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EFFECTS OF LSD AND 2-BROMO LSD ON STRIATAL DOPAC LEVELS

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Summary

Administration of d-lysergic acid diethylamide (LSD) and its analogue 2-bromo lysergic acid diethylamide (BOL) resulted in a shortlasting increase of 3,4-dihydroxyphenylacetic acid (DOPAC) levels in the rat striatum. BOL was more potent than LSD in the dose range 0.5-4.0 mg/kg. Since there was a concomitant increase in the striatal *in vivo* tyrosine hydroxylation as measured by DOPA accumulation after decarboxylase inhibition, our findings suggest that LSD and BOL increase the impulse flow in the nigro-neostriatal pathway probably by central dopamine (DA) receptor antagonism. However, 4 hrs after LSD the DOPAC level was decreased, while the DOPA accumulation was not. Thus the effect of LSD on the dopaminergic system appears not to be limited to a pure receptor antagonism. The possibility also exists that the effect of LSD on the nigro-neostriatal DA pathway is secondary to its effect on the central 5-hydroxytryptaminergic system.

The mechanisms of action of the psychotomimetic agent d-lysergic acid diethylamide (LSD) are still not known. This especially applies to the effects of LSD on the central catecholaminergic (CA) system. Recently, however, it was demonstrated that LSD could affect dopaminergic receptor function. Thus in animal models, where the dopaminergic pathways have degenerated after 6-hydroxydopamine (6-OHDA) treatment, LSD, like dopamine (DA), has an agonist function on postsynaptic DA receptors (1, 2). Furthermore, LSD has been demonstrated to increase the production of cyclic AMP in homogenates from DA-rich parts of the rat brain (3, 4, 5, 6). These results suggest that LSD is a central DA agonist. The increase in the adenylate cyclase activity could be inhibited by DA receptor blocking agents such as haloperidol (4, 5). However, when DA was added to the homogenates (3, 4, 5), or when LSD was administered *in vivo* (6), LSD was found to inhibit the DA-mediated increase in the production of cyclic AMP. The LSD-analogue 2-bromo-LSD (BOL) did not increase cyclic AMP production *in vitro*, but BOL like LSD inhibited the increased cyclic AMP production after DA (4, 5, 6). These findings most probably indicate that LSD has antagonistic as well as agonistic properties but BOL only antagonistic properties at central DA receptors. It has furthermore been shown that LSD

could bind to both agonist and antagonist receptor sites in a membrane model. BOL, however, could bind only to antagonist receptor sites (7).

In the rat LSD has been reported to decrease the homovanillic acid (HVA) content in the striatum and to delay the α-methyl-p-tyrosine-induced disappearance of DA in the teldiencephalon, suggesting a decreased DA-turnover in the central nervous system (3). On the other hand, we have previously found an increased *in vivo* tyrosine hydroxylation in rat brain after LSD and BOL administration as measured by DOPA accumulation after central decarboxylase inhibition (8). Administration of LSD has also been demonstrated to increase the tyrosine and DA levels in the rat brain, suggesting an increased DA-synthesis (9).

In order to get some further knowledge of the effects of LSD and BOL on the central dopaminergic system we now present data on the striatal DOPAC levels after treatment with LSD and BOL. Short-term changes in brain levels of DOPAC appear to provide a useful index of alterations in the functional activity of central dopaminergic neurons (10). For comparison we also include data on some other drugs known to affect the function of the DA pathways, as well as data on the striatal DOPA accumulation after LSD and BOL.

Materials and Methods

Male Sprague-Dawley rats (Anticimex, Sollentuna, Sweden) weighing 190-293 g were used in all experiments. The rats were fed on Ewos-Anticimex commercial type pelleted diet, R3 and had access to water ad libitum except during the experiments, when only water was available. A group of six rats was kept in each cage. The experiments were performed between 9 a.m. and 1 p.m. at a room temperature of 21-25° C.

The following drugs were used: apomorphine HCl (Sandoz, Basle), D-amphetamine SO_4 (Siegfried, Zofingue, Switzerland), 2-bromo lysergic acid diethylamide bitartrate, BOL (Sandoz, Basle), haloperidol (Leo, Helsingborg), clorgyline HCl (May & Baker, Essex), benztropine mesylate (Merck, Sharp and Dohme, Rahway, N.J.), lysergic acid diethylamide tartrate, LSD (Sandoz, Basle), reserpine (Ciba-Geigy, Basle). 3-hydroxybenzylhydrazine HCl, NSD 1015 was kindly synthezised by Dr. B. Magnusson, Department of Organic Chemistry, University of Umeå, Sweden. Doses of amphetamine, 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 reagents used were of reagent grade quality. The solutions were made up just before injection. All drugs were administered i.p.

