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THE EFFECT OF LYSERGIC ACID DIETHYLAMIDE (LSD) AND 2-BROMO-LYSERGIC ACID DIETHYLAMIDE (BOL) ON THE STRIATAL DOPA ACCUMULATION: INFLUENCE OF CENTRAL 5-HYDROXY-TRYPTAMINERGIC PATHWAYS

SVEN-ÅKE PERSSON and HÅKAN JOHANSSON

Departments of Pharmacology and Physiology, University of Umeå, S-901 87 Umeå (Sweden)

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SUMMARY

Selective chronic lesions of the dorsal raphe nucleus or combined lesions of the dorsal and median raphe nuclei did not significantly change the in vivo tyrosine hydroxylation in the striatum as measured by the DOPA accumulation after decarboxylase inhibition. Neither did acute combined lesions of the raphe nuclei, nor did electrical stimulation of the dorsal raphe nucleus have any significant effect. *p*-Chloroamphetamine (PCA, 20 mg/kg, i.p.) and *p*-chlorophenylalanine (PCPA, 400 mg/kg, i.p.), known inhibitors of the 5-hydroxytryptamine (5-HT) synthesis, significantly decreased the DOPA accumulation. The increase in DOPA accumulation observed after LSD (0.5 mg/kg, i.p.) or BOL (0.5 mg/kg, i.p.) was seemingly unaffected by pretreatment with PCA or PCPA and also after lesion of the dorsal raphe nucleus. The results suggest that the effect of LSD or BOL on the DOPA accumulation in the striatum is not mediated via a 5-hydroxytryptaminergic control mechanism originating in the dorsal raphe nucleus. A control mediated via the median raphe nucleus cannot be excluded, since LSD did not increase the DOPA accumulation after combined chronic raphe lesions. Such a control would also be in agreement with our previous results suggesting that the DOPA generation after LSD is controlled by 5-HT receptors.

INTRODUCTION

The psychotomimetic agent D-lysergic acid diethylamide (LSD) has been shown to have electrophysiological effects on the central 5-hydroxytryptamine (5-HT)-containing neurons[2,13] as well as electrophysiological and biochemical effects at 5-hydroxytryptaminergic synapses[3,10,13]. LSD has also been found to interact with

central dopamine (DA)[8] and noradrenaline (NA)[3,11,25] pathways. Data presented in recent biochemical[7,24,27,28] and behavioral[16,23] studies suggest that LSD stimulates central DA receptors. However, it has also been reported that LSD in vitro could act both as a DA antagonist[7,24,27,28] as well as a DA agonist. The 2-bromo derivative of LSD, 2-bromolysergic acid diethylamide (BOL), on the other hand, appears to have only antagonistic effect on DA receptors[27,28]. It has been shown in previous papers[14,22] that both LSD and BOL increased the in vivo tyrosine hydroxylation as measured by the accumulation of DOPA in the whole brain and in the striatum of the intact rat after central decarboxylase inhibition. BOL appeared to be more effective than LSD.

The aim of the present investigation was to find out whether LSD could influence the DOPA accumulation in the striatum via 5-hydroxytryptaminergic pathways[4,21] or whether this effect was a more direct effect, i.e. mediated via central DA receptors. Additional biochemical information is also provided concerning the interaction between central 5-HT pathways and the nigro-neostriatal DA pathway.

MATERIALS AND METHODS

Male Sprague–Dawley rats (Anticimex, Sollentuna, Sweden) weighing 208–298 g at beginning of the experiments were used. They were housed in groups of 8 animals in a makrolon cage measuring 550 × 330 × 200 mm. Food (Ewos-Anticimex commercial type pelleted diet, R3) and water were available ad libitum, except under the surgical and injection procedures. The experiments were performed at an ambient room temperature of 24 ± 1 °C. Most lesions were performed in diethylether anesthesia with atropine (0.2 mg/kg, i.p.) as premedication. The acute lesions and the stimulations were performed in pentobarbital (Nembutal, Abbott, 60 mg/kg, i.p.) anesthesia without premedication. Two of 78 operated animals died.

