CLINICAL STUDIES WITH LSD-25 AND TWO SUBSTANCES RELATED TO SEROTONIN*

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PART I

CLINICAL REACTIONS OF NORMAL HUMAN SUBJECTS GIVEN LSD-25 AND AN ANTI-METABOLITE OF SEROTONIN (1 BENZYL-2, 5-DIMETHYLSEROTONIN HYDROCHLORIDE) (BAS)

INTRODUCTION

IN 1953, Twarog and Page (1) demonstrated that 5-hydroxytryptamine (serotonin) is present in relatively high concentrations in the brain. At about the same time Gaddum (2) and Woolley and Shaw (3) observed that LSD is a highly potent serotonin antagonist in such tissues as rat uterus and hypothesized that the hallucinogenic effect of LSD might be due to interference in the action of serotonin.

Following these discoveries, several workers attempted to test this hypothesis on a clinical level in humans. Montanari and Tonini (4) reported the successful use of intramuscular serotonin as an antagonist to the psychological effects of LSD-25 in humans. In contrast to these findings, Sjoerdsma, Kornetsky and Evarts (5) reported the administration of LSD-25 in two patients who suffered from malignant carcinoid. Both patients experienced all the usual effects of LSD-25.

There is an obvious contradiction between the two latter reports. The validity of the study by Montanari is open to question, however, since serotonin is rapidly destroyed by amine oxidases in the blood (6), and if injected, probably would not reach the brain in effective concentrations. Furthermore, serotonin does not cross the blood brain barrier in appreciable amounts. In the carcinoid patients, on the other hand, a large, continuous output of serotonin obviates the first objection, but the blood brain barrier problem remains.

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Gaddum (7) has tested this hypothesis himself in cats. Serotonin was injected directly into the cerebral ventricles. This was followed by LSD administration. The LSD reactions did not appear to be significantly affected by the serotonin. This result is highly significant, and casts considerable doubt on the basic hypothesis; yet observations of psychological changes in animals are necessarily crude, and allow little scope for noting subtle changes. Thus, in spite of these important findings, it would seem desirable to carry out further clinical studies in humans.

Gaddum originally advanced his hypothesis after it had been observed that LSD and serotonin are antagonists *in vitro*, on such tissues as rat uterus. More recently it has been demonstrated by Delay and Thuillier (8) that this antagonism exists only with relatively high concentrations of LSD. When LSD concentrations are lower, at levels corresponding to those exerting psychotic effects in man, it potentiates the effects of serotonin on rat uterus. Woolley and Shaw (9) have also reported that LSD has effects similar to those of serotonin on tissues such as oligodendroglia from the brain, and on the heart beat of the clam, *Venus mercenaria*.

With these findings in view, if we are to make inferences about the relationship between serotonin and LSD in mental functioning it would seem possible that LSD acts by potentiating the effects of serotonin normally found in the brain. If this should be true, then a specific antagonist towards serotonin would be expected to block the psychological effects of LSD.

Woolley and Shaw (10) have reported on an orally active benzyl analogue of serotonin (1-benzyl-2, 5-dimethylserotonin hydrochloride) referred to as BAS. This substance acts as an anti-metabolite to serotonin. Presumably it is bound by the same substrate as serotonin, thereby preventing serotonin from acting. Wilkins (12) reported the use of this substance in hypertensive medical patients, in whom it causes moderate lowering of blood pressure. Side-effects include sedation or sleepiness, some slowing of the pulse, mild nasal stuffiness and abdominal cramps. In these studies, doses of 40 to 160 mg. a day were used.

In view of these considerations, the authors considered it worth while to test BAS as a potential antagonist to the psychological effects of LSD. Since our studies were concluded, other studies reporting the clinical use of BAS have been reported (13, 14). Since the work described in this paper was completed (in 1957), a paper by Isbell, *et al.* (20) has appeared in which a similar experiment using BAS with LSD-25 is reported. Isbell also found the BAS to have no influence on the LSD reactions.

Procedure

In the pilot studies, BAS was given in divided doses—the first dose of 100 to 200 mg. was given on the night preceding the experiment, and the second dose approximately ½ to 1 hour before LSD administration. BAS was not found to exert any effect on the LSD reaction.

After pilot studies,* using eight subjects, the following study was performed. Six "normal" male volunteers were selected for the study. One of three drug

* Tables II and III show the independent ratings of both observers in terms of relative severity of the reactions. Drugs were all administered on a double-blind basis. Both raters correspond with one another and with the schedule of administration, only in Column 3. In Columns 1 and 2 the observer's ratings are only in 50 per cent. agreement, which is no better than chance. It will also be seen that observers' ratings in Columns 1 and 2 correspond no better with Table I than with each other.

