LSD 220/BOL 14

Brit.J.Pharmacol. 11, 313 (1956)

THE ANTAGONISM BETWEEN 5-HYDROXYTRYPTAMINE AND CERTAIN DERIVATIVES OF LYSERGIC ACID

BY

E. C. SAVINI

From the Department of Pharmacology, University of Edinburgh

(RECEIVED MAY 5, 1956)

The high pharmacological activity of 5-hydroxytryptamine (5-HT) and its wide distribution in the body suggest that it may play a part in normal physiological processes, but this is still a matter for speculation. Very small amounts of 5-HT cause vasoconstriction in the perfused ear of a rabbit, and this tissue was used in the work which first led to the isolation of 5-HT. It has been suggested that an important action of 5-HT may be to modify the responses of tissues to other active substances. The effects on the rabbit's ear of 5-HT combined with various other drugs have been studied by Gaddum and Hameed (1954). The experiments described below are an extension of this work.

METHODS

Rabbits were killed by a blow on the head and bled out. Both ears were removed with a sharp scalpel. The central auricular artery was cleaned and cannulated as described by Page and Green (1948). The ears were perfused at room temperature through a polythene cannula. Two reservoirs were used so that alternative fluids could be perfused by adjusting a two-way stopcock. The ear was fixed on a tilted draining board and perfusate was collected in a glass tube from which it ran to the drop timer (Gaddum and Kwiatkowski, 1938). In the tracings the height of the record indicates the time interval between drops.

The perfusion fluid was that recommended by Page and Green (1948) for the study of vasoconstrictors and had the following composition (g./l.): NaCl 8.2, KCl 0.84, $CaCl_2.2H_2O$ 0.04, $MgCl_2.6H_2O$ 0.06, $NaHCO_3$ 0.4, glucose 1. To each litre was added 10 ml. of phosphate containing 4 parts of M-K_2HPO_4 to 1 part of M-KH_2PO_4.

The ears were more sensitive on the second and third days than on the first day, after being left overnight in the refrigerator.

Sandoz Products Ltd. kindly presented supplies of lysergic acid diethylamide (LSD) and (+)-2-bromolysergic acid diethylamide (Brom LSD). Eli Lilly and Company kindly presented the angiotonin. Other drugs used were ergometrine maleate (B.D.H.) and vasopressin (Pitressin, Parke Davis and Co.), morphine, and methadone.

The doses, which, owing to a misunderstanding and contrary to the usual custom, are given in terms of the weights of the salts, were injected in a uniform volume of 0.1 ml. at regular time intervals. The injections were made slowly through the rubber cap of an injection tube similar to that described by Gaddum and Kwiatkowski (1938) and connected with the polythene cannula. The perfusion fluid entered the air space in this tube in drops, and the rate of injection was regulated so as to keep the size of the drop approximately constant. In this way changes in flow due to changes of pressure in the cannula can be avoided. The "dose-ratio" is the ratio of the dose of 5-HT producing an effect in the presence of the antagonist to the dose producing the same effect in its absence.

RESULTS

The doses were kept small in order to avoid changes in the sensitivity of the preparation. The intervals between injections were 5–10 min. in order to avoid tachyphylaxis, which was particularly liable to occur with 5-HT and tryptamine. When these precautions were taken the sensitivity was reasonably constant and suitable effects were produced by the following doses: adrenaline, 0.5–1 ng.; noradrenaline, 1–2 ng.; 5-HT, 1–10 ng.; tryptamine, 10–100 ng.; pitressin, 1 mU.; and angiotonin, 0.1 unit.

Potentiation

When 5-HT and adrenaline were given together the vasoconstrictor effect was larger than had been expected. A systematic investigation was therefore made of the combined action of various pairs of vasoconstrictor drugs. The drugs used were 5-HT, tryptamine, adrenaline, and noradrenaline. These four drugs can be combined in pairs in 6 possible ways and each of those pairs was studied separately. The two drugs in each pair were first given separately and the concentrations were adjusted until roughly equal effects were produced by 0.1 ml. of solutions of the two drugs. These two equivalent solutions were then mixed in equal volumes, and 0.1 ml. of

