Reprinted from the QUARTERLY JOURNAL OF EXPERIMENTAL PHYSIOLOGY,
VOL. XXXVIII, 1953.

A STUDY OF THE VASCULAR ACTIONS OF 5-HYDROXYTRYPTAMINE, TRYPTAMINE, ADRENALINE AND NORADRENALINE.

By K. H. GINZEL and S. R. KOTTEGODA.[1]
From the Department of Pharmacology, Oxford University.

(*Received for publication 8th July* 1953.)

IN previous studies on the vascular responses to 5-hydroxytryptamine (HT) and tryptamine [Rapport, Green and Page, 1948; Reid, 1951; Reid and Rand, 1951; Reid, 1952; Page, 1952], comparisons between the actions of these substances and those of adrenaline and noradrenaline have been made only incidentally. To investigate the relative potencies of these substances on different circulatory regions, experiments were performed on the isolated perfused lung, on the renal circulation of the cat, on the hind leg of the cat and the dog, and on the vessels of the rabbit's ear.

METHODS.

Measurement of Renal Blood Flow.—All experiments on cats were performed under chloralose anæsthesia (80 mg./kg. intravenous). The animal was eviscerated and in some experiments both suprarenal glands tied off. Heparin (3500 units/kg.) was given intravenously. After cleaning the renal vein throughout its length and tying off its tributaries, a cannula was inserted. A second cannula was put into the jugular vein and these two veins were connected by a length of polythene tubing interrupted by a glass T-piece. The venous outflow was measured through the middle limb of the T-piece. For intra-arterial injections a metal cannula was inserted into the central stump of the superior mesenteric artery. When determining the effect of intra-arterial injections a clamp was placed on the aorta in the lower abdomen. When a constant blood pressure was required, a compensator of the type described by Krayer and Verney [1936] was connected to the side arm of the blood pressure cannula. It consisted of a litre bottle containing 500 ml. of 50 per cent dextran in Locke's solution connected to a stoneware jar of 20 l. capacity in which the requisite air pressure was maintained.

Observations on Pulmonary Vessels.—The method employed was a modification of that described by Dale and Schuster [1928]. The cat under ether anæsthesia was heparinized (500 units/kg.) and bled out in stages with alternate injections of adrenaline and Locke's solution.

[1] On study leave from the Faculty of Medicine, University of Ceylon.

The blood collected was filtered through muslin and heparin (500 units/100 ml.) added to it.

A cannula was made from a boiling tube of 2 cm. diameter into the bottom of which a glass tube was sealed. This tube turned horizontally and ended in a nose which was inserted into the pulmonary artery. The blood was pumped into the vertical boiling tube through a rubber stopper and stood at a height of about 17 cm. above the artery. The air space above the blood was connected to a piston recorder which recorded the changes in the air space as the blood ran more quickly or more slowly into the vessels.

Hind Limb Perfusion: Dog or Cat.—The preparation of the hind leg for perfusion was essentially the same as that described by Dale and Richards [1918]. Blood for the limb perfusion was oxygenated by perfusion through the lungs with a separate pump. When cat limbs were perfused, a second cat provided additional blood for the perfusion, and its lungs were used for oxygenation. The outflow from the legs was measured by means of a modification of Stephenson's method [1948], using a piston recorder.

Experiments on the Vessels of the Rabbit's Ear.—These were made either by the method of Gaddum and Kwiatkowski [1938] using a Gaddum drop-timer, or observations were made on the isolated ear according to the method described by Burn and Robinson [1951] where Stephenson's recorder was used. HT was used in the form of serotonin creatinine sulphate. All doses of substances injected, unless otherwise stated, refer to the salt.

Results.

Pulmonary Vessels of the Cat.—In all experiments HT and tryptamine caused an increase in the resistance of the pulmonary circulation. A comparison between the constrictor action of these two substances and that of adrenaline and noradrenaline on the pulmonary vessels is shown in fig. 1. The doses of 5-hydroxytryptamine and tryptamine were appreciably smaller (5–10 μg.) than those of adrenaline and noradrenaline (50–100 μg.) necessary to produce a similar degree of vasoconstriction. The effects of noradrenaline and of adrenaline, however, were much more prolonged. Adrenaline sometimes had an initial dilator action preceding the constriction (fig. 1 (*b*)). The relative equipotent doses of these substances from 7 experiments were as follows:—

5-Hydroxytryptamine (base).	Tryptamine.	Adrenaline.	Noradrenaline.
1	2–4	10–20	10–20

In one experiment where the sensitivity to HT was unusually low its effect was not greater than that of adrenaline or of noradrenaline.

