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Rotational Behavior in Dopamine Nigrostriatal Denervated Rats: Effects of a Wide Range of Time Intervals Between Apomorphine Administrations

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CASAS, M., G. PRAT, P. ROBLEDO AND F. JANÉ. *Rotational behavior in dopamine nigrostriatal denervated rats: Effects of a wide range of time intervals between apomorphine administrations.* PHARMACOL BIOCHEM BEHAV **62**(3) 481–485, 1999.—Previous studies using the turning behavior animal model have shown both increases and decreases in rotational behavior following successive administrations of dopamine agonists. To clarify the results obtained with this model, we studied the variability of rotational behavior after repeated challenges with low doses of apomorphine (0.05 mg/kg) at different time intervals ranging between 2 h and 14 days. Results show a decrease in the total number of turns with time intervals of 2, 6, and 12 h between administrations, and an increase in the total number of turns when apomorphine was administered once every 24 h. In contrast, when animals were tested at 7- and 14-day intervals, a stable number of turns in successive challenges was obtained. These results suggest that when successive injections of dopamine agonists are administered at sufficiently long intervals of time, the neuroadaptations that take place due to repeated drug exposure may not be apparent. These findings are relevant for the design of future experiments using this model. © 1999 Elsevier Science Inc.

Turning behavior Animal model Rotational behavior Sensitization Tolerance
Dopamine agonists Time interval between administrations

THE turning behavior model is one of the most useful animal models for studying the function of the dopaminergic neurotransmitter system "in vivo" (16). Currently, this model is applied to screen potential therapeutic drugs to be introduced in neurology and psychiatry (8,23,26), and to assess the efficacy of neuronal grafts in transplantation procedures [for review, see (27)]. In this model, unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal dopaminergic pathway produce an imbalance of dopamine between the two corpora striata, followed by the development of postsynaptic dopamine receptor supersensitivity in the lesioned side. Treatment with direct dopamine agonists such as apomorphine, leads to contralateral rotational behavior in denervated animals. Conversely, treatment with indirect dopamine agonists such as amphetamine causes ipsilateral rotation (25,27,28,33).

A number of factors can influence dopamine agonist-induced contralateral rotation including the degree of dopamine depletion in the nigrostriatal system (11,12), the time elapsed between 6-OHDA–induced lesions and screening with agonists (19), priming and conditioning effects (4,13,18,21,29), as well as, the time interval between successive drug administrations.

With respect to the influence of time interval between repeated apomorphine administrations, it has been shown that at small intervals (<24 h), rotational behavior decreases (6), and at greater intervals (>24 h), rotational behavior increases

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(6,22). However, because none of these studies have examined rotational behavior in a wide range of time intervals, the question still remains as to the reproducibility of the turning behavior animal model.

To clarify this issue, in the present study, we examined the variability in rotational behavior appearing in unilaterally 6-OHDA–lesioned rats after repeated challenges with apomorphine, using intervals of time between administrations ranging from 2 h to 14 days.

METHOD

Animals

Male Sprague–Dawley rats were used in all groups. Subjects were housed eight to a cage, with free access to rat chow and water. They were maintained in a temperature-controlled environment (21 ± 1°C) on a 12 L:12 D cycle (lights on at 0800 h) when not in experimental sessions. The experiment was carried out in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) for care and use of laboratory animals.

Surgical Procedure

Rats weighing 150 g ± 15 g were anaesthetized with sodium pentobarbitone (40 mg/kg IP) and placed in a David Kopf stereotaxic frame with the incisor bar set at 2.4 mm (15). The animals were injected unilaterally with 8 μg of 6-OHDA in a total volume of 4 μl using a Hamilton syringe with a conically shaped needle (outer diameter of 0.4 mm), at an infusion rate of 1 μl/min. The injection was aimed at the medial forebrain bundle (A −4.4, L −1.2, V −7.8 mm) calculated from bregma and dura. This lesion has been shown to extensively denervate the dopaminergic nigrostriatal system (32).

Rotational Behavior

Rotational behavior was recorded using a computerized system (Panlab, S. A., Barcelona, Spain). Animals were allocated individually into clear plastic hemispherical bowls (40-cm diameter), attached to a harness and connected to photoelectric detectors. Rotational behavior was monitored every 2 min for 1 h.

