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Pharmacological Characterization of DM232 (Unifiram) and DM235 (Sunifiram), New Potent Cognition Enhancers

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ABSTRACT

DM232 (unifiram) and DM235 (sunifiram) are potent cognition-enhancers, which are four order of magnitude more potent than piracetam. These compounds, although not showing affinity in binding studies for the most important central receptors or channels, are able to prevent amnesia induced by modulation of several neurotransmission systems. These compounds are able to increase the release of acetylcholine from rat cerebral cortex, and, as far as unifiram is concerned, to increase the amplitude of fEPSP in rat hippocampal slices. *In vitro* experiments, performed on hippocampal slices, also supported the hypothesis of a role of the AMPA receptors for the cognition-enhancing properties of unifiram and sunifiram.

INTRODUCTION

Cognition enhancing drugs can be defined as drugs which facilitate attention, acquisition, storage and retrieval of information, attenuating the impairment of cognitive functions associated with age and age-related pathologies such as mild cognitive impairment (MCI) (69) and the cognitive aspects of neurodegenerative diseases such as Alzheimer's disease (AD) and other dementias (25,45). MCI refers to patients with memory deficits who do not yet fulfil the criteria for dementia, but are at risk of conversion. According to this definition, MCI would represent a transitional stage between normal aging and initial neurodegeneration and appears to be the most suitable stage for early drug treatment.

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Given the intrinsic complexity of the human brain and the number of neurotransmitter systems involved in learning and memory (40), the approach to develop cognition enhancing drugs can hardly be any better than a reductionist one, where the physiological events that regulate cognitive functions are considered separately, often on the basis of working hypotheses. Yet, the future in this field does not look bright as most of the approaches designed to face cognitive impairment, in particular that related to AD, have not provided satisfactory solutions (43).

Neurotransmitter replacement remains one of the most used approaches. Among the many receptors involved in cognition (40), the cholinergic system continues to be at the center of attention (14,46,52) being the target of several attempts to develop new cognition enhancing drugs (59,63), as suggested by the controversial cholinergic hypothesis (38,56,62). As a matter of fact, four drugs directly descending from this hypothesis, acting as acetylcholinesterase inhibitors, have been introduced in therapy: tacrine, donepezil, rivastigmine, and galantamine, while a few others are in development (1,36,44,55). However, the benefits obtained with these drugs are limited in time and their use has been recently questioned (2). A fifth drug also belonging to the class of neurotransmitter receptor modulators, memantine, a NMDA receptor antagonist, has obtained approval for AD treatment (55). Among others, histaminergic (3), σ_1 (35), and glutamatergic (9) receptor systems continue to be explored as targets for cognition enhancing drugs. In particular, allosteric modulators of the AMPA receptor (6,20,24,39) seem to be promising as neuroprotectors and cognition enhancing agents. Piracetam-like compounds, also called nootropics, characterized by a 2-pyrrolidinone structure, also interact, directly or indirectly, with receptors but they suffer from the lack of a common, generally accepted mechanism of action, which has precluded so far a wide acceptance of these drugs as useful medicines. Ironically, the very low toxicity of this class of compounds is itself a problem since it has been considered the result of insufficient activity, even if they are active in most preclinical assays and, at least in some clinical trials, their therapeutic efficacy has been found significant (10,18,20,21).

As far as AD is concerned, a few other working hypotheses have flanked the cholinergic one: the inflammation and cholesterol hypothesis, the oxidative damage theory and the amyloid hypothesis have prompted the evaluation of the effects of non steroidal antiinflammatory drugs (NSAID) (64,70), statins (that inhibit cholesterol biosynthesis) (16,53, 65), antioxidant molecules (15,29) and compounds that can modulate the enzymes controlling the production of β-amyloid protein. Indeed, this last hypothesis, suggesting that cognition impairment is induced by the formation and accumulation of a metabolic product of APP (amyloid precursor protein) called Aβ, is the most popular, at present. Aβ peptides result from the hydrolytic action of two enzymes, called β and γ secretases, that operate at the level of the cell membrane. Oligomerization and fibrillation of Aβ gives origin to the plaques that are a typical feature of AD. Today it is believed that the soluble oligomers of amyloid proteins (17,47), rather than the formed fibrils that accumulate in the disease, cause neuronal toxicity. Molecules that depress the action of β and γ secretases or impair oligomerization and plaque formation would then be useful to control AD (5,7,34,66). However, so far, attempts to develop effective cognition enhancing drugs have been unsuccessful and, as mentioned above, only a few compounds, mainly AChE inhibitors, have been approved.

