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TURNING IN MFB-LESIONED RATS AND ANTAGONISM OF NEUROLEPTIC-INDUCED CATALEPSY AFTER LISURIDE AND LSD

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Summary

The effects of lisuride, d-lysergic acid diethyl amide (LSD) and apomorphine were studied in rats with unilateral destruction of nigro-striatal nerve terminals either with 6-hydroxydopamine (6-OHDA) or 5,6-dihydroxytryptamine (5,6-DHT). Lisuride at the dose of 50 $\mu g\ kg^{-1}$ i.p. induced contralateral turning for more than 4 hours while the circling induced by LSD (200 $\mu g\ kg^{-1}$) and apomorphine (1 mg kg^{-1}) persisted for only one hour. Lisuride, a compound stimulating both dopamine (DA) and 5-hydroxytryptamine(5-HT) receptors induced a more intense turning in 6-OHDA than in 5,6-DHT lesioned rats. This might indicate a modulation of 5-HT on rotational behavior. Haloperidol (1 mg kg^{-1} i.p.) antagonized both lisuride- and LSD-induced turning. LSD, and much more persistently lisuride, counteracted the prochlorperazine-induced catalepsy. These findings correlate with the biochemical data indicating that lisuride is a very potent agonist at central dopaminergic receptors.

Introduction

Biochemical and neuroendocrinological evidence indicates that lisuride, a semi-synthetic ergot-derivative used as an antimigraine agent (1), and the hallucinogen d-lysergic acid diethylamide tartrate (LSD) are potent agonists at dopamine (DA) as well as at 5-hydroxytryptamine (5-HT) receptors in the brain (2,3,4,5,6,7,8,9,10).

In the present investigation we studied lisuride, LSD and a relatively specific DA agonist i.e. apomorphine, for their ability to induce contralateral rotation in rats with unilateral chemical lesion in the medial forebrain bundle (MFB) induced by either 6-hydroxydopamine (6-OHDA) or 5,6-dihydroxytryptamine (5,6-DHT). The aim was to compare the potencies of these drugs in producing circ-

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ling and to elucidate the possible role of the 5-HT system in turning behavior. The two types of chemical lesion were chosen because DA and NA are preferentially depleted after 6-OHDA, whereas 5,6-DHT mainly decreases the levels of DA and 5-HT (11,12). As is well-known, the intracerebral local injection of these neurotoxins, which are rapidly taken up by monoaminergic fibers, results in a much more selective damage than electrolytic degeneration (13). However, the use of neurotoxic compounds for lesioning the cerebral monoaminergic systems raises the still unsolved problem of the degree of specificity of these lesions (13,14). From previous studies performed in our laboratory it appears that a certain selectivity of fiber degeneration results from unilateral injection in the MFB of 5,6-DHT and 6-OHDA respectively, provided these neurotoxins are applied in appropriate doses and concentrations (11,12). Therefore, these two animal models appear to be adequate for pharmacological characterization of compounds interacting with various monoaminergic systems. This approach could also provide some clues for defining the interaction between 5-HT and DA neurons which is suggested by various pieces of experimental evidence (15,16,17).

The recently proposed model of a lesion restricted to 5-HT fibers (18) using 5,7-dihydroxytryptamine (5,7-DHT) appears inadequate to study the effect of 5-HT in circling since the rotation induced by 5-HT agonists in this animal model is much less intense (and therefore difficult to quantify) than that observed after DA agonists in nigrostriatal lesions. Moreover, according to our experience, the lesions induced by 5,7-DHT seem to be rather unspecific (6). Therefore, we though that a more suitable condition to clarify the issue of 5-HT and DA interaction in turning behaviour would be a model in which intense turning is induced in nigrostriatal-lesioned rats by drugs which stimulate both DA and 5-HT receptors in the absence as well as in the presence of intact 5-HT pathways. As an additional approach we looked for a possible antagonism of prochlorperazine-induced catalepsy by lisuride and LSD.

