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A Comparison of Drug-induced rotation in rats lesioned in the Medial Forebrain Bundle with 5,6-Dihydroxytryptamine or 6-Hydroxydopamine

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Abstract Drug-induced rotational behaviour was studied in two groups of rats with differing chemical lesion of the right medial forebrain bundle (MFB). 6-hydroxydopamine (6-OH-DA), 3.5 μg, injected in one group, induced a marked lowering of dopamine (DA) and noradrenaline (NA) in the right hemiforebrain. 5,6-Dihydroxytryptamine (5,6-HT), 10 μg, injected in a second group, produced a profound and long-lasting depletion of 5-hydroxytryptamine (5-HT) and DA, but not of NA.

Rotational behaviour induced in both groups by DA receptor agonists (apomorphine, piribedil, L-DOPA, ergometrine, ergocornine, 2-bromo-α-ergocryptine, ergocristine, methylergometrine) and agents releasing DA (*d*-methamphetamine, methylphenidate) were qualitatively identical and quantitatively very similar, suggesting a minor role of 5-HT striatal terminals in these experimental conditions. LSD induced contralateral rotation by direct stimulation of the DA receptor, while L-5-hydroxytryptophan (L-5HTP) was inactive.

Introduction

For the study of the relationship between neurotransmitters derangement and Parkinson's disease, animal models based on localized lesions in the extrapyramidal system have been developed in recent years (35, 4, 32, 51). A common feature of these models is the unilateral impairment of the dopaminergic nigrostriatal pathway by either electrolytic (4) or chemical (48, 49) or functional lesions (26). The resulting imbalance between the dopaminergic activity in the intact and in the impaired striatum leads to a spontaneous or drug-induced rotational behaviour. So far the approach imitating best the situation in Parkinson's disease has been the chemical lesion induced by stereotaxic injection

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in the vicinity of dopamine (DA) cell bodies of the substantia nigra (SN) or of the DA axons at the level of the area ventralis tegmenti or of the lateral hypothalamus with small amounts of 6-hydroxydopamine (6-OH-DA) (49). Degeneration induced by 6-OH-DA resulted in a highly selective depletion of DA, accompanied by a decrease of noradrenaline (NA) when the injected site was close to noradrenergic fibres or cell bodies (49). However, Parkinson's disease is associated not only with a deficiency of DA but also with a reduction of 5-hydroxytryptamine (5-HT) in the striatum, while significant alterations of the NA level are present mainly in the hypothalamus (17, 6, 24). Moreover, according to recent findings in different experimental animals, the striatal dopaminergic function seems to be interrelated in some way with a 5-hydroxytryptaminergic mechanism (22, 9). Therefore an animal model with a unilateral concomitant lowering of DA and 5-HT appears to reflect more closely the monoaminergic derangement observed in Parkinson's disease and to be more appropriate for the study of a possible functional relationship between these two neurotransmitters in the striatum.

Recently it has been shown that 5,6-dihydroxytryptamine (5,6-HT) stereotaxically injected into one medial forebrain bundle (MFB) of rats produced a long-lasting depletion of both DA and 5-HT, but not of NA (42, 43). Hence, in the present study, the spontaneous and drug-induced rotational behaviour of rats with lesions induced by 5,6-HT was compared to that occurring in animals injected in the MFB with 6-OH-DA.

For the comparison of the two animal models drugs were used that were already known to act on the dopaminergic junction either presynaptically (methamphetamine, methylphenidate) or postsynaptically (apomorphine, L-DOPA, piribedil) (4, 18, 10, 51). Some ergot alkaloids and derivatives that have recently been shown to stimulate directly DA receptors (11, 52) were included. To explore a possible involvement of the 5-HT system in rotational behaviour we studied LSD and N,N-dimethyltryptamine, hallucinogenic indole derivatives postulated to stimulate 5-HT receptors in the central nervous system (25, 46) and reported to induce stereotyped behaviour in rats (15). BOL-148, a congener of LSD devoid of hallucinogenic activity (40, 41) and a potent antagonist of 5-HT in peripheral tissues, as well as quipazine, a compound postulated to stimulate central 5-HT receptors (38) and possibly also interacting with the DA system (21) were also investigated.