In 2000, Manetti and coworkers (30) reported the synthesis and preliminary pharmacological evaluation of a series of 1,4-diazabiciclo[4.3.0]nonan-9-ones endowed with potent

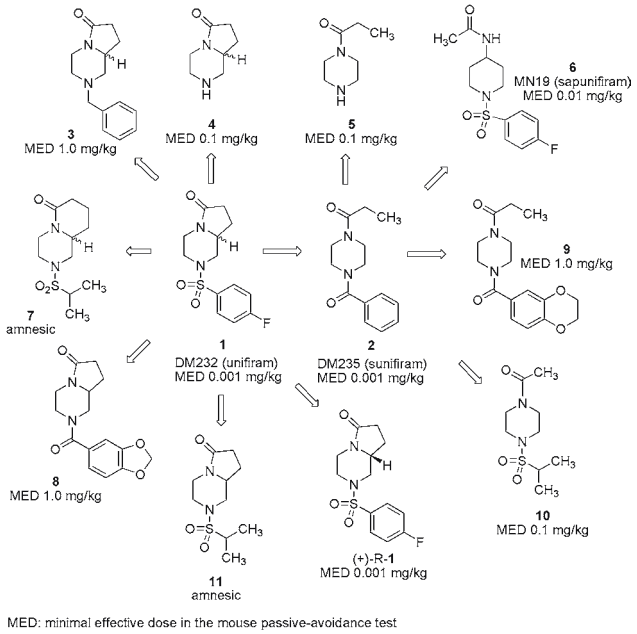

Fig. 1. Chemical structures and minimal effective doses (MED) of DM232, DM235, and related compounds in the mouse passive avoidance test.

nootropic activity on mouse passive avoidance. These compounds are somehow related to piracetam as they have a 2-pyrrolidinone cycle but seem to belong to a different class of compounds, since they are much more potent than piracetam and piracetam-like compounds and the 2-pyrrolidinone ring does not appear to be necessary for nootropic activity. In fact their piperazine analogs, where the 2-pyrrolidinone ring has been cleaved, maintain the same high potency as nootropics (31). Two compounds emerged from the research of Manetti and coworkers: DM232 (unifiram) and DM235 (sunifiram), which were chosen for further studies. The present review will describe the chemical and pharmacological studies that have been performed on these two molecules.

CHEMISTRY AND STRUCTURE-ACTIVITY RELATIONSHIPS

The synthetic methods to obtain DM232 (**1**, 4-(4-fluorobenzenesulfonyl)-1,4-diazabicyclo[4.3.0]nonan-9-one, unifiram) are reported in ref. (30) and (58); DM235 (**2**, 1-benzoyl-4-propionylpiperazine, sunifiram), its product of simplification, was synthesized according to the procedure reported in ref. 31. These derivatives (Fig. 1), characterized by two amide moieties, are the most potent compounds among a series of analogs carrying different acyl, benzoyl, or benzenesulfonyl residues (30,31,58). All the synthesized compound were initially screened by means of the mouse passive-avoidance test in order to select the most interesting compounds for further pharmacological characterization. Although a large number of compounds have been synthesized and tested, the *in vivo* screening procedure, necessary to select nootropic compounds, does not lead to sound

structure-activity relationships due to the fact that the biological activity can be the consequence of both pharmacokinetic and pharmacodynamic properties, which may be differently affected by structural modifications. Nevertheless, extensive structural changes have been performed on unifiram and sunifiram in order to improve their potency and possibly to understand their mechanism of action (30–33,57,58).

The replacement of one of the acyl groups by an alkyl moiety gives compounds (i.e., **3**) which are still active, although three orders of magnitude less potent than the parent molecules (30); moreover, the removal of one of the nitrogen substituents gives compounds (**4**, **5**) which are possible metabolites, showing cognition enhancing properties but at 100-fold higher doses (58). Both modifications introduce a basic nitrogen into the molecules, thus changing the pharmacokinetic properties; their lower potency indicates that the diamidic structure is requested for high nootropic activity in this class of substances.

Moving one of the piperazine nitrogen atoms out of the six-membered ring gave potent cognition enhancers, the most interesting compound being **6** (MN19, sapunifiram), which shows a minimal effective dose (0.01 mg/kg) close to that of sunifiram (32). The homologation of the five-membered 2-oxopyrrolidine ring is detrimental for activity since the corresponding 1,5-diazabicyclo[4.4.0]decan-10-ones are some hundred times less potent than the parent compounds. However, compound **7**, which carries an isopropylsulfonyl moiety, showed interesting amnesic activity (58).

