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Role of Dopaminergic Mechanisms in the Stimulatory Effects of MK-801 Injected Into the Ventral Tegmental Area and the Nucleus Accumbens

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NARAYANAN, S., D. WILLINS, A. DALIA, L. WALLACE AND N. URETSKY. *Role of dopaminergic mechanisms in the stimulatory effects of MK-801 injected into the ventral tegmental area and the nucleus accumbens.* PHARMACOL BIOCHEM BEHAV **54**(3) 565–573, 1996. – The bilateral administration of 10 μg of (+)MK-801, but not (−)MK-801, into either the VTA or the N.Ac. stimulated locomotor activity. The stimulation induced by (+)MK-801 at both sites was inhibited by reserpine (5 mg/kg, SC) and the D_1 antagonist, SCH 23390 (0.1 mg/kg, SC). Eticlopride (0.03 mg/kg, SC), a D_2 antagonist, inhibited the stimulation produced by MK-801 in the VTA but not in the N.Ac. Baclofen (32 ng), a $GABA_B$ receptor agonist, injected into the VTA inhibited the stimulatory response to MK-801 injected systemically, into the VTA, or into the N.Ac., but did not significantly inhibit spontaneous locomotion or the stimulatory response to apomorphine (5 mg/kg, SC). These observations suggest that the stimulatory effects of MK-801 in the VTA and the N.Ac. are dependent on endogenous dopamine. In addition, the effects produced by MK-801 injected into the VTA closely resemble those produced by the systemic administration of low doses of MK-801, suggesting that this is the primary site of action of MK-801.

MK-801 Ventral tegmental area Nucleus accumbens Dopamine Baclofen

MK-801 ((±)-5-methyl-10,11-dihydro-[^{5}H]-dibenzo[a,d] cyclohepten-5,10-imine) is a noncompetitive antagonist of *N*-methyl-D-aspartate (NMDA) receptors and has potent psychoactive effects (9,10). When MK-801 is injected systemically into rodents at low doses (0.05 to 0.3 mg/kg), it produces a stimulation of coordinated locomotor activity (25,29,37,41, 44). This stimulation is inhibited by the depletion of endogenous dopamine stores with reserpine (37,44) and by dopamine receptor blocking agents administered either systemically (11,25,29,30,44) or directly into the nucleus accumbens (N.Ac.) (44). In addition, MK-801 has been shown to produce an increase in dopamine turnover in various brain regions (25,35). Finally, the systemic administration of MK-801 has been shown to increase the firing rate of dopaminergic neurons, as recorded from the ventral tegmental area (VTA) (14,27,45). These observations suggest that the stimulation of locomotion produced by the systemic administration of MK-801 is mediated by endogenous dopamine through the activation of dopaminergic receptors in the N.Ac.

However, there is also evidence that MK-801-stimulated locomotion can be produced by dopamine-independent mechanisms. Thus, the systemic administration of high doses of MK-801 has been reported to stimulate locomotor activity in reserpine-pretreated mice (6,7). In addition, it has recently been reported that the destruction of dopaminergic neurons in the N.Ac. by the injection of 6-hydroxydopamine into this site did not inhibit the locomotor stimulation produced by MK-801 (29). This latter observation is in agreement with the find-

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ing that the administration of MK-801 directly into the N.Ac. produces a stimulation of locomotor activity, which is not blocked by haloperidol (34).

Recent studies have been concerned with determining the neural site where MK-801 may act to produce locomotor stimulation. The administration of MK-801 into either the N.Ac., which contains dopaminergic terminals, or the ventral tegmental area, which contains dopaminergic cell bodies, elicited an increase in locomotor activity (20,34). The stimulation produced by the administration of MK-801 in the N.Ac. was not attenuated by haloperidol, suggesting the involvement of a dopamine-independent mechanism. However, the effects of other dopaminergic antagonists on this response were not studied. In addition, none of the studies reported so far have looked at the involvement of dopamine in the behavioral activation elicited by MK-801 administration into the VTA. Therefore, the goal of the present work was to further assess the importance of dopamine in the behavioral stimulation produced by MK-801 by studying the role of dopaminergic mechanisms in the locomotor stimulation produced by the administration of MK-801 into dopaminergic terminal sites in the nucleus accumbens and dopaminergic cell body sites in the VTA. The results suggest that dopaminergic mechanisms are involved in the behavioral effects produced by injections of MK-801 into both sites. In addition, the characteristics of the behavioral response to the administration of MK-801 into the VTA closely resemble those produced by the administration of MK-801 systemically.

METHOD

Administration of Drugs Into the Brain

Male Sprague-Dawley rats (Harlan Inc., Indianapolis, IN), weighing 200-350 g, were housed four animals per cage in a temperature-controlled environment (23 ± 1°C) with a 12 D : 12 L cycle. The animals were provided with food and water ad lib. All procedures involving animals were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Laboratory Animal Care and Use Committee. Animals were habituated to the locomotor activity test cages for at least 30 min prior to surgery. For intracranial administration of drugs, animals were first anesthetized with a halothane-oxygen mixture and mounted on a stereotaxic frame (David Kopf Instruments, Tujunga, CA). Drugs or saline in a volume of 0.5 μl were injected bilaterally into the VTA and/or the N.Ac. according to either the coordinates described by the atlas of Pellegrino et al. (32) (tooth bar +5 mm; VTA: AP −3.4 from bregma, L ±1.8, DV −8.5 from skull surface with needle angled at 6°; N.Ac.: AP +3.4 from bregma, L ±1.8, DV −7.5 from the surface of the skull) or the atlas of Paxinos and Watson (31) (tooth bar −3.3 mm; VTA: AP +4.2 relative to intraaural line, L ±2.3, DV −8.6 from skull surface with needle angled at 10°; N.Ac.: AP +10.2 relative to the intraaural line; L ±1.2, DV −7.2 from skull surface). The solutions were injected over 1 min, using a 1 μl Hamilton syringe (Hamilton Co., Reno, NV). After injection, the needle was left at the injection site for an additional minute to enable the drug to diffuse from the injection site. After the surgery, rats were placed in their home cage and allowed 5-10 min to recover from the halothane and regain their righting reflex. There appeared to be no lasting effect of halothane on motor function, because as soon as the animals recovered, they began to move about the cage in a coordinated manner.

