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Sensitization of Rotation Behavior in Rats With Unilateral 6-Hydroxydopamine or Kainic Acid-Induced Striatal Lesions

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NORMAN, A. B., L. M. WYATT, J. P. HILDEBRAND, M. KOLMONPUNPORN, C. A. MOODY, M. N. LEHMAN AND P. R. SANBERG. *Sensitization of rotation behavior in rats with unilateral 6-hydroxydopamine or kainic acid-induced striatal lesions*. PHARMACOL BIOCHEM BEHAV **37**(4) 755–759, 1990.—Following unilateral 6-hydroxydopamine (6-OHDA) lesions of the substantia nigra or unilateral kainic acid (KA) lesions of the striatum rats displayed rotation behavior in response to apomorphine (0.25 mg/kg SC or 1 mg/kg SC for the 6-OHDA- and KA-lesioned rats respectively). Three to five days following the initial apomorphine challenge rats were challenged under identical conditions with the same dose of apomorphine received previously. Both 6-OHDA- and KA-lesioned rats demonstrated a significant increase in the total number of rotations. Following a subsequent challenge with apomorphine, rats showed further increases in the total number of rotations. With the second and the subsequent apomorphine challenges there were significant increases in the maximal number of rotations, a significant decrease in the time of onset of rotation behavior and in some cases an increase in the duration of the rotation behavior. These increases in rotation behavior following repeated challenges with apomorphine indicate a supersensitivity to dopamine receptor agonists distinct from that elicited by lesions and chronic antagonist treatments. Furthermore, the utility of the rotation behavior model for testing the efficacy of dopaminergic agonists might be compromised if repeated challenges in individual animals are employed.

Apomorphine Rotation behavior Supersensitivity 6-Hydroxydopamine Excitotoxin lesions Striatum Kainic acid

DOPAMINE deafferentation of the basal ganglia elicits an increase in the sensitivity of neurons to dopamine receptor agonists (23,24) and a concomitant upregulation of postsynaptic dopamine receptors (7, 8, 23). Following unilateral deafferentation of the basal ganglia, the behavioral response to dopamine receptor agonists is rotation produced by the asymmetry in dopaminergic neurotransmission with one side of the brain being hypersensitive while the contralateral side remains normosensitive. Following excitotoxin lesions of the striatum, the loss of postsynaptic neurons which express dopamine receptors produces a deficit in dopaminergic neurotransmission and following unilateral lesions, the asymmetry in dopaminergic neurotransmission again results in rotation behavior in response to dopamine receptor agonists (15, 16, 19).

Rotation behavior in response to dopamine receptor agonists in dopaminergic deafferented or in excitotoxin-lesioned rats is consequently widely used in the investigation of dopamine-mediated responses in the basal ganglia. During our preliminary investigations of apomorphine-induced rotation behavior in 6-hydroxydopamine (6-OHDA)- and in kainic acid (KA)-lesioned rats, we observed an increase in the rotation behavior following repeated challenges with apomorphine (25). We report herein that repeated challenges with the dopamine receptor agonist apomorphine produce a significant increase in the behavioral effects of

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TABLE 1

EFFECT OF REPEATED CHALLENGES WITH APOMORPHINE ON THE TOTAL NUMBER OF ROTATIONS IN RATS WITH UNILATERAL 6-HYDROXYDOPAMINE OR KAINIC ACID-INDUCED LESIONS

	Total Number of Rotations			
	Trial 1	Trial 2	Trial 3	Trial 4
6-OHDA (n=9)	236 ± 93	554 ± 147	884 ± 213*	940 ± 220*
Kainic Acid (n=20)	26 ± 21	96 ± 23*	137 ± 27†	N.A.