Methods

Animals and criterion of selection.

The stereotaxic operation was performed on SPF male rats from the stock Füllinsdorf albino with a weight of 150 ± 5 g. After 10–12 days the operated animals were tested for rotation activity by injecting apomorphine (3 mg/kg i.p.). For further circling studies only those responding with at least

20 turns/2 min (10–15 min after apomorphine injection) were used. Approximately 50 % of the operated animals failed at this criterion and were discarded.

Stereotaxic lesion.

The animals were operated in a David Kopf stereotaxic instrument under anaesthesia with sodium pentobarbitone (45 mg/kg i.p.). Some of the animals were anaesthetized with halothane in order to study the spontaneous behaviour immediately after the operation.

Intracerebral unilateral (right side) injections of 5,6-HT (10 μg as base in 10 μl solvent) or 6-OH-DA (3.5 μg as base in 4 μl solvent) were carried out through a stainless-steel cannula (diameter 0.3 mm) connected to a microsyringe.

As solvent, Ringer solution containing 0.02 % ascorbic acid was used. The liquid was injected at a speed of 2 μl/min for 5,6-HT and 1 μl/min for 6-OH-DA by means of a high precision infusion pump, following a technique already described (43).

In order to deposit 5,6-HT or 6-OH-DA in the vicinity of NA, DA and 5-HT pathways in the MFB we used the following coordinates: A + 3.8 mm, L (right) − 0.9 mm, V − 3.3 mm according to the Atlas of König and Klippel (28) as previously described (43).

Histological controls were randomly performed only in order to confirm the localization of the cannula. We found a good correlation between the correct localization of the cannula and the positivity of the selection criterion of apomorphine-induced rotation.

Recording of rotational behaviour

The experiments were performed on 85 rats (63 injected with 5,6-HT and 22 with 6-OH-DA).

The circling behaviour was measured as the number of turns performed by an animal during a 2 min period. The rats were placed individually in perspex cages (base 35 cm × 20 cm, height 15 cm), immediately after injection of drugs. The measurement was started 5 min after the drug administration and performed every 5 min for a period of 2 min, extending over 1 hr, or longer, if necessary.

Biochemical analysis.

10–12 or 170 days after the lesion the animals were killed by decapitation and two pooled hemitelediencephala were analyzed for 5-HT, DA and NA (44).

Drugs and solutions.

The following drugs were used: *d*-methamphetamine HCl, methylphenidate (phenyl (α-piperidil) acetic acid methylester · HCl), LSD (*d*-lysergic acid diethylamide bitartrate), L-Dopa (3,4-dihydroxy-L-phenylalanine methylester

· HCl), 5-hydroxy-L-tryptophan ethylester · HCl, benserazide (1-DL-seryl-2-(2,3,4-trihydroxybenzyl) hydrazine · HCl), apomorphine · HCl, ergocornine bimaleate, methylergometrine bimaleate, mescaline (3,4,5-trimethoxyphenethylamine · HCl), PCPA (*p*-chloro-D,L-phenylalanine methylester · HCl) were dissolved in distilled water. Ergometrine, ergocristine, 2-Br-α-ergocryptine methansulfonate (CB-154), methysergide (N-methyl-*d*-lysergic acid butanolamide), BOL-148 (2-bromo-*d*-lysergic acid diethylamide bitartrate), DMT (N,N-dimethyl-tryptamine-HCl), quipazine (2-(1-piperazinyl) quinoline maleate) and piribedil (1-(2″-pyrimidil)-4-piperonylpiperazine) were suspended in 5 % gum acacia.

All substances used were of analytical grade. The reported doses of drugs refer to the salt and were injected i.p. in a volume of 0.2 ml/100 g. One week without treatment was allowed between drug administrations.

Results

Monoamine levels after injection of MFB with 5,6-HT.

