Br. J. Pharmac. (1973), **49**, 543–553.

The continuous bioassay of the release and disappearance of histamine in the circulation

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Summary

1. The blood-bathed organ technique was used for the continuous assay of histamine in circulating blood.

2. Longitudinal strips of cat or kitten terminal ileum detect concentrations of histamine of 2–5 ng/ml ; these are below the threshold at which hypotension is induced in dogs.

3. The cat or kitten terminal ileum also contract to bradykinin. However, the simultaneous use of a cat jejunum strip, which is selectively sensitive to bradykinin, will distinguish between histamine and bradykinin.

4. The utility of the method for detecting and quantitating circulating histamine was exemplified by measuring: (a) its half-life in the circulation, (b) its disappearance during a passage through the vascular beds of lungs, liver and lower limbs, and (c) its release into the circulation by compound 48/80.

Introduction

Several vasoactive hormones, including peptides, amines and prostaglandins have been assayed continuously in the circulating blood by the blood-bathed organ technique (see Vane, 1969). For example, angiotensin II has been assayed by the rat colon (Regoli & Vane, 1964, 1966), catecholamines by the rat stomach strip and chick rectum (see Vane, 1966), bradykinin by the cat jejunum (Ferreira & Vane, 1967a) and prostaglandins by the rat stomach strip, chick rectum and rat colon (Ferreira & Vane, 1967b). This paper describes the reactions of strips of kitten and cat terminal ileum. These are selectively sensitive to histamine and bradykinin and when used at the same time as other assay tissues, allow the specific detection and continuous assay of histamine in the circulating blood.

Methods

Selection of an assay organ for histamine

A search for a suitable assay tissue for histamine was made amongst isolated tissues of four laboratory animals. Ileum, taenia-caecum, ascending colon, descending colon (Mikos & Vane, 1967) and portal vein were taken from the guinea-pig. Stomach strip (Vane, 1957), ileum, proximal colon (Regoli & Vane, 1964), descending colon, uterus and spirally cut aortic strip were taken from

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the rat. Ileum, taenia-coli, descending colon and spirally cut aortic strip were taken from the rabbit. Longitudinal muscle strips of duodenum, jejunum (Ferreira & Vane, 1967a), mid-ileum, terminal ileum, colon, spiral strips of pulmonary artery and pulmonary vein were taken from adult cats and from kittens. Segments of cat intestine were stored in Krebs solution in a refrigerator at 4° C for 1–3 days. Kitten tissues were used from animals killed on the day of the experiment. Three or four tissues were superfused (Gaddum, 1953) in series with Krebs solution at 10 or 15 ml/min and when necessary with blood from the animal as described previously (Vane, 1964, 1969). Sometimes six tissues were used, arranged in series in two banks of three. The initial load on the tissues was 1–3 grams. Contractions were recorded by auxotonic levers (Paton, 1957), mechanically with a magnification of 16 times on smoked kymograph paper, or electronically by transducers (Harvard heart/smooth muscle transducers) on a multi-channel pen recorder (Beckman Offner or Watanabe).

Experiments with blood-bathed organs

Dogs (9·5–20 kg) of either sex were used. Some dogs were anaesthetized with halothane; anaesthesia was then maintained with chloralose (100 mg/kg intravenously). Others were anaesthetized by intravenous pentobarbitone (30 mg/kg). Anaesthesia was supplemented when necessary with pentobarbitone (5–10 mg/kg intravenously or intramuscularly).

The trachea was cannulated and the animal was ventilated mechanically. Polyethylene cannulae were tied into the carotid or femoral artery and into the external jugular or femoral vein for the removal and replacement of blood. Heparin (1,000 i.u./kg) was injected intravenously. Mean arterial blood pressure was recorded by a transducer connected to the side arm of the arterial cannula (1 mmHg = 1·333 mbar).

The assay tissues were superfused at 10–15 ml/min, initially with Krebs solution and then with blood from the dog. The blood was collected in a reservoir and returned continuously by gravity into the jugular or femoral vein. The assay tissues were calibrated either by infusing the test substances into the stream of blood after it had left the animal or by intravenous or intra-arterial infusions.

