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Nucleus Accumbens NMDA Antagonist Decreases Locomotor Activity Produced by Cocaine, Heroin or Accumbens Dopamine, But Not Caffeine

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PULVIRENTI, L., N. R. SWERDLOW AND G. F. KOOB. *Nucleus accumbens NMDA antagonist decreases locomotor activity produced by cocaine, heroin or accumbens dopamine, but not caffeine.* PHARMACOL BIOCHEM BEHAV **40**(4) 841–845, 1991.—Glutamatergic afferents to the nucleus accumbens (NAC) have been suggested to modulate psychostimulant-induced locomotor activation. The purpose of this study was 1) to determine the importance of nucleus accumbens N-methyl-D-aspartate (NMDA) glutamate receptors in the control of psychostimulant-induced locomotion, 2) to determine whether NMDA receptor modulation of psychostimulant-induced locomotion occurs presynaptic to or postsynaptic to NAC dopamine terminals, and 3) to determine whether NMDA receptors also modulate opiate- and caffeine-induced locomotor activation. For this purpose, rats treated with cocaine (10 mg/kg IP), dopamine (20 μg directly into the NAC), heroin (0.5 mg/kg SC), or caffeine (10 mg/kg SC) were challenged with intra-NAC microinfusion of 2-amino-5-phosphonovaleric acid (APV), a selective NMDA receptor antagonist. APV reduced locomotor activation induced by cocaine, heroin and intra-NAC dopamine, but not caffeine. These results suggest that NAC glutamate modulates psychomotor stimulation at the level of the NAC through an interaction with the integrated output of this region.

Nucleus accumbens Dopamine NMDA Cocaine Heroin Locomotor activity

THE nucleus accumbens of the ventral striatum (NAC) is a limbic structure that has been implicated in locomotor activity, reward and affective behavior (16, 24, 32). Dopamine terminals within the NAC originating from cell bodies in the ventral tegmental area are a critical substrate for the locomotor activating properties of psychostimulant drugs such as cocaine and amphetamine (14,15). The psychomotor activation induced by low doses of heroin, in contrast, depends upon activation of opiate receptors within the NAC (1) and does not seem to require the integrity of dopamine terminals (34), while caffeine-induced hyperlocomotion seems to depend upon activation of extra-accumbens structures (31).

Anatomical studies have shown that the NAC receives a consistent neuronal input from allocortical structures such as the hippocampal formation and the amygdaloid complex (12,13) and these afferents are glutamatergic in nature (6). According to electrophysiological evidence, these fibers may subserve reciprocal modulation with dopamine terminals within the NAC (36,37), and behavioral observations suggest that endogenous glutamate neurotransmission may modulate spontaneous and drug-induced locomotor activity (19, 20, 26). Furthermore, biochemical studies have shown that intra-NAC infusion of glutamate or glutamate agonists induces release of dopamine, both in vitro, in accumbens slices (9,28), and in vivo, as revealed by microdialysis (20), and this may correlate with the concomitant behavioral activation (5).

It was recently reported that fibers of hippocampal origin and dopamine terminals converge onto the same postsynaptic neuron within the NAC (33), and this is consistent with electrophysiological findings (36,37). In addition, such hippocampal afferents have been observed to establish presumptive presynaptic contact with dopaminergic fibers (30). This provides the ultrastructural basis for a glutamate-dopamine interaction within the NAC and leaves open the possibility that glutamate neurotransmission may modulate dopamine function, both at the presynaptic and postsynaptic level, and may modulate dopamine-independent psychomotor stimulation.

To test these hypotheses, the role of NAC glutamate neurotransmission in modulating the psychomotor activation produced by drugs that stimulate NAC dopamine neurotransmission at the pre- and postsynaptic level was evaluated. Locomotor activity was stimulated by cocaine, a reuptake blocker of dopamine from

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the presynaptic terminals (8), or by intra-NAC infusion of dopamine, in rats treated with intra-NAC infusion of 2-amino-5-phosphonovaleric acid (APV), a glutamate antagonist at the N-methyl-D-aspartate (NMDA) receptor site (4). Locomotion also was stimulated by heroin or caffeine in other accumbens APV-treated rats in order to evaluate the selectivity of glutamate modulation of accumbens dopamine mechanisms. The present results show that blockade of NMDA receptors in the NAC selectively reduces locomotor activation induced by cocaine, intra-NAC dopamine or heroin, but not by caffeine. This suggests a role of glutamate, through NMDA receptors, on the integrated function of the NAC.