When injections were repeated within a short time, responses were reduced, but 10 min. after injection of 200 μg. the response to 10 μg. returned to its original size.

The vasoconstrictor action of HT or tryptamine was not increased by previous injections of either tyramine (500 μg.) or paredrine (*p*-(2-aminopropyl)-phenol) (1 mg.), but was slightly reduced. Both tyramine and paredrine exerted a slight vasoconstrictor action. In three other experiments ephedrine and paredrine in concentrations up to 2×10^{-5} in the perfusing blood failed to enhance the action of either HT or tryptamine.

Lysergic acid diethyl amide (L.S.D.), in concentrations of 10^{-6} to 3×10^{-6}, abolished or reduced greatly the vasoconstrictor action of 100 to 200 μg. HT. A record from one of these experiments is shown in fig. 2. Injection of HT several times at suitable intervals both before and after L.S.D. showed that this reduction was not due to the previous doses of HT. Injections of 100 to 200 μg. L.S.D. caused a brief dilatation of the pulmonary vessels. Ergotamine blocked the action of HT and tryptamine in concentrations greater than 10^{-5}, which reversed the actions of adrenaline and noradrenaline. L.S.D., however, in the concentrations which reduced the action of HT and tryptamine, had little effect on the actions of adrenaline and noradrenaline.

Effect on Blood Vessels of the Perfused Hind Leg of Cat and Dog.— The constrictor effects of HT and tryptamine in the vessels of the hind leg of the cat and dog were much weaker than those of adrenaline and noradrenaline. There was, again, great variation in different experiments. Thus in the cat (5 expts.) adrenaline and noradrenaline were 10 to 500 times stronger than HT, and in the dog (2 expts.) the former were 10 to 100 times stronger than HT. Generally in the cat and in the dog adrenaline and noradrenaline were equally vasoconstrictor; in one cat adrenaline was four times less effective than noradrenaline. In the cat tryptamine was either as effective as HT or was about one-third as active. In the dog HT was about ten times more active than tryptamine. These figures refer to the maximum constriction; the duration of constrictor responses was much greater for HT than for adrenaline and noradrenaline. Tryptamine occupied an intermediate position. A comparison of these substances in the cat is illustrated in fig. 3. While the sensitivity of the vessels to adrenaline and noradrenaline altered little throughout the experiment, the responses to HT and to tryptamine always declined with time. Thus in an experiment in a dog, 30 μg. HT was at first rather greater than 1 μg. adrenaline; 2 hours later, however, 30 μg. HT was rather less than 1 μg. adrenaline; 10 minutes later than this 100 μg. HT caused a rise equal to 1 μg. adrenaline. In another experiment 100 μg. tryptamine suppressed the action of smaller amounts of HT and tryptamine without affecting the response to adrenaline. The suppression persisted for 20 minutes.

After injections of even large amounts of HT the return of the previous responses occurred usually, though not always, within 10 minutes. The amount of HT or tryptamine necessary to suppress the effect of either substance differed in different experiments, being from 1 to 5 times the test dose. L.S.D. in a concentration of 10^{-6} in the perfusion fluid completely abolished not only the effect of 30 μg. HT, but also of nearly 10 times this dose (fig. 4). In this concentration of L.S.D. the action of an equi-effective dose of adrenaline was only slightly diminished.

Comparison of Effects in the Lungs and the Hind Leg.—The high constrictor potency of HT on the pulmonary vessels of the dog compared with its weaker action on the vessels of the hind leg and the reverse relation for the action of noradrenaline at these sites is well seen in fig. 5. In fig. 5, injections 1, 2 and 3 were made into the pulmonary artery. At 2, 100 μg. HT caused a greater pulmonary constriction than did 50 μg. noradrenaline at 3. But when these substances had travelled through the lungs and passed on their way to the hind leg, the rise in femoral artery pressure after 2 was very slight compared with the large rise of pressure after 3. Even the small dose of 0·1 μg. noradrenaline injected into the pulmonary artery at 1, which did not cause pulmonary constriction, produced as much constriction when it reached the femoral artery as was produced after 2.

