ELSEVIER

European Journal of Pharmacology 272 (1995) 203–209

ejp

Putative cognition enhancers reverse kynurenic acid antagonism at hippocampal NMDA receptors

Anna Pittaluga, Roberto Pattarini, Maurizio Raiteri *

Istituto di Farmacologia e Farmacognosia, Viale Cembrano 4, 16148 Genova, Italy

Received 2 June 1994; revised MS received 12 September 1994; accepted 18 October 1994

Abstract

Oxiracetam, aniracetam and D-cycloserine, three putative cognition enhancers, were examined in a functional assay for NMDA receptors. Rat hippocampal slices or synaptosomes were labeled with [^{3}H]noradrenaline and exposed to NMDA or glutamate in superfusion. NMDA (100 μM) elicited a remarkable rise (about 500%) in the release of [^{3}H]noradrenaline from slices. The effect of NMDA was antagonized by the glutamate receptor blocker, kynurenic acid. The antagonism by 100 μM kynurenate was reduced by submicromolar concentrations of oxiracetam and totally reversed by 1 μM of the drug. The concentration-antagonism curve for kynurenic acid was shifted to the right in the presence of 0.2 or 1 μM oxiracetam. Aniracetam and D-cycloserine, as well as glycine and D-serine, behaved similarly to oxiracetam: all compounds, tested at 1 μM, reversed the antagonism by 100 μM kynurenate of the NMDA-evoked [^{3}H]noradrenaline release. In superfused hippocampal synaptosomes, 100 μM NMDA or glutamic acid elicited the release of [^{3}H]noradrenaline; the evoked release was enhanced by glycine, but not by oxiracetam. In this preparation 1 μM glycine or 1 μM oxiracetam prevented the antagonism by kynurenate of the NMDA- or the glutamate-evoked [^{3}H]noradrenaline release. As kynurenic acid is an *endogenous* glutamate receptor antagonist whose brain levels are known to increase in conditions associated to cognitive deficits, it is proposed that the putative cognition enhancers tested may act in vivo by relieving the antagonism produced by excessive endogenous kynurenate.

Keywords: D-Cycloserine; Oxiracetam; Aniracetam; NMDA receptor; Noradrenaline release; Kynurenic acid

1. Introduction

An increasing number of compounds have been proposed to facilitate acquisition, consolidation and retrieval of information and/or to ameliorate the deficits of such cognitive functions associated with aging or dementias (Sarter, 1991).

Investigations of the neuronal mechanisms involved in the effects of putative cognition enhancers have been largely focussed on central cholinergic transmission, due to the likely involvement of acetylcholine in cognition processes (Bartus et al., 1982). Indeed many compounds have been proposed to act through inhibition of acetylcholinesterase, activation of muscarinic receptors or increase of acetylcholine release, although other putative cognition enhancers have been implicated in a wide variety of mechanisms (Sarter, 1991).

As the importance of glutamate in the processes of learning and memory has emerged more recently than that of acetylcholine, only in few instances have putative cognition enhancers been postulated to interact with glutamatergic transmission. On the other hand, the glutamatergic system appears to represent a biological substrate particularly suitable to the action of cognition-enhancing agents. Long-term potentiation that is regarded as a neuronal model to study memory formation depends upon the function of glutamate receptors of the NMDA type (Bliss and Collingridge, 1993). Antagonists at the NMDA receptors display amnesic properties (Danysz and Wroblewski, 1989; Belfiore et al., 1992) and activation of NMDA receptors appears necessary for certain kinds of learning (Davis et al., 1992).

Interestingly, the mammalian brain contains, as a physiological metabolite, kynurenic acid, a broad spectrum antagonist at ionotropic glutamate receptors, particularly those of the NMDA type (Kessler et al., 1989;

* Corresponding author. Tel. 39 10 3532651, fax 39 10 3993360.

SSDI 0014-2999(94)00641-5

Stone et al., 1989). The levels of kynurenate are highest in the human brain, where they reach micromolar concentrations (Moroni et al., 1988a; Turski et al., 1988). As abnormally elevated concentrations of kynurenic acid may occur in conditions associated with cognitive deficits (Moroni et al., 1988b; Heyes et al., 1990; Gramsbergen et al., 1992), we have postulated that some cognition enhancers may act by relieving kynurenate antagonism at NMDA receptors.

To test this hypothesis we employed a functional assay for NMDA receptors based on the enhancement of [^{3}H]noradrenaline release elicited by NMDA or glutamate added to hippocampal slices or synaptosomes (Pittaluga and Raiteri, 1990, 1992; Fink et al., 1992). The effect of NMDA receptor activation was prevented by kynurenic acid but this antagonism was reversed by submicromolar concentrations of oxiracetam, aniracetam and D-cycloserine, three putative cognition enhancers.

2. Materials and methods

2.1. Animals and brain tissue

Adult male rats (Sprague Dawley; 200–250 g) were housed at constant temperature (22 ± 1°C) and relative humidity (50%) under a regular light-dark schedule (light 7 a.m.–7 p.m.). Food and water were freely available. The animals were killed by decapitation and the hippocampi were rapidly removed.

