The “Kynurenate Test,” a Biochemical Assay for Putative Cognition Enhancers

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ABSTRACT

Some putative cognition enhancers (oxiracetam, aniracetam and d-cycloserine) were previously shown to prevent the kynurenic acid antagonism of the N-methyl-D-aspartate (NMDA)-evoked norepinephrine (NE) release in rat hippocampal slices. This functional in vitro assay was further characterized in the present work. d-Serine, a glutamate coagonist at the NMDA receptor glycine site, concentration-dependently (EC(50) = 0.1 μM) prevented the kynurenic acid (100 μM)-evoked [3H]NE release. L-Serine was inactive up to 10 μM. The γ-aminobutyric acidB (GABA(B) receptor antagonist CGP 36742, reported to improve cognitive performance, potently prevented the kynurenic acid antagonism. The activity of CGP 36742 (1 μM) appeared to be unaffected by 10 μM (−)-baclofen, a GABA(B) receptor agonist; furthermore, CGP 52432, a GABA(B) antagonist more potent than CGP 36742, but reportedly devoid of nootropic properties, was inactive in the “kynurenate test.” The novel putative cognition enhancer CR2249, but not its enantiomer CR2361, also potently prevented the kynurenic acid antagonism. In contrast, linopirdine, nicotine and tacrine were inactive. In rat hippocampal synaptosomes glycine and d-cycloserine enhanced the NMDA-evoked [3H]NE release, whereas oxiracetam and CR2249 did not. These four compounds were all similarly effective in preventing kynurenate antagonism, both in slices and in synaptosomes. The NMDA potentiation caused by glycine (0.1–100 μM) was not affected by 100 μM oxiracetam, which suggested that drugs active in the “kynurenate test” may bind to sites different from the glycine site of the NMDA receptor. To conclude, the “kynurenate test” is an in vitro assay useful in the identification and characterization of putative cognition enhancers acting via NMDA receptors.

In a recent work (Pittaluga et al., 1995) it was found that submicromolar concentrations of the putative cognition enhancers oxiracetam, aniracetam and d-cycloserine could counteract kynurenic acid antagonism of the NMDA-evoked NE release from rat hippocampal slices. These interactions were subsequently tested with [3H]MK-801 ([3H]dizocilpine) binding in rat forebrain membranes (Hamelin and Lehmann, 1995): low micromolar d-cycloserine or aniracetam decreased kynurenate-induced inhibition of [3H]MK-801 binding, which supported the findings of Pittaluga et al. (1995).

Although the above-mentioned results are compatible with the idea that some cognition enhancers may function via NMDA receptors, several questions remain. In particular, how nootropics believed not to act through the glutamate system behave in the “kynurenate test” is unknown. Compounds not expected to provide positive responses in the kynurenate test, for instance amnesic drugs, have not yet been examined. As for the drugs seemingly acting through the NMDA receptor, i.e., oxiracetam, aniracetam and d-cycloserine, it is unclear to what site(s) on the NMDA receptor complex these compounds may bind to prevent kynurenate antagonism of the NMDA-mediated effect. In the study carried out with hippocampal slices (Pittaluga et al., 1995), the inhibition by kynurenate of the NMDA-evoked [3H]NE release could be relieved not only by oxiracetam, aniracetam or d-cycloserine, but also by glycine, which suggested involvement of the glycine site of the NMDA receptor. However, preliminary experiments with synaptosomes showed that oxiracetam, differently from glycine, was unable to activate the NMDA receptor glycine site. Thus the site(s) where nootropics bind to prevent kynurenate antagonism may differ from the glycine site of the NMDA receptor.