In some studies, intracranial injections were made using chronically implanted cannulas. Rats were anesthetized with xylazine (15 mg/kg, IP) and ketamine (60 mg/kg, IP) and 22 gauge guide cannulas (Plastic One, Roanoke, VA) were implanted bilaterally 2 mm above the VTA, using the coordinates of Pellegrino et al. (32) described above. The cannulas were attached to the skull with stainless steel screws and cranioplastic cement (Plastic One). After surgery, wires were placed into the guide cannulas to keep them patent. On the day of drug injections, the wires were removed, and a 28 gauge inner injector cannula (Plastic Products, Roanoke, VA) was lowered to the VTA extending 2.0 mm below the guide cannula tip. A drug solution of 0.5 μl volume was infused bilaterally for a period of one minute into the VTA on each side. After the infusion, the inner cannula was removed, the wires reinserted into the guide cannula, and the animals were placed in locomotor activity cages.

Measurement of Locomotor Activity

Following recovery, locomotor activity was monitored in activity cages (Columbus Instruments Inc., Columbus, OH), which were ventilated Plexiglas boxes, measuring 42 sq. cm and 20 cm high and containing a 12 × 12 grid of infrared beams, 3.5 cm apart and 5 cm from the bottom of the cages. Ambulatory counts were recorded when two consecutive beams were interrupted.

Histology

After each experiment, the brains were fixed in 10% formalin for at least 24 h and then frozen. They were sectioned using a Cryo-Cut Microtome (American Optical Corp., Buffalo, NY), and the positions of the markings left by the needle tips were determined and recorded.

For some animals, the locations of the needle tips aimed at the VTA were compared to the locations of tyrosine hydroxylase positive cells (dopaminergic cells). To accomplish this, coronal sections of the midbrain and sagittal brain sections were processed for tyrosine hydroxylase immunohistochemistry using a modification of the procedure of Beck et al. (2). The animals were perfused with 40% paraformaldehyde to fix the brain. Sections were then made using the Cryo-Cut Microtome and incubated for 24 h with antityrosine hydroxylase monoclonal antibody (Boehringer, Chicago, IL) (1 : 1000), diluted in phosphate-buffered saline (PBS) that contained 2% bovine serum albumin, 0.2% Triton X-100, 0.02% sodium azide, and 10% horse serum. The sections were washed with PBS and then incubated for 1 h with biotinylated secondary antibody (mouse IgG, Vector Labs, Burlingame, CA) dissolved in PBS containing 0.2% Triton X-100 and 10% horse serum. The sections were then treated with a mixture of avidin-biotin-peroxidase and incubated for 1 h. The sections were then treated with 6% diaminobenzidene solution containing 0.1% hydrogen peroxide for 2-3 min, and then washed and mounted on gelatin-coated slides. The slides were examined with a Nikon microscope.

Drugs

d-Amphetamine sulfate and reserpine were obtained from Sigma Chemicals Co. (St. Louis, MO). MK-801 hydrogen maleate, baclofen, eticlopride HCl, and SCH 23390 HCl were purchased from Research Biochemicals Inc. (Natick, MA). Reserpine was initially dissolved in a minimum amount of glacial acetic acid and adjusted to volume with water. All the other drug solutions were made in 0.9% saline.

Statistical Analysis

Data are expressed as the mean ± SEM. Statistical analysis was done using an unpaired Student's *t*-test, with a level of $p < 0.05$ being considered significant.

RESULTS

Effect of MK-801 Administered Into the VTA or the N.Ac. on Locomotor Activity: Effect of Reserpine Pretreatment

The bilateral administration of 10 μg (30 nmol) of (+)MK-801 into the VTA or the N.Ac. produced a marked stimulation of locomotor activity, while the administration of 10 μg of the less active enantiomer, (−)MK-801, had essentially no effect (Table 1). To determine the importance of endogenous dopamine in the locomotor stimulation produced by the local administration of (+)MK-801, rats were pretreated with saline or reserpine (5 mg/kg, IP) 18–24 h before the bilateral injection of 10 μg of (+)MK-801 into either the VTA or N.Ac. Locomotor activity was recorded for 1 h. Reserpine (5 mg/kg, IP) almost completely inhibited the locomotor stimulation produced by MK-801 in the VTA and produced a 55% inhibition of the locomotor stimulation produced by MK-801 in the N.Ac. (Fig. 1).

Effect of the Systemic Administration of the D_1 Receptor Antagonist, SCH 23390, or the D_2 Antagonist, Eticlopride, on MK-801-Stimulated Locomotor Activity

Rats were pretreated with either saline, SCH 23390 (0.1 mg/kg, SC), or eticlopride (0.03 mg/kg, SC) 20 min prior to the injection of MK-801 (10 μg) into either the VTA or the N.Ac., and locomotor activity was recorded for 1 h. These doses of the antagonists were chosen because they were shown previously to inhibit the locomotor stimulation produced by the systemic administration of MK-801 (44). Both of the DA receptor antagonists were found to significantly inhibit the stimulatory effect produced by the administration of MK-801 into the VTA (Fig. 2). However, only SCH 23390, the D_1 receptor antagonist, inhibited the stimulatory effect of MK-801 injected into the N.Ac. (Fig. 3). Eticlopride, at a dose that antagonized the stimulatory effect of systemically administered MK-801 (44), did not inhibit the stimulatory response to intraaccumbal MK-801 administration (Fig. 3).

TABLE 1

EFFECT OF ADMINISTRATION OF (+)MK-801 AND (−)MK-801 INTO THE VTA AND THE N.Ac. ON LOCOMOTOR ACTIVITY

	Locomotor Activity (Mean ± SEM)	
Drug	VTA	N.Ac.
Saline	612 ± 73	743 ± 96
(+)MK-801	11906 ± 685*	8602 ± 935*
(−)MK-801	716 ± 205	759 ± 203

Rats were injected bilaterally with saline, 10 μg of (+)MK-801, or 10 μg of (−)MK-801 in either the VTA or the N.Ac., and locomotor activity was recorded for 1 h. Each value is the mean ± SEM of four determinations.
*$p < 0.01$.

