The Interaction of Lisuride, an Ergot Derivative, with Serotonergic and Dopaminergic Receptors in Rabbit Brain[1]

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ABSTRACT

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The interaction of lisuride (Lysenyl, Spofa), an ergot derivative, with serotonergic and dopaminergic receptors and with adenylate cyclase was studied in homogenates of rabbit brain. In frontal cortex, lisuride interacts with serotonin receptors as shown by its ability to compete with [^{3}H]serotonin, [^{3}H]spiroperidol and [^{3}H]lysergic acid diethylamide for their receptor binding sites, with respective IC_{50} values of 14, 1.0 and 3.7 nM. The IC_{50} for displacement of [^{3}H]spiroperidol by lisuride in frontal cortex was increased by the GTP analog, 5'-guanylyl-imidodiphosphate, indicating an agonist-like interaction. Lisuride is extraordinarily potent in stimulating serotonin-sensitive adenylate cyclase in this brain region, with maximal stimulations occurring at 0.1 nM lisuride. In caudate nucleus, lisuride interacted with both serotonergic and dopaminergic receptor sites as labeled by [^{3}H]serotonin, [^{3}H]lysergic acid diethylamide and [^{3}H]2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene, with IC_{50} values ranging from 2.0 to 7 nM. Lisuride did not stimulate adenylate cyclase in caudate nucleus. In summary, lisuride is a very potent stimulator of serotonin-sensitive adenylate cyclase in rabbit frontal cortex and can interact with serotonin and dopamine receptor binding sites in rabbit cortex and caudate nucleus.

There is increasing evidence that both the dopaminergic and serotonergic transmitter systems are involved in several neurological and behavioral disorders (Hornykiewicz, 1978; Garver and Davis, 1979). Many of the ergot alkaloids have been shown to interact with these systems (Corrodi *et al.*, 1975; Goldstein *et al.*, 1978; Makman *et al.*, 1979; Silbergeld and Hruska, 1979). The erogts can act as agonists, partial agonist-antagonists or as antagonists (Corrodi *et al.*, 1973; Fuxe *et al.*, 1978). This diversified spectrum of action may be due to selectivity of each ergot or class of ergot for one receptor type or for specific states of a receptor. Lisuride (lisuride hydrogen maleate, Lysenyl, Spofa) is a semisynthetic ergot derivative (Zikan and Semonsky, 1960) that was first used clinically as an antimigraine agent. Lisuride has dopaminergic agonist action in mice and rats (Horowski and Wachtel, 1976, 1979) and is one of the most potent agents known to lower serum prolactin levels in humans (Horowski *et al.*, 1978). It has also been shown to be potentially useful in the treatment of acromegaly, an effect postulated due to a dopamine agonist action (Liuzzi *et al.*, 1978). In addition, lisuride has been shown to interact with peripheral and central serotonin systems, both as an agonist (DaPrada *et al.*, 1977; Kehr, 1977) and as an antagonist (Podvalova and Dlabac, 1972). Lisuride has been shown to decrease serotonin (5-HT) turnover in rat brain (Pieri *et al.*, 1978).

In this report, we describe the effect of lisuride on the specific receptor binding of radioactively labeled 5-HT, spiroperidol, *d*-lysergic acid diethylamide (LSD) and 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) in rabbit frontal cortex and caudate nucleus. We also describe the influence of lisuride on the dopamine (DA)- and 5-HT-stimulated adenylate cyclases in rabbit brain.

Methods

Male rabbits (New Zealand White), 2 to 3.5 kg, were sacrificed by air injection into an ear vien. Brain regions were rapidly dissected on ice. Tissue was used fresh for assay of adenylate cyclase activity and either used fresh or after storage at −90°C for studies of receptor binding.