A better characterization of the kynurenate test is clearly required to establish its potential usefulness as a functional in vitro assay in the development of cognition-enhancing agents. To this aim, we included in the present study several putative cognition enhancers believed to act through mechanisms not directly involving NMDA receptors, such as nico-

ABBREVIATIONS: NE, norepinephrine; NMDA, N-methyl-D-aspartate; CGP 36742, (3-aminopropyl)-n-butylphosphinic acid; CGP 52432, [3-[[3,4-dichlorophenyl] methylj[amino]propyl] [diethoxymethyl] phosphinic acid; CR2249, (S)-4-amino-5-[[4,4-dimethylcyclohexyl]amino]-5-oxo-pentanoic acid; GABA, γ-aminobutyric acid.
tine, linopirdine (Zaczek and Saydoff, 1993), tacrine (Farlow et al., 1992; Sahakian et al., 1993) and the GABA<sub>B</sub> receptor antagonist CGP 36742 (Mondadori et al., 1993; Olpe et al., 1993; Fröstl et al., 1995). In addition, the recently described putative cognition enhancer CR2249 (Garofalo et al., 1996), a drug proposed to interact with NMDA receptors (Lanza et al., 1997), was tested. Finally, we used hippocampal synaptosomes to investigate the possible site of action of the compounds displaying activity in the kynurenate test.

Materials and Methods

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 sacrificed by decapitation and the hippocampi were rapidly removed and placed in physiological salt solution (see below) at 2–4°C.

Release Experiments from Slices

Slices (0.4 mm thick) were prepared from the ventro-medial hippocampus with a McIlwain tissue chopper and then labeled with 0.08 μM [3H]NE, 20 min at 37°C, in a physiological salt solution with the following composition (mM): NaCl, 125; KCl, 3; MgSO<sub>4</sub>, 1.2; CaCl<sub>2</sub>, 1.2; Na<sub>H</sub>PO<sub>4</sub>, 1; NaHCO<sub>3</sub>, 22; glucose, 10 (aeration with 95% O<sub>2</sub> and 5% CO<sub>2</sub>); pH 7.2 to 7.4. The incubation medium contained 0.1 μM 6-nitroquipazine, a serotonin uptake inhibitor, to prevent false labeling of serotonergic terminals. After washing with tracer-free medium, slices were transferred to parallel superfusion chambers (one slice per chamber) and superfused at 1 ml/min, at 37°C, with a semisynaptic medium from which Mg<sup>2+</sup> was added concomitantly with NMDA, except in the experiments of kynurenate antagonism in which the drugs were added together with the antagonist, 8 min before NMDA. At the end of superfusion, samples and superfused slices (solubilized with Soluene) were then collected. This ratio was compared with 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; multiple comparisons were made with Dunnett’s test. The [3H]-radioactivity released when slices or synaptosomes were exposed to 100 μM NMDA had previously been analyzed chromatographically and shown to consist largely (about 90%) of authentic [3H]NE (Fink et al., 1992; Pittaluga and Raiteri, 1992; Pittaluga et al., 1995).

Chemicals

[3H]Norepinephrine (specific activity, 39 Ci/mmol) was purchased from Amersham Radiochemical Centre (Buckinghamshire, UK). Kynurenic acid, D-serine, L-serine, D-cycloserine, (−)-scopolamine and nicotine were obtained from Sigma Chemical Co (St. Louis, MO); NMDA was purchased from Torris Cookson (Bristol, UK). The following substances were gifts from the companies indicated: (−)-baclofen, CGP 36742, CGP 52432 and tacrine (Ciba Geigy, Basel, Switzerland); aniracetam (Prodotti Roche, Milan, Italy); 6-nitroquipazine maleate (Duhref, Amsterdam, The Netherlands); linopirdine (The Du Pont Merck Pharmaceutical Company, Wilmington, DE); CR2249 and CR2361 (Rotta Research Laboratory, Monza, Italy).

Results

As a first step toward establishing the selectivity of the kynurenate test, we investigated the possible effects of scopolamine, a known amnesic drug, and of (−)-baclofen, a GABA<sub>B</sub> receptor agonist also endowed with amnesic activity (Swartzwelder et al., 1987; Castellano et al., 1990; Carletti et al., 1993). Rat hippocampal slices, prelabeled with [3H]NE, were superfused in the absence or presence of NMDA; when added at 100 μM, the excitatory amino acid increased by about 500% the release of the [3H]catecholamine (table 1); this effect was strongly reduced by 100 μM kynurenic acid. According to the expectation, neither scopolamine, added at 100 μM, nor (−)-baclofen, added at 10 μM, reduced the kynurenate antagonism. The table also shows that scopolamine did not modify the effect of NMDA in the absence of kynurenate, whereas (−)-baclofen (10 μM) inhibited significantly (by about 30%) the release of [3H]NE evoked by NMDA.

