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Lisuride-Induced Mounting Behavior and Effects on the Monoaminergic System in Rat Brain

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This chapter briefly reviews our main neurochemical and behavioral findings with lisuride and D-lysergic acid diethylamide (LSD) on the monoaminergic system in rat brain. The experiments discussed deal with the agonistic activities of lisuride and LSD at pre- and postsynaptic dopamine (DA) and 5-hydroxytryptamine (5-HT) receptor sites. Evidence for an α-adrenoceptor-blocking activity of lisuride and LSD is also provided. Special attention is given to lisuride-induced mounting of rats and to the monoaminergic mechanisms sustaining this behavior.

MATERIALS AND METHODS

The neurochemical studies were carried out with male albino rats (Fü-SPF, 150 to 200 g). Prepuberal and adult female albino rats (Fü-SPF) were used in the behavioral experiments. Lisuride hydrogen maleate [5(R)-8(S)] and its 5(R)-8(R) epimer [in the following referred to as 8(R) epimer] and LSD bitartrate [5(R)-8(R), Sandoz Ltd.; in the following referred to as LSD] were dissolved in saline and administered orally or injected by various routes. Doses refer to the salts throughout. In the *in vitro* experiments (Fig. 3, below), small rat striatal slices were incubated at 37°C with ^{3}H-dopamine (New England Nuclear, NET-131, specific activity 33.4 Ci/mmol) in oxycarbon-saturated Krebs–Ringer buffer (6 mM in K^+) containing 10 mM glucose, 0.1 mM ascorbic acid, and 100 mM pargyline. The superfusion experiments were performed as described in Fig. 3. In addition to lisuride and LSD, pergolide mesylate (Eli Lilly & Co.) was tested in combination with molindone·HCl (Endo Laboratories). The monoamines and their metabolites were determined fluorimetrically in either single whole brains (6,11,12,16,17) or brain regions from one rat each (18). Female-to-female mounting was quantified by observing groups of three rats each kept in Macrolon cages; the number of mounts with pelvic thrusts per active rat were counted during 2 hr after lisuride injection or after grouping animals pretreated with *p*-chlorophenylalanine (p-CPA). (For further details see Fig. 5 and ref. 3.) Statistical significance for differences between experimental groups were calculated by Student's *t*-test.

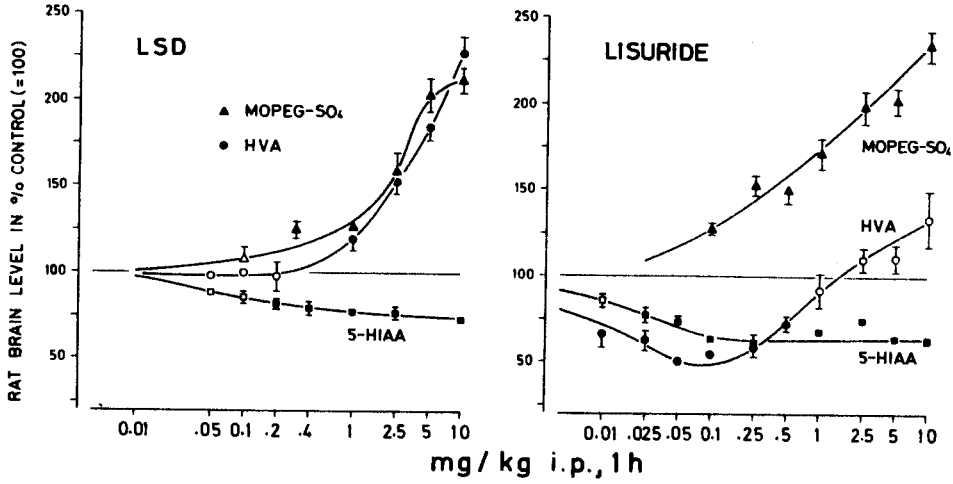

FIG. 1. LSD- and lisuride-induced dose-dependent changes in rat whole brain level of MOPEG-SO_4, homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA). Given are the means (± SEM if greater than size of symbol) of 5 to 10 single values. Statistical signficance for difference from control: *open symbols*, $p > 0.05$; *filled symbols*, at least $p < 0.05$. Absolute control values in nanograms per gram of fresh tissue, average, $N \geq 16$: MOPEG-SO_4 128; HVA 83; 5-HIAA 416.

