Cells were counted, and the area of analysis was measured. Density of cells (neurons, astrocytes, microglial cells) was calculated as cells/mm^2 in striatum and cortex, on confocal z projections of ten scans (total 12 µm inside the section).

3.17 Determination of TNF-α and IL-10 plasma levels

The levels of Tumor Necrosis Factor-α (TNF-α) pro-inflammatory cytokine and of interleukine-10 (IL-10) a regulatory cytokine, were measured on aliquots (100µl) of plasma using commercial ELISA kits (Rat TNF-α Platinum ELISA, Catalogue no: BMS622, Affymetrix eBioscience, Vienna, Austria; Rat IL-10 Platinum ELISA, Catalogue no: BMS629, Affymetrix eBioscience, Vienna, Austria), following the protocol provided by the manufacturer. Results are expressed as pg of protein/ml of plasma.

3.18 Statistical analysis

Data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test, Repeated Measures two-way analysis of variance (ANOVA) followed by Tukey post-hoc test and by unpaired Student's t test as specified in text and in figure legends. A value of $p<0.05$ was considered statistically significant. The statistical analysis was performed utilizing GraphPad Prism.

4. Results

Section I. Adenosine A_{2B} receptor and SphK/S1P signaling axis control maturation of OPC *in vitro*.

4.1 Adenosine A_{2B} receptor activation stimulates $SphK_1$ phosphorylation in cultured OPCs

Electrophysiology experiments in our laboratory demonstrated that adenosine A_{2B} receptor activation by 10μM BAY60-6583 inhibited voltage-dependent K^+ currents in cultured OPCs. In the attempt to identify a putative cross-talk between A_{2B} receptor and SphK/S1P signaling pathway, we studied whether an acute, 10 minutes, BAY60-6583 application (10 μM) in purified OPC cultures, could possibly affect the activation of one or both isoforms of SphK, $SphK_1$ and $SphK_2$, known to be expressed in oligodendroglial cells (Saini et al., 2005). Since it has been previously demonstrated that $SphK_1$ and $_2$ activation require phosphorylation and then translocation to the plasma membrane, where the substrate is located (Pitson et al., 2003; Hait et al., 2007), we analyzed the phosphorylation of both enzymes after 10 minutes BAY60-6583 challenge. Results presented here clearly show a significant increase in the phosphorylation of $SphK_1$, but not $SphK_2$, in cells exposed for 10 minutes to 10 μM BAY60-6583, as reported in Figure 33 (A and B).

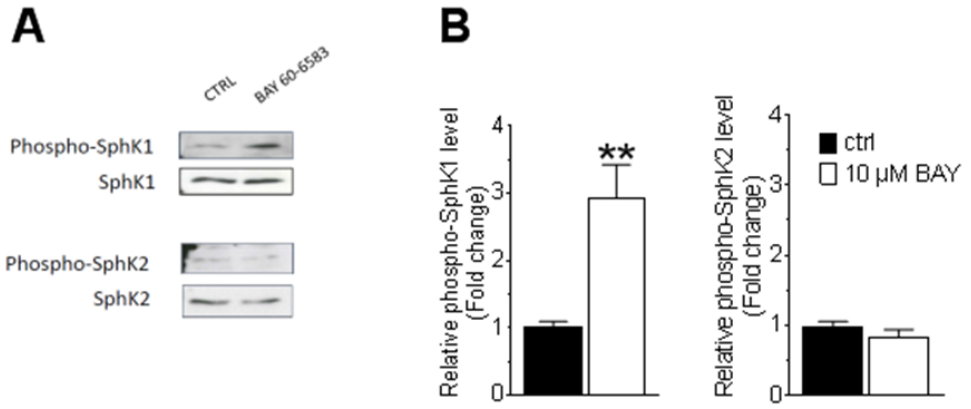


Figure 33. The A_{2B} receptor agonist BAY60-6583 stimulates SphK1 phosphorylation in cultured OPCs. A) Western Blot analysis of phospho-$SphK_1$ (upper panel) and phospho-$SphK_2$ (lower panel) in total cell lysates of OPC cultures demonstrates that 10 min application of 10 µM BAY60-6583 in cultured OPCs enhances $SphK_1$ phosphorylation. B) In the histogram, band intensity corresponds to the phosphorylated form of $SphK_1$ or $SphK_2$ and is normalized to $SphK_1$ or $SphK_2$ total content. Data are mean ± SEM of three independent experiments, -fold change over control, set as 1. Densitometric analyses are reported as fold change above control (Ctrl).

4.2 Adenosine A_{2B} receptor and SphK activation modulate oligodendrocyte maturation in vitro.

To study oligodendroglial cell maturation *in vitro*, OPC cells were led to differentiate into mature OLGs by growing for 3, 7 and 10 days in DM. As shown in figure 34 A, a significant and time dependent increase in MAG and Mbp expression was found, compared to OPC (open bar), that was maximal at 7 days (black bar). Moreover, we dissected the time-dependent expression of the adenosine A_{2B} receptor in OLG compared to control OPC and found out that the expression of the receptor increases by the time of incubation with DM (Figure 34 B). The expression of A_{2B} receptor was not affected by FTY720-P treatment, whereas, interestingly, it was dramatically decreased by a 7 days exposure to VPC96047 in respect to control untreated OLGs (black bar). Since $S1P_5$ displays a time-dependent expression during OLG differentiation, we performed Real-Time PCR to measure $S1P_5$ expression at 3, 7 and 10 days, and confirmed previous results reporting an increase of the receptor during differentiation from OPC towards OLG, turning to be the most expressed S1P receptor in mature OLGs (Yu et al., 2004; Jung et al., 2007; Novgorodov et al., 2007).

Moreover, $S1P_5$ mRNA levels were not significantly changed after 7 day-treatment with FTY720-P nor VPC96047 (Figure 34 C, orange bar, purple bar).

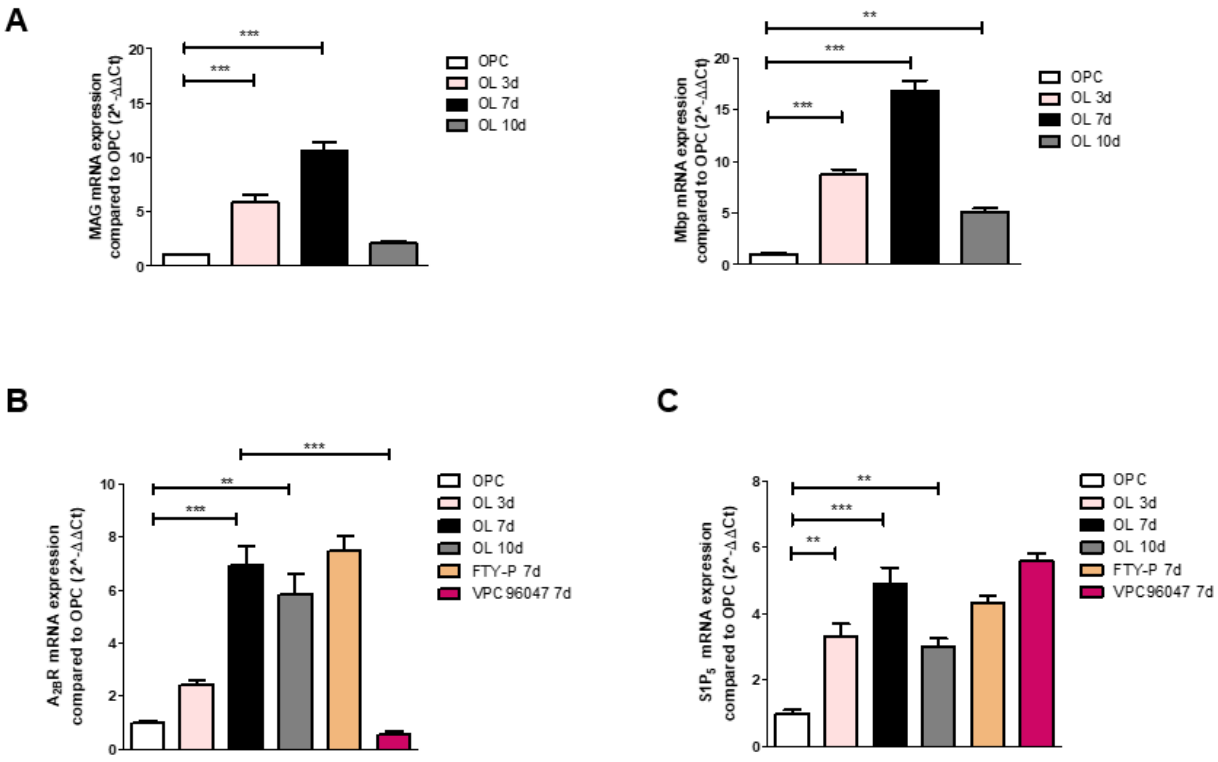


Figure 34. Time course of oligodendrocyte marker expression, A_{2B} receptor and $S1P_5$ receptor during differentiation from OPC to OL. A) Gene expression analysis of oligodendrocyte differentiation markers MAG and Mbp was performed by Real Time-PCR in OPC and 3, 7 and 10 day-differentiation (OL 3d, 7d, 10d) employing specific rat primers, as reported in table 3, using SYBR green probe. Each measurement was carried out in triplicate. The 2^(−ΔΔCT) method was applied as a comparative method of quantification and data were normalized to β–actin expression. B) A_{2B} receptor expression, analyzed employing specific rat primers and SYBR Green probe, was reported in OPC and after 3, 7, 10 days (t3, t7, t10) of DM, as 2^(-ΔΔCT) normalized to β–actin and compared to the control OPC. OL 7d were challenge with or without 1 µM FTY720-P and 500 nM VPC96047 (FTY720-P 7d and VPC96047 7d). C) $S1P_5$ receptor expression, analyzed employing specific rat primers, was reported as 2^-ΔΔCT, normalized to β–actin. OL at t7 were challenge with or without 1 µM FTY720-P and 500 nM VPC96047 (FTY720-P t7 and VPC96047 t7). Data are means ± SEM of three independent experiments performed in triplicate. Statistical analysis was performed using One-way ANOVA, followed by Bonferroni post-hoc test, *p<0.05, **p<0.01, ***p<0.001.

Finally, we investigated the involvement of the A_{2B} receptor agonists in oligodendroglial cell maturation in vitro, alone or in combination with S1P analogues or SphK inhibitors.

According to the results reported in the figure 34, we selected 7 days of DM as the reference time to analyze OPC differentiation under different conditions.

When OPCs were grown in DM for 7 days in the presence of the A_{2B} receptor agonist BAY60-6583 (10 µM), the compound prevented the increase of either MAG or Mbp expression in comparison to 7 day OLGs (Figure 35 A, red bar compared to open bar), indicating that A_{2B} receptor stimulation counteracts OPC maturation towards OLGs. A similar result was obtained with the other A_{2B} agonist, P453 (500nM) (Figure 35 A, brown bar).

The opposite effect in comparison to A_{2B} receptor stimulation was observed when OPCs were grown in the presence of the pan SphK-inhibitor VPC96047 or the selective SphK1-inhibitor VPC96091 (both at 500 nM, dark grey, dark green, respectively). In these two experimental groups, a further increase in MAG and Mbp expression was observed in comparison to 7 day OLGs, thus indicating that SphK inhibition promotes OPC maturation. Of note, neither of the two SphK inhibitors tested were able to prevent BAY60-6583- nor P453-mediated inhibition of OPC differentiation, demonstrating that A_{2B} receptor stimulation overrides the pro-differentiating effect of SphK inhibition.

The S1P analogue FTY720-P, at a concentration of 1 µM, mimicked the effect of BAY60-6583 in inhibiting oligodendroglial differentiation (Figure 35 A, purple bar).

Among the S1P receptors possibly involved in modulating OPC maturation, we tested the role of $S1P_1$ receptor by applying the selective $S1P_1$ agonist SEW2871 (1 µM) and antagonist W146 (10 µM). As reported in figure 35 B and C, neither of them affected OLG differentiation nor 10 µM W146 ruling out a role for $S1P_1$ in this process.

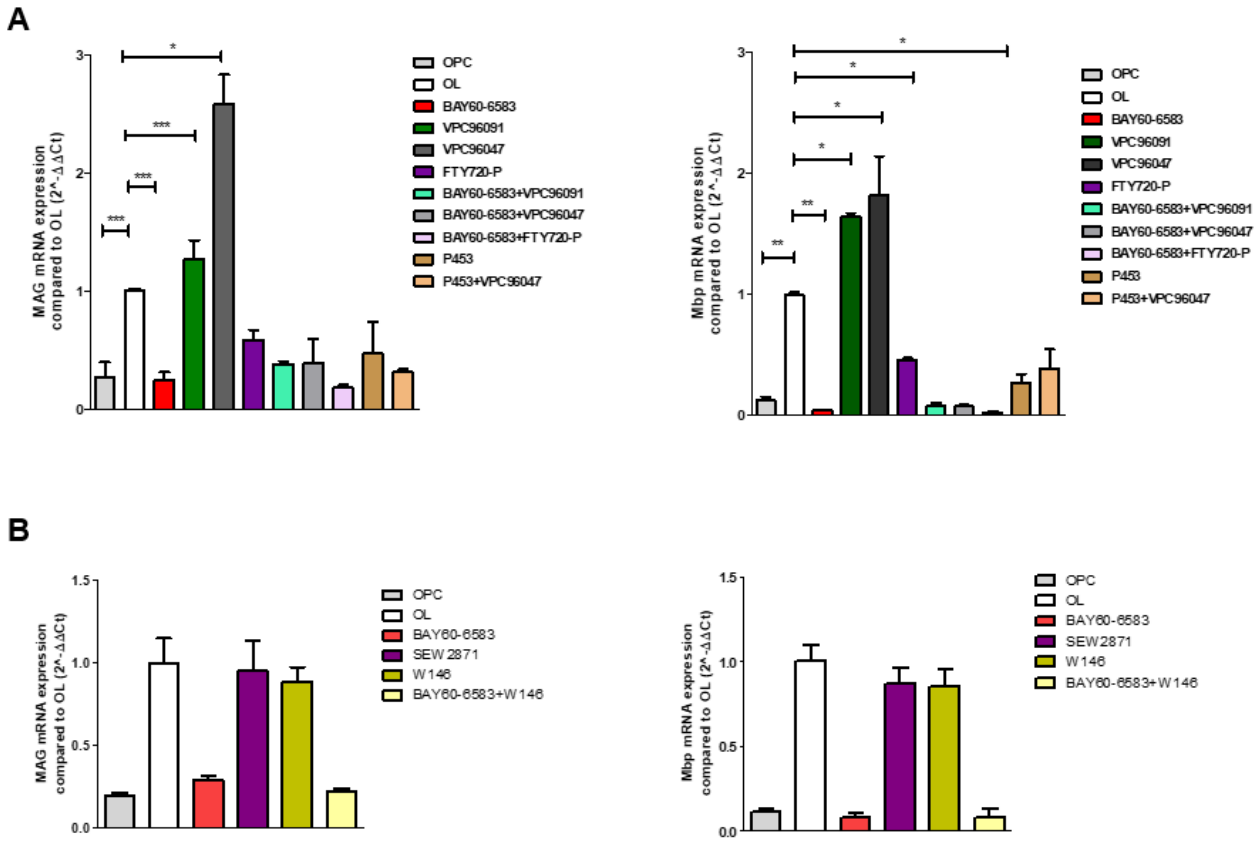


Figure 35. Adenosine A_{2B} receptor and SphK/S1P mediated effects on oligodendrocyte differentiation in vitro. A) Gene expression analysis of oligodendrocyte differentiation markers MAG and Mbp by Realtime-PCR in OPC and after 7 days of DM (t7 OL) in the absence or presence of: BAY60-6583 (10 µM), VPC96047 (500 nM), VPC96091 (500 nM), FTY720-P (1 µM) and P453 (500 nM). The quantification of MAG and Mbp mRNA was performed by Real-Time PCR employing specific primers as reported in materials and methods section using SYBR green probe. Each measurement was carried out in triplicate. The 2^(−ΔΔCT) method was applied as a comparative method of quantification and data were normalized to β–actin expression. B) Gene expression analysis of oligodendrocyte differentiation markers MAG and Mbp by Realtime-PCR in OPC and after 7 days of DM (OL 7d) in the absence or presence of: BAY60-6583 (10 µM), SEW2871 (1 µM), W146 (10 µM). The quantification of MAG and Mbp mRNA was performed by Real-Time PCR as described above. Data are means ± SEM of three independent experiments performed in triplicate.
Statistical analysis was performed by One-way ANOVA followed by Bonferroni post-hoc test: *p<0.05; **p<0.01; ***p<0.001.

4.3 Effects of adenosine A_{2B} receptor silencing on S1P pathway and oligodendrogenic differentiation.

In order to clarify whether the effects on OPC maturation and differentiation can be attributed to A_{2B} receptor-mediated effects, we performed A_{2B} silencing by RNA interference. (Figure 36).

After 48 hours silencing, about 50% A_{2B} receptor was downregulated ($p<0.01$). Of note, A_{2B} downregulation increased OPC differentiation (increase of CNPase, a myelin-marker and decrease of NG2 level) ($p<0.05$; $p<0.001$). These data consistent with our previous results demonstrating that A_{2B} agonists inhibits OPC maturation towards OLGs. Moreover, cells transfected with A_{2B}-siRNA showed a striking increase in S1P lyase levels, the enzyme responsible for of S1P catabolism (black bar, $p<0.01$).

A_{2B} downregulation also decreased S1P production (reduction of SphK levels, $p<0.01$) and $S1P_5$ receptor expression ($p<0.01$).

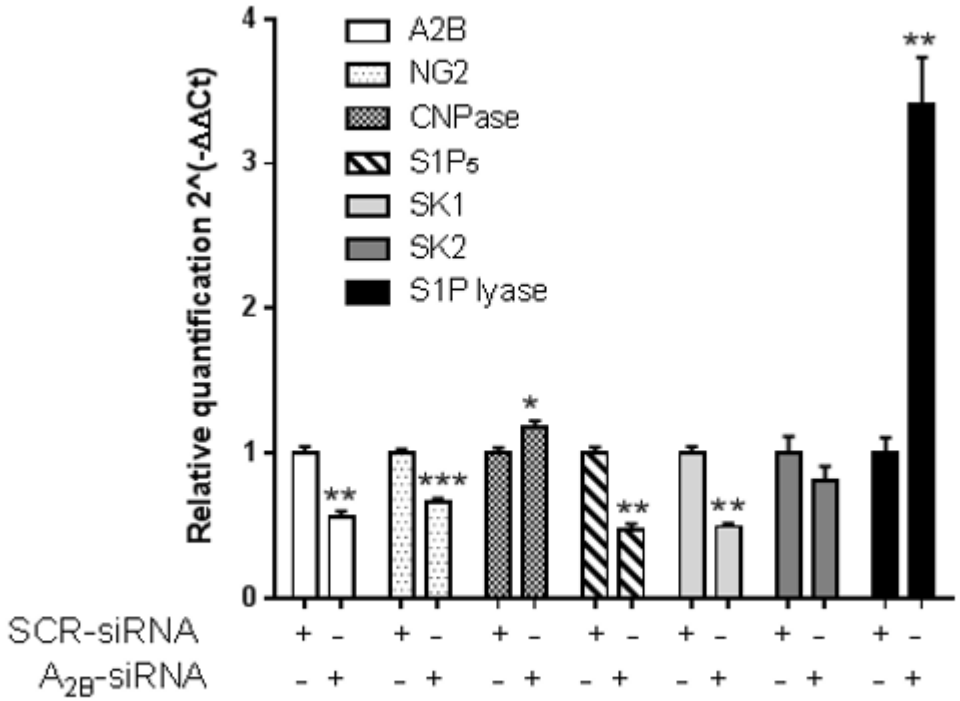


Figure 36. Adenosine A_{2B} receptor silencing in cultured OPCs by small interference RNA (siRNA), facilitates cell maturation and interferes with S1P signaling. Relative quantitative mRNA analysis was performed by Real-Time PCR in OPC transfected with SCR-siRNA or with A2B-siRNA; the content of housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was analyzed in parallel. Results are expressed as fold changes according to the 2^-DDCT method, utilizing as calibrator the expression of each gene in scrambled siRNA-transfected cells. Data are means ± SEM of three independent experiments performed in triplicate. *p<0,05, **p<0,01, ***p<0,001; unpaired Student's t-test.

Section II. Adenosine A_{2B} receptor agonist, BAY60-6583, is protective in a rat model of transient cerebral ischemia.

4.4 Effect of treatment with the adenosine A_{2B} receptor agonist on neurological deficit after tMCAo

Figure 37 A shows that in the mNSS test, sham-operated rats had a neurological score of 0.1-0.6 in the period from 1 to 7 days after tMCAo, showing any neurological deficit. Twenty-four hours after tMCAo, vehicle-treated rats showed a clear neurological deficit with a neurological score of 10.6±0.9 (mean±SEM) that defines a moderate injury. The neurological impairment spontaneously recovered 5 and 7 days after tMCAo. Five days after tMCAo the neurological score was reduced to 6.6±0.7, seven days after tMCAo, the neurological score was further reduced to 5.8±1.1. Chronic treatment with the A_{2B} agonist, BAY60-6583, at the dose of 0.1 mg/kg, significantly reduced the neurological deficit at each time point after tMCAo. Repeated Measures two-way ANOVA calculated for the two factors: treatment and time after tMCAo, showed that treatment factor ($F_{2,16}$=37.2; p<0.0001), time factor ($F_{2,32}$=17.0; p<0.0001) and interaction between treatment and time ($F_{4,32}$=3.9; p<0.009) were statistically significant. The Tukey post hoc test indicated that sham-operated rats had a neurological score significantly different from vehicle-treated and BAY60-6583-treated rats at each time point (1, 5, 7 days after tMCAo, p<0.0001-0.03). The chronic treatment with BAY60-6583 significantly reduced the neurological deficit with respect to vehicle-treated rats at 1, 5 and 7 days (p<0.0005-0.05) after tMCAo.

4.5 Effect of treatment with the adenosine A_{2B} receptor agonist on body weight loss after tMCAo.

Twenty-four hours after operation, sham-operated rats were not affected in weight, then they increased in body weight as evaluated up to 7 days after tMCAo. On the contrary, vehicle-treated rats lost 17.60±4.5 g 1 day after tMCAo, 14.0±3.6 g 5 days after tMCAo and 0.6±4.1 g 7 days after tMCAo (Figure 37 B). Treatment with the A_{2B} receptor agonist, BAY60-6583, did not significantly modify the body weight loss at any time point after tMCAo respect to vehicle-treated rats. Repeated Measures two-way ANOVA, calculated for the two factors: treatment and time after tMCAo, showed that treatment factor ($F_{2,15}$ = 29.7; p <0.0001), time factor ($F_{2,30}$

= 68.5; p <0.0001) and the interaction between treatment and time ($F_{4,30}$ = 13.7; p <0.0001) were statistically significant. The Tukey post hoc test indicated that in sham-operated rats, body weight was different with respect to vehicle-treated and BAY60-6583-treated rats at each time point (p <0.0001-0.0002) after tMCAo.

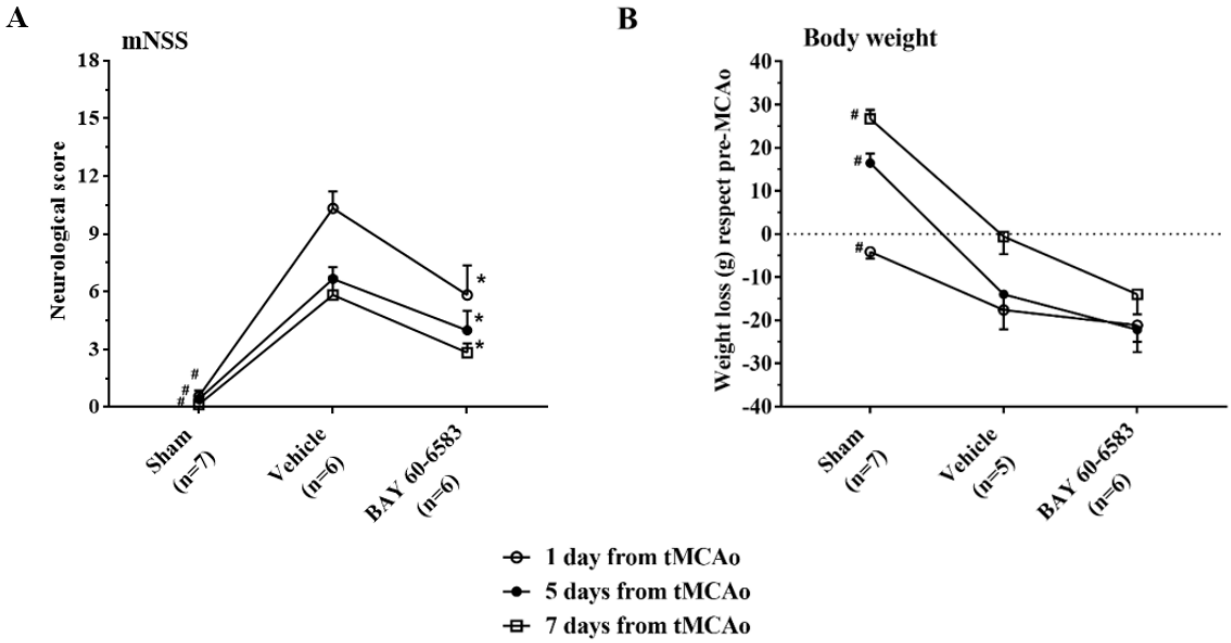


Figure 37. Effect of chronic treatment with BAY60-6583 (0.1 mg/kg i.p.) on neurological deficit (A) and body weight loss (B). Data are expressed as mean±SEM of "n" rats. A) mNSS test: the score is evaluated before and after 1, 5 and 7 days from tMCAo in each rat group. Repeated Measures two-way ANOVA followed by Tukey post hoc test: #p<0.0001-0.03 sham-operated versus chronic BAY60-6583- and vehicle-treated rats; *p<0.0005-0.05 chronic BAY60-6583-treated versus vehicle-treated rats. B) Body weight loss: sham-operated rats increase their body weight in the period after the operation. The body weight loss of tMCAo rats was calculated as the mean±SEM of the difference between body weight at each time point and pre-operation body weight. Repeated Measures two-way ANOVA followed by Tukey post hoc test: #p<0.0001-0.0002 sham-operated versus chronic BAY60-6583 and vehicle-treated rats.

4.6. Effect of treatment with the adenosine A_{2B} receptor agonist on brain ischemic damage after tMCAo

Figure 38 shows the extent of ischemic damage evaluated as infarct area (Figure 38 A, C) and infarct volume (Figure 38 B, D) in ischemic striatum and cortex of vehicle- and BAY60-6583-treated rats 7 days after tMCAo.

Chronic treatment with BAY60-6583, at the dose of 0.1 mg/kg significantly reduced the infarct in both areas. The striatal and cortical infarct volume were reduced by 44.7% and 46.3%

respectively. (Unpaired Student's t-test: $p<0.05$-0.001; Figure 38 B, D). Sham-operated rats did not show any damage.

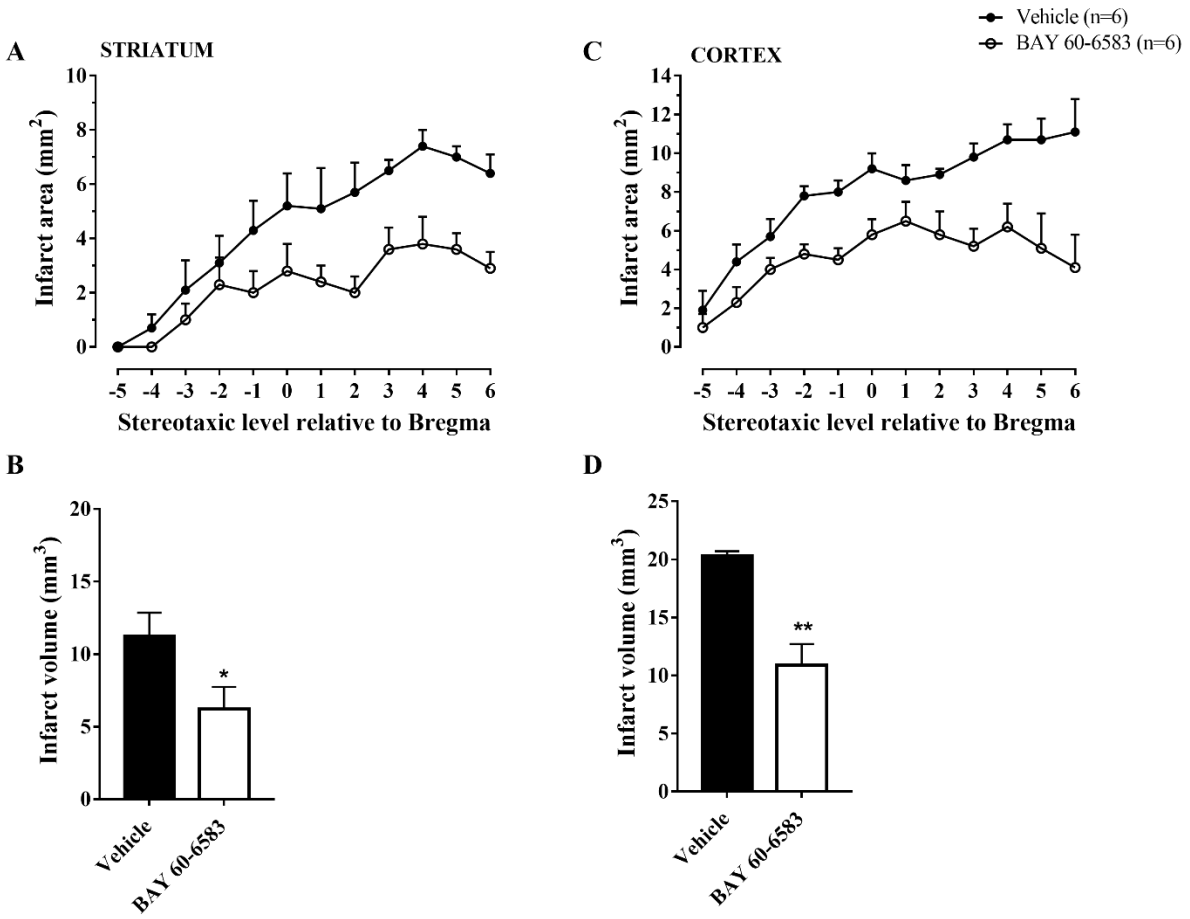


Figure 38. Effect of chronic treatment with BAY60-6583 (0.1mg/kg) on infarct area (A, C) and infarct volume (B, D) in the striatum and cortex 7 days after tMCAo. Data are mean±SEM of infarct area measured at 12 predetermined coronal levels through the brain of "n" rats. Bregma=0 (König et al., 1964). Bar graphs show the infarct volume calculated as mean±SEM in the striatum and cortex. Unpaired Student's t-test: *$p<0.05$; **$p<0.001$ vs vehicle-treated rats.

