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Disruption of Cocaine Self-Administration Following 6-Hydroxydopamine Lesions of the Ventral Tegmental Area in Rats

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ROBERTS, D. C. S. AND G. F. KOOB. *Disruption of cocaine self-administration following 6-hydroxydopamine lesions of the ventral tegmental area in rats.* PHARMAC. BIOCHEM. BEHAV. 17(5)901–904, 1982.—6-Hydroxydopamine-induced destruction of dopaminergic terminals in the nucleus accumbens have been shown previously to disrupt cocaine and amphetamine self-administration. We sought to determine whether lesions of the DA cell bodies in the ventral tegmental area (VTA) which give rise to the DA innervation of the n. accumbens, would also disrupt cocaine self-administration behavior. Rats were trained to self-inject cocaine (0.75 mg/kg) for 4 hr/day. After a stable baseline was established, one group of rats received bilateral injections of 6-OHDA (4 μg/1 μl) into the VTA. Control rats received vehicle injections. When retested on the fifth day post-lesion, all of the 6-OHDA treated animals showed a long lasting reduction in cocaine intake. Three animals did not reinitiate cocaine self-administration after the lesion, although each showed stable post-lesion responding for apomorphine. The surgery had no effect on cocaine self-administration in control animals. These data support the hypothesis that dopaminergic mechanisms are necessary for the normal expression of cocaine self-administration.

Cocaine Self-administration 6-OHDA lesions Ventral tegmental area

GREAT strides have been made in identifying the mechanisms of action of the psychomotor stimulants, amphetamine and cocaine. In vitro studies have shown that amphetamine causes the release of noradrenaline (NA) and dopamine (DA) and both stimulants block the reuptake of these catecholamines. These effects result in a potentiation of the action of NA and DA at their respective synapses [6,13]. Amphetamine and cocaine are therefore indirect acting agonists, requiring the intact presynaptic catecholamine (CA) element.

The ability to selectively destroy catecholamine cell bodies, fiber systems and terminals through the use of 6-hydroxydopamine (6-OHDA) has made possible the investigation of the anatomical location of the behavioral effects of stimulants such as locomotor activity [8], stereotypy [9] and anorexia [1].

In an effort to identify the specific CA pathways which may be responsible for cocaine reinforcement, we have embarked on a systematic study of the effects of 6-OHDA lesions to CA systems on cocaine self-administration. In our initial study [17], we reported that lesions to the dorsal and ventral NA projections in the mid-brain had no effect on the rate or pattern of cocaine self-administration.

By contrast, injections of 6-OHDA to the nucleus accumbens caused a marked disruption of cocaine self-administration. In a subsequent report [18], it was observed that this behavior could recover to pre-lesion levels unless a sufficient DA loss was achieved. However, if the animals were pretreated with a monoamine oxidase inhibitor to enable a more complete depletion of DA, and if the animals were tested after degeneration was complete, extinction-like behavior was observed. Self-administration of apomorphine was not affected in these same animals. Lyness *et al.* [12] have also reported a similar effect on amphetamine self-administration following 6-OHDA lesions of the nucleus accumbens. These data suggest that the DA innervation of the nucleus accumbens, or a DA projection through it, is necessary for the normal expression of amphetamine and cocaine reinforcement.

This hypothesis would therefore predict that lesions to the DA cell bodies which innervate the nucleus accumbens and limbic cortex should also disrupt cocaine self-administration. We now report that 6-OHDA lesions to these cells in the ventral tegmental area (VTA) do affect cocaine intake, however the degree of this disruption may not be due to denervation of the nucleus accumbens.

METHOD

Male Wistar rats (Charles River) were 23 hr/day food deprived for several days, then trained to press a lever for food reward (Noyes Pellets). This procedure facilitated subsequent acquisition of drug self-administration by increasing the likelihood of lever pressing behavior. Each rat was then anesthetized with a combination of chloral hydrate and pentobarbitol (Equithesin) and implanted with a jugular cannula as previously described [17]. Briefly, a silastic tube was inserted into the vein, led subcutaneously to the back, and brought out at the mid-scapula level. The cannula was fed through a protective spring to a fluid swivel which was in turn connected to a syringe pump. The animals were housed in individual test chambers with ad lib food and water, for the duration of the experiment.

