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5-HYDROXYTRYPTAMINE. PHARMACOLOGICAL ACTION AND DESTRUCTION IN PERFUSED LUNGS.

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The isolation of the powerful vasoconstrictor substance 5-hydroxytryptamine [Rapport, Green and Page, 1948 *a*, *b* and *c*; Rapport, 1949] was an important advance in the study of the old problem of the pharmacological activity of shed blood [see Gaddum, 1936; Reid and Bick, 1942; Zucker, 1944]. This substance (HT) appears to be mainly responsible for the vasoconstrictor action of beef serum on the perfused rabbit's ear, but is not the only vasoconstrictor substance found in blood; adrenaline, noradrenaline, vasopressin, renin and hypertensin must sometimes be present, and other vasoconstrictors may be there too. According to Bayliss and Ogden [1933], the vasoconstrictor action of defibrinated blood on the perfused kidney of a dog is due to two substances, one of which appears quickly, acts briefly, and is stable in blood, but is removed by perfusion through lungs and kidneys, while the other only appears after 1½ to 2 hours and has a much more prolonged action. The first of these substances may well be HT, but not the second. The smooth muscle-contracting substance (SMC) detected by Zucker [1944] in blood causes vasoconstriction in a perfused cat's tail and excites various other smooth muscles, but has no action on the perfused rabbit's ear, and cannot therefore be HT. The pharmacological effects of serum should not be attributed to HT unless the HT-equivalent is found to be constant when estimated by more than one method.

The work described here was done because experiments by one of us (J. H. G.) had indicated that HT causes pulmonary vasoconstriction in the anæsthetized cat.

Method.

The method was similar to that used by Daly [1938] for perfusing monkey's lungs. Cats were anæsthetized with chloralose intraperitoneally (approx. 70 mg./kg.), given 1 ml. of heparin intravenously (5000 units per ml. BDH) and then bled from a cannula in the carotid

[1] Part of this work was done during tenure of a Medical Research Council Studentship for training in research methods.

artery. If heparin is omitted there is a risk that subsequent perfusion of the lungs will fail because of intravascular clotting. Immediately after death the chest was opened and cannulæ tied into the pulmonary artery, left auricle and trachea. Ligatures were tied to close off all extrapulmonary systemic vessels through which blood might escape by way of the bronchial vascular system. At this stage the lungs were removed from the chest and transferred to a respiratory chamber where perfusion and ventilation by negative pressure were begun. In some of the later experiments the lungs were ventilated by positive pressure from a Starling "Ideal" pump. The maximum ventilating pressure was kept constant at 8 to 10 cm. water by the overflow system devised by Konzett and Rössler [1940]. The airtight chamber containing the lungs was used as a plethysmograph and lung ventilation recorded by a Krogh spirometer.

Perfusion with the heparinized blood already collected was maintained at constant volume inflow. In experiments where the effects of HT on the lungs were observed, two flow rates were used, 95 ml. per min. with small and 125 ml. per min. with larger cats. In later experiments on destruction of HT during perfusion the rates ranged from 20 to 200 ml. per min. Blood temperature was kept constant at 33° C. A Dale Schuster pump was adapted for small volumes by using a small rubber teat as pulsator and fitting it with a glass pump chamber. The glass parts of the pump containing rubber flutter valves were fitted with Quickfit joints. With these modifications the total capacity of the pump and connections was 10 to 15 ml. All glass surfaces in contact with blood were coated with a film of silicone (General Electric Drifilm 9987—or 5 per cent solution of BTH "Teddol" in carbon tetrachloride). Pulmonary arterial pressure, venous reservoir volume and tidal air were recorded. The venous reservoir volume is an indirect measure of lung blood volume provided allowances are made for capacity changes in the manometers [Daly, 1938]. Glucose (0·5 ml., 20 per cent solution) and heparin (0·2 ml.) were added at intervals to the venous reservoir.

The substances tested were injected into the rubber tubing leading into the pulmonary artery cannula through a long two-headed needle constructed so that the test dose could be washed in by a following injection of saline. The dead space of the needle was between 0·1 and 0·15 ml., and the total volume of injection was kept constant at 0·4 ml. except on a few occasions when larger doses of the test substances were employed. The same routine was followed for each injection, which lasted 3 to 5 sec. The interval between injections was usually 5 minutes.

