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α_2 Adrenergic Agonists Prevent MK-801 Neurotoxicity

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Antagonists of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor are of considerable interest for various neurotherapeutic purposes, including preventing neuronal degeneration in stroke and CNS trauma, suppressing neuropathic pain and preventing the development of tolerance to opiate analgesics. Unfortunately, NMDA antagonists can cause potentially serious side effects, including acute neurodegenerative changes in corticolimbic regions of the adult rat brain and psychotic reactions in adult humans. We have been investigating the mechanisms underlying the neuropathological changes in rat brain and exploring methods of suppressing or preventing such changes. Here we report that α_2 adrenergic agonists can prevent NMDA antagonist neurotoxicity. Therefore, administering α_2 adrenergic agonists together with NMDA antagonists may be a valuable strategy for preventing adverse side effects of NMDA antagonists and making these agents safer for various neurotherapeutic purposes. ***[Neuropsychopharmacology 12:347–349, 1995]***

KEY WORDS: *Clonidine; α_2 adrenergic receptor; Neurotoxicity; MK-801; Phencyclidine; NMDA antagonists*

Accumulating evidence implicates the NMDA subtype of glutamate receptor in many important CNS functions, and hyperactivation of NMDA receptors by endogenous glutamate is thought to play a role in various disorders of the CNS. Thus, NMDA receptor antagonists are currently under development as drugs for preventing neuronal degeneration in stroke and CNS trauma (Choi 1988; Olney 1989), and recent evidence suggests that these agents may also be useful for suppressing neuropathic pain (Davar et al. 1991; Kristensen et al. 1992; Yamamoto and Yaksh 1992) and preventing the development of tolerance to opiate analgesics (Marek et al. 1991; Trujillo and Akil 1991). A practical obstacle to the use of NMDA antagonists as neurotherapeutic drugs is the fact that they cause potentially serious side effects, including acute neurodegenerative changes in corticolimbic regions of the adult rat brain (Fix et al. 1993; Olney et al. 1989, 1991) and psychotic reactions in adult humans (Grotta 1994; Kristensen et al. 1992). We have been investigating mechanisms underlying the neuropathological changes induced in rat brain by the powerful NMDA antagonist, MK-801, and exploring methods of suppressing or preventing such changes (Farber et al. 1993, 1995; Olney et al. 1991; Olney and Farber 1994; Price et al. 1994). Here we report the new finding that α_2 adrenergic agonists can prevent MK-801 neurotoxicity.

METHODS

Adult female rats received MK-801 (0.5 mg/kg) subcutaneously (SC), together with one of several test agents (clonidine, xylazine, guanabenz, p-iodoclonidine) intraperitoneally (IP) at various doses. Control animals received MK-801 (0.5 mg/kg SC) and either

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dimenthyl sulfoxide (DMSO) or saline, the vehicles used to dissolve the test agents. For each test agent, at least 17 rats were used and at least four doses tested. The dose of MK-801 employed has previously been shown to induce in all treated rats a fully developed neurotoxic reaction consisting of acute vacuole formation in the majority of pyramidal neurons in layers III and IV of the posterior cingulate and retrosplenial (PC/RS) cortices. The animals were sacrificed 4 hours after treatment for histopathological evaluation by previously described methods (Olney et al. 1991), and the number of vacuolated PC/RS neurons were counted on each side of the brain at a rostrocaudal level immediately posterior to where the corpus callosum ceases decussating across the midline (approximately 5.6 mm caudal to Bregma; Paxinos and Watson 1986). In previous work (Farber et al. 1993) we have found that the toxic reaction approaches maximal severity at this level and does not vary much in severity from one animal to another. Percent reduction in neurotoxicity was calculated by dividing the mean number of vacuolated neurons in a given experimental group by the mean number in the MK-801 controls. The result was subtracted from 1 and multiplied by 100. Linear regression analysis was used to determine an ED_{50} (dose of a given compound that reduced the mean number of vacuolated neurons to 50% of the control mean) with the 25th and 75th percentiles defining the confidence limits.

In a second set of experiments designed to corroborate that the α_2 receptor was the site of action for the neuroprotective drugs, adult female rats received MK-801 (0.5 mg/kg SC) plus a combination of various doses of the α_2 agonist xylazine (IP) and a fixed dose of the α_2 antagonist yohimbine (1 mg/kg IP). The brains were processed and the severity of damage assessed as in the first set of experiments. Results were analyzed by analysis of covariance (ANCOVA), where the dose of xylazine was the covariate and the presence of yohimbine served as the classification (treatment) variable. The homogeneity of slopes assumption was tested by determining whether there was a significant interaction between the covariate and the treatment variable.