Inactivation of histamine in circulating blood

An incubation circuit (Ferreira & Vane, 1967c) was made of a length of silicone tubing of 3 mm internal bore and of 40 ml capacity; it was kept at 37° C by a water bath. Blood from an artery first passed through this tubing at 10 ml/min and then superfused the assay organs. Histamine was infused for 3–5 min at different points in the circuit so it was in contact with the circulating blood for 1–4 min before being assayed. The contractions of the assay tissues were then compared with those produced by infusions given into the bathing blood close to the tissues so that there was no delay.

Half-life of histamine in the circulation

The rate of relaxation of the terminal ileum after stopping an intravenous infusion was compared with the rate of relaxation produced when the concentration of histamine in the blood bathing the ileum declined exponentially with different

time constants (Ferreira & Vane, 1967d). The exponential decay of the concentration of histamine in the blood was produced by infusing histamine into the blood with an infusion pump, driven by a Servomex MC43 motor controller coupled with condensers of different capacities which, by discharging through a resistance, regulated the negative feed back voltage which controlled the motor (Ferreira & Vane, 1967d).

Disappearance of histamine in one circulation through vascular beds

To measure the disappearance in a particular vascular bed, histamine was infused for 5–8 min into the arterial inflow of the vascular bed. The plateau contractions of the assay tissue produced by these infusions were compared with contractions produced by similar histamine infusions into the blood leaving the vascular bed. For example, if an arterial input infusion of 1 (μg/kg)/min induced a contraction of the assay tissue similar to that produced by 0·5 (μg/kg)/min given into the venous output there must have been a continuous disappearance of 50% of the histamine in the vascular bed under study.

Disappearance of histamine in the lung was measured by comparing infusions made into the superior vena cava with those made into the base of the ascending aorta or the left ventricle. In these experiments blood for assay was taken from the femoral artery.

To study the disappearance of histamine in the liver or the lower limbs, infusions made into the portal vein or the femoral artery were compared with those made into the superior vena cava. The assay organs were bathed in carotid arterial blood.

The disappearance of histamine in the complete circulation was measured by making infusions into the ascending aorta via a coaxial polyethylene catheter (Ferreira & Vane, 1967d). The cardiac output was sampled for assay just above the aortic valves whereas infusions were made through the second catheter, the tip of which was a few millimetres downstream. In this way, the infused substance mixed with the total cardiac output and was exposed to a complete circulation before reaching the site of blood sampling. These infusions were compared with those made into the superior vena cava.

Drugs

The following drugs were used: acetylcholine perchlorate (British Drug Houses), adenosine triphosphate (Sigma), (−)-adrenaline bitartrate (British Drug Houses), ileu^5-angiotensin I (Wellcome Research Laboratories), angiotensin II-amide (Hypertensin, CIBA), bradykinin (Parke-Davis), burimamide (Smith, Kline and French Laboratories Ltd.), histamine acid phosphate (Burroughs Wellcome), 5-hydroxytryptamine creatinine sulphate (May and Baker), mepyramine maleate (May and Baker), oxytocin (Syntocinon, Sandoz), pentagastrin (Pentavlon, ICI), pentolinium tartrate (May and Baker), prostaglandin E_2 (Upjohn), prostaglandin $F_{2\alpha}$ (Upjohn), partially purified slow-reacting substance in anaphylaxis (SRS-A), substance P (1 mg=13·2 u), vasopressin (Pitressin, Parke-Davis) and the histamine liberator, compound 48/80 (Wellcome Research Laboratories). Doses of salts are expressed in terms of the base.

Results

Sensitivity of tissues superfused with Krebs solution

Initially, tissues were tested for their sensitivity to histamine in Krebs solution by injections rather than infusions.

Rat tissues were relatively insensitive to histamine. Injections of histamine (up to 3 μg) produced a small contraction or no response on the rat stomach strip, the ileum, the uterus, the proximal and the distal colons.

Histamine (0·02–0·5 μg) contracted the portal vein of the guinea-pig, spirally cut aortae of the rat and rabbit, and spirally cut pulmonary artery and pulmonary vein of the cat. Histamine also induced contractions of the intestinal segments of the guinea-pig, cat and rabbit but the dose necessary to produce a measurable height of contraction (1–1·5 cm) in these tissues varied considerably. Thus, the threshold dose was 0·01–0·04 μg for the guinea-pig preparations, 0·05–0·5 μg for those of the cat and 1–4 μg for those of the rabbit.

