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CHEMICALLY-INDUCED ALTERATIONS IN THE BEHAVIORAL EFFECTS OF LSD-25

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Abstract—When LSD-25 is administered to rats trained to press a bar for food, a dose-dependent period of no responding occurs. After pretreatment with reserpine, tetrabenazine, or tranylcypromine and after recovery from behavioral effects of these compounds, the response to ED_{90} or threshold doses of LSD-25 was markedly enhanced. Such effects were correlated with a change in levels of brain serotonin and norepinephrine; i.e., they occurred 5 hr after tetrabenazine when the amines were only 12% to 14% below normal. Fifteen mg benzquinamide/kg affected neither the LSD-25 response nor the levels of amines, whereas 30 μg chlorpromazine/kg attenuated the effect of 130 μg LSD-25/kg. The relationship of these findings to mechanisms regulating brain amines is discussed.

SOME psychoactive drugs are known to influence the level and distribution of biogenic amines such as serotonin (5-HT) or norepinephrine (NE) in the brain.[1–6] The extent to which drug-induced behavioral and physiological effects are mediated through such action is not clear. With LSD-25, the onset of autonomic, behavioral, and EEG changes in the rat is correlated with a change in the level and distribution of amines, but it is not known whether a shift in amines is both necessary and sufficient to account for these results.[7] A link between drug response and mechanisms governing binding and release of 5-HT and NE seems plausible in the case of reserpine where biochemical changes as well as behavioral and autonomic disturbances persist beyond the period in which the drug can be detected.[1, 8–10]

The following experiments were undertaken to determine whether alterations in the status of brain amines such as those observed after reserpine would influence the behavioral effects of LSD-25. While these studies cannot prove a causal relationship, they might demonstrate a correlation between parameters of amine metabolism and behavioral changes induced by LSD-25.

The behavioral effects of LSD-25 in rats trained to press a bar for food were observed, and the levels of 5-HT and NE in brain were later altered by pretreatment with tranquillizing agents—i.e., reserpine, tetrabenazine (Nitoman), benzquinamide (Quantril), chlorpromazine, or with the monoamine oxidase (MAO) inhibitor, tranylcypromine (Parnate). After evidence of recovery from the behavioral effects of these compounds, rats were again tested with LSD-25 so that the action of the drug before and after pretreatment was compared in the same animals. Most of the drugs were selected because they can alter the level and distribution of brain amines. Tetrabenazine and benzquinamide were selected because they are chemically related but are reported to have different effects on the levels of amines.[11–14] Chlorpromazine

(CPZ) was selected because, in small doses, it can apparently block certain behavioral effects of LSD-25.[15]

METHODS

Subjects. The subjects in both the behavioral and assay experiments were male albino rats of Sprague–Dawley strain obtained from Holzman Co., Madison, Wis. These animals had received neither drugs nor behavioral training before the beginning of the investigation. They were maintained on a diet of moderately restricted food intake consisting of about 10 g of Wayne Laboratory Chow in addition to whatever liquid food they obtained during experimental sessions. Water was always available in individual home cages. They were tested after they weighed at least 250 g. The animals thus were not acutely or chronically starved and continued to gain weight throughout the course of the experiments.

Apparatus. The apparatus consisted of two commercially built chambers (R. Gerbrands Co.) of identical construction. A single lever was located in the center of one side of each box, and a translucent panel through which a house light appeared during experimental sessions was mounted directly above the lever. A force of 10 to 15 g activated the lever, and a dipper delivered 0·05 ml of a liquid diet consisting of sweetened milk, eggs, and vitamins into a feeding cup to the right of the lever.

The experimental chambers were housed in light- and sound-attenuated boxes. All experimental contingencies were programmed automatically by relay and timing circuits in an adjoining room. Responses were recorded on electromagnetic counters and cumulative recorders.

Procedures

Biochemical studies. The effects of tetrabenazine, benzquinamide, and reserpine upon brain 5-HT and NE were studied on 180-day-old rats. In this laboratory, neither weight nor diet influences the control values of the amines. Norepinephrine and 5-HT were assayed in the same brain by fluorescence spectrometry with the method of Mead and Finger.[16] There were no differences in brain weight attributable to the experimental conditions. Control rats were assayed with each group of drug-treated rats. The data were expressed as millimicrograms of amine per gram wet weight, from which statistical evaluation and percentage change were calculated.