RESULTS AND DISCUSSION OF NEUROCHEMICAL AND ELECTROPHYSIOLOGICAL STUDIES

Effects on the Noradrenergic System

In rat whole brain, lisuride dose-dependency decreased the level of norepinephrine intraperitoneally (NE) to a moderate extent (by approximately 30% below the control value 0.5 to 1 hr after 1 mg/kg i.p.), whereas LSD had a similar but smaller effect at 1 mg/kg i.p. (9,14). Dose-response curves showed that lisuride and, less potently, LSD induced an increase of 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MOPEG-SO_4), the first significant effects being seen with 0.1 and 0.3 mg/kg i.p., respectively (Fig. 1). This rise was prominent in the frontal cortex, limbic forebrain, and hypothalamus but absent in the striatum (9). The increment of MOPEG-SO_4 in rat whole brain after lisuride (9) and LSD (H. H. Keller, *unpublished data*) was completely prevented by a low dose of the α_2-adrenoceptor agonist clonidine. NE-stimulated cAMP formation in limbic slices was found to be potently inhibited by lisuride and less potently by LSD (14). All these results demonstrate that lisuride and, to the same extent but less potently, LSD increase the NE turnover in brain as a consequence of blocking α-adrenoceptors. The α-blockade, at least by lisuride, is stereospecific, as lisuride's 8(R) epimer had very little effect on MOPEG-SO_4 (Table 1). In addition, lisuride at a concentration as low as 10^{-8}M enhanced the K^+-provoked output of ^{3}H-NE from rat cerebral cortex slices

TABLE 1. *Monoamine metabolite levels in rat whole brain 1 hr after lisuride 8(R) epimer*

Lisuride dose (mg/kg i.p.)	Cerebral content (% of control)*		
	MOPEG-SO_4	HVA	5-HIAA
0.1	102.9 ± 5.1	96.7 ± 4.2	90.8 ± 2.5**
1.0	113.4 ± 3.1*	100.2 ± 4.4	90.4 ± 1.6*

*Control = 100%. Means ± SEM, N = 9 to 12. Significance vs. control: *$p < 0.01$; **$p < 0.001$.

(H. H. Keller, *unpublished*). Whether this latter effect is due to the blockade of presynaptic NE receptors or partly due to a releasing effect of lisuride for NE remains to be elucidated.

Effects on the Serotonergic System

Lisuride and LSD at low and high doses were reported to induce a statistically significant, although minor, increase of the 5-HT level in rat brain (7,9,13). Both ergolines decreased the whole brain level of 5-hydroxyindoleacetic acid (5-HIAA) in a dose-dependent manner, whereby lisuride was more potent than LSD by a factor of about 10, the first significant effects being seen with 0.025 and 0.2 mg/kg i.p., respectively (Fig. 1). As neither drug inhibits monoamine oxidase or blocks the neuronal uptake of 5-HT to any considerable extent, the decreased level of 5-HIAA reflects the agonistic effect of these drugs at 5-HT receptors in the brain (7,9). In order to determine if there is a direct effect on 5-HT neurons, lisuride or LSD was microinfused stereotactically at the high total amount of 20 μg into the raphe medianus and dorsalis of halothane-anesthetized rats, and the level of 5-HIAA was determined in the forebrain (13). Such treatment resulted in a marked reduction of 5-HIAA after lisuride, but little if any diminution was seen with LSD. When methiothepin, a potent blocker of 5-HT receptors in the brain at pre- and postsynaptic sites (2), was injected i.p., the 5-HIAA level was increased significantly. This accumulation of 5-HIAA was completely prevented by stereotactically injected lisuride, whereas LSD had no such effect. Therefore, lisuride seems to stimulate preferentially somatic 5-HT autoreceptors, leading to a diminished release of 5-HT, whereas with LSD such an effect could hardly be demonstrated (13).

