Pharmacology Biochemistry & Behavior, Vol. 9, pp. 609–613. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Rotational Behaviour Induced by Theophylline in 6-OHDA Nigrostriatal Denervated Rats Is Dependent on the Supersensitivity of Striatal Dopaminergic Receptors

MIQUEL CASAS,[1] SERGI FERRÉ, JOSEP CADAFALCH, JOSEP M. GRAU AND FRANCESC JANÉ

Laboratori de Neuropsicofarmacología i Programa Sant Pau-CITRAN
Fundació d'Investigació de la Santa Creu i Sant Pau
Hospital de Sant Pau (Medical School, U.A.B.), Barcelona, Spain

Received 4 September 1987

CASAS, M., S. FERRÉ, J. CADAFALCH, J. M. GRAU AND F. JANÉ. *Rotational behaviour induced by theophylline in 6-OHDA nigrostriatal denervated rats is dependent on the supersensitivity of striatal dopaminergic receptors.* PHARMACOL BIOCHEM BEHAV 33(3) 609–613, 1989.—We studied apomorphine- and theophylline-induced rotational behaviour in rats with a unilateral 6-hydroxydopamine lesion of the dopaminergic nigrostriatal pathway. It was seen that there was a direct correlation between the number of apomorphine- and theophylline-induced contralateral turns. These data suggest the existence of a relationship between theophylline-induced rotational behaviour and the degree of supersensitivity of the striatal dopaminergic receptors. Because the rotational behaviour induced by theophylline is in the same direction as dopaminergic agonists, contralateral to the nigrostriatal pathway lesion, these results suggest the possibility of a direct dopaminergic agonism of methylxanthines.

Theophylline Apomorphine Rotation Behavioural supersensitivity

IN the rat, a unilateral lesion of the nigrostriatal pathway with 6-hydroxydopamine (6-OHDA) produces deviation of the animal towards the lesioned side (18). This deviation changes to intense rotational behaviour in the same direction following the administration of amphetamine (18), which causes dopamine (DA) release in the intact striatum. Amphetamine-induced turning behaviour contrasts with that induced by direct DA agonists, such as apomorphine or L-dopa, which produce intense rotation contralateral to the lesioned hemisphere. This observation led Ungerstedt to suggest that denervation causes striatal DA receptor supersensitivity. This hypothesis is supported by radioligand binding studies (3) and by studies of striatal DA-sensitive adenylate-cyclase activity (16). Currently, this animal model is widely used in the search of new substances with direct or indirect DA agonist activity (9).

Ungerstedt and Herrera-Marschitz (19) and Hefti *et al.* (11) have demonstrated that to obtain apomorphine-induced turning behaviour in the rat, unilateral striatal DA-depletion of at least 90% is needed. In addition, it has been found that there is a direct correlation between the degree of unilateral striatal DA-denervation and the total number of apomorphine-induced contralateral turns (19). As a consequence, total contralateral rotation (TCR) induced by apomorphine can be used to determine in vivo the level of denervation-induced supersensitivity of the striatal dopamine receptors.

Methylxanthines potentiate apomorphine-induced rotational behaviour (6), and when administered alone, produce intense rotational behaviour in the same direction as that produced by direct dopamine agonists (10, 20, 21), which suggests that they can act as dopamine agonists. The possibility that methylxanthines are direct dopamine agonists is not yet well established, although some reports support this hypothesis (10, 14, 20, 21). More commonly accepted mechanisms are: inhibition of phosphodiesterase

[1]Requests for reprints should be addressed to Dr. Miquel Casas, Laboratori de Neuropsicofarmacología, Hospital de Sant Pau, Avda. San Antoni María Claret 167, 08025 Barcelona, Spain.