Materials and Methods

Unilateral lesions of the right MFB were performed in female rats (SPF, Füllinsdorf albino stock) by local injections of 6-OHDA (3.5 µg as base in 4 µl) or 5,6-DHT (10 µg as base in 10 µl). Both neurotoxic compounds were dissolved in Ringer's solution containing 0.02 % ascorbic acid (12). After 10-15 days the rats were challenged with apomorphine (1 mg kg^{-1} i.p.). Animals rotating towards the intact side (contralateral turning) for about 1 hour were selected for further investigations. The circling was recorded continuously in an automatic rotameter (12), one week after the selection with apomorphine ; the animals were employed only in one experimental run. In some experiments haloperidol (1 mg kg^{-1}) was given i.p. 30 min. before lisuride or LSD.

In intact male rats of the same strain, maximal catalepsy was induced by injecting 15 mg kg^{-1} i.p. prochlorperazine methanesulfonate. The animals were considered to be cataleptic if the

homolateral limbs, when crossed by the experimenter, remained in this unnatural position for at least 10 sec. Catalepsy as defined above was present in all animals between the third and the sixth hour after prochlorperazine. Therefore, lisuride and LSD were administered in three different doses 3 hours after prochlorperazine, using different groups of animals for each treatment. 5,6-DHT and 6-OHDA were obtained from Regis, prochlorperazine methanesulfonate (dissolved in water) from Roche, haloperidol (dissolved in 1 % lactic acid) from Janssen, d-lysergic acid diethylamide tartrate from Sandoz, lisuride hydrogen maleate from Spofa (Prague) and apomorphine hydrochloride from Siegfried (Zofingen, Switzerland).

All compounds were injected i.p. and their doses refer to the salt. Statistical analysis was performed using the Kolmogorov-Smirnov-test, two-tailed (19).

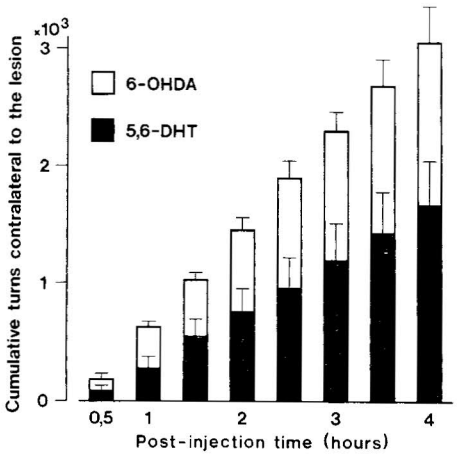

FIG. 1

Turning response to lisuride (50 μg kg^{-1}) of rats lesioned unilaterally in the MFB with 6-hydroxydopamine (6-OHDA) or 5,6-dihydroxytryptamine (5,6-DHT) expressed as cumulative counts.
Each column represents the mean ± SE of the total turns, at any given time, (N = 6).
Significance : 6-OHDA versus 5,6-DHT, $p < .05$ for all post-injection times.

TABLE 1

Cumulative contralateral turns induced by three dopamine agonists in rats lesioned by unilateral injection of 6-hydroxydopamine (Column A) or 5,6-dihydroxytryptamine (Column B) in the MFB.

post-injection time (minutes)	LSD (200 $\mu g \cdot kg^{-1}$)		APOMORPHINE (1 mg Kg^{-1})		LISURIDE (50 $\mu g\ kg^{-1}$)		HALOPERIDOL (1 mg kg^{-1}) + LISURIDE (50 $\mu g\ kg^{-1}$)
	A (N=10)	B (N=9)	A (N=16)	B (N=12)	A_1 (N=5)	B (N=5)	A_2 (N=4)
0 30	567 ±53	355 ±42	357 ±44	217 ±35	243 ±16	49 ±14	26 ±11
0 60	896 ±97	805 ±99	606 ±55	572 ±89	632 ±35	328 ±97	33 ±14

A vs B, 30 min: $p < .05$ 60 min: NS | A vs B, 30 min: NS 60 min: NS | A_1 vs B, 30 min: $p < .01$ 60 min: $p < .05$ | A_1 vs A_2, 30 min : $p < .05$ 60 min : $p < .01$

LSD , apomorphine and lisuride were injected and measurement of turning immediately started. In some experiments haloperidol was injected 30 minutes before lisuride. Control animals receiving only the vehicles exhibited less than 10 rotations in the first minutes after injection, these turns were not substracted from the values in the Table. Values are means ± SE.