RESULTS

All control animals developed a neurotoxic reaction to MK-801 (mean number of vacuolated neurons per section = 223 ± 10.5 SEM; n = 26). Of the agents tested, clonidine was the most potent at preventing the neurotoxic reaction. From 0.01 to 1.0 mg/kg, it dose-dependently suppressed MK-801-induced neurotoxicity (ED_{50} = 0.044 mg/kg; Table 1). Higher doses (5–50 mg/kg) tended to be less effective providing only 50% to 75% protection (data not shown). Xylazine (1–5 mg/kg) was the least potent in preventing the reaction

Table 1. Efficacy of α_2 Adrenergic Agonists in Blocking MK-801 Neurotoxicity

Test Compound	ED_{50} (mg/kg IP)	Confidence Limits (25th and 75th percentiles)
Clonidine	0.044	(0.0088–0.22)
Guanabenz	0.21	(0.13–0.33)
p-Iodoclonidine	1.3	(0.29–5.8)
Xylazine	2.4	(1.4–4.2)

(ED_{50} = 2.4 mg/kg; Table 1), but it did not appear to lose efficacy at higher doses (10–100 mg/kg; data not shown). Guanabenz (ED_{50} = 0.21 mg/kg) and p-iodoclonidine (ED_{50} = 1.3 mg/kg) conferred protection with a potency intermediate between the other two agents (Table 1). Doses higher than the ED_{100} were not tested for the latter two agents.

When a fixed dose of the α_2 antagonist, yohimbine (1 mg/kg IP), was administered together with various doses of xylazine to MK-801-treated rats, it significantly interfered with xylazine's protection [$F(1,42)$ = 9.44, p = .004] and shifted the blocking curve to the right (Figure 1). This effect is consistent with a competitive interaction between xylazine and yohimbine at an α_2 receptor site. A similar shifting of the curve to the right was seen in preliminary experiments with a small number of animals treated with yohimbine (1 mg/kg IP) and clonidine (n = 4) or in

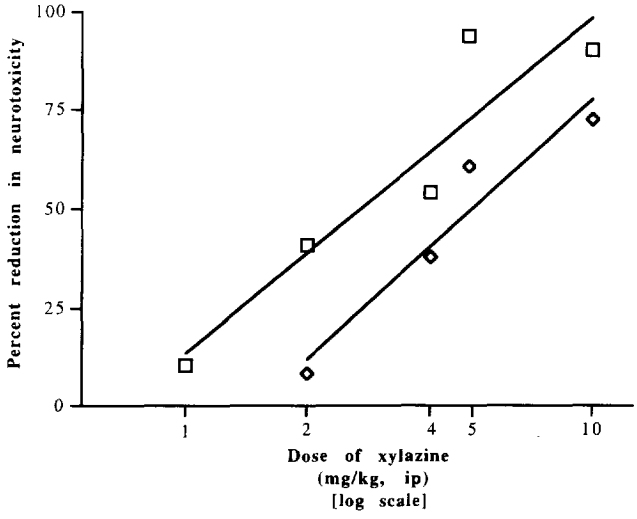

Figure 1. Inhibition by yohimbine of xylazine's protection against MK-801 neurotoxicity. Data points represent the percent reduction in neurotoxicity (see Methods for formula). Xylazine, an α_2 agonist, dose-dependently prevented MK-801's neurotoxic effect. Yohimbine, an α_2 antagonist, significantly interfered with xylazine's protection [$F(1,42)$ = 9.44, p = .004] as is indicated by shifting of the dose-response curve to the right. *Squares:* MK-801 (0.5 mg/kg) plus xylazine; *diamonds*: MK-801 (0.5 mg/kg) plus xylazine plus yohimbine (1 mg/kg).

animals treated with the α_2 antagonist, rauwolscine (5 mg/kg IP), and clonidine ($n = 4$).

DISCUSSION

Our prior findings (Olney et al. 1991; Price et al. 1994) indicate that NMDA antagonist neurotoxicity is mediated by a disinhibition principle in which blockade of NMDA receptors that normally drive GABAergic neurons abolishes the tonic inhibitory mechanism by which these GABAergic neurons regulate the release of excitatory transmitters (both acetylcholine and glutamate) at muscarinic and non-NMDA glutamate receptors on PC/RS neurons. Our present finding that α_2 receptor agonists block this neurotoxic reaction suggests that α_2 receptors are a critical component of the circuitry through which the neurotoxic reaction is mediated. Tentatively we postulate that norepinephrine functions like GABA in this circuit to inhibit the release of an excitatory transmitter that is instrumental in producing the neurotoxic reaction. Blockade of NMDA receptors by MK-801 abolishes the inhibitory action of both GABA and norepinephrine, thus allowing excessive release of excitatory transmitter as the proximal mechanism triggering the neurotoxic reaction. Administration of an α_2 agonist restores inhibitory control and prevents excessive release of excitatory transmitter, thereby preventing the neurotoxic reaction.

The order of potencies of the α_2 agonists in blocking MK-801 neurotoxicity does not correspond well with their order of binding affinities for the α_2 receptor, but this might be explained by factors such as the relative abilities of these compounds to penetrate the central nervous system. It is interesting that clonidine was maximally active at a low dose and appeared to lose activity at higher doses. This might be explained by clonidine having potent action at an α_2 receptor and less potent activity at a second receptor that functions to oppose α_2 receptor activity. For example, clonidine binds to α_1 receptors, but with an affinity weaker than it displays for α_2 receptors (Doxey et al., 1985).

A potentially important implication of our findings is that α_2 agonists can possibly be used to prevent the neurotoxic side effects of NMDA antagonists, thereby making NMDA antagonists safer for a number of neurotherapeutic purposes.

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