The animals were killed by a guillotine. The brain was rapidly removed and the striata were dissected on ice, weighed and homogenized in cold 0.1 M HCl. The extract was frozen (-80° C) overnight. DOPAC was then extracted and assayed spectrofotofluorimetrically (11, 12). Striatal tissue from two rats was pooled. Cerebellar tissue of about the same weight as the pooled striata was used for determination of recovery and tissue blank in each experiment. The recovery of 100 - 250 ng added to tissue blanks was 63.5 ± 1.1 % (mean ± S.E.M., n = 33). In some rats striatal

DOPA-accumulation after central decarboxylase inhibition was measured. This technique appears to be a good index of the *in vivo* tyrosine hydroxylation (13). DOPA was isolated and assayed spectrophotofluorimetrically (14) with some modification (15). The recovery of 500 ng DOPA taken through the entire procedure was 72.1 ± 0.8 % (mean ± S.E.M., n = 93). All values were corrected for the recovery in each experiment.

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

Results

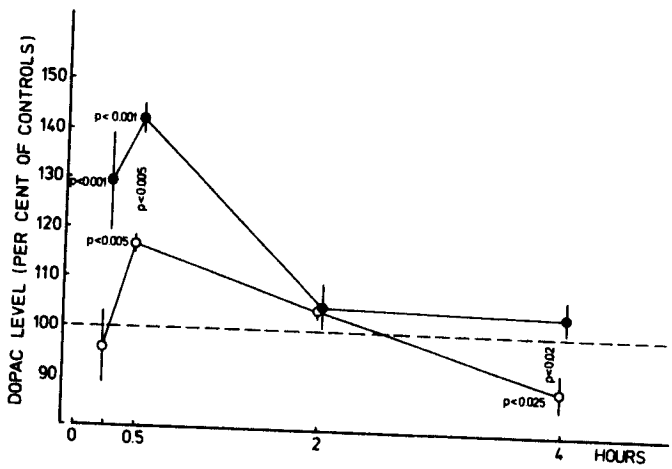

Fig. 1

DOPAC levels at various times after the administration of LSD (○) or BOL (●) (0.5 mg/kg i.p.). Each point represents the mean ± S.E.M. of 3-6 determinations. The control value is 100 ± 2 % (mean ± S.E.M., n = 12). The horizontal p-values indicate significant differences between DOPAC levels after LSD or BOL and the control DOPAC level. The vertical p-values indicate significant differences between DOPAC levels after LSD and after BOL.

LSD as well as BOL increased the striatal DOPAC levels (Fig. 1). Two hours after the administration of the drugs the DOPAC concentrations were found to be normalized. However, the DOPAC level was significantly decreased 4 hrs after the administration of LSD.

Figure 2 shows that the increased DOPAC levels after LSD and BOL were dose-dependent with a minimal effective dose of 0.05 mg/kg. Furthermore LSD was found to be less potent than BOL in increasing

the DOPAC concentration.

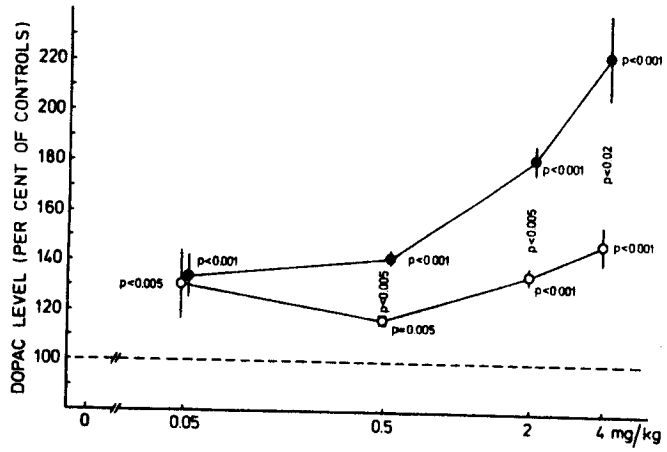

Fig. 2

DOPAC levels (0.5 hr) after various doses of LSD (○) or BOL (●). Each point represents the mean ± S.E.M. of 3-6 determinations. The control value is 100 ± 2 % (mean ± S.E.M., n = 12). The horizontal p-values indicate significant differences between DOPAC levels after LSD or BOL and the control DOPAC level. The vertical p-values indicate significant differences between DOPAC levels after LSD and after BOL.

In agreement with other workers (10), we found a decreased DOPAC concentration in the striatum after the central (DA) agonist apomorphine and after amphetamine (Table 1). The MAO-inhibitor clorgyline and benztropine, an inhibitor of DA uptake (16) also decreased the DOPAC concentration. The DA antagonist haloperidol increased the DOPAC level in striatum, and so did reserpine, which depletes the granular DA stores.

