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EFFECT OF D-LSD AND RELATED COMPOUNDS ON RELEASE OF NOREPINEPHRINE-H^3 AND SEROTONIN-H^3 EVOKED FROM BRAIN SLICES BY ELECTRICAL STIMULATION

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ABSTRACT

Slices of brain accumulate monoamines when incubated with tritiated compounds. Mild electrical-field stimulation, which causes depolarization of nerve membranes, induces a striking increase in the rate of release of tritiated amines. The effects of psychotomimetic and related drugs on the evoked release of norepinephrine-H^3 and serotonin-H^3 have been examined. The addition of D-LSD to the superfusing medium diminished release of both tritiated amines while mescaline, L-acetyl-D-LSD and N,N-dimethyltryptamine lowered release of serotonin-H^3 only. The non-hallucinogenic L-LSD and BOL exerted no inhibitory effect. It is suggested that the mode of action of D-LSD involves a specific interplay between D-LSD and serotonin by one or more mechanisms.

INTRODUCTION

A good deal of evidence has been accumulated which supports the view that lysergic acid diethylamide (LSD) and related compounds may affect biogenic amines in the central nervous system *(Giarman and Freedman, 1965; Cohen, 1967)*. Investigations of an antagonism between serotonin and LSD or other psychotomimetic agents have been primarily directed toward an examination of effects of the drug on modifying the action on metabolism of these amines *(Gaddum, 1953;*

Appel and Freedman, 1964; Marchbanks, 1967; Rosenerans, Lovell and Freedman, 1967), the electrophysiology of special sensory systems *(Evarts, 1957; Key 1965)* or on the physiology of amine-containing neurons *(Aghajanian, Foote and Sheard, 1968; Kawai and Yamamoto, 1968; Bunna and Anderson, 1968)*.

Slices of brain actively accumulate norepinephrine-H^3 *(Dengler et al., 1962)* and serotonin-H^3 *(Schanberg, 1963)* where the labeled transmitter is largely localized in nerve endings *(Lenn, 1967; Ross and Renyi, 1967)*. The passage of an electrical stimulus through such slices has been found to displace membrane potentials of constituent cells *(Hillman, Campbell and Mallwain, 1963)* and to markedly accelerate efflux of labeled monoamines into a superfusing medium. The remarkably good agreement between the effects of drugs on uptake of biogenic amines in vitro and their effects in vivo *(Shore, 1962)* supports the view that events in slices of cerebral tissue may have relevance to function in intact brain. Electrically induced release of norepinephrine *(Baldessarini and Kopin, 1967)* and serotonin *(Chase, Breese and Kopin, 1967)* from mammalian brain slices has recently been used as a model for studying putative transmitter release from brain. This technique has been used to examine the effects of D-LSD and related psychotomimetic compounds on release of monoamines from brain slices.

The addition of D-LSD to the superfusing medium diminished release of both tritiated amines, while mescaline, L-acetyl-D-LSD, and N,N-dimethyltryptamine (NNMT) lowered release of serotonin-H^3 only. The non-hallucinogenic L-lysergic acid diethylamide (L-LSD) and 2,brom-lysergic acid diethylamide (BOL) exerted no inhibitory effect.

METHODS

Adult male Sprague-Dawley rats weighing 150 to 200 grams were killed by cervical fracture, and their brains were rapidly removed. Slices of tissue were prepared from the brain as follows: A coronal section of brain was made

about 4 mm from the anterior pole of the cerebral hemispheres, and a slice about 0.4 to 0.5 mm thick was cut from the posterior portion. The slice was placed on a piece of wet (Krebs-Ringer bicarbonate solution) filter paper, and a disc of tissue 3 mm in diameter (weighing about 20 mg) was punched out of the area corresponding to the striatum. Such discs were placed in 20 ml of a supplemented Krebs-Ringer solution *(Krebs, 1950)*, saturated with 5% carbon dioxide in oxygen and maintained at 37° C. Norepinephrine-H^3 (NE-H^3)(8 c/mmole, 25 ng/ml) or serotonin-H^3 (5HT-H^3)(2 c/mmole, 50 ng/ml) was added.* Incubation was continued for 30 minutes, after which the tissues were transferred to glass superfusion chambers.

