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THE DETECTION AND ESTIMATION OF BRADYKININ IN THE CIRCULATING BLOOD

BY

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It has always been difficult to estimate small concentrations of bradykinin in the circulating blood. Usually, therefore, indirect methods have been used which measure the activation of kinin-releasing enzymes in plasma and lymph (Hilton & Lewis, 1956; Fox & Hilton, 1958; Edery & Lewis, 1962) or the bradykininogen concentration in plasma (Armstrong, Keele & Stewart, 1960; Lecomte, 1961; Diniz & Carvalho, 1963; Brocklehurst & Lahiri, 1963). Bradykinin itself has been detected in plasma in shock induced by peptone and by proteolytic enzymes, in anaphylaxis (Beraldo, 1950; Brocklehurst & Lahiri, 1962; Corrado, 1964) and in the flush phase of the carcinoid syndrome (Oates, Melmon, Sjoerdsma, Gillespie & Mason, 1964). These observations involved the removal of considerable amounts of blood (up to 30 ml.) followed by laborious chemical processing.

In this paper we describe a method utilizing the blood-bathed organ technique (Vane, 1964) by which bradykinin can be continuously estimated in the circulation at the time of its formation. We have also studied the influence of a bradykinin potentiating factor (Ferreira, 1965) on the detection of circulating bradykinin.

METHODS

Bradykinin was detected with isolated organs superfused (Gaddum, 1953) either with Krebs solution or with blood (Vane, 1964). Three organs were superfused in cascade with the same stream of fluid which was maintained at 37° C by a water jacket and supplied by a roller pump at 15 ml./min. The movements of the organs were recorded on smoked kymograph paper using auxotonic levers (Paton, 1957) of 16:1 magnification and with an initial load on the tissues of 1–4 g.

Isolated organs

The animals were killed by stunning and bleeding through the carotid arteries. The part of the intestine required was removed, washed and suspended for superfusion in a polypropylene jacket. The following tissues were used: from the rat the stomach strip (Vane, 1957), duodenum (Horton, 1959) and ascending colon (Regoli & Vane, 1964); from the rabbit the taenia coli, duodenum, middle and terminal ileum and longitudinal strips of caecum; from the guinea-pig the duodenum, jejunum, ileum and taenia coli; from the cat, longitudinal strips about 4–6 mm wide and 4–7 cm long were cut from the duodenum, the jejunum, the ileum and the caecum; cat jejunum has previously been

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used to detect kinins by Schachter (1956) and by Erspamer & Erspamer (1962). The tissues were bathed in Krebs solution of the following composition (in g/l. of distilled water): NaCl 6.9, KCl 0.35, $CaCl_2.6H_2O$ 0.5, KH_2PO_4 0.16. $MgSO_4.7H_2O$ 0.29. Glucose 1 and $NaHCO_3$ 2.1. This solution was gassed with a mixture of 95% oxygen and 5% carbon dioxide.

Preparation of animals

Cats of either sex, weighing 1.5–4 kg were anaesthetized with ethylchloride and ether and anaesthesia was then maintained with chloralose (80 mg/kg intravenously). Dogs of either sex, weighing 5–12 kg, were anaesthetized first with halothane; anaesthesia was then maintained with chloralose (100 mg/kg intravenously). The trachea was cannulated to facilitate artificial respiration. Polyethylene cannulae were tied into a femoral or carotid artery, and a femoral or jugular vein. Heparin (1,000 u./kg intravenously) was injected through the venous cannula. To superfuse the tissues, blood was taken from the cannulated artery, pumped by the roller pump at 15 ml./min over the three isolated organs, collected in a reservoir and then returned to the vein either by gravity or by a second channel in the roller pump. In some experiments, the tissues were superfused with venous blood obtained by pushing a polyethylene catheter *via* a femoral vein into the vena cava.

In experiments designed to measure the inactivation of bradykinin by blood, a piece of silicone tubing of 3 mm internal diameter and sufficiently long to contain 15 ml. was maintained at 37° C in a water bath and included in the external circuit between the cannula supplying the blood and the tissues. In this way, injections or infusions could be made either directly to the blood bathed tissues (I.B.B.) or after incubation with the circulating blood for time intervals of up to 1 min, as it passed through the extended external circuit (" incubating circuit "). Blood pressure was measured with a mercury manometer from a side arm on the arterial cannula.