We next investigated if the previously observed ability of D-serine to prevent kynurenate antagonism (Pittaluga et al., 1995) displayed stereoselectivity. As illustrated in figure 1, D-serine, but not L-serine, potent and concentration-dependently protected the [3H]NE-releasing effect of NMDA from the kynurenate antagonism. Concentrations of D-serine as low as 0.01 μM tended to attenuate the antagonism brought about by 100 μM kynurenate; the antagonism was halved at about 0.1 μM D-serine and completely relieved by 1 μM D-serine. In contrast, L-serine was ineffective when added at

<table>
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<tr>
<th>TABLE 1</th>
<th>Effects of (−)-scopolamine or (−)-baclofen on the [3H]NE release induced in rat hippocampal slices by NMDA alone or in the presence of kynurenic acid (KYNA)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMDA (100 μM)</td>
<td>NMDA (100 μM) + KYNA (100 μM)</td>
</tr>
<tr>
<td>Controls</td>
<td>482 ± 59</td>
</tr>
<tr>
<td>(−)-Scopolamine (100 μM)</td>
<td>595 ± 70</td>
</tr>
<tr>
<td>(−)-Baclofen (10 μM)</td>
<td>350 ± 25&lt;sup&gt;d&lt;/sup&gt;</td>
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<sup>*</sup> Data (expressed as percent increase over basal) are the means ± S.E.M. of four to six experiments run in triplicate. For experimental details, see “Materials and Methods.”

<sup>a</sup> P < .01 vs. NMDA.

<sup>b</sup> P < .05 vs. NMDA.
concentrations 2 to 3 orders of magnitude higher than the active concentrations of d-serine.

In a previous work (Ransom and Deschenes, 1988), carried out under apparently similar experimental conditions, d-serine was found to stereoselectively block the inhibitory effect of kynurenate on NMDA-evoked \([3^H]NE\) release from rat hippocampal slices. However, in that study, the EC\(_{50}\) value of d-serine was 14 \(\mu M\), i.e., much higher than that (~0.1 \(\mu M\)) estimated from our data. To clarify the discrepancy, experiments were performed in which slices were exposed to d-serine, kynurenate and NMDA under different conditions. As shown in figure 2, when d-serine was added to the slices concomitantly with NMDA, but 45 min after kynurenate, no reduction of the kynurenate antagonism could be seen at a concentration (1 \(\mu M\)) at which d-serine completely blocked kynurenate in the conditions used in the present work (d-serine and kynurenate concomitantly added 45 min before NMDA); actually, about 20 \(\mu M\) d-serine was required to prevent the kynurenate antagonism by 50%.

Drugs thought to positively affect cognition through mechanisms not strictly and directly related to glutamate transmission were then investigated in the kynurenate test. Linopirdine, nicotine and tacrine all were unable to prevent the inhibition of the NMDA-evoked \([3^H]NE\) release caused by kynurenic acid, when added to hippocampal slices at 1 or 10 \(\mu M\) (table 2). The three drugs, tested at 10 \(\mu M\), did not enhance the effect of NMDA either. At 100 \(\mu M\), nicotine and tacrine, but not linopirdine, elicited their own increase of \([3^H]NE\) release.

Mondadori et al. (1993) reported that CGP 36742, a selective GABA\(_B\) receptor antagonist, can improve the cognitive performance of several animal species. As shown in figure 3, the kynurenate inhibition of the NMDA-evoked release of \([3^H]NE\) was potently counteracted by CGP 36742. The drug completely abolished the antagonism by 100 \(\mu M\) kynurenic acid at concentrations ranging between 0.3 and 1 \(\mu M\). CGP 36742 (1 \(\mu M\)) had no significant effect, on its own, on the NMDA-evoked \([3^H]NE\) release (table 3). The table also shows that the GABA\(_B\) receptor agonist (–)-baclofen, added at 10 \(\mu M\), only slightly decreased the activity of 1 \(\mu M\) CGP 36742.
in the kynurenate test. The kynurenate inhibition of the NMDA-evoked \[^{3}H\]NE release was unaffected by 10 \(\mu M\) \((2)^{-}\)-baclofen. On the other hand, the GABA\(_B\) receptor agonist, added at 10 \(\mu M\), depressed the effect of NMDA in the absence of kynurenate; however, this effect of \((2)^{-}\)-baclofen persisted in the presence of 1 \(\mu M\) CGP 36742 (table 3).