Receptor binding studies. Tissue was homogenized at a concentration of 10 mg/ml (wet weight) in ice-cold 0.05 M Tris-HCl buffer (pH 7.4 at 37°C) by using a Brinkmann Polytron, setting 6, for 10 sec. The homogenate was then centrifuged at 20,000 × *g* for 10 min. The supernatant was discarded and the pellet washed once by resuspension in the inital volume of Tris-HCl. Resuspension was accomplished by vortexing the pellet in the buffer since at this stage resuspension by polytron sometimes resulted in a loss of specific binding sites. The resuspended tissue was then centrifuged at 20,000 × *g* for 10 min and the final pellet was resuspended by vortexing directly in a reaction mixture solution at an approximate tissue concentration of 2 mg of wet weight per 0.4 ml. For [^{3}H]-5-HT, [^{3}H]LSD and [^{3}H]spiroperidol binding, the reaction mixture contained 0.05 M Tris-HCl (pH 7.2 at 37°C), 120 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 1 μM pargyline and 0.05% ascorbic acid. For [^{3}H]ADTN binding, the reaction mixture contained

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0.05 M Tris-HCl (pH 7.2 at 37°C) and 1 mM $MnCl_2$. The final tissue suspension was preincubated at 37°C for 10 min, then cooled on ice for 10 min. Aliquots (400 μl) of the tissue suspension were then incubated, each with 50 μl of appropriate concentration of ^3H and 50 μl of drug or other substance to be tested. Incubation time was 10 min at 37°C in a slowly shaking water bath for studies of [^{3}H]-5-HT and [^{3}H]ADTN and 20 min for studies of [^{3}H]spiroperidol and [^{3}H]LSD. Final concentration of ^3H in each assay was: 3 nM for [^{3}H]-5-HT, 2 nM for [^{3}H]ADTN and 1 nM for [^{3}H]spiroperidol and [^{3}H]LSD. The assay was terminated by rapid filtration through Whatman GF/B filters using a Millipore filtering apparatus. The filters were washed three times with 2 ml of cold 0.05 M Tris-HCl, pH 7.7. Radioactivity on the filters was extracted in 5 ml of New England Nuclear Formula 950A scintillation fluid and counted in a Packard 3375 liquid scintillation spectrometer at a tritium counting efficiency of 35 to 41%. Specific binding was defined as the difference between the total number of counts on a filter in the presence of a saturating concentration of cold ligand: 1 μM 5-HT for [^{3}H]-5-HT binding, 1 μM LSD for [^{3}H]LSD binding, 1 μM (+)-butaclamol for [^{3}H]ADTN binding and 10 μM spiroperidol for [^{3}H]spiroperidol binding. [^{3}H]ADTN binding sites were shown to be stereospecific by the lack of ability of (−)-butaclamol to displace bound [^{3}H]ADTN.

Adenylate cyclase assay. Assays to determine activity of adenylate cyclase were carried out in triplicate as previously described (Mishra *et al.*, 1974). Tissue was homogenized in cold 0.32 M Tris-maleate buffer (pH 7.4) with 0.8 mM ethylene glycol-bis-(β-aminoethyl ether)-N,N'-tetraacetic acid by hand in an all glass homogenizer. The final dilution of tissue in the assay was 1:240 for frontal and limbic cortex and 1:200 for caudate nucleus. The final incubation medium contained 80 mM Tris-maleate (pH 7.4), 10 mM theophylline, 2 mM $MgSO_4$ and 0.5 mM Na_2ATP. Incubation time of tissue with hormone was 2.5 min in a shaking water bath at 30°C. Cyclic adenosine 3',5'-monophosphate (cyclic AMP) determinations were done by using a protein binding assay (Brown and Makman, 1973). Protein was measured by the method of Lowry *et al.* (1951).

Materials. [^{3}H]-5-HT (25-26 Ci/mmol), [^{3}H]ADTN (25-33 Ci/mmol) and [^{3}H]LSD (18 Ci/mmol) were obtained from the New England Nuclear, Boston, MA., [^{3}H]spiroperidol (20 Ci/mmol) was obtained from Amersham/Searle Corporation, Des Plaines, IL. Lisuride hydrogen maleate was donated by R. Horowski (Schering Corporation, Kenilworth, NJ). ADTN was a gift from J. M. McDermed (Burroughs Wellcome and Company, Research Triangle Park, NC), and haloperidol from Janssen Pharmaceuticals, Beerse, Belgium. Other compounds were obtained through standard commercial sources.