2.2. Release from slices

Coronal slices (0.40 mm thick) were prepared from the ventro-medial hippocampus using a McIlwain tissue chopper. Slices were labeled with 0.08 μM [^{3}H]noradrenaline, 20 min at 37°C, in a medium having the following composition (mM): 125 NaCl, 3 KCl, 1.2 $CaCl_2$, 1.2 $MgSO_4$, 1 NaH_2PO_4, 22 $NaHCO_3$ and 10 glucose (aerated with 95% O_2 and 5% CO_2 at 37°C), pH 7.2–7.4. The incubation medium contained 0.1 μM of the serotonin uptake inhibitor, 6-nitroquipazine, to prevent possible false labeling of serotonergic terminals. After washing with tracer-free medium, slices were transferred to parallel superfusion chambers (1 slice/chamber) and superfused at 1 ml/min, at 37°C, with a medium from which Mg^{2+} ions were omitted. After 60 min of superfusion to equilibrate the system, nine 5-min samples were collected. Samples and superfused slices (solubilized with Soluene) were then counted for radioactivity.

NMDA was added for 5 min starting at min 75 of superfusion; kynurenic acid, oxiracetam, aniracetam, D-serine, glycine or D-cycloserine was added 45 min before NMDA.

2.3. Release from synaptosomes

Crude hippocampal synaptosomes were prepared according to Gray and Whittaker (1962) with some modifications. Briefly, the tissue was homogenized in 40 volumes of 0.32 M sucrose, buffered to pH 7.4 with phosphate; the homogenate was first centrifuged (5 min, 1000 × g) and the synaptosomal fraction was isolated from the supernatant by centrifugation (20 min, 12000 × g). The synaptosomal pellet was then resuspended with standard medium and incubated for 15 min at 37°C with 0.03 μM [^{3}H]noradrenaline and 6-nitroquipazine (0.1 μM). At the end of the incubation period, identical aliquots of the synaptosomal suspension were distributed in parallel superfusion chambers maintained at 37°C (Raiteri et al., 1974). Superfusion was carried out, at a rate of 0.6 ml/min, for a total period of 48 min. Starting at $t = 36$ min, four 3-min fractions were collected. The synaptosomes were exposed to NMDA or glutamate starting at the end of the first fraction collected up to the end of the superfusion; oxiracetam or glycine was added concomitantly with the glutamate receptor agonists in the experiments reported in Fig. 3 while, in the experiments shown in Fig. 4, oxiracetam or glycine was added, alone or with kynurenic acid, 8 min before the agonists. At the end of the experiments, the fractions collected and the superfused synaptosomes were counted for radioactivity.

2.4. Calculation

The amount of tritium released into each superfusate fraction was expressed as a percentage of the total tissue tritium content at the start of the respective collection period. Drug effects were evaluated by calculating the ratio between the percent efflux in the fraction corresponding to the maximal effect and the efflux in the first fraction collected. This ratio was compared to the corresponding ratio obtained under control conditions. A two-tailed Student's t-test was used to analyze the significance of the difference between two means. EC_{50} and IC_{50} values for agonists and antagonists were determined from curves obtained using a function-fitting routine (software Sigma Plot, version 5.0).

In one set of experiments the [^{3}H]noradrenaline present in superfusate fractions, in slices and in synaptosomes was determined chromatographically according to Smith et al. (1975).

2.5. Drugs

[^{3}H]Noradrenaline (specific activity 39 Ci/mmol) was purchased from Amersham Radiochemical Centre (Buckinghamshire, UK). Kynurenic acid, glutamic acid,

glycine, D-serine and D-cycloserine were obtained from Sigma Chemical Co. (St. Louis, MO, USA); NMDA, 7-Cl-kynurenate and D-(−)-2-amino-5-phosphonopentanoic acid (D-AP5) were purchased from Tocris Neuramin (Bristol, UK). The following drugs were gifts from the companies indicated: 6-nitroquipazine (Duphar BV, Health Products Division, Weesp, Netherlands), oxiracetam (SmithKline Beecham, Milan, Italy) and aniracetam (Prodotti Roche, Milan, Italy).

3. Results

Rat hippocampal slices were labeled with [^{3}H]noradrenaline and then exposed in superfusion to 100 μM NMDA for 5 min. The glutamate receptor agonist elicited a remarkable rise (about 500%; Fig. 1) in the release of tritium; the NMDA-evoked overflow of radioactivity consisted largely (about 90%) of authentic [^{3}H]noradrenaline. When the slices were pretreated with 100 μM kynurenic acid, the effect of 100 μM NMDA on [^{3}H]noradrenaline release was reduced by 70% (Fig. 1). Oxiracetam, added at varying concentrations (0.01–1 μM) concomitantly with 100 μM kynurenate, concentration dependently restored the effect of 100 μM NMDA. The concentration of oxiracetam able to reverse by 50% the kynurenate antagonism amounted to 0.17 μM; complete recovery of the NMDA effect could be observed when oxiracetam was added at 1 μM (Fig. 1).