METHOD

Male albino Wistar rats (N=83, 240–260 g, Charles River Laboratories) were housed in groups of three, and exposed to a normal 12-hour light-dark cycle, with free access to food and water. All rats were anesthetized with pentobarbital [50 mg/kg intraperitoneally, (IP)] and secured in a Kopf stereotaxic instrument with the toothbar 5 mm above the interaural line. The animals were then implanted with bilateral 23-gauge 10-mm steel cannulae aimed 3 mm above the NAC at coordinates AP +3.2, L ±1.7, DV −4.8 (skull surface), which were fastened to the skull with dental cement and sealed with a 10-mm stylet wire.

Locomotor testing for all animals began one week after surgery using 16 wire cages as described previously (11). Each cage measured 36×25×20 cm with twin photocell beams across the long axis 2 cm above the cage floor. One day before testing, all animals were familiarized with the photocell cages by placing them individually into the cages for 180 min.

On a testing day, animals were placed in the photocell cages for 90 min, at which time their activity levels decreased to essentially below measurable levels. Subsequently, all animals received an injection of either cocaine (10 mg/kg IP), heroin (0.5 mg/kg SC), dopamine (20 μg/1 μl, directly into the NAC) or caffeine (10 mg/kg, base, SC). All animals then received intra-NAC microinfusion of either saline (1 μl/side) or APV (3.0 μg/1 μl/side total of 6 μg/rat) (RBI, MA). An additional group of 8 rats were injected with cocaine and tested with 1.5 μg/1 μl/side of APV (total of 3 μg/rat). All intra-NAC infusions were performed through 30-gauge injectors fashioned to extend 3 mm beyond the ventral tip of the cannula. All drugs were dissolved in physiological saline vehicle. Immediately following the above treatments, animals were replaced in the photocell cages and locomotor activity was recorded for 180 min. These doses were chosen based on behavioral observations in other studies (1, 26, 27, 36). Each experimental group consisted of seven to eight different animals.

Following completion of behavioral testing, all animals were sacrificed by overdose of pentobarbital, and perfused through the heart with cold 10% formalin/saline. The brains were then removed and 30 μ frozen sections were cut in a frontal plane using a rotary microtome. Cannulae sites were assessed without knowledge of behavioral results.

Data were analyzed using ANOVA with repeated measures on the time factor. When there was a main effect with no drug × time interactions, no individual means comparisons were made at each time point, but the overall drug effect was represented in the insert box of the total (3 hours) drug response. When there was a drug × time interaction but no main effect then individual means comparisons were conducted at each time point and the lack of an overall group effect was shown in the insert box of the total (3 hours) drug response.