Results

Interaction of lisuride with receptor binding sites in rabbit frontal cortex and caudate nucleus. In rabbit frontal cortex, lisuride was found to interact with the binding sites for all ligands tested (fig. 1). As shown in table 1, lisuride displaced [^{3}H]-5-HT with an IC_{50} similar to 5-HT. Lisuride also displaced [^{3}H]spiroperidol and was actually slightly more potent than spiroperidol itself. For both of these ligands, the Hill coefficients for lisuride were significantly less than 1. Lisuride was more potent than 5-HT in displacing [^{3}H]LSD. The Hill coefficients for lisuride and LSD displacement of [^{3}H]LSD are both nearly 1.

In rabbit caudate nucleus, the ability of lisuride to displace [^{3}H]-5-HT was similar to that in frontal cortex (fig. 2A). Lisuride was also potent in displacing the DA agonist [^{3}H]ADTN from binding sites in caudate nucleus (fig. 2B). The IC_{50} values and Hill coefficients for displacement of these ligands are given in table 2. For both ligands, the Hill coefficients for displacement by lisuride was significantly less than 1.

[^{3}H]LSD binding sites in rabbit caudate nucleus were found to be both dopaminegic and serotonergic. Of the total specifically bound [^{3}H]LSD, only 40% or 34 ± 3 fmol/mg of protein could be displaced by high concentrations of DA. Lisuride, in contrast, could displace all of the specific [^{3}H]LSD binding sites (fig. 2C). At 1 μM, lisuride displaced 100% of the total specific binding sites or 90 ± 5 fmol/mg of protein [^{3}H]LSD. At this same concentration, 5-HT displaced 60 ± 2 fmol/mg of protein [^{3}H]LSD. In the presence of 0.3 μM DA, a maximally effective concentration, 1 μM lisuride and 5-HT could displace 61 ± 3 and 51 ± 5 fmol/mg of protein [^{3}H]LSD, respectively. This indicates that at this concentration lisuride could interact with both the dopaminergic and serotonergic components of [^{3}H]LSD binding. In contrast, 5-HT was interacting mainly

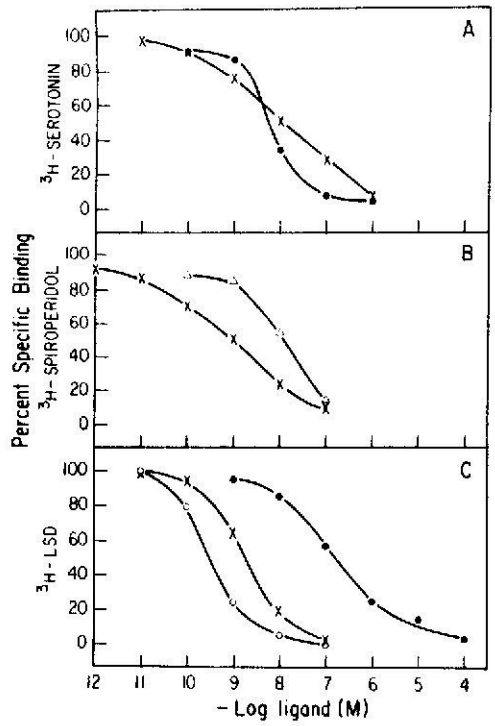

Fig. 1. Competition by ligands for specific [^{3}H]-5-HT (A), [^{3}H]spiroperidol (B) and [^{3}H]LSD (C) binding sites in rabbit frontal cortex. Homogenates were prepared and assayed as described in "Methods." Values for the total bound and specifically displaced radioactivity in femtomoles per milligram of protein were, respectively, 43 ± 2 and 19 ± 2 for [^{3}H]-5-HT, 94 ± 11 and 61 ± 6 for [^{3}H]spiroperidol and 112 ± 11 and 57 ± 11 for [^{3}H]LSD. Each point is the mean of four to five separate experiments, each experiment assayed in triplicate. ×——×, lisuride; ●——●, serotonin; △——△, spiroperidol; ○——○, LSD.