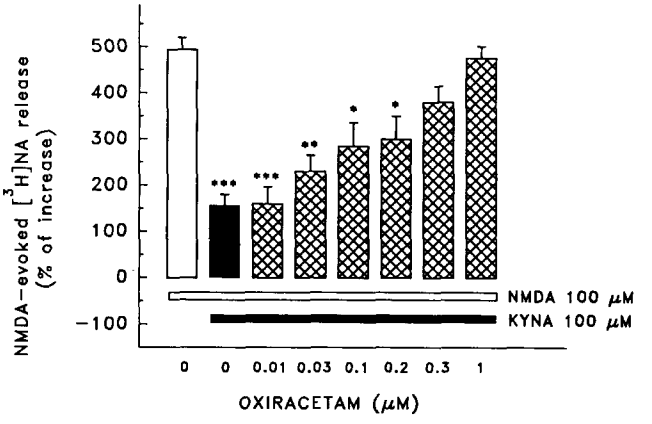

Fig. 1. Effect of oxiracetam on the kynurenic acid antagonism of the NMDA-induced [^{3}H]noradrenaline release from rat hippocampal slices. Slices were superfused as indicated in Materials and methods with Mg^{2+}-free medium; oxiracetam and kynurenic acid were added together 45 min before NMDA. See Materials and methods for other technical details. The basal [^{3}H]noradrenaline release corresponded to 0.69 ± 0.09%/5 min of the total tritium content. Empty bar = NMDA (100 μM); solid bar = NMDA (100 μM) + kynurenic acid (100 μM); cross-hatched bars = NMDA (100 μM) + kynurenic acid (100 μM) + oxiracetam (concentrations as indicated). Data are means ± S.E.M. of five to six experiments run in triplicate. * $P < 0.05$ vs. NMDA; ** $P < 0.01$ vs. NMDA; *** $P < 0.001$ vs. NMDA. [^{3}H]NA = [^{3}H]noradrenaline; KYNA = kynurenic acid.

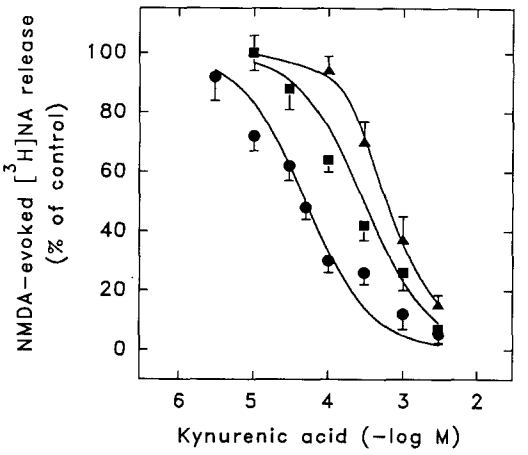

Fig. 2. Antagonism of the NMDA-evoked [^{3}H]noradrenaline release from hippocampal slices by kynurenic acid alone (●) or in the presence of 0.2 μM (■) or 1 μM (▲) oxiracetam. Slices, prelabeled with [^{3}H]noradrenaline, were exposed to 100 μM NMDA. The NMDA-evoked release was antagonized by varying concentrations of kynurenic acid. Oxiracetam (0.2 or 1 μM) shifted to the right the kynurenic acid curve. For other technical details see Materials and methods. Data are means ± S.E.M. of five to seven experiments run in triplicate. [^{3}H]NA = [^{3}H]noradrenaline.

Fig. 2 illustrates the concentration-antagonism curves for kynurenic acid in the absence and in the presence of oxiracetam. Kynurenic acid antagonized concentration dependently the [^{3}H]noradrenaline-releasing effect brought about by 100 μM NMDA in hippocampal slices. The IC_{50} value (concentration of kynurenic acid causing 50% antagonism of the NMDA effect) amounted to 50.0 ± 6.5 μM. The concentration-antagonism curve for kynurenate was progressively shifted to the right in the presence of increasing concentrations of oxiracetam. The calculated IC_{50} values for kynurenic acid in the presence of 0.2 μM or 1 μM oxiracetam amounted to 299.8 ± 35.7 μM and to 585.8 ± 76.9 μM, respectively.

Table 1
Effect of oxiracetam, aniracetam, D-cycloserine, glycine and D-serine on the [^{3}H]noradrenaline release induced in rat hippocampal slices by NMDA alone or in the presence of kynurenic acid (KYNA)

	NMDA (100 μM)	NMDA (100 μM) + KYNA (100 μM)
Controls	494 ± 26	157 ± 24 [a]
Oxiracetam (1 μM)	478 ± 20	475 ± 26 [b]
Aniracetam (1 μM)	486 ± 61	482 ± 67 [c]
D-Cycloserine (1 μM)	471 ± 61	472 ± 67 [c]
Glycine (1 μM)	484 ± 20	466 ± 20 [b]
D-Serine (1 μM)	489 ± 30	457 ± 14 [b]

Data (expressed as percent increase over basal) are means ± S.E.M. of three to five experiments run in triplicate. [a] $P < 0.001$ vs. NMDA; [b] $P < 0.001$ vs. NMDA + KYNA; [c] $P < 0.01$ vs. NMDA + KYNA.