The authors conclude from this that the BAS administered produced no consistent effect in either direction on the severity of the LSD reactions.

combinations was administered to each subject, on each of three experimental days, one week apart. Each subject received all three drug combinations on separate days.

The design employed is shown in Table I.

TABLE I

Table Shows that on Any Experimental Day there were Two Subjects Receiving Each Drug Combination

		Drugs		
Day		Column 1	Column 2	Column 3
		LSD+Placebo	LSD+BAS	BAS+Placebo
1	.. .. ..	A, B	C, D	E, F
2	.. .. ..	D, F	B, E	A, C
3	.. .. ..	C, E	A, F	B, D

Subjects labelled A, B, C, D, E, F.

TABLE II

Ratings of Rater 1 (GDK)

Day		Column 1	Column 2	Column 3
		Most Severe	Medium Severe	Least Severe or None
1	.. .. ..	A, D	B, C	E, F
2	.. .. ..	B, E	D, F	A, C
3	.. .. ..	C, F	A, E	B, D

TABLE III

Ratings of Rater 2 (EC)

Day		Column 1	Column 2	Column 3
		Most Severe Reaction	Medium Severe Reaction	Least Severe Reaction or None
1	.. .. ..	B, D	A, C	E, F
2	.. .. ..	E, F	B, D	A, C
3	.. .. ..	A, C	E, F	B, D

LSD was administered orally in doses of 100 micrograms dissolved in distilled water. BAS was given orally in two doses. Two hundred milligrams was given one-half hour before LSD, and another 100 mg. was given one-half hour following LSD administration. Double-blind procedure was followed.

Each subject was rated three to five times on each experimental day by each of two psychiatric observers, working independently, and all were interviewed on the following day. Ratings were recorded on a rating sheet which allows for quantitative and qualitative ratings of physiological and psychological changes. Both objective and subjective factors were considered.

This method of rating has been used with considerable success by the authors in another study (15). We have found that where differences in relative severity of response exist, they can be reliably detected.

With the design which was followed, it was possible to compare subjects against one another, and each subject against himself with regard to severity of reactions on the various experimental days.

If BAS acted as an antagonist to LSD, then we should have expected subjects receiving combinations of LSD and BAS to be rated as having milder

reactions than those receiving only LSD. If, by any chance, BAS potentiated the action of LSD, then we should expect the LSD-BAS subjects to have the more severe reactions. Administration of BAS by itself gave us a chance to see what effects, if any, this drug had on psychological functioning.

The effects of more prolonged premedication of BAS upon the LSD reaction was studied in two subjects. Each was given BAS, 100 mg. q.i.d., for three successive days. On the fourth day each received 200 mg. BAS, followed by 1 microgram per kg. of LSD-25. Each subject received 1 mcg./kg. LSD on another occasion, without BAS, for comparison.

Results

Both raters were 100 per cent. successful in distinguishing the 6 BAS-placebo responses from the others, as would be expected: neither rater succeeded in distinguishing the LSD-BAS reactions from the LSD-placebo reactions with any greater degree of accuracy than might be expected from chance. Nor did the raters agree any better with one another in this (see Tables II and III). In the smaller study, in which two subjects were given more prolonged dosage of BAS, the BAS produced slight drowsiness, but no effect on the severity of the LSD reaction was observed, subjectively or objectively.

Discussion

Since, in other studies, we have been able to accurately rate the relative severity of LSD reactions, it appears rather clear that BAS had no significant effect on the reactions of these subjects to LSD.

The physiological significance of this finding is not yet clear, unfortunately, since it is not known with certainty whether BAS crosses the blood brain barrier. The symptoms of drowsiness from BAS reported by our subjects and by Wilkins's patients strongly suggest that it does, but this is not conclusive evidence. Further evidence, however, has come recently from Rudy, *et al.* (16) and from Rinaldi (17). The former author has reported a tranquillizing action of BAS, resembling reserpine effects in chronic schizophrenic patients, while Rinaldi has shown that BAS causes EEG changes in rabbits when administered intravenously, and in some humans (three out of fifteen) when administered orally.

There is also some evidence to the contrary. Woolley and Shaw (18) have shown that BAS injected peripherally in mice produces no behavioural effects. When injected into the brain of the animal, however, it causes distinct behavioural changes. This suggests that BAS does not normally cross the blood brain barrier, but that it is active when introduced directly into the brain, at least in mice.

Gaddum (19) himself, has already observed that BOL (2-Brom-LSD) and a variety of other substances which antagonize serotonin lack its potency as a psychotomimetic agent. Gaddum further indicates that these observations "diminish the value of the existing evidence favouring this theory" (i.e. that LSD acts by influencing serotonin action in the brain).

If we assume that BAS does cross the blood brain barrier in effective concentrations, our results cast further doubt on the theory.