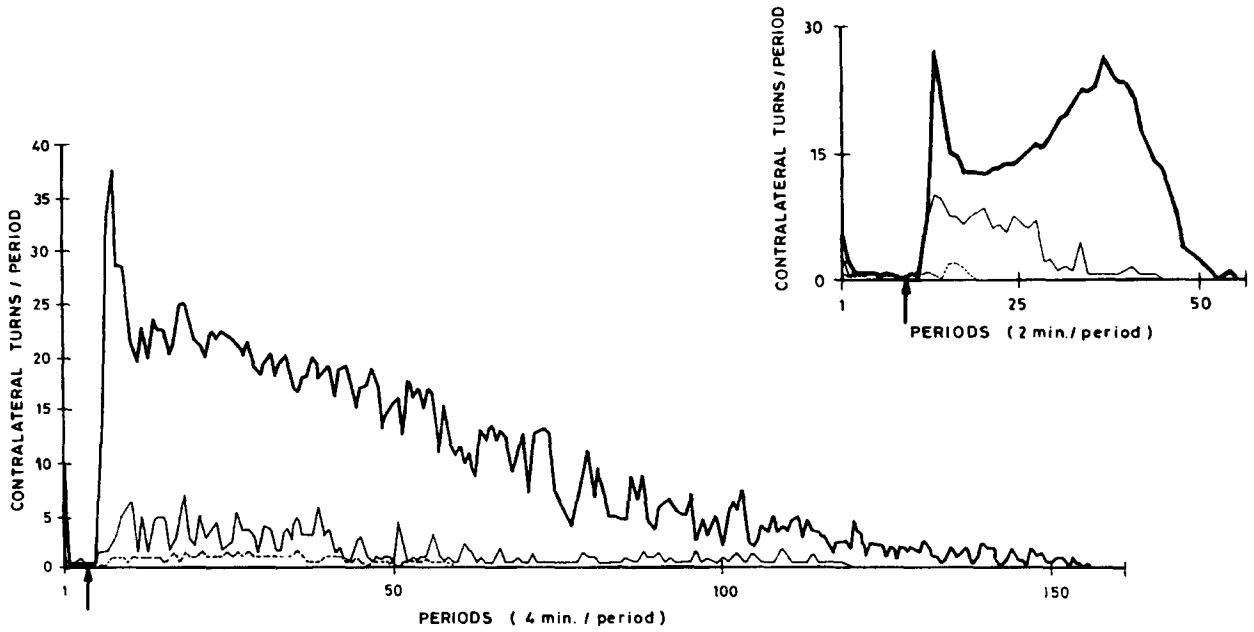

FIG. 1. Rotational patterns induced by apomorphine and theophylline in the three different groups described in the text. Each curve shows the means of total contralateral turns/period of a group (broken curve=1st group; light continuous curve=2nd group; dense continuous curve=3rd group) (n=20/group). Apomorphine-induced contralateral turns are represented in the upper graphic (x-axis=2 minutes/period). Theophylline-induced contralateral turns are represented in the lower curves (x-axis=4 minutes/period. Arrows points to the injection time and before this time conditioned rotational behaviour can be observed.

(2), calcium blockade (8), adenosine antagonism (17) and catecholamine release (1).

We have studied the relationship between rotational behaviour induced by apomorphine and theophylline in rats with a unilateral nigrostriatal pathway 6-OHDA lesion. Our aim has been to contribute to clarify the mechanisms by which methylxanthines induce turning behaviour in rats with unilateral nigrostriatal pathway lesion.

METHOD

Animals

One hundred male Sprague-Dawley rats were used. The animals were maintained in a temperature- and humidity-controlled environment with a 12-hr light/dark cycle and they had acces to water and food ad lib.

Surgical Procedure

Rats weighing 145–160 g were given lesions of the left nigrostriatal dopaminergic pathway [coordinates in respect to bregma: A −4.4, L −1.2, V −7.8, into the area ventralis tegmenti, which contains the bundle of axons leaving the nigral dopaminergic cell bodies; according to the König and Klippel atlas (13)] by means of stereotaxic injection of 8 μg 6-OHDA dissolved in 4 μl 0.02% ascorbic acid solution, with an injection rate of 1 μl/min under pentobarbital IP anesthesia.

