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INTERACTION OF SUBSTANCE P AND OTHER SMOOTH MUSCLE STIMULANTS WITH LSD-25 ON THE GUINEA PIG ILEUM (1)

BY

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It has been reported (KRIVOY, 1957) that lysergic acid diethylamide (LSD-25), in concentrations of 1 μg/ml or less, potentiated the stimulatory effect of the polypeptide substance P on the isolated guinea pig ileum. Under similar conditions, the effect of histamine was not potentiated. It was speculated that this interaction might have some relationship to the pharmacological actions of LSD-25. It was also demonstrated that LSD-25 was a potent, selective inhibitor of the substance P destroying enzyme from guinea pig brain and this suggested the possibility that the inhibition of this enzyme was probably the mechanism of the potentiation of the effect of Substance P on the guinea pig ileum.

The present study was undertaken to extend the interesting observations of KRIVOY (1957) to other hallucinogens, and to further investigate the mechanism of this potentiation.

METHODS

Isolated guinea pig ileum. Distal strips of ileum were suspended in a 3 ml bath containing oxygenated Tyrode's solution maintained at 35° C. Responses were recorded on a kymograph with an isotonic

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frontal writing lever. The lever magnification and weight, and the lengths of the intestinal strips were kept constant so that strip to strip results were comparable. The time of contact for agonists on the intestinal strips was 1 to 2 minutes with a total time interval of 4 to 5 minutes between responses. Test drugs were added to the organ bath 2 minutes prior to the agonists.

Intestinal substance P destroying enzyme preparation. Acetone-dried powders of distal guinea pig ileum were used as the enzyme source. The powder was stored at 0° C until ready for use, at which time it (2.5 or 10 mg/ml) was ground with Tyrode's solution and centrifuged for 30 minutes at 5° C and 4,000 rev./min.

To test the substance P destroying activity of the intestinal extract, and any modification by LSD-25, the following mixture was prepared and incubated at 37° C in a Dubnoff Metabolic Shaker: substance P (24 units or 6 mg/ml in Tyrode's solution) 0.5 ml; 0.1 or 0.25 ml of the supernatant from the centrifuged enzyme solution; 0.25 ml of an LSD-25 solution (to give a final concentration of 0.1 μg/ml LSD-25) to be tested for inhibition of enzymatic activity, and Tyrode's solution, if necessary, to make a total volume of 1.0 ml.

Enzymatic activity was terminated by placing the incubation mixtures in a boiling water bath for 5 minutes. After cooling, the mixtures were centrifuged to obtain a clear supernatant. Residual substance P activity remaining in the supernatants was determined by bioassay on guinea pig ileum in the presence of atropine (0.1 μg/ml) and tripelennamine (0.5 μg/ml). The standard preparation of substance P solution to be used for the bioassay was carried through the incubation in exactly the same way as the enzyme mixtures, and was also placed in a boiling water bath for 5 minutes following incubation. When testing the effect of LSD-25 on the enzyme, LSD-25 (0.1 μg/ml) was present in the standard substance P solution.

Substance P content of brain tissue. The extraction and estimation of substance P in brain tissue was carried out according to the method of AMIN, CRAWFORD and GADDUM (1954).

Chemicals and substance P standards. The standard preparation of substance P was obtained through the courtesy of Dr. G. F. CARTLAND of the Upjohn Company and contained 4 units/mg. The bradykinin was prepared according to the method of PRADO, MONIER, PRADO and FROMAGEOT (1956). Substance A was prepared according to the method of WALASZEK and HUGGINS (1959).

The drugs used were *d*-lysergic acid diethylamide tartrate (LSD-25), 2-brom-*d*-lysergic acid diethylamide tartrate (BOL-148), acetylcholine bromide, histamine diphosphate, tripelennamine hydrochloride, atropine sulfate, ibogaine hydrochloride, harmine hydrochloride, harmaline hydrochloride, dimethyltryptamine, bufotenine, adrenochrome, mescaline sulfate and N-methyl-3-piperidyl benzilate (JB-336). Concentrations of acetylcholine and histamine are expressed as the base. All other concentrations are expressed as the salts.