Because the vascular tissues and the intestinal segments of the guinea-pig and of the cat were more sensitive to histamine than other preparations, further experiments were carried out with the blood-bathed organ technique.

Sensitivity to histamine of tissues superfused with blood

Superfusion of the tissues with blood caused them to contract. The vascular tissues remained contracted, but the guinea-pig tissues gradually relaxed again. With these preparations there was also a decrease in sensitivity to histamine so that ten to twenty times the dose had to be given to produce the same effect. This reduction in sensitivity, together with a lack of specificity for histamine, made these tissues unsuitable for assay of histamine in circulating blood. However, cat and kitten intestinal tissues, after the initial contraction caused by blood superfusion had waned, maintained their sensitivity to histamine. Furthermore, the sensitivity tended to increase during the course of an experiment. The sensitivity of cat and kitten tissues to histamine was in descending order, as follows: terminal ileum (last 10 cm)>colon>mid-ileum>jejunum>duodenum. The terminal ileum freshly removed from cats often showed vigorous spontaneous activity. This was reduced by storage in the refrigerator at 4° C for one or two days. Storage for more than two days also reduced the sensitivity to histamine, although the sensitivity to bradykinin was retained. Fresh kitten tissues showed spontaneous activity but this did not interfere with the assay (see Fig. 1) when the strips were cut long and thin (10 cm×0·2 cm). In both cat and kitten tissues histamine (2–40 ng/ml) induced contractions which were well sustained: even during infusions for as long as 10 min there was no tachyphylaxis. Discrimination between different concentrations was good and there was a steep dose-response curve (Figures 1 and 2).

With these preparations, it was possible to detect histamine in the circulating blood when it was infused intravenously at rates of 0·3–0·7 (μg/kg)/min, which induced little or no vasodepressor effect. After cessation of the infusions the assay tissues relaxed again (Figure 1).

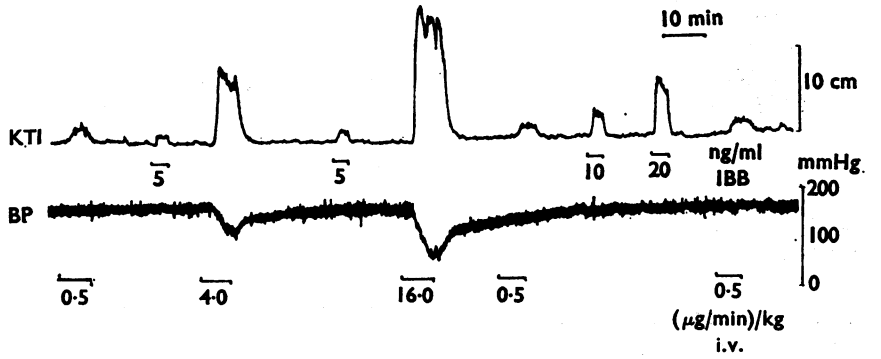

FIG. 1. A kitten terminal ileum (KTI) was bathed in arterial blood from a 15 kg dog. Histamine was infused intravenously at three different rates. The KTI detected circulating histamine after an infusion of 0·5 (μg/kg)/min, which had no effect on arterial blood pressure (BP). Higher infusion rates cause hypotension and greater contractions of the KTI. After the infusion of histamine (16 μg/kg)/min was stopped, the KTI relaxed well *before* the hypotensive effect of histamine was over, suggesting that the prolonged hypotensive response was not maintained by histamine circulating in the blood. IBB=in bathing blood. Time 10 min; vertical scales 10 cm and mmHg.

Specificity of the cat and kitten terminal ileum

The reactions of cat terminal ileum to various substances were tested first in Krebs solution and then in circulating blood. As well as histamine (0·1–0·5 μg), three other substances also contracted the cat terminal ileum bathed in Krebs solution: bradykinin (0·002–0·01 μg), SRS-A (2·5 μg) and acetylcholine (0·01–0·06 μg). When the cat ileum was bathed in circulating blood, bradykinin was 5–10 times more potent than histamine (on a weight basis) whereas SRS-A was 10 times and acetylcholine 15 times less potent.