Since experimental evidence suggests an interaction of these substances with AMPA receptor (11), the structures of unifiram and sunifiram were hybridized with those of ampakines (67), but the introduction of the 5-piperonyloyl,1,4-benzodioxan-6-carbonyl or 6-quinoxaloyl residues was not successful since the compounds were much less active (for instance, compounds **8** and **9**) or inactive in the mouse passive-avoidance test (58). However, the replacement of the benzoyl group on the piperazine with the isopropylsulfonyl moiety, which is present on the AMPA positive allosteric modulators LY395153 and LY404187 (68), gave a compound (**10**) which shows good cognition enhancing properties with a minimal effective dose of 0.1 mg/kg (mouse passive-avoidance test) (58).

Surprisingly, the same modification on unifiram gave a compound (**11**, MC68) showing amnesic properties with a potency similar to scopolamine; the amnesia induced by MC68 was completely reversed by unifiram in a dose-dependent way (58).

Since unifiram has a stereogenic center, the two enantiomers were synthesized starting from (*R*)- and (*S*)-5-hydroxymethyl-2-pyrrolidinone as chiral precursors, and obtained with enantiomeric excess higher than 99.9%. In all the performed assays (mouse passive avoidance test, social learning test, ACh release), the (*R*) form ((+)-R-**1**) displayed higher potency than its (*S*) enantiomer, being able to elicit comparable effects at 3- to 10-fold lower doses. The study of enantioselectivity was performed also on the enantiomers of the amnesic drug **11**, but in this molecule the (*R*) and (*S*) enantiomers showed the same potency when tested in the passive-avoidance test (33).

IN VIVO STUDIES

Antiamnesic Activity

Sunifiram and unifiram prevented amnesia induced by scopolamine, after i.p. and p.o. administration, in the mouse passive avoidance test (Fig. 2), their minimal effective doses

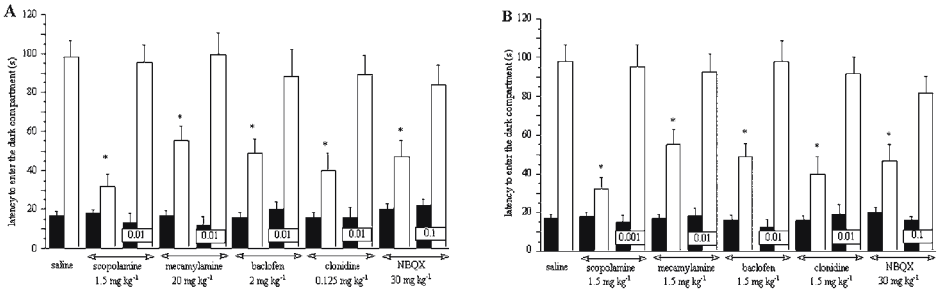

Fig. 2. Antiamnesic effect of unifiram (panel A) and sunifiram (panel B) on amnesia induced by scopolamine, mecamylamime, baclofen, clonidine, NBQX in the mouse passive avoidance test. The dose of unifiram and sunifiram administered is listed in each column. Vertical lines represent S.E.M.; $*P < 0.05$ in comparison with saline-treated group.

(MDE) being 0.001 mg/kg i.p. and 0.01 mg/kg p.o. The antiamnesic effect of the investigated compounds was comparable to that exerted by well known nootropic drugs such as piracetam (30 mg/kg i.p.), aniracetam (100 mg/kg p.o.) and rolipram (30 mg/kg p.o.), with a potency 1000–10,000 times higher.

Unifiram and sunifiram were also able to prevent the amnesia induced by the nicotinic antagonist mecamylamine, the $GABA_B$ agonist baclofen, the α_2 agonist clonidine and the AMPA receptor antagonist NBQX in the passive avoidance test (Fig. 2).

In the rat Morris watermaze test, the administration of scopolamine inhibited the reduction of escape latency in both acquisition and retention/retraining test. Sunifiram and unifiram, administered 20 min before each daily acquisition training, were able to prevent scopolamine-induced memory impairment (Fig. 3) (12,13).

In the passive avoidance test, an improvement in cognition of animals which have no memory impairment is difficult to demonstrate. As a matter of fact, well known nootropic drugs, such as piracetam, aniracetam and rolipram or cholinomimetics, do not show any memory facilitation in normal animals (4,19). A procognitive activity of sunifiram and unifiram was unmasked by using a social learning test, performed according to Mondadori et al. (37). Unifiram and sunifiram exerted beneficial effects on cognitive performance in the social learning test by prolonging the time normally required by rats to delete mnemonic information (12,31).

