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Circling induced by dopamine uptake inhibitors*

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Analysis of the circling behaviour induced in the rat with a unilateral 6-hydroxydopamine lesion of the substantia nigra has proved to be of great interest in the study of drugs acting on catecholamine neural systems. This animal at rest tends to assume an asymmetric posture with the head, body and tail describing a gentle curve concave on the lesioned side. Administration of amphetamine accentuates the postural asymmetry and induces vigorous circling towards the side of the lesion; that is, ipsiversive circling. In contrast, apomorphine and L-dopa induce circling in the opposite direction (contraversive).

Evidence has been presented by Ungerstedt, Butcher & others (1969), Arbuthnott & Crow (1971), and Ungerstedt (1971), that the response to amphetamine reflects the asymmetric release of dopamine from nigrostriatal nerve endings in the striatum. Since these nerve endings have almost completely degenerated on the side of the nigral lesion, little or no dopamine release can occur on that side, whereas the release occurs normally on the unlesioned side. The animal turns away from the side of greater striatal dopamine activity and consequently, after amphetamine, it turns ipsiversively towards the side of the lesion.

* Supported in part by the Clinical Research Center for Parkinson's and Allied Diseases, NIH Grant NS-11631-3.

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This interpretation of the mechanism of action of amphetamine in the circling rat suggests that drugs which inhibit the uptake of dopamine but do not cause its release should also cause ipsiversive turning in this animal model. Several drugs have recently become available which make it possible to test this hypothesis. Nomifensine, introduced recently as an antidepressant, is a potent inhibitor of dopamine as well as of noradrenaline uptake (Kruse, Hoffman & others, 1977). The new anorexic and stimulant drugs mazindol (5-hydroxy-5-*p*-chlorophenyl-2,3-dihydro-5H-imidazo-(2,1-α)isoindole and dita (3′,4′-dichloro-2-(2-imidazolin-2yl-thio) acetephenone hydrobromide) were recently shown in our laboratories to be extremely weak releasers but very potent inhibitors of both dopamine and noradrenaline uptake (Heikkila, Cabbat & Mytilineou, 1977). In the present study, we compared the behavioural effect of these three dopamine uptake inhibitors with that of amphetamine in rats with unilateral nigral lesions.

Sprague-Dawley rats, 150–175 g, were subjected to a unilateral chemical nigrotomy performed essentially as described by Ungerstedt (1971) with minor modifications. A solution of 6-hydroxydopamine HBr containing 8 μg/4 μl was injected into the rostro-medial portion of the left substantia nigra under Brevital (Rx) anaesthesia in a David Kopf model 900 stereotactic apparatus. Lesion coordinates derived from the König & Klippel atlas (1963) were A 3·0, L 1·8 and V −2·6. The animals

were subsequently tested for their rotational response to amphetamine at monthly intervals. For this purpose, each animal was placed in a hemispheric plastic bowl 40 cm in diameter and the number of complete 360° turns was counted continuously for a minimum of 3 h following the intraperitoneal (i.p.) injection of (±)-amphetamine sulphate, 2·5 mg kg^{-1}.

Three months after lesioning, six animals were selected who executed at least 500 turns in 2 h following amphetamine. These animals also rotated contraversively following the intraperitoneal administration of apomorphine and L-dopa. The rotational response of each animal to mazindol, dita, nomifensine, amphetamine and saline was then determined. The sequence of drug or saline was randomized in accordance with a latin square design. A rest period of 2 to 4 days separated consecutive injections. Drugs were administered intraperitoneally in an aqueous solution. Mazindol was dissolved in dilute HCl and the pH of the solution was then titrated to 5·5 with NaOH. Nomifensine and dita were dissolved in warm water. Amphetamine was dissolved in U.S.P. sterile water for injection at room temperature. The dosages employed, calculated as the free base, were (mg kg^{-1}) mazindol, 5; dita, 11·7; nomifensine, 3·4 and (±)-amphetamine, 2·5. These doses were found to be optimal after several preliminary trials. The physiological saline control (0·9% NaCl) injection was 1 ml kg^{-1}, a volume equal to that of the above drug injections.

All six test animals exhibited sporadic single turns to the left before administration of any drug and also following the intraperitoneal injection of physiological saline 1 ml kg^{-1}. The mean total number of turns completed in 3 h following saline was 69, s.d. 63 turns (mean with 1 s.d.).

After the injection of (±)-amphetamine, the animals began to circle vigorously to the left (ipsiversively) within several minutes. Circling reached a mean velocity of 10 turns min^{-1} 15 to 30 min after injection, reached a peak of 13 turns min^{-1} 1·5 h after injection and then began to decline. There were still about 8 turns min^{-1} 3·0 h after injection.

All three dopamine uptake inhibitors also produced vigorous circling to the left. As may be seen from the data summarized in Table 1 and shown graphically in Fig. 1, mazindol and nomifensine produced circling nearly comparable in intensity and duration to that induced by amphetamine. In comparison, circling following dita was less vigorous and had largely subsided 2 h after injection. After all three test drugs, the animals exhibited behavioural signs typical of amphetamine including piloerection, exophthalmos and increased sniffing, grooming and exploratory activity.

