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Magnesium Selectively Inhibits N-Methyl-Aspartic Acid-Induced Hypermotility After Intra-Accumbens Injection

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DONAZANTI, B. A. AND N. J. URETSKY. *Magnesium selectively inhibits N-methyl-aspartic acid-induced hypermotility after intra-accumbens injection.* PHARMACOL BIOCHEM BEHAV 20(2) 243–246, 1984.—The excitatory amino acids, N-methyl-aspartic acid, kainic acid and quisqualic acid have been shown to produce a marked increase in locomotor activity after bilateral injection into the rat nucleus accumbens. The intra-accumbens injection of magnesium inhibited the hypermotility response produced by N-methyl-aspartic acid in a dose-dependent manner. However, magnesium had no significant inhibitory effect on the increase in motility produced by either kainic acid or quisqualic acid. In contrast to magnesium, calcium produced a weak inhibitory action on N-methyl-aspartic acid-induced hypermotility. These data suggest that in the nucleus accumbens, at least two receptor types (N-methyl-aspartic acid/magnesium-sensitive and non-N-methyl-aspartic acid/magnesium-insensitive receptors) are present which can mediate the stimulation of locomotor activity produced by excitatory amino acids.

Excitatory amino acids Nucleus accumbens Hypermotility Kainic acid Quisqualic acid
N-methyl-aspartic acid

THE nucleus accumbens is a forebrain structure which has been postulated to be involved in both psychotic disorders [3,14] and the control of motor function [16,17]. This region is innervated by an ascending dopaminergic neuronal pathway originating in the ventral tegmental area [10]. The bilateral injection of dopamine directly into the nucleus accumbens produces an increase in motility which is blocked by dopamine receptor antagonists [17]. The nucleus accumbens is also believed to be innervated by glutamatergic neuronal pathways derived from both allocortex and frontal neocortex and may also contain glutamatergic/aspartatergic interneurons [10]. Recent studies have shown that the bilateral injection of glutamate analogs in the nucleus accumbens produces, like dopamine, a hypermotility response which can be blocked by dopamine receptor antagonists [1,7]. This suggests that excitatory amino acid-induced hypermotility is mediated through the release of dopamine and subsequent stimulation of dopamine receptors within the nucleus accumbens.

N-Methyl-aspartic acid (NMA), kainic acid (KA) and quisqualic acid (QA) have been previously shown to cause the excitation of single neurons in the vertebrate central nervous system [15,21]. The electrophysiological responses to these compounds appear to be mediated by different mechanisms, since they are preferentially inhibited by different antagonists [4, 15, 21]. NMA, KA and QA can produce a marked stimulation of locomotor activity after their injection into the nucleus accumbens [7]. However, it is not clear whether these neuroexcitants act by the same or different mechanisms at this brain site. Numerous organic antagonists of excitatory amino acids, which are effective in microiontophoretic studies, have not been found to be useful in defining the effects of excitatory amino acids on motor function since these antagonists have been shown to be either ineffective in inhibiting the behavioral responses to excitatory amino acids [1,11] or to produce motor effects by themselves which are similar to those elicited by excitatory amino acids [6,19].

In isolated spinal cord preparations, magnesium (Mg) has been shown to selectively depress the excitatory responses of frog motoneurons to NMA while having little or no effect on responses to KA or QA [2, 8, 9]. Similar responses were also obtained *in vivo* in the cat spinal cord [5]. This observation provided the first evidence to suggest that more than one type of excitatory amino acid receptor may exist in the vertebrate central nervous system. In the present study, we report that the intra-accumbens administration of Mg inhibits the hypermotility response induced by NMA but had no effect on control locomotor activity or on the hypermotility responses induced by KA and QA.

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TABLE 1

EFFECTS OF $MgSO_4$ ON NMA- AND KA-INDUCED HYPERMOTILITY

Treatment	None	+ Mg 7.5 (nmoles)	+ Mg 15 (nmoles)
Control (0.5 μl)	180 ± 23 (6)	256 ± 51 (3)	154 ± 55 (3)
NMA (16.9 nmoles)	4172 ± 570 (4)*	2335 ± 502 (8)**	1240 ± 389 (5)**
KA (0.07 nmoles)	6369 ± 1197 (4)*	5361 ± 1409 (4)	7397 ± 1754 (4)

Rats were injected bilaterally into the nucleus accumbens with NMA or KA alone or in combination with Mg. Doses represent the amount injected on each side. Motility was recorded for 1 hour, starting 10 minutes after injection. Values represent the mean ± SEM for the number of observations shown in parentheses.

*$p<0.05$ with respect to control. **$p<0.05$ with respect to NMA or KA alone.

