3109

Psychopharmacology 59, 113–116 (1978)

Psychopharmacology

al.

Effects of LSD and BOL on the Catecholamine Synthesis and Turnover in Various Brain Regions

Sven-Åke Persson

Department of Pharmacology, University of Umeå, S-901 87 Umeå, Sweden

Abstract. In the rat, lysergic acid diethylamide (LSD) 0.5 mg/kg and 2-bromo lysergic acid diethylamide (BOL) 0.5 mg/kg increased the rate of the striatal in vivo tyrosine hydroxylation as measured by the DOPA accumulation after decarboxylase inhibition. Neither LSD nor BOL significantly changed the DOPA accumulation in the olfactory tubercle, a dopamine-rich part of the limbic system. LSD but not BOL increased the DOPA accumulation in the cerebral cortex and in the brain stem. LSD and BOL appeared not to alter the rate of α-MT-induced disappearance of DA or of NA in the whole brain, nor did they change the rate of the α-MT-induced disappearance of DA in the striatum. It is suggested that in the striatum LSD and BOL block autoreceptors (presynaptic receptors) regulating the tyrosine hydroxylation. These receptors may be DA receptors, but may also be 5-HT- or LSD-sensitive receptors.

The regional differences observed between LSD and BOL suggest that LSD in the cerebral cortex and in the brain stem increases the DOPA accumulation by mechanism other than that functioning in the striatum. One possible explanation is that LSD and BOL may differ in their effects on 5-hydroxytryptaminergic systems in the cerebral cortex and in the brain stem.

Key words: LSD – BOL – DOPA – Tyrosine hydroxylation – Noradrenaline – Dopamine – Antagonist – Autoreceptors

In some early investigations into the effect of the psychotomimetic agent d-lysergic acid diethylamide (LSD-25, LSD), the drug markedly activated the sympathetic nervous system in animals (Rothlin, 1957). Later, LSD was found to decrease cerebral noradrenaline (NA) levels (Freedman, 1963; Sugrue, 1969) and cerebral dopamine (DA) levels (Diaz et al., 1968). However, increased cerebral DA levels after LSD have also been reported (Smith et al., (1975). Relatively few studies on the cerebral catecholamine turnover have been reported. High doses of LSD increased the disappearance of NA in the rat brain after treatment with α-methyl-p-tyrosine methylester (α-MT) (Andén et al., 1968). LSD did not affect the disappearance of DA. Da Prada et al. (1975) found that LSD decreased the disappearance of DA in the rat teldiencephalon after α-MT and that LSD also decreased the homovanillic acid (HVA) level in the striatum and in the eyes, suggesting a decreased DA turnover after LSD.

We reported an increased rate of the in vivo tyrosine hydroxylation as measured by the DOPA accumulation after decarboxylase inhibition in the rat brain after LSD as well as after its 2-bromo analogue, 2-bromo lysergic acid diethylamide (BOL 148, BOL) (Hollunger and Persson, 1975). LSD and BOL were also found to increase the in vivo tyrosine hydroxylation in the DA-rich striatum (Persson, 1977a), suggesting an increased DA synthesis or turnover. Our findings were further supported by the observation that both LSD and BOL increased the striatal DOPAC levels (Persson, 1977b).

The present study provides more information on the effects of LSD and BOL on the DOPA accumulation in various brain regions. The influence of LSD and BOL on the rate of α-MT-induced disappearance of DA and NA in the whole brain and on the rate of α-MT-induced disappearance of DA in the striatum was investigated.

Materials and Methods

Animals. Male Sprague-Dawley rats (Anticimex, Sollentuna, Sweden) weighing 198–294 g were used in all experiments. They were fed the Ewos-Anticimex commercial-type pelleted diet, R3, and had free access to food and water. During the experiments the rats had free access only to water.