Animal Selection

To select the successfully denervated animals, 30 days postsurgery all rats were challenged four times with a small dose of apomorphine (0.05 mg/kg), at intervals of minimum 14 days. This dose of apomorphine was chosen because in previous studies it has been shown that it is sufficient to induce rotational behavior (33). Rats showing more than 250 complete 360° turns in the two last tests with apomorphine were used in the experiments. Several authors have demonstrated that at least 90% dopamine depletion is needed for apomorphine to induce contralateral rotational behavior (11,12). Although no biochemical or histological verification of the lesions was conducted in the present study, recent unpublished observations in our laboratory show that in a separate group of 60 rats with identical unilateral 6-OHDA lesions, the mean levels of DA and DOPAC in the lesioned striatum were decreased to 99 and 97%, respectively, compared to the nonlesioned striatum.

Drugs

Apomorphine HCl (Sigma, St. Louis, MO) was dissolved in physiological saline. The solution was prepared daily, it was protected from the light at all times in order to avoid oxidation, and it was kept at 4°C when not in use. The dose of apomorphine was calculated as free base and injected subcutaneously (SC) in a volume of 1 ml/kg into the left flank. The 6-OHDA HCl (Sigma) was dissolved in physiological saline containing 0.2% ascorbic acid.

General Procedure

Forty-nine unilaterally well-denervated rats were randomly allocated into six experimental groups. Fourteen days after the last apomorphine administration during the selection process, five consecutive administrations of apomorphine (0.05 mg/kg) were given at different time intervals: group 1 ($n = 8$): 2-h intervals; group 2 ($n = 9$): 6-h intervals; group 3 ($n = 8$): 12-h intervals; group 4 ($n = 8$): 24-h intervals; group 5 ($n = 8$): 7-day intervals; group 6 ($n = 8$): 14-day intervals. All animals were removed from the rotometers and returned to their home cages between injections. Apomorphine was always given 10 min after the animal was placed in the rotometer to ensure that conditioned rotation had extinguished.

Data Analysis

Barlett-Box test for homogeneity of variances was performed. Since no homogeneity of variances was observed, $F(75, 3225) = 1.627$, $p < 0.001$, a Kruskall–Wallis analysis of variance was used to compare the rotational behavior between groups for each apomorphine administration, followed by a Mann–Whitney *U*-test for comparisons between means when the overall analysis was found to be significant. Analysis of variance for repeated measures by means of polynomial contrast was performed to examine the evolution of the rotational behavior in each group for successive apomorphine administrations.

RESULTS

Rotational behavior for the different groups tested with successive injections of apomorphine is shown on Fig. 1. Results show no significant differences in rotational behavior between any of the groups in the first apomorphine administration ($\chi^2 = 2.74$, $p > 0.05$), revealing that the initial behavioral sensitivity was similar for all the groups tested. Significant differences were found between groups as of the second administration [second administration ($\chi^2 = 23.46$, $p < 0.0004$); third administration ($\chi^2 = 26.10$, $p < 0.001$); fourth administration ($\chi^2 = 25.10$, $p < 0.001$); and fifth administration ($\chi^2 = 31.57$, $p < 0.0001$)].

Further analysis showed that on the second apomorphine administration, group 4, tested at 24-h intervals, rotated significantly more than all the other groups ($U \leqslant 8$, $p \leqslant 0.009$). On the third administration, group 4 also showed a greater number of rotations than all the other groups ($U \leqslant 7$, $p \leqslant 0.009$), and groups 1 and 2, tested at 2- and 6-h intervals rotated significantly less that group 5, tested at 7-day intervals ($U \leqslant 15$, $p \leqslant 0.05$). On the fourth administration, group 4, again, rotated significantly more than all the other groups ($U \leqslant 11$, $p \leqslant 0.003$), and group 1, tested at 2-h intervals showed significantly less rotational behavior than groups 5 and 6, tested at 7- and 14-day intervals. Finally, on the fifth administration, group 4, tested at 24-h intervals, rotated significantly more than the rest of the groups, and groups 1 and

