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Separate Neural Substrates of the Locomotor-Activating Properties of Amphetamine, Heroin, Caffeine and Corticotropin Releasing Factor (CRF) in the Rat[1]

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SWERDLOW, N. R. AND G. F. KOOB. *Separate neural substrates of the locomotor-activating properties of amphetamine, heroin, caffeine and corticotropin releasing factor (CRF) in the rat.* PHARMACOL BIOCHEM BEHAV **23**(2) 303–307, 1985.—Destruction of dopamine terminals within the nucleus accumbens (N.Acc.) with 6-hydroxydopamine blocked the locomotor activating properties of d-amphetamine, but not caffeine or corticotropin-releasing factor (CRF). Infusion of muscimol into the region of nucleus accumbens efferent terminals in the substantia innominata and lateral preotic region (SI/LPO) blocked amphetamine-, but not caffeine- or CRF-stimulated locomotion. These muscimol infusions also blocked heroin-stimulated locomotion. These results suggest that amphetamine, acting through a process dependent on N.Acc. dopamine transmission, stimulates locomotion by decreasing GABAergic activity within this N.Acc.-SI/LPO projection; heroin, known to act through a process dependent on N.Acc. opiate receptor activation, also stimulates locomotion by decreasing GABAergic activity within the SI/LPO; caffeine and CRF produce their activation through different neural substrates.

Locomotor activity Amphetamine Heroin Caffeine Corticotropin releasing factor

THE locomotor-activating properties of several indirect psychostimulants are believed to result largely from their ability to increase dopamine (DA) transmission within the nucleus accumbens (N.Acc.). Destruction of dopamine-containing terminals within the N.Acc. following intracerebral injections of 6-hydroxydopamine (6-OHDA) eliminates amphetamine- [8] and cocaine-induced locomotor activation [9]; these lesions also block the locomotor-activating properties of methylphenidate [9], as well as the activation that follows injections of endorphin-compounds into the ventral tegmentum [7,16].

In contrast, other psychostimulants produce locomotor activation that is independent of changes in N.Acc. DA activity. Caffeine, a phosphodiesterase inhibitor, and scopolamine, a muscarinic antagonist, maintain their locomotor-activating properties following 6-OHDA-induced destruction of DA terminals within the N.Acc. [7] as does the opiate agonist heroin [19]. Indirect evidence also suggests that corticotropin-releasing factor (CRF) produces locomotor activation independent of N.Acc. DA transmission: CRF-activation, like caffeine but not amphetamine, is antagonized only by cataleptic doses of the DA antagonist alpha-flupenthixol, and the time course and behavioral profile of CRF-activation resemble those of caffeine, but not amphetamine [11].

Much evidence suggests that locomotor activation following increases in DA transmission within the N.Acc. can be disrupted by increases in GABAergic activity within N.Acc. efferent terminal regions in the ventral pallidum, including the substantia innominata and lateral preoptic area (SI/LPO). Locomotor activation that follows infusion of DA into the N.Acc. is blocked by concomitant infusion of GABA into the SI/LPO [12]. A similar blockade of N.Acc. DA-stimulated activity results from infusion of either GABA or EOS (which inhibits GABA degradation) into regions adjacent to the SI/LPO, within the ventral globus pallidus [14]. Also, infusion of the GABA agonist muscimol into the SI/LPO disrupts the locomotor activation that results from apomorphine-induced stimulation of supersensitive DA-receptors following 6-OHDA-induced denervation of the N.Acc. [18]. One

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explanation for these findings is that N.Acc. DA-stimulated locomotor activity results from a decrease in GABA released from terminals within the SI/LPO; the resulting decrease in GABAergic transmission is opposed by treatments (e.g., GABA, EOS or muscimol) that increase or simulate increases in SI/LPO GABAergic activity. This notion is consistent with existing biochemical [3, 4, 20] and electrophysiological [5,13] data regarding the existence and functional properties of N.Acc.-SI/LPO GABAergic efferents. It is not known, however, whether such a decrease in GABAergic transmission within the SI/LPO serves as a common neural substrate for some or all psychostimulant action.