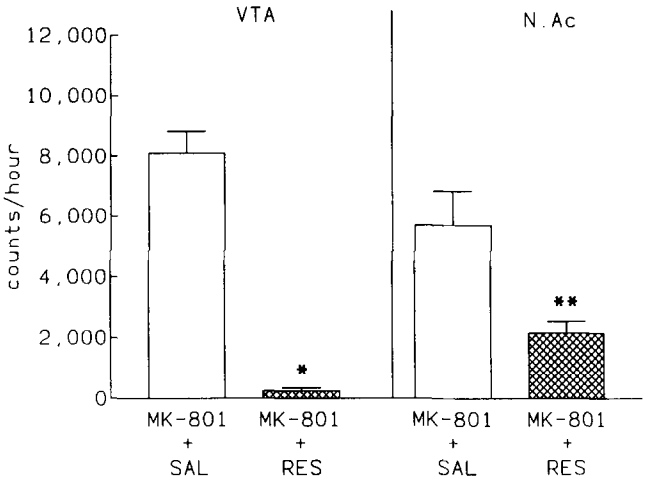

FIG. 1. Effect of reserpine on the locomotor stimulation produced by MK-801 injected into the VTA or into the N.Ac. Rats were treated with reserpine (5 mg/kg, IP) or vehicle, and 18–24 h later injected with MK-801 (10 μg/side) in the VTA or in the N.Ac. Locomotor activity was recorded every 10 min for 1 h. Each value represents the mean ± SEM of four to six animals. *$p < 0.01$ and **$p < 0.05$.

Effect of the Administration of Baclofen Into the VTA on the Locomotor Stimulation Produced by Systemically Administered MK-801 and Apomorphine

The $GABA_B$ receptor agonist, baclofen, inhibits the activity of dopaminergic neurons projecting from the midbrain to

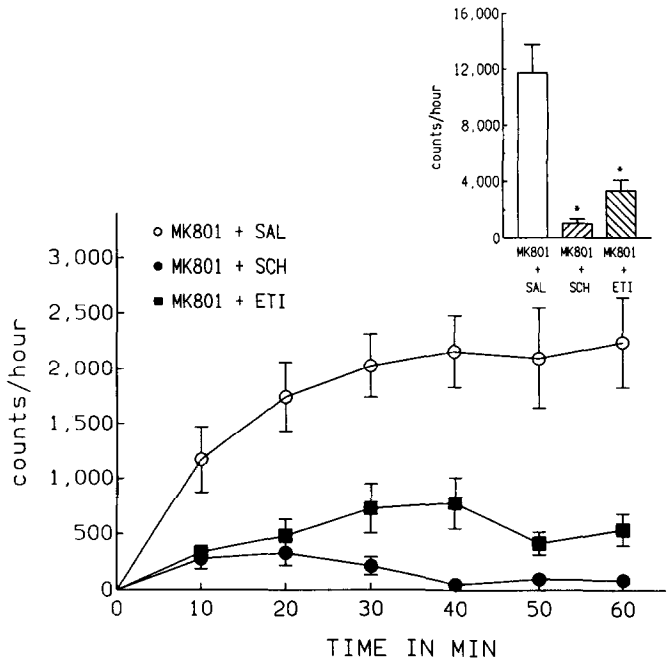

FIG. 2. Effect of D_1-antagonist SCH 23390 and D_2-antagonist eticlopride on the change in locomotor activity over time after the injection of MK-801 into the VTA. Rats were treated with SCH 23390 (0.1 mg/kg, SC) or eticlopride (0.03 mg/kg, SC) or vehicle and 20 min later injected with MK-801 (10 μg/side) in the VTA. Locomotor activity was recorded every 10 min for 1 h. Each value represents the mean ± SEM of four to six animals. *$p < 0.01$.

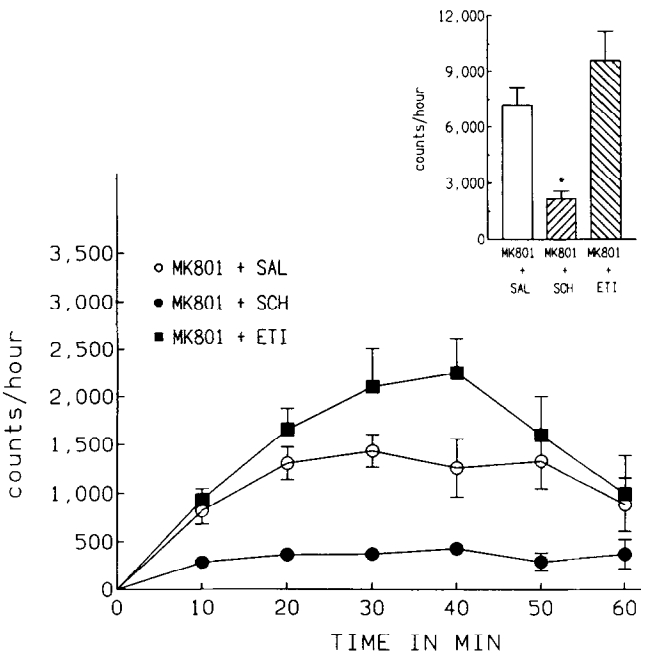

FIG. 3. Effect of D_1-antagonist SCH 23390 and D_2-antagonist eticlopride on the change in locomotor activity over time after the injection of MK-801 into the accumbens. Rats were treated with SCH 23390 (0.1 mg/kg, SC) or eticlopride (0.03 mg/kg, SC) or vehicle and 20 min later injected with MK-801 in the N.Ac. Locomotor activity was recorded every 10 min for 1 h. Each value represents the mean ± SEM of four to six animals. $^*p < 0.01$.

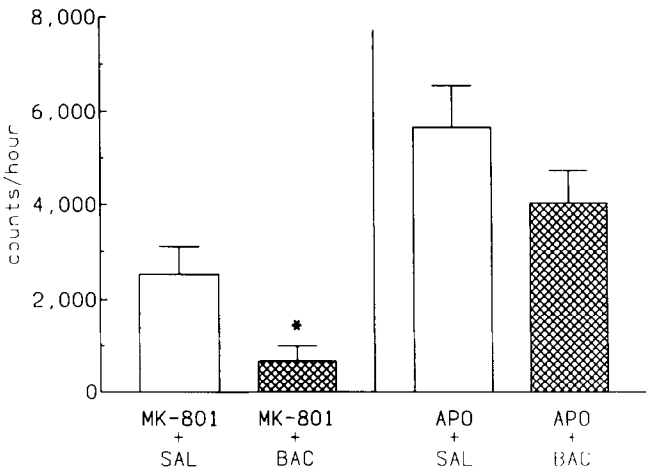

FIG. 4. Effect of baclofen injected into the VTA on the locomotor stimulation produced by the systemic administration of MK-801 (0.1 mg/kg, IP) or apomorphine (5 mg/kg, SC). Rats were injected with baclofen (0.15 nmol/side) or vehicle into the VTA and 5 min later injected with either MK-801 (0.1 mg/kg, IP) or apomorphine (5 mg/kg, SC). Locomotor activity was recorded every 10 min for 1 h. Each value represents the mean ± SEM of 5–10) animals. $^*p < 0.05$.