Estimates of the concentration of HT in plasma were made by a method developed by Amin, Crawford and Gaddum [1952]. The blood was collected in siliconed centrifuge tubes standing in ice, and quickly centrifuged. A measured volume of plasma was then mixed with

19 volumes of cold acetone. The resulting precipitate was removed in a centrifuge and the acetone extract dried *in vacuo*. The residue was dissolved in de Jalon's solution [Gaddum, Peart and Vogt, 1949] and compared with a standard solution of HT by its action on a rat's uterus suspended in 3 ml. of de Jalon's solution containing atropine (1 mg./l.) at 30° C.

A synthetic preparation of 5-hydroxytryptamine creatinine sulphate was presented by Messrs. Upjohn. Doses are given in terms of the base, by dividing the weight of the salt by 2·3. Dihydroergotamine and lysergic acid diethylamide were presented by Messrs. Sandoz.

Results.

HT, although a powerful vasoconstrictor, does not always cause a rise of blood pressure. In our preliminary experiments with anæsthetized cats the usual effect was a fall of carotid pressure, sometimes followed by a rise when large doses were injected later in the experiment. Both pressor and depressor effects have also been observed by others [Rapport, Green and Page, 1948*d*; Freyburger *et al.*, 1952; Page, 1952; Reid, 1952]. We thought it possible that the initial depressor effect in our experiments was due to a rise of resistance in the pulmonary circulation. A record of the pressure in a branch of the pulmonary artery showed a rise of pressure within 3 sec. after the injection of HT into the jugular vein, followed 12 sec. later by a fall of carotid pressure. The time relations suggested that the second effect was a consequence of the first. These experiments are not recorded in detail since similar conclusions have already been reached by Reid [1952].

The speed with which the pulmonary pressure rose suggested direct action of the pulmonary vessels, and this was confirmed by experiments on perfused lungs. In all these experiments the intra-arterial injection of HT produced both broncho- and vaso-constriction (fig. 1). The reduction in tidal air caused by doses of 10 to 20 μg. varied from 12 to 80 per cent. The vasomotor response was also variable. The sensitivity of the blood vessels and bronchi were not associated. When the pulmonary arterial pressure was at a constant base-line, the maximum variation between responses to successive injections of the same amount of HT was 22 per cent.

Experiments on anæsthetized dogs [Freyburger *et al.*, 1952] and on cats [Reid, 1952] and isolated guinea-pig ileum [Gaddum, 1953*a*] showed that the sensitivity of the reactive tissues to successive injections of HT decreased at least temporarily, but in isolated lungs we did not observe an effect of this kind with minimum test intervals of 4 min. This was true of both bronchomotor and vasomotor responses.

An injection of adrenaline given immediately before HT abolished or reduced the bronchoconstrictor response in all our experiments, but

had a less constant effect on the vasomotor response. The changes in the bronchoconstrictor response due to adrenaline and their time relations are illustrated by the result in fig. 2. In this experiment 20 μg. of adrenaline was injected; before this HT had reduced tidal air by 20 per cent: 5 min. later it had almost no effect but within 35 min. its activity had returned to the previous level. We have observed this effect on the bronchi with doses of adrenaline ranging from 10 to 50 μg.

Fingl and Gaddum [1953] have observed that some of the effects of HT are antagonized by dihydroergotamine (DHE). In three experiments DHE was therefore added to the blood reservoir after a number of consistent responses to HT had been obtained, and the effect on subsequent injections of HT was then observed. The effect of DHE in a concentration of 1 μg. per ml. blood was first to reverse and then to abolish the vasomotor response to 20 μg. of HT. Larger doses could still produce vasoconstriction though the response was small. The bronchoconstrictor response was completely abolished. Lysergic acid diethylamide (LSD), which is structurally related to the ergot alkaloids, was found by Gaddum [1953 *b*] to antagonize the effects of HT on the rat's uterus. It also antagonized the effects of HT on the lungs in a concentration of 1 μg. per ml. blood. This result is illustrated in fig. 3, which also shows that the lungs still responded to the bronchoconstrictor action of histamine.

Adrenaline itself caused a rise in pulmonary arterial pressure in four experiments, a fall in two experiments, and in one experiment a rise in the early stage of perfusion and later a fall. It always caused bronchodilatation although the changes in tidal air were small. After DHE adrenaline invariably produced vasodilatation and the bronchodilatation normally observed.