As shown in Table 1, aniracetam, D-cycloserine, glycine and D-serine appear to behave like oxiracetam in the system examined. All these compounds, tested at 1 μM, allowed full recovery of the NMDA(100 μM)-evoked [^{3}H]noradrenaline release in the presence of 100 μM kynurenic acid. At the concentration used (1 μM), none of the drugs on their own, increased significantly the NMDA-evoked overflow of [^{3}H]noradrenaline. When submaximal concentrations (0.2 μM) of glycine and oxiracetam were combined, the reversal of the kynurenate antagonism did not seem to show either synergistic or reciprocally occlusive characteristics. The glycine (0.2 μM) reversal amounted to $36 \pm 7\%$ ($n = 3$) and that by 0.2 μM oxiracetam to $40 \pm 9\%$ ($n = 3$); when combined, the two compounds prevented the kynurenate antagonism by $80 \pm 12\%$ ($n = 3$), a value similar to the one that can be extrapolated for 0.4 μM oxiracetam from Fig. 1.

When hippocampal synaptosomes, prelabeled with [^{3}H]noradrenaline, were exposed in superfusion to 100 μM NMDA (in Mg^{2+}-free medium, top panel) or to 100 μM glutamate (in Mg^{2+}-containing medium; see Pittaluga and Raiteri, 1992) the release evoked by the excitatory amino acids was increased concentration dependently by glycine ($EC_{50} = 0.3$ μM in the case of NMDA and 3.7 μM in the case of glutamate). In contrast, oxiracetam was ineffective up to 1000 μM (Fig. 3).

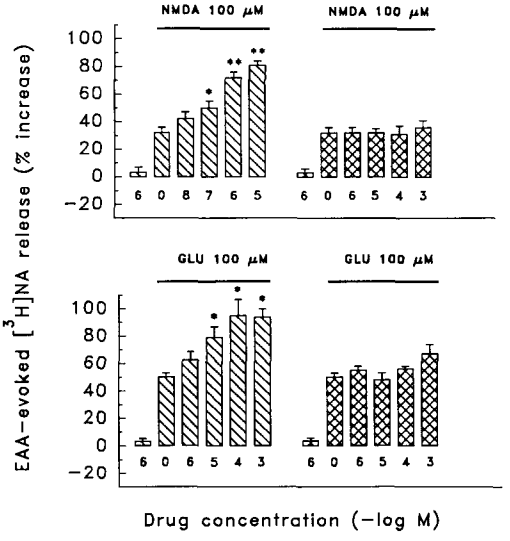

Fig. 3. Effects of glycine or oxiracetam on the NMDA or on the glutamate-induced release of [^{3}H]noradrenaline from rat hippocampal synaptosomes superfused respectively with Mg^{2+}-free (NMDA stimulation) or Mg^{2+}-containing (glutamate stimulation) medium as described in Materials and methods. The basal tritium release corresponded to $1.88 \pm 0.12\%$ of the total synaptosomal tritium content. About 85% of the tritium overflow consisted of authentic [^{3}H]noradrenaline. Hatched bars = with glycine added. Cross-hatched bars = with oxiracetam added. EAA = excitatory amino acid (NMDA or glutamate); GLU = glutamic acid; [^{3}H]NA = [^{3}H]noradrenaline. The concentrations of glycine or oxiracetam are indicated as −log M. The data shown in the upper left panel (effects of glycine on the NMDA-evoked [^{3}H]noradrenaline overflow) are taken from Pittaluga et al. (1993). The other results are means ± S.E.M. of four to five experiments run in triplicate. * $P < 0.05$ vs. respective controls; ** $P < 0.01$ vs. respective controls.

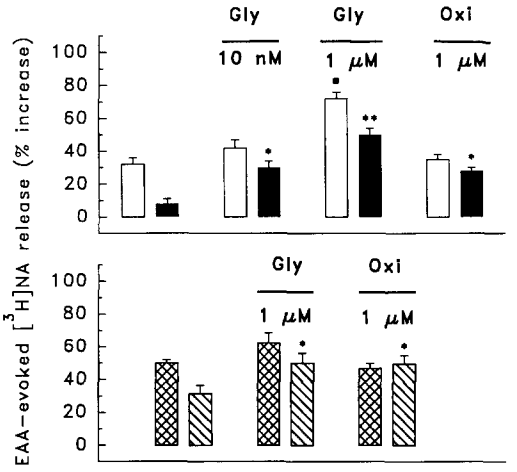

Fig. 4. Effects of glycine (Gly) or oxiracetam (Oxi) on the kynurenic acid antagonism of the release of [^{3}H]noradrenaline elicited by NMDA (upper panel) or by glutamic acid (lower panel) in superfused hippocampal synaptosomes. Open bars: NMDA (100 μM); solid bars: NMDA (100 μM) + kynurenic acid (100 μM); cross-hatched bars: glutamate (100 μM); hatched bars: glutamate (100 μM) + kynurenic acid (100 μM). EAA = excitatory amino acid (NMDA or glutamate); [^{3}H]NA = [^{3}H]noradrenaline. Data are means ± S.E.M. of three to five experiments run in triplicate. ■ $P < 0.05$ vs. NMDA; * $P < 0.05$ vs. NMDA + kynurenic acid; ** $P < 0.01$ vs. NMDA + kynurenic acid.