To characterize the cytoarchitecture of the ischemic cortex and striatum 7 days after tMCAo, ischemic tissue was stained by H&E (Figure 39 A-F). Seven days after transient ischemia, H&E staining showed a decrease in staining intensity in vehicle-treated rats (Figure 39 C, D) compared to the sham-operated rats (Figure 39 A, B). The typical cytoarchitecture of these two regions (for a description see Danner and Pfister, 1981) was lost. In the striatum, the white matter *fascicula* (*f*) were no more recognizable; in the fronto-parietal cortex the columnar organization was not appreciable and very numerous heterochromatic small nuclei were present. Figure 39 E, F shows that chronic administration of BAY60-6583 at the dose of 0.1 mg/kg, was associated with a recovery of staining intensity and with a reduction of heterochromatic small nuclei staining in both brain regions.

The cytoarchitecture of the white matter *fascicula* was recognizable in the dorsal corpus striatum (Figure 39 E) and the columnar organization was appreciable in the fronto-parietal cortex (Figure 39 F). Quantitative analysis showed that chronic BAY60-6583 treatment significantly reduced heterochromatic nuclei number in the ischemic striatum ($p<0.01$; Fig. 39 G) and in the ischemic cortex ($p<0.005$; Fig. 39 H).

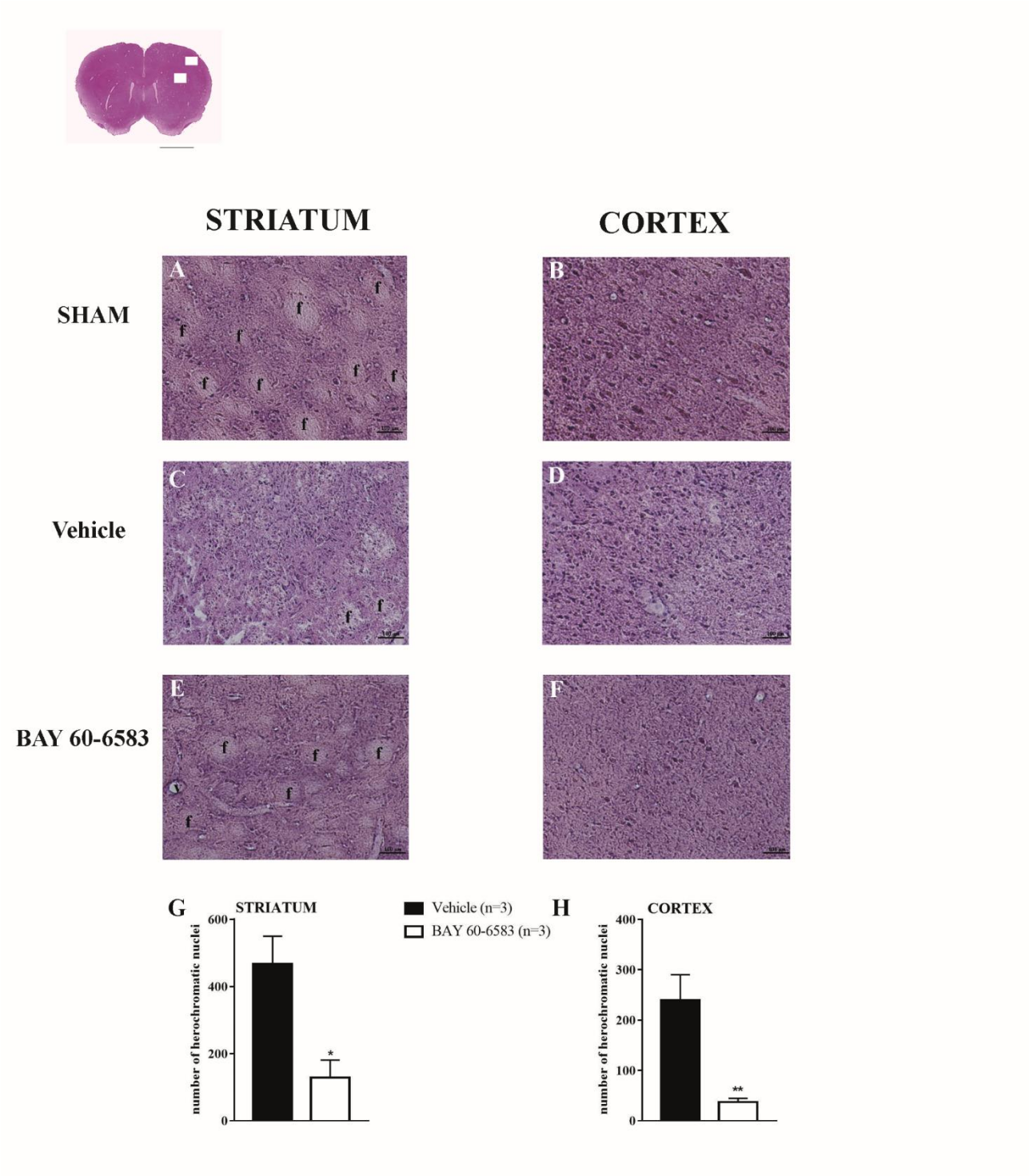


Figure 39. Effect of chronic treatment with BAY60-6583 (0.1 mg/kg i.p.) on cytoarchitecture of the ischemic striatum and cortex after tMCAo. Upper part: representative photomicrograph of a histological section of control rat (at Bregma + 1.5mm) (König et al., 1964). The two white boxes indicate the ischemic striatal and cortical area reported in the magnifications. Scale bar=2mm). Sham-operated rats: (A) in the dorsal striatum, the typical caudate-putamen cytoarchitecture is appreciable, numerous transversally sectioned white matter *fascicula* (*f*) are surrounded by gray matter containing diverse type of neurons, distinct on the basis of their size and shape (Danner et al., 1981); (B) in the fronto-parietal cortex the typical columnar organization is appreciable. Vehicle-treated ischemic rats: (C) in most parts of the ischemic dorsal striatum, the cytoarchitecture is completely lost. The distinction between white and gray matter is no more appreciable; a remarkable increase of the interstitial spaces and numerous

heterochromatic small nuclei are present; (D) in the ischemic fronto-parietal cortex the columnar organization is hardly visible, the interstitial spaces are enlarged and dilatated and numerous heterochromatic small nuclei are present. BAY60-6583-treated ischemic rats: note the recovery of the staining intensity and the decrease of heterochromatic small nuclei; (E) in the ischemic dorsal striatum, the cytoarchitecture of the white matter *fascicula* is again recognizable; (F) in the ischemic fronto-parietal cortex, the columnar organization is appreciable. Scale bar=100µm. Bar graphs represent the mean±SEM of the number of heterochromatic nuclei per optical field (40X) present at Bregma=0 coronal level of three rats, in ischemic striatum (G) and ischemic cortex (H). Un paired Student's t-test: *p<0.02; **p<0.005 vs. vehicle-treated rats.

4.7 Effect of treatment with the adenosine A_{2B} receptor agonist on neuronal damage in striatum after tMCAo

To estimate the neuronal damage caused by tMCAo in the striatal boundary zone, we performed fluorescent confocal immunohistochemical analysis using neuron specific anti-NeuN antibody in control slices taken from sham-operated rats, in slices taken from vehicle-treated rats and in slices taken from BAY60-6583-treated rats.

Representative images (each obtained stacking 10 consecutive confocal scans, z step of 1.2 µm, total thickness 12 µm) of NeuN immunostaining in the striatal boundary zone are shown in Figure 40 A-C. Figure 40 E shows the density of $NeuN^+$ cells in the striatum of sham-operated, vehicle- and BAY60-6583-treated rats. Density of $NeuN^+$ striatal neurons significantly decreased in vehicle-treated rats in comparison with sham-operated rats (*p<0.05).

The treatment with BAY60-6583 reverted this effect in a statistically significant manner (#p<0.05).

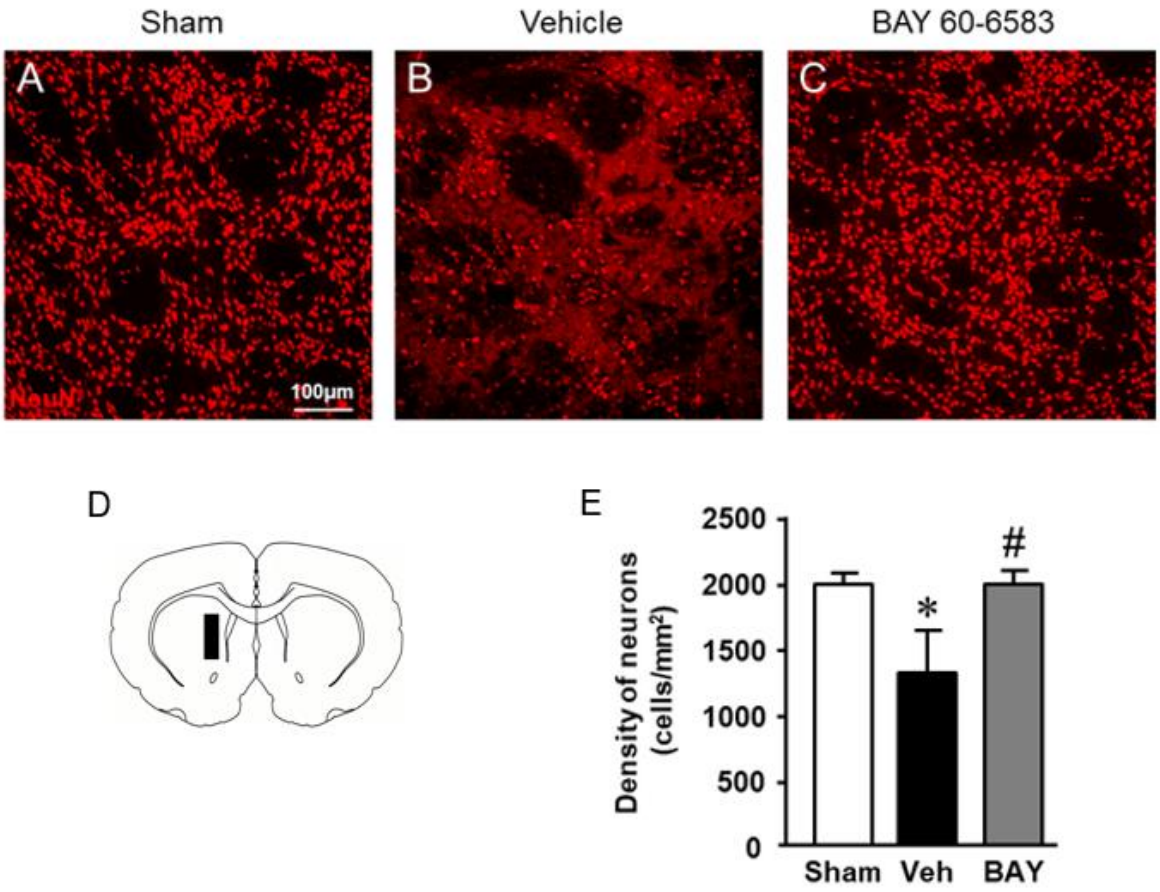


Figure 40. Analysis of neurons in the striatal boundary zone of sham-operated, vehicle- and BAY60-6583-treated rats 7 days after tMCAo. Schematic brain picture (D) indicate the region of interest (dark box) where microphotographs of ischemic boundary zone were captured.
A-C: Representative confocal photomicrographs of NeuN immunostaining of neurons (red) in striatum of a sham (A), a vehicle (B) and a BAY60-6583 (C) rats. Scale bar: 100 µm. E: Quantitative analysis of neurons/mm^2 in striatal boundary zone of sham, vehicle and BAY 60-6583-treated rats. Data reported in all graph bars are expressed as mean±SEM. One-way ANOVA and Newman-Keuls Multiple Comparison Test: *$p<0.05$, vehicle-treated rats vs sham-operated rats; #$p<0.05$ BAY60-6583-treated rats vs vehicle-treated rats.

4.8 Characterization of neuronal damage in the striatum after tMCAo

A closer examination of the neurons in the striatal boundary zone with confocal microscopy indicated the presence of many damaged neurons in both vehicle- and BAY60-6583-treated rats seven days after tMCAo. Representative images (each obtained stacking 10 consecutive confocal scans, z step 1.2 µm each, total thickness 12µm) of NeuN immunostaining in the striatal boundary zone are shown in Figure 41 A-C. Magnifications of the framed areas, shown in figure 41 B1-C1, shows the altered morphology of striatal neurons after tMCAo, in

comparison to those of the sham-operated rat as shown in panel 41 A1. Indeed, in the striatal boundary zone of vehicle- and BAY60-6583-treated rats, we observed many $NeuN^+$ neurons that lost their $NeuN^+$ nuclear immunofluorescence, an index of damaged nuclei, while $NeuN^+$ immunofluorescence persisted in the cytoplasm (Figure 41, white arrows). We defined these neurons as Low Density Nucleus neurons, "LDN neurons" (Fusco et al., 2018). It has been demonstrated that these damaged neurons are apoptotic thanks to cytochrome C staining (Fusco et al., 2018).

In order to better characterize this phenomenon, we performed the quantitative analysis of LDN neurons in sham-operated, vehicle- and BAY60-6583-treated rats (Figure 41 E).

This analysis demonstrated that the percentage of LDN neurons was significantly increased in vehicle-treated rats compared with sham-operated rats (**$p<0.01$ vehicle-treated vs sham-operated rats). Furthermore BAY60-6583 significantly reverted the percentage increase in comparison to vehicle treated rats (#$p<0.05$ BAY60-6583-treated vs vehicle-treated rats).

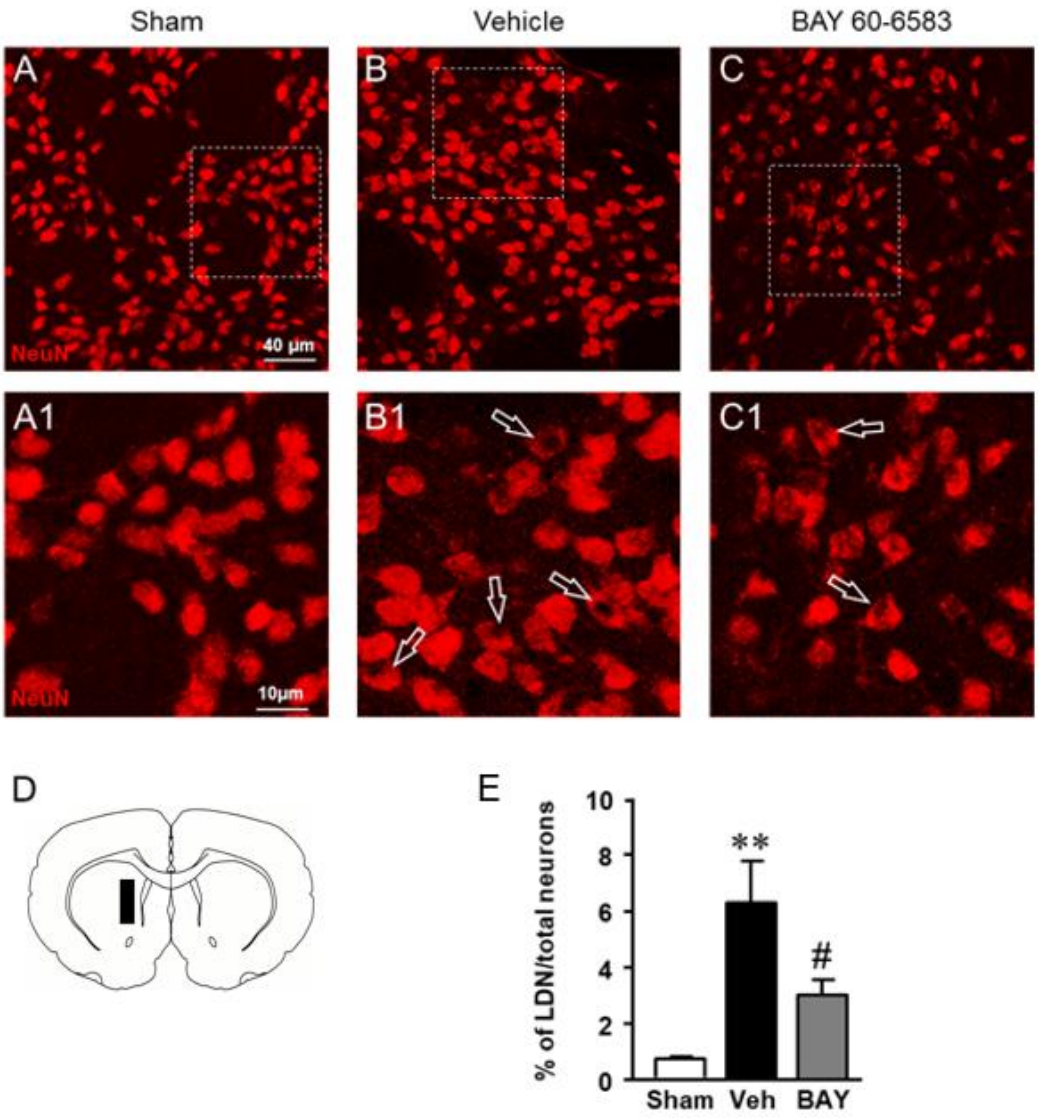


Figure 41. Analysis of damaged neurons in striatum 7 days after tMCAo. Schematic brain picture (D) indicate the region of interest (dark box) where microphotographs of ischemic boundary zone were captured. A-C1: Representative confocal microphotographs of NeuN immunostaining of neurons (red) in the striatal boundary zone of sham-operated (A), vehicle- (B), and a BAY 60-6583-treated (C) rats. Scale bar: 40 µm. Panels A1, B1 and C1 shows the magnification of neurons framed in Panels A, B and C. Scale bar: 10 µm. Note the presence of many LDN neurons (open arrows) in the striatum of vehicle (B1) and BAY60-6583 (C1). E: Quantitative analysis of percent of LDN neurons on total neurons. Percent of LDN neurons was significantly higher in striatum of vehicle vs sham rats. BAY60-6583 reverted this significant increase. All data in the graphs are expressed as mean±SEM. One-way ANOVA and Newman Multiple Comparison Test, **p<0.01 vehicle vs sham-operated rats; #p<0.05 BAY60-6583-treated rats vs vehicle-treated rats.

4.9 Effect of treatment with the adenosine A_{2B} receptor agonist on microglia morphology in the striatum after tMCAo

Total microglia cells were identified in the striatal boundary zone using the fluorescent immunostaining for IBA1, as shown by the representative images of Figure 42 A-C, each obtained stacking 10 consecutive confocal z scans (1.2µm each, total thickness 12 µm) through the thickness of striatal boundary zone. Figure 42 shows the magnifications of the framed areas in panels A1-C1. Quantitative analysis of IBA1-positive cells in Figure 42 E revealed that microglia cells increase in vehicle-treated rats in comparison with sham-operated rats (+95% vehicle vs sham) 7 days after tMCAo, although not in a statistically significant manner. The qualitative analysis shows that in vehicle-treated rats there is a strong pattern of microglia activation (magnification B1): microglial cells appeared with hypertrophic branches, round-shaped and ameboid morphology in comparison with those of sham-operated rats, that are in a resting ramified state (Figure 42 A1). BAY60-6583 reverted these morphological changes reducing the strong pattern of activation. As shown in figure C1, microglia cells belonging to BAY60-6583-treated rats have similar morphology to that of sham-operated rats (Figure A1).

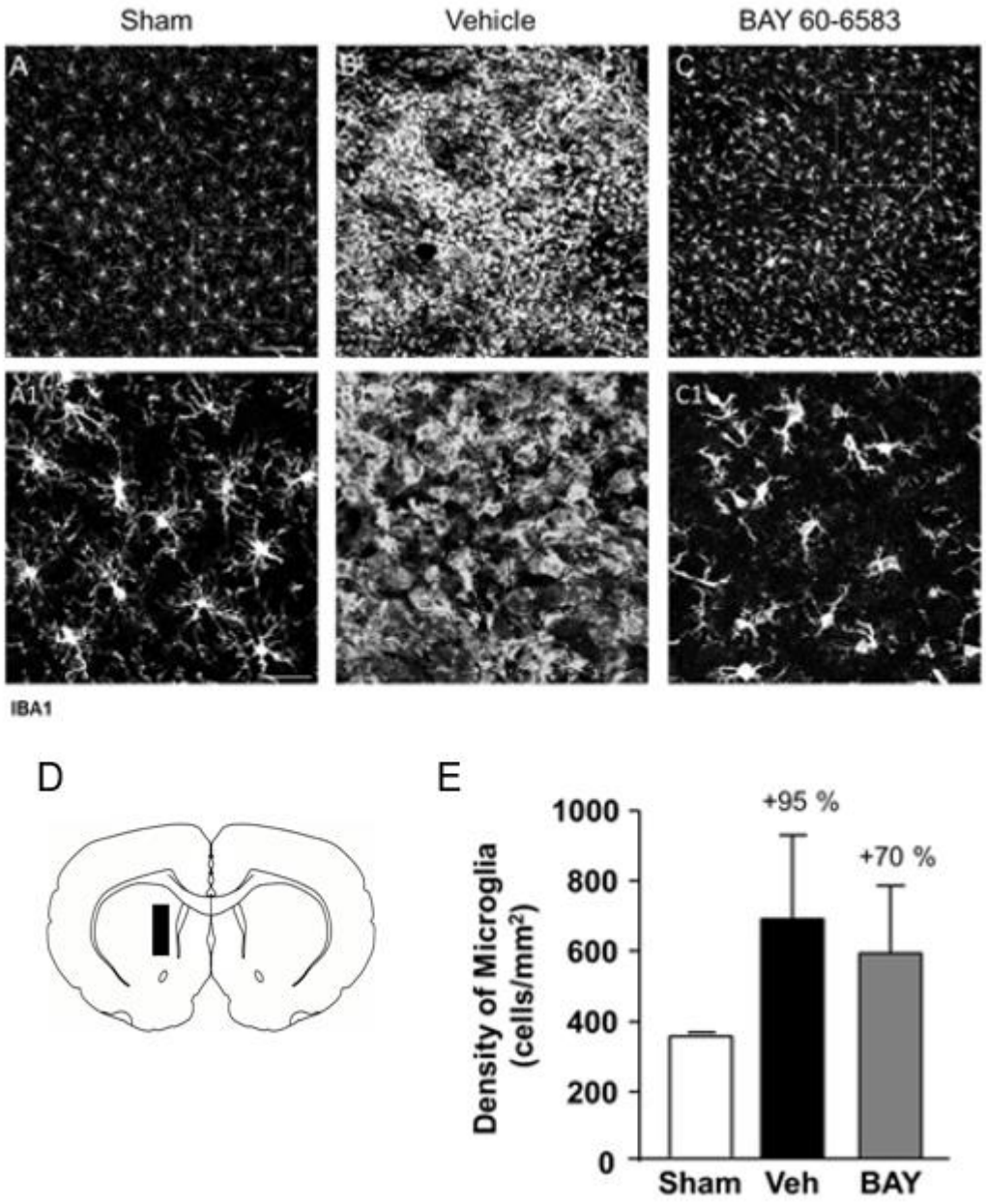


Figure 42. Analysis of microglia in the striatal boundary zone of sham-operated, vehicle- and BAY 60-6583-treated rats 7 days after tMCAo. Schematic brain picture (D) indicate the region of interest (dark box) where microphotographs of ischemic boundary zone were captured. A-C1: Representative confocal photomicrographs of IBA1 immunostaining of microglia (white) in striatal boundary zone of sham-operated (A), vehicle- (B) and BAY60-6583-treated rats (C). Scale bar: 100 µm. Panels A1, B1 and C1 show the magnification of neurons framed in Panels A, B and C. Scale bar: 10 µm. E: Quantitative analysis of IBA1 positive microglia/mm^2 in striatal boundary zone of sham-operated, vehicle- and BAY60-6583-treated rats. All data in the graphs are expressed as mean±SEM.

4.10 Effect of treatment with the adenosine A_{2B} receptor agonist on astrocytes morphology in the striatum after tMCAo

Astrocytes were immunolabelled in the striatal boundary zone with anti-GFAP antibody.
Figure 43 A-C show the representative images, each obtained stacking 10 consecutive confocal z scans (1.2 µm each, total thickness 12 µm) through the thickness of striatal boundary zone.
Panels 43 A1-C1 show the magnifications of the framed areas in figures 43 A-C. Figure 43 B1 shows that 7 days after tMCAo the density and morphology of striatal astrocytes of vehicle-treated rats was significantly different from that of the sham-operated rats (Figure 43 A1). Indeed, in vehicle-treated rats, astrocytes morphology is strongly altered: they are smaller with shorter and highly fragmented branches. Chronic treatment with BAY60-6583, at the dose of 0.1 mg/kg, reverted the alterations caused by ischemia in the morphology of astrocytes (Figure 43 C1): astrocytes in BAY60-6583-treated rats show a morphology mostly similar to that of sham-operated rats.
Quantitative analysis of $GFAP^+$ astrocytes reported in Figure 43 E, shows that the density of $GFAP^+$ astrocytes decreased significantly (-75% vehicle-treated rats vs sham-operated rats) in vehicle-treated rats in comparison to sham-operated rats (*$p<0.05$ vehicle-treated vs sham-operated rats). In slices taken from BAY60-6583-treated rats, the loss of astrocytes was reduced (-32% BAY60-6583-treated rats vs sham-operated rats) although the difference respect to vehicle-treated rats was not different in a statistically significant manner.

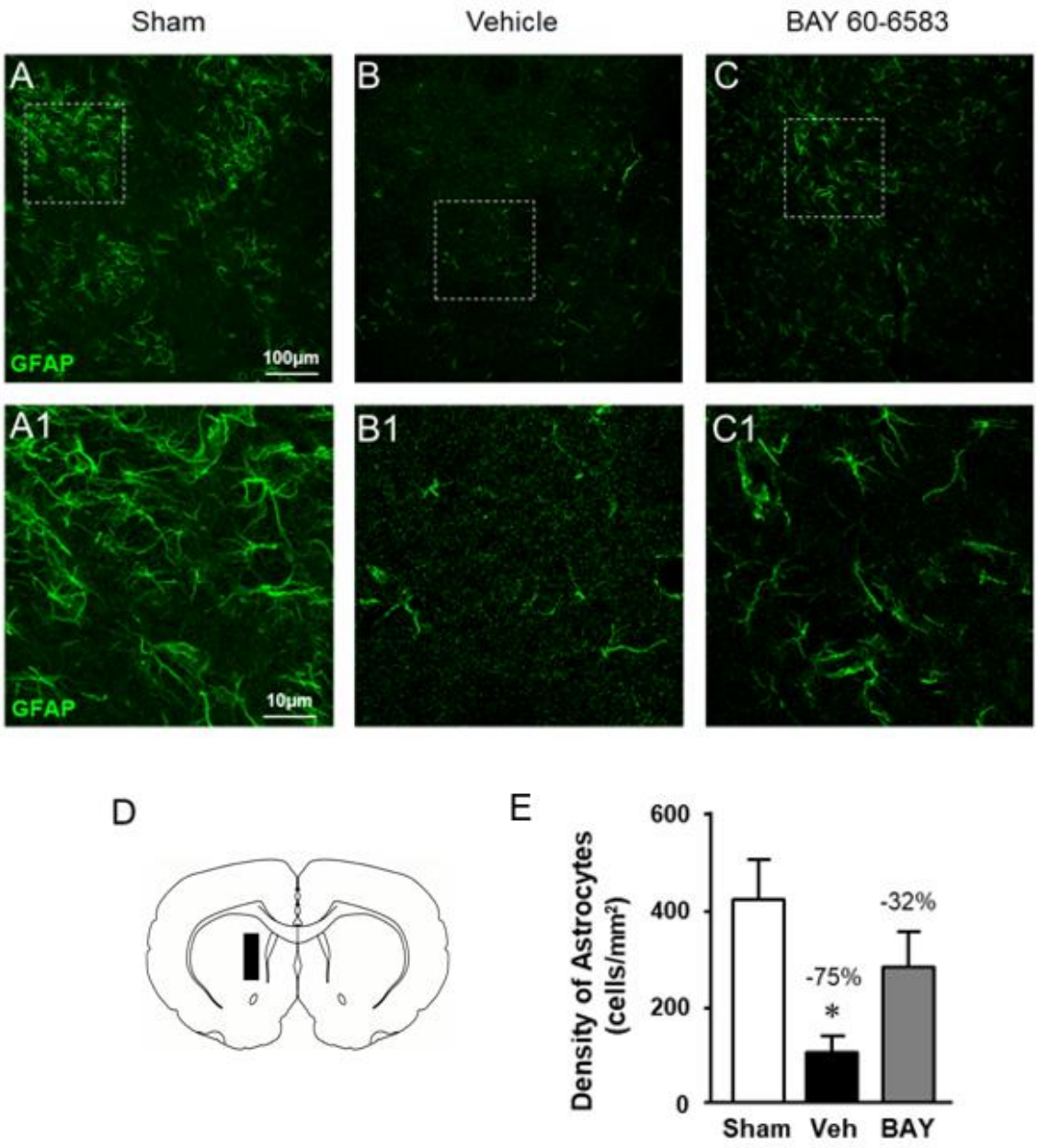


Figure 43. Analysis of astrocytes in striatal boundary zone of sham-operated, vehicle- and BAY60-6583- treated rats 7 days after ischemia. Schematic brain picture (D) indicate the region of interest (dark box) where microphotographs of ischemic boundary zone were captured. A-C: Representative confocal photomicrographs of GFAP immunostaining of astrocytes (green) in the striatum of a sham-operated (A), vehicle- (B), and a BAY60-6583- (C) treated rats. Scale bar: 100 µm. Panels A1, B1 and C1 show the magnification of areas framed in panels A, B and C. Scale bar: 10 µm. E: Quantitative analysis of GFAP positive astrocytes/mm^2 in striatum of sham vehicle and BAY60-6583 treated rats. Astrocytes were significantly less numerous in striatum of vehicle vs sham rats: One-way ANOVA and Newman Multiple Comparison Test, *$p<0.05$ vehicle-treated vs sham-operated. All data in the graphs are expressed as mean±SEM.