Drugs

The following drugs were used, doses of salts being expressed as base: acetylcholine perchlorate (British Drug Houses), (−)adrenaline bitartrate (British Drug Houses), synthetic angiotensin (" Hypertensin Ciba "), synthetic bradykinin (Parke-Davis), Dextran (6% w/v in 0.9% w/v sodium chloride solution, Benger Labs), synthetic eledoisin (ELD 90, Sandoz), heparin (Pularin, Evans), histamine acid phosphate (Burroughs Wellcome), synthetic kallidin (Sandoz), (−)noradrenaline bitartrate (British Drug Houses), oxytocin (Pitocin, Parke-Davis), trypsin (L. Light), vasopressin (Pitressin, Parke-Davis) and bradykinin potentiating factor (BPF) prepared as described by Ferreira (1965). A " slow reacting substance in anaphylaxis " (SRS-A) was kindly given by Dr. H. O. J. Collier and Substance P (1 mg=13.2 u.) by Professor F. Lembeck. Prostaglandin E_2 and $F_2\alpha$ were kindly supplied by Upjohn.

RESULTS

Of all the blood-bathed organs tested, only the rat duodenum and strips of cat jejunum responded to injections of bradykinin (5–30 ng). The rat duodenum relaxes to bradykinin given by injection (Horton, 1959); but during an infusion the response was not maintained (Fig. 1). Thus, although sensitive, the tissue was not suitable for detecting prolonged changes of bradykinin concentration. A further disadvantage was the similarity between the response to bradykinin and that of catecholamines which may be released by circulating bradykinin (Feldberg & Lewis, 1964; Staszewska-Barczak & Vane, 1965).

Strips of cat jejunum responded to bradykinin by contraction; the response was proportional to the dose and was well maintained (Fig. 1). The preparations usually responded to as little as 0.5 ng bradykinin/ml. and sometimes to 0.1 ng/ml.; they exhibited no tachyphylaxis. Contractions of cat jejunum bathed in cat's blood to injections of bradykinin were slightly greater than when bathed in Krebs solution. When

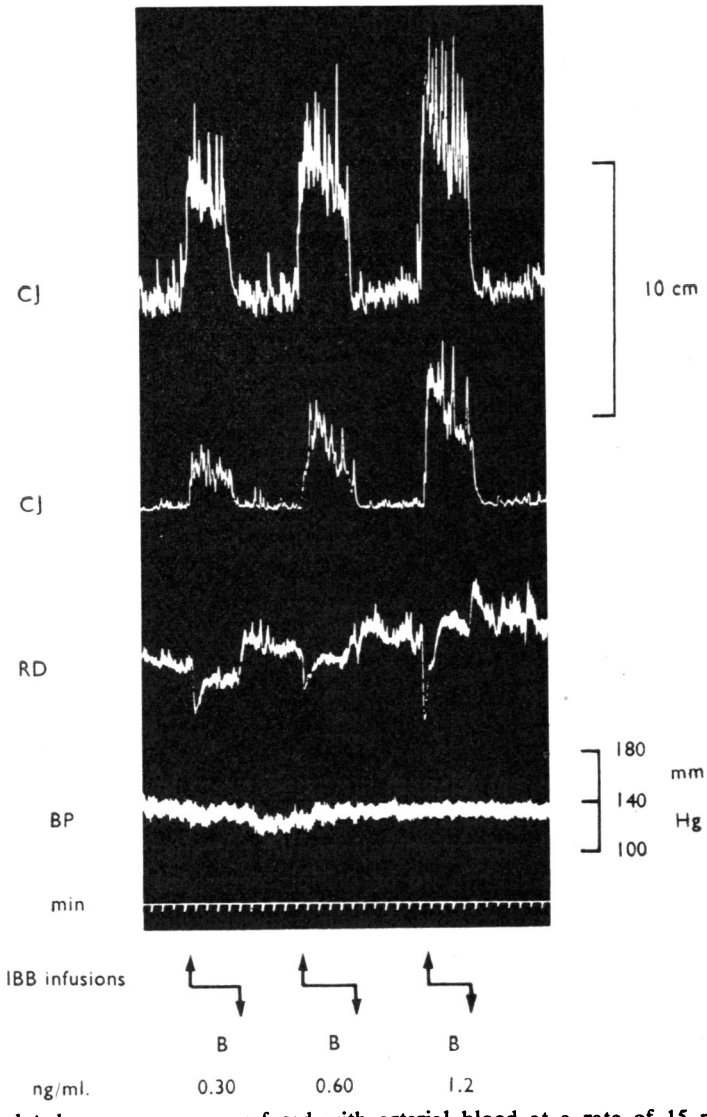

Fig. 1. The isolated organs were superfused with arterial blood at a rate of 15 ml./min, from a cat anaesthetized with chloralose. Responses of two strips of cat jejunum (CJ) and a rat duodenum (RD) to infusions of bradykinin (B) to give final concentrations in the bathing blood (IBB) of 0.3, 0.6 and 1.2 ng/ml. The blood pressure (BP) is in mm Hg. Both strips of cat jejunum responded with a maintained contraction to the bradykinin infusions whereas the relaxation of the rat duodenum rapidly declined. Time in min: vertical scale, 10 cm.

bathed in dog's blood the sensitivity was slightly reduced and the spontaneous activity was greater; both factors made it more difficult to measure small concentrations.