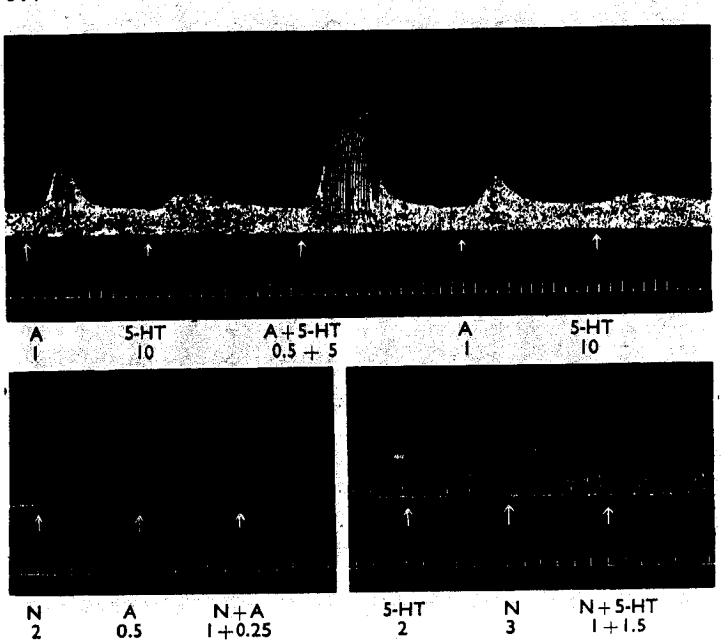

FIG. 1.—Outflow from rabbit's ear. Effects of adrenaline (A), 5-hydroxytryptamine (5-HT), and noradrenaline (N). Doses in ng. Combination of two half-doses of adrenaline and 5-HT shows potentiation. Other pairs of drugs show simple addition.

the mixture, containing half of the original dose of each drug, was injected. When the effect so produced is equal to the original effect, as it would be if the two solutions contained the same drug, the combined action is additive; when the combined effect is larger, there is potentiation. This technique was used by Best, Dale, Dudley, and Thorpe (1927) to show that the depressor effect was potentiated when histamine and choline were injected together into cats.

In the present experiments on the rabbit's ear, potentiation occurred when 5-HT was combined with adrenaline (Fig. 1). This effect was not invariably obtained, but in 13 out of a total of 15 satisfactory experiments with different ears the effect of the combined half-doses was larger than the effect of either of the single doses. The reason for the two failures is not known, but in both cases the initial sensitivity to 5-HT was high, and most of the best results were obtained when it was low. It may be that it is more difficult to sensitize ears which are already sensitive. The sensitivity to adrenaline varied much less.

This potentiation appeared to be exceptional, since none of the other five pairs of drugs showed a similar effect. When tryptamine or noradrenaline was combined with any of the other drugs the effects appeared to be purely additive. When the initial effects were equal the combined effect was equal to them both. When the initial effects were not quite equal the combined effects were greater than one and less than the other (Fig. 1).

Ginzel and Kottegoda (1953) have described potentiation between tryptamine and noradrenaline on the rabbit's ear, but the conditions of their experiments were different. A comparatively large dose of noradrenaline (100 ng.) caused a small vasoconstriction which became larger 30 min. after a large dose of tryptamine. It is thus evident that the potentiation between 5-HT and adrenaline is not unique, but it appears to be especially easy to demonstrate.

LSD

The observation that LSD is a powerful antagonist of 5-HT (Gaddum and Hameed, 1954) has been confirmed. Perfusion of a concentration of 1 μg./l. had a marked effect, increasing for about 2 hr. When low concentrations of LSD are perfused for a short time the response to 5-HT may return after washing (Gaddum and Hameed, 1954). When higher concentrations (20–100 μg./l.) were perfused for longer times, LSD caused an irreversible effect which was not abolished even by washing for 3 hr.

With these higher concentrations the antagonism was unsurmountable (Gaddum, Hameed, Hathway, and Stephens, 1955), so that even large doses of

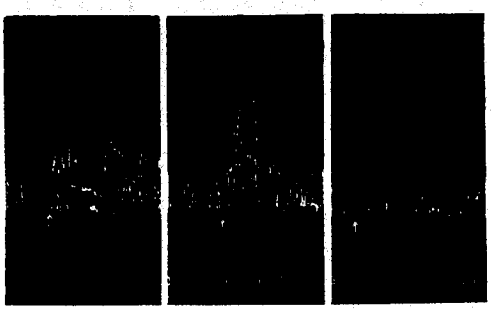

FIG. 2.—Outflow from rabbit's ear. Vasoconstriction due to LSD (1 μg.) and ergometrine, E (0.2 μg.), but not Brom LSD (100 μg.).

5-HT had no action. LSD itself causes vasoconstriction (Ginzel and Kottegoda, 1953), which was sufficient to interfere seriously with the experiment when 100 μg./l. of LSD was present in the perfusion fluid; otherwise even a single small injection (1 μg. in 0.1 ml.) caused a transient vasoconstriction (Fig. 2) with no obvious change in the sensitivity to 5-HT given subsequently.

The effect of tryptamine (100 ng.) was also depressed by LSD in similar concentrations.

