UNIVERSITÀ
DEGLI STUDI
FIRENZE

DOTTORATO DI RICERCA IN
FARMACOLOGIA, TOSSICOLOGIA E TRATTAMENTI INNOVATIVI

CICLO XXVII

COORDINATORE Prof. Elisabetta Teodori

Pharmacological characterisation of behavioural and metabolic effects of 3-iodothyronamine (T_1AM) and 3-iodothyroacetic acid (TA_1), endogenous end products of thyroid hormone metabolism.

Settore Scientifico Disciplinare BIO/14

Dottorando
Dott. Manni Maria Elena

Tutore Scientifico
Prof. Raimondi Laura

Tutore Teorico
Prof. Lodovici Maura

Coordinatore
Prof. Teodori Elisabetta

Anni 2012/2014

INDEX

1. **GENERAL INTRODUCTION** 4

1.1 Thyroid Hormone *4*

1.2 Thyroid Hormone and the central nervous system *7*

1.3 Thyroid Hormone and Food Intake *9*

1.4 The non classical THs *14*

Tetrac and Triac *14*

Thyronamines *15*

T1AM metabolism *27*

1.5 Histaminergyc System *30*

Histamine and Thyroid Axis *33*

Histamine and Memory *34*

Histamine and Pain *35*

2. **AIM** 37

3. **METHODS** 38

3.1 Animals *38*

HDC$^{-/-}$ mice *38*

3.2 Detection of endogenous TA1 and T1AM in mice brain *39*

3.3 I.c.v. injection technique *39*

3.4 Evaluation of food consumption *40*

3.5 Glycemia and collection of samples for T1AM plasma level determination *40*

3.6 Insulin Tolerance test *41*

3.7 Plasma levels of thyroid hormones *41*

3.8 Behavioral tests *42*

3.9 T1AM-activated signaling in the brain of mice *44*

3.10 Statistical analysis *45*

4 **RESULTS** 46

4.1 T1AM produced hypo- and hyperphagia *46*

4.2 T1AM rapidly induced hyperglycemia *48*

4.3-1 T1AM-induced hyperglycemia increased insulin resista-nce *50*

4.3-2 T1AM induced hyperglycemia was prevented by exena-tide treatment *53*

4.4 T1AM injection reduced fT3 plasma levels *55*

4.5 T1AM presented systemic bioavailability *57*

4.6 T1AM behaves as a modulator of the hypothalamus-pancreas-thyroid axes in mice *59*

4.7 T1AM effects on memory and pain *62*

4.8 T1AM improved memory acquisition: the object recogni-tion task *64*

4.9 T1AM increased exploratory mice activity: the hole-board test *66*

4.10 T1AM increased pain sensitivity *68*

4.11 Clorgyline pretreatment modifies behavioural effects of T1AM *70*

4.12 T_1AM pro-learning effect does not include the muscarinic pathway: T1AM revert scopolamine-induced amnesia *72*

4.13 Reversion of scopolamine amnesia depends T1AM-oxidative deamination *74*

4.14 T1AM induced ERK1/2 phosphorylation at different ce-rebral areas *76*

4.15 T1AM is an endogenous memory enhancer and hyperal-gesic compound 78

4.16 Pharmacological effects of 3-iodothyroacetic acid (TA1), the oxidative metabolite of T1AM 80

4.17 TA1 reverts scopolamine-induced amnesia *82*

4.18 Histaminergic antagonists pretreatment prevents stimu-lation of memory, hyperalgesia and increase of plasma glyce-mia *84*

4.19 The hyperalgesic and hyperglycemic effects of T1A are prevented in $HDC^{-/-}$ mice *87*

4.20 Detection of endogenous T1AM and TA1 in the brain of euthyroid (CD1), $HDC^{+/+}$ and $HDC^{-/-}$ mice *89*

4.21 TA1 shares with T1AM some behavioural effects *91*

5 **CONCLUSIONS** 93

6. **REFERENCES** 98

1. GENERAL INTRODUCTION

1.1 Thyroid Hormone

First isolated in 1914 by Kendall (Kendall EC., 1919), and first synthesized in 1925 by Harrington (Harrington CR 1926) thyroxine (T4) is a classic hormone that is used worldwide to treat millions of patients with thyroid disorders. During the past decades much progress has been made in the understanding of thyroid hormone (TH) physiology and a substantial part of TH biology has been elucidated. T4 is the main secretory product of the thyroid gland. In humans, it comprises ~80% of the THs secreted, the remaining ~20% being secreted as triiodothyronine (T3). T4 has only limited affinity for the nuclear thyroid hormone receptors (TRs) as compared with T3, which is regarded the primary biologically activeform. In order to become bio-active, T4 has to be converted to T3 by outer-ring deiodination. Furthermore, both T4 and T3 can be inactivated by inner-ring deiodination.

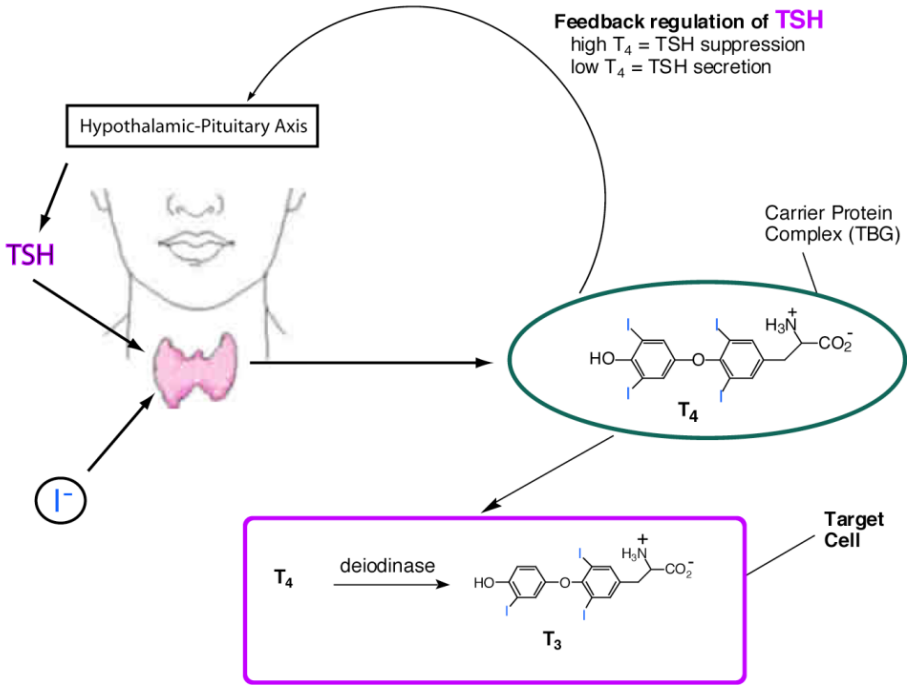


Fig. 1 Schematic immage of thyroid hormones production.

These reactions are catalysed by the iodothyronine deiodinases type 1, 2 and 3 (D1, D2 and D3), that are expressed in a multitude of peripheral tissues, each deiodinase with its specific tissue distribution. Outer ring deiodination, i.e. the activating pathway, is catalysed by D1 and D2. Inner ring deiodination of T4 and T3 to lower iodothyronines that have no affinity for the TRs, *i.e.* the inactivating pathway, is catalysed by both D1 and D3 (Bianco AC., 2002). Thyroidal TH secretion is regulated via a classical central negative feedback mechanism. Thyrotropin-releasing hormone (TRH) is synthesized by neurons in the paraventricular nucleus (PVN) of the hypothalamus and reaches the anterior pituitary via the median eminence and the portal system. In the anterior pituitary TRH stimulates the secretion of thyroid stimulating hormone (TSH or thyrotropin) from the thyrotropes. TSH is the major factor stimulating thyroid hormone synthesis and secretion by the thyroid gland. In turn, both circulating T3 and T4 exert an inhibitory effect on the release of TRH and TSH from the hypothalamus and the pituitary, respectively. The nuclear thyroid hormone receptors (TR) are products of the TRα and TRβ genes. These receptors are members of the ligand-dependent transcription modulator family. This implies that upon intra-nuclear binding of T3 to a TR and via interaction with several co-factors, the complex binds to a thyroid hormone responsive element (TRE) in the promoter region of a TH-responsive gene, ultimately affecting gene transcription (Yen PM 2001). Many actions of TH can be explained by this transcriptional mechanism of action. TH transport across the cell membrane is required, since both deiodinases and TRs are located intracellularly. In recent years, several specific TH transporters have emerged, such as the monocarboxylate transporters (MCT) and the organic anion transporting polypeptide (OATP) family. Of the latter family, OATP1C1 appears to be critical for transport of T4 across the blood-brain-barrier (Visser WE., 2008).

1.2 Thyroid Hormone and the central nervous system

Although a pivotal role of thyroid hormones in the developing mammalian brain has been long established and extensively documented (Bernal J., 2007), the adult central nervous system (CNS) was generally assumed to be a thyroid hormone-insensitive organ until the 1980s. A number of findings in more recent literature have casted doubt upon this assumption and have gradually led to the general acceptance of the notion that the adult brain is a highly TH-sensitive organ. This notion is in line with the well-known psychomotor and cognitive dysfunction often observed in adult-onset thyroid disorders, especially hypothyroidism (Dugbartey AT., 1998). By use of radio-labeled iodothyronines, the group of dr. M.B. Dratman showed for the first time that THs can be transported across the blood-brain-barrier and choroid plexus in rats (Dratman MB., 1991). Further studies showed uptake and concentration of radio-labeled T3 in nerve terminals (Dratman MB., 1982), pointing to a new role for THs as (precursors for) amino-acid neurotransmitters or neuro-modulators (Dratman MB., 1996). A molecular basis for an active transport mechanism for THs in the brain was provided by the identification of TH-specific transporters both in neurons and in capillaries of the choroid plexus (Friesema EC., 2003). The existence of active thyroid hormone transport in the human brain may explain why concentrations of free T4 (FT4) and free T3 (FT3) are within the same range, or even h igher, in the cerebrospinal fluid (CSF) as compared with plasma. THs and several TH derivatives are widely distributed in the CNS. For instance, both T4 and T3 concentrations have been reported in the pmol/g range in rat hypothalamus (Pinna G., 2002). These tissue concentrations are similar to T4 and T3 concentrations in rat liver, which is considered to be a major TH target tissue. Importantly, also the thyroid hormone receptors TR α and TR β, as well as the deiodinating enzymes D2 and D3, are widely distributed both in the rat and human CNS, including the hypothalamus (Alkemade A., 2005). It has been estimated that more than 75% of neuronal T3 is derived from conversion of T4 to T3 by D2, underlining the importance of D2 in regulating T3 bio-availability in the CNS (Crantz FR., 1982). A phenomenon pointing to an important functional role of T3 in the CNS is the efficient homeostatic mechanism in the brain ensuring notably stable local T3-tissue concentrations in the face of pathological changes in systemic TH status. For example, when rats are rendered hypothyroid by thyroidectomy, D2 activity in the cerebral

cortex rapidly increases, promoting the local conversion of T4 into T3, and this can be prevented by the administration of systemic TH replacement at the same time. Similar adaptive mechanisms have been reported in the hypothalamus, where hypothyroidism elevates D2 mRNA expression and activity, whereas thyrotoxicosis decreases local D2 levels (Tu HM., 1997). In addition, the TH inactivating enzyme D3 is highly T3-responsive throughout the CNS, as evidenced by a dose-dependent induction during thyrotoxicosis (Escobar-Morreale HF., 1997). These TH dependent adaptations in local deiodinase enzyme expression result in remarkably stable tissue T3 concentrations in the CNS over a wide range of systemic TH conditions. Furthermore, under non-pathological conditions, hypothalamic deiodinase levels also show marked fluctuations in association with physiologic processes and stimuli such as day-night rhythmicity (Kalsbeek A., 2005), seasonal *(i.e.* photoperiod) changes (Yoshimura T., 2002) and food availability (Diano S.,1998). However, in most of these processes it remains unclear if deiodinase regulated TH bio-availability in the hypothalamus may play a regulatory role in the (metabolic) adaptation of the organism to these conditions, or if these represent epiphenomena (Lechan, 2005). Recently, with the advances in functional neuro-imaging techniques, there have been several clinical reports describing metabolic changes in the CNS associated with altered TH status in patients (Constant EL., 2001), possibly explaining the neuro-cognitive symptoms in these patients. Interestingly, even in subclinical hypothyroidism, which may be regarded as a subtle thyroid hormone deficit, fMRI revealed malfunction of brain areas critical for working memory, which could be ameliorated by thyroxine supplementation (Zhu DF., 2006).

1.3 Thyroid Hormone and Food Intake

The role of TH in the regulation of lipid and glucose metabolism has been the subject of study ever since the recognition of the link between thyroid function and body weight. The effects of TH on metabolism are among the foremost actions of TH in vertebrates. This is illustrated by the profound alterations in (energy) metabolism during hyper- and hypothyroidism, and also by the metabolic adaptations of the HPT-axis to physiologic stressors such as food deprivation and critical illness (Lechan RM, 2006). Hypermetabolism is one of the hallmarks of thyrotoxicosis, reflected in an increase of resting energy expenditure (REE). This increased REE can be measured in humans by indirect calorimetry, assessing whole body O2 consumption and CO2 production. In fact, this was an important tool in the diagnosis of thyrotoxicosis before the development of sensitive radioimmunoassays for T4 and TSH. To ensure replenishment of macronutrients, appetite is simultaneously stimulated during thyrotoxicosis, which is obviously advantageous. There are recent data to suggest that this hyperphagia represents a direct TH effect in the hypothalamic ventromedial nucleus, which is involved in appetite regulation (Kong WM, 2004). In addition, TH may affect appetite indirectly via the hypothalamic neuropeptide Y (NPY)/ Agouti related peptide (AGRP) system that is critical for appetite regulation (Ishii S, 2003). The alterations in glucose metabolism during thyrotoxicosis have been extensively studied.

The major central nervous system (CNS) areas important in the regulation of appetite are the hypothalamus and brainstem. The hypothalamus interprets and integrates afferent signals from the periphery and brainstem to modulate efferent signals that regulate food intake and energy expenditure. Neural and hormonal peripheral signals communicate information including acute nutritional states and energy stores. The hypothalamus is subdivided into a number of interconnecting nuclei, including the paraventricular nucleus (PVN), the ventromedial nucleus (VMN), and the arcuate nucleus (ARC), which are particularly important in regulating energy homeostasis.

It is well established that the hypothalamic-pituitary-thyroid (HPT) axis regulates body weight. Thyroid hormones are known to effect metabolic rate. Thyroid dysfunction can have clinically significant consequences on appetite and body weight. Hypothyroidism classically causes reduced basal energy expenditure (M. Wolf ,1996) with weight gain

(N.Manji, 2006). Conversely, hyperthyroidism increases energy expenditure and reduces body weight (Klieverik LP., 2009). Traditionally, it has been assumed that it is this reduced body weight that drives the hyperphagia that can be a presenting feature in hyperthyroidism. However, recent evidence suggests that the HPT axis may play a direct role in the hypothalamic regulation of appetite, independent of effects on energy expenditure. Classically, hypothalamic thyrotropin-releasing hormone (TRH) stimulates thyroid-stimulating hormone (TSH) release from the anterior pituitary gland, which then stimulates the release of both thyroid hormones, triiodothyronine (T3) and thyroxine (T4). Reports suggest that all of these signalling molecules can directly influence food intake (Kong WM., 2004). Improved understanding of the role of the HPT axis and thyroid hormone in appetite may identify new targets for anti obesity agents.

There are well-characterised effects of fasting on hypothalamic TRH expression. This is primarily thought to downregulate the HPT axis in periods of limited food availability thus reducing food intake. However, TRH has been reported to have direct anorectic effects, suggesting it may regulate food intake independent of effects on the HPT axis. In rodents, central administration of TRH reduces food intake; similar effects on food intake are seen following peripheral administration. TSH has also been shown to reduce food intake when injected centrally into rats. There is evidence that TSH from the pars tuberalis is involved in the photoperiodic response in birds and rodents, and it is thus possible that TSH is involved with the seasonal alterations in food intake and body weight that occur in some species. The hyperphagia associated with hyperthyroidism may be a result of thyroid hormones acting directly on CNS appetite circuits. T3 directly stimulates food intake at the level of the hypothalamus. In rodent models, peripheral and central hypothalamic administration of T3 increases food intake (Ishii S, 2003).

Hormone	Effect on food intake
TRH	↓
TSH	↓
T3	↑

Fig. 2 Effect of Thyroid Hormone on food intake.

Thyroid hormone derivatives have also been implicated in the regulation of appetite. G protein-coupled trace amine-associated receptor 1 (TAAR1) is expressed in the rat hypothalamus and is associated with the regulation of energy homeostasis. Thyroid hormone derivative 3-iodothyronamine (T1AM), an endogenous biogenic amine, is a potent agonist of TAAR1. Rodent studies show that T1AM significantly increases food intake in rats, when administered intraperitoneally, ICV, or directly into the ARC (Dhillo WS., 2009).

However, the physiological relevance of these effects remains unknown.

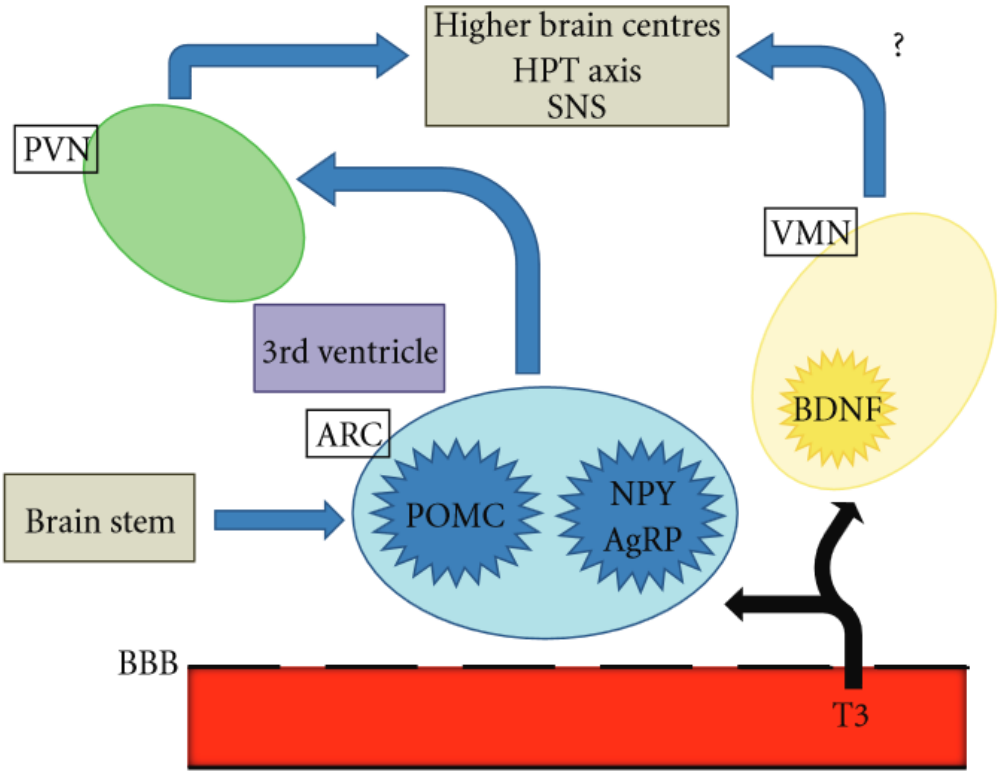


Fig. 3 Schematic diagram of central appetite regulation. T3 can access the hypothalamus and brainstem via the incomplete blood brain barrier. PVN: paraventricular nucleus; ARC: arcuate nucleus; VMN: ventromedial nucleus; BBB: blood-brain barrier; T3: triiodothyronine; POMC: Pro-opiomelanocortin; NPY: neuropeptide Y; AgRP: agouti-related protein; BDNF: brain-derived neurotrophic factor; HPT: hypothalamic-pituitary thyroid; SNS: sympathetic nervous system.

1.4 The non classical THs

Tetrac and Triac

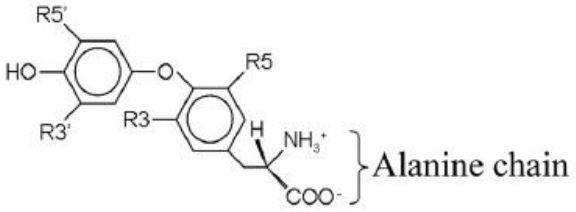
In humans, the amount of Triac produced by the liver and other tissues accounts for about 14% of T3 metabolism (Siegrist-Kaiser & Burger 1994). Triac is weakly TRb-selective, with a 1.5-fold affinity for TRb (Schueler et al . 1990). Triac has been used to suppress thyroid-stimulating hormone (TSH) secretion in TH-resistant patients (Kunitake et al . 1989) and to increase metabolic rate in obese patients (Dumas et al . 1982). It has been shown to be more potent than T3 as both α-β-adrenergic stimulator of uncoupling protein 1 and inducer of lipoprotein lipase mRNA, D3 activity, and mRNA (Medina-Gomez et al . 2003). Triac inhibits expression and secretion of leptin in rat primary white and brown adipocytes with a potency similar to that of T3 (Medina-Gomez et al., 2004). The use of Tetrac as a potential substitute for T4 has been studied in the treatment of myxedema and for its ameliorating effect on peripheral lipid metabolism in humans. The effects are similar to those of T4, but require higher dosing (Lerman, 1956). Tetrac is currently used in the clinic for the treatment of TH resistance (Anzai et al., 2012). Therapeutic doses of Triac to treat pituitary and thyroid disorders exceed those required for T4 and T3 (Sherman & Ladenson 1992, Bracco et al., 1993), a property attributed to its short half-life in humans and rodents (Pittman et al., 1980; Moreno et al., 1994). Classic THs are transported within the cell by TH transporters (Visser, 2013). Tetrac does not seem to depend on active transport, at least by the most abundant transporter MCT8. Tetrac can replace T3 to restore normal fetal mouse brain development in MCT8-null mice (Horn et al., 2013).

Thyronamines

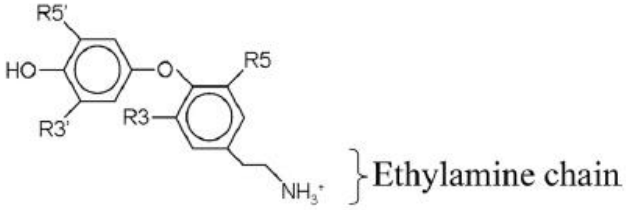
There is growing evidence that T_3 and T_4 may trigger non-genomic effects, resulted from their binding to plasma membrane, cytoplasm, or mitochondrial receptors that leads to a rapidly regulation of cell functions (Bassettt et al., 2003; Farach-Carson and Davis, 2003), even if the molecules that mediate these non-genomic effects remain obscure. In this respect, interesting perspectives arrived from the identification of some thyroid hormone metabolites, the so-called thyronamines, a series of primary amines with different degree of iodination.

The structures of the thyronamines (TAMs) differ from T4 and deiodinated TH derivatives by the absence of a carboxylate group on the alanine side chain.

A Thyronine (TH)



B Thyronamine (TAM)



Analyte	R3	R5	R3'	R5'
T_0(AM)	H	H	H	H
3-T_1(AM)	I	H	H	H
3'-T_1(AM)	H	H	I	H
3,5-T_2(AM)	I	I	H	H
3,3'-T_2(AM)	I	H	I	H
3',5'-T_2(AM)	H	H	I	I
T_3(AM)	I	I	I	H
rT_3(AM)	I	H	I	I
T_4(AM)	I	I	I	I

Fig. 4 A, Structure of THs. B, Structure and nomenclature of TAMs. R, Variable residue.

3-Iodothyro-namine (T1AM) and T0AM have been detected in vivo (Scanlan et al., 2004; DeBarber et al., 2008) in the serum of rodents and humans (Saba et al., 2010; Hoefig et al., 2011), in rat liver, brain, and heart (Chiellini et al., 2007; Saba et al., 2010).

Concentrations of T_1AM in rat serum and tissues

Tissue	T_1AM pmol/g	T_0AM pmol/g	T3 pmol/g	T4 pmol/g
Heart	6.60±1.36	ND	0.54±0.10	0.68±0.17
Liver	92.92±28.46	20.68±0.25	4.71±0.82	14.47±2.98
Kidney	36.08±10.42	6.01±1.00	7.14±0.89	13.02±2.81
Muscle	25.02±6.95	0.27±0.16	0.82±0.16	1.81±0.44
Stomach	15.46±6.93	ND	3.26±0.69	15.99±9.17
Lung	5.61±1.53	ND	4.44±1.48	24.80±12.22
Serum	0.30±0.03	0.04±0.03	1.40±0.07	49.57±4.77

Fig. 5 Data represent mean±SEM of 5 samples per group. Serum concentrations are actually expressed as pmol/ml. ND, not detectable

Data from Piehl et al. (2008) present a role for deiodinases in TAM biosynthesis, defining biosynthetic pathways for T1AM and T0AM with T4 as a pro-hormone. Seemingly in contrast, a recently developed method to detect T1AM and T0AM in tissues and plasma (Ackermans et al., 2010) failed to reproduce the above data. Using rats treated with (13)C-labeled T4, the authors could detect in vivo conversion of T4 to T3 but not to T1AM in plasma or brain samples, neither any endogenous T1AM nor T0AM was detected in the plasma from rats and plasma and in thyroid tissue from humans. Indeed, iodothyronine decarboxylation to iodothyronamines has not been demonstrated directly, and the aromatic amino acid decarboxylase was shown to be unable to catalyse iodothyronine decarboxylation (Hoefig et al., 2012). In line with this, data from Hackenmueller et al. (2012) suggest that T1AM is not an extrathyroidal metabolite of T4, yet is produced within the thyroid by a process that requires a sodium–iodide symporter and thyroperoxidase, the same biosynthetic factors necessary for T4 synthesis. These data shed new light on the pathways potentially involved in T1AM production and imply that the enzymatic conversion of iodothyronine to iodothyronamine is not simple. Steady-state physiological T1AM serum concentrations are similar to those of T3, and tissue concentrations of its metabolite, T0AM, exceed T4 and T3 metabolites by two- and 20-fold respectively (Hart et al., 2006; Chiellini et al., 2007). Physiological receptor(s) of TAMs remain to be identified. In TR receptor binding/gene activation assays, T1AM showed no affinity for TRb and TRa, and inability to modulate nuclear TR-mediated transactivation (Chiellini et al., 1998). Studies surrounding TAM association with other receptors concluded that neither T0AM nor 3-T1AM activated Gαs-coupled dopamine D1 and β2 adrenergic receptors (Scanlan et al., 2004). T1AM, however, was found to be a potent agonist of trace amine-associated receptor 1 (TAAR1), an orphan G protein-coupled receptor (Zucchi et al., 2006). Rat and mouse TAAR1 are activated by T1AM, with EC50 values of 14 and 112 nM respectively. The T1AM ligand pharmacophore that activates TAAR1 was later characterized (Hart et al., 2006; Tan et al., 2007; 2008; Snead et al., 2008). TAARs are a family (from isoform 1 to isoform 9, of which three out of the nine members are pseudogenes Vanti (2003) of G-protein coupled receptor (GPCRs) which are target for several trace biogenic amines, precursors or byoproducts of neurotransmitter synthesis also found in some foods, whose activation provides a rapid on-off mechanism. TAARs are mainly expressed in the cardiovascular and in the central nervous system. At this latter site, TAARs' activation is supposed to be

involved in neurological disorders like bipolar disease, schizophrenia, depression and Parkinson's disease and, in consequence, potential new important therapeutic targets. The interaction of T1AM with TAAR was first demonstrated in 2004 and considered responsible for the cardiac inotropic effect of T1AM (Frascarelli_et al., 2008). In addition to this, TAAR activation might also be responsible for the hyperglycemia and the hypothermia induced by T1AM. However, Panas et al (2010) have demonstrated that T1AM-induced hypothermia is maintained in TAARs knockout mice and studies with radioactive T1AM have revealed a tissue distribution of radioactivity which does not match with that of TAAR (Chiellini et al., 2012).