the striatum and the N.Ac. (22,28,33,37). In addition, baclofen injected into the VTA inhibits spontaneous locomotor activity (40) and the stimulation of locomotion induced by amphetamine or cocaine (19). To determine whether the administration of baclofen into the VTA inhibited the effects of MK-801 administered systemically, either baclofen (32 ng–0.15 nmol) or vehicle was bilaterally administered into the VTA 5 minutes before MK-801 (0.1 mg/kg, IP). Baclofen markedly inhibited the locomotor stimulation produced by MK-801 (Fig. 4). As a control, the effect of the direct acting dopaminergic agonist, apomorphine (5 mg/kg, SC) was determined in animals pretreated with baclofen (0.15 nmol) or vehicle in the VTA. Baclofen pretreatment produced only a small decrease in the locomotor response to apomorphine, which was not significantly different from the vehicle-treated control group (Fig. 4). In agreement with the results of Kalivas (19), baclofen at the dose of 0.15 nmol injected into the VTA did not significantly inhibit locomotor activity relative to that of control animals injected with vehicle (443 ± 239 activity counts for baclofen treated animals compared to 447 ± 231 for control).

Effect of Baclofen on the Locomotor Stimulation Produced by the Administration of MK-801 Into the VTA or the N.Ac.

Rats were injected bilaterally with baclofen (0.15 nmol) or vehicle together with MK-801 (10 μg) into the VTA. In a second experiment, baclofen (0.15 nmol) or vehicle was administered bilaterally into the VTA immediately prior to the bilateral administration of MK-801 (10 μg) into the N.Ac. In both experiments, locomotor activity was recorded for 1 h. Baclofen, injected into the VTA, inhibited the locomotor stimulation produced by MK-801 administered into either the VTA or the N.Ac. (Fig. 5).

Effect of Baclofen, Reserpine, and SCH 23390 on the Locomotor Stimulation Produced by MK-801 Administered Into the VTA Using Chronic Cannula

To determine whether the halothane or the surgical procedure affected the locomotor response to MK-801, rats were implanted with chronic cannulas over the VTA, as described in the methods section, and injected into the VTA with saline and baclofen (0.15 nmol/side) 5 min prior to saline and MK-801 (10 μg/side). Some of the animals injected with MK-801 received reserpine (5 mg/kg, IP) 18–24 h prior or SCH 23390 (0.1 mg/kg, SC) 20 min prior to the administration of MK-801 into the VTA. Locomotor activity was recorded for 1 h. As we have found in experiments using halothane as the anesthetic, MK-801 injected into the VTA produced a marked stimulation of locomotion (Fig. 6). This stimulation was reversed by either the administration of baclofen into the VTA immediately prior to MK-801 administration or by reserpine or SCH 23390 administered systemically (Fig. 6).

Histology

Figures 8 and 9 show representative photomicrographs of the regions of the VTA and the N.Ac., where the needle tips were located. For the VTA, the tips were found to be located medial to the substantia nigra in the region of tyrosine hydroxylase positive cells. For the N.Ac. the needle tips were located slightly medial to the anterior commissures. The needle tip markings from animals that were injected using the coordinates of Pellegrino et al. (32) were located at similar sites as markings from animals injected using the coordinates from Paxinos and Watson (31). In confirmation of this, animals, injected with MK-801 and baclofen into the VTA and the

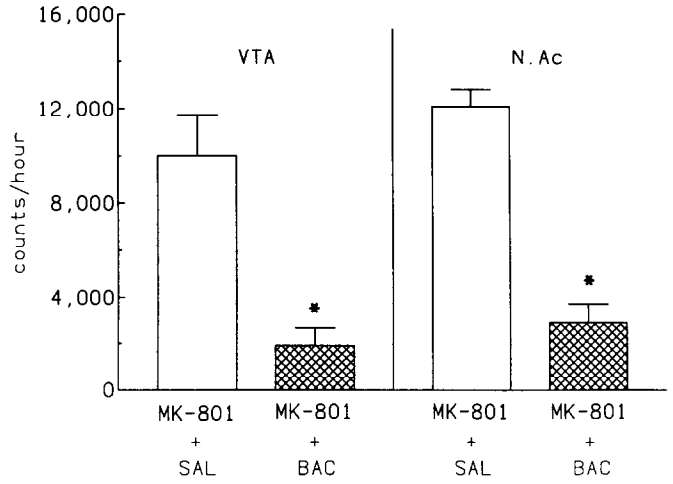

FIG. 5. Effect of baclofen injected into the VTA on the locomotor stimulation produced by MK-801 (10 μg/side) injected into the VTA or into the N.Ac. Rats were coadministered MK-801 (10 μg/side) with either baclofen (0.15 nmol/side) or vehicle in the VTA, or with baclofen (0.15 nmol/side) or vehicle in the VTA followed by MK-801 (10 μg/side) in the accumbens and the locomotor activity recorded every 10 min for 1 h. Each value represents the mean ± SEM of seven animals. *$p < 0.01$.

N.Ac. using either set of coordinates, displayed similar behavioral effects (data not shown). Consequently, the data using both sets of coordinates were combined for this study.

In one study, an error was made in the measurement of the coordinates for the VTA, and drugs were injected bilaterally into sites located 1.0 to 1.5 mm caudal to the standard VTA site described in the methods section. Histological examination of this injection site using a tyrosine hydroxylase immunoassay showed that the injections into the caudal VTA region were medial to the substantia nigra and in close proximity to dopaminergic cells (Fig. 8b). The results obtained from injections of drugs into the caudal VTA were comparable to

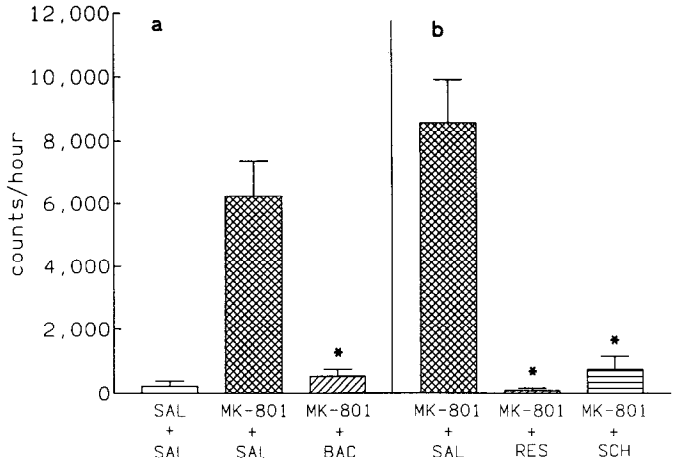

FIG. 6. Effect of baclofen, reserpine, and SCH 23390 on the locomotor stimulation produced by MK-801 administered into the VTA using chronic cannula. (a) Rats were injected into the VTA with saline or baclofen (0.15 nmol/side) and 5 min later injected with MK-801 (10 μg/side) or saline. Locomotor activity was recorded every 10 min for 1 h. (b) Rats were injected with saline, reserpine (5 mg/kg, IP) 18–24 hours prior or SCH 23390 20 min prior to the administration of MK-801 (10 μg/side) into the VTA. Locomotor activity was recorded every 10 min for 1 h. Each value represents the mean ± SEM of three to six animals. *$p < 0.01$.