The compound \[3-L[(3,4-dichlorophenyl) methyl]amino]pro pyl\] (diethoxymethyl) phosphinic acid (CGP 52432), a GABAB receptor antagonist more potent than CGP 36742 in several tests, but reported to be devoid of cognition-enhancing properties (Bittiger \textit{et al.}, 1996), displayed no activity in the kynurenate test up to 10 \(\mu M\) (fig. 4).

A novel substance, CR2249, has been recently reported to display cognition-enhancing activity in different behavioral paradigms (Garofalo \textit{et al.}, 1996). As illustrated in figure 5, CR2249 potently prevented the kynurenate antagonism of the NMDA-evoked \[^{3}H\]NE release in hippocampal slices. In contrast, the enantiomer CR2361 appeared to be about 2 orders of magnitude less potent than CR2249. At the concentrations used, CR2249 had no effect, on its own, on the NMDA-evoked \[^{3}H\]NE release (not shown).

Although kynurenic acid is a nonselective NMDA receptor antagonist (Stone, 1993), the stereoselectivity of \(D\)-serine in preventing the kynurenate antagonism suggests involvement of the glycine site. If this is the case, the nootropic drugs shown to be effective in the kynurenate test may mimic glycine and \(D\)-serine as NMDA coagonists. To verify this idea, experiments were performed with hippocampal superfused synaptosomes, an experimental set-up in which indirect ef-

**Table 3**

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<thead>
<tr>
<th></th>
<th>NMDA (100 (\mu M))</th>
<th>NMDA (100 (\mu M)) + KYNA (10 (\mu M))</th>
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</thead>
<tbody>
<tr>
<td>Controls</td>
<td>530 ± 51</td>
<td>147 ± 36(^b)</td>
</tr>
<tr>
<td>CGP36742 (1 (\mu M))</td>
<td>494 ± 25</td>
<td>486 ± 20(^c)</td>
</tr>
<tr>
<td>Baclofen (10 (\mu M))</td>
<td>390 ± 35(^d)</td>
<td>152 ± 73(^b)</td>
</tr>
<tr>
<td>CGP36742 (1 (\mu M)) + baclofen (10 (\mu M))</td>
<td>399 ± 53(^d)</td>
<td>397 ± 42(^c)</td>
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\(^*\) Data (expressed as percent increase over basal) are means ± S.E.M. of four to six experiments run in triplicate.

\(^{b}\) P < .01 vs. NMDA.

\(^{c}\) P < .01 vs. NMDA + KYNA.

\(^{d}\) P < .05 vs. NMDA.

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**Fig. 3.** Effect of CGP 36742 on the kynurenic acid (KYNA) antagonism of the NMDA-induced \[^{3}H\]NE release from rat hippocampal slices. CGP 36742, at the concentrations indicated, did not affect, on its own, the basal release of \[^{3}H\]NE. For experimental details, see "Materials and Methods." Data are means ± S.E.M. of three to five experiments run in triplicate. * P < .05 vs. NMDA; ** P < .01 vs. NMDA; # P < .05 vs. NMDA + KYNA; ## P < .01 vs. NMDA + KYNA.

**Fig. 4.** Effect of CGP 52432 on the kynurenic acid (KYNA) antagonism of the NMDA-induced \[^{3}H\]NE release from rat hippocampal slices. For experimental details, see "Materials and Methods." Data are means ± S.E.M. of three to five experiments run in triplicate. * P < .01 vs. NMDA.