Dorsal raphe lesions

A craniotomy was performed over the midbrain raphe nuclei and a glass-insulated, platinum-iridium wire electrode (free tip 0.8 mm) was stereotaxically lowered to the depth of the dorsal raphe nucleus (coordinates, according to König and Klippel[17], were A 0.16, L 0, H −1.0). An electrolytical lesion was then made at this site by applying a 2–3 mA DC (anodal) for 15–20 sec. The indifferent electrode was a silver pin in contact with the cerebral surface. Control rats of two different kinds were used. In some rats lesions were made 2 mm lateral to the midline, and in some rats an electrode was implanted without passage of any current. The extent of the lesions was, in all cases, determined from serial frozen sections of the midbrains (after fixation in Holt and Hick's buffered formaldehyde solution) stained with toluidine blue or cresyl violet. A typical dorsal raphe lesion is shown in Fig. 1.

Combined lesions of the dorsal and median raphe nuclei

In these cases, one lesion was made at the coordinates given above, using 5 mA DC for 15–20 sec, and then another lesion was similarly performed at the level of the

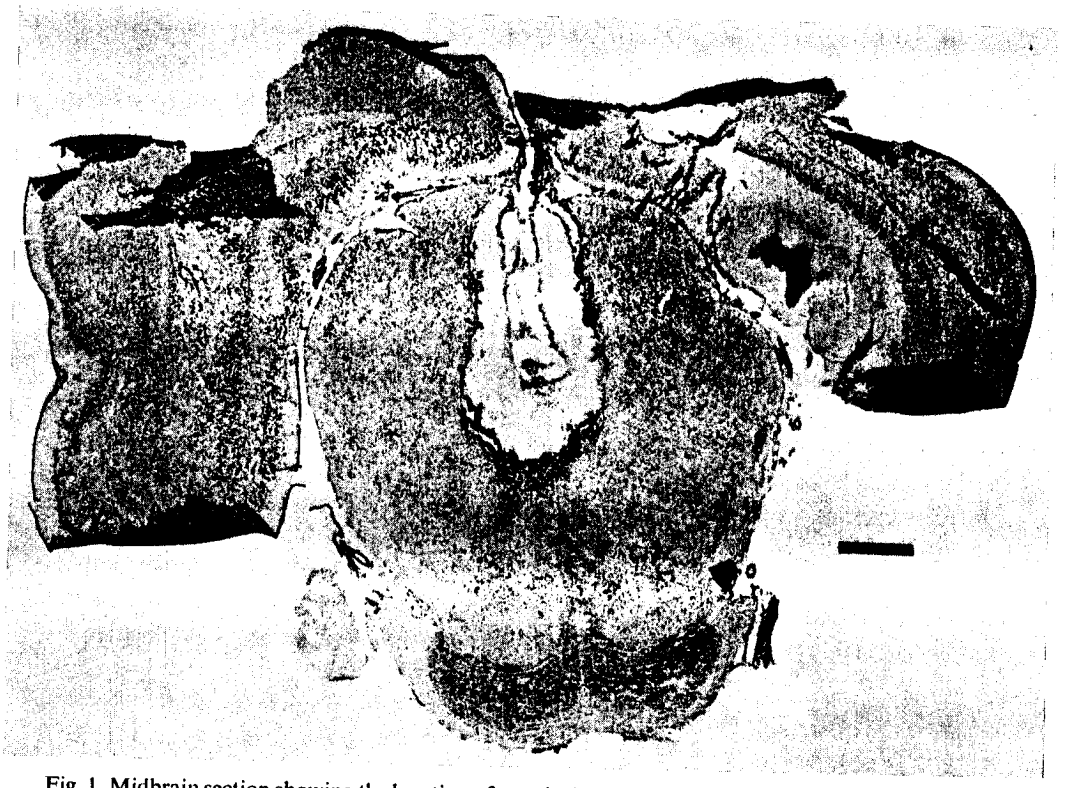

Fig. 1. Midbrain section showing the location of a typical dorsal raphe lesion. Calibration: 1 mm (bar).