The glass superfusion chambers consisted of open-end tubes (0.5 cm diameter, 2.5 cm length) mounted vertically by sealing the open ends into holes of the upper and lower plates of a rectangular clear lucite box. The lucite box served as a jacket through which flowed water at 37° C. The open ends of the superfusion chambers were sealed with small rubber plungers, each of which held a small metal tube connected to a polyethylene tube through which the superfusing solution could be introduced (at the bottom) and collected (from the top). Platinum wires (No. 22) which ended in flat coils (about 0.4 mm diameter) served as electrodes and were adjusted so that the tissue slice rested between the electrodes, which were placed about 1 mm apart.

Fresh warmed oxygenated medium was pumped through the chambers at a rate of about 0.3 mm/min so that the fluid surrounding the tissue was rapidly replaced (volume of chamber was less than 0.4 ml). When spontaneous efflux of radioactivity into the effluent fluid diminished to low, relatively constant levels (20 min), rectangular DC pulsatile stimulation (9 mA, 100 cycles/sec,

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*Both labeled compounds were purchased from the New England Nuclear Corp. (Boston, Massachusetts).

4.0 msec duration) from a Grass S-4 stimulator through a constant-current control unit was applied through the platinum electrodes for a total of one minute. Effluent superfusate was collected during two-minute intervals immediately before and during stimulation and assayed for total tritium by liquid scintillation spectroscopy.

The effect of drugs on release of labeled amine was studied. The following drugs were used: D-lysergic acid bitartrate (D-LSD), L-lysergic acid bitartrate (L-LSD), 2,brom-D-lysergic acid diethylamide bitartrate (BOL), mescaline HCl, L-acetyl-D-2-lysergic acid diethylamide bitartrate (L-acetyl-LSD) and N,N-dimethyltryptamine (NNMT).

RESULTS

Effect of D-LSD on release of tritiated monoamines from slices of brain. As previously described *(Baldessarini and Kopin 1967; Chase, Breese and Kopin, 1967)* electrical field stimulation resulted in a striking release of tritium from brain slices previously incubated with norepinephrine-H^3 or serotonin-H^3 consisting mainly of the unmetabolized amine. When D-LSD was present in the superfusate, there was no alteration in the rate of spontaneous efflux of labeled amines from striatum during the interval just before stimulation. However, stimulation-induced release of both amines was diminished in the presence of the hallucinogen.

When the concentration of D-LSD was reduced to 10^{-6} M, inhibition of serotonin-H^3 release was still evident but release of norepinephrine-H^3 was unimpaired. Efflux of neither amine was altered at drug levels of 10^{-7} M.

Effect of psychotomimetic agents on inhibition of stimulation-evoked monoamine-H^3 release. A variety of psychotomimetic agents and related compounds were tested for their effect on evoked amine release. Only D-LSD

diminished evoked release of norepinephrine-H^3 and serotonin-H^3. At appropriate concentrations, the less potent hallucinogens, mescaline, L-acetyl-LSD and N,N-dimethyltryptamine, did diminish release of serotonin-H^3 but not norepinephrine-H^3. D-LSD, at a dose level which gives comparable inhibition of serotonin-H^3 release, fails to diminish release of norepinephrine-H^3 (Table I); but at much higher levels it can inhibit the release of the catecholamine (Fig. 1). The inactive hallucinogens, L-LSD and BOL, did not inhibit release of either monoamine.