The following substances in a concentration up to 50 μg/ml had no effect on the cat terminal ileum but sometimes gave small contractions of the kitten terminal ileum; angiotensin II, 5-hydroxytryptamine and prostaglandins E_2 and $F_{2\alpha}$. Oxytocin and vasopressin had no effect in either preparation in concentrations up to 20 mu/ml. The preparations were contracted by acetylcholine (1–10 ng/ml) when bathed in Krebs solution but when bathed with blood concentrations 10–20 times greater were needed. Hyoscine (100 ng/ml) completely blocked the effect of acetylcholine. Adrenaline (5–10 ng/ml) relaxed the terminal ileum preparations but concentrations as high as 100 ng/ml were needed to diminish contractions induced by histamine. The contractions caused by high concentrations of 5-hydroxytryptamine (200–400 ng/ml) were only partially blocked by methysergide (2 μg/ml). Burimamide, an H_2-receptor antagonist (Black, Duncan, Durant, Ganellin & Parsons, 1972) in concentrations up to 5 μg/ml did not inhibit the contractions induced by histamine.

Thus cat preparations were more specific than those from the kitten for detection of histamine in the circulating blood. However, kitten strips treated with mepyramine, used in a parallel assay with untreated tissues, allowed the detection of other substances which may be released into the circulation together with histamine. Infusions of mepyramine (2 μg/ml) into the blood superfusing strips of terminal ileum did not alter their tone thus indicating that there is no detectable histamine in the circulation. The presence of other circulating hormones such

as 5-hydroxytryptamine, angiotensin II, prostaglandins and bradykinin was also monitored by adding other assay tissues such as the rat stomach strip, rat colon, chick rectum and cat jejunum strips (see Vane, 1969; Regoli & Vane, 1966 and Ferreira & Vane, 1967a, b).

Stability of histamine in circulating blood

Usually cat and kitten terminal ileum detected changes in concentrations of 2–10 ng/ml. Histamine (10–50 ng/ml) was incubated with the circulating blood for up to 4 minutes. In 5 experiments there was no change in the activity. An experiment showing the stability of histamine in blood is illustrated in Figure 2. Infusions close to the tissues giving final concentrations of 5, 10 or 20 ng/ml caused graded contractions of the kitten terminal ileum. No change in the height of contractions was detected when the same amounts of histamine were in contact with blood for an extra 3 minutes.

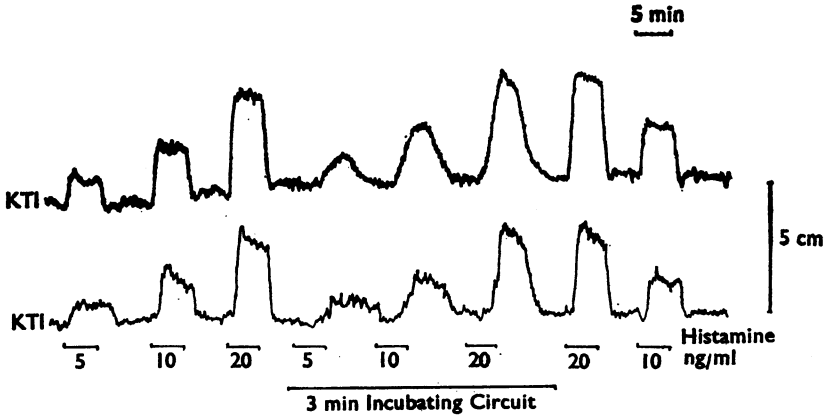

FIG. 2. Stability of histamine in circulating blood. Two longitudinal strips of kitten terminal ileum were superfused with carotid blood from a 15 kg dog. Histamine was infused either directly into the blood bathing the tissues or into an incubating circuit which increased the contact time of histamine with blood by 3 minutes. No destruction of histamine was detected. Time 5 min; vertical scale 5 cm.

TABLE 1. *Disappearance of histamine in some vascular beds of the dog*

Vascular bed	Dog	Weight (kg)	Histamine* infusions (μg/kg)/min	% Disappearance
Lung	1	11·5	1·5–3·0	0
	2	12·0	0·8–1·6	0
	3	20·0	1·0–2·0	0
	4	16·0	1·2–2·4	0
Liver	3	20·0	2·5–5·0	50
	5	10·0	2·0–4·0	50
Lower limbs	2	12·0	1·0–2·0	50
	6	16·0	2·0–5·0	60
	7	20·0	2·5–5·0	50
	8	15·0	1·2–2·6	50
All vascular beds	3	20·0	0·25–0·5	60
	9	9·5	0·20–0·4	50
	10	18·0	0·25–0·5	63

* Calibrating infusions were made into the blood leaving the vascular bed. They are quoted to indicate the amount of histamine used for measuring the histamine disappearance in each vascular bed.