10–12 days after unilateral injection of MFB with 5,6-HT, a selective depletion of 5-HT and DA was observed in the lesioned telediencephalon (as compared to the intact side) of animals not selected on the basis of their rotational response to apomorphine (43). In rats selected according to their response to apomorphine, 5-HT and DA were still lowered respectively by 70 % and 93 % 170 days after the lesion, whereas NA was only slightly affected (Fig. 1, a, b).

Postural and locomotor asymmetry in rats lesioned with 5,6-HT or 6-OH-DA.

Rats injected with either 5,6-HT or 6-OH-DA showed marked postural asymmetry and rotation ipsilateral to the lesion, on recovery from halothane anaesthesia; pinching the tail enhanced these anomalies. This spontaneous behaviour slowly disappeared during the subsequent 10 days.

In addition a rotation, lasting 2–3 min and directed contralaterally to the lesion, appeared 3–4 weeks after the lesion in response to sudden stress (e.g. placing the animal in a new environment or handling for injection) and persisted afterwards.

On the basis of the early ipsilateral and the late contralateral asymmetric behaviours, animals injected with 6-OH-DA could not be differentiated from those lesioned with 5,6-HT.

Circling after the lesion with 5,6-HT.

Drugs inducing contralateral rotation (towards the non-lesioned side). After 5,6-HT, the application of apomorphine (1 mg/kg) induced a rotation which started approximately 5 min (about 20 turns in 2 min) and ceased 90–120 min after the injection (Fig. 2A). The intensity of the turning behaviour using