TABLE 1

Inhibition of [^{3}H]-5-HT, [^{3}H]spiroperidol and [^{3}H]LSD binding in rabbit frontal cortex by lisuride and other agents

IC_{50} represents the concentration required for 50% displacement of specifically bound ^3H ligand. Values represent the mean ± S.E.M. for four to five separate experiments.

Displacer	IC_{50}	Hill Coefficient
	nM	
	[^{3}H]-5-HT	
5-HT	8.8 ± 0.3	1.00 ± 0.12
Lisuride	14.0 ± 5.0	0.56 ± 0.06
	[^{3}H]Spiroperidol	
Spiroperidol	8.5 ± 2.0	0.83 ± 0.09
Lisuride	1.0 ± 0.3	0.40 ± 0.02
	[^{3}H]LSD	
LSD	1.8 ± 1.0	0.96 ± 0.05
Lisuride	3.7 ± 0.9	0.91 ± 0.05
5-HT	300 ± 120	0.57 ± 0.06

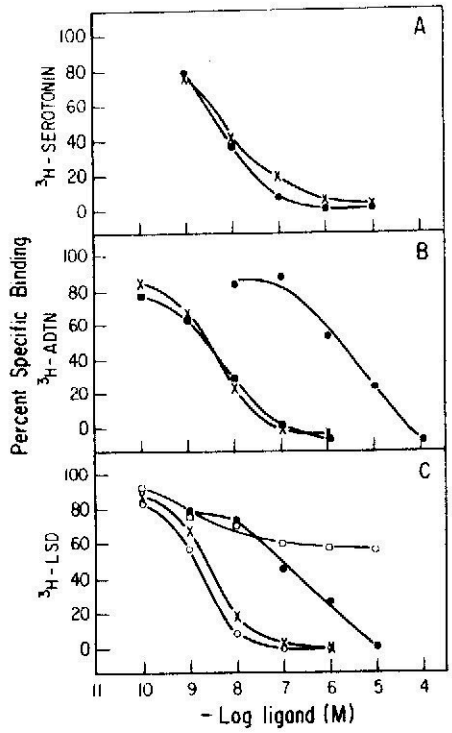

Fig. 2. Competition by ligands for specific [^{3}H]-5-HT (A), [^{3}H]ADTN (B) and [^{3}H]LSD, (C) binding sites in rabbit caudate nucleus. Homogenates were prepared and assayed as described in "Methods." Values for the total bound and specifically displaced radioactivity in femtomoles per milligram of protein were, respectively, 57 ± 3 and 33 ± 2 for [^{3}H]-5-HT, 100 ± 7 and 46 ± 5 for [^{3}H]ADTN and 159 ± 6 and 97 ± 8 for [^{3}H]LSD. Each point is the mean of three to five separate experiments, each assayed in triplicate. ×——×, lisuride; ●——●, serotonin; □——□, dopamine; ■——■, ADTN; ○——○, LSD.

TABLE 2

Inhibition of [^{3}H]-5-HT, [^{3}H]ADTN and [^{3}H]LSD binding in rabbit caudate nucleus by lisuride and other agents

Values represent the mean ± S.E.M. for four to five separate experiments.