Effect of Sunifiram and Unifiram on Animal Behavior

Sunifiram (1 mg/kg i.p.) and unifiram (0.1 mg/kg i.p.) reduced the duration of hypnosis induced by pentobarbital in mice without modifying the induction time of hypnosis.

Sunifiram and unifiram produced their maximal antiamnesic and procognitive effects without any visible modification in gross behavior of mice or rats. Moreover, mice treated with unifiram and sunifiram at the same doses retained motor coordination in the rotating rod test [method of Kuribara et al. (27)] (Table 1)(11). The spontaneous motility of mice, evaluated by the Animex apparatus, as well as the exploratory behavior, studied by the hole-board test, were normal after unifiram and sunifiram administration (12,13).

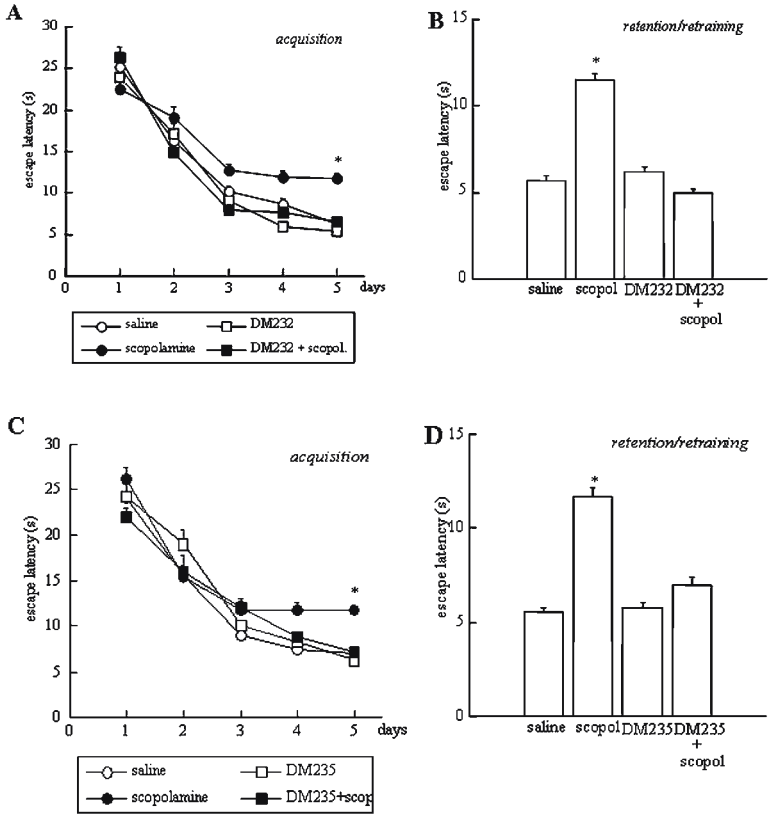

Fig. 3. The effect of DM232 (unifiram) (0.1 mg/kg i.p.) and DM235 (sunifiram) (0.1 mg/kg i.p.) on spatial reference memory in the Morris watermaze test. **Panel A**: Effect of unifiram on scopolamine (0.8 mg/kg i.p.)-induced impairment of rat acquisition. Task acquisition is reflected as a decrease in escape latency. $*P < 0.05$ in comparison with saline-treated rats. Vertical lines represent S.E.M. **Panel B**: Effect of unifiram on scopolamine-induced impairment in the retention/retraining day. $*P < 0.05$ in comparison with saline-treated rats. Vertical lines represent S.E.M. **Panel C**: Effect of sunifiram on scopolamine (0.8 mg/kg i.p.)-induced impairment of rat acquisition. Task acquisition is reflected as a decrease in escape latency. $*P < 0.05$ in comparison with saline-treated rats. Vertical lines represent S.E.M. **Panel D**: Effect of sunifiram on scopolamine-induced impairment in the retention/retraining day. $*P < 0.05$ in comparison with saline-treated rats. Vertical lines represent S.E.M. Reproduced from refs. 12,13 with kind permission of John Wiley & Sons and of Springer Science and Business Media.

Effect of Unifiram and Sunifiram on Central Cholinergic Transmission

Unifiram and sunifiram modulate cholinergic transmission in the cerebral cortex of freely moving rats. As shown by microdialysis experiment, at the dose of 0.01 mg/kg, both compounds are able to increase the release of ACh; sunifiram shows higher potency, being able to double the neurotransmitter's concentration in the CNS 45 min after injection, while the increase produced by unifiram at the same dose is lower (about 40%). Both compounds are inactive at higher doses (1 mg/kg) (31). In the same test, (*R*)-DM232 ((+)-R-**1**) showed a 10-fold higher potency than its (*S*)-enantiomer ((–)-S-**1**) (33).

