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CIRCULATORY EFFECTS OF 5-HYDROXYTRYPTAMINE

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The existence of a vasoconstrictor agent in serum or defibrinated blood has been known for a period of about 80 years. The first noteworthy progress in the identification of the active substance was made by Rapport, Green & Page (1948*a*, *b*) who isolated the vasoconstrictor principle, naming it serotonin, and by Rapport (1949) who identified it as 5-hydroxytryptamine. This compound, which has since been synthesized by Hamlin & Fischer (1951) and by Speeter, Heinzelmann & Weisblat (1951), has been shown to be chemically identical with the substance isolated by Rapport.

Recently (Reid & Rand, 1951; Rand & Reid, 1952), we reported the actions of the vasoconstrictor partially purified by the method of Rapport *et al.* (1948*a*). All these actions appeared to be due to a single substance, a conclusion which was further supported by the fact that they were mimicked by those of one group of compounds—the indolalkylamines. So far as the vascular effects in the cat are concerned it was vasoconstrictor in the hind-limb and kidney, and liberated adrenaline from the suprarenal gland; but did not appear to be a very potent pressor agent. Its pressor effect was usually preceded and followed by a depression. Previously the action on the cat's blood pressure was not discussed in detail because of the possibility that impurities may have been responsible for the depressor phases, although it had been shown (Reid, 1951) that tryptamine caused similar changes in blood pressure. More recently (Reid & Rand, 1952), a sample of synthetic 5-hydroxytryptamine, kindly given us by Dr Rapport, was shown to have all the muscle stimulating and vascular actions of the partially purified substance. Many of these actions are possibly of no importance physiologically; their importance lies in the fact that they may be used to identify, by pharmacological tests, the active substance in tissue extracts. On the other hand, the vascular effects demand more attention because this substance is liberated from platelets and has a possible importance in haemostasis and in vascular accidents. Indeed it has been

suggested (Moolten, Vroman, Vroman & Goodman, 1949) that serotonin formed from platelets in the peripheral circulation acts locally as a factor in maintaining arteriolar tone.

With further supplies of 5-hydroxytryptamine it has become possible to investigate its vascular effects in more detail.

METHODS

These have been described previously (Reid, 1951) or are referred to in the appropriate section under 'Results'. Cats were anaesthetized with chloralose (100 mg/kg). Usually the vagi were divided and the animals artificially ventilated. For outflow experiments heparin was used as an anticoagulant and the outflow recorded by means of Gaddum's outflow recorder.

The salt used was the creatinine sulphate; doses are expressed in terms of the free base, 5-hydroxytryptamine.

RESULTS

Fig. 1 shows the effect produced by the injection of 36μg of 5-hydroxytryptamine. There is initially a transient fall of blood pressure succeeded by a pressor response which is followed in turn by a depression lasting for several minutes.

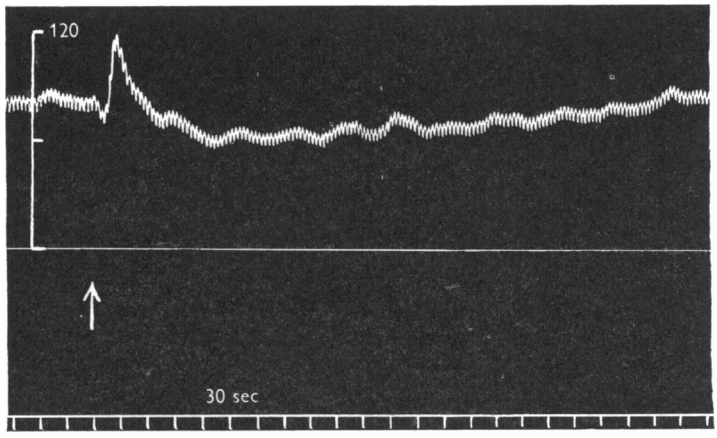

Fig. 1. Cat, 4·0 kg. Chloralose. Vagotomized. Artificial ventilation. Record of carotid blood pressure. At arrow 36μg of 5-hydroxytryptamine injected intravenously. Time scale in 30 sec.

While this is a common and apparently characteristic type of response to doses ranging from 10 to 200μg, the particular pattern seen in this figure may be distorted by exaggeration or diminution of one or other of the three phases. Thus the initial depression is often absent; yet sometimes it is the dominant feature (cf. first response in Fig. 11), and in one experiment the blood pressure fell rapidly towards zero and did not recover. Again the pressor phase may dominate the picture and be unassociated with one or both of the depressor phases (cf. Figs. 5 and 6); it may be of longer or shorter duration (for the same

magnitude of rise) than as shown in Fig. 1, it may fall away rapidly as though interrupted by the processes which are responsible for the succeeding depressor phase, it may be insignificant or it may appear during a period of depression as a small rise which does not reach the pre-injection level of blood pressure. The second period of depression may be the dominant feature succeeding a slight pressor response or continuing on from the initial fall, the whole response being a depressor one. On the other hand it may be absent. When present, it varies in duration from 3 min to over 20 min, and in magnitude may be much greater than in Fig. 1 (cf. Figs. 7 and 10).

The responses referred to occur whether or not the vagi are divided or atropine has been given. The pressor response may be abolished, or reduced in magnitude by a previous intravenous injection of yohimbine hydrochloride (1–2 mg/kg).