Rats received either unilateral 6-OHDA (8 μg) lesions of the substantia nigra or unilateral KA (5 nmol) lesions of the anterior striatum. Eleven days following the 6-OHDA lesion or 28 days following the KA lesion rats were injected with apomorphine (0.25 mg/kg SC or 1 mg/kg SC for the 6-OHDA- and KA-lesioned rats, respectively) and the rotations counted visually for the total duration of the rotations. The procedure was repeated for each individual rat a further two or three times with a three- to five-day interval between trials. Values represent the mean ± S.E.M. total number of rotations from the number of animals shown in parentheses. Significantly different from Trial 1, *$p<0.05$, †$p<0.01$, ANOVA.

apomorphine in both 6-OHDA- and KA-lesioned rats.

METHOD

Surgical Procedures

Male Sprague-Dawley rats (200–300 g) were used in all experiments. Rats were housed with free access to food and water on a 12-h light/dark cycle. All testing was during the light phase of the cycle. Rats were anesthetized with pentobarbital (65 mg/kg, IP) and mounted in a Kopf stereotaxic frame. One group of rats received an injection of 8 μg 6-OHDA (Sigma Chemical Co.) in 4 μl 0.9% saline containing 0.02% ascorbate. The solution was injected over 4 min and the needle left in place an additional 5 min before withdrawal. Stereotaxic coordinates were AP = 4.4 mm posterior, ML = 0.9 mm relative to bregma, and DV = 7.5 mm from dura according to atlas of Paxinos and Watson (17). The incisor bar was set at 2.3 mm below the interaural line. A second group of rats were lesioned with KA (5 nmol; Sigma Chemical Co.) in 1 μl 0.9% saline. The solution was injected over 1 min and the needle left in place an additional 2 min before withdrawal. Stereotaxic coordinates were AP = 1.5 mm anterior, ML = 2.3 mm relative to bregma, and DV = 5.1 mm from dura. The incisor bar was set at 0 mm.

Behavioral Testing

Behavioral testing began for the 6-OHDA-lesioned rats after eleven or thirty days and for the KA-lesioned rats after four to six weeks.

Animals were individually placed in an open field environment consisting of a Plexiglas box (dimensions: 30 × 30 cm) and left to habituate for 20–30 min. Animals were then injected with apomorphine (Sigma Chemical Co.) dissolved in normal saline containing 0.07% ascorbate. 6-OHDA-lesioned rats were administered 0.25 mg/kg apomorphine, SC; KA-lesioned rats were administered 1.0 mg/kg, SC. Rotations were continuously counted visually in five-minute periods until all rotation behavior ceased. Rotations were defined as complete 360° turns and were reported as the net difference between the two directions. Apomorphine-induced rotation behavior was measured for each rat under identical conditions at three- to five-day intervals until there was no further significant increase in the number of rotations. Three to five days following the last apomorphine challenge rats were challenged with the vehicle solution alone under identical conditions.

Statistical analysis of rotational behavior used analysis of variance (ANOVA).

RESULTS

6-OHDA-Lesioned Rats

When the rats were placed into the open field environment, no spontaneous rotation behavior was observed during the 20–30-min habituation period. Following all injections with apomorphine (0.25 mg/kg), the rats displayed rotations contralateral to the lesioned side. As shown in Table 1, the total mean number of rotations was 236 following the first challenge with apomorphine. Three days following the initial challenge, the procedure was repeated exactly as before using an identical dose of apomorphine from the same batch of drug. The rats again rotated contralaterally, but the mean number of rotations increased to 554. Following a third identical apomorphine challenge, the number of rotations significantly increased ($p<0.05$) from those observed following the first and second trials. Following the fourth challenge with apomorphine, there was no significant increase in the total number of rotations from that observed in trial three.