Kallidin (0.2–2 ng/ml.) and eledoisin (0.8–8 ng/ml.) also contracted the cat jejunum but, as shown in Table 1, the tissue was relatively insensitive to other substances likely to be circulating in blood. A preparation of SRS-A was compared with bradykinin on

guinea-pig ileum and strips of cat jejunum, using a 10 ml. isolated organ bath. To give a contraction equivalent to 10 ng bradykinin, 2 μg SRS-A was needed on the ileum but 2 mg on the cat jejunum.

TABLE 1

RESPONSES OF ISOLATED STRIPS OF CAT JEJUNUM TO INJECTIONS OF SOME ENDOGENOUS SUBSTANCES

The doses represent those needed to elicit a contraction of 3 cm or a relaxation of 0·5 cm lever movement. " Small contraction " or " Small relaxation " indicates a lever movement smaller than 3 cm or 0·5 cm respectively

	Superfusion fluid			
	Krebs		Cat or dog blood	
Substances	Dose	Observed response	Dose	Observed response
Bradykinin	5–20 ng	Contraction	5–30 ng	Contraction
Kallidin	5–20 ng	Contraction	5–30 ng	Contraction
Eledoisin	20–30 ng	Contraction	30–50 ng	Contraction
Acetylcholine	10–30 ng	Contraction	20–30 ng	Contraction
5-Hydroxytryptamine	400 ng	Contraction	Up to 1 μg	None
Histamine	Up to 1 μg	None or small contraction	Up to 1 μg	None or small contraction
Adrenaline	200 ng	Relaxation	Up to 1 μg	None or small relaxation
Noradrenaline	200 ng	Relaxation	Up to 1 μg	None
Angiotensin	Up to 1 μg	None	Up to 1 μg	None
Vasopressin	Up to 5 u.	None	Up to 5 u.	None
Oxytocin	Up to 5 u.	None	Up to 5 u.	None
SRS-A	Up to 1 mg	None	Up to 1 mg	None
Substance P	Up to 13 u.	None	Up to 13 u.	None
Prostaglandin E_2	Up to 0·5 μg	None	Up to 1 μg	None
Prostaglandin $F_2\alpha$	Up to 0·5 μg	None	Up to 1 μg	None

Since catecholamines relax cat jejunum it was necessary to test its response to bradykinin in their presence. Concentrations of adrenaline (10 ng/ml.) which were always detected by the rat stomach strip preparations did not change the resting tone of the cat jejunum ; however, its sensitivity to bradykinin decreased by about one-half.

Because of its sensitivity and relative specificity towards the actions of bradykinin-like peptides the cat jejunum was chosen to detect bradykinin in circulating blood. The sensitivity to bradykinin of the cat jejunum bathed in Krebs solution increases with storage (Erspamer & Erspamer, 1962). Preparations that had been kept in Krebs solution in the refrigerator for 18–48 hr were more sensitive than the freshly prepared tissue also when subsequently bathed in blood. This fact made it convenient to take jejunum from the cat at the end of an experiment for storage and use in subsequent experiments.

Inactivation of bradykinin by blood

To demonstrate the inactivation of bradykinin in circulating dog's blood, the incubating circuit was used. Bradykinin was infused into the external circuit either directly to the tissues (into the bathing blood—IBB) or into the circuit so that the bradykinin was in contact with the blood for an extra 60 sec (Fig. 2). Infusions of bradykinin into the incubating circuit had to be 2.5 times greater than infusions IBB, showing that much more than 50% of the bradykinin was inactivated within 60 sec. BPF was then infused into the external circuit to give a concentration of 10 μg/ml. In Krebs solution this concentration of BPF did not potentiate the effects of bradykinin on the cat jejunum though in

