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GABA and Glutamate Interact in the Substantia Innominata/Lateral Preoptic Area to Modulate Locomotor Activity

PAUL E. SHREVE* AND NORMAN J. URETSKY†[1]

**University of Nebraska Medical Center, Department of Pharmacology*
600 South 42nd Street, Omaha, NE 68198-6260
†The Ohio State University, College of Pharmacy
500 W. 12th Avenue, Columbus, OH 43210

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SHREVE, P E AND N J URETSKY *GABA and glutamate interact in the substantia innominata/lateral preoptic area to modulate locomotor activity* PHARMACOL BIOCHEM BEHAV **38**(2) 385–388, 1991 —Previous studies have shown that excitatory amino acid agonists or GABAergic antagonists injected into the substantia innominata/lateral preoptic area (SI/LPO) can produce the stimulation of coordinated locomotor activity The purpose of the present study was to determine whether GABAergic and glutamatergic mechanisms in the SI/LPO interact to regulate locomotor activity The stimulation of locomotor activity produced by the bilateral injection into the SI/LPO of 0 5 μg of AMPA, a potent quisqualic acid receptor agonist, was antagonized by the coinjection of muscimol (25 ng) Similarly, the stimulation of locomotor activity produced by picrotoxin, an inhibitor of the effects of GABA, was antagonized by the coinjection of DNQX, which has been shown to inhibit the behavioral effects of both kainic acid and quisqualic acid, or a high dose of GAMS (25 μg), which has been shown to inhibit the behavioral effects of both AMPA and N-methyl-D-aspartic acid In contrast, a lower dose of GAMS (5 μg), which selectively inhibited the locomotor stimulation produced by AMPA, or D-α-aminoadipic acid, at a dose (10 μg) which selectively inhibited the locomotor stimulation produced by N-methyl-D-aspartic acid, did not inhibit the effects of picrotoxin However, the combination of both GAMS (5 μg) and D-α-aminoadipic acid (10 μg) produced a marked inhibition of the response to picrotoxin These results suggest that the hypermotility response elicited by picrotoxin can only be antagonized when more than one subtype of excitatory amino acid receptor is antagonized and support the concept that excitatory amino acid receptors and GABAergic receptors in the SI/LPO interact to regulate locomotor activity

Glutamate GABA Substantia innominata Preoptic area Locomotor activity NMDA

THE substantia innominata/lateral preoptic area (SI/LPO) is a subpallidal region which appears to be strategically located between the nucleus accumbens and motor areas of the brain (15,17). The anatomical organization of the SI/LPO is such that it receives a large GABAergic projection from the nucleus accumbens (5, 8, 9, 12), a forebrain region which is thought to be involved in the integration of motivational and motor behavior (7,10). Recent evidence suggests that this GABAergic projection from the nucleus accumbens to the SI/LPO is involved in the hypermotility produced by drugs which act in the nucleus accumbens (4, 8, 9, 12). Thus it has been shown that the injection of GABA or muscimol into the SI/LPO inhibited the locomotor activity produced by activation of dopamine (8, 16, 18), opioid (18), or excitatory amino acid receptors (13) in the nucleus accumbens. These observations suggest that the hypermotility responses produced by increases in dopaminergic, opioid, and glutamatergic neurotransmission in the nucleus accumbens may be mediated by a decrease in GABAergic activity in the SI/LPO Therefore, it is proposed that the injection of GABA or muscimol into SI/LPO would increase the activation of GABAergic receptors at this site and decrease hypermotility responses. In contrast to the effects of muscimol, the injection into the SI/LPO of picrotoxin, an inhibitor of the effects of endogenous GABA, has been shown to stimulate locomotor activity (8, 13, 17).

Glutamate, an excitatory neurotransmitter, is also found in the SI/LPO. Autoradiographic studies have shown that the SI/LPO contains excitatory amino acid binding sites that are thought to represent receptors for glutamate (3,11). Synaptosomes prepared from the SI/LPO have been shown to release endogenous glutamate in a calcium-dependent manner in response to depolarization and to possess high-affinity uptake sites for L-glutamate and D-aspartate (1) The location of the cells of origin of these glutamatergic nerve terminals may be in the amygdala, a region of the limbic system (2).

