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RESPONSE OF CENTRAL MONOAMINERGIC NEURONS TO LISURIDE: COMPARISON WITH LSD

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Summary

The response of brain serotonergic (dorsal raphe), noradrenergic (locus coeruleus) and dopaminergic (pars compacta, substantia nigra) neurons to lisuride hydrogen maleate, a non-hallucinogenic ergot, was studied in the rat using extracellular single cell recording techniques. As has been previously reported for LSD, minute intravenous infusions of lisuride (1-5 μg/kg) produced a complete but reversible suppression of raphe unit spontaneous firing. A similar depressant response was noted when lisuride was applied to raphe units by microiontophoresis. In contrast, locus coeruleus neurons were accelerated by the drug at somewhat higher doses (25-50 μg/kg). Pars compacta neurons demonstrated a predominately depressant response to lisuride but many of the cells tested were only partially suppressed and a few units were accelerated. It is suggested that the marked alterations in central monoamine turnover which have been observed with lisuride are directly paralled by changes in impulse flow in monoaminergic neurons. The fact that lisuride has powerful suppressant effects on central serotonergic neurons but no psychotomimetic actions in man challenges the "serotonin theory" of hallucinogensis; however, other pharmacological properties may account for lisuride's lack of hallucinogenic effects. Further studies with lisuride may provide insight into those drug characteristics critical to the presence or absence of hallucinogenic action.

Lisuride is an ergoline derivative which is structurally similar to the potent hallucinogen d-lysergic acid diethylamide (LSD). However, unlike LSD, lisuride does not produce hallucinations in man and, in fact, has recently been evaluated for use in the prophylaxis of migraine headache (1,2) and in the treatment of hyperprolactinemic states (3,4). The effects of lisuride on biochemical measures of monoaminergic neuronal function have been extensively investigated. In common with some but not all chemically related ergot alkaloids (5,6), lisuride reduces serotonin (5-HT) and dopamine (DA) turnover and, at higher doses, increases norepinephrine (NE) turnover (7-9). Lisuride's effects on 5-HT and DA turnover are particularly impressive because of the drug's extereme potency and relatively long duration of action. These and other biochemical observations have lead to the conclusion that lisuride is a potent 5-HT and DA receptor agonist and a somewhat weaker NE receptor antagonist.

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Behavioral studies provide support for the concept that lisuride stimulates brain 5-HT (10,11) and DA (6,12-15) receptors. In very low doses lisuride causes profound behavioral disruptions in experimental animals which have been variously interpreted as dopamine stereotypies (6,12) or the "serotonergic syndrome" (11). The drug is more potent than the dopaminergic agonist apomorphine in producing contralateral turning in rats with lesions of the nigrostriatal DA system (6,14,15) or in inducing emesis in dogs (12).

In view of lisuride's striking neuropharmacological and behavioral activity we felt it would be of interest to examine its effects on monoaminergic neurons in a more direct fashion. In addition, the observation that lisuride lacks psychotomimetic activity but nevertheless appears to exhibit pharmacological properties qualitatively similar to LSD prompted us to compare these two ergots in order to gain further insight into the mechanism of hallucinogenesis.

Therefore, in the present study using extracellular unit recording techniques, we determined the acute effects of lisuride on the activity of serotonergic neurons of the dorsal raphe, noradrenergic neurons of the locus coeruleus and dopaminergic neurons of the substantia nigra (pars compacta).

Materials and Methods

Fifty-five male Sprague-Dawley rats (Charles River Laboratories, Wilmington, Mass.) weighing 210-280 g were used in these experiments. The animals were anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic apparatus. Additional injections of chloral hydrate were given as needed during the course of the experiment. Body temperature was maintained at 35-37°C by a thermostatically controlled heating pad.

