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Effect of LSD-25 and 1-Methyl-*d*-Lysergic Acid Butanolamide on Rat Brain and Platelet Serotonin Levels

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Abstract. Factorial analysis of rat cerebral 5-HT values observed following intraperitoneal LSD-25 and UML (1-methyl-*d*-lysergic acid butanolamide) showed that the highly significant decrease induced by both drugs was independent of dose and that the effect of both drugs was the same. Increased platelet 5-HT values were also obtained with each drug. These findings suggest that the psychotomimetic effect of LSD-25 is not due to changes in cerebral 5-HT, since a relationship can probably be postulated between such an effect in man and the effect of LSD-25 on conditioned behaviour in the rat. It is also felt that the fact that these drugs decrease and increase cerebral and platelet values respectively may be of assistance in the interpretation of data relating to platelet 5-HT patterns in man under psychiatric conditions and during treatment with psychotropic drugs.

Key words: LSD-25 — UML — 5-HT — Brain — Platelets — Behaviour — Rat.

Earlier personal work (Torre and Fagiani, 1968) has shown that 0.1 mg/kg LSD-25 improves the performance of rats conditioned to the avoidance reflex 10′ after its administration, whereas 0.5 mg/kg has a statistically significant opposite effect. 1-methyl-*d*-lysergic acid butanolamide (UML), however, causes no significant changes when used at the same doses. This substance has the same chemical formula and antiserotonin action as LSD-25, but displays no psychotomimetic effect in man.

It was clear, therefore, that LSD-25 had a marked two-stage effect, whereas its apsychotomimetic companion was without influence on a conditioned reflex at either a low or a high dose. Further work (Torre, 1971) showed that as much as 3 mg/kg UML was ineffective when administered alone, though its association with 0.5 mg/kg LSD-25 inhibited or suppressed the performance depression induced by the latter drug. This suggested that the effect of LSD-25 is not a result of its antiserotonin action. In this connection, however, it must be remembered that the label "antiserotoninic" refers essentially to the neurovegetative and visceral action of this monoamine and not its presumably neurohormo-

nal effect on the CNS. It may be, therefore, that these two drugs act in the same way in one area and differently in another.

The present paper reports a comparison of changes in rat cerebral and extra-cerebral (platelet) serotonin (5-HT) following the administration of LSD-25 and UML[1]. Platelets are very rich in 5-HT and it was felt that their position as a kind of reserve gave good reason to suppose that variations in their 5-HT levels were indicative for the body as a whole. Comparison between cerebral and other sites is of interest in that clinical attention has recently been directed to platelet 5-HT as an index of psychological disturbance and the effect of psychotropic drugs on behaviour and the CNS. Lastly, there are no literature data for the effect of LSD-25 and UML on rat platelet 5-HT, apart from our preliminary report (Torre, M., Bogetto and Torre, E., 1972).

Data relating to the effect of LSD-25 on brain 5-HT *in vivo*, on the other hand are numerous, though differences in the experimental conditions employed (i.v., intraperitoneal or oral administration, dose administered (20—1300 μg/kg), time between administration and examination) or in the system of determination used (biological or spectrophotofluorimetrical), have resulted in their lack of uniformity.

Few workers have subjected their results to statistical analysis. It need hardly be stated that percent increases, especially those of modest dimensions, cannot show whether the variations observed were casual or indeed due to the drug employed.

The following findings may be briefly noted: 1. decreased brain 5-HT after LSD-25 in the mouse (Ruckebusch, Roche, and Schurch, 1965; biological method); 2. no action on the part of LSD-25 in the rabbit (Brodie, Shore, and Pletscher, 1956), mouse (Schubert, Nyback, and Sedvall, 1970) or rat (Tonge and Leonard, 1971, spectrophotofluorimetry), 3. increase not assessed statistically and certainly not significant in some cases in the rat (Giarman and Schaunberg, 1962, increase of 25%: biological method) (Diaz and Huttunen, 1971, increase of 13% following chronic administration of 20 μg/day).

A statistically significant increase, however, was obtained in the rat with intraperitoneal 250 μg/kg by Matin and Vijayvargiya (1967). Withdrawal was made 30′ after injection and a biological method was employed. Freedman (1961) reported a significant increase with 130 μg/kg (biological determination using quahaug heart) and with 500 μg/kg (spectrophotofluorimetry, withdrawal after 60′). Lastly, Rosecrans, Lovell and Freedman (1967) noted an increase with a significant peak at 30′ following 200 μg/kg i.v., while neither before nor after that time was the increase significant. A significant increase at 30′ was also observed with

1 Supplied as Delysid and Deserril (Sandoz).

260 μg/kg intraperitoneally, whereas 560 and 1300 μg/kg produced an increase after only 15′.