As shown in Fig. 1, following the initial challenge with apomorphine, some of the rats began rotating within the first five-min interval, though it was not significantly different from zero ($p>0.05$). This was followed by a rapid increase in the number of rotations until the mean peak magnitude was reached during the third to the fifth five-min period. Following subsequent challenges with apomorphine, where there was a significant increase in the total number of rotations, it can be seen (Fig. 1) that this was due to an increase in the number of rotations observed in the first five-min period ($p<0.01$) followed by an increase in the peak magnitude of rotation behavior ($p<0.01$). It was further observed that the time from injection of the drug to the beginning of rotation was significantly decreased with succeeding trials and was significantly different for each of the trials. The mean ± S.E.M. five-min periods during which rotations began were: trial 1: 1.7 ± 0.2; trial 2: 1.1 ± 0.1; trial 3: 1 ± 0; trial 4: 1 ± 0 (trials 2, 3 and 4

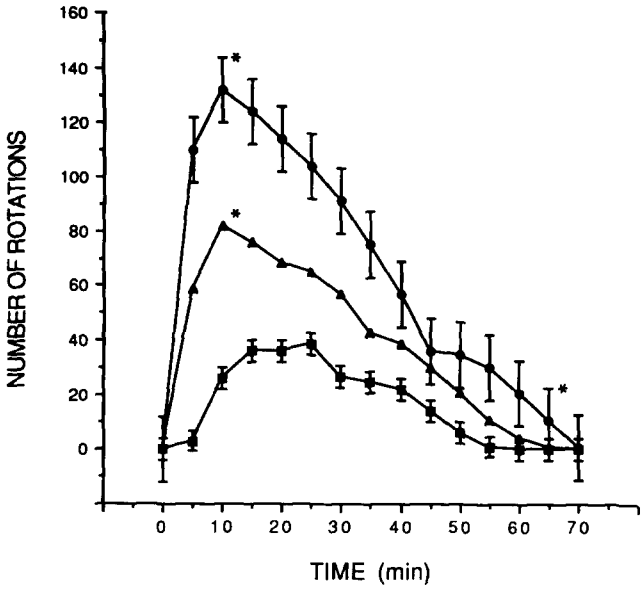

FIG. 1. Effect of repeated challenges with apomorphine on rotation behavior in rats with unilateral 6-hydroxydopamine lesions of the substantia nigra. Three- to five-day intervals separated each drug challenge trial. The dose of apomorphine was 0.25 mg/kg SC. Values represent the mean ± S.E.M. rotations contralateral to the side of the lesion minus any ipsilateral rotations observed in each five-min period. The data is from nine individual rats. ■ = Trial 1, ▲ = Trial 2 and ● = Trial 4 (Trial 3 was omitted for clarity and did not differ significantly from Trial 4). The standard error bars are omitted from Trial 2 for clarity. *Significantly different from Trial 1, $p<0.01$, ANOVA.

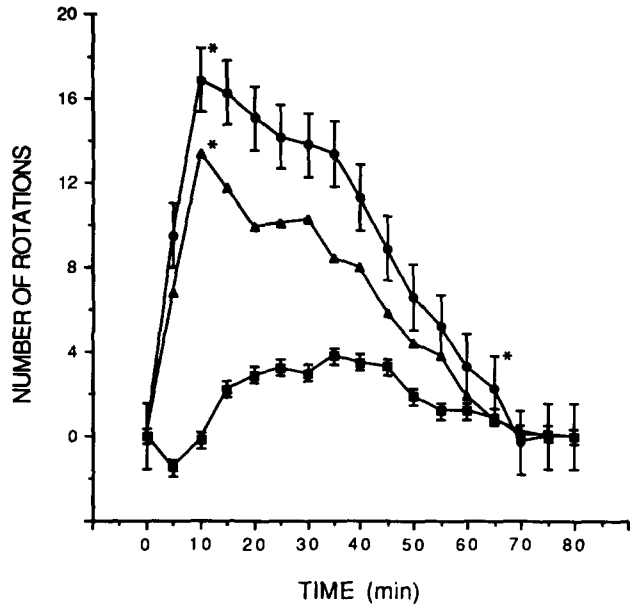

FIG. 2. Effect of repeated challenges with apomorphine on rotation behavior in rats with unilateral kainic acid lesions of the striatum. Three- to five-day intervals separated each drug challenge trial. The dose of apomorphine was 1 mg/kg SC. Values represent the mean ± S.E.M. rotations contralateral to the side of the lesion minus any ipsilateral rotations observed in each five-min period. The data is from 20 individual rats. ■ = Trial 1, ▲ = Trial 2 and ● = Trial 3. The standard error bars are omitted from Trial 2 for clarity. *Significantly different from Trial 1, $p<0.01$, ANOVA.