TABLE 1. Effect of unifiram and sunifiram in the mouse rota-rod test

Treatment, i.p.	Endurance time (sec)			
	Before treatment	At minutes after treatment		
		15	30	45
Saline	104.1 ± 7.3	101.8 ± 8.2	111.4 ± 7.0	107.8 ± 8.2
Unifiram, 0.1 mg/kg	108.9 ± 7.5	99.6 ± 7.2	104.2 ± 8.4	101.6 ± 7.4
Unifiram, 10 mg/kg	112.6 ± 7.1	101.2 ± 8.5	108.0 ± 9.6	110.1 ± 7.6
Sunifiram, 0.1 mg/kg	113.6 ± 6.4	102.1 ± 7.1	111.1 ± 6.4	110.5 ± 7.2
Sunifiram, 10 mg/kg	105.8 ± 7.0	100.1 ± 7.5	108.4 ± 7.9	101.0 ± 8.3

Data are reported as means ± S.E.M.
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TABLE 2. Dose-response curve of both unifiram and sunifiram in the mouse hot-plate test

Treatment, i.p.	Licking latency in mice (sec)			
	Before treatment	At minutes after treatment		
		60	90	120
Saline i.p.	14.7 ± 0.9	15.5 ± 1.2	16.3 ± 1.1	14.2 ± 1.3
Unifiram, 0.001 mg/kg	14.5 ± 0.8	16.2 ± 1.4	15.6 ± 1.3	15.7 ± 1.5
Unifiram, 0.01 mg/kg	15.0 ± 1.1	19.7 ± 1.6^	21.3 ± 1.0^	16.3 ± 1.4
Unifiram, 0.1 mg/kg	14.9 ± 0.8	22.3 ± 1.4*	23.8 ± 2.1*	18.5 ± 1.4
Unifiram, 1 mg/kg	15.0 ± 0.9	17.2 ± 1.9	18.5 ± 2.2	16.7 ± 1.8
Sunifiram, 0.001 mg/kg	14.8 ± 0.7	17.2 ± 2.3	15.9 ± 1.9	17.2 ± 2.0
Sunifiram, 0.01 mg/kg	15.8 ± 1.8	17.3 ± 2.4	22.3 ± 1.6*	16.8 ± 2.2
Sunifiram, 0.1 mg/kg	16.5 ± 1.9	23.8 ± 1.8*	23.2 ± 1.5*	18.1 ± 2.1
Sunifiram, 1 mg/kg	14.5 ± 1.7	15.3 ± 1.3	16.5 ± 1.2	15.3 ± 1.9

Compounds were injected i.p. Each point represents the mean of at least 10 mice.
*$P < 0.01$; ^$P < 0.05$ in comparison with saline controls.

As expected from their ACh-releasing properties, unifiram and sunifiram show analgesic activity. In the mouse hot-plate test, unifiram and sunifiram are able to increase the pain threshold after i.p. administration in a dose-dependent manner; the maximal effect was obtained between 15 and 30 min after treatment (Table 2). The dose-response data in both cases indicate a bell-shaped relationship.

IN VITRO STUDIES

Norepinephrine Release from Brain Slices

Exposure of rat hippocampal slices, prelabelled with [^{3}H]NE, to 100 μM NMDA elicited an increase of tritium release previously shown to mainly account for unmetabolized norepinephrine (48). As shown in Fig. 4A, the effect of NMDA was largely prevented