Each day a removeable lever was introduced to the cage which produced, when depressed, a 4 sec infusion of cocaine (0.75 mg/kg/injection). Each infusion was signalled by a 20 sec stimulus light. Responses made during this period had no programmed effect. All animals were given daily access to cocaine until a stable pattern of self-administration behaviour was acquired. This was defined as a regular response rate with less than 15% variability in cocaine intake on four consecutive days. The animals were then prepared for 6-OHDA or control treatments. The cannulae were flushed with saline and the rats received no drugs on the day prior to surgery. All animals were pretreated with pargyline (50 mg/kg) one half hour prior to induction of anesthesia with Equithesin, after which the rats were introduced to a Kopf stereotaxic instrument, equipped with blunt (guinea pig) ear bars.

Rats in the experimental group received 6-OHDA hydrobromide (4 μg/1 μl, dose expressed as the free base) aimed at the ventral tegmental area. The injection coordinates were as follows: 2.8 mm anterior to the interaural line, $\pm$0.5 mm lateral to the midline, and 8.7 mm ventral to the skull surface. The incisor bar was positioned 5.0 mm above the interaural line which resulted in the animals head being held in the plane of section of Pellegrino and Cushman [14]. Bilateral injections were made simultaneously through parallel 30 ga needles held 1.0 mm apart, at a rate of 1.0 μl/2 min. The approximate weight of the animals at the time of the stereotaxic procedure was 350 g. Four control animals were treated identically except that the 6-OHDA was omitted from the injection solution.

The animals were returned to the test chambers and allowed to recover for four days without access to drug. The cannulae were flushed daily with saline. On the fifth day post-lesion, animals were again given the opportunity to self-administer cocaine. Testing continued for several weeks, depending on the patency of the cannulae. A number of animals were dropped from the study because they did not show stable responding prior to surgery, or became unhealthy due to clots in the heart caused by the cannula implants. These animals were replaced, and data are based on 14 experimental rats which remained healthy with patent cannulae for at least seven days of post-lesion testing. The animals were then sacrificed by decapitation, and the brain rapidly removed and dissected on ice. Dopamine was measured in the nucleus accumbens by the method of Coyle and Henry [1].

RESULTS

6-Hydroxydopamine infusions into the ventral tegmental area were found to produce a decrease in cocaine intake in all animals tested. However, the degree to which each animal was affected was extremely variable. Three animals ceased taking cocaine after the lesion, only one of these displayed behavior somewhat resembling extinction, that is, an increase in response rate followed by cessation (left panel, Fig. 1). The other two simply stopped after one or two days after the drug was available following the lesion. Each of these three animals appeared healthy. All were offered apomorphine (0.1 mg/kg/injection) to self-administer, and each self-injected this drug in a steady and reliable fashion. Because these rats were not tested for apomorphine self-administration prior to the lesion, it was not possible to determine whether the lesion produced an alteration in rate of apomorphine intake. One of the three animals which ceased cocaine self-administration subsequently also displayed regular heroin intake.

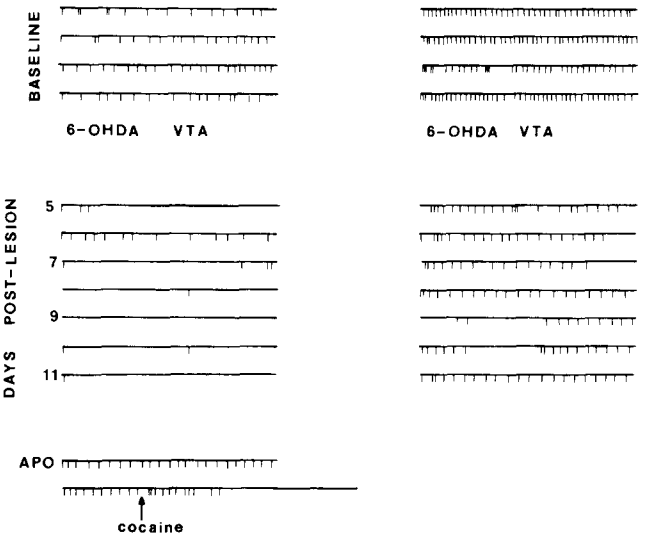

FIG. 1. Event records of cocaine self-administration behavior from two rats which received bilateral injections of 6-OHDA into the ventral tegmental area. Each line represents one daily 4 hr session. Each pen deflection represents a drug infusion. The bottom two lines show apomorphine self-administration. Cocaine was substituted for apomorphine at the time indicated by the arrow.