TAAR$_1$

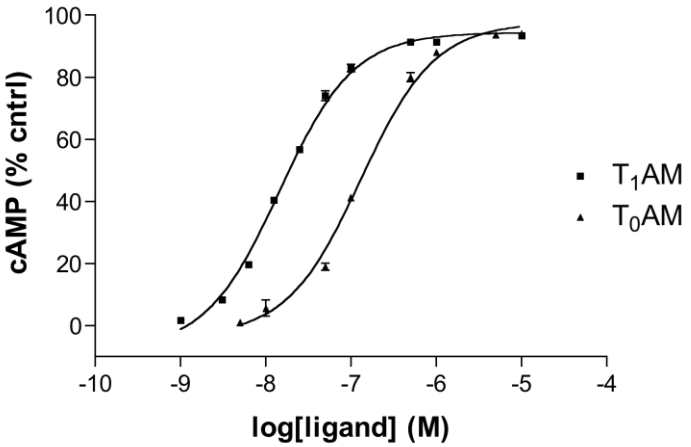


Fig. 6 Scanlan *et al.*, *Nature Med.* (2004) **10**, 638

TAAR$_1$-KO MICE

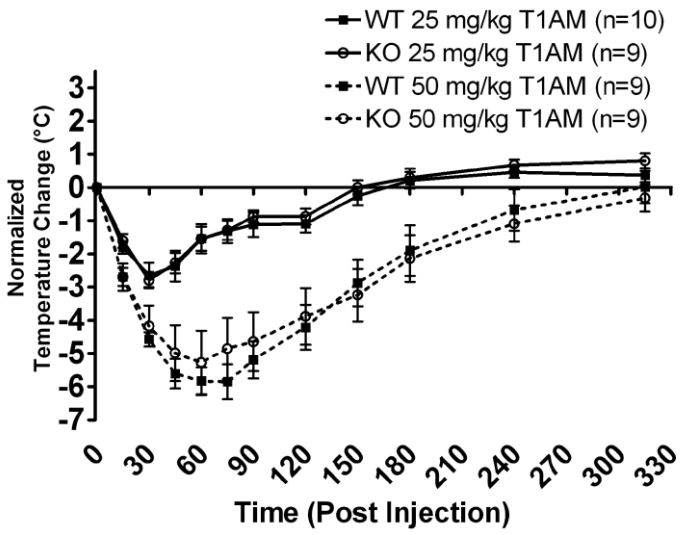


Fig. 7 Panas *et al. J. Neurosci. Res.* (2010) **88**, 196

Both studies argued in opposition to the hypothesis that T1AM might represent an endogenous ligand for TAARs. In addiction to putative action of TAAR1, there is evidence that thyronamines my also act through other signalling pathways. For istance, Regard et al. demonstrated that T1AM acts at the α_2 adrenergic receptor in pancreatic beta cells to produce hyperglycemia and hypoinsulinemia. T1AM was found to bind specifically to α_2 adrenergic receptor with Ki of 0.36 μM. Furthermore, in α_{2A} adrenergic receptor knock out mouse model, T1AM-induced hyperglycemia was abolished, indicating that the physiological effects of T1AM may be mediated, et least in part, via adrenergic signalling pathways. Thyronamines have also been suggested to modulate the function of other neurotransmitters. Snead et al. have demonstrated, in both synaptosomal preparations and heterologous expression systems, that thyronamines act as specific dopamine and norepinephrine transporter reuptake inhibitors, as well as inhibitors of the uptake of monoamines into synaptic vesicles by vesicular monoamine transporter 2. Each of these transporters appears to have a slinghthly different profile of sensitivity to a various thyronamines. It remains unclear whether the pharmacologic effects of thyronamine are related to direct effects of thyronamines on TAAR1 versus the α_{2A} adrenergic receptor, by modulation of relase of catecholamine neurotrasmitters or by some alternate pathway, not yet described.

Ianculescu et al. (2009) reported that the cellular uptake of T1AM occurs via specific, saturable, and inhibitable transport mechanisms that are sodium and chloride independent, pH dependent, TAM specific, and do not involve candidate transporters of monoamines, organic cations, or THs. By a novel RNAi screening method, eight transporters of interest were identified. Knockdown resulted in T1AM transport in HeLa cells, but the physiological role of these transporters remains unknown. Studies using COS-1 cells transfected with multispecific OATPs, 1A2, 1B3, and 1C1, and the specific TH transporters, MCT8 and MCT10, proved that T1AM differentially inhibits T3 and T4 cellular uptake by these transporters (Ianculescu et al., 2010). Notably, T1AM also inhibits both T3 and T4 uptake via MCT8, the most specific TH transporter. T1AM has no effect on TH transport by OATP1B3 and MCT10.

In mice, Scanlan et al. (2004) showed that a single i.p. injection of T1AM rapidly induced an ~10°C drop in body temperature that peaked 1 h after injection an dose dependently disappeared after 4–6 h. The same authors further showed that T1AM reduction on cardiac performance was a direct effect and independent of T1AM-induced hypothermia. In a rat working heart preparation held at 37°C, introduction of

T1AM into the perfusion buffer resulted in large and immediate decreases in both heart rate and systolic aortic pressure. Additional studies on the heart have further supported direct actions of T1AM on this organ (Chiellini et al., 2007; Frascarelli et al., 2008). A single i.p. dose of T1AM dramatically switched fuel utilization away from carbohydrates and toward lipids (Braulke et al., 2008). Siberian hamsters (Phodopus sungorus), a hibernating rodent species, and mice completely shifted their respiratory quotients (RQ) from a normal, mixed carbohydrate and lipid value (0.90 for hamsters and 0.83 for mice) to a complete and persistent lipid-related RQ value of ~ 0.7 with elevated urin ketone content. The RQ effect (4.5 h after injection) lagged behind hypothermia, bradycardia, or hyperglycemia (1 h after injection). I.v. infusion with a low T1AM dose (0.5 mg/kg) into non fasted naive rats rapidly increased endogenous glucose production and plasma glucose, plasma glucagon, and corticosterone, but did not affect plasma insulin (Klieverik et al., 2009).

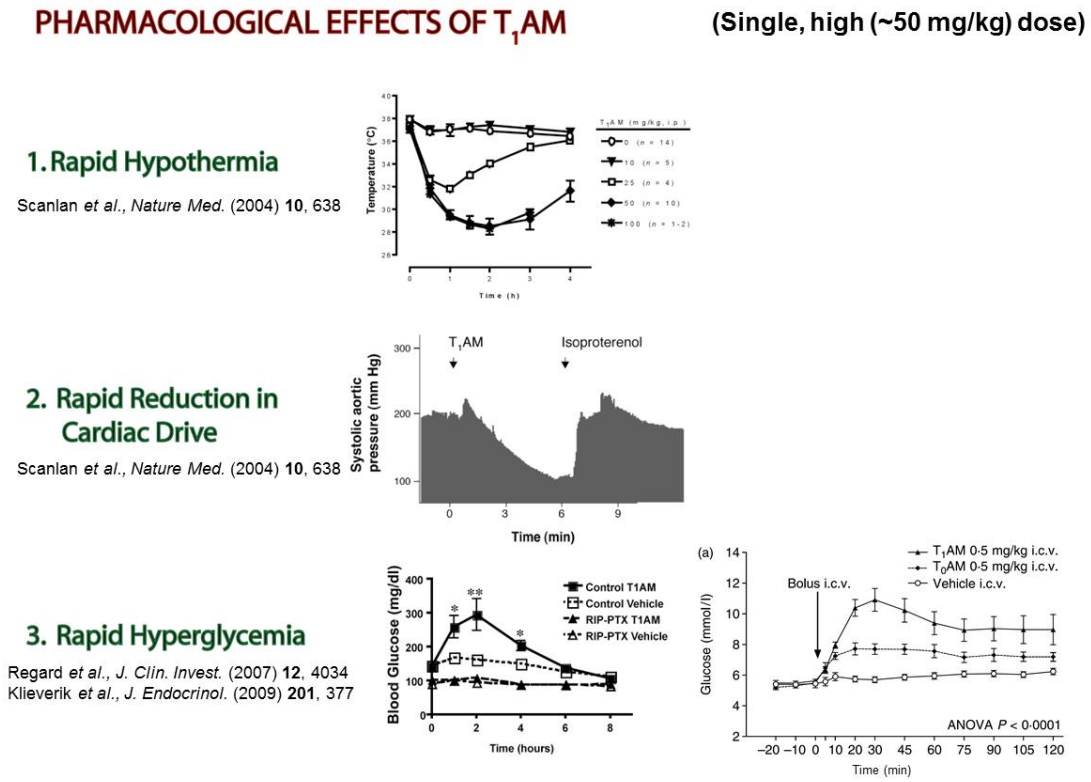


Fig. 8 Pharmacological effects of single high dose of T_1AM.

It is known to possess a central effect, namely hypophagia, as well as peripheral effects of raised plasma glucose levels and reduced peripheral insulin sensitivity (the latter being also seen after i.p. injection (Braulke et al., 2008, Klieverik et al., 2009)), accompanied by pancreatic insulin production. Non fasted, drug-naive rats (Klieverik et al., 2009) treated with T1AM (100 mg/kg) acutely increased endogenous glucose production and hyperglucagonemia. T0AM had a similar effect that was less profound (Klieverik et al., 2009). This same group had previously demonstrated that thyroid hormone could modulate liver glucose production, potentially mediated by sympathetic hypothalamic pathways initiated in the paraventricular nucleus of the hypothalamus. They now hypothesize that the thyronamines also mediate their metabolic effects via central mechanisms, likely via the hypothalamus.

Based on these experments, an emerging theory is that many of the functions of T1AM may be mediated centrally. An addiction example of central actions of T1AM were presented in a study by Dhillo et al. (2009), wherein very low doses of T1AM were suggested to act as orexigenic factors potentially mediated by the arcuate nucleus of the hypothalamus. Intraperitoneal injection of T1AM resulted in an increase in food intake only at a very low dose, 4 nmol/kg (1.3µg/kg), and was not associated with a change in metabolic rate (VO2) or activity level. Both i.c.v. and intra-arcuate nuclear administration of T1AM were also associated with increases in food intake, suggesting that T1AM may be acting centrally to mediate its orexigenic effect, although there was not a clear dose response for the effect. C-fos was followed as a marker of cellular activity and there was an increase in arcuate nucleus straining in T1AM treated animals. This suggests that T1AM may exert at least some effects in specific regions of the brain.

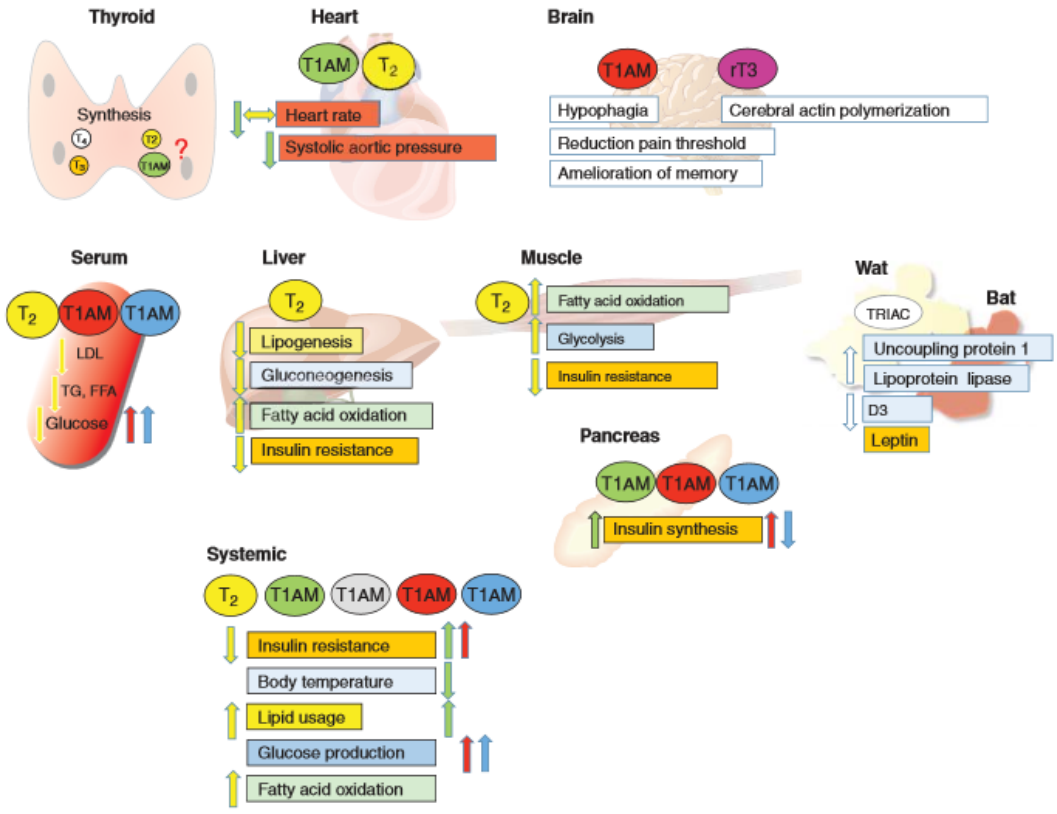


Fig. 9 Thyroid synthesis of thyroid hormones (THs) and administration effects of nonclassical THs. Question mark, thyroid synthesis of T2 and T1AM is currently still under debate. Administration of non-classical THs indicated in color. White, Triac incubation in rat primary cultures; yellow, i.p. administration of T2 in rats on a high-fat diet; green, i.p. or i.v. administration of T1AM in mice; grey, i.v. administration of T1AM in mice; red, i.c.v. administration of T1AM to fasted mice; blue, i.c.v. administration of T1AM to mice; and purple, rT3 administration to mice. Arrows pointing upward, upregulation; arrows pointing downward, downregulation; double horizontal arrows, no change; and absence of arrows, no effect reported to date.

T1AM metabolism

According to evidence obtained in vitro and in vivo, T1AM is rather a good substrate for amine oxidases (membrane-bound and mitochondrial monoamine oxidases, SSAO and MAO respectively) enzyme operating an oxidative deamination on the primary amine producing the corresponding aldehyde/acid, hydrogen peroxide and ammonia. Of course, the different cell localisation of such enzyme activities might impact on T1AM concentration at target(s). Moreover, the intracellular metabolism of T1AM can be considered as a reaction of nonmicrosomial Phase 1 metabolism. Oxidative deamination is not the only intracellular fate of T1AM. Infact, once inside cells, T1AM can be deiodinated to thyronamine (T0AM), another primary amine substrate for SSAO and MAO (Fig.10).

At the moment, similar pharmacological features for T1AM and for T0AM have been described. Instead, evidence are lacking on the involvement of some product(s) of T1AM oxidative deamination on T1AM pharmacological effects. In addition to the oxidative deamination, T1AM may be also sulphonylated, thus suggesting that this pathway may conclude T1AM activity by favouring its renal excretion (Phase II metabolism without Phase1). We have produced evidence that inhibition of MAO activity may influence T1AM pharmacological effects in rodents treated in vivo. Then, if from one side tissue levels of T1AM may change according to the expression levels of enzyme of its metabolism, from the other, T1AM tissue levels may also change following pharmacological inhibition of enzyme activities. In this respect it would be interesting the measure T1AM plasma levels in animal models of thyroid dysfunctions, in diabetes and in other chronic inflammatory diseases accompanied by alteration of SSAO and/or MAO activity.

From the pathways of T1AM metabolism, derives that T1AM oxidation may locally increase reactive oxygen (ROS) and carbonyl (CRS) species levels and that some of its metabolites might have its own pharmacological/physiological features.

If it is well known the role of ROS and RCS in cell signalling and in unbalancing cell redox state, the potential of acid derivatives of T1AM and T0AM in activating acid-sensitive cell mechanisms remains an attracting view in the definition of T1AM features. In particular, acid compounds are modulators of specific channels (ASIC) for the modulation of cells of the ENaC/degenerin superfamily of cation channels that in

the recent years have drawn increasing attention as potential therapeutic targets in a variety of diseases and conditions which have acidosis as a common determinant. In fact, activation of homomeric ASIC1 channels by H+, leads to elevations of intracellular calcium and then to cell death. Because of this, therapeutic opportunities of modulators of ASIC channels ranges from hypertension, stroke, anxiety disorders and pain associated with ASIC activity (Quadri et al., 2012). It is just the pain one of the most interesting features of T1AM.

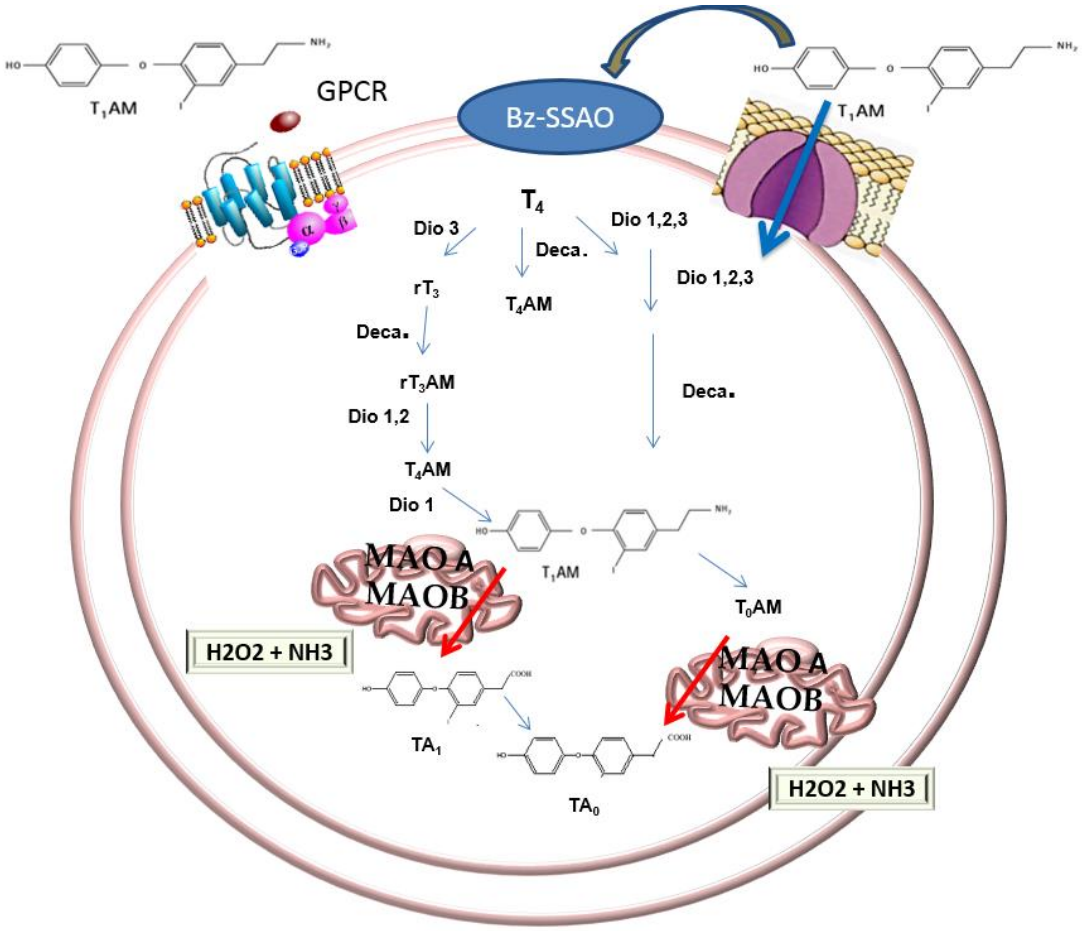


Fig. 10 Thyronamine metabolism.

1.5 Histaminergyc System

Histamine (CID 774) is synthesized from the aminoacid histidine through oxidative decarboxylation by histidine-decarboxylase (HDC; EC 4.1.1.22), a pyridoxal 5-phosphate (PLP)-dependent enzyme (Fleming JV., 2004) found in many species and highly conserved throughout the animal kingdom from mollusc, insect, rodent, to human (Almeida AP., 1981). Restricted and cell-specific expression of HDC in peripheral tissues is controlled at both transcriptional (DNA methylation) (Hutcheon B., 1994; Suzuki-Ishigaki S., 2000) and posttranslational levels [ubiquitin-proteasome (Olmo MT., 2000), caspases]. Little is known about specific regulation of HDC gene expression in the brain. However, neuroactive peptides, such as gastrin and pituitary adenylate cyclase-activating polypeptide (PACAP) (McLaughlin JT., 2004), steroids, such as glucocorticoids (Karlstedt K., 1999), and other factors control HDC gene promoter activity and also protein degradation in various tissues and contexts (e.g., oxidative stress) (Ai W., 2004). The rate of histamine synthesis, in contrast to that of other biogenic amines, is determined by the bioavailability of the precursor; histidine is taken up into the cerebrospinal fluid and neurons through L-amino acid transporters (Fig. 11). HDC activity can be inhibited by α-fluoromethylhistidine (α-FMH), a suicide substrate leading to a marked depression of histamine levels (Kollonitsch J., 1978). α-FMH has proven a useful tool to study histaminergic functions (Garcia M, Floran B., 1997) but is difficult to synthesize and not commercially available at present. Neuronal histamine is stored in cell somata and especially in axon varicosities (Diewald L., 1997), where it is carried into vesicles by exchange of two protons through the vesicular monoamine transporter VMAT-2 and released upon arrival of action potentials (Erickson JD., 1996). The level of histamine in brain tissue is somewhat lower than that of other biogenic amines, but its turnover is considerably faster (in the order of minutes) and varies with functional state (Dismukes K., 1974). Brain histamine levels measured with implanted microdialysis tubes exhibit a marked circadian rhythmicit in accordance with the firing of histamine neurons during waking (Mochizuki T., 1992). Extracellular histamine levels in the preoptic/anterior hypothalamus follow the oscillations of different sleep stages [wakefulness > non-rapid eye movement (REM) sleep >REM sleep], but invariant histamine levels during sleep deprivation suggest that histamine may relay circadian rather than homoeostatic sleep

drive (Porkka-Heiskanen T., 1994). Philippu and Prast (Philippu A.,1991) have demonstrated a direct correlation between histamine levels in the hypothalamus and behavioral state by electroencephalography. Synthesis and release of histamine are controlled by feedback through H3 autoreceptors located on somata and axonal varicosities (Arrang JM., 1997). Furthermore, the release of histamine is affected by transmitters impacting histamine neuron firing and/or release from varicosities bearing inhibitory m1muscarinic, α2-adrenergic, and peptidergic receptors (Arrang JM., 1991). Inactivation of histamine in the extracellular space of the CNS is achieved by methylation through neuronal histamine *N*-methyltransferase (HNMT; EC 2.1.1.8) (Barnes WG., 2002) (Fig. 11). Histamine methylation requires *S*-adenosyl-methionine as the methyl donor (Schwartz JC., 1991). Blockers of HNMT reduce tele-methylhistamine and increase histamine levels in the brain. Histamine hardly passes the blood-brain barrier, but HNMT is also found in the walls of blood vessel where blood-borne histamine and histamine released from mast cells is methylated and inactivated (Nishibori M., 2000). Moreover, a vectorial transport system (shuttle) from the brain to the vasculature may help to drain neuronal histamine after excessive surges. Tele-methylhistamine in the brain undergoes oxidative deamination through a monoamine oxidase (MAO-B) to *t*-methyl-imidazoleacetic acid (Schwartz JC., 1991). The main histamine-degrading enzyme in peripheral tissues (gut, connective tissues) and in invertebrates is diamine oxidase (DAO), which directly converts histamine into imidazoleacetic acid. DAO activity in the brain is negligibly low under basal conditions, but when HNMT is inhibited may represent a salvage pathway for production of imidazoleacetic acid, an effective GABAA receptor agonist (Prell GD., 1997).

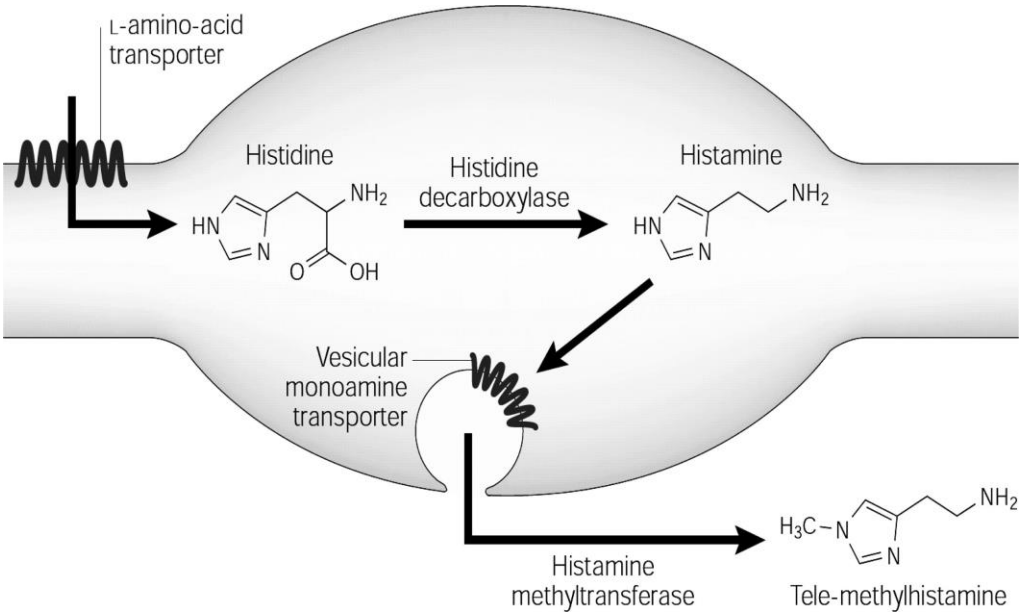


Fig. 11 Histamine synthesis and metabolism. Histidine is taken up in a varicosity and decarboxylated; histamine is transported into a vesicle, released, and methylated.

Four metabotropic histamine receptor types (H1RH4R) have been cloned so far. H1-H3R are expressed in abundance in the brain. H4R occurs mainly in peripheral tissues (De Esch IJ., 2005). All metabotropic histamine receptors (H1RH4R) belong to the rhodopsin-like family of G proteincoupled receptors (GPCR) (Hill SJ., 1997). Each receptor consists of seven large transmembrane-spanning elements with prototypic domains determining agonist binding specificity and activation (Jongejan A., 2005), G protein coupling and constitutive activity (Gbahou F., 2003), as well as covalent modifications (e.g., through phosphorylation by proteinkinases), homo- and heterodimerization, trafficking and membrane anchoring, as well as receptor sensitization and desensitization (e.g., through agonist-induced internalization) (Kuramasu A., 2006). A high degree of molecular and functional heterogeneity achieved through different transcriptional and posttranscriptional processing (splice variants) is prototypic for the H3R, which is largely confined to the nervous system (Leurs R., 2005).

Histamine and Thyroid Axis

Thyroid functions, as mentioned previously, play a role in energy metabolism, thermogenesis, and bone physiology. TRH is synthesized in preoptic, paraventricular, and periventricular neurons, from where it is transported and released into the hypophysial portal circulation. The majority of the Tubero mammillary neurons (TMN) are excited by TRH (Mignot E., 2002), and hypothalamic neuronal histamine in turn has predominantly inhibitory effects on the hypothalamo-pituitary-thyroid (HPT) axis (Knigge U., 1991). Histamine decreases TRH release and TSH plasma levels through H2R in both hypothalamic and pituitary targets. Cimetidine facilitates cold-induced and TRH-induced TSH responses (Nakada K., 1990). Systemic L-thyroxine administration, along with rises in T3 and T4 levels, increases cortical 5-HT and histamine content, whereas carbimazole treatment lowers histamine, glutamate, and 5-HT levels, suggesting a T3/T4-mediated negative feedback on TRH production by histamine. TRH is also a cotransmitter of glutamatergic neurons located in DMH (Chou TC., 2003) and serotonergic neurons in the raphe implicated in TRH-induced suppression of food intake by histamine (Gotoh K., 2007) and effects on behavioral state (Riehl J., 2000).