0033-3158/78/0059/0113/$01.00

Table 1. DOPA accumulation in various parts of the brain

Treatment	DOPA ng/g ± SEM (*N*)					
	Eye	Cortex	Brain stem	Hypo-thalamus	Striatum	Olfactory tubercle
NaCl	9 ± 4 (4)	68 ± 6 (6)	144 ± 14 (6)	228 ± 30 (6)	1169 ± 49 (6)	1101 ± 80 (5)
LSD	13 ± 4 (3)	88 ± 6 (5)	218 ± 18 (4)	358 ± 57 (4)	1688 ± 75 (5)	1076 ± 183 (5)
BOL	12 ± 2 (4)	78 ± 11(4)	124 ± 11 (4)	189 ± 21 (4)	2323 ± 232 (4)	1336 ± 52
Comparisons between the various treatments	*P*-values					
NaCl vs. LSD	NS	$P < 0.05$	$P < 0.02$	NS	$P < 0.001$	NS
NaCl vs. BOL	NS	NS	NS	NS	$P < 0.001$	NS
LSD vs. BOL	NS	NS	$P < 0.005$	$P < 0.05$	$P < 0.025$	NS

NaCl (0.9%) LSD (0.5 mg/kg) or BOL (0.5 mg/kg) were administered 30 min before sacrifice. The decarboxylase inhibitor NSD 1015 (100 mg/kg) was administered to all animals 20 min before sacrifice

Drugs. The following drugs were used: 2-bromo lysergic acid diethylamide bitartrate, BOL (Sandoz AG, Basle), *d*-lysergic acid diethylamide tartrate (LSD, Sandoz AG, Basle), D,L-α-methyl-p-tyrosine methyl-ester HCL, α-MT, H44/68 (Astra AB, Södertälje). 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. All drugs were dissolved in 0.9% saline just before injection. All drug administrations were i. p.

Experimental. The experiments were performed between 9 a.m. and 12:30 p.m. at a room temperature of 21 – 26° C. DOPA accumulation after central decarboxylase inhibition was used as an index of the in vivo tyrosine hydroxylation (Carlsson et al., 1972). The rats were killed with a guillotine. The brains were rapidly removed and dissected on ice. The dissection was performed mainly according to Glowinski and Iversen (1966). In the experiments in which DOPA was measured, brain parts from three rats were pooled. Striata from two rats were pooled for the determination of DA. The whole brain derterminations of DA and NA were performed on a single brain. The brains or brain parts were weighed and either analyzed immediately or frozen (– 70° C) for not more than 3 days before the chemical analysis.

DOPA was then isolated and measured fluorimetrically by means of the technique of Kehr et al. (1972) with some modifications (Persson, 1977a). In order to get rid of disturbing fluorescent material when isolating DOPA from extract of whole brains or pooled brain parts, the extract was adsorbed on acid-washed Al_2O_3 (Rentzhog, 1972). After homogenization of the tissue in 0.4-M $HClO_4$ and centrifugation at 14,000 g, 0° C, for 10 min, the pH of the extract was adjusted with K_2CO_3 to about 4. After another centrifugation to spin down perchlorate, the pH was adjusted to 8.6 and catechols adsorbed on 400 mg Al_2O_3 prewashed with 0.1-M acetate buffer pH 8.6. The adsorption procedure was performed in small bottles under continuous shaking for 5 min. After washings with glass-distilled water, DOPA was eluted with 2 + 1 ml 0.1-M H_3PO_4. The eluate was then put on a small column containing Amberlite CG 120 (Na^+) and DOPA was isolated as described by Persson (1977a). The recovery of 500 ng DOPA taken through the entire procedure was 62.0 ± 1.2% (mean ± SEM, $N = 17$).

Dopamine was isolated and determined spectrophotofluorimetrically according to Atack and Magnusson (1970) and Atack (1973).

Noradrenaline was isolated according to Atack and Magnusson (1970) and determined spectrophotofluorimetrically (Kehr et al., 1976).

In the experiments in which DA and NA were measured in the whole brain, the recovery of 500 ng taken through the entire procedure was for DA 76.6 ± 7% (mean ± SEM, $N = 11$ and NA 63.1 ± 1.9% (mean ± SEM, $N = 19$).

Since the DA recovery in the experiments in which DA was measured in the striatum was about 90%, no correction for recovery was used in these experiments. All other values are corrected for recovery.

Statistical Treatment. Student's *t*-test was used to assess the significance of the results.

Results

In agreement with earlier findings (Persson, 1977a), LSD (0.5 mg/kg) and BOL (0.5 mg/kg) increased the DOPA accumulation in the rat striatum (Table 1). However, LSD and BOL did not significantly change the DOPA accumulation in the olfactory tubercle, a DA-rich part of the limbic system. In the NA-rich cerebral cortex LSD but not BOL significantly increased the accumulation in the brain stem. The DOPA accumulation in the hypothalamus and in the eyes was not significantly changed after the administration of LSD or BOL.