4.11 Effect of treatment with the adenosine A_{2B} receptor agonist on cytokines plasma levels after tMCAo.

Seven days after tMCAo, plasma levels of pro-inflammatory cytokine TNF-α were significantly increased in vehicle-treated rats as compared to sham-operated rats (One-way ANOVA: $p<0.01$; Figure 44 A), while the plasma level of IL-10, a regulatory cytokine with anti-inflammatory action was reduced in vehicle-treated rats as compared to sham-operated rats (Figure 44 B). Chronic treatment with BAY60-6583, at the dose of 0.1 mg/kg, significantly decrease TNF-α levels and significantly increase the plasma level of IL-10 (One-way ANOVA: $p<0.001$-0.01).

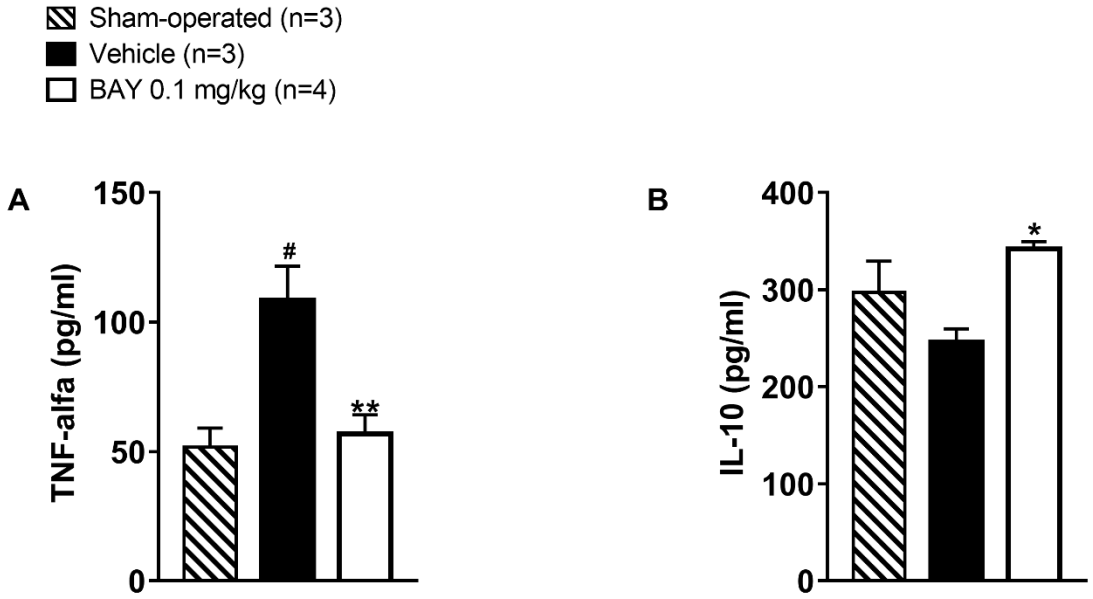


Figure 44. Effect of chronic treatment with BAY60-6583 (0.1 mg/kg i.p.) on TNF-α (A) and IL-10 (B) plasma levels. Results are expressed as pg of protein/ml of plasma and values are mean±SEM. One-way ANOVA: #$p<0.01$ vs sham-operated rats; *$p<0.01$; **$p<0.001$vs vehicle-treated rats.

4.12 Effect of treatment with the adenosine A_{2B} receptor agonist on blood cell infiltration after tMCAo.

Two days after tMCAo, HIS-48-positive cells (granulocytes) were detected in the cortical and striatal ischemic core (Figure 45), while seven days after ischemia, granulocytes were anymore detectable in the ischemic tissue (data not shown). No HIS-48-positive cells were present in the cortical and striatal tissue of sham-operated rats.

Chronic treatment with BAY60-6583 (0.1 mg/kg) clearly reduced HIS-48-positive cells in cortex and to some extent also in the striatum core. The number of HIS-48-positive cells was reduced in a statistically significant way in cortical ischemic core after treatment with BAY60-6583 (mean±SEM.: 121.2±4.2 cells/optical field in vehicle- vs 93.3±3.7 cells/optical field in BAY60-6583-treated rats; Unpaired Student's t test: $p<0.004$), but not in the striatal ischemic core (mean±SEM.: 104.05±8.61 cells/optical field in vehicle- vs 90.4±4.3cells/optical field in BAY60-6583 -treated rats; Unpaired Student's t test: $p<0.18$) (Figure 45 B).

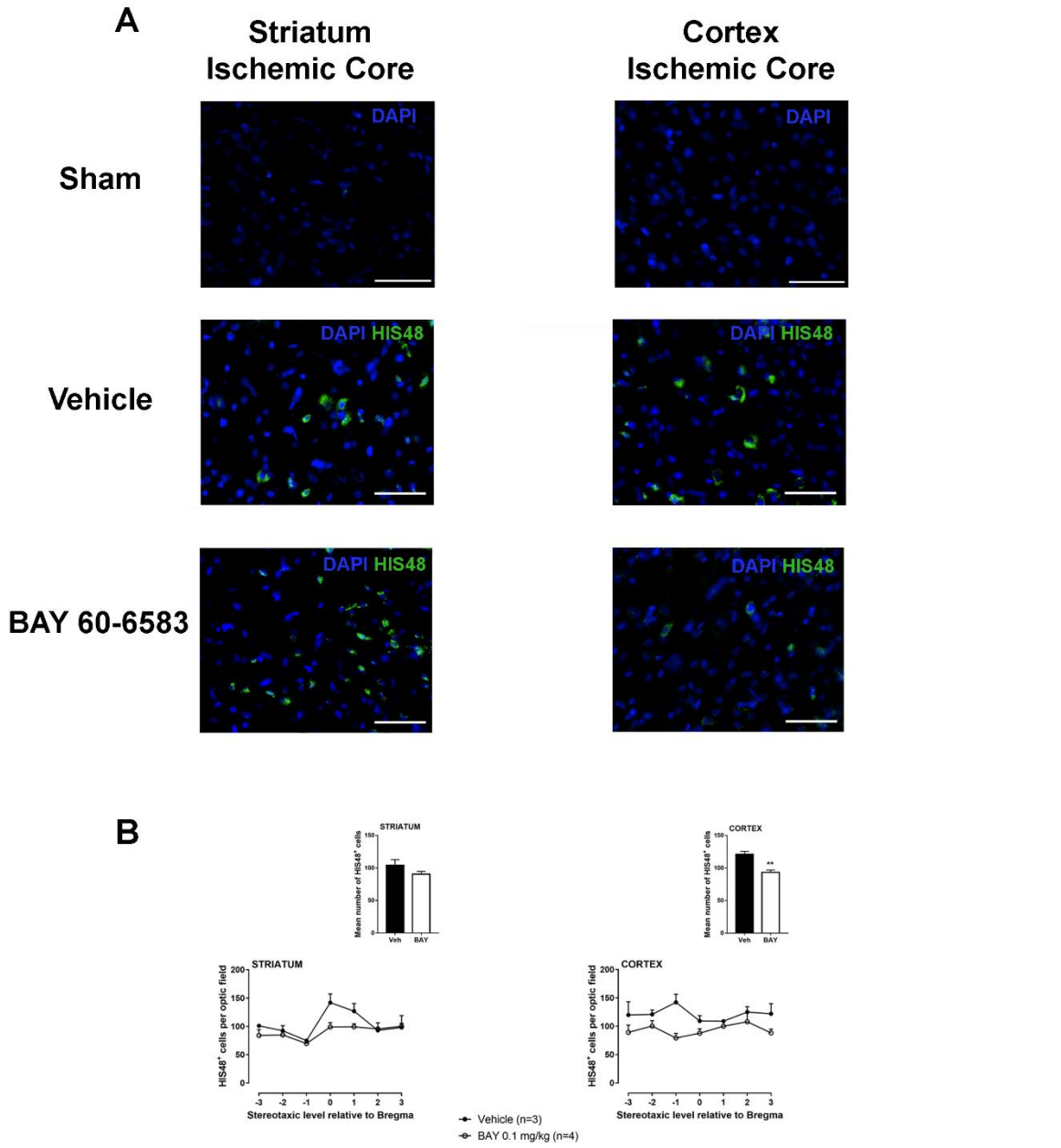


Figure 45. A: representative microphotographs of HIS-48 positive cells detected in the striatal and cortical core of sham-operated, vehicle-and BAY60-6583-treated rats acquired with Olympus BX63 microscope equipped with CellSens Dimension software (Olympus, Germany) at 20X magnification. Scale bar: 50µm. BAY60-6583 reduces HIS-48-positive cells in cortex and striatum. B: Bar graphs represent the mean±SEM of the number of HIS-48 positive cells per optical field (20X) counted in seven coronal levels through the brain of "n" rats taking Bregma=0 (König et al., 1964) in ischemic striatum and in ischemic cortex. Unpaired Student's t-test: **p<0.004.

Section III. Carbonic Anhydrase Inhibitors (CAIs) for management of cerebral ischemia

4.13 Effect of treatment with CAIs on neurological deficit after pMCAo.

The mNSS test was performed according to Chen et al. (2001) before and 24 hours after pMCAo. Figure 46 shows that sham-operated rats had a neurological score of 0.6±0.3, 24 hours after pMCAo. Twenty-four hours after pMCAo, vehicle-treated rats showed a clear neurological deficit with a neurological score of 14.0±0.7 (mean±SEM) that defines a severe injury. Sub-chronic treatment with both carbonic anhydrase inhibitors, acetazolamide (4.4 mg/kg i.p.) and AN11-740 (1.0 mg/kg i.p.) respectively, reduced by 28.6% and by 41.4% respectively the neurological deficit 24 hours after pMCAo (One-way ANOVA: $p<0.001$-0.0001) in a statistically significant manner.

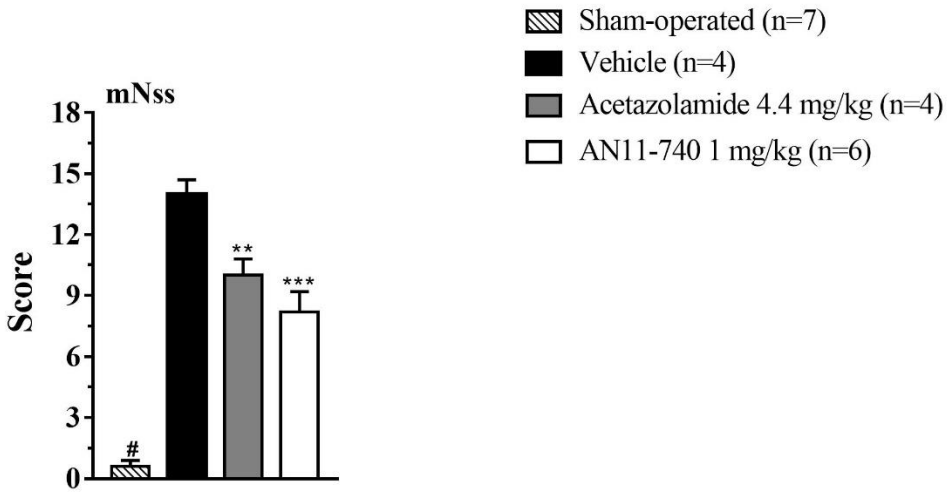


Figure 46. Effect of sub-chronic treatment with acetazolamide and AN11-740 on neurological deficit. Data are expressed as mean±SEM of "n" rats. mNSS test: the score is evaluated before and after 24 hours from pMCAo in each rat group. One-way ANOVA: **$p<0.001$-***$p<0.0001$ vs vehicle-treated rats.

4.14 Effect of treatment with CAIs on brain ischemic damage after pMCAo.

Figure 47 shows the extent of ischemic damage evaluated as infarct area (Figure 47 A, C) and infarct volume (Figure 47 B, D) in ischemic striatum and cortex of vehicle-, acetazolamide and AN11-740-treated rats 24 hours after pMCAo. Sub-chronic treatment with both acetazolamide (4.4 mg/kg i.p.) and AN11-740 (1.0 mg/kg i.p.) significantly reduced the infarct volume in ischemic cortex by 72.4% and 83.7%, respectively (One-way ANOVA: $p<0.001$) and in

ischemic striatum by 48.1% and 54.1%, respectively (One-way ANOVA: $p<0.001$). Sham-operated rats did not show any damage.

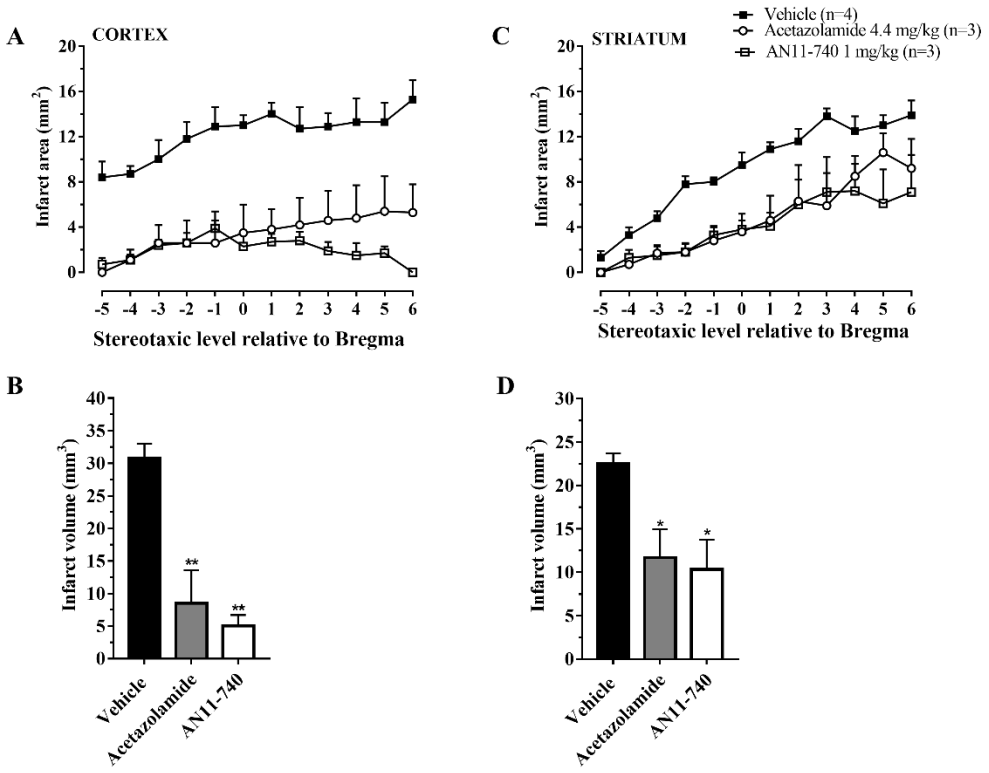


Figure 47. Effect of sub-chronic treatment with acetazolamide and AN11-740 on infarct area (A, C) and infarct volume (B, D) in the cortex and striatum 24 hours after pMCAo. Data are mean±SEM of infarct area measured at 12 predetermined coronal levels through the brain of "n" rats. Bregma=0 (König et al., 1964). Bar graphs show the infarct volume calculated as mean±SEM in the striatum and cortex. One-way ANOVA: *p<0.01- **p<0.001 vs vehicle-treated rats.

4.15 Effect of treatment with CAIs on neuronal damage after pMCAo.

Twenty-four hours after pMCAo, the extent of neuronal damage in ischemic striatum and cortex was assessed by immunohistochemistry using anti-NeuN antibody. Representative images of NeuN immunostaining of each group were shown in figure 48 A-H. Twenty-four hours after pMCAo, $NeuN^+$ cells decreased in both ischemic areas in vehicle-treated rats (Figure 48 C-D). Quantitative analysis (Figure 48 I-L) demonstrated that sub-chronic treatment with acetazolamide and AN11-740 significantly increased the number on $NeuN^+$ cells in ischemic cortex (mean±SEM.: 81.75±4.4 cells/optical field in vehicle- vs 158±34.7 cells/optical field in acetazolamide-treated rats and vs 132±8.4 cells/optical field in AN11-740-treated rats, One-way ANOVA: $p<0.01$) and also in the ischemic striatum (mean±SEM.: 90±11.6 cells/optical field in vehicle- vs 146±3.6 cells/optical field in acetazolamide-treated rats and vs 148.3±20.6 cells/optical field in AN11-740-treated rats, One-way ANOVA:$p<0.01$).

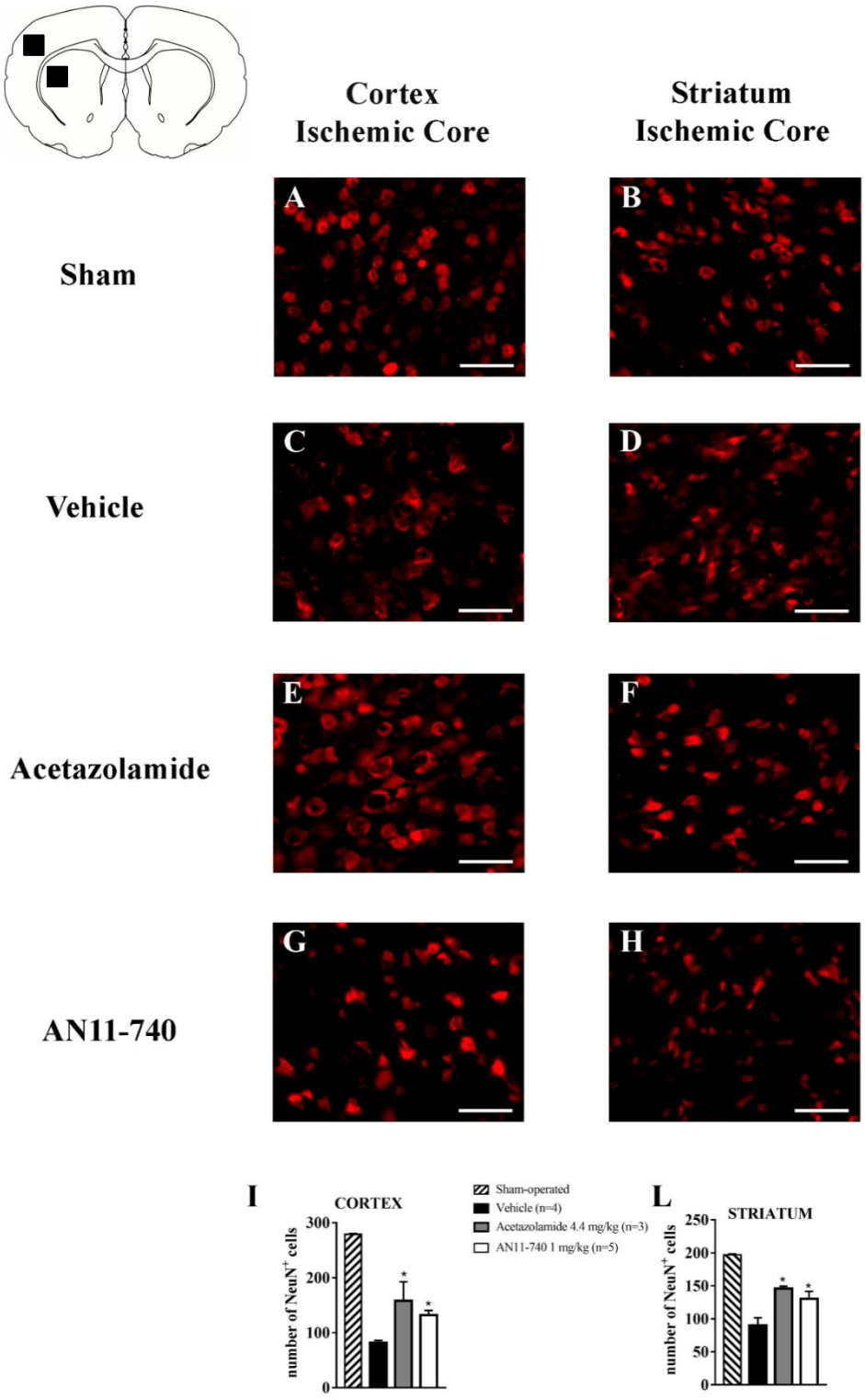

Cortex
Ischemic Core
Striatum
Ischemic Core
Sham
Vehicle
Acetazolamide
AN11-740
A
B
C
D
E
F
G
H
I
CORTEX
number of NeuN+ cells
300
200
100
0
Sham-operated
Vehicle (n=4)
Acetazolamide 4.4 mg/kg (n=3)
AN11-740 1 mg/kg (n=5)
L
STRIATUM
number of NeuN+ cells
250
200
150
100
50
0

Figure 48. Effect of sub-chronic treatment with acetazolamide and AN11-740 on neuronal damage in cortex and striatum 24 hours after pMCAo. Upper part: schematic brain picture indicates the regions of interest (dark box) where microphotographs of ischemic core in cortex and striatum were captured.
A-H: representative microphotographs of neurons (red) cortex and in striatum of sham-operated (A-B), vehicle- (C-D) acetazolamide-(E-F) and AN11-740-(G-H) treated rats acquired with Olympus BX63 microscope equipped with Cell-Sens Dimension software (Olympus, Germany). I-L: quantitative analysis of number NeuN positive cells in ischemic cortex and striatum. Bar graphs represent mean±SEM of the number of neurons per optical field (20X). One-way ANOVA: $^{*}p<0.01$ vs vehicle-treated rats.

4.16 Effect of treatment with CAIs on cytokines plasma levels after pMCAo.

Twenty-four hours after pMCAo, we evaluated plasma levels of pro-inflammatory cytokine TNF-α. In vehicle-treated rats TNF-α plasma levels significantly increased as compared to sham-operated rats (one-way ANOVA: $p<0.001$; Figure 49 A) while the plasma level of IL-10, a regulatory cytokine with anti-inflammatory action was reduced in vehicle-treated rats as compared to sham-operated rats (Figure 49 B). Sub-chronic treatment with both carbonic anhydrase inhibitors, acetazolamide and AN11-740, at the dose of 4.4 mg/kg and 1.0 mg/kg, respectively, didn't modify neither TNF-α nor IL-10 plasma levels 24 hours after pMCAo (Figure 49 B).

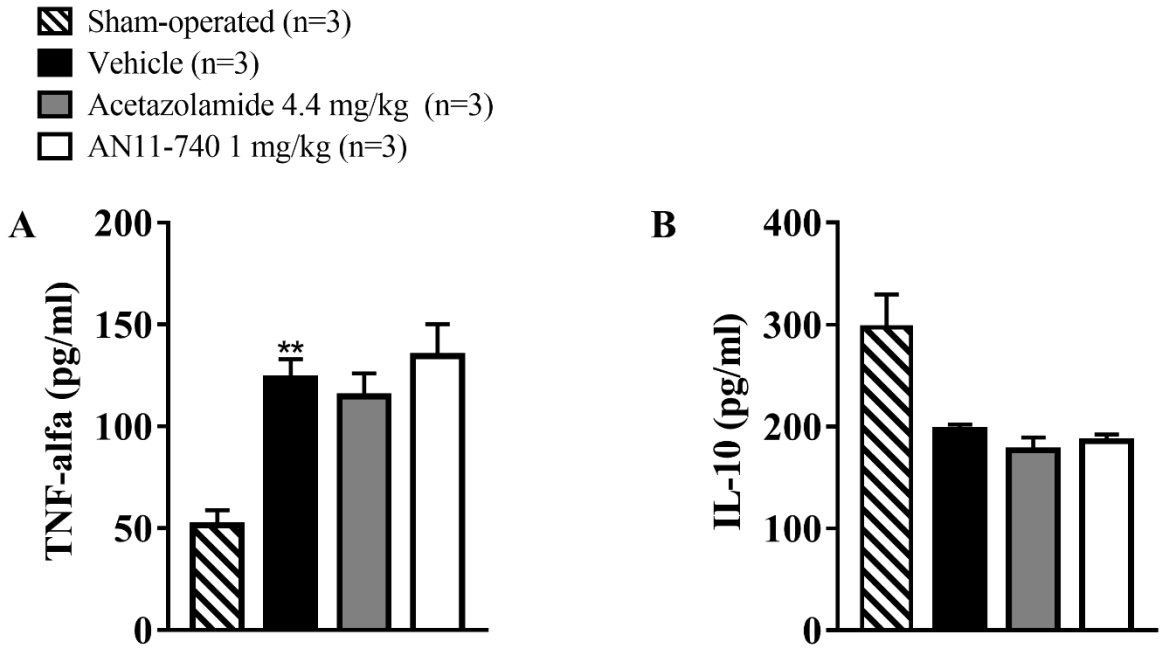


Figure 49. Effect of sub-chronic treatment with acetazolamide and AN11-740 on TNF-α (A) and IL-10 (B) plasma levels. Results are expressed as pg of protein/ml of plasma and values are mean±SEM. One-way ANOVA: $^{**}p<0.001$ vs sham-operated rats.

5. Discussion.

Section I. Adenosine A_{2B} receptor and SphK/S1P signaling axis control maturation of OPC *in vitro*.

The present work provides the first description of a role of adenosine A_{2B} receptor subtype in oligodendroglial cell cultures.

Adenosine is known to participate to a number of OPC functions, from cell migration to myelin production (Fields et al., 2002; Coppi et al., 2015). Our group recently contributed to address this issue by demonstrating that adenosine A_{2A} receptor stimulation counteracts oligodendroglial cell differentiation *in vitro* by inhibiting outward sustained I_K currents in cultured OPCs, which are known to be necessary to OPC differentiation (Gallo et al., 1996).

In the present work, we demonstrate that also A_{2B} receptor subtype is involved in OPC maturation: indeed, the selective A_{2B} agonist, BAY60-6583 at 10µM concentration, inhibited *in vitro* OPC differentiation when added for 7 days in the culture medium of these cells.

The SphK/S1P signaling pathway is known to be a pleiotropic mechanism in modulating a variety of cell functions, also in the oligodendroglial lineage.

It has already been shown that these two signaling pathways, A_{2B} receptor and SphK/S1P, interact in a different cell system (i.e. human erythrocytes) (Sun et al., 2015).

We provide here the first demonstration of an interplay between SphK and adenosine A_{2B} receptor activation in OPC maturation *in vitro*, since an acute application of the A_{2B} receptor agonist BAY60-6583 promotes $SphK_1$ phosphorylation. The effect was specific on this enzyme isoform because no differences were found in the phosphorylated form of $SphK_2$.

We focused on elucidating the functional role of $SphK_1$/A_{2B} receptor interaction through the use of SphK or S1P receptor ligands.

We found that the two SphK inhibitors tested, VPC96047 (which affect both isoforms, $SphK_1$ and $_2$) and VPC96091 (that selectively inhibits $SphK_1$), prompted OPC development into myelin-producing cells, because the expression of myelin markers such as MAG and Mbp after 7 days of *in vitro* differentiation was increased when oligodendroglial cultures were grown in the presence of these compounds. Of note, the effect of unselective SphK inhibitor VPC96047, which prevents both $SphK_1$ and $SphK_2$ activation, was much more pronounced than that obtained in the presence of selective $SphK_1$ inhibitor VPC96091, indicating an involvement of both enzyme isoforms in OPC maturation. This result is in accordance with previous works demonstrating that the mitogenic effect of PDGF in these cells requires is mediated by the activation of SphK (Soliven et al., 2003). Of note, when cells were grown in the presence of BAY60-6583 co-applied with either of the two SphK inhibitors, the effect of BAY60-6583

prevailed since OPC differentiation was inhibited. This result indicates that SphK activation is not required for A_{2B}-mediated signaling.

To date, among S1P receptors, we do not have a valid candidate to be involved in OPC maturation. We can only rule out the involvement of $S1P_1$ subtype since neither selective agonism nor antagonism was found to affect OPC maturation nor to counteract A_{2B}-mediated effects.

An additional proof that A_{2B} receptors are critical modulators of OPC maturation resides in the fact that this receptor subtype is upregulated during cell differentiation. Interestingly, SphK inhibition potently reduced A_{2B} mRNA levels in OPCs at 7 days of differentiation. This result confirms that a cross-talk exists between A_{2B} and SphK/S1P pathways and indicates a possible positive effect of the sphingolipid on A_{2B} receptor expression.

$S1P_5$ receptor is also overexpressed during oligodendrogliogenesis but, differently from A_{2B}, its upregulation is not modified by VPC96047.

FTY720 is the first oral MS therapeutic agent and the first human medicine to be approved that targets S1P receptors. Emerging evidence from preclinical studies demonstrate that mechanisms independent of peripheral immune effects contribute significantly to the efficacy of FTY720 in models of MS. Indeed, FTY720, which is lipophilic, is able to cross the BBB into the CNS and after its conversion by $SphK_2$, FTY720-P has been detected in the cerebrospinal fluid at sub-nanomolar levels (Foster et al., 2007). In addition, recent data using neural lineages from conditional $S1P_1$ knockout demonstrated a crucial action of FTY720 in reducing the severity of an animal model of MS, EAE.

Results from *in vitro* studies have shown that the effects of FTY720-P on cultured oligodendrocyte lineage cells are affected by developmental stage, treatment concentration and duration (Novgorodov et al., 2007; Miron et al., 2008). Cultured rodent OPC treated with FTY720 are protected from apoptosis induced by inflammatory cytokines and microglial activation (Coelho et al., 2007).

The differentiation of OPC in our model is reduced after the treatment with high (μM) concentrations of FTY720-P in agreement with previous results obtained in rat cultured OPCs (Coelho et al., 2007; Miron et al., 2008).

In order to further clarify the effect of the A_{2B} receptor activation on OPC maturation and its interaction with SphK/S1P pathway, we performed the silencing of A_{2B} receptor in OPC cells culture by RNA interference (RNAi). As reported by RT-PCR, transfected cells showed specific silencing of the A_{2B} receptor gene. These results show that RNA silencing of the A_{2B} receptor gene changed S1P pathway by increasing S1P lyase level and decreasing $S1P_3$, $S1P_5$

and ShpK1 expression. Moreover, A_{2B} silencing also accelerated OPC maturation, as demonstrated by the significant decrease of NG2 (which labels undifferentiated OPCs) and increase of CNPase (which labels post-mitotic OLGs) expressions. These results are consistent with the fact that A_{2B} agonist inhibit oligodendroglial maturation.