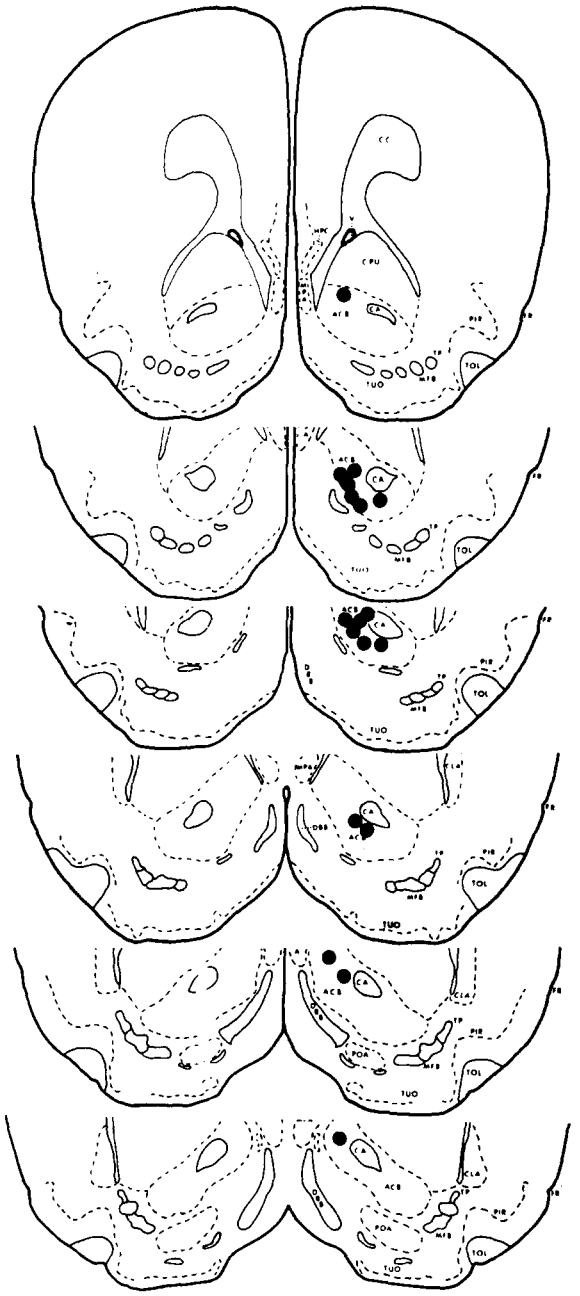

FIG. 1. Histological localization of cannulae placement for 18 rats randomly chosen from the cocaine experiment. Cannulae were implanted bilaterally but unilateral localization is shown for clarity. For coordinates see text.

RESULTS

The site of injection, as determined by histological examination, is shown in Fig. 1. Cannulae, in general, fell within the NAC at locations 2.4 to 3.4 anterior to bregma.

Figure 2 shows the effect of intra-NAC microinfusion of APV on locomotor activity induced by cocaine (10 mg/kg IP). ANOVA analysis revealed that there was a significant main ef-

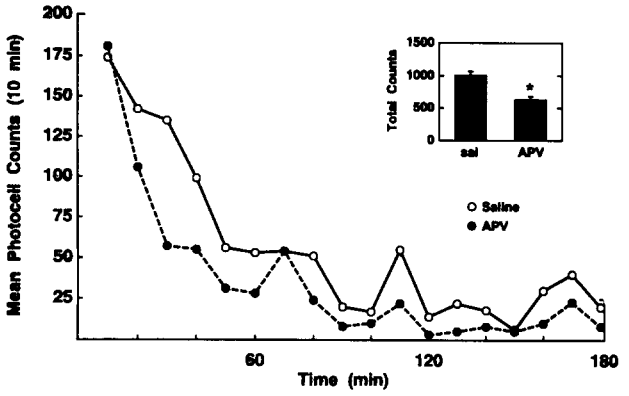

FIG. 2. Locomotor response after injection of cocaine (10 mg/kg IP). Ordinate refers to total photocell counts (mean ± SEM) for each 10-min period of a 180-min test. Animals received bilateral intra-NAC microinfusion of either physiological saline (open circle) or APV (6.0 μg/rat total, solid circle). Inset shows the mean total counts for 180 min ± SEM. *$p<0.01$.

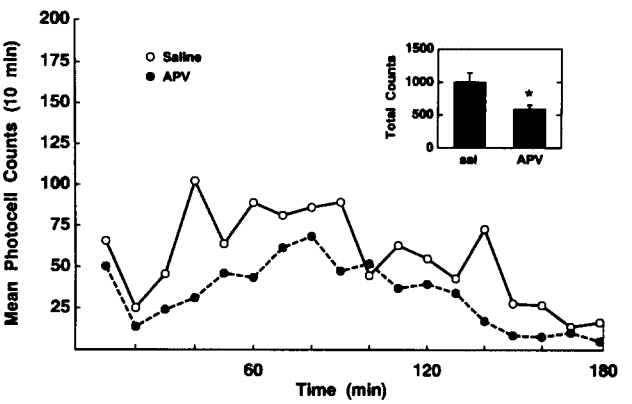

FIG. 4. Locomotor response after injection of heroin (0.5 mg/kg SC). Ordinate refers to total photocell counts (mean ± SEM) for each 10-min period of a 180-min test. Animals received bilateral intra-NAC microinfusion of either physiological saline (open circle) or APV (6.0 μg/rat total, solid circle). Inset shows the mean total counts for 180 min ± SEM. *$p<0.01$.

fect of drug, $F(1,12)=9.94$, $p<0.01$, a main effect of time, $F(1,17)=17.53$, $p<0.01$, with no significant drug × time interaction, $F(1,17)<1$, NS. Similar effects were observed with a dose of 1.5 μg APV, data not shown. Figure 3 shows the effect of microinfusion of APV on hyperactivity induced by intra-NAC dopamine (20 μg). ANOVA revealed that there was no main effect of drug, $F(1,14)=1.875$, $p>0.05$, a main effect of time, $F(17,238)=18.151$, and significant drug × time interaction, $F(17,238)=6.016$, $p<0.01$. Individual means analysis of each 10-min period using a simple main effects F-test (36) revealed significant differences at 20–60 min postinjection (see Fig. 3). These same sample main effects F-tests, performed because of the significant drug × time interaction, failed to reveal a significant difference between the groups at any of the later time points.