The HT-Equivalent of the Plasma.—The method used for estimating HT in the plasma is thought to be fairly specific. Histamine does not cause contraction of the rat's uterus. Ordinary amounts of acetylcholine are inactive in the presence of the concentration of atropine used in the bath. Oxytocin and substance P are insoluble in 95 per cent acetone. Potassium could not be present in sufficient amounts to affect the result. The only substance known to be present in the body in sufficient concentrations which contracts the rat's uterus, is active in the presence of atropine, and soluble in 95 per cent acetone, is HT. The conclusion that the effects were really due to HT was tested by the use of specific antagonists. In one experiment 0·1 μg. of LSD was added to the bath at the end of the experiment. In the presence of this drug the effects of HT and of extract were completely abolished, but the muscle still gave a large response to 1 mU of oxytocin (fig. 4*b*).

There is no real reason to doubt that HT actually was present in the amounts shown in Table I, but since the evidence is not conclusive, the results are presented as HT-equivalents instead of HT-concentrations.

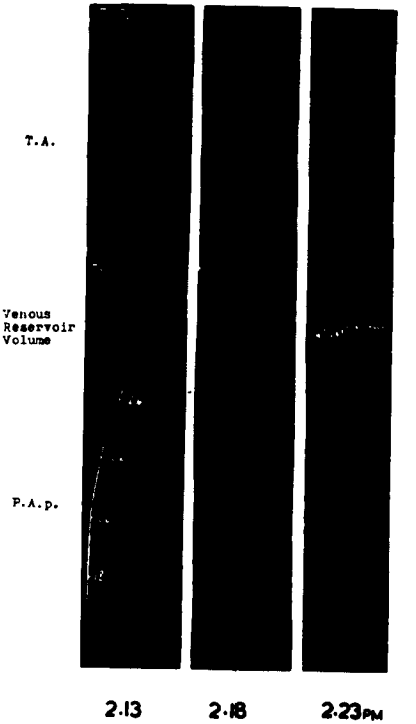

FIG. 1.—Isolated perfused lungs. HT intra-arterial injection 20 μg. 5-hydroxytryptamine. Negative pressure ventilation. TA tidal-air. P.A.P. pulmonary arterial pressure.

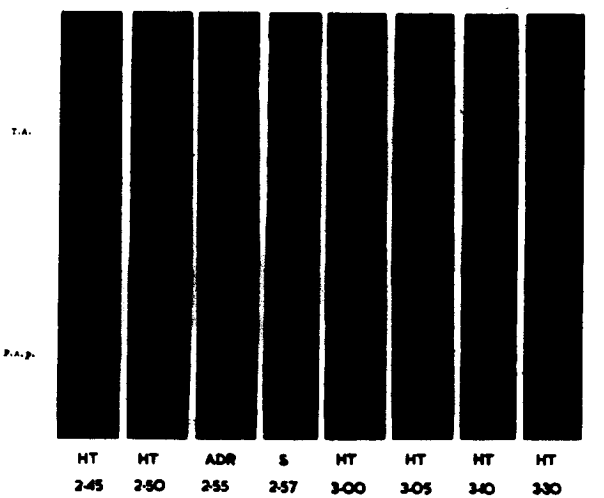

FIG. 2.—Isolated perfused lungs. Effect of adrenaline on the response to 5-hydroxytryptamine.

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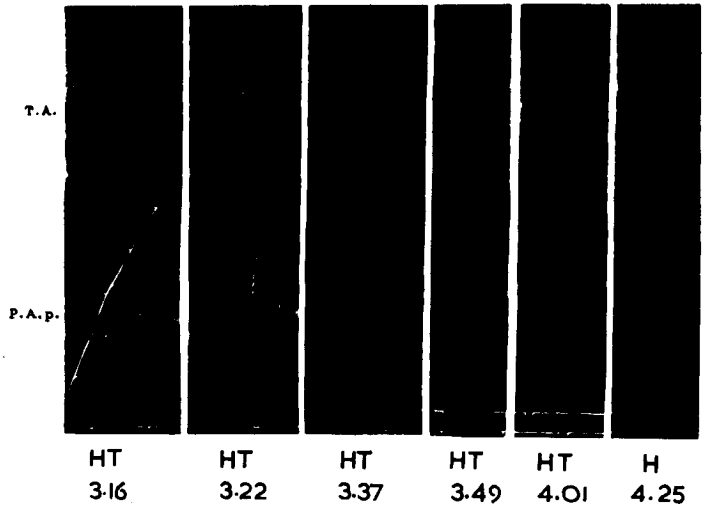

FIG. 3.—Isolated perfused lungs. HT intra-arterial injection 10 μg. 5-hydroxytryptamine. H 20 μg. histamine. At 3.31 p.m. lysergic acid diethylamide added to venous reservoir. Find concentration 1 μg./ml.