These behavioural data confirm and supplement previous observations on the effect of nomifensine by Pycock & others (1976) working with striatal-lesioned mice, those of Costall, Kelly & Naylor (1975) based on rats with electrolytic lesion of the nigrostriatal pathways

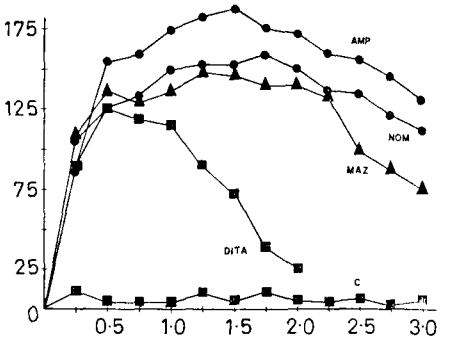

FIG. 1. Number of 360° turns executed in successive 15 min periods following intraperitoneal administration of amphetamine (AMP), nomifensine (NOM), mazindol (MAZ), dita and physiological saline (C). Data represent mean of six animals. Ordinate: No of 360° turns/15 min. Abscissa: Time (h).

and also those of Setler (P. Setler, personal communication) on 6-OH dopamine nigral-lesioned rats. Our data also confirm the observations of Zambotti, Carruba & others (1976) on the response of the nigral-lesioned rat to mazindol. All these authors noted ipsiversive rotational activity in animals with various types of lesions of the nigrostriatal dopamine pathways. Zambotti & others (1976) recorded that mazindol produced rotation comparable to that induced by amphetamine and concluded that mazindol probably had a similar mechanism of action. However, mazindol, nomifensine and dita differ from amphetamine in being uptake inhibitors rather than releasing agents.

Taken together, all these data serve to identify an additional site and mechanism of drug action which can induce rotational behaviour in the rodent with a selective unilateral lesion of the dopamine nigrostriatal pathways. The present data further show that uptake inhibitors are functionally comparable to even a very potent releaser of dopamine such as amphetamine. Both classes of drugs depend upon presynaptic dopamine stores and produce their behavioural effects by increasing the amount of dopamine available in the synaptic

Table 1. *Mean total number of ipsiversive 360° turns completed (with 1 s.d.) by nigral-lesioned rats (n = 6) after the various treatments studied.* Experiments with dita were concluded at 2 h (see Fig. 1).

Drug	Dose mg kg^{-1}	Cumulative number of turns 2 h	3 h
Saline control (1 ml)	—	54 (50)	69 (63)
Amphetamine	2·5	1282 (501)	1867 (704)
Mazindol	5	1078 (254)	1470 (263)
Nomifensine	3·4	1123 (287)	1625 (509)
Dita	11·7	674 (148)	—

cleft or in the striatal neuropil for interaction with dopamine receptor sites. Consequently, they produce a similar response in the rotating animal model. The striatum on the side of the lesion is depleted of dopamine so that the major effect of an uptake inhibitor or of a releasing agent occurs on the intact side. Accordingly, the animal in either case rotates away from the side of greater striatal dopamine activity and towards the lesioned side.

Although the rotating animal model cannot distinguish between these two classes of indirect dopamine agonists, it nevertheless adds a dimension to the study of the 'remote' analogues of amphetamine. The efficacy of the three drugs failed to correlate with their observed potency in inhibiting dopamine uptake *in vitro*. Mazindol is a more potent dopamine uptake inhibitor than nomifensine or dita, the latter two being approximately equivalent. Heikkila & others (1977) found ED50 values (point of 50% inhibition of uptake), for the three drugs of 2·8, 8·5 and 7·8 $\times 10^{-7}$ M respectively. Mazindol was indeed more potent in the rotating rat than dita but nomifensine proved equivalent to mazindol. Possibly the divergent results reflect differences in drug metabolism. For example, several active metabolites of nomifensine are formed *in vivo* (Kruse & others, 1977), one of which is equipotent with nomifensine itself in inhibiting dopamine uptake.

The dopamine uptake inhibitors we have studied also inhibit the uptake of noradrenaline and 5-hydroxytryptamine. Thus the possibility that these monoamines may modulate the circling response in animals with lesions of the dopamine nigrostriatal system cannot be excluded. However, the fact that dopamine uptake inhibitors cause circling in these animals whereas desipramine and amitriptyline, potent inhibitors of noradrenaline and 5-HT uptake, do not (Christie & Crow, 1973; Pycock & others, 1976) is consistent with the growing body of evidence relating rotational behaviour in this animal model primarily to dopamine neural systems.

May 22, 1978

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GABA involvement in neuroleptic-induced catalepsy

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Neurophysiological (cf. Stevens, Wilson & Foote, 1974; Dray & Straughan, 1976) and biochemical (Kim, Bak & others, 1971; Bartholini & Stadler, 1977; Cheramy, Nieoullon & Glowinski, 1977; Lloyd, Shemen & Hornykiewicz, 1977) evidence indicates that a GABA-mediated mechanism is involved in the regulation of the dopaminergic nigrostriatal tract. Results of behavioural experiments also suggest this regulation, but the data are more difficult to interpret (Stevens & others, 1974; Dray, Fowler & others, 1977; Olpe, Schellenberg & Koella, 1977, Scheel-Kruger, Arnt & Magelund, 1977). However, amongst other parameters, neuroleptic-induced catalepsy has been studied. Thus, benzodiazepines (which have been suggested to act via a GABA-ergic mechanism) as well as aminooxyacetic acid (AOAA), a GABA-transaminase inhibitor, potentiate neuroleptic-induced catalepsy. Furthermore, *p*-chloro-β-phenyl-GABA, which is a structural analogue of GABA (although its mechanism of action is at present unclear), also potentiates this syndrome (Kääriäinen, 1976; Keller, Schafner & Haefely, 1976). The experiments reported here were to study the effects of direct and indirect GABA agonists or antagonists on the catalepsy induced by various neuroleptics.

Male Sprague-Dawley CD COBS rats (180–220 g; Charles River, France) were used. Catalepsy measurements (four-cork test, Worms & Lloyd, 1978) were performed in a quiet laboratory, with the temperature maintained constant at 20° ± 1°. In each experiment 6 rats were used per dose. Dose schedules used were:

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