Observations on Renal Vessels of the Cat.—In 9 experiments the relative potency of HT, adrenaline and noradrenaline on the renal outflow were examined. An intravenous dose of about 50 μg. adrenaline or noradrenaline caused a 50 per cent reduction in the renal outflow. Even in doses of 200 μg. HT did not decrease the outflow more than 20 per cent. Such an effect was usually produced with a dose of about 20 μg. noradrenaline. When the injections were made intra-arterially, usually 10 μg. noradrenaline produced a 50 per cent reduction in the outflow, while doses of HT from 20 to 300 μg. caused reductions in outflow varying from 10 to 40 per cent. A great variability in the effects of HT injected intra-arterially was observed, the degree of vasoconstriction changing considerably with different experiments. In experiments where the suprarenal glands were ligated the effect of HT was not significantly altered. Tolazoline in doses sufficient to reverse the actions of adrenaline or noradrenaline did not abolish or reverse the vasoconstriction caused by HT. Tryptamine had to be used in doses more than ten times those of HT to produce a noticeable effect on the renal vessels. A desensitizing action of HT to its own action was again observed.

Effect on Vessels of the Rabbit's Ear.—In perfusing the vessels of the rabbit's ear by the method of Gaddum and Kwiatkowski [1938], it was found that single injections of 1 μg. tryptamine caused a prolonged vasoconstriction equal to that caused by 0·2 μg. HT. Noradrenaline or adrenaline in a dose of 0·2 μg. produced a short-lasting effect of the

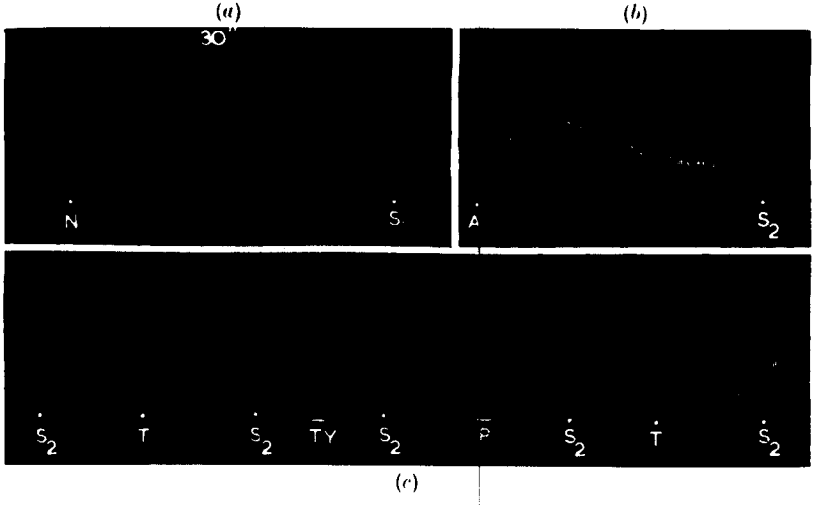

FIG. 1.—Pulmonary vessels of cat. Record in this and fig. 2 is the pressure in the pulmonary vessels. In these two experiments injections were made into pulmonary artery cannula.

(*a*) At N 50 μg. noradrenaline caused a rise of pulmonary pressure in two stages; an initial sharp rise followed by a more prolonged rise of pressure. At S_1 10 μg. HT caused a brief rise of pulmonary pressure.

(*b*) 100 μg. adrenaline injected at A caused an initial vasodilatation followed by a prolonged vasoconstriction. At S_2 5 μg. HT were injected.

(*c*) Tryptamine in a dose of 10 μg. injected at T caused an effect equal to that of 5 μg. HT injected at S_2. At TY 0·5 mg. tyramine and at P 1 mg. paredrine injected; neither substance potentiated either tryptamine or HT.