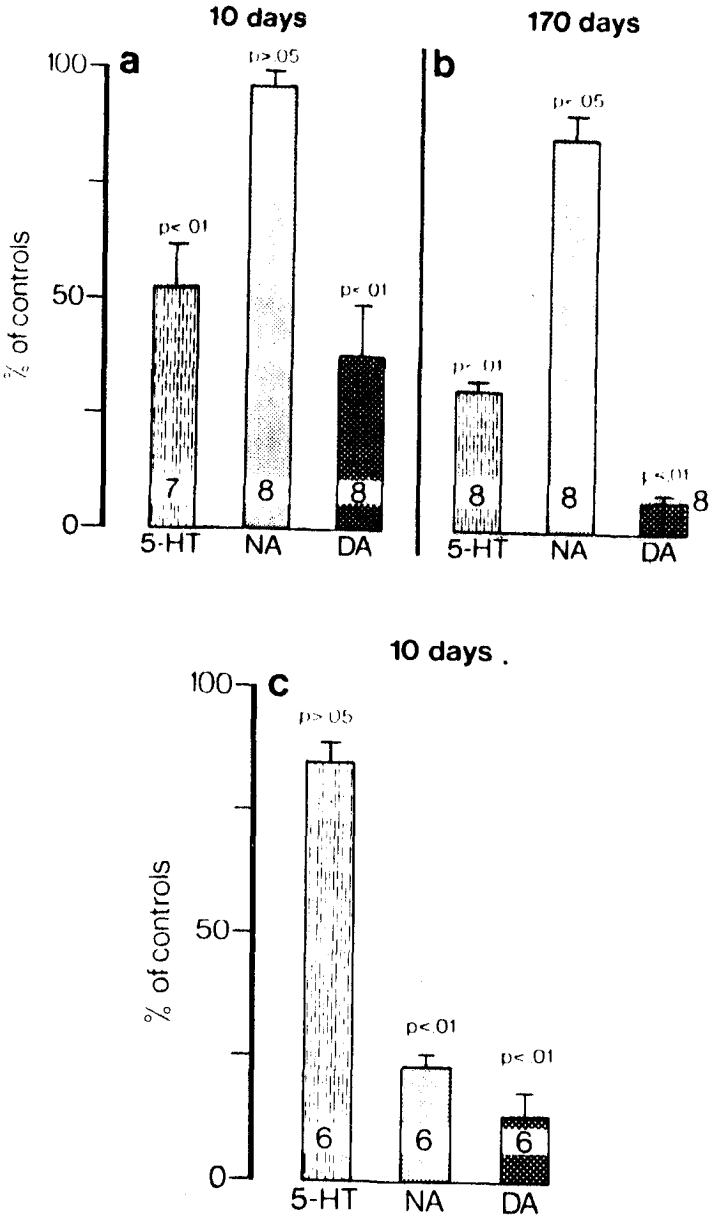

FIG. 1

Levels of 5-HT, NA and DA in the lesioned telediencephalon 10 days (a) and 170 days (b) after 5,6-HT (10 μg/10 μl solvent) or 10 days (c) after 6-OH-DA (3.5 μg/4 μl solvent) unilaterally applied in the MFB. The columns indicate the means with S.E.M., in per cent of the values found in sham-injected animals (= 100 %). Student's *t*-test. The *number of determinations* (each from two pooled hemitelediencephala), as well as the statistical significance, are given in each column. No significant changes were observed in the hemisphere contralateral to the lesion.

Absolute values in ng/g tissue of sham-injected controls:
a: 5-HT, 439 ± 28 (9); NA, 378 ± 7 (8); DA, 1068 ± 84 (8).
b: 5-HT, 462 ± 24 (8); NA, 470 ± 12 (8); DA, 706 ± 5 (8).
c: 5-HT, 359 ± 6 (7); NA, 314 ± 11 (7); DA, 864 ± 46 (7).

apomorphine in amounts higher than 1 mg/kg was not dose-dependent, due to the concomitant appearance of other stereotype signs interfering with turning.

Piribedil (25 mg/kg) elicited rotation 5 min after drug application, with relatively stable and long-lasting (up to 6 hr) turning (Fig. 2 B). L-DOPA (15–125 mg/kg) after pretreatment with benserazide at a dose (50 mg/kg) that inhibits selectively extracerebral DOPA decarboxylase produced a rotation of rapid onset and of 120 min duration (10 animals). LSD (0.2 mg/kg) induced circling approximately 5 min after the injection which ceased 60 min afterwards (Fig. 2 C). After repeated application of LSD (0.2 mg/kg once a day for 5 days), no reduction or suppression of turning was noticed. Ergometrine (5 mg/kg) initiated 15–30 min after application a long-lasting rotation (up to 180 min). The peak of rotational activity (30 turns/2 min) was reached approximately 1 hr after drug injection (Fig. 2D). Ergocornine (5 mg/kg) elicited a long-lasting (more than 3 hr) turning, beginning 5–10 min after the injection (Fig. 2 E). 2-bromo-α-ergocryptine (5 mg/kg) initiated turning between 70 and 120 min after administration, with a duration of more than 5 hr. Methylergometrine (5 mg/kg) was also able to induce rotation, reaching its peak (about 40 turns/2 min) 35 min after injection, with a duration longer than 1 hr (7 animals). All 7 animals died 5–6 hr after the injection, while all unoperated animals survived after the same dose. 1 mg/kg was not toxic but also unable to elicit rotation.

Drugs inducing ipsilateral rotation (towards the lesioned side): *d*-Methamphetamine (3 mg/kg) elicited turning which began about 10 min after injection and lasted longer than 60 min. Methylphenidate (10 mg/kg) induced rotation with a somehow quicker onset than *d*-methamphetamine, an essentially similar duration of action, and an intensity reaching a plateau of about 10 turns/2 min after 20–30 min (Fig. 2 F).

Drugs not inducing circling: Quipazine (10 to 20 mg/kg) evoked a pronounced asymmetry towards the lesioned side but no rotational behaviour. BOL-148 (20 mg/kg), DMT (1 to 7.5 mg/kg), mescaline (5 to 20 mg/kg) and methysergide (30 mg/kg) were inactive, up to 3 hr after the injection.

Effect of PCPA or L-5HTP on turning.