Reserpine (from ampules supplied by Ciba) was administered so that rats received a total of 1·4 mg of the drug/kg over a period of four days. On days 1 and 2, 0·2 mg of the drug/kg was administered i.p., and 0·5 mg reserpine/kg was given on days 3 and 4. Control rats received equivalent volumes of physiological saline during the four treatment days, and all animals were sacrificed 96 hr after the termination of drug administration.

With tetrabenazine (TBZ), 5 mg was suspended in 2·5 mg cellulose for each ml of distilled water. Twenty-eight rats were given an i.p. dose of 4 mg/kg. Control rats were given injections of the same suspension without drug. Seven experimental and two or three control animals were sacrificed together at 1, 5, 8, and 16 hr after treatment. These procedures were repeated with a dose of 20 mg TBZ/kg. A suspension of benzquinamide was prepared in the same manner, and 15 rats were given 15 mg benzquinamide/kg and sacrificed along with controls treated with an equivalent

volume of the cellulose medium; the rats were sacrificed at 1, 5, and 8 hr after treatment. Fifty mg benzquinamide/kg was given to eight additional rats which were sacrificed along with the controls at 1 or 8 hr after i.p. injection.

Behavioral studies. Subjects for the behavioral studies were always run 7 days per week, 30 min per day. The animals were trained to press a lever such that every thirtieth response was followed by 3-sec access to liquid food (FR 30). Experiments were begun only after many weeks of stable performance on the fixed-ratio schedule. Dose and time parameters were selected on the basis of preliminary studies in our laboratory and previously reported results. Drugs or equivalent volumes of control solutions (isotonic saline or cellulose) were given i.p. either immediately before an animal's daily session or at times specified below. Each animal served as its own control; at least 10 days of FR 30 without drugs were interspersed between treatments. This interval was sufficient to permit recovery from any effects on the amines induced by LSD-25 and to insure loss of any tolerance that might be induced by the drug. Two doses of LSD-25 were used; a threshold dose of 40 μg/kg or 130 μg/kg. The higher dose was found to produce a reliable effect on fixed-ratio schedules[17] in 90–100% of rats of this strain. Since dose and time parameters necessarily differ according to the specific compounds tested, such details will be summarized as the results are presented.

RESULTS

Biochemical experiments

The effects of various doses of tetrabenazine, benzquinamide, and reserpine on brain 5-HT and NE assayed with fluorescence spectrometry are shown in Table 1.

TABLE 1. EFFECTS OF TRANQUILLIZING DRUGS ON BRAIN 5-HT AND NE

Hours after treatment		Tetrabenzaine (4 mg/kg) M*	S.D.	Tetrabenzaine (20 mg/kg) M	S.D.	Benzquinamide (15 mg/kg) M	S.D.	Benzquinamide (50 mg/kg) M	S.D.	Reserpine (1·4 mg/kg)† M	S.D.
1	5-HT	86	10	51	7	99	5	94	6		
	NE	93	10	51	7	99	1	87	9		
		N = 7		N = 4		N = 3		N = 4			
5	5-HT	88	5	66	4	90	4	105	2		
	NE	86	8	58	7	98	5	88	22		
		N = 7		N = 4		N = 7		N = 7			
8	5-HT	104	5	73	16	98	1	109	3		
	NE	97	7	67	13	100	5	101	5		
		N = 7		N = 7		N = 3		N = 4			
16	5-HT	99	8	92	8						
	NE	102	6	79	24						
		N = 7		N = 7							
96	5-HT									59	2
	NE									39	4
										N = 3	

* M = Mean per cent of control level: control values of 50 rats for HT, 530μg/g (S.D. 42); NE, 525 μg/g (S.D. 47). S.D. = Standard deviation. N = Number of animals tested.

† In four daily doses; 0·20 mg/kg on days 1 and 2, 0·5 mg/kg on days 3 and 4

The mean per cent depletion at various hours after injection, the standard deviations of these measures, and the number of animals tested at each dose and time are indicated.

