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THE EFFECT OF LSD AND 2-BROMO LSD ON THE STRIATAL DOPA ACCUMULATION AFTER DECARBOXYLASE INHIBITION IN RATS

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LSD and BOL (0.125–0.5 mg/kg) were equipotent in increasing the in vivo tyrosine hydroxylation in the striatum as measured by the accumulation of DOPA after inhibition of neuronal decarboxylase. However, with 2–4 mg/kg doses, the maximum effect of BOL was larger than that of LSD. LSD and BOL antagonized the apomorphine-induced decrease of DOPA accumulation, without affecting the haloperidol-induced increase. LSD like apomorphine inhibited the increase of DOPA accumulation seen after reserpine, cerebral hemisection and after γ-butyrolactone (GBL). The effect of apomorphine in rats given GBL was blocked by haloperidol, but not by BOL and promethazine, whereas that of LSD was inhibited by haloperidol, BOL, and promethazine.

These findings suggest that LSD and BOL directly affect nigro-neostriatal dopamine neurons. LSD therefore appears to be a partial agonist and BOL a pure antagonist at dopamine autoreceptors. It is proposed in addition that LSD activates and BOL blocks 5-HT receptors that control DOPA formation.

Dopamine receptors Striatum DOPA BOL LSD γ-Butyrolactone

1. Introduction

d-Lysergic acid diethylamide (LSD) has been demonstrated to produce electrophysiological and biochemical changes in the 5-hydroxytryptaminergic (5-HT) pathways (Freedman, 1961; Aghajanian et al., 1968; Andén et al., 1968). In addition, LSD has been found to affect catecholaminergic (CA) mechanisms. High doses of LSD have e.g. been reported to decrease the norepinephrine (NE) levels in rat brain (Freedman, 1963; Sugrue, 1969). Some reports have suggested an increased turnover of the rat brain catecholamines after LSD (Andén et al., 1968; Persson, 1970; Stolk et al., 1974).

Recently we reported that both LSD and its bromo derivative 2-bromo lysergic acid diethylamide (BOL) increased the accumulation of DOPA after decarboxylase inhibition in the rat brain (Hollunger and Persson, 1975). Furthermore Smith et al. (1975) found that LSD increased tyrosine and dopamine (DA) levels in the rat brain but decreased DA levels in the rat brain after LSD have also been reported (Diaz et al., 1968).

LSD may stimulate CA receptors directly. Thus in the rotational model of Ungerstedt and Arbuthnott (1970) LSD behaves like a striatal DA receptor agonist (Pieri et al., 1974). LSD has also been shown to be an agonist at limbic postsynaptic DA receptors after 6-hydroxydopamine (6-OHDA)-induced degeneration of the DA nerve terminals (Kelly and Iversen, 1975).

LSD has also been found to stimulate the adenylate cyclase from striatum and other DA receptor-rich structures (Von Hungen et al., 1974, 1975; Da Prada et al., 1975; Spano et al., 1975). This stimulation could be blocked by DA receptor antagonists such as haloperidol and chlorpromazine (Von Hungen et al., 1974, 1975). BOL, on the other hand, had only an antagonistic effect on the DA-sensitive

adenylate cyclase. The stimulation of adenylate cyclase after LSD was clearly shown to be submaximal compared to the effect of DA (Da Prada et al., 1975; Spano et al., 1975). That LSD has to be considered as a partial DA receptor agonist was further shown by its inhibitory effect on the adenylate cyclase maximally stimulated by DA (Von Hungen et al., 1974, 1975; Spano et al., 1975; Da Prada et al., 1975). Direct binding studies on suggested DA receptors (Creese et al., 1975) lend further support for the characterization of LSD as a partial agonist and of BOL as a pure antagonist.

In the present investigation we have studied the effect of LSD and BOL on the accumulation of DOPA in the striatum of rats with a functionally intact nigro-neostriatal pathway and after pharmacological and surgical interruption of the impulse flow in this pathway.

2. Materials and methods

2.1. Animals

Male Sprague—Dawley rats (Anticimex, Sollentuna, Sweden) weighing 188–298 g were used in all experiments. They were fed the Ewos-Anticimex commercial type pelleted diet, R3. During the experiments the rats had free access only to water.

2.2. Drugs

The following drugs were used: apomorphine HCl (Sandoz, Basle), 2-bromo lysergic acid diethylamide bitartrate, BOL (Sandoz *, Basle), haloperidol (Leo *, Helsingborg), gamma-butyrolactone, GBL (Sigma, St. Louis, Mo.), d-lysergic acid diethylamide tartrate, LSD (Sandoz *, Basle), promethazine chloride, Lergigan® (Recip, Stockholm), reserpine, Serpasil® (Ciba, Basle). 3-Hydroxybenzylhydrazine HCl, NSD 1015 was kindly synthesized by Dr. B. Magnusson, Department of Organic Chemistry, University of Umeå, Sweden. Doses of LSD and BOL refer to the base. Haloperidol was dissolved in 5.5% glucose after acidification with 200 µl glacial acetic acid. All other drugs were dissolved in 0.9% saline. The solutions were made up just before injection. All drugs were administered i.p.