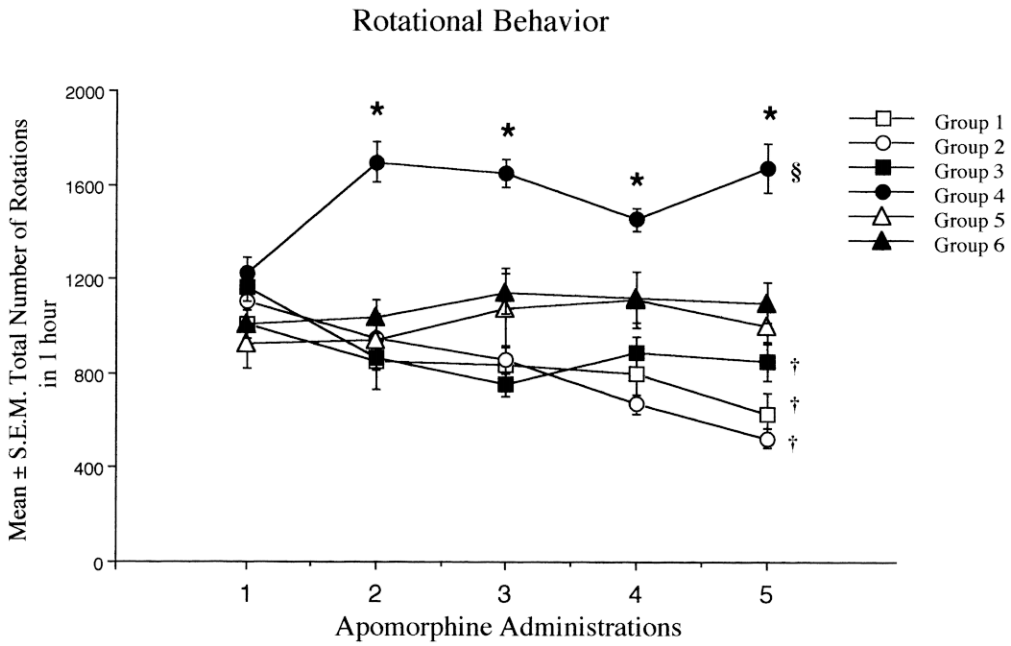

FIG. 1. Effects of time interval between repeated apomorphine (0.05 mg/kg SC) administrations on rotational behavior in rats with unilateral 6-OHDA lesions. Group 1 ($n = 8$): 2-h interval; group 2 ($n = 9$): 6-h interval; group 3 ($n = 8$): 12-h interval; group 4 ($n = 8$): 24-h interval.; group 5 ($n = 8$): 7-day interval; group 6 ($n = 8$): 14-day interval. Data are shown as means ± SEM. *$p < 0.05$, Mann–Whitney U for comparisons between means for each apomorphine administration. Significant linearly ascending trend §$p < 0.05$, and significant descending trend †$p < 0.02$.

2, tested at 2 and 6 h rotated significantly less than groups 5 and 6, tested at 7 and 14 days ($U \leqslant 12$, $p \leqslant 0.003$).

The analysis of variance for repeated measures showed statistical significance in all groups except group 6 [group 1, $F(4, 28) = 11.0$, $p < 0.001$; group 2, $F(4, 32) = 24.82$, $p < 0.001$; group 3, $F(4, 28) = 8.42$, $p < 0.001$; group 4, $F(4, 28) = 13.35$, $p < 0.001$; group 5, $F(4, 28) = 2.79$, $p < 0.05$; group 6, $F(4, 28) = 1.48$, $p > 0.05$], revealing that rotational behavior changed significantly in most groups with successive apomorphine administrations, except in the group tested at 14-day intervals where stable responses were observed. In group 5, tested at 7-day intervals, although a significant variability was found in the statistical analysis, only a small percentage of change was observed between the first and last apomorphine administration (see Table 1).