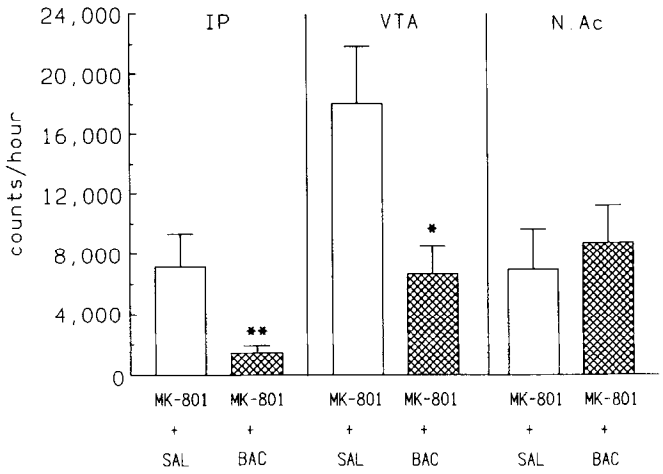

FIG. 7. Effect of baclofen injected into the caudal border of the VTA on the locomotor stimulation produced by the administration of MK-801 either systemically, into the VTA or into the N.Ac. Rats were either pretreated with or coadministered baclofen (0.15 nmol/side) or vehicle in the VTA and 5 min later were treated with either MK-801 (0.1 mg/kg, IP), or MK-801 (10 μg/side) in the VTA or in the N.Ac. and the locomotor activity was recorded every 10 min for 1 h. Each value represents a mean ± SEM of five to nine animals. $*p < 0.01$ and $**p < 0.05$.

those obtained from drug administration into the more central VTA site described in the Method section. Thus, the administration of MK-801 into the caudal VTA site produced an intense stimulation of locomotion, which was inhibited by reserpine pretreatment (data not shown), indicating the involvement of dopaminergic mechanisms. In addition, baclofen administered into this site inhibited the stimulant response to MK-801 injected either systemically or into the VTA (Fig. 7). Unexpectedly, baclofen, when injected into the caudal VTA site, did not inhibit the locomotor response to MK-801 injected into the N.Ac. (Fig. 7). This observation suggests that the locomotor stimulatory effects produced by the administration of MK-801 into the N.Ac. may depend upon the activity of those dopamine neurons with perikarya located at sites in the central region of the VTA but not in the caudal border of the VTA.

DISCUSSION

The results of this study show that MK-801 administered either systemically, into the N.Ac., or into the VTA produces a locomotor stimulatory response, which appears to require endogenous dopamine. Thus, reserpine, which depletes brain tissue of dopamine by over 95% (1,3,8,18), completely antagonized the locomotor response to MK-801 administered systemically (44) and inhibited the effects of MK-801 in the VTA by over 90% and in the N.Ac. by 55% (Fig. 1). In addition, the D_1 antagonist, SCH 23390, at a dose shown to inhibit the locomotor stimulation produced by systemic MK-801 (26,44), inhibited the response to MK-801 administered into either the VTA or the N.Ac. (Figs. 2 and 3). These observations suggest that endogenous dopamine plays an important role in the actions of MK-801 administered systemically, into the VTA, or into in the N.Ac.

The stimulatory effect of systemic MK-801 appears to resemble most closely that produced by the administration of MK-801 into the VTA. Thus, locomotor stimulation elicited by MK-801 given by either method was inhibited by a D_1 antagonist, a D_2 antagonist, baclofen given in either of two VTA sites, or by dopamine depletion produced by reserpine. In contrast, the response to MK-801 given into the N.Ac. was different in that it was not attenuated by a D_2 antagonist or by baclofen given in the caudal VTA site. In addition, the N.Ac. response was less sensitive to inhibition by reserpine. Such observations suggest that an action of MK-801 in the VTA provides a major contribution to the stimulant effect produced by the systemic administration of the low dose of 0.1 mg/kg MK-801 used in this study. This is consistent with observations that the systemic administration of MK-801 increases the firing rate of dopaminergic neurons, as recorded from the ventral tegmental area (VTA) (14,27,45). This increased activity of dopaminergic neurons would be expected to increase dopaminergic neurotransmission in the nucleus accumbens, resulting in the stimulation of locomotor activity.

The mechanism of the locomotor stimulatory effect of MK-801 acting in the VTA is unclear but appears to be mediated through antagonism of NMDA receptors, because the administration of competitive NMDA antagonists into the VTA also stimulates locomotor activity (13). In addition, locomotor stimulation was produced only by the active enantiomer, (+)MK-801. It has been demonstrated that systemically administered MK-801 can stimulate the electrophysiological activity of meso-accumbal dopaminergic neurons (14,27,45). Such an effect would be expected to lead to an increase in DA neurotransmission in the N.Ac. It is unlikely that the stimulatory effect of MK-801 is mediated by a direct action of this drug on dopaminergic neurons, because NMDA, itself, has been shown to stimulate these neurons (36,42,45). Therefore, MK-801, by antagonizing NMDA receptor activation, should inhibit rather than stimulate neuronal activity. One