To date this is the first characterization of the role of adenosine A_{2B} receptor in oligodendrocyte cells in which we demonstrated that a direct cross-talk exists between A_{2B} receptor and SphK/S1P signaling axis in inhibiting OPC maturation. Further investigations will be needed in order to clarify the molecular mechanism involved.

Section II. Adenosine A_{2B} receptor agonist, BAY60-6583, is protective in a rat model of transient cerebral ischemia.

Our results demonstrate that the selective adenosine A_{2B} receptor agonist, BAY60-6583, chronically and systemically administered improves the neurological deficit, reduces the ischemic brain damage, counteract the neurodegeneration, and microglia activation and tend to protect from astrocyte death seven days after tMCAo. Moreover, BAY60-6583 reduces blood cell infiltration in ischemic areas two days after tMCAo.

The chronic treatment with BAY60-6583 at the dose of 0.1 mg/kg protects from the neurological deficit as evaluated up 7 days after tMCAo but does not protect ischemic rats from the body weight loss. On this regard it is worth mentioning that adenosine released from fat cells in adipose tissue (Schwabe et al., 1975; Capogrossi et al., 1986) participates in the regulation of adipocyte function (Eisenstein et al., 2014). In particular, stimulation of A_{2B} receptor inhibits adipogenesis (Gharibi et al., 2012). This effect might account for the lack of the chronic treatment with BAY60-6583 in protecting from the body weight loss.

BAY60-6583 reduces significantly the volume of the ischemic infarct both in the cortex and striatum. Immunohistochemical analysis of neurons, in the striatum of ischemic rats, 7 days after MCAo induction revealed remarkable alterations in comparison with sham-operated rats. We found a significant decrease of total neuron density and a significative increment of the low-density nuclei (LDN) neurons. Fusco and colleagues (Fusco et al., 2018) had characterized LDN neurons as damaged and apoptotic neurons, a clear sign of an ischemic insult. BAY60-6583 significantly prevented the loss of neurons and reduced the percent of LDN neurons on total neurons, a clear sign of its protective role against neurodegeneration. Indeed, seven days after ischemia, H&E staining shows that chronic treatment with BAY60-6583 has reconstituted the cortex and striatum cytoarchitecture and has decreased heterochromatic small nuclei in the ischemic area. Melani et colleagues (Melani et al., 2014) have reported that 7 days after ischemia the heterochromatic small nuclei belong to activated astrocytes and microglia. It is today well known that after an ischemic event, over-activation of microglia is detrimental and these resident immune cells are involved in the neuroinflammation process (Schwartz, 2003; Kriz, 2006; Block et al., 2007). Seven days after ischemia, the chronic treatment of BAY60-6583 has definitely reduced the morphological features of microglia activation (i.e. the amoeboid state). Adenosine A_{2B} receptor is expressed on microglia and their stimulation reduces TNF-α levels in primary microglia cultures (Merighi et al., 2015) and augments the

release of IL-10 by murine microglial cells (Koscsó et al., 2012). Moreover, adenosine A_{2B} receptor is also expressed on astrocytes where its levels increase 24 hours after transient ischemia (Li et al., 2017). The chronic treatment of BAY60-6583 tended to decrease the loss of astrocytic cells. In a model *in vitro* of hypoxia/reoxygenation, A_{2B} receptor stimulates the increase of IL-6 from astrocytes (Maeda et al., 1994) and it was demonstrated that IL-6 exerts neuroprotective actions in a model of permanent MCAo (Loddick et al., 1998). Interestingly, analysis of astrocytes gave an unexpected result: after ischemia, astrocytes density decreased and their morphology was altered with shorter and thinner branches, suggesting a clasmatodendrotic process.

Astrocyte clasmatodendrosis is induced *in vitro* by mild acidosis, a microenvironmental condition commonly associated with aging (Mercatelli et al., 2016) and ischemia (Hulse et al., 2001). BAY60-6583 showed a tendency to revert this effect. Further analyses are necessary to better investigate this phaenomenon. On the all, the previous observations support that the protective effect of chronic BAY60-6583 treatment along the days after ischemia can be attributed to direct agonism of A_{2B} receptor located on rat microglial cells and on astrocytes cells.

Besides microglia and astrocytes, A_{2B} receptor is present also on endothelial cells where their expression increases after transient ischemia (Li et al., 2017). It has been reported that stimulation of A_{2B} receptor reduces vascular leak in a model of hypoxia in mice (Eckle et al., 2008 a). After ischemia, it is known that damage evolves in time and space (Dirnagl et al., 1999) and it is today accepted that after a first excitotoxic damage, a secondary damage characterized by neuroinflammation further damages the brain tissue. In the days after ischemia, activated blood cells infiltrate brain tissue and by secreting cytotoxic factors contribute to further enlarge the ischemic damage. A_{2B} receptor localized on blood immune cells, i.e. neutrophils and lymphocytes (Gessi et al., 2005; Eckle et al., 2008 a) exert anti-inflammatory actions, inhibiting vascular adhesion (Yang et al., 2006) and inflammatory cells migration (Wakai et al., 2001; Konrad et al., 2012).

Our observation that 2 days after tMCAo the A_{2B} agonist, BAY60-6583, has significantly reduced granulocyte infiltration in cortex, supports that A_{2B} receptor activation on endothelial and blood cells is involved in counteracting neutrophils infiltration and then inflammation of brain parenchyma. In agreement A_{2B} receptor KO mice, exposed to hypoxia, exhibit increased neutrophils infiltration in the brain (Eckle et al., 2007 a, 2008 a).

Interestingly, seven days after ischemia an inflammatory parameter such as TNF-α is increased in the peripheral plasma and that chronic administration of the adenosine A_{2B} receptor agonist,

BAY60-6583, clearly reduced TNF-α in the plasma. Such reduction might reflect a central effect of BAY60-6583 that reduces pro-inflammatory cytokine produced in the brain and reaching peripheral blood. It is also possible that TNF-α reduction in the plasma reflect a direct peripheral mechanism of action of BAY60-6583 that reduces neutrophils activation. On the other hand, stroke and inflammation are strictly interrelated. Brain ischemia induces profound inflammatory changes in the periphery (Szigeti et al., 2015). Inflammatory changes in peripheral organs (especially the lungs and the gut) as early as 2 hours after tMCAo in mice, has been detected by whole-body-SPECT-based imaging protocol (Szigeti et al., 2015).

Such peripheral inflammatory changes on their turn might contribute to a worse recovery after stroke. The precise cellular-molecular mechanisms underlying are unclear, but likely reflect a vicious circle responsible of inflammatory mediator production and of the blood brain barrier (BBB) function. Overall our results stress the key research questions of the predictive value of blood biomarkers in stroke and suggest that BAY60-6583 by controlling a secondary inflammatory damage, represents a new interesting target after brain ischemia.

A previous study has reported that intravenous treatment with the selective A_{2B} receptor agonist BAY60-6583 (1 mg/kg), at the start of reperfusion after brain ischemia induced by tMCAO, at an early time after ischemia, that is 24 hours later, has reduced the lesion volume, has attenuated brain swelling and BBB disruption. In the presence of tPA (administered after ischemic stroke to dissolve intravascular clots), BAY 60-6583 also has reduced tPA induced haemorrhages after ischemia (Li et al., 2017). The neurovascular protection afforded by BAY 60-6583 was related to stimulation of the inhibitor of matrix, the metalloproteinase-1 (TIMP-1), to inhibition of tPA-induced matrix metalloprotease (MMP) activation, and prevention of tight junction protein degradation. Since MMPs by degradation extracellular matrix complex and tight junction proteins on endothelial cells (Tsuji et al., 2005; Yepes et al., 2009), can increase BBB permeability, protection afforded by A_{2B} agonist after MCAo was largely attributed to protection of the endothelium.

The present study points toward the possibility that stimulation of adenosine A_{2B} receptors located on central neural cells, on vascular cells and on blood cells attenuate neuroinflammation that develops days after ischemia. Importantly, A_{2B} receptor agonists might be adjuvant to the today accepted pharmacological strategy of tPA administration, a promising strategy for decreasing the risk of haemorrhages during treatment for ischemic stroke.

Section III. Carbonic Anhydrase Inhibitors (CAIs) for management of cerebral ischemia.

In this work, we report that the two carbonic anhydrase inhibitors (CAIs) acetazolamide and AN11-740, sub-chronically administered at the dose of 4.4 mg/kg i.p and 1.0 mg/kg i.p. respectively, improve neurological deficit and reduce ischemic brain damage 24 hours after pMCAo in the rat.

A previous paper reported that several inhibitors of CA were able to reduce neurological deficit but not significantly the ischemic damage 24 hours after pMCAo (Di Cesare Mannelli et al., 2016). Results obtained in the present work show, 24 hours after pMCAo, a definite volume damage in striatum and cortex. In the same areas, the immunohistochemical analysis revealed a clear decrease of neuron number. Acetazolamide and AN11-740 have clearly protected neurons from damage and from the decrease in number of neurons. Protection is definite in the cortex and in striatum.

Evidence that hypoxic microenvironments elicit the expression of specific isoforms of CA (in particular CA IX and CA XII) through the hypoxia inducible factor (Wykoff et al., 2000) stress the relevance of CA in ischemia and supports the importance of CAIs as protective drugs after ischemia. Indeed, under ischemia, the lack of oxygen caused by hypoxia leads to a switch from aerobic to anaerobic glucose metabolism, characterized by increased production of lactic acid and a lowered intracellular pH (Swietach et al., 2007) in neurons and glial cells (Obara et al., 2008). CA isoforms, IX and XII are highly concentrated in glial cells both intracellularly and membrane-bound (Tong et al., 2000; Svichar et al., 2006) and contribute to pH regulation both in physiological and pathological conditions. Astrocytes act as key players in pH homeostasis in the brain (Bélanger et al., 2009). Glial CA converts neuron derived CO_2 to bicarbonate and protons which are driven out of the glial cell by a Na^+/HCO_3 co-transporter and monocarboxylate transporters. In the extracellular space, CA catalyzes the reaction of bicarbonate with a proton to recycle CO_2, which is instrumental for buffering extracellular pH (Tong et al., 2000). A pH dysregulation has been related to a spectrum of pathological conditions (Obara et al., 2008). CAIs by reducing H^+ concentration and thus contributing to the pH homeostasis (Supuran, 2008; Pettersen et al., 2015) can protects from damage especially neurons which are particularly sensitive to pH decrease (Obara et al., 2008).

Maintenance of pH homeostasis in the CNS is of key importance for proper execution and regulation of neurotransmission, and deviations from this homeostasis is a crucial factor in the

mechanism underlying a spectrum of pathological conditions (Obara et al., 2008). It is known that changes in the intracellular concentration of protons may affect the release of neurotransmitters. Lowering of pH results in increased release of dopamine (Cannizzaro et al., 2003; Pittaluga et al., 2005), noradrenaline and serotonine from rat brain synaptosomes (Pittaluga et al., 2005). Although no direct evidence links pH changes to excitatory amino acid efflux, it is thinkable that maintenance of pH homeostasis by CAIs in reducing excitatory amino acids outflow from neurons, might protect from ensuing excitotoxicity.

Previous results obtained in our laboratory, (unpublished results) have shown that the carbonic anhydrase inhibitors acetazolamide and AN11-740 tested in an *in vitro* model of ischemia induced by OGD (oxygen and glucose deprivation, OGD) in the CA1 area of rat hippocampus, delay significantly the onset of anoxic depolarization (AD), a phenomenon closely related to cell damage and death (Pugliese et al., 2006). The evidence that NMDA receptors are essential to AD initiation and propagation (Somjen, 2001), supports the possibility that carbonic anhydrase inhibitors delays AD by removing the OGD-induced potentiation of glutamate transmission, thus reducing the participation of glutamate in triggering the AD. This result obtained in an *in vitro* model of acute OGD-induced depolarization is in favor that carbonic anhydrase inhibitors protect *in vivo* from the functional and tissue damage because of a direct central effect that reduces excitotoxicity and precocious brain depolarization induced by the lack of O_2 and glucose.

We also must consider that CAIs acetazolamide and AN11-740 in view of their diuretic effect, could also reduce brain edema and therefore can mitigate the brain damages induced by cerebral ischemia. The presence of edema is a key aggravating factor in ischemic stroke, brain volume is limited by the rigidity of the skull, so that even little volume increase lead to high intracranial pressure and compression of neural tissue and vasculature (Klatzo, 1985). Rodent brains that have undergone middle cerebral artery occlusion (MCAo) develop brain edema and therefore swelling during the acute and subacute phases of ischemic injury (Katzman et al., 1977). Guo et al. (Guo et al., 2012) demonstrated that after intracerebral hemorrhage in the rat, acetazolamide reduced brain edema, neuronal death and neurological deficits. Currently, CAIs are used to reduce body fluid volume and edema in pathologies such as glaucoma (Masini et al., 2013; Scozzafava et al., 2014).

Interestingly, 24 hours after pMCAo, a pro-inflammatory cytokine such as TNF-α is increased while levels of IL-10, a regulatory cytokine with an anti-inflammatory action, was reduced in the plasma of vehicle-treated rats as compared to sham-operated rats. Results are in agreement with previous results that have reported an increase of TNF- α in the peripheral plasma 6 and

24 hours after pMCAo in mice (Clausen et al., 2017) and 7 days after tMCAo in the rat (Dettori et al., 2018). Significant increases of TNF-α serum levels were also found in patients with acute stroke, reaching the peak values on day 7 (Intiso et al., 2004) in comparison with controls.
Modifications of inflammatory markers in the blood likely reflect cytokines that are produced in the brain and that reach the systemic circulation. On their turn peripheral inflammatory cytokines might contribute to control damage after stroke. Precise cellular-molecular mechanisms underlying are unclear, but likely reflect a vicious circle responsible of endothelial cell and BBB permeability changes bringing to blood cell infiltration and further brain damage (what is called the secondary damage after stroke). Thus, a TNF-α increase and an IL-10 decrease represent valuable markers over time of the brain damage following an ischemic insult (Jickling et al., 2011) Treatment with acetazolamide and AN11-740 did neither modify TNF-α or IL-10 levels in the plasma. Likely 24 hours after stroke is a too early time after damage to appreciate a protective effect of the CAIs from such damage markers.

6. References

Abbracchio, M. P.; Brambilla, R.; Ceruti, S.; Kim, H. O.; von Lubitz, D. K.; Jacobson, K. A.; Cattabeni, F., 1995: G protein-dependent activation of phospholipase C by adenosine A3 receptors in rat brain. *Molecular pharmacology*., **48**, 1038–1045.

Adachi, K.; Kohara, T.; Nakao, N.; Arita, M.; Chiba, K.; Mishina, T.; Sasaki, S.; Fujita, T., 1995: Design, synthesis, and structure-activity relationships of 2-substituted-2-amino-1,3-propanediols: Discovery of a novel immunosuppressant, FTY720. *Bioorganic and Medicinal Chemistry Letters*., **5**, 853–856.

Aggarwal, M.; Kondeti, B.; McKenna, R., 2013: Anticonvulsant/antiepileptic carbonic anhydrase inhibitors: a patent review. *Expert opinion on therapeutic patents*., **23**, 717–724.

Agresti, C.; Meomartini, M. E.; Amadio, S.; Ambrosini, E.; Serafini, B.; Franchini, L.; Volonté, C.; Aloisi, F.; Visentin, S., 2005: Metabotropic P2 receptor activation regulates oligodendrocyte progenitor migration and development. *GLIA*.

Aho, K.; Harmsen, P.; Hatano, S.; Marquardsen, J.; Smirnov, V. E.; Strasser, T., 1980: Cerebrovascular disease in the community: results of a WHO collaborative study. *Bulletin of the World Health Organization*., **58**, 113–130.

Albers, G. W.; Goldstein, L. B.; Hess, D. C.; Wechsler, L. R.; Furie, K. L.; Gorelick, P. B.; Hurn, P.; Liebeskind, D. S.; Nogueira, R. G.; Saver, J. L., 2011: Stroke treatment academic industry roundtable (STAIR) recommendations for maximizing the use of intravenous thrombolytics and expanding treatment options with intra-arterial and neuroprotective therapies. *Stroke*., **42**, 2645–2650.

Almeida, T.; Rodrigues, R. J.; de Mendonça, A.; Ribeiro, J. A.; Cunha, R. A., 2003: Purinergic P2 receptors trigger adenosine release leading to adenosine A2A receptor activation and facilitation of long-term potentiation in rat hippocampal slices. *Neuroscience*., **122**, 111–121.

Anderson, C. M.; Xiong, W.; Young, J. D.; Cass, C. E.; Parkinson, F. E., 1996: Demonstration of the existence of mRNAs encoding N1/cif and N2/cit sodium/nucleoside cotransporters in rat brain. *Brain research. Molecular brain research*., **42**, 358–361.

Andiné, P., (n.d.) Involvement of adenosine in ischemic and postischemic calcium regulation. *Molecular and chemical neuropathology*., **18**, 35–49.

Antonioli, L.; Csóka, B.; Fornai, M.; Colucci, R.; Kókai, E.; Blandizzi, C.; Haskó, G., 2014: Adenosine and inflammation: what's new on the horizon? *Drug Discovery Today*., **19**, 1051–1068.

Asahi, M.; Wang, X.; Mori, T.; Sumii, T.; Jung, J. C.; Moskowitz, M. A.; Fini, M. E.; Lo, E. H., 2001: Effects of matrix metalloproteinase-9 gene knock-out on the proteolysis of blood-brain barrier and white matter components after cerebral ischemia. *The Journal of neuroscience : the official journal of the Society for Neuroscience*., **21**, 7724–7732.

Astrup, J.; Siesjö, B. K.; Symon, L., 1981: Thresholds in cerebral ischemia — the ischemic penumbra. *Stroke*.

Attali, B.; Wang, N.; Kolot, A.; Sobko, A.; Cherepanov, V.; Soliven, B., 1997: Characterization of delayed rectifier Kv channels in oligodendrocytes and progenitor cells. *The Journal of neuroscience : the official journal of the Society for Neuroscience*., **17**, 8234–8245.

Back, T.; Hoehn, M.; Mies, G.; Busch, E.; Schmitz, B.; Kohno, K.; Hossmann, K. A., 2000:

Penumbral tissue alkalosis in focal cerebral ischemia: Relationship to energy metabolism, blood flow, and steady potential. *Annals of Neurology*., **47**, 485–492.

Balatoni, B.; Storch, M. K.; Swoboda, E. M.; Schönborn, V.; Koziel, A.; Lambrou, G. N.; Hiestand, P. C.; Weissert, R.; Foster, C. A., 2007: FTY720 sustains and restores neuronal function in the DA rat model of MOG-induced experimental autoimmune encephalomyelitis. *Brain research bulletin*., **74**, 307–316.

Baldwin, S. A.; Mackey, J. R.; Cass, C. E.; Young, J. D., 1999: Nucleoside transporters: molecular biology and implications for therapeutic development. *Molecular Medicine Today*., **5**, 216–224.

Barateiro, A.; Fernandes, A., 2014: Temporal oligodendrocyte lineage progression: in vitro models of proliferation, differentiation and myelination. *Biochimica et biophysica acta*., **1843**, 1917–1929.

Barone, F. C.; Feuerstein, G. Z., 1999: Inflammatory mediators and stroke: new opportunities for novel therapeutics. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*., **19**, 819–834.

Barone, F. C.; Irving, E. A.; Ray, A. M.; Lee, J. C.; Kassis, S.; Kumar, S.; Badger, A. M.; Legos, J. J.; Erhardt, J. A.; Ohlstein, E. H.; Hunter, A. J.; Harrison, D. C.; Philpott, K.; Smith, B. R.; Adams, J. L.; Parsons, A. A., 2001a: Inhibition of p38 mitogen-activated protein kinase provides neuroprotection in cerebral focal ischemia. *Medicinal Research Reviews*.

Barone, F. C.; Irving, E. A.; Ray, A. M.; Lee, J. C.; Kassis, S.; Kumar, S.; Badger, A. M.; Legos, J. J.; Erhardt, J. A.; Ohlstein, E. H.; Hunter, A. J.; Harrison, D. C.; Philpott, K.; Smith, B. R.; Adams, J. L.; Parsons, A. A., 2001b: Inhibition of p38 mitogen-activated protein kinase provides neuroprotection in cerebral focal ischemia. *Medicinal research reviews*., **21**, 129–145.

Barres, B. A.; Koroshetz, W. J.; Swartz, K. J.; Chun, L. L.; Corey, D. P., 1990: Ion channel expression by white matter glia: the O-2A glial progenitor cell. *Neuron*., **4**, 507–524.

Barry, M. A.; Eastman, A., 1992: Endonuclease activation during apoptosis: the role of cytosolic Ca2+ and pH. *Biochemical and biophysical research communications*., **186**, 782–789.

Bélanger, M.; Magistretti, P. J., 2009: *The role of astroglia in neuroprotection*. *Dialogues Clin Neurosci*., Vol. 11.

Berger, T.; Schnitzer, J.; Orkand, P. M.; Kettenmann, H., 1992: Sodium and Calcium Currents in Glial Cells of the Mouse Corpus Callosum Slice. *The European journal of neuroscience*., **4**, 1271–1284.

Betti, M.; Catarzi, D.; Varano, F.; Falsini, M.; Varani, K.; Vincenzi, F.; Dal Ben, D.; Lambertucci, C.; Colotta, V., 2018: The aminopyridine-3,5-dicarbonitrile core for the design of new non-nucleoside-like agonists of the human adenosine A2B receptor. *European Journal of Medicinal Chemistry*., **150**, 127–139.

Beukers, M. W.; Chang, L. C. W.; von Frijtag Drabbe Künzel, J. K.; Mulder-Krieger, T.; Spanjersberg, R. F.; Brussee, J.; IJzerman, A. P., 2004: New, Non-Adenosine, High-Potency Agonists for the Human Adenosine A_{2B} Receptor with an Improved Selectivity Profile Compared to the Reference Agonist *N*-Ethylcarboxamidoadenosine. *Journal of Medicinal Chemistry*., **47**, 3707–3709.

Bianchi, V.; Spychala, J., 2003: Mammalian 5′-Nucleotidases. *Journal of Biological Chemistry*., **278**, 46195–46198.

Biber, K.; Fiebich, B. L.; Gebicke-Härter, P.; van Calker, D., 1999: Carbamazepine-Induced Upregulation of Adenosine A1-Receptors in Astrocyte Cultures Affects Coupling to the Phosphoinositol Signaling Pathway. *Neuropsychopharmacology*., **20**, 271–278.

Birnbaumer, L., 1992: Receptor-to-effector signaling through G proteins: Roles for βγ dimers as well as α subunits. *Cell*.

Block, M. L.; Zecca, L.; Hong, J. S., 2007: Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nature Reviews Neuroscience*., **8**, 57–69.

Boda, E.; Viganò, F.; Rosa, P.; Fumagalli, M.; Labat-Gest, V.; Tempia, F.; Abbracchio, M. P.; Dimou, L.; Buffo, A., 2011: The GPR17 receptor in NG2 expressing cells: focus on in vivo cell maturation and participation in acute trauma and chronic damage. *Glia*., **59**, 1958–1973.

Bolaños, J. P.; Almeida, A., 1999: Roles of nitric oxide in brain hypoxia-ischemia. *Biochimica et biophysica acta*., **1411**, 415–436.

Borea, P. A.; Gessi, S.; Bar-Yehuda, S.; Fishman, P., 2009: A3 adenosine receptor: Pharmacology and role in disease. *Handbook of Experimental Pharmacology*.

Borea, P. A.; Varani, K.; Vincenzi, F.; Baraldi, P. G.; Tabrizi, M. A.; Merighi, S.; Gessi, S., 2014: The A3 Adenosine Receptor: History and Perspectives. *Pharmacological Reviews*., **67**, 74–102.

Borea, P. A.; Gessi, S.; Merighi, S.; Vincenzi, F.; Varani, K., 2018: Pharmacology of adenosine receptors: The state of the art. *Physiological Reviews*., **98**, 1591–1625.

Bowes, M. P.; Rothlein, R.; Fagan, S. C.; Zivin, J. A., 1995: Monoclonal antibodies preventing leukocyte activation reduce experimental neurologic injury and enhance efficacy of thrombolytic therapy. *Neurology*., **45**, 815–819.

Bralet, J.; Schreiber, L.; Bouvier, C., 1992: Effect of acidosis and anoxia on iron delocalization from brain homogenates. *Biochemical Pharmacology*., **43**, 979–983.

Brand, A.; Vissiennon, Z.; Eschke, D.; Nieber, K., 2001: Adenosine A(1) and A(3) receptors mediate inhibition of synaptic transmission in rat cortical neurons. *Neuropharmacology*., **40**, 85–95.

Braun, N.; Lenz, C.; Gillardon, F.; Zimmermann, M.; Zimmermann, H., 1997: Focal cerebral ischemia enhances glial expression of ecto-5'-nucleotidase. *Brain research*., **766**, 213–226.

Bridges, A. J.; Bruns, R. F.; Ortwine, D. F.; Priebe, S. R.; Szotek, D. L.; Trivedi, B. K., 1988: N6-[2-(3,5-Dimethoxyphenyl)-2-(2-methylphenyl)-ethyl]adenosine and Its Uronamide Derivatives. Novel Adenosine Agonists with Both High Affinity and High Selectivity for the Adenosine A2 Receptor. *Journal of Medicinal Chemistry*.

Brinkmann, V.; Davis, M. D.; Heise, C. E.; Albert, R.; Cottens, S.; Hof, R.; Bruns, C.; Prieschl, E.; Baumruker, T.; Hiestand, P.; Foster, C. A.; Zollinger, M.; Lynch, K. R., 2002: The immune modulator FTY720 targets sphingosine 1-phosphate receptors. *The Journal of biological chemistry*., **277**, 21453–21457.

Brinkmann, V.; Cyster, J. G.; Hla, T., 2004: FTY720: sphingosine 1-phosphate receptor-1 in the control of lymphocyte egress and endothelial barrier function. *American journal of*

transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons., **4**, 1019–1025.

Brinkmann, V., 2007: Sphingosine 1-phosphate receptors in health and disease: Mechanistic insights from gene deletion studies and reverse pharmacology. *Pharmacology and Therapeutics*.

Brodie, C.; Blumberg, P. M.; Jacobson, K. A., 1998: Activation of the A2A adenosine receptor inhibits nitric oxide production in glial cells. *FEBS letters*., **429**, 139–142.

Brunkhorst, R.; Vutukuri, R.; Pfeilschifter, W., 2014: Fingolimod for the treatment of neurological diseases—state of play and future perspectives3. *Frontiers in Cellular Neuroscience*.

Bruns, R. F.; Fergus, J. H.; Badger, E. W.; Bristol, J. A.; Santay, L. A.; Hartman, J. D.; Hays, S. J.; Huang, C. C., 1987: Binding of the A1-selective adenosine antagonist 8-cyclopentyl-1,3-dipropylxanthine to rat brain membranes. *Naunyn-Schmiedeberg's Archives of Pharmacology*.

Bua, S.; Di Cesare Mannelli, L.; Vullo, D.; Ghelardini, C.; Bartolucci, G.; Scozzafava, A.; Supuran, C. T.; Carta, F., 2017: Design and Synthesis of Novel Nonsteroidal Anti-Inflammatory Drugs and Carbonic Anhydrase Inhibitors Hybrids (NSAIDs-CAIs) for the Treatment of Rheumatoid Arthritis. *Journal of medicinal chemistry*., **60**, 1159–1170.

Burke, S. P.; Nadler, J. V, 1988: Regulation of glutamate and aspartate release from slices of the hippocampal CA1 area: effects of adenosine and baclofen. *Journal of neurochemistry*., **51**, 1541–1551.

Burnstock, G.; Fredholm, B. B.; Verkhratsky, A., 2011: Adenosine and ATP receptors in the brain. *Current topics in medicinal chemistry*., **11**, 973–1011.

Busa, W. B.; Nuccitelli, R., 1984: Metabolic regulation via intracellular pH. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*., **15**, 409–438.

Butt, A. M.; Duncan, A.; Hornby, M. F.; Kirvell, S. L.; Hunter, A.; Levine, J. M.; Berry, M., 1999: Cells expressing the NG2 antigen contact nodes of Ranvier in adult CNS white matter. *Glia*., **26**, 84–91.

Butt, A. M.; Kiff, J.; Hubbard, P.; Berry, M., 2002: Synantocytes: New functions for novel NG2 expressing glia. *Journal of Neurocytology*.

Canazza, A. P. Ms.; Minati, L. P. Ce. Cp. Cs.; Boffano, C. M.; Parati, E. M.; Binks, S. B. B., 2014: Experimental Models of Brain Ischemia: A Review of Techniques, Magnetic Resonance Imaging, and Investigational Cell-Based Therapies. *Frontiers in Neurology*., **5**, 19.

Cannizzaro, C.; Monastero, R.; Vacca, M.; Martire, M., 2003: [3H]-DA release evoked by low pH medium and internal H+ accumulation in rat hypothalamic synaptosomes: involvement of calcium ions. *Neurochemistry international*., **43**, 9–17.

Capogrossi, M. C.; Di Girolamo, M.; Zimring, H.; Crandall, D.; Francendese, A.; Israili, Z. H., 1986: Adenosine release from isolated rat adipocytes: influence of fat cell concentration and cell size. *Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, N.Y.)*., **182**, 15–22.

Carbonell, T.; Rama, R., 2007: Iron, oxidative stress and early neurological deterioration in ischemic stroke. *Current medicinal chemistry*., **14**, 857–874.

Carpenter, B.; Nehmé, R.; Warne, T.; Leslie, A. G. W.; Tate, C. G., 2016: Structure of the adenosine A2A receptor bound to an engineered G protein. *Nature*., **536**, 104–107.

Carta, F.; Supuran, C. T.; Scozzafava, A., 2012: Novel therapies for glaucoma: a patent review 2007 - 2011. *Expert opinion on therapeutic patents*., **22**, 79–88.

Carta, F.; Supuran, C. T., 2013: Diuretics with carbonic anhydrase inhibitory action: a patent and literature review (2005 - 2013). *Expert opinion on therapeutic patents*., **23**, 681–691.

