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THE PRESERVATION OF SUBSTANCE P BY LYSERGIC ACID DIETHYLAMIDE

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Lysergic acid diethylamide (LSD) potentiated the response of guinea-pig ileum to substance P but not to histamine. It also inhibited the disappearance of substance P when incubated with guinea-pig brain extract but not when incubated with chymotrypsin. Eserine, morphine, mescaline, chlorpromazine, ergometrine, strychnine and 2 bromo-LSD did not have this effect. Oxytocin was not destroyed by brain extract. The inhibition of the destruction of substance P by LSD could be antagonized by 2 bromo-LSD. This effect of LSD may have some relation to its pharmacological actions.

This paper concerns an evaluation of lysergic acid diethylamide (LSD) and various other drugs for their ability to inhibit the enzymatic destruction of substance P *in vitro*. The study was initiated when, during the course of an investigation of the contractile response of plain muscle to substance P, it was observed that LSD, in concentrations below 10^{-6} (Fig. 1), potentiated the action of this polypeptide on guinea-pig ileum, whereas the action of histamine was not potentiated. Since Gullbring (1943) described an enzyme capable of destroying substance P at a fairly rapid rate, it seemed possible that the potentiation was due to the preservation of substance P from enzymatic destruction.

METHODS

Acetone-dried powders of guinea-pig brain were used as a source of the enzyme. The extract was found to be thermolabile, and consequently the powder was stored in a deep freeze until ready for use, at which time an aliquot was ground with Tyrode (1 to 2 mg./ml.) and centrifuged for 2 hr. at 4° and 2,000 rev./min. The optimal *p*H for activity of this enzyme was found to lie between 6 and 7 by the method described below. This fact suggested that this system was similar to that described by Gullbring.

The substance P used in these experiments was prepared by Dr. T. B. B. Crawford of this laboratory. It was extracted from horse intestine by the method described by Amin, Crawford and Gaddum (1954), and contained 13 units/mg. Part of this was further purified by the method of Pernow (1953), and contained 60 units/mg. Experiments were conducted with both of these preparations.

To test the potency of the brain extract and the modifications of its activity by pharmacological agents, the following mixture was prepared and incubated at 37°. Substance P (5 units/ml. in Tyrode) 0.5 ml.; 0.1 ml. of the brain extract supernatant; 0.1 ml. of the drug or drugs to be tested for inhibition of enzymatic activity, and Tyrode solution to make up 1.0 ml. In order to slow the reaction to a rate which could be measured accurately and to mitigate the disappearance of the LSD in the brain extract (Rothlin, 1956), the quantities of the brain extract were less than those used by Gullbring. After incubation the sample was cooled and an aliquot assayed on guinea-pig ileum in terms of a substance P standard in the presence of atropine 10^{-7}. Except where noted, drugs were applied to the guinea-pig ileum for bioassay purposes for a period of 30 sec. In certain experiments chymotrypsin (0.1 ml. of 5×10^{-6}) was used in place of the brain extract. The composition of the incubation mixture was kept constant in all other respects.

The guinea-pigs used as a source of enzyme and for bioassay purposes weighed between 150 and 200 g.

LSD and 2-bromo-lysergic acid diethylamide (BrLSD) were kindly presented by Sandoz Products. Crystalline chymotrypsin was obtained from the Armour Laboratories.

RESULTS

The LSD potentiation of substance P was determined on the guinea-pig ileum. In these experiments the preparation was exposed to standard concentrations of substance P for periods of 50 sec. In some experiments alternate doses of histamine and substance P were applied. After obtaining a constant response, LSD was introduced into the bath 1 to 2 min. prior to the histamine or substance P. Fig. 1 illustrates a typical result: the response of the guinea-pig ileum to substance P was potentiated, whereas that to histamine was

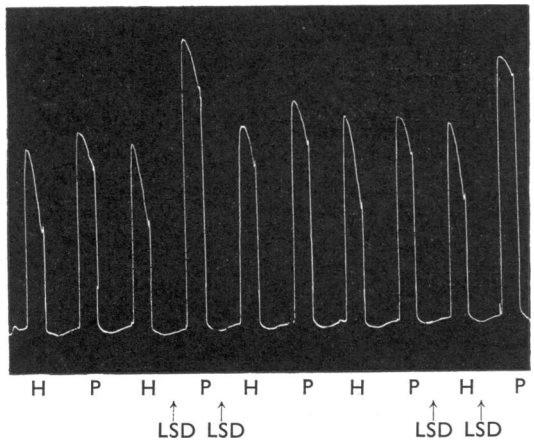

FIG. 1.—Guinea-pig ileum. 3 ml. bath. Potentiation of substance P but not of histamine by LSD. H=histamine, 15 ng.; P=substance P, 0.25 units. At the arrows, 0.1 ml. LSD 10^{-8} was introduced into the bath 1.5 min. before H or P.

not. The potentiation appeared within 1.5 min. and was reversible. If the duration of exposure to substance P was decreased to 30 sec. in these experiments, potentiation was not observed.