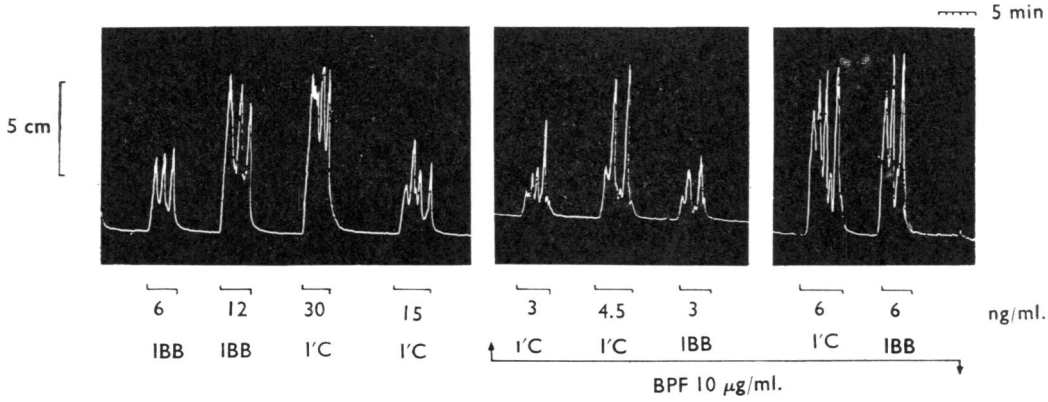

Fig. 2. A strip of cat jejunum was superfused at 15 ml./min with arterial blood from a dog anaesthetized with chloralose. The reactions are to infusions of bradykinin IBB, at rates to give initial concentrations (ng/ml.) as shown. The first panel shows that when the infusion of bradykinin is given into the incubating circuit to give a contact time with blood increased by 1 min (1′ C) more than 50% the bradykinin is destroyed. In the presence of BPF (10 μg/ml.) smaller concentrations of bradykinin now contract the cat jejunum and little or no bradykinin is destroyed by contact with blood. Time in min: vertical scale 5 cm.

concentrations of more than 30 μg/ml. a slight potentiation could be observed. This infusion of BPF completely prevented the inactivation of bradykinin in the blood. At the same time, the tissues apparently became more sensitive to bradykinin infusions; this was probably related to the fact that there was a 10 sec delay between IBB infusions and the arrival of the drug at the organs. In every experiment in which it was measured (Table 2) much more than 50% of the bradykinin was inactivated within 1 min. BPF given either by intravenous injection to the animal or by infusion into the incubating circuit greatly decreased this inactivation.

TABLE 2

INFLUENCE OF BPF ON THE RECOVERY OF BRADYKININ INCUBATED WITH CIRCULATING BLOOD

The percentage recovery of bradykinin was obtained by infusing bradykinin into the bathing blood or into the 1-min incubating circuit and comparing the responses of the cat jejunum. BPF (1 mg/kg) was administered by single intravenous injection to the animals in the first three experiments. In the remaining experiments BPF was infused into the incubating circuit to give a final concentration of 10 μg/ml. in the bathing blood

	% Recovery of bradykinin after 1 min		
	Before BPF (A)	In presence of BPF (B)	Ratio B/A
Dog	25	100	4
Dog	25	100	4
Dog	10	50	5
Dog*	40	100	2·5
Cat	25	75	3

* Experiment illustrated in Fig. 2.

Detection of bradykinin given intravenously

Table 3 shows results of experiments in which the effects of intravenous infusions and injections of bradykinin were compared with those of infusions and injections given

TABLE 3

DETECTION OF BRADYKININ IN THE ARTERIAL BLOOD AFTER INTRAVENOUS ADMINISTRATION

For single doses the figures represent the intravenous dose necessary to give a detectable response of the blood-bathed cat jejunum. For infusions the infusion rate given is calculated from the experimental results to correspond to that which would give a carotid blood concentration of 1 ng/ml.

Animal	Weight (kg)	I.V. dose to give detectable response ng/kg/injection (A)	BPF 1 mg/kg I.V. +	After BPF; I.V. dose to give detectable response ng/kg/injection (B)	Ratio A/B
Dog	10	10,000		300	33
Dog	11	3,000		650	4·6
Cat	2·0	800		115	7
Cat	2·5	500		50	10
		I.V. infusion to give arterial concn. of 1 ng/ml. ng/kg/min		After BPF; I.V. infusion to give arterial concn. of 1 ng/ml. ng/kg/min	
Cat	2·5	400	10 μg/ml. IBB	40	10
Cat	3·0	490	10 μg/ml. IBB	75	6·5
Cat	2·5	2,000	10 μg/ml. IBB	160	12·5
Cat	2·7	750	10 μg/ml. IBB	120	6·2

