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Effects of putative cognition enhancers on the NMDA receptor by [^{3}H]MK801 binding

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Abstract

Piracetam, aniracetam, and D-cycloserine were tested for their ability to reduce inhibition of [^{3}H]MK801 (dizocilpine) binding by 100 μM kynurenate. Piracetam (100 μM–1 mM) failed to reduce inhibition by kynurenate but stimulated [^{3}H]MK801 binding in the absence of kynurenate. In contrast, D-cycloserine (30 μM–1 mM) and aniracetam markedly reduced this inhibition by kynurenate. Thus, cognition enhancers might function via at least some subtypes of NMDA receptors.

Keywords: NMDA (*N*-methyl-D-aspartate); Kynurenate; Nootropic

Investigations on the neuronal mechanisms involved in central glutamatergic transmission have largely focused on the NMDA receptor due to the putative involvement of glutamate in cognitive processes (Vender et al., 1994). Antagonists of NMDA receptors disrupt performance, whereas some agonists at NMDA receptors were found to enhance performance in learning models. Thus, the hypothesis emerges that enhancement of the NMDA receptor tone increases cognitive performance.

Pittaluga et al. (1995) found that some cognition enhancers of the piracetam class relieved kynurenate inhibition of NMDA- and glutamate-evoked norepinephrine release from hippocampal slices. Kynurenate is a physiological metabolite localized in the mammalian brain and a relatively non-selective antagonist at the NMDA receptor complex as well as other receptors (Stone et al., 1987). With aging this metabolite increases in content in brain (Moroni et al., 1988) and causes an age-related decrease in glutamate-stimulated norepinephrine release (Pittaluga et al., 1993). It is a novel notion that some nootropics may act via the NMDA receptor complex. The present investigation was carried out to test these same interactions with [^{3}H]MK801 (dizocilpine) binding in neuronal membranes, a simpler system than intact noradrenergic terminal in slices.

Rat forebrains were homogenized in 50 vols. of Hepes (20 mM, pH = 7.7)/EDTA (1 mM), using a polytron (Mixer Homogenizer, OMNI International, Waterbury, CT, USA). Following centrifugation (18 000 $\times g$ for 20 min), the pellet was resuspended in 50 vols. of Hepes/EDTA. This step was repeated 5 more times and the final pellet was frozen at −20°C overnight. The following day, the pellet was thawed, resuspended in 50 vols. of Hepes (20 mM, pH = 7.7), and centrifuged (same parameters). This operation was repeated 2 more times and the final suspension was frozen at −70°C for at least 5 days prior to the binding assay. On the day of the assay, the membrane suspension was thawed, centrifuged (same parameters), then resuspended in 250 vols. of Hepes.

[^{3}H]MK801 (22 Ci/mmol; Dupont Medical Products, Wilmington, NJ, USA) binding assays (final volume of 2 ml containing 0.15 mg of membrane proteins and 5 nM [^{3}H]MK801) were terminated following 2 h incubation at room temperature by filtration (rinsed 2 × 5 ml Hepes, 20 mM, pH 7.7) over polyethyleneimine pre-treated (0.05%) Whatman GF/B filters (non-specific binding, representing about 15% of the