Fig. 4 shows that glycine or oxiracetam, added at 1 μM, prevented in synaptosomes also the antagonism by kynurenic acid (100 μM) of the [^{3}H]noradrenaline release evoked by NMDA or glutamic acid. Similar results were obtained when the NMDA recognition site antagonist, D-AP5, was used instead of kynurenic acid. Glycine (1 μM) or oxiracetam (1 μM) could reverse the antagonism of the glutamate (100 μM)-evoked [^{3}H]noradrenaline release caused by 10 pM D-AP5 (Pittaluga and Raiteri, 1992) in superfused hippocampal synaptosomes. The data, expressed as percent increase of [^{3}H]noradrenaline release over basal (means ± S.E.M. of three to four experiments in triplicate), are the following: glutamate (50.1 ± 3.1); glutamate + D-AP5 (28.4 ± 3.2; $P < 0.01$ vs. glutamate); glutamate + glycine (62.3 ± 6.6); glutamate + D-AP5 + glycine (49.7 ± 5.2); glutamate + oxiracetam (46.9 ± 3.1); glutamate + D-AP5 + oxiracetam (44.8 ± 6.9).

4. Discussion

More than 80 putative cognition enhancers were listed in a recent review article (Sarter, 1991). Although it seems that the optimistic predictions based

on animal behavioral pharmacology have often been followed by dissatisfying clinical findings, results of studies in human volunteers and in patients with age-associated cognitive dysfunctions or dementia seem to support the view that pharmacological cognition enhancement may become a valuable therapeutic intervention.

Results from diverse experimental approaches have suggested that the cognitive impairments associated with aging or with degenerative diseases involve disruption of cholinergic functions (see Bartus et al., 1982). Accordingly, many putative cognition enhancers are thought to act via cholinergic mechanisms. Several compounds were tested in clinical settings but only a few, i.e. those stimulating the activity of presynaptic cholinergic neurons (reviewed by Sarter, 1991), appear to be of clinical significance.

Impairments of glutamatergic transmission may also represent a major event underlying cognitive dysfunctions. The NMDA receptor has been implicated in the induction of long-term potentiation in the hippocampus (Bliss and Collingridge, 1993), an area which has been associated with certain forms of learning (see Davis et al., 1992). Oxiracetam, a nootropic drug which, like piracetam, aniracetam and pramiracetam belongs to the pyrrolidone class (Moos et al., 1988), was found to prevent the amnesia caused in rats by intracerebroventricular administration of competitive NMDA receptor antagonists (Paoli et al., 1990; Belfiore et al., 1992). All these piracetam-like drugs have been reported to amplify long-term potentiation in various hippocampal subfields (Olpe and Lynch, 1982; Satoh et al., 1988; Pugliese et al., 1990; Asztely et al., 1992; Libri et al., 1994), although important differences have been observed among these compounds. These differences concern not only the hippocampal subfield (CA1 or CA3) where a given drug amplifies long-term potentiation but also the glutamate receptor type involved. Aniracetam, for instance, was reported to interact with ionotropic non-NMDA receptors (Ito et al., 1990; Isaacson and Nicoll, 1991) although concentrations 2–3 orders of magnitude higher than those of oxiracetam acting at NMDA receptors (Pugliese et al., 1990) were required. Thus, if the piracetam-like drugs seem to interact with glutamatergic transmission, there is only poor understanding of the mechanism(s) of the interaction. Indeed these pyrrolidone derivatives also exhibit a generalized stimulatory influence on cholinergic transmission (Pugsley et al., 1983; Pepeu and Spignoli, 1989). Moreover, their memory-enhancing effects have been proposed to depend on endogenous corticosteroids, as these effects can be eliminated by adrenalectomy (Mondadori et al., 1990).

D-Cycloserine is an isoxazolidone derivative known as a partial agonist of the glycine site on the NMDA receptor (Hood et al., 1989; Henderson et al., 1990). Results from behavioral animal studies have provided evidence that D-cycloserine positively influences cognitive processes (Monahan et al., 1989; Flood et al., 1992; Schuster and Schmidt, 1992; Thompson et al., 1992). The drug was shown to effectively counteract the age-related deficit of the NMDA receptors mediating the enhancement of noradrenaline release in rat hippocampus (Pittaluga et al., 1993). In postmortem brain membranes of patients with Alzheimer's disease, D-cycloserine facilitated activation of NMDA receptors (Chessell et al., 1991). Finally, D-cycloserine was reported to improve learning in a human model of the cognitive deficits associated with aging and dementia (Wesnes et al., 1991).

When tested in the functional assay for NMDA receptors chosen in the present work, oxiracetam, aniracetam and D-cycloserine all displayed similar effects: at a concentration as low as 1 μM, the three putative cognition enhancers completely reversed the ability of 100 μM kynurenic acid to block the noradrenaline-releasing effect of NMDA in hippocampal slices (Table 1).

To shed light on the mechanism of action of the three compounds, we tested glycine and D-serine, two known agonists at the glycine site on the NMDA receptor (Johnson and Ascher, 1987; Kleckner and Dingledine, 1988), in the same functional assay and under the same experimental conditions as used with the putative cognition enhancers. Although inactive on their own on the NMDA-evoked effect, possibly due to the presence of consistent concentrations of endogenous glycine in slices (but not in superfused synaptosomes where endogenous glycine is continuously removed; see below), glycine and D-serine behaved like oxiracetam, aniracetam and D-cycloserine in the presence of 100 μM kynurenic acid (Table 1). In fact, the antagonism of NMDA-evoked release of noradrenaline was completely reversed by 1 μM of either glycine or D-serine. The complete reversal of the kynurenate antagonism seen at such low concentration suggests that all the compounds tested may act selectively at a common site.