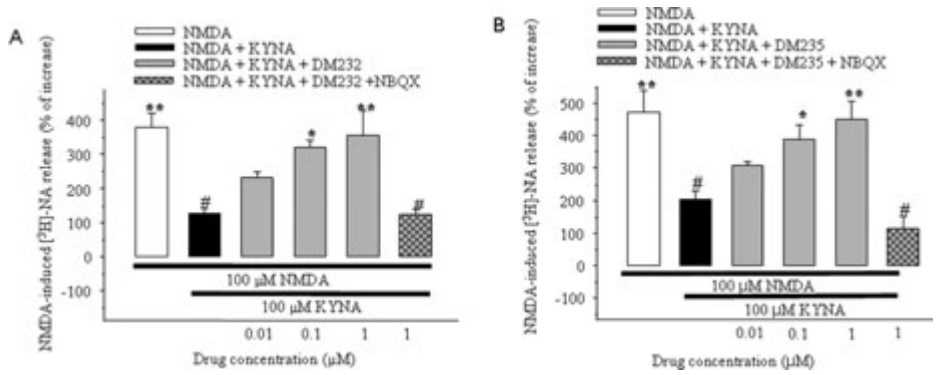

Fig. 4. Panel A: Effects of DM232 (unifiram), alone or in the presence of NBQX, in the kynurenate test. Rat hippocampal slices were labelled with [^{3}H]NE and superfused with Mg^{2+}-free medium. Unifiram was added together with kynurenic acid (KYNA) and tritium release was then provoked by NMDA. In some experiments NBQX was added concomitantly with KYNA. Empty bars: 100 µM NMDA; solid bars: NMDA + 100 µM KYNA; gray bars: NMDA+KYNA+DM232 (concentration as indicated); cross hatched bars: NMDA + KYNA + 1 µM DM232 + 5 µM NBQX. Data are means ± S.E.M. of 5–7 experiments run in triplicate (three superfusion chambers for each condition). $^{\#}P < 0.01$ vs. NMDA; $^{*}P < 0.05$ vs. NMDA + KYNA; $^{**}P < 0.01$ vs. NMDA + KYNA. **Panel B**: Effects of DM235 (sunifiram), alone or in presence of NBQX, in the kynurenate test. Slices were superfused with Mg^{2+}-free medium from $t = 0$ min of superfusion. In some experiments NBQX was added concomitantly with KYNA, at $t = 30$ min till the end of superfusion. Empty bars: 100 µM NMDA; solid bars: NMDA + 100 µM KYNA; gray bars: NMDA + KYNA + DM235 (concentration as indicated); cross hatched bars: NMDA + KYNA + 1 µM DM235 + 5 µM NBQX. Data are means ± S.E.M. of 4–7 experiments run in triplicate (three superfusion chambers for each condition). $^{\#}P < 0.01$ vs. NMDA; $^{*}P < 0.05$ vs. NMDA + KYNA; $^{**}P < 0.01$ vs. NMDA + KYNA. Reproduced from ref. 11 with kind permission of Springer Science and Business Media.

when 100 µM kynurenic acid (KYNA), inactive on its own, was present. Addition of nanomolar concentrations of unifiram significantly reverses KYNA antagonism. The effect of unifiram was concentration-dependent ($EC_{50} \leq 0.1$ µM) and almost complete at 1 µM (11). A similar attenuation of the KYNA antagonism was also observed in slices exposed to sunifiram (Fig. 4B). At the concentrations used, none of the drugs affected the basal tritium outflow. The compounds also failed to affect the release of [^{3}H]NE induced by depolarizing stimuli other than NMDA receptor activation.

The possible involvement of AMPA receptors in the effects of unifiram and sunifiram in the kynurenate test was then assessed by determining the sensitivity of these reversals to the selective AMPA receptor antagonist NBQX. When applied at 5 µM, NBQX prevented the [^{3}H]NE release induced by AMPA (100 µM), but not the 100 µM NMDA-mediated tritium release from rat hippocampal slices (48).

NBQX, inactive on its own, antagonizes the unifiram reversal of the KYNA antagonism of the NMDA-evoked tritium release (Fig. 4A) as well as the sunifiram-mediated effect (Fig. 4B), suggesting involvement of AMPA receptors in the effects of both compounds (11).

Electrophysiological Studies

In a series of experiments, the effects of different concentrations of unifiram on *in vitro* synaptic transmission were studied with extracellular recordings of fEPSP in the CA1