2.3. Experimental

2.3.1. General

The experiments were performed between 0.900–11.00 a.m. at a room temperature of 23–26°C. The body temperature was monitored frequently by means of a telethermometer with the thermistor probe (YSI 44018) inserted 5 cm from the anal orifice. The lowest temperature observed was 36.1°C.

In some rats cerebral hemisection at the level of the caudal hypothalamus was performed under light diethylether anesthesia according to Bédard et al. (1972). In vivo tyrosine hydroxylation was measured according to Carlsson et al. (1972). The decarboxylase inhibitor 3-hydroxybenzylhydrazine, NSD 1015 was administered 20 min before killing the rats. The rate of DOPA accumulation was constant for at least 20 min after the administration of the inhibitor. There are good reasons to believe that the rate of DOPA accumulation is a reliable measure of the rate of DOPA synthesis (Carlsson et al., 1972). The rats were killed with a guillotine. The brains were rapidly removed and dissected on ice. The striata were reached by opening the lateral ventricles from above. With the guidance of the exposed upper surface of the nucleus caudatus, a part mainly containing nucleus caudatus and putamen was dissected out.

2.3.2. DOPA determination

DOPA was isolated and measured fluorimetrically by means of the technique of Kehr et al. (1972a) with some modifications. One pair of striata was weighed and homogenized in 5 ml 0.4 M icecold $HClO_4$ containing 100 mg EDTA and 0.1 ml 5% $Na_2S_2O_5$. The

homogenate was centrifuged (14,000 *g*) at 0°C for 10 min. The supernatant was adjusted to pH 2 with 2.5 M K_2CO_3 and put on a small column (4.0 I.D. × 40 mm) containing Amberlite CG 120 II Na^+. The resin was prepared as described by Häggendal (1962). The column was then treated according to the following procedure: (1) 10 ml 0.1 M sodium phosphate buffer pH 6.5 containing 0.1% EDTA; (2) 3 ml H_2O; (3) the extract (pH 2) was passed through the column; (4) 2 × 5 ml H_2O; (5) 5 ml 0.1 M sodium citrate-phosphate buffer pH 2.5; (6) 5 ml H_2O; (7) 1 ml 0.1 M sodium citrate-phosphate buffer pH 4.5 (discarded); (8) 3 ml 0.1 M sodium citrate-phosphate buffer pH 4.5 (eluted DOPA).

DOPA was then quantitated as in the method of Kehr et al. (1972a) modified in that 10 M NaOH was used instead of 5 M NaOH in the final step. This modification was found to give an increased fluorescence reading but a relatively smaller increase of the blank readings. Twice, the reagent blank fluorescence corresponded to about 2.5 ng DOPA. The fluorescence reading of the sample and that of the tissue blank from one pair of untreated striata was about the same as the reading of the reagent blank. The recovery of 500 ng DOPA taken through the entire procedure was 72.1 ± 0.8% (mean ± S.E.M., n = 93) and all values were corrected for recovery.

2.4. Statistical analysis

The significance of the results was assessed using the two-tailed Student's *t*-test. A *p*-value of less than 0.05 was considered significant.

3. Results

3.1. Striatal DOPA accumulation after LSD and BOL administration. Time and dose relations

The accumulation of striatal DOPA induced by NSD 1015 was already significantly increased 15 min after the administration of LSD (2 mg/kg) and BOL (2 mg/kg) (fig. 1). The maximum increase of DOPA accumulation was seen 30 min after LSD and BOL. No increase of the accumulation of DOPA was observed 2 h after LSD and BOL.

The minimum effective dose of both LSD and BOL was 0.125 mg/kg (fig. 2). Within the 0.125–0.500 mg/kg dose range the increases in DOPA accumulation after LSD administration did not differ significantly from those obtained after treatment with BOL. However, within the 2–4 mg dose range, the administration of BOL resulted in increases of the DOPA accumulation at least twice as large as those obtained after LSD treatment. The differences were highly significant ($p < 0.005$–0.001). The DOPA levels after BOL (2–4 mg/kg) were of the same order as those reached after haloperidol (0.5 mg/kg). Increasing the LSD dose from 0.125 to 4 mg/kg produced no further increase in the DOPA accumulation. Thus with LSD it was not possible to reach the maximum DOPA accumulation seen after high doses of BOL or after haloperidol. In agreement with earlier results (Kehr et al., 1975) there was a dose-dependent increase in DOPA accumulation after haloperidol treatment and a dose-

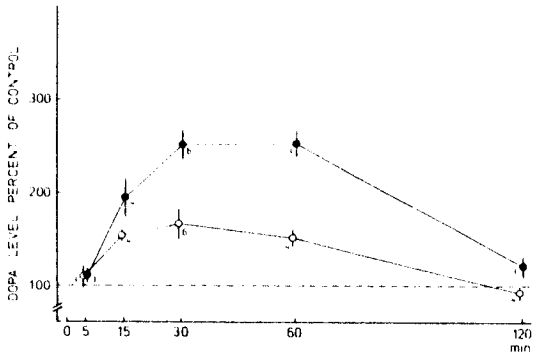

Fig. 1. Striatal DOPA accumulation after LSD (○ 2 mg/kg) and BOL (● 2 mg/kg). Each point represents the mean ± S.E.M. The numbers indicate the number of individual experiments. The control value is 1042 ± 77 ng/g. The abscissa indicates the time after administration of LSD or BOL. All animals were given NSD 1015 (100 mg/kg) 20 min before sacrifice.