Both tetrabenazine and reserpine reduced the levels of the two biogenic amines in the brain. An analysis of variance of the levels of both 5-HT and NE following tetrabenazine revealed that dose, hours after injection, and the interaction of dose and hours were all significant ($P < 0{\cdot}001$). Figure 1 shows graphically how the average level of amine depletion is functionally related to dose and to time.

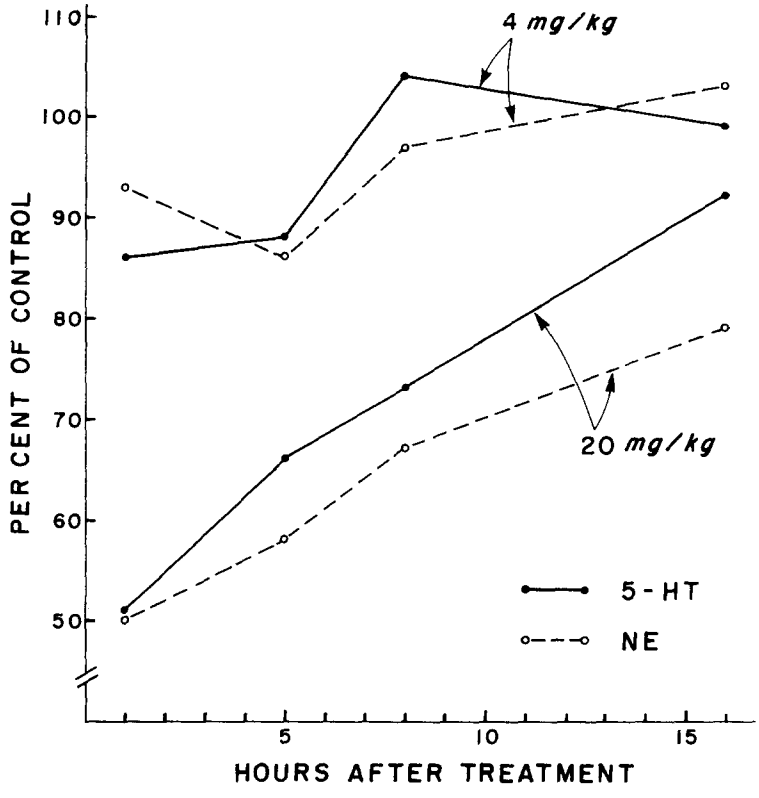

FIG. 1. Mean relative concentrations of 5-HT and NE in brain as a function of time after two doses of tetrabenazine.

Since the effect of altered amine levels on the behavioral response to LSD-25 was measured 5 hr after tetrabenazine or benzquinamide, and 96 hr after reserpine, it is important to know whether the amine levels are, in fact, reliably lower than controls at these times. Five hr after 4 mg TBZ/kg, 5-HT and NE levels are depleted to 86–88% of normal (Fig. 1). These relatively small changes are significant, as indicated by 't' tests ($P < 0{\cdot}01$ for both 5-HT and NE). On the other hand, benzquinamide has no effect on levels of brain amines in any of the doses tested (Table 1). Levels of 5-HT and NE are significantly below controls ($P < 0{\cdot}01$) 96 hr after reserpine.

Behavioral studies

Dose–effect relationships of LSD-25 on various fixed-ratio schedules have been described in detail elsewhere.[17] The drug induces a dose-dependent period of no responding. Typically, 40μg LSD-25/kg produces little or no disruption of bar pressing (record B, Fig. 2). Since an animal on FR generally responds at its normal