The cause of the initial fall of blood pressure

The initial steep fall of systemic blood pressure is due primarily to vasoconstriction in the pulmonary circulation. Following the systemic intravenous injection of 10–110 μg of 5-hydroxytryptamine the pressure in the pulmonary artery rises and simultaneously the pressure in the carotid artery falls. At the same time there is a fall of pulmonary venous pressure and a rise of pressure in the right auricle. These pressure changes indicate that the rise of pressure in the pulmonary artery is due to an increase in resistance and is not cardiac in origin. This is further shown by the fact that when injections are made into a pulmonary vein so as to by-pass the lungs the rise of pulmonary arterial pressure is absent or when present is weaker and occurs after the systemic arterial pressure has already begun to rise. Also when injections are made into a pulmonary vein the initial fall of systemic pressure is regularly absent. Fig. 2 shows the effect on the pulmonary and carotid arterial pressures of injecting 36 μg of 5-hydroxytryptamine into a pulmonary vein and into a systemic vein respectively. In the former case the rise in systemic pressure is greater and more prolonged and the initial depression is absent (cf. also Fig. 8).

Fig. 3 shows the changes in right auricular pressure which are associated with the initial changes in arterial blood pressure.

These records are from a cat which had been decapitated under chloralose anaesthesia. They show that as the systemic arterial pressure falls the right auricular pressure rises and as the systemic arterial pressure subsequently rises, the auricular pressure returns to normal.

When the initial fall of arterial pressure is greater and more prolonged, cardiac impairment may contribute to it. This is indicated by the fact that the pressure in the pulmonary artery after rising steeply is interrupted by a fall towards zero. In one experiment with a cat from which the adrenals had been

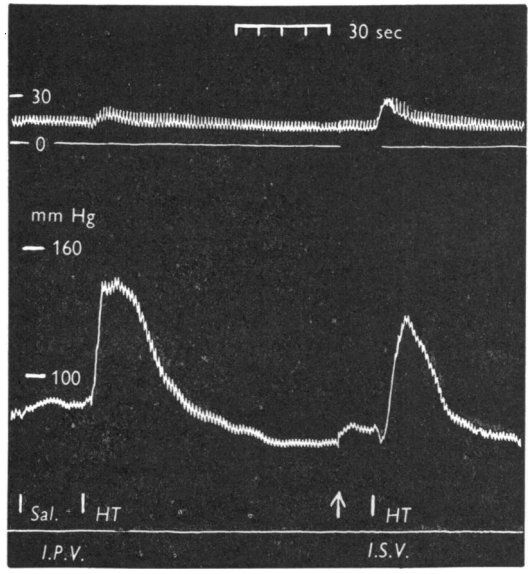

Fig. 2. Cat, 2·9 kg. Chloralose. Vagotomized. Thorax open. Artificial ventilation. Upper tracing: pressure in pulmonary artery. Lower tracing: pressure in carotid artery. The first two injections were made through a pulmonary vein as denoted by *I.P.V.* and the third through the saphenous vein as denoted by *I.S.V.* At *Sal.*, 1·0 ml of 0·9% (w/v) sodium chloride and at *HT*, 36 μg of 5-hydroxytryptamine were injected. At the arrow the drum was stopped for 4 min. Time in 30 sec.

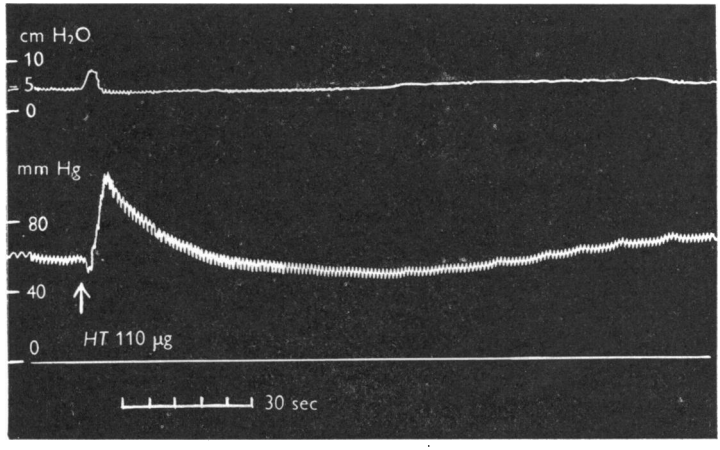

Fig. 3. Cat, 2·0 kg. Chloralose followed by decapitation. Upper record: pressure in right auricle. Lower record: pressure in carotid artery. At arrow 110 μg of 5-hydroxytryptamine injected intravenously. Time in 30 sec.

removed the blood pressure fell rapidly, death resulting in 3–4 min; on opening the thorax, the right heart was engorged and the left heart relatively empty. The failure of the heart in such instances is probably attributable to the impaired coronary flow resulting from the low arterial pressure, a direct vasoconstrictor action of the drug on the coronary vessels possibly contributing. When the adrenals are intact an initial profound fall of arterial pressure may after several minutes give place to a rapid rise with increase in heart rate due evidently to the release of adrenaline secondary to anoxia (cf. Fig. 9).

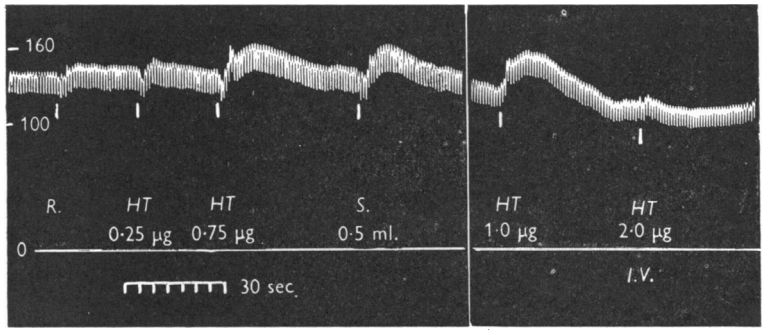

Fig. 4. Cat, 2·8 kg. Chloralose. Vagotomized. Eviscerated, aorta tied below renal arteries which were also ligated. Record of carotid blood pressure. Except for last injection which was given intravenously injections made into cut stump of superior mesenteric artery. *HT*, 5-hydroxytryptamine in doses as indicated. *S*, 0·5 ml. of serum prepared from this cat 45 min previously. *R*, 1·0 ml. of Ringer's solution. Time in 30 sec.