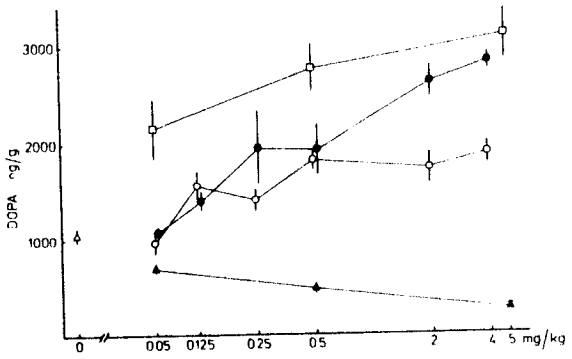

Fig. 2. Striatal DOPA accumulation after different doses of LSD (○), BOL (●), apomorphine (▲) and haloperidol (□). Haloperidol was administered 80 min before sacrifice. LSD, BOL and apomorphine were administered 30 min before sacrifice. Control animals (△) received saline 30 min before sacrifice. NSD 1015 (100 mg/kg) was given to all rats 20 min before sacrifice. Each point represents the mean ± S.E.M. of 6–9 determinations.

dependent decrase after apomorphine (fig. 2). The minimum effective dose was the same for both apomorphine and haloperidol (0.05 mg/kg).

Combined administration of various doses of LSD and BOL did not result in a DOPA accumulation that was statistically different from that obtained after the administration of BOL alone (table 1).

3.2. The influence of apomorphine and haloperidol on the increased striatal DOPA accumulation produced by LSD and BOL

The effect of LSD and BOL on the apomorphine-induced inhibition of DOPA accumulation is shown in table 2. LSD and BOL (0.5 mg/kg) were equally effective in antagonizing the apomorphine-induced decrease of DOPA accumulation. After 2 mg/kg, more DOPA accumulated than in the saline controls. BOL was, however, much more effective than LSD. In fact the DOPA level after combined treatment with apomorphine (0.5 mg/kg) and BOL (2 mg/kg) was the same as after treatment with BOL alone (2 mg/kg). When the apomorphine dose was increased to 5 mg/kg, the decrease in the DOPA concentration was still dose-dependently counteracted by LSD and BOL (0.5–2.0 mg/kg).

LSD and BOL (2 mg/kg) did not affect the striatal DOPA accumulation in rats treated with haloperidol (5 mg/kg).

3.3. The effect of LSD and BOL on the increased DOPA accumulation induced by reserpine

Reserpine has been shown to increase the DOPA accumulation after decarboxylase

TABLE 1

Striatal DOPA accumulation after various doses of LSD and BOL or combinations of LSD and BOL. The combination of LSD and BOL was given in the same syringe. LSD, BOL and LSD + BOL were administered 30 min before killing the rats. NSD 1015 (100 mg/kg) was given to all animals 20 min before sacrifice.

Drug (mg/kg)	DOPA ng/g ± S.E.M.	n	Difference from the combined treatment
LSD 0.125	1560 ± 157	6	N.S.
BOL 0.125	1386 ± 106	6	N.S.
LSD 0.125 + BOL 0.125	1725 ± 127	6	—
LSD 0.250	1397 ± 106	6	$p < 0.02$
BOL 0.250	1951 ± 380	6	N.S.
LSD 0.250 + BOL 0.250	2218 ± 255	6	—
LSD 2.0	1726 ± 163	6	$p < 0.001$
BOL 2.0	2630 ± 169	6	N.S.
LSD 2.0 + BOL 2.0	2879 ± 169	6	—

TABLE 2

The effects of apomorphine and haloperidol on striatal DOPA accumulation after LSD and BOL. Haloperidol was administered 80 min and promethazine 45 min before killing the rats. Apomorphine, BOL and LSD were administered 30 min before sacrifice. When apomorphine was combined with LSD or BOL, it was given 40 min before sacrifice. NSD 1015 was administered to all rats 20 min before sacrifice.