TABLE 1

Treatment	Dose mg/kg	n	DOPAC ng/g ± S.E.M.	Differ from controls p-values
NaCl		12	1107 ± 27	-
LSD-25	0.5	3	1294 ± 18	< 0.02
BOL-148	0.5	3	1574 ± 33	< 0.001
Apomorphine	2.5	4	788 ± 75	< 0.001
Amphetamine	5.0	4	439 ± 11	< 0.001
Haloperidol	0.5	4	3519 ± 247	< 0.001
Reserpine	5.0	3	1529 ± 231	< 0.005
Clorgyline	5.0	3	531 ± 157	< 0.001
Benztropine	25	3	958 ± 46	< 0.05

TABLE 2

Time after administration of LSD and BOL		Drug	Dose (mg/kg)	n	DOPA accumulation ng/g ± S.E.M.	Comparisons between the various treatments (p-values)
0.5 h	a	NaCl 0.9 %		8	1042 ± 77	-
	b	LSD-25	0.5	6	1823 ± 113	a-b < 0.001 b-c N.S.
	c	BOL-148	0.5	9	1924 ± 131	a-c < 0.001
	d	LSD-25	2.0	6	1726 ± 163	a-d < 0.005 d-e < 0.005
	e	BOL-148	2.0	6	2630 ± 169	a-e < 0.001
2.0 h	f	LSD-25	0.5	6	816 ± 45	a-f < 0.05 f-g < 0.01
	g	BOL-148	0.5	6	1045 ± 54	a-g N.S.
	h	LSD-25	2.0	4	957 ± 78	a-h N.S. h-i N.S.
	i	BOL-148	2.0	3	1276 ± 119	a-i N.S.
4.0 h	j	LSD-25	0.5	6	906 ± 46	a-j N.S. j-k N.S.
	k	BOL-148	0.5	6	1003 ± 65	a-k N.S.
	l	LSD-25	2.0	3	1107 ± 45	a-l N.S. l-m N.S.
	m	BOL-148	2.0	3	895 ± 90	a-m N.S.

DOPA accumulation at various times after the administration of LSD or BOL (0.5 and 2.0 mg/kg). NSD 1015 was administered 20 min before sacrifice.

DOPAC levels 30 min after administration of different drugs known to affect the central dopaminergic system.

DOPA accumulation was increased 0.5 hr after both LSD and BOL (0.5 and 2.0 mg/kg) (Table 2). BOL was more potent than LSD at a dose level higher than 0.5 mg/kg. No increased DOPA accumulation was observed 2-4 hrs after administration of LSD or BOL. Two hrs after LSD (0.4 mg/kg) the DOPA accumulation was significantly decreased as compared with controls. However, the significance was on the 5 % level and furthermore no decreased DOPA accumulation was seen 4 hrs after LSD.

Discussion

It is generally agreed that DA agonists decrease, while DA antagonists increase, DA turnover. Taking the rate of DOPA accumulation after central decarboxylase inhibition as an index of DA turnover we concluded in a previous work (15) that there was no indication of a DA receptor stimulating effect of LSD in intact rats. The discrepancy between this biochemical finding and the results of functional studies (1, 2) may be accounted for by LSD in those experiments acting on supersensitive DA receptors and/or by LSD being only a partial DA agonist. Our findings that the DOPAC levels in the striatum were increased after LSD and BOL are in full agreement with our previous findings of an increased DOPA accumulation after LSD and BOL (15). However, a decrease of the striatal HVA concentration has been reported 2-8 hrs after the administration of LSD. In addition the α-methyl-p-tyrosine-induced disappearance of DA in the teldiencephalon was delayed 2 and 4 hrs after the administration of LSD (3). These findings suggest a decreased DA-turnover in the central nervous system and were taken as evidence for a central DA receptor stimulating effect of LSD. Our finding that the DOPAC concentration was decreased 4 hrs after administration of LSD also suggests a decreased DA-turnover at this time. However, the DOPA-accumulation was not decreased. In addition the brain level of LSD at that time must be very low (17). Although it seems possible that the decreased HVA and DOPAC levels and the delayed disappearance of DA after α-methyl-p-tyrosine indicate a decreased DA-turnover, these late effects probably do not represent a direct effect of LSD but perhaps only reveal a late compensatory mechanism.

The increased DOPAC-levels after LSD and BOL observed in the present study suggest an increased impulse flow in the nigro-neostriatal pathway secondary to a postsynaptic DA-receptor blockade (18). However, the increased DOPAC levels may at least partly be a result of an increased DA-synthesis not reflecting an increased neuronal activity, but only an increased DA-synthesis due to an antagonistic effect of LSD and BOL on the postulated presynaptic DA-receptors. Evidence for such an antagonistic effect of LSD and BOL has previously been presented (15).

There is, however, an alternative explanation to the increased DOPAC levels in the striatum. LSD has been shown to act on 5-HT-containing neurons and in high doses on 5-hydroxytryptaminergic synapses (19). We have proposed that LSD and BOL may affect the DA-synthesis also via 5-HT-receptors (15). Furthermore evidence has been presented for a direct inhibitory, probably 5-hydroxytryptaminergic, input from the median raphe nucleus to the substantia nigra (20). LSD may thus turn off the raphe neurons which

inhibit the neurons in the substantia nigra and thereby produce an increased impulse flow in the nigro-neostriatal pathway.

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