The effect of intra-NAC microinfusion of APV on heroin-induced locomotion is shown in Fig. 4. ANOVA revealed that there was a significant main effect of drug, $F(1,13)=5.151$, $p<0.05$, a significant main effect of time, $F(17,221)=5.151$, $p<0.01$, with no significant drug × time interaction ($F<1$). The effect of intra-NAC APV on caffeine-induced locomotion is shown in Fig. 5. ANOVA revealed that there was no significant main effect of drug ($F<1$), a significant main effect of time, $F(17,221)=15.314$, $p<0.01$, with no significant drug × time interaction ($F<1$).

DISCUSSION

The present report shows that endogenous glutamate activity within the NAC modulates the psychomotor activation induced by cocaine, NAC dopamine, and heroin, but not caffeine. Locomotor activity produced by cocaine, dopamine directly injected into the NAC and heroin were attenuated by direct administra-

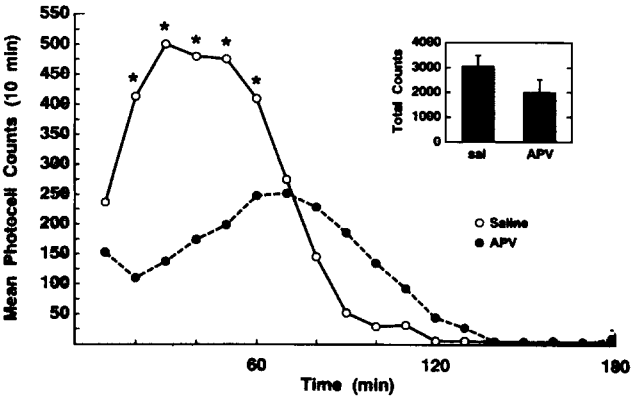

FIG. 3. Locomotor response after bilateral intra-NAC microinfusion of dopamine (20 μg). Ordinate refers to total photocell counts (mean ± SEM) for each 10-min period of a 180-min test. Animals received bilateral intra-NAC microinfusion of either physiological saline (open circle) or APV (6.0 μg/rat total, solid circle). Inset shows the mean total counts for 180 min ± SEM. *$p<0.01$ simple main effects.

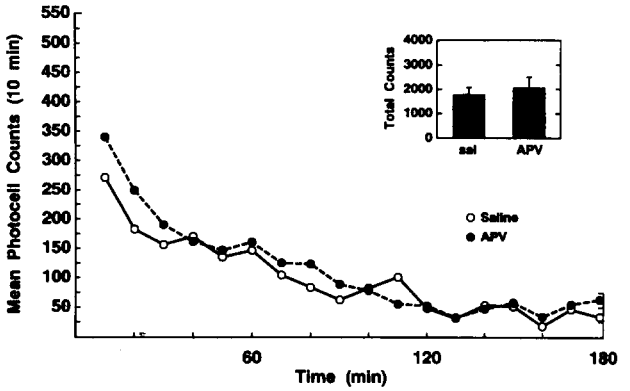

FIG. 5. Locomotor response after injection of caffeine (10 mg/kg, base, SC). Ordinate refers to total photocell counts (mean ± SEM) for each 10-min period of a 180-min test. Animals received bilateral intra-NAC microinfusion of either physiological saline (open circle) or APV (6.0 μg/rat total, solid circle). Inset shows the mean total counts for 180 min ± SEM.

tion into the NAC of the NMDA antagonist, APV. Caffeine-induced locomotor activity was not altered by NAC administration of APV.

The locomotor activating properties of cocaine appear to depend largely upon activation of the dopaminergic system within the NAC. This conclusion is based on the observations that both lesions of the dopamine terminals within the NAC using the neurotoxin 6-hydroxydopamine and blockade of postsynaptic dopamine receptors reduce cocaine-induced hyperactivity (14,17).