Drug (mg/kg)	DOPA ng/g ± S.E.M. (n)	Comparisons between various treatments p-values		
a NaCl 0.9%	1042 ± 77 (8)			
b Apomorphine 0.5	472 ± 51 (6)	a—b < 0.001	b—c < 0.005	
c Apomorphine 5.0	249 ± 31 (6)	a—c < 0.001		
d Haloperidol 0.5	2764 ± 240 (6)	a—d < 0.001	d—e N.S.	
e Haloperidol 5.0	3105 ± 257 (6)	a—e < 0.001		
f Promethazine 10	1005 ± 36 (3)	a—f N.S.		
g LSD 0.5	1823 ± 113 (6)	a—g < 0.001	g—h N.S.	
h LSD 2.0	1726 ± 163 (6)	a—h < 0.005		
i BOL 0.5	1924 ± 131 (9)	a—i < 0.001	i—j < 0.01	
j BOL 2.0	2630 ± 169 (6)	a—j < 0.001		
k Apomorphine 0.5 + LSD 0.5	823 ± 76 (3)	b—k < 0.01		k—m N.S.
l Apomorphine 0.5 + LSD 2.0	1358 ± 29 (3)	b—l < 0.001	h—l N.S.	l—n < 0.001
m Apomorphine 0.5 + BOL 0.5	781 ± 28 (3)	b—m < 0.005		
n Apomorphine 0.5 + BOL 2.0	2888 ± 111 (3)	b—n < 0.001	j—n N.S.	
o Apomorphine 5.0 + LSD 0.5	617 ± 82 (3)	c—o < 0.005		o—q N.S.
p Apomorphine 5.0 + LSD 2.0	1091 ± 98 (8)	c—p < 0.001		p—r N.S.
q Apomorphine 5.0 + BOL 0.5	594 ± 42 (3)	c—q < 0.001		
r Apomorphine 5.0 + BOL 2.0	828 ± 190 (9)	c—r < 0.05		
s Haloperidol 0.5 + apomorphine 0.5	2593 ± 718 (3)	d—s N.S.		
t Haloperidol 5.0 + LSD 2.0	3095 ± 227 (6)	e—t N.S.	t—u N.S.	
u Haloperidol 5.0 + BOL 2.0	2741 ± 116 (6)	e—u N.S.		

inhibition. This increase can be inhibited by the DA receptor agonist, apomorphine (Carlsson, 1975). We found that LSD (0.5 mg/kg) and apomorphine (0.05 mg/kg) inhibited the increase in DOPA accumulation both 4 and 24 h after the administration of reserpine, 5 mg/kg (table 3). BOL had no effect on the increased DOPA accumulation after reserpine.

3.4. The effect of LSD and BOL on the DOPA accumulation after interruption of the impulse flow in the nigro-neostriatal pathway by cerebral hemisection

Cerebral hemisection has been shown to cause a pronounced but shortlasting increase in the DOPA accumulation after decarboxylase inhibition. This increase can be counteracted

TABLE 3

Effects of apomorphine and LSD on striatal DOPA accumulation after a high dose of reserpine. Reserpine (5 mg/kg) was administered 4 or 24 h before sacrifice. Apomorphine, BOL, LSD or NaCl were administered 30 min before killing the rats. NSD 1015 (100 mg/kg) was administered to all animals 20 min before sacrifice.

Pretreatment dose mg/kg	Treatment dose mg/kg	DOPA ng/g ± S.E.M. (n)	Comparisons between the various treatments *p*-values	
Reserpine 5	a. NaCl	4332 ± 199 (5)		
4 h	b. Apomorphine 0.05	2583 ± 665 (3)	a—b < 0.02	b—c N.S.
	c. LSD 0.5	1916 ± 394 (3)	a—c < 0.001	
Reserpine 5	e. NaCl	2618 ± 136 (6)		
24 h	f. Apomorphine 0.05	1223 ± 156 (6)	e—f < 0.001	f—g N.S.
	g. LSD 0.5	1148 ± 76 (5)	e—g < 0.001	
	h. BOL 0.5	2465 ± 243 (6)	e—h N.S.	

by the DA receptor agonist, apomorphine (Kehr et al., 1972b). We now found that LSD (0.5 mg/kg) also inhibited the increase of DOPA accumulation on the sectioned side (table 4). On the unsectioned side, LSD increased the DOPA level by about 35%, but the difference from saline was not significant. BOL did not inhibit the increase in DOPA accumulation on the sectioned side but increased the DOPA accumulation on the unsectioned side.

3.5. Modifications of the increased DOPA accumulation produced by inhibiting the impulse flow in the nigro-neostriatal pathway by γ-butyrolactone (GBL)

GBL has been shown to inhibit the impulse flow in the nigro-neostriatal pathway. Under such conditions no dopamine (DA) seems to be released, with the consequence that DOPA synthesis is increased (Roth et al., 1973; Walters et al., 1975) possibly because DA autoreceptors are not activated. In the present study, the increase of DOPA accumulation by GBL was confirmed (fig. 3). In agreement with others (Roth et al., 1973; Walters and Roth, 1974) we found that the increased DOPA accumulation could be inhibited by apomorphine. This inhibition was dose-dependent. LSD (0.5 mg/kg) also inhibited this increased DOPA accumulation, while haloperidol, BOL and promethazine did not have any effect on the DOPA level after GBL. When the dose of LSD was raised above 0.5 mg/kg, the rats did not survive. The effect of apomorphine on the DOPA accumulation after GBL has been shown to be counteracted

TABLE 4

Effects of LSD on striatal DOPA accumulation after cerebral hemisection. Hemisection was performed 30 min before sacrifice. LSD, BOL or NaCl were injected immediately after the hemisection. NSD 1015 (100 mg/kg) was administered to all animals 20 min before sacrifice.

