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Circling Behavior in Rats with Partial, Unilateral Nigro-Striatal Lesions: Effect of Amphetamine, Apomorphine, and DOPA

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HEFTI, F., E. MELAMED, B. J. SAHAKIAN AND R. J. WURTMAN. *Circling behavior in rats with partial, unilateral nigro-striatal lesions: Effects of amphetamine, apomorphine and DOPA*. PHARMAC. BIOCHEM. BEHAV. 12(2) 185–188, 1980.—Partial, unilateral lesions of the nigro-striatal tract were produced in rats by injecting various quantities of 6-hydroxydopamine into the substantia nigra. The extent of each animal's lesion was estimated by comparing tyrosine hydroxylase activities in its lesioned and control striata. L-DOPA and apomorphine induced contralateral (i.e., away from the lesion) circling behavior only in rats in which more than 90% of the nigro-striatal system had been destroyed. In contrast, d-amphetamine caused turning in the ipsilateral direction when as few as 50% of the nigro-striatal neurons had been destroyed.

Circling behavior Rats Nigro-striatal neurons Dopamine Apomorphine DOPA Amphetamine 6-Hydroxydopamine

THE rotational behavior observed in rats with unilateral lesions of the dopaminergic (DA) nigro-striatal tract, induced by injecting 6-hydroxydopamine (6-OH-DA) in the substantia nigra, is widely used to test the effects of drugs on DA mechanisms [4,6]. DA-receptor agonists, like apomorphine, cause the animals to turn contralaterally (i.e., away from the lesioned side); this is attributed to the direct stimulation of supersensitive postsynaptic receptors on the lesioned side [16]. L-DOPA (after having been decarboxylated in surviving DA neurons or in other striatal cells) also elicits contralateral circling in such animals, presumably by increasing the amounts of DA ipsilateral to the lesion [16]. In contrast, amphetamine causes ipsilateral circling by acting presynaptically, presumably by releasing DA from nigro-striatal terminals, which are more abundant on the unlesioned side [15,16]. The circling behavior caused by asymmetric activation of DA receptors can be influenced by lesions in other brain areas [4,6] and appears to be mediated by striato-nigral projections [10]; however, the exact neuronal mechanism leading to its expression remains unknown. In most investigations, rotational behavior has been studied in animals with near-total nigro-striatal lesions. We now describe the induction of turning behavior by apomorphine, DOPA, and amphetamine in rats with partial nigro-striatal lesions of varying degrees of severity. Each animal was tested with all three drugs, and the extent of lesions produced in any particular animal ranged from virtually none to virtually total. We observe that much smaller lesions render rats susceptible to amphetamine-induced ipsilateral circling than those needed to produce the contralateral circling induced by drugs acting directly on DA receptors.

METHOD

Male Sprague-Dawley rats (150–200 g; Charles River Breeding Laboratories, Wilmington, MA) had free access to water and Big Red Rat Chow (Charles River). To produce unilateral nigro-striatal lesions with various degrees of severity, 74 rats were injected with different amounts of 6-OH-DA into the right anteromedial substantia nigra (anteroposterior level A2420, according to the atlas of König and Klippel [7], −2.6 mm dorsoventral, 1.6 mm mediolateral). Eight, 4, 2, 1, or 0.5 μg of 6-OH-DA (6-OH-DA·HBr free base [Sigma Chemical Company, St. Louis, MO] dissolved in 4 μl of saline containing 0.2 mg/ml ascorbic acid) were injected into each animal over a 2-min period, using a 10 μl syringe (Unimetrics; Bradford Scientific, Marblehead, MA); the syringe was retrieved 3 min after the injection was terminated. Nine additional animals received injections of 8 μg of 6-OH-DA into the substantia nigra, immediately followed by a second injection of 4 μg of 6-OH-DA (in 2 μl saline) into the right medial forebrain bundle at the level of the posterior hypothalamus (anterior level A 3990, −2.7 mm dorsoventral, 1.8 mm mediolateral [8]). Between 6 and 14 days after the 6-OH-DA injections, the rats were tested for circling behavior in a metal cylinder (35 cm dia.) with a flat bottom. Ungerstedt [15,16] has shown that circling behavior is fully de-