were significantly different from trial 1, $p<0.01$, ANOVA). By the fourth trial, the peak rotation behavior of 138 rotations/5-min period, which was significantly different ($p<0.01$) from that observed following the first challenge with apomorphine, was reached during the second five-min period. Furthermore, the duration of rotations was significantly greater in trials 3 and 4 compared to trial 1. The mean ± S.E.M. duration of rotations was: trial 1: 40 ± 5 min; trial 2: 51 ± 6 min; trial 3: 57 ± 5 min; trial 4: 61 ± 5 min.

The injection of the same volume of vehicle under identical conditions produced no rotation behavior in any of the rats.

Kainic Acid-Lesioned Rats

Similar to the results observed in the 6-OHDA-lesioned rats, there was no spontaneous rotation behavior following the placement of the rats into the open field environment. Following the injection with apomorphine (1.0 mg/kg SC), the rats displayed rotations contralateral to the lesion to produce a total mean number of rotations of 26 following the initial challenge with apomorphine (Table 1). Following the second injection with apomorphine, three to five days following the first trial, the total number of contralateral rotations was significantly increased from that observed in the first trial ($p<0.05$). Similarly, following the third trial, there was a significant increase in the total number of rotations to 137 from that observed following the second challenge ($p<0.01$). Interestingly, the rats in these present studies and in our previous studies (15) turned contralateral to the lesioned striatum in contrast to other reports (19) where rats with unilateral KA lesions of the striatum turned ipsilateral to the lesioned striatum in response to apomorphine. The precise explanation for these discrepancies is unclear at present. However, preliminary indications are that the anterior-posterior lesion coordinates are critical factors determining the direction of apomorphine-induced rotation behavior (26). Lesions of the anterior striatum (as used in the present studies) appear to elicit contralateral rotations, while lesions of the posterior striatum appear to elicit ipsilateral rotations in response to apomorphine (26).

As shown in Fig. 2, following the second challenge with apomorphine, some rats began rotating within the first 5 minutes postinjection and there was a rapid increase in rotation behavior until the peak magnitude of 12 was reached during the third 5-min period. Following the third challenge with the same dose of apomorphine, the number of rotations observed during the first 5-min period was significantly increased ($p<0.01$). The peak response of 22 was also significantly greater ($p<0.05$) than that seen following the initial challenge. There was no significant difference between the peak response in trial 3 from that observed in trial 2. The interval in which this peak response occurred was also not significantly different between trials 2 and 3. It was further observed that the time from injection of the drug to the beginning of rotation was significantly decreased. The mean ± S.E.M. five-min period in which rotations began were: trial 1: 1.8 ± 0.2; trial 2: 1.3 ± 0.1; trial 3: 1.1 ± 0.1 (trials 2 and 3 were significantly different than trial 1, $p<0.05$ and $p<0.01$, respectively). Furthermore, the duration of rotations was significantly greater in trial 3 compared to trial 1 ($p<0.01$, ANOVA). The mean ± S.E.M. duration of rotations was: trial 1: 44 ± 5 min; trial 2: 51 ± 4 min; trial 3: 59 ± 2 min.

The injection of vehicle alone produced no rotation behavior in any of the rats.

DISCUSSION

Sensitization to the behavioral effects of drugs such as amphetamine (20), cocaine (18) or diazepam (1) has been demonstrated in rats following repeated challenges. Sensitization (also termed behavioral facilitation) in the apomorphine-induced climbing behavior of mice following repeated administration of apomorphine has also been observed (10). Furthermore, repeated administration of apomorphine to rats induces a sensitization to the locomotor and stereotypical responses (11–14). This behavioral facilitation appears to be dopamine receptor-mediated and can be blocked by concomitant administration of dopamine receptor antagonists (10,12).

