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RAPID COMMUNICATION

The NMDA Receptor Complex Modulates Clonidine-Induced Increases in Growth Hormone Levels in Rats

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MAZZOLA-POMIETTO, P., C. S. AULAKH AND D. L. MURPHY. *The NMDA receptor complex modulates clonidine-induced increases in growth hormone levels in rats.* PHARMACOL BIOCHEM BEHAV **50**(2) 305-307, 1995.—Intraperitoneal administration of clonidine (50 μg/kg) produced increases in growth hormone levels in male Wistar rats. Pretreatment with NMDA receptor antagonists including (±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP/NMDA site), ifenprodril (polyamine site), and dizocilpine maleate (MK-801) or phencyclidine (PCP) (channel blockers) did not have any significant effect on clonidine-induced increases in growth hormone levels. In contrast, pretreatment with 5,7-dichlorokynurenic acid and 6,7-dinitroquinoxaline-2,3-dione (DNQX) (NMDA receptor-associated glycine site antagonists) significantly attenuated clonidine-induced increases in growth hormone levels. Attenuation of clonidine's effect on growth hormone levels by NMDA receptor-associated glycine site antagonists appears most likely due to an interaction between their effects on the NMDA receptor complex with growth hormone releasing factor.

5,7-Dichlorokynurenic acid DNQX Dizocilpine Glycine Growth hormone releasing factor

GROWTH hormone (GH) secretion in the rat is under the control of both growth hormone releasing factor (GHRF) and somatostatin. GHRF stimulates GH release, whereas somatostatin inhibits it (12). The release of GHRF and somatostatin is, in turn, controlled by a complex interaction of multiple central nervous system neurotransmitters and several neuropeptides (18). The central noradrenergic system has been shown to play a dominant stimulatory role on GH secretion. Administration of clonidine (α_2-adrenergic agonist) stimulates GH secretion (13), whereas inhibition of norepinephrine synthesis or blockade of α_2-adrenergic receptors lowers plasma GH levels and eliminates the spontaneous episodic growth hormone surges in male rats (16). Besides the noradrenergic system, there is evidence that *N*-methyl-D-aspartic acid (NMDA) and nonNMDA excitatory amino acid receptor subtypes also modulate GH secretion (17). Single doses of *N*-methyl-D,L-aspartate (NMA) increase plasma growth hormone in monkeys (9) and dose dependently increases plasma growth levels in sheep (8). Blockade of NMDA receptors by the noncompetitive NMDA antagonist dizocilpine (MK-801) has been reported to induce a long lasting reduction of growth rate, pituitary content of GH, and basal and GHRF-stimulated GH release in immature female rats (20).

The purpose of the present study was to investigate whether clonidine-induced increases in GH levels might be modulated by NMDA receptors. Therefore, we studied the effects of various NMDA receptor antagonists on clonidine-induced increases in plasma GH levels in rats.

MATERIALS AND METHODS

Male Wistar rats obtained from Charles River (Kingston, NY) and weighing approximately 250 g were used. The animals were housed in a temperature-controlled (22 ± 1°C)

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room with a 12-h light dark cycle (lights on at 0600). The animals had free access to Purina rat chow (Ziegler Co., Gardners, PA) and water at all times. Separate groups of animals were used for each NMDA antagonist studied.

Vehicle (saline or dimethyl sulfoxide) or each antagonist was injected intraperitoneally (IP) 30 min before administering saline or clonidine (50 μg/kg). This dose of clonidine was selected based on our previous work (2). Animals were sacrificed 30 min after injection of saline or clonidine between 1100 and 1130. Clonidine was injected IP on the side opposite the antagonist injection.

The rats were sacrificed by decapitation, and trunk blood was collected in centrifuge tubes containing 0.5 ml of EDTA. After centrifugation, plasma samples were collected and stored at −70°C. The plasma concentrations of GH were measured by radioimmunoassay as described elsewhere (7).