TABLE 1

PERCENT CHANGES, INCREASES (+) AND DECREASES (−) IN ROTATIONAL BEHAVIOR INDUCED BY APOMORPHINE BETWEEN THE FIRST AND THE LAST ADMINISTRATION

Group	Time Interval	% Change
Group 1 ($n = 8$)	2 hours	(−) 38.15
Group 2 ($n = 9$)	6 hours	(−) 52.40
Group 3 ($n = 8$)	12 hours	(−) 26.82
Group 4 ($n = 8$)	24 hours	(+) 36.41
Group 5 ($n = 8$)	7 days	(+) 7.34
Group 6 ($n = 8$)	14 days	(+) 9.34

A significant linearly descending trend was observed in group 1, tested at 2-h intervals ($t^2 = 25.10$, $p < 0.003$), group 2, tested at 6-h intervals ($t^2 = 46.38$, $p < 0.001$), and group 3, tested at 12-h intervals ($t^2 = 8.82$, $p < 0.02$), showing decreased responding with repeated apomorphine injections. In contrast, a significant linearly ascending trend was found for group 4, tested at 24-h intervals ($t^2 = 5.75$, $p < 0.05$), showing increased responding to successive apomorphine injections. No significant trend was obtained for group 5, tested at 7-day intervals. Table 1 summarizes the percent change in total number of rotations between the first and the last apomorphine administration for each group.

DISCUSSION

The turning behavior animal model has contributed to increase the knowledge of the functioning of the dopaminergic system, and has provided a useful tool to screen potential new dopaminergic agonists that can serve as therapeutical agents in the treatment of Parkinson's disease.

One of the limitations of the turning behavior animal model is the fact that animals are previously screened for their rotational response to apomorphine. Indeed, priming effects to a single or to repeated administrations of dopamine agonists have been described (6,9,14,18,21,22). In the present study, rats had previous administrations of apomorphine; therefore, we cannot underestimate the possible influence of priming effects in the results presented here.

Nevertheless, our results provide clear evidence showing that in primed rats, a highly stable number of turns can be obtained with successive apomorphine administrations to the same animals when the interval of administrations is between

7 and 14 days. These results confirm data from one study showing good reproducibility of rotational behavior when animals are given successive apomorphine injections at intervals of 14 days (34). In contrast, if primed rats are tested repeatedly at 24-h intervals, an increased number of rotations is observed with successive challenges of apomorphine. On the other hand, if rats are tested at small time intervals of 2, 6, and 12 h, a decrease in rotational behavior is observed. These findings suggest that time interval between injections may be a critical factor affecting dopamine agonists-induced contralateral rotational behavior in this animal model.

In this study, the increase in rotational behavior observed with apomorphine challenges at 24-h intervals may involve both D_2 and /or D_1 receptor mechanisms because apomorphine is a mixed D_1/D_2 receptor agonist. In fact, recent data shows an increase in rotational behavior in response to daily injections of the D_2 agonist quinpirole or to the mixed D_1/D_2 agonist l-DOPA in apomorphine-primed rats (1,24). In addition, priming may change transduction mechanisms at the level of D_1 receptors (2,20) that may influence the effects of subsequent D_1 receptor stimulation with apomorphine on rotational behavior.

Our results also showed decreased rotational behavior in response to successive apomorphine challenges when given at short intervals of time (<24 h). Similar results have been obtained in 6-OHDA–lesioned rats (10), and mice (35), and in monkeys treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (17). These results are in line with one study showing that repeated administration with the D_1 agonist, SKF 38393, results in decreased rotational behavior associated with decreased D_1 receptor binding in the striatum and substantia nigra of denervated rats (7). Similarly, in another study, dopamine denervated rats repeatedly treated with the novel dopamine D_1 receptor agonist A-85653, showed decreased rotational behavior and c-*fos* induction in the striatum (1).

Finally, conditioning factors may have also influenced the results obtained in this study because it has been shown that increased response to dopamine agonists in 6-OHDA–lesioned rats may be due to conditioned environmental cues. In fact, several studies report that conditioned rotational behavior appears immediately when the animal is placed in the rotometer, and lasts approximately 2 or 3 min. This behavior, however, is not observed in successive challenges, and its maximal expression is when animals are challenged after a long period of drug withdrawal (3,5,29–31). In our study we minimized these conditioned effects by administering apomorphine after a period of time sufficiently long to favor extinction of this conditioned behavior, a procedure similar to the one used by other authors (14,21). Nevertheless, in our experiment conditioning effects cannot be ruled out completely because conditioning can also take place to drug-related cues.

In conclusion, we observed a stable rotational behavior with 7- and 14-day intervals between apomorphine administrations. These findings provide new data relevant for the design of future studies that use this model to screen for potential therapeutic drugs in the treatment of neurological and psychiatric diseases.

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