**Fig. 5.** Effects of CR2249 and CR2361 on the antagonism by kynurenic acid (KYNA) of the NMDA-induced \[^{3}H\]NE release from hippocampal slices. At the concentrations tested CR2249 and CR2361 had no effect, on their own, on the basal release or on the NMDA-evoked release of tritium. For experimental details, see "Materials and Methods." Data are means ± S.E.M. of three to five experiments run in triplicate. * P < .01 vs. NMDA; # P < .01 vs. NMDA + KYNA.
fects are minimized, essentially because the endogenous compounds released are immediately removed by the superfusion fluid. In fact, exogenous glycine can not be easily studied as an NMDA coagonist in brain slices because unknown, but relevant, concentrations of endogenous glycine are present at the NMDA receptors. These studies are possible with superfused synaptosomes, however, because of the rapid removal of endogenous glycine. As shown in figure 6A, glycine concentration-dependently increased the effect of 100 μM NMDA on the synaptosomal release of [3H]NE (the activity of NMDA alone is known to be caused by low nanomolar glycine contamination present in the water: see Johnson and Ascher, 1987; Pittaluga and Raiteri, 1990). The putative cognition enhancer d-cycloserine mimicked glycine, although with lower efficacy (fig. 6B). In contrast, oxiracetam (1 μM–1 mM) or CR2249 (1–100 μM) was unable to enhance the NMDA effect (fig. 6, C and D). On the other hand, glycine, d-cycloserine, oxiracetam and CR2249 all displayed activity in the kynurenate test in slices (Pittaluga et al., 1986; Miserendino et al., 1995; present work) and also prevented the kynurenate antagonism in superfused synaptosomes (fig. 6). In particular, oxiracetam and CR2249 were effective against kynurenate at concentrations (≥1 μM) that are at least 2 to 3 orders of magnitude lower than those found unable to activate the NMDA receptor glycine site.

Finally, the presence of 100 μM oxiracetam in the medium superfusing hippocampal synaptosomes did not interfere with the potentiation by glycine (0.1–100 μM) of the NMDA-evoked [3H]NE release (fig. 7), which suggested that oxiracetam does not bind at all or at the glycine site.

**Discussion**

The glutamatergic system is thought to play a major role in the processes of learning and memory. Long-term potentiation, that is regarded as a neuronal model to study memory function, depends on the activation of glutamate receptors of the NMDA type (Bliss and Collingridge, 1993). Antagonists of NMDA receptors display amnesic properties (Morris et al., 1986; Miserendino et al., 1990) and activation of NMDA receptors appears necessary for certain kinds of learning (Davis et al., 1992).

The noradrenergic neurons of the locus ceruleus have long been known to be involved in the regulation of attention and memory (McGaugh, 1989; Harley, 1991; Izquierdo et al., 1993 and references therein). 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). Interactions between glutamatergic and noradrenergic systems have been described (see Huang and Kandel, 1996 and references therein). Of particular relevance to cognition processes may be the NMDA-induced enhancement of NE release shown to occur in hippocampus and neocortex under in vitro (Jones et al., 1987; Vezzani et al., 1987; Ransom and Deschenes, 1988; Gonzales and Woodward, 1990; Pittaluga and Raiteri, 1990, Fink et al., 1992; Pittaluga et al., 1995) and in vivo (Lehmann et al., 1992) conditions.

Kynurenic acid is an antagonist at ionotropic glutamate receptors, particularly those of the NMDA type, that is present in the mammalian brain as a physiological metabolite (Kessler et al., 1989; Stone, 1993). The levels of this endogenous glutamate receptor antagonist are highest in the human brain (Moroni et al., 1988a; Turski et al., 1988) and may become abnormally elevated under conditions associated with cognitive deficits (Moroni et al., 1988b; Heyes et al., 1990; Gramsbergen et al., 1992). In a recent work we postulated that some cognitive enhancers may act by preventing antagonism by endogenous kynurenate at NMDA receptors (Pittaluga et al., 1995). To test this hypothesis, we used a functional in vitro assay for NMDA receptors, here referred to as the “kynurenate test,” in which an effect of NMDA (elevation of NE release in hippocampal slices) is antagonized by kynurenic acid and putative cognition enhancers are tested for their ability to counteract the kynurenate antagonism. The findings that submicromolar concentrations of oxiracetam, aniracetam and d-cycloserine could prevent the antagonism produced by concentrations of kynurenate at least 2 orders of magnitude higher than those of the nootropics (Pittaluga et al., 1995), together with the consideration...
that these drugs (for instance, aniracetam) often need to be added at 100 to 1000 μM to affect glutamatergic transmission in in vitro electrophysiological experiments related to cognitive phenomena (Ito et al., 1990; Isaacson and Nicoll, 1991), prompted us to further characterize the kynurenate test as a functional assay potentially useful in the study of cognition-enhancing compounds.