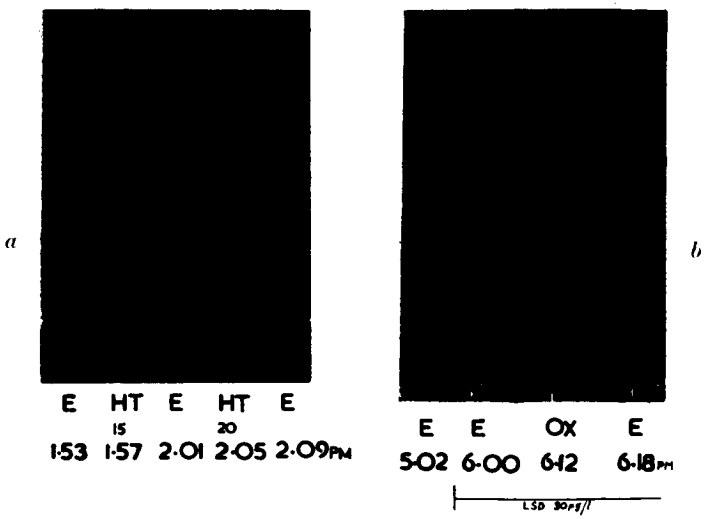

FIG. 4*a*.—Isolated rat uterus. Assay of HT-equivalent of plasma. E = Extract equivalent to 0·015 ml. blood. HT = 5-hydroxytryptamine. Small figures give doses in nanograms (ng.) of the base.

FIG. 4*b*.—E = Extract of different blood sample from that used in fig. 4*a*. Ox. = 1 mU oxytocin. Lysergic acid diethylamide (LSD) 30 μg./l. added at vertical mark.

When blood was run directly from the artery of the anæsthetized cat into a cooled siliconed centrifuge tube and quickly centrifuged the HT-equivalent of the plasma was low (25 μg./l.), but when blood was collected without special precautions the HT-equivalent rose in a few minutes to about 1000 μg./l. These observations are in keeping with the view that HT is normally present in platelets and is easily

TABLE I.

(Time in minutes—HT equivalent (μg./l.).)

Experiment.	1.		2.		3.		4.		5*a*.		5*b*.	
	Time	HT	Time	HT	Time	HT	Time	HT	Time	HT	Time	HT
	0	1100	0	750	0	750	0	333	0	1000	..	..
					8	750	12	167	22	575	..	..
					15	450	24	42	44	230	..	..
					60	100	60	10	..	..	..	..
HT added to increase concentration by amount shown (μg./l.)	1	(1000)	1	(5000)	..		..		..		0	(1000)
	2	1600	2	6000	..		..		..		1	1200
	17	1300	18	2500	..		..		..		21	30
Initial blood vol. (ml.)	..		..		100		145		135		120	
Circulation rate (ml./min.)	..		..		26		66		20		200	
Halving time (min.)	..		..		20		11		22		4	
Per cent removed per circulation ($100k$)	..		..		13		14		21		10	

released [Reid and Bick, 1942; Humphrey and Jaques, 1953]. In experiments 1 and 2 known amounts of HT were added to the reservoir at the beginning of the experiment. This produced an increase in the HT of the plasma which did not differ significantly from the expected increase. Experiment 2 shows that the lungs did remove this added HT. In experiments 3 and 4 no HT was added, but when the lungs were perfused the HT-equivalent of the plasma fell.

In experiment 5 the lungs were first perfused at 20 ml. per min. and the fall of the HT-equivalent of the plasma was followed. HT was then added to the blood and the rate of perfusion was increased to 200 ml. per min. The rate of disappearance of HT was then found to be increased.

In experiments 3–5 the logarithm of the HT-equivalent was plotted against time and the points were found to lie approximately on straight lines. These results therefore indicate that the fall was exponential.

