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THE ANTAGONISM OF THE VASCULAR EFFECTS OF 5-HYDROXYTRYPTAMINE BY BOL 148 AND SODIUM SALICYLATE IN THE HUMAN SUBJECT

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2-Bromo-(+)-lysergic acid diethylamide (BOL 148) is a specific antagonist of the constrictor response of the forearm and hand blood vessels to intra-arterial infusions of 5-hydroxytryptamine. This has been shown to be a direct action in the tissue concerned, since the antagonism was as effective when the drug was given by local arterial injection as when given intravenously. Sodium salicylate is also a specific antagonist of 5-hydroxytryptamine, but its action is indirect, occurring only when the general serum level was raised above 10 or 20 mg.%; local limb concentrations of 30 to 40 mg.% had no antagonistic effect. The mechanism of the antagonistic effect of salicylate is not known. It is not related to the associated hyperventilation but is possibly due to the secondary release of, or synergism with, some other substance, probably a hormone.

A number of substances including lysergic acid derivatives have been shown by Gaddum and Hameed (1954) and other workers to antagonize the action of 5-hydroxytryptamine (5-HT) on isolated smooth muscle preparations. The effect of some of these substances on the response of the blood pressure to intravenous 5-HT has also been studied in animals (Page and McCubbin, 1953; Salmoiraghi, McCubbin, and Page, 1957) and in man (Spies and Stone, 1952).

The vascular responses in the upper limbs of normal human subjects to intra-arterial injections of 5-HT have been described (Roddie, Shepherd, and Whelan, 1955). In the present paper a comparison is made of the antagonistic effect on these responses of 2-bromo-(+)-lysergic acid diethylamide bitartrate (BOL 148) with that of sodium salicylate, which has been shown by Kelemen (1957) to diminish the oedema of the paw of the rat produced by locally injected 5-HT.

MATERIALS AND METHODS

The investigations were carried out on ourselves, our colleagues, and medical students. The subject rested on a couch for at least 30 min. before the first observation was made. The room temperature was kept at a constant level in the range 22 to 25°. An indwelling needle was inserted into the left brachial artery in the antecubital fossa and through it either 0.9% saline or a solution of the drug was infused at a rate of 4 ml./min. using a mechanically driven syringe and a length of polythene tubing. Measurements of forearm blood flow were made by venous occlusion plethysmography using the water-filled, mechanically stirred plethysmographs described by Greenfield (1954), the water temperature being 34 to 35°.

Changes in the circulation through the skin of the finger were followed using the heat flow discs described by Hatfield (1950). These were applied to the finger pulp using Nobecutane (Evans) and the whole hand inserted into a plethysmograph, the temperature of which was carefully maintained at 29°. In most experiments control observations were made on the opposite limb.

5-Hydroxytryptamine creatinine sulphate (Sandoz) was infused intra-arterially for periods of 5 to 10 min. at rates of 1, 4, or 16 μg./min. Solutions were prepared in 0.9% saline so that the dose for 1 min. was contained in 4 ml. The antagonists were administered intravenously into an antecubital vein or intra-arterially by the same needle as the 5-HT. The intravenous doses were 1.5 mg. of 2-bromo-(+)-lysergic acid diethylamide bitartrate in 1 to 2 min. and 3 to 4 g. of sodium salicylate (B.D.H.) in 100 to 150 ml. saline over 3 to 5 min. The intra-arterial doses were 10 μg./min. of BOL 148 and 20 mg./min. of sodium salicylate given for 3 to 10 min. before and the 5 min. during the 5-HT infusion. These arterial doses were calculated to give approximately the same local blood concentration arriving in the forearm as prevailed during the intravenous infusions. In 6 experiments sodium salicylate was given intra-arterially in larger doses of 40 to 200 mg./min. for periods up to 20 min. In some experiments serum salicylate levels were de-

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termined by the method of Keller (1947), and respiratory movements recorded using stethographs around the chest and abdomen (Shepherd, 1951 ; Dornhorst and Leathart, 1952).