METHOD

Surgical Procedure

Male Sprague-Dawley rats (175–200 g) were anesthetized with chloral hydrate (420 mg/kg, intraperitoneally) and placed in a stereotaxic frame (David Knopf Inst., CA). Holes were then drilled on each side of the skull for injection into the nucleus accumbens (A 9.4; L ±2.4) [12]. The animals were then returned to their home cage. On the following day, the rats were reanesthetized with a halothane/oxygen mixture and returned to the stereotaxic frame for the injection of drugs into the nucleus accumbens. After exposing the skull, the needle (o.d.=0.46 mm) of a 5 μl Unimetrics syringe was inserted at a 10° angle (to avoid puncturing the ventricles) into the holes previously drilled in the skull to a depth of V −1.0 [12] and 0.5 μl volume of solution was injected bilaterally over a 1 minute period. The microsyringe was left in place for an additional minute to allow diffusion of the solution away from the needle. After the injections, the skin incision was closed with wound clips and covered with lidocaine ointment (5%). The rats recovered from anesthesia within 5 minutes after the removal of halothane. Each rat was injected only once.

Monitoring Locomotor Activity

After the intra-accumbens injections, the rats were placed in motor activity cages (Opto-Varimex-Minor, Columbus Inst., OH) and allowed 10 minutes to adapt to the environment. The cages contained 12×12 i.r. beams passing at a height of 5 cm from the bottom of the cage through a ventilated Plexiglas box measuring 42 cm square and 20 cm high. Locomotor activity was recorded as the number of times two consecutive beams, 3.5 cm apart, were interrupted per hour. The data were printed out by a digital counter.

The motor activity of control and drug-treated animals was always determined in parallel between 10 a.m. and 5 p.m. in an isolated environmental room maintained at a temperature of 22±1°C.

Drugs

The following compounds were purchased from Sigma Chemical Co. (St. Louis, MO): N-methyl-DL-Aspartic acid, kainic acid and quisqualic acid were dissolved in saline and adjusted to pH 7.0 with 1 N NaOH. $MgCl_2$, $MgSO_4$ and $CaCl_2$ were also dissolved in saline. Doses shown refer to the amount injected on each side of the nucleus accumbens. Control animals were injected with an equal volume (0.5 μl) of saline vehicle.

Statistics

Data were expressed as the mean and standard error of the mean (SEM). Significant differences were evaluated using the two-tailed Mann-Whitney U-test, with a level of $p<0.05$ being considered significant. Percent inhibitions were calculated as follows:

$$\left[\frac{(\text{Agonist} - \text{Control}) - ([\text{Antagonist} + \text{Agonist}] - \text{Antagonist})}{(\text{Agonist} - \text{Control})}\right] \times 100.$$

Histology

After each experiment, the rats were decapitated and their brains rapidly removed and fixed in a 10% formalin solution for 48 hours. Frozen sections (80 μ thick) were sliced using a Cryo-Cut Microtome (American Optical Corp., Buffalo, NY) to check the location of the injection needle.

Results

The intra-accumbens administration of NMA (16.9 nmoles), KA (0.07 nmoles) and QA (2.6 nmoles) produced an intense increase in locomotor activity when compared to saline-treated controls (Table 1; Figs. 1 and 2). The addition of $MgSO_4$ (7.5 and 15 nmoles) or $MgCl_2$ (15 nmoles) to the injection solutions did not produce a significant change in the locomotor activity of saline-treated rats (Table 1; Fig. 1) or in the hypermotility responses induced by KA (Table 1; Fig. 2) and QA (Fig. 2). In contrast, $MgSO_4$ (7.5 and 15 nmoles) produced a significant dose-dependent inhibition of NMA-induced hypermotility (48 and 75% inhibition, respectively; Table 1). The hypermotility response induced by NMA was also inhibited (90%) by $MgCl_2$ (15 nmoles), suggesting that the inhibitory effect was due to the positive Mg ion and not the associated anion (e.g., SO_4; Fig. 1).

In order to determine whether calcium (Ca), a divalent cation like Mg, possesses similar antagonistic activity, $CaCl_2$ (15 nmoles) was injected into the nucleus accumbens alone or in combination with NMA. Figure 1 shows that $CaCl_2$ produced a much weaker inhibitory effect (37%) than Mg on NMA-induced hypermotility. Ca, when added alone, did not change the response of saline-treated controls (Fig. 1).