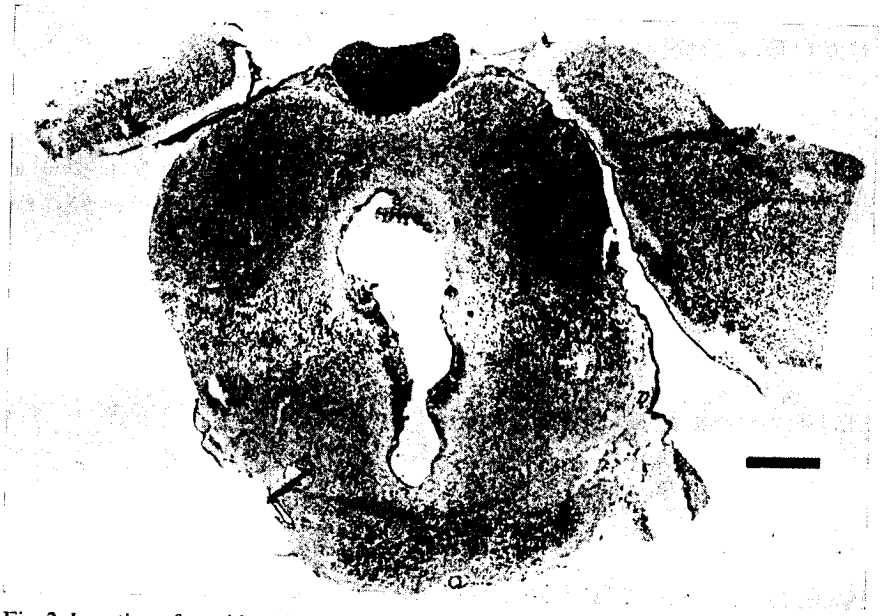

Fig. 2. Location of combined lesions of the dorsal and median raphe nuclei. Calibration: 1 mm (bar).

median raphe nucleus (A 0.16, L 0, H —2.6). Controls and histology as above. A typical combined raphe lesion is shown in Fig. 2.

Dorsal raphe stimulation

Stimulation in the dorsal raphe nucleus was performed via electrolytically-sharpened, glass-insulated, platinum-iridium wire electrodes with a free tip of 100–150 μm (tip diameter approx. 10 μm) and an impedance of 5–80 kΩ at 1 kHz. The electrode was placed at the coordinates given above. The indifferent electrode was a silver wire in contact with the cerebral surface through the craniotomy. The stimulator delivered constant current pulses (cathodal, 0.2 msec) via isolation units. The current used was 0.2 and 0.5 mA at 300–600 Hz (high-frequency stimulation) and 1 mA at 20 Hz (low-frequency stimulation). The stimulation was applied for 30 min. The positions of the stimulating electrode-tips were, at the end of the stimulation, marked with positive current (1–2 mA, pulse duration 200 msec, current duration 10–15 sec), and the electrode placements were histologically controlled. For comparison some stimulations were made without lesions, so that both the possibility for the stimulation to cause a lesion, and the possibility for the final lesion to have any effect, could be excluded. The controls were either stimulated for 30 min 2 mm lateral to the midline or made just by implanting an electrode at the level of the dorsal nucleus for 30 min.