The purpose of the present study was to examine whether this N.Acc. DA-SI/LPO GABAergic circuitry serves as a general neural substrate for psychostimulant-induced locomotor activation. Specifically, three distinct stimulants—amphetamine, caffeine and corticotropin-releasing factor (CRF) were tested for stimulant action following destruction of DA terminals within the N.Acc., and following infusion of muscimol into the SI/LPO. In addition, heroin-stimulated locomotion, which is not blocked by destruction of N.Acc. DA terminals [19] was examined following muscimol infusion into the SI/LPO.

METHOD

Fifty-six male albino Wistar rats (250–300 g, Charles River Laboratories) were housed in groups of three, and exposed to a normal 12 hr light-dark cycle, with free access to food and water. All rats were anesthetized with pentobarbital (50 mg/kg, IP) and secured in a Kopf stereotaxic instrument with the toothbar 5 mm above the interaural line. The rats were divided into three groups. One group of rats received infusion of either 6-OHDA (8 μg/2 μl, expressed as free base in 0.1 mg/ml ascorbic acid in saline; n=8) or vehicle (0.1 mg/ml ascorbic acid in saline; n=8) into the N.Acc. through 30 ga cannulae at the rate of 1 μl/3 min aimed at coordinates (from bregma) AP +3.2, L ±1.7, DV −7.8 (skull surface). Each animal was then implanted with a 23 ga 7 mm steel cannulae aimed 1 mm above the lateral ventricle at coordinates AP −0.6, L−2.0, DV −3.2 (skull surface), which was fastened to the skull with dental cement and sealed with a 7 mm stylet wire. A second group of animals (n=32) was implanted with bilateral 23 ga 10 mm steel cannulae aimed 3 mm above the SI/LPO at coordinates AP +1.2, L ±1.8, DV −5.6 (skull surface), which were fastened and sealed as above. A third group of animals (n=8) was implanted with both a single cannula aimed above the lateral ventricle as above, and bilateral cannulae aimed above the SI/LPO as above.

Behavioral Testing

Locomotor testing for all animals began one week after surgery using sixteen wire cages as described previously [6]. 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 began, all animals were familiarized with the photocell cages by placing them individually into the cages for 180 min.

On each testing day, animals were placed in the photocell cages for 90 min. Animals that had received infusions of either 6-OHDA or vehicle into the N.Acc. were then treated with either 0.75 mg/kg d-amphetamine sulfate, 10.0 mg/kg caffeine sulfate, or 1 μg CRF on three testing days, each separated by three non-testing days. Amphetamine and caffeine were administered SC in a saline vehicle (1 ml/kg), while CRF was administered intracerebroventricularly in a 2 μl volume. For CRF administration, the wire cannula stylet was replaced with a 30 ga 8 mm steel injector attached to a 1 meter length of plastic tubing filled with CRF, and injected using gravity to propel the infusate [17]. For intracerebral injections, muscimol or saline were injected (1 μl over 2 min) through 30 ga needles fashioned to extend 3 mm beyond the ventral tip of the cannula. These specific doses and methods of administration were chosen because they produce a robust locomotor activation of approximately equivalent magnitude [11,17].

Animals that had been implanted with bilateral cannulae over the SI/LPO were separated into two groups and treated on two consecutive days with either amphetamine (n=8) or caffeine (n=8) given SC following pretreatment with intracerebral 10 ng muscimol or saline vehicle. In order to avoid interpretative difficulties related to repeated opiate treatments, the remaining animals implanted with cannulae over the SI/LPO were treated on one day with heroin (0.25 mg/kg SC) following pretreatment with intracerebral 10 ng muscimol (n=8) or saline vehicle (n=8). Animals (n=8) that had been implanted with both SI/LPO and ICV cannulae were treated with CRF ICV as above on two consecutive days following pretreatment with either muscimol or saline into the SI/LPO. This dose of muscimol has been demonstrated to significantly decrease the locomotor response to apomorphine in N.Acc. 6-OHDA pretreated animals [17]. The order of pretreatment for all animals was balanced such that an equal number of animals within each group received muscimol or saline on each testing day.