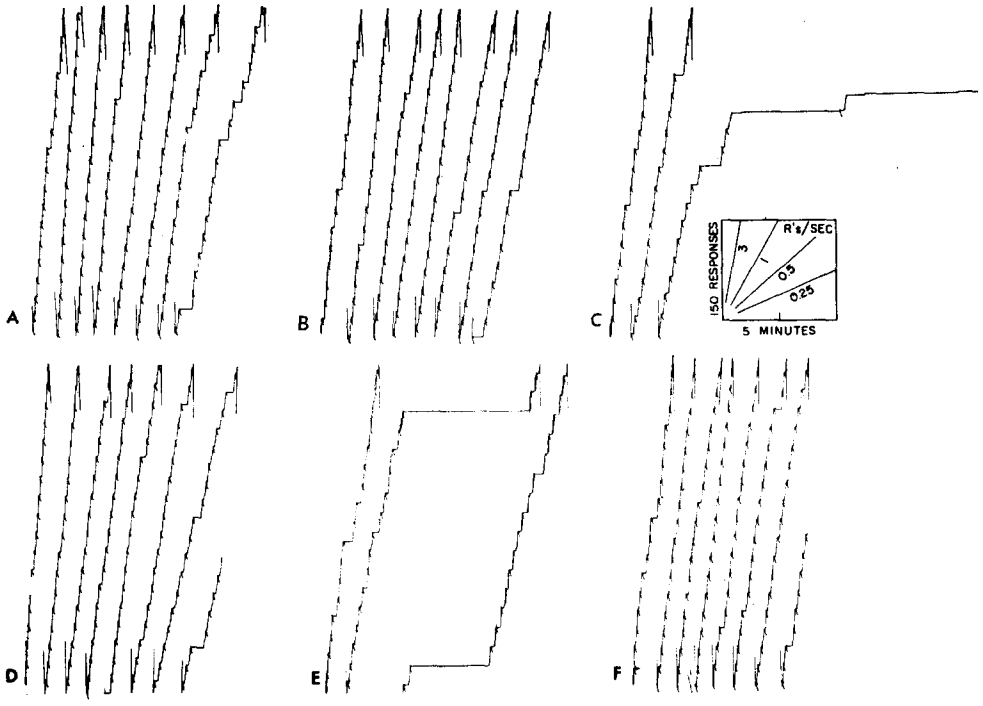

FIG. 2. Cumulative response curves for a single rat working for food on FR 30. Records A–F are described in the text. The slopes of all these curves are proportional to the animal's rate of response and vertical 'pips' indicate reinforcements. Successive segments were compressed to save space.

rate or not at all after LSD-25, the average rate for each 30-min session reflects the length of the period of no responding. The rate for 12 animals treated with 40 μg LSD-25/kg was 122 responses per minute (R's/min; Table 2). Sixteen rats which were given an equivalent volume of isotonic saline had an average rate of 128 R's/min. This difference in rates is clearly small and not significant.

In contrast to 40 μg LSD-25/kg, 130 μg/kg produces a period of no responding which lasts for about 20 min.[17] For 16 rats given 130 μg, the average rate was 34 R's/min (Table 2).

Reserpine. Six rats trained on FR 30 were given 40 μg LSD-25/kg immediately prior to a 30-min session. After at least ten days of no drug administration, the animals were given a total of 1·4 mg reserpine/kg in five days of chronic treatment. Record C in Fig. 2 shows the depressed performance immediately after the fifth daily dose of reserpine. At this time, ptosis and the parasympathetic effects usually observed with this drug (miosis, diarrhea, etc.) were in evidence. The animals were run on FR for the next three days during which saline injections were given. Their behavior gradually

returned to normal by the third day after termination of reserpine treatment (record D, Fig. 2). In general, ptosis and signs of parasympathetic stimulation were frequently observed *prior* to any change in objective measures. With the onset of measurable behavioral effects, the ptosis syndrome was almost invariably present but disappeared *before* recovery from objective behavioral effects. Forty μg LSD-25/kg was given

TABLE 2. EFFECT OF ALTERED AMINE MECHANISMS ON LSD-25*

Treatment	Mean rate (R's/min)	S.D.	N	t'_d	P
NaCl	128	50	16		
40 μg LSD-25/kg	122	36	12†		
40 μg LSD-25/kg (5 hr post 15 mg Benz/kg)	89	36	6	0·29	n.s.
40 μg LSD-25/kg (5 hr post 4 mg TBZ/kg)	32	30	6	3·62	$<$0·02
40 μg LSD-25/kg (4 days post 1·0 mg res/kg)	41	17	6	6·73	$<$0·01
130 μg LSD-25/kg	34	22	16†		
130 μg LSD-25/kg (6 hr post 1·5 mg tran/kg)	2	1·5	4	4·07	$<$0·02
130 μg LSD-25/kg (1 hr post 30 μg CPZ/kg)	88	59	8	2·73	$<$0·05

* Abbreviations: Benz = benzquinamide; TBZ = tetrabenazine; res = reserpine; tran = tranylcypromine; CPZ = chlorpromazine; R's/min = responses per minute. t'_d = Value of 't' test for repeated measures on same subject; each t'_d based upon its own appropriate control. n.s. = Not significant.
† Pooled over independent subjects.