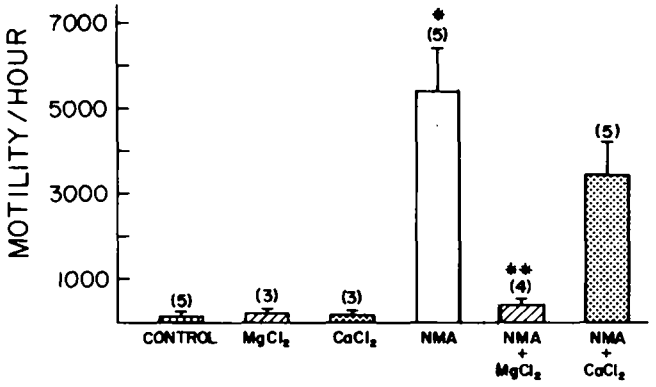

FIG. 1. Effect of $MgCl_2$ and $CaCl_2$ on NMA-induced hypermotility. Rats were injected into the nucleus accumbens on each side with $MgCl_2$ (15 nmoles/0.5 μl) or $CaCl_2$ (15 nmoles/0.5 μl) alone or in combination with NMA (16.9 nmoles/0.5 μl). Motility was recorded for 1 hour, starting at 10 minutes after injection. Each bar represents the mean and SEM for the number of observations indicated in the parentheses. $*p<0.05$ with respect to control. $**p<0.05$ with respect to NMA alone.

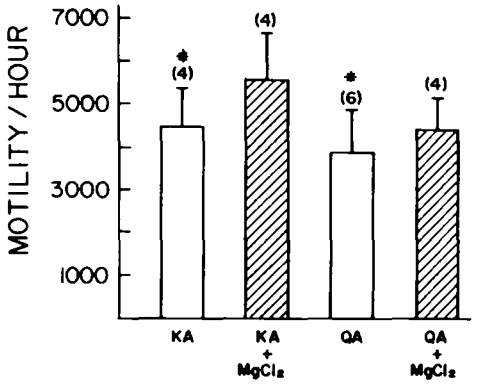

FIG. 2. Effect of $MgCl_2$ on KA- and QA-induced hypermotility. Rats were injected into the nucleus accumbens on each side with KA (0.07 nmoles/0.5 μl) or QA (2.6 nmoles/0.5 μl) alone or in combination with $MgCl_2$ (15 nmoles/0.5 μl). Motility was recorded for 1 hour, starting at 10 minutes after injection. Each bar represents the mean and SEM for the number of observations indicated in the parentheses. $*p<0.05$ with respect to control (140 ± 25 counts/hour; $N=5$).

DISCUSSION

In studies on the isolated frog or rat spinal cord, low concentrations of Mg (0.5–1 mM) were found to selectively inhibit motoneuron depolarization produced by NMA but not that produced by KA or QA [2, 8, 9]. Similar results were also obtained when Mg was applied by microiontophoresis onto cat spinal neurons *in vivo* [5]. These findings suggested that at least two receptor types, a NMA/Mg-sensitive receptor and a non-NMA/Mg-insensitive receptor, exist for excitatory amino acids in the vertebrate central nervous system.

In the present study, the intra-accumbens injection of Mg inhibited NMA-induced hypermotility but not that produced by either QA or KA. In addition, the intra-accumbens injection of Ca was much less effective than Mg in inhibiting the effects of NMA. These results are consistent with the spinal cord studies [2, 5, 8, 9] and suggest that there are at least two receptor types for excitatory amino acids in the nucleus accumbens, one of which is specifically sensitive to Mg.

The mechanism by which Mg produces its inhibitory action is unknown. The effect of Mg on NMA-induced hypermotility does not appear to be caused by a direct chemical interaction between divalent cations and amino acids because: (1) the amount of interaction between Mg and excitatory amino acids is small at physiological pH [18], (2) the inhibitory effect of Mg on NMA-induced hypermotility is relatively selective since Ca produced a much weaker inhibition (Fig. 1), (3) the inhibitory effect of Mg was selective for NMA, since it did not inhibit the effects of the other amino acids, KA and QA (Fig. 2). Based on previous studies on isolated frog spinal neurons [4], Mg may act in a non-competitive manner to lower the affinity of the receptor for NMA or may act at the receptor-ionophore coupling process to reduce the effectiveness of the receptor-agonist interaction.

Recent behavioral data suggest that excitatory amino acids may produce their hypermotility response through the release of dopamine and subsequent stimulation of dopamine receptors within the nucleus accumbens [1,7]. These findings are supported by *in vitro* studies which show L-glutamic acid, an endogenous neuroexcitant, to release dopamine from accumbal slices [13,20]. This effect of glutamic acid was inhibited by Mg [13], suggesting a possible interaction at NMA receptors within the nucleus accumbens. In the present studies, Mg selectively inhibited the response produced by NMA but not that produced by QA and KA. Thus, in the nucleus accumbens, it appears that at least two excitatory amino acid receptor types (NMA/Mg-sensitive and non-NMA/Mg-insensitive receptors) are present which are capable of regulating dopaminergic neurotransmission. The finding that Mg alone does not affect spontaneous locomotor activity suggests that NMA receptor stimulation in the nucleus accumbens does not function tonically to control dopamine-induced changes in motor function.

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