In this context, the results obtained with hippocampal synaptosomes appear to provide some useful information. Noradrenergic nerve terminals in rat hippocampus possess ionotropic NMDA and non-NMDA receptors, activation of which elicits the release of noradrenaline. NMDA can evoke release not only in Mg^{2+}-free medium, but also in Mg^{2+}-containing medium provided that the non-NMDA receptors are concomitantly activated. Accordingly, glutamic acid (which activates both receptor types) can evoke, in the presence of Mg^{2+} ions, release of noradrenaline due in part to NMDA receptor and in part to non-NMDA receptor activation (Pittaluga and Raiteri, 1992; Raiteri et al., 1992). As illustrated in Fig. 3, addition of glycine

to synaptosomes enhanced the noradrenaline-releasing effects of NMDA (EC_{50} = 0.3 μM) and, when added at higher concentrations (EC_{50} = 3.7 μM), the effect of glutamate; in contrast, oxiracetam was ineffective up to 1 mM, suggesting that the compound is not a glycine site agonist. Oxiracetam (10 μM) did not prevent the enhancement of the glutamate effect caused by 1–100 μM glycine (data not shown), which tends to exclude the possibility that the drug is a glycine site ligand. On the other hand (Fig. 4) both glycine and oxiracetam, added at 1 μM, could prevent the antagonism by kynurenic acid of the NMDA or the glutamate effect on the release of [^{3}H]noradrenaline. It should be noted that glycine and oxiracetam could 'protect' NMDA receptor activation not only against the broad spectrum antagonist, kynurenic acid, but also against D-AP5, a selective blocker at the NMDA recognition site.

As one explanation for these results, it could be hypothesized that glycine has a dual action: (a) the well known allosteric modulation of the NMDA recognition site (Johnson and Ascher, 1987); (b) a 'protective' action against kynurenic acid following binding to a site different from the strychnine-insensitive site. Oxiracetam and, possibly, aniracetam would *only* act at the latter hypothetical novel site, while D-cycloserine would *also* act at the strychnine-insensitive site, as a partial agonist (Hood et al., 1989; Henderson et al., 1990). The shifts of the kynurenate concentration-antagonism curve caused by oxiracetam (Fig. 2) suggest that the interaction between kynurenic acid and oxiracetam is competitive. Clearly, further investigation is required to understand the interaction of the putative cognitive enhancers examined with the NMDA receptor complex. It will be particularly important to establish how low concentrations of these drugs, but also of glycine, can 'disarm' much higher concentrations of the NMDA receptor antagonist, kynurenic acid. As to the above-mentioned electrophysiological actions of aniracetam at ionotropic non-NMDA receptors, it should be noted that they could be observed only at concentrations of aniracetam between 100 and 1000 μM. This leaves open the possibility that the drug, when tested at much lower concentrations, might also prove to be effective on NMDA-mediated currents.

To conclude, our results suggest a common neurochemical mechanism for oxiracetam, aniracetam and D-cycloserine. At very low concentrations (1 μM or less) all three drugs can prevent kynurenic acid from blocking the NMDA-mediated noradrenaline release in the hippocampus. As mentioned in the introductory statement, kynurenic acid is an endogenous glutamate receptor antagonist, the brain levels of which are highest in humans (Moroni et al., 1988a; Turski et al., 1988) and can increase significantly in conditions associated with cognitive impairments including aging (Moroni et al., 1988b; Gramsbergen et al., 1992) and HIV-1 infection (Heyes et al., 1990). The noradrenergic neurons of the locus coeruleus are involved in the regulation of many brain functions such as attention and memory (McGaugh, 1989; Harley, 1991) and NMDA receptors are present on these neurons also at the somadendritic level (Olpe et al., 1989). In Alzheimer's type dementia there is a marked reduction in the noradrenergic innervation of the target fields, including the hippocampus (Chan-Palay, 1991; German et al., 1992). Thus abnormally elevated concentrations of kynurenic acid may impair glutamate-mediated functions involved in mnesic processes, such as stimulation of noradrenergic transmission. The putative cognition enhancers, oxiracetam, aniracetam and D-cycloserine, may act by relieving the glutamate receptor antagonism brought about by kynurenic acid released in excess into the extracellular space.

Acknowledgements

This work was supported by grants from the Italian M.U.R.S.T. and the Italian C.N.R. The authors wish to thank Mrs. Maura Agate for assistance in the preparation of the manuscript.

References

Asztely, F., E. Hanse, H. Wigstrom and B. Gustafsson, 1992, Aniracetam-evoked potentiation does not interact with long-term potentiation in the CA1 region of the hippocampus, Synapse 11, 342.

Bartus, R.T., R.L. Dean III, B. Beer and A.S. Lippa, 1982, The cholinergic hypothesis of geriatric memory dysfunction, Science 217, 408.

Belfiore, P., F. Ponzio, R. Biagetti, C. Berettera, M. Magnani and O. Pozzi, 1992, Oxiracetam prevents the hippocampal cholinergic hypofunction induced by the NMDA receptor blocker AP7, Neurosci. Lett. 143, 127.