Summary

A study was carried out to test whether an anti-serotonin in the form of a chemical analogue of serotonin (1 benzyl-2, 5-dimethylserotonin hydrochloride),

referred to as BAS, would antagonize the psychological effects of LSD-25. A modified Latin Square design was followed, in which 6 normal subjects each received LSD+placebo; LSD+BAS; and BAS+placebo on three separate experimental days. Double-blind procedure was followed. In a smaller study two subjects were given LSD-25 following more prolonged premedication with BAS. Subjects were rated for relative severity of response with each of the drug combinations. LSD-BAS responses were not significantly different from LSD-placebo responses. Though there are conflicting reports regarding the question of whether BAS crosses the blood brain barrier, recent evidence suggests that it does. If we assume that this is true, these results cast doubt on the hypothesis that LSD-25 acts by affecting serotonin action in the brain.

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PART II

CLINICAL REACTIONS OF NORMAL HUMAN SUBJECTS GIVEN 5-HYDROXYTRYPTOPHAN (A SEROTONIN PRECURSOR) AND LSD-25

Udenfriend *et al.* (1) have reported a method of significantly increasing brain levels of serotonin (5-hydroxytryptamine, 5-HT), in animals by administration of 5-hydroxytryptophan (5-HTP), a serotonin precursor. This compound is decarboxylated in the brain to form serotonin. Serotonin itself penetrates the blood brain barrier to only a slight degree. Udenfriend has also reported that 5-HTP produces effects in animals resembling the effects of LSD-25 (2). Davidson *et al.* (3) has demonstrated that in man also, 5-HTP is converted to serotonin, by recovering increased amounts of serotonin and 5-hydroxyindole-acetic acid (5-HIAA) in the urine after parenteral administration of 5-HTP: (5-HTP→5-HT→5-HIAA). 5-HTP was administered to 11 subjects by this author. In 10 subjects, a dose of 50 mg. or less was the maximum which could be given because of gastro-intestinal side-effects. In one subject a dose of 120 mg. was reached. No central nervous system effects were reported. They concluded that this dose of 5-HTP was inadequate for increasing brain serotonin to levels at which mental changes might be expected.

Bessman *et al.* (4) reported on the electroencephalographic effects of 10 mg. of intravenous 5-HTP in patients who were in hepatic coma. Slow activity was modified in the direction of a more normal pattern. 5-HIAA was found in the urine. This study offers further evidence that 5-HTP enters the brain, is converted to serotonin, and that neurophysiological effects can be produced. It suggests further, that serotonin has a role in normal brain functioning. It is of interest that a dose of only 10 mg. was effective in these patients.

As noted in Part I there has been much speculation regarding the interrelationship between serotonin, and LSD-25 in the brain. The present study was designed with two purposes. The first was to test the hypothesis that LSD effects in the brain are related to some type of interaction with serotonin (5). The second purpose was to discern whether relatively large doses of 5-HTP produce any psychological effects in normal subjects.

Method

A pilot experiment was undertaken to study possible interaction between LSD-25 and 5-HTP. Two volunteers were administered 50 mcg. of LSD-25 orally and 50 mg. of 5-HTP intravenously at weekly intervals as follows: one week 5-HTP followed by LSD-25, 15 minutes after the 5-HTP infusion was completed; and one week LSD-25 followed by 5-HTP 60 minutes later; and one week LSD-25 alone.

Following this a more controlled study was undertaken, employing four subjects and a double-blind procedure. (The double-blind was not entirely successful, however, since 5-HTP could usually be identified by the G.I. symptoms it produced.) The dose of LSD-25 administered orally, was decreased to 30 mcg. since with this amount, effects are usually just perceptible. Consequently any enhancement of LSD-25 effects by 5-HTP could be more readily discerned. The dose of 5-HTP was increased to 150 mg., administered by slow intravenous drip. (One subject received only 120 mg. because of persistent nausea and vomiting.) LSD was given following completion of the infusion. The subjects were not informed as to what drugs were to be used, but were told that their behaviour might be affected by either of the drugs. Each volunteer received placebo (i.v. saline) plus LSD-25 one week, and 5-HTP plus LSD-25 the following week. Urinary serotonin and 5-HIAA were determined for two consecutive four-hour periods beginning at the start of the i.v. infusions (Table I).