LSD (0.5 mg/kg) and BOL (0.5 mg/kg) did not significantly change the disappearance of DA or NA from the whole brain 1 and 2 h after the administration of α-MT (Table 2).

In the DA-rich striatum neither LSD nor BOL significantly changed the α-MT-induced disappearance of DA (Table 3). Unexpectedly, BOL (0.5 mg/kg) raised the striatal DA levels.

Discussion

The present study confirms our earlier observations that BOL, and to a lesser extent LSD, increase the rate

Table 2. Effects of LSD and BOL on the α-MT-induced disappearance of dopamine and noradrenaline in the whole brain

Treatment	Dopamine ng/g ± SEM (*N*)	Noradrenaline ng/g ± SEM (*N*)
a) No treatment	853 ± 90 (8)	360 ± 34 (5)
b) LSD + NaCl	968 ± 21 (3)	443 ± 37 (3)
c) BOL + NaCl	998 ± 13 (2)	429 ± 23 (3)
d) NaCl + α-MT 1 h	620 ± 22 (6)	302 ± 20 (4)
e) LSD + α-MT 1 h	672 ± 39 (6)	246 ± 14 (4)
f) BOL + α-MT 1 h	652 ± 31 (6)	327 ± 13 (5)
g) NaCl + α-MT 2 h	347 ± 35 (8)	210 ± 7 (4)
h) LSD + α-MT 2 h	375 ± 39 (8)	159 ± 47 (2)
i) BOL + α-MT 2 h	339 ± 33 (8)	208 ± 27 (3)
Comparisons between the various treatments	*P* values	
a − b; a − c; b − c	NS, NS, NS	NS, NS, NS
a − d; a − g	$P<0.05$, $P<0.001$	NS, $P<0.01$
d − e; d − f; e − f	NS, NS, NS	NS, NS, $P<0.005$
g − h; g − i; h − i	NS, NS, NS	NS, NS, NS

D,L-α-methyltyrosine methylester (α-MT, 250 mg/kg) was administered 1 or 2 h before sacrifice. NaCl (0.9%), LSD (0.5 mg/kg) or BOL (0.5 mg/kg) were administered 15 min before α-MT, i.e., 1 h 15 min or 2 h 15 min before sacrifice

Table 3. Effects of LSD and BOL on the α-MT-induced disappearance of dopamine in the striatum

Treatment	Dopamine ng/g ± SEM (*N*)
a) No treatment	7179 ± 121 (5)
b) NaCl + NaCl	6928 ± 294 (5)
c) LSD + NaCl	6615 ± 421 (3)
d) BOL + NaCl	8204 ± 189 (3)
e) NaCl + α-MT	4878 ± 130 (3)
f) LSD + α-MT	4508 ± 59 (2)
g) BOL + α-MT	4413 ± 187 (3)
Comparisons between the various treatments	
a − b	*P* values
b − c; b − d; c − d	NS
a − e; b − e	NS, $P<0.025$, $P<0.05$
e − f; e − g; f − g	$P<0.001$, $P<0.005$
	NS, NS, NS

D,L-α-methyltyrosine methylester (α-MT, 250 mg/kg) was administered 1 h before sacrifice. NaCl (0.9%), LSD (0.5 mg/kg), or BOL (0.5 mg/kg) were administered 15 min before α-MT, i.e., 1 h 15 min before sacrifice

of the in vivo tyrosine hydroxylation in the striatum as measured by the DOPA accumulation after inhibition of the L-aromatic amino acid decarboxylase (Persson, 1977a). The main new result of the present study is that the effects of BOL and LSD in several brain regions investigated differed from their effects in the striatum. BOL thus seemed to have only small, if any, effects on the DOPA accumulation in cerebral cortex, brain stem, hypothalamus, and olfactory tubercle. In contrast, LSD significantly increased the DOPA accumulation in cerebral cortex and in the brain stem. LSD also tended to increase the DOPA accumulation in the hypothalamus. Remarkably, LSD appeared not to have any effect in the olfactory tubercle. The stimulating effect of BOL and LSD on the in vivo tyrosine hydroxylation in the striatum observed in our earlier report (Persson, 1977a) was thought to be due to a blockade of DA receptors regulating the tyrosine hydroxylation. At these receptors BOL probably is a pure antagonist and LSD a partial agonist. We also found evidence suggesting a 5-hydroxytryptaminergic control of the tyrosine hydroxylation in the nigro-neostriatal pathway.