Table I

INHIBITION OF STIMULATION-EVOKED AMINE RELEASE FROM RAT BRAIN SLICES

		Percent Inhibition	
	Concentration	Norepinephrine-H^3	Serotonin-H^3
D-LSD	2×10^{-6} M	0	30 ± 6
L-LSD	2×10^{-3} M	0	0
BOL	1×10^{-3} M	0	0
Mescaline	2×10^{-4} M	0	24 ± 4
Acetyl-D-LSD	2×10^{-4} M	0	32 ± 3
NNMT	2×10^{-3} M	0	68 ± 13

Slices of striatum were incubated with labeled amines, superfused and stimulated as indicated in the text. Drug abbreviations are listed in Methods section. Results are expressed as percent inhibition of control release ± SEM.

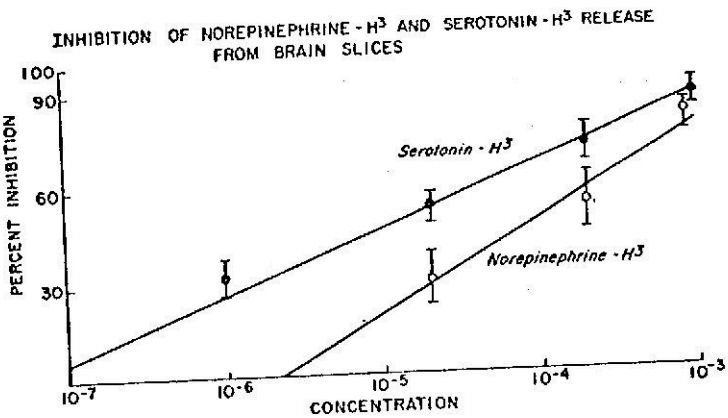

Figure 1. *Inhibition by D-LSD of stimulation-evoked amine release.* Slices of striatum were incubated with labeled amines, superfused and stimulated as indicated in the text. Results are expressed as percent inhibition of release ± SEM at concentrations of D-LSD indicated on the abscissa.

DISCUSSION

Various psychoactive drugs alter biogenic amine metabolism in brain as well as in peripheral tissues *(Coppen, 1967; Glowinski and Axelrod, 1965; Schanberg, Schildkraut and Kopin, 1967)*. *Gaddum's (1953)* report of the antagonistic effect of D-LSD (10^{-9} g/L) on the action of serotonin in contracting smooth muscle first implicated this amine in the action of hallucinogens. D-LSD has the typical action of ergot alkaloids in contracting smooth muscle, particularly of the uterus, and it may therefore activate some receptors. The diverse effects of D-LSD and some evidence for involvement of central biogenic mechanisms in these effects have been extensively reviewed *(Giarman and Freedman, 1965)*. D-LSD causes a small but significant increase in cerebral serotonin with a decrease in 5-hydroxyindole acetic acid (5HIAA), the major metabolite of serotonin *(Rosencrans, Lovell and Freedman, 1967)*.

Electrically induced convulsions decrease serotonin concentration and increase 5HIAA in brain *(Hinesly, Norton and Aprison, 1968)*. The suggestion that D-LSD decreases discharge of serotonin from neurons containing this amine has received support from the elegant studies of *Aghajanian et al. (1968)*, who showed that minute doses of D-LSD reversibly depress the firing rate of serotonin-containing cells in rat brain in vivo. It was suggested that the depressed firing rate of these neurons was a consequence of D-LSD action on serotonin receptors, with consequent indirect neuronal feedback depressing the activity of the serotonergic neurons.