Displacer	IC_{50}	Hill Coefficient
	nM	
	[^{3}H]-5-HT	
5-HT	7.5 ± 1.5	0.91 ± 0.10
Lisuride	7.0 ± 0.8	0.66 ± 0.05
	[^{3}H]ADTN	
ADTN	4.5 ± 1.5	0.51 ± 0.03
Lisuride	3.9 ± 1.3	0.60 ± 0.01
5-HT	2600 ± 700	0.61 ± 0.08
	[^{3}H]LSD	
LSD	1.8 ± 0.7	0.93 ± 0.04
Lisuride	2.0 ± 1.0	0.98 ± 0.09
5-HT	68 ± 15	0.40 ± 0.03

with the DA-insensitive component of [^{3}H]LSD binding. In addition, the sites remaining after displacement by 0.3 μM DA were further characterized as serotonergic by their sensitivity to cinanserin, a 5-HT antagonist (Ruben *et al.*, 1964) (data not shown).

Effect of lisuride on adenylate cyclase activity. Table 3 demonstrates the effect of DA and 5-HT on the formation of cyclic AMP in homogenates of rabbit frontal cortex, anterior limbic cortex and caudate nucleus. All three regions show DA-stimulated activity. 5-HT is able to stimulate adenylate cyclase activity in both frontal cortex and anterior limbic cortex but is inactive in caudate nucleus. Figure 3 compares the effect of lisuride on the adenylate cyclase activity in these same brain regions. Similar to 5-HT, lisuride stimulated cyclic AMP production in both cortical regions but was inactive in caudate nucleus. The cortical areas appeared to be quite sensitive to lisuride with significant stimulations occurring at concentrations of lisuride as low as 10^{-10} M. As shown, the maximal stimulation achieved by lisuride and 5-HT were found to be similar as opposed to the larger percentage of stimulation obtained with DA.

Effect of antagonists on lisuride-stimulated adenylate cyclase in rabbit frontal cortex. To evaluate whether or not lisuride was acting at 5-HT and/or DA receptors, we compared the ability of molindone and haloperidol to antagonize the stimulatory effects of lisuride on adenylate cyclase.

Molindone has been shown to be highly selective in its ability to antagonize 5-HT-stimulated adenylate cyclase in monkey brain (Ahn and Makman, 1978b). In the present studies, molindone was able to effectively antagonize the 5-HT-stimulated adenylate cyclase activity in rabbit frontal cortex. Molindone (1 μM) inhibited the stimulation produced by 100 μM 5-HT by 78% (data not shown).

Although haloperidol like other dopamine antagonists can block both DA- and 5-HT-stimulated adenylate cyclase activity, it has been shown to be more selective than were several other antagonists for blocking the DA-stimulated activity (Ahn and Makman, 1978a). Haloperidol is a particularly potent antagonist of DA receptors coupled to adenylate cyclase in cortex (Brockaert *et al.*, 1977; Ahn and Makman, 1978a).

As shown in figure 4, in rabbit frontal cortex low concentrations of molindone were effective in antagonizing the stimulation produced by a supermaximal concentration of lisuride (10^{-7} M). Molindone could only inhibit the DA-stimulated activity at concentrations equimolar to or greater than DA. Haloperidol was able to antagonize both the lisuride and DA stimulations.

Effect of guanine nucleotides on lisuride displacement of [^{3}H]spiroperidol. The effect of the guanine nucleotide analog, 5'-guanylyl-imidodiphosphate [Gpp(NH)p], on the lisuride displacement of [^{3}H]spiroperidol in frontal cortex is shown in table 4. In the presence of 10 μM Gpp(NH)p, the ability of lisuride to displace [^{3}H]spiroperidol decreased significantly. The Hill coefficient for lisuride also changed from a value less than 1 in the absence of Gpp(NH)p toward 1 in the presence of Gpp(NH)p. A similar shift of Hill coefficients for other ligands in the presence of guanine nucleotides has been shown by other investigators (Hegstrand *et al.*, 1979). This effect of Gpp(NH)p implies an agonist-like interaction of lisuride with the receptor sites. By comparison, the displacement caused by the antagonist molindone is not affected by Gpp(NH)p.

Discussion

We have reported here the ability of lisuride to interact with several radioactively labeled ligands in rabbit frontal cortex and caudate nucleus. We have also demonstrated the ability of very low concentrations of lisuride to stimulate adenylate cyclase activity in rabbit cortical regions and presented evidence indicating that this stimulation involves 5-HT receptors rather than DA receptors.