Behavioural Evaluation

Rotational behaviour was measured with automated rotometers that allow the continuous recording of turns to the right and to the left, by means of a detector using infrared photocell barriers (19). Four weeks after surgery rats were injected with apomorphine 0.05 mg/kg SC four times with one-week intervals, because, as Ungerstedt and Herrera-Marschitz (19) have pointed out, repeated tests are necessary to get a reproducible apomorphine-induced rotational behaviour. Consequently, we take into account the average of the two last apomorphine-induced TCR for calculations. Once the rat is introduced in the rotometer conditioned rotational behaviour can be observed, which consists of a few numbers of rotations which last up to 3 minutes. To avoid interference between conditioned and pharmacological rotational behaviour, drugs (apomorphine or theophylline) were administered 20 minutes after the animal had been introduced in the rotometer.

We selected those animals which could be included into three groups: 1st group—Rats with a TCR induced by apomorphine 0.05 mg/kg SC fewer than 10 turns and with a TCR induced by apomorphine 0.5 mg/kg SC greater than 100 turns. 2nd group—Rats with a TCR induced by apomorphine 0.05 mg/kg SC between 100 and 200 turns. 3rd group—Rats with a TCR induced by apomorphine 0.05 mg/kg SC greater than 400 turns. The purpose for this selection was to obtain three groups with different denervation-induced supersensitivities of the striatal dopamine receptors. The first group did not differ from naive rats in respect with TCR induced by apomorphine 0.05 mg/kg SC, but because a single higher dose of apomorphine 0.5 mg/kg SC, given one week following the fourth test with apomorphine 0.05 mg/kg, could elicit a contralateral rotational behaviour, the presence of a low supersensitivity of the striatal dopamine receptors was inferred. Because rats from the second and third group did not

need a higher dose of apomorphine to demonstrate contralateral rotation, we inferred that they have a greater supersensitivity of the striatal dopamine receptors than rats from the first group. The difference in supersensitivity of the striatal dopamine receptors between the second and third group is inferred from their difference in TCR induced by apomorphine 0.05 mg/kg SC, following the studies of Ungerstedt and Herrera-Marschitz that show a correlation between the degree of unilateral DA denervation of the striatum and the number of TCR induced by apomorphine 0.05 mg/kg SC (19).

Four weeks after the last apomorphine administration we determined the TCR induced by a single dose of theophylline 30 mg/kg SC in the three groups.

Drugs

All drugs were from Sigma. Apomorphine (HCl) and theophylline (anhidrous crystals) were dissolved in warm physiological saline solution. All doses were injected SC in a volume of 1 mg/kg body weight.

Statistics

For statistical analysis we used the software MICROSTAT. Statistical tests are specified in each case.

RESULTS

Figure 1 shows the rotational patterns induced by apomorphine and theophylline in the three different groups. Conditioned rotational behaviour can be appreciated after the animal has been introduced in the rotometer (first and second periods). Following drug injection the animal begins to show a rotational behaviour aproximately 5 minutes after apomorphine and 10 minutes after theophylline administration, which rules out the existence of an injection-induced rotational behaviour. In the three groups the rotational behaviour induced by theophylline (30 mg/kg) has a much longer duration than that produced by apomorphine (0.05 mg/kg). Rats from the third group show a "two-peak" rotational pattern induced by apomorphine and an "initial-peak" rotational pattern induced by theophylline.

Study of the Differences Between Apomorphine-Induced TCR and Theophylline-Induced TCR Among the Different Groups

Apomorphine- and theophylline-induced TCR were greater in group 2 than in group 1 (Mann-Whitney U-test: $p<0.0001$ and $p<0.02$ respectively) and also greater in group 3 than in group 2 (Mann-Whitney U-test: $p<0.0001$ in both cases) (Fig. 2).