Bliss, T.V.P. and G.L. Collingridge, 1993, A synaptic model of memory: long-term potentiation in the hippocampus, Nature 361, 31.

Chan Palay, V., 1991, Alterations in the locus coeruleus in dementias of Alzheimer's and Parkinson's disease, Prog. Brain Res. 88, 625.

Chessell, I.P., A.W. Procter, P.T. Francis and D.M. Bowen, 1991, D-Cycloserine, a putative cognitive enhancer, facilitates activation of the *N*-methyl-D-aspartate receptor-ionophore complex in Alzheimer brain, Brain Res. 565, 345.

Danysz, W. and J.T. Wroblewski, 1989, Amnesic properties of glutamate receptor antagonists, Neurosci. Res. Commun. 5, 9.

Davis, S., S.P. Butcher and R.G.M. Morris, 1992, The NMDA receptor antagonist D-2-amino-5-phosphonopentanoate (D-AP5) impairs spatial learning and LTP in vivo at intracerebral concentrations comparable to those that block LTP in vitro, J. Neurosci. 12, 21.

Fink, K., R. Schultheiss and M. Göthert, 1992, Stimulation of noradrenaline release in human cerebral cortex mediated by *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors, Br. J. Pharmacol. 106, 67.

Flood, J.F., J.E. Morley and T.H. Lanthorn, 1992, Effect on memory processing by D-cycloserine, an agonist of the NMDA/glycine receptor, Eur. J. Pharmacol. 221, 249.

German, D.C., K.F. Manaye, C.L. White, D.J. Woodward, D.D. McIntire, W.K. Smith, R.N. Kalaria and D.M. Mann, 1992, Disease-specific patterns of locus coeruleus cell loss, Ann. Neurol. 32, 667.

Gramsbergen, J.B., W. Schmidt, W.A. Turski and R. Schwarcz, 1992, Age-related changes in kynurenic acid production in rat brain, Brain Res. 588, 1.

Gray, E.G. and V.P. Whittaker, 1962, The isolation of nerve endings from brain: an electron-microscopic study of cell fragments derived by homogenization and centrifugation, J. Anat. 96, 79.

Harley, C., 1991, Noradrenergic and locus coeruleus modulation of the perforant path-evoked potential in rat dentate gyrus supports a role for the locus coeruleus in attentional and memorial processes, Prog. Brain Res. 88, 307.

Henderson, G., J.W. Johnson and P. Ascher, 1990, Competitive antagonists and partial agonists at the glycine modulatory site of the mouse *N*-methyl-D-aspartate receptor, J. Physiol. 430, 189.

Heyes, M.P., B.J. Brew, R.W. Price and S.P. Markey, 1990, Cerebrospinal fluid quinolinic acid and kynurenic acid in HIV-1 infection, in: Neurotoxicity of Excitatory Amino Acids, ed. A. Guidotti (Raven Press, New York) p. 217.

Hood, W.F., R.P. Compton and J.B. Monahan, 1989, D-Cycloserine: a ligand for the *N*-methyl-D-aspartate coupled glycine receptor has partial agonist characteristics, Neurosci. Lett. 98, 91.

Isaacson, J.S. and R.A. Nicoll, 1991, Aniracetam reduces glutamate receptor desensitization and slows the decay of fast excitatory synaptic currents in hippocampus, Proc. Natl. Acad. Sci. USA 88, 10936.

Ito, I., S. Tanabe, A. Kohda and H. Sugiyama, 1990, Allosteric potentiation of quisqualate receptors by a nootropic drug aniracetam, J. Physiol. 424, 533.

Johnson, J.W. and P. Ascher, 1987, Glycine potentiates the NMDA response in cultured mouse brain neurons, Nature 325, 529.

Kessler, M., T. Terramani, G. Lynch and M. Baudry, 1989, A glycine site associated with *N*-methyl-D-aspartic acid receptors: characteristics and identification of a new class of antagonists, J. Neurochem. 52, 1319.

Kleckner, N.W. and R. Dingledine, 1988, Requirement for glycine in activation of *N*-methyl-D-aspartate receptors expressed in Xenopus oocytes, Science 241, 835.

Libri, V., A.M. Scorsa, G. Dawe and G. Nisticò, 1994, Long-term potentiation of synaptic transmission in rodents: effects of pramiracetam sulfate, Pharmacol. Commun. 4, 125.

McGaugh, J.L., 1989, Involvement of hormonal and neuromodulatory systems in the regulation of memory storage, Annu. Rev. Neurosci. 12, 255.

Monahan, J.B., G.E. Handelmann, W.F. Hood and A.A. Cordi, 1989, D-Cycloserine, a positive modulator of the *N*-methyl-D-aspartate receptor, enhances performance of learning task in rats, Pharmacol. Biochem. Behav. 34, 649.

Mondadori, C., A. Bhatnagar, J. Borkowski and A. Hausler, 1990, Involvement of a steroidal component in the mechanism of action of piracetam-like nootropics, Brain Res. 506, 101.

Moos, W.H., R.E. Davis, R.D. Schwarz and E.R. Gamzu, 1988, Cognition activators, Med. Res. Rev. 8, 353.