In accordance with these findings are the results of electrophysiological experiments (14). The spontaneous multiunit activity in raphe dorsalis was much more markedly reduced by intravenously injected lisuride (by about 50%) than by LSD (about 18%). In this respect, too, the effect of lisuride was stereospecific, as its 8(R) epimer was completely inactive (14). Likewise, the single unit activity in rat raphe was decreased by intravenous lisuride much more markedly and for a longer period of time than by i.v. LSD (14). Finally, both lisuride and LSD have been shown to inhibit the 5-HT-stimulated formation of cAMP (15).

Effects on the Dopaminergic System

In accordance with the results of other investigators (7), we found that lisuride and LSD caused a small but statistically significant increase of the DA level in rat whole brain (13). Lisuride injected at low doses (0.1 mg/kg i.p., 0.05 mg/kg intracerebroventricularly) caused a marked reduction of homovanillic acid (HVA) in rat brain which occurred rapidly (maximum effect after 60 min) and lasted for 3 to 4 hr (8,10). The decrement of HVA in rat brain after lisuride injected i.p. or given orally was markedly enhanced and prolonged in rats pretreated with the microsomal enzyme inhibitor proadifen (8,10). All of the above-mentioned findings reflect the agonistic effect of lisuride at central DA receptors and strongly suggest that lisuride, like other ergot alkaloids, exerts its DA agonistic effect by itself and not through active metabolites (10). Dose-response curves over a wide dose range revealed (Fig. 1) that the level of HVA after low doses of LSD was either slightly reduced (13) or, as in the present study, essentially unchanged. Therefore, as judged by the HVA level, an agonistic effect of LSD at DA receptors is difficult to demonstrate. With 1 mg/kg i.p. and higher doses there was even a moderate to marked increase of this metabolite (Fig. 1), which probably reflects the antagonistic action of LSD at DA receptors and/or a DA-releasing effect. In line with these latter two possibilities is the finding that the K^+-induced release of ^{3}H-DA from superfused rat striatal slices was increased by 3×10^{-6}M LSD (H. H. Keller, *unpublished data*). The effect of lisuride was clearly biphasic. Low doses (i.e., 0.01 to 0.1 mg/kg i.p.) markedly decreased HVA, indicating a reduction in DA turnover due to stimulation of DA receptors (Fig. 1) (9). At higher doses this effect gradually diminished, whereas HVA levels even slightly above control were observed (Fig. 1). It is of interest that this biphasic effect was prominent in the frontal cortex, less pronounced in the limbic forebrain, and hardly detectable in the striatum (Fig. 2). Thus, this dual effect seems to occur in the mesocortical (and mesolimbic) but not in the nigrostriatal dopaminergic pathway. The biphasic effect on the HVA level shown here for the first time with lisuride probably reflects the drug's DA receptor agonistic effect at low doses and its antagonistic effect at high doses. The interaction of lisuride at central DA receptors is stereospecific, as the lisuride 8(R) epimer had no effect on the HVA level (Table 1).

The changes in HVA do not allow one to conclude whether lisuride stimulates pre- or postsynaptic DA receptors. Postsynaptic DA receptor stimulation in the brain is indicated by the dopaminergic behavioral signs (see below) and by lisuride's ability to induce contralateral rotation in rats unilaterally lesioned in the medial forebrain bundle (14). Also, lisuride and LSD both stimulate the DA-sensitive adenylate cyclase and antagonize DA-stimulated cyclic AMP formation (for detailed discussion, see ref. 14).