Treatment	DOPA ng/g ± S.E.M. (n)		Comparisons between the various treatments *p*-values		
	Sectioned side	Unsectioned side			
NaCl 0.9%	a 2647 ± 400 (4)	c 901 ± 232 (4)	a—c < 0.01	a—b < 0.05	a—e N.S.
LSD 0.5 mg/kg	b 1573 ± 129 (4)	d 1247 ± 112 (4)	b—d N.S.	c—d N.S.	
BOL 0.5 mg/kg	e 1915 ± 215 (4)	f 2058 ± 158 (3)	e—f N.S.	c—f < 0.02	

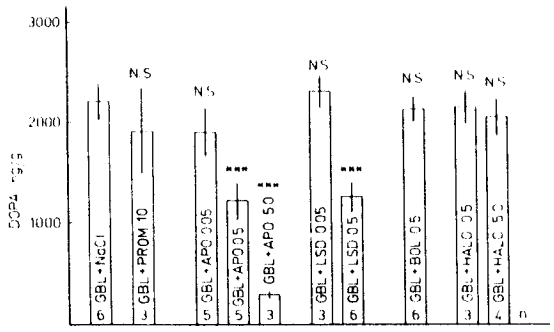

Fig. 3. Influence of apomorphine (APO) and LSD on the striatal DOPA accumulation after GBL. Haloperidol (HALO) was administered 80 min, promethazine (PROM) 45 min and GBL (750 mg/kg) 35 min before sacrifice. APO, BOL, LSD and NaCl were administered 30 min before sacrifice. All rats were treated with NSD 1015 (100 mg/kg) 20 min before sacrifice. Doses of APO, BOL, LSD, HALO and PROM are shown in the histograms. All doses are expressed in mg/kg. The results are presented as mean ± S.E.M. Significantly different from GBL + NaCl. $***p < 0.005$.

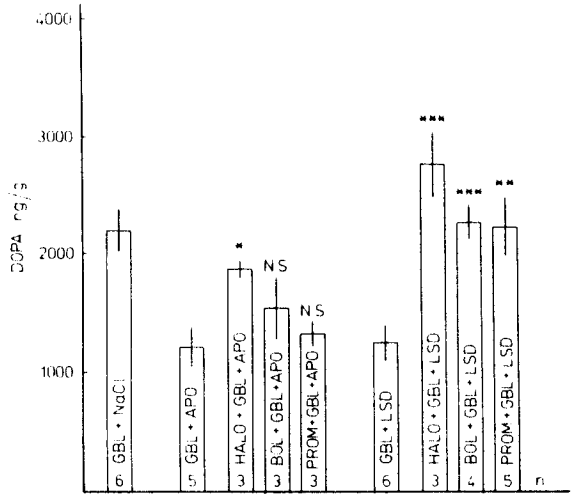

Fig. 4. Effects of haloperidol (HALO), BOL and promethazine (PROM) on DOPA accumulation after treatment with GBL and apomorphine (APO) and with GBL and LSD. HALO (0.5 mg/kg), BOL (2 mg/kg) and PROM (10 mg/kg) were administered 45 min before killing the rats. (GBL (750 mg/kg) was administered 35 min, and APO (0.5 mg/kg) and LSD (0.5 mg/kg) 30 min before sacrifice. All rats were treated with NSD 1015 (100 mg/kg) 20 min before sacrifice. The results are presented as mean ± S.E.M. Significantly different from GBL + APO or GBL + LSD respectively. $*p < 0.05$, $**p < 0.01$, $***p < 0.005$.

by DA receptor antagonists with presynaptic activity (e.g. haloperidol), but not by DA receptor antagonists without presynaptic activity (Walters and Roth, 1976; Roth et al., 1975). In agreement with earlier reports (Walters and Roth, 1976) we found that haloperidol antagonized the effect of apomorphine on DOPA accumulation (fig. 4). BOL and promethazine did not significantly alter the effect of apomorphine. Haloperidol also counteracted the LSD-induced decrease in DOPA accumulation after GBL. It is important to note that both BOL and promethazine effectively antagonized this LSD effect.

4. Discussion

In the present study our earlier observation (Hollunger and Persson, 1975) that administration of LSD and BOL increased the rate of DOPA accumulation in the whole rat brain has been found to be true for the striatum. Studies by Carlsson (1975) and Roth et al. (1975) indicate that an increased accumulation may result from either an acceleration of the impulse flow in the nigroneostriatal pathway or a reduction of the concentration of DA in the synaptic cleft (e.g. after interruption of the impulse flow in the pathway by surgical or pharmacological means or after depletion by reserpine). Our finding that BOL antagonized the inhibitory effect of the DA agonist, apomorphine, on DOPA accumulation strongly suggests that BOL is acting by blocking DA receptors. This interpretation is also in agreement with the finding (Von Hungen et al., 1974; 1975) that BOL, like haloperidol and other neuroleptics, effectively antagonized the DA-induced activation of the adenylate cyclase from DA-receptor-rich parts of the brain including the striatum. A common point of attack for BOL and haloperidol is also indicated by the

fact that the combined effect of maximal doses of these two substances did not exceed the effect of either one given alone.