It is unclear, however, whether the functional modulatory effect of glutamate within the NAC on cocaine locomotion that has been previously reported (26) could be attributed to a pre- or postsynaptic locus of action. Anatomical studies have provided evidence for convergence of glutamatergic hippocampal afferents and tyrosine hydroxylase-positive terminals onto the same postsynaptic medium spiny neurons within the NAC (30,33). Indeed, the ultrastructural features of hippocampal afferents are consistent with a pathway mediating excitatory input (30) and electrophysiological evidence supports that NAC neurons show excitatory response to hippocampal simulation (34). However, axo-axonal interrelationships between tyrosine hydroxylase-positive and hippocampal afferents have also been described in the form of close apposition between the terminals (30,33). Such axo-axonal appositions have been suggested to be sites of functional interaction at the presynaptic level with the NAC. Indeed, neurochemical studies have shown that glutamate agonists induce release of dopamine in vitro in accumbens slices and in vivo using microdialysis (9, 20, 28), and, finally, the hyperactivity induced by intra-NAC infusion of glutamate agonists seems to be reduced by concomitant intra-NAC administration of a dopamine antagonist (5).

Earlier work, however, has focused mostly on the effects of pharmacological doses of glutamate or glutamate agonists (often in the millimolar range) and the physiological relevance of these findings has been questioned (21). The present study addressed this issue using a glutamate antagonist (APV) in an attempt to assess the physiological role of NAC glutamate neurotransmission on drug-induced activation. From the results of this study it appears that, although a dopamine component may be important for the expression of glutamate effects within the NAC (7), endogenous glutamate afferents may exert a tonic action on the NAC, playing a "permissive" facilitatory role on the integrated output of this nucleus.

Opiate drugs are known to stimulate locomotor activity at low doses (2). Although opiates are thought to release dopamine within the NAC, probably through activation of opiate receptors within the ventral tegmental area (3,10), opiate-induced locomotion can be reduced by the dopamine receptor antagonist fluphenazine only at cataleptic doses (31), and opiate-induced locomotion is relatively unaffected by 6-hydroxydopamine lesions of the NAC (34). Also, the activating properties of heroin are effectively antagonized by intra-NAC microinfusion of the opiate receptor antagonist methylnaloxonium (1), and many of the opiate receptors in the striatum are not linked to the dopamine system since a large proportion of these receptors are spared by neurotoxic lesions of the dopamine system (25). All this suggests that activation of opiate receptors located on non-dopaminergic neurons is a critical link in opiate-induced hyperactivity, and that dopamine neurotransmission is not a critical substrate for these opiate effects. The findings of the present study indicate therefore that glutamate neurotransmission may modulate opiate-dependent behavioral activation, as well as cocaine- and dopamine-induced activation.

Cocaine is known to possess reinforcing effects [for review, see (16)]. Using a model of intravenous self-administration in rats, it has been suggested that the acute rewarding properties of cocaine depend on the integrity of dopamine terminals within the NAC (23,29). It was recently reported that APV, at the same doses used in this study, reduced the rewarding properties of intravenously self-administered cocaine (27). This suggests the possibility that glutamate neurotransmission may modulate the role of NAC dopamine activity in drug reinforcement as well as drug-induced locomotion.

Finally, APV did not significantly modify caffeine-induced locomotor stimulation. Caffeine is thought to stimulate locomotion via extra-accumbens structures (31), and this finding suggests that APV does not decrease cocaine, dopamine and heroin locomotion simply by impairing general motor functions. Furthermore, Hamilton et al. (7) showed that microinfusion of APV alone within the NAC, at the same dose and under similar conditions as in the present study, had no significant effect on spontaneous locomotor activity (see ref. 7, Fig. 2). Indeed, APV seemed to produce a slight, but nonsignificant increase in locomotor activity in that study. This provides further evidence against a nonspecific cataleptogenic effect of glutamate antagonists at the level of the NAC.

The NAC is part of the limbic striatum and has been implicated as a substrate of drug reward and motivated behavior (16, 23, 31). Given their anatomical connections, allocortical afferents originating from the amygdala and the hippocampus have been proposed as possible candidates for functional modulation of information processing within the NAC. The present data further support such hypotheses and underlie the functional importance of a possible modulation of the integrated output of the NAC by endogenous glutamate neurotransmission. The functional link of the allocortico-limbic circuitry to other forebrain structures and the possible role of the hippocampo- and amygdalo-accumbens glutamatergic pathway in the translation of motivation to action (20) remain matters for future investigation.

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