Drugs

The drugs, clonidine hydrochloride, (±)-3-(2-carboxypiperazin-4-yl)-propyl-1 phosphonic acid ((±)CPP), 5,7-dichlorokynurenic acid (5,7-DCKA), DNQX, ifenprodril tartrate, dizocilpine [(+)MK-801] maleate, and phencyclidine (PCP) hydrochloride (Research Biochemicals, Inc., Natick, MA) were used in the study. DNQX, 5,7-DCKA, and PCP were dissolved in dimethyl sulfoxide (DMSO). All other drugs were dissolved in 0.9% saline. The volume injected was 0.1 ml/100 g of body weight. All drug doses given in the text refer to the salt. The selection of the doses for each antagonist was based on previously published literature (14,19).

Data Analysis

For statistical analysis, various NMDA antagonists were grouped according to their site of action. The data were analyzed using one way analysis of variance accompanied by contrasts specified a priori comparing each antagonist group plus clonidine vs. saline plus clonidine. All data are presented as means ± SEM.

RESULTS

The schematic representation of the NMDA receptor complex [modified from Barnes and Henley, 1992 (3)] along with the site of action of various NMDA receptor antagonists used in the present study is illustrated in Fig. 1. Clonidine in these experiments increased plasma GH levels from a baseline of 312 ± 110 ng/ml to 728 ± 171 ng/ml. Pretreatment with either a 0.1 mg/kg dose of dizocilpine (538 ± 125 ng/ml) or 1 mg/kg dose of PCP (642 ± 90 ng/ml) yielded modest but nonsignificant reductions [$F(3, 22) = 1.31$, $p > 0.05$] of these clonidine-induced increases in GH levels. Similarly, pretreatment with 1 mg/kg doses of either CPP (598 ± 120 ng/ml) or ifenprodril (505 ± 125 ng/ml) also did not have significant effects [$F(3, 22) = 1.39$, $p > 0.05$]. In contrast, pretreatment with a 1 mg/kg dose of either 5,7-DCKA or DNQX significantly attenuated clonidine-induced increases in GH levels [$F(3, 22) = 3.27$, $p < 0.05$] (Fig. 2).

DISCUSSION

The demonstration of enhanced secretion of GH in rats following clonidine administration in the present study is consistent with several earlier reports (2,12). In a recent report from this laboratory, we have demonstrated that clonidine stimulates GH secretion by activation of α_2-adrenergic heteroreceptors present on 5-HT nerve terminals which, in turn, enhance 5-HT activity via stimulation of postsynaptic 5-HT_{2C} receptors [formerly designated 5-HT_{1C} receptors (10)] to promote GHRF release and increase plasma GH levels (2).

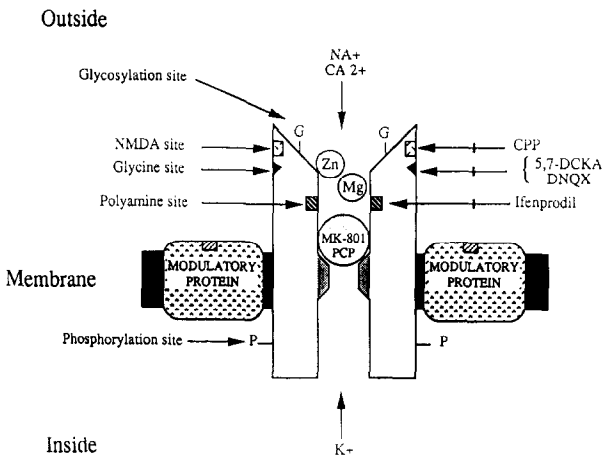

FIG. 1. The schematic representation of NMDA receptor complex showing the site of action of various NMDA receptor antagonists used in the present study [modified from Barnes and Henley, 1992 (3)].