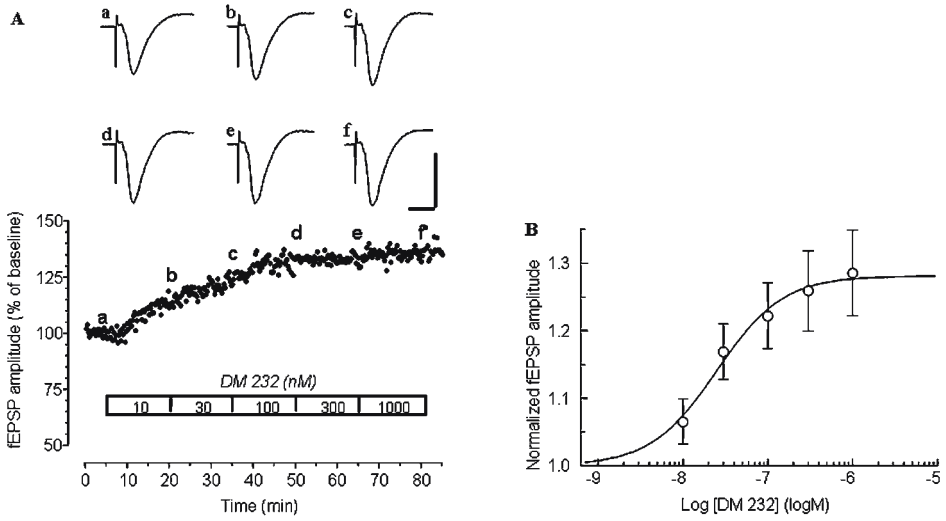

Fig. 5. Effects of DM232 (unifiram) on fEPSP evoked by electrical stimulation of the stratum radiatum in the CA1 hippocampal region. **Panel A**: The graph shows the time-course of the effects of increasing concentrations of DM 232 (10, 30, 100, 300, 1000 nM) on the amplitude of fEPSP, in a typical experiment. Each concentration was applied for 15 min before switching to the next higher (cumulative concentration- response protocol). *Upper panel*: Traces are averages of five consecutive responses taken at times indicated by corresponding letters in the graph recorded in control conditions (a) and in the presence of DM232 (b, c, d, e, and f). Calibration bars: 1 mV, 10 msec. **Panel B**: Concentration-response relationships for DM232. Individual points correspond to the mean ± S.E.M. ($n = 4$) of values of integrals normalized by taking the control as unity. The continuous lines are the best least-squares fits of the logistic equation: $1/(1 + (EC_{50}/[DM232])nH)$, where EC_{50} is the half-maximally effective concentration and nH is the Hill coefficient. Reproduced from ref. 11 with kind permission of Springer Science and Business Media.

region of rat hippocampal slices (11). As illustrated in Fig. 5A, in a typical experiment application of unifiram (from 10 nM to 1 μM) increased the amplitude of fEPSP in a concentration-dependent manner. This effect was not reversible after washout of the drug (up to 30 min, data not shown). Similar results were obtained in seven out of ten slices examined and the percentage increase was of 13 ± 4% at 10 nM ($P < 0.05$) and 34 ± 5% at 1 μM ($P < 0.001$) in comparison to respective control values.

In four slices, the cumulative concentration-response curve for the increase in fEPSP amplitude by unifiram was constructed. Estimation of the EC_{50} values (27 ± 6 nM) was calculated from the best fit of the experimental data to a logistic function (Fig. 5B).

Binding Studies

Unifiram and sunifiram, at 1 μM, did not reveal any affinity towards the most important central receptors, such as serotoninergic, dopaminergic, muscarinic, nicotinic, adrenergic, glutamatergic, histaminergic, opiod, or GABAergic.

Other Studies

It has recently been reported (41) that sunifiram, as well as other nootropic drugs (piracetam, levetiracetam, aniracetam) and endogenous neuropeptides (TRH), is able to anta-

gonize the inhibition of glucose transport by barbiturates, diazepam, melatonin, and endogenous neuropeptide galanin in human erythrocytes *in vitro*. The potencies of nootropic drugs in opposing scopolamine-induced memory loss correlate with their potencies in antagonizing pentobarbital inhibition of erythrocyte glucose transport *in vitro*. Sunifiram at low doses competitively antagonizes the effect of barbiturate, while at high doses it directly inhibits glucose transport.

The possibility that unifiram and sunifiram could display anticonvulsant activity was investigated using pentylenetetrazole (90 mg/kg s.c.) for chemically induced convulsions. Unifiram and sunifiram do not exhibit any protection against convulsions up to the maximal employed dose of 1 mg/kg i.p. Both compounds were administered i.p. 30 min before the test.

MECHANISM OF ACTION

Unifiram and sunifiram were able to prevent amnesia induced by the administration of scopolamine, mecamylamine, baclofen, and clonidine. Thus, both compounds counteract amnesia induced by modulation of different neurotransmission systems. Furthermore, binding studies demonstrated that unifiram and sunifiram, at 1 μM, did not reveal any affinity towards muscarinic and nicotinic receptors, nor for the most important central receptors. The lack of receptorial affinity is also a characteristic feature of most of the nootropic compounds. These drugs, with the only exception of nefiracetam, which shows high affinity for the $GABA_A$ receptors, do not seem to act at any well-characterized receptor system (19).

Unifiram and sunifiram induce a procognitive activity as demonstrated by the results obtained in the social learning test.