It has been proposed that [^{3}H]spiroperidol primarily labels 5-HT receptors in cortex (Creese and Snyder, 1978a; Leysen *et al.*, 1978; Peroutka and Snyder, 1979). [^{3}H]LSD binding has

TABLE 3

Effect of DA and 5-HT on adenylate cyclase activity in rabbit brain

Conc. of Amine (M)	% Stimulation of Activity Over Basal[a]					
	Frontal cortex		Anterior limbic cortex		Caudate nucleus	
	DA	5-HT	DA	5-HT	DA	5-HT
M	pmol cyclic AMP mg/protein/2.5 min					
10^{-8}	4 ± 3 (4)		9 ± 3 (4)		11 ± 7 (8)	
10^{-7}	26 ± 3 (10)	10 ± 4 (2)	19 ± 10 (4)	4 ± 3 (4)	34 ± 5 (3)	0 ± 0 (3)
10^{-6}	29 ± 6 (10)	26 ± 4 (3)	62 ± 20 (7)	17 ± 2 (3)	42 ± 7 (8)	0 ± 0 (3)
10^{-5}	82 ± 6 (10)	60 ± 16 (3)	80 ± 12 (9)	36 ± 15 (5)	75 ± 12 (8)	5 ± 3 (5)
10^{-4}	120 ± 22 (12)	56 ± 12 (12)	108 ± 12 (9)	65 ± 7 (5)	84 ± 12 (13)	4 ± 2 (5)

[a] Basal activity was 103 ± 8, 100 ± 4 and 158 ± 15 pmol of cyclic AMP/mg of protein/2.5 min (mean ± S.E.M.), respectively, for frontal cortex, anterior limbic cortex and caudate nucleus. Number of separate experiments in parentheses.

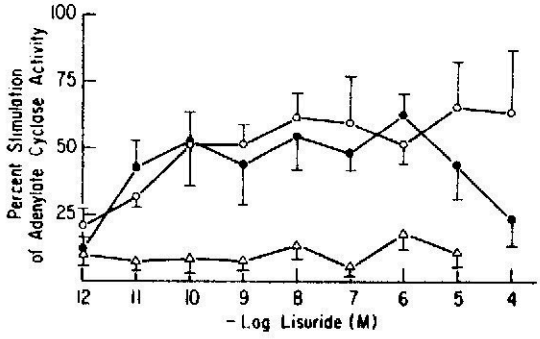

Fig. 3. Stimulation of adenylate cyclase activity in homogenates of rabbit brain regions. Homogenates were prepared and assayed as described in "Methods." Basal activities were 103 ± 8, 100 ± 4 and 166 ± 16 pmol of cAMP per mg of protein per 2.5 min (mean ± S.E.M.), respectively, for frontal cortex, anterior limbic cortex and caudate nucleus. Each point represents the mean ± S.E.M. of 7 to 13 experiments, each assayed in triplicate. ●——●, frontal cortex; ○——○, anterior limbic cortex; △——△, caudate nucleus.

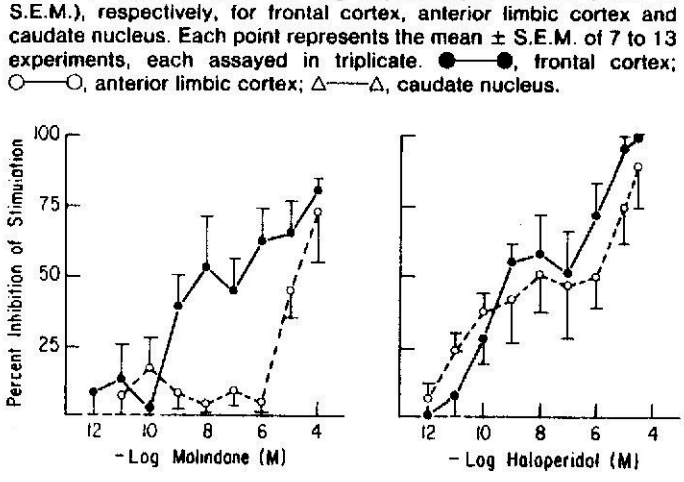

Fig. 4. Inhibition of stimulation of adenylate cyclase activity produced by 10^{-7} M lisuride and 10^{-5} M dopamine in rabbit frontal cortex. Each point is the mean ± S.E.M. of three to five separate experiments, each assayed in triplicate. ●——●, lisuride; ○——○, dopamine.