The nature of the pressor response

Unlike tryptamine, 5-hydroxytryptamine liberates adrenaline from the suprarenal glands, as can be demonstrated by the method of Feldberg & Minz (1931). Fig. 4 shows the effect of injecting 0·25, 0·75 and 1·0 μg into the cut stump of the superior mesenteric artery of an eviscerated cat in which the aorta was tied below the renal arteries which were also ligated. The effect of 0·5 ml. of cat serum is shown for comparison. The intravenous injection of 2·0 μg had a slight depressor action only. While this direct action on the suprarenal gland probably contributes to the pressor effect of large doses given intravenously, the drug has an independent pressor action, since changes in blood pressure similar to those already described occur in adrenalectomized animals.

Venous outflow experiments. A vasoconstrictor action of 5-hydroxytryptamine has been demonstrated in the hind-limb, in the whole of the vascular field supplied by the aorta distal to the inferior mesenteric artery, in the splanchnic area and in the kidney. When the drug in doses of 1 μg or more is injected into the femoral artery through the cut stump of a muscular branch the outflow from the femoral vein increases transiently followed by a decrease

lasting up to about 5 min. When the drug is given intravenously to the adrenalectomized cat the outflow decreases during the initial depressor phase and may remain reduced throughout the remainder of the blood-pressure response or may increase somewhat during the earlier part of the pressor phase though proportionately less than the rise of pressure.

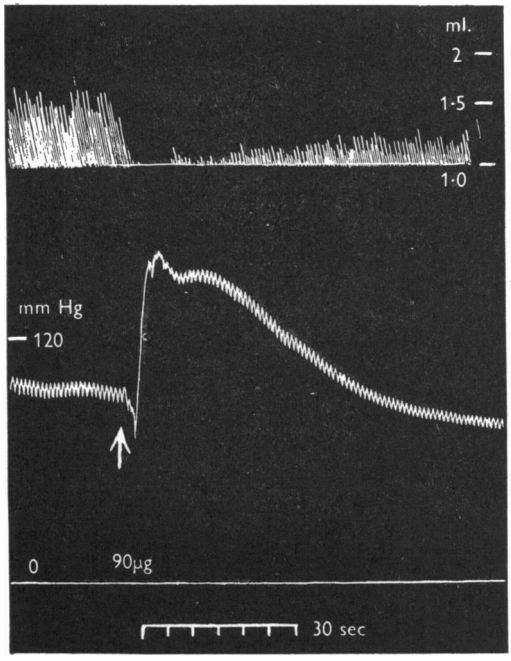

Fig. 5. Cat, 4·1 kg. Chloralose. Vagotomized. Artificial ventilation. Eviscerated and adrenalectomized. Upper trace: outflow from vena cava in mid-abdomen. Lower traces: carotid blood pressure. At arrow 90μg of 5-hydroxytryptamine injected intravenously. Time in 30 sec.

Fig. 5 shows records obtained from an experiment on an eviscerated and adrenalectomized cat. The outflow from the inferior vena cava in the mid-abdomen was returned to a reservoir connected to the inferior vena cava just below the renal veins. The aorta was occluded for 5 min during the insertion of the caval cannulae. The upper tracing shows the outflow from the hind-part of the animal and the lower tracing the carotid blood pressure. The intravenous injection of 90μg of 5-hydroxytryptamine caused a diminished outflow beginning with the initial depression and continuing during the pressor phase. Following the latter the blood pressure returned to a level lower than it was before injection, and neither the pressure nor the venous outflow had returned to the pre-injection level in 20 min. Unfortunately the recorder in this experiment did not respond to volumes less than about 1 ml.; nevertheless, the

experiment clearly indicates a decrease in blood flow with a very marked pressor response. In this same experiment the injection of 2 μg into the cut stump of the inferior mesenteric artery caused a decrease in outflow with a negligible change in blood pressure.

Fig. 6 shows the outflow from the portal vein of an adrenalectomized cat; the aorta was occluded above the coeliac artery for 4 min during the insertion of the portal cannula. The portal blood was returned to the right external

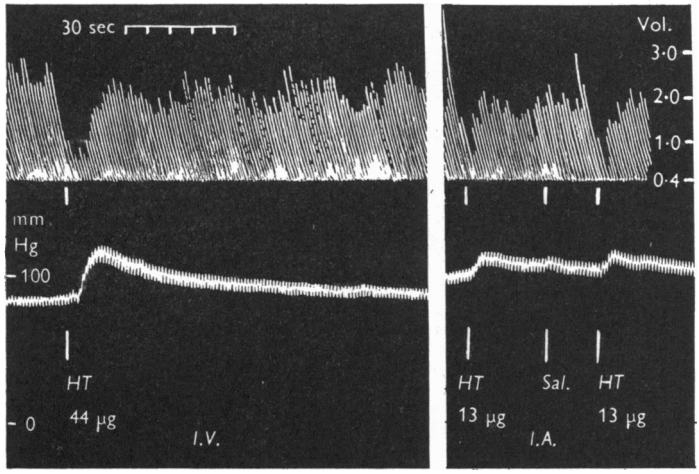

Fig. 6. Cat, 2·7 kg. Chloralose. Vagotomized. Artificial ventilation. Adrenalectomized. Upper trace: outflow from portal vein. Lower trace: carotid blood pressure. In the first panel 44 μg of 5-hydroxytryptamine was injected intravenously. In the second panel injections were made into the lower thoracic aorta through fine plastic tubing pushed up the aorta from an iliac artery. *HT*, 5-hydroxytryptamine. *Sal.*, 1·0 ml. of 0·9% saline. Time in 30 sec.

jugular vein, the liver receiving no portal flow. The drug was injected intravenously or into the lower thoracic aorta through a fine plastic tubing inserted via the left iliac artery. Intravenous injection of 44 μg caused a rise of pressure with a diminished outflow; intra-arterial injection of 13 μg caused a transient increase followed by a decrease in outflow.