Study of Correlation Between Apomorphine-Induced TCR and Theophylline-Induced TCR

As the Kolmogorov-Smirnov test showed that we could discard a normal distribution ($p<0.05$) of some results (apomorphine-induced TCR in group 3), statistical analyses were performed with nonparametric tests. We found a direct correlation between apomorphine-induced TCR and theophylline-induced TCR (Spearman's correlation test: $p<0.05$, $r_s=.76$) (Fig. 3).

DISCUSSION

To explain the cause of rotational behaviour induced by methylxanthines in the rat with a unilateral nigrostriatal dopamine pathway lesion, basically three hypotheses have been postulated: phosphodiesterase inhibition (6), adenosine antagonism (5) and dopamine agonism (10, 14, 20, 21).

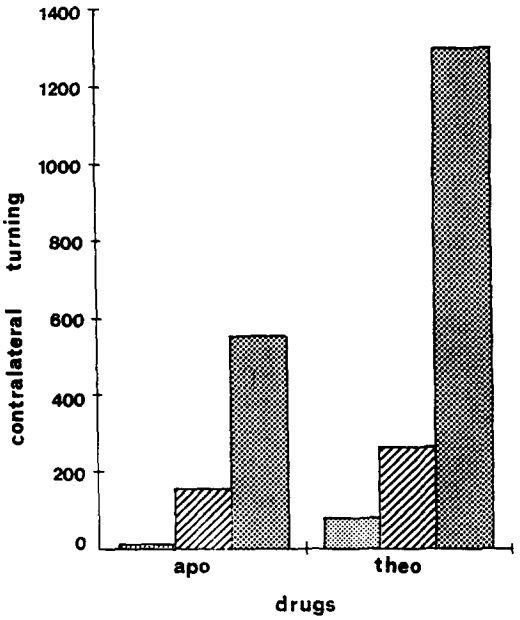

FIG. 2. Medians of apomorphine- and theophylline-induced TCR in the first (left bars), second (medial bars) and third group (right bars) (n=20/group). Characteristics of groups are described in the text.

As the existence of a striatal dopamine-sensitive adenylate-cyclase was known (12), the ability of methylxanthines to inhibit phosphodiesterase was initially thought to be the most probable cause of methylxanthine-induced turning behaviour (6). However, it was demonstrated that methylxanthines produced this behaviour without reaching cerebral concentrations sufficient to inhibit phosphodiesterase (4) and, furthermore, that some phosphodiesterase inhibitors did not produce rotational behaviour (4).

Green *et al.* (7) and Freedholm *et al.* (5) have shown that adenosine inhibits striatal dopamine activation. This could explain the methylxanthine-induced potentiation of rotational behaviour induced by direct dopamine agonists, because methylxanthines are adenosine antagonists (17). However, it is not likely that the purinergic hypothesis could explain the rotational behaviour produced by the administration of isolated methylxanthines, because 8-phenyltheophylline, a more potent adenosine antagonist than caffeine and theophylline, does not produce rotational behaviour (5).

The hypothesis of a dopamine agonism of methylxanthines is based on studies that demonstrate that in the rat with unilateral 6-OHDA nigrostriatal pathway lesion, methylxanthines potentiate apomorphine-induced turning behaviour (6) and produced contralateral turning when administered alone (10, 20, 21), and that dopamine blockade inhibits turning behaviour induced by methylxanthines (10, 20, 21).

Taking into account that there exist experimental data which demonstrate the existence of a correlation between the degree of unilateral nigrostriatal pathway lesion and the number of contralateral turns induced by apomorphine