The effects of adrenaline (0.5–1 ng.) and noradrenaline (1–2 ng.) were actually increased by LSD (10 μg./l.) so that the recorded height of the rise on the record was often 2–5 times as large as it had been.

Ergometrine

The action of ergometrine resembled that of LSD. It antagonized 5-HT when perfused for 1 hr. in a concentration of 1 μg./l. In a concentration of 10 μg./l. the dose-ratio was about 10, so that doses of 5-HT had to be increased 10 times to reproduce the original effects. Ergometrine appeared to be 2–5 times less active than LSD.

The effect of tryptamine was abolished in much the same way as that of 5-HT.

These results do not agree with conclusions reached by Gaddum and Hameed (1954), and the cause of this discrepancy is unknown, but clear evidence of antagonism has now been consistently obtained in 6 experiments. It is possible that the preparation of ergometrine used by Gaddum and Hameed had become inactive.

These experiments were complicated by the direct vasoconstrictor action of ergometrine itself which appeared to be about 5 times as great as that of LSD. This was clearly shown when 0.2 μg. of ergometrine was injected in 0.1 ml. (Fig. 2). When a concentration of 1 μg./l. was perfused it caused marked vasoconstriction.

The effects of adrenaline and noradrenaline were not definitely affected by ergometrine, even in high concentrations (100–1,000 μg./l.).

Brom LSD

Cerletti and Rothlin (1955) found that Brom LSD was slightly more active than LSD as an antagonist of 5-HT on rat's uterus or perfused rat's kidney. It was also as active an anti-5-HT as LSD in various other tests. It had no action, however, on the brain of man or mouse, and Gaddum and Vogt (1956) found that it failed to cause sham rage in cats such as followed equivalent doses of LSD. These results weaken the evidence for the theory that the actions of LSD on the brain are due to interference with the physiological action of 5-HT on the brain, and it is therefore of interest to study other differences between the actions of these two substances.

Brom LSD was an active antagonist of 5-HT in the rabbit's ear, but it was clearly less active than LSD. It is not easy to give a precise figure for the

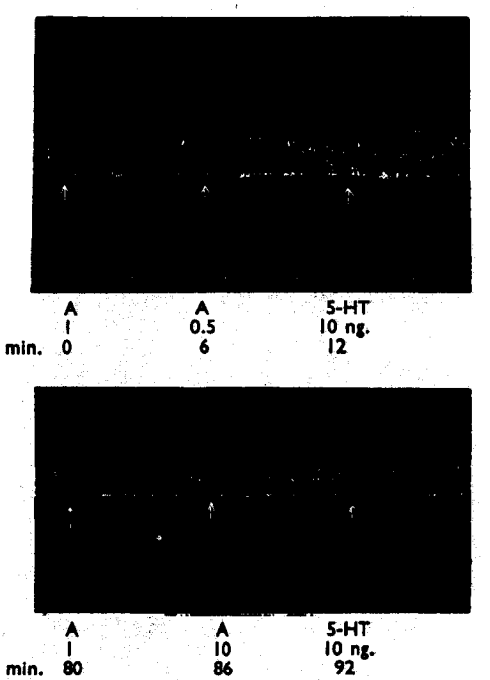

FIG. 3.—Outflow from rabbit's ear. Brom LSD (100 μg./l.). From 50 min. onwards. Doses in ng. Vasoconstrictor effects of both adrenaline (A) and 5-HT diminished.

ratio of the activities, because the action of LSD increased with time for as long as 2 hr., whereas that of Brom LSD was complete sooner, but in experiments where equilibrium conditions seemed to have been reached with both drugs 1 μg./l. of LSD had about the same action (dose ratio 50) as 10 μg./l. of Brom LSD. These results suggest that in these conditions after prolonged perfusion LSD is 10 times as active as Brom LSD.

Brom LSD also differed from both of the other drugs in the following respects:

(1) It never caused vasoconstriction in any dose tested. Thus 100 μg. in a single injection (Fig. 2) had no effect, whereas 1 μg. of LSD caused definite vasoconstriction. A concentration of 2,000 μg./l. was perfused continuously without causing any vasoconstriction.

(2) A concentration of 100 μg./l. reduced the effects of adrenaline and noradrenaline considerably, whereas this same concentration of LSD increased the effects of these drugs. Some antagonism to adrenaline (dose ratio 2–3) was seen after 10 μg./l., and with 1,000 μg./l. the effect was considerable (dose ratio 100–200). It was not possible to test such high concentrations of the other drugs, owing to vasoconstriction.

(3) The antagonistic actions of Brom LSD appeared to develop and disappear more rapidly than those of LSD or ergometrine, and recovery was complete, which was not so with LSD.