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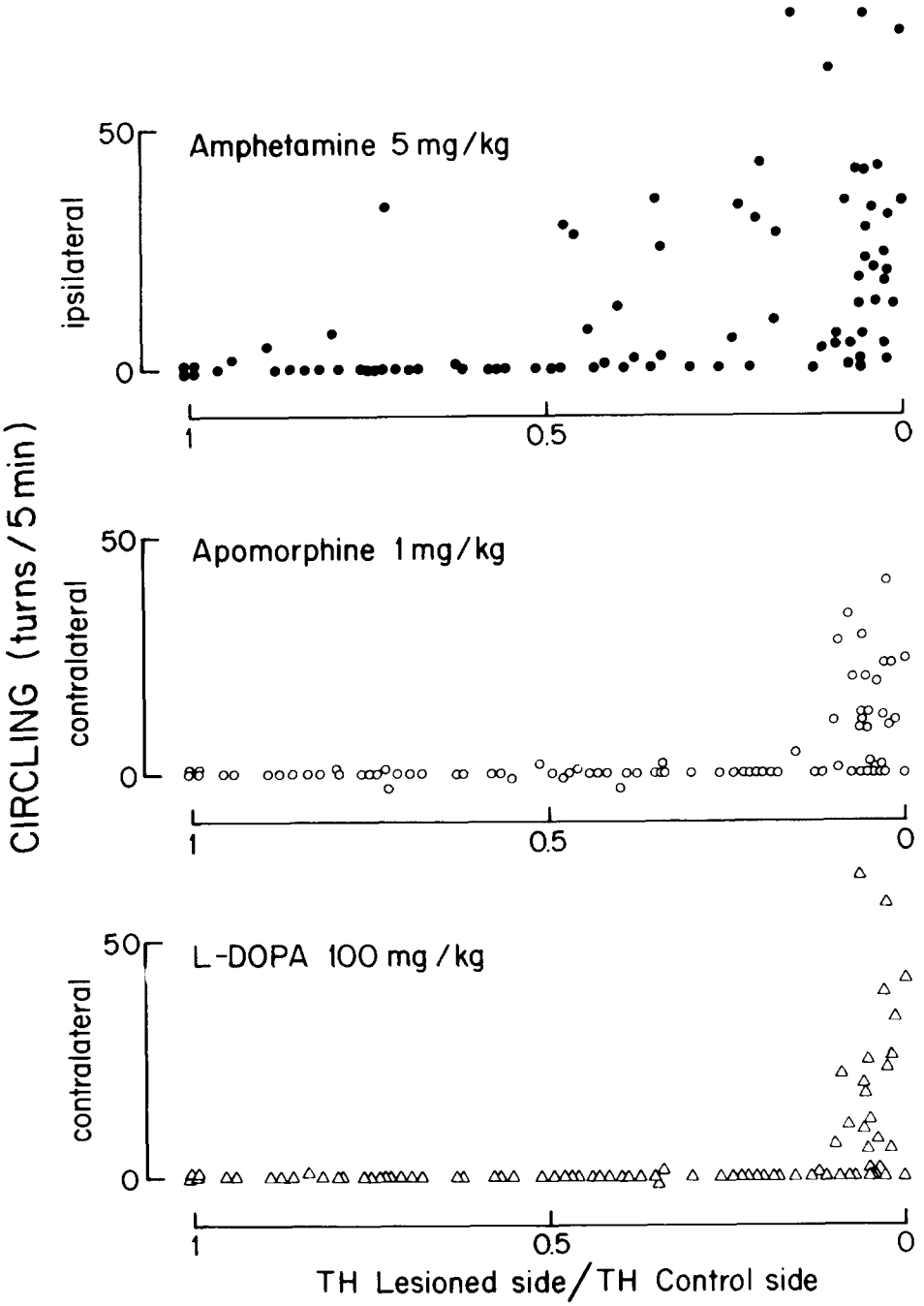