[1]Requests for reprints should be addressed to Norman J Uretsky

We have recently studied the effects of excitatory amino acids on locomotor activity. We found that the direct injections of the excitatory amino acids, α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA), kainic acid, and N-methyl-D-aspartic acid, into the SI/LPO produce dose-dependent increases in locomotor activity, which could be selectively antagonized by excitatory amino acid antagonists (14) These results are consistent with the concept that the stimulation of locomotor activity produced by neuronal mechanisms in the nucleus accumbens may be mediated by either a decrease in GABAergic and/or an increase in glutamatergic neurotransmission in the SI/LPO

In the present study, we examined the possibility that GABAergic and glutamatergic receptors in the SI/LPO may interact to regulate locomotor activity. We observed that the locomotor activity produced by the injection of AMPA into the SI/LPO was inhibited by the coadministration of muscimol. We also observed that the locomotor activity produced by injection of picrotoxin into the SI/LPO was inhibited by the coadministration of various excitatory amino acid antagonists Although the mechanism of this interaction between GABA and glutamate in the SI/LPO is presently not known, these results provide preliminary evidence to suggest that these two neurotransmitters may either directly or indirectly oppose each other in this region of the brain to regulate locomotor activity.

METHOD

Surgical Procedure

Male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN), weighing 150–190 g, were lightly anesthetized with a halothane/oxygen mixture and placed in a stereotaxic apparatus (David Kopf Inst., CA). A midline incision was made in the scalp and holes were drilled on each side of the skull to facilitate bilateral injections in the SI/LPO. The coordinates for the SI/LPO were 6.8 mm anterior to the intraaural line, 1.8 mm lateral to the sagittal suture, and 2.0 mm above the intraaural line (6). Drugs or vehicle were injected in a 0.5 μl volume at a rate of 0.5 μl/min. The needle was left in place for an additional 1 min to allow for diffusion of the solution. After the injection, the needle was removed, and the incision was sutured and swabbed with 5% (w/v) lidocaine ointment.

After the injections into the SI/LPO, the anesthesia was turned off and animals recovered from anesthesia within 5–10 min. The halothane/oxygen mixture allows the use of a stereotaxic instrument to guide the injection of drugs into the SI/LPO. It is unlikely that the behavioral effects of the test drugs were changed by the anesthetic mixture, since in previous studies, amphetamine injected into the nucleus accumbens and muscimol and picrotoxin injected into the SI/LPO produced the same behavioral effects in animals anesthetized with halothane/oxygen anesthesia as in conscious animals that were injected using chronic cannulae (4, 8, 16, 17).

Monitoring Locomotor Activity

After recovery, the animals were placed in motor activity cages (Opto Varimex-Minor, Columbus, Inst., OH) and allowed 10 min to adapt to the cages. The motor activity was monitored and recorded as previously described (13) The animals were observed for convulsions, rearing, or any other nonambulatory behavior during all recording sessions. All testing was done between 8 00 a m. and 4 00 p.m. in an isolated environmental room maintained at a temperature of 22 ± 1°C. Prior to the day of the experiment, the animals were housed, four to a cage, in an air-conditioned room kept at 20–21°C with an automatic light-dark cycle (light on 6:00 a.m –6:00 p.m).

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 μm thick) were sliced using a Cryo-Cut Microtome (American Optical Corp., Buffalo, NY) to check the location of the needle tips. When the tips of the needle were found to be outside the SI/LPO, the locomotor activity readings of the animals were not used for the study.

Drugs

D-α-Aminoadipic acid and muscimol were purchased from Sigma Chemical Co (St. Louis, MO). α-Amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) was purchased from Research Biochemicals Inc (Natick, MA). 6,7-Dinitro-quinoxaline-2,3-dione (DNQX) and γ-glutamylaminomethylsulfonate (GAMS) were obtained from Tocris Chemicals (Essex, England)

All drugs were dissolved in saline and adjusted to pH 7.4 with 1 N NaOH. The doses shown refer to the amount injected on each side of the SI/LPO. Control animals were injected with an equal volume (0.5 μl) of saline.

Statistics

Data were expressed as the mean and standard error of the mean (SEM). The effects of drugs and saline treatment were evaluated statistically using the nonparametric Kruskal-Wallis one-way analysis of variance followed by the one-tailed Mann-Whitney U-test, with a level of $p<0.05$ being considered significant

RESULTS

Effect of Muscimol on AMPA-Stimulated Locomotor Activity in the SI/LPO

The purpose of this experiment was to determine the effect of muscimol, a GABA-A receptor agonist, on the hypermotility produced by the injection of AMPA into the SI/LPO. The bilateral injection of AMPA (0.5 μg) into the SI/LPO elicited a marked stimulation of coordinated locomotor activity The injection of muscimol (25 ng) with AMPA produced a significant 82% inhibition of this AMPA-stimulated hypermotility while having no discernible effect on normal locomotor activity (Fig 1). This dose of muscimol did not produce a cataleptic state which is in agreement with a previous study (13).