In the present studies we have shown that D-LSD, but not L-LSD, inhibits electrical field stimulation-induced release of serotonin from rat brain slices. The lowest dose of D-LSD which significantly reduced such release was 10^{-6} M (Fig. 1). This is considerably greater than the level of D-LSD which inhibits firing of serotonergic neurons *(Aghajanian, Foote and Sheard, 1968)* but less than the dose levels required to demonstrate changes in serotonin metabolism *(Rosencrans, Lovell and Freedman, 1967)*. At this dose level, evoked release of norepinephrine from brain slices was unaffected. The L-isomer of LSD and BOL, which are relatively impotent hallucinogens, failed to inhibit evoked release of serotonin, while other hallucinogenic compounds, mescaline and N,N-dimethyltryptamine, inhibited release of serotonin without influencing norepinephrine release. Other drugs (e.g. chlorpromazine) which inhibit release of serotonin, also inhibit release of norepinephrine to about the same extent at comparable dose levels. Thus, the hallucinogenic compounds appear to selectively inhibit release of serotonin without greatly influencing release of norepinephrine.

Numerous investigators have now described an interaction between D-LSD and related drugs and neuronal systems sensitive to serotonin. The demonstration of impaired firing of serotonergic fibres *(Aghananian, Foote and*

Sheard, 1968; Aghajanian and Weiss, 1968) and the findings of *Gaddum (1953)* suggest that D-LSD may act at receptor loci while our studies and those of *Appel and Freedman (1964)* and of *Kawai and Yamamoto (1968)* suggest inhibition of release of the indoleamine from presynaptic sites. These results support the contention that the mode of action involves a specific interplay between D-LSD and serotonin by one or more mechanisms.

REFERENCES

1. Aghajanian, G.K.; Foote, W.E. and Sheard, M.H. (1968): Science 161, 706.
2. Aghajanian, G.K. and Weiss, B.L. (1968): Nature 220, 795.
3. Appel, J.B. and Freedman, D.X. (1964): Biochem. Pharmac. 13, 861.
4. Baldessarini, R.J. and Kopin, I.J. (1967): J. Pharmac. Exp. Ther. 156, 31.
5. Banna, N.R. and Anderson, E.G. (1968): J. Pharmac. Exp. Ther. 162, 319.
6. Chase, T.N.; Breese, G.R. and Kopin, I.J. (1967): Science 157, 1461.
7. Cohen, S. (1967): Ann. Rev. Pharmac. 7, 301.
8. Coppen, A. (1967): Br. J. Psychiat. 113, 1237.
9. Dengler, H.; Michaelson, I.; Spiegel, H. and Titus, E. (1962): Int. J. Neuropharmac. 1, 23.
10. Evarts, E.V. (1957): Proc. Ass. Res. Nerv. Ment. Dis. 36, 347.
11. Gaddum, J.H. (1953): J. Physiol. 121, 15.
12. Giarman, N.J. and Freedman, D.X. (1965): Pharmac. Rev. 17, 1.
13. Glowinski, J. and Axelrod, J. (1965): J. Pharmac. Exp. Ther. 149, 43.
14. Hillman, H.H.; Campbell, W.J. and McIlwain, H. (1963): J. Neurochem. 10, 325.
15. Hinesly, R.K.; Norton, J.A. and Aprison, M.H (1968): J. Psychiat. Res. 6, 143.
16. Kawai, N. and Yamamoto, C. (1968): Brain Res. 7, 325.
17. Key, B.J. (1965): Br. Med. Bull. 21, 30.
18. Krebs, H.A. (1950): Biochim. Biophys. Acta 4, 249.

19. Lenn, N.A. (1967): Am. J. Anat. 120, 377.

20. Marchbanks, R.M. (1967): Biochem. Pharmac. 16, 1971.

21. Rosecrans, J.A.: Lovell, R.A. and Freedman, D.X. (1967): Biochem. Pharmac. 16, 2011.

22. Ross, S.B. and Renyi, A.L. (1967): Life Sci. 6, 1407.

23. Schanberg, S.M. (1963): J. Pharmac. Exp. Ther. 139, 191.

24. Schanberg, S.M.; Schildkraut, J.J. and Kopin, I.J. (1967): Biochem. Pharmac. 16, 39.

25. Shore, P.A. (1962): Pharmac. Rev. 14, 531.