Disappearance of histamine in one circulation through some vascular beds

The concentration of histamine was at least halved during one passage through the limbs, liver and all vascular beds of the dog. Table 1 summarizes the results. Figure 3 shows that the disappearance of histamine in one circulation through all vascular beds is 50–75% (exp. 10, Table 1). There was no disappearance of histamine during one passage through the lungs (four experiments). Figure 4 illustrates one of these experiments (exp. 4, Table 1) in which histamine infused at two different rates into the superior vena cava (1·2 and 2·4 (μg/kg)/min) produced similar contractions of the assay organ to those caused by equal infusions into the root of the aorta.

The half-life of histamine in the circulation

The half-life of histamine in the circulation was measured in three dogs by comparing the fall in histamine concentration in the arterial blood (as shown by the rate of relaxation of the assay tissues) with responses of the assay organs obtained by making the concentration of histamine in the blood bathing the tissues decay expon-

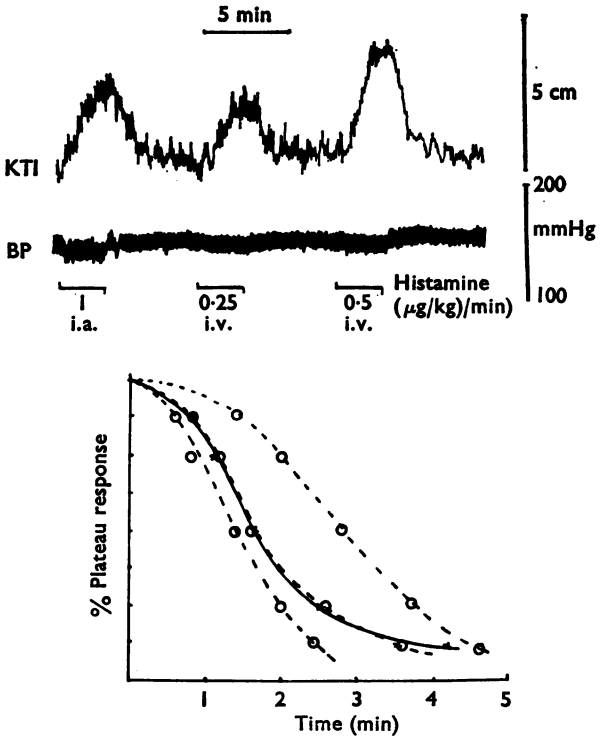

FIG. 3. Disappearance of histamine during one circulation through all vascular beds (upper panel) and the half-life of histamine in the circulation (lower panel). The upper panel shows an experiment in which a disappearance of 63% of the intra-arterial infusion of histamine occurred in one circulation (experiment 10, Table 1). In the lower panel, the lines were plotted from the decay of contractions of a kitten terminal ileum (KTI) bathed in arterial blood from a dog (15 kg) after histamine was infused intravenously (solid line) or in calibrating doses into the blood bathing the tissues (dotted lines). The relaxation of the KTI when the concentration of histamine was made to decay exponentially with a time constant of 0, 27 and 45 s are clearly distinguishable. The relaxation after an intravenous infusion (1·3 (μg/kg)/min) of histamine followed the middle curve, indicating a half-life of 27 seconds.