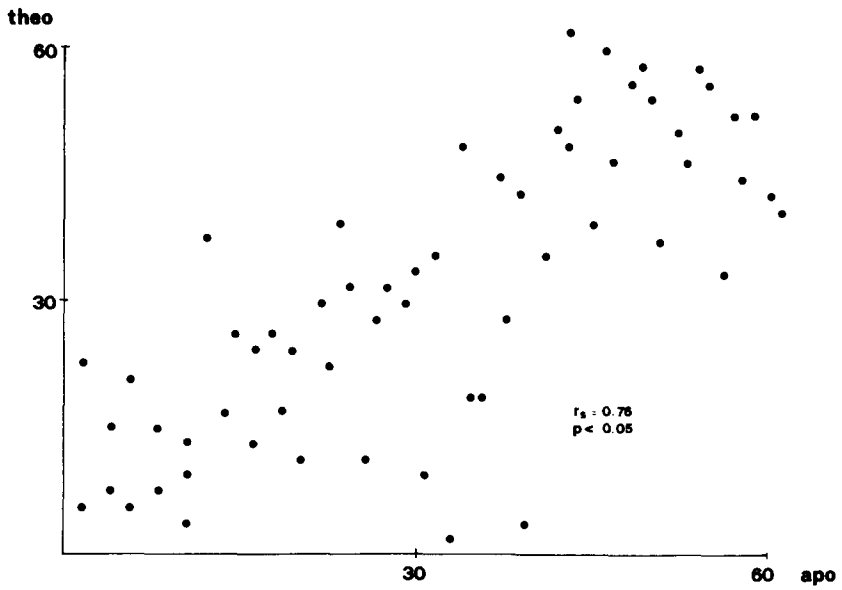

FIG. 3. Correlation between ranks of apomorphine- and theophylline-induced TCR (n=60).

(19), rotational behaviour induced by apomorphine can be a suitable measure to determine the degree of denervation-induced supersensitivity of the striatal dopamine receptors. In this study we have shown a correlation between the number of apomorphine-induced turns and theophylline-induced turns, which suggests that there is a correlation between the level of supersensitivity of the striatal dopamine receptors and the number of theophylline-induced turns contralateral to the nigrostriatal pathway lesion. The existence of a probable relationship between theophylline-induced rotational behaviour and the degree of sensitivity of striatal dopamine receptors supports the hypothesis that methylxanthines could act directly on these receptors.

In this study we also have found that animals with greater TCR induced by apomorphine show a "two-peak" rotational pattern, which has been described as being associated to a very good unilateral lesion of the nigrostriatal pathway. This pattern is only observed in rats with more than 95% striatal dopamine depletion (19). In addition, animals with a greater TCR induced by theophylline also present a distinctive pattern, an "initial-peak" rotational behaviour. Consequently, the existence of an "initial-peak" rotational pattern induced by theophylline indicates the existence of a good unilateral striatal denervation.

More studies are needed to be certain that methylxanthines, besides their already known actions, also act as dopamine agonists and, consequently, that this action can have pharmacological implications: Methylxanthines could have a role in the treatment of Parkinson's disease and neuroleptic-induced parkinsonism. They also had to be taken into account in the management of psychotic patients, because of the possible interference with dopamine antagonists, and in lactation, due to their possible inhibition on prolactin release, which is tonically inhibited by the dopaminergic tuberoinfundibular pathway (15).

ACKNOWLEDGEMENTS

Excellent technical assistance by A. Rubio and S. Lopez is acknowledged. Work supported by grants from FISS (84/1073), from SANDOZ S.A.E. and from CITRAN (1987).

REFERENCES

1. Bellet, S.; Roman, L.; De Castro, O.; Kim, K. E.; Kershbaum, A. Effect of coffee ingestion on catecholamine release. Metabolism 18:288–291; 1969.
2. Butcher, R. W.; Sutherland, E. W. Adenosine 3',5'-phosphate in biological materials. J. Biol. Chem. 237:1244–1250; 1962.
3. Creese, I.; Burt, D. R.; Snyder, S. H. Dopamine receptor binding enhancement accompanies lesion-induced behavioural supersensitivity. Science 197:596–598; 1977.
4. Fredholm, B. B.; Fuxe, K.; Agnati, L. Effect of some phosphodiesterase inhibitors on central dopamine mechanisms. Eur. J. Pharmacol. 38:31–38; 1976.
5. Fredholm, B. B.; Herrera-Marschitz, M.; Jonzon, B.; Lindstrom, K. and Ungerstedt, U. On the mechanism by which methylxanthines enhance apomorphine-induced rotation behaviour in the rat. Pharmacol. Biochem. Behav. 19:535–541; 1983.
6. Fuxe, K.; Ungerstedt, U. Action of caffeine and theophylline on supersensitive dopamine receptors: considerable enhancement of receptor response to treatment with dopa and dopamine receptor agonist. Med. Biol. 52:48–54; 1974.
7. Green, R. D. Proudfit, H. K.; Young, S. M. H. Modulation of striatal dopaminergic function by local injection of 5-N'-carboximideadenosine. Science 218:58–61; 1982.