However, as shown earlier *in vivo* (7), recent results obtained *in vitro* suggest that lisuride is also a potent agonist at presynaptic DA receptors (Fig. 3). The potent neuroleptic molindone at 10^{-7}M enhanced the potassium-evoked release of ^{3}H-DA from superfused small rat striatal slices. If simultaneously added with molindone, lisuride antagonized the enhancing effect of molindone in a concentration-dependent

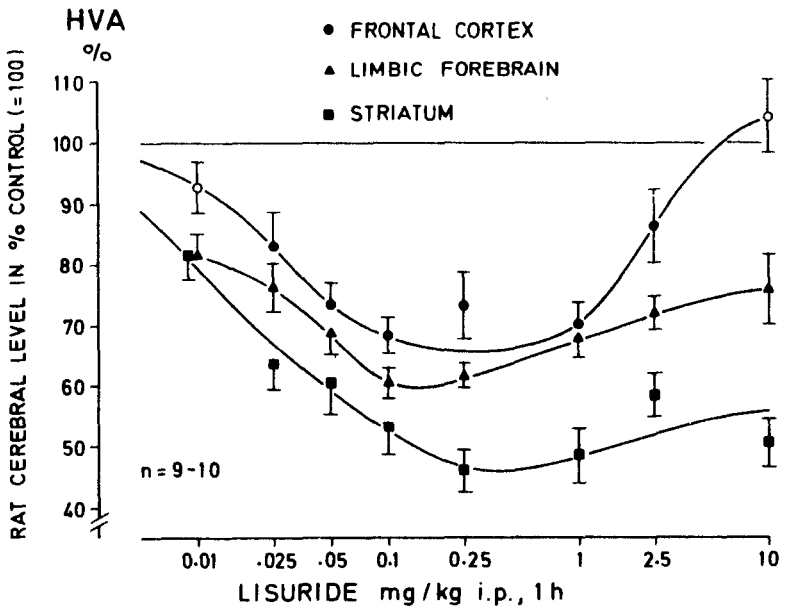

FIG. 2. Dose-dependent changes of the HVA level in three rat brain areas induced by lisuride. Given are the means with SEM. Statistical significance for difference from control: *open symbols:* $p > 0.05$; *filled symbols:* at least $p < 0.05$. Absolute control values in nanograms per gram of fresh tissue, means ± SEM, $N = 16$: frontal cortex 69.8 ± 2.5; limbic forebrain 200.2 ± 10.4; striatum 438.0 ± 19.0.

manner, a first significant effect being seen with 10^{-8}M. Essentially the same effect was obtained with pergolide, whereas with LSD only some slight effect was seen with 10^{-7}M. From these data we conclude that: (a) the enhanced release of DA obtained with molindone is the consequence of blocking presynaptic DA receptors, as has been recently found *in vivo* (1), and (b) these two ergolines antagonize the presynaptic effect of molindone, although we have not been able so far to show in this *in vitro* system that lisuride and pergolide per se inhibit potassium-evoked DA release consistently. Thus, lisuride and pergolide stimulate presynaptic DA receptors, whereas LSD is weak in this respect.

Behavioral Characteristics

In the rat, lisuride induces a number of behavioral signs probably related to the stimulation of postsynaptic dopamine receptors in the brain, i.e., increased locomotor activity and stereotyped sniffing, gnawing, and boxing position. Also, lisuride displays some signs associated with 5-HT receptor stimulation, e.g., head weaving, tremor, increased muscle tone, and Straub tail. In addition, unlike LSD, lergotrile, pergolide, and bromocriptine, lisuride induces a very marked masculine mounting behavior in grouped rats. This behavior is observed both in males and, even more pronounced, females (3).