The following drugs were used: 2-bromolysergic acid diethylamide bitartrate, BOL; D-lysergic acid diethylamide tartrate, LSD (Sandoz, Basle); *p*-chloro-DL-amphetamine HCl, PCA; *p*-chloro-DL-phenylalanine methylester HCl, PCPA (Sigma, St. Louis, Mo.); 3-hydroxybenzylhydrazine HCl, NSD 1015, was kindly synthesized by Dr. B. Magnusson, Department of Organic Chemistry, University of Umeå, Sweden. The drugs were dissolved in 0.9% saline immediately before injection. All injections were given intraperitoneally. Doses of BOL and LSD refer to the base. The in vivo tyrosine hydroxylation was measured by the DOPA accumulation after central decarboxylase inhibition[6]. There are good reasons to believe that short-time DOPA accumulation is a measure of the tyrosine hydroxylation in vivo[6]. DOPA was quantified essentially according to Kehr et al.[15] with slight modification[22]. The striata from one rat were pooled for the determination. The recovery of 500 ng added to striatal tissue was 71.3 $\pm$ 1.0% (mean $\pm$ S.E.M., n = 25). For statistical analysis we used the two-tailed Student's *t*-test. A P value of < 0.05 was considered significant.

RESULTS

Table I shows that the DOPA accumulation in the striatum in rats with chronic dorsal raphe lesions or in rats with combined chronic dorsal and median raphe lesions did not significantly differ from that in sham-operated controls. Both LSD and BOL increased the DOPA accumulation in comparison with saline controls, in sham-operated rats and in rats with chronic dorsal raphe lesions. However, in rats with both dorsal and median raphe lesions, the DOPA accumulation after LSD did not significantly differ from that in saline-treated rats. The DOPA accumulation after LSD

TABLE I

DOPA accumulation in the striatum after selective electrolytic lesions of the raphe neurons

The lesions were performed at least 14 days before sacrifice. NaCl, LSD and BOL were administered 30 min and NSD 1015, 100 mg/kg, i.p., 20 min before sacrifice. DOPA ng/g ± S.E.M. (n). n.s. = not significant.

Treatment	*Sham-operated controls*	*Dorsal raphe lesions*	*Median and dorsal raphe lesions*
NaCl 0.9%	(a) 849 ± 149 (4)	(d) 913 ± 97 (10)	(g) 986 ± 22 (4)
LSD 0.5 mg/kg	(b) 1748 ± 76 (3)	(e) 1650 ± 120 (4)	(h) 1273 ± 151 (5)
BOL 0.5 mg/kg	(c) 2342 ± 361 (3)	(f) 2201 ± 441 (4)	(i) 1894 ± 224 (4)
Comparisons between the different treatments	(a–b) $P < 0.005$	(d–e) $P < 0.005$	(g–h) n.s.
	(a–c) $P < 0.01$	(d–f) $P < 0.005$	(g–i) $P < 0.01$
	(b–c) n.s.	(e–f) n.s.	(h–i) $P < 0.05$
	(a–d) n.s.	(a–g) n.s.	(d–g) n.s.
	(b–e) n.s.	(b–h) $P < 0.05$	(e–h) n.s.
	(c–f) n.s.	(c–i) n.s.	(f–i) n.s.

was significantly less in rats with combined dorsal and median raphe lesions than in sham-operated rats. After treatment with BOL, the DOPA accumulation in rats with combined dorsal and median raphe lesions did not significantly differ from that in sham-operated animals. The DOPA accumulation after BOL was larger than after LSD. However, a significant difference, as regards DOPA accumulation, between LSD and BOL was found only in rats with combined raphe lesions.

PCA as well as PCPA significantly decreased the striatal DOPA accumulation as compared with controls (Table II). LSD and BOL increased the striatal DOPA accumulation in rats pretreated with PCA or PCPA as well as in the saline controls. Both in the PCA-treated rats and in the controls, BOL was more effective than LSD.

TABLE II

DOPA accumulation in the striatum after treatment with PCA and PCPA

PCA (20 mg/kg, i.p.) was administered 2 h and PCPA (400 mg/kg, i.p.) 48 h before sacrifice. NSD 1015 (100 mg/kg, i.p.) was administered 20 min before sacrifice. DOPA ng/g ± S.E.M. (n). n.s. = not significant.

