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SOME OBSERVATIONS ON THE INTERACTION OF RESERPINE AND LSD

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Central actions of reserpine have been attributed to a liberation of 5-hydroxytryptamine (serotonin) (Brodie, Pletscher and Shore, 1955). The following experimental results support such an interpretation:

1) Reserpine decreases the serotonin content of the brain while increasing the urinary excretion of 5-hydroxyindolacetic acid, a major metabolite of serotonin (Brodie, Pletscher and Shore, 1955; Pletscher, Shore and Brodie, 1956; Shore, Silver and Brodie, 1955a).

2) Serotonin has a slight sedative effect and potentiates barbiturate hypnosis; it exerts in this respect an action similar to that of reserpine. A further parallelism between the two drugs is evident in the finding that a potent inhibitor of serotonin, lysergic acid diethylamide (LSD-25), antagonizes not only the potentiating effect of serotonin on barbiturate hypnosis but also that of reserpine (Shore, Silver and Brodie, 1955a, b). Barbiturate-induced hypnosis itself is not inhibited by LSD (Shore, Silver and Brodie, 1955a, b; Taeschler and Rothlin, 1956).

Although both reserpine and serotonin potentiate barbiturate hypnosis, the two drugs manifest profound differences in central actions, notably regarding the pattern of sedation and regarding EEG effects on the rabbit (unpublished data).

BOL-148 (2-brom-lysergic acid diethylamide) inhibits serotonin at least to the same extent as LSD but differs fundamentally in other peripheral and central effects from LSD (Cerletti and Rothlin, 1955; Cerletti, 1956). A comparison of the action of LSD and BOL on the barbiturate-potentiating effect on serotonin and reserpine was undertaken in an effort to answer the question whether the antagonistic effect of LDS on reserpine is actually due to its anti-serotonin effect and whether the potentiating action of the barbiturate hypnosis by reserpine and serotonin is based on a common mechanism.

METHODS. Barbiturate potentiation was assayed in mice of a uniform strain. In all experiments a threshold dose of thiopental (20 mgm./kgm. intravenously), which induced an average sleeping time of 0.7 minutes, was administered; maximal sleeping time observed after this dose of thiopental was 1.3 minutes. Following pretreatment with the potentiating substances (serotonin and reserpine) alone or together with the inhibitors (LSD and BOL), the hypnotic effect of 20 mgm./kgm. thiopental was determined. The degree of barbiturate potentiation was expressed by the percentage of the total number of mice which show a hypnosis of more than two minutes. Thus the results were evaluated by an "all or none" reaction rather than by the average sleeping time. Drug-induced changes of spontaneous motor activity were measured by means of activity cages according to Aschoff's method (Aschoff, 1951), which was slightly modified.

RESULTS. Fig. 1 illustrates the results with LSD and BOL on the serotonin-induced thiopental potentiation. Both compounds show an equal inhibitory

potency with minimal effective doses of 200 microgm./kgm. subcutaneously. Despite equal serotonin antagonism, BOL, however, does not inhibit the reserpine-induced thiopental potentiation (fig. 2). Using a large dose of reserpine the degree of barbiturate hypnosis had to be expressed by the average sleeping time

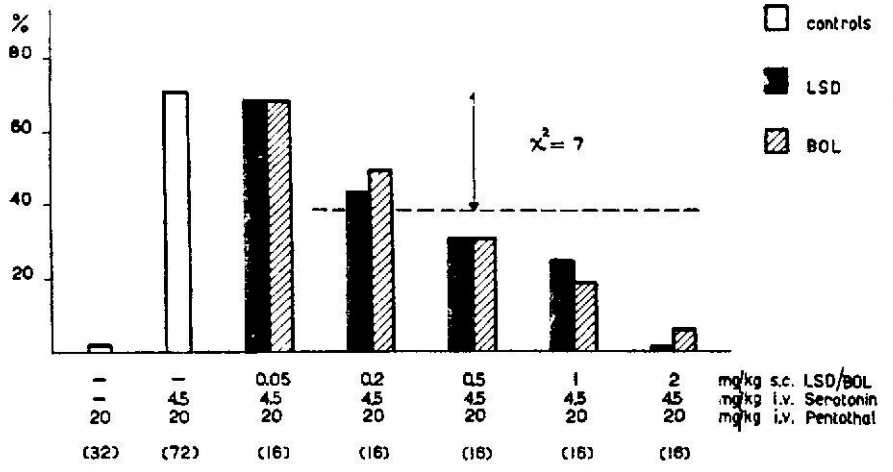

FIG. 1. Effect of LSD-25 and BOL-148 on the potentiation of thiopental by serotonin in mice. Ordinate: percentage of mice which show a hypnosis of more than two minutes. Abscissae: doses of LSD or BOL, respectively, with the corresponding control values. Numbers in parentheses represent the number of animals used with each substance. $\chi^2 = 7$ gives the confidence limit of 99 per cent. LSD and BOL were administered subcutaneously 20 minutes prior to serotonin which was injected intravenously 10 minutes before thiopental.