RESULTS

Subjective Effects.—During intra-arterial infusion of 5-HT a tingling sensation was experienced in the forearm and hand with doses of 4 and 16, and usually with 1 μg./min. This was reduced or abolished by BOL 148 given either intra-arterially or intravenously and by sodium salicylate given intravenously. No symptoms were noted during administration of BOL 148 by either route, but two subjects complained of tinnitus after an intravenous injection of 4 g. of sodium salicylate. Intra-arterial infusions of sodium salicylate were well tolerated up to a concentration of 50 to 100 mg. in 4 ml. saline/min. ; when the concentration was increased to 100–200 mg., a burning sensation was noted along the course of the brachial artery and its branches in the forearm. In two subjects, intravenous infusion of sodium salicylate in a concentration of 3 to 4 g.% in saline caused pain along the course of the vessel, which ceased abruptly when saline was flushed through. On no occasion did sodium salicylate intra-arterially (12 subjects) or intravenously (12 subjects) cause thrombosis or have any other residual effects on the vessels perfused.

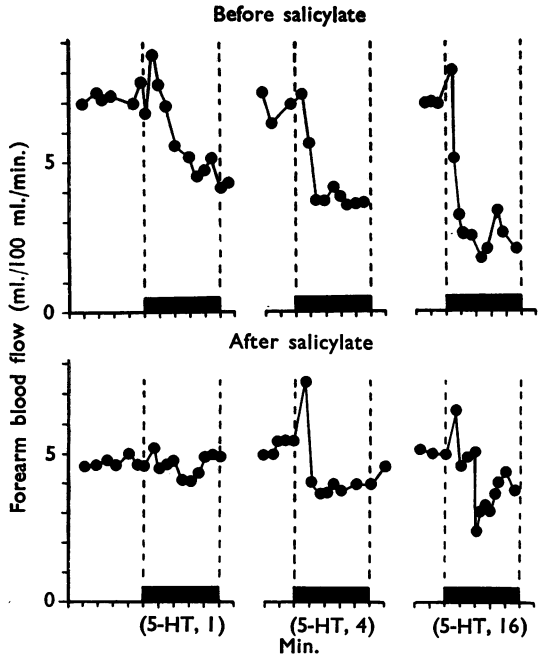

FIG. 1.—The forearm blood-flow changes in response to infusion of 5-HT into the brachial artery before and after 3 g. of sodium salicylate intravenously. The flow is expressed in ml./100 ml./min. The solid rectangles represent the periods of infusion of 5-HT and the numerals in parentheses below them indicate the doses in μg./min. A 10 to 15 min. interval elapsed between each infusion.

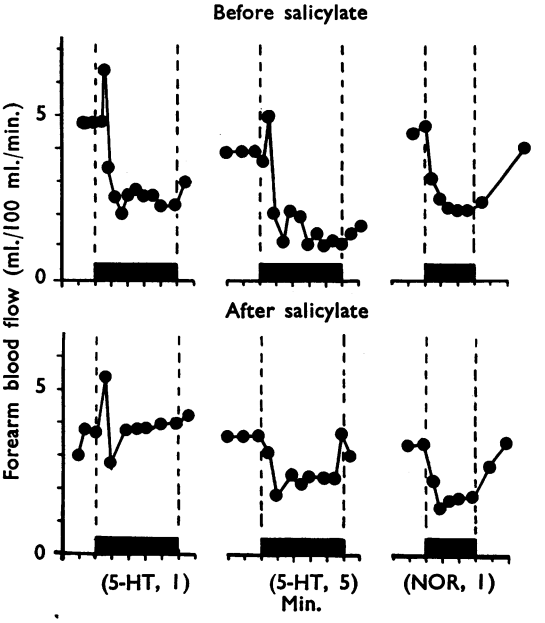

FIG. 2.—The forearm blood flow changes in response to infusions of 5-HT and noradrenaline (NOR) into the brachial artery before and after 3 g. of sodium salicylate intravenously. See Fig. 1 for explanation of symbols.

Forearm Blood Flow.—The changes in forearm blood flow during intra-arterial infusions of 5-HT have already been described (Roddie *et al.*, 1955), and these were confirmed in the present study. At all doses, there was usually an initial transient increase in blood flow lasting for ¾ to 1 min. Subsequently the blood flow fell below the resting level by approximately 30% with 1 μg./min., 50% with 4 μg./min. and 70% with 16 μg./min. (averages of 30 experiments). These levels of flow were reached after 2 to 3 min. of the infusion and persisted as long as it continued. On returning to saline the flow gradually rose to that on the control side over 10 to 20 min. Repeated doses at 10 to 20 min. intervals gave approximately the same response on each occasion.