To investigate the action of the drug on the renal vessels experiments of the following type were performed: The cat was eviscerated and the adrenal veins ligated or the adrenals were removed. The aorta and inferior vena cava were tied below the renal vessels. A cannula directed cranially was inserted into the vena cava immediately below the entrance of the renal veins and connected to delivery tubing which was for the time being closed with a clip. The aorta above the renal arteries was temporarily occluded and the vena cava tied above the entry of the renal veins. A reservoir and cannula were connected to the cava above the proximal ligature. The aorta and delivery tubing were unclipped and the blood which now flowed through the caval cannula was returned via the

outflow recorder to the venous reservoir. The left ovarian or spermatic vein was tied off and the small amount of blood which still returned by anastomotic channels to the lower abdominal part of the vena cava was also delivered by means of cannula and tubing to the venous reservoir. The effect of an injection of 5-hydroxytryptamine was variable, but usually the blood flow fell during the initial depression, rose during the rise of arterial pressure and then

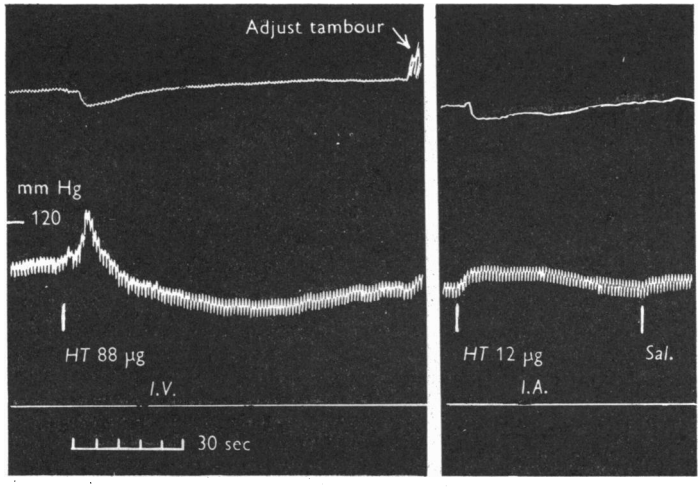

Fig. 7. Cat, 2·0 kg. Chloralose. Vagotomized. Artificial ventilation. Upper record: hind-limb volume. Lower record: carotid blood pressure. In first panel 88 μg of 5-hydroxytryptamine injected intravenously. In second panel injections given through opposite iliac artery. *HT*, 5-hydroxytryptamine, *Sal.*, 1·0 ml. of 0·9% saline. Time in 30 sec. Interval between panels, 12 min.

decreased for a period of up to several minutes. Consideration of the changes in flow and in arterial pressure indicated an increase in resistance in the kidney. An injection of 20–100 μg of 5-hydroxytryptamine into the cut stump of the superior mesenteric artery caused a slight decrease in outflow lasting for up to a few minutes.

Four such experiments have been performed, and they suggest that 5-hydroxytryptamine has a weaker vasoconstrictor action in the kidney than elsewhere.

Plethysmograph experiments. The volume of the hind-limb decreased when 5-hydroxytryptamine was injected intravenously, and this decrease in volume continued as a rule for a short time after the pressor phase and sometimes right throughout the ensuing depression. When the drug was injected into the vascular supply of the limb through the opposite iliac artery a small dose (1–20 μg) caused a shrinkage in volume without much change in arterial blood pressure. Fig. 7 shows the changes in leg volume and in arterial blood pressure after injection of the drug intravenously and intra-arterially respectively.

The transient increase in outflow from the femoral or portal vein (cf. Fig. 6), which may be seen following the intra-arterial injection of 5-hydroxytryptamine, is probably explainable by the squeezing out of the blood contained in the contracting vessels (the part played by the spleen so far as the outflow from the portal vein is concerned was not explored). However, the plethysmograph experiments show a slight but quite definite increase in limb volume following the intra-arterial injection of 5-hydroxytryptamine, an effect which is not given by the injection of saline alone. Whether this is due to a transient active dilatation or to a passive dilatation due to constriction first occurring in the venules, it is not possible to state.

Relationship of the heart to the pressor response. The action of the drug on the heart has been studied by Langendorff's perfusion method and cardiometry.

When the heart is perfused by Langendorff's method, the injection of 4–200μg of 5-hydroxytryptamine into the Ringer solution proximal to the cannula increases the amplitude and rate of the heart beat and also the coronary flow. These results resemble those observed by Laidlaw (1912) in experiments on the action of tryptamine on the perfused rabbit's heart.

Cardiometric methods in the 'intact' animal do not indicate that an increase in cardiac output contributes significantly to the pressor response. A glass cardiometer was placed around the heart below the auriculo-ventricular ring; or the pericardium itself, into the apex of which a large tracheal cannula was tied, served as the cardiometer chamber, the sac being drawn taut by traction on the cannula. Recording was done by means of a large loose tambour. Provided one bears in mind that the recorded amplitude may be influenced by changes in rate as well as by changes in the stroke output of the heart, the records can be used to give a qualitative indication of changes in cardiac output.

Following the intravenous injection of 40–150μg of 5-hydroxytryptamine into an adrenalectomized cat there is initially an increase in the 'stroke volume', and an increase in diastolic volume of the heart. When the pressure in the pulmonary artery is recorded also, it can be seen that the initial increase in 'stroke volume' occurs simultaneously with the rise of pulmonary and the fall of systemic arterial pressures. The apparent increase in stroke volume cannot be due to an action of the drug upon the heart because it is slight or absent when injections are made into the pulmonary vein, and seems therefore to be related in some way to the distension of the right side of the heart which results from pulmonary vasoconstriction.