Lowering of the cerebral 5-HT by PCPA (4 consecutive daily i.p. injections of 100 mg/kg) did not result in rotational behaviour. Administered 16 hr after the last injection of PCPA, *d*-methamphetamine (5 mg/kg) induced ipsilateral rotation, while LSD (0.2 mg/kg) and apomorphine (1 mg/kg) induced contralateral rotation. These turning curves did not show any appreciable difference in comparison to those obtained without pretreatment of PCPA. Administration of 100 mg/kg L-5HTP to animals pretreated with benserazide (50 mg/kg) did not result in any locomotor effect. In addition this treatment was unable to modify the turning behaviour elicited by LSD (0.2 mg/kg), apomorphine (1 mg/kg) or *d*-methamphetamine (5 mg/kg) injected 60 min afterwards.

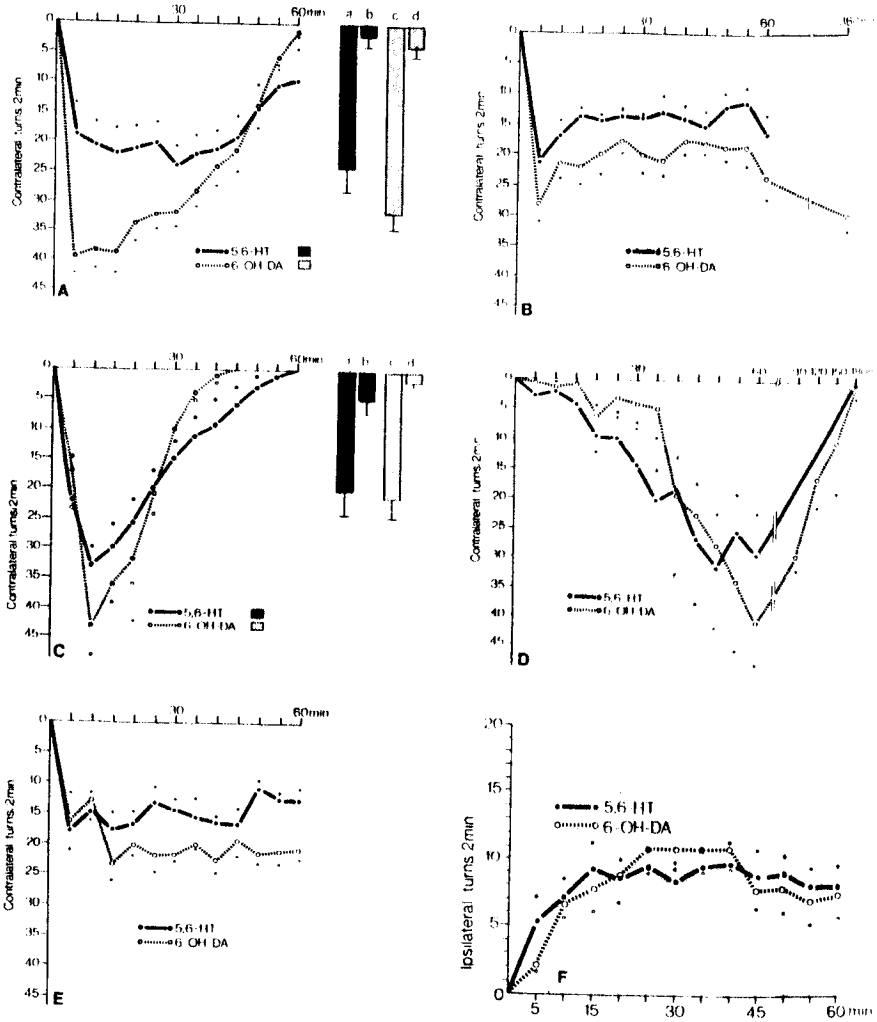

FIG. 2

Contralateral rotation elicited by apomorphine (1 mg/kg i.p.) (A); piribedil (25 mg/kg i.p.) (B); LSD (0.2 mg/kg i.p.) (C); ergometrine (5 mg/kg i.p.) (D); ergocornine (5 mg/kg i.p.) (E), and ipsilateral rotation after methylphenidate (10 mg/kg i.p.) (F), in rats lesioned in the right MFB with 6-OH-DA (dotted line) or 5,6-HT (solid line).