A maximum effective dose of LSD was only about half as effective as BOL in increasing DOPA accumulation in the striatum. Assuming that LSD acts on the same receptors as does its bromo derivative and taking into account the evidence for a DA receptor stimulating action of LSD (Pieri et al., 1974; Da Prada et al., 1975; Kelly and Iversen, 1975; Spano et al., 1975), this difference most probably indicates that in vivo LSD has agonistic as well as antagonistic effects on DA receptors. The antagonistic property would also explain the finding that LSD antagonized the apomorphine effect on DOPA accumulation.

In intact rats, the supposed agonistic effect of LSD did not manifest itself by a lower rate of DOPA accumulation than in non-treated rats but only as a lower rate than after BOL and haloperidol. In fact, it seems doubtful whether LSD acts as a DA receptor agonist in functionally intact dopaminergic systems. The finding of a decrease in homovanillic acid in the striatum and a delayed disappearance of DA after tyrosine hydroxylase inhibition (Da Prada et al., 1975) suggests that LSD directly stimulates DA receptors. However, these changes appear hours after administration of LSD, when the LSD concentration in the brain is very low (Rosecrans et al., 1967). In the experiments of Pieri et al. (1974) and Kelly and Iversen (1975) demonstrating an agonistic effect of LSD in striatum and n. accumbens respectively, the dopaminergic innervation was destroyed by 6-hydroxydopamine. Grabowska et al. (1974) could only show an LSD-induced increase of locomotor activity after reserpine administration. In all these experiments, the concentration of DA at the postsynaptic receptor must have been very low. The same is true for the in vitro experiments directly demonstrating an agonistic effect of LSD on the DA-sensitive adenylate cyclase.

A more direct demonstration of a DA receptor activating effect of LSD in DOPA accumulation experiments is provided by the finding that LSD, like apomorphine, antagonized the increase of DOPA accumulation induced by reserpine. The increase of DOPA accumulation after the surgical interruption of the nigro-neostriatal pathway was also antagonized.

There are reasons to believe that the feedback regulation of DOPA synthesis in DA neurons is mediated in two ways: via postsynaptic receptors on target cells of DA neurons over neuronal loops controlling the impulse flow in the dopaminergic neurons and more directly via autoreceptors (Roth et al., 1975; Carlsson, 1975). Roth et al. (1975) have made use of the finding that GBL effectively blocks the impulse flow in the nigro-neostriatal pathway (Roth et al., 1973) to eliminate the control exerted by the target cells for differentiation between drug effects on pre- and postsynaptic DA receptors.

Using this model, we found that both LSD and apomorphine inhibited the GBL-induced increase in DOPA accumulation. It can thus be concluded that LSD has an action on the DA neuron itself. Haloperidol (0.5 mg/kg) antagonized the apomorphine effect, while BOL (2 mg/kg) had no significant effect. Since as little as 0.5 mg/kg BOL effectively antagonized the apomorphine effect in rats with an intact feedback mechanism, it could be argued that the BOL effect is predominantly postsynaptic. However, any functional effect of BOL in the dopaminergic systems has, as far as we are aware, never been reported.

It is possible that the postulated activation by LSD of DA receptors on target cells of DA neurons is not reflected to the degree expected by changes in DOPA accumulation, because other effects of LSD, such as 5-HT-like effects, may counteract the changes in DA synthesis.

That BOL has a presynaptic blocking effect is evident from the finding that the LSD effect in the GBL model was effectively blocked by BOL as well as by haloperidol. It is likely that the BOL effect on DOPA

synthesis is predominantly due to its presynaptic effect.

A surprising finding was that promethazine was as effective as BOL in inhibiting the LSD effect on GBL-treated rats. The dose used (10 mg/kg) has never been shown to have any central DA-receptor antagonistic effects. Since neither promethazine nor BOL antagonized the apomorphine effect after GBL, it seems that there are presynaptic receptors sensitive to LSD but not to apomorphine and with an affinity to BOL and promethazine.

Assuming that the BOL effect on the DOPA synthesis in normal rats, i.e. rats not treated with GBL, was at least partly mediated by inhibition of the effect of DA on presynaptic receptors, our results could be explained if LSD were acting on two different types of receptors. One of these would be the DA receptor on which LSD behaves as a partial agonist and which is blocked by BOL. The other postulated receptor would be blocked by promethazine (and probably also by BOL). This receptor does not seem to be activated under normal conditions since promethazine does not have any effects on DOPA synthesis in normal rats. Promethazine, like BOL, has been suggested to have antiserotonergic properties (Garattini and Valzelli, 1965); both substances can displace LSD from specific binding sites in various parts of the brain including striatum (Bennett and Aghajanian, 1974; Bennett and Snyder, 1975). Since biochemical and histochemical studies have indicated the existence of serotonergic pathways innervating the striatum (Fuxe, 1965; Andén et al., 1965; 1966), the postulated LSD-sensitive receptor, which is blocked by promethazine, may thus be a 5-HT receptor.