Methods

In our first series of experiments, LSD-25 and UML were administered intraperitoneally (0.5 mg/kg) and blood and brain removed 10′ later. As already stated, LSD-25 produces a significant depression of conditioned performance at this time, while UML is without effect. The controls received an identical quantity of saline. In the second series, 0.1 mg/kg LSD and 3 mg/kg UML were employed.

Four groups of 8 male Wistar rats were used, together with 19 controls. Blood was obtained following decapitation and the brain was removed as quickly as possible. 5-HT values were determined on the whole brain following section at the lower part of the myelo-encephalon.

The method of Ackroyd (1964) was used to isolate and count platelets. Spectrophotofluorimetry was carried out with an Aminco-Bowman apparatus, using Crosti and Luchelli's modification (1962) of Weissbach's method (1958). Shore and Olin's method (1958) was used for brain 5-HT identification.

Results

The results in the first series of experiments (Table 1) show that LSD produces a statistically significant fall in cerebral 5-HT and a highly significant platelet 5-HT increase in the same animals. UML also led to a barely significant decrease in cerebral and a highly significant increase in platelet 5-HT values.

Table 1

Animal	Brain (5-HT ng/g)			Platelets (5-HT ng/10^9 platelets)		
	Controls	LSD (0.5 mg/kg)	UML (0.5 mg/kg)	Controls	LSD (0.5 mg/kg)	UML (0.5 mg/kg)
1	340	294	263	215	946	1.071
2	340	325	309	269	810	876
3	356	325	340	634	828	652
4	386	340	356	234	627	630
5	386	356	371	303	564	570
6	402	371	371	94	669	624
7	402	385	402	715	445	413
8	417	402	417	140	520	877
9	433			593		
10	495			666		
11	557			474		
Mean and S.D.	410 ± 66	350 ± 36	354 ± 50	394 ± 227	676 ± 172	714 ± 214
Student's t		2.348 $P<0.05$	2.014 $P<0.1$		2.946 $P<0.01$	3.120 $P<0.01$

Table 2

Animal	Brain (5-HT ng/g)		
	Controls	LSD (0.1 mg/kg)	UML (3 mg/kg)
1	358	254	291
2	380	313	336
3	380	328	336
4	403	373	336
5	403	388	358
6	403	388	358
7	425	403	380
8	515	350	380
Mean and S.D.	408 ± 48	350 ± 50	347 ± 29
Student's t		2.382 $P < 0.05$	3.108 $P < 0.01$

As stated at the outset, 0.1 mg/kg LSD-25 improves conditioned avoidance reflex performance, whereas 3 mg/kg simultaneously administered UML blocks the performance-depressing effect of a very much larger LSD dose. Investigation of the cerebral 5-HT aspect of these changes in our second series of experiments (Table 2) showed that both drugs produced a highly significant fall in values at these doses.

Analysis using Student's t index indicated that the effect was the same in both cases.

Factorial analysis of variance on the influence of various doses of the two drugs on cerebral 5-HT, using three criteria (A = treatment; B = drug; C = dose) gave the following results:

A: $F = 22.71$, $P < 0.01$: highly significant effect of treatment irrespective of the drug employed or the dose used.

A×C: $F = 0.016$: purely casual differences between the two doses employed (both drugs).

B×C: $F = 0.017$: non-significant differences between the effects of the two drugs and their respective doses.

It was clear, therefore, that both drugs were responsible for a highly significant fall in cerebral 5-HT values at either dose.

Discussion

LSD- and UML-induced decreases and increases in cerebral and platelet 5-HT values respectively are of both clinical and pharmacological interest in the interpretation of platelet 5-HT patterns in man under psychiatric conditions and during treatment with psychotropic drugs.

The difference between the cerebral and extra-cerebral picture may perhaps be explained by suggesting that both drugs block 5-HT utilization, but do not interfere with its production, resulting in enhanced platelet storage. Alternatively, the increased 5-HT value in the platelets could be caused by equilibrium of a raised plasma 5-HT due to the effect of LSD and UML, which is not found in the brain.

It is clear that no relationship exists between conditioned reflex behaviour and cerebral 5-HT, since both drugs effect 5-HT values in the same way, whereas LSD-25's influence on behaviour is dose-dependent, while UML is without effect. It has been suggested that only one drug influences 5-HT turnover. This involves a complicated and unconvincing interpretation, wherein LSD is presumed to effect turnover in such a way as to diminish 5-HT values, while UML either has no effect, or else acts in such a way that the same 5-HT value (i.e. not the start value) is obtained. Lastly, our results do not agree with the view that the psychotomimetic action of LSD-25 is the result of changes in cerebral 5-HT, since it is probable that a relationship exists between such action in man and the effect of the drug on rat behaviour.

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