In some experiments, drugs were administered by means of a 25-gauge hypodermic needle placed in a lateral tail vein. In these studies, extracellular action potentials were monitored with single-barrel glass micropipettes. The electrodes were broken back to a tip diameter of 1-2 μm and were filled with a 2 M NaCl solution containing 2% pontamine sky blue. Electrodes with impedances of 3-6 MΩ were used for recording.

For application of drugs by microiontophoresis, five-barrel micropipettes were prepared as described by Haigler and Aghajanian (16). The center (recording) barrel was filled with the NaCl-dye solution and the side barrels were loaded with 5-HT creatinine sulfate (0.04 M, pH 4), lisuride hydrogen maleate (0.01 or 0.001 M in 0.1 M NaCl, pH 4-5) and LSD bitartrate (0.001 M in 0.1 M NaCl, pH 4). LSD and lisuride were diluted ionically to reduce their transport numbers. Although the extent of the ionic dilution should theoretically produce a corresponding reduction in the transport number, this assumption has been verified only for LSD (16). In experiments with raphe neurons a 100-fold ionic dilution was routinely used; this dilution was necessary from a practical standpoint because of the extreme sensitivity of these cells to the two ergolines. The remaining side barrel always contained 4 M NaCl and was used for automatic current balancing (17). Electrodes with recording barrel impedances of 3.5-5 MΩ were usually suitable for simultaneous recording and drug ejection. A retaining current of -10 nA was applied to the drug barrels between ejection periods.

In preparation for recording, a burr hole was drilled in the skull overlying the appropriate brain region and the dura was retracted with a hooked needle. The microelectrode was lowered into the brain by means of a hydraulic microdrive. Electrode signals were amplified and displayed on an oscilloscope. The filtered output from the oscilloscope was passed to an audiomoniter and to an electronic counter with a period of 10 sec. The trigger of the

counter could be adjusted to register only the action potentials of the individual unit under study. The analog output of the counter was used to drive the pen of a graphic recorder.

The stereotaxic coordinates and methods of cell identification have been described previously. In brief, serotonergic neurons of the dorsal raphe were found on the midline, approximately 0.3-0.5 mm anterior to the lambdoidal suture. Units were usually encountered 5.5-6.5 mm ventral to the skull surface and could be tentatively identified by their slow, regular firing pattern (typically 0.2-2 spikes/sec) and wide, positive-negative spike shape (1-2 msec in duration). Such units have been identified as serotonergic on the basis of histochemical and electrophysiological criteria [see Aghajanian and Wang (18)].

For recording from the locus coeruleus, the electrode was positioned 1.1 mm lateral and 1.2 mm posterior to lambda. The nucleus was located according to the procedure outlined by Aghajanian et al. (19). Neurons of the locus coeruleus also exhibited a slow, regular firing pattern (0.5-4 spikes/sec) and wide spike shape. All cells responded to compression of the contralateral hind paw with a brief burst of firing followed by a short quiescent period.

Units of the pars compacta were encountered 3.0 mm anterior and 2.0 mm lateral to lambda (20). Presumed DA cells [Type I units as described by Guyenet and Aghajanian (21)] were typically found 7-7.5 mm below the skull surface and were tentatively identified by their slow rate (2-6 spikes/sec) and wide, biphasic action potentials (2-3 msec). Some of these cells displayed a bursting pattern.

The anatomical location of all units studied was verified after the recording session by passing a 20 nA negative current through the recording barrel for 30-60 min thus deposition a spot of blue dye at the site of the electrode tip. The animals were then perfused through the heat with 10% buffered formalin and coronal serial sections of the appropriate brain regions were cut, mounted, stained with cresyl violet and counterstained with neutral red. Only cells which were subsequently confirmed to be within the boundaries of the nucleus of interest were included in the study.

Quantitative data are expressed as the mean ± S.E.M. Treatments were compared using non-parametric methods (Mann-Whitney $\bar{U}$ test or Kruskal-Wallis one-way analysis of variance).