A reasonably safe conclusion to be drawn from the present work is that both BOL and LSD have presynaptic effects in the nigroneostriatal pathway. From functional studies by other workers (Pieri et al., 1974; Kelly and Iversen, 1975) it appears that LSD also has a postsynaptic DA-receptor-stimulating action. It is however doubtful whether these effects appear under normal conditions, at least we were not able to demonstrate the decreased DOPA accumulation expected after LSD in intact rats if it stimulated postsynaptic and/or presynaptic DA receptors. The reason for this could be that so many of the DA receptors are normally occupied by the pure agonist DA that the same degree of receptor stimulation cannot be achieved with the partial agonist LSD even with complete receptor occupation. Moreover, the agonistic and antagonistic potencies of LSD at postsynaptic and presynaptic DA receptors may be different. The possibility cannot be excluded, however, that in species other than the rat, normal postsynaptic DA-receptor activation is so low that administration of LSD can lead to a further activation. This may explain that certain psychotic symptoms after LSD can disappear at least partly after treatment with haloperidol and other DA-receptor-blocking neuroleptics (Isbell and Logan, 1957).

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References

Aghajanian, G.K., W.E. Foote and M.H. Sheard, 1968, Lysergic acid diethylamide: sensitive neuronal units in the midbrain raphe, Science 161, 706.

Andén, N.-E., A. Dahlström, K. Fuxe and K. Larsson, 1965, Mapping out of catecholamine and 5-hydroxytryptamine neurons innervating the telencephalon and diencephalon, Life Sci. 4, 1275.

Andén, N.-E., A. Dahlström, K. Fuxe, K. Larsson, L. Olsson and U. Ungerstedt, 1966, Ascending monoamine neurons to the telencephalon and diencephalon, Acta Physiol. Scand. 67, 313.

Andén, N.-E., H. Corrodi, K. Fuxe and T. Hökfelt, 1968, Evidence for a central 5-hydroxytryptamine receptor stimulation by lysergic acid diethylamide, Brit. J. Pharmacol. 34, 1.

Bédard, P., A. Carlsson and M. Lindqvist, 1972, Effect of a transverse cerebral hemisection on 5-hydroxytryptamine metabolism in the rat brain, Naunyn-Schmiedeb. Arch. Pharmacol. 272, 1.

Bennett, J.L. and G.K. Aghajanian, 1974, D-LSD binding to brain homogenates: possible relationship to serotonin receptors, Life Sci. 15, 1935.

Bennett, J.P., Jr. and S.H. Snyder, 1975, Stereospecific binding of D-lysergic acid diethylamide (LSD) to brain membranes: relationship to serotonin receptors, Brain Res. 94, 523.

Carlsson, A., 1975, Receptor-mediated control of dopamine metabolism, in: Pre- and Postsynaptic Receptors, eds. E. Usdin and W.E. Bunney, Jr. (Marcel Dekker Inc., New York) p. 49.

Carlsson, A., J.N. Davis, W. Kehr, M. Lindqvist and C.V. Atack, 1972, Simultaneous measurement of tyrosine and tryptophan hydroxylase activities in brain in vivo using an inhibitor of the aromatic amino acid decarboxylase, Naunyn-Schmiedeb. Arch. Pharmacol. 275, 153.

Creese, I., D.R. Burt and S.H. Snyder, 1975, The dopamine receptor: Differential binding of d-LSD and related agents to agonist and antagonist states, Life Sci. 17, 1715.

Da Prada, M., A. Saner, W.P. Burkard, G. Bartholini and A. Pletscher, 1975, Lysergic acid diethylamide: evidence for stimulation of cerebral dopamine receptors, Brain Res. 94, 67.

Diaz, P.M., S.H. Ngai and E. Costa, 1968, Factors modulating brain serotonin turnover, in: Advances in Pharmacology, Vol. 6, part B, eds. S. Garattini and P.A. Shore (Academic Press, New York) p. 75.

Freedman, D.X., 1961, Effects of LSD-25 on brain serotonin, J. Pharm. Exptl. Therap. 134, 160.

Freedman, D.X., 1963, Psychotomimetic drugs and brain biogenic amines, Amer. J. Psychiat. 119, 843.

Fuxe, K., 1965, The distribution of monoamine terminals in the central nervous system, in: Evidence for the existence of monoamine neurons in the central nervous system, Acta Physiol. Scand. 64, Suppl. 247, 37.

Garattini, S. and L. Valzelli, 1965, Serotonin (Elsevier Publ. Comp., Amsterdam) p. 88.

Grabowska, M., L. Antkiewicz and J. Michaluk, 1974, The influence of LSD on locomotor activity in reserpinized mice, Pol. J. Pharmacol. Pharm. 26, 499.