FIG. 1. Rotational behavior in rats with partial, unilateral lesions of the nigro-striatal tract induced by injecting various amounts of 6-OH-DA into the anteromedial substantia nigra. The extent of the lesion is expressed as ratio between the striatal TH activities of the injected side and the contralateral side in each animal. Circling (total number of complete, full-body turns) was counted during the 5-min interval between 15 and 20 min after apomorphine injection, and between 40 and 45 min after amphetamine or L-DOPA injection.

veloped 3 days after unilateral 6-OH-DA lesions. The total number of full-body turns (complete rotations) was observed and counted for a 5-min interval, i.e., between 15 and 20 min after apomorphine injection (1 mg/kg IP) and between 40 and 45 min after d-amphetamine (5 mg/kg IP) and L-DOPA (100 mg/kg IP) injections. These observation intervals corresponded to those of maximal drug effects, as reported earlier [15–17]. Drugs (Sigma) were dissolved in saline; 0.2 mg/ml of ascorbic acid were added to the apomorphine solution. Each drug was tested once in each animal, with at least 2 days intervening between injections. The sequence of drugs given any animal was randomized. Rats were killed 2 weeks after the 6-OH-DA injections; brains were removed, corpora striata were frozen on dry ice, and tyrosine hydroxylase (TH) activity in each was assayed [13,18].

RESULTS

The dose of 6-OH-DA injected into each rat's substantia nigra paralleled the subsequent reductions in its striatal TH activity ($r=0.84$; $p<0.01$); however, at doses between 0.5 and 4 μg, the within-group variations in TH activity were large. In rats receiving two 6-OH-DA injections into both the substantia nigra and the medial forebrain bundle, striatal TH activity was reduced by more than 94% compared to that in the control side. In another study, we showed that striatal TH activity can be used in place of DA levels to assess the extent of the nigro-striatal lesion in any particular animal: TH activities and DA levels exhibit a linear correlation ($r=0.91$) in lesioned rat striata (Hefti *et al.*, in preparation).

Apomorphine and DOPA, the postsynaptically active drugs [16], induced contralateral circling only in animals (20 out of 30 rats in this group) in which TH activity in the lesioned side had been reduced by more than 90% compared to that of the control side (Fig. 1). Amphetamine, which acts presynaptically to release DA from DA terminals [11,15], was able to induce ipsilateral circling in animals with considerably less severe nigro-striatal lesions. Out of 30 animals with reductions in TH activity exceeding 90%, 25 circled after this drug. However, when TH activity had been reduced only by 50–90%, 14 of 26 animals exhibited circling behavior after amphetamine injection, while only one circled also after apomorphine, and none circled after DOPA injection; no circling behavior was observed in the other 12 animals in this group. The mean decrease in TH activities of all animals circling after amphetamine but not after apomorphine or DOPA administration was 73.6 ± 5.0% (mean ± SEM, n=25); among animals circling after all three drugs, TH activity was reduced by 95.4 ± 0.9% (n=13; different from previous group by $p<0.001$; t-test).

The test doses of these drugs produced equal intensities of turning behavior. Among animals circling after all three drugs, amphetamine induced 23.6 ± 4.1 (n=13), apomorphine 21.3 ± 2.3, and DOPA 21.6 ± 4.1 turns/5 min (mean ± SEM). Seven animals circling only after amphetamine administration were given a higher dose of apomorphine (5 mg/kg) to assess whether the test dose (1 mg/kg) might not have reached the threshold level needed to induce circling behavior. Amphetamine induced 34.9 ± 12.1 ipsilateral turns/5 min (mean ± SEM) in this group; however, none of the animals showed contralateral turning even after the higher dose of apomorphine.