Pretreatment/ treatment	*NaCl 0.9%*	*PCA 20 mg/kg*	*PCPA 400 mg/kg*
NaCl 0.9%	(a) 1034 ± 49 (6)	(d) 742 ± 51 (6)	(g) 787 ± 26 (6)
LSD 0.5 mg/kg	(b) 1369 ± 86 (6)	(e) 936 ± 51 (6)	(h) 981 ± 60 (6)
BOL 0.5 mg/kg	(c) 2095 ± 242 (6)	(f) 1377 ± 57 (6)	(i) 1159 ± 127 (6)
Comparisons between the different treatments	(a–b) $P < 0.01$	(d–e) $P < 0.025$	(g–h) $P < 0.02$
	(a–c) $P < 0.005$	(d–f) $P < 0.001$	(g–i) $P < 0.02$
	(b–c) $P < 0.02$	(e–f) $P < 0.001$	(h–i) n.s.
	(a–d) $P < 0.005$	(a–g) $P < 0.005$	(d–g) n.s.
	(b–e) $P < 0.005$	(b–h) $P < 0.005$	(e–h) n.s.
	(c–f) $P < 0.02$	(c–i) $P < 0.01$	(f–i) n.s.

TABLE III

DOPA accumulation in the striatum after acute selective lesions of the raphe neurons

The lesions were performed one hour before sacrifice. NSD 1015 (100 mg/kg, i.p.) was administered 20 min before sacrifice. DOPA ng/g ± S.E.M. (n). n.s. = not significant.

Treatment	*Anesthetized controls*	*Sham-operated controls*	*Median and dorsal raphe lesions*
	(a) 921 ± 100 (4)	(b) 590 ± 59 (3)	(c) 868 ± 111 (4)
Comparisons between the different treatments	(a–b) $P < 0.05$ (a–c) n.s.	(b–c) n.s.	

TABLE IV

DOPA accumulation in the striatum after high-frequency stimulation of the dorsal raphe neurons

The duration of the stimulation was 30 min. NSD 1015 (100 mg/kg, i.p.) was administered 20 min before sacrifice. DOPA ng/g ± S.E.M. (n). n.s. = not significant.

Treatment	*Anesthetized controls*	*Sham-operated controls*	*Stimulation 0.2 mA*	*Stimulation 0.5 mA*
	(a) 921 ± 100 (4)	(b) 676 ± 105 (3)	(c) 1008 ± 167 (4)	(d) 886 ± 140 (3)
Comparisons between the different treatments	(a–b) n.s. (a–c) n.s. (a–d) n.s.	(b–c) n.s. (b–d) n.s.	(c–d) n.s.	

TABLE V

DOPA accumulation in the striatum after low-frequency stimulation of the dorsal raphe neurons

The duration of the stimulation was 30 min. NSD 1015 (100 mg/kg, i.p.) was administered 20 min before sacrifice. DOPA ng/g ± S.E.M. (n). n.s. = not significant.

Treatment	*Sham-operated controls*	*Stimulation 1 mA*	*Stimulation 1 mA (electrode localized with a lesion)*
	(a) 825 ± 68 (6)	(b) 1175 ± 272 (6)	(c) 972 ± 199 (6)
Comparisons between the different treatments	(a–b) n.s. (a–c) n.s.	(b–c) n.s.	

Such a difference between LSD and BOL was not observed in the PCPA-treated rats.

Table III demonstrates the effect of acute combined dorsal and median raphe lesions on the DOPA accumulation in the striatum. These acute lesions did not significantly change the DOPA accumulation in comparison with the DOPA accumulation in anesthesized controls or sham-operated controls.

High-frequency (300–600 Hz) stimulation of the dorsal raphe neurons (0.2 and 0.5 mA) did not result in a significant change in the DOPA accumulation in comparison with the DOPA accumulation observed in sham controls or in barely anesthesized animals (Table IV). Neither did low-frequency stimulation (1 mA at 20 Hz) with or without a lesion to mark the electrode tip change the DOPA accumulation as compared with sham controls (Table V).