Immediately following the above treatments, animals were replaced into the photocell cages, and locomotor activity was recorded for 180 min.

Statistical Analysis

Locomotor activity within N.Acc. 6-OHDA- and vehicle-injected animals was compared using a two factor analysis of variance (ANOVA) with the 6-OHDA and vehicle groups forming the independent factor, and time the repeated measure. Analyses of amphetamine, caffeine and CRF-Locomotion were conducted using a two factor ANOVA with repeated measures on both factors with subjects forming one repeated measure, and time the other. Analysis of heroin locomotion was conducted using a two factor ANOVA with repeated measures on time. Significance was taken at the $p<0.05$ level.

Histology and Biochemistry

Following completion of behavioral testing, all 6-OHDA- and vehicle-injected sham animals were decapitated and the N.Acc. was removed from coronal slices and stored at −70°C until assayed for dopamine using electrochemical detection following separation by high pressure liquid chromatography [9]. All other 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 and stained with cresyl violet. Cannulae sites were assessed without knowledge of the behavioral results.

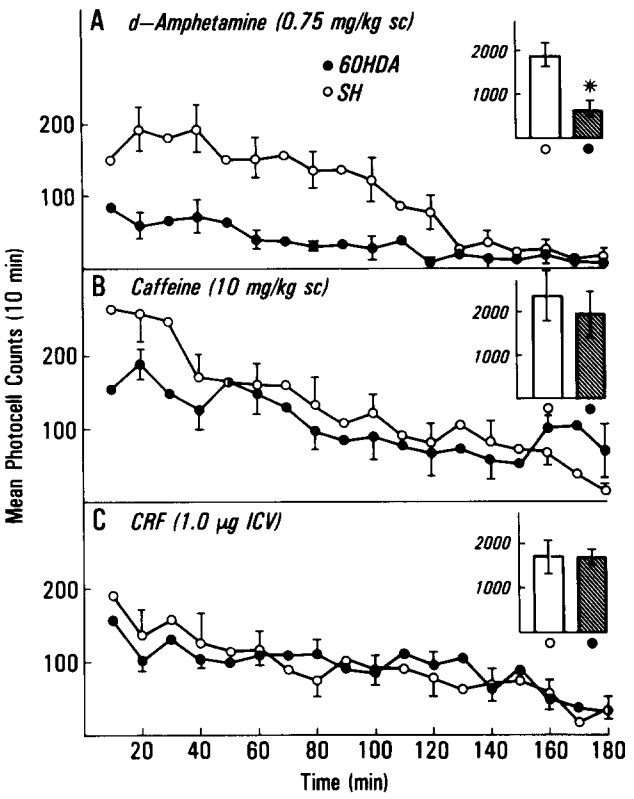

FIG. 1. Locomotor response to (A) 0.75 mg/kg d-amphetamine, (B) 10 mg/kg caffeine and (C) 1.0 μg CRF in rats following sham (○) or 6-OHDA (●) lesions of the nucleus accumbens. Ordinate refers to total photocell counts for each 10 min period of a 180 min test. Insets refer to total photocell counts (means±S.E.M.) for 180 min.