Results

As shown in Table 1, rats lesioned with 6-OHDA or 5,6-DHT in the MFB responded to LSD (200 μg Kg^{-1}) and apomorphine (1 mg kg^{-1}) with a turning behavior similar in quality and intensity (except LSD at 30 minutes), i.e. by rotating away from the lesion (contralateral turning) for about 1 hour. After this time only a small percentage (20-30 %) of the animals displayed a discontinued rotation ; this last part of the recording was therefore disregarded.

Lisuride at 50 μg kg^{-1} elicited a much longer-lasting (more than 4 hours) contralateral rotation than LSD and apomorphine in all animals tested (Fig. 1).

Lisuride was much more effective in 6-OHDA-treated than in 5,6-DHT-lesioned rats over the whole time course of the rotational response (Table 1, Fig. 1).

The smaller dose of lisuride that induced turning (duration about 1 hour) in 6-OHDA-lesioned rats was 10 μg kg^{-1}. After 100 μg kg^{-1} the animals rotated only slightly more than after 50 μg kg^{-1} (4068 ± 819 against 3063 ± 303 turns in 4 hours).

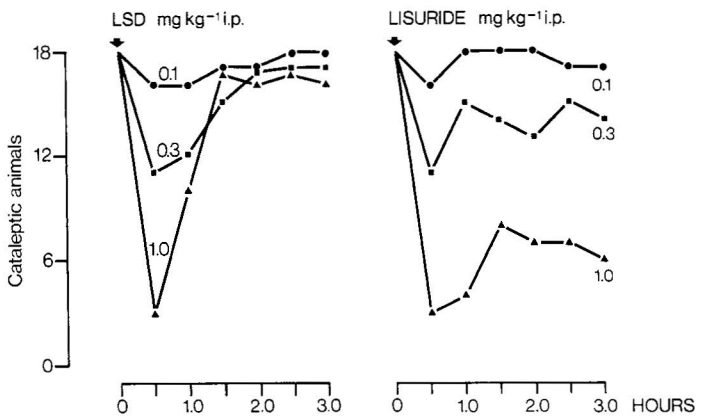

FIG. 2

Effect of d-lysergic acid diethylamide (LSD) and lisuride hydrogen maleate (lisuride) on prochlorperazine-induced catalepsy.
LSD and lisuride were injected at three different doses 3 hours after prochlorperazine methanesulfonate (15 mg kg^{-1} i.p.) using 18 rats for each dose.

As indicated in Table 1, haloperidol (1 mg kg^{-1}) injected 30 minutes before lisuride (50 μg kg^{-1}) prevented the induction of turning. As reported in Fig. 2, LSD and lisuride antagonized in a dose-dependent way the catalepsy induced by prochlorperazine. With both compounds the antagonism was seen shortly after the injection with a peak effect at 30 minutes after drug treatment. The effect of LSD was short-lasting (about 1 hour) whereas lisuride at 1 mg kg^{-1} was active for at least 3 hours. Considering the peak anticataleptic effect, LSD and lisuride were equipotent.