Carta, F.; Di Cesare Mannelli, L.; Pinard, M.; Ghelardini, C.; Scozzafava, A.; McKenna, R.; Supuran, C. T., 2015: A class of sulfonamide carbonic anhydrase inhibitors with neuropathic pain modulating effects. *Bioorganic and Medicinal Chemistry*., **23**, 1828–1840.

Casey, J. R.; Grinstein, S.; Orlowski, J., 2010: Sensors and regulators of intracellular pH. *Nature Reviews Molecular Cell Biology*., **11**, 50–61.

Chang, A.; Nishiyama, A.; Peterson, J.; Prineas, J.; Trapp, B. D., 2000: NG2-positive oligodendrocyte progenitor cells in adult human brain and multiple sclerosis lesions. *The Journal of neuroscience : the official journal of the Society for Neuroscience*., **20**, 6404–6412.

Chen, J.; Sanberg, P. R.; Li, Y.; Wang, L.; Lu, M.; Willing, A. E.; Sanchez-Ramos, J.; Chopp, M., 2001: Intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats. *Stroke*., **32**, 2682–2688.

Chiba, K.; Hoshino, Y.; Suzuki, C.; Masubuchi, Y.; Yanagawa, Y.; Ohtsuki, M.; Sasaki, S.; Fujita, T., 1996: FTY720, a novel immunosuppressant possessing unique mechanisms. I. Prolongation of skin allograft survival and synergistic effect in combination with cyclosporine in rats. *Transplantation proceedings*., **28**, 1056–1059.

Chin, T. Y.; Hwang, H. M.; Chueh, S. H., 2002: Distinct effects of different calcium-mobilizing agents on cell death in NG108-15 neuroblastoma × glioma cells. *Molecular Pharmacology*., **61**, 486–494.

Chittajallu, R.; Chen, Y.; Wang, H.; Yuan, X.; Ghiani, C. A.; Heckman, T.; McBain, C. J.; Gallo, V., 2002: Regulation of Kv1 subunit expression in oligodendrocyte progenitor cells and their role in G1/S phase progression of the cell cycle. *Proceedings of the National Academy of Sciences of the United States of America*., **99**, 2350–2355.

Chittajallu, R.; Aguirre, A. A.; Gallo, V., 2005: Downregulation of platelet-derived growth factor-alpha receptor-mediated tyrosine kinase activity as a cellular mechanism for K+-channel regulation during oligodendrocyte development in situ. *The Journal of neuroscience : the official journal of the Society for Neuroscience*., **25**, 8601–8610.

Choi, D. W., 1990: Possible mechanisms limiting N-methyl-D-aspartate receptor overactivation and the therapeutic efficacy of N-methyl-D-aspartate antagonists. *Stroke*., **21**, III20-2.

Choi, J. W.; Gardell, S. E.; Herr, D. R.; Rivera, R.; Lee, C. W.; Noguchi, K.; Teo, S. T.; Yung, Y. C.; Lu, M.; Kennedy, G.; Chun, J., 2011: FTY720 (fingolimod) efficacy in an animal model of multiple sclerosis requires astrocyte sphingosine 1-phosphate receptor 1 (S1P1) modulation. *Proceedings of the National Academy of Sciences of the United States of America*., **108**, 751–756.

Choi, J. W.; Chun, J., 2013: Lysophospholipids and their receptors in the central nervous system. *Biochimica et biophysica acta*., **1831**, 20–32.

Chopp, M.; Li, Y.; Jiang, N.; Zhang, R. L.; Prostak, J., 1996: Antibodies against adhesion molecules reduce apoptosis after transient middle cerebral artery occlusion in rat brain. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism.*, **16**, 578–584.

Choukèr, A.; Thiel, M.; Lukashev, D.; Ward, J. M.; Kaufmann, I.; Apasov, S.; Sitkovsky, M. V.; Ohta, A., 2008: Critical role of hypoxia and A2A adenosine receptors in liver tissue-protecting physiological anti-inflammatory pathway. *Molecular Medicine.*, **14**, 116–123.

Chun, J., 1999: Lysophospholipid receptors: implications for neural signaling. *Critical reviews in neurobiology.*, **13**, 151–168.

Ciruela, F.; Ferré, S.; Casadó, V.; Cortés, A.; Cunha, R. A.; Lluis, C.; Franco, R., 2006: Heterodimeric adenosine receptors: a device to regulate neurotransmitter release. *Cellular and Molecular Life Sciences.*, **63**, 2427–2431.

Clark, W. M.; Lauten, J. D.; Lessov, N.; Woodward, W.; Coull, B. M., 1995: The influence of antiadhesion therapies on leukocyte subset accumulation in central nervous system ischemia in rats. *Journal of molecular neuroscience : MN.*, **6**, 43–50.

Clark, W. M.; Lessov, N.; Lauten, J. D.; Hazel, K., 1997: Doxycycline treatment reduces ischemic brain damage in transient middle cerebral artery occlusion in the rat. *Journal of molecular neuroscience : MN.*, **9**, 103–108.

Clausen, B. H.; Lundberg, L.; Yli-Karjanmaa, M.; Martin, N. A.; Svensson, M.; Alfsen, M. Z.; Flæng, S. B.; Lyngsø, K.; Boza-Serrano, A.; Nielsen, H. H.; Hansen, P. B.; Finsen, B.; Deierborg, T.; Illes, Z.; Lambertsen, K. L., 2017: Fumarate decreases edema volume and improves functional outcome after experimental stroke. *Experimental Neurology.*, **295**, 144–154.

Coelho, R. P.; Payne, S. G.; Bittman, R.; Spiegel, S.; Sato-Bigbee, C., 2007: The immunomodulator FTY720 has a direct cytoprotective effect in oligodendrocyte progenitors. *The Journal of pharmacology and experimental therapeutics.*, **323**, 626–635.

Connolly, E. S.; Winfree, C. J.; Springer, T. A.; Naka, Y.; Liao, H.; Yan, S. D.; Stern, D. M.; Solomon, R. A.; Gutierrez-Ramos, J. C.; Pinsky, D. J., 1996: Cerebral protection in homozygous null ICAM-1 mice after middle cerebral artery occlusion. Role of neutrophil adhesion in the pathogenesis of stroke. *The Journal of clinical investigation.*, **97**, 209–216.

Cooper, A. J.; Kristal, B. S., 1997: Multiple roles of glutathione in the central nervous system. *Biological chemistry.*, **378**, 793–802.

Coppi, E.; Maraula, G.; Fumagalli, M.; Failli, P.; Cellai, L.; Bonfanti, E.; Mazzoni, L.; Coppini, R.; Abbracchio, M. P.; Pedata, F.; Pugliese, A. M., 2013a: UDP-glucose enhances outward K(+) currents necessary for cell differentiation and stimulates cell migration by activating the GPR17 receptor in oligodendrocyte precursors. *Glia.*, **61**, 1155–1171.

Coppi, E.; Cellai, L.; Maraula, G.; Pugliese, A. M.; Pedata, F., 2013b: Adenosine A_2A receptors inhibit delayed rectifier potassium currents and cell differentiation in primary purified oligodendrocyte cultures. *Neuropharmacology.*, **73**, 301–310.

Coppi, E.; Cellai, L.; Maraula, G.; Dettori, I.; Melani, A.; Pugliese, A. M.; Pedata, F., 2015: Role of adenosine in oligodendrocyte precursor maturation. *Frontiers in Cellular Neuroscience.*, **9**.

Corradetti, R.; Lo Conte, G.; Moroni, F.; Passani, M. B.; Pepeu, G., 1984: Adenosine

decreases aspartate and glutamate release from rat hippocampal slices. *European journal of pharmacology*., **104**, 19–26.

Corsi, C.; Melani, A.; Bianchi, L.; Pepeu, G.; Pedata, F., 1999: Striatal A2A adenosine receptors differentially regulate spontaneous and K+-evoked glutamate release in vivo in young and aged rats. *Neuroreport*., **10**, 687–691.

Corsi, C.; Melani, A.; Bianchi, L.; Pedata, F., 2000: Striatal A2A adenosine receptor antagonism differentially modifies striatal glutamate outflow in vivo in young and aged rats. *Neuroreport*., **11**, 2591–2595.

Corti, F.; Cellai, L.; Melani, A.; Donati, C.; Bruni, P.; Pedata, F., 2013: Adenosine is present in rat brain synaptic vesicles. *Neuroreport*., **24**, 982–987.

Costenla, A. R.; Lopes, L. V; de Mendonça, A.; Ribeiro, J. A., 2001: A functional role for adenosine A3 receptors: modulation of synaptic plasticity in the rat hippocampus. *Neuroscience letters*., **302**, 53–57.

Crack, P. J.; Taylor, J. M., 2005: Reactive oxygen species and the modulation of stroke. *Free radical biology & medicine*., **38**, 1433–1444.

Crawford, A. H.; Stockley, J. H.; Tripathi, R. B.; Richardson, W. D.; Franklin, R. J. M., 2014: Oligodendrocyte progenitors: adult stem cells of the central nervous system? *Experimental neurology*., **260**, 50–55.

Cristalli, G.; Lambertucci, C.; Marucci, G.; Volpini, R.; Dal Ben, D., 2008: A2A adenosine receptor and its modulators: overview on a druggable GPCR and on structure-activity relationship analysis and binding requirements of agonists and antagonists. *Current pharmaceutical design*., **14**, 1525–1552.

Cunha, R. A.; Johansson, B.; Fredholm, B. B.; Ribeiro, J. A.; Sebastião, A. M., 1995: Adenosine A2A receptors stimulate acetylcholine release from nerve terminals of the rat hippocampus. *Neuroscience letters*., **196**, 41–44.

Cunha, R. A., 2001: Adenosine as a neuromodulator and as a homeostatic regulator in the nervous system: different roles, different sources and different receptors. *Neurochemistry international*., **38**, 107–125.

d'Alcantara, P.; Ledent, C.; Swillens, S.; Schiffmann, S. N., 2001: Inactivation of adenosine A2A receptor impairs long term potentiation in the accumbens nucleus without altering basal synaptic transmission. *Neuroscience*., **107**, 455–464.

Dai, S. S.; Zhou, Y. G.; Li, W.; An, J. H.; Li, P.; Yang, N.; Chen, X. Y.; Xiong, R. P.; Liu, P.; Zhao, Y.; Shen, H. Y.; Zhu, P. F.; Chen, J. F., 2010: Local glutamate level dictates adenosine A2A receptor regulation of neuroinflammation and traumatic brain injury. *The Journal of neuroscience : the official journal of the Society for Neuroscience*., **30**, 5802–5810.

Danner, H.; Pfister, C., 1981: [The cytoarchitecture of the rat globus pallidus]. *Journal fur Hirnforschung*., **22**, 47–57.

Dariush, M.; J, B. E.; S, G. A.; K, A. D.; J, B. M.; Mary, C.; Sarah, de F.; Jean-Pierre, D.; J, F. H.; J, H. V.; D, H. M.; E, J. S.; M, K. B.; T, L. D.; H, L. J.; D, L. L.; Simin, L.; H, M. R.; B, M. D. et al., 2015: Heart Disease and Stroke Statistics—2015 Update. *Circulation*., **131**, e29–e322.

Dawson, M. R. L.; Polito, A.; Levine, J. M.; Reynolds, R., 2003: NG2-expressing glial

progenitor cells: an abundant and widespread population of cycling cells in the adult rat CNS. *Molecular and cellular neurosciences*., **24**, 476–488.

De Castro, F.; Bribián, A., 2005: The molecular orchestra of the migration of oligodendrocyte precursors during development. *Brain Research Reviews*.

Dean, J. M.; Moravec, M. D.; Grafe, M.; Abend, N.; Ren, J.; Gong, X.; Volpe, J. J.; Jensen, F. E.; Hohimer, A. R.; Back, S. A., 2011: Strain-specific differences in perinatal rodent oligodendrocyte lineage progression and its correlation with human. *Developmental neuroscience*., **33**, 251–260.

Deckert, J.; Jorgensen, M. B., 1988: Evidence for pre- and postsynaptic localization of adenosine A1 receptors in the CA1 region of rat hippocampus: a quantitative autoradiographic study. *Brain research*., **446**, 161–164.

Dettori, I.; Gaviano, L.; Melani, A.; Lucarini, L.; Durante, M.; Masini, E.; Pedata, F., 2018: A selective histamine H4 receptor antagonist, JNJ7777120, is protective in a rat model of transient cerebral ischemia. *Frontiers in Pharmacology*., **9**.

Di Cesare Mannelli, L.; Micheli, L.; Carta, F.; Cozzi, A.; Ghelardini, C.; Supuran, C. T., 2016: Carbonic anhydrase inhibition for the management of cerebral ischemia: in vivo evaluation of sulfonamide and coumarin inhibitors. *Journal of Enzyme Inhibition and Medicinal Chemistry*., **31**, 894–899.

Di Paola, R.; Melani, A.; Esposito, E.; Mazzon, E.; Paterniti, I.; Bramanti, P.; Pedata, F.; Cuzzocrea, S., 2010: Adenosine A2A receptor-selective stimulation reduces signaling pathways involved in the development of intestine ischemia and reperfusion injury. *Shock (Augusta, Ga.)*., **33**, 541–551.

Dirnagl, U.; Iadecola, C.; Moskowitz, M. A., 1999: Pathobiology of ischaemic stroke: An integrated view. *Trends in Neurosciences*., **22**, 391–397.

Dixon, A. K.; Gubitz, A. K.; Sirinathsinghji, D. J.; Richardson, P. J.; Freeman, T. C., 1996: Tissue distribution of adenosine receptor mRNAs in the rat. *British journal of pharmacology*., **118**, 1461–1468.

Dobolyi, A.; Reichart, A.; Szikra, T.; Nyitrai, G.; Kékesi, K. A.; Juhász, G., 2000: Sustained depolarisation induces changes in the extracellular concentrations of purine and pyrimidine nucleosides in the rat thalamus. *Neurochemistry international*., **37**, 71–79.

Dobrek, Ł.; Thor, P., 2011: Glutamate NMDA receptors in pathophysiology and pharmacotherapy of selected nervous system diseases. *Postepy Higieny i Medycyny Doswiadczalnej*.

Dong, Y.; Benveniste, E. N., 2001: Immune function of astrocytes. *Glia*., **36**, 180–190.

Donnan GA, Fisher M, Macleod M, D. S., 2008: Stroke. - PubMed - NCBI. *Lancet*.

Draper-joyce, C. J.; Khoshouei, M.; Thal, D. M.; Liang, Y. lynn; Nguyen, A. T. N.; Furness, S. G. B.; Venugopal, H.; Baltos, J. anne; Plitzko, J. M.; Danev, R.; Baumeister, W., 2018: Structure of the adenosine-bound human adenosine A 1 receptor – G i complex.

Drury, A. N.; Szent-Györgyi, A., 1929: The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart. *The Journal of physiology*., **68**, 213–237.

Dunwiddie, T. V; Hoffer, B. J., 1980: Adenine nucleotides and synaptic transmission in the in

vitro rat hippocampus. *British journal of pharmacology*., **69**, 59–68.

Dunwiddie, T. V, 1984: Interactions between the effects of adenosine and calcium on synaptic responses in rat hippocampus in vitro. *The Journal of physiology*., **350**, 545–559.

Dunwiddie, T. V, 1985: The physiological role of adenosine in the central nervous system. *International review of neurobiology*., **27**, 63–139.

Dunwiddie, T. V; Fredholm, B. B., 1997: Adenosine neuromodulation. In: KA, J. & J. MF (eds.), *Purinergics approaches in experimental therapeutics*. New York, NY: Wiley-Liss, pp. 359–382.

Dux, E.; Fastbom, J.; Ungerstedt, U.; Rudolphi, K.; Fredholm, B. B., 1990: Protective effect of adenosine and a novel xanthine derivative propentofylline on the cell damage after bilateral carotid occlusion in the gerbil hippocampus. *Brain research*., **516**, 248–256.

Eckle, T.; Krahn, T.; Grenz, A.; Köhler, D.; Mittelbronn, M.; Ledent, C.; Jacobson, M. A.; Osswald, H.; Thompson, L. F.; Unertl, K.; Eltzschig, H. K., 2007a: Cardioprotection by Ecto-5′-Nucleotidase (CD73) and A_{2B} Adenosine Receptors. *Circulation*., **115**, 1581–1590.

Eckle, T.; Krahn, T.; Grenz, A.; Köhler, D.; Mittelbronn, M.; Ledent, C.; Jacobson, M. A.; Osswald, H.; Thompson, L. F.; Unertl, K.; Eltzschig, H. K., 2007b: Cardioprotection by ecto-5′-nucleotidase (CD73) and A2B adenosine receptors. *Circulation*.

Eckle, T.; Faigle, M.; Grenz, A.; Laucher, S.; Thompson, L. F.; Eltzschig, H. K., 2008a: A2B adenosine receptor dampens hypoxia-induced vascular leak. *Blood*., **111**, 2024–2035.

Eckle, T.; Grenz, A.; Laucher, S.; Eltzschig, H. K., 2008b: A2B adenosine receptor signaling attenuates acute lung injury by enhancing alveolar fluid clearance in mice. *Journal of Clinical Investigation*.

Edsall, L. C.; Pirianov, G. G.; Spiegel, S., 1997: Involvement of sphingosine 1-phosphate in nerve growth factor-mediated neuronal survival and differentiation. *The Journal of neuroscience : the official journal of the Society for Neuroscience*., **17**, 6952–6960.

Eisenstein, A.; Ravid, K., 2014: G Protein-coupled receptors and adipogenesis: A focus on adenosine receptors. *Journal of Cellular Physiology*.

Ekdahl, C. T.; Kokaia, Z.; Lindvall, O., 2009: Brain inflammation and adult neurogenesis: the dual role of microglia. *Neuroscience*., **158**, 1021–1029.

Ekholm, A.; Katsura, K.; Siesjo, B. K., 1991: Tissue lactate content and tissue PCO2 in complete brain ischaemia: Implications for compartmentation of H+. *Neurological Research*., **13**, 74–76.

El Waly, B.; Macchi, M.; Cayre, M.; Durbec, P., 2014: Oligodendrogenesis in the normal and pathological central nervous system. *Frontiers in neuroscience*., **8**, 145.

Elmore, S., 2007: Apoptosis: A Review of Programmed Cell Death. *Toxicologic Pathology*.

Eltzschig, H. K.; Thompson, L. F.; Karhausen, J.; Cotta, R. J.; Ibla, J. C.; Robson, S. C.; Colgan, S. P., 2004: Endogenous adenosine produced during hypoxia attenuates neutrophil accumulation: coordination by extracellular nucleotide metabolism. *Blood*., **104**, 3986–3992.

Englert, M.; Quitterer, U.; Klotz, K. N., 2002: Effector coupling of stably transfected human A3 adenosine receptors in CHO cells. *Biochemical Pharmacology*., **64**, 61–65.

Fang, Z. Z.; Tosh, D. K.; Tanaka, N.; Wang, H.; Krausz, K. W.; O'Connor, R.; Jacobson, K. A.; Gonzalez, F. J., 2015: Metabolic mapping of A3 adenosine receptor agonist MRS5980. *Biochemical pharmacology*., **97**, 215–223.

Fausther, M.; Lecka, J.; Soliman, E.; Kauffenstein, G.; Pelletier, J.; Sheung, N.; Dranoff, J. A.; Sévigny, J., 2012: Coexpression of ecto-5'-nucleotidase/CD73 with specific NTPDases differentially regulates adenosine formation in the rat liver. *American journal of physiology. Gastrointestinal and liver physiology*., **302**, G447-59.

Feoktistov, I.; Biaggioni, I., 1997: Adenosine A2B receptors. *Pharmacological reviews*., **49**, 381–402.

Feoktistov, I.; Ryzhov, S.; Zhong, H.; Goldstein, A. E.; Matafonov, A.; Zeng, D.; Biaggioni, I., 2004: Hypoxia modulates adenosine receptors in human endothelial and smooth muscle cells toward an A2B angiogenic phenotype. *Hypertension (Dallas, Tex. : 1979)*., **44**, 649–654.

Ferre, S.; von Euler, G.; Johansson, B.; Fredholm, B. B.; Fuxe, K., 1991: Stimulation of high-affinity adenosine A2 receptors decreases the affinity of dopamine D2 receptors in rat striatal membranes. *Proceedings of the National Academy of Sciences of the United States of America*., **88**, 7238–7241.

Fiebich, B. L.; Biber, K.; Lieb, K.; van Calker, D.; Berger, M.; Bauer, J.; Gebicke-Haerter, P. J., 1996: Cyclooxygenase-2 expression in rat microglia is induced by adenosine A2a-receptors. *Glia*., **18**, 152–160.

Fields, R. D.; Stevens-Graham, B., 2002, October 18: Neuroscience: New insights into neuron-glia communication. *Science*.

Fields, R. D.; Burnstock, G., 2006: Purinergic signalling in neuron-glia interactions. *Nature reviews. Neuroscience*., **7**, 423–436.

Folbergrová, J.; Zhao, Q.; Katsura, K. I.; Siesjö, B. K., 1995: N-tert-butyl-α-phenylnitrone improves recovery of brain energy state in rats following transient focal ischemia. *Proceedings of the National Academy of Sciences of the United States of America*., **92**, 5057–5061.

Foster, C. A.; Howard, L. M.; Schweitzer, A.; Persohn, E.; Hiestand, P. C.; Balatoni, B.; Reuschel, R.; Beerli, C.; Schwartz, M.; Billich, A., 2007: Brain penetration of the oral immunomodulatory drug FTY720 and its phosphorylation in the central nervous system during experimental autoimmune encephalomyelitis: Consequences for mode of action in multiple sclerosis. *Journal of Pharmacology and Experimental Therapeutics*., **323**, 469–476.

Franco, R.; Canela, E. I.; Bozal, J., 1986: Heterogeneous localization of some purine enzymes in subcellular fractions of rat brain and cerebellum. *Neurochemical research*., **11**, 423–435.

Franklin, R. J. M.; Ffrench-Constant, C., 2008: Remyelination in the CNS: from biology to therapy. *Nature reviews. Neuroscience*., **9**, 839–855.

Fredholm, B.; Cunha, R.; Svenningsson, P., 2003a: Pharmacology of Adenosine A2A Receptors and Therapeutic Applications. *Current Topics in Medicinal Chemistry*., **3**, 413–426.

Fredholm, B. B.; Lindgren, E.; Lindstrom, K.; Nordstedt, C., 1987: α-Adrenoceptor stimulation, but not muscarinic stimulation, increases cyclic AMP accumulation in brain slices due to protein kinase C mediated enhancement of adenosine receptor effects. *Acta Physiologica Scandinavica*., **131**, 543–551.

Fredholm, B. B.; Dunwiddie, T. V, 1988: How does adenosine inhibit transmitter release? *Trends in pharmacological sciences*., **9**, 130–134.

Fredholm, B. B.; Arslan, G.; Halldner, L.; Kull, B.; Schulte, G.; Wasserman, W., 2000: Structure and function of adenosine receptors and their genes. *Naunyn-Schmiedeberg's archives of pharmacology*., **362**, 364–374.

Fredholm, B. B.; IJzerman, A. P.; Jacobson, K. A.; Klotz, K. N.; Linden, J., 2001: International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacological reviews*., **53**, 527–552.

Fredholm, B. B.; Svenningsson, P., 2003b: Adenosine-dopamine interactions: development of a concept and some comments on therapeutic possibilities. *Neurology*., **61**, S5-9.

Fredholm, B. B.; Chen, J. F.; Masino, S. A.; Vaugeois, J. M., 2005: ACTIONS OF ADENOSINE AT ITS RECEPTORS IN THE CNS: Insights from Knockouts and Drugs. *Annual Review of Pharmacology and Toxicology*., **45**, 385–412.

Fredholm, B. B.; Chern, Y.; Franco, R.; Sitkovsky, M., 2007: Aspects of the general biology of adenosine A2A signaling. *Progress in neurobiology*., **83**, 263–276.

Fredholm, B. B.; IJzerman, A. P.; Jacobson, K. A.; Linden, J.; Muller, C. E., 2011: International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and Classification of Adenosine Receptors--An Update. *Pharmacological Reviews*., **63**, 1–34.

Fujino, M.; Funeshima, N.; Kitazawa, Y.; Kimura, H.; Amemiya, H.; Suzuki, S.; Li, X. K., 2003: Amelioration of experimental autoimmune encephalomyelitis in Lewis rats by FTY720 treatment. *The Journal of pharmacology and experimental therapeutics*., **305**, 70–77.

Fumagalli, M.; Daniele, S.; Lecca, D.; Lee, P. R.; Parravicini, C.; Douglas Fields, R.; Rosa, P.; Antonucci, F.; Verderio, C.; Letizia Trincavelli, M.; Bramanti, P.; Martini, C.; Abbracchio, M. P., 2011: Phenotypic changes, signaling pathway, and functional correlates of GPR17-expressing neural precursor cells during oligodendrocyte differentiation. *Journal of Biological Chemistry*.

Furukawa, K.; Fu, W.; Li, Y.; Witke, W.; Kwiatkowski, D. J.; Mattson, M. P., 1997: The actin-severing protein gelsolin modulates calcium channel and NMDA receptor activities and vulnerability to excitotoxicity in hippocampal neurons. *Journal of Neuroscience*., **17**, 8178–8186.

Fusco, I.; Ugolini, F.; Lana, D.; Coppi, E.; Dettori, I.; Gaviano, L.; Nosi, D.; Cherchi, F.; Pedata, F.; Giovannini, M. G.; Pugliese, A. M., 2018: The Selective Antagonism of Adenosine A2B Receptors Reduces the Synaptic Failure and Neuronal Death Induced by Oxygen and Glucose Deprivation in Rat CA1 Hippocampus in Vitro. *Frontiers in Pharmacology*., **9**, 399.

Gallo, V.; Armstrong, R. C., 1995: Developmental and growth factor-induced regulation of nestin in oligodendrocyte lineage cells. *Journal of Neuroscience*.

Gallo, V.; Zhou, J. M.; McBain, C. J.; Wright, P.; Knutson, P. L.; Armstrong, R. C., 1996: Oligodendrocyte progenitor cell proliferation and lineage progression are regulated by glutamate receptor-mediated K+ channel block. *Journal of Neuroscience*.

Gao, Z. G.; Kim, S. K.; Biadatti, T.; Chen, W.; Lee, K.; Barak, D.; Kim, S. G.; Johnson, C. R.; Jacobson, K. A., 2002: Structural determinants of A(3) adenosine receptor activation: nucleoside ligands at the agonist/antagonist boundary. *Journal of medicinal chemistry*., **45**,

4471–4484.

Garau, A.; Bertini, R.; Colotta, F.; Casilli, F.; Bigini, P.; Cagnotto, A.; Mennini, T.; Ghezzi, P.; Villa, P., 2005: Neuroprotection with the CXCL8 inhibitor repertaxin in transient brain ischemia. *Cytokine*., **30**, 125–131.

García-Nafría, J.; Lee, Y.; Bai, X.; Carpenter, B.; Tate, C. G., 2018: Cryo-EM structure of the adenosine A2A receptor coupled to an engineered heterotrimeric G protein. *eLife*., **7**, 1–19.

Gard, A. L.; Pfeiffer, S. E., 1989: Oligodendrocyte progenitors isolated directly from developing telencephalon at a specific phenotypic stage: Myelinogenic potential in a defined environment. *Development*.

Gebicki, J. M.; Bielski, B. H. J., 1981: Comparison of the Capacities of the Perhydroxyl and the Superoxide Radicals To Initiate Chain Oxidation of Linoleic Acid. *Journal of the American Chemical Society*., **103**, 7020–7022.

Genovese, T.; Melani, A.; Esposito, E.; Mazzon, E.; Di Paola, R.; Bramanti, P.; Pedata, F.; Cuzzocrea, S., 2009: The selective adenosine A2A receptor agonist CGS 21680 reduces JNK MAPK activation in oligodendrocytes in injured spinal cord. *Shock*.

Gessi, S.; Varani, K.; Merighi, S.; Cattabriga, E.; Pancaldi, C.; Szabadkai, Y.; Rizzuto, R.; Klotz, K. N.; Leung, E.; Mac Lennan, S.; Baraldi, P. G.; Borea, P. A., 2005: Expression, Pharmacological Profile, and Functional Coupling of A2B Receptors in a Recombinant System and in Peripheral Blood Cells Using a Novel Selective Antagonist Radioligand, [3H]MRE 2029-F20. *Molecular Pharmacology*., **67**, 2137–2147.

Gharibi, B.; Abraham, A. A.; Ham, J.; Evans, B. A. J., 2012: Contrasting effects of A1 and A2b adenosine receptors on adipogenesis. *International Journal of Obesity*.

Gibson, C. L., 2013, September: Cerebral ischemic stroke: Is gender important? *Journal of Cerebral Blood Flow and Metabolism*.

Gibson, E. M.; Purger, D.; Mount, C. W.; Goldstein, A. K.; Lin, G. L.; Wood, L. S.; Inema, I.; Miller, S. E.; Bieri, G.; Zuchero, J. B.; Barres, B. A.; Woo, P. J.; Vogel, H.; Monje, M., 2014: Neuronal activity promotes oligodendrogenesis and adaptive myelination in the mammalian brain. *Science*.

Giovannini, M. G.; Scali, C.; Prosperi, C.; Bellucci, A.; Vannucchi, M. G.; Rosi, S.; Pepeu, G.; Casamenti, F., 2002: β-amyloid-induced inflammation and cholinergic hypofunction in the rat brain in vivo: Involvement of the p38MAPK pathway. *Neurobiology of Disease*., **11**, 257–274.

Glukhova, A.; Thal, D. M.; Nguyen, A. T.; May, L. T.; Sexton, P. M.; Christopoulos, A.; Scammells, P. J., 2017: Structure of the Adenosine A 1 Receptor Reveals the Basis for Subtype Selectivity Article Structure of the Adenosine A 1 Receptor Reveals the Basis for Subtype Selectivity, 867–877.

Goldman, J. E.; Geier, S. S.; Hirano, M., 1986: Differentiation of astrocytes and oligodendrocytes from germinal matrix cells in primary culture. *The Journal of neuroscience : the official journal of the Society for Neuroscience*., **6**, 52–60.

Gomes, C.; Ferreira, R.; George, J.; Sanches, R.; Rodrigues, D. I.; Gonçalves, N.; Cunha, R. A., 2013: Activation of microglial cells triggers a release of brain-derived neurotrophic factor (BDNF) inducing their proliferation in an adenosine A2A receptor-dependent manner: A2A receptor blockade prevents BDNF release and proliferation of microglia. *Journal of*

Neuroinflammation., **10**, 780.