Intravenous Administration of Antagonists.—Fig. 1 shows the results of a typical experiment in which the forearm blood flow was measured during intra-arterial infusions of 5-HT before and after intravenous administration of 3 g. of sodium salicylate. The initial transient increase in flow

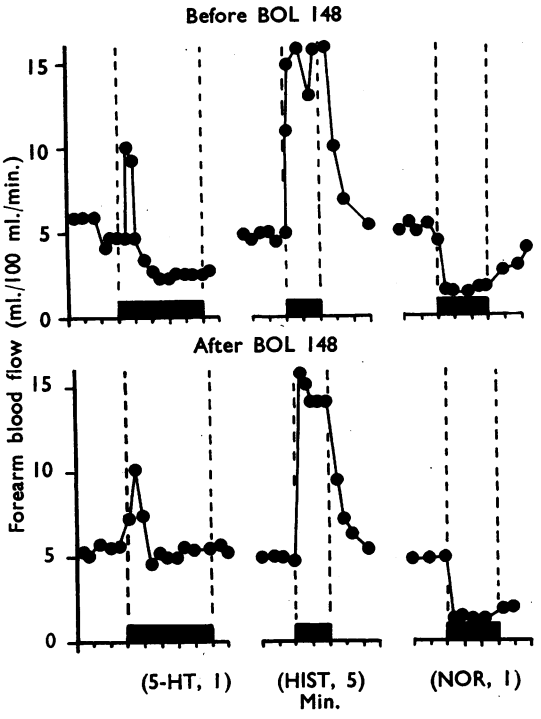

FIG. 3.—The forearm blood flow changes in response to infusions of 5-HT, histamine, and noradrenaline into the brachial artery before and after 1.5 mg. of BOL 148 intravenously. See Fig. 1 for explanation of symbols.

was not consistent in this experiment, but it was not abolished by the sodium salicylate. The fall in flow produced by 1 μg./min. of 5-HT was almost completely abolished, while the responses to 4 μg./min. and 16 μg./min. were considerably reduced. That this antagonistic effect of sodium salicylate was a specific one and not a generalized inhibition of the smooth muscle of the blood vessels is shown by the fact that the response to 5-HT was abolished or reduced by a dose of sodium salicylate which did not affect the response to noradrenaline (Fig. 2). The antagonistic effect of intravenous administration of BOL 148 on the response to 1 μg./min. of 5-HT intra-arterially is shown in Fig. 3. The initial transient dilatation was not affected, but the constrictor response was abolished. The dilator effect of histamine and the constrictor effect of noradrenaline, however, were not altered. The results of all experiments in which the responses to 1, 4, and 16 μg./min. of 5-HT were determined before and after BOL 148 and sodium salicylate intravenously are shown in Fig. 4. The level of forearm blood flow during the last 2 min. of the 5 min. infusion has been expressed as a % of the pre-infusion level corrected for any general fluctuations in flow by reference to the level in the control forearm (Duff, 1952; Greenfield and Patterson, 1954). Each pair of points in each graph represents a separate experiment. It is clear that the two drugs given in this way have an approximately equal antagonistic effect on the responses of the forearm vessels to intra-arterial 5-HT.

In two experiments in which the same dose of 5-HT was given at intervals after the antagonist, the inhibition of the constrictor effect was still present after 1½ to 2 hr.