Table I

Urinary Excretion of 5-HIAA and Serotonin Following 5-HTP or Saline Administration
(Subjects received LSD-25 in combination with 5-HTP or saline on each occasion)

Subject	Date	Drug	Dose	Urinary Serotonin* 1st Period	Urinary Serotonin* 2nd Period	Urinary 5-HIAA* 1st Period	Urinary 5-HIAA* 2nd Period
A ..	11/3/58	5-HTP	120 mg.	↑	↑	↑	↑
	18/3/58	Saline	200 cc.	Norm	Norm	Norm	Norm
B ..	11/3/58	Saline	200 cc.	Norm	Norm	Norm	Norm
	18/3/58	5-HTP	150 mg.	↑	↑	↑	↑
C ..	11/3/58	Saline	200 cc.	Norm	Norm	Norm	Norm
	18/3/58	5-HTP	150 mg.	↑	—	↑	—
D ..	11/3/58	5-HTP	150 mg.	↑	↑	↑	↑
	18/3/58	Saline	200 cc.	Norm	Norm	Norm	Norm

* Although quantitative determinations of urinary serotonin and 5–HIAA were done, the results are not listed quantitatively because of technical difficulties which led to minor inaccuracies in the results.

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After completion of these studies, one of the four subjects who appeared to have a psychological reaction to 5-HTP was administered a larger dose (200 mg.) of 5-HTP intravenously, and observed clinically (see Results).

Results

All four subjects in the controlled study had a significant rise of both serotonin and 5-HIAA excretion after 5-HTP administration. No G.I. effects from 5-HTP were reported by either of the subjects used in the pilot study. Among the other four subjects, two vomited and a third experienced nausea. Regarding the effects of 5-HTP on the LSD reactions; both subjects in the pilot study reported that the LSD reaction was slightly stronger when 5-HTP was given. The experimenters could see no significant difference, however. Among the other four subjects, two reported slightly stronger LSD reactions from the LSD-5-HTP combination, while the other two reported slightly more severe reactions from LSD-placebo. The experimenters could not distinguish between the LSD-5-HTP and LSD-placebo combinations except by the nausea produced by 5-HTP.

One volunteer (C) during placebo i.v. (before LSD administration) noted some difficulty in concentration (reading, Serial 7 subtraction), felt "woozy", and had twitching of his left index finger. These effects lasted approximately 45 minutes. Another subject reported mental effects during 5-HTP administration. These consisted of restlessness, difficulty in concentrating, and a mild feeling of timelessness.

As mentioned above, the volunteer who had mental effects following the 5-HTP infusion was again administered 5-HTP intravenously. Nausea was reported early after the start of the experiment, but this was controlled by slowing the rate of the infusion. After 180 minutes, a total dose of 200 mg. of 5-HTP was administered. The infusion was stopped because of persistent nausea and imminent vomiting. The subject again noted "dizziness", restlessness, impairment of concentration and in addition reported "after images". These changes were entirely subjective. Within one hour after the infusion was completed all psychological symptoms had abated, and did not recur. A blood pressure increase was observed fifteen minutes after the termination of the intravenous drip. The highest blood pressure recorded was 170/110 at this time, gradually decreasing to control levels (130/80) three hours later. No electrocardiographic abnormalities occurred.

Discussion

Because of the limited number of subjects included in the study only tentative conclusions can be reached. The results indicate, however that 5-HTP has little or no effect on the qualitative or quantitative aspects of the LSD reaction, at least in the doses employed. As noted above, it has been shown that 5-HTP is converted to serotonin in the brain. In view of this, these results further weaken the hypothesis that LSD produces its psychological effects via some type of interaction with serotonin or its receptors in the brain.

The one subject who reported subjective psychological effects from 5-HTP alone is worthy of comment. We have observed several other subjects who were not included in this study who also reported mild, transient effects of the same sort from 5-HTP, but not from placebo controls. Since such effects are sporadic —subjective and fleeting—it is difficult to determine whether or not one is dealing with a "will-o-the-wisp" or some genuine effects of the drug. (It will

be noted that one subject reported similar effects from the saline control.) The question probably could be answered, if larger doses of 5-HTP could be given. This might be accomplished if a substance were found which would block the peripheral, but not the central effects of 5-HTP and/or serotonin. For the time being, the matter is open to conjecture.

Summary

Six subjects (2 in a pilot study and 4 in a later study) were given doses of LSD-25 in combination with 5-hydroxytryptophan (5-HTP), a serotonin precursor, which raises brain serotonin levels. Saline placebo was also administered on separate occasions as a control for the effects of 5-HTP. Urine samples were collected for determinations of serotonin and 5-hydroxy-indoleacetic acid (5-HIAA). The chief purpose of the study was to test the hypothesis that 5-HTP by increasing brain serotonin levels would alter the severity of the LSD reactions. Secondarily, the authors were interested in observing any possible psychological effects of 5-HTP itself.

The results were as follows:

1. Serotonin and 5-HIAA levels in the urine increased in each case following 5-HTP.

2. 5-HTP was not observed to produce any definite or consistent effects on LSD reactions.

3. One subject reported vague psychological effects from saline placebo, and another reported mild psychological effects from 5-HTP.

The need is indicated for further studies to elucidate the possible psychological effects of 5-HTP.

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