Gomes, C. A. R. V.; Vaz, S. H.; Ribeiro, J. A.; Sebastião, A. M., 2006: Glial cell line-derived neurotrophic factor (GDNF) enhances dopamine release from striatal nerve endings in an adenosine A2A receptor-dependent manner. *Brain Research*., **1113**, 129–136.

Gonçalves, M. L.; Cunha, R. A.; Ribeiro, J. A., 1997: Adenosine A2A receptors facilitate 45Ca2+ uptake through class A calcium channels in rat hippocampal CA3 but not CA1 synaptosomes. *Neuroscience letters*., **238**, 73–77.

González-Fernández, E.; Sánchez-Gómez, M. V.; Pérez-Samartín, A.; Arellano, R. O.; Matute, C., 2014: A3 Adenosine receptors mediate oligodendrocyte death and ischemic damage to optic nerve. *Glia*., **62**, 199–216.

Greene, R. W.; Haas, H. L., 1991: The electrophysiology of adenosine in the mammalian central nervous system. *Progress in neurobiology*., **36**, 329–341.

Gu, L. Z.; Huang, B. S.; Shen, W.; Gao, L.; Ding, Z. Z.; Wu, H. W.; Guo, J., 2013: Early activation of nSMase2/ceramide pathway in astrocytes is involved in ischemia-associated neuronal damage via inflammation in rat hippocampi. *Journal of Neuroinflammation*., **10**.

Gubitz, A. K.; Widdowson, L.; Kurokawa, M.; Kirkpatrick, K. A.; Richardson, P. J., 1996: Dual signalling by the adenosine A2a receptor involves activation of both N- and P-type calcium channels by different G proteins and protein kinases in the same striatal nerve terminals. *Journal of neurochemistry*., **67**, 374–381.

Guo, F.; Hua, Y.; Wang, J.; Keep, R. F.; Xi, G., 2012: Inhibition of Carbonic Anhydrase Reduces Brain Injury After Intracerebral Hemorrhage. *Translational Stroke Research*., **3**, 130–137.

Hagberg, H.; Andersson, P.; Lacarewicz, J.; Jacobson, I.; Butcher, S.; Sandberg, M., 1987: Extracellular adenosine, inosine, hypoxanthine, and xanthine in relation to tissue nucleotides and purines in rat striatum during transient ischemia. *Journal of neurochemistry*., **49**, 227–231.

Hait, N. C.; Bellamy, A.; Milstien, S.; Kordula, T.; Spiegel, S., 2007: Sphingosine kinase type 2 activation by ERK-mediated phosphorylation. *The Journal of biological chemistry*., **282**, 12058–12065.

Halassa, M. M.; Fellin, T.; Haydon, P. G., 2009: Tripartite synapses: roles for astrocytic purines in the control of synaptic physiology and behavior. *Neuropharmacology*., **57**, 343–346.

Hallenbeck, J. M., 1996: Significance of the inflammatory response in brain ischemia. *Acta neurochirurgica. Supplement*., **66**, 27–31.

Hamidi, S.; Avoli, M., 2015: Carbonic anhydrase inhibition by acetazolamide reduces in vitro epileptiform synchronization. *Neuropharmacology*., **95**, 377–387.

Han, B. H.; Holtzman, D. M., 2000: BDNF protects the neonatal brain from hypoxic-ischemic injury in vivo via the ERK pathway. *The Journal of neuroscience : the official journal of the Society for Neuroscience*., **20**, 5775–5781.

Harada, J.; Foley, M.; Moskowitz, M. A.; Waeber, C., 2004: Sphingosine-1-phosphate induces proliferation and morphological changes of neural progenitor cells. *Journal of neurochemistry*., **88**, 1026–1039.

Harms, H. H.; Wardeh, G.; Mulder, A. H., 1978: Adenosine modulates depolarization-induced release of 3H-noradrenaline from slices of rat brain neocortex. *European journal of pharmacology*., **49**, 305–308.

Harms, H. H.; Wardeh, G.; Mulder, A. H., 1979: Effects of adenosine on depolarization-induced release of various radiolabelled neurotransmitters from slices of rat corpus striatum. *Neuropharmacology*., **18**, 577–580.

Haskó, G.; Kuhel, D. G.; Chen, J. F.; Schwarzchild, M. A.; Deitch, E. A.; Mabley, J. G.; Marton, A.; Szabò, C., 2000: Adenosine inhibits IL-12 and TNF-α production via adenosine A 2a receptor-dependent and independent mechanisms. *The FASEB Journal*., **14**, 2065–2074.

Haskó, G.; Linden, J.; Cronstein, B.; Pacher, P., 2008: Adenosine receptors: therapeutic aspects for inflammatory and immune diseases. *Nature Reviews Drug Discovery*., **7**, 759–770.

Heese, K.; Fiebich, B. L.; Bauer, J.; Otten, U., 1997: Nerve growth factor (NGF) expression in rat microglia is induced by adenosine A2a-receptors. *Neuroscience Letters*., **231**, 83–86.

Hettinger, B. D.; Lee, A.; Linden, J.; Rosin, D. L., 2001: Ultrastructural localization of adenosine A2A receptors suggests multiple cellular sites for modulation of GABAergic neurons in rat striatum. *The Journal of comparative neurology*., **431**, 331–346.

Hewett-Emmett, D., 2000: Evolution and distribution of the carbonic anhydrase gene families. *The Carbonic Anhydrases*. Birkhäuser Basel, Basel, pp. 29–76.

Hinz, S.; Lacher, S. K.; Seibt, B. F.; Muller, C. E., 2014: BAY60-6583 Acts as a Partial Agonist at Adenosine A2B Receptors. *Journal of Pharmacology and Experimental Therapeutics*., **349**, 427–436.

Hollingsworth, E. B.; Sears, E. B.; Daly, J. W., 1985: An activator of protein kinase C (phorbol-12-myristate-13-acetate) augments 2-chloroadenosine-elicited accumulation of cyclic AMP in guinea pig cerebral cortical particulate preparations. *FEBS Letters*., **184**, 339–342.

Horner, P. J.; Power, A. E.; Kempermann, G.; Kuhn, H. G.; Palmer, T. D.; Winkler, J.; Thal, L. J.; Gage, F. H., 2000: Proliferation and differentiation of progenitor cells throughout the intact adult rat spinal cord. *The Journal of neuroscience : the official journal of the Society for Neuroscience*., **20**, 2218–2228.

Hossmann, K. A., 1994: Viability thresholds and the penumbra of focal ischemia. *Annals of neurology*., **36**, 557–565.

Huang, J.; Agus, D. B.; Winfree, C. J.; Kiss, S.; Mack, W. J.; McTaggart, R. A.; Choudhri, T. F.; Kim, L. J.; Mocco, J.; Pinsky, D. J.; Fox, W. D.; Israel, R. J.; Boyd, T. A.; Golde, D. W.; Connolly, E. S., 2001: Dehydroascorbic acid, a blood-brain barrier transportable form of vitamin C, mediates potent cerebroprotection in experimental stroke. *Proceedings of the National Academy of Sciences of the United States of America*., **98**, 11720–11724.

Huang, J.; Upadhyay, U. M.; Tamargo, R. J., 2006: Inflammation in stroke and focal cerebral ischemia. *Surgical Neurology*., **66**, 232–245.

Hulse, R. E.; Winterfield, J.; Kunkler, P. E.; Kraig, R. P., 2001: Astrocytic clasmatodendrosis in hippocampal organ culture. *GLIA.*, **33**, 169–179.

Hunsucker, S. A.; Mitchell, B. S.; Spychala, J., 2005: The 5′-nucleotidases as regulators of nucleotide and drug metabolism. *Pharmacology & Therapeutics*., **107**, 1–30.

Iadecola, C.; Anrather, J., 2011: Stroke research at a crossroad: asking the brain for directions. *Nature neuroscience*., **14**, 1363–1368.

Intiso, D.; Zarrelli, M. M.; Lagioia, G.; Di Rienzo, F.; Checchia De Ambrosio, C.; Simone, P.; Tonali, P.; Cioffi Dagger, R. P., 2004: Tumor necrosis factor alpha serum levels and inflammatory response in acute ischemic stroke patients. *Neurological sciences : official journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology*., **24**, 390–396.

Irving, E. A.; Barone, F. C.; Reith, A. D.; Hadingham, S. J.; Parsons, A. A., 2000a: Differential activation of MAPK/ERK and p38/SAPK in neurones and glia following focal cerebral ischaemia in the rat. *Brain research. Molecular brain research*., **77**, 65–75.

Irving, E. A.; Barone, F. C.; Reith, A. D.; Hadingham, S. J.; Parsons, A. A., 2000b: Differential activation of MAPK/ERK and p38/SAPK in neurones and glia following focal cerebral ischaemia in the rat. *Molecular Brain Research*., **77**, 65–75.

Ishii, I.; Fukushima, N.; Ye, X.; Chun, J., 2004: Lysophospholipid receptors: signaling and biology. *Annual review of biochemistry*., **73**, 321–354.

Jaakola, V. P.; Griffith, M. T.; Hanson, M. A.; Cherezov, V.; Chien, E. Y. T.; Lane, J. R.; Ijzerman, A. P.; Stevens, R. C., 2008: The 2.6 angstrom crystal structure of a human A2A adenosine receptor bound to an antagonist. *Science*., **322**, 1211–1217.

Jacobson, K. A.; Nikodijevic, O.; Ji, X. duo; Berkich, D. A.; Eveleth, D.; Dean, R. L.; Hiramatsu, K. I.; Kassell, N. F.; van Galen, P. J. M.; Lee, K. S.; Bartus, R. T.; Daly, J. W.; LaNoue, K. F.; Maillard, M., 1992: Synthesis and Biological Activity of N6-(p-Sulfophenyl)alkyl and N6-Sulfoalkyl Derivatives of Adenosine: Water-Soluble and Peripherally Selective Adenosine Agonists. *Journal of Medicinal Chemistry*.

Jacobson, K. A.; Nikodijević, O.; Padgett, W. L.; Gallo-Rodriguez, C.; Maillard, M.; Daly, J. W., 1993: 8-(3-Chlorostyryl)caffeine (CSC) is a selective A2-adenosine antagonist in vitro and in vivo. *FEBS Letters*.

Jaillard, C.; Harrison, S.; Stankoff, B.; Aigrot, M. S.; Calver, A. R.; Duddy, G.; Walsh, F. S.; Pangalos, M. N.; Arimura, N.; Kaibuchi, K.; Zalc, B.; Lubetzki, C., 2005: Edg8/S1P5: an oligodendroglial receptor with dual function on process retraction and cell survival. *The Journal of neuroscience : the official journal of the Society for Neuroscience*., **25**, 1459–1469.

James, S.; Richardson, P. J., 1993: Production of adenosine from extracellular ATP at the striatal cholinergic synapse. *Journal of neurochemistry*., **60**, 219–227.

Jarvis, M. F.; Schulz, R.; Hutchison, A. J.; Do, U. H.; Sills, M. A.; Williams, M., 1989: [3H]CGS 21680, a selective A2 adenosine receptor agonist directly labels A2 receptors in rat brain. *The Journal of pharmacology and experimental therapeutics*., **251**, 888–893.

Jeon, S. J.; Rhee, S. Y.; Ryu, J. H.; Cheong, J. H.; Kwon, K.; Yang, S. I.; Park, S. H.; Lee, J.; Kim, H. Y.; Han, S. H.; Ko, K. H.; Shin, C. Y., 2011: Activation of Adenosine A2A Receptor Up-Regulates BDNF Expression in Rat Primary Cortical Neurons. *Neurochemical Research*., **36**, 2259–2269.

Jeong, L. S.; Jin, D. Z.; Kim, H. O.; Shin, D. H.; Moon, H. R.; Gunaga, P.; Chun, M. W.; Kim, Y. C.; Melman, N.; Gao, Z. G.; Jacobson, K. A., 2003: N6-substituted D-4′-thioadenosine-5′-methyluronamides: Potent and selective agonists at the human A3 adenosine receptor. *Journal of Medicinal Chemistry*.

Ji, X.; Kim, Y. C.; Ahern, D. G.; Linden, J.; Jacobson, K. A., 2001: [3H]MRS 1754, a selective antagonist radioligand for A(2B) adenosine receptors. *Biochemical pharmacology*., **61**, 657–663.

Ji, X. D.; Gallo-Rodriguez, C.; Jacobson, K. A., 1994: A selective agonist affinity label for A3 adenosine receptors. *Biochemical and biophysical research communications*., **203**, 570–576.

Jickling, G. C.; Sharp, F. R., 2011: Blood Biomarkers of Ischemic Stroke. *Neurotherapeutics*., **8**, 349–360.

Jones, P. A.; Smith, R. A.; Stone, T. W., 1998: Protection against kainate-induced excitotoxicity by adenosine A2A receptor agonists and antagonists. *Neuroscience*., **85**, 229–237.

Jung, C. G.; Kim, H. J.; Miron, V. E.; Cook, S.; Kennedy, T. E.; Foster, C. A.; Antel, J. P.; Soliven, B., 2007: Functional consequences of S1P receptor modulation in rat oligodendroglial lineage cells. *Glia*., **55**, 1656–1667.

Káradóttir, R.; Hamilton, N. B.; Bakiri, Y.; Attwell, D., 2008: Spiking and nonspiking classes of oligodendrocyte precursor glia in CNS white matter. *Nature neuroscience*., **11**, 450–456.

Kataoka, H.; Sugahara, K.; Shimano, K.; Teshima, K.; Koyama, M.; Fukunari, A.; Chiba, K., 2005: FTY720, sphingosine 1-phosphate receptor modulator, ameliorates experimental autoimmune encephalomyelitis by inhibition of T cell infiltration. *Cellular & molecular immunology*., **2**, 439–448.

Katsura, K. I.; Kurihara, J.; Siesjö, B. K.; Wieloch, T., 1999: Acidosis enhances translocation of protein kinase C but not Ca2+/calmodulin-dependent protein kinase II to cell membranes during complete cerebral ischemia. *Brain Research*., **849**, 119–127.

Katsura, K. ichiro; Kristián, T.; Siesjö, B. K., 1994: Energy metabolism, ion homeostasis, and cell damage in the brain. *Biochemical Society Transactions*., **22**.

Katzman, R.; Clasen, R.; Klatzo, I.; Meyer, J. S.; Pappius, H. M.; Waltz, A. G., 1977: Report of Joint Committee for Stroke Resources. IV. Brain edema in stroke. *Stroke*., **8**, 512–540.

Kerr, J. F. R.; Wyllie, A. H.; Currie, A. R., 1972: Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. *British Journal of Cancer*., **26**, 239–257.

Kessey, K.; Trommer, B. L.; Overstreet, L. S.; Ji, T.; Mogul, D. J., 1997: A role for adenosine A2 receptors in the induction of long-term potentiation in the CA1 region of rat hippocampus. *Brain research*., **756**, 184–190.

Kim, H. J.; Miron, V. E.; Dukala, D.; Proia, R. L.; Ludwin, S. K.; Traka, M.; Antel, J. P.; Soliven, B., 2011: Neurobiological effects of sphingosine 1-phosphate receptor modulation in the cuprizone model. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*., **25**, 1509–1518.

Kim, M.; Spelta, V.; Sim, J.; North, R. A.; Surprenant, A., 2001: Differential assembly of rat purinergic P2X7 receptor in immune cells of the brain and periphery. *The Journal of biological chemistry*., **276**, 23262–23267.

Kim, Y. C.; Ji, X.; Melman, N.; Linden, J.; Jacobson, K. A., 2000: Anilide derivatives of an 8-phenylxanthine carboxylic congener are highly potent and selective antagonists at human A(2B) adenosine receptors. *Journal of medicinal chemistry*., **43**, 1165–1172.

Kimura, A.; Ohmori, T.; Ohkawa, R.; Madoiwa, S.; Mimuro, J.; Murakami, T.; Kobayashi, E.; Hoshino, Y.; Yatomi, Y.; Sakata, Y., 2007: Essential roles of sphingosine 1-phosphate/S1P1 receptor axis in the migration of neural stem cells toward a site of spinal cord injury. *Stem cells (Dayton, Ohio)*., **25**, 115–124.

Kitagawa, H.; Mori, A.; Shimada, J.; Mitsumoto, Y.; Kikuchi, T., 2002: Intracerebral adenosine infusion improves neurological outcome after transient focal ischemia in rats. *Neurological research*., **24**, 317–323.

Kitakaze, M.; Minamino, T.; Node, K.; Komamura, K.; Hori, M., 1996: Activation of ecto-5'-nucleotidase and cardioprotection by ischemic preconditioning. *Basic research in cardiology*., **91**, 23–26.

Klatzo, I., 1985: Brain oedema following brain ischaemia and the influence of therapy. *British Journal of Anaesthesia*., **57**, 18–22.

Knutson, P.; Ghiani, C. A.; Zhou, J. M.; Gallo, V.; McBain, C. J., 1997: K+ channel expression and cell proliferation are regulated by intracellular sodium and membrane depolarization in oligodendrocyte progenitor cells. *The Journal of neuroscience : the official journal of the Society for Neuroscience*., **17**, 2669–2682.

Koeppen, M.; Eckle, T.; Eltzschig, H. K., 2011: Interplay of hypoxia and A2B adenosine receptors in tissue protection. *Advances in pharmacology (San Diego, Calif.)*., **61**, 145–186.

Koistinaho, M.; Koistinaho, J., 2002: Role of p38 and p44/42 mitogen-activated protein kinases in microglia. *Glia*., **40**, 175–183.

König, B. F. R.; KLIPPEL, R. A.; ZEMAN, W., 1964: The Rat Brain. A Stereotaxic Atlas. *Journal of Neuropathology and Experimental Neurology*.

König JFR, K. R., 1967: *The rat atlas: A stereotaxic atlas of the forebrain and lower parts of the brain stem*. (Williams and Wilkins, Ed.). Baltimore.

Kono, M.; Allende, M. L.; Proia, R. L., 2008, September: Sphingosine-1-phosphate regulation of mammalian development. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*.

Konrad, F. M.; Witte, E.; Vollmer, I.; Stark, S.; Reutershan, J., 2012: Adenosine receptor A2b on hematopoietic cells mediates LPS-induced migration of PMNs into the lung interstitium. *American journal of physiology. Lung cellular and molecular physiology*., **303**, L425-38.

Koscsó, B.; Csóka, B.; Selmeczy, Z.; Himer, L.; Pacher, P.; Virág, L.; Haskó, G., 2012: Adenosine augments IL-10 production by microglial cells through an A2B adenosine receptor-mediated process. *Journal of immunology (Baltimore, Md. : 1950)*., **188**, 445–453.

Kostopoulos, G. K.; Limacher, J. J.; Phillis, J. W., 1975: Action of various adenine derivatives on cerebellar Purkinje cells. *Brain research*., **88**, 162–165.

Kreutzberg, G. W.; Barron, K. D., 1978: 5'-Nucleotidase of microglial cells in the facial nucleus during axonal reaction. *Journal of neurocytology*., **7**, 601–610.

Kristián, T.; Siesjö, B. K., 1996: Chapter 2 Changes in Ionic Fluxes During Cerebral Ischaemia, pp. 27–45.

Kriz, J., 2006: Inflammation in Ischemic Brain Injury: Timing Is Important. *Critical Reviews™ in Neurobiology*., **18**, 145–157.

Kulesskaya, N.; Võikar, V.; Peltola, M.; Yegutkin, G. G.; Salmi, M.; Jalkanen, S.; Rauvala, H., 2013: CD73 Is a Major Regulator of Adenosinergic Signalling in Mouse Brain. (Eckle, T., Ed.)*PLoS ONE*., **8**, e66896.

Kumar, A.; Aakriti; Gupta, V., 2016: A review on animal models of stroke: An update. *Brain Research Bulletin*., **122**, 35–44.

Kummer, J. L.; Rao, P. K.; Heidenreich, K. A., 1997: Apoptosis induced by withdrawal of trophic factors is mediated by p38 mitogen-activated protein kinase. *Journal of Biological Chemistry*., **272**, 20490–20494.

Kuno, A.; Critz, S. D.; Cui, L.; Solodushko, V.; Yang, X. M.; Krahn, T.; Albrecht, B.; Philipp, S.; Cohen, M. V.; Downey, J. M., 2007: Protein kinase C protects preconditioned rabbit hearts by increasing sensitivity of adenosine A2b-dependent signaling during early reperfusion. *Journal of Molecular and Cellular Cardiology*., **43**, 262–271.

Kuroda, Y.; Saito, M.; Kobayashi, K., 1976: Concomitant changes in cyclic AMP level and postsynaptic potentials of olfactory cortex slices induced by adenosine derivatives. *Brain research*., **109**, 196–201.

Lakhan, S. E.; Kirchgessner, A.; Tepper, D.; Leonard, A., 2013: Matrix metalloproteinases and blood-brain barrier disruption in acute ischemic stroke. *Frontiers in Neurology*., **4 APR**.

Lana, D.; Melani, A.; Pugliese, A. M.; Cipriani, S.; Nosi, D.; Pedata, F.; Giovannini, M. G., 2014: The neuron-astrocyte-microglia triad in a rat model of chronic cerebral hypoperfusion: Protective effect of dipyridamole. *Frontiers in Aging Neuroscience*., **6**.

Lappas, C. M.; Day, Y. J.; Marshall, M. A.; Engelhard, V. H.; Linden, J., 2006: Adenosine A2A receptor activation reduces hepatic ischemia reperfusion injury by inhibiting CD1d-dependent NKT cell activation. *The Journal of experimental medicine*., **203**, 2639–2648.

Latini, S.; Pazzagli, M.; Pepeu, G.; Pedata, F., 1996a: A2 adenosine receptors: Their presence and neuromodulatory role in the central nervous system. *General Pharmacology*.

Latini, S.; Corsi, C.; Pedata, F.; Pepeu, G., 1996b: The source of brain adenosine outflow during ischemia and electrical stimulation. *Neurochemistry international*., **28**, 113–118.

Latini, S.; Pazzagli, M.; Pepeu, G.; Pedata, F., 1996c: A2 adenosine receptors: their presence and neuromodulatory role in the central nervous system. *General pharmacology*., **27**, 925–933.

Latini, S.; Bordoni, F.; Pedata, F.; Corradetti, R., 1999: Extracellular adenosine concentrations during *in vitro* ischaemia in rat hippocampal slices. *British Journal of Pharmacology*., **127**, 729–739.

Latini, S.; Pedata, F., 2001a: Adenosine in the central nervous system: release mechanisms and extracellular concentrations. *Journal of neurochemistry*., **79**, 463–484.

Latini, S.; Pedata, F., 2001b: Adenosine in the central nervous system: Release mechanisms and extracellular concentrations. *Journal of Neurochemistry*.

Le Stunff, H.; Galve-Roperh, I.; Peterson, C.; Milstien, S.; Spiegel, S., 2002: Sphingosine-1-phosphate phosphohydrolase in regulation of sphingolipid metabolism and apoptosis. *The Journal of cell biology*., **158**, 1039–1049.

Lebon, G.; Warne, T.; Edwards, P. C.; Bennett, K.; Langmead, C. J.; Leslie, A. G. W.; Tate, C. G., 2011: Agonist-bound adenosine A2A receptor structures reveal common features of

GPCR activation. *Nature*., **474**, 521–525.

Lecca, D.; Ceruti, S.; Fumagalli, M.; Abbracchio, M. P., 2012: Purinergic trophic signalling in glial cells: Functional effects and modulation of cell proliferation, differentiation, and death. *Purinergic Signalling*., **8**, 539–557.

Ledoux, S.; Runembert, I.; Koumanov, K.; Michel, J. B.; Trugnan, G.; Friedlander, G., 2003: Hypoxia Enhances Ecto-5′-Nucleotidase Activity and Cell Surface Expression in Endothelial Cells. *Circulation Research*., **92**, 848–855.

Lee, Y. C.; Chien, C. L.; Sun, C. N.; Huang, C. L.; Huang, N. K.; Chiang, M. C.; Lai, H. L.; Lin, Y. S.; Chou, S. Y.; Wang, C. K. L.; Tai, M. H.; Liao, W. L.; Lin, T. N.; Liu, F. C.; Chern, Y., 2003: Characterization of the rat A2A adenosine receptor gene: a 4.8-kb promoter-proximal DNA fragment confers selective expression in the central nervous system. *The European journal of neuroscience*., **18**, 1786–1796.

Lennmyr, F.; Karlsson, S.; Gerwins, P.; Ata, K. A.; Terént, A., 2002: Activation of mitogen-activated protein kinases in experimental cerebral ischemia. *Acta Neurologica Scandinavica*., **106**, 333–340.

Levi, G.; Gallo, V.; Ciotti, M. T., 1986: Bipotential precursors of putative fibrous astrocytes and oligodendrocytes in rat cerebellar cultures express distinct surface features and 'neuron-like' γ-aminobutyric acid transport. *Proceedings of the National Academy of Sciences of the United States of America*., **83**, 1504–1508.

Levine, J. M.; Reynolds, R., 1999: Activation and proliferation of endogenous oligodendrocyte precursor cells during ethidium bromide-induced demyelination. *Experimental Neurology*.

Levine, S. R., 2004: Pathophysiology and therapeutic targets for ischemic stroke. *Clinical Cardiology*., **27**, 12–24.

Li, Q.; Han, X.; Lan, X.; Hong, X.; Li, Q.; Gao, Y.; Luo, T.; Yang, Q.; Koehler, R. C.; Zhai, Y.; Zhou, J.; Wang, J., 2017: Inhibition of tPA-induced hemorrhagic transformation involves adenosine A2b receptor activation after cerebral ischemia. *Neurobiology of disease*., **108**, 173–182.

Li, Y.; Oskouian, R. J.; Day, Y. J.; Rieger, J. M.; Liu, L.; Kern, J. A.; Linden, J., 2006: Mouse spinal cord compression injury is reduced by either activation of the adenosine A2A receptor on bone marrow-derived cells or deletion of the A2A receptor on non-bone marrow-derived cells. *Neuroscience*., **141**, 2029–2039.

Ligon, K. L.; Kesari, S.; Kitada, M.; Sun, T.; Arnett, H. A.; Alberta, J. A.; Anderson, D. J.; Stiles, C. D.; Rowitch, D. H., 2006: Development of NG2 neural progenitor cells requires Olig gene function. *Proceedings of the National Academy of Sciences of the United States of America*., **103**, 7853–7858.

Linden, J.; Taylor, H. E.; Robeva, A. S.; Tucker, A. L.; Stehle, J. H.; Rivkees, S. A.; Fink, J. S.; Reppert, S. M., 1993: Molecular cloning and functional expression of a sheep A3 adenosine receptor with widespread tissue distribution. *Molecular pharmacology*., **44**, 524–532.

Linden, J., 1994: Cloned adenosine A3 receptors: pharmacological properties, species differences and receptor functions. *Trends in pharmacological sciences*., **15**, 298–306.

Linden, J., 2005: Adenosine in tissue protection and tissue regeneration. *Molecular*

pharmacology., **67**, 1385–1387.

Loddick, S. A.; Turnbull, A. V.; Rothwell, N. J., 1998: Cerebral Interleukin-6 is Neuroprotective during Permanent Focal Cerebral Ischemia in the Rat. *Journal of Cerebral Blood Flow & Metabolism*., **18**, 176–179.

Lomelino, C.; McKenna, R., 2016: Carbonic anhydrase inhibitors: a review on the progress of patent literature (2011–2016). *Expert Opinion on Therapeutic Patents*., **26**, 947–956.

Lopes, L.; Rebola, N.; Pinheiro, P.; Richardson, P.; Oliveira, C.; Cunha, R., 2003: Adenosine A3 receptors are located in neurons of the rat hippocampus. *Neuroreport*., **14**, 1645–1648.

Lu, X. C. M.; Williams, A. J.; Wagstaff, J. D.; Tortella, F. C.; Hartings, J. A., 2005: Effects of delayed intrathecal infusion of an NMDA receptor antagonist on ischemic injury and peri-infarct depolarizations. *Brain Research*., **1056**, 200–208.

Lucas, S. M.; Rothwell, N. J.; Gibson, R. M., 2006: The role of inflammation in CNS injury and disease. *British journal of pharmacology*., **147 Suppl 1**, S232-40.

Maceyka, M.; Milstien, S.; Spiegel, S., 2009, April: Sphingosine-1-phosphate: The Swiss army knife of sphingolipid signaling. *Journal of Lipid Research*.

Macrez, R.; Ali, C.; Toutirais, O.; Le Mauff, B.; Defer, G.; Dirnagl, U.; Vivien, D., 2011: Stroke and the immune system: from pathophysiology to new therapeutic strategies. *The Lancet Neurology*., **10**, 471–480.

Maeda, Y.; Matsumoto, M.; Hori, O.; Kuwabara, K.; Ogawa, S.; Yan, S. D.; Ohtsuki, T.; Kinoshita, T.; Kamada, T.; Stern, D. M., 1994: Hypoxia/reoxygenation-mediated induction of astrocyte interleukin 6: a paracrine mechanism potentially enhancing neuron survival. *The Journal of experimental medicine*., **180**, 2297–2308.

Majno, G.; Joris, I., 1995: Apoptosis, oncosis, and necrosis: An overview of cell death. *American Journal of Pathology*.

Maki, T.; Gottschalk, R.; Ogawa, N.; Monaco, A. P., 2005: Prevention and cure of autoimmune diabetes in nonobese diabetic mice by continuous administration of FTY720. *Transplantation*., **79**, 1051–1055.

Mandala, S.; Hajdu, R.; Bergstrom, J.; Quackenbush, E.; Xie, J.; Milligan, J.; Thornton, R.; Shei, G. J.; Card, D.; Keohane, C.; Rosenbach, M.; Hale, J.; Lynch, C. L.; Rupprecht, K.; Parsons, W.; Rosen, H., 2002: Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. *Science (New York, N.Y.)*., **296**, 346–349.

Mandala, S. M.; Thornton, R.; Galve-Roperh, I.; Poulton, S.; Peterson, C.; Olivera, A.; Bergstrom, J.; Kurtz, M. B.; Spiegel, S., 2000: Molecular cloning and characterization of a lipid phosphohydrolase that degrades sphingosine-1- phosphate and induces cell death. *Proceedings of the National Academy of Sciences of the United States of America*., **97**, 7859–7864.