Histamine and Memory

Histaminergic modulation of learning and memory is evident from lesions and pharmacological interventions in the tuberomamillary (Klapdor K., 1994) and other decisive brain regions and from studies in histamine and histamine receptor-deficient mice (Liu L., 2007). Confusingly, histamine can have both inhibitory and facilitatory effects on learning and memory. Seemingly conflicting evidences may be explained by differences in species and gender but also context- and task-inherent reinforcement contingencies, particularly novelty.

Histamine-deficient mice lack the ability to stay awake in a novel environment associated with defects in hippocampal theta rhythm, cortical activation, and episodic object memory (Parmentier R., 2002). Novelty-induced arousal reinforces learned appetitive behaviors, such as conditioned place preference, and novelty detection and comparator functions have been attributed to the hippocampus, where histamine exerts powerful effects (Selbach O., 2007). HDC-KO mice show improved negatively reinforced performance in a water-maze and retention of contextual fear memory, along with enhanced hippocampal CA1 LTP before and decreased LTP after training (Liu L., 2007). Injection of histamine (icv) immediately after training normalizes conditioned contextual fear responses. Acute histamine infusion into the CA1 region of rats immediately after training, but not later, enhances consolidation of inhibitory avoidance memory through an H2R-dependent mechanism. This suggests a narrow time window at which histamine reinforces episodic memory and learned behaviors (Dere E., 2003). Thioperamide (an H3R inverse agonist) enhances memory retention when administered after acquisition. In the amygdala, H3R activation enhances consolidation of fear memory, and H3R antagonists impair fear memory but through protean agonism may also facilitate it (Baldi E., 2005). Systemic administration of *R*-α-methylhistamine, an H3R agonist, improves spatial memory in rats (Rubio S., 2002). Thus brain histamine, associated with heightened states of vigilance, is required to learn the new, which (through remembrance of things past) implies discrimination and comparison of what, where, and when in previous and novel contexts (novelty detection) and consolidation of episodic memory (through mechanisms of synaptic plasticity).

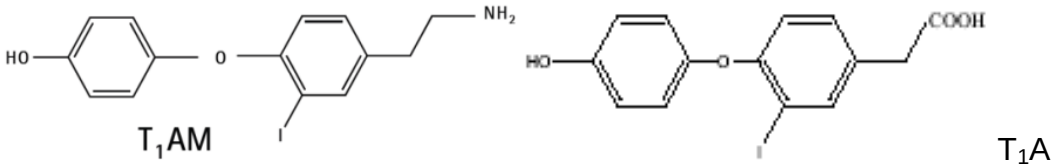
Histamine and Pain

Histamine mediates itch and modulates pain in the periphery and in the CNS. Broad functional overlap but also a striking anatomical and molecular specificity characterizes these distinct sensations (Ikoma A., 2006). In the periphery histamine specifically activates and sensitizes itch-specific nociceptive C fibers. Itch and pain appear to employ similar molecular and mechanistic signatures but exhibit largely antagonistic interactions and recruit distinct neural pathways (Andrew D., 2001). Both histamine and opioids can generate itch, while scratch-induced pain and antidepressants with antihistaminic properties can abolish itch (Sawynok J., 2001). In contrast to histamine actions on nociceptive fibers, the central histamine system plays a role in antinociception and stress-induced analgesia (Hough LB., 2000). Antihistaminic properties of antidepressants may in turn contribute to the analgesic effects of these drugs (Sawynok J., 2001). Central sites of itch and pain modulation by histamine include first-order itch-specific lamina I neurons in the dorsal horn of the spinal cord and spinothalamic itch sensitive pathways up to higher order subcortical and cortical circuitries (Mochizuki H., 2003). Histamine applied into the cerebral ventricles or periaquaeductal grey is analgesic. Analgesic and hyperalgesic effects of central histamine are mediated through H2R and H1R, respectively, in keeping with altered pain sensitivity in H1R- and H2R-KO mice (Mobarakeh JI., 2005). Analgesic or nociceptive effects of many neuropeptides rely on histaminergic transmission. Morphine can increase the release and metabolism of brain histamine when applied systemically or more locally in the periaqueductal grey (Barke KE., 1994) and slightly depolarizes TMN neurons, whereas the opioid peptide nociceptin causes a hyperpolarization (Eriksson KS., 2000), which may contribute to the antagonism of opioid-induced analgesia. Histamine release has been shown to be under the control of facilitatory presynaptic μ-opioid receptors and inhibitory μ opioid receptors; the latter are also gating GABAergic inputs on TMN neurons by orexins/ hypocretins (Eriksson KS., 2004). Hypocretin-induced antinociception is naloxone insensitive but enhanced in H1R- or H2R-KO mice and under pharmacological blockade of H1R and H2R (Mobarakeh JI., 2005). Reductions in brain histamine levels by administration of α-FMH or H3R agonists promote nociception. Increases in brain histamine produced by loading with L-histidine or application of HNMT inhibitors or H3R antagonists have

analgesic effects (Malmberg-Aiello P., 1994, 1998). H3R represent a promising target in pain therapy (Cannon KE., 2007).

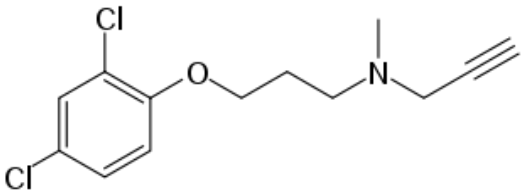
2. AIM

In order to get further insight the relation between TH and thyronamines, during my Ph.D. program, I was involved in the exploration of the behavioral and metabolic effects induced by pharmacological administration of low doses of T_1AM and T_1A.



T_1AM T_1A

We started studying the effect of T1AM and T1A on feeding, on plasma glycemia, learning and pain sensitivity, and to better ascertain the role of T_1AM metabolism in its pharmacological effects, we repeated our observation in animals pre-treated with clorgyline (2.5 $mg \cdot kg^{-1}$) an inhibitor of MAO.



Clorgyline

3. METHODS

3.1 Animals

Male mice (CD1 strain; 20–30 g) from Harlan-Nossan (Italy) were used. Five mice were housed per cage. Cages were placed in the experimental room 24 h before the test for adaptation. Animals were kept at 23±1°C with a 12 h light–dark cycle (light on at 07:00 h) and were fed a standard laboratory diet with water ad libitum. Experiments and animal use procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996). The experimental protocols were approved by the Animal Care Committee of the Department of Pharmacology, University of Florence, in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS no. 123) and the European Communities Council Directive of 24 November 1986 (86/609/EEC). The authors further attest that all efforts were made to minimize the number of animals used and their suffering.

$HDC^{-/-}$ mice

Histidine decarboxylase ($HDC^{-/-}$) knock-out and their wild type littermate mice ($HDC^{+/+}$; 129/Sv background; 18-22 g of body weight) were a kind gift from Dr. Ohtsu, Japan (Ohtsu et al., 2001). The animals were housed by four to six in standard transparent laboratory cages in a temperature-controlled room (22 ± 1°C) and kept in a 12 h light-dark cycle (lights on at 7 am) with access to food and water ad libitum.

Detection of the HDC gene

The genotypes of the null mice used with respect to the HDC gene were confirmed at the mice birth amplifying the DNA prepared from tale biopsies by polymerase chain reactions (PCR) prepared and visualized according to Anaclet et al. (2009).

3.2 Detection of endogenous TA1 and T1AM in mice brain

Four mice from each of the CD1, HDC+/+ and HDC -/- strain (20–30 g) were sacrificed by cervical dislocation. The brains were isolated and quickly frozen at -80°C. TA1 and T1AM were then assayed by HPLC coupled to tandem mass spectrometry, as described previously (Saba et al, 2010), with the only difference, that tissue was homogenized with ultrasounds, extracted with acetonitrile and then washed with hexane to remove lipids.

3.3 I.c.v. injection technique

I.c.v. administration was performed under light ether anaesthesia according to the method described by Haley and McCormick (1957) with minor modifications. The depth of anesthesia was checked by monitoring respiratory rate (which was reduced within 2 min) and testing the lack of pain response to gentle pressure on the hind paws. The head of the anesthetized mouse was grasped firmly and the needle of a 10 µl microsyringe (Hamilton) was inserted perpendicularly 2 mm through the skull into the brain. Ten microliters of solution were then slowly injected (in 20 s) into a lateral ventricle. The injection site was 1 mm to the left from the midpoint on a line drawn through to the anterior base of the ears. Immediately after needle removal, the animal remained quiet for approximately 1 min and then resumed its normal activity. To ascertain that solutions were administered exactly into the cerebral ventricle, some mice were injected with 10 µl of 1:10 India ink and their brains were examined macroscopically after sectioning. The accuracy of the injection technique was confirmed, since 95% of injections turned out to be correct.

3.4 Evaluation of food consumption

Mice (5 per cage) were starved for 16 h (from 5 pm to 9 am), but water was available *ad libitum.*

A weighed amount of food (standard laboratory pellets) was then given and the amount consumed (evaluated as the difference between the original amount and the food left in the cage without littermate, including spillage) was measured at 30, 60, 90, 120 min after i.c.v. (3nd ventricle) administration to anestethised mice of 10 µl of 0.5% DMSO wv^{-1}in saline solution (vehicle) or T1AM (1.3, 6.6, 13.2, 20 and 26 µg kg^{-1} prepared in vehicle), with an accuracy of 0.1 g. Both T1AM and vehicle-injected mice received i.p. treatment with saline solution or with clorgyline (2.5 mg kg^{-1}; Sigma-Aldrich, St Louis, MO, USA) 30 min before T1AM i.c.v. injections. Results are the mean ± SEM of two experiments carried out 10 animals for each injection.

3.5 Glycemia and collection of samples for T1AM plasma level determination

Blood samples were collected from the tail vein of 16 h fasted mice (from 5 pm to 9 am) pre-treated i.p. with clorgyline (2.5 $mgkg^{-1}$) or with saline, 30 min before i.c.v. of vehicle (n=15) or T1AM 1.3 (n=15) or T1AM 20 μgkg^{-1} (n=15). Five mice in each group were sacrificed by decapitation 30 min after T1AM injection to collect the blood and the brains in order to measure plasma T1AM tissue levels.

In another set of experiments, glycemia was monitored in the blood collected from the tail vein of 4 h (from 8 am to 12 am) fasted mice who received T1AM (1.3 μgkg^{-1} i.c.v) and saline (i.p.), or T1AM (1.3 µgkg-^{1} i.c.v) and Exenatide (0.25 $mgml^{-1}$ i.p.), or vehicle i.c.v. and Exenatide (0.25 $mgml^{-1}$ i.p.) (n=5 in each group).

Glycemia was evaluated by a glucorefractometer at 0, 15, 30 and 60 min after i.c.v. injections.

3.6 Insulin Tolerance test

An i.p. insulin tolerance test was performed in 4 h fasting mice (from 8 am to 12 am), a condition chosen to avoid hypoglycemia following insulin treatment.

Three experimental groups were considered (n=5 mice per group): one group was injected i.c.v. with vehicle, one with 1.3 $\mu g kg^{-1}$ T1AM, and one with T1AM 20 $\mu g\ kg^{-1}$ T1AM under light ether anaesthesia. All these animals received insulin (1 $U kg^{-1}$ Humulin R Regular, Ely Lilly) i.p. 10 min after i.c.v. injections.

Small blood samples from the lateral tail vein were collected to measure glycemic levels at time 0, 15, 30, 60, 90 and 120 min after i.p. insulin injection. A glucose solution (33% w v^{-1}) was prepared in order to correct symptomatic hypoglycemia. Results are shown as glucose concentration (mg dl^{-1}) and as the difference from the baseline value.

3.7 Plasma levels of thyroid hormones

To evaluate free plasma three iodothyroxine (fT3), thyroxine (fT4) and thyroid stimulating hormone (TSH) levels, 3 sets of experiments were performed on 4 h fasted mice (n=5 mice in each experimental group):

Exp. 1: mice pre-treated with saline i.p., 30 min before i.c.v. injection with vehicle, 1.3 $\mu g kg^{-1}$ T1AM, or 20 $\mu g kg^{-1}$ T1AM

Exp. 2: mice pre-treated with clorgyline (2.5 mg kg^{-1}) i.p., 30 min before i.c.v. injection with vehicle or 1.3 $\mu g kg^{-1}$ T1AM.

Exp 3: mice treated with exenatide (0.25 $mg ml^{-1}$; i.p.) before i.c.v. injection of vehicle or with 1.3 $\mu g.kg^{-1}$ T1AM.

All the animals were sacrificed by decapitation within 60 min after i.c.v. injections. The blood was collected and centrifuged at 3000xg x 20 min at room temperature to prepare plasma.

Circulating fT3, fT4 and TSH levels were quantified in 100 µl of mouse plasma, by a solid-phase, two-site chemiluminescent immunometric assay (Immunolite 2000; Siemens Corp., Tarrytown, NY). The manufacturer's reference limit were 2.3-4.2 $pg ml^{-1}$, 0.5 $nmol L^{-1}$ and 0.40 $mU L^{-1}$ respectively for fT3, fT4 and FSH. The assay was

validated by unchanged plasma fT3 and fT4 levels recovered over 1:10 plasma. Intra-assay variability was in the range of 8%.

3.8 Behavioral tests

The passive avoidance paradigm

The test was performed according to the step-through method described by Jarvik and Kopp (1967). The apparatus consists of a two-compartment acrylic box with a lighted compartment connected to a darkened one by a guillotine door. Mice receive a non-painful punishment consisting of a fall (from 40 cm) into a cold water bath (10 °C), since the dark chamber was constructed with a pitfall floor. Mice receive the punishment when entering the dark room in the training session and remember it in the session 1 h later or on the following day. Before trials, mice were removed from the cage and injected i.c.v. (3^{nd} ventricle) with 10 µl of vehicle, T1AM (0.13, 0.40, 1.32 and 4 µg kg^{-1} prepared in vehicle; n=15 for each treatments), or TA1 (0.4, 1.32 and 4 µg kg^{-1}; n=20 for each treatments). In another set of experiments, mice were pre-treated with or intraperitoneal (i.p.) injection of clorgyline (2.5 $mgkg^{-1}$) or scopolamine (0.3 $mgkg^{-1}$; Sigma–Aldrich, St. Louis, MO, USA) or saline a s.c. injection of saline or of pyrilamine (10 $mgkg^{-1}$; Sigma–Aldrich, St. Louis, MO, USA) or zolantidine (5 $mgkg^{-1}$; Sigma–Aldrich, St. Louis, MO, USA), H_1-antagonist and H_2-antagonists respectively and, after 15 min, they all were injected i.c.v. with vehicle or T1AM or TA1. In all cases, the training session was performed 15 min after T1AM or TA1 i.c.v. injection placing mice on an illuminated platform (60 W, 840 lux) and allowing them to enter the dark compartment. The extent of punishment memory was expressed as the time (sec) spent in the light portion during the training and the retention sessions. Retention sessions were performed 1 h and 24 h after the training session. In the 1 h and 24 h tests each animal was placed on the platform and the latency to enter the dark compartment was measured up to a maximum of 300 s.

T1AM and TA1, kindly provided by Dr. Thomas Scanlan (Portland, OR, USA), were dissolved in vehicle.

The novel object recognition task

The experiments were carried out as described by Ennaceur and Delacour (1988), with minor modifications. Male mice were subjected to the procedure separately and care was taken to remove any olfactory/taste cues by cleaning the arena and test objects with alcohol between trials and mice. Each mouse completed one session with three successive trials. In trial 1, habituation phase, the mouse was placed in the center of the empty "open-field" arena placed in a sound-attenuated room under dimmed lighting for 2 min. On the following day, mice (n=10) were injected with T1AM 1.32 μgkg^{-1} 30 min before the second trials. During trial 2, the sample phase, two of the same objects (such as cubs or cylinders plastic shapes) were placed in opposite corners of the box and the mouse was allowed to explore the objects. After 24 h, each mouse was again placed in the test arena for 5 min, respectively (t3), in the presence of one of the familiar and a novel object, and the time spent exploring both objects was again recorded. The presentation order and position of the objects (left/right) was randomized to prevent bias from order or place preference. Each trial was 5 min with a 3 min inter-trial interval (ITI; this time point was chosen to allow for the largest behavioral window to detect performance variance in aged mice). The mouse was removed from the testing box and placed in a holding cage during the ITI. Object exploration was defined as the state in which the mouse's nose was kept within 2 cm of the object. Locomotor activity in the arena was also recorded. The most effective dose of T1AM 1.32, μgkg^{-1} was selected on the basis of our previous experiments (Manni, 2011).

The Hole-board

The hole-board test was performed according to Galeotti (2006). The test consisted of a 40-cm square plane with 16 flush mounted cylindrical holes (3 cm diameter) distributed four by four in an equidistant, grid-like manner. Mice were placed on the center of the board one by one and allowed to move about freely for a period of 10 min each. Two electric eyes, crossing the plane from midpoint to midpoint of opposite sides, thus dividing the plane into four equal quadrants, automatically signaled the movement of the animal (counts in 5 min) on the surface of the plane (locomotor activity). Miniature

photoelectric cells, in each of the 16 holes, recorded (counts in 5 min) the exploration of the holes (exploratory activity) by the mice. The animals were injected i.c.v. with vehicle or T1AM at the dosages of 0.4, 1.32 or 4 μgkg^{-1} (n=10 in each group) and tested 30 min after i.c.v. injections.

The hot plate

After introducing mice to a hot-plate device (51,5 ±1°C), the latency of a flinching or jumping response was measured. The cut-off time was set at 45 s to minimize skin damages. The animals were injected i.c.v. with vehicle or T1AM (0.13, 0.4, 1.32, $4\mu gkg^{-1}$; n=10 in each group) or T1A (0.4, 1.32, 4 μgkg^{-1}; n=20 in each group). Measurements were performed 15 min after i.c.v. injections.

3.9 T1AM-activated signaling in the brain of mice

Preparation of mouse brain tissues

Male mice (mean weigh, 23 ± 3 g), were removed from the cage and injected i.c.v. (3^{nd} ventricle) with 10 µl of DMSO wv^{-1}in saline solution (vehicle; Veh.) or T1AM (0.044, 0.13 and 1.32, µg kg^{-1} prepared in Veh.). Mice (n=5 for each treatments) were then sacrificed by decapitation, within 30 min from i.c.v. injections. The skull was opened with scissors and the brain surface was cooled down by exposing 1–2 s in situ to liquid nitrogen vapors. Brain areas required to conduct Western blotting experiments were dissected to separate specific areas; the brain was removed and the forebrain was divided into two parts: frontal cortex and the diencephalon. Hippocampus and amigdala were then quickly removed. The hypothalamus was separated from thalamus, and all tissues were flash-frozen in liquid nitrogen and stored at -80 °C until usage.

Brain areas were homogenized in an homogenization buffer containing (in mM): 50 Tris–HCl (pH 7.5), 150 NaCl, 1 EDTA, 5 sodium pyrophosphate (NaPP), 10 β-glicerophosphate, 1 Na_3VO_4, 0.2 phenylmethylsulfonyl fluoride (PMSF), 25 µg/ml leupeptin, 10 µg/ml aprotinin, 0.1% SDS. Homogenates were then centrifuged at 1000

x g for 10 min at 4°C to remove cell debris and supernatants were used for western-blot analysis (brain lysates).

Western blot analysis

Brain areas or hippocampal slice lysates (20 μg), prepared as described above, were separated on 10% SDS–PAGE and transferred into nitrocellulose membranes (120 min at 100 V) using standard procedures. Membranes were blocked in PBST (PBS containing 0.1% Tween) containing 5% non-fat dry milk for 60 min. Following washing, blots were incubated overnight at 4°C with specific antibody against ERK1/2 phosphorylated on Thr^{202}/Tyr^{204} (pERK1/2, Cell Signalling Technology). The primary antibody was diluited in TTBS containing 3% albumin. After being washed with PBS containing 0.1% Tween, the nitrocellulose membrane was incubated with polyclonal goat anti-rabbit horseradish peroxidase-conjugated secondary antisera (1:2.000, diluited in TTBS containing 5% non-fat dry milk) and left for 1 h at room temperature. Blots were then extensively washed and developed using an enhanced chemiluminescence detection system (Pierce, Italy). Exposition and developing time used was standardized for all the blots. Densitometric analysis of scanned images was performed on a Macintosh iMac computer using the public domain NIH Image program. α-tubulin was used as loading control. Protein concentration was quantified using Bradford's method (protein assay kit, Bio-Rad Laboratories, Italy).

3.10 Statistical analysis

Data were expressed as mean ± SEM of independent experiments. Statistical analysis was performed by one-way ANOVA, followed by Student-Newman-Keuls multiple comparison *post hoc* test; the threshold of statistical significance was set at $p<0.05$. Data analysis was performed by GraphPad Prism 5.0 statistical program (GraphPad software, San Diego CA USA).

4 RESULTS

4.1 T1AM produced hypo- and hyperphagia

Increasing doses of T1AM (1.3, 6.5, 13, and 20 μgkg^{-1}; n=10 mice for each dose) produced opposite effects on feeding. In particular, after 120 min the total amount of food eaten (gmouse^{-1}) by mice receiving 1.3 and 20 μgkg^{-1} T1AM was significantly ($P<0.05$) lower (2.74 ± 0.084) and higher (3.83 ± 0.02), respectively, than the control value (3.29 ± 0.08). The hypophagic effect of T1AM 1.3 μgkg^{-1} derived from increased satiety occurring within the first 30 min after injection. In fact, mice injected with T1AM 1.3 μgkg $^{-1}$ almost stopped feeding within the first 30 min, while those injected with T1AM 20 μgkg^{-1} and with vehicle reached satiety within 60 min. Both effects on feeding reached the threshold of significance vs. vehicle-injected mice after 60 min after injection, and the difference persisted for the whole duration of the experiment (120 min) (Fig. 1, panel A).

T1AM effects on feeding behavior of fasted mice did not show a linear dose-effect relationship but rather a biphasic response.

In mice pre-treated with clorgyline (2.5 mg kg^{-1}; Ilani et al 2000), the hypophagic effect of T1AM 1.3 μmol kg^{-1} was conserved, and it reached the threshold of statistical difference vs. vehicle-injected mice within the first 30 min after injection (Fig. 1, panel B), while satiety occurred within 60 min. Conversely, the hyperphagia produced by 20 μmol kg^{-1} T1AM was completely prevented (Fig. 1, panel B).

Clorgyline did not interfere with food intake of fasted mice injected with vehicle.

All further investigations were performed using T1AM dosages able to produce effects on feeding, namely 1.3 and 20 μmol kg^{-1}.

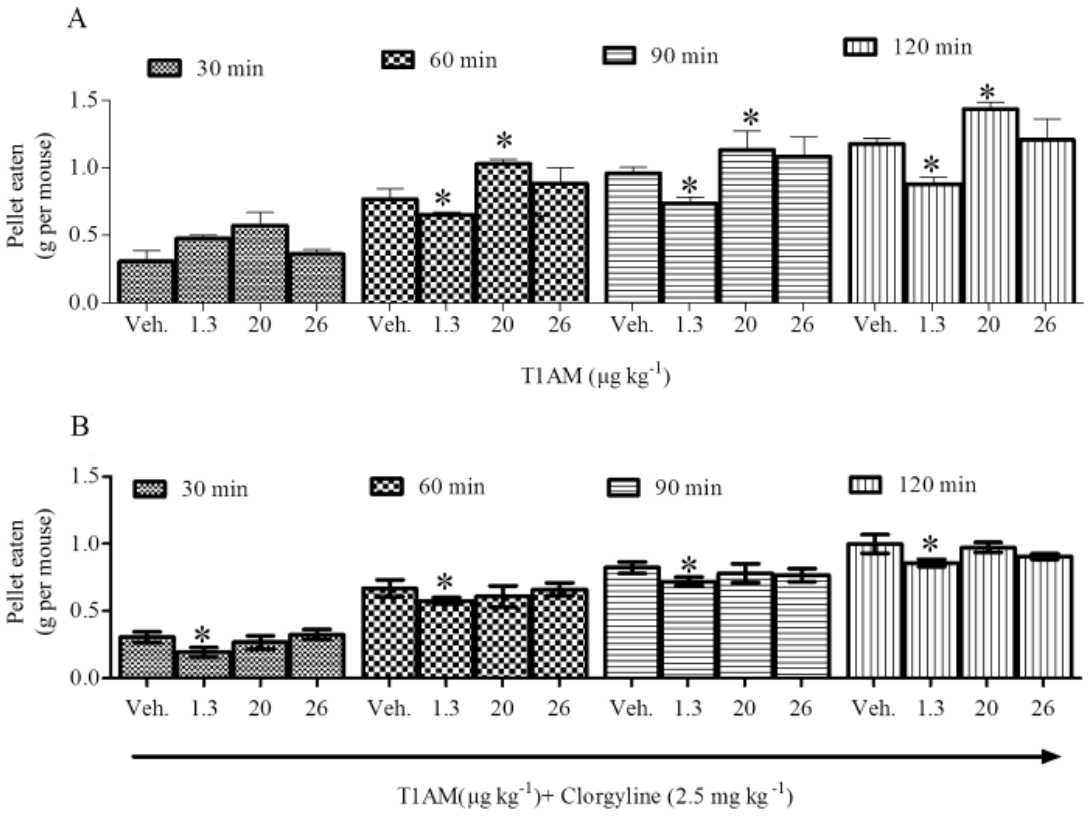


Fig.1 The i.c.v. administration of T1AM resulted in hypo- and hyperphagia: the effect of clorgyline pre-treatment. 16 h fasting mice were pre-treated i.p. with saline solution (panel A) or with clorgyline 2.5 mg kg^{-1} (Panel B). After 30 min, mice (n=10) were injected i.c.v. with vehicle or with T1AM (from 1.3 to 26 µg $kg^{-1;}$ n= 10 mice for each dose), mice were then allowed food and water ad libitum. The amount of food consumed after 30, 60, 90 and 120 min following T1AM or vehicle injection was evaluated. Results are expressed as means ± SEM of 2 experiments. Clorgyline pre-treatment did not affect mice feeding behavior compared to vehicle-injected mice. *P<0.05 vs. vehicle

4.2 T1AM rapidly induced hyperglycemia

Despite opposite effects on feeding, both 1.3 and 20 μg kg^{-1} T1AM rapidly (within 15 min) induced hyperglycemia (Fig. 2) in fasted mice which persisted up to 60 min after injection (data not shown).

Clorgyline (2.5 mg kg^{-1}) pre-treatment strongly affected T1AM-induced hyperglycemia, since both doses of T1AM became ineffective (Fig. 2). Interestingly, after 60 min after receiving 20 μg Kg^{-1} T1AM, the animals actually showed very low levels of glycemia (47.7 ± 5.6 mg dl^{-1}).

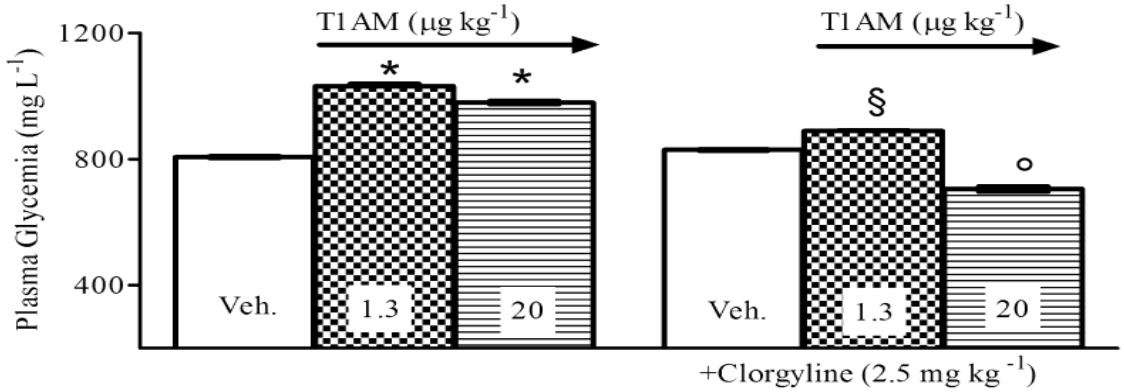


Fig. 2 T1AM injected i.c.v. increases plasma glycemia: the effect of clorgyline pre-treatment. The blood was collected from the tail vein of 2 groups (n=5 each) of mice (fasted for 16 h) pre-treated i.p. with clorgyline (2.5 mgkg^{-1}) or with saline, 30 min before i.c.v. injection of vehicle or T1AM 1.3 and 20 μg kg^{-1}. Glycemia was evaluated by a glucorefractometer at 0, 15, 30 and 60 min after i.c.v. injections. Results are the means ± SEM of two experiments carried out on 5 mice for each experimental setting. The value of glycemia measured 15 min after T1AM injection is shown. *P<0.05 vs. vehicle i.c.v. and saline i.p; § and ° P<0.05 vs. T1AM 100 and 1500 pmol mouse $^{-1}$ respectively in the absence of clorgyline pre-treatment.