Fig. 8 shows a series of responses from an adrenalectomized cat. The upper tracing is the cardiometer record (using the pericardial sac as a cardiometer chamber); the middle and lower tracings are of the pulmonary and carotid arterial blood pressures respectively. The first injection of 110μg caused an

increase in 'stroke volume' simultaneously with the fall of systemic pressure and the rise of pulmonary pressure. This was shortly followed by a decrease in 'stroke volume'. Apart from an initial increase, lasting for about 1 min, heart rate was unchanged. Later in the experiment (see second panel, Fig. 8) injection into the saphenous vein caused initial changes in the cardiometer tracing which were an exaggeration of those shown in the first panel. Through-

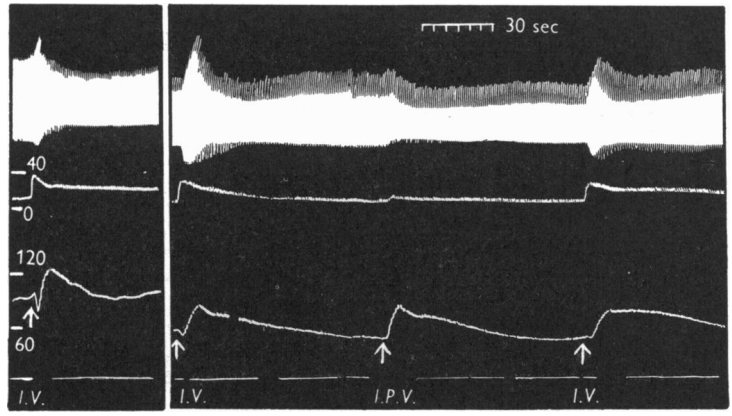

Fig. 8. Cat, 3·0 kg. Chloralose. Vagotomized. Thorax open. Artificial ventilation. Adrenals removed. Upper trace: cardiometer record using pericardial sac as cardiometer chamber. The upper end of the excursion represents 'diastolic volume'. Middle trace: pressure in pulmonary artery. Lower trace: carotid blood pressure. At the arrows 110 μg of 5-hydroxytryptamine was given intravenously as denoted by *I.V.* or into a pulmonary vein as denoted by *I.P.V.* Between the panels is an interval of 19 min during which two similar injections were made, one into a pulmonary vein and the other into a systemic vein, and the cardiometer was adjusted. Time in 30 sec.

out the experiments three separate injections were made into the pulmonary vein, each of which caused a reduction in stroke volume. The action of one such injection is seen in the second panel.

In a previous paper (Reid, 1951) the pressor action of tryptamine was ascribed partly to vasoconstriction and partly to a cardiodynamic action. The evidence for the latter consisted mainly in the increased amplitude of pulsation in the mercury manometer connected with the right ventricle or pulmonary artery. The present results suggest that this apparent dynamic action may not be due to a direct action of tryptamine on the heart, a conclusion which is supported by cardiometric experiments which have since been carried out with tryptamine.

Fig. 9 shows the cardiometer and systemic arterial pressure records from a cat with intact adrenals. In this experiment there is an initial steep fall of pressure following the injection of 100μg of 5-hydroxytryptamine, associated with a slight increase in recorded 'stroke output' especially following the first injection. The fall of blood pressure is interrupted by a rise which does not

reach the initial level and is succeeded by a further fall associated with diminution in the stroke output. This effect is interrupted after a few minutes by a sharp increase in blood pressure and of pulse rate due, presumably, to the release of adrenaline consequent upon the impaired blood supply to the central nervous system. Following this, once again the stroke volume and the blood pressure are reduced before recovery finally takes place.

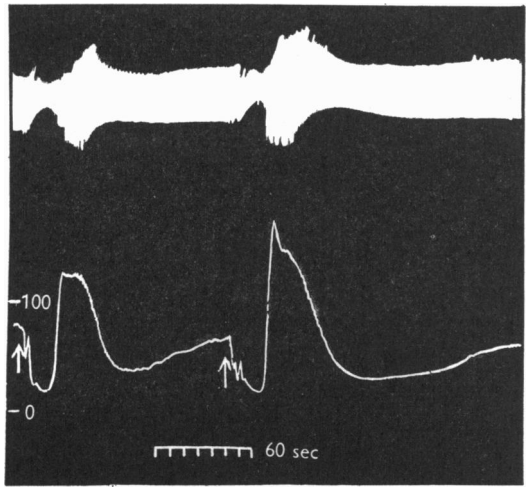

Fig. 9. Cat, 3·4 kg. Chloralose. Vagotomized. Thorax open. Artificial ventilation. Adrenals intact. Upper trace: cardiometer record. Lower trace: carotid blood pressure. At arrows 100 μg of 5-hydroxytryptamine injected intravenously. Time in minutes.

The secondary depressor phase

This has not been satisfactorily interpreted. Theoretically it may be due to decrease in either the peripheral resistance or the left ventricular output. Reduction of peripheral resistance may result from a direct vasodilator action or from an indirect action mediated through the nervous system or through the liberation of a vasodilator agent. No evidence of direct or indirect vasodilatation has been observed in venous outflow or plethysmograph experiments.

The following experiments also lend no support for indirect action mediated through the nervous system. As Fig. 3 indicates, the secondary depressor response occurs in decapitated animals. Injection into the carotid artery does not elicit a depressor response, whether or not the sinuses are denervated. In one experiment the vagus nerves were divided, the sinuses were denervated and an injection of 12 μg was made through a fine hypodermic needle into the left carotid artery. This was without effect on the blood pressure. The same dose injected through the saphenous vein gave a particularly good secondary

depressor response. This experiment indicates that the depressor phase is not due to an action of 5-hydroxytryptamine in that part of the nervous system supplied by a carotid artery; it also shows that the depressor response following intravenous injection does not depend on the carotid sinus mechanism. In one experiment in which the thorax was open an injection of 100 μg was given headwards through a fine hypodermic needle in the innominate artery so that it might enter that part of the nervous system supplied by a vertebral artery. Again there was no depressor phase in the response. Indeed intra-arterial injection has never evoked it except in one experiment (Fig. 10), when injection was made through a plastic catheter passed up the aorta from the iliac artery so that it opened close to the aortic valves.