Margheri, F.; Ceruso, M.; Carta, F.; Laurenzana, A.; Maggi, L.; Lazzeri, S.; Simonini, G.; Annunziato, F.; Del Rosso, M.; Supuran, C. T.; Cimaz, R., 2016: Overexpression of the transmembrane carbonic anhydrase isoforms IX and XII in the inflamed synovium. *Journal of enzyme inhibition and medicinal chemistry*., **31**, 60–63.

Markus, H. S., 2004: Cerebral perfusion and stroke. *Journal of neurology, neurosurgery, and psychiatry*., **75**, 353–361.

Martin, R. L.; Lloyd, H. G.; Cowan, A. I., 1994: The early events of oxygen and glucose deprivation: setting the scene for neuronal death? *Trends in neurosciences*., **17**, 251–257.

Masini, E.; Carta, F.; Scozzafava, A.; Supuran, C. T., 2013a: Antiglaucoma carbonic anhydrase inhibitors: A patent review. *Expert Opinion on Therapeutic Patents*.

Masini, E.; Carta, F.; Scozzafava, A.; Supuran, C. T., 2013b: Antiglaucoma carbonic anhydrase inhibitors: a patent review. *Expert opinion on therapeutic patents*., **23**, 705–716.

Matloubian, M.; Lo, C. G.; Cinamon, G.; Lesneski, M. J.; Xu, Y.; Brinkmann, V.; Allende, M. L.; Proia, R. L.; Cyster, J. G., 2004: Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. *Nature*., **427**, 355–360.

Matsumoto, K.; Graf, R.; Rosner, G.; Shimada, N.; Heiss, W. D., 1992: Flow thresholds for extracellular purine catabolite elevation in cat focal ischemia. *Brain research*., **579**, 309–314.

Mazzon, E.; Esposito, E.; Impellizzeri, D.; DI Paola, R.; Melani, A.; Bramanti, P.; Pedata, F.; Cuzzocrea, S., 2011: CGS 21680, an agonist of the adenosine (A2A) receptor, reduces progression of murine type II collagen-induced arthritis. *The Journal of rheumatology*., **38**, 2119–2129.

McGiffert, C.; Contos, J. J. A.; Friedman, B.; Chun, J., 2002: Embryonic brain expression analysis of lysophospholipid receptor genes suggests roles for s1p1 in neurogenesis and s1p1-3 in angiogenesis. *FEBS Letters*., **531**, 103–108.

Medina-Pulido, L.; Molina-Arcas, M.; Justicia, C.; Soriano, E.; Burgaya, F.; Planas, A. M.; Pastor-Anglada, M., 2013: Hypoxia and P1 receptor activation regulate the high-affinity concentrative adenosine transporter CNT2 in differentiated neuronal PC12 cells. *The Biochemical journal*., **454**, 437–445.

Meghji, P., 1993: Storage, release, uptake, and inactivation of purines. *Drug Development Research*., **28**, 214–219.

Meibom, D.; Albrecht-Küpper, B.; Diedrichs, N.; Hübsch, W.; Kast, R.; Krämer, T.; Krenz, U.; Lerchen, H. G.; Mittendorf, J.; Nell, P. G.; Süssmeier, F.; Vakalopoulos, A.; Zimmermann, K., 2017: Neladenoson Bialanate Hydrochloride: A Prodrug of a Partial Adenosine A1 Receptor Agonist for the Chronic Treatment of Heart Diseases. *ChemMedChem*., **12**, 728–737.

Melani, A.; Pantoni, L.; Corsi, C.; Bianchi, L.; Monopoli, A.; Bertorelli, R.; Pepeu, G.; Pedata, F., 1999a: Striatal outflow of adenosine, excitatory amino acids, gamma-aminobutyric acid, and taurine in awake freely moving rats after middle cerebral artery occlusion: correlations with neurological deficit and histopathological damage. *Stroke*., **30**, 2448–2454; discussion 2455.

Melani, A.; Pantoni, L.; Corsi, C.; Bianchi, L.; Monopoli, A.; Bertorelli, R.; Pepeu, G.; Pedata, F., 1999b: Striatal outflow of adenosine, excitatory amino acids, γ-aminobutyric acid, and taurine in awake freely moving rats after middle cerebral artery occlusion: Correlations with neurological deficit and histopathological damage. *Stroke*.

Melani, A.; Gianfriddo, M.; Vannucchi, M. G.; Cipriani, S.; Baraldi, P. G.; Giovannini, M. G.; Pedata, F., 2006a: The selective A2A receptor antagonist SCH 58261 protects from neurological deficit, brain damage and activation of p38 MAPK in rat focal cerebral ischemia. *Brain Research*., **1073**–**1074**, 470–480.

Melani, A.; Gianfriddo, M.; Vannucchi, M. G.; Cipriani, S.; Baraldi, P. G.; Giovannini, M.

G.; Pedata, F., 2006b: The selective A2A receptor antagonist SCH 58261 protects from neurological deficit, brain damage and activation of p38 MAPK in rat focal cerebral ischemia. *Brain Research*.

Melani, A.; Cipriani, S.; Vannucchi, M. G.; Nosi, D.; Donati, C.; Bruni, P.; Giovannini, M. G.; Pedata, F., 2009: Selective adenosine A2a receptor antagonism reduces JNK activation in oligodendrocytes after cerebral ischaemia. *Brain*.

Melani, A.; Corti, F.; Stephan, H.; Müller, C. E.; Donati, C.; Bruni, P.; Vannucchi, M. G.; Pedata, F., 2012a: Ecto-ATPase inhibition: ATP and adenosine release under physiological and ischemic in vivo conditions in the rat striatum. *Experimental Neurology*., **233**, 193–204.

Melani, A.; Corti, F.; Stephan, H.; Müller, C. E.; Donati, C.; Bruni, P.; Vannucchi, M. G.; Pedata, F., 2012b: Ecto-ATPase inhibition: ATP and adenosine release under physiological and ischemic in vivo conditions in the rat striatum. *Experimental Neurology*.

Melani, A.; Corti, F.; Cellai, L.; Giuliana Vannucchi, M.; Pedata, F., 2014: Low doses of the selective adenosine A2A receptor agonist CGS21680 are protective in a rat model of transient cerebral ischemia. *Brain Research*., **1551**, 59–72.

Menn, B.; Garcia-Verdugo, J. M.; Yaschine, C.; Gonzalez-Perez, O.; Rowitch, D.; Alvarez-Buylla, A., 2006: Origin of oligodendrocytes in the subventricular zone of the adult brain. *Journal of Neuroscience*., **26**, 7907–7918.

Mercatelli, R.; Lana, D.; Bucciantini, M.; Giovannini, M. G.; Cerbai, F.; Quercioli, F.; Zecchi-Orlandini, S.; Delfino, G.; Wenk, G. L.; Nosi, D., 2016: Clasmatodendrosis and b-amyloidosis in aging hippocampus. *FASEB Journal*., **30**, 1480–1491.

Merighi, S.; Borea, P. A.; Stefanelli, A.; Bencivenni, S.; Castillo, C. A.; Varani, K.; Gessi, S., 2015: A2a and a2b adenosine receptors affect HIF-1α signaling in activated primary microglial cells. *Glia*., **63**, 1933–1952.

Meyerhof, W.; Müller-Brechlin, R.; Richter, D., 1991: Molecular cloning of a novel putative G-protein coupled receptor expressed during rat spermiogenesis. *FEBS letters*., **284**, 155–160.

Michaelis, M. L.; Michaelis, E. K.; Myers, S. L., 1979: Adenosine modulation of synaptosomal dopamine release. *Life sciences*., **24**, 2083–2092.

Mies, G.; Iijima, T.; Hossmann, K. A., 1993: Correlation between peri-infarct dc shifts and ischaemic neuronal damage in rat. *NeuroReport*.

Mincione, F.; Scozzafava, A.; Supuran, C., 2007: The Development of Topically Acting Carbonic Anhydrase Inhibitors as Antiglaucoma Agents. *Current Topics in Medicinal Chemistry*., **7**, 849–854.

Mirabet, M.; Mallol, J.; Lluis, C.; Franco, R., 1997: Calcium mobilization in Jurkat cells via A_{2b} adenosine receptors. *British Journal of Pharmacology*., **122**, 1075–1082.

Miron, V. E.; Jung, C. G.; Kim, H. J.; Kennedy, T. E.; Soliven, B.; Antel, J. P., 2008a: FTY720 modulates human oligodendrocyte progenitor process extension and survival. *Annals of neurology*., **63**, 61–71.

Miron, V. E.; Schubart, A.; Antel, J. P., 2008b: Central nervous system-directed effects of FTY720 (fingolimod). *Journal of the neurological sciences*., **274**, 13–17.

Mishiro, K.; Ishiguro, M.; Suzuki, Y.; Tsuruma, K.; Shimazawa, M.; Hara, H., 2012: A broad-spectrum matrix metalloproteinase inhibitor prevents hemorrhagic complications

induced by tissue plasminogen activator in mice. *Neuroscience*., **205**, 39–48.

Mitra, P.; Oskeritzian, C. A.; Payne, S. G.; Beaven, M. A.; Milstien, S.; Spiegel, S., 2006: Role of ABCC1 in export of sphingosine-1-phosphate from mast cells. *Proceedings of the National Academy of Sciences of the United States of America*., **103**, 16394–16399.

Mizugishi, K.; Yamashita, T.; Olivera, A.; Miller, G. F.; Spiegel, S.; Proia, R. L., 2005: Essential role for sphingosine kinases in neural and vascular development. *Molecular and cellular biology*., **25**, 11113–11121.

Mizushima, T.; Ito, T.; Kishi, D.; Kai, Y.; Tamagawa, H.; Nezu, R.; Kiyono, H.; Matsuda, H., 2004: Therapeutic effects of a new lymphocyte homing reagent FTY720 in interleukin-10 gene-deficient mice with colitis. *Inflammatory Bowel Diseases*., **10**, 182–192.

Mogul, D. J.; Adams, M. E.; Fox, A. P., 1993: Differential activation of adenosine receptors decreases N-type but potentiates P-type Ca2+ current in hippocampal CA3 neurons. *Neuron*., **10**, 327–334.

Montaner, J.; Alvarez-Sabín, J.; Molina, C.; Anglés, A.; Abilleira, S.; Arenillas, J.; González, M. A.; Monasterio, J., 2001: Matrix metalloproteinase expression after human cardioembolic stroke: temporal profile and relation to neurological impairment. *Stroke*., **32**, 1759–1766.

Moreau, J. L.; Huber, G., 1999: Central adenosine A(2A) receptors: an overview. *Brain research. Brain research reviews*., **31**, 65–82.

Müller, C. E.; Jacobson, K. A., 2011: Recent developments in adenosine receptor ligands and their potential as novel drugs. *Biochimica et Biophysica Acta (BBA) - Biomembranes*., **1808**, 1290–1308.

Mullershausen, F.; Craveiro, L. M.; Shin, Y.; Cortes-Cros, M.; Bassilana, F.; Osinde, M.; Wishart, W. L.; Guerini, D.; Thallmair, M.; Schwab, M. E.; Sivasankaran, R.; Seuwen, K.; Dev ¶, K. K., 2007: Phosphorylated FTY720 promotes astrocyte migration through sphingosine-1-phosphate receptors. *J. Neurochem.*, **102**, 1151–1161.

Murray, C. J. L.; Vos, T.; Lozano, R.; Naghavi, M.; Flaxman, A. D.; Michaud, C.; Ezzati, M.; Shibuya, K.; Salomon, J. a.; Abdalla, S.; Aboyans, V.; Abraham, J.; Ackerman, I.; Aggarwal, R.; Ahn, S. Y.; Ali, M. K.; Alvarado, M.; Anderson, H. R.; Anderson, L. M. et al., 2012: Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: A systematic1. Murray CJL, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 . *The Lancet*., **380**, 2197–2223.

Nadareishvili, Z. G.; Li, H.; Wright, V.; Maric, D.; Warach, S.; Hallenbeck, J. M.; Dambrosia, J.; Barker, J. L.; Baird, A. E., 2004: Elevated pro-inflammatory CD4+CD28- lymphocytes and stroke recurrence and death. *Neurology*., **63**, 1446–1451.

Naito, Y.; Lowenstein, J. M., 1981: 5'-Nucleotidase from rat heart. *Biochemistry*., **20**, 5188–5194.

Neri, D.; Supuran, C. T., 2011: Interfering with pH regulation in tumours as a therapeutic strategy. *Nature Reviews Drug Discovery*., **10**, 767–777.

Nijnik, A.; Clare, S.; Hale, C.; Chen, J.; Raisen, C.; Mottram, L.; Lucas, M.; Estabel, J.; Ryder, E.; Adissu, H.; Adams, N. C.; Ramirez-Solis, R.; White, J. K.; Steel, K. P.; Dougan, G.; Hancock, R. E. W., 2012: The Role of Sphingosine-1-Phosphate Transporter Spns2 in Immune System Function . *The Journal of Immunology*., **189**, 102–111.

Nishiyama, A.; Lin, X. H.; Giese, N.; Heldin, C. H.; Stallcup, W. B., 1996: Interaction between NG2 proteoglycan and PDGF alpha-receptor on O2A progenitor cells is required for optimal response to PDGF. *Journal of neuroscience research*., **43**, 315–330.

Nishiyama, A.; Chang, A.; Trapp, B. D., 1999: NG2+ glial cells: a novel glial cell population in the adult brain. *Journal of neuropathology and experimental neurology*., **58**, 1113–1124.

Nishiyama, A.; Watanabe, M.; Yang, Z.; Bu, J., 2002: Identity, distribution, and development of polydendrocytes: NG2-expressing glial cells. *Journal of Neurocytology*.

Nonaka, H.; Ichimura, M.; Takeda, M.; Nonaka, Y.; Shimada, J.; Suzuki, F.; Yamaguchi, K.; Kase, H., 1994: KF17837 ((E)-8-(3,4-dimethoxystyryl)-1,3-dipropyl-7-methylxanthine), a potent and selective adenosine A2 receptor antagonist. *European journal of pharmacology*., **267**, 335–341.

Noshita, N.; Sugawara, T.; Fujimura, M.; Morita-Fujimura, Y.; Chan, P. H., 2001: Manganese Superoxide Dismutase Affects Cytochrome c Release and Caspase-9 Activation After Transient Focal Cerebral Ischemia in Mice. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*., **21**, 557–567.

Novgorodov, A. S.; El-Alwani, M.; Bielawski, J.; Obeid, L. M.; Gudz, T. I., 2007: Activation of sphingosine-1-phosphate receptor S1P5 inhibits oligodendrocyte progenitor migration. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*., **21**, 1503–1514.

O'Sullivan, S.; Dev, K. K., 2017, February: Sphingosine-1-phosphate receptor therapies: Advances in clinical trials for CNS-related diseases. *Neuropharmacology*.

Obara, M.; Szeliga, M.; Albrecht, J., 2008: Regulation of pH in the mammalian central nervous system under normal and pathological conditions: facts and hypotheses. *Neurochemistry international*., **52**, 905–919.

Odashima, M.; Bamias, G.; Rivera-Nieves, J.; Linden, J.; Nast, C. C.; Moskaluk, C. A.; Marini, M.; Sugawara, K.; Kozaiwa, K.; Otaka, M.; Watanabe, S.; Cominelli, F., 2005: Activation of A2A adenosine receptor attenuates intestinal inflammation in animal models of inflammatory bowel disease. *Gastroenterology*., **129**, 26–33.

Ohta, K.; Graf, R.; Rosner, G.; Heiss, W. D., 2001: Calcium ion transients in peri-infarct depolarizations may deteriorate ion homeostasis and expand infarction in focal cerebral ischemia in cats. *Stroke*.

Okasa, Y.; Ozawa, S., 1980: Inhibitory action of adenosine on synaptic transmission in the hippocampus of the guinea pig in vitro. *European Journal of Pharmacology*., **68**, 483–492.

Ong, W. Y.; Levine, J. M., 1999: A light and electron microscopic study of NG2 chondroitin sulfate proteoglycan-positive oligodendrocyte precursor cells in the normal and kainate-lesioned rat hippocampus. *Neuroscience*., **92**, 83–95.

Ongini, E.; Adami, M.; Ferri, C.; Bertorelli, R., 1997: Adenosine A2A receptors and neuroprotection. *Annals of the New York Academy of Sciences*., **825**, 30–48.

Osinde, M.; Mullershausen, F.; Dev, K. K., 2007: Phosphorylated FTY720 stimulates ERK phosphorylation in astrocytes via S1P receptors. *Neuropharmacology*., **52**, 1210–1218.

Othman, T.; Yan, H.; Rivkees, S. A., 2003: Oligodendrocytes express functional A1

adenosine receptors that stimulate cellular migration. *Glia*., **44**, 166–172.

Pak, M. A.; Haas, H. L.; Decking, U. K.; Schrader, J., 1994: Inhibition of adenosine kinase increases endogenous adenosine and depresses neuronal activity in hippocampal slices. *Neuropharmacology*., **33**, 1049–1053.

Patel, A.; Craig, R. H.; Daluge, S. M.; Linden, J., 1988: 125I-BW-A844U, an antagonist radioligand with high affinity and selectivity for adenosine A1 receptors, and 125I-azido-BW-A844U, a photoaffinity label. *Molecular Pharmacology*.

Paterniti, I.; Melani, A.; Cipriani, S.; Corti, F.; Mello, T.; Mazzon, E.; Esposito, E.; Bramanti, P.; Cuzzocrea, S.; Pedata, F., 2011: Selective adenosine A2Areceptor agonists and antagonists protect against spinal cord injury through peripheral and central effects. *Journal of Neuroinflammation*.

Pazzagli, M.; Pedata, F.; Pepeu, G., 1993: Effect of K+ depolarization, tetrodotoxin, and NMDA receptor inhibition on extracellular adenosine levels in rat striatum. *European journal of pharmacology*., **234**, 61–65.

Peakman, M. -C; Hill, S. J., 1994: Adenosine A2B-receptor-mediated cyclic AMP accumulation in primary rat astrocytes. *British Journal of Pharmacology*.

Pedata, F.; Pepeu, G.; Spignoli, G., 1984: Biphasic effect of methylxanthines on acetylcholine release from electrically-stimulated brain slices. *British journal of pharmacology*., **83**, 69–73.

Pedata, F.; Pazzagli, M.; Tilli, S.; Pepeu, G., 1990: Regional differences in the electrically stimulated release of endogenous and radioactive adenosine and purine derivatives from rat brain slices. *Naunyn-Schmiedeberg's archives of pharmacology*., **342**, 447–453.

Pedata, F.; Latini, S.; Pugliese, A. M.; Pepeu, G., 1993: Investigations into the adenosine outflow from hippocampal slices evoked by ischemia-like conditions. *Journal of neurochemistry*., **61**, 284–289.

Pedata, F.; Corsi, C.; Melani, A.; Bordoni, F.; Latini, S., 2001: Adenosine extracellular brain concentrations and role of A2A receptors in ischemia. *Annals of the New York Academy of Sciences*., **939**, 74–84.

Pedata F.; Pugliese A.M.; Coppi E.; Popoli P.; Morelli M.; Schwarzschild M.A.; Melani A., 2007: Adenosine in the Central Nervous System: Effects on Neurotransmission and Neuroprotection. *Immunology, Endocrine & Metabolic Agents in Medicinal Chemistry*., **7**, 304–321.

Pedata, F.; Pugliese, A. M.; Sebastião, A. M.; Ribeiro, J. A., 2010: Adenosine A3 receptor signaling in the central nervous system. *A3 Adenosine Receptors from Cell Biology to Pharmacology and Therapeutics*. Springer Netherlands, pp. 165–188.

Pedata, F.; Pugliese, A. M.; Coppi, E.; Dettori, I.; Maraula, G.; Cellai, L.; Melani, A., 2014: Adenosine A 2A Receptors Modulate Acute Injury and Neuroinflammation in Brain Ischemia. *Mediators of Inflammation*., **2014**, 1–16.

Pei, Z.; Pang, S. F.; Cheung, R. T. F., 2002: Pretreatment with melatonin reduces volume of cerebral infarction in a rat middle cerebral artery occlusion stroke model. *Journal of pineal research*., **32**, 168–172.

Pettersen, E. O.; Ebbesen, P.; Gieling, R. G.; Williams, K. J.; Dubois, L.; Lambin, P.; Ward, C.; Meehan, J.; Kunkler, I. H.; Langdon, S. P.; Ree, A. H.; Flatmark, K.; Lyng, H.; Calzada,

M. J.; Peso, L. Del; Landazuri, M. O.; Görlach, A.; Flamm, H.; Kieninger, J. et al., 2015: Targeting tumour hypoxia to prevent cancer metastasis. From biology, biosensing and technology to drug development: the METOXIA consortium. *Journal of enzyme inhibition and medicinal chemistry*., **30**, 689–721.

Phillips, E.; Newsholme, E. A., 1979: Maximum activities, properties and distribution of 5' nucleotidase, adenosine kinase and adenosine deaminase in rat and human brain. *Journal of neurochemistry*., **33**, 553–558.

Phillis, J. W.; Edstrom, J. P.; Kostopoulos, G. K.; Kirkpatrick, J. R., 1979: Effects of adenosine and adenine nucleotides on synaptic transmission in the cerebral cortex. *Canadian Journal of Physiology and Pharmacology*., **57**, 1289–1312.

Phillis, J. W.; O' regan, M. H.; Perkins, L. M., 1993: Effect of adenosine receptor agonists on spontaneous and potassium-evoked acetylcholine release from the in vivo rat cerebral cortex. *Brain Research*., **605**, 293–297.

Phillis, J. W.; Smith-Barbour, M.; O'Regan, M. H.; Perkins, L. M., 1994: Amino acid and purine release in rat brain following temporary middle cerebral artery occlusion. *Neurochemical research*., **19**, 1125–1130.

Phillis, J. W.; Smith-Barbour, M.; O'Regan, M. H., 1996: Changes in extracellular amino acid neurotransmitters and purines during and following ischemias of different durations in the rat cerebral cortex. *Neurochemistry international*., **29**, 115–120.

Phillis, J. W., 2004: Adenosine and adenine nucleotides as regulators of cerebral blood flow: roles of acidosis, cell swelling, and KATP channels. *Critical reviews in neurobiology*., **16**, 237–270.

Piao, C. S.; Kim, J. B.; Han, P. L.; Lee, J. K., 2003: Administration of the p38 MAPK inhibitor SB203580 affords brain protection with a wide therapeutic window against focal ischemic insult. *Journal of Neuroscience Research*., **73**, 537–544.

Pinard, M. A.; Boone, C. D.; Rife, B. D.; Supuran, C. T.; McKenna, R., 2013: Structural study of interaction between brinzolamide and dorzolamide inhibition of human carbonic anhydrases. *Bioorganic and Medicinal Chemistry*., **21**, 7210–7215.

Pitson, S. M.; Moretti, P. A. B.; Zebol, J. R.; Lynn, H. E.; Xia, P.; Vadas, M. A.; Wattenberg, B. W., 2003: Activation of sphingosine kinase 1 by ERK1/2-mediated phosphorylation. *The EMBO journal*., **22**, 5491–5500.

Pittaluga, A.; Segantini, D.; Feligioni, M.; Raiteri, M., 2005: Extracellular protons differentially potentiate the responses of native AMPA receptor subtypes regulating neurotransmitter release. *British Journal of Pharmacology*., **144**, 293–299.

Plum, F., 1983: What causes infarction in ischemic brain?: The Robert Wartenberg Lecture. *Neurology*., **33**, 222–233.

Popoli, P.; Betto, P.; Reggio, R.; Ricciarello, G., 1995: Adenosine A2A receptor stimulation enhances striatal extracellular glutamate levels in rats. *European journal of pharmacology*., **287**, 215–217.

Porter, N. A., 1984: Chemistry of lipid peroxidation. *Methods in enzymology*., **105**, 273–282.

Prince, D. A.; Stevens, C. F., 1992: Adenosine decreases neurotransmitter release at central synapses. *Proceedings of the National Academy of Sciences of the United States of America*.,

89, 8586–8590.

Pringle, N. P.; Mudhar, H. S.; Collarini, E. J.; Richardson, W. D., 1992: PDGF receptors in the rat CNS: during late neurogenesis, PDGF alpha-receptor expression appears to be restricted to glial cells of the oligodendrocyte lineage. *Development (Cambridge, England)*., **115**, 535–551.

Puffinbarger, N. K.; Hansen, K. R.; Resta, R.; Laurent, A. B.; Knudsen, T. B.; Madara, J. L.; Thompson, L. F., 1995: Production and characterization of multiple antigenic peptide antibodies to the adenosine A2b receptor. *Molecular pharmacology*., **47**, 1126–1132.

Pugliese, A. M.; Latini, S.; Corradetti, R.; Pedata, F., 2003: Brief, repeated, oxygen-glucose deprivation episodes protect neurotransmission from a longer ischemic episode in the in vitro hippocampus: role of adenosine receptors. *British journal of pharmacology*., **140**, 305–314.

Pugliese, A. M.; Coppi, E.; Spalluto, G.; Corradetti, R.; Pedata, F., 2006: A3 adenosine receptor antagonists delay irreversible synaptic failure caused by oxygen and glucose deprivation in the rat CA1 hippocampus in vitro. *British journal of pharmacology*., **147**, 524–532.

Pugliese, A. M.; Coppi, E.; Volpini, R.; Cristalli, G.; Corradetti, R.; Jeong, L. S.; Jacobson, K. A.; Pedata, F., 2007: Role of adenosine A3 receptors on CA1 hippocampal neurotransmission during oxygen-glucose deprivation episodes of different duration. *Biochemical Pharmacology*., **74**, 768–779.

Radi, R.; Beckman, J. S.; Bush, K. M.; Freeman, B. A., 1991: Peroxynitrite oxidation of sulfhydryls. The cytotoxic potential of superoxide and nitric oxide. *The Journal of biological chemistry*., **266**, 4244–4250.

Raff, M. C.; Miller, R. H.; Noble, M., (n.d.) A glial progenitor cell that develops in vitro into an astrocyte or an oligodendrocyte depending on culture medium. *Nature*., **303**, 390–396.

Rama, R.; Garcia Rodriguez, J. C., 2012: Excitotoxicity and Oxidative Stress in Acute Ischemic Stroke. *Acute Ischemic Stroke*. InTech.

Rao, T. S.; Lariosa-Willingham, K. D.; Lin, F. F.; Palfreyman, E. L.; Yu, N.; Chun, J.; Webb, M., 2003: Pharmacological characterization of lysophospholipid receptor signal transduction pathways in rat cerebrocortical astrocytes. *Brain research*., **990**, 182–194.

Rao, T. S.; Lariosa-Willingham, K. D.; Lin, F. F.; Yu, N.; Tham, C. S.; Chun, J.; Webb, M., 2004: Growth factor pre-treatment differentially regulates phosphoinositide turnover downstream of lysophospholipid receptor and metabotropic glutamate receptors in cultured rat cerebrocortical astrocytes. *Int. J. Devl Neuroscience*., **22**, 131–135.

Regli, F.; Yamaguhi, T.; Waltz, A. G., 1971: Responses of Surface Arteries and Blood Flow of Ischemic and Nonischemic Cerebral Cortex to Aminophylline comma Ergotamine Tartrate comma and Acetazolamide. *Stroke*., **2**, 461–470.

Resta, R.; Yamashita, Y.; Thompson, L. F., 1998: Ecto-enzyme and signaling functions of lymphocyte CD73. *Immunological reviews*., **161**, 95–109.

Ribeiro, J. A.; Sebastião, A. M.; de Mendonça, A., 2002: Adenosine receptors in the nervous system: pathophysiological implications. *Progress in neurobiology*., **68**, 377–392.

Rivers, L. E.; Young, K. M.; Rizzi, M.; Jamen, F.; Psachoulia, K.; Wade, A.; Kessaris, N.; Richardson, W. D., 2008: PDGFRA/NG2 glia generate myelinating oligodendrocytes and

piriform projection neurons in adult mice. *Nature neuroscience*., **11**, 1392–1401.

Rivkees, S. A.; Price, S. L.; Zhou, F. C., 1995: Immunohistochemical detection of A1 adenosine receptors in rat brain with emphasis on localization in the hippocampal formation, cerebral cortex, cerebellum, and basal ganglia. *Brain research*., **677**, 193–203.

Roberts, E. L., 1999: pH and Brain Function . Kai Kaila , Bruce R. Ransom . *The Quarterly Review of Biology*.

Rosin, D. L.; Robeva, A.; Woodard, R. L.; Guyenet, P. G.; Linden, J., 1998: Immunohistochemical localization of adenosine A2A receptors in the rat central nervous system. *The Journal of comparative neurology*., **401**, 163–186.

Rosin, D. L.; Hettinger, B. D.; Lee, A.; Linden, J., 2003: Anatomy of adenosine A2A receptors in brain: morphological substrates for integration of striatal function. *Neurology*., **61**, S12-8.

Rothhammer, V.; Kenison, J. E.; Tjon, E.; Takenaka, M. C.; de Lima, K. A.; Borucki, D. M.; Chao, C. C.; Wilz, A.; Blain, M.; Healy, L.; Antel, J.; Quintana, F. J., 2017: Sphingosine 1-phosphate receptor modulation suppresses pathogenic astrocyte activation and chronic progressive CNS inflammation. *Proceedings of the National Academy of Sciences of the United States of America*., **114**, 2012–2017.

Rousselet, E.; Kriz, J.; Seidah, N. G., 2012: Mouse model of intraluminal MCAO: cerebral infarct evaluation by cresyl violet staining. *Journal of visualized experiments : JoVE*.

Sacco, R. L.; Ellenberg, J. H.; Mohr, J. P.; Tatemichi, T. K.; Hier, D. B.; Price, T. R.; Wolf, P. A., 1989: Infarcts of undetermined cause: the NINCDS Stroke Data Bank. *Annals of neurology*., **25**, 382–390.

Sacco, R. L.; Benjamin, E. J.; Broderick, J. P.; Dyken, M.; Easton, J. D.; Feinberg, W. M.; Goldstein, L. B.; Gorelick, P. B.; Howard, G.; Kittner, S. J.; Manolio, T. A.; Whisnant, J. P.; Wolf, P. A., 1997: Risk Factors - Stroke. *Stroke*., **28**, 1507–1517.