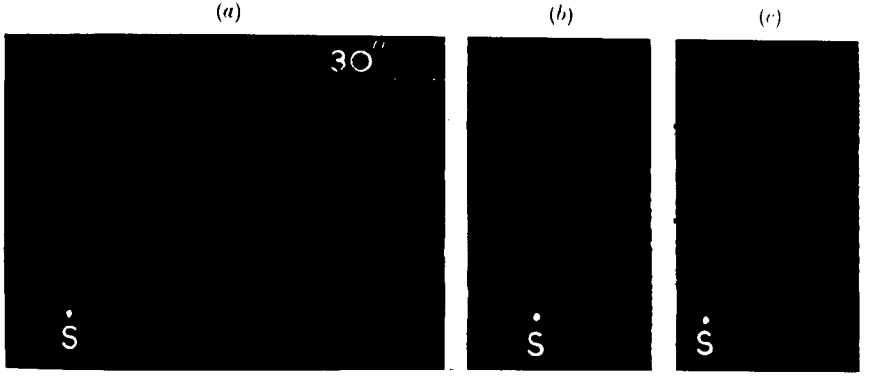

FIG. 2.—Pulmonary vessels of cat. 100 μg. HT injected at S in all cases. Between (*a*) and (*b*) is an interval of 15 minutes, during which L.S.D. in a concentration of 2×10^{-6} was present in the perfusing blood. In (*b*) and (*c*) the constrictor action of HT was greatly reduced in the presence of L.S.D. There was an interval of 10 minutes between (*b*) and (*c*).

[*To face page* 228

FIG. 3.—Cat hind leg perfusion records: above is the outflow (Stephenson recorder); below is the pressure. At N 0·1 μg. noradrenaline caused a rise of pressure nearly equal to that caused by 50 μg. HT injected at S. The effect of HT is more prolonged than that of noradrenaline. At N the same dose of noradrenaline was followed by 50 μg. tryptamine.

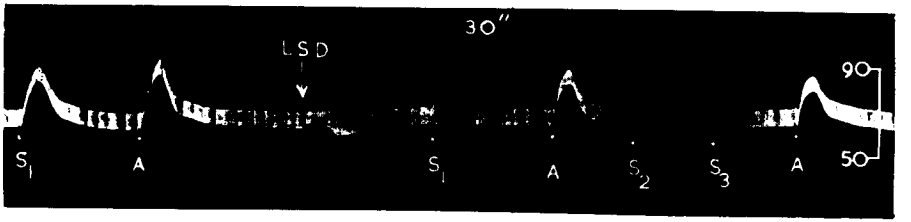

FIG. 4.—Dog hind leg perfusion. Pressure in arterial cannula. 30 μg. HT injected at S_1 produced an effect equal to that of 1 μg. adrenaline injected at A. At L.S.D. lysergic acid diethylamide was added to the venous reservoir to produce a concentration of 10^{-6}. Doses of HT of 30, 100 and 250 μg. at S_1, S_2 and S_3 respectively were without effect. The response to 1 μg. adrenaline (at A) was, however, only slightly reduced.

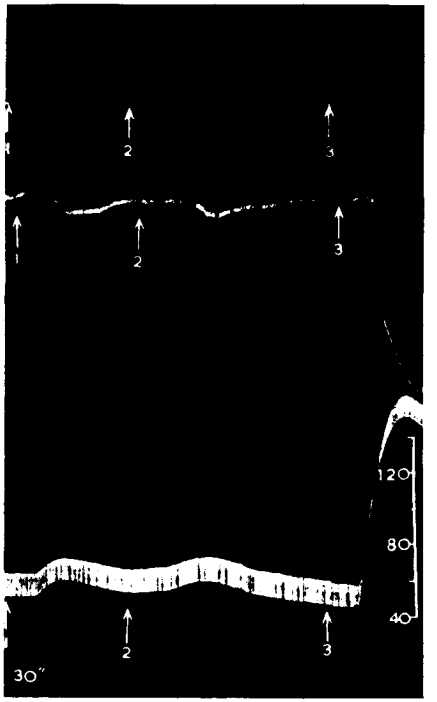

FIG. 5.—Dog hind leg and lung perfusion. Upper record is pulmonary arterial pressure; middle record is outflow from hind leg; bottom record is pressure in arterial cannula of hind leg. Injections 1, 2 and 3 made into pulmonary arterial cannula. At 1, 0·5 μg. noradrenaline had no effect on the pulmonary vessels but caused a constriction in the limb vessels about 60 sec. later. At 2, 100 μg. HT caused a sharp rise in pulmonary pressure and an action on the vessels of the hind leg equal to that caused by noradrenaline at 1. At 3, 50 μg. noradrenaline had a smaller action than 100 μg. HT on the lung vessels, but had a strong constrictor action on the vessels of the hind leg.