On the abscissa is indicated the time after drug application. On the ordinate, the intensity of the circling is given by the number of turns/2 min counted every 5 min (means of 5-10 animals).

The S.E.M.'s are indicated by small points or circles. The effect of haloperidol (1 mg/kg i.p.) injected 15 min after apomorphine and 10 min after LSD is shown in A and C on the right-hand side.

The bars labelled a and c indicate the turning activity in the two groups of rats, 30 min after apomorphine or 25 min after LSD respectively and the bars labelled b and d the turning activity under the effect of haloperidol. Mean ± S.E.M. (N = 10) are given. In all cases the differences between a, b and c, d are significant ($p < 0.001$, Student's t-test two-tailed).

Monoamine levels after injection of MFB with 6-OH-DA.

6-OH-DA (3.5 μg in 4 μl) injected into the MFB elicited a quite selective and marked decrease of the telediencephalic NA and DA (by 78 % and 87 % respectively) 10–12 days after application, whereas the 5-HT level was only slightly affected (Fig. 1 C).

Drug-induced circling after 6-OH-DA lesion.

Apomorphine (Fig. 2 A), piribedil (Fig. 2 B), L-DOPA, LSD (Fig. 2 C), ergometrine (Fig. 2 D), ergocornine (Fig. 2 E), *d*-methamphetamine and methylphenidate (Fig. 2 F), at the same dose used as in the 5,6-HT-lesioned rats produced rotation patterns, similar in onset and direction to those observed in 5,6-HT-injected animals. In addition ergocristine (5 mg/kg) elicited contralateral turning approximately 30 min after application and lasting more than 2 hr.

Sham-operated animals.

Animals in which the MFB was injected with 5-HT and DA equimolar to 10 μg 5,6-HT and 3.5 μg 6-OH-DA in 10 and 4 μl solvent respectively, showed no tendency to rotate after apomorphine (5 mg/kg), LSD (0.2 mg/kg i.p.) and *d*-methamphetamine (5 mg/kg).

Effect of haloperidol on drug-induced rotation.

Haloperidol (1 mg/kg i.p.) administered at the time of peak rotational activity of apomorphine (Fig. 2 A), LSD (Fig. 2 C), ergometrine and *d*-methamphetamine, significantly reduced the rotational behaviour in both groups of lesioned animals.

Discussion

As reported previously, in rats injected into one MFB with 5,6-HT, the level of DA and 5-HT in the lesioned telediencephalon was markedly decreased 10 days after application: this effect was related to anterograde fibre degeneration (43). This finding contrasts with that after the intraventricular application of 5,6-HT which induced a quite selective depletion of 5-HT (42). The lowering of DA and 5-HT is still present and marked 170 days after the local intracerebral injection, indicating a long-lasting and probably irreversible damage. The effect of 5,6-HT seems to be restricted to DA and 5-HT neurons since the level of NA is only slightly changed.

On the other hand, the fact that both DA and NA but not 5-HT are strongly depleted after application of 6-OH-DA at the same site in MFB, supports the inference that the two neurotoxic agents induce a quite specific fibre degenera-

tion. The conclusion that 5,6-HT, at appropriate doses, does not destroy the NA system is reinforced by the finding that this agent, in contrast to 6-OH-DA (unpublished results), when applied in the NA dorsal bundle, does not produce a lowering of NA (43).

In addition the two neurotoxic agents applied in the MFB at lower concentrations (2 μg 5,6-HT/10 μl or 1.7 μg 6-OH-DA/4 μl) induced biochemical changes and rotational responses to apomorphine and *d*-methamphetamine comparable to those obtained by injecting higher concentrations (10 μg 5,6-HT/10 μl or 3.5 μg 6-OH-DA/4 μl) (42, 43 and unpublished results). With these lower concentrations, according to Sotelo *et al.* (45) and Agid *et al.* (2), it can be assumed, at least for 6-OH-DA, that a quite elective degeneration takes place. The determination of the acetylcholine level and of the acetylcholinesterase activity in the rat striatum (3.5 μg/4 μl 6-OH-DA in MFB) and the glutamic acid decarboxylase and choline acetylase activity (10 μg 5,6-HT/10 μl in MFB) do not indicate an unspecific damage of the cholinergic or gabaergic fibres (Möhler and Stadler, personal communications).