From the present work it is apparent that such a control mechanism seems not to exist in that part of the mesolimbic system, which ends in the olfactory tubercle. LSD and BOL did not significantly change the DOPA accumulation in the eyes. However, the DOPA levels in the eyes are at the lower limit of the sensitivity of the present method of determination. The finding that LSD but not BOL increased the DOPA accumulation in the cerebral cortex and in the brain stem is not easily explained within the framework of the above hypothesis. Maybe the dominating effect of LSD on the DOPA generation in these parts of the brain is secondary to an effect of LSD on central 5-hydroxytryptaminergic pathways.

It is well known that blockade of postsynaptic receptors on target cells of DA-neurons increases the DA turnover (Cheramy et al., 1970; Andén et al., 1972). In the present work there were no indications that BOL and LSD increased DA turnover in the striatum or in the whole brain as measured by the rate of disappearance of DA after α-MT. These negative findings suggest that BOL and LSD exert their blocking activity predominantly at presynaptic receptors (autoreceptors) (Carlsson, 1975; Roth et al., 1975; Persson, 1977a). BOL increased the DA concentration in the striatum, a finding that may reflect an increased DA synthesis and/or turnover. Our finding that LSD did not change the DA turnover in the whole brain agrees with earlier observations in the rat (Andén et al., 1968) and the mouse (Menon et al., 1977). However, we were unable to observe an increased rate of disappearance of NA in the whole brain as observed by Andén et al. (1968).

Da Prada et al. (1975) reported that LSD decreased the α-MT-induced disappearance of DA in the teldiencephalon. They also reported that LSD decreased HVA in the eyes and in the striatum. In contrast, we found that LSD did not significantly change the α-MT-

induced disappearance of DA in the striatum. In addition we have in the present study and in earlier studies (Persson, 1977a, 1977b) demonstrated that LSD and BOL increased the DOPA accumulation in the striatum. Furthermore, we found that LSD and BOL increased the DOPAC levels in the striatum (Persson, 1977b).

The present study confirms our earlier findings that both LSD and BOL increase the in vivo tyrosine hydroxylation in the striatum (Persson, 1977a, 1977b). The finding that LSD but not BOL increased the DOPA accumulation in the cerebral cortex and in the brain stem suggests that LSD primarily affects 5-hydroxytryptaminergic pathways and may be explained by the observed differences between the drugs in their effects on central 5-HT neurons (Aghajanian et al., 1970; Aghajanian, 1976). The increased DOPA accumulation after LSD and BOL probably means that LSD and BOL block DA receptors. The unchanged DA turnover in the striatum suggests that LSD and BOL block autoreceptor (presynaptic receptors) (Carlsson, 1975; Roth et al., 1975; Persson, 1977a). These receptors may be DA receptors, but they may also be 5-HT- and/or LSD-sensitive receptors (Lovell and Freedman, 1976; Persson, 1977a). The present investigation gives no evidence for a DA-receptor-stimulating effect of LSD.

Acknowledgements. The author is greatly indebted to Miss Indra Svensson and Miss Inger Johansson for skilful technical assistance. The author thanks Astra AB, Södertälje, and Sandoz AG, Basle, for generous gifts of drugs.

References

Aghajanian, G. K., Foote, W. E., Sheard, M. H.: Action of psychotogenic drugs on single midbrain raphe neurons. J. Pharmacol. Exp. Ther. **171**, 178–187 (1970)

Aghajanian, G. K.: LSD and 2-bromo-LSD: comparison of effects on serotonergic neurones and on neurones in two serotonergic projection areas, the ventral lateral geniculate and amygdala. Neuropharmacology **15**, 521–528 (1976)

Andén, N.-E., Corrodi, H., Fuxe, K., Hökfelt, T.: Evidence for a central 5-hydroxytryptamine receptor stimulation by lysergic acid diethylamide. Br. J. Pharmacol **34**, 1–7 (1968)

Andén, N.-E., Corrodi, H., Fuxe, K.: Effect of neuroleptic drugs on central catecholamine turnover assessed using tyrosine and dopamine-β-hydroxylase inhibitors. J. Pharm. Pharmacol. **24**, 177–182 (1972)

Atack, C. V., Magnusson, T.: Individual elution of noradrenaline (together with adrenaline), dopamine, 5-hydroxytryptamine and histamine from a single strong cation exchange column, by means of mineral acid-organic solvent mixtures. J. Pharm. Pharmacol. **22**, 625–627 (1970)