also been demonstrated to be primarily serotonergic in cortex (Leysen *et al.*, 1978). The ability of lisuride to displace [^{3}H]-5-HT, spiroperidol and LSD in cortex therefore supports the site of action of lisuride as serotonergic. However, the interaction of lisuride with [^{3}H]LSD in frontal cortex is different from that with these other ligands as demonstrated by the Hill coefficients obtained: 0.91 for [^{3}H]LSD; 0.40 for [^{3}H]spiroperidol; and 0.56 for [^{3}H]-5-HT. LSD binding sites in cortex, although described primarily as serotonergic, do show some differences in drug affinities from [^{3}H]-5-HT binding sites (Bennett and Snyder, 1976). In brain synaptosomal membranes, Fillion *et al.*, (1977)

TABLE 4

Effect of Gpp(NH)p on ligand displacement of [^{3}H]spiroperidol in rabbit frontal cortex

Each value represents the mean ± SEM for four to seven separate experiments each assayed in triplicate.

	IC_{50}	Hill Coefficient
	Lisuride (nM)	
Control	0.59 ± 0.15	0.38
+10 μM Gpp(NH)p	5.1 ± 1.2*	0.86
	Molindone (μM)	
Control	6.1 ± 1.1	0.31
+10 μM Gpp(NH)p	12.0 ± 6.0	0.27

* $P < 0.01$ as determined by Students *t* test.

demonstrated that the high affinity binding sites for [^{3}H]-5-HT were not identical with those for [^{3}H]LSD. Although [^{3}H]LSD might bind to several different serotonergic receptor types, the data presented in this study indicate that lisuride is not a selective agent for any one site. [^{3}H]-5-HT binding to two distinct serotonergic sites in crude brain membranes has been demonstrated (Fillion *et al.*, 1977). If lisuride were interacting with only one subsite or possessed different affinities for each subsite of the bound [^{3}H]-5-HT, then the resulting displacement curve would yield a low Hill coefficient as was obtained in this study. Similar considerations might also apply to [^{3}H]spiroperidol binding.

Studies of transmitter-stimulated adenylate cyclase further support a serotonergic site of action of lisuride. In frontal cortex, lisuride stimulates adenylate cyclase and this stimulation is antagonized by molindone. The high selectivity of molindone as an antagonist of 5-HT-stimulated adenylate cyclase in the present study is indicated by the several orders of magnitude of greater concentrations of molindone required to inhibit the DA as opposed to 5-HT-stimulated activity. Also, the maximal percentage of stimulation obtained by lisuride in cortex is less than that obtained by DA but similar to that of 5-HT (table 3). Finally, both lisuride and 5-HT are active in cortical regions but not in caudate nucleus. In contrast, DA is stimulatory in all these regions. The influence of lisuride on 5-HT-stimulated activity in cortex would appear to resemble that of LSD, a compound we have previously reported to stimulate adenylate cyclase in monkey cortex primarily or perhaps entirely by interaction with 5-HT receptors (Ahn and Makman, 1979).