FIG. 6.—Outflow from perfused rabbit's ear (method of Gaddum and Kwiatkowski). Figures at the bottom denote time of injection. At N in all instances 0·1 μg. noradrenaline injected. Tryptamine in a concentration of $2{\cdot}5 \times 10^{-6}$ present in the perfusion fluid during the period marked T. Tryptamine is seen to cause constriction. After tryptamine, action of noradrenaline is greatly enhanced. At N', nearly 2 hours after tryptamine, 0·05 μg. noradrenaline produced the same effect as 0·1 μg. injected before tryptamine.

same degree (1 expt.). When tryptamine and HT were added to the perfusion fluid a noticeable decrease in the outflow appeared at a concentration of 10^{-7}. In the presence of either of these substances in this concentration the effect of single injections of adrenaline or noradrenaline was not modified. When a higher concentration was used (*e.g.* $2{\cdot}5 \times 10^{-6}$ in the experiment shown in fig. 6), an increase in the effect of noradrenaline and adrenaline was observed after the tryptamine was removed and its strong vasoconstrictor effect had worn off. HT had an action similar to that of tryptamine. In the vessels of the isolated rabbit's ear perfused by the method of Burn and Robinson [1951], in 6 experiments it was found that the constrictor action of HT was equal to, or 2 to 5 times greater than, that of adrenaline. In 3 experiments it was 300 to 1000 times more constrictor than adrenaline. The relationship between the constrictor potencies of HT and adrenaline was not changed when tested on the second day of perfusion. Tryptamine was about 10 to 25 times less effective than HT in causing constriction. Hexamethonium in doses of 150 to 200 μg. abolishing the constriction caused by equipotent doses of nicotine [Kottegoda, 1953] did not modify the constriction caused by HT or tryptamine. When tolazoline was added to the perfusing fluid so as to reverse the effect of adrenaline, that of HT or tryptamine remained unaltered. Repeated injections of HT given within a short time of each other resulted in a gradual decline of the vascular response, while the sensitivity to adrenaline remained unaltered.

The blocking action of L.S.D. towards HT observed in other preparations could not be satisfactorily investigated in the isolated ear because it had by itself a constrictor action.

Discussion.

The results of these experiments indicate that the relative constrictor potencies of HT and adrenaline and noradrenaline vary widely according to the vascular region studied. In the pulmonary vessels of the dog and cat, and in the vessels of the rabbit's ear, HT was generally a more powerful constrictor than adrenaline or noradrenaline. On the other hand, adrenaline and noradrenaline were more effective than HT in causing constriction in the vessels of the hind leg of the dog and cat and in the renal vessels of the cat. Tryptamine was usually $\frac{1}{2}$ to $\frac{1}{50}$th as active as HT. The relationship between equi-effective doses of these substances often depended on the stage of the experiment, as the effect of HT declined with time while those of adrenaline and noradrenaline remained unaltered. The change may have been due in part to the desensitizing action of HT and tryptamine on their own action, which was observed in all experiments. This has been observed

in other tissues for HT and for tryptamine by previous workers [Laidlaw, 1912; Reid, 1952; Freyburger *et al.*, 1952; Gaddum, 1953 *a*].

Both tryptamine [Blaschko, Richter and Schlossmann, 1937; Pugh and Quastel, 1937] and HT [Blaschko, 1952; Freyburger *et al.*, 1952] have been shown to be substrates of amine oxidase. However, when high doses of a substrate or inhibitor of amine oxidase like tyramine [Hare, 1928], paredrine [Alles and Heegaard, 1943] and ephedrine [Blaschko, Richter and Schlossmann, 1937] were present in the cat lung perfusion, no modification of the responses to adrenaline, noradrenaline, tryptamine or HT was observed. Similarly, high doses of HT or tryptamine did not influence the responses to adrenaline in this preparation. In the vessels of the rabbit's ear both tyramine and tryptamine often caused an increase of the responses to adrenaline and noradrenaline.