Following the lesions, eleven of the fourteen animals continued to self-administer cocaine at a reduced rate. An example of this response is shown in the right hand panel of Fig. 1. These animals showed a reduced rate on the first day of testing (post-lesion day 5), and this rate did not change for the duration of testing. In one animal, this reduced rate was identical six weeks after the 6-OHDA treatment.

Pharmacological pretreatment with DA receptor blockers or inhibition of CA synthesis have been widely reported to increase stimulant self-administration [3, 4, 15, 19, 20, 21], and these data have been interpreted as evidence of dopaminergic involvement in psychomotor stimulant reward [20,21]. Modulation of stimulant intake appears to be specific to DA systems, as treatments which block NE receptors [4,6], destroy NE pathways [17] or inhibit serotonin synthesis [PCPA, Roberts and Fibiger, unpublished observations], fail to specifically affect drug intake.

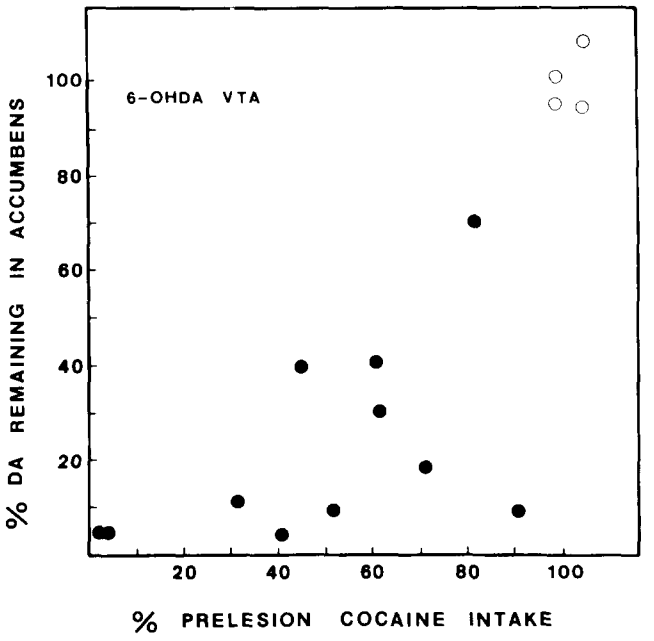

FIG. 2. Relationship between the rate of cocaine self-administration following bilateral infusions of 6-OHDA into the VTA and the dopamine (DA) content of the nucleus accumbens. Open circles represent control animals. The Pearson r was calculated to be 0.79 with all values included, and r=0.51 with control values omitted.

The four animals which received vehicle injection into the VTA showed no alteration in self-administration behavior, and continued a reliable and stable pattern of cocaine intake.

The neurochemical effect of the 6-OHDA lesions was assessed by measuring the reduction of DA from the nucleus accumbens. Three tissue samples were lost in processing; two from animals which reduced their intake to 40% and 55% and one from the animal which showed extinction. Analysis of the remaining samples revealed a depletion of DA to 22.4±6.7% of control levels (2.0 ng/mg protein).

The depletion of DA from the accumbens was somewhat variable. In an effort to find a relationship between the effectiveness of the lesion and disruption of cocaine self-administration, a correlational analysis was undertaken. The percentage of DA remaining in the accumbens and the average post-lesion cocaine intake (expressed as a percentage of pre-lesion rate) was found to be r=0.79 ($p<0.05$, $df=13$). These data are presented graphically in Fig. 2. If the control animals are excluded from the analysis the correlation falls to r=0.51 ($df=10, p>0.05$). It is apparent from the figure that several animals sustained a severe depletion of DA yet continued to take cocaine at high rates.