Effect of DNQX, GAMS, or D-α-Aminodipic Acid on Picrotoxin-Stimulated Locomotor Activity in the SI/LPO

The purpose of this experiment was to determine the effect of various excitatory amino acid antagonists on the hypermotility produced by the injection of picrotoxin, an inhibitor of the effects of GABA, into the SI/LPO. Picrotoxin (0 5 μg) was bilaterally injected into the SI/LPO in the presence and absence of the excitatory amino acid antagonists, GAMS, DNQX, or D-α-aminoadipic acid. DNQX (1 μg), which has been shown previously to inhibit the hypermotility responses produced by AMPA and kainic acid (14), produced a significant 64% inhibition of picrotoxin-stimulated locomotor activity (Fig. 2B). GAMS at a dose of 5 μg, which has been found to selectively antagonize the hypermotility elicited by AMPA (unpublished observations), did not produce a significant inhibition of picrotoxin-induced locomotor

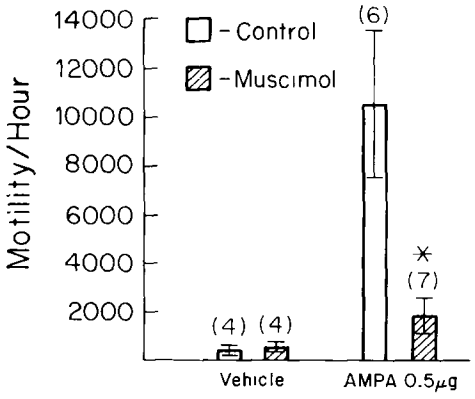

FIG 1 Effect of muscimol on AMPA-stimulated locomotor activity after bilateral injection into the SI/LPO A solution of AMPA (0 5 μg) or vehicle with or without muscimol (25 ng) was injected in a 0 5 μl volume The animals were placed in motor activity cages and locomotor activity recorded for 1 h Control refers to rats injected with either vehicle or AMPA given alone Each point represents the mean ± SEM for the number of observations in parentheses *$p<0$ 05 with respect to control

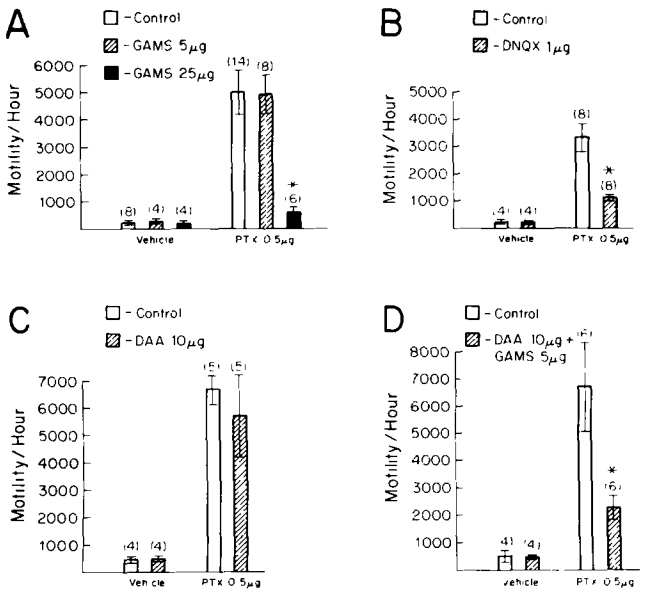

FIG 2 Effect of excitatory amino acid antagonists on picrotoxin (PTX)-stimulated locomotor activity after bilateral injection into the SI/LPO A solution of PTX (0 5 μg) or vehicle with and without (A) GAMS (5 or 25 μg), (B) DNQX (1 μg), (C) D-α-aminoadipic acid (DAA, 10 μg), or (D) GAMS (5 μg) and DAA (10 μg) The solutions were injected in a 0 5 μl volume and the animals were placed in motor activity cages and locomotor activity recorded for 1 h Control refers to rats injected with either PTX or vehicle given alone Each value represents the mean ± SEM for the number of observations in parentheses Kruskal-Wallis analysis of variance, H = 13 25, $p<0$ 05 for GAMS (5 or 25 μg) *$p<0$ 05 with respect to control