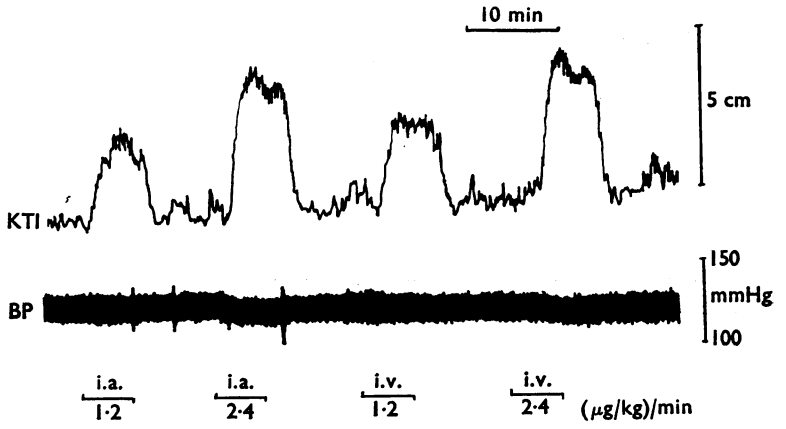

FIG. 4. Disappearance of histamine in the pulmonary vasculature of an anaesthetized dog (16 kg, Table 1, experiment 4). The upper tracing was obtained from a kitten terminal ileum (KTI) superfused with femoral arterial blood. Intra-arterial infusions (i.a.) were made by a catheter placed into the ascending aorta and the intravenous infusions (i.v.) were made into the jugular vein. There was no disappearance of histamine during its passage through the lungs. Time 10 min; vertical scales 5 cm and mmHg.

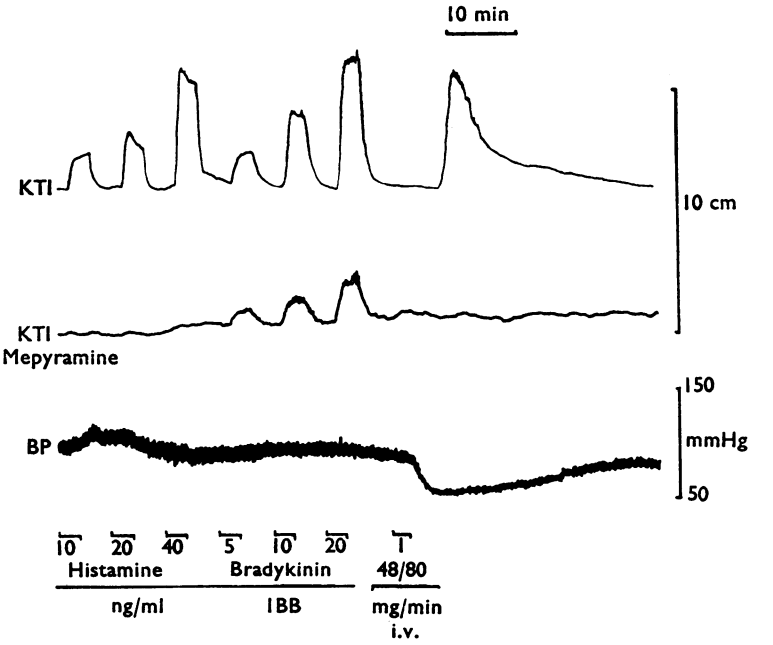

FIG. 5. Release of histamine by compound 48/80. Two strips of kitten terminal ileum (KTI) were superfused with arterial blood from a 13 kg dog. One (middle tracing) was treated with mepyramine. The untreated KTI contracted to histamine at 10, 20 and 40 ng/ml and both contracted to bradykinin (5, 10 and 20 ng/ml). Compound 48/80 (1 mg/min) was infused intravenously as shown. The peak concentration of histamine released into the circulation was about 40 ng/ml. There was no evidence for bradykinin release. IBB=in bathing blood. Time 10 min; vertical scales 10 cm and mmHg.

entially. A match was obtained with curves having half-lives of 20, 27 and 27 s respectively. In one of these experiments (plotted in Fig. 3) the decay of histamine in the circulation follows a curve the time constant of which was 27 seconds. In another experiment the mean blood pressure (150 mmHg) was lowered to 50–60 mmHg by pentolinium (5 mg/kg i.v.) but there was no increase in the half-life of histamine in the circulation.

Release of histamine by compound 48/80

Injections of up to 100 μg of compound 48/80 or infusions giving a final concentration of 10 ng/ml in the fluid bathing the tissues (blood or Krebs solution) had no direct effect on the assay tissues. Intravenous injections (0·5–1·0 mg/kg; 4 dogs) or infusions (50–100 μg/min for 1–4 min; 13 dogs) produced a fall in the arterial blood pressure and a concomitant contraction of the strips of cat or kitten terminal ileum (Figure 5). Calibration showed concentrations of histamine greater than 40 ng/ml in 5 animals and of 10–40 ng/ml in the others. Adrenaline was also detected in the circulation, shown by relaxation of the rat stomach strip, but its time of appearance was delayed in comparison to the release of histamine. Confirmation that the material contracting the terminal ileum strips was histamine was obtained by blocking one of the assay strips with an antihistamine. Infusions of mepyramine were made directly into the bathing blood for 10–30 min only to avoid accumulation in the dog with consequent recirculation affecting the other assay tissues. The absence of contraction of the cat jejunum or the terminal ileum strip treated with mepyramine showed that there was no release of bradykinin during the hypotension caused by compound 48/80 (Figure 5).