Another way in which indirect reduction in peripheral resistance could conceivably occur is by the liberation of a vasodilating agent. The liberation of adrenaline from the suprarenal gland cannot be responsible for the secondary depressor phase for it is met with in the adrenalectomized animal. The liberation of histamine seems to be excluded by the fact that it still occurs after intravenous administration of diphenhydramine (benadryl) or mepyramine (anthisan).

So far as reduction in left ventricular output is concerned this could result (1) from a reduction in venous inflow due to trapping of blood elsewhere in the circulation, or (2) from impaired performance of the cardiac muscle. It has been shown already that accumulation of blood in the right heart and in the pulmonary arterial tree is concerned with the initial fall of blood pressure, but the records do not suggest that it is concerned with the later fall. Likewise, obstruction in the hepatic circulation seems to be excluded by observations of the pressure in the portal vein. This is unaffected, or rises, following the intravenous injection of 5-hydroxytryptamine, but the rise of portal pressure is greater when it is given via a systemic vein than when it is given directly into the portal circulation. Experiments in man, described below, indicate that it is a potent venoconstrictor, but plethysmograph experiments already described do not show a late increase in volume of the cat's hind-limb which could be attributable to venous obstruction.

By a process of exclusion, therefore, the evidence points to impaired performance of cardiac muscle as being responsible for the secondary depressor phase. This could be brought about by coronary vasoconstriction. The cardiometer experiments do indicate a fall in the output of the heart but it does seem surprising that a greater rise of venous pressure is not recorded during the second depressor phase, especially when the fall of blood pressure is as great as in Fig. 10.

Fig. 10 shows the pressure in the carotid artery and in the right auricle of a cat with open thorax. The first response resulted from the introduction of 24 μg of 5-hydroxytryptamine into the aorta through a plastic catheter

inserted through the iliac artery the tip coming to rest (as disclosed at autopsy) close to the aortic valves and the opening of the coronary arteries. The second injection was given through a pulmonary vein. The right auricular

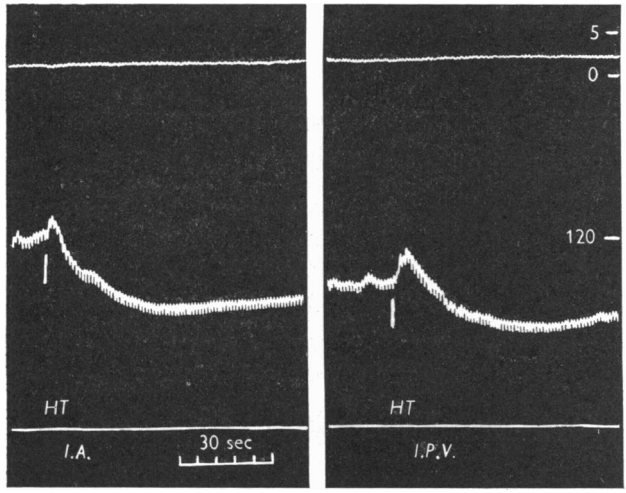

Fig. 10. Cat, 3·0 kg. Chloralose. Vagotomized. Thorax open. Artificial ventilation. Records of right auricular pressure (upper trace) and of carotid blood pressure (lower trace). At *HT*, 24 μg of 5-hydroxytryptamine was injected. In the first panel it was delivered by fine plastic tubing close to the origin of the coronary arteries; in the second panel it was given into a pulmonary vein. Time in 30 sec.

pressure rose by less than 1·0 cm of saline. With depressor responses such as that illustrated in Fig. 1, the right auricular pressure rose as a rule only about 0·5 cm.

Such a slight rise of right auricular pressure in association with a marked fall of systemic arterial pressure makes one hesitate to ascribe it wholly to cardiac depression. It is possible that peripheral effects mediated through the central nervous system may be partly responsible for the secondary depressor phase, and that the experiments so far have failed to detect them.

The effect of successive injections

Laidlaw (1912) described a decreased pressor response to tryptamine in the cat following repeated injections. Earlier work from this laboratory with both tryptamine and with partially purified serum vasoconstrictor showed that sometimes the systemic pressor responses became less following two or three injections; sometimes there was little change. On the other hand, the pressor response in the lesser circulation regularly declined. At that time the question of tachyphylaxis was not discussed. Considering the present experiments with 5-hydroxytryptamine, it appears that the results cannot be adequately

expressed simply by saying that the blood-pressure response in the cat shows tachyphylaxis. The results show that vasoconstriction in the hind-limb and vasoconstriction in the pulmonary circulation become less active with repeated doses. While decreased responsiveness of systemic vessels will tend to decrease the systemic pressor response, decreased responsiveness of pulmonary vessels may tend to increase it. To this must be added the possi-

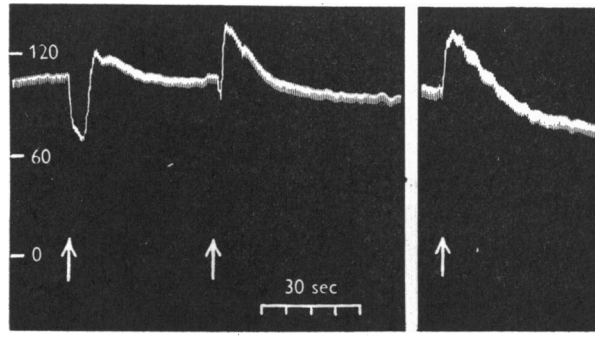

Fig. 11. Cat, 2·0 kg. Chloralose. Vagotomy. Artificial ventilation. Adrenalectomy. Carotid blood pressure. At arrows three successive injections of 50 μg of 5-hydroxytryptamine were made intravenously. Interval between panels 5 min. Time in 30 sec.

bility that change in sensitivity may vary from one vascular field to another. In view of these considerations, one would expect that the pattern of response to an intravenous injection may change with successive injections; such is in fact the case. Sometimes this consists mainly in a reduction of the pressor response (cf. Laidlaw's results with tryptamine), sometimes the outstanding feature is that the initial depressor response decreases. Fig. 11 shows three successive injections of 50 μg of 5-hydroxytryptamine into an eviscerated adrenalectomized cat. With the first injection the initial depression dominates the picture, by the third it has disappeared.