Discussion

The present results indicate that lisuride has a much longer duration of action than LSD, since it provokes contralateral rotation for more than 4 hours in rats lesioned unilaterally in the nigrostriatal pathways and antagonizes the neuroleptic-induced catalepsy for at least 3 hours. Induction of turning and anticataleptic properties are to be expected primarily with compounds that directly stimulate DA receptors, as is the case for apomorphine (12,20). The longer duration of action of lisuride in both the turning and the anticatalepsy model could be related to a much longer half-life of lisuride in the rat brain as compared to that of LSD, which has been determined to be 30-40 min. (21). Haloperidol, which is considered to block predominantly DA receptors (22,23), prevented circling induced by either lisuride or LSD (Table 1, 11). In addition, lisuride decreases more markedly the HVA level in the brain than LSD without producing substantial changes of DA level (2,3,4). These findings and the effects induced on the adenylate cyclase (7,24) indicate that both drugs stimulate the DA receptors. Therefore, the turning observed after lisuride and LSD and which is prevented by haloperidol seems to be induced by stimulation of a DA mechanism. However the possibility that the 5-HT system modulates in some way the rotation initiated by DA stimulation, has to be considered seriously. In fact lisuride, more than LSD, also reduces the 5-HIAA levels in the brain, suggesting a pre- and/or post-synaptic agonistic action at 5HT receptor sites (2,3,4,7,24).

The careful comparison of the rotational action of lisuride in our two animal models shows that the number of turns induced was higher in animals lesioned with 6-OHDA than in those injected with 5,6-DHT. This quantitatively different circling responses cannot be attributed to a more pronounced degree of DA supersensitivity after 6-OHDA versus 5,6-DHT lesion since the turning induced by apomorphine was very similar in both animal models (Table 1). Evidence exists that the 5-HT system could inhibit the activity of DA neurones. For instance, 5-HT receptor stimulation elicited by 5-hydroxytryptophan (5-HTP) antagonizes apomorphine-induced hypermotility (25). Moreover, 5-HTP inhibited apomorphine-induced circling behavior in nigrostriatal lesioned rats (26) and mice (27). This suggests that 5-HT is modulating the turning behavior in a direction opposite to DA, as recently proposed (26, 27). Assuming that 5-HT and DA receptors become supersensitive after 5,6-DHT, the net effect of stimulating two neuronal systems acting in opposition would be a less marked rotation than that

seen in animals lesioned with 6-OHDA, in which the 5-HT system is not affected (Table 1, Fig. 1). In fact, during the first 30 minutes after injection of either lisuride or LSD, lower frequency and a smaller total number of rotations was observed in rats lesioned with 5,6-DHT than in those lesioned with 6-OHDA (Table 1). Therefore we assume that 5-HT receptor stimulation by lisuride or LSD attenuates the circling induced by the part of the molecule acting as agonist on DA receptors.

The recent finding that 5-HT agonists induced a contralateral turning in rats lesioned with 5,7-DHT (18) needs further support, since this circling is much less intense and, therefore, more difficult to quantify than that observed after DA agonists in nigrostriatal lesions. Moreover, in 5,7-DHT-lesioned rats apomorphine (3 mg/kg i.p.) elicited a clear ipsilateral rotation (unpublished results) indicating a complex answer to the drug possibly due to unspecificity of the lesion as also observed by our recent biochemical studies using this neurotoxin (6).

Regarding the antagonism of the prochlorperazine-induced catalepsy (see Fig. 2) by lisuride and LSD, the possible link between DA and 5-HT systems must also be considered.
The reduced cataleptic effect of neuroleptics reported after raphe lesions or p-chlorophenylalanine (28) points towards a 5-HT modulation of the catalepsy. It seems unlikely, however, that the anticataleptic action of lisuride and LSD is due to a reduced activity of 5-HT neurons by autoreceptor stimulation. In fact, both compounds are anticataleptic at relatively high doses, which are considered to stimulate pre- as well as post-synaptic 5-HT receptors. Therefore, it is more likely that both compounds antagonize the cataleptsy by a direct action on the DA receptors.

In conclusion the present data, together with other biochemical and neuroendocrinological findings, clearly indicate that lisuride stimulates DA receptors. In addition, the decrease of the 5-HT turnover observed after lisuride supports a concomitant stimulation of 5-HT receptors as in the case for LSD and other ergot-derivatives (2,3,4,24). Stimulation of 5-HT receptors appears to attenuate rotational responses to DA agonists.