Intra-arterial Administration of Antagonists.—When BOL 148 was given intra-arterially in a dose of 10 μg./min. for 5 min. before and the 5 min. during the 5-HT infusion, a similar antagonism was exhibited. That this was due to the local, and not the general, effect of the drug is shown by the experiment illustrated in Fig. 5. In this ex-

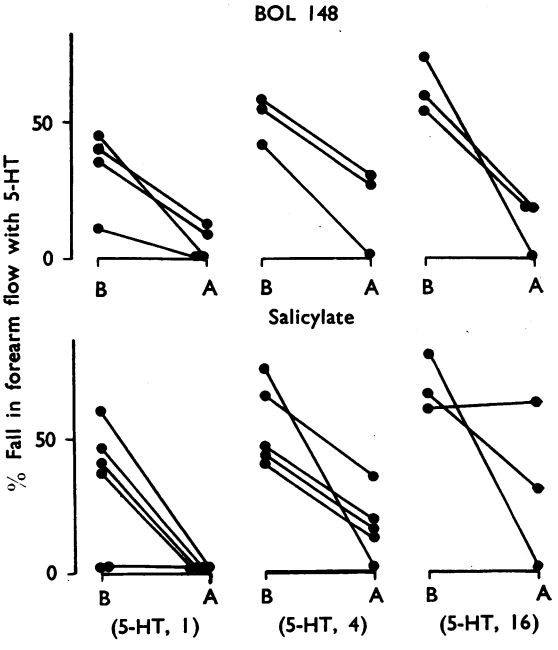

FIG. 4.—The results of all experiments in which the forearm blood flow changes in response to intra-arterial 5-HT were determined before and after 1.5 mg. of BOL 148 intravenously (upper graphs), or 3 to 4 g. of sodium salicylate intravenously (lower graphs). % fall in flow is calculated from the flow in the last 2 min. of the infusion, and the flow that would have been expected if the infusion had not been given. The latter is derived by correcting the pre-infusion level for general fluctuations in vasomotor tone by reference to the control forearm. B, before, and A, after, BOL 148 or sodium salicylate. Each pair of points joined by a line represents one experiment. See Fig. 1 for explanation of symbols.

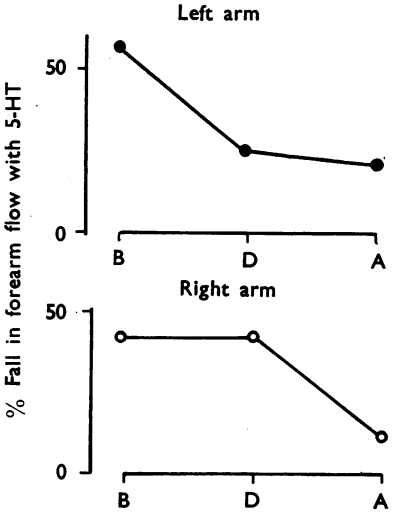

FIG. 5.—The responses of the left (upper graph) and right (lower graph) forearm vessels to the intra-arterial infusions of 5-HT, 4 μg./min., determined simultaneously (B) before, (D) during the infusion of 10 μg./min. of BOL 148 into the left brachial artery, and (A) after 1.5 mg. BOL 148 intravenously. The response is expressed as % fall in flow as in Fig. 4.

artery of each arm, and the responses of the forearm flows to 4 μg./min. of 5-HT measured simultaneously on the two sides. BOL 148 was then given intra-arterially into the left side at the rate of 10 μg./min. for 10 min., and during the second 5 min. the bilateral 5-HT infusion was repeated. The response on the left side was greatly reduced, but the right side responded as before. When, however, 1.5 mg. of BOL 148 was given intravenously and the bilateral infusions of 5-HT again repeated, the response on the right side was reduced to approximately the same level as the left.

Sodium salicylate was only effective intra-arterially when given in doses which caused the general blood level to approach that achieved in the intravenous experiments. Doses as high as 100 mg./min. for 10 min. had no effect on the response to 5-HT. In the experiment illustrated in Fig. 6, the response to 4 μg./min. 5-HT was recorded. Sodium salicylate was then given intra-arterially at the rate of 20 mg./min. for 6 min. and for the last 5 min. the 5-HT infusion was repeated. The response was unchanged even though the venous blood returning from the arm contained 40 mg./100 ml. of sodium salicylate. At this time the general blood level was 2.5 mg./100 ml., but when it was raised to 33 mg./100 ml. by the intravenous administration of 4 g. of sodium salicylate the response to a further 4 μg./min. of 5-HT was greatly reduced. The results of this and 6 similar experiments on different subjects are shown in Fig. 7. In one subject the constrictor response to 5-HT was not affected either by intra-arterial or by intravenous sodium salicylate. In the others the response was reduced or abolished only when the sodium salicylate was given intravenously. These findings suggest that the antagonistic effect of sodium salicylate is not a local one on the tissues but due to some indirect action of the drug.