We have examined apomorphine-induced rotation behavior in both unilaterally 6-OHDA-lesioned and unilaterally kainic acid-lesioned rats and have found an increase in the total number of rotations following repeated challenges. This is in agreement with the data presented by Coward (6) where repeated apomorphine challenges of 6-OHDA-lesioned rats produced increases in the number of rotations, although these data were not discussed. A similar report also demonstrated an increase in apomorphine-induced rotation behavior in 6-OHDA-lesioned rats following repeated apomorphine challenges (2). We have extended these studies and demonstrated that this sensitization to apomorphine-induced rotation behavior was characterized by an increase in the magnitude of the peak response in addition to a significant effect on the time of initiation of the behavioral response. There was also an increase in the duration of the rotation behavior following repeated challenges with apomorphine. Interestingly, these effects were evident in both the 6-OHDA- and KA-lesioned rats which display rotational behavior due to both supersensitive and hyporesponsive dopamine receptors respectively.

Other investigators have reported that a conditioned learning behavior can produce an increase in rotation behavior or induce spontaneous rotations in rats with unilateral 6-OHDA lesions (3–5, 21, 22). This is unlikely to account for the sensitization phenomenon observed in our experiments, as no spontaneous rotations were observed when rats were placed in the testing area. Furthermore, when the rats were injected with vehicle three to five days following the last apomorphine challenge, no rotation behavior was observed in any animal. It has also been demonstrated that the behavioral sensitization to apomorphine in unlesioned rats develops with repeated challenges in the absence of drug associated contextual environmental stimuli (13).

It may also be argued that an ongoing development of the lesion may account for the increases in rotation behavior observed in our and other studies in 6-OHDA-lesioned rats. However, we have also observed the same sensitization phenomenon when we waited 28 days following the 6-OHDA lesion before the first challenge with apomorphine (data not shown). Furthermore, Staunton et al. (23) found that apomorphine-induced rotation behavior reached its maximum at 3 days after unilateral 6-OHDA lesions. That we found a similar sensitization behavior in KA-lesioned rats, where testing began 5 weeks after lesioning, also argues against this hypothesis.

Chronic treatment with antagonists of dopamine receptors elicits a supersensitivity to dopamine receptor agonists which is concomitant with an increase in the number of dopamine receptors (8). Furthermore, following unilateral dopamine deafferentation of the striatum, the rotation behavior elicited by apomorphine and other dopamine receptor agonists is due to supersensitivity mediated by the dopamine receptors in the lesioned striatum (7, 23, 24). The increases in rotation behavior following repeated administration of apomophine in rats which already have supersensitive dopamine receptors in one striatum indicate that an additional supersensitivity occurs. Thus the supersensitivity to the behavioral effects of apomorphine elicited by 6-OHDA lesions and repeated challenges with apomorphine must be produced via distinct mechanisms.

The sensitization to apomorphine-induced rotation following repeated challenges might have important implications for studies where dose-response curves to receptor agonists are performed in individual animals over a protracted period. Furthermore, it is possible that treatments with multiple dopamine receptor agonists may elicit a "cross-supersensitivity." Therefore, procedures such as drug screening for compounds with dopamine receptor agonist activity should take into consideration the effects of multiple challenges with both the same and with different dopaminergic agonists in individual animals. In addition, the sensitization to apomorphine-induced rotation behavior following 6-OHDA lesions and KA lesions must also be taken into consideration when using such lesions to assess the efficacy of neural transplants into these unilateral rat models of Parkinson's disease (9) and Huntington's disease (15,16). The repeated challenges with apomorphine, which are necessary in such studies, may complicate the interpretation of the observed responses following transplantation. However, it is possible that the decrease in the number of apomorphine-induced rotations following transplantation may represent an underestimate of the true effect of the transplants if there was sensitization of the response to the second drug challenge.

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