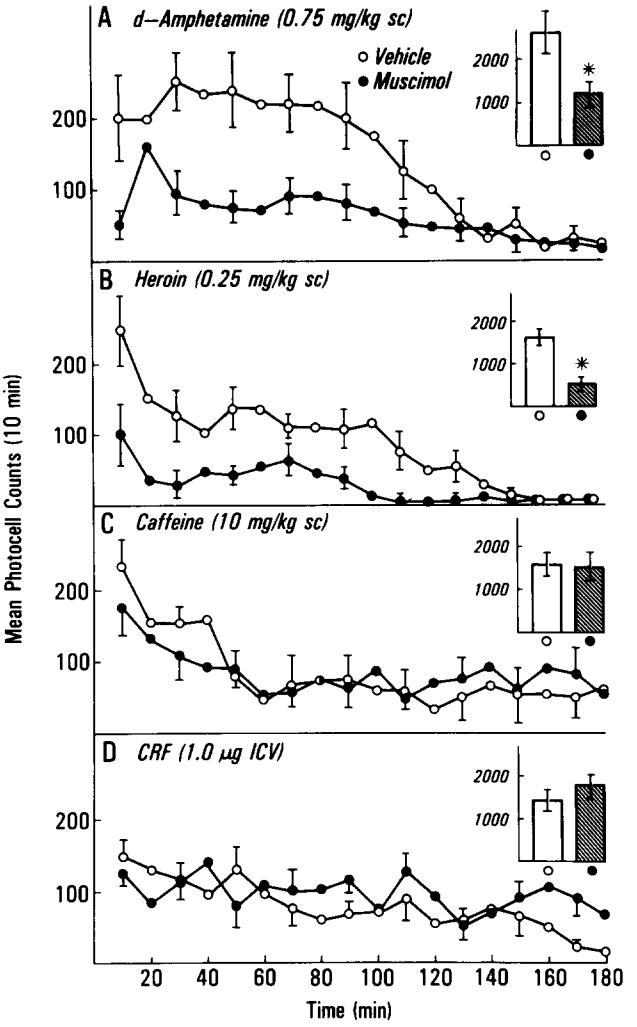

FIG. 2. Locomotor response to (A) 0.75 mg/kg d-amphetamine, (B) 0.25 mg/kg heroin, (C) 10 mg/kg caffeine and (D) 1.0 μg CRF in rats following injection of vehicle (○) or 10 ng muscimol (●) into the SI/LPO region. Ordinate refers to total photocell counts for each 10 min period of a 180 min test. Insets refer to total photocell counts (means±S.E.M.) for 180 min.

RESULTS

The locomotor response to amphetamine, caffeine and CRF in 6-OHDA- and vehicle-injected animals is seen in Fig. 1. Compared to vehicle-injected controls, 6-OHDA-injected rats showed a significantly decreased locomotor response to amphetamine over the 180 min test period, $F(1,15)=10.27$, $p<0.01$ (Fig. 1A), but not to caffeine, $F(1,15)<1$, NS (Fig. 1B) or CRF, $F(1,15)<1$, NS (Fig. 1C). There was, however, a significant lesion × time interaction in caffeine-treated animals, $F(17,238)=2.50$, $p<0.02$, and subsequent analysis revealed that the locomotor response to caffeine in 6-OHDA-injected animals was significantly depressed compared to vehicle-injected controls over the first 40 min of the test, $F(1,15)=5.06$, $p<0.05$. Interestingly, there was a significant positive correlation of 0.61 between the amount of activity observed in all rats during this initial 40 min period and that observed during the pre-caffeine habituation period ($df=14$, $p<0.05$, Pearson correlation), suggesting that the initial depressed response in 6-OHDA-injected animals might not be specific to caffeine treatment, but instead might be a more general lesion effect [13]. 6-OHDA-injected animals averaged 83% depletion of dopamine (11.83±6.7 vs. 63.8±1.7 ng/mg protein; $t(8)=11.68$, $p<0.005$), 63% depletion of DOPAC (10.13±7.5 vs. 27.27±0.3 ng/mg proteins; $t(8)=3.83$, $p<0.005$) and 27% depletion of norepinephrine (1.00±0.3 vs. 1.47±0.1 ng/mg proteins; $t(8)=0.88$, NS) in the nucleus accumbens, compared to vehicle-injected controls. Depletion levels in the anterior caudate were 44.7% for dopamine (80.01±13.1 vs. 144.7±15.5 ng/mg protein; $t(8)=2.64$, $p<0.05$), and 39.3% for DOPAC (34.07±6.1 vs. 56.09±8.1 ng/mg protein; $t(8)=1.92$, NS) compared to vehicle-injected controls; there was no detectable norepinephrine depletion in the anterior caudate.