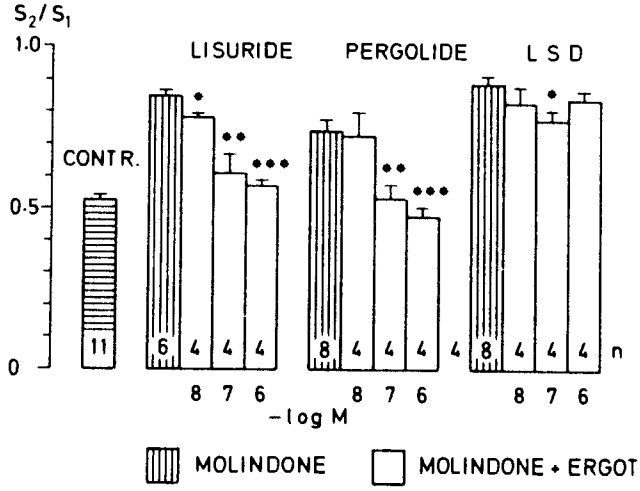

FIG. 3. Enhancement by molindone of the potassium-induced release of ^{3}H-dopamine from rat striatal slices and antagonism of this effect by three ergolines. Rat striatal slices (0.2 × 0.2 × 1.5 mm) previously loaded for 30 min with ^{3}H-dopamine (2×10^{-8} M) in the presence of 10^{-5} M pargyline were continuously superfused at 37°C with oxycarbon-saturated Krebs–Ringer buffer containing 10^{-5} M cocaine as DA uptake inhibitor. The radioactivity then released into the superfusion medium was counted in 16 consecutive fractions of 3 min each. During the first (S_1) and second (S_2) stimulation periods of 1 min at the beginning of fractions 3 and 12, the K^+ concentration was raised to 15 mM, which resulted in an immediate release of radioactivity that returned to the basal level in the following two fractions. The results are expressed as the ratio of the net output over the basal release in S_2 and S_1. In control experiments (CONTR., superfusion medium only), the average ratio was about 0.5. Molindone either alone or in combination with one of the ergots was added to the medium at the beginning of fraction 8, i.e., 12 min before stimulation S_2. The columns represent means ± SEM. Molindone vs. control: $p < 0.001$; molindone vs. molindone + ergot: $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

FIG. 4. Masculine mounting behavior between two female prepuberal rats. Both the mounting animal and the animal being mounted were injected 30 min earlier with lisuride 1 mg/kg i.p. The photograph was taken during the short period of mounting accompanied by vigorous and frequent pelvic thrusts. The mounting rat characteristically shows grasping with the forepaw, active eye closure, and pinnae adduction.

Figure 4 shows two female rats, both of which have been injected with lisuride 1 mg/kg i.p. The sequence of events normally consists of a short foreplay (i.e., approaching from behind and anogenital exploration), then mounting with frequent vigorous pelvic thrusts, and, after dismounting, licking of the animal's own genitals. This behavior is an impressive imitation of the masculine copulatory behavior. Under the action of lisuride, the same rat occasionally either mounts or is being mounted by an other animal. Both events occur at about the same frequency. This behavior can be quantified easily by observing groups of three rats per cage and counting the number of mounts with pelvic thrusts for each animal. Mounting was induced in females with lisuride 0.2 mg/kg i.p., and its frequency increased with incremental doses (4). At high doses, frequencies up to about 60 mounts during 2 hr have been observed (Fig. 5). Other previously described (3,4) features include the following: Lisuride induces mounting even more readily in prepuberal females than in adult females or adult males. It can be seen as early as 18 days after birth, i.e., even in weanling rats. It occurs only in rats, and not in any other animal species so far studied, i.e., mice, hamsters, guinea pigs, rabbits, and cats. It can be directed not only to other rats but also toward individuals of other animal species (e.g., guinea pigs) or even toward dead animals or a dummy. It seems to be independent of sex hormones, as it also occurred in gonadectomized rats. However, pituitary factors do seem to play a role, since hypophysectomized rats showed no mounting at all (4). Considering all these characteristics, lisuride-induced mounting, in our mind, does not truly reflect male copulatory behavior. We believe, rather, that it is a special kind of inborn stereotype associated with several elements of masculine sexual behavior.