4.3-*1* T1AM-induced hyperglycemia increased insulin resistance

We also tested whether the hyperglycemia induced by T1AM was associated with a reduction of insulin peripheral sensitivity. To this aim, we ran an i.p. insulin tolerance test in mice starved for 4 h, as described in "Methods". The condition of 4 h fasting was chosen to avoid severe hypoglycemia. Peripheral insulin sensitivity was significantly reduced after injection of 1.3 μgkg^{-1} T1AM, while at the dose of 20 μg kg^{-1} the response to insulin was not different from that obtained in vehicle-injected animals (Fig. 3, panel A and B).

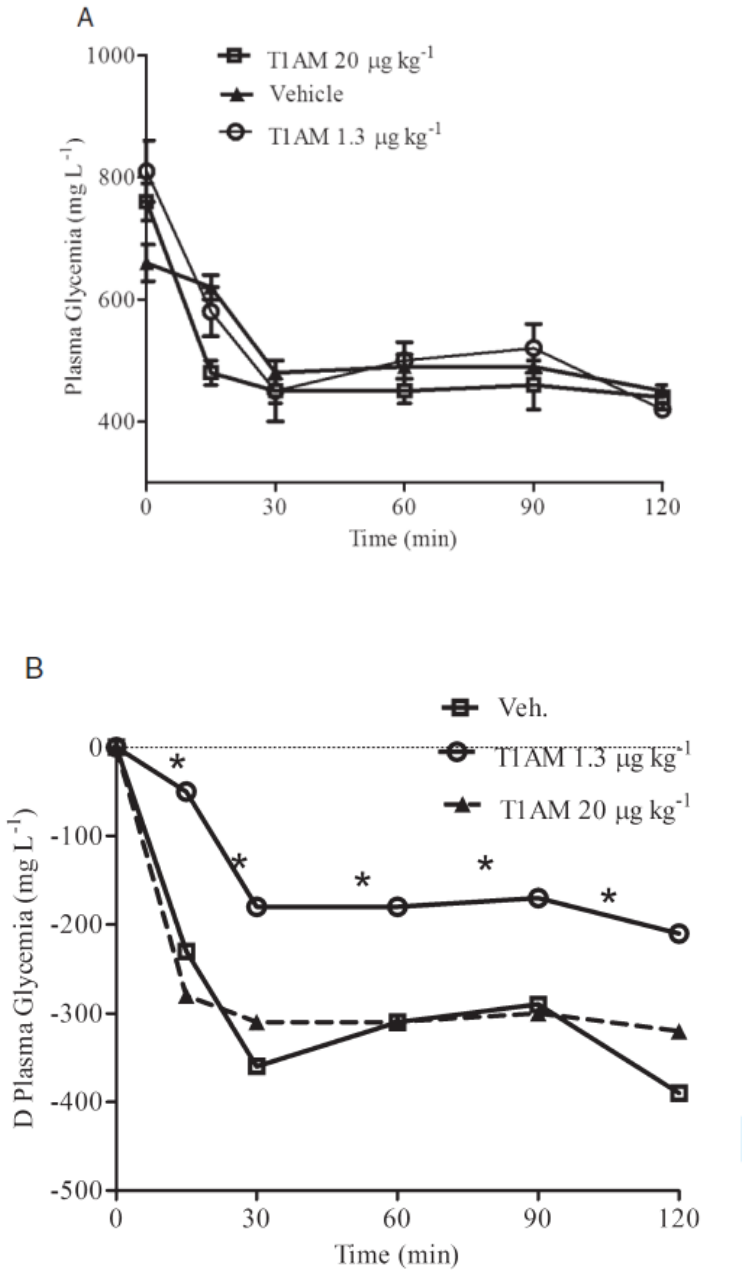


Fig. 3 T1AM 1.3 µgkg^{-1} reduces peripheral insulin sensitivity 15 mice fasted for 4 h, were divided into 3 groups (5 mice each). One group was injected i.c.v. with T1AM 1.3 µg kg^{-1}, one with T1AM and 20 µg kg^{-1} and both i.p. with insulin (1 Ukg^{-1}). The remaining group was injected i.c.v. with vehicle and i.p. with insulin (1Ukg^{-1}). Small blood samples from the lateral tail vein were collected to measure glycemic levels at 0, 15, 30, 60, 90 and 120 min after i.p. insulin treatment. Results are shown as glucose levels (mg dl^{-1}) and as the difference between glycemia at one time point and the value at T=0 (Δ mg dl^{-1}). Results represent the means ±

SEM of the values of two experiments carried on 5 mice for each time point. * P<0.05 vs. vehicle

4.3-*2* T1AM induced hyperglycemia was prevented by exenatide treatment

In order to evaluate whether the hyperglycemia induced by 1.3 μg kg^{-1} T1AM involved glucagon secretion, we repeated the previous experiments in mice treated i.p. with exenatide (0.25 $mgml^{-1}$; Byetta, Ely Lilly; n=5), a glucagon-like peptide-1 analog which prevents glucagon release with a low risk of hypoglycemia. As shown in Fig. 4, the hyperglycemia induced by T1AM (1.3 μg kg^{-1}) was prevented in mice treated with exenatide.

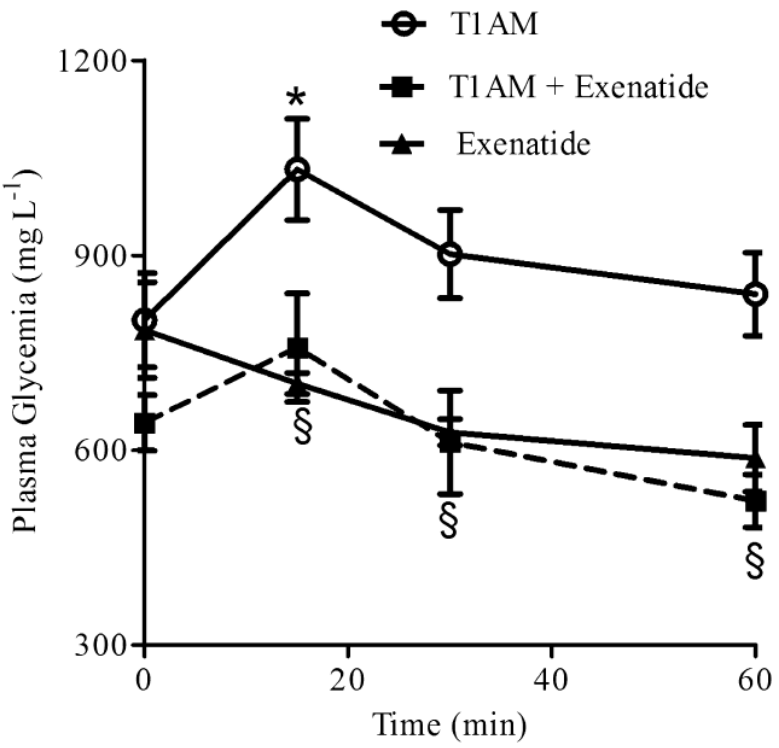


Fig. 4 Exenatide treatment prevented T1AM (1.3µg kg^{-1})-induced increase of plasma glycemia. Glycemia was also monitored in the blood collected from the tail vein of 4 h (from 8 am to 12 am) fasted mice (n=5 mice for each injection) treated as described in the "Methods". Mice received T1AM 1.3 µg kg $^{-1}$ i.c.v and saline i.p., or T1AM 1.3 µg kg^{-1} i.c.v and exenatide (0.25 mg ml^{-1}) i.p., or vehicle (Veh.) i.c.v. and exenatide (0.25 mg ml^{-1}) i.p. Results represent the means ± SEM of the values of two experiments carried out on 5 mice for each time point.

* P<0.05 vs. basal glycemia level (T=0); § P<0.05 vs. T1AM in the absence of exenatide.

4.4 T1AM injection reduced fT3 plasma levels

Subsequently, we verified whether T1AM doses active on food intake and on glucose homeostasis were able to modify plasma T3 levels. Following injection of 1.3 μgkg^{-1} T1AM the fT3 plasma concentration was significantly reduced, while no change was observed with 20 μg kg^{-1} T1AM. This T1AM effect on fT3 was not detected in mice treated with exenatide i.p. (2.4 ± 0.087 and 2.20 ± 0.184 pmoles L^{-1} respectively) or in those pre-treated with clorgyline 30 min before T1AM injection. Plasma fT4 levels averaged 33.05±1.55 pmol L^{-1} in the vehicle group, and were not significantly modified in T1AM 1.3 and 20 μg kg^{-1} injected mice (30.67±1.96 and 31.66 ± 3.75 respectively) as well as in exenatide (37.00 ± 2.88) and clorgyline (29.66 ± 1.45) i.p. treated mice. TSH levels measured in vehicle and T1AM-injected mice stabilized closed to the limit of the method (4 ± 2 mU ml^{-1}).

	Brain levels		Plasma levels	
Treatment	**T_3** ng/g	**T_4** ng/g	**fT_3** pmol/L	**fT_4** pmol/L
Vehicle N=6	**2.28** ±0.8	**2.60** ±0.35	**2.34** ±0.09	**33.05** ±1.55
T_1AM (1.3 µg/kg) N=6	**2.31**±0.12	**2.60** ± 0.35	***1.82** ±0.11	**30.60** ±1.96

Table 1 T_1AM injection reduced fT_3 plasma levels. T_3 and T_4 brain and plasma levels were assayed by HLPC tandem mass spectrometry following i.c.v. injection of vehicle or T1AM 1.3 µgkg^{-1}. *; $p<0.05$ vs. vehicle

4.5 T1AM presented systemic bioavailability

T1AM given i.c.v. showed systemic bioavailability, suggesting spillover from the central nervous system. As shown in Table 2, the percentage of amine recovered was around 0.2 % of the amount injected at both the low and the high dose (1.3 and 20 μgkg^{-1} respectively). This result suggested that only a small amount of the injected T1AM can reach the periphery and that the mechanism responsible for its transport into the blood is not simple diffusion but rather a carrier-mediated mechanism. In mice with MAO blockade, systemic bioavailability of endogenous and pharmacological injected T1AM increased remarkably (Table2). Under these experimental conditions, the bioavailability of 1.3 $\mu g\ kg^{-1}$ T1AM increased only twice, while with 20 μgkg^{-1} T1AM a 40-fold increase was observed. The yield of T1AM recovery was calculated on a volume of 1.5 ml of blood for each mouse (Lee and Blaufox, 1985).

T1AM (µg·kg^{-1})	pmol·mL^{-1}	Total pmol	Yield (%)
Vehicle	ND		
1.3	0.19	0.28	0.215
20	1.5	2.25	0.15
Veh +clorgyline	0.05	0.082	0.082
1.3+clorgyline	0.35	0.52	0.52
20+clorgyline	57.05	85.58	5.7

Table 2 T1AM level in the systemic circulation. T1AM plasma levels were assayed as described in the Metods section following i.c.v. injection of vehicle or 1.3 and 20 µg kg-1. Results were opteined from pooled sample (five mice for each group) collected from16 h fasted mice. Veh,vehicle. The dose of clorgyline used was 2.5 mg kg-1.

4.6 T1AM behaves as a modulator of the hypothalamus-pancreas-thyroid axes in mice

We observed that i.c.v. injection of 1.3 μg kg^{-1} T1AM in fasted mice produced central and peripheral effects, including hypophagia, hyperglycemia, and reduction of plasma fT3 levels. All these effects were not linearly dependent on T1AM dosage. Infact, the hypophagia vanished at doses higher than 1.3 μg kg^{-1} and it was replaced by hyperphagia at 20 μg kg^{-1}. The latter dose was also ineffective on plasma fT3 levels. This biphasic response might account for the different results reported in the literature in *ad libitum* fed mice (Dhillo et al., 2009; Hettinger et al, 2010).

After clorgyline pre-treatment, the hypophagic effect was preserved, while hyperphagia was prevented. This observation might imply that: i) increased adrenergic drive due to amine oxidase inhibition can overcome the anti-adrenergic effect of T1AM, and ii) since T1AM itself is a substrate of amine oxidases, T1AM catabolites like 3-iodothyroacetic acid or thyroacetic acid might be involved in neuronal effects or iii) increased T1AM levels might induce a rapid desensitization of the target (s).

As a novel and interesting finding we report that T1AM i.c.v. injected had systemic bioavailability. Even if we have tested only two T1AM doses, our results suggest that T1AM entry into the blood is mediated by a transporter rather than representing simple diffusion. Whatever the mechanism may be, the occurrence of T1AM in the systemic circulation suggests that peripheral effects should be expected following i.c.v. T1AM administration. Systemic bioavailability increased remarkably under conditions of MAO inhibition, indicating intracellular deamination as a prominent metabolic pathway of T1AM. Therefore, in the presence of the MAO inhibitor clorgyline, T1AM central and peripheral levels increase, mimicking the effects of higher T1AM doses. This is another possible reason why after clorgyline pretreatment of 20 μg kg^{-1}, T1AM was ineffective in modifying feeding as it occurred with higher T1AM dosages (26 μgkg^{-1}) in the absence of clorgyline.

Peripheral actions are the likely cause for the effects of T1AM on plasma glucose, since in pancreatic cells T1AM has been reported to induce glucagon secretion and decrease insulin secretion (Regard et al., 2007). The hyperglycemic effect of 1.3 μg kg^{-1} was associated with a transient reduction in peripheral insulin sensitivity and was

prevented by exenatide, suggesting a major role for glucagon secretion; while at 20 μg $kg^{-1,}$ reduced insulin secretion might also be involved. Notably our results indicate that T1AM has a high affinity for the pancreas, and hyperglycemia was achieved at plasma concentrations of T1AM on the order of 0.35 nM with regard to the hyperphagic effect, T1AM was unable to produce hyperglycemia in mice pre-treated with clorgyline. Again, this might imply that increased sympathetic drive counteracting the effect of T1AM depends on glucagon release, since a biphasic effect of T1AM on insulin secretion has been described: low concentrations inhibited insulin secretion, possibly through alpha2 adrenergic receptors, but high concentration had a stimulatory effect, possibly mediated by TAAR1 (Regard et al, 2007; Winzell and Ahren, 2007). An alternative possibility is that glucagon release may be stimulated by T1AM catabolites produced through oxidative deamination.

T1AM (1.3 μg kg^{-1}) also reduced systemic fT3 levels. This effect was prevented by exenatide, supporting a role for glucagon. Consistent with this hypothesis, inhibition of liver deiodinase activities by glucagon has been reported (Mitchell et al., 1986) (Kabadi et al., 1988) (Langer et al., 1991). No change in fT3 was observed after 20 μgkg^{-1} T1AM, which might be explained by increased insulin secretion, since insulin is known to stimulate liver deiodinase activities (Sato et al., 1984). Another hint regarding the role of the pancreas is the observation that changes in fT3 were prevented by clorgyline pre-treatment. Glucagon might represent the factor linking T3 plasma levels with hyperglycemia and might even contribute to changes in mice feeding patterns (Valassi et al., 2008).

In summary, we demonstrated that a single low dose of T1AM decreases mice feeding and plasma fT3 levels, while increasing plasma glucose and raising insulin resistance. T1AM appears to be a potent amine stimulating the central nervous system, the pancreas and, in turn, the liver where it might affect fT3 production and/or degradation.

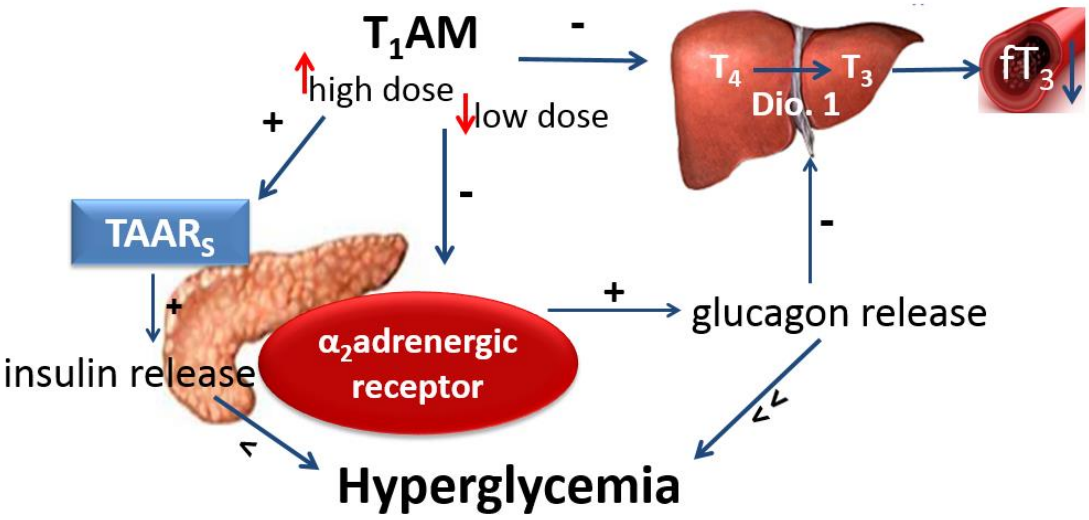


Fig. 5 Graphic rappresentation of T_1AM pharmacological effcts. T_1AM appears to be a potent amine stimulating the central nervous system, the pancreas and, in turn, the liver where it might affect fT3 production and/or degradation.

4.7 T1AM effects on memory and pain

As described in the "Methods" section, a retention session of the passive voidance test was performed 1 h and 24 h after T1AM injection. Following administration of 0.4 and 0.13 μgkg^{-1} T1AM, mice showed a trend to increase the latency to enter the dark compartment either in the 1h or 24 h retention session, but the difference vs the control group did not reach the threshold of significance. However, a significantly higher ($P<0.001$) latency to enter the dark compartment was observed after 1.3 and 4 μgkg^{-1} T1AM, both in the 1 h and 24 h retention sessions (Fig. 6).

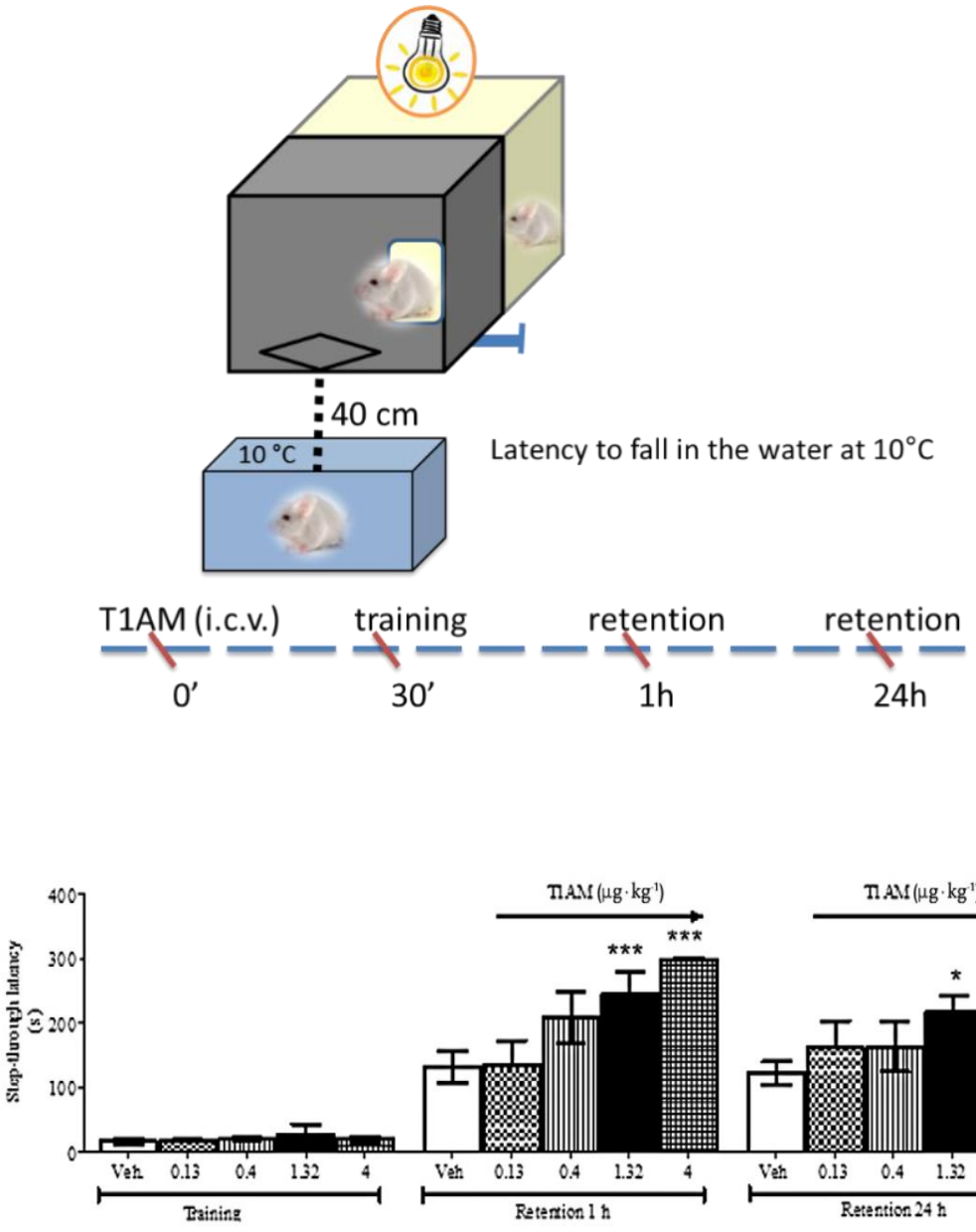


Fig. 6 Mice were injected i.c.v. with T1AM (0.13, 0.4, 1.32 and 4 µgkg-1; 20 mice for each dose) or with vehicle and subjected to the passive avoidance test according to the scheme drawn. Results presented are the mean ± SEM of 5 different experiments, each run on 5 mice for dose. *P<0.05 vs. vehicle

4.8 T1AM improved memory acquisition: the object recognition task

Since 1.32 μgkg^{-1} was the lowest effective dose in the passive avoidance test, we next verified whether the pro-cognitive effect of this dose was confirmed in another test, the novel object recognition task, which is based on the natural tendency of rodents to explore unfamiliar objects (Ennaceur, 1988).

As shown in Fig. 7, mice treated with vehicle spent the same time to explore the familiar and the novel object, while mice injected i.c.v. with 1.3 μgkg^{-1} T1AM showed significantly ($P < 0.001$) enhanced exploratory preference for the novel object in the 24-h retention test (Fig. 7, panel A). At this dose, T1AM did not increase locomotor activity (Fig. 7, panel B).

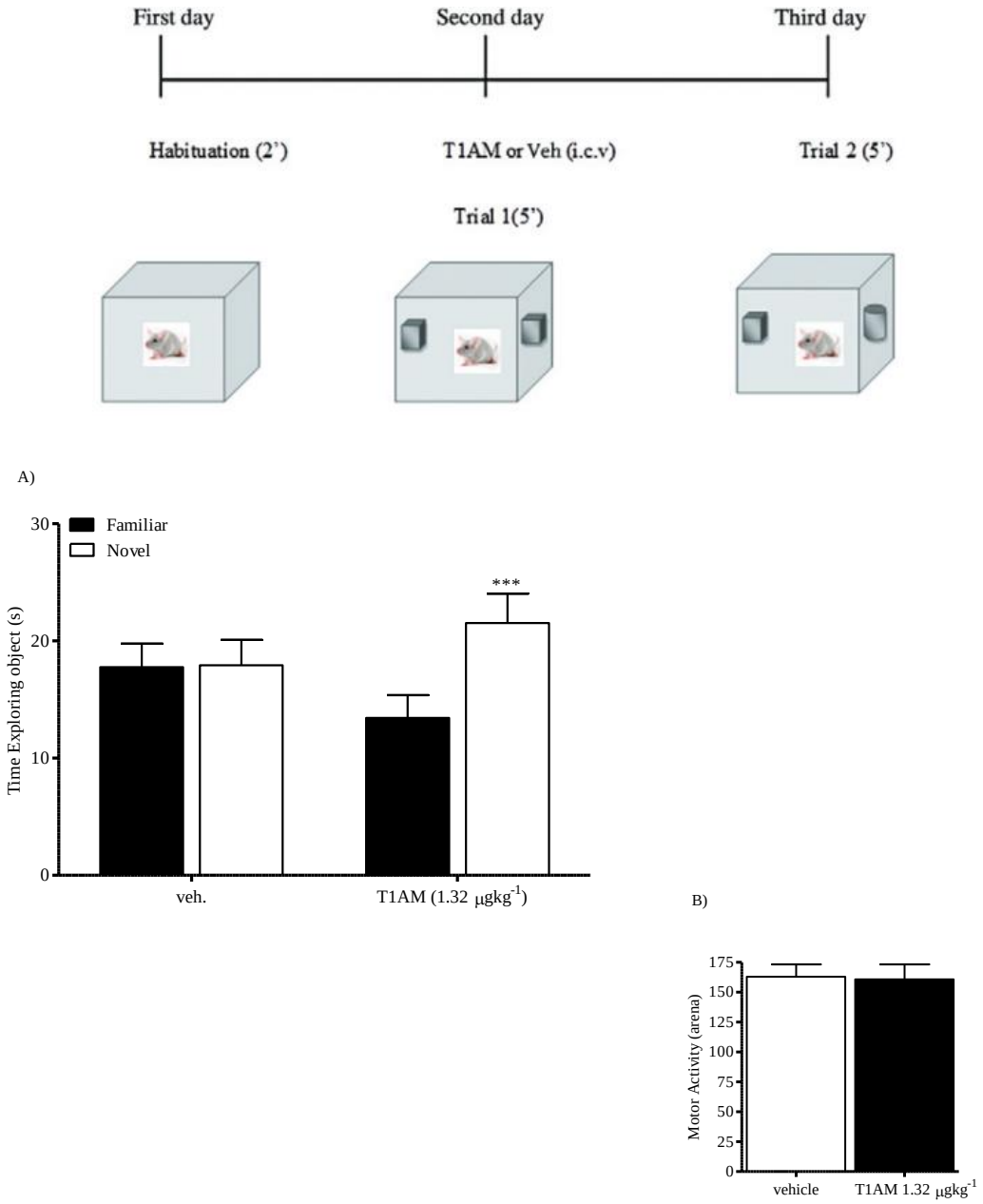


Fig. 7 The object recognition task was performed on fed mice i.c.v. injeceted at day 2 with T1AM 1.32 μgkg-1. Retention session was performed 24 later (Panel A). Mice locomotor activity in the arena was also monitored (Panel B). Results are the mean ± SEM of values obtained from three different experiments each using 5 mice. ***P<0.001 vs. vehicle

4.9 T1AM increased exploratory mice activity: the hole-board test

We also investigated whether increased memory acquisition induced by T1AM was associated with increased animal curiosity. To this aim, mice injected i.c.v. with T1AM 0.04, 0.13 and 1.3 and 4 μgkg^{-1}) were located on the hole-board platform and their behavior was observed. At the highest doses tested, namely 1.3 and 4 μgkg^{-1}, T1AM significantly increased mice exploratory activity, while at 0.13 μgkg^{-1} no significant effect was produced (Fig. 8).