Hollunger, G. and S.-Å. Persson, 1975, The effect of LSD and 2-bromo LSD on the DOPA accumulation after central and peripheral decarboxylase inhibition, European J. Pharmacol. 31, 156.

Häggendal, J., 1962, On the use of strong exchange resins for determinations of small amounts of catecholamines, Scand. J. Clin. Lab. Invest. 14, 537.

Isbell, H. and C.R. Logan, 1957, Studies on the diethylamide of lysergic acid (LSD-25), Arch. Neurol. Psychiat. 77, 350.

Kehr, W., A. Carlsson and M. Lindqvist, 1972a, A method for the determination of 3,4-dihydroxyphenylalanine (DOPA) in brain, Naunyn-Schmiedeb. Arch. Pharmacol. 274, 273.

Kehr, W., A. Carlsson, M. Lindqvist, T. Magnusson and C. Atack, 1972b, Evidence for a receptor-mediated feedback control of striatal tyrosine hydroxylase activity, J. Pharm. Pharmacol. 24, 744.

Kehr, W., A. Carlsson and M. Lindqvist, 1975, Biochemical aspects of dopamine agonists, in: Advances in Neurology, Vol. 9, eds. D. Calne, T.N. Chase and A. Barbeau (Raven Press, New York) p. 185.

Kelly, P.H. and L.L. Iversen, 1975, LSD as an agonist at mesolimbic dopamine receptors, Psychopharmacologia 45, 221.

Persson, T., 1970, Drug induced changes in ^{3}H-catecholamine accumulation after ^{3}H-tyrosine, Acta Pharmacol. Toxicol. 28, 378.

Pieri, L., M. Pieri and W. Haefely, 1974, LSD as an agonist of dopamine receptors in the striatum, Nature 252, 586.

Rosecrans, J.A., R.A. Lovell and D.X. Freedman, 1967, Effects of lysergic acid diethylamide on the metabolism of brain 5-hydroxytryptamine, Biochem. Pharmacol. 16, 2011.

Roth, R.H., J.R. Walters and G.K. Aghajanian, 1973, Effect of impulse flow on the release and synthesis of dopamine in the rat striatum, in: Frontiers in Catecholamine Research, eds. E. Usdin and S. Snyder (Pergamon Press, New York) p. 567.

Roth, R.H., J.R. Walters, L.C. Murrin and V.H. Morgenroth, III, 1975, Dopamine neurons: role of impulse flow and presynaptic receptors in the regulation of tyrosine hydroxylase, in: Pre- and Postsynaptic Receptors, eds. E. Usdin and W.E. Bunney (Marcel Dekker Inc., New York) p. 5.

Smith, R.C., W.O. Boggan and D.X. Freedman, 1975, Effects of single and multiple dose LSD on endogenous levels of brain tyrosine and catecholamines, Psychopharmacologia 42, 271.

Spano, P.F., K. Kumakura, G.C. Tonon, S. Govoni and M. Trabucchi, 1975, LSD and dopamine-sensitive adenylate cyclase in various rat brain areas, Brain Res. 93, 164.

Stolk, J.M., J.D. Barchas, M. Goldstein, W. Boggan and D.X. Freedman, 1974, A comparison of psychotomimetic drug effects on rat brain norepinephrine metabolism, J. Pharmacol. Exptl. Therap. 189, 42.

Sugrue, M.F., 1969, A study of the role of noradrenaline in behavioural changes produced in the rat by psychotomimetic drugs, Brit. J. Pharmacol. 35, 243.

Ungerstedt, U. and G.W. Arbuthnott, 1970, Quantitative recording of rotational behavior in rats after 6-hydroxy-dopamine lesions of the nigrostriatal dopamine system, Brain Res. 24, 485.

Von Hungen, K., S. Roberts and D.F. Hill, 1974, LSD as an agonist and antagonist at central dopamine receptors, Nature 252, 588.

Von Hungen, K., S. Roberts and D.F. Hill, 1975, Interactions between lysergic acid diethylamide and dopamine-sensitive adenylate cyclase systems in rat brain, Brain Res. 94, 57.

Walters, J.R. and R.H. Roth, 1974, Dopaminergic neurons: drug-induced antagonism of the increase in tyrosine hydroxylase activity produced by cessation of impulse flow, J. Pharmacol. Exptl. Therap. 191, 82.

Walters, J.R. and R.H. Roth, 1976, Pre- and postsynaptic actions of antipsychotic drugs, in: Antipsychotic Drugs: Pharmacodynamics and Pharmacokinetics, eds. G. Sedvall, B. Uvnäs and Y. Zotterman (Pergamon Press, Oxford) p. 147.

Walters, J.R., B.S. Bunney and R.H. Roth, 1975, Piribedil and apomorphine: pre- and postsynaptic effects on dopamine synthesis and neuronal activity, in: Advances in Neurology, Vol. 9, eds. D. Calne, T.N. Chase and A. Barbeau (Raven Press, New York) p. 273.