96 hr after the final reserpine injection, and the enhanced effect of pretreatment on the response to this dose of LSD-25 is shown in record E of Fig. 2. The average rate during this treatment with 40 μg LSD-25/kg was only 41 R's/min (Table 2) which is significantly lower than 122 R's/min on the first LSD-25 day ($P < 0{\cdot}01$). Behavior was normal when saline was given on the fifth day after reserpine (record F, Fig. 2). The same rats showed no differences during the first and second treatments with 40 μg LSD-25/kg when saline was given in place of the four reserpine treatments.

Tetrabenazine and benzquinamide. Six rats previously tested with 40 μg LSD-25/kg were later given 4 mg TBZ/kg or an equivalent volume of the cellulose control. This dose of tetrabenazine was found to reliably induce a depression of bar pressing, which disappeared at 5 hr.[18] The rats were nevertheless run for 10 min, 5 hr after either tetrabenazine or control injection, to determine whether or not the behavioral effects of the pretreatment compound had terminated. No impairment of responding was observed during these 10-min control periods. Forty μg LSD-25/kg was then given, and the animals pretreated with tetrabenazine showed an enhanced response to LSD-25, i.e., rates following LSD-25 after tetrabenazine pretreatment were significantly lower ($P < 0{\cdot}02$) than rates after cellulose pretreatment (Table 2). Several rats were also given 40 μg LSD-25/kg at 10, 15, and 24 hr after 4 mg TBZ/kg. At these times, amine levels were roughly normal (Fig. 1), and no changes in the LSD-25 effect were observed. There thus appears to be a period at about 5 hr after pretreatment with tetrabenazine when amine levels are altered and the response to LSD-25 is enhanced.

Another group of six rats previously tested with 40 μg LSD-25/kg was pretreated 10 days later with 15 mg benzquinamide/kg or cellulose and tested according to the same procedure as that used with tetrabenazine. While the pretreatment compound did induce disruptions in behavior, these effects were dissipated by the time the animals were exposed to the 10-min control period (5 hr after pretreatment). After 40 μg LSD-25/kg, bar pressing rates were lower after benzquinamide pretreatment than after cellulose, but this difference was not significant ($P < 0{\cdot}05$).

Tranylcypromine. Four rats were given 130 μg LSD-25/kg 6 hr after pretreatment with 1·5 mg tranylcypromine/kg or saline controls (when response rates were normal). Table 2 shows that the MAO inhibitor significantly prolonged the response to LSD-25 ($P < 0{\cdot}02$).

Chlorpromazine. Eight rats which previously had shown a reliable response to 130 μg LSD-25/kg were treated with 30 μg CPZ/kg. This dose of the phenothiazine had no observable effects on behavior. The animals were given an injection of 130 μg LSD-25/kg 1 hr after pretreatment with chlorpromazine and run on FR 30. The average rate of bar pressing (Table 2) was significantly higher after LSD-25 when the rats had been pretreated with chlorpromazine than when the same animals were similarly pretreated with saline ($P < 0{\cdot}05$). It was therefore concluded that a low dose of chlorpromazine can attenuate the response to LSD-25 in a free-responding situation; Ray and Marrazzi reported similar results with the same dose of chlorpromazine when LSD-25 effects were measured on the latency of drinking behavior.[15]

DISCUSSION

These experiments demonstrate that prior treatment with reserpine, tetrabenazine, or tranylcypromine prolongs the period of no responding induced by LSD-25 on a fixed-ratio schedule of food reinforcement. A low dose of chlorpromazine attenuates the same effect, and benzquinamide does not alter the response to LSD-25. Although residual microquantities of the pretreatment compound could be present, the likelihood that these results are contingent upon an interaction of two exogenous compounds was minimized by allowing behaviour to recover to base line levels between drug administrations. With reserpine and tetrabenazine the characteristic array of autonomic effects had largely dissipated by the time FR behaviour returned to normal, but the level of biogenic amines in the brain was still significantly depressed. It was during this time that altered response to the test dose of LSD-25 was observed. These facts and the results with the MAO inhibitor suggest that the enhanced behavioral response to LSD-25 is contingent upon drug-induced shifts in the status of amines (or on mechanisms regulating their normal equilibria) rather than upon any predominant interaction of two drugs.