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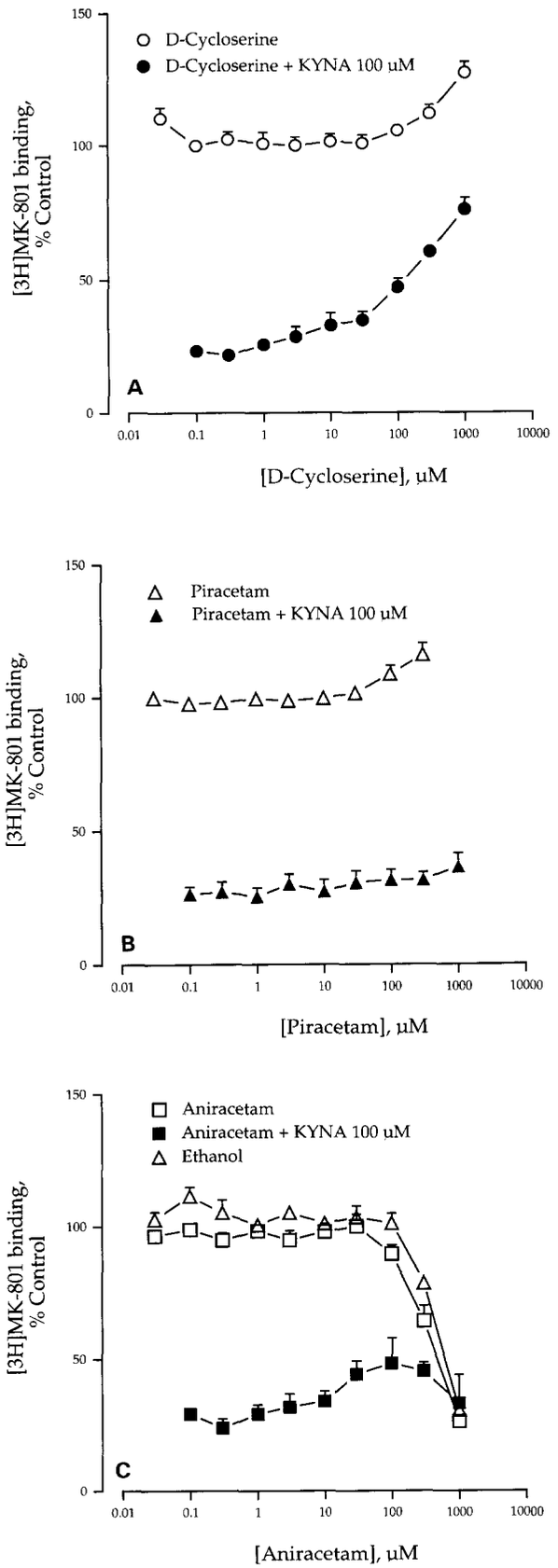

total binding, was determined with ketamine 3.65 mM). All compounds were dissolved in Hepes except aniracetam (1 mM), which was dissolved in pure ethanol. Membrane-bound radioactivity was determined by standard liquid scintillation counting techniques.

D-Cycloserine alone (30 μM–1 mM) increased [^{3}H]MK801 binding (Fig. 1A), maximally to 28% at 1 mM. D-Cycloserine (10 μM–1 mM) also reduced kynurenate-induced inhibition with a much more robust and potent effect. D-Cycloserine (1 mM) tripled the amount of specific binding in the presence of 100 μM kynurenate. Piracetam alone (100 μM–1 mM) enhanced [^{3}H]MK801 binding (Fig. 1B), to 16% of control at 1 mM. Aniracetam alone had no effects on [^{3}H]MK801 binding (Fig. 1C) but maximally stimulated binding (by 88% at 100 μM) in the presence of 100 μM kynurenate. However, the effect of aniracetam in the presence of kynurenate could not be accurately determined at concentrations above 100 μM due to the vehicle (Fig. 1C).

All of the putative cognition enhancers examined here stimulated [^{3}H]MK801 binding, suggesting that this may be a common mechanism of action for their psychopharmacological effects. First and foremost, these results support the findings of Pittaluga et al. (1995), who noted comparable effects on NMDA-stimulated norepinephrine release in the presence of kynurenate. Our findings concerning D-cycloserine and aniracetam agree qualitatively with their findings, but were less robust. This may be explained because noradrenergic terminals most likely contained a different population of NMDA receptor complexes. Moreover, their study involved hippocampal slices rather than isolated membranes, the brain slice being a more complex system, and the noradrenergic terminal possibly containing additional receptors sensitive to kynurenate and/or piracetam analogs (e.g., Chapman et al., 1993). However, in the context of Alzheimer's disease and potentially other learning disorders, these results have important implications for the design and use of more effective cognition enhancers.

Fig. 1. Effects of putative cognitive enhancers on specific [^{3}H]MK801 binding in the absence or presence of kynurenate: A: Effects of D-cycloserine (DCS) on specific [^{3}H]MK801 binding in the absence or presence of kynurenate. B: Effects of piracetam on specific [^{3}H]MK801 binding in the absence or presence of kynurenate. C: Effects of aniracetam and ethanol on specific [^{3}H]MK801 binding in the absence of kynurenate. Effects of aniracetam on specific [^{3}H]MK801 binding in the presence of kynurenate. Each point represents the mean with S.E.M. of quadruplicate determinations.

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