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References

1. W.M. HERRMANN, R. HOROWSKI, K. DAMREHL, V. KRAMER and K.LURATI, Headache 17 54-60 (1977).
2. W. KEHR, Eur. J. Pharmacol. 41 261-273 (1973).
3. H.H. KELLER, W.P. BURKARD and M. DA PRADA, Experientia 33 806 (1977).
4. H.H. KELLER, W.P. BURKARD, L.PIERI, E.P. BONETTI and M. DA PRADA, in Symposium on dopamine, Southampton, Raven Press (in the press).

5. M. PIERI, R. SCHAFFNER and W. HAEFELY, Experientia 33 808 (1977).
6. M. DA PRADA, L. PIERI, H.H. KELLER, M. PIERI and E.P. BONETTI in Conference on serotonin neurotoxins, Ann. N.Y. Acad. Sci. (in the press).
7. M. DA PRADA, A. SANER, W.P. BURKARD, G. BARTHOLINI and A. PLETSCHER, Brain Res. 94 67-73 (1975).
8. L. PIERI, M. PIERI and W. HAEFELY, Nature 252 586-588 (1974).
9. N.E. ANDÉN, H. CORRODI, K. FUXE and T. HÖKFELT, Brit. J. Pharmac. 34 1-7 (1968).
10. R. HOROWSKI, F. NEUMANN and K.J. GRAEF, J. Pharm. Pharmacol. 27 532-534 (1975).
11. A. SANER, L. PIERI, J. MORAN, M. DA PRADA and A. PLETSCHER, Brain Res. 76 109-117 (1974).
12. M. PIERI, L. PIERI, A.SANER, M. DA PRADA and W. HAEFELY, Arch. int. Pharmacodyn. Ther. 217 118-130 (1975).
13. H.G. BAUMGARTEN, A. BJÖRKLUND, A. NOBIN, E. ROSENGREN and H.G. SCHLOSSBERGER, Acta Physiol. Scand. Suppl. 429 1-60 (1975).
14. S. GERSON and R.J. BALDERSSARINI, J. Pharm. Pharmac. 26 71-73 (1974).
15. R.B. HOLMAN and M. VOGT, J. Physiol. 223 243-254 (1972).
16. B.L. JACOBS, Psychopharmacologia, 39 81-86 (1974).
17. M.A. GEYER, A. PUERTO, D.B. MENKES, D.S. SEGAL and A.J. MANDELL, Brain Res. 106 257-270 (1976).
18. B.L. JACOBS, S.M. SIMON, D.D. RUIMY and M.E. TRULSON, Brain Res. 124 271-281 (1977).
19. R.C. CAMPBELL, Statistische Methoden für Biologie und Medizin (P.53) Georg Thieme Verlag, Stuttgart (1971).
20. A.M. ERNST, Psychopharmacologia 10 316-323 (1967).
21. J.A. ROSENCRANS, R.A. LOVELL, D.X. FREEDMAN, Biochem. Pharmacol. 16, 2011-2021 (1967).
22. N.-E. ANDÉN, B.-E. ROOS and B. WERDINIUS, Life Sci. 3 149-158 (1964).
23. A. CARLSSON and M. LINDQVIST, Acta Pharmacol. Toxicol. 20 140-144 (1963).
24. L. PIERI, H.H. KELLER, W.BURKARD and M. DA PRADA, Nature, in the press.
25. M. GRABOWSKA, L. ANTKIEWICZ, J. MAJ and J. MICHALUK, Pol. J. Pharmacol. Pharm. 25 29-39 (1973).
26. T.J. CROW and J.L. WADDINGTON, Brit. J. Pharmac. 60 265P-266P (1977).
27. J.A. MILSON, C.J. PYCOCK, Brit. J. Pharmac. 56 77-85 (1976).
28. W. GUMULKA, W. KOSTOWSKI and A. CZLONKOWSKI, Pharmacology, 10 363-372 (1973).