DISCUSSION

The present results show that 6-OHDA infusion into the VTA can cause severe and long lasting reductions in cocaine self-administration. These data would seem to support the hypothesis that dopaminergic systems may be critically involved in the rewarding effects of psychomotor stimulants. In an earlier report [18] 6-OHDA infusions into the nucleus accumbens were shown to cause extinction of cocaine self-administration. We have since observed that similar infusions of 6-OHDA dorsal to the accumbens, or into the head of the caudate have no effect on cocaine self-administration (Roberts and Koob, unpublished observation). These data therefore indicate that the DA innervation of the accumbens, or a DA projection through this nucleus, is critical for the normal expression of cocaine reward.

The DA cell bodies in the ventral tegmental area (VTA) are the principal source of DA innervation of the accumbens and the DA fibers of passage through it. We hypothesized, therefore, that lesions of this area should also disrupt cocaine self-administration. The present results support this hypothesis. In all animals, reductions in cocaine intake were observed; in some animals, cocaine no longer supported self-administration behavior.

In the present study we used depletion of DA in the nucleus accumbens as an index of the degree of damage to the mesolimbic system. If the DA innervation of the accumbens is indeed critical for maintenance of normal self-administration behavior, then the DA depletion should correlate with the degree of change in cocaine intake. Contrary to this prediction, there was a positive but nonsignificant correlation and several animals with severe accumbens DA loss showed near normal cocaine intake. These data indicate the possibility that the DA innervation of some other structure or combination of structures is critical. Possibly the DA innervation of the olfactory tubercle, frontal cortex or amygdala contribute to behavioral effects of cocaine. Analysis of DA content in these areas following accumbens or DA lesions which disrupt cocaine self-administration will be required before this question can be addressed directly.

One other possible explanation should be noted. Hökfelt *et al.* [4] have reported that the mesolimbic DA system may also contain cholecystokinin (CCK), a peptide putative neurotransmitter. Some, but not all, of the DA cells in the VTA also contain CCK, and further, 6-OHDA lesions deplete forebrain CCK. While it is not yet known if cocaine and/or amphetamine have effects on CCK release or turnover, CCK may be important to the action of these psychomotor stimulants. It is possible that CCK and not DA depletion is critical to the reinforcing effects of cocaine and previous correlations of DA depletion and disruption of cocaine self-administration simply reflect the close association of CCK and DA cell bodies.

Colpaert *et al.* [5] have presented pharmacological evidence that the discriminative stimulus properties of cocaine may not be dopaminergic and suggest a possible role for β-phenylethylamine. Whether the discriminative stimulus and rewarding properties of cocaine are both subserved by the same neurochemical mechanisms is unknown, however both properties would appear necessary for the normal maintenance of self-administration behaviour. Manipulation of either the discriminability of the drug injection or of its rewarding effects must necessarily affect drug intake.

Finally the present demonstration that VTA lesions produce an apparent diminution in cocaine intake are in apparent contrast to the work of Le Moal *et al.* [11]. They showed that rats with radio frequency (r.f.) lesions of the VTA displayed a hypersensitivity to amphetamine and showed a more rapid acquisition of amphetamine self-administration behavior than controls. These differences are most probably accounted for by the lesioning procedures employed in the two studies. Le Moal *et al.* [11] point out that their r.f. lesion produces an imcomplete loss of DA cells

which may induce an overactivity in the remaining neurones. The 6-OHDA procedure, on the other hand, produces a more complete and selective destruction of DA cell bodies. Koob *et al.* [10] have recently studied the differences between r.f. and 6-OHDA lesions of the VTA with respect to spontaneous and drug-induced locomotor activity. It was confirmed that rats with r.f. lesion of the VTA remain sensitive to the stimulant actions of amphetamine, however additional damage to the DA system by 6-OHDA injection into either the nucleus accumbens or VTA produced a hyposensitivity to amphetamine.

The present results indicate that the VTA may be critical for psychomotor stimulant reward, in that near complete destruction of the DA cell bodies can cause cessation of cocaine self-administration. Partial destruction following r.f. lesions may produce a hypersensitivity to stimulant drugs and, as Le Moal *et al.* [11] have suggested, provide a neurobiological model for predisposition to drug abuse.

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