Assuming that compounds displaying activity in the kynurenate test, like oxiracetam, aniracetam, and D-cycloserine, act via NMDA receptors, it was clearly important to examine the responses in the test of behaviorally active compounds that are thought to act through non-glutamatergic mechanisms, such as the “cholinergic” drugs. A role for cholinergic transmission in learning and memory has long been suspected (Bartus et al., 1982), mainly because of the fact that administration of the muscarinic receptor antagonist scopolamine causes amnesia in humans and animals.

Tacrine is thought to display cognition-enhancing activity through cholinomimetic mechanisms. In particular, the drug is a cholinesterase inhibitor which has been in use clinically to alleviate the cognitive deficits of patients with Alzheimer’s disease (Davis et al., 1992; Farlow et al., 1992; Sahakian et al., 1993). Tacrine neither potentiated the activity of NMDA nor could it decrease the kynurenate antagonism of the NMDA-evoked NE release. When added at relatively high concentrations, the drug caused [3H]NE release on its own, although whether this effect occurs directly or indirectly is difficult to say.

The cognition-enhancing properties of nicotine are well recognized (see, for a review, Arneric et al., 1995). Studies of transmitter release have shown that nicotine can elicit release of acetylcholine (Thomas et al., 1993; Marchi and Raiteri, 1996). As reported in table 2, nicotine was inactive in the kynurenate test, up to 10 μM. At this concentration, the drug was also unable to modify the effect of NMDA. Nicotine, added to hippocampal slices at higher concentrations, increased on its own the release of NE, in keeping with previous observations in hippocampal slices (Snell and Johnson, 1989) and synaptosomes (Clarke and Reuben, 1996). Considering the involvement of both the cholinergic and noradrenergic systems in the regulation of attention and memory, the NE-releasing activities of nicotine and tacrine, together with their ability to enhance extracellular acetylcholine concentration, may play important roles.

Linopirdine, a drug proposed for symptomatic treatment of Alzheimer’s disease, has been shown to enhance release of acetylcholine, dopamine and serotonin (Nickolson et al., 1990). The compound could not elicit release of NE from rat hippocampal slices, however (Zaczek et al., 1993). Our data (table 2) confirm the inability of linopirdine to enhance the release of NE from hippocampal slices. Similar to tacrine and nicotine, linopirdine was inactive in the kynurenate test. On the other hand, performance improvements in several tests of learning and memory in rats have been attributed to modulation by linopirdine of cholinergic transmission (Zaczek and Saydoff, 1993; Fontana et al., 1994).

Based on the negative responses of cholinergic cognition enhancers in the kynurenate test, it would seem that the NMDA-induced release of NE in hippocampal slices is independent of the activation of cholinergic receptors. It may be worth recalling that NMDA failed to evoke acetylcholine release from slices of rat hippocampus (Lehmam and Scatton, 1982). On the other hand, interactions between glutamatergic and cholinergic systems in learning and memory are likely to occur. Scopolamine-induced amnesia in human volunteers was found to be alleviated by D-cycloserine (Jones et al., 1991), a partial agonist at the glycine site of NMDA receptors (Hood et al., 1989; Monahan et al., 1989), clearly active in the kynurenate test (Pittaluga et al., 1995). Other nootropics such as oxiracetam and aniracetam, also active in the kynurenate test, were reported to prevent scopolamine-induced amnesia and to enhance cholinergic transmission in rats (Pepeu and Spignoli, 1989; for a review see Sarter, 1991). The lack of effect of tacrine, nicotine and linopirdine in the kynurenate test raises the possibility that interactions between cholinergic and glutamatergic systems occur downstream from the NMDA-mediated step.