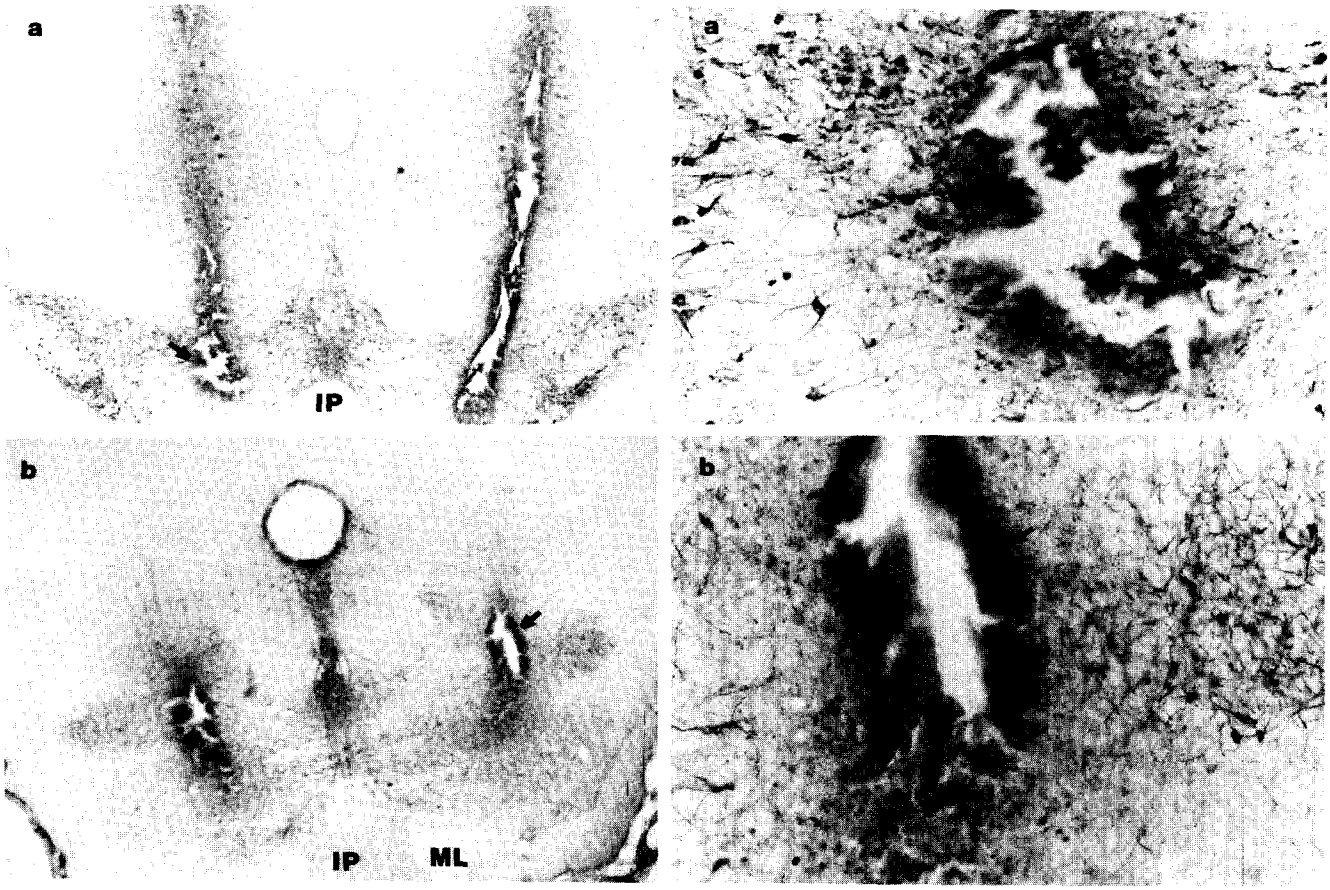

FIG. 8. Representative photomicrograph of a tyrosine hydroxylase (TH) immunostained section at 2.76× and 13.8× magnification showing the sites of injection. (a) Central region of the VTA: the lower magnification shows that the needle tip was located medial to the substantia nigra (SN) and lateral and slightly dorsal to the interpeduncular nucleus (IP). The arrow indicates the site shown at higher magnification. The higher magnification shows that the needle tip was in the region of TH immunopositive cells. (b) Caudal region of the VTA: the lower magnification micrograph shows that the tip of the needle tip was located lateral and slightly dorsal to the IP and dorsal to the medial lemniscus (ML). The arrow indicates the site shown at higher magnification. The higher magnification micrograph shows that the lesion produced by the needle tip is in the vicinity of TH immunopositive cells.

explanation that has been suggested (45) is that glutamatergic neurons that project to the VTA may activate NMDA receptors, producing a stimulation of inhibitory GABAergic interneurons and/or collaterals of efferent GABAergic neurons projecting from the VTA. The resultant enhancement of GABA release would activate $GABA_B$ receptors on mesolimbic dopaminergic neurons, inhibiting their firing rate. Consistent with this hypothesis are the recent observations that local perfusion of the VTA with high concentrations of NMDA can decrease the firing rate of VTA DA neurons in vitro and inhibit DA release in the N.Ac., measured in vivo using microdialysis in halothane-anesthetized animals (43). According to this hypothesis, MK-801, by antagonizing NMDA receptors, would inhibit the excitation of GABAergic interneurons, thereby disinhibiting meso-accumbal dopamine neurons.

Baclofen, a $GABA_B$ receptor agonist, when injected into the VTA inhibits the activity of dopamine neurons (16,22, 28,33) and the locomotor stimulant effects of cocaine, amphetamine, and DAMGO, a mu receptor opioid agonist (19). In the present study, baclofen, injected into the VTA, inhibited the locomotor stimulation produced by MK-801 injected either systemically, into the VTA, or into the N.Ac. (Figs. 4 and 5). In contrast, baclofen did not inhibit normal locomotor activity and produced a relatively small inhibition of the stimulatory effect of apomorphine (Fig. 4), a dopamine receptor agonist whose effects presumably do not require activation of dopaminergic neurons. These observations suggest that the stimulant effects of MK-801 are dependent upon the uninterrupted activity of meso-accumbal dopamine neurons and provide further evidence that the stimulatory effects of MK-801 are dependent upon endogenous dopamine neurotransmission in the N.Ac.