Fig. 2 shows that when substance P was incubated with the brain extract it was progressively inactivated, whereas substance P incubated with LSD and the brain extract was spared. The brain extract, with or without LSD, did not interfere with assays for substance P. Nearly full activity was found in the presence of fresh brain extract (zero time) or in the presence of extract that had been incubated for 2 hr. (encircled points).

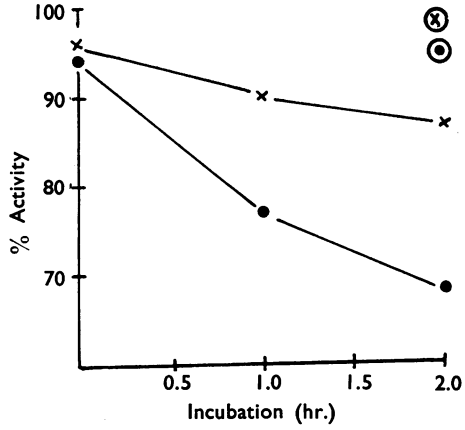

FIG. 2.—The % activity remaining after incubating substance P for various periods with brain extract without LSD (●) and in the presence of LSD 10^{-8} (X). The points enclosed in circles show the results obtained in control experiments when substance P was added after incubation.

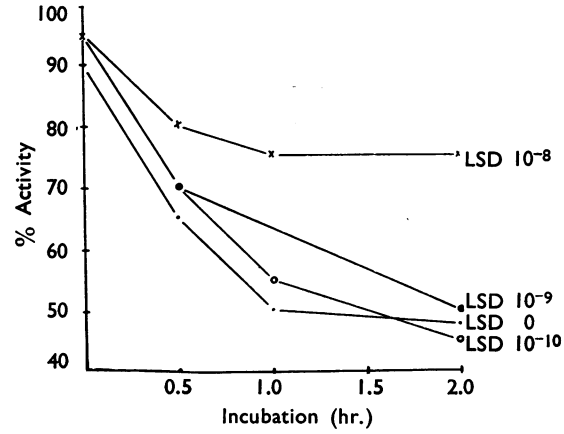

FIG. 3.—The % activity remaining after incubation of substance P with brain extract in the presence of various concentrations of LSD.

Fig. 3 illustrates the effects of various doses of LSD on the speed of inactivation of substance P. The ED50 appears to lie between 10^{-8} and 10^{-9}. It should be noted that although the destruction of substance P appeared to cease in this experiment it was more frequently found that, given a proper relationship between substrate and enzyme, the substance P activity was continuously reduced as long as it was followed.

It is important to note that, if either the period of incubation was increased or too much brain extract was used in the incubation mixture, the two curves representing the mixtures with and without LSD tended to converge. In the latter case, this could proceed at a very rapid rate, so that after the first few minutes of incubation no difference appeared between the variously treated incubation mixtures. This phenomenon is probably due to the disappearance of LSD in the brain extract (Rothlin, 1956).

The following drugs were also investigated for their ability to inhibit the destruction of substance P: BrLSD, ergometrine, mescaline, eserine, chlorpromazine and strychnine. As may be seen in Table I, the only one of these drugs which inhibited the destruction of substance P was LSD.

Guinea-pig brain extract appeared to be incapable of destroying oxytocin. The oxytocin activity was measured on the rat uterus. The brain extract produced a small increase in the effect of oxytocin on this preparation. The oxytocin equivalent of the mixture before incubation was 120% of the theoretical value and this figure was not significantly changed by incubation for 1 or 2 hr. In a parallel experiment substance P was about 50% inactivated in 2 hr.

TABLE I

COMPARISON OF THE INFLUENCE OF VARIOUS DRUGS ON THE RATE OF DISAPPEARANCE OF SUBSTANCE P INCUBATED WITH ACETONE DRIED POWDERS OF GUINEA-PIG BRAIN

The % activity remaining after incubation is given in terms of a substance P standard. Nil indicates that substance P was incubated with brain extract in the absence of any drugs.