activity (Fig. 2A). A further increase in the dose of GAMS (25 μg) produced a significant 86% inhibition of the hypermotility produced by picrotoxin. D-α-Aminoadipic acid (10 μg), which has been shown previously to selectively inhibit the hypermotility response produced by the injection of N-methyl-D-aspartic acid into the SI/LPO (14), did not produce a significant effect on the locomotor activity elicited by picrotoxin (Fig. 2C). However, the combination of GAMS (5 μg) and D-α-aminoadipic acid (10 μg), at doses which were ineffective in inhibiting picrotoxin when administered alone, produced a significant 66% inhibition of picrotoxin induced hypermotility (Fig. 2D). In these experiments, the injection of these excitatory amino acid antagonists, alone, into the SI/LPO did not produce changes in locomotor activity as compared to vehicle-injected controls.

DISCUSSION

Previous studies have shown that the stimulation of excitatory amino acid (glutamatergic) receptors, as well as the antagonism of GABAergic-mediated responses in the SI/LPO, can produce a stimulation of coordinated locomotor activity. Thus the injection of the excitatory amino acids, AMPA, kainic acid, and N-methyl-D-aspartic acid, into the SI/LPO produced marked stimulations of coordinated locomotor activity which were selectively antagonized by excitatory amino acid antagonists (14). Additionally, it has been shown that the injection of picrotoxin, an inhibitor of the effects of GABA, into the SI/LPO stimulated locomotor activity (8, 13, 16, 18). Indeed, it has been suggested that a decrease in GABAergic transmission in the SI/LPO, is involved in the stimulation of locomotor activity initiated by stimulation of opioid, dopamine, and glutamate receptors in the nucleus accumbens (8, 13, 16, 18).

Therefore, it appears that GABAergic and glutamatergic neural components in the SI/LPO may interact in some manner to regulate locomotor activity This possibility was examined in the present study by first determining the effect of muscimol on the locomotor activity produced by the injection of AMPA, a quisqualic acid receptor agonist, into the SI/LPO. In the present study, the hypermotility response elicited by the stimulation of quisqualic acid receptors in the SI/LPO was significantly inhibited by the administration of muscimol, a GABA-A receptor agonist. These results initially suggested that the stimulation of excitatory amino acid receptors in the SI/LPO may produce locomotor activity by either directly or indirectly opposing the effects of GABA in this region

We also examined the effect of excitatory amino acid antagonists on the locomotor activity produced by the injection of picrotoxin, an inhibitor of the effects of GABA, into the SI/LPO. In the present study, DNQX, at a dose which selectively inhibited the hypermotility responses to kainic acid and AMPA (but not to N-methyl-D-aspartic acid) in the SI/LPO (14), significantly inhibited the stimulation of locomotor activity produced by picrotoxin. GAMS produced a significant inhibition of the hypermotility elicited by picrotoxin, but only at a high dose of 25 μg, which has been shown previously to antagonize the hypermotility effects of *both* AMPA and N-methyl-D-aspartic acid, but not to kainic acid (14) In contrast, a lower dose of GAMS (5 μg), which has been shown to selectively inhibit the hypermotility response to AMPA, did not inhibit the locomotor activity elicited by picrotoxin Similarly, D-α-aminoadipic acid (10 μg), which has been shown to selectively inhibit the hypermotility response to N-methyl-D-aspartic acid (14), also did not significantly change the stimulation of locomotion produced by picrotoxin. Thus these results suggest that the hypermotility elicited by picrotoxin can only be antagonized by the inhibition of more than one subtype of excitatory amino acid receptor. This concept is further supported by the present results which show that the coadministration of D-α-aminoadipic acid (10 μg) and the lower dose of GAMS (5 μg) significantly inhibited the hypermotility response to picrotoxin.

The present findings are in agreement with previous studies which have shown that a decrease in GABAergic or an increase in glutamatergic neurotransmission in the SI/LPO leads to a coordinated hypermotility response (8, 13, 14, 16, 18). The results of the present study suggest that GABAergic and glutamatergic receptors may directly or indirectly oppose each other in the SI/LPO and may both play a role in modulating locomotor activity initiated by neuronal mechanisms in the nucleus accumbens, a region of the forebrain which has been postulated to play a role in goal-oriented behavior (7,10). Thus the present results suggest that GABA and glutamate in the SI/LPO may interact in some manner to modulate goal-oriented behavior

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