The effects of MK-801 administered into the N.Ac. differed from those elicited by MK-801 administered systemically or into the VTA. Thus, the effects of intraaccumbens administration were not inhibited by the D_2 receptor antagonist, eticlopride (Fig. 3) or by baclofen administered into the caudal VTA site. The apparent lack of activation of D_2 receptors is compatible with the observations of Raffa et al. (34), who found that the response to MK-801 injected into the N.Ac. was not inhibited by haloperidol, which is primarily a D_2 re-

ceptor antagonist (39). However, the effects produced by the direct injection of MK-801 in the N.Ac. still appear to require endogenous dopamine, since they are attenuated by reserpine and a D_1 antagonist. It is unlikely that the locomotor response to MK-801 injected into the N.Ac. is caused by the release of dopamine from nerve terminals at this site because the locomotor activity elicited by amphetamine and cocaine, which act by increasing dopamine levels in the synapse, is inhibited by D_2 antagonists (4,12). It, therefore, appears that the locomotor response to intraaccumbal MK-801 is mediated by an enhanced activation of D_1 dopaminergic receptors, but the mechanism is unclear. Perhaps MK-801 can sensitize D_1 receptors to dopamine, enabling them to act independently of D_2 receptors, as is observed in some cases of supersensitivity (5). Consistent with this hypothesis is the recent observation that systemic administration of MK-801 enhances the in vivo binding of [^{3}H]-SCH 23390 in the striatum (21). In addition, MK-801 was shown to enhance the stimulant effect of a D_1 agonist, but not a D_2 agonist, in monoamine-depleted mice (15,38)

FIG. 9. Representative photomicrograph of a cresyl violet stained section showing the location of the needle tip in the nucleus accumbens (N.Ac.). The needle tip was located just medial to the anterior commissures (AC).

Most of the reports indicating that MK-801 stimulates locomotor activity independent of dopaminergic mechanisms utilize doses that are 10-fold higher (7) than those used in the present study. However, a recent report that 6-hydroxydopamine-induced lesions of the N.Ac. did not block the stimulation produced by systemic administration of low doses of MK-801 (29) points toward mechanisms other than enhanced dopamine neurotransmission in the behavioral effects of MK-801. At present, we cannot reconcile our observations with these findings. The effects of MK-801 could, in part, involve serotonergic neurons, because ipsapirone, a 5-HT_{1A} receptor ligand, has been reported to antagonize some of the behavioral effects of systemic MK-801 (23). Future studies should examine the role of serotonergic mechanisms in the effects produced by the local administration of MK-801 into the VTA and the N.Ac. and determine whether the chronic loss of dopamine neurons after 6-hydroxydopamine might alter the behavioral response to MK-801.

In conclusion, the present study shows that the VTA and the N.Ac. are sites of action for MK-801-induced stimulation of locomotion. The effects of MK-801 at both sites are dependent on endogenous dopamine. The effects of MK-801 in the VTA closely resemble those of systemic administration of low doses of MK-801, suggesting the VTA as a primary target following systemic administration. However, the N.Ac. is also likely to be involved and may become increasingly important at higher systemic doses of MK-801.

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REFERENCES

1. Anden, N. E. Effects of reserpine and a tyrosine hydroxylase inhibitor on monoamine levels in different regions of the rat central nervous system. Eur. J. Pharmacol. 1:1–5; 1967.
2. Beck, K. D.; Knusel, B.; Pasinetti, G.; Michel, P. P.; Zawadzka, H.; Goldstein, M.; Hefti, F. Tyrosine hydroxylase mRNA expression by dopaminergic neurons in culture: Effect of 1-methyl-4-phenylpyridinium treatment. J. Neurochem. 57:527–532; 1991.
3. Bertler, A. Effect of reserpine on the storage of catecholamines in the brain. Acta Physiol. Scand. 51:75–83; 1961.
4. Boldry, R. C.; Willins, D. L.; Wallace, L. J.; Uretsky, N. J. The role of endogenous dopamine in the hypermotility response to intra-accumbens AMPA. Brain Res. 559:100–108; 1991.
5. Breese, G. R.; Napier, T. C.; Mueller, R. A. Dopamine-agonist induced locomotor activity in rats treated with 6-hydroxydopamine at differing ages: Functional supersenstivity of D_1 dopamine receptors in neonatally lesioned rats. J. Pharmacol. Exp. Ther. 234:447–455; 1985.
6. Carlsson, M.; Carlsson, A. The NMDA antagonist MK-801 causes marked locomotor stimulation in monoamine-depleted mice. J. Neural Transm. 75:221–226; 1989.
7. Carlsson, M.; Carlsson, A. Dramatic synergism between MK-801 and clonidine with respect to locomotor stimulatory effect in monoamine-depleted mice. J. Neural Transm. 77:64–71; 1989.
8. Carlsson, A.; Lindqvist, M.; Magnusson, T. 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. Nature 180:1200; 1957.
9. Clineschmidt, B. V.; Martin, G. E.; Bunting, P. R.; Papp, N. L. Central sympathomimetic activity of (±)-5-methyl-10,11-dihy-