The locomotor responses to amphetamine, caffeine, heroin and CRF following injection of saline or 10 ng muscimol into the SI/LPO region are seen in Fig. 2. Within-subject comparison revealed that muscimol injections significantly decreased the locomotor response to amphetamine, $F(1,7)=11.01$, $p<0.02$, and heroin, $F(1,15)=10.59$, $p<0.01$, but not to caffeine, $F(1,7)<1$, NS, or CRF, $F(1,7)<1$, NS. There were no significant treatment × time interactions. Histological examination (Fig. 3) revealed some variability in cannula placements, but all injector tips could be localized within the SI/LPO region. Necrotic changes in surrounding tissue were typically limited to the ventral extension of the internal capsule and the postero-lateral extent of the bed

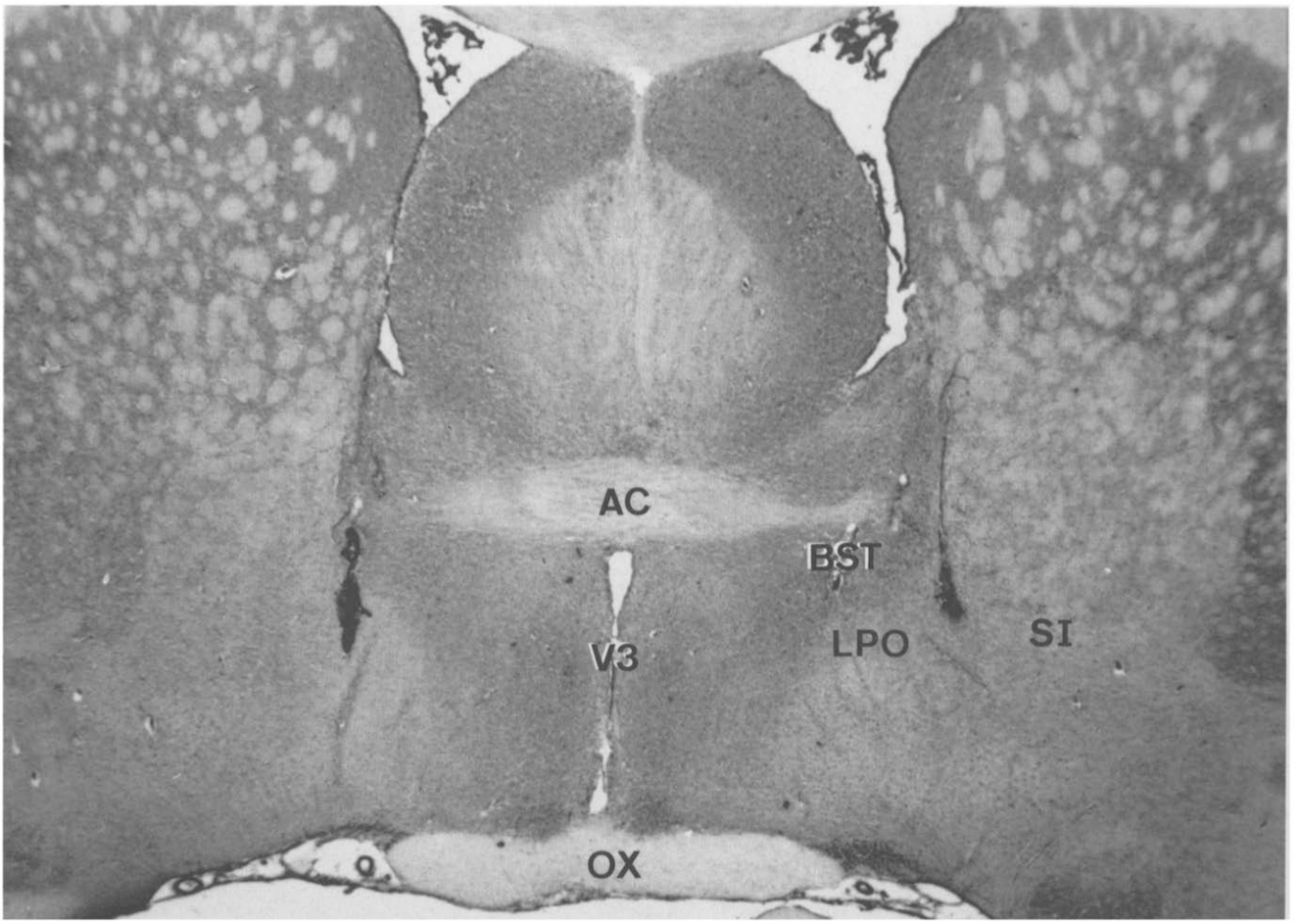

FIG. 3. Photomicrograph of typical injection sites within the SI/LPO region. Extent of necrotic damage varied between animals, with incidental involvement of the ventral globus pallidus, anterior commissure (AC) and bed nucleus of the stria terminalis (BST) similar to this representative section. Other abbreviations: V3—third ventricle; OX—optic chiasm.

nucleus of the stria terminalis. No extensive damage to the globus pallidus was detected in any animal.

DISCUSSION

The present results are consistent with numerous reports that selective depletion of N.Acc. DA blocks amphetamine stimulated locomotor activity. This often replicated finding [6, 8, 9] supports the hypothesis that amphetamine-locomotion results from the release of DA at N.Acc. presynaptic terminals onto DA receptors located within the N.Acc. Our results also support the notion [6], however, that this mechanism cannot be generalized to all psychostimulant-induced activation, since both caffeine- and CRF-induced locomotor activity are not blocked by N.Acc. DA depletion.

The effects of muscimol injections into the SI/LPO on psychostimulant-induced locomotor activity are strikingly similar to those produced by depletion of N.Acc. DA, i.e., amphetamine-, but not caffeine- or CRF-stimulated locomotion is blocked by these injections. These findings suggest that the differences in mechanism of action of these three psychostimulants extend beyond the level of N.Acc. DA transmission. Since amphetamine-locomotion is opposed by GABA-agonist activity in N.Acc. efferent terminal fields in the SI/LPO, it is possible that this locomotion results from a decrease in GABAergic transmission within the SI/LPO region. This mechanism may serve as a general