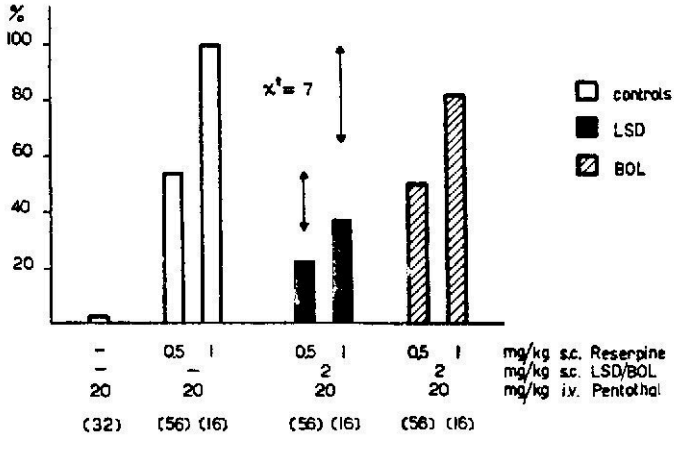

FIG. 2. Effect of LSD-25 and BOL-148 on the potentiation of thiopental by reserpine in mice. Ordinate: percentage of mice which show a hypnosis of more than two minutes. Abscissae: doses of reserpine, LSD or BOL, respectively, and thiopental. Numbers in parentheses represent the number of animals used with each substance. $\chi^2 = 7$ gives the confidence limit of 99 per cent. Reserpine was administered 140 minutes before thiopental. LSD and BOL were injected 120 minutes after reserpine (= 20 minutes before thiopental).

of the mice. Fig. 3 illustrates the pronounced inhibitory effect of LSD on the thiopental potentiation by 2 mgm./kgm. of reserpine. These experiments also suggest that an equal dose of LSD (2 mgm./kgm.) antagonizes larger doses of reserpine more markedly than lower reserpine doses. A dose of 0.7 mgm./kgm. of LSD did not inhibit the potentiating effect of 0.5 mgm./kgm. reserpine. Minimal effective doses of LSD for antagonizing reserpine are, therefore, five to ten times higher than those antagonizing serotonin. Additional experiments showed that the difference between LSD and BOL is not related to the length of time between the administration of LSD (or BOL) and reserpine. These experiments fail to support the assumption that the inhibitory effect of LSD on the reserpine-induced potentiation of thiopental is due to its anti-serotonin activity. It rather appears that this effect of LSD is related to some other mechanism.

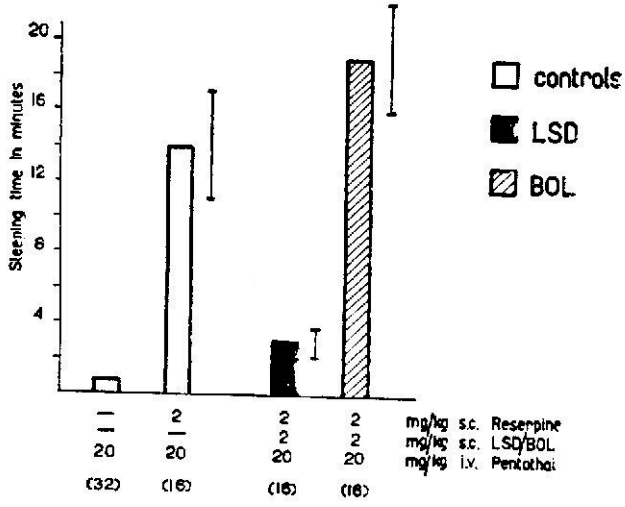

FIG. 3. Effect of LSD-25 and BOL-148 on the reserpine-induced potentiation of thiopental in mice. Ordinate: Average sleeping time in minutes. Abscissae: Doses of reserpine, LSD or BOL, respectively, and thiopental. Numbers in parentheses represent the number of animals. I indicates the standard error. Interval of injections as in fig. 2.