The effect of intradermal and subcutaneous injections in man

The intradermal injection of 5-hydroxytryptamine in concentrations up to 1 in 2×10^5 were without visible action in the skin over the front of the forearm. Concentrations greater than this (up to 1 in 10^4) may produce a red reaction lasting 15 min to 45 min; there is neither itching nor wheal formation. When the subcutaneous veins are visible and raised above the surrounding skin, a subcutaneous or intradermal injection causes them to disappear for distances up to several centimetres from the site of injection. This effect has been observed on the volar surface of the forearm in six subjects and on the dorsum of the foot in a sympathectomized subject; it comes on within a few minutes, reaches its maximum in about 5 min and persists for periods up to

60 min. The smallest dose giving such an effect was 0·1 μg and quite marked responses were caused by amounts as small as 1 μg.

The demarcation between the region of constriction and the region where the vein appears fully patent may be very sharp indeed so that as one traces the full vein heartwards towards the site of injection it seems to come to an abrupt stop as though the blood were held up by a valve. The constriction may be so intense that massage of the vein with the finger cannot force blood onwards past the contriction, and raising the pressure in a sphygmomanometer cuff to 60 mm does not cause distension of the constricted regions of the veins.

DISCUSSION

Earlier work (Reid & Rand, 1951) showed that partially purified preparations of the vasoconstrictor of beef serum caused both pressor and depressor responses in the systemic blood pressure of the cat. The present results indicate that both pressor and depressor phases can be attributable to one substance in such preparations namely, 5-hydroxytryptamine. The mechanism by which these phases are produced has already been discussed with the presentation of the results. There is, however, an aspect which has not received attention. The intravenous injection of 10–200 μg of 5-hydroxytryptamine into cats under chloralose anaesthesia with intact vagi and breathing naturally, causes a brief period of apnoea lasting up to half a minute. When respiration recommences the rate is usually increased and there is bronchoconstriction as evidenced by the increased excursion of the intrapleural pressure, decreased excursion of respiratory movement and increase in the volume of the thorax. The bronchoconstriction and the associated increase in respiratory rate occur also in animals with divided vagi. These respiratory effects will be described more fully elsewhere; to what extent they may be responsible for or may modify the circulatory effects has not been fully explored, but it is clear that vasoconstriction in the hind-limb, in the bowel and in the kidney as well as the liberation of adrenaline from the suprarenal gland are caused by the intra-arterial injection into the appropriate field of doses too small to influence respiration. Constriction of the bronchioles may conceivably contribute to the pressor response in the lesser circulation. It is not considered to be a significant factor in this respect because characteristic responses of both systolic and pulmonary arterial blood pressures occurred in experiments in which the artificial ventilation of the lungs as seen through the open thorax wall appeared to be undisturbed by the injection of 5-hydroxytryptamine.

One of the most striking features about the action of this substance is the contrast between its great activity on isolated tissues and its relative weakness as a pharmacodynamic agent when injected intravenously. For example, the guinea-pig, mouse or rabbit intestine, the rat uterus and the isolated arterial

ring of carotid artery of the sheep give good responses to concentrations of 1 in 10^8 (Reid & Rand, 1952). This degree of pharmacological activity is comparable with that of histamine on the guinea-pig intestine. Rapport *et al.* (1948*b*) likewise found serotonin to be more actively vasoconstrictor in the isolated perfused rabbit's ear than adrenaline. Further, the experiments dealing with its local action on human veins after subcutaneous injection indicate that it is an exceedingly potent venoconstrictor. Why, on the other hand, it should be such a weak pressor agent is not clear. Its weak pressor action may be attributable to the possibility that its constrictor action is exerted mainly on the venous side of the circulation but the plethysmograph experiments provide no evidence for this. Another possibility may be that it possesses actions such as pulmonary vasoconstriction, coronary vasoconstriction, or actions mediated through the central nervous system (although not actually demonstrated) which modify the effects of systemic vasoconstriction. In any case direct arterial injection into the blood supply of the hind-limb, the bowel and the kidney has indicated that the threshold dose causing vasoconstriction is relatively high, probably 10 to 100 times greater than that of noradrenaline similarly injected, although no systematic comparison of these drugs has as yet been made. Experiments in which such intra-arterial injections are made are not strictly comparable with those in which the continuous perfusion of isolated organs with defibrinated blood first drew the attention of physiologists to this vasoconstrictor agent; in the former case the 5-hydroxytryptamine is in the field of injection only transiently before being swept on by the blood stream. This probably prevents its exerting its full vasoconstrictor action, because isolated organs such as the artery strip respond only after an appreciable latency of several seconds. Furthermore, it is possible that it is destroyed more rapidly when injected intravascularly *in vivo* than when it is administered to tissues *in vitro* bathed with Ringer solution. When it is applied locally extravascularly as in the experiments in the human forearm it probably persists for a longer time.