Alteration of the status of brain amines similarly produces enhanced effects in human subjects given LSD-25 48 hr after a single dose of reserpine.[19] Aprison and Ferster[20] showed that a change in the behavioral response of pigeons to 5-HTP occurs throughout the period of MAO inhibition induced by 150 mg iproniazid/kg; after 5-HTP, changes of 5-HT levels in specific brain areas correlated with behavioral changes.[21] In human subjects, the effects of tryptophan were enhanced after pretreatment with iproniazid,[22] and in mice the motor effects of amphetamine were increased during the phase of repletion following reserpine.[23]

The question arises as to what order of magnitude of change in amine levels could conceivably be of biological significance. With reserpine, a 50% depletion of 5-HT and with tetrabenazine, a 12–14% depletion (80 mμg/g or less) are correlated with enhancement of the effects of a threshold dose of LSD-25. The ED_{90} dose of LSD-25 on FR 30 (130 μg/kg) induces a rise of approximately 20% in 5-HT levels. The onset of these effects on behavior and on 5-HT are correlated, but increasing the dose of LSD-25 does not lead to a proportional increase in the level of 5-HT in brain. Recent studies in this laboratory show that the fixed-ratio effects of LSD-25 occur and continue largely during the period of drug-induced elevation of 5-HT—i.e., as total levels are increasing. Similarly, in the present study, the disappearance of sedative effects of reserpine was not reflected in large changes in levels of 5-HT, nor was the appearance of sedative effects in tryptophan-deficient animals given reserpine correlated with a change in level of 5-HT.[24] Thus, statistical correlation of amine level and behavioral change does not describe biological mechanisms; neither does the order of magnitude of change in total levels (a consideration which has not vitiated interest in the role of acetylcholine in brain). Many relevant biochemical factors in addition to large or small changes or level have been suggested by experiments with LSD-25.[25]

Two classes of phenomena are to be explained: the role of amines in regulating the sequence of responses to particular patterns of central excitation and the role of amines or related compounds in inducing such excitation. This study and those cited indicate that once excitation is induced by an appropriate agent, the subsequent pattern of general effects can be enhanced if the amine-regulating mechanisms are altered. Under normal conditions, the amines may regulate patterns of response to an average unexpectable range of central excitations, but there is no direct evidence to support such a hypothesis. Certain unusual nonpharmacological conditions which activate and mobilize the rat (e.g., a cold swim)[7, 26] induce changes in amines that correlate with onset of and recovery from the induced exhaustion. Similarly, certain drugs such as LSD-25 which alter amines produce a pattern of central excitation. There is, however, no clear-cut evidence in these studies to indicate whether or not it is the amines that directly initiate the pattern of physiological excitation which, in turn, could induce further behavioral and biochemical changes.

With 30 μg CPZ/kg, no behavioral changes were evident, but the response to 130 μg LSD-25/kg was attenuated. The relationship of this effect of chlorpromazine to the level and distribution of amines is not clear. Small doses are said to block the action of serotonin in isolated organs,[27] but the mechanism is not clear and no effects of 30 μg CPZ/kg on levels of 5-HT or on LSD-25-induced binding of 5-HT could be reliably measured in brain with fluorescence spectrometry.[7] Doses above 2 mg/kg regularly induced a rise of 30–40 mμg/g in brain 5-HT;[7] this result may be related to a drug-induced decrease in permeability which has been demonstrated in several test systems.[28] Recent studies in this laboratory show that doses of 2–20 mg CPZ/kg do not influence the uptake of LSD-25 in the brain. Thus, the mechanism by which 30 μg CPZ/kg might affect either brain amines or the behavioral response to 130 μg LSD-25/kg cannot be specified. It is possible that this small dose of chlorpromazine causes an adrenergic blockade in local areas of brain. In this respect it is of interest that, among the autonomic changes induced by LSD-25, the sympathetically mediated responses are those that show tolerance.[29]

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