substrate of behavioral activation resulting from enhanced N.Acc. DA activity, since increases in SI/LPO GABAergic stimulation also oppose the locomotor activation that follows direct infusion of DA into the N.Acc. [12,14], as well as the "supersensitive" locomotor response to the DA agonist apomorphine following 6-OHDA-induced denervation of the N.Acc. [18]. Consistent with this mechanism is the finding that injection of the GABA antagonist picrotoxin into the SI/LPO region produces a dose-dependent behavioral activation that is opposed by concomitant injection of GABA [12].

The finding that muscimol injections into the SI/LPO antagonize heroin-stimulated locomotor activity suggests that GABAergic mechanisms within the SI/LPO might serve as one substrate of the behavioral activation produced by opiates. Previous studies have demonstrated that heroin-stimulated locomotion in the rat is antagonized by blockade of opiate receptors within the N.Acc. [1], but not by destruction of N.Acc. DA terminals with 6-OHDA [19]. Apparently, while neural substrates of opiate- and DA-mediated behavioral activation remain dissociable at the level of the N.Acc., both systems may effect similar changes within the ventral pallidum.

The present study provides evidence for some differentiation in the neural mechanisms of psychostimulant action. Thus, amphetamine-activation may depend on two specific sequential neural events: an increase in DA transmission within the N.Acc. and a subsequent inhibition of GABAergic transmission within the SI/LPO region. Heroin-activation, which is known to result from its action on opiate receptors within the N.Acc. [1], also may depend on a subsequent inhibition of GABAergic transmission within the SI/LPO region. In contrast, caffeine and CRF must stimulate locomotor activation independent of these neural changes. Caffeine is a phosphodiesterase inhibitor, but perhaps more importantly an adenosine antagonist and thus its site and mechanism of action may be related to central adenosine receptors [2]. In contrast, the site and mechanism of action of CRF remain unknown, but available evidence suggests that CRF might act totally independent of brain dopamine systems [11]. The similarity of the pharmacological and behavioral profiles of CRF- and caffeine-activation as described previously [10], together with the similarities revealed in the present study suggest that hypotheses as to a common mechanism of action should be considered.

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