A potent local action with a relatively weak systemic action would be in keeping with the hypothetical role of this substance in haemostasis. The question may be raised as to whether systemic effects such as have been described ever arise from the liberation of 5-hydroxytryptamine in the body. Inspection of Fig. 4 indicates that the sample of cat serum injected at *S* has a serotonin content of 1·5 μg/ml., and this result was confirmed by matching on the isolated spiral strip of sheep carotid artery. So far a systematic examination of sera for their serotonin content has not been made; two other samples of cat serum contained respectively 3 and 4 μg/ml. If, for sake of argument, we choose the lowest figure, viz. 1·5 μg/ml. then assuming that all the serotonin is released during clotting and that the haematocrit value for cat blood is $33\frac{1}{3}$% (Dale & Laidlaw, 1919), 1·5 ml. of blood will yield 1·5 μg. With a blood

volume of 50 ml./kg (Dale & Laidlaw, 1919) a cat weighing 3 kg would contain in its circulation 150 μg of serotonin. If a large fraction of this amount were released rapidly, particularly during passage through the lungs, it is quite within the bounds of possibility that occasionally an animal in which this took place would suffer respiratory disturbances and severe circulatory failure due to pulmonary vasoconstriction. It is possible that such an event may take place in anaphylactic shock. So far there is no published evidence that serotonin is released in anaphylactic shock but some experiments of Bender (1943) are consistent with it. Bender showed that in anaphylaxis in a number of animal species, including the cat, the pupil of the denervated eye constricts. Such an effect on the pupil is produced by intracarotid injections of indolalkylamines; it is not produced by histamine nor by the slow reacting substance of Feldberg, Holden & Kellaway (1938) (Reid & Rand, unpublished experiments).

An enormous release of serotonin like that which has just been considered must be an uncommon event if, indeed, it ever occurs; on the other hand, the question may be raised as to how much is released by the daily wastage of platelets. The experiments of Lawrence & Valentine (1947), and a consideration of earlier work which is reviewed by them, indicate that the total platelet mass is renewed every 2–5 days, or that platelets are destroyed at the rate of 2500/mm^3/hr. An average platelet count in the cat is 400,000/mm^3 (Lawrence & Valentine, 1947), from which it follows that a fraction of the order of $\frac{1}{160}$ of the platelets is lost per hour. Therefore, assuming that the normal wastage of platelets does release the active substance, only about 1–2 μg of serotonin is released per hour. In view of the magnitude of the doses required to cause vasoconstriction in the various vascular fields of the cat this makes it unlikely that the natural destruction of platelets throughout the body plays any part in the normal maintenance of arteriolar tone as has been suggested (Moolten *et al.* 1949).

It should be borne in mind that the platelet's life ends with its utilization, and many more are likely to be used up locally in association with haemostasis than are destroyed elsewhere in the body where no haemostatic mechanism is for the time being called into action. The volume occupied by the platelets is, according to Tocantins (1938), of the order of 0·5% of the blood volume, although he gives no figures for the cat as such. This means that the concentration of serotonin in the platelets must be of the order of 1 μg/0·005 ml. This means that where platelets agglutinate, the liberation of their serotonin will result in an exceedingly high local concentration. Isolated artery strips give good responses in concentrations of only 1 in 100 million. M. B. Zucker (1947) has demonstrated local vasoconstriction of incised vessels in the rat's mesentery in association with the platelet plug, and nearby vessels also show constriction presumably by diffusion of an active substance. On the other hand, Akers (1951) has been unable to find these phenomena in experiments

with the cheek pouch of the hamster. It is difficult to reconcile these two groups of observations unless perchance the importance of a locally acting vasoconstrictor hormone varies from species to species.

Prolongation of the bleeding time is so commonly associated in clinical medicine with a low platelet count that it is hard not to believe that the cause of the prolonged bleeding is the absence of a vasoconstrictor agent which the platelets are known to liberate. The failure of the active substance, 5-hydroxytryptamine, to constrict the vessels responsible for skin colour might appear to make such a hypothesis unlikely but it has been shown (M. B. Zucker, 1947) that haemostasis of capillary bleeding takes place in a different manner from that of bleeding from larger vessels, and it has been shown (H. D. Zucker, 1949) that prolongation of the bleeding time in thrombocytopaenia does not occur when injury is restricted to the capillaries. The intense venoconstriction observed in the human forearm may at first sight appear to be more likely to prolong bleeding from that part of the vascular tree distal to the constricted veins. It must be borne in mind, however, that following injury, although distant vessels may be constricted by diffusion of the active substance the maximum effect will be on those responsive vessels which are nearest to the site of injury.

The substance, 5-hydroxytryptamine has recently turned up in another context, for Erspamer & Asero (1951) have identified it with enteramine, a substance derived from the enterochromaffin cells, so that it may prove to have functions other than those which one would suspect from its occurrence in the blood platelets. Erspamer states that 'serotonin is nothing but circulating enteramine'. This, however, is unlikely because serotonin does not appear in serum until after clotting.

SUMMARY

1. The intravenous injection of 5-hydroxytryptamine in doses ranging from 10 to 200 μg causes, in the chloralosed cat, an initial fall of systemic arterial pressure followed by a rise and by a more prolonged fall. This pattern is varied as a result of exaggeration or diminution of any one of these three phases. The response occurs irrespective of whether or not the vagi are divided or the cat is atropinized; it occurs also in the spinal cat. The pressor phase is reduced by yohimbine.

2. The initial fall is associated with an increase in resistance in the pulmonary circulation.

3. The drug has a direct stimulating action on the suprarenal medulla.

4. The pressor phase of the response to intravenous injection occurs after adrenalectomy and is due to vasoconstriction which has been demonstrated in the hind-limb, the intestine and the kidney.

5. The cause of the secondary fall of arterial pressure has not been satisfactorily elucidated.

6. In the human forearm the intradermal or subcutaneous injection causes a slight red reaction without itching or whealing. Intradermal or subcutaneous injections cause venoconstriction for several centimetres around the site of injection.

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