Confirming the findings of Freyburger and his co-workers [1952], we found that the constrictor action of HT was much less affected by antiadrenaline substances (tolazoline and ergotamine) than those of adrenaline or noradrenaline. On the other hand, L.S.D. was much more effective in antagonizing the vascular actions of HT than those of adrenaline and noradrenaline. This behaviour of L.S.D. towards 5-hydroxytryptamine has been recently described by Gaddum [1953 *b*], and we learn that similar observations to ours on the vascular antagonism have been made by Gaddum, Hebb, Silver and Swan [1953].

Summary.

1. 5-Hydroxytryptamine (HT) is a more powerful constrictor of the pulmonary vessels of the dog and the cat and of the vessels of the rabbit's ear than adrenaline or noradrenaline.

2. Adrenaline and noradrenaline are more powerful constrictors of the hind leg vessels of the dog and the cat and of the renal vessels of the cat than HT.

3. HT is usually between 2 to 50 times as active as tryptamine.

4. Lysergic acid diethyl amide (L.S.D.) is a strong antagonist of the constrictor action of HT in both the lung and the leg vessels and has only a weak effect on the actions of adrenaline and noradrenaline. Ergotamine and tolazoline are relatively ineffective in modifying the vascular actions of HT.

5. Since HT is a substrate of amine oxidase, we expected to find that an inhibitor of this enzyme such as paredrine would potentiate the action of HT. This was not observed. However, tryptamine was found to potentiate the action of noradrenaline.

6. Desensitization to HT and to tryptamine by themselves was constantly observed.

ACKNOWLEDGMENTS.

We wish to thank Professor J. H. Burn for suggesting this investigation and for the advice given throughout. We are grateful to Professor E. Rothlin for a sample of the diethylamide of lysergic acid, and to Dr. R. K. Richards for a sample of serotonin creatinine sulphate.

REFERENCES.

ALLES, G. A., and HEEGAARD, E. V. (1943). *J. biol. Chem.* **147,** 487.
BLASCHKO, H. (1952). *Biochem. J.* **52,** x.
BLASCHKO, H., RICHTER, D., and SCHLOSSMANN, H. (1937). *Biochem. J.* **31,** 2187.
BURN, J. H., and ROBINSON, J. A. (1951). *Brit. J. Pharmacol.* **6,** 110.
DALE, H. H., and RICHARDS, A. N. (1918). *J. Physiol.* **52,** 110.
DALE, H. H., and SCHUSTER, E. H. J. (1928). *J. Physiol.* **64,** 356.
FREYBURGER, W. A., GRAHAM, B. E., RAPPORT, M. M., SEAY, P. H., GOVIER, W. M., SWOAP, O. F., and VANDER BROOK, M. J. (1952). *J. Pharmacol.* **105,** 50.
GADDUM, J. H. (1953 *a*). *J. Physiol.* **119,** 363.
GADDUM, J. H. (1953 *b*). *J. Physiol.* **121,** 15 *P.*
GADDUM, J. H., HEBB, C. O., SILVER, A., and SWAN, A. A. B. (1953). *Quart. J. exp. Physiol.* **38,** 255.
GADDUM, J. H., and KWIATKOWSKI, H. (1938). *J. Physiol.* **94,** 87.
HARE, M. L. C. (1928). *Biochem. J.* **22,** 968.
KOTTEGODA, S. R. (1953). *Brit. J. Pharmacol.* **8,** 156.
KRAYER, O., and VERNEY, E. B. (1936). *Arch. exp. path. Pharmak.* **180,** 75.
LAIDLAW, P. P. (1912). *Biochem. J.* **6,** 141.
PAGE, I. H. (1952). *J. Pharmacol.* **105,** 58.
PUGH, C. E. M., and QUASTEL, J. H. (1937). *Biochem. J.* **31,** 2306.
RAPPORT, M. M., GREEN, A. A., and PAGE, I. H. (1948). *Science,* **108,** 329.
REID, G. (1951). *Aust. J. exp. Biol. med. Sci.* **29,** 101.
REID, G. (1952). *J. Physiol.* **118,** 435.
REID, G., and RAND, M. (1951). *Aust. J. exp. Biol. med. Sci.* **29,** 401.
STEPHENSON, R. P. (1948). *J. Physiol.* **107,** 162.