Drugs were obrained from the following sources: lisuride hydrogen maleate, Schering AG, Berlin; d-lysergic acid diethylamide bitartrate, NIDA; serotonin creatinine sulfate, Sigma Chemical Co., St. Louis, Mo.; piperoxane HCl, Rhone-Poulenc, Paris. Intravenous drug dosages are expressed in terms of the weights of the salts and were administered in 0.9% NaCl. Lisuride solutions were prepared fresh daily as the drug is readily oxidised.

Results

Raphe Neurons

When administered intravenously, lisuride produced a rapid inhibition of raphe unit spontaneous activity (N = 12; Fig. 1A). Slowing was observed in doses as low as 0.4 µg/kg and 1-5 µg/kg was usually sufficient to produce a transient cessation of firing. At these doses, partial recovery of 15-50% of pre-drug baseline activity was attained within 20 min.

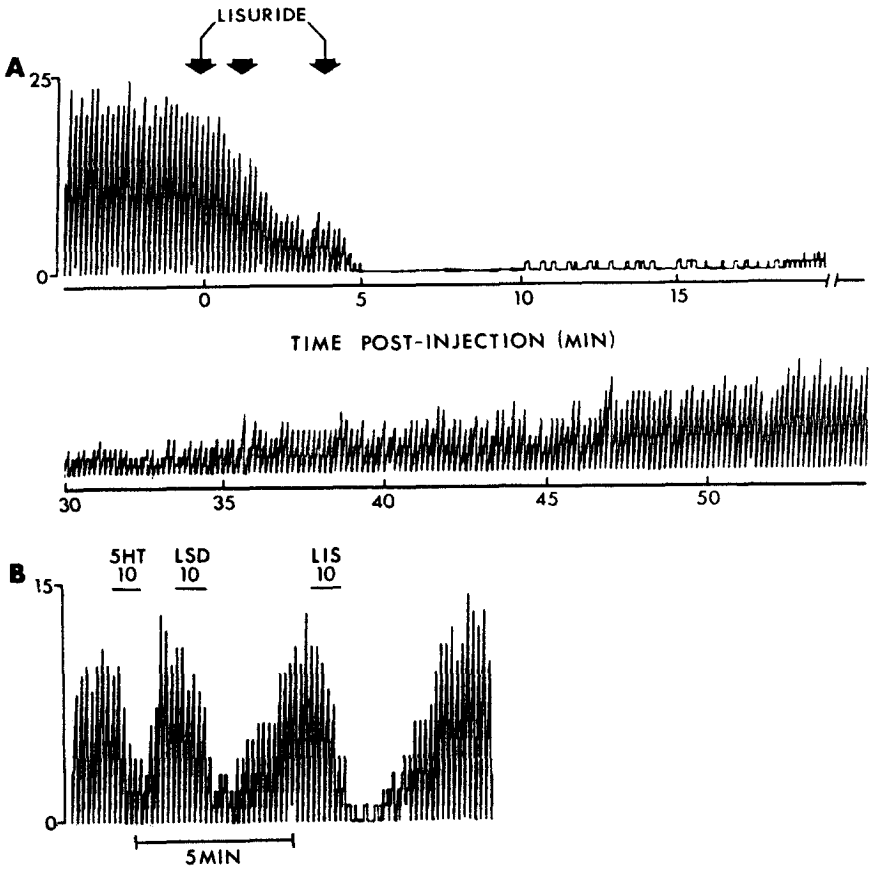

FIG. 1

Response of dorsal raphe neurons to intravenous (A) and microiontophoretic (B) lisuride. In A, partial suppression of spontaneous activity is obtained with 1 µg/kg cumulative dose of lisuride (first and second arrows). An additional 0.5 µg/kg infusion (third arrow) produces a transient cessation of firing. Partial recovery takes place during the 1 hour period following the initial injection. In B, the depressant response to iontophoretic lisuride (LIS) is compared with the responses to 5-HT and LSD. Note the prolonged time for recovery with LSD and lisuride. Duration of iontophoretic ejection is indicated by bars above record; all ejections +10 nA. Unit activity is expressed in the form of an integrated rate histogram. Ordinate indicates number of spikes per 10 sec epoch.