Discussion

The longitudinal strips of kitten or cat terminal ileum were sensitive enough to detect and assay histamine in the circulating blood. The sensitivity was such that the assay tissues contracted to infusions of histamine which had no hypotensive effect. The main disadvantage of these tissues is their high sensitivity to bradykinin. Thus, to distinguish between histamine and bradykinin, the cat terminal ileum should always be used in parallel assay with a cat jejunum strip, which is selectively sensitive to bradykinin (Ferreira & Vane, 1967a), or a terminal ileum strip rendered insensitive to histamine by an antihistamine drug. The latter procedure has the additional advantage that it provides corroborative evidence that the substance being assayed is indeed histamine. In this paper, a kitten terminal ileum treated with mepyramine together with an untreated preparation illustrated well the release of histamine and lack of release of bradykinin by compound 48/80 (Figure 5). Treatment with mepyramine also showed that normally there was no detectable histamine in the circulating blood.

The disappearance of histamine in blood, in some vascular beds and in the whole animal, as well as a determination of its half-life in the circulation was measured to exemplify the utility of the method for quantitating circulating histamine. The incubation of histamine in blood for up to 4 min showed no loss of activity. These experiments accord with the current view regarding the unimportance of the blood for inactivation of histamine in the circulation. Histamine activity of plasma is low (Best & McHenry, 1930) and the diffusion of histamine

into the cell fraction is too slow (Lindell & Viske, 1961 ; Born, Day & Stockbridge, 1967) to account for the rapid disappearance of histamine from the circulation (Best & McHenry, 1930 ; Halpern, Neveu & Wilson, 1959). In this paper, by the use of a continuous assay of circulating histamine, a half-life of 20–30 s (Fig. 3) was found. These results are compatible with the 50% disappearance of histamine during one circulation (15–20 s) in all vascular beds of the dog (Table 1, Figure 3). Halpern *et al.* (1959) also found that more than 50% of circulating histamine disappears within 30 s in the rat. This rapid disappearance of histamine from the circulation must be due to its removal or inactivation by tissues (Rose & Brown, 1938 ; Emmelin, 1951) and, as indicated in this paper, does not depend on blood pressure.

Our results show that the pulmonary circulation and heart chambers play no part in the clearance of circulating histamine. This result may appear to contradict those observations in which significant amounts of radioactivity (though smaller than those of kidneys, liver and striated muscle) could be detected in the lung after administration of labelled histamine (Halpern *et al.*, 1959 ; Robinson & Green, 1964 ; Nilsson, Lindell, Schayer & Westling, 1959). However, in heart-lung preparations, negligible disappearance of histamine was observed when compared with heart-lung-liver preparations (Steggerda, Essex & Mann, 1935 ; Lilja & Lindell, 1961).

Histamine disappears during passage through the vascular beds of liver and hind legs (Best & McHenry, 1930 ; Steggerda, Essex & Mann, 1935 ; Emmelin, 1951). The bulk of the histamine taken up by tissues is catabolized *in situ*. However, some tissues may release it back to the circulation when it will be metabolized by other tissues or excreted by the kidneys (Emmelin, 1951 ; Halpern *et al.*, 1959 ; Green, 1967).

Our assay tissues for detection are more sensitive to circulating histamine than is the systemic blood pressure of the dog. However, after the cessation of an intravenous infusion of histamine, the assay tissues return to baseline quicker than does the blood pressure. This may indicate that the prolonged hypotensive effect is either due to the release of another hypotensive endogenous material or to an uptake mechanism which, by the slow release of histamine, maintains an effective concentration at the vascular receptors.

We thank the Wellcome Trust for a grant and Ciba, ICI, Parke-Davis, Wellcome Research Laboratories, Upjohn and Smith, Kline and French Laboratories for drugs. S. H. F. wishes to thank Dr. Ivoni Bareicha for assistance during the preliminary phase of this study carried out in the Dept. of Pharmacology, Faculty of Medicine, Ribeirao Prêto, Brazil.

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(*Received March 22*, 1973)