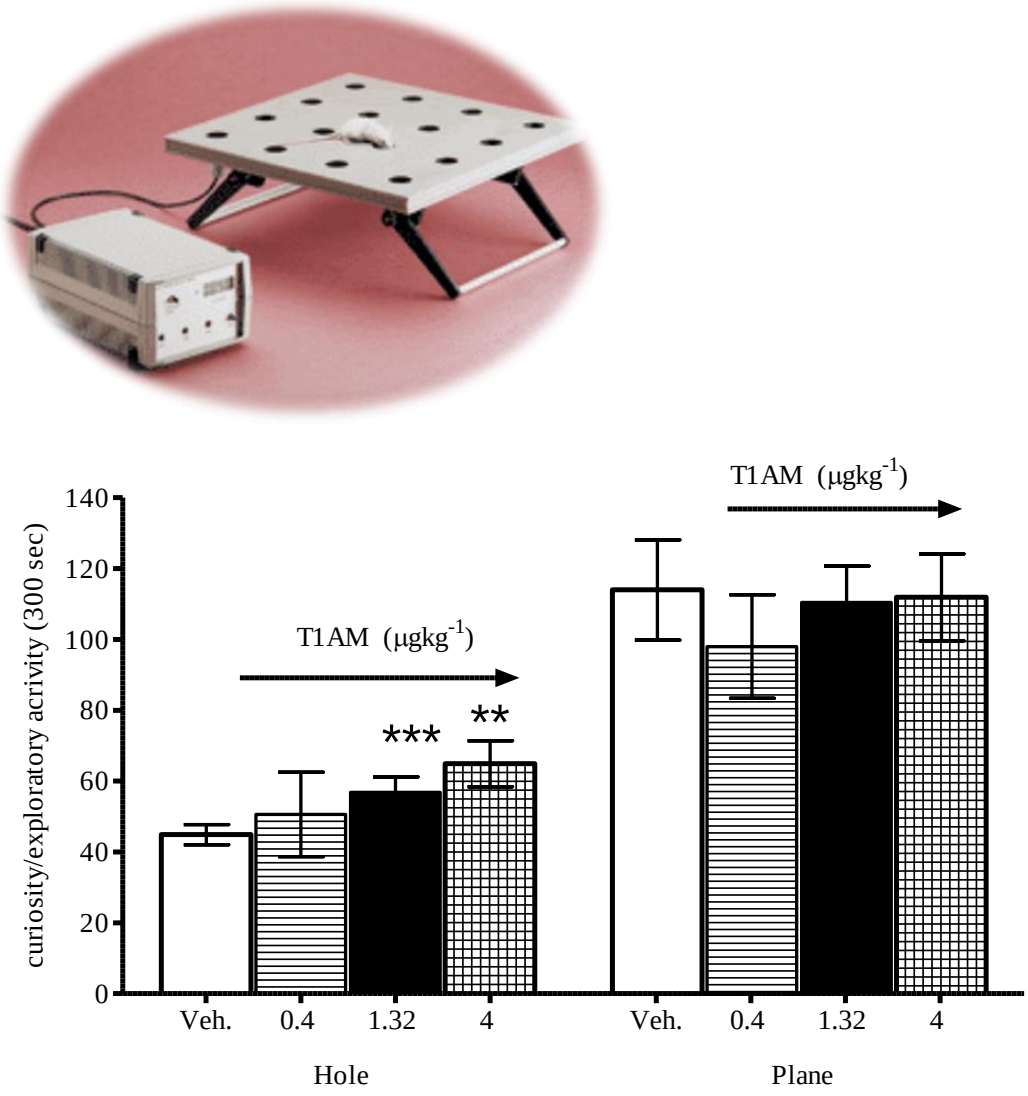


Fig. 8 Mice were injected i.c.v. with T1AM (0.4, 1.32 and 4 μgkg-1). After 30 min, the animals were put on the hole-board platform and movements and exploratory activities were monitored as described in "Methods". Results are the mean ± SEM of three different experiments each using 5 mice for every T1AM doses. *P<0.05 vs. vehicle

4.10 T1AM increased pain sensitivity

Since the passive avoidance and the object recognition tests may be affected by analgesic drugs, we next wondered whether T1AM had any effect in increasing pain threshold. To this aim we investigated the latency to licking of mice which were injected i.c.v. with T1AM and then located on the hot plate. As it is shown in Fig. 9, at the doses, which proved to be effective in the object recognition and passive avoidance tests (0.4, 1.3 and 4 μgkg^{-1}), T1AM significantly reduced the threshold of pain perception to hot insults. Interestingly, the hyperalgesic effect of T1AM was not dose-related and it was not observed with 0.04 μgkg^{-1} T1AM.

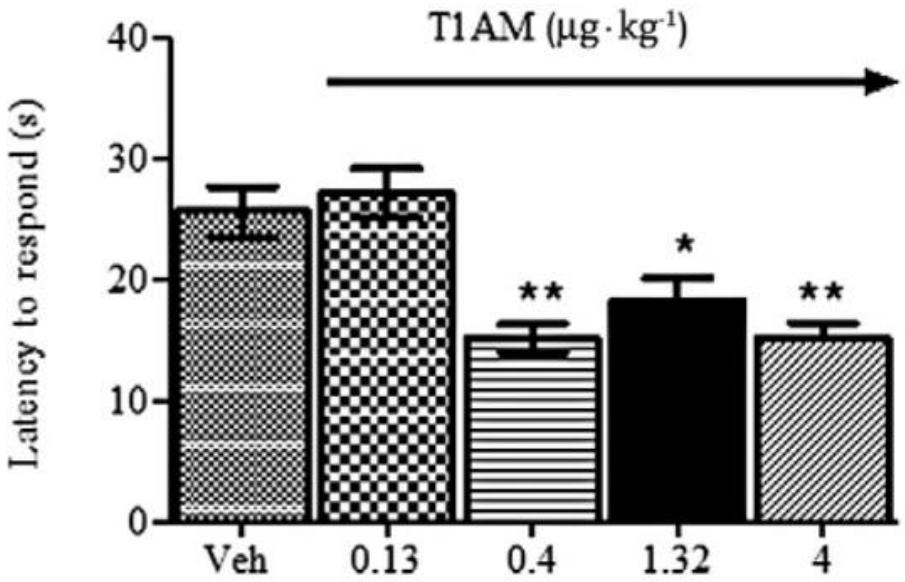
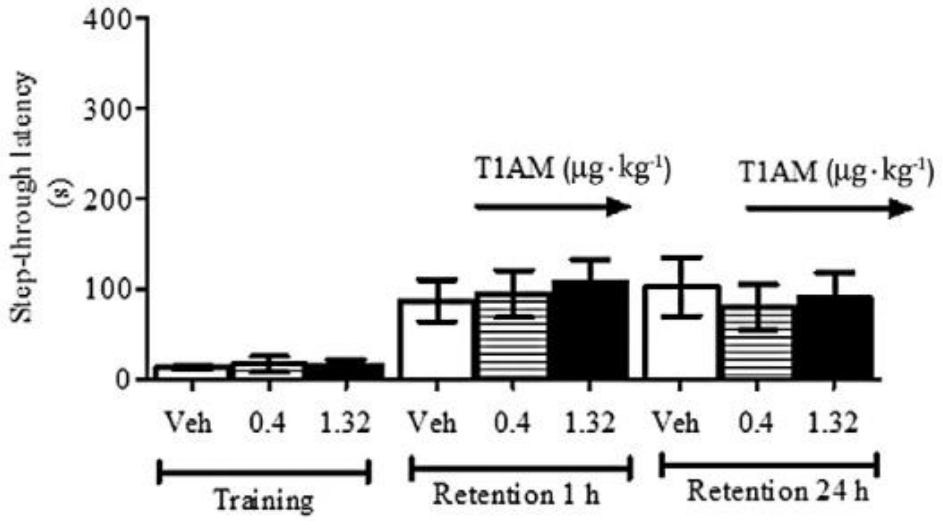


Fig. 9 Mice injected were located on the hot-plate 30 min after i.c.v. injection of T1AM (0.13, 04, 1.32 and 4 µgkg-1). The latency to respond to the insult was evaluated as described in Methods. Results are the mean ± SEM of three different experiments each evaluating the response of 5 mice for each T1AM dose. *P<0.05 vs. vehicle

4.11 Clorgyline pretreatment modifies behavioural effects of T1AM

Oxidative deamination by amine oxidases appears to be the chief metabolic pathway for T1AM (Wood et al., 2009; Saba et al., 2010), while other possible modifications are represented by deiodination (Piehlet al., 2008) and sulphorylation (Pietsch et al., 2007). Therefore, it seemed interesting to investigate whether the pro-learning and hyperalgesic effects of T1AM were affected by the MAO inhibitor clorgyline, which was administered i.p. at a dose of 2.5 mg·kg-1 (preliminary experiments showed that i.p. injection of vehicle had no effect either in the vehicle group or in the T1AM-treated group). In the passive avoidance paradigm, the effect of T1AM was lost after pretreatment with clorgyline (Fig. 10A). With regard to pain threshold experiments (Fig. 10B), in the presence of clorgyline T1AM still produced a hyperalgesic action, but the dose–response relationship was modified, because a significant effect was detected only with 0.134 µg·kg-1 T1AM ($P < 0.05$ vs. vehicle), but not with higher concentrations. Notably, clorgyline treatment per se appeared to increase pain sensitivity, because latencies were in general lower ($P < 0.05$) than those observed in the previous set of experiments (Fig.9).

A



B

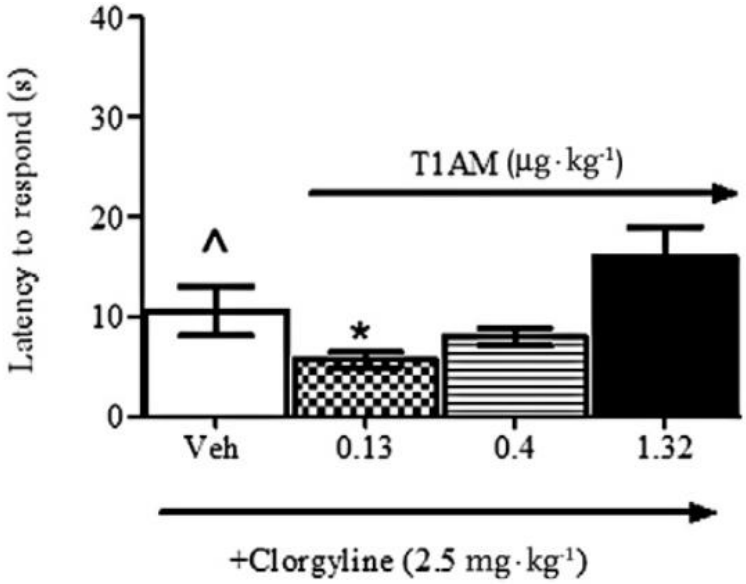


Fig. 10 A/B Mice pretreated with clorgyline (2.5 mg·kg-1, i.p.), received either vehicle (Veh) or T1AM (0.4 or 1.32 µg·kg-1) i.c.v and after 30 min were subjected to the passive avoidance test (10 A) or were plased on the hot plate (10 B) as described in the Methods section. Results are expressed as mean SEM; n=10 in each group**.**

4.12 T_1AM pro-learning effect does not include the muscarinic pathway: T1AM revert scopolamine-induced amnesia

Memory acquisition and retention is a complex behaviour controlled by an organized network of integrated signalling including the muscarinic pathway. In line with this, muscarinic receptor antagonists are useful pharmacological tools to produce a model of amnesia. Accordingly, we injected i.c.v. T_1AM or vehicle to mice pre-treated with scopolamine i.p. with 0.3 $mgkg^{-1}$ or saline solution. This dose of scopolamine was devoid of analgesic and sedative effects (data not shown), but produced amnesia. At this condition, mice injected with T_1AM i.c.v. (0.4, 1.32 and 4 μgkg^{-1}) recalled properly in the retention session performed 1 but also 4 h following the training session with a complete recover from amnesia (Fig. 11).

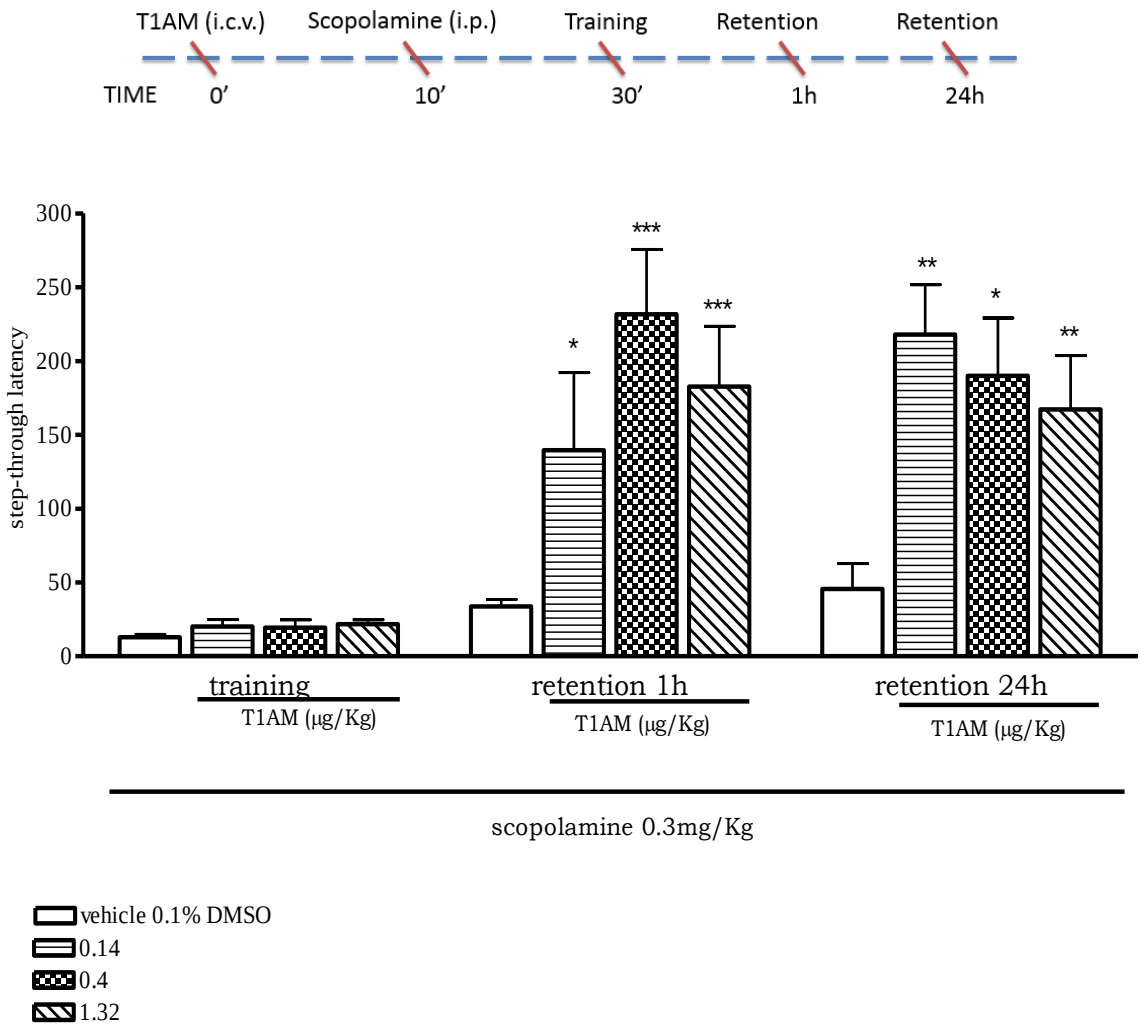


Fig. 11 In another set of experiments, T1AM (0.14, 0.4 or 1.32 µgkg^{-1}) or vehicle (Veh.) were injected i.c.v. in mice pre-treated i.p. with scopolamine (0.3 mgkg^{-1}) or saline. Mice were then subjected to the passive avoidance test as described in "Methods". Results are expressed as mean ± SEM; n=20 in each group. *P<0.05, **P<0.01, ***P<0.001 vs. vehicle.

4.13 Reversion of scopolamine amnesia depends T1AM-oxidative deamination

In mice pre-treated i.p. with clorgyline (2.5 $mgkg^{-1}$) 30 min before i.p. scopolamine (0.3 $mgkg^{-1}$), T1AM (0.14, 0.4 and 1.32 μgkg^{-1}) given i.c.v. failed to revert the scopolamine-induced amnesia (Fig. 12). This result suggested that T1AM pro-learning but also anti-amnesic features depended, at least in part, by amine oxidative degradation pathways.

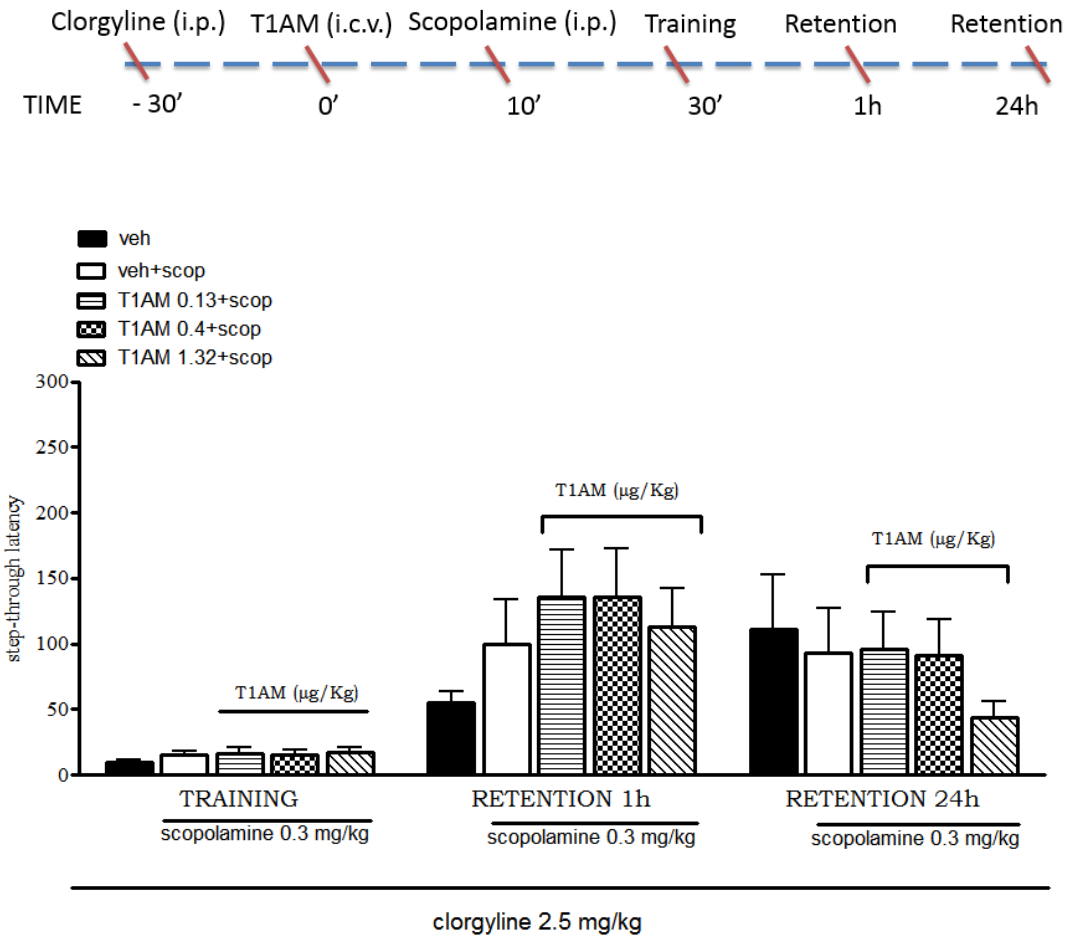
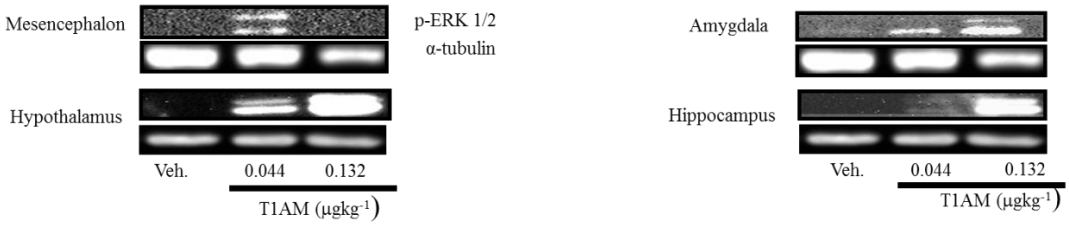


Fig. 12 T1AM-reversion of scopolamine-induced amnesia is prevented by clorgyline pre-treatment of mice Mice were pre-treated with clorgyline (2.5 mgkg-1) 30 min before scopolamine (0.3 $mgkg^{-1}$) i.p. or saline and, after 10 min, they were injected i.c.v. with T1AM (0.13, 0.4 or 1.32 or 4 μgkg^{-1}) or vehicle. The passive avoidance task was then repeated as described in "Methods". Results are expressed as mean ± SEM; n=10 in each group.

4.14 T1AM induced ERK1/2 phosphorylation at different cerebral areas

Memory acquisition and storage are typically associated with increased ERK phosphorylation. So in parallel experiments, we measure in specific brain areas the activation of typical signaling proteins involve in memory acquisition. After treatment with T1AM at the doses 10 fold and 33 fold lower than the lowest dose proved to be effective on memory acquisition and retention, p-ERK is significantly higher than observed in the vehicle group (Fig. 13, panel A e B).

A)



B)

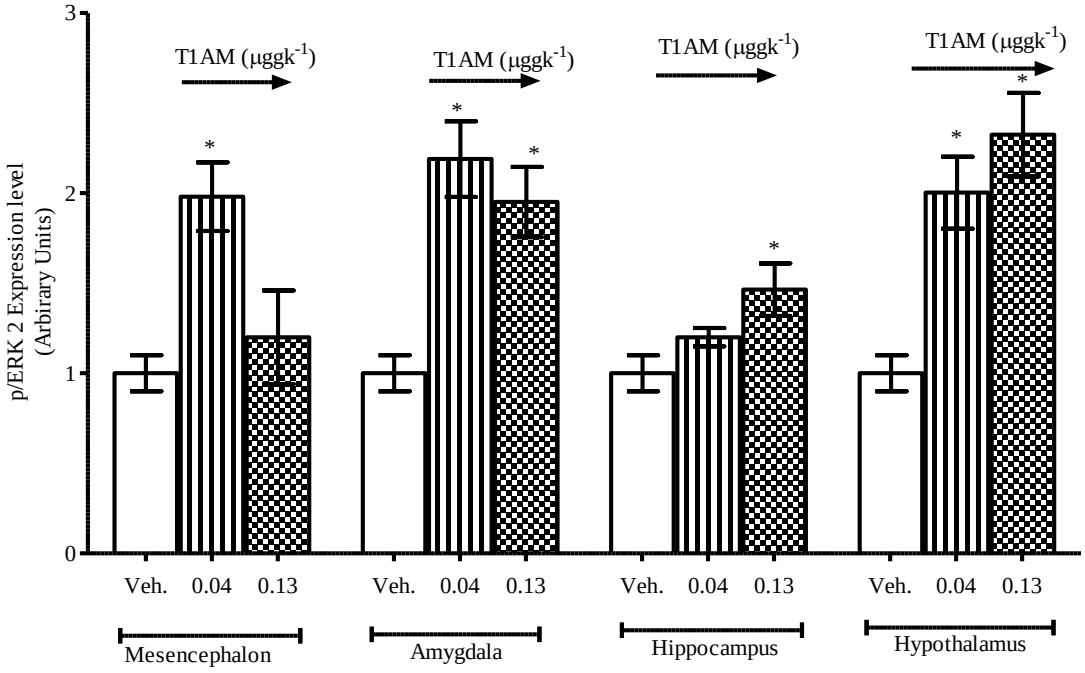


Fig. 13 Thirty minutes after i.c.v injection of T1AM (0.04, 0.13 mg·kg-1) or vehicle, mice (n= 3 per group) were killed and brain regions separated as described in the Methods section. Immunodetection for pERK was carried out on protein lysates from each region separated on SDS-PAGE gels. Results are the mean SEM of the densitometry of three different gels. (A) Results of a typical experiment. (B) Cumulative results. *P<0.05 versus vehicle.

4.15 T1AM is an endogenous memory enhancer and hyperalgesic compound

We report for the first time that T1AM behaves as a memory enhancer in the euthyroid mice if given i.c.v.. This effect is achieved by stimulating animal curiosity without increasing locomotor activity or producing any analgesic effect.

Since, memory effects require the synthesis of new proteins and therefore imply a transcriptional action, we have evaluated if T1AM can activate transduction pathways leading to transcriptional effects and known to play a role in memory retention. We found that T1AM activates pERK in hippocampus and other brain regions. The observation that pERK2 activation in hippocampus does not follow a close dose relationship suggests that pERK2 is a sensitive target of T1AM and that an amnesic effect might possibly be elicited even at doses lower than 0.13 $\mu g kg^{-1}$.

Another interesting finding is the discovery that i.c.v. T1AM reduced pain threshold. This effect might also be related to ERK2 activation (Ji at al., 2009). In particular, we observed increased pERK2 in the amigdala, the central nucleus identified as a site of nociception induced by peripheral injury, where ERK2 activation is associated with increased pain perception (Kolber, 2010; Schicho, 2005). As for memory acquisition, the mechanism of the hyperalgesic effect of T1AM is unknown. On the basis of its putative effect on alpha-2 adrenergic receptors (Regard, 2007), T1AM might inhibit the release of analgesic mediators (Hu, 2007). While thyroid hormones may affect pain threshold (Guasti, 2007) their brain levels where not affected by T1AM injection, so T3 and T4 are unlikely to be involved in T1AM-dependent hyperalgesia.

In cell cultures and in isolated hearts, T1AM showed a short half-life largely due to cellular uptake and oxidative deamination to 3-iodothyroacetic acid (Saba et al., 2010). This observation was confirmed in our experimental setting. By 30 min after its i.c.v. injection, the brain T1AM concentration had decreased from a nominal value of 231 pmol·g-1 to 13 pmol·g-1, consistent with its degradation and/or transport into the systemic circulation. Therefore, we wondered whether T1AM metabolite(s) might contribute to its effects. To ascertain the role of oxidative deamination, we repeated the passive avoidance and the hot plate tests in animals pretreated with clorgyline, which has been reported to increase T1AM concentration and to abolish the production of deaminated derivatives, particularly of 3-iodothyroacetic acid (Saba et al., 2010; Manni

et al., 2012). Under these conditions, in the passive avoidance test, the response to T1AM was markedly reduced and did not reach the threshold of statistical significance. In the pain threshold experiments, a significant response was still observed, but the dose–response relationship was modified, as the dose of 0.13 mg·kg-1, which was ineffective in the absence of clorgyline, turned out to be hyperalgesic, while this effect disappeared at higher doses. These results suggest either that T1AM acts on rapid desensitizing targets, or that some of its effects are mediated by deaminated derivatives, such as 3-iodothyroacetic acid.

An additional feature of the amine is its ability to revert amnesia induced by scopolamine, outstanding the peculiarities of this compound which is endowed of pro-learning but also anti-amnesic effects, two features which are hard to find going hand in hand, and it indicates that muscarinic pathway is not included in amine mechanism. Furthermore, in the presence of scopolamine, T1AM reverted amnesia just at a dose of 0.4 μgkg^{-1} which was instead inactive as pro-learning (Manni et al., 2013), thus suggesting that removalof the muscarinic pathway abolish the releaseof an amnesic mediator.

Furthermore as for the pro-learning effect, also scopolamine-induced amnesia was prevented in mice pre-treated with clorgyline, thus suggesting T1A formation is a mediator of T1AM anti-amnesic effect.

4.16 Pharmacological effects of 3-iodothyroacetic acid (TA1), the oxidative metabolite of T1AM

The effect of TA1 on learning

All the effect of TA1 were studied injecting doses equimolar to those of T1AM.