Besides its serotonin antagonism, LSD produces in rabbits a syndrome of central sympathetic excitation consisting of hyperthermia, hyperglycemia, mydriasis, etc. (Rothlin *et al.*, 1956). LSD also elicits in mice a slight stimulation of spontaneous activity, a potentiation of the excitatory effects of amphetamine and a mydriasis which can be inhibited by adrenergic blocking agents. BOL lacks this stimulating action. As a mild sedative it antagonizes the amphetamine-induced excitation and also the mydriatic effects in mice. It does not produce hyperthermia or hyperglycemia in rabbits. The possibility should, therefore, be considered that the antagonistic effect of LSD on the thiopental potentiation by reserpine is not primarily due to its serotonin blocking activity but rather to its central stimulatory effect. Such an assumption is supported by the fact that amphetamine (1 or 3 mgm./kgm. subcutaneously) antagonizes the reserpine-

TABLE 1

Effect of amphetamine on the reserpine-induced thiopental potentiation in mice

Five-tenths mgm./kgm. of reserpine was given subcutaneously in all experiments 100 minutes before amphetamine or saline, which were administered 20 minutes before thiopental (20 mgm./kgm. intravenously).

Number of animals	Dose of amphetamine s.c.	Per cent of mice which show hypnosis of more than two minutes
	mgm./kgm.	
48	—	71*
48	1.0	29*
48	3.0	25
24	Control with thiopental alone	0

* = $\chi^2 > 7$, *i.e.* these values fall outside the 99 per cent confidence limits.

TABLE 2

Effect of LSD-25 (2 mgm./kgm. s.c.) on the activity of mice

Pretreatment	Interval	Total activity per mouse per 30 minutes	Number of animals
	hours		
Reserpine, 5 mgm./kgm. s.c.	20	49	36
Saline = Control	20	7	36

The activity (arbitrary units) was measured 20 minutes after LSD during a period of 90 minutes.

induced thiopental potentiation in the same manner as LSD (table 1) without interfering with the barbiturate hypnosis itself. Since LSD and these low doses of amphetamine do not influence the thiopental hypnosis itself, a specific interference of LDS and amphetamine with reserpine is suggested, namely that reserpine enhances the slight stimulatory effect of LSD or amphetamine. Indeed, pretreatment of mice with a relatively large dose of reserpine administered twenty hours before LSD potentiates greatly the slight excitatory effect of LSD. Table 2 illustrates such results obtained in the activity cage. Amphetamine excitation is not regularly potentiated by reserpine. A slight and short lasting potentiating effect can, however, be obtained if the time of pretreatment is more than twenty hours. This difference could possibly point to a difference in the central site of action of LSD and amphetamine, respectively. The activity of mice receiving BOL is not influenced or further depressed by pretreatment with reserpine.

After the completion of this study, Salmoiraghi *et al.* (1956) reported that BOL inhibits the potentiating effect of serotonin, as well as that of reserpine on hexobarbital hypnosis. The doses of BOL used by these investigators (10 mgm./kgm. i.p.) are considerably higher than the doses used in the present study (2 mgm./kgm. s.c.). In view of the greater toxicity of BOL (LD_{50} = 20 mgm./kgm. i.v. in mice) as compared to LSD (LD_{50} = 67 mgm./kgm. i.v.), high doses of BOL

have been avoided in the present study, and emphasis was put on a comparison of the specific activity of LSD and BOL on serotonin- and reserpine-induced barbiturate potentiation. Thus it can be concluded that BOL has in high doses a certain antagonistic action toward the barbiturate potentiating effect of reserpine, but that it is in this respect much weaker than LSD.

SUMMARY

LSD antagonizes the potentiating effect of reserpine on barbiturate hypnosis in mice. This effect of LSD is probably due to its own central stimulatory action. Amphetamine, likewise, antagonizes the effect of reserpine on barbiturate hypnosis.

The excitatory effects of LSD in mice are markedly enhanced by pretreatment with reserpine.

BOL, an equipotent inhibitor of serotonin which lacks the central stimulatory effect of LSD, does not antagonize the sedative- and barbiturate-potentiating action of reserpine although it blocks the serotonin-induced potentiation of barbiturates to the same extent as LSD.

A common mechanism of action of reserpine and injected serotonin in mice regarding their barbiturate potentiation must therefore be questioned.

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