However, the possible encroachment on other ascending and/or descending neuronal pathways by the neurotoxic agent cannot be completely ruled out (36, 37), although the difference in findings between this author and others (7) could be explained by the fact that the latters have used a 5 times less concentrated solution (2 μg/μl) than that found to produce "nonspecific histopathological changes" (10 μg/μl) (7).

Therefore, considering all the points discussed above, it can be reasonably concluded that the application of the two neurotoxic agents in MFB provides two models in which a decrease of DA is associated in one case with depletion of 5-HT and in the other case with a depletion of NA. Animals of both groups manifested a similar short-lasting spontaneous postural and locomotor asymmetry (tendency to deviate or rotate towards the lesioned side), which might be interpreted as a result of a transient predominance of DA activity in the intact hemisphere, compensating functionally with time.

On the other hand, the "paradoxical" rotation, appearing in response to stress 3–4 weeks after the operation, might be connected with the accelerated turnover of DA observed in the few dopaminergic neurons that are still intact after intranigral injection of 6-OH-DA (1). In fact, the approximately 3.500 DA neurons of each substantia nigra have an enormous number of terminals with a high degree of divergence, i.e. a single cell in the striatum probably receives DA terminals from different DA neurons (5). An enhanced release of DA within such a network in response to stress on the side of denervation supersensitivity could result in abrupt, very short, dopaminergic predominance on this side.

In conclusion, considering spontaneous postural and locomotor asymmetry and paradoxical rotation, both groups of rats behaved similarly to animals lesioned with 6-OH-DA in the substantia nigra (50, 51).

Drug-induced rotational behaviour in both animal models was investigated using as criteria the direction, duration and intensity of rotation to be expected

on the basis of the pre-synaptic (release) and the post-synaptic (denervation supersensitivity) mechanism of action of drugs acting on dopaminergic junctions (4, 51).

Injections of *d*-methamphetamine, methylphenidate, apomorphine, piribedil and L-DOPA produced comparable rotation in rats with MFB lesioned by either 6-OH-DA or 5,6-HT. The responses were similar to those reported by Ungerstedt *et al.* (51) for rats injected into the SN with 6-OH-DA. Haloperidol, a catecholaminergic blocking agent, reduced, as was to be expected, the turning response also in our experimental conditions.

In agreement with previous studies (30) our data seem to exclude an inhibitory role of the 5-HT system on turning. On the other hand, as already postulated (50), a NA mechanism might be involved in rotational response, since apomorphine and piribedil induced a slightly higher rotational response in 6-OH-DA than in 5,6-HT-lesioned rats (Fig. 2 A, B). However, the significance of this difference is questionable because the two neurotoxic agents did not produce identical damage to DA neurons (see Fig. 1).

Our results show that in both models the peptide-type ergot alkaloid ergocornine and its analogue 2-Br-α-ergocryptine were able to induce contralateral rotations similar to that found previously with a lesion of the substantia nigra (11). Also with ergocornine 6-OH-DA lesioned rats turned more than 5,6-HT-lesioned animals. The amine ergot alkaloid ergometrine induced a long-lasting contralateral circling that was antagonized by haloperidol, indicating dopaminergic agonist properties as previously reported (52). Ergometrine and its analogue methylergometrine at the dose able to induce rotation (5 mg/kg) and which was not toxic in normal animals, produced a mortality of 40–50 % in the lesioned rats. The lethality was further increased when these drugs were applied together with haloperidol and therefore in this case the turning antagonism could not be studied in extenso. Ergocristine, another ergot derivative capable of inducing circling, showed a less pronounced toxicity (about 20 %). The ergot alkaloids and derivatives tested had a markedly longer-lasting effect than apomorphine, with the notable exception of LSD. The contralateral circling induced by LSD was similar in onset, intensity and duration in rats injected in MFB with 5,6-HT and with 6-OH-DA. This suggests that LSD acts primarily by stimulating DA receptors. This assumption is strongly substantiated by the blockade of the rotational effects of LSD by haloperidol. A presynaptic site of action of LSD seems improbable, although a slight depletion of DA by this drug has been reported (14). In fact, 4 hr after α-methyl-L-tyrosine (200 mg/kg i.p.), no diminution of LSD-induced rotation was seen, despite the pronounced sedation and catecholamines depletion in the whole CNS (34). In addition two groups of 8 rats (lesioned in MFB with 5,6-HT or 6-OH-DA, respectively) pretreated 16 hr before with 7.5 mg/kg i.p. reserpine showed a contralateral circling after 0.2 mg/kg i.p. LSD similar to animals without pretreatment (unpublished results). The duration of the LSD-induced contralateral rotation was rather short and agrees quite well with the half-life of the drug in the brain of rats. In