The i.c.v. injection of TA1 doses close to its physiological levels (0.4, 1.32 or 4 μgkg^{-1}) produced a biphasic effect on mice memory. As shown in Fig. 14 (panel A), at the lowest dose (0.4 μgkg^{-1}) TA1 behaved as an amnestic compound in 1 h and 24 h retention sessions. On the contrary, at the doses of 1.32 and 4 μgkg^{-1}, mice remained in the light portion of the box for a significantly longer time in the 1 h retention session. Interestingly, and differently from what occurred with T1AM, memory consolidation was not observed in the 24 h retention session (Fig. 14, panel A).

In addition, 15 min after TA1 (0.4, 1.32 and 4 μgkg^{-1}) injection, mice showed hyperalgesia to the hot stimulus (Fig. 14, panel B) and, at the doses of 1.32 and 4 μgkg^{-1}, higher plasma glycemia than control value (Fig. 14, panel C).

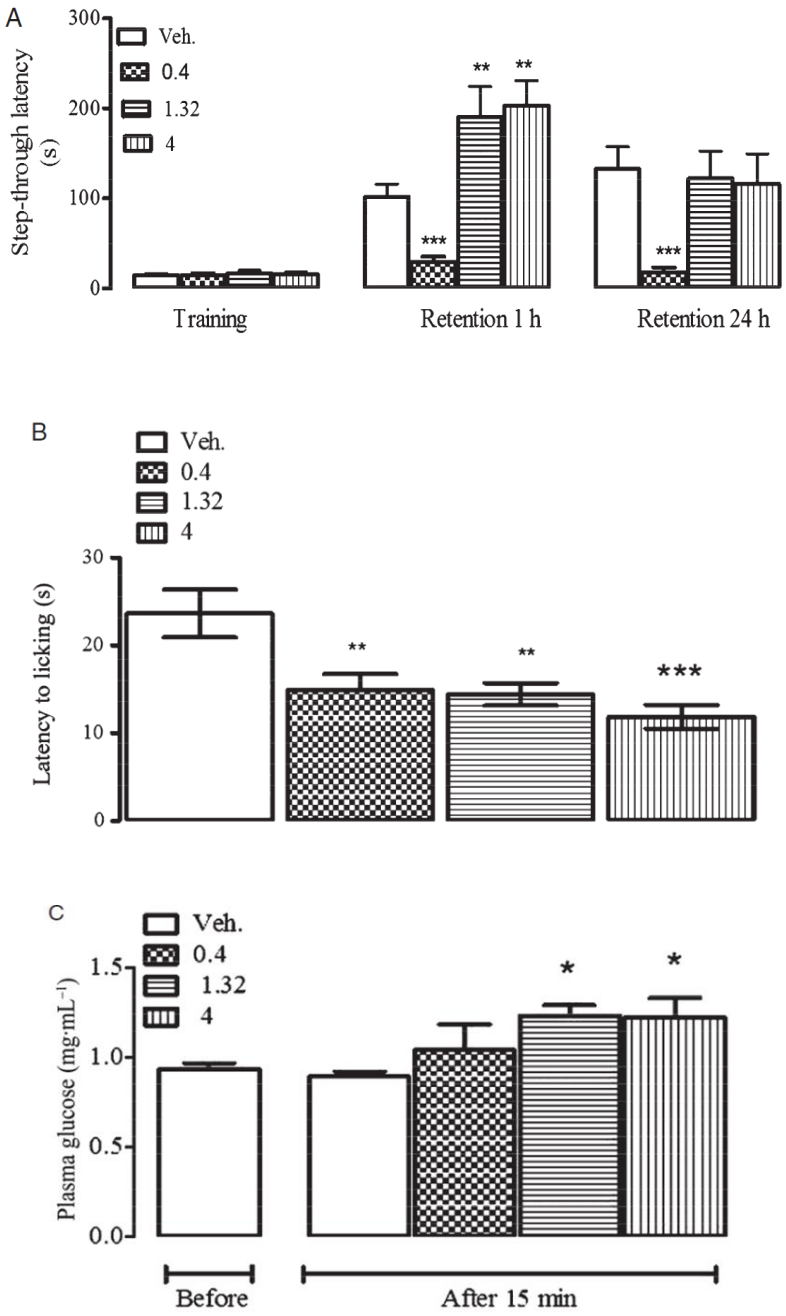


Fig. 14 TA1 modifies learning, reduces pain threshold and increases plasma glycemia in mice. Mice (n=20 for each groups of animals) were injected i.c.v. with TA1 (0.4, 1.32 or 4 μgkg^{-1}) or with vehicle (Veh.) and, after 15 min, subjected to the training test of the passive avoidance task (panel A) or put on the hot plate to measure their pain threshold (panel B). **P<0.01 and ***P<0.001 vs. Veh. Plasma glycemia was also measured 15 min after TA1 injection in the blood collected from the tail veins of 4h starved mice (n=10 for reach groups of animals). Each test was carried on as described in "Methods" and results are expressed as mean ± SEM; *P<0.05 vs. Veh

4.17 TA1 reverts scopolamine-induced amnesia

Memory acquisition and retention is a complex behaviour controlled by an organized network of integrated signalling including the muscarinic pathway. Because of this, scopolamine injection in rodents is often used to obtain an experimental model of amnesia. In order to investigate further the TA1 effects, we next verified whether TA1 could revert scopolamine-induced amnesia.

In mice pre-treated i.p. with scopolamine, at a dose which produced amnesia (Rush, 2008) without inducing analgesia or sedative effects (0.3 $mgkg^{-1}$), the i.c.v. injection of 1.32, but not 4 μgkg^{-1}, TA1 completely reverted the amnesia, giving consolidation in the 1 h and also in the 24 h retention sessions (Fig.15).

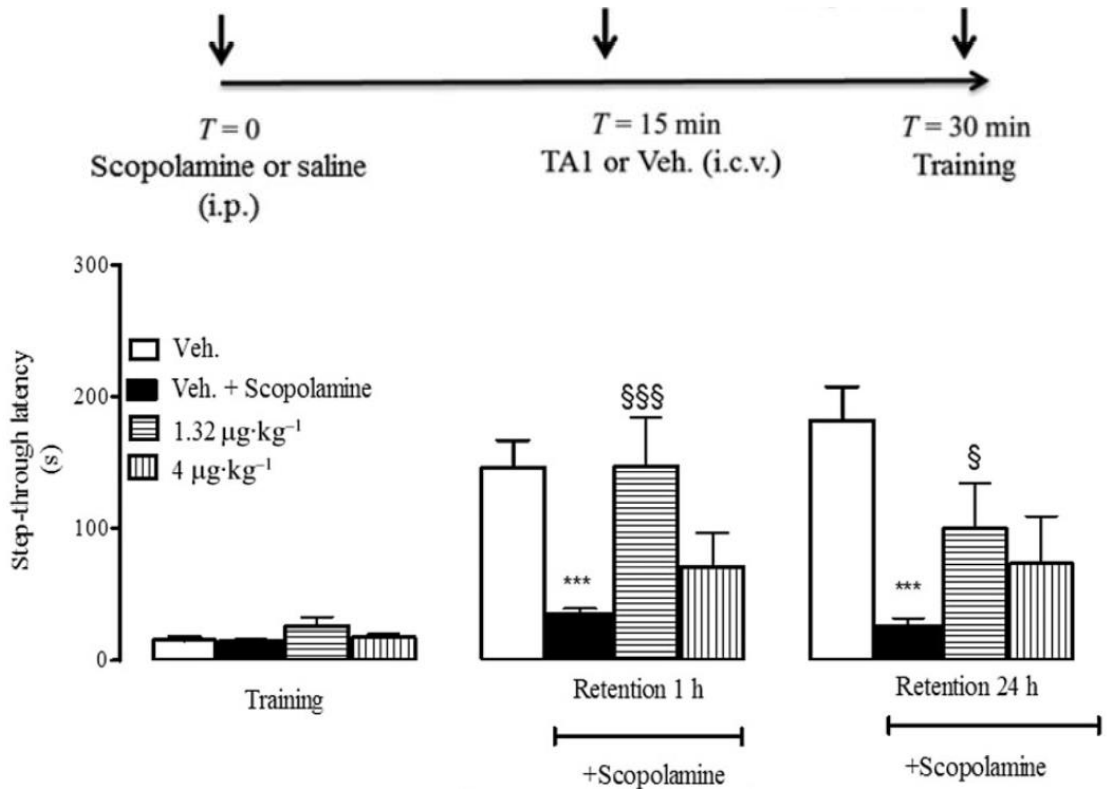


Fig. 15 TA1 reverts scopolamine-induced amnesia. In another set of experiments, TA1 1.32 and 4 μgkg^{-1} (n=10 for each dose) or vehicle (Veh.) were injected i.c.v. in mice (n=20 for each groups of animals) pretreated i.p. with scopolamine (0.3 $mgkg^{-1}$ n= 30) or saline. Mice were then subjected to the passive avoidance test as described in "Methods". Results are expressed as mean ± SEM. §P<0.05 and §§§P<0.001 vs. Veh.+scopolamine, ***P<0.001 vs. Veh.

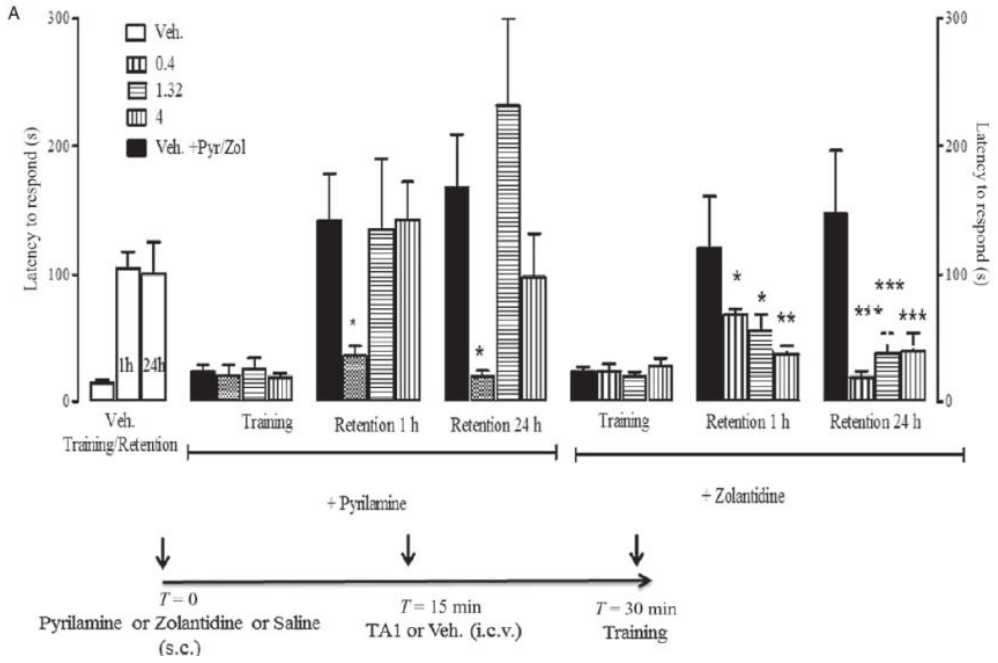
4.18 Histaminergic antagonists pretreatment prevents stimulation of memory, hyperalgesia and increase of plasma glycemia

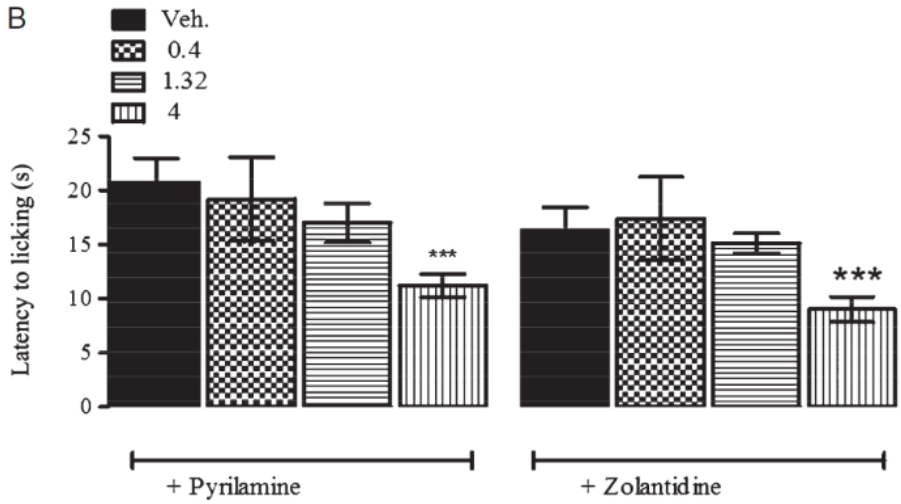
TA1 i.c.v. injection was performed in mice pre-treated s.c. with saline or pyrilamine (10 $mgkg^{-1}$), or zolantidine (5 $mgkg^{-1}$), antagonists of H_1 and H_2 type receptors respectively. As shown in Fig. 16 (panel A, B and C), pre-treatment with anti-histaminergic drugs *per se* did not modify memory acquisition, pain threshold or plasma glycemia, but it deeply affected the behavioural responses induced by TA1.

Pyrilamine pre-treatment abolished the pro-learning effect of 1.32 and 4 μgkg^{-1} TA1, while the amnesic effect of 0.4 μgkg^{-1} TA1 was maintained (Fig. 16, panel A). In mice pre-treated with zolantidine, TA1, turned out to be amnesic at all the doses studied (Fig. 16, panel A).

Pre-treatment with pyrilamine or zolantidine also affected the hyperalgesic and the hyperglycemic effect of TA1. In particular, irrespective of the histaminergic antagonist used, hyperalgesia was evident only at the highest dose of 4 μgkg^{-1} (Fig. 16, panel B) and plasma glycemia did not raise significantly, at any of the doses injected (Fig. 16, panel C).

Taken together these results suggested that stimulation of memory, hyperalgesia and increase of plasma glycemia might have a common mechanism involving the histaminergic system.


A
Veh.
0.4
1.32
4
Veh. +Pyr/Zol
Latency to respond (s)
300
200
100
0
1h
24h
Veh.
Training/Retention
Training
Retention 1 h
Retention 24 h
Training
Retention 1 h
Retention 24 h
+ Pyrilamine
+ Zolantidine
T = 0
Pyrilamine or Zolantidine or Saline
(s.c.)
T = 15 min
TA1 or Veh. (i.c.v.)
T = 30 min
Training


B
Veh.
0.4
1.32
4
Latency to licking (s)
25
20
15
10
5
0
***
***
+ Pyrilamine
+ Zolantidine

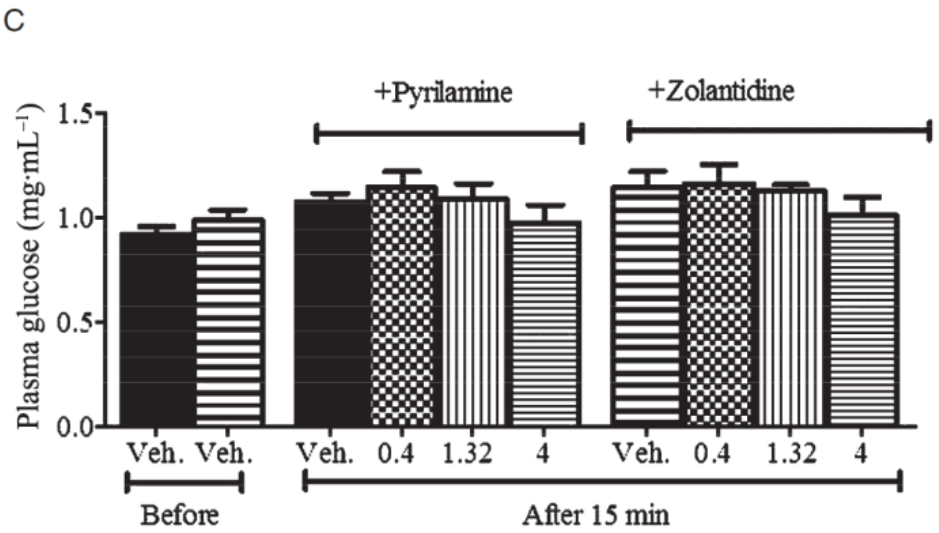


Fig. 16 The effects of H_1 and H_2 antagonists on TA1-induced modification of learning, pain threshold and plasma glycemia in mice Mice (n=20 for each groups of animals) were pre-treated s.c. with a single injection of pyrilamine (10 $mgkg^{-1}$) or zolantidine (5 $mgkg^{-1}$, 15 min before the i.c.v. injection of T1A (0.4, 1.32 and 4 μgkg^{-1}) or vehicle (Veh.). After 15 min from TA1 or vehicle (Veh.) injection, mice were subjected to the training session of the passive avoidance task (panel A), or put on the hot plate (panel B). Plasma glycemia was also measured 15 min after TA1 (0.4, 1.32 and 4 μgkg^{-1}) i.c.v. injection in the blood collected from the tail veins of 4 h starved mice (n=10 for each treatment) (Panel C). Each test was carried on as described in "Methods" and results are expressed as mean ± SEM. *P<0.05, **P<0.01 ,***P<0.001 vs. Veh.

4.19 The hyperalgesic and hyperglycemic effects of T1A are prevented in HDC$^{-/-}$ mice

Histidine decarboxylase (HDC) is the rate limiting step in the synthesis of histamine. Notwithstanding the availability of pharmacological inhibitors of HDC, the genetic silencing of the HDC gene represents a strategy to obtain a mouse depleted of histamine. Because of this, HDC$^{-/-}$ mice might represent a suitable model to investigate the participation of the histaminergic system in TA1 effects. In HDC$^{-/-}$ mice, i.c.v. injected TA1 (1.32 and 4 μgkg^{-1}) did not produce any significant reduction of pain threshold (Fig. 17, panel C) nor any increase in plasma glycemia (Fig. 17, panel D). On the contrary, HDC$^{+/+}$ mice responded to the same doses of TA1 with a significant increase of plasma glycemia and a reduction of pain threshold (Fig. 17, panel A and B).

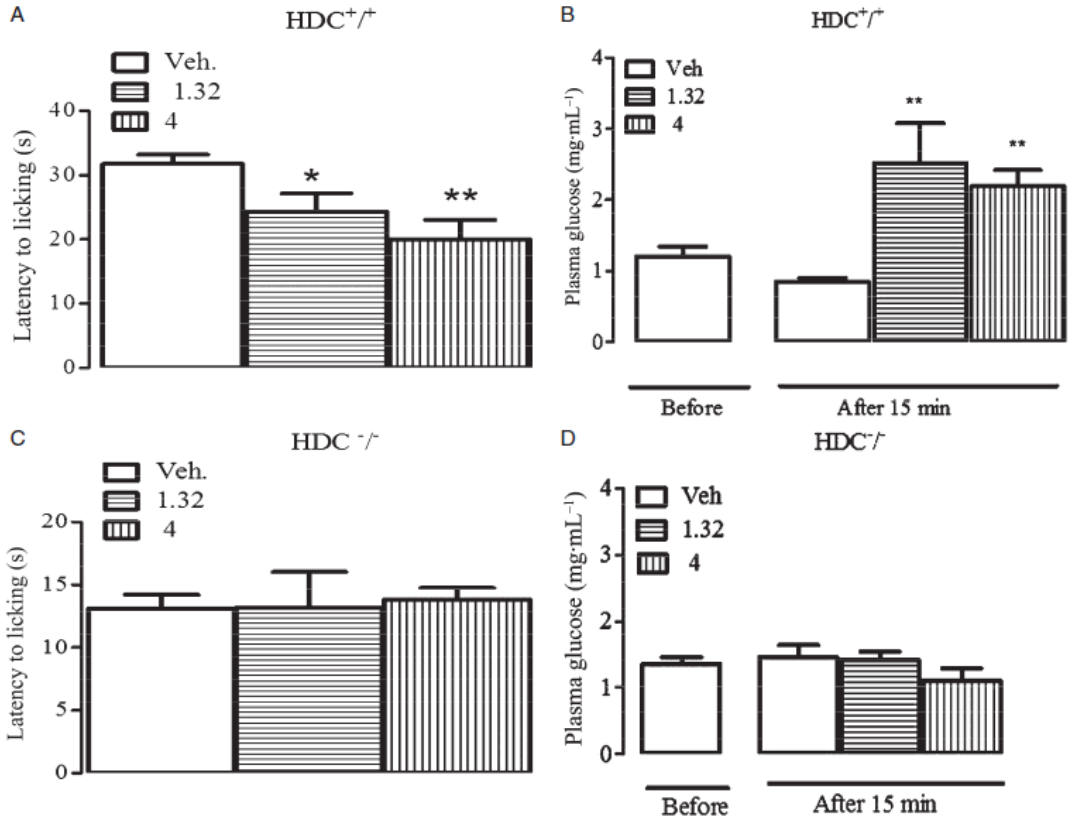


Fig. 17 T1A failed to induce hyperglycemia and to raise plasma glycemia when injected i.c.v. in HDC$^{-/-}$ mice. TA1 (1.32 and 4 μgkg^{-1}), or vehicle (Veh.), were injected i.c.v. in HDC$^{-/-}$ and HDC$^{+/+}$ mice (n=10 for each groups of animals). After 15 min, pain threshold to hot stimulus (panel A and C) was evaluated as described in "Methods". Plasma glycemia was evaluated in HDC$^{-/-}$ and HDC$^{+/+}$ mice (panel B and D) (n=10 for each groups of animals) fasted for 4 h before and 15 after the i.c.v. injection of TA1 (1.32 and 4 μgkg^{-1}) or vehicle (Veh.) as described in "Methods". Results are presented as the mean ± SEM. *P<0.05 vs. Veh.; **P<0.01vs. Veh.

4.20 Detection of endogenous T1AM and TA1 in the brain of euthyroid (CD1), $HDC^{+/+}$ and $HDC^{-/-}$ mice

Mice brains were analysed for the presence of TA1 and T1AM by HPLC coupled to mass spectrometry, as described in "Methods". We observed that both T1AM and its oxidative derivative, TA1, were endogenously occurring reaching concentrations of 48.6±17.7 and 0.8±0.2 $pmolg^{-1}$ of tissue, corresponding to 17.3±6.2 and 0.30±0.06 μgkg^{-1} (n=3), respectively. So, TA1 concentration represented about 1.6% of T1AM concentration. Interestingly, the WT $HDC^{+/+}$ mice exhibited significantly lower brain levels of T1AM (0.22 ± 0.03 $pmolg^{-1}$ of tissue; n=4, $P<0.001$) than found in CD1 mice and the level of TA1 were below the limit of detection. In $HDC^{-/-}$ mice, neither T1AM nor TA1 could be detected in brain tissue.

	T1AM (pmol/g of tissue)	TA1 (pmol/g of tissue)
CD1	48.6± 17.7	0.8±0.2
HDC +/+	0.22±0.03	**ND**
HDC -/-	ND	**ND**

Table 3 T1AM and TA1 brain levels were assayed by HLPC tandem mass spectrometry.

4.21 TA1 shares with T1AM some behavioural effects

We here provide, for the first time, evidence indicating that TA1, is endogenously occurring in the brain of mice representing 1.6% of its putative precursor T1AM. In these animals, pharmacologically injected TA1 produced rapid (within 15 min) effects including modification of memory, pain threshold and plasma glycemia. All these effects were modulated by histamine receptor antagonists. Furthermore, TA1 failed to raise plasma glycemia and to induce hyperalgesia when injected in mice bearing deletion of HDC gene. Because of this, this data support evidence indicating that the effects elicited by TA1 (1.32 and 4 μgkg^{-1}) are mediated by the release of histamine. In particular, the effect on memory was dose-related being TA1 amnesic at 0.4 μgkg^{-1} and stimulating learning at 1.32 and 4 μgkg^{-1}. Interestingly, this latter effect was not retained 24 h after training. Overall, these results show relevant differences between TA1 and T1AM: at the lowest dose studied (0.4 μgkg^{-1}) T1AM did not modify learning while, at higher doses (1.32 and 4 μgkg^{-1}) it improved memory also in the 24 h retention session. The reason of the different effect of T1AM and TA1 on memory is not clear. While it is possible that direct TA1 administration ensured at cell target(s) higher concentrations than obtained by T1AM oxidative deamination, it seems likely that the effects of T1AM are not entirely mediated by TA1. Instead, TA1-induced hyperalgesia and hyperglycemia were linearly dose related.

All these findings indicated TA1 as a potential novel neuromediator, and encouraged to exploit its mechanism of action.

Then, since modification of memory, increase of plasma glycemia and reduction of pain threshold were already reported following i.c.v injection of histamine and of selective histamine receptor agonists in rodents (Nishibori et al., 1999; Galeotti et al., 2004), we decided to verify the effect of anti-histaminergic drugs on TA1 pharmacological effects. The data show that the pro-learning, hyperalgesic and hyperglycemic effects induced by TA1 were prevented by H_1 or H_2 receptor antagonists, thus suggesting the involvement of histamine.

It is rather well known that histamine and histaminergic drugs produced controversial effect on memory depending on the brain region where they are injected, on the type of test used and on the receptor subtype recruited (Kohler et al., 2011). At this experimental settings it was not possible to locate the sites where histamine release

might occur since i.c.v. injection of TA1 ensures distribution of the drug throughout the central nervous system. However, we observed that pre-treatment of mice with pyrilamine abolished the stimulation of memory by 1.32 and 4 μgkg^{-1} TA1, whereas the amnesic effect of 0.4 μgkg^{-1} TA1 was maintained. On the other hand, at condition of zolantidine pre-treatment, TA1 turned out to be amnesic at all doses. Several hypothesis can be proposed to explain these results including that i) H_1 and H_2 receptors might control the release of mediators with opposite effects on memory, ii) under condition of H_2 or H_1 blockade, activation of H_3 receptors might prevail reducing the release of histamine or of other mediators stimulating memory (Korotkova et al., 2005; Murotani et al., 2009; Hough et al., 2011) iii) TA1 might have intrinsic activity at H_3 receptors.

In addiction, TA1 (1.32 μgkg^{-1}) reverted scopolamine-induced amnesia, producing a long-lasting memory consolidation which was also evident in the 24 h retention session. This latter result is in line with data reporting that muscarinic receptors (M4) negatively control histamine release (Nakamura et al., 2012). In addition, since at 4 μgkg^{-1} the reversion of amnesia was not evident there is the possibility that, at this dose of T1A, histamine released induced rapid desensitization or have opposite effects stimulating also receptors releasing amnesic mediators. Pyrilamine and zolantidine pre-treatment also affected hyperalgesia and hyperglycemia induced by TA1. In particular, histaminergic antagonists shifted the dose-response curve toward the right for the hyperalgesic effects of TA1 so that hyperalgesia was evident only at the highest dose (4 μgkg^{-1}).

Additional evidence indicating that the hyperlagesic and hyperglycemic effects of TA1 were linked to histamine release were obtained in $HDC^{-/-}$ mice. In this animal model, TA1 injection failed to increase glycemia and to reduce pain threshold to the hot stimulus. Interestingly, the hyperglycemic effect of TA1 was higher in $HDC^{+/+}$ than in CD1 mice. At the moment, we do not have any plausible explanation for this result.

Histamine is among the main mediators of inflammatory pain and itching and it acts as a nociceptive mediator with a mechanism which is not completely understood. Even if histamine has been found to potentiate the activity of acid-sensitive channels, including TRPV1 (Kajihara et al., 2010), at present there is no direct evidence that this receptor is involved in TA1 effects, although further investigations will be necessary to ascertain the potential role of TRPV1.

5 CONCLUSIONS

In my thesis I have demonstrated that a single low dose of T_1AM, closed to its tissue levels, is able to decrease mice feeding and plasma fT_3 levels, while increasing plasma glucose and raising insulin resistance. T_1AM appears to be a potent amine stimulating the CNS, the Pancreas and, in turn, the Liver where it might affect fT_3 production and/or degradation. Most of the studies on T1AM have been produced in rodents. It is interesting to note that while the definition of T1AM pharmacological features is on-going, the dose of the amine tested reduced progressively. Infact, starting from the early studies where Scanlan et al., (2004) observed T1AM hypothermia at 50 mg/kg of T1AM given i.p., we reported a lower dose of 1.32 μg/kg that given i.c.v. was enough in modifying animal feeding behaviour (Manni et al., 2012).