The site of action of lisuride in rabbit caudate nucleus appears to involve both 5-HT and DA receptors. Lisuride is a potent displacer of [^{3}H]ADTN, which has been shown to label DA receptors (Creese and Snyder 1978b; Seeman *et al.*, 1979). [^{3}H] LSD binding in calf caudate has been demonstrated to involve

both serotonergic and dopaminergic receptor types (Burt *et al.*, 1976). In rabbit, we find this also to be the case, with approximately 40% of the specific [^{3}H]LSD binding sites to be dopaminergic and the rest serotonergic. We have shown that lisuride is able to interact with both classes of [^{3}H]LSD binding sites. The displacement curve obtained by lisuride results in a Hill coefficient near 1, suggesting that lisuride interacts with similar affinities with both types of sites. This also appears to be the case for LSD itself, as has been reported previously for calf caudate (Burt *et al.*, 1976).

In frontal cortex, the ability of lisuride to displace [^{3}H]spiroperidol is significantly decreased by Gpp(NH)p. Guanine nucleotides and their analogs have been shown to decrease the affinity of agonist ligands for receptor binding sites, while having no effect on the binding of antagonists (Maguire *et al.*, 1976; Zahniser and Molinoff, 1978; Creese *et al.*, 1979). This effect of Gpp(NH)p indicates an agonist interaction of lisuride at these receptor sites and is consistent with the ability of lisuride to stimulate adenylate cyclase in this brain region.

There is evidence suggesting that guanine nucleotides are needed to couple adenylate cyclase activation with the binding of agonist ligands to membrane receptor sites (Salomon *et al.*, 1975; Levitski, 1976; Iyengar *et al.*, 1979). In caudate nucleus, a dependence upon guanine nucleotides for stimulation of adenylate cyclase by DA can be demonstrated readily in washed membrane preparations (Roufogalis *et al.*, 1976). Also, in crude homogenates of rat caudate nucleus, pergolide 8β-8-[(methylthio) methyl]-6-propylergoline), an ergot with DA-agonist activity *in vivo* (Fuller *et al.*, 1979), will stimulate cyclic AMP formation in the absence of added GTP, but this activity is further enhanced in the presence of GTP (Goldstein *et al* 1980). The stimulatory effect of pergolide in this system has also been shown to involve DA receptors (Rosenfeld *et al.*, 1980). However, in this same system, lisuride is not able to stimulate cyclic AMP formation even in the presence of GTP (Rosenfeld *et al.*, 1980).

The inability of lisuride to activate DA-sensitive adenylate cyclase in caudate is similar to that of other ergot derivatives such as lergotrile, bromocriptine and LSD. All of these compounds are able to antagonize this activity (Kebabian *et al.*, 1977; Makman, 1977; Markstein *et al.*, 1978; Ahn and Makman, 1979). Lisuride has also been demonstrated to antagonize DA-stimulated activity in rat striatum (Pieri *et al.*, 1978). This further implies that the previously reported *in vivo* effects of lisuride on nigral-neostriatal DA receptors, *i.e.*, induction of sterotypic behavior in mice (Horowski and Wachtel, 1976), are not due to direct stimulation of DA receptors coupled to adenylate cyclase. It should also be mentioned that LSD is a potent antagonist of DA-stimulated adenylate cyclase not only in caudate nucleus but in other central nervous system regions as well, including cortex (Ahn and Makman, 1979). Whether or not lisuride is also an antagonist of cortical DA-stimulated adenylate cyclase remains to be established.

There is now considerable evidence indicating that not all DA receptor sites assessed by radioligand binding are coupled to adenylate cyclase (Kebabian and Calne, 1979; Makman *et al.*, 1979). Also, with respect to 5-HT receptors, Peroutka and Snyder (1979) have reported that in rat cerebral cortex there are at least two distinct classes of 5-HT receptors, selectively labeled by [^{3}H]-5-HT and [^{3}H]spiroperidol, with those labeled by [^{3}H]-5-HT resembling that of the 5-HT-stimulated adenylate cyclase. [^{3}H]LSD will label both sites. The ability of lisuride to displace all of the radioligands studied and to stimulate 5-HT-sensitive adenylate cyclase suggests that in rabbit cortex lisuride interacts with both those 5-HT receptors coupled to adenylate cyclase and with those not coupled to adenylate cyclase.

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