When applied by microiontophoresis with currents ranging from 6-20 nA, lisuride depressed the firing of 25 raphe units tested. Twenty units were recorded from while ejecting currents of 9-12 nA. At these currents, one min pulses of lisuride produced a 70-100% (mean 92%) reduction in baseline rate

TABLE I

Comparison of the Response of Raphe Neurons to Iontophoretically Applied Lisuride, LSD, and 5-HT

Drug	Numbers Of Cells Tested	Iontophoretic Current (nA)	T_{50}[1] (sec)	Mean Inhibition[2] (percent)	Mean Time For Recovery[3] (sec)
Lisuride	20	10.0 ± 0.2	30 ± 3	92 ± 3	304 ± 40†
LSD	17	10.1 ± 0.1	25 ± 3	90 ± 3	149 ± 25
5-HT	17	10.1 ± 0.1	18 ± 2*	88 ± 4	21 ± 6

One min iontophoretic pulses (9-12 nA) were applied to single units in the dorsal raphe. Recording techniques are described in Materials and Methods. All cells demonstrated a depressant response to the drugs, but not every cell was tested with each drug.

1. Time after onset of ejection at which firing was reduced to 50% of pre-ejection baseline rate.
2. Maximum depression of baseline rate obtained.
3. Time after end of drug pulse to regain stable firing rate.
* Significantly different from lisuride or LSD, $P < 0.05$.
† All treatments significantly different, $P < 0.001$

(Table 1). Thirteen of the 20 cells were inhibited completely for at least one 10 sec epoch. Higher iontophoretic currents produced a more profound suppression of activity. The onset and magnitude of the response to LSD was not statistically different from that produced by lisuride (Table 1). However, the initial response to 5-HT was slightly faster than that to lisuride or LSD ($P < 0.05$). Compared with 5-HT, both ergolines also caused a relatively more prolonged depression of firing. Following 1 min, 10 nA iontophoretic pulses of drug, maximum recovery was attained after 3-9 min with lisuride and 1-4 min with LSD. The mean time for recovery with lisuride was significantly longer than for LSD ($P < 0.001$).

Locus Coeruleus Neurons

Intravenous lisuride produced an acceleration in the firing of all 8 cells tested in the locus coeruleus. The maximum increase in rate was 20 to 500% (median 144%) above pre-drug baseline firing and this was obtained with doses of lisuride between 25 and 50 µg/kg (Fig. 2A). The degree of acceleration was highly dependent upon the cell's initial rate and the general level of anesthesia. Cells firing at or below 1.3 spikes/sec typically showed the greatest activation with lisuride while those units with faster initial firing rates were affected only slightly. After a plateau had been reached where additional doses of lisuride did not further activate the cells, the α-antagonist piperoxane (0.5 mg/kg) was able to produce an additional increase in firing rate (N = 3).

Lisuride had little or no effect on the firing of most locus coeruleus neurons when applied by iontophoresis (N = 15). Even when prepared at a