The results obtained with GABAA receptor antagonists are of particular interest. The cognitive performances of mice, rats and monkeys were reported to be improved by CGP 36742 in tests covering diverse manifestations of learning and memory (Mondadori et al., 1993; Fröstl et al., 1995). As shown in figure 3, CGP 36742 displayed potent activity in the kynurenate test. Because of the great selectivity of the drug for the GABA_A type of the GABA receptor (Olpe et al., 1993), the behavioral activities of CGP 36742 have been attributed to the blockade of GABA_A receptors (Mondadori et al., 1993; Fröstl et al., 1995; Bittiger et al., 1996). Although reports of the memory-disturbing effects of the GABA_A receptor agonist (--)-baclofen (Swartzwelder et al., 1987; Castellano et al., 1990; Carletti et al., 1993) would lend support to this view, the data obtained with CGP 52432 appear controversial. In fact, although the lack of effect of this compound in the kynurenate test is in line with the absence of effects on cognitive functions (Bittiger et al., 1992; Farlow et al., 1996), the findings that the affinity at GABAB sites, as measured in vitro by binding assays (Bittiger et al., 1996), was much higher for CGP 52432 (IC_{50} = 0.07 μM) than for CGP 36742 (IC_{50} = 32 μM), together with data showing that the latter antagonist displayed relatively low potency in various experimental paradigms (Olpe et al., 1993; Fröstl et al., 1995; Bittiger et al., 1996), raises the question of whether GABA_B receptors are...
the site of action of CGP 36742 as a cognition enhancer. Two additional observations made in the present work appear to weaken the idea of an involvement of GABA<sub>B</sub> receptors. As shown in table 2, (−)-baclofen significantly inhibited the NMDA-evoked [³H]NE release (which might account for the reported amnesic activity of the drug), but the effect of the GABA<sub>B</sub> agonist could not be prevented by CGP 36742 added at a concentration (1 μM) that completely inactivated the kynurenate antagonism of the NMDA-evoked [³H]NE release. Moreover, addition of (−)-baclofen at 10 μM was unable to compete with 1 μM CGP 36742 in the kynurenic test. On the other hand, GABA<sub>B</sub> receptors appear to be pharmacologically heterogeneous (Bonanno and Raiteri, 1993). Thus the possibility of CGP 36742 to block one of the subtypes of the GABA<sub>B</sub> receptor with unsuspectedly high affinity cannot presently be excluded.

The great potency of the drugs displaying activity in the kynurenic acid test is compatible with the idea that these drugs directly interfere with the binding of kynurenic acid to the NMDA receptors. The data showing that low micromolar d-cycloserine or aniracetam decreased the kynurenic acid (100 μM)-induced inhibition of [³H]MK-801 binding to rat forebrain membranes (Hamelin and Lehmann, 1995) strongly support the view of a nootropic-kynurenic acid interplay occurring at the NMDA receptor. Previous data with oxiracetam suggest that its interaction with kynurenic acid is competitive in nature (Pittaluga et al., 1995).

To shed light on the site of action of nootropics on the NMDA receptor complex we compared d- and l-serine in the kynurenic acid test and found that d-serine, but not the l-isomer, potently prevented the kynurenic acid antagonism of the NMDA-evoked [³H]NE release. Moreover, as illustrated in figure 2, the high potency of d-serine here observed (EC<sub>50</sub> = 0.1 μM) appears to depend on its addition to the slices together with kynurenic acid and before NMDA. In fact, the potency of d-serine was much lower (−200-fold) when the amino acid was added concomitantly with NMDA, 45 min after kynurenic acid. Thus it seems that submicromolar concentrations of d-serine can “prevent” the antagonism of 100 μM kynurenic acid, whereas much higher concentrations of the amino acid are necessary to “reverse” this antagonism. Although the underlying mechanisms are not clear, it may be worthwhile recalling that d-serine and glycine were found to oppositely affect agonist and antagonist binding at NMDA receptors (Monaghan et al., 1988). Based on these findings, pretreatment with d-serine may reduce binding of kynurenic acid and increase that of NMDA, thus facilitating prevention of kynurenic acid antagonism. d-Serine has long been known as an agonist at the glycine site of the NMDA receptor (Kleckner and Dingledine, 1988), where it potentiates NMDA efficacy somewhat more potently than oxiracetam or CR2249 (fig. 6) is compatible with this view. Although the present results strengthen the idea that the kynurenic test represents a useful assay in the study of cognition enhancers, we hope that the impressive potency of the drugs tested will attract the interest of molecular neurobiologists willing to reproduce the system in cells transected with NMDA receptor subunits with the aim of identifying the “nootropic sites” possibly present on NMDA receptors.

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References


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