Up to know it is not known whether T1AM may have systemic bioavailability when given by oral route. Systemic bioavailability is instead obtained following i.c.v. administration. By using this route we were able to demonstrate that plasma levels of T1AM does not increase in parallel with the dose injected thus suggesting the involvement of a saturable transporter.

The finding regarding hyperglycemic effect and feeding can be closed to endocrine disorders where hyperglycemia is a common manifestation. Interestingly enough, endogenous tissue levels of T_1AM were found increased in Diabetic patients (Galli et al., 2012). It is interesting to note that diabetes often clusters with hypothyroidism, a condition which might produce modification of T_1AM tissue levels.

The picture we have drawn indicates that T1AM is a molecule with a very interesting non-genomic mechanism of action . Since these effects cluster in endocrine disorders, including thyroid dysfunctions (Fierabracci et al., 2011) and diabetes (Chari et al., 2010), the assay of tissue and plasma T1AM levels in experimental models of thyroid diseases and diabetes might open new paths for diagnosis and/or therapy of such diseases. In addition, prevention of the metabolic effects of T1AM might be included among the therapeutic, or side effects, of MAO inhibitors.

It is well known that thyroid gland plays important regulatory functions in tissue developmental and cell differentiation throughout all phases of life from gestation to aging. Disturbed action of the gland is linked with major health problems concerning diseases of thyroid hormones' target organs including the heart and vessels, the brain,

the bone. In line with this, hypo and hyper-functioning of the thyroid with different clinical manifestations and ethiogenesis, are currently identified as the endocrinological ground of several neurological and cardiac degenerative diseases with metabolic bases including diabetes, heart failure, obesity. Because of this, thyroid disorders represent, together with diabetes, a growing health-economical problem (Biondi and Cooper 2008).

Regulators of cell differentiation/cell de-differentiation process, such as thyroid hormones (TH) or analogues seem to represent novel and interesting therapeutic approaches against diabetes complication including heart failure. The idea is that restoration of a physiological TH and its receptors levels might counteract progression of heart disease by different mechanisms including: the modulation of myocardial gene expression and perfusion, improvement in haemodynamics, re-activation of adult phenotype and correct insulin sensitivity. This perspective is attractive even if most of the biology and pharmacological features of TH in the failing heart remain to be address. In this respect, the relation between TH and TH-derivatives, including thyronamines, might represent a new frontier to explore in order to exploit future pharmacological strategies, to anticipate serious side effects or pharmacological interactions of thyroid hormone-based therapies.

There is no doubt that the identification of such target(s) remains an crucial issue to make light on the physiological function(s) of the amine.

To the best of our knowledge, this is the first report indicating that the central effects of T1AM include the regulation of complex behavioural functions involved in learning and pain perception. These actions were associated with an increase in local T1AM concentration of about one order of magnitude, suggesting a novel potential physiological role of endogenous T1AM and/or its deaminated derivative(s).

Thyroid hormones are essential for the development of mammalian brain and maintenance of optimal cognitive ability in different periods of life (Bauer *et al*., 2008). In adulthood, thyroid dysfunction leads to neurological and behavioural abnormalities, including memory impairment. Adult-onset hypothyroidism is also associated with clinically relevant cognitive dysfunctions such as psychotic behaviour, hallucinations, confusion and learning defects (Rivas and Naranjo, 2007). Central hypothyroidism has been reported in patients with Alzheimer's disease (Sampaolo *et al*., 2005) and the analysis of different experimental models suggests that the effects on cognition rely on hippocampal modifications. In the present work, we demonstrated that T1AM, an

endogenous compound related to thyroid hormones, stimulates the acquisition of memory in the mouse and that this effect does not involve significant modifications of brain thyroid hormone levels. It should be noted, thyroid hormone levels found in the brains of our mice were similar to those demonstrated by Escobar-Morreale *et al*. (1996) and Pinna *et al*. (2002). Due to these novel effects, our results suggest that pharmacological administration of T1AM might be useful in neurodegenerative and endocrine disorders associated with memory deficits.

In addiction we here provide, for the first time, evidence that endogenous TA1 was found in the brain of CD1 mice, representing 1.6% of its putative precursor T1AM. In these animals, exogenous T1AM and TA1, injected i.c.v., produced rapid (within 15 min) effects including modification of memory, of nociceptive threshold and of plasma glucose.

TA1 effects were modulated by histamine H1 and H2 receptor antagonists. Furthermore, TA1 failed to raise plasma glucose and to induce hyperalgesia when injected in mice lacking histamine, indicating that these effects of TA1 (1.32 and 4 μg·kg−1) were mediated by histamine release. Since TA1 derives from oxidative deamination (by constitutive MAO activity) and/or deiodination of amine derivative thyroid hormone metabolites, T3 intracellular bioavailability potentially represents the limiting step in TA1 formation. We here present TA1 as a novel endogenous thyromimetic whose behavioral and metabolic effects reveal the existence of a signaling pathway integrating thyroid and histaminergic system.

The interaction between thyroid and histamine could have clinical relevance in explaining the occurrence of some symptoms associated with thyroid dysfunction. In addition, our results indicate oxidative deamination of T1AM as a limiting step in amine pharmacological effects. Accordingly, since deiodinase and MAO activities are over-expressed in pathological conditions and, notably, they are the target of drugs, endogenous levels of TA1 may change under pathological conditions or pharmacological treatments. Even if translation of experimental results to patients is always hard and dangerous, our results suggest that therapies targeting such enzymes deserve special attention and care.

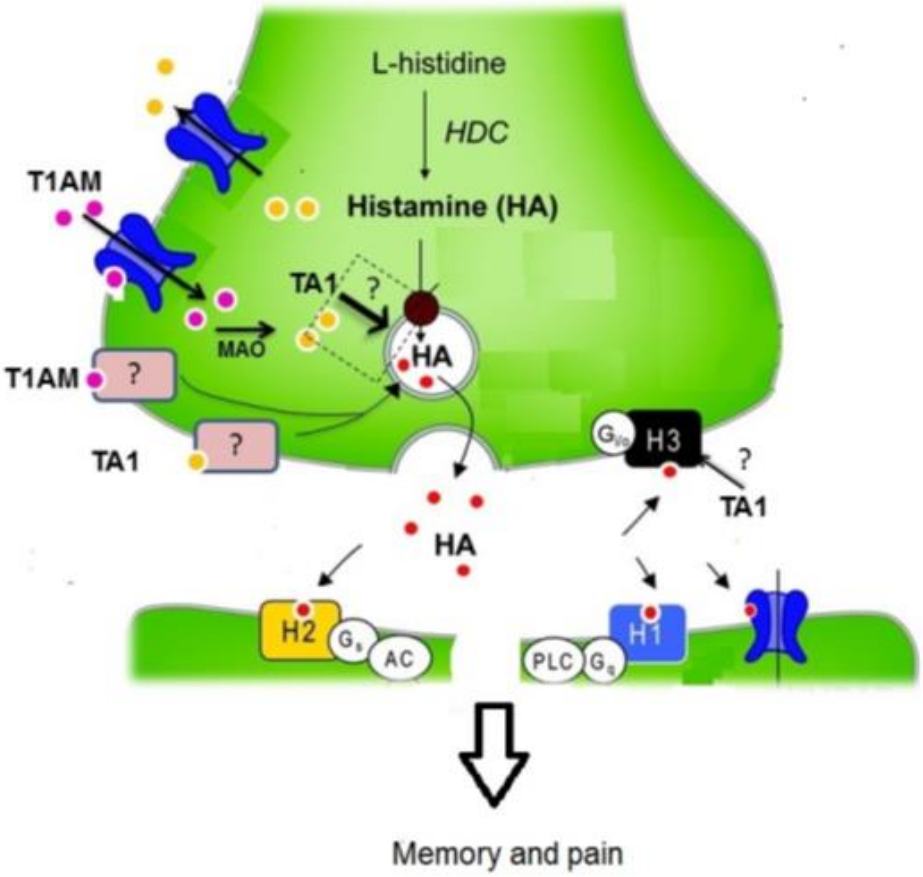


Fig. 1 3-iodothyronamine (T1AM) and 3-iodothyroacetic acid (TA1) interaction at histaminergic neurons. T1AM (pink circles) could interact at membrane receptors stimulating histamine (HA, red circles) release but also converted intracellular to TA1 (yellow circles). TA1 could induce HA release by producing vescicle trafficking or interacting at autoreceptors.

So far, thyromimetic compounds devoid of genomic activity are extensively studied for their potential therapeutic usefulness in ameliorating hyperlipidemia and its associated cardiovascular risk (Tancevski et al., 2011). We here present TA1 as a novel endogenous thyromimetic whose (non genomic) behavioural and metabolic effects have revealed the existence of a signalling pathway integrating thyroid and the histaminergic system. It is well established that 1-4% of patients with thyroid diseases experience generalized pruritus, without flare (Ward and Bernhard, 2005), whose pathophysiology is largely unknown and which can be relieved by anti histaminergic treatments (Hiramanek 2004). Interestingly, hypothyroid patients also show a reduced pain threshold (Guieu et al., 1993). About of this, the further study of interaction between thyronamines and histamine release might have clinical relevance in explaining some symptoms, including itch, associated with thyroid dysfunctions (Hiramanek, 2004).

6. REFERENCES

Ackermans MT, Klieverik LP, Ringeling P, Endert E, Kalsbeek A & Fliers E (2010) An online solid-phase extraction-liquid chromatography–tandem mass spectrometry method to study the presence of thyronamines in plasma and tissue and their putative conversion from 13C6-thyroxine. Journal of Endocrinology 206 327–334.

Agretti P, De Marco G, Russo L, Saba A, Raffaelli A, Marchini M, Chiellini G, Grasso L, Pinchera A, Vitti P, Scanlan TS, Zucchi R, Tonacchera M (2011) 3-Iodothyronamine metabolism and functional effects in FRTL5 thyroid cells. J Mol Endocrinol47:23-32.

Ai W, Liu Y, Langlois M, Wang TC. (2004) Kruppel-like factor 4 (KLF4) represses histidine decarboxylase gene expression through an upstream Sp1 site and downstream gastrin responsive elements. *J Biol Chem* 279: 8684–8693

Alkemade A, Vuijst CL, Unmehopa UA, Bakker O, Vennstrom B, Wiersinga WM, Swaab DF, Fliers E (2005) Thyroid hormone receptor expression in the human hypothalamus and anterior pituitary. J Clin Endocrinol Metab 90:904-912

Almeida AP, Beaven MA. (1981) Phylogeny of histamine in vertebrate brain. *Brain Res* 208: 244–250

Andrew D, Craig AD. **(**2001) Spinothalamic lamina I neurons selectively sensitive to histamine: a central neural pathway for itch. *Nat Neurosci* 4: 72–77

Anzai R, Adachi M, Sho N, Muroya K, Asakura Y & Onigata K (2012) Long-term 3,5,30 triiodothyroacetic acid therapy in a child with hyperthyroidism caused by thyroid hormone resistance: pharmacological study and therapeutic recommendations. Thyroid 22 1069–1075

Arrang JM, Garbarg M, Lancelot JC, Lecomte JM, Pollard H, Robba M, Schunack W, Schwartz JC. (1987) Highly potent and selective ligands for histamine H3-receptors. *Nature* 327: 117–123.

Arrang JM, Garbarg M, Schwartz JC. (1983) Auto-inhibition of brain histamine release mediated by a novel class (H3) of histamine receptor. *Nature* 302: 832–837

Arrang JM, Gulat-Marnay C, Defontaine N, Schwartz JC. (1991)Regulation of histamine release in rat hypothalamus and hippocampus by presynaptic galanin receptors. *Peptides* 12: 1113–1117

Axelband F., Dias J., FerraO FM, and Einicker-Lamas M. (2011) Nongenomic Signaling Pathways Triggered by Thyroid Hormones and Their Metabolite 3-Iodothyronamine on the Cardiovascular System. J Cell Physiol 226: 21–28.

Baldi E, Bucherelli C, Schunack W, Cenni G, Blandina P, Passani MB. (2005) The H3 receptor protean agonist proxyfan enhances the expression of fear memory in the rat. *Neuropharmacology* 48: 246–251

Barke KE, Hough LB. (1994) Characterization of basal and morphineinduced histamine release in the rat periaqueductal gray. *J Neurochem* 63: 238–244

Barnes WG, Hough LB. (2002). Membrane-bound histamine *N*-methyltransferase in mouse brain: possible role in the synaptic inactivation of neuronal histamine. *J Neurochem* 82: 1262–1271

Bassett JH, Harvey CB & Williams GR (2003) Mechanisms of thyroid hormone receptor-specific nuclear and extra nuclear actions. Molecular and Cellular Endocrinology 213 1–11.

Bauer M, Goetz T, Glenn T, Whybrow PC (2008). The thyroid-brain interaction in thyroid disorders and mood disorders. J Neuroendocrinol 20: 1101–1114.

Becskei C,. Lutz TA, and Riediger T (2010). Reduced fasting-induced activation of hypothalamic arcuate neurons is associated with hyperleptinemia and increased leptin sensitivity in obese mice. *Am J Physiol Regul Integr Comp Physiol* 299: R632–R641.

Bernal J (2007) Thyroid hormone receptors in brain development and function. Nat Clin Pract Endocrinol Metab 3:249-259

Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR (2002) Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocr Rev 23:38-89

Biondi B, Cooper DS (2008) The clinical significance of subclinical thyroid dysfunction. Endocr Rev, 29:76-131.

Bly M (2005) Examination of the trace amine-associated receptor 2 (TAAR2). Schizophrenia Research 80: 367–368.

Borgioni S, Chiellini G, Suffredini S, Cerbai E, Zucchi R (2007). 3-Iodothyronamine affects calcium handling in isolated rat cardiomyocytes. J Mol Cell Cardiol 42:S21

BraccoD, MorinO, SchutzY, Liang H, JequierE&BurgerAG (1993) Comparison of the metabolic and endocrine effects of 3,5,30-triiodothyroacetic acid and thyroxine. Journal of Clinical Endocrinology and Metabolism 77 221–228.

Braulke LJ, Klingenspor M, DeBarber A, Tobias SC, Grandy DK, Scanlan TS & Heldmaier G (2008) 3-Iodothyronamine: a novel hormone controlling the balance between glucose and lipid utilisation. Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology 178 167–177.

Cannon KE, Leurs R, Hough LB.(2007)Activation of peripheral and spinal histamine H(3) receptors inhibits formalin-induced inflammation and nociception, respectively. *Pharmacol Biochem Behav* 88: 122–129,.

Chari M, Lam CKL, Lam TKT (2010). Hypothalamic Fatty Acid Sensing in the Normal and Disease States. Editors Montmayeur JP, le Coutre J, *In: Fat Detection: Taste, Texture, and Post Ingestive Effects*.. Chapter 20.Frontiers in Neuroscience.

Chen G, Chen P, Tan H, Ma D, Dou F, Feng J *et al*. (2008). Regulation of the NMDA receptor-mediated synaptic response by acetylcholinesterase inhibitors and its impairment in an animal model of Alzheimer's disease. Neurobiol Aging 29: 1795–1804.

Chiellini G, Apriletti JW, Yoshihara HA, Baxter JD, Ribeiro RC & Scanlan TS (1998) A high-affinity subtype-selective agonist ligand for the thyroid hormone receptor. Chemistry & Biology 5 299–306.

Chiellini G, Frascarelli S, Ghelardoni S, Carnicelli V, Tobias SC, DeBarber A et al (2007) Cardiac effects of 3-iodothyronamine: a new aminergic system modulating cardiac function. FASEB J21:1597–1608.

Chou TC, Scammell TE, Gooley JJ, Gaus SE, Saper CB, Lu J. (2003) Critical role of dorsomedial hypothalamic nucleus in a wide range of behavioral circadian rhythms. *J Neurosci* 23: 10691–10702

Chu XP, Papasian CJ, Wang JQ, Xiong ZG (2011) Modulation of acid-sensing ion channels: molecular mechanisms and therapeutic potential. Int J Physiol Pathophysiol Pharmacol 3:288-309.

Constant EL, de Volder AG, Ivanoiu A, Bol A, Labar D, Seghers A, Cosnard G, Melin J, Daumerie C (2001) Cerebral blood flow and glucose metabolism in hypothyroidism: a positron emission tomography study. J Clin Endocrinol Metab 86:3864-3870

Coppola A, Liu ZW, Andrews ZB, Paradis E, Roy MC, Friedman JM, et al (2007). A central thermogenic-like mechanism in feeding regulation: an interplay between arcuate nucleus T3 and UCP2. *Cell Metab*. 5:21-33.

Crantz FR, Silva JE, Larsen PR (1982) An analysis of the sources and quantity of 3,5,3'-triiodothyronine specifically bound to nuclear receptors in rat cerebral cortex and cerebellum. Endocrinology 110:367- 375

Cumero S, Fogolari F, Domenis R, Zucchi R, Mavelli I, Contessi S (2012) Mitochondrial F(0) F(1) -ATP synthase is a molecular target of 3-iodothyronamine, an endogenous metabolite of thyroid hormone. Br J Pharmacol 166:2331-47.

D'Andrea G, Nordera G, Pizzolato G, Bolner A, Colavito D, et al. (2010) Trace amine metabolism in Parkinson's disease: Low circulating levels of octopamine in early disease stages. Neuroscience Letters 469: 348–351.

De Esch IJ, Thurmond RL, Jongejan A, Leurs R. (2005) The histamine H4 receptor as a new therapeutic target for inflammation. *Trends Pharmacol Sci* 26: 462–469.

DeBarber AE, Geraci T, Colasurdo VP, Hackenmueller SA & Scanlan TS (2008) Validation of a liquid chromatography–tandem mass spectrometry method to enable quantification of 3-iodothyronamine from serum. Journal of Chromatography. A 1210 55–59.

Dere E, Souza-Silva MA, Topic B, Spieler RE, Haas HL, Huston JP. **(**2003) Histidine-decarboxylase knockout mice show deficient nonreinforced episodic object memory, improved negatively reinforced water-maze performance, increased neo- and ventro-striatal dopamine turnover. *Learn Mem* 10: 510–519

Dhillo WS, Bewick GA, White NE, Gardiner JV, Thompson EL, Bataveljic A, et al.(2009). The thyroid hormone derivative 3-iodothyronamine increases food intake in rodents. *Diabetes Obes Metab*. 11:251-60.

Diano S, Naftolin F, Goglia F, Horvath TL (1998) Fasting-induced increase in type II iodothyronine deiodinase activity and messenger ribonucleic acid levels is not reversed by thyroxine in the rat hypothalamus. Endocrinology 139:2879-2884

Diewald L, Heimrich B, Busselberg D, Watanabe T, Haas HL. (1997) Histaminergic system in co-cultures of hippocampus and posterior hypothalamus: a morphological and electrophysiological study in the rat. *Eur J Neurosci* 9: 2406–2413.

Dismukes K, Snyder SH. (1974) Histamine turnover in rat brain. *Brain Res* 78: 467–481

Doyle KP, Suchland KL, Ciesielski TM, Lessov NS, Grandy DK, Scanlan TS *et al*. (2007). Novel thyroxine derivatives, thyronamine and 3-iodothyronamine, induce transient hypothermia and marked neuroprotection against stroke injury. Stroke 38: 2569–2576.

Dratman MB, Crutchfield FL, Schoenhoff MB (1991) Transport of iodothyronines from bloodstream to brain: contributions by blood:brain and choroid plexus:cerebrospinal fluid barriers. Brain Res 554:229-236

Dratman MB, Futaesaku Y, Crutchfield FL, Berman N, Payne B, Sar M, Stumpf WE (1982) Iodine-125-labeled triiodothyronine in rat brain: evidence for localization in discrete neural systems. Science 215:309-312

Dratman MB, Gordon JT (1996) Thyroid hormones as neurotransmitters. Thyroid 6:639-647

Duan J, Martinez M, Sanders AR, Hou C, Saitou N, et al. (2004) Polymorphisms in the Trace Amine Receptor 4 (TRAR4) Gene on Chromosome 6q23.2 Are Associated with Susceptibility to Schizophrenia. The American Journal of Human Genetics 75: 624–638.

Dugbartey AT 1998 Neurocognitive aspects of hypothyroidism. Arch Intern Med 158:1413-1418

Dumas P, Autissier N, Loireau A & Michel R (1982) Effects of 3,5,30-triiodothyroacetic acid (TRIAC) on protein metabolism of genetically obese or non-obese Zucker rats. Comptes Rendus des Se´ances de la Socie´te´ de Biologie et de Ses Filiales 176 178–183.

Ennaceur A, Delacour J (1988). A new one-trial test for neurobiological studies of memory in rats. 1: behavioral data. Behav Brain Res 31: 47–59.

Erickson JD, Schafer MK, Bonner TI, Eiden LE, Weihe E. (1996) Distinct pharmacological properties and distribution in neurons and endocrine cells of two isoforms of the human vesicular monoamine transporter. *Proc Natl Acad Sci USA* 93: 5166–5171

Eriksson KS, Sergeeva OA, Selbach O, Haas HL. (2004) Orexin (hypocretin)/ dynorphin neurons control GABAergic inputs to tuberomammillary neurons. *Eur J Neurosci* 19: 1278–1284.

Eriksson KS, Stevens DR, Haas HL. (2000) Opposite modulation of histaminergic neurons by nociceptin and morphine. *Neuropharmacology* 39: 2492–2498.

Escobar-Morreale HF, Obregon MJ, Hernandez A, Escobar dR, Morreale dE (1997) Regulation of iodothyronine deiodinase activity as studied in thyroidectomized rats infused with thyroxine or triiodothyronine. Endocrinology 138:2559-2568

Fleming JV, Fajardo I, Langlois MR, Sanchez-Jimenez F, Wang TC. (2004) The C-terminus of rat L-histidine decarboxylase specifically inhibits enzymic activity and disrupts pyridoxal phosphatedependent interactions with L-histidine substrate analogues. *Biochem J* 381: 769–778.

Frascarelli S, Chiellini S, Ghelardoni S, Ronca-Testoni S, GrandyDK, Scanlan TS et al. (2008) Cardioprotection by 3-iodothyronamine, a new endogenous chemical messenger. J Mol Cell Cardiol 44:773 (abstract).

Frascarelli S, Ghelardoni S, Chiellini G, Vargiu R, Ronca-Testoni S, Scanlan TS, Grandy DK & Zucchi R (2008) Cardiac effects of trace amines: pharmacological characterization of trace amine associated receptors. European Journal of Pharmacology 587 231–236.

Friesema EC, Ganguly S, Abdalla A, Manning Fox JE, Halestrap AP, Visser TJ (2003) Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter. J Biol Chem 278:40128-40135

Galeotti N, Bartolini A, Ghelardini C (2006). Blockade of intracellular calcium release induces an antidepressant-like effect in the mouse forced swimming test. Neuropharmacology 50: 309–316.

Galeotti N, Malmberg-Aiello P, Bartolini A, Schunack W, Ghelardini C (2004) H1-receptor stimulation induces hyperalgesia through activation of the phospholipase C-PKC pathway. Neuropharmacology 47:295-303.

Galli E, Marchini M, Saba A, Berti S, Tonacchera M, Vitti P *et al*. (2012). Detection of 3-iodothyronamine in human patients: a preliminary study. J Clin Endocrinol Metab 97: E69–E74.

Garcia M, Floran B, Arias-Montano JA, Young JM, Aceves J (1997). Histamine H3 receptor activation selectively inhibits dopamine D1 receptor-dependent [3H]GABA release from depolarization-stimulated slices of rat substantia nigra pars reticulata. *Neuroscience* 80: 241–249.

Gbahou F, Rouleau A, Morisset S, Parmentier R, Crochet S, Lin JS, Ligneau X, Tardivel-Lacombe J, Stark H, Schunack W, Ganellin CR, Schwartz JC, Arrang JM. (2003) Protean agonism at histamine H3 receptors in vitro and in vivo. *Proc Natl Acad Sci USA* 100: 11086–11091.

Gompfa S H, Greenberg J H, Aston-Jones G, IanculescuA G, Scanlan TS, Dratman MB (2010). 3-Monoiodothyronamine: The rationale for its action as an endogenous adrenergic-blocking neuromodulator. *Brain Res* 1351 : 130–140.

Gotoh K, Fukagawa K, Fukagawa T, Noguchi H, Kakuma T, Sakata T, Yoshimatsu H. (2007) Hypothalamic neuronal histamine mediates the thyrotropin-releasing hormone-induced suppression of food intake. *J Neurochem* 103: 1102–1110.

Gribble FM, Phil. DBM B.Ch (2010) α_{2A}-Adrenergic Receptors and Type 2 DiabetesN Engl J Med; 362:361-36

Guasti L, Marino F, Cosentino M, Cimpanelli M, Rasini E, Piantanida E *et al*. (2007). Pain perception, blood pressure levels, and peripheral benzodiazepine receptors in patients followed for differentiated thyroid carcinoma: a longitudinal study in hypothyroidism and during hormone treatment. Clin J Pain 23: 518–523.

Guieu R, Harley JR, Blin O, Pouget J, Serratrice G. (1993) Nociceptive threshold in hypothyroid patients. Acta Neurol.;15(3):183-8.

Hackenmueller SA, Marchini M, Saba A, Zucchi R & Scanlan TS (2012) Biosynthesis of 3 iodothyronamine (T1AM) is dependent on the sodium–iodide symporter and thyroperoxidase but does not involve extrathyroidal metabolism of T4. Endocrinology 153 5659–5667.

Hackenmueller SA, Scanlan TS (2012) Identification and quantification of 3-iodothyronamine metabolites in mouse serum using liquid chromatography-tandem mass spectrometry. Endocrinology 1256:89-97.

Haley Tj, McCormick Wg, (1957). Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. *Br J Pharmacol Chemother*. 12:12-5.

Harington CR (1926) Chemistry of Thyroxine: Constitution and Synthesis of Desiodo-Thyroxine. Biochem J 20:300-313

Hart ME, Suchland KL, Miyakawa M, Bunzow JR, Grandy DK & Scanlan TS (2006) Trace amine-associated receptor agonists; synthesis and evaluation of thyronamines and related analogues. Journal of Medicinal Chemistry 49 1101–1112.

Hettinger BD, Schuff K, Marks D, Scanlan TS (2010) 3-iodothyronamine (T1AM) causes weight loss in mice via reduction in food consumption. 14th International Thyroid Congress, Paris, France: OC-141 (Abstract)

Hill SJ, Ganellin CR, Timmerman H, Schwartz JC, Shankley NP, Young JM, Schunack W, Levi R, Haas HL. (1997) International Union of Pharmacology. XIII. Classification of histamine receptors. *Pharmacol Rev* 49: 253–278.

Hoefig CS, Ko¨hrle J, Brabant G, Dixit K, Yap J, Strasburger CJ & Wu Z (2011) Evidence for extrathyroidal formation of 3-iodothyronamine in humans as provided by a novel monoclonal antibody-based chemiluminescent serum immunoassay. Journal of Clinical Endocrinology and Metabolism 98 1864–1872.

Hoefig CS, Renko K, Piehl S, Scanlan TS, Bertoldi M, Opladen T, Hoffmann GF, Klein J, Blankenstein O, Sc weizer U et al. (2012) Does the aromatic L-amino acid decarboxylase contribute to thyronamine biosynthesis? Molecular and Cellular Endocrinology 349 195–201.

Horn S, Kersseboom S, Mayerl S, Mu¨ller J, Groba C, Trajkovic-Arsic M, Ackermann T, Visser TJ & Heuer H (2013) Tetrac can replace thyroid hormone during brain development in mouse mutants deficient in the thyroid hormone transporter mct8. Endocrinology 154 968–679.

Hough LB, Nalwalk JW, Barnes WG, Leurs R, Menge WM, Timmerman H, Wentland M. (2000) A third life for burimamide. Discovery and characterization of a novel class of non-opioid analgesics derived from histamine antagonists. *Ann NY Acad Sci* 909: 25–40.

Hough LB, Rice FL (2011) H3 receptors and pain modulation: peripheral, spinal, and brain interactions. J Pharmacol Exp Ther. 336:30-37

Hu HJ, Alter BJ, Carrasquillo Y, Qiu CS, Gereau RW 4th (2007). Metabotropic glutamate receptor 5 modulates nociceptive plasticity via extracellular signal-regulated kinase-Kv4.2 signaling in spinal cord dorsal horn neurons. J Neurosci 27: 13181–13191.