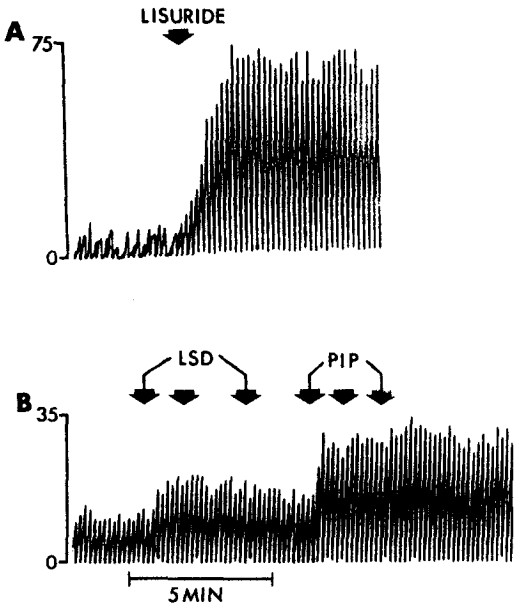

FIG. 2

Response of locus coeruleus neurons to intravenous lisuride (A) and LSD (B). In A, infusion of lisuride (25 µg/kg) causes a marked acceleration of slowly firing unit. In B, LSD (25 µg/kg) induces a moderate increase in rate but additional infusions (25, 50 µg/kg) produce little further response. Piperoxane (0.5, 0.5, 1 mg/kg) causes an additional increment in rate even after a plateau has been reached with LSD. Ordinate indicates spikes per 10 sec epoch.

10-fold ionic dilution rather than the 100-fold dilution used when recording in the raphe, 9 of 11 cells tested showed no response. The remaining 2 cells were accelerated slightly.

In confirmation of a previous report (26), LSD produced a slight activation of locus coeruleus neurons when administered intravenously. Of 4 cells tested, all were accelerated (25-100%, median 38%) by LSD. Full activation was obtained with 25 µg/kg of the drug. Additional doses caused no further increase in rate but piperoxane (0.5 mg/kg) was still highly active (Fig. 2B).

Substantia Nigra (Zona Compacta) Neurons

Lisuride had a variable effect on presumed dopamine neurons in the zona compacta of the substantia nigra. Of 11 cells tested in naive animals, 10 were slowed and 1 was accelerated by the drug. Five of the slowed cells exhibited a complete, longlasting depression of activity with 25-100 µg/kg lisuride (Fig. 3A). The remainder were partially (11-50%) depressed by the initial dose of the drug but did not respond to additional doses (Fig. 3B). Spontaneously active units with characteristics of DA cells were occasionally encountered in the vicinity of cells which were slowed or stopped by lisuride. These cells were often accelerated (24-213%, N = 4) in a dose dependent fashion by additional intravenous infusions of lisuride.

Discussion

As has been previously demonstrated for LSD (22,23,24), extremely small doses of lisuride produced a rapid, dose dependent suppression of serotonergic

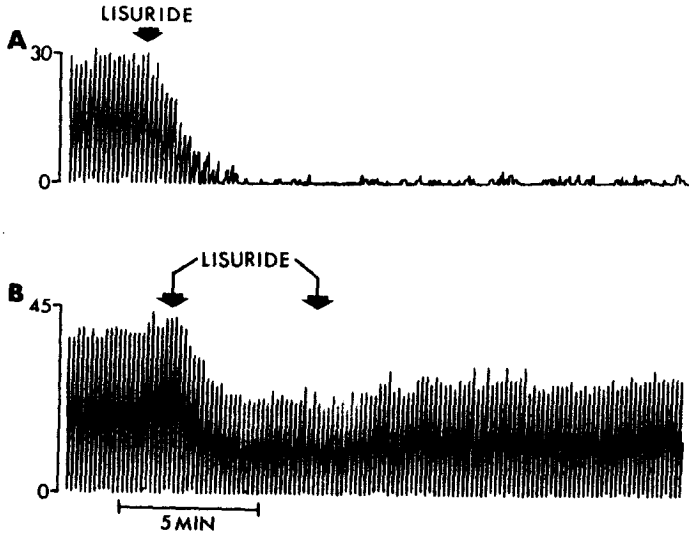

FIG. 3

Variable response of substantia nigra (pars compacta) neurons to intravenous lisuride. A. Lisuride (25 µg/kg) produces a near complete, long lasting suppression of firing. B. Initial injection of lisuride (80 µg/kg) induces a partial suppression of activity but an additional injection (80 µg/kg) produces no further decrease in rate. Ordinate indicates spikes per 10 sec epoch.

neurons in the rat dorsal raphe. On the basis of the molar intravenous dose required to achieve a complete inhibition of spontaneous activity, lisuride is approximately 5-10 times as potent as LSD and is therefore the most highly active drug described to date to which these neurons respond. Following the completion of these studies, Laurent and Pieri (27) reported a comparison of the effects of intravenous lisuride and LSD on dorsal raphe neuronal activity. Although these authors recorded from multiple rather than single raphe units, their conclusion, that lisuride is considerably more potent than LSD in depressing firing, was similar to ours.