Atack, C. V.: The determination of dopamine by a modification of the dihydroxyindole fluorimetric assay. Br. J. Pharmacol **48**, 699–714 (1973)

Carlsson, A., Davis, J. N., Kehr, W., Lindqvist, M., Atack, C. V.: Simultaneous measurement of tyrosine and tryptophan hydroxylase activities in brain *in vivo* using an inhibitor of the aromatic amino acid decarboxylase. Naunyn Schmiedebergs Arch. Pharmacol. **275**, 153–168 (1972)

Carlsson, A.: Receptor-mediated control of dopamine metabolism. In: Pre- and postsynaptic receptors, E. Usdin and W. E. Bunney, Jr. eds., pp. 49–65. New York: Marcel Dekker 1975

Cheramy, A., Besson, M. J., Glowinski, J.: Increased release of dopamine from striatal dopaminergic terminals in the rat after treatment with a neuroleptic: thioproperazine. Eur. J. Pharmacol. **10**, 206–214 (1970)

Da Prada, M., Saner, A., Burkard, W. P., Bartholini, G., Pletscher, A.: Lysergic acid diethylamide: evidence for stimulation of cerebral dopamine receptors. Brain Res. **94**, 67–73 (1975)

Diaz, P. M., Ngai, S. H., Costa, E.: Factors modulating brain serotonin turnover. In: Advances in pharmacology, vol. 6, part B, S. Garattini and P. A. Shore, eds., pp. 75–92. New York: Academic Press 1968

Freedman, D. X.: Psychotomimetic drugs and brain biogenic amines. Am. J. Psychiatry **119**, 843–850 (1963)

Glowinski, J., Iversen, L. L.: Regional studies of catecholamines in the rat brain. I. J. Neurochem. **13**, 655–669 (1966)

Hollunger, G., Persson, S. Å.: The effect of LSD and 2-bromo LSD on the DOPA accumulation after central and peripheral decarboxylase inhibition. Eur. J. Pharmacol. **31**, 156–158 (1975)

Kehr, W., Carlsson, A., Lindqvist, M.: A method for the determination of 3,4-dihydroxyphenylalanine (DOPA) in brain. Naunyn-Schmiedebergs Arch. Pharmacol. **274**, 273–280 (1972)

Kehr, W., Lindqvist, M., Carlsson, A.: Distribution of dopamine in the rat cerebral cortex. J. Neural Trans. **38**, 173–180 (1976)

Lovell, R. A., Freedman, D. X.: Stereospecific receptor sites for d-lysergic acid diethylamide in rat brain: effects of neurotransmitters, amine antagonists, and other psychotropic drugs. Mol. Pharmacol. **12**, 620–630 (1976)

Menon, M. K., Clark, W. G., Masuoka, D. T.: Possible involvement of the central dopaminergic system in the antireserpine effect of LSD. Psychopharmacology **52**, 291–297 (1977)

Persson, S. Å.: The effect of LSD and 2-bromo LSD on the striatal DOPA accumulation after decarboxylase inhibition in rats. Eur. J. Pharmacol. **43**, 73–83 (1977a)

Persson, S. Å.: Effects of LSD and 2-bromo LSD on striatal DOPAC levels. Life Sci. **20**, 1199–1206 (1977b)

Rentzhog, L.: Double isotope dilution derivative technique for measurement of catecholamines. Acta Physiol. Scand. [Suppl.] 377 (1972)

Roth, R. H., Walters, J. R., Murrin, L. C., Morgenroth, V. H., III: Dopamine neurons: role of impulse flow and presynaptic receptors in the regulation of tyrosine hydroxylase. In: Pre- and postsynaptic receptors, E. Usdin and W. E. Bunney, Jr., eds., pp. 5–48. New York: Marcel Dekker 1975

Rothlin, E.: Pharmacology of lysergic acid diethylamide and some of its related compounds. J. Pharm. Pharmacol. **9**, 569–587 (1957)

Smith, R. C., Boggan, W. O., Freedman, D. X.: Effects of single and multiple dose LSD on endogenous levels of brain tyrosine and catecholamines. Psychopharmacologia (Berl.) **42**, 271–276 (1975)

Sugrue, M. F.: A study of the role of noradrenaline in behavioural changes produced in the rat by psychotomimetic drugs. Br. J. Pharmacol. **35**, 243–252 (1969)

Received December 14, 1977