The halving time was estimated graphically and is given in the table The figures show that the more rapid the circulation the more rapid wa the fall of HT-equivalent.

The figures in the last row of the table were calculated as follows:–

x = concentration of HT.
k = proportion of HT removed by the lungs in one circuit.
V = volume of blood in the circulation (ml.).
v = circulation rate (ml. per min.).

The rate of disappearance of HT $= \frac{d(x\mathrm{V})}{dt} = -kvx.$

By integration $\log_e x = -kvt/\mathrm{V}$.
If the halving time is t', then

$$k = 0{\cdot}693\ \mathrm{V}/vt' \quad (\text{where } 0{\cdot}693 = \log_e 2).$$

The estimates of $100k$ obtained in this way indicate, as might have been expected, that when the circulation was rapid the percentage of HT removed in each circuit was less than when the circulation was slow. The data are, however, insufficient to establish this point with certainty.

Discussion.

Our results with perfused lungs confirm the evidence that HT causes pulmonary vasoconstriction and bronchoconstriction and show that such effects can be caused by a direct action on the lungs. Both were antagonized by ergot alkaloids.

These effects resemble the vasoconstrictor action of defibrinated blood in perfused lungs from rabbits [Tribe, 1914; von Euler, 1932], cats [Newton, 1932] and dogs [Daly, 1938], and the bronchoconstrictor action in perfused lungs from cats [Newton, 1932], guinea-pigs and dogs [Hemingway, 1931 *a*; Daly, 1938]. Both appear to be due to substances liberated in the blood after it has been removed from the body. There is evidence that HT is normally present in platelets [Reid and Bick, 1942] and may be liberated when these break up. These effects of shed blood may therefore all be due to HT, and this theory is supported by the fact that the effects on the dog's pulmonary vessels and the guinea-pig's bronchi were antagonized by ergotoxine [Daly, 1938]. The new facts about HT and ergot alkaloids provide an easy explanation of this antagonism which seemed strange when first found. On the other hand, the experiments described here were all done with cats and scarcely justify the discussion of effects on other animals. If the estimates of the HT-equivalent really do represent concentrations of HT, the amount added to the reservoir in experiment 1 of the table was about equal to that already present. The fact that

this caused increased vasoconstriction and bronchoconstriction shows that concentrations similar to those occurring naturally are effective. It is thus reasonable to conclude that this effect of shed blood on cats' lungs is mainly due to HT.

There can be little doubt that many of the other vasoconstrictor effects of shed blood are also due to HT. For example, Heymans, Bouckaert and Moraes [1932] found that the vasoconstrictor effects of defibrinated blood on perfused dogs' heads, frogs' legs and rabbits' ears were antagonized by ergotamine; these effects were probably due to HT. On the other hand, some of the vasoconstrictor effects of shed blood are not completely suppressed by dihydroergotamine [Hebb and Linzell, 1951]. This fact confirms the conclusion discussed above that shed blood may contain other vasoconstrictor substances besides HT.

The estimates shown in the table were made because it is possible that HT has been responsible for some of the difficulties encountered by those who have used defibrinated blood in perfusion experiments. Experiments on dogs' kidneys perfused with blood were much hindered by "vasotonins", until it was shown that they disappeared if a heart-lung preparation was included in the circuit [Verney and Starling, 1922; Eichholtz and Verney, 1924]. The lungs played a part in this detoxication of the blood, and even the kidneys themselves had some such effect [Hemingway, 1931*b*; Bayliss and Ogden, 1933]. There is no reason to believe that the lungs remove vasotonins more rapidly than other tissues, but they do remove them, and if HT is the main vasotonin, it should disappear when the blood is perfused through lungs. Our results confirm that this does happen.

Summary.

1. 5-Hydroxytryptamine (HT) caused vasoconstriction and bronchoconstriction in cats' lungs perfused with blood. These actions were antagonized by dihydroergotamine or lysergic acid diethylamide.

2. The HT-equivalent of the plasma was estimated by extraction with acetone and assay on rat's uterus in comparison with synthetic HT. This was low immediately after bleeding, but rose rapidly to about 1 mg. per litre.

3. The HT-equivalent of the plasma fell exponentially during perfusion with a halving time of 4–20 min.

4. When the rate of circulation was increased the rate of disappearance of HT rose.

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