Results

The effect of various hallucinogens on the response of the isolated guinea pig ileum to substance P. Figure 1 illustrates the characteristic potentiation, on the isolated guinea pig ileum, of substance P, but not histamine, by LSD-25 (0.1 μg/ml). This confirms the work of Krivoy (1957), who reported this interaction previously. The potentiation was usually maximal with lower concentrations of LSD-25, e.g. 0.1 to 0.2 μg/ml,

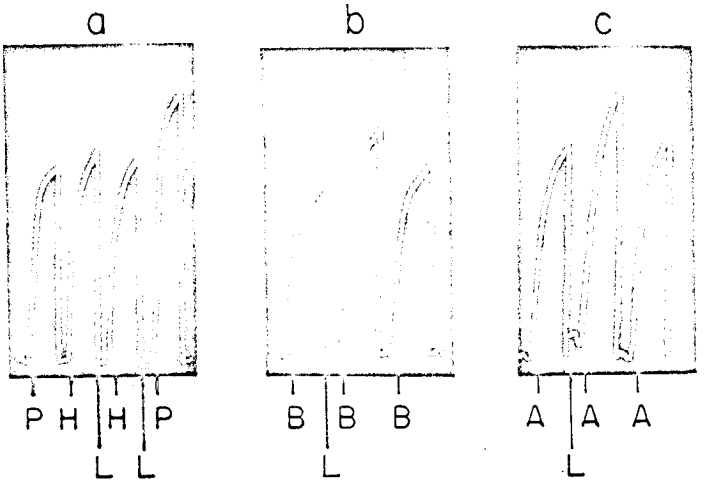


Fig. 1

Isolated guinea pig ileum: the potentiation of responses to substance P, bradykinin and substance A by LSD-25. At P, substance P (0.2 U/ml); at B, bradykinin (50 μg/ml); at A, substance A (3 μg/ml); at H, histamine (0.005 μg/ml); at L, LSD-25 (0.1 μg/ml).

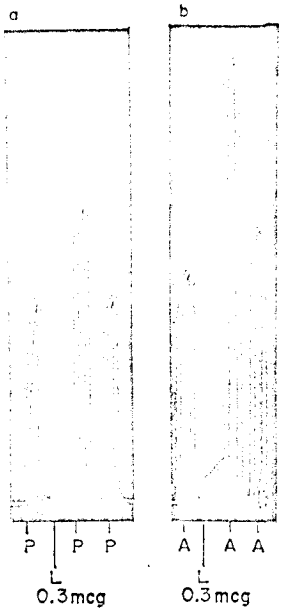
and increasing the concentration to 1.0 μg/ml did not cause increased potentiation. The potentiation of substance P by LSD-25 was confirmed in all 10 experiments performed.

We have also been able to demonstrate that LSD-25 was able to potentiate the stimulatory effect of substance P on the isolated rabbit

intestine. The magnitude of this potentiation was, however, not as large as that seen on the guinea pig ileum. The vasodepressor effect of Substance P on rabbit blood pressure was not potentiated by LSD-25. A slight antagonism to substance P responses was observed in four experiments, however, responses to other agonists were also depressed.

BOL-148 and the following hallucinogens in a wide range of concentrations were tested against the response of the guinea pig ileum to substance P: bufotenine, ibogaine, harmine, harmaline, adrenochrome, dimethyltryptamine, mescaline and N-methyl-3-piperidyl benzilate. Potentiation of substance P was not observed and in most cases slight antagonism was found.

The effect of LSD-25 on the response of the isolated guinea pig ileum to other smooth muscle stimulants. Since potentiation of substance P effects on the guinea pig ileum was not a general property of hallucinogens, a more detailed study of the LSD-25 potentiation was carried out. To test the specificity of the substance P potentiation, LSD-25 was tested against substance A and bradykinin, two pharmacologically active polypeptides which are devoid of central nervous system effects. Figure 1 illustrates the potentiation of bradykinin and substance A, respectively, by LSD-25 (0.1 μg/ml).



