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PRELIMINARY NOTE

BEHAVIORAL REACTION OF RATS PRETREATED WITH RESERPINE TO LSD-25

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Summary—Behavioral patterns and brain monoamine levels were studied with rats receiving either a combination of 0·1–2·0 mg/kg of LSD and 5 mg/kg of reserpine 20 hr before LSD-25 or of 1 mg/kg of reserpine given for 7 days, the last dose again preceding LSD-25 by 20 hr. With the lower doses of LSD-25 only the blepharospasm and sedation induced by reserpine were overcome; with the higher doses of that drug, the rats reacted with excitement to the point of convulsions, became aggressive and hostile and also vocalized. Two hours after the administration of LSD-25 (1 mg/kg), some of the rats were killed for brain monoamine determinations. Both with the acute and chronic administration of reserpine the brain levels of norepinephrine, dopamine and serotonin were depleted. LSD-25 elevated serotonin and decreased norepinephrine and dopamine brain levels. High doses of the non-hallucinogenic methysergide, given after reserpine, evoked behavioral changes similar to those of the lowest doses of LSD-25 in counteracting the reserpine-induced sedation but not the excitement.

MORPURGO and THEOBALD (1966) have reported a peculiar behavioral pattern induced by the administration of amphetamine in rats pretreated with reserpine: enhanced motor activity and increased social interaction. We performed similar behavioral experiments but used LSD-25 in doses from 0·1–2·0 mg/kg i.p. in rats pretreated with reserpine either after a single administration of 5 mg/kg s.c., or after 7 days of 1 mg/kg of reserpine. LSD-25 was given 20 hr after the last dose of reserpine. We also examined the non-hallucinogenic drug methysergide, which acts like LSD-25 peripherally though not necessarily centrally. Female albino rats ranging in weight from 140–180 g were kept in groups of three, confined in stainless steel cages. They were isolated in a small room with a constant temperature of 24°C, where the behavioral observations were made. Three rats with different pretreatments were studied simultaneously after placing them on a metal tray, 35 cm wide × 55 cm in length and 5 cm in depth. Their reactions to simple stimuli, such as touching with a pencil or a finger, were checked at 5 min intervals following the administration of LSD-25 in the same dose. Of these three animals one was pretreated 20 hr before with one dose of 5 mg/kg reserpine, the second with seven daily doses of 1 mg/kg reserpine, and the third control rat received saline solution. In some instances rectal temperature was measured, the thermometer being inserted in the rectum for 1 min. All drugs were dissolved in distilled water and injected in volumes that were kept constant. Reserpine phosphate was injected i.m. in a volume of 1 ml/kg, LSD-25 tartrate or methysergide bimaleate, each 5 ml/kg i.p. Control rats received equal volumes of saline solution administered as above. Brain norepinephrine, dopamine and serotonin were measured in some animals.

Two hours after the administration of LSD-25 (1 mg/kg i.p.), the rats were decapitated, the brains were removed, weighed and homogenized in ice cold 0·4 perchloric acid using a Branson sonifier. Norepinephrine was estimated according to the method of BERTLER *et al.* (1958). Dopamine was estimated according to CARLSSON and WALDECK (1958) as modified by CARLSSON and LINDQUIST (1962). Serotonin was estimated by the method of BERTLER (1961) as modified by CARLSSON and LINQUIST (1962).

RESULTS

The results following the administration of 1 mg/kg of LSD-25 are summarized in Table 1. The behavioral activity induced in rats pretreated with reserpine consisted of increased motor activity and exploratory behavior. The lower doses of LSD-25 (0·1 mg/kg) antagonized only the reserpine-induced blepharospasm and sedation. This antagonism appeared rapidly and lasted from 40–60 min. LSD-25 doses of 0·5–1·0 mg/kg and especially of 2 mg/kg rapidly induced a peculiar reaction pattern in rats far different from that of LSD-25 without reserpine premedication. The animals showed a greatly decreased threshold to peripheral stimulation, were ceaselessly in motion, sometimes circling to one side and sometimes to the other, were aggressive, hostile and vocalized. In contrast, in the doses of LSD-25 used in rats pretreated with saline solution, the threshold was comparatively higher to peripheral stimulation; moreover, they were not aggressive, but were hyperactive though noticeably less so than the rats receiving the combination. None of the latter group vocalized.

In rats receiving reserpine chronically, by the seventh day of treatment spontaneous behaviour had returned almost to normal and only some blepharospasm remained. The administration of LSD-25 promptly eliminated the blepharospasm and evoked behavioral changes similar to those of the rats injected with one dose of reserpine only; the aggressiveness was substantially lower however. Methysergide (2 mg/kg i.p.) also antagonized blepharospasm and sedation but produced none of the other behavioral changes noticed with LSD-25. The rectal temperatures after LSD-25 did not seem to change significantly.