fact, a peak of rotational activity was observed at a time when the maximal concentration of the drug in brain was reached, 10–15 min after i.p. injection (39). The rotation decreased gradually and ceased after 50–60 min, which is a little longer than the time of the disappearance of LSD from the rat brain, reported to be 30 min (39). Biochemical results indicating reduction of DA turnover (13) tend to further support that ergometrine and LSD stimulate DA receptors.

The possible role of 5-HT on rotation activity is not clear, since there are some indications that a 5-HT mechanism could be inhibitory on locomotion in rats (29, 33) and that in the cat 5-HT locally applied in the anteroventral area of the head of the caudate elicits a peculiar sterotyped behaviour (9). Moreover biochemical findings in the rat show that brain 5-HT and 5-hydroxyindolacetic acid could be increased by apomorphine, but only in the case dopaminergic receptors were not blocked by spiroperidol and pimozide (22). However, using rats lesioned in MFB with 5,6-HT we were unable to demonstrate a possible inhibitory role of 5-HT on turning, since pretreatment with PCPA in one case or with L-5HTP in the other, did not "per se" induce circling and did not modify the rotational effect of *d*-methamphetamine, apomorphine and LSD, although these pretreatments were quite effective in decreasing or increasing respectively the rat brain 5-HT (27, 12).

We were unable to induce circling either by drugs supposed to stimulate (N,N-dimethyltryptamine, quipazine, L-5HTP) or block (BOL-148 and methysergide) central 5-HT receptors, or by mescaline, a phenylethylamine with hallucinogenic properties. However, quipazine induced a strong postural asymmetry towards the lesioned side that is not readily explainable even in the light of a possible interaction with DA receptors (21).

Unlike the tolerance in behavioural and autonomic effects reported to occur with LSD (20), we were unable to change the rotational response to LSD by 5 subsequent daily injections of 200 μg of the drug.

The finding that LSD has DA receptors stimulating properties seems to be of particular interest since so far this potent hallucinogenic drug in man was generally regarded as merely acting on 5-HT neurons (19, 3). On this basis the psychotomimetic properties of LSD which, as well known, are counteracted by neuroleptic drugs (15, 31) should be considered also in the light of a dopaminergic implication. In this connection a possible involvement of the dopaminergic terminals of the limbic cortex (47, 23) and/or of the retina (16) could be of importance in the mechanism underlying the occurrence of hallucinations after LSD in man.

In conclusion our data obtained with a new, more differentiating rotational model confirm and extend the notion that many ergot alkaloids and derivatives act as DA agonists. Therefore, a thorough investigation of other ergot analogues could probably lead to less toxic and more active compounds. The experimental approach used here suggests that NA but not 5-HT pathways could modulate turning behaviour.

In general, considering the recent findings of Butcher *et al.* (8), the point of the selectivity of neurotoxic effect of 6-OH-DA is still controversial and needs further assessment. Anyhow, due to the biochemical and pharmacological findings reported, we feel that the dopaminergic system may play a major role in circling behaviour in the rat; however it cannot be excluded that other mechanisms might be involved in the integration of nervous activity subserving turning behaviour (37).

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