The presence of atropine diminished but did not abolish the potentiation of polypeptide responses by LSD. Occluding the ends of the intestinal strip by tying them off did not influence the potentiation. Figure 2 illustrates the potentiation of substance P in an experiment where the ends of the intestinal strip were occluded.

FIG. 2

Isolated guinea pig ileum: The potentiation of responses to substance P and acetylcholine by LSD-25 (conc. present in 3 ml bath). At P, substance P (0.05 U/ml); at A, acetylcholine (0.03 μg/ml) and at L, LSD-25 (0.1 μg/ml).

LSD-25 potentiated the response of the guinea pig ileum to the stimulatory effects of acetylcholine. Quantitatively, LSD-25 evoked a

greater potentiation of acetylcholine than of substance P. Figure 2 illustrates the potentiation of acetylcholine by LSD-25. The threshold concentration of LSD-25 giving potentiation of acetylcholine in the most sensitive preparation was 0.066 μg/ml. As was the case with the substance P potentiation, after reaching maximal potentiation, further increases in LSD-25 did not result in greater potentiation. The maximal potentiations thus obtained were not the maximal contractile responses obtainable from the strips of ileum. The potentiated responses of the polypeptides and acetylcholine were readily reversed by washing the ileum with fresh Tyrode's. At least four experiments were carried out for each of the points mentioned above.

The effect of LSD-25 on the substance P content of rabbit brain. If LSD-25 inhibits the enzyme that destroys substance P it would follow that there should be an increased quantity of substance P in the brain following the administration of LSD-25. Accordingly, the effect of 2 hours pre-treatment with 0.05 mg/kg LSD-25 on the content of substance P in the brain was evaluated in rabbits. BOL-148 which differs from LSD-25 in that it does not have the same psychological effect (CERLETTI and ROTHLIN, 1955), and saline were used as controls. It was found the pretreatment of rabbits with LSD-25 and BOL-148 had no significant effect on the substance P content of rabbit hypothalamus and caudate nucleus. These results are summarized in Table I.

TABLE I

The Effect of LSD-25 and BOL-148 on the Substance P Content of Rabbit Hypothalamus and Caudate Nucleus

Treatment	Dose	No. of Animals	Hypothalamus Units/g ± S.E.	No. of Animals	Caudate Nucleus Units/g ± S.E.
LSD-25	0.05 mg/kg	17	114 ± 17	18	85 ± 13
BOL-148	1.0 mg/kg	9	139 ± 31	9	96 ± 20
Controls		11	116 ± 14	10	89 ± 15

Effect of LSD-25 on the substance P destroying activity of guinea pig ileum. Using the enzyme preparation from guinea pig ileum it was not possible to show any effect of LSD-25 (0.1 μg/ml) on its substance P destroying activity. Incubations were carried out for 2 hours. Pre-

incubation of the enzyme with LSD-25 (0.1 μg/ml) for 15 minutes, without substrate, did not change the results. Figure 3 illustrates the results of two experiments, one with 0.25 mg of enzyme extract equivalent per flask, and the other with 2.5 mg.

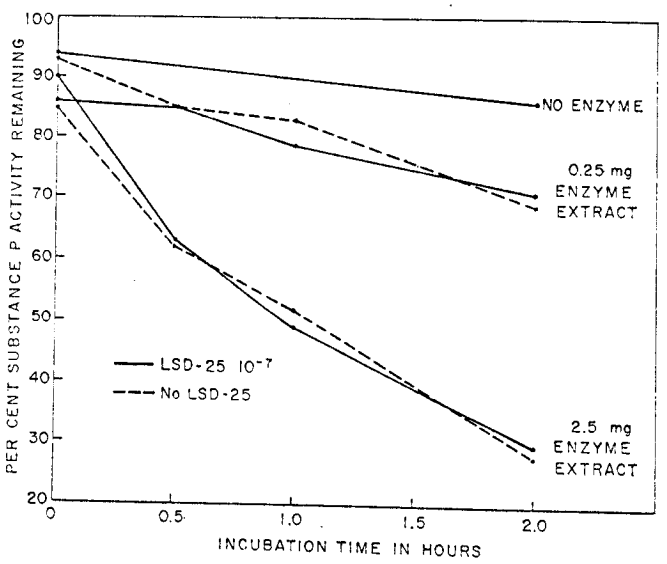


FIG. 3

The effect of LSD-25 on the destruction of substance P by an acetone dried powder enzyme preparation from guinea pig ileum.