DISCUSSION

Rotational behavior was initially observed by Anden *et al.* [2] in rats with unilateral electrolytic lesions that destroyed the nigro-striatal pathway. In subsequent studies, Ungerstedt [15,16] showed that the unilateral injection of the neurotoxin 6-OH-DA into the substantia nigra produced both rotational behavior and the loss of catecholamine-induced histofluorescence in the striatum. In mice, apomorphine and DOPA induced circling only in animals in which striatal DA levels had been reduced by more than 80% [14]. Rats with lesions of various degrees, produced by injecting 8 μg of 6-OH-DA in different locations near the nigro-striatal neurons, circled in response to amphetamine or apomorphine only if DA levels, analyzed subsequently, were reduced by at least 60% [3]. In our study, using rats injected with various amounts of 6-OH-DA into the same location within the substantia nigra, slightly larger reductions in DA terminals were needed to allow turning after apomorphine or DOPA administration. The nigro-striatal neurons have a limited projection area within the striatum, and there is a topographical relationship between the position of the cell bodies in substantia nigra and the projection areas in the striatum [8,9]. The method we used to produce lesions, i.e., injecting 6-OH-DA into the anteromedial substantia nigra, where the axons assemble, probably creates lesions that are more homogenous than those caused by injecting a large amount in marginal areas. This latter technique probably destroys the dopaminergic input to a limited but well-circumscribed area within the striatum. Receptor supersensitivity within such an area might then be sufficient to allow drug-induced circling behavior.

Our study with partial lesions characterizes for the first time lesioned animals in which amphetamine, but not apomorphine or DOPA, can induce turning behavior. In rats exhibiting this response to amphetamine, TH activity in striata ipsilateral to the lesions was reduced by 50–100%. It has been reported that amphetamine can induce circling behavior in even unlesioned animals [5]. However, this phenomenon probably does not account for our findings, since the frequency of turning was much lower in normal rats [5] than in rats with partial lesions given the same dose of amphetamine. Furthermore, amphetamine did not induce significant circling behavior in our animals with mild nigro-striatal lesions (TH activity reduced by 0–25%; Fig. 1). It does not appear that apomorphine's or DOPA's failure to cause turning in partly lesioned animals was due to the use of subthreshold drug doses, since even a very high dose of apomorphine (5 mg/kg) did not induce circling. Furthermore, the doses used in our study have been reported to produce near-maximal intensity of circling behavior [16,17], and in rats responding to all three drugs, equal amounts of turning were elicited by all three drugs. Partial lesions of the nigro-striatal tract increase DA synthesis [1] and release (Hefti *et al.*, in preparation) in the surviving neurons, which are thereby able to compensate partially for the neuronal loss. The present study's results suggest that this mechanism is able to prevent the development of postsynaptic DA-receptor supersensitivity in animals with lesions destroying up to 90% of the DA neurons. In rats with partial 6-OH-DA-induced lesions, we found compensatory increases in DA released from surviving neurons when lesions have TH activity reduced by 40% or more (Hefti *et al.*, in preparation), close to the extent of lesioning necessary to allow circling after amphetamine administration only. Amphetamine reportedly releases endogenous DA, preferentially from a newly synthesized pool [11]. The remaining neurons of a partly-lesioned nigro-striatal system might already be synthesizing

and releasing transmitter at full capacity, thus limiting amphetamine's additional effect. The resulting imbalance in DA release by amphetamine within intact and partially-lesioned striata would then cause circling toward the lesioned side.

Compensatory increases in DA release also appear to occur in surviving nigro-striatal neurons in patients with Parkinson's disease [12]. Hence, rats with partial, unilateral nigro-striatal lesions of varying severity might be good experimental models for moderate forms of this neurological disorder.

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