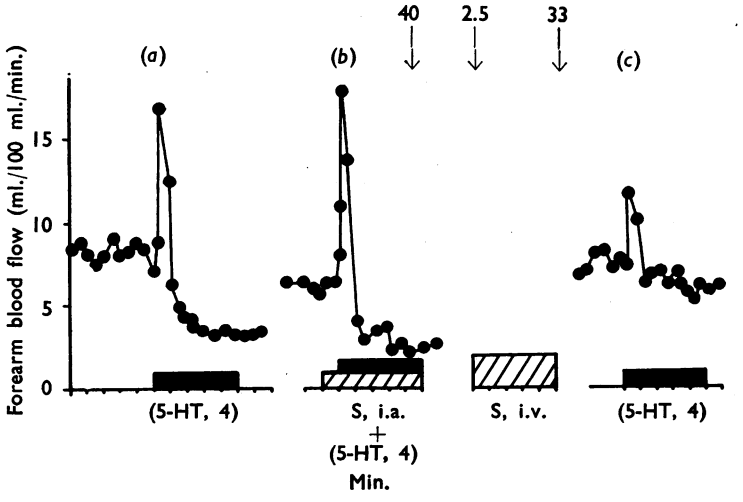

FIG. 6.—The changes in the left forearm flow (ml./100 ml./min.) in response to the intra-arterial infusion of 4 μg./min. of 5-HT (*a*) before, (*b*) during the infusion of 20 mg./min. of sodium salicylate into the same brachial artery (S, i.a.), and (*c*) after 4 g. sodium salicylate intravenously (S, i.v.). The arrows represent the time at which blood samples were taken from a left antecubital vein, and the numerals the serum sodium salicylate content in mg.%.

Hand Blood Flow.—Changes in hand blood flow were followed by measurement of heat flow from the skin of the pulp of the finger. The plethysmographic method was not used because of the difficulty in interpretation of hand inflow curves as a consequence of reduction in the distensibility of the hand vessels (Roddie *et al.*, 1955 ; Glover, Greenfield, Kidd, and Whelan, 1957). In 4 experiments, the level of heat flow was determined during intra-arterial infusions of 5-HT both before and after intravenous injection of 2 g. of sodium salicylate. 5-HT caused a fall in heat elimination and this constrictor response

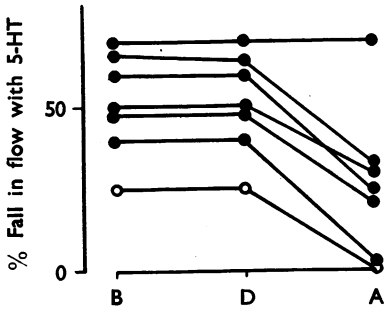

FIG. 7.—The results of seven experiments of the type illustrated in Fig. 6. The response to 5-HT intra-arterially is expressed as a % fall in flow. Solid circles represent a dose of 4 μg./min. of 5-HT; open circles a dose of 1 μg./min. of 5-HT. B, before administration of sodium salicylate. D, during the intra-arterial infusion of 20 mg./min. of sodium salicylate. A, after the intravenous injection of 3 to 4 g. of sodium salicylate.

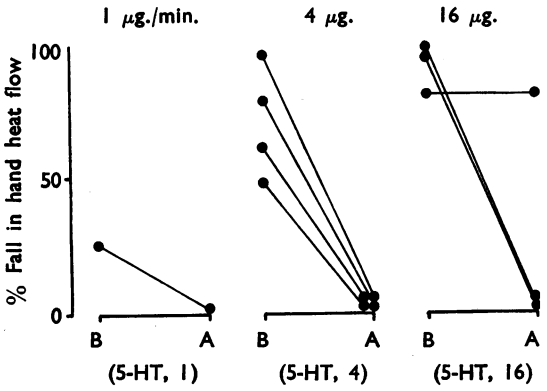

FIG. 8.—The results of experiments in which the heat flow from the finger skin during intra-arterial infusions of 1, 4, or 16 μg./min. of 5-HT was determined before (B) and after (A) 2 g. sodium salicylate intravenously. The response is expressed as % fall in heat elimination and is calculated as in Fig. 4.

was abolished by sodium salicylate on all but one occasion, when the response to 16 μg./min. was unaffected (Fig. 8). It is concluded that the vessels of the hand respond to 5-HT and to sodium salicylate in the same way as do those of the forearm.