(4) The action of large doses of Brom LSD was surmountable by large doses of agonist, but those of large doses of LSD and ergometrine often became unsurmountable.

Morphine

Morphine has been found (Kosterlitz and Robinson, 1955) to have some antagonistic action to 5-HT on the guinea-pig's ileum. No sign of any such effect has been seen in experiments with the rabbit's ear. A concentration of 1 mg./l. had no apparent effect, but when 10 mg./l. was perfused it caused some vasoconstriction and increased the effect of a whole series of vasoconstrictor drugs—adrenaline, noradrenaline, 5-HT, tryptamine, vasopressin, and angiotonin.

Discussion

The potentiation observed when adrenaline and 5-HT were given together was unexpected, and it was surprising that none of the 5 other pairs of drugs showed a similar effect. Results which may be due to the same mechanism were obtained by Lecomte (1953), who injected these drugs intravenously in cats under allobarbitone anaesthesia and found that 5-HT increased and prolonged the effect of adrenaline injected during the next 30 min. Tryptamine did not have this action either in Lecomte's experiments or in those recorded here.

The comparison between LSD, Brom LSD, and ergometrine is of interest because of the actions of LSD and ergometrine on the brain. LSD causes disorders of perception and personality (Stoll, 1947) and sham rage (Gaddum and Vogt, 1956); ergometrine causes sham rage (Brown and Dale, 1935), but psychological changes such as those caused by LSD are not among its recognized effects. Brom LSD appears to have neither effect (Cerletti and Rothlin, 1955; Gaddum and Vogt, 1956), at any rate when tested in equivalent doses. Brom LSD thus differs from the other two drugs both in its failure to cause sham rage and in its failure to cause vasoconstriction in the perfused rabbit's ear. It is possible that these two actions are related to one another.

The activity of Brom LSD relative to LSD was less than that found by Cerletti and Rothlin (1955) in other tissues. This ratio varies according to the conditions, and it is therefore just possible that the failure of Brom LSD to cause psychological changes in man was due to inadequate dosage, but this is not very likely, since it was tested in 20 times the dose found effective when LSD was used.

Summary

1. Potentiation occurred when 5-HT and adrenaline were injected together in the perfused ear of a rabbit.

2. Lysergic acid diethylamide (LSD) and ergometrine in low concentrations (1 μg./l.) both antagonized the vasoconstrictor action of 5-HT or tryptamine, but not that of adrenaline or noradrenaline.

3. In higher concentrations these two drugs have a direct vasoconstrictor action themselves.

4. 2-Bromo-lysergic acid diethylamide in low concentrations (1 μg./l.) also antagonized 5-HT, but it differed from LSD and ergometrine in the following ways:

(*a*) It did not cause vasoconstriction.

(*b*) It antagonized adrenaline and noradrenaline.

(*c*) Its action developed more quickly and recovery was complete on washing.

(*d*) It was always possible to surmount the antagonism with large doses of 5-HT.

This work was carried out during the tenure of a Fellowship awarded by the International Union of Pharmacologists. It is a pleasure to acknowledge my indebtedness to Riker Laboratories for establishing this Fellowship.

I am very grateful to Professor J. H. Gaddum, F.R.S., for his hospitality, constant help, and advice.

References

Best, C. H., Dale, H. H., Dudley, H. W., and Thorpe, W. V. (1927). *J. Physiol.*, **62**, 397.

Brown, G. L., and Dale, H. H. (1935). *Proc. Roy. Soc.* B, **118**, 446.

Cerletti, A., and Rothlin, E. (1955). *Nature, Lond.*, **176**, 785.

Gaddum, J. H., and Hameed, K. A. (1954). *Brit. J. Pharmacol.*, **9**, 240.

—— Hameed, K. A., Hathway, D. E., and Stephens, F. F. (1955). *Quart. J. exp. Physiol.*, **40**, 49.

—— and Vogt, M. (1956). *Brit. J. Pharmacol.*, **11**, 175.

—— and Kwiatkowski, H. (1938). *J. Physiol.*, **94**, 87.

Ginzel, K. H., and Kottegoda, S. R. (1953). *Quart. J. exp. Physiol.*, **38**, 225.

Kosterlitz, H. W., and Robinson, J. A. (1955). *J. Physiol.*, **129**, 18P.

Lecomte, J. (1953). *Arch. int. Physiol.*, **61**, 84.

Page, I. H., and Green, A. A. (1948). *Methods in Medical Research*, **1**, 123.

Stoll, W. A. (1947). *Schweiz. Arch. Neurol. Psychiat.*, **60**, 279.