Saini, H. S.; Coelho, R. P.; Goparaju, S. K.; Jolly, P. S.; Maceyka, M.; Spiegel, S.; Sato-Bigbee, C., 2005: Novel role of sphingosine kinase 1 as a mediator of neurotrophin-3 action in oligodendrocyte progenitors. *Journal of neurochemistry*., **95**, 1298–1310.

Saito, A.; Hayashi, T.; Okuno, S.; Nishi, T.; Chan, P. H., 2004: Modulation of the Omi/HtrA2 signaling pathway after transient focal cerebral ischemia in mouse brains that overexpress SOD1. *Brain research. Molecular brain research*., **127**, 89–95.

Sala-Newby, G. B.; Freeman, N. V; Skladanowski, A. C.; Newby, A. C., 2000: Distinct roles for recombinant cytosolic 5'-nucleotidase-I and -II in AMP and IMP catabolism in COS-7 and H9c2 rat myoblast cell lines. *The Journal of biological chemistry*., **275**, 11666–11671.

Sala-Newby, G. B.; Newby, A. C., 2001: Cloning of a mouse cytosolic 5'-nucleotidase-I identifies a new gene related to human autoimmune infertility-related protein. *Biochimica et biophysica acta*., **1521**, 12–18.

Sánchez-Moreno, C.; Dashe, J. F.; Scott, T.; Thaler, D.; Folstein, M. F.; Martin, A., 2004: Decreased Levels of Plasma Vitamin C and Increased Concentrations of Inflammatory and Oxidative Stress Markers after Stroke. *Stroke*.

Sarges, R.; Howard, H. R.; Browne, R. G.; Lebel, L. A.; Seymour, P. A.; Koe, B. K., 1990: 4-Amino[1,2,4]triazolo[4,3-a]quinoxalines. A Novel Class of Potent Adenosine Receptor

Antagonists and Potential Rapid-Onset Antidepressants. *Journal of Medicinal Chemistry*., **33**, 2240–2254.

Sattin, A.; Rall, T. W., 1970: The effect of adenosine and adenine nucleotides on the cyclic adenosine 3', 5'-phosphate content of guinea pig cerebral cortex slices. *Molecular pharmacology*., **6**, 13–23.

Schiffmann, S. N.; Libert, F.; Vassart, G.; Vanderhaeghen, J. J., 1991: Distribution of adenosine A2 receptor mRNA in the human brain. *Neuroscience Letters*., **130**, 177–181.

Schindler, M.; Harris, C. A.; Hayes, B.; Papotti, M.; Humphrey, P. P., 2001: Immunohistochemical localization of adenosine A1 receptors in human brain regions. *Neuroscience letters*., **297**, 211–215.

Schoen, S. W.; Kreutzberg, G. W.; Singer, W., 1993: Cytochemical Redistribution of 5′-Nucleotidase in the Developing Cat Visual Cortex. *European Journal of Neuroscience*., **5**, 210–222.

Schulte, G.; Fredholm, B. B., 2003: Signalling from adenosine receptors to mitogen-activated protein kinases. *Cellular signalling*., **15**, 813–827.

Schwabe, U.; Ebert, R.; Erbler, H. C., 1975: Adenosine release from fat cells: effect on cyclic AMP levels and hormone actions. *Advances in cyclic nucleotide research*., **5**, 569–584.

Schwartz, M., 2003: Macrophages and microglia in central nervous system injury: Are they helpful or harmful? *Journal of Cerebral Blood Flow and Metabolism*.

Schwarzschild, M. A.; Chen, J. F.; Ascherio, A., 2002: Caffeinated clues and the promise of adenosine A(2A) antagonists in PD. *Neurology*., **58**, 1154–1160.

Sciotti, V. M.; Roche, F. M.; Grabb, M. C.; Van Wylen, D. G., 1992: Adenosine receptor blockade augments interstitial fluid levels of excitatory amino acids during cerebral ischemia. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*., **12**, 646–655.

Scozzafava, A.; Supuran, C. T., 2014: Glaucoma and the applications of carbonic anhydrase inhibitors. *Sub-Cellular Biochemistry*.

Sebastião, A. M.; Ribeiro, J. A., 1996: Adenosine A2 receptor-mediated excitatory actions on the nervous system. *Progress in neurobiology*., **48**, 167–189.

Shinpo, K.; Kikuchi, S.; Moriwaka, F.; Tashiro, K., 1999: Protective effects of the TNF-ceramide pathway against glutamate neurotoxicity on cultured mesencephalic neurons. *Brain Research*., **819**, 170–173.

Shneyvays, V.; Zinman, T.; Shainberg, A., 2004: Analysis of calcium responses mediated by the A3 adenosine receptor in cultured newborn rat cardiac myocytes. *Cell Calcium*., **36**, 387–396.

Shneyvays, V.; Leshem, D.; Zinman, T.; Mamedova, L. K.; Jacobson, K. A.; Shainberg, A., 2005: Role of adenosine A_1 and A_3 receptors in regulation of cardiomyocyte homeostasis after mitochondrial respiratory chain injury. *American Journal of Physiology-Heart and Circulatory Physiology*., **288**, H2792–H2801.

Silverman, D. N.; Vincent, S. H., 1983: Proton transfer in the catalytic mechanism of carbonic anhydrase. *CRC critical reviews in biochemistry*., **14**, 207–255.

Silverman, D. N.; Lindskog, S., 1988: The Catalytic Mechanism of Carbonic Anhydrase: Implications of a Rate-Limiting Protolysis of Water. *Accounts of Chemical Research*., **21**, 30–36.

Simon, C.; Götz, M.; Dimou, L., 2011: Progenitors in the adult cerebral cortex: Cell cycle properties and regulation by physiological stimuli and injury. *GLIA*.

Sitkovsky, M. V.; Lukashev, D.; Apasov, S.; Kojima, H.; Koshiba, M.; Caldwell, C.; Ohta, A.; Thiel, M., 2004: P hysiological C ontrol of I mmune R esponse and I nflammatory T issue D amage by H ypoxia -I nducible F actors and A denosine A_{2A} R eceptors. *Annual Review of Immunology*., **22**, 657–682.

Soliven, B.; Bae, H.; Attali, B.; Sobko, A.; Iwase, T.; Ma, L., 2003: PDGF upregulates delayed rectifier via Src family kinases and sphingosine kinase in oligodendroglial progenitors.

Soliven, B.; Miron, V.; Chun, J., 2011: The neurobiology of sphingosine 1-phosphate signaling and sphingosine 1-phosphate receptor modulators. *Neurology*., **76**, S9-14.

Somjen, G. G., 2001: Mechanisms of Spreading Depression and Hypoxic Spreading Depression-Like Depolarization. *Physiological Reviews*., **81**, 1065–1096.

Sommer, I.; Schachner, M., 1981: Monoclonal antibodies (O1 to O4) to oligodendrocyte cell surfaces: an immunocytological study in the central nervous system. *Developmental biology*., **83**, 311–327.

Sontheimer, H.; Kettenmann, H., 1988: Heterogeneity of potassium currents in cultured oligodendrocytes. *Glia*.

Sontheimer, H.; Trotter, J.; Schachner, M.; Kettenmann, H., 1989: Channel expression correlates with differentiation stage during the development of Oligodendrocytes from their precursor cells in culture. *Neuron*., **2**, 1135–1145.

Sorensen, S. D.; Nicole, O.; Peavy, R. D.; Montoya, L. M.; Lee, C. J.; Murphy, T. J.; Traynelis, S. F.; Hepler, J. R., 2003: Common signaling pathways link activation of murine PAR-1, LPA, and S1P receptors to proliferation of astrocytes. *Molecular pharmacology*., **64**, 1199–1209.

Spera, P. A.; Ellison, J. A.; Feuerstein, G. Z.; Barone, F. C., 1998: IL-10 reduces rat brain injury following focal stroke. *Neuroscience letters*., **251**, 189–192.

Spignoli, G.; Pedata, F.; Pepeu, G., 1984: A1 and A2 adenosine receptors modulate acetylcholine release from brain slices. *European journal of pharmacology*., **97**, 341–342.

Spychala, J., 2000: Tumor-promoting functions of adenosine. *Pharmacology and Therapeutics*., Vol. 87pp. 161–173.

Stevens, B.; Fields, R. D., 2000: Response of Schwann cells to action potentials in development. *Science (New York, N.Y.)*., **287**, 2267–2271.

Stevens, B.; Porta, S.; Haak, L. L.; Gallo, V.; Fields, R. D., 2002: Adenosine: a neuron-glial transmitter promoting myelination in the CNS in response to action potentials. *Neuron*., **36**, 855–868.

Stewart, M.; Steinig, A. G.; Ma, C.; Song, J. P.; McKibben, B.; Castelhano, A. L.; MacLennan, S. J., 2004: [3 H]OSIP339391, a selective, novel, and high affinity antagonist radioligand for adenosine A 2B receptors. *Biochemical Pharmacology*., **68**, 305–312.

Stoll, G.; Jander, S.; Schroeter, M., 1998: Inflammation and glial responses in ischemic brain lesions. *Progress in Neurobiology*., **56**, 149–171.

Stone, T. W.; Behan, W. M. H., 2007: Interleukin-1beta but not tumor necrosis factor-alpha potentiates neuronal damage by quinolinic acid: protection by an adenosine A2A receptor antagonist. *Journal of neuroscience research*., **85**, 1077–1085.

Sudlow, C. L.; Warlow, C. P., 1997: Comparable studies of the incidence of stroke and its pathological types: results from an international collaboration. International Stroke Incidence Collaboration. *Stroke*., **28**, 491–499.

Sugawara, T.; Noshita, N.; Lewén, A.; Gasche, Y.; Ferrand-Drake, M.; Fujimura, M.; Morita-Fujimura, Y.; Chan, P. H., 2002: Overexpression of copper/zinc superoxide dismutase in transgenic rats protects vulnerable neurons against ischemic damage by blocking the mitochondrial pathway of caspase activation. *The Journal of neuroscience : the official journal of the Society for Neuroscience*., **22**, 209–217.

Sun, B.; Bachhawat, P.; Chu, M. L. H.; Wood, M.; Ceska, T.; Sands, Z. A.; Mercier, J.; Lebon, F.; Kobilka, T. S.; Kobilka, B. K., 2017: Crystal structure of the adenosine A 2A receptor bound to an antagonist reveals a potential allosteric pocket. *Proceedings of the National Academy of Sciences*., **114**, 2066–2071.

Sun, K.; Zhang, Y.; Bogdanov, M. V.; Wu, H.; Song, A.; Li, J.; Dowhan, W.; Idowu, M.; Juneja, H. S.; Molina, J. G.; Blackburn, M. R.; Kellems, R. E.; Xia, Y., 2015: Elevated adenosine signaling via adenosine A2B receptor induces normal and sickle erythrocyte sphingosine kinase 1 activity. *Blood*., **125**, 1643–1652.

Supuran, C. T.; Scozzafava, A., 2000: Carbonic-anhydrase inhibitors and their therapeutic potential. *Expert Opinion on Therapeutic Patents*.

Supuran, C. T.; Scozzafava, A., 2002: Applications of carbonic anhydrase inhibitors and activators in therapy. *Expert Opinion on Therapeutic Patents*.

Supuran, C. T., 2008: Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. *Nature reviews. Drug discovery*., **7**, 168–181.

Supuran, C. T., 2016: Carbonic anhydrase inhibition and the management of neuropathic pain. *Expert review of neurotherapeutics*., **16**, 961–968.

Supuran, C. T.; Alterio, V.; Di Fiore, A.; D' Ambrosio, K.; Carta, F.; Monti, S. M.; De Simone, G., 2018: Inhibition of carbonic anhydrase IX targets primary tumors, metastases, and cancer stem cells: Three for the price of one. *Medicinal Research Reviews*., **38**, 1799–1836.

Svenningsson, P.; Hall, H.; Sedvall, G.; Fredholm, B. B., 1997: Distribution of adenosine receptors in the postmortem human brain: An extended autoradiographic study. *Synapse*., **27**, 322–335.

Svenningsson, P.; Le Moine, C.; Fisone, G.; Fredholm, B. B., 1999: Distribution, biochemistry and function of striatal adenosine A2A receptors. *Progress in neurobiology*., **59**, 355–396.

Svichar, N.; Esquenazi, S.; Waheed, A.; Sly, W. S.; Chesler, M., 2006: Functional demonstration of surface carbonic anhydrase IV activity on rat astrocytes. *Glia*., **53**, 241–247.

Swanson, T. H.; Drazba, J. A.; Rivkees, S. A., 1995: Adenosine A1 receptors are located

predominantly on axons in the rat hippocampal formation. *The Journal of Comparative Neurology*., **363**, 517–531.

Swietach, P.; Vaughan-Jones, R. D.; Harris, A. L., 2007, June: Regulation of tumor pH and the role of carbonic anhydrase 9. *Cancer and Metastasis Reviews*.

Szabó, C.; Scott, G. S.; Virág, L.; Egnaczyk, G.; Salzman, A. L.; Shanley, T. P.; Haskó, G., 1998: Suppression of macrophage inflammatory protein (MIP)-1alpha production and collagen-induced arthritis by adenosine receptor agonists. *British journal of pharmacology*., **125**, 379–387.

Szigeti, K.; Horváth, I.; Veres, D. S.; Martinecz, B.; Lénárt, N.; Kovács, N.; Bakcsa, E.; Márta, A.; Semjéni, M.; Máthé, D.; Dénes, Á., 2015: A novel SPECT-based approach reveals early mechanisms of central and peripheral inflammation after cerebral ischemia. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism.*, **35**, 1921–1929.

Takagi, Y.; Nozaki, K.; Sugino, T.; Hattori, I.; Hashimoto, N., 2000: Phosphorylation of c-Jun NH2-terminal kinase and p38 mitogen-activated protein kinase after transient forebrain ischemia in mice. *Neuroscience Letters*., **294**, 117–120.

Takahashi, M.; Fujita, M.; Asai, N.; Saki, M.; Mori, A., 2018: Safety and effectiveness of istradefylline in patients with Parkinson's disease: interim analysis of a post-marketing surveillance study in Japan. *Expert opinion on pharmacotherapy*., **19**, 1635–1642.

Takeda, K.; Ichiijo, H., 2002, November 1: Neuronal p38 MAPK signalling: An emerging regulator of cell fate and function in the nervous system. *Genes to Cells*.

Takigawa, T.; Alzheimer, C., 1999: G protein-activated inwardly rectifying K+ (GIRK) currents in dendrites of rat neocortical pyramidal cells. *The Journal of physiology*., **517 (Pt 2**, 385–390.

Takigawa, T.; Alzheimer, C., 2002: Phasic and tonic attenuation of EPSPs by inward rectifier K+ channels in rat hippocampal pyramidal cells. *The Journal of physiology*., **539**, 67–75.

Tan, D. X.; Manchester, L. C.; Reiter, R. J.; Qi, W. B.; Karbownik, M.; Calvo, J. R., 2000: Significance of melatonin in antioxidative defense system: reactions and products. *Biological signals and receptors*., **9**, 137–159.

Tebano, M. T.; Martire, A.; Potenza, R. L.; Grò, C.; Pepponi, R.; Armida, M.; Domenici, M. R.; Schwarzschild, M. A.; Chen, J. F.; Popoli, P., 2008: Adenosine A(2A) receptors are required for normal BDNF levels and BDNF-induced potentiation of synaptic transmission in the mouse hippocampus. *Journal of neurochemistry*., **104**, 279–286.

Tendera, M.; Gaszewska-Zurek, E.; Parma, Z.; Ponikowski, P.; Jankowska, E.; Kawecka-Jaszcz, K.; Czarnecka, D.; Krzemińska-Pakuła, M.; Bednarkiewicz, Z.; Sosnowski, M.; Kilama, M. O.; Agrawal, R., 2012: The new oral adenosine A1 receptor agonist capadenoson in male patients with stable angina. *Clinical Research in Cardiology*., **101**, 585–591.

Terada, N.; Banno, Y.; Ohno, N.; Fujii, Y.; Murate, T.; Sarna, J. R.; Hawkes, R.; Zea, Z.; Baba, T.; Ohno, S., 2004: Compartmentation of the mouse cerebellar cortex by sphingosine kinase. *The Journal of comparative neurology*., **469**, 119–127.

Thiry, A.; Dogne, J. M.; Supuran, C.; Masereel, B., 2007: Carbonic Anhydrase Inhibitors as Anticonvulsant Agents. *Current Topics in Medicinal Chemistry*., **7**, 855–864.

Tibbles, L. A.; Woodgett, J. R., 1999, August 15: The stress-activated protein kinase pathways. *Cellular and Molecular Life Sciences*.

Toman, R. E.; Spiegel, S., 2002: Lysophospholipid receptors in the nervous system. *Neurochemical research*., **27**, 619–627.

Tong, C. K.; Brion, L. P.; Suarez, C.; Chesler, M., 2000: *Interstitial Carbonic Anhydrase (CA) Activity in Brain Is Attributable to Membrane-Bound CA Type IV*.

Tosh, D. K.; Finley, A.; Paoletta, S.; Moss, S. M.; Gao, Z. G.; Gizewski, E. T.; Auchampach, J. A.; Salvemini, D.; Jacobson, K. A., 2014: In vivo phenotypic screening for treating chronic neuropathic pain: modification of C2-arylethynyl group of conformationally constrained A3 adenosine receptor agonists. *Journal of medicinal chemistry*., **57**, 9901–9914.

Traut, T. W., 1994: Physiological concentrations of purines and pyrimidines. *Molecular and cellular biochemistry*., **140**, 1–22.

Traystman, R. J.; Kirsch, J. R.; Koehler, R. C., 1991: Oxygen radical mechanisms of brain injury following ischemia and reperfusion. *Journal of Applied Physiology*., **71**, 1185–1195.

Trincavelli, M. L.; Marroni, M.; Tuscano, D.; Ceruti, S.; Mazzola, A.; Mitro, N.; Abbracchio, M. P.; Martini, C., 2004: Regulation of A2B adenosine receptor functioning by tumour necrosis factor a in human astroglial cells. *Journal of neurochemistry*., **91**, 1180–1190.

Trincavelli, M. L.; Melani, A.; Guidi, S.; Cuboni, S.; Cipriani, S.; Pedata, F.; Martini, C., 2008: Regulation of A2A adenosine receptor expression and functioning following permanent focal ischemia in rat brain. *Journal of Neurochemistry*., **104**, 479–490.

Trump, B. F.; Berezesky, I. K.; Chang, S. H.; Phelps, P. C., (n.d.) The pathways of cell death: oncosis, apoptosis, and necrosis. *Toxicologic pathology*., **25**, 82–88.

Tsuji, K.; Aoki, T.; Tejima, E.; Arai, K.; Lee, S. R.; Atochin, D. N.; Huang, P. L.; Wang, X.; Montaner, J.; Lo, E. H., 2005: Tissue plasminogen activator promotes matrix metalloproteinase-9 upregulation after focal cerebral ischemia. *Stroke*., **36**, 1954–1959.

van Calker, D.; Müller, M.; Hamprecht, B., 1979: Adenosine regulates via two different types of receptors, the accumulation of cyclic AMP in cultured brain cells. *Journal of neurochemistry*., **33**, 999–1005.

Van Doorn, R.; Van Horssen, J.; Verzijl, D.; Witte, M.; Ronken, E.; Van Het Hof, B.; Lakeman, K.; Dijkstra, C. D.; Van Der Valk, P.; Reijerkerk, A.; Alewijnse, A. E.; Peters, S. L. M.; De Vries, H. E., 2010: Sphingosine 1-phosphate receptor 1 and 3 are upregulated in multiple sclerosis lesions. *Glia*., **58**, 1465–1476.

Van Veldhoven, P. P.; Gijsbers, S.; Mannaerts, G. P.; Vermeesch, J. R.; Brys, V., 2000: Human sphingosine-1-phosphate lyase: cDNA cloning, functional expression studies and mapping to chromosome 10q22(1). *Biochimica et biophysica acta*., **1487**, 128–134.

Varani, K.; Gessi, S.; Dionisotti, S.; Ongini, E.; Andrea Borea, P., 1998: [^{3} H]-SCH 58261 labelling of functional A_{2A} adenosine receptors in human neutrophil membranes. *British Journal of Pharmacology*., **123**, 1723–1731.

Verkhratsky, A. N.; Trotter, J.; Kettenmann, H., 1990: Cultured glial precursor cells from mouse cortex express two types of calcium currents. *Neuroscience letters*., **112**, 194–198.

Von Lubitz, D. K., 1999: Adenosine and cerebral ischemia: therapeutic future or death of a brave concept? *European journal of pharmacology*., **365**, 9–25.

Voors, A. A.; Düngen, H. D.; Senni, M.; Nodari, S.; Agostoni, P.; Ponikowski, P.; Bax, J. J.; Butler, J.; Kim, R. J.; Dorhout, B.; Dinh, W.; Gheorghiade, M., 2017: Safety and Tolerability of Neladenoson Bialanate, a Novel Oral Partial Adenosine A1 Receptor Agonist, in Patients With Chronic Heart Failure. *Journal of Clinical Pharmacology*., **57**, 440–451.

Wakai, A.; Wang, J. H.; Winter, D. C.; Street, J. T.; O'Sullivan, R. G.; Redmond, H. P., 2001: Adenosine inhibits neutrophil vascular endothelial growth factor release and transendothelial migration via A2B receptor activation. *Shock (Augusta, Ga.)*., **15**, 297–301.

Watterson, K.; Sankala, H.; Milstien, S.; Spiegel, S., 2003: Pleiotropic actions of sphingosine-1-phosphate. *Progress in lipid research*., **42**, 344–357.

Webb, M.; Tham, C. S.; Lin, F. F.; Lariosa-Willingham, K.; Yu, N.; Hale, J.; Mandala, S.; Chun, J.; Rao, T. S., 2004: Sphingosine 1-phosphate receptor agonists attenuate relapsing-remitting experimental autoimmune encephalitis in SJL mice. *Journal of neuroimmunology*., **153**, 108–121.

Wei, W.; Du, C.; Lv, J.; Zhao, G.; Li, Z.; Wu, Z.; Haskó, G.; Xie, X., 2013: Blocking A2B adenosine receptor alleviates pathogenesis of experimental autoimmune encephalomyelitis via inhibition of IL-6 production and Th17 differentiation. *Journal of immunology (Baltimore, Md. : 1950)*., **190**, 138–146.

White, P. J.; Rose'Meyer, R. B.; Hope, W., 1996: Functional characterization of adenosine receptors in the nucleus tractus solitarius mediating hypotensive responses in the rat. *British journal of pharmacology*., **117**, 305–308.

Williams, T. C.; Jarvis, S. M., 1991: Multiple sodium-dependent nucleoside transport systems in bovine renal brush-border membrane vesicles. *The Biochemical journal*., **274 (Pt 1**, 27–33.

Williamson, A. V; Compston, D. A.; Randall, A. D., 1997: Analysis of the ion channel complement of the rat oligodendrocyte progenitor in a commonly studied in vitro preparation. *The European journal of neuroscience*., **9**, 706–720.

Windrem, M. S.; Nunes, M. C.; Rashbaum, W. K.; Schwartz, T. H.; Goodman, R. A.; McKhann, G.; Roy, N. S.; Goldman, S. A., 2004: Fetal and adult human oligodendrocyte progenitor cell isolates myelinate the congenitally dysmyelinated brain. *Nature medicine*., **10**, 93–97.

Woodruff, T. M.; Thundyil, J.; Tang, S. C.; Sobey, C. G.; Taylor, S. M.; Arumugam, T. V, 2011: Pathophysiology, treatment, and animal and cellular models of human ischemic stroke. *Molecular neurodegeneration*., **6**, 11.

Wu, L. G.; Saggau, P., 1994: Adenosine inhibits evoked synaptic transmission primarily by reducing presynaptic calcium influx in area CA1 of hippocampus. *Neuron*., **12**, 1139–1148.

Wykoff, C. C.; Beasley, N. J.; Watson, P. H.; Turner, K. J.; Pastorek, J.; Sibtain, A.; Wilson, G. D.; Turley, H.; Talks, K. L.; Maxwell, P. H.; Pugh, C. W.; Ratcliffe, P. J.; Harris, A. L., 2000: Hypoxia-inducible expression of tumor-associated carbonic anhydrases. *Cancer research*., **60**, 7075–7083.

Xia, Z.; Dickens, M.; Raingeaud, J.; Davis, R. J.; Greenberg, M. E., 1995: Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. *Science (New York, N.Y.)*., **270**, 1326–1331.

Xu, J.; Guo, S.; Jia, Z.; Ma, S.; Li, Z.; Xue, R., 2013: Additive effect of prostaglandin E2 and adenosine in mouse experimental autoimmune encephalomyelitis. *Prostaglandins & other lipid mediators*., **100**–**101**, 30–35.

Xu, K.; Bastia, E.; Schwarzschild, M., 2005: Therapeutic potential of adenosine A2A receptor antagonists in Parkinson's disease. *Pharmacology & Therapeutics*., **105**, 267–310.

Yamagata, K.; Tagami, M.; Torii, Y.; Takenaga, F.; Tsumagari, S.; Itoh, S.; Yamori, Y.; Nara, Y., 2003: Sphingosine 1-phosphate induces the production of glial cell line-derived neurotrophic factor and cellular proliferation in astrocytes. *GLIA.*, **41**, 199–206.

Yang, D.; Zhang, Y.; Nguyen, H. G.; Koupenova, M.; Chauhan, A. K.; Makitalo, M.; Jones, M. R.; St Hilaire, C.; Seldin, D. C.; Toselli, P.; Lamperti, E.; Schreiber, B. M.; Gavras, H.; Wagner, D. D.; Ravid, K., 2006: The A2B adenosine receptor protects against inflammation and excessive vascular adhesion. *The Journal of clinical investigation*., **116**, 1913–1923.

Yang, D.; Chen, H.; Koupenova, M.; Carroll, S. H.; Eliades, A.; Freedman, J. E.; Toselli, P.; Ravid, K., 2010: A new role for the A2b adenosine receptor in regulating platelet function. *Journal of thrombosis and haemostasis : JTH*., **8**, 817–827.

Yang, H.; Avila, M. Y.; Peterson-Yantorno, K.; Coca-Prados, M.; Stone, R. A.; Jacobson, K. A.; Civan, M. M., 2005: The cross-species A3 adenosine-receptor antagonist MRS 1292 inhibits adenosine-triggered human nonpigmented ciliary epithelial cell fluid release and reduces mouse intraocular pressure. *Current eye research*., **30**, 747–754.

Yawo, H.; Chuhma, N., 1993: Preferential inhibition of oω-conotoxin-sensitive presynaptic Ca2+ channels by adenosine autoreceptors. *Nature*., **365**, 256–258.

Yenari, M. A.; Kunis, D.; Sun, G. H.; Onley, D.; Watson, L.; Turner, S.; Whitaker, S.; Steinberg, G. K., 1998: Hu23F2G, an Antibody Recognizing the Leukocyte CD11/CD18 Integrin, Reduces Injury in a Rabbit Model of Transient Focal Cerebral Ischemia. *Experimental Neurology*., **153**, 223–233.

Yepes, M.; Roussel, B. D.; Ali, C.; Vivien, D., 2009, January: Tissue-type plasminogen activator in the ischemic brain: more than a thrombolytic. *Trends in Neurosciences*.

Yilmaz, G.; Granger, D. N., 2008: Cell adhesion molecules and ischemic stroke. *Neurological research*., **30**, 783–793.

Young, K. M.; Psachoulia, K.; Tripathi, R. B.; Dunn, S. J.; Cossell, L.; Attwell, D.; Tohyama, K.; Richardson, W. D., 2013: Oligodendrocyte dynamics in the healthy adult CNS: evidence for myelin remodeling. *Neuron*., **77**, 873–885.

Yu, L.; Frith, M. C.; Suzuki, Y.; Peterfreund, R. A.; Gearan, T.; Sugano, S.; Schwarzschild, M. A.; Weng, Z.; Fink, J. S.; Chen, J. F., 2004: Characterization of genomic organization of the adenosine A2A receptor gene by molecular and bioinformatics analyses. *Brain research*., **1000**, 156–173.

Yu, W. P.; Collarini, E. J.; Pringle, N. P.; Richardson, W. D., 1994: Embryonic expression of myelin genes: Evidence for a focal source of oligodendrocyte precursors in the ventricular zone of the neural tube. *Neuron*., **12**, 1353–1362.

Zawadzka, M.; Rivers, L. E.; Fancy, S. P. J.; Zhao, C.; Tripathi, R.; Jamen, F.; Young, K.; Goncharevich, A.; Pohl, H.; Rizzi, M.; Rowitch, D. H.; Kessaris, N.; Suter, U.; Richardson, W. D.; Franklin, R. J. M., 2010: CNS-resident glial progenitor/stem cells produce Schwann cells as well as oligodendrocytes during repair of CNS demyelination. *Cell stem cell*., **6**, 578–590.

Zetterström, T.; Fillenz, M., 1990: Adenosine agonists can both inhibit and enhance in vivo striatal dopamine release. *European journal of pharmacology*., **180**, 137–143.

Zhang, R.; Chopp, M.; Zhang, Z.; Jiang, N.; Powers, C., 1998: The expression of P- and E-selectins in three models of middle cerebral artery occlusion. *Brain research*., **785**, 207–214.

Zhao, Z.; Francis, C. E.; Ravid, K., 1997: An A3-Subtype Adenosine Receptor Is Highly Expressed in Rat Vascular Smooth Muscle Cells: Its Role in Attenuating Adenosine-Induced Increase in cAMP. *Microvascular Research*., **54**, 243–252.

Zhu, Y.; Yang, G. Y.; Ahlemeyer, B.; Pang, L.; Che, X. M.; Culmsee, C.; Klumpp, S.; Krieglstein, J., 2002: Transforming growth factor-beta 1 increases bad phosphorylation and protects neurons against damage. *The Journal of neuroscience : the official journal of the Society for Neuroscience*., **22**, 3898–3909.

Zimmermann, H., 1992a: 5'-Nucleotidase: Molecular structure and functional aspects. *Biochemical Journal*.

Zimmermann, H., 1992b: 5'-Nucleotidase: molecular structure and functional aspects. *The Biochemical journal*., **285 (Pt 2)**, 345–365.

Zimmermann, H., 2000, November: Extracellular metabolism of ATP and other nucleotides. *Naunyn-Schmiedeberg's Archives of Pharmacology*.