dro-[^{5}H]-dibenzo[a,d] cyclohepten-5,10-imine (MK-801), a substance with potent anticonvulsant, central sympathomimetic, and apparent anxiolytic properties. Drug. Dev. Res. 2:134–145; 1982.
10. Clineschmidt, B. V.; Martin, G. E.; Bunting, P. R. Anticonvulsant activity of (±)-5-methyl-10,11-dihydro-[^{5}H]-dibenzo[a,d] cyclohepten-5,10-imine (MK-801), a substance with potent anticonvulsant, central sympathomimetic, and apparent anxiolytic properties. Drug Dev. Res. 2:123–134; 1982.
11. Dall'Olio, R.; Gandolfi, O.; Montanaro, N. Effect of chronic treatment with dizocilpine (MK-801) on the behavioral response to dopamine receptor agonists in the rat. Psychopharmacology (Berlin) 107:591–594; 1992.
12. Dall'Olio, R.; Roncada, P.; Vaccheri, A.; Gandolfi, O.; Montanaro, N. Synergistic blockade of some dopamine mediated behaviors by (−)sulpiride and SCH 23390 in the rat. Psychopharmacology (Berlin) 98:342–346; 1989.
13. Dawbarn, D.; Pycock, C. J. Motor effects following application of putative excitatory amino acid antagonists to the region of the mesencephalic dopamine cell bodies in the rat. Naunyn Schmiedebergs Arch Pharmacol. 318:100–104; 1981.
14. French, E. D.; Ceci, A. Noncompetitive *N*-methyl-D-aspartate antagonists are potent activators of ventral tegmental A_{10} dopamine neurons. Neurosci. Lett. 119:159–162; 1990.
15. Goodwin, P.; Starr, B. S.; Starr, M. S. Motor responses to dopamine D_1 and D_2 agonists in the reserpine-treated mouse are affected differentially by the NMDA receptor antagonist MK-801. J. Neural Transm. 4:15–26; 1992.
16. Grace, A. A.; Bunney, B. S. The control of the firing pattern of nigral dopamine neurons: burst firing. J. Neurosci. 4:2877–2890; 1984.
17. Hiramattsu, M.; Cho, A. K.; Nabeshima, T. Comparison of the behavioral and biochemical effects of the NMDA receptor antagonist MK-801 and phencyclidine. Eur. J. Pharmacol. 166:359–366; 1989.
18. Hiroi, N.; White, N. M. The reserpine-sensitive dopamine pool mediates (+)-amphetamine-conditioned reward in the place preference paradigm. Brain Res. 510:33–42; 1990.
19. Kalivas, P. W.; Duffy, P.; Eberhardt, H. Modulation of A_{10} dopamine neurons by aminobutyric acid agonists. J. Pharmacol. Exp. Ther. 253:858–866; 1990.
20. Kalivas, P. W.; Alesdatter, J. E. Involvement of *N*-methyl-D-aspartate receptor stimulation in ventral tegmental area and amygdala in behavioral sensitization to cocaine. J. Pharmacol. Exp. Ther. 267:486–495; 1993.
21. Kobayashi, K.; Inoue, O. An increase in the in vivo binding of [^{3}H]-SCH 23390 induced by MK-801 in the mouse striatum. Neuropharmacology 32:341–348; 1993.
22. Lacey, M. G.; Mercuri, N. B.; North, R. A. On the potassium conductance increase activated by $GABA_B$ and dopamine D_2 receptors in rat substantia nigra neurons. J. Physiol. (London) 401: 435–437; 1988.
23. Loscher, W.; Honack, D. The behavioral effects of MK-801 in rats: Involvement of dopaminergic, serotonergic, and noradrenergic systems. Eur. J. Pharmacol. 215:199–208; 1992.
24. Loscher, W.; Annies, R.; Honack, D. The *N*-methyl-D-aspartate receptor antagonist MK-801 induces increased in dopamine and serotonin metabolism in several brain regions of rats. Neurosci. Lett. 128:191–194; 1991.
25. Maj, J.; Rogoz, Z.; Skuza, G. Locomotor hyperactivity induced by MK-801 in rats. Pol. J. Pharmacol. Pharm. 43:449–458; 1991.
26. Martin, P.; Svensson, A.; Carlsson, A.; Carlsson, M. L. On the roles of dopamine D_1 vs. D_2 receptors for the hyperactivity response elicited by MK-801. J. Neural Transm. 95:113–121; 1994.
27. Murase, S.; Mathe, J. M.; Grenhoff, J.; Svensson, T. H. Effects of dizocilpine (MK-801) on rat midbrain dopamine cell activity: Differential actions on firing pattern related to anatomical localization. J. Neural Transm. 91:13–25; 1993.
28. Olpe, H. R.; Koella, W. P.; Wolf, P.; Haas, H. L. The action of baclofen on neurons of the substantia nigra and of the ventral tegmental area. Brain Res. 134:577–580; 1977.
29. Ouagazaal, A.-M.; Nieoullon, A.; Amalric, M. Locomotor activation induced by MK-801 in the rat: Postsynaptic interactions with dopamine receptors in the ventral striatum. Eur. J. Pharmacol. 251:229–236; 1994.
30. Ougagazal, A.; Nieoullon, A.; Amalric, M. Effects of dopamine D_1 and D_2 receptor blockade on MK-801-induced hyperlocomotion in rats. Psychopharmacology (Berlin) 111:427–434; 1993.
31. Paxinos, G.; Watson, C. The rat brain in sterotaxic coordinates. New York: Academic Press; 1986.
32. Pellegrino, L. K.; Pellegrino, A. S.; Cushman, A. J. A stereotaxic atlas of the rat brain. New York: Plenum Press; 1979.
33. Pinnock, R. D. Hyperpolarizing action of baclofen on neurons in the rat substantia nigra slice. Brain Res. 332:337–340; 1984.
34. Raffa, R.; Ortegon, M. E.; Robisch, D. M.; Martin, G. E. In vivo demonstration of the enhancement of MK-801 by *l*-glutamate. Life Sci. 44:1593–1599; 1989.
35. Rao, T. S.; Kim, H. S.; Lehmann, J.; Martin, L. L.; Wood, P. L. Interactions of the phencyclidine receptor agonist MK-801 with the dopaminergic system; regional studies in the rat. J. Neurochem. 54:1157–1162; 1990.
36. Seutin, V.; Johnson, S. W.; North, R. A. Effect of dopamine and baclofen on *N*-methyl-D-aspartate-induced burst firing in rat ventral tegmental neurons. Neuroscience 58:201–206; 1994.
37. Starr, M. S.; Starr, B. S. Comparison of the effects of NMDA and AMPA antagonists on the locomotor activity induced by selective D_1 and D_2 dopamine agonists in reserpine-treated mice. Psychopharmacology (Berlin) 114:469–476; 1994.
38. Svensson, A.; Carlsson, A.; Carlsson, M. L. Differential locomotor interactions between dopamine D_1/D_2 receptor agonists and the NMDA receptor antagonist dizocilpine in monoamine depleted mice. J. Neural Transm. 90:199–217; 1992.
39. Tamminga, C. A.; Gerlach, J. New neuroleptics and experimental antipsychotics in schizophrenia. In: Meltzer, H. Y., ed. Psychopharmacology: The third generation of progress. New York: Raven Press; 1987:1129–1140.
40. Tanner, T. GABA-induced locomotor acivity in the rat after bilateral injection into the ventral tegmental area. Neuropharmacology 18:441–446; 1979.
41. Tricklebank, M. D.; Singh, L.; Oles, R. J.; Preston, C.; Iversen, S. D. The behavioral effect of MK-801: A comparison with antagonists acting noncompetitively and competitively at the NMDA receptor. Eur. J. Pharmacol. 167:127–135; 1989.
42. Wang, T.; French, E. D. Effects of phencyclidine on spontaneous and excitatory amino acid-induced activity of ventral tegmental dopamine neurons: An extracellular in vitro study. Life Sci. 53: 49–56; 1993.
43. Wang, T.; O'Connor, W. T.; Ungerstedt, U.; French, E. D. *N*-methyl-D-aspartic acid biphasically regulates the biochemical and electrophysiological response of A_{10} dopamine neurons in the ventral tegmental area: In vivo microdialysis and in vitro electrophysiological studies. Brain Res. 666:255–262; 1994.
44. Willins, D. L.; Narayanan, S.; Wallace, L. J.; Uretsky, N. J. The role of dopamine and AMPA/kainate receptors in the nucleus accumbens in the hypermotility response to MK-801. Pharmacol. Biochem. Behav. 46:881–887; 1993.
45. Zhang, J.; Chiodo, L. A.; Freeman, A. S. Electrophysiological effects of MK-801 on rat nigrostriatal and mesoaccumbal dopaminergic neurons. Brain Res. 590:153–163; 1992.