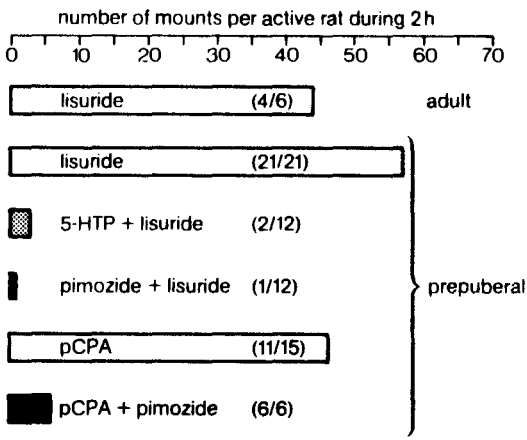

FIG. 5. Drug-induced female-to-female mounting of adult (40 to 60 days) and prepuberal (21 to 38 days) rats. Mounting was counted in groups of three animals each during the first 2 hr after lisuride or after grouping p-CPA-injected rats previously kept isolated (for details, see ref. 3). The columns represent the mean number of mounts per active animal; the numbers in parentheses are the active animals/total number of rats tested in the experimental group. Lisuride (1.5 mg/kg i.p.) was injected either alone or after various pretreatments (for details, see ref. 3). (From ref. 3, with permission.)

Monoaminergic Mechanisms Possibly Underlying Mounting

Lisuride-induced mounting, although being much more pronounced, resembles that observed after repeated injections of p-CPA, after cerebral indolectomy by 5,6- and 5,7-dihydroxytryptamine, and after L-DOPA plus a peripheral L-DOPA decarboxylase inhibitor (5). Figure 5 demonstrates that pretreatment with the 5-HT precursor amino acid 5-hydroxytryptophan almost completely abolished female-to-female mounting. Blockade of the DA receptors by pimozide greatly reduced mounting provoked by lisuride as well as by p-CPA (3). More evidence for an inhibitory role of 5-HT stems from experiments in which lisuride-induced mounting was dose-dependently reduced or even abolished by the 5-HT agonist quipazine, trazodone and its metabolite *m*-chlorophenylpiperazine, and by potent inhibitors of neuronal 5-HT uptake, including chlorimipramine, citalopram, paroxetine, and Ro 11-2465, all of which enhance serotonergic neurotransmission by increasing 5-HT in the synaptic cleft (4).

CONCLUSIONS

The neurochemical and electrophysiological experiments presented show that lisuride stimulates presynaptic 5-HT receptors more markedly than LSD. The dose-dependent changes of the cerebral HVA levels induced by lisuride and LSD indicate that both ergolines have mixed agonistic/antagonistic properties at DA receptors in the brain. In addition, the experiments with rat striatal slices labeled with ^{3}H-DA provide evidence that lisuride and pergolide stimulate presynaptic DA receptors more potently than LSD. In addition, lisuride and LSD, like other ergot alkaloids, block α-adrenoceptors at higher doses. All these interactions of lisuride at 5-HT, DA, and NE receptors are stereospecific. The behavioral experiments described show also that, similarly to p-CPA, lisuride induces intensive mounting in rats, and that this behavior is suppressed, both by DA receptor blockers and 5-HT agonists, or 5-HT uptake inhibitors. They support the view that lisuride-induced mounting is subserved by simultaneous DA stimulation and a reduction in 5-HT input. The differences between lisuride and LSD in their actions on the various monoaminergic systems are, however, quantitative rather than qualitative. They do not appear to be sufficient to explain why only lisuride induces mounting in rats and why LSD is such a powerful hallucinogen in humans (13).

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