Moroni, F., P. Russi, G. Lombardi, M. Beni and V. Carlà, 1988a, Presence of kynurenic acid in the mammalian brain, J. Neurochem. 51, 177.

Moroni, F., P. Russi, V. Carlà and G. Lombardi, 1988b, Kynurenic acid is present in the rat brain and its content increases during development and aging process, Neurosci. Lett. 94, 145.

Olpe, H.-R. and G. Lynch, 1982, The action of piracetam on the electrical activity of the hippocampal slice preparation: a field potential analysis, Eur. J. Pharmacol. 80, 415.

Olpe, H.-R., M.W. Steinmann, F. Brugger and M.F. Pozza, 1989, Excitatory amino acid receptors in rat locus coeruleus. An extracellular in vitro study, Naunyn-Schmied. Arch. Pharmacol. 339, 312.

Paoli, F., G. Spignoli and G. Pepeu, 1990, Oxiracetam and D-pyroglutamic acid antagonize a disruption of passive avoidance behaviour induced by the *N*-methyl-D-aspartate receptor antagonist 2-amino-5-phosphonovalerate, Psychopharmacology 100, 130.

Pepeu, G. and G. Spignoli, 1989, Nootropic drugs and brain cholinergic mechanisms, Prog. Neuropsychopharmacol. Biol. Psychiat. 13 Suppl., S77.

Pittaluga, A. and M. Raiteri, 1990, Release-enhancing glycine-dependent presynaptic NMDA receptors exist on noradrenergic terminals of hippocampus, Eur. J. Pharmacol. 191, 231.

Pittaluga, A. and M. Raiteri, 1992, *N*-Methyl-D-aspartic acid (NMDA) and non-NMDA receptors regulating hippocampal norepinephrine release. III. Changes in the NMDA receptor complex induced by their functional cooperation, J. Pharmacol. Exp. Ther. 263, 327.

Pittaluga, A., E. Fedele, C. Risiglione and M. Raiteri, 1993, Age-related decrease of the NMDA receptor-mediated noradrenaline release in rat hippocampus and partial restoration by D-cycloserine, Eur. J. Pharmacol. 231, 129.

Pugliese, A.M., R. Corradetti, L. Ballerini and G. Pepeu, 1990, Effect of the nootropic drug oxiracetam on field potentials of rat hippocampal slices, Br. J. Pharmacol. 99, 189.

Pugsley, T.A., B.P. Poschel, D.A. Danws, Y.H. Shih and I. Melvyn, 1983, Some pharmacological and neurochemical properties of a new cognition activator agent, Piracetam (Ci-879), Psychopharmacol. Bull. 19, 721.

Raiteri, M., F. Angelini and G. Levi, 1974, A simple apparatus for studying the release of neurotransmitters from synaptosomes, Eur. J. Pharmacol. 25, 411.

Raiteri, M., B. Garrone and A. Pittaluga, 1992, *N*-Methyl-D-aspartic acid (NMDA) and non-NMDA receptors regulating hippocampal norepinephrine release. II. Evidence for functional cooperation and for coexistence on the same axon terminal, J. Pharmacol. Exp. Ther. 260, 238.

Sarter, M., 1991, Taking stock of cognition enhancers, Trends Pharmacol. Sci. 12, 456.

Satoh, M., K. Ishihara and H. Katsuki, 1988, Different susceptibilities of long-term potentiation in CA3 and CA1 regions of guinea-pig hippocampal slices to nootropic drugs, Neurosci. Lett. 93, 236.

Schuster, G.M. and W.J. Schmidt, 1992, D-Cycloserine reverses the working memory impairment of hippocampal-lesioned rats in a spatial learning task, Eur. J. Pharmacol. 224, 97.

Smith, J.E., J.D. Lane, P.A. Shea, W.J. McBride and M.H. Aprison, 1975, A method for concurrent measurement of picomole quantities of acetylcholine, choline, dopamine, norepinephrine, serotonin, 5-hydroxytryptophan, 5-hydroxyindoleacetic acid, tryptophan, tyrosine, glycine, aspartate, glutamate, alanine and gamma-aminobutyric acid in single tissue samples from different areas of rat central nervous system, Anal. Biochem. 64, 149.

Stone, T.W., N.R. Burton and D.A.S. Smith, 1989, Electrophysiology of quinolinic acid, kynurenic acid, and related compounds in the CNS, in: Quinolinic acid and the Kynurenine, ed. T.W. Stone (CRC Press, Boca Raton, FL, USA) p. 113.

Thompson, L.T., J.R. Moskal and J.F. Disterhoft, 1992, Hippocampus-dependent learning facilitated by a monoclonal antibody or D-cycloserine, Nature 359, 638.

Turski, W.A., M. Nakamura, W.P. Todd, B.K. Carpenter, W.O. Wetsell, Jr. and R. Schwarcz, 1988, Identification and quantification of kynurenic acid in human brain tissue, Brain Res. 454, 164.

Wesnes, K., R.W. Jones and J. Kirby, 1991, The effects of D-cycloserine, a glycine agonist, in a human model of the cognitive deficits associated with ageing and dementia, Br. J. Clin. Pharmacol. 31, 577.