Hutcheon B, Puil E, Spigelman I. (1993) Histamine actions and comparison with substance P effects in trigeminal neurons. *Neuroscience* 55: 521–529.

Ianculescu AG, Friesema EC, Visser TJ, Giacomini KM & Scanlan TS (2010) Transport of thyroid hormones is selectively inhibited by 3-iodothyronamine. Molecular BioSystems 6 1403–1410.

Ianculescu AG, Giacomini KM, Scanlan TS (2009). Identification and characterization of 3-iodothyronamine intracellular transport. *Endocrinology*. 150:1991-9.

Ikoma A, Steinhoff M, Stander S, Yosipovitch G, Schmelz M. (2006) The neurobiology of itch. *Nat Rev Neurosci* 7: 535–547.

Ilani T, Lamensdorf I, Finberg JP (2000). Selective monoamine oxidase subtype inhibition and striatal extracellular dopamine in the guinea-pig. *Br J Pharmacol*. 130:1992-8.

Ishibory M, Itoh Y, Oishi R and Saeki K (1990) Effect of Microinjection of Histamine into the Brain on Plasma Levels of Epinephrine and Glucose in Freely Moving Rats. J of Pharmacol 54: 257-263

Ishii S, Kamegai J, Tamura H, Shimizu T, Sugihara H, Oikawa S (2003) Hypothalamic neuropeptide Y/Y1 receptor pathway activated by a reduction in circulating leptin, but not by an increase in circulating ghrelin, contributes to hyperphagia associated with triiodothyronine-induced thyrotoxicosis. Neuroendocrinology 78:321-330

Jarvik ME, Kopp R (1967). An improved one-trial passive avoidance learning situation. Psychol Rep 21: 221–224.

Ji RR, Gereau RW 4th, Malcangio M, Strichartz GR (2009). MAP kinase and pain. Brain Res Rev 60: 135–148.

Jongejan A, Bruysters M, Ballesteros JA, Haaksma E, Bakker RA, Pardo L, Leurs R. (2005) Linking agonist binding to histamine H1 receptor activation. *Nat Chem Biol* 1: 98–103

Kabadi UM, Premachandra BN (1988). Decline of T3 and elevation in reverse T3 induced by hyperglucagonemia*:* changes in thyroid hormone metabolism, not altered release of thyroid hormones. *Horm Metab Res.* 20:513-6.

Kajihara Y, Murakami M, Imagawa T, Otsuguro K, Ito S, Ohta T (2010) Histamine potentiates acid-induced responses mediating transient receptor potential V1 in mouse primary sensory neurons. Neuroscience 166:292-304.

Kalsbeek A, Buijs RM, van SR, Kaptein E, Visser TJ, Doulabi BZ, Fliers E (2005) Daily variations in type II iodothyronine deiodinase activity in the rat brain as controlled by the biological clock. Endocrinology 146:1418-1427

Karlstedt K, Sallmen T, Eriksson KS, Lintunen M, Couraud PO, Joo F, Panula P. Lack of histamine synthesis and downregulation of H1 and H2 receptor mRNA levels by dexamethasone in cerebral endothelial cells. *J Cereb Blood Flow Metab* 19: 321–330

Kendall EC (1919) Isolation of the Iodine Compound Which Occurs in the Thyroid . J.Biol.Chem. 39, 125-147

Kida S, Josselyn SA, Peña de Ortiz S, Kogan JH, Chevere I, Masushige S *et al*. (2002). CREB required for the stability of new and reactivated fear memories. Nat Neurosci 5: 348–355.

Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG (2010). NC3Rs Reporting Guidelines Working Group. Br J Pharmacol 160:1577–1579.

Klapdor K, Hasenohrl RU, Huston JP. (1994) Facilitation of learning in adult and aged rats following bilateral lesions of the tuberomammillary nucleus region. *Behav Brain Res* 61: 113–116.

Klieverik LP, Foppen E, Ackermans MT, Serlie MJ, Sauerwein HP, Scanlan TS, Grandy DK, Fliers E & Kalsbeek A (2009) Central effects of thyronamines on glucose metabolism in rats. Journal of Endocrinology 201 377–386.

Klieverik LP, Foppen E, Ackermans MT, Serlie MJ, Sauerwein HP, Scanlan TS et al. (2009). Central effects of thyronamines on glucose metabolism in rats. *J Endocrinol*. 201:377-86.

Knigge U, Warberg J. (1991) The role of histamine in the neuroendocrine regulation of pituitary hormone secretion. *Acta Endocrinol* 124: 609–619.

Kohler CA, da Silva WC, Benetti F, Sartori Bonini J (2011) Histaminergic mechanisms for modulation of memory systems Neural Plast..

Kollonitsch J, Perkins LM, Patchett AA, Doldouras GA, Marburg S, Duggan DE, Maycock AL, Aster SD. (1978) Selective inhibitors of biosynthesis of aminergic neurotransmitters. *Nature* 274: 906– 908.

Kong WM, Martin NM, Smith KL, Gardiner JV, Connoley IP, Stephens DA, Dhillo WS, Ghatei MA, Small CJ, Bloom SR (2004) Triiodothyronine stimulates food intake via the hypothalamic ventromedial nucleus independent of changes in energy expenditure. Endocrinology 145:5252-5258

Korotkova TM, Sergeeva OA, Ponomarenko AA, Haas HL (2005) Histamine excites noradrenergic neurons in locus coeruleus in rats. Neuropharmacology 49:129-34.

Kunitake JM, Hartman N, Henson LC, Lieberman J, Williams DE, Wong M& Hershman JM (1989) 3,5,30-Triiodothyroacetic acid therapy for thyroid hormone resistance. Journal of Clinical Endocrinology and Metabolism 69 461–466.

Kuramasu A, Sukegawa J, Yanagisawa T, Yanai K. (2006) Recent advances in molecular pharmacology of the histamine systems: roles of C-terminal tails of histamine receptors. *J Pharmacol Sci* 101: 7–11.

Lechan RM, Fekete C (2005) Role of thyroid hormone deiodination in the hypothalamus. Thyroid 15:883-897

Lechan RM, Fekete C (2006) The TRH neuron: a hypothalamic integrator of energy metabolism. Prog Brain Res 153:209-235

Lerman J (1956) The activity of dl-tribromthyronine. Journal of Clinical Endocrinology and Metabolism 16 1395–1397.

Leurs R, Bakker RA, Timmerman H, de Esch IJ. (2005) The histamine H3 receptor: from gene cloning to H3 receptor drugs. *Nat Rev Drug Discov* 4: 107–120.

Liu L, Ji F, Liang J, He H, Fu Y, Cao M (2012). Inhibition by dexmedetomidine of the activation of spinal dorsal horn glias and the intracellular ERK signaling pathway induced by nerve injury. Brain Res 1427: 1–9.

Liu L, Zhang S, Zhu Y, Fu Q, Zhu Y, Gong Y, Ohtsu H, Luo J, Wei E, Chen Z. (2007) Improved learning and memory of contextual fear conditioning and hippocampal CA1 long-term potentiation in histidine decarboxylase knock-out mice. *Hippocampus* 17: 634–641.

Lyles GA,Callingham BA. (1979) Selective influences of age and thyroid hormones on type A monoamine oxidase of the rat heart. J Pharm Pharmacol.

Malmberg-Aiello P, Lamberti C, Ghelardini C, Giotti A, Bartolini A. (1994) Role of histamine in rodent antinociception. *Br J Pharmacol* 111: 1269–1279.

Malmberg-Aiello P, Lamberti C, Ipponi A, Bartolini A, Schunack W. (1998) Evidence for hypernociception induction following histamine H1 receptor activation in rodents. *Life Sci* 63: 463–476.

Manni ME, Bigagli E, Lodovici M, Zazzeri M, Raimondi L (2012) The protective effect of losartan in the nephropathy of the diabetic rat includes the control of monoamine oxidase type A activity. Pharmacol Res 65:465-71.

Manni ME, De Siena G, Saba A, Marchini M, Bigagli E, Cinci L, et al (2012) 3-iodothyronamine: a modulator of the hypothalamus-pancreas-thyroid axes in mouse. Br J Pharmacol 166:650-8.

Manni ME, De Siena G, Saba A, Marchini M, Landucci E, Gerace E, Zazzeri M, Musilli C, Pellegrini-Giampietro D, Matucci R, Zucchi R, Raimondi L **(**2013) Pharmacological effects of 3-iodothyronamine (TA1M) in mice include facilitation of

memory acquisition and retention and reduction of pain threshold. Br J Pharmaco*l* 168:354-62.

Masini-Repiso AM, Bonaterra M, Spitale L, Di Fulvio M, Bonino MI, Coleoni AH, Orgnero-Gaisán E (2004) Ultrastructural localization of thyroid peroxidase, hydrogen peroxide-generating sites, and monoamine oxidase in benign and malignant thyroid diseases. Hum Pathol 35:436-46.

McGrath J, Drummond G, Kilkenny C, Wainwright C (2010). Guidelines for reporting experiments involving animals: the ARRIVE guidelines. Br J Pharmacol 160: 1573–1576.

McLaughlin JT, Ai W, Sinclair NF, Colucci R, Raychowdhury R, Koh TJ, Wang TC. (2004) PACAP and gastrin regulate the histidine decarboxylase promoter via distinct mechanisms. *Am J Physiol Gastrointest Liver Physiol* 286: G51–G59.

Medina-Gomez G, Hernandez A, Calvo RM, Martin E & Obregon MJ (2003) Potent thermogenic action of triiodothyroacetic acid in brown adipocytes. Cellular and Molecular Life Sciences 60 1957–1967.

Metszaros Z, Szombathy T, Raimondi L, Karadi I, Romics L, and K Magyar (1999) Elevated serum levels of semicarbazide-sensntive amine oxidase activity in Non-Insulin-Dependent Diabetes Mellitus: Correlation With Body Mass Index and Serum Triglyceride. Metabolism *48:* 113-117.

Mignot E, Taheri S, Nishino S. (2002) Sleeping with the hypothalamus: emerging therapeutic targets for sleep disorders. *Nat Neurosci* 5 *Suppl*: 1071–1075.

Mitchell MA, Raza A (1986).The effects of glucagon and insulin on plasma thyroid hormon*e* levels in fed and fasted domestic fowls. *Comp Biochem Physiol A Comp Physiol*.85:217-23.

Mobarakeh JI, Takahashi K, Sakurada S, Nishino S, Watanabe H, Kato M, Naghdi N, Yanai K. (2005) Enhanced antinociception by intracerebroventricularly administered orexin A in histamine H1 or H2 receptor gene knockout mice. *Pain* 118: 254–262.

Mochizuki H, Tashiro M, Kano M, Sakurada Y, Itoh M, Yanai K. (2003).Imaging of central itch modulation in the human brain using positron emission tomography. *Pain* 105: 339–346.

Mochizuki T, Yamatodani A, Okakura K, Horii A, Inagaki N, Wada H. (1992) Circadian rhythm of histamine release from the hypothalamus of freely moving rats. Physiol Behav 51: 391–394.

Moreno M, Kaptein E, Goglia F & Visser TJ (1994) Rapid glucuronidation of tri- and tetraiodothyroacetic acid to ester glucuronides in human liver and to ether glucuronides in rat liver. Endocrinology 135 1004–1009.

Morreale de Escobar G (1996). Only the combined treatment with thyroxine and triiodothyronine ensures euthyroidism in all tissues of the thyroidectomized rats. Endocrinology 137: 2490–2502.

Murotani T, Ishizuka T, Nakazawa H, Wang X, Mori K, Sasaki K, Ishida T, Yamatodani (2010) Possible involvement of histamine, dopamine, and noradrenalin in the periaqueductal gray in electroacupuncture pain relief. Brain Res 1306:62-68.

Murphy BA, Jochnowitz N, Fakira K, Gagen K, Contie S, Lorsignol A et al. (2009). Fasting enhances the response of arcuate neuropeptide Y-glucose-inhibited neurons to decreased extracellular glucose**.** *Am J Physiol Cell Physiol.*296:C746-56.

M. Wolf, A. Weigert, and G. Kreymann, (1996) “Body composition and energy expenditure in thyroidectomized patients during short-term hypothyroidism and thyrotropin-suppressive thyroxine therapy,” European Journal of Endocrinology, vol. 134,168–173.

N.Manji,K.Boelaert,M.C.Sheppard,R.L.Holder,S.C. Gough, and J. A. Franklyn, (2006) "Lack of association between serum TSH or free T4 and body mass index in euthyroid subjects," Clinical Endocrinology, 125–128.

Nakada K, Mitsuma T, Furusawa A, Maeda Y, Morise K. (1990) The effects of histamine on the concentrations of immunoreactive thyrotropin- releasing hormone in the stomach and hypothalamus in rats. *Gastroenterol Jpn* 25: 425–431.

Nakamura M, Jang IS (2012) Muscarinic M4 receptors regulate GABAergic transmission in rat tuberomammillary nucleus neurons Neuropharmacology. 63:936-44.

Nishibori M, Itoh Y, Oishi R And Saeki K (1987) Mechanism of the Central Hyperglycemic Action of Histamine inMice1. J Pharm Exp Therap 241: 582-586.

Nishibori M, Tahara A, Sawada K, Sakiyama J, Nakaya N, Saeki K. (2000) Neuronal and vascular localization of histamine *N*-methyltransferase in the bovine central nervous system. *Eur J Neurosci* 12: 415–424.

Ohtsu H, Tanaka S., Terui T, Hori Y, Makabe-Kobayashi Y, Pejler G, Buzas E, Kovacs P, Csaba G, Kittel A, Okada M, Hara M, Mar L, Numayama-Tsuruta K, Ishigaki-Suzuki S, Ohuchi K, Ichikawa A, Falus A, Watanabe T, and Nagy A (2001) Mice lacking histidine decarboxylase exhibit abnormal mast cells. FEBS Lett 502: 53–56.

Olmo MT, Urdiales JL, Pegg AE, Medina MA, Sanchez-Jimenez F. (2000).In vitro study of proteolytic degradation of rat histidine decarboxylase. *Eur J Biochem* 267: 1527–1531.

Pae CU, Drago A, Kim JJ, Patkar AA, Jun TY, et al. (2010) TAAR6 variations possibly associated with antidepressant response and suicidal behavior. Psychiatry R

Pae CU, Drago A, Mandelli L, De Ronchi D, Serretti A (2009) TAAR 6 and HSP-70 variations associated with bipolar disorder. Neurosci Lett 465: 257–261.

Panas HN, Lynch LJ, Vallender EJ, Xie Z, Chen GL, Lynn SK, et al (2010). Normal thermoregulatory responses to 3-iodothyronamine, trace amines and amphetamine-like psychostimulants in trace amine associated receptor 1 knockout mice. *J Neurosci Res*.88:1962-9.

Parmentier R, Ohtsu H, Djebbara-Hannas Z, Valatx JL, Watanabe T, Lin JS. Anatomical, physiological, pharmacological characteristics of histidine decarboxylase knock-out mice: evidence for the role of brain histamine in behavioral and sleep-wake

Paulmann N, Grohmann M, Voigt JP, Bert B, Vowinckel J, Bader M et al. (2009). Intracellular serotonin modulates insulin secretion from pancreatic beta-cells by protein serotonylation. *PLoS Biol*. 71000229.

Peng S, Zhang Y, Ren B, Zhang J, Wang H (2011). Effect of ketamine administration on memory consolidation, p-CREB and c-fos expression in the hippocampal slices of minor rats. Mol Biol Rep 38: 2401–2407.

Petrovic N, Cvijic G, Djordjevic J, Davidovic V (2005) The activities of antioxidant enzymes and monoamine oxidase and uncoupling protein 1 content in brown fat of hypo- and hyperthyroid rats. Ann NY Acad Sci 1040:431-5.

Philippu A, Prast H. (1991) Patterns of histamine release in the brain. *Agents Actions* 33: 124–125.

Piehl S, Heberer T, Balizs G, Scanlan TS, Smits R, Koksch B *et al*. (2008). Thyronamines are isozyme-specific substrates of deiodinases. Endocrinology 149: 3037–3045.

Piehl S, Hoefig CS, Scanlan TS, Köhrle J (2011). Thyronamines –past, present, and future. Endocr Rev 32: 64–80.

Pietsch CA, Scanlan TS, Anderson RJ (2007). Thyronamines are substrates for human liver sulfotransferases. Endocrinology 148: 1921–1927.

Pinna G, Brödel O, Visser T, Jeitner A, Grau H, Eravci M *et al*. (2002). Concentrations of seven iodothyronine metabolites in brain regions and the liver of the adult rat. Endocrinology 143:1789–1800.

Pino R, Failli P, Mazzetti L, and Buffoni F (1997) Monoamine oxidase and semicarbazide-sensitive amine oxidase activities in isolated cardiomyocytes of spontaneously hypertensive rats. Biochem Mol Med 62 :188–196 .

Pittenger C, Huang YY, Paletzki RF, Bourtchouladze R, Scanlin H, Vronskaya S *et al*. (2002). Reversible inhibition of CREB/ATF transcription factors in region CA1 of the dorsal hippocampus disrupts hippocampus-dependent spatial memory. Neuron 34:447–462.

Pittman CS, Buck MW, and Chambers JB (1972) Urinary Metabolites of '4C-Labeled Thyroxine in Man. J. Clin Invest 51:1759-1766.

Pittman CS, Shimizu T, Burger A & Chambers JB Jr (1980) The nondeiodinative pathways of thyroxine metabolism: 3,5,30,5-tetraiodothyroacetic acid turnover in normal and fasting human subjects. Journal of Clinical Endocrinology and Metabolism 50 712–716.

Porkka-Heiskanen T, Tuomisto L, Ylinen M, Stenberg D. (1994) The effect of REM sleep deprivation on histamine concentrations in different brain areas. *Life Sci* 54: 1719–1726.

Prell GD, Morrishow AM, Duoyon E, Lee WS. (1997) Inhibitors of histamine methylation in brain promote formation of imidazoleacetic imidazoleacetic acid, which interacts with GABA receptors. *J Neurochem* 68: 142–151.

Prengel H, Brodel H, Hiedra L, Pinna G, Eravci M, Meinhold H et al (2000). Effects of tranylcypromine on thyroid hormon metabolism and concentrations in rat brain. *Neuropharmacology* 39: 99-104.

Qadri YJ,. Rooj AK, Fuller CM. (2012) ENaCs and ASICs as therapeutic targets. *Am J Physiol Cell Physiol* 302: C943–C965

.

Raimondi L, Banchelli G, Sgromo L, Pirisino R, Ner M, Parini A et al. (2000). Hydrogen peroxide generation by monoamine oxidases in rat white adipocytes: role on cAMP production. *Eur J Pharmacol*. 395:177-82.

Regard JB, Kataoka H, Cano DA, Camerer E, Yin L, Zheng YW *et al*. (2007). Probing cell type-specific functions of Gi *in vivo* identifies GPCR regulators of insulin secretion. J Clin Invest 117: 4034–4043.

Revel FG, Moreau JL, Gainetdinov RR, Bradaia A, Sotnikova TD, Mory R *et al*. (2011). TAAR1 activation modulates monoaminergic neurotransmission, preventing hyperdopaminergic and hypoglutamatergic activity. Proc Natl Acad Sci USA 108:8485–8490.

Riehl J, Honda K, Kwan M, Hong J, Mignot E, Nishino S. (2000) Chronic oral administration of CG-3703, a thyrotropin releasing hormone analog, increases wake and decreases cataplexy in canine narcolepsy. *Neuropsychopharmacology* 23: 34–45.

Rivas M, Naranjo JR (2007) Thyroid hormones, learning and memory. Genes Brain Behav Suppl 1:40-4. Review

Roberson ED, Sweatt JD (1999). A biochemical blueprint for long-term memory. Learn Mem 6: 381–388. Review.

Rollema H, Wilson GG, Lee TC, Folgering JH, Flik G (2011). Effect of co-administration of varenicline and antidepressants on extracellular monoamine concentrations in rat prefrontal cortex**.** *Neurochem Int.* 58:78-84.

Rubio S, Begega A, Santin LJ, Arias JL. (2002) Improvement of spatial memory by (*R*)-alpha-methylhistamine, a histamine H(3)-receptor agonist, on the Morris water-maze in rat. *Behav Brain Res* 129: 77–82.

Rush DK (1988) Scopolamine amnesia of passive avoidance: a deficit of information acquisition. Behav Neural Biol 50:255-74.

Saba A, Chiellini G, Frascarelli S, Marchini M, Ghelardoni S, Raffaelli A et al. (2010). Tissue distribution and cardiac metabolism of 3-iodothyronamine. *Endocrinology*. 151:5063-73.

Sampaolo S, Campos-Barros A, Mazziotti G, Carlomagno S, Sannino V, Amato G *et al*. (2005). Increased cerebrospinal fluid levels of 3,3′,5′-triiodothyronine in patients with Alzheimer's disease. J Clin Endocrinol Metab 90: 198–202.

Sato K, Mimura H, Han DC, Tsushima T, Shizuma K (1984). Ontogenesis of iodothyronine-5'-deiodinase. Induction of 5'-deiodinating activity by insulin, glucocorticoid and thyroxine in cultured fetal mouse liver. *J Clin Invest.* 74: 2254-62.

Sawynok J, Esser MJ, Reid AR. (2001) Antidepressants as analgesics: an overview of central and peripheral mechanisms of action. *J Psychiatry Neurosci* 26: 21–29.

Scanlan TS (2009). Minireview: 3-iodothyronamine (T1AM): a new player on the thyroid endocrine team? Endocrinology 150: 1108–1111.

Scanlan TS, Suchland KL, Hart ME, Chiellini G, Huang Y, Kruzich PJ, Frascarelli S, Crossley DA, Bunzow JR, Ronca-Testoni S, Lin ET, Hatton D, Zucchi R, Grandy DK (2004) 3-Iodothyronamine is an endogenous and rapid-acting derivative of thyroid hormone. Nat Med 10:638-42.

Schicho R, Liebmann I, Lippe IT (2005). Extracellular signal-regulated kinase-1 and -2 are activated by gastric luminal injury in dorsal root ganglion neurons via N-methyl-D-aspartate receptors. Neuroscience 134: 505–514.

Schreckenberger MF, Egle UT, Drecker S, Buchholz HG, Weber MM, Bartenstein P, Kahaly GJ (2006) Positron emission tomography reveals correlations between brain metabolism and mood changes in hyperthyroidism. J Clin Endocrinol Metab 91:4786-4791

Schueler PA, Schwartz HL, Strait KA, Mariash CN & Oppenheimer JH (1990) Binding of 3,5,30-triiodothyronine (T3) and its analogs to the in vitro translational products of c-erbA protooncogenes: differences in the affinity of the a- and b-forms for the acetic acid analog and failure of the human testis and kidney a-2 products to bind T3. Molecular

Schwartz JC, Arrang JM, Garbarg M, Pollard H, Ruat M. (1991) Histaminergic transmission in the mammalian brain. *Physiol Rev* 71: 1–51.

Selbach O, Stehle J, Haas HL. (2007) Hippocampal long-term synaptic plasticity is controlled by histamine, hypocretins (orexins) and clock genes. *Soc Neurosci Abstr* 928.13.

Siegrist-Kaiser C & Burger AG (1994) Modification of the side chain of thyroid hormones. In Thyroid Hormone Metabolism, pp 17–198. Eds SY Wu & TJ Visser TJ. Boca Raton: CRC Press, Inc.

Smith CD, Ain KB (1995) Brain metabolism in hypothyroidism studied with 31P magnetic-resonance spectroscopy. Lancet 345:619-620

Snead AN, Santos MS, Seal RP, Miyakawa M, Edwards RH & Scanlan TS (2007). Thyronamines inhibit plasma membrane and vesicular monoamine transport. *ACS Chemical Biology*. 2: 390-398.

Suzuki-Ishigaki S, Numayama-Tsuruta K, Kuramasu A, Sakurai E, Makabe Y, Shimura S, Shirato K, Igarashi K, Watanabe T, Ohtsu H. The mouse L-histidine decarboxylase gene: structure and transcriptional regulation by CpG methylation in the promoter

Tomita K And Lardy HA (1960). Enzymic Conversion of Iodinated Thyronines to Iodinated Thyroacetic Acids. The Journal of Bioloqical Chemistry 235: 3292-3297.

Toyota H, Dugovic C, Koehl M, Laposky AD, Weber C, Ngo K, Wu Y, Lee DH, Yanai K, Sakurai E, Watanabe T, Liu C, Chen J, Barbier AJ, Turek FW, Fung-Leung WP,

Lovenberg TW(2002) Behavioral characterization of mice lacking histamine H(3) receptors. Mol Pharmacol 62:389-97.

Tu HM, Kim SW, Salvatore D, Bartha T, Legradi G, Larsen PR, Lechan RM (1997) Regional distribution of type 2 thyroxine deiodinase messenger ribonucleic acid in rat hypothalamus and pituitary and its regulation by thyroid hormone. Endocrinology 138:3359-3368

Valassi E, Scacchi M, Cavagnini F(2008). Neuroendocrine control of food intake. *Nutrition, Metabolism & Cardiovascular Diseases* 18: 158-168.

Vanti WB, Nguyen T, Cheng R, Lynch KR, George SR, et al. (2003) Novel human G-protein-coupled receptors. Biochem Biophys Res Commun 305: 67–71.

Visser WE, Friesema EC, Jansen J, Visser TJ (2008) Thyroid hormone transport in and out of cells. Trends Endocrinol Metab 19:50-56

Vollinga RC, deKoning JP, Jansen FP, Leurs R, Menge WMPB, Timmerman H (1994) A new potent and selective histamine H3 receptor agonist, 4-(1H-imidazol-4-yl-methyl)piperidine (immepip). J Med Chem 37:332–338.

Ward JR, and Bernhard JD (2005) Willan's itch and other causes of pruritus in the elderly International Journal of Dermatology, 44 , 267–273.

Whitaker-Azmitia PM, Zhang X, Clarke C (1994). Effects of gestational exposure to monoamine oxidase inhibitors in rats: preliminary behavioral and neurochemical studies. Neuropsychopharmacology 11: 125–132.

Winzell MS and Ahren B (2007). G-protein-coupled receptors and islet function-implications for treatment of type 2 diabetes. *Pharmacol and Therapeutics.* 116: 437-448.

Wood WJ, Geraci T, Nilsen A, DeBarber AE, Scanlan TS (2009) Iodothyronamines are oxidatively deaminated to iodothyroacetic acids in vivo. Chembiochem. 10:361-365.

Wood WJ, Geraci T, Nilsen A, DeBarber AE, Scanlan TS (2009). Iodothyronamines are oxidatively deaminated to iodothyroacetic acids *in vivo*. Chembiochem 10: 361–365.

Yang SN, Bjelke B, Narváez JA, Cintra A, Agnati LF, Fuxe K(1995). Counteraction of NYP-induced c-Fos expression in the nucleus tractus solitarii by alpha 2 receptor agonists. *Neuroreport*. 20:384-8.

Yen PM (2001) Physiological and molecular basis of thyroid hormone action. Physiol Rev 81:1097-1142

Yoshimura T, Yasuo S, Watanabe M, Iigo M, Yamamura T, Hirunagi K, Ebihara S (2003) Light-induced hormone conversion of T4 to T3 regulates photoperiodic response of gonads in birds. Nature 426:178-181

Youdim MB, Weinstock M, (2004). Therapeutic applications of selective and non-selective inhibitors of monoamine oxidase A and B that do not cause significant tyramine potentiation. *Neurotoxicology*. 25:243-50.

Zhu DF, Wang ZX, Zhang DR, Pan ZL, He S, Hu XP, Chen XC, Zhou JN (2006) fMRI revealed neural substrate for reversible working memory dysfunction in subclinical hypothyroidism. Brain 129:2923-2930

Zucchi R, Chiellini G, Scanlan TS, Grandy DK (2006) Trace amine-associated receptors and their ligands. Br J Pharmacol 149:967-78.