8. Hagiwara, S.; Byerly, L. "Calcium channel." Annu. Rev. Neurosci. 4:69–125; 1981.
9. Herrera-Marschitz, M. Neuropharmacology and functional anatomy of the basal ganglia. Stockholm, Karolinska Institute. Thesis, 1986:1–79.
10. Herrera-Marschitz, M.; Casas, M.; Ungerstedt, U. Caffeine produces contralateral rotation in rats with unilateral denervation: Comparison with apomorphine induced responses. Psychopharmacology (Berlin) 94:38–45; 1988.
11. Hefti, F.; Melamed, E.; Sahakian, B. J.; Wurtman, R. J. Circling behaviour in rats with partial, unilateral nigro-striatal lesions: Effect of amphetamine, apomorphine and DOPA. Pharmacol. Biochem. Behav. 12:185–188; 1980.
12. Kebabian, J. W.; Petzold, G. L.; Greengard, P. Dopamine-sensitive adenylate cyclase in the caudate nucleus of rat brain and its similarity to the dopamine receptor. Proc. Natl. Acad. Sci. USA 69:2145–2149; 1972.
13. König, J. F. R.; Klippel, R. A. The rat brain. A stereotaxic atlas of the forebrain and lower parts of the brain stem. New York: R. E. Krieger Publishing Co. Inc.; 1963.
14. Lundberg, D. B.; Breese, G. R.; Mueller, R. A. Aminophylline may stimulate respiration in rats by activation of dopaminergic receptors. J. Pharmacol. Exp. Ther. 217:215–221; 1981.
15. MacLeod, R. M. Regulation of prolactin release. In: Martini, L.; Ganong, W. F., eds. Frontiers in neuroendocrinology. Vol. 4. New York: Raven Press; 1976:169–194.
16. Mishra, R. K.; Marshall, A. M.; Varmuza, S. L. Supersensitivity in rat caudate nucleus: Effects of 6-hydroxydopamine on the time course of dopamine receptor and cyclic AMP changes. Brain Res. 200:47–57; 1980.
17. Snyder, S. H.; Katims, J. J.; Annau, Z.; Bruns, R. F.; Daly, J. W. Adenosine receptors and behavioural actions of methylxanthines. Proc. Natl. Acad. Sci. USA 78:3260–3264; 1981.
18. Ungerstedt, U. Postsynaptic supersensitivity after 6-hydroxydopamine induced degeneration of the nigrostriatal dopamine system in the rat brain. Acta Physiol. Scand. 82(Suppl. 367):69–93; 1971.
19. Ungerstedt, U.; Herrera-Marschitz, M. Behavioural pharmacology of dopamine receptor mechanisms. In: Stjärne, L.; Hedqvist, P.; Lagerkrantz, H.; Welmalm, A., eds. Chemical Neurotransmission: 75 years. New York: Academic Press; 1981:85–93.
20. Ungerstedt, U.; Herrera-Marschitz, M.; Casas, M. Are apomorphine, bromocriptine, and the methylxanthines agonists at the same dopamine receptor? In: Gessa, G. L.; Corsini, G. U., eds. Apomorphine and other dopaminomimetics. New York: Raven Press; 1981:85–93.
21. Watanabe, H.; Ikeda, M.; Watanabe, K. Properties of rotational behaviour produced by methylxanthine derivatives in mice with unilateral striatal 6-hydroxydopamine-induced lesions. J. Pharmacol. Dynam. 4:301–307; 1981.