DISCUSSION

Biochemical[7,24,27,28] and behavioral[16,23] studies indicate that LSD has an agonistic effect on DA receptors. There is also evidence that LSD directly activates central 5-HT pathways[2,3,13]. Furthermore, there appear to be connections between the central raphe neurons and the striatum[4,21]. In addition 5-HT and LSD-sensitive receptors have recently been demonstrated in the striatum[5,20]. Therefore, it was of great interest to elucidate to what extent the effect of LSD on the DA synthesis in the striatum was influenced by central 5-HT pathways. We could not find a significant change in DOPA accumulation in the striatum, neither after chronic lesions of the dorsal raphe nucleus, nor after combined lesions of the dorsal and median raphe nuclei. These results are in agreement with the findings of other authors, who could not observe any significant change in tyrosine hydroxylase activity after chronic raphe lesions[12,18]. We were also unable to demonstrate a change in DOPA accumulation after acute raphe lesions or after stimulation of the dorsal raphe nucleus. However, after treatment with PCA or PCPA, in doses which have been reported to decrease the brain 5-hydroxytryptamine (5-HT) levels, a decrease in DOPA accumulation in the striatum was observed. These findings, in contrast to the findings above, suggest that a decrease in 5-HT levels results in a decreased DOPA accumulation in the striatum. However, PCA and PCPA may affect the DOPA accumulation by other mechanisms than by inhibition of 5-HT synthesis. They could, for example, directly or indirectly affect the tyrosine hydroxylation[19,26].

In agreement with earlier findings[14,22] both LSD and BOL increased the striatal DOPA accumulation in intact animals. These increases seemed unimpaired by previous pretreatment with PCA and PCPA and also by lesion of the dorsal raphe nucleus. Since LSD activates central 5-HT receptors[3,13], acute stimulations or electrolytic lesions of the raphe nuclei might imitate the short-time effect of LSD on the 5-hydroxytryptaminergic pathways. If this assumption is correct the DOPA accumulation should have been increased after acute lesions and stimulations of the raphe nuclei. However, the DOPA accumulation after acute lesions and stimulations was not significantly changed. These findings suggest that the increased DOPA accumulation in the striatum after LSD is not mediated via 5-hydroxytryptaminergic pathways.

The present study thus leads us to the conclusion that the 5-hydroxytryptaminergic input from the raphe neurons to the striatum does not play a role for the in vivo tyrosine hydroxylation in this DA-rich brain region. It furthermore indicates that the effects of LSD and BOL on the DOPA accumulation in the striatum are not mediated via central 5-hydroxytryptaminergic pathways originating in the dorsal raphe nucleus. Hence, LSD and BOL, probably to a very large extent, act via postsynaptic DA receptors and/or autoreceptors (presynaptic receptors).

However, involvement of 5-hydroxytryptaminergic mechanisms in the effect of LSD on the striatal DOPA accumulation cannot be excluded, since LSD did not increase the DOPA accumulation after chronic lesions of both the dorsal and median raphe nuclei. BOL, on the other hand, still increased the DOPA accumulation after these lesions. This difference between LSD and BOL could be explained by the findings that LSD is a strong activator of central 5-HT receptors, while BOL only has a slight effect on these receptors[1,3]. Further support for involvement of 5-hydroxytryptaminergic mechanisms in the effects of LSD on the DOPA accumulation is our previous finding that the effect of LSD in rats treated with gamma-butyrolactone (GBL) was counteracted by 5-HT antagonists[22]. Recently evidence for an inhibitory input from the median raphe nucleus to the substantia nigra has been presented[9]. LSD could thus increase the DOPA accumulation in the striatum by stopping the impulse flow in this inhibitory pathway. Furthermore, other workers have demonstrated LSD- and 5-HT-sensitive receptor sites in the striatum[5,20] which may be a structural base for the effect of LSD in this brain region.

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