In the present study, pretreatment with CPP, MK-801, PCP, or ifenprodril did not attenuate clonidine's effect on GH levels, although slight but statistically nonsignificant reductions were observed. CPP is a competitive antagonist that acts at the NMDA site on the NMDA receptor complex (3). Dizocilpine and PCP are noncompetitive NMDA receptor antagonists, which act as channel blockers, but ifenprodril is a NMDA receptor antagonist at the polyamine site [Barnes and Henley, 1992 (3)]. Failure of dizocilpine pretreatment to attenuate clonidine's effect on GH levels was somewhat unanticipated in view of two previous reports in which chronic treatment (0.2 mg/kg, b.i.d. × 10) with dizocilpine was shown to significantly reduce GHRF content in the median eminence, GHRF mRNA levels in the hypothalamus and K^+-induced GH secretion from anterior pituitary fragments of immature male rats (6) and to reduce pituitary GH content,

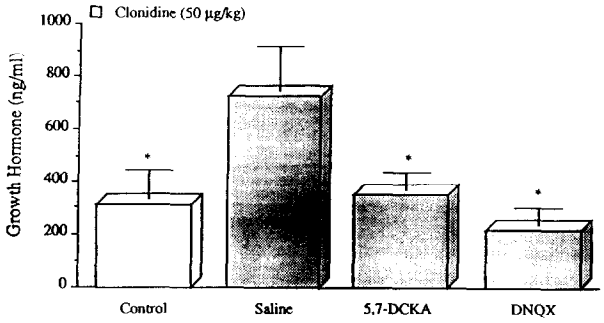

FIG. 2. Effects of pretreatment with 5,7-dichlorokinurenic acid (5,7-DCKA, 1 mg/kg), DNQX (1 mg/kg) or saline on clonidine-induced increases in growth hormone (ng/ml) level. Values are expressed as means ± SEM from 5–9 animals. *$p < 0.05$, significantly different from saline plus clonidine-treated animals.

basal and GHRH-stimulated GH release in vitro, and plasma somatomedin C levels in immature female rats (20). However, acute effects of dizocilpine were apparently not investigated in those studies. It is possible that higher doses of CPP, PCP, dizocilpine, and ifenprodril may have attenuated clonidine-induced increases in GH levels in the present study. However, it is of note that the doses of dizocilpine, PCP, and CPP used in the present study have previously been shown to counteract the long lasting 5-HT_{1A} receptor-induced attenuation of postsynaptic responses (19) and decrease NMDA receptor mRNA levels (14). In contrast to dizocilpine and other NMDA antagonists, pretreatment with 5,7-DCKA and DNQX significantly attenuated clonidine-induced increases in GH levels in the present study. 5,7-DCKA has been reported to be a potent antagonist at the NMDA receptor-associated strychnine-insensitive glycine binding site (4). Similarly, DNQX has also been shown to antagonize NMDA receptor-mediated responses by competing at this glycine modulatory site (5,11,15). Therefore, attenuation of clonidine's effect on GH levels by 5,7-DCKA and DNQX appears most likely due to an interaction at the glycine site of the NMDA receptor complex.

The mechanism by which NMDA antagonists acting at the glycine site attenuate clonidine-induced increases in GH levels is not clear. As mentioned earlier in the discussion, clonidine stimulates GH secretion by activation of α_2-adrenergic heteroreceptors present on 5-HT nerve terminals that, in turn, enhance 5-HT activity via stimulation of 5-HT_{2C} receptors to promote GHRF and increase plasma GH levels. We are not aware of any report in the literature demonstrating an interaction of NMDA receptor antagonists at α_2-adrenergic receptors or 5-HT_{2C} receptors. On the other hand, there are a few reports suggesting that the site of action of excitatory amino acids on GH secretion is in the hypothalamus, and not the pituitary gland, and involves an interaction with the specific GH regulatory hormones, i.e., GHRF and/or somatostatin (1,6). Destruction of the arcuate nucleus, where the cell bodies of GHRF-producing neurons are located, completely abolished the GH releasing effect of NMA and, furthermore, NMA-induced increases in GH levels in newborn rats were counteracted by administration of antibodies to GHRF (1). In another study, chronic treatment with a NMDA receptor antagonist, dizocilpine, produced significant decreases in hypothalamic GHRF mRNA levels (6). Thus, the most likely explanation for the attenuation of clonidine's effect on GH levels by 5,7-DCKA and DNQX in the present study may be due to an interaction between their effects on the NMDA receptor complex with GHRF and/or somatostatin in the hypothalamus. However, an interaction at α_2-adrenergic heteroreceptors or 5-HT_{2C} receptors can not be ruled out. Only further experimentation will clarify this issue.

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