Drug	% Activity Remaining After Incubation
LSD 10^{-8}	100
BrLSD 10^{-7}	70
Nil	73
Strychnine 10^{-6}	73
Morphine 10^{-6}	75
Eserine 10^{-6}	74
Nil	75
Chlorpromazine 10^{-6}	62
Nil	60
Ergometrine 10^{-7}	74
Nil	73
Mescaline 10^{-6}	74
Nil	71

Incubation of LSD and substance P with chymotrypsin in place of guinea-pig brain extract showed that LSD offered no protection to substance P against destruction by chymotrypsin. After 30 min., the residual activity was estimated as 60% both when LSD was present and when it was absent. After 60 min., the estimates were 32% and 30% respectively.

BrLSD resembles LSD in its antagonism to 5-HT, but differs from it in that it does not have the same psychological effects (Cerletti and Rothlin, 1955), and may even antagonize those of LSD (Ginzel and Mayer-Gross, 1956). It was therefore tested for its effects on substance P. When it was directly applied to guinea-pig ileum in concentrations up to 10^{-6}, it neither increased nor decreased the response to substance P. When added to brain extract, it did not alter the effect of this extract on substance P, but when both LSD and BrLSD were present the inhibitory effect of LSD on the enzyme was prevented, so that substance P was inactivated. Evidence for this is shown in Fig. 4.

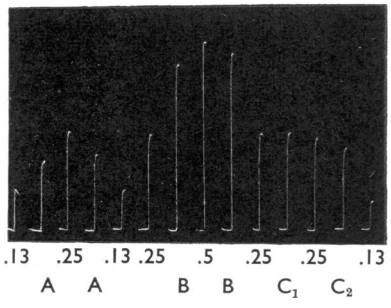

FIG. 4.—Guinea-pig ileum. 3 ml. bath. Responses to substance P after incubation with brain extracts (A, B, C_1, and C_2) compared with those to a standard preparation of substance P, the dose of which in units is indicated by the numerals. At A, 0.5 units (0.2 ml.) without an inhibitor. Note partial destruction. At B, 0.5 units with LSD 2×10^{-9}. Inhibition of destruction is evident. At C_1 0.55 units and at C_2 0.5 units each with LSD 2×10^{-9} and BrLSD 5×10^{-9}. There is now inhibition of the inhibition.

In this experiment, substance P was added to the brain extract in a concentration of 2.5 units/ml. Duplicate estimates of the substance P equivalent after incubation without inhibitors were 0.86 and 0.93 units/ml. In the presence of LSD (2×10^{-9}) the estimates were 2.1 and 2.3. In the presence of both LSD (2×10^{-9}) and BrLSD (5×10^{-9}) the estimates were 1.1 and 1.1. The inactivation was thus almost completely inhibited by LSD and this inhibition was mostly inhibited by BrLSD.

DISCUSSION

It seems evident from these results that LSD can protect substance P from enzymatic destruction. This is not due to the action of LSD on cholinesterase (Thompson, Tickner and Webster, 1955) since eserine does not have a similar action on the destruction of substance P. The specificity of LSD as an inhibitor of the destruction of substance P is noteworthy; BrLSD and ergometrine are inactive. A similar specificity is encountered in their activity as hallucinogens.

The evidence that BrLSD is an antagonist to LSD, both *in vivo* and *in vitro*, suggests that the observed relationship between substance P and LSD may be related to the effects of LSD on the brain.

Various attempts have been made to detect an effect of substance P on the brain. According to Pernow (1955) the cerebral intraventricular injection of substance P can cause rage in otherwise mild-tempered cats. In a single experiment of the same sort, Gaddum and Krivoy (unpublished observation) were unable definitely to confirm this observation, even by employing doses up to 500 units. However, more evidence must be obtained on this point before a definite conclusion can be reached. In the extensive experiments of Zetler (1956), the effect of substance P on the motor activity of mice was generally sedative.

This discussion of the relationship of these *in vitro* findings to *in vivo* observations is complicated by the fact that the effect of LSD on the destruction of substance P is lessened by increasing the duration of incubation. This may perhaps be due to destruction of LSD, but there is no direct evidence that LSD was destroyed in these experiments. The effect of LSD was also diminished

when large amounts of brain extract were used. This may have been due either to the destruction of LSD or to the fact that the total amount of LSD then became inadequate to block a sufficient number of receptors in the enzyme.

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