Biochemical determination of brain serotonin, norepinephrine and dopamine, performed in rats killed two hours after the administration of LSD-25 (1 mg/kg i.p.), showed that the concentration of serotonin in the brain was elevated, whereas those of norepinephrine and dopamine were diminished. This is in agreement with previous findings of FREEDMAN (1960, 1961) and FREEDMAN and GIARMAN (1962). Both the elevation of serotonin as well as the decrease of norepinephrine were statistically significant. In the rats pretreated with one dose or seven daily doses of reserpine, the levels of serotonin and especially those of norepineprine and dopamine were greatly diminished. The administration of LSD-25 did not antagonize the reserpine depletion of serotonin nor potentiate the depletion of norepinephrine and dopamine.

DISCUSSION

Many authors have studied the interaction between the effects of LSD-25 and reserpine. Reserpine enhances the impairment of performance after LSD-25 in hungry rats, climbing up a rope in order to obtain food (WINTER and FLATAKER, 1956), as well as the EEG activation induced by LSD-25 in curarized rabbits (HIMWICH, 1959). According to other authors, LSD-25 abolishes the reserpine-induced prolongation of sleeping time after hexobarbital (SHORE *et al.*, 1955). The biochemical studies of brain serotonin levels have

TABLE 1. EFFECT OF LSD-25 IN ACUTE OR CHRONIC RATS PRETREATED WITH RESERPINE

No. of rats	Reserpine (mg/kg s.c.)	Time interval (hr)	LSD-25 (mg/kg i.p.)	Pharmacology: Time of peak effect (min)	Pharmacology: Behavior pattern	Pharmacology: No. rats	Biochemistry: Brain amines (μg/g fresh tissues) 5-HT	NE	Dopamine
10	5	20	1	15–30	Running, jumping, very touchy, attempt to escape, vocalized, piloerection, aggressive, low threshold to peripheral stimulation	3	0·06±0·01	0·00±0·00	0·03±0·01
10	7 days 1	20	1	20–30	Tremor, decreased motor activity, alternate stamping of forefeet, slight hostility	3	0·04±0·01	0·01±0·00	0·06±0·01
10	None	—	1	15	Slight, tremor, low motor activity, piloerection, not aggressive	4	0·56±0·10	0·24±0·01	0·40±0·13
10	5	20	None	—	Very passive, blepharospasm, cataleptic	4	0·04±0·01	0·01±0·01	0·08±0·02
10	7 days 1	20	None	—	Low motor activity, slight blepharospasm	4	0·03±0·01	0·02±0·01	0·12±0·04
10	Control				Normal reaction	8	0·33±0·05	0·41±0·04	0·90±0·05

*Figures represent average ± SEM. Rats were killed 2 hr after the administration of LSD-25 or saline solution in control trials.

shown that reserpine evokes long-lasting depletion, whereas LSD-25 elevates brain serotonin levels and partly antagonizes the reserpine-induced depletion of that monoamine (FREEDMAN, 1960, 1961, 1963; FREEDMAN *et al.*, 1962, 1964). In contrast to serotonin, norepinephrine was only partially reduced by high doses of LSD-25 (FREEDMAN, 1960). We repeated these biochemical tests in some rats treated with high doses of LSD-25 and were able to confirm the results of FREEDMAN *et al.* (1962, 1964), i.e. an increase of serotonin and a reduction of norepinephrine. Furthermore, we determined the levels of dopamine and found that these levels were also lowered by LSD-25.

However, none of the authors referred to above has described the surprising change in the behavior evoked by LSD-25 in rats pretreated with reserpine. In our experiments, it was necessary to use large doses of LSD-25 to evoke these peculiar and specific behavioral reactions, similar only in the degree of excitement to those described by MORPURGO and THEOBALD (1966) in rats receiving the combination of reserpine and amphetamine. The social interaction was notably absent with the combination of reserpine and LSD-25.

Changes in the effect of LSD-25 after pretreatment with reserpine have been described for human beings (ISBELL and LOGAN, 1957). After the pretreatment with relatively high dose of reserpine (5·0–7·5 mg p.o. or i.m.) the administration of LSD-25 evoked very unpleasant and unusual hallucinations (hissing sound in the back of the head culminating in an expanding flash of light). The neurological and autonomic effects of tremor and nausea respectively were also enhanced. In our review of the literature we did not find studies of drug-induced changes on behavior as modified by LSD-25. It is surprising that in our experiments, rats chronically pretreated with reserpine, LSD-25 did not provoke the peculiar changes in behavior seen after the administration of the single dose of reserpine. In considering possible explanations for the divergent behavioral results, we may suggest that though the levels of stored catecholamines and serotonin were practically the same for the single high doses of reserpine as for the lower chronic dose, it is possible that 20 hr after the administration of a single dose of 5 mg/kg of reserpine the level of free amines was higher than that which was obtained after 7 days of chronic pretreatment with 1 mg/kg reserpine. Perhaps the changes in behaviour after the single dose of reserpine are associated with the interactions between the higher levels of free amines and LSD-25 than the lower ones with the more prolonged pretreatment. It is also known that reserpine can evoke localized seizures in rhinencephalic structures and that LSD-25 prolongs such seizures (KILLAM and KILLAM, 1957). Perhaps with repeated doses of reserpine the seizure effect drops out.

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