The results obtained by electrophysiological recording *in vitro* demonstrate that unifiram brought about a long lasting increase of neurotransmission in the CA1 region of rat hippocampal slices. This effect is concentration-dependent and not reversible upon interruption of drug application. Our data provide experimental evidence supporting the proposition that the long-lasting synaptic enhancements produced by unifiram are similar to the hippocampal LTP, that represents a model for a cellular mechanism related to learning and memory (61). Unifiram, through unknown mechanism(s), might enhance either the release of putative neurotransmitters such as glutamate, as already demonstrated for FK960, a putative cognitive enhancer in the hippocampus (22), or the response to glutamate at postsynaptic level probably on AMPA receptors, since NMDA receptors contribute little to the generation of fEPSP evoked by low frequency stimulation in the presence of physiological concentrations of Mg^{2+} (42).

More recently, the involvement of the AMPA receptors in the antiamnesic and procognitive activity of unifiram and sunifiram has been postulated. Both compounds reversed the impairment of memory processes induced by the AMPA antagonist NBQX in the passive avoidance paradigm. *In vitro* experiments, performed on hippocampal slices, also supported the hypothesis of a role of the AMPA receptors for unifiram and sunifiram.

Unifiram and sunifiram have been predicted to act as ampakine-like compounds and, as a direct consequence, they should be expected to ameliorate amnesic conditions through AMPA/kainate receptor-mediated mechanisms (28,60), as well as to reverse memory

impairments chemically induced by administering AMPA/kainate receptor antagonists (8,26,54).

The hypothesis that unifiram and sunifiram exert their antiamnesic effect through the activation of AMPA-mediated effects is also supported by *in vitro* results in which both compounds produced a NBQX-sensitive reversal of the kynurenate-induced antagonism in the "kynurenate test." In 1995, a biochemical test for evaluation of cognition enhancers acting through glutamate receptors of the N-methyl-D-aspartate (NMDA) type was proposed (50). In this test, called "the kynurenate test," nootropics are evaluated for their ability to attenuate kynurenate antagonism of the NMDA-evoked NE release from rat hippocampal slices. It was, therefore, postulated that learning and memory improvements obtained with some nootropics might be associated with a relief of the antagonism exerted by the endogenous compounds at glutamate receptors, especially the NMDA receptor complex subtypes. Several compounds were found to be active in this test: some of these drugs relieved the kynurenate antagonism probably by acting directly on the NMDA receptor (i.e., D-cycloserine, oxiracetam, CR2249) (50,51) while other compounds reverted the kynurenate antagonism through indirect mechanisms, involving receptor-receptor interaction (49).

One possible explanation for the DM-induced AMPA-mediated reversal of the "kynurenate test" is that AMPA receptors might influence NMDA receptor function, by directly modulating their activity. Actually, AMPA and NMDA receptors are colocalized on noradrenergic terminals and they reciprocally influence their functions. Another possible explanation considers that, once slices are exposed to 100 μM NMDA, a release of endogenous glutamate occurs in the biophase that might induce AMPA receptor desensitization (reviewed by Holman and Heinemann (23)). The desensitization is prevented, possibly, by the presence of an ampakine-like compound, leading to a reinforcement of the AMPA-mediated effect and, therefore, to an apparent reversal of the kynurenate antagonism.

Finally, it could be proposed that unifiram and sunifiram might influence the AMPA-induced release of neurotransmitters other than norepinephrine, which may in turn facilitate NMDA receptor functions.

The amelioration of memory processes induced by unifiram and sunifiram is obtained without any induction of side effects. Both compounds, at the highest effective doses, did not impair motor coordination, as revealed by the rota-rod test, nor modify spontaneous motility, as indicated by the Animex apparatus and the hole board test. Furthermore, unifiram and sunifiram, at a dose 1000-times higher than the minimal effective dose, are still devoid of any alteration of behavioral parameters.

In conclusion, unifiram and sunifiram represent new antiamnesic and procognitive compounds, which can be supposed to belong to the class of nootropic drugs. As a matter of fact, these compounds show not only a chemical structure similarity with piracetam (30), but they also exhibit a pharmacological profile comparable to that of nootropics. Unifiram and sunifiram are endowed with the main pharmacological properties of piracetam-like compounds: facilitation of memory processes, lack of toxicity and side effects, and lack of affinity towards the most important central receptors. However, unifiram and sunifiram differ from nootropics in their potency. Even if they exert the same pharmacological effects, unifiram and sunifiram are at least 1000 times more potent than the most active nootropic drugs, such as oxiracetam, nefiracetam, etiracetam, aniracetam (19). These observations lead to consideration of unifiram and sunifiram as promising compounds for the treatment of human cognitive deficits.

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