DISCUSSION

The following facts seem to mitigate against the proposal that the potentiation of substance P by LSD-25 on the guinea pig ileum and the inhibition of substance P destroying enzymes from guinea pig brain by LSD-25 bear some relationship to the *in vivo* effect of LSD-25. (1) LSD-25 was the only hallucinogen, among 9 tested, which potentiated the response of the guinea pig ileum to substance P. (2) The potentiation of the intestinal response to substance P by LSD-25 was non-specific and extended to two other pharmacologically active polypeptides, bradykinin and substance A, which are devoid of effects on the central nervous system. Acetylcholine effects were also potentiated. (3) LSD-25, had no effect on the concentration of substance P in rabbit hypothalamus and caudate nucleus. (4) Most of the previous experimental work with substance P has shown that its central actions are sedative in nature (ZETLER, 1956; STERN and MILIN, 1959). It would

be unusual, therefore, if an excitatory drug like LSD-25 produced its effect indirectly through substance P. (5) The mechanism of the potentiation of substance P by LSD-25 on the guinea pig ileum does not seem to be due to inhibition of intestinal substance P destroying enzymes by LSD-25.

Since LSD-25, in high concentrations, consistently evoked weak contractions of the guinea pig ileum, the possibility was considered that the potentiation is simply a summation of the stimulatory effects of LSD-25 and the other agonists. This possibility is rendered unlikely by the observations that the potentiations were consistently obtained with concentrations of LSD-25 which did not cause stimulation. Moreover, when utilizing stimulating concentrations of LSD-25, the potentiations were not greater than with non-stimulatory concentrations of LSD-25, and the potentiations did not increase in proportion to the amount of stimulation by LSD-25.

Since LSD-25, is a selective inhibitor of pseudo cholinesterase (THOMPSON, 1955; EVANS, 1960) it seems likely that the potentiation of exogenous acetylcholine by LSD-25, on the guinea pig ileum is due to inhibition of intestinal pseudo cholinesterase. The concentrations of LSD-25, as reported by THOMPSON (1955) and EVANS (1960), causing 50 % inhibition of plasma pseudo cholinesterase, i.e., 0.2 to 0.3 μg/ml, are within the range utilized in this work for the potentiation of acetylcholine and the polypeptides. The potentiation of acetylcholine by LSD-25 on the guinea pig ileum may be similar to the potentiation of acetylcholine contractions of the rat uterus (DRASKOCI, 1960) by concentrations of LSD-25 (0.001 to 0.0001 μg/ml) which had no stimulatory effects of their own.

Although inhibition of intestinal pseudo cholinesterase by LSD-25 may explain the potentiation of acetylcholine, the mechanism of potentiation of the polypeptides is not known. If an inhibition of intestinal pseudo cholinesterase by LSD-25 is also involved in the potentiation of the polypeptides, it would have to be assumed that these polypeptides exert at least part of their intestinal stimulatory action by a release of endogenous intestinal acetylcholine. However, no evidence is presently available for a release of acetylcholine being involved in the actions of the polypeptides.

SUMMARY

The potentiation by LSD-25 of the response of the isolated guinea pig ileum to substance P, but not to histamine, was confirmed. This poten-

tiation was not specific for substance P as it extended to substance A, bradykinin, and acetylcholine. Rabbits pretreated with LSD-25 showed no change in the substance P content of the hypothalamus and caudate nucleus. The potentiation of substance P was not due to an inhibition of intestinal substance P destroying enzymes by LSD-25. It was suggested that LSD-25 may potentiate acetylcholine responses by inhibiting intestinal pseudo cholinesterase. Although the mechanism of potentiation of the polypeptides by LSD-25 is unknown it may involve inhibition of intestinal pseudo cholinesterase.

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