In microiontophoretic experiments, both lisuride and LSD produced a complete suppression of most raphe units studied but, for equivalent iontophoretic currents, the recovery with lisuride was more prolonged. These results suggest that lisuride, like LSD, has a direct agonist action at 5-HT-sensitive receptors on serotonergic neurons ["5-HT autoreceptors"; see Aghajanian (25)]. However, since drugs interacting with central adrenoceptors have been found to indirectly influence the activity of raphe serotonergic neurons (26,28,29), the possibility that lisuride's action is in whole or in part mediated via an action at adrenergic receptor sites cannot be excluded. In any case, the response of raphe neurons appears to be highly selective since units in the locus coeruleus were never depressed by the drug.

The effect of lisuride on brain serotonergic neurons as determined in these physiological experiments corresponds well with what would be predicted

on the basis of previous biochemical studies. Thus, lisuride has been demonstrated to produce a long lasting decrease in 5-HT turnover (7-9) which appears to be paralleled by a reduction in impulse flow along serotonergic neurons.

In contrast to its depressant effects in the raphe, lisuride, in somewhat higher doses, caused an activation of noradrenergic neurons in the locus coeruleus. This activation is consistent with the increased turnover of NE reported after correspondingly higher doses in biochemical studies (7-9).

Pharmacological agents with α-adrenoceptor blocking properties, such as piperoxane, which activate the locus coeruleus (30) have been reported to produce anxiety or fear in human subjects (31,32). It is therefore of interest to note that one of the most prominent side effects of lisuride is its anxiety producing or "dysphoric" action (33).

LSD also caused an activation of locus coeruleus neurons and it too has been reported to accelerate NE turnover, but both the physiological and biochemical effects were less pronounced than with lisuride (8,9).

Lisuride's action on dopaminergic neurons was not as clearcut as its effects on the other two monoaminergic systems. The majority of cells tested were depressed by low doses of the drug but in many cases this response was only partial and a small proportion of the cells were excited. Christoph et al. (33) have reported very similar effects of LSD on dopamine cells.

Lisuride and LSD are also reported to have potent effects on DA turnover, however, unlike with the 5-HT or NE systems, the degree of the response is not related to dose in a simple fashion. Low doses of both drugs markedly reduced DA synthesis and release (7,8) but with higher doses an increase toward baseline turnover was noted (7). It is conceivable that the subpopulation of excited cells noted in the present study and by Christoph et al. could account for this biphasic dose-effect relationship.

What implication do these observations have for a theory of hallucinosis? The most striking aspect of lisuride's action on monoaminergic neurons was its consistent and exceedingly potent depression of raphe actitivty. It has been proposed that psychedelic agents such as LSD and other indoleamine hallucinogens exert their profound psychological effects via a direct suppression of central 5-HT neurons which, in turn, disinhibits neurons in postsynaptic areas receiving a tonic inhibitory serotonergic input (35). As previously suggested (8), the fact that lisuride is not a hallucinogen challanges this theory of hallucinogenesis. On the other hand, pharmacological agents which inhibit raphe firing indirectly by altering 5-HT availability [tricyclic antidepressants, MAO inhibitors; see Aghajanian and Wang (18)] or by acting at adrenergic receptor sites (26-28) are not hallucinogens. Lisuride might share some properties with these indirectly acting agents and therefore would not express hallucinogenic activity. In any case, it will be of theoretical interest to investigate further pharmacological differences between lisuride and LSD which could reveal drug actions critical for psychotomimetic activity.

Acknowledgements

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