DISCUSSION

Lysergic acid and its derivatives including 2-bromo-(+)-lysergic acid diethylamide bitartrate have been shown to antagonize the effects of 5-HT on isolated smooth muscle preparations (Gaddum and Hameed, 1954), but these substances have been found to be without effect on the symptoms of patients suffering from carcinoid tumour (Snow, Lennard-Jones, Curzon, and Stacey, 1955). The results of the present experiments demonstrate that BOL 148 is an effective antagonist of certain of the vascular responses to 5-HT in the limbs of normal human subjects. The antagonism is displayed whether the BOL 148 is given intravenously, or locally by intra-arterial injection into the limb segment concerned. It is concluded that the antagonistic action is dependent upon a direct effect of BOL 148 at the site of action of the 5-HT, namely, the smooth muscle of the blood vessels. The antagonism is not a consequence of a non-specific inhibition of smooth muscle, because the dilator response of the vessels of the part to histamine and the constrictor response to noradrenaline can be shown to be unaffected at a time when the response to 5-HT has been abolished or considerably diminished by BOL 148.

The antagonistic action of sodium salicylate on the vascular responses of the forearm vessels to 5-HT is likewise a specific one, but, in contrast to that of BOL 148, it appears to be an indirect action. Introduction of sodium salicylate locally into the limb is only effective if given in amounts large enough to circulate and to raise the serum level to 20 to 25 mg.% or more. Smaller doses, though providing a high local blood concentration, were without effect on the responses to 5-HT. In the intra-arterial experiments care was taken to achieve as far as possible the same local conditions in the forearm as during the intravenous experiments, by maintaining the sodium salicylate level in the perfusing blood at approximately the same level and by administering it for the same period before and during the 5-HT infusion.

The present experiments provide no explanation of the mechanism of the indirect antagonistic effect of sodium salicylate. Sodium salicylate given intravenously has been shown to stimulate respiration and thus cause a rise in blood *p*H (Graham and Parker, 1948). That this effect of sodium salicylate is unlikely to be the cause of its antagonism to 5-HT is shown by the result of the following experiment. In a normal subject, the respiratory movements were recorded on a smoked drum from stethographs applied around the chest and abdomen, and the responses of the forearm vessels to intra-arterial 5-HT were determined. When an intravenous injection of 4 g. of sodium salicylate was given both the rate and depth of respiration were increased for a period of 10 to 15 min. Towards the end of this time, and while the respiration was still increased, the doses of 5-HT were repeated and the responses were found to be reduced by the usual amount. On a subsequent occasion the same subject hyperventilated for the same period of time at a rate and

depth somewhat greater than before, as judged by " playing back " to him the previous record (Dornhorst and Whelan, 1953 ; Barcroft, Gaskell, Shepherd, and Whelan, 1954). The responses of the forearm vessels to the 5-HT infusions were the same during as before the period of hyperventilation, showing that the effects of hyperventilation of a degree greater than that due to sodium salicylate provoked no antagonism of the response to 5-HT.

It is possible that sodium salicylate or one of its derivatives releases, activates or combines with some other substance already present in the body but not in the forearm, such as the secretion of an endocrine gland. It has long been recognized that sodium salicylate has a cortisone-like effect, but it has been shown that in man this is not due to an increase in the plasma level of adrenal cortical hormone (Bayliss and Steinbeck, 1954). Kelemen (1957) has shown that salicylate antagonizes the oedema of the paw in the rat produced by locally injected 5-HT in the intact but not in the adrenalectomized animal. This suggests that salicylates depend in some way on the adrenal corticoids for some of their actions, and it seems likely that a similar synergism plays a part in the antagonism of the vascular effects of 5-HT in man.

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