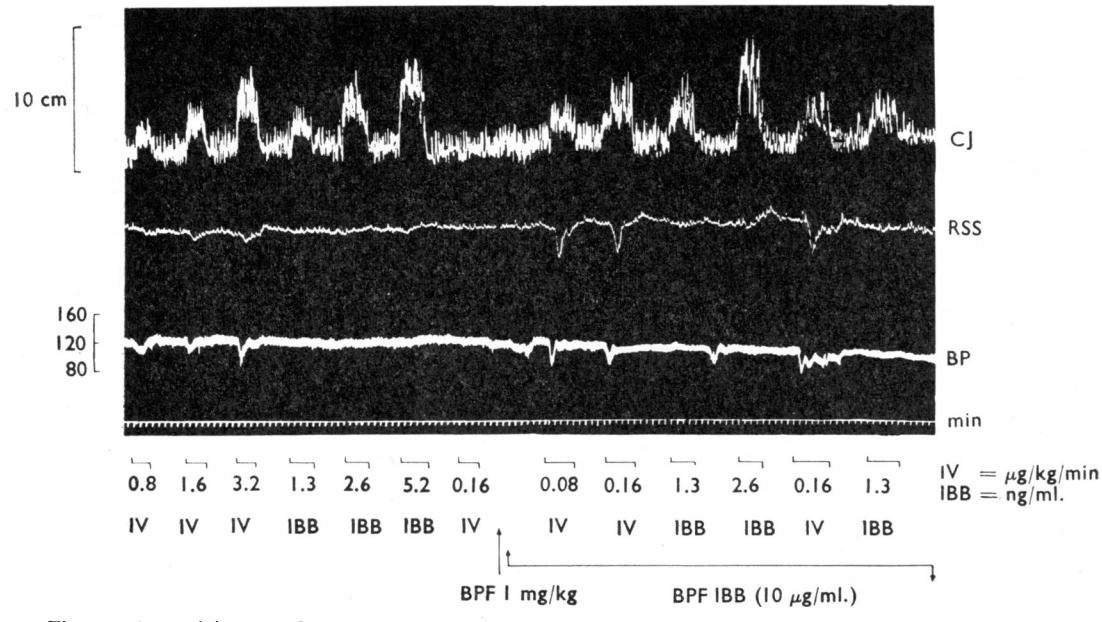

Fig. 3. A cat jejunum (CJ) and rat stomach strip (RSS) were superfused at 15 ml./min with arterial blood from a cat anaesthetized with chloralose. Intravenous infusions of bradykinin of 1.6 and 3.2 μg/kg gave responses of the cat jejunum intermediate to the effects induced by infusions of bradykinin into the bathing blood (IBB) to give concentrations of 1.3, 2.6 and 5.2 ng/ml. respectively. After BPF (1 mg/kg IV and then a continuous infusion IBB to give a concentration of 10 μg/ml.) much lower rates of intravenous infusion of bradykinin (0.08 and 0.16 μg/kg/min) induced responses on the cat jejunum. Now 0.16 μg/kg/min matched the response of 1.3 ng/ml. given IBB. Note also that these small intravenous infusions now elicited release of catecholamine into the circulation, as shown by the relaxation of RSS. Time in min: vertical scales 10 cm and in mm Hg.

directly into the blood superfusing the organs. This indicated the sensitivity of the method to the intravenous administration or to the release of bradykinin, and permitted the calculation of bradykinin concentrations in the arterial blood. Figure 3 shows such an experiment. Before BPF intravenous bradykinin (3.2 μg/kg/min) produced a carotid blood concentration of between 2.6 and 5.2 ng/ml. or approximately 4 ng/ml.; 0.8 μg/kg/min gave less than 1.3 ng/ml. After BPF (1 mg/kg intravenously and then an infusion of 10 μg/ml. IBB) an intravenous infusion of bradykinin (0.16 μg/kg/min) gave a carotid blood concentration of 1.3 ng/ml. Thus, by preserving bradykinin in the circulation the BPF increased the sensitivity of the method about tenfold. Figure 3 also shows that intravenous bradykinin (0.16 μg/kg/min) did not release sufficient adrenaline to be detected by the rat stomach strip preparation. After BPF however, 0.08 μg/kg/min intravenously released adrenaline, as was shown by the relaxation of the rat stomach strip.

Superfusion with venous blood. In some experiments, the cat jejunum preparations were superfused with venous blood. No difference could be detected in spontaneous activity of the jejunum or in its reactions to bradykinin.

Formation of endogenous bradykinin

(a) *Release by contact with glass.* Figure 4 shows the effects of passing blood through a small tube containing glass beads (1 g of 0.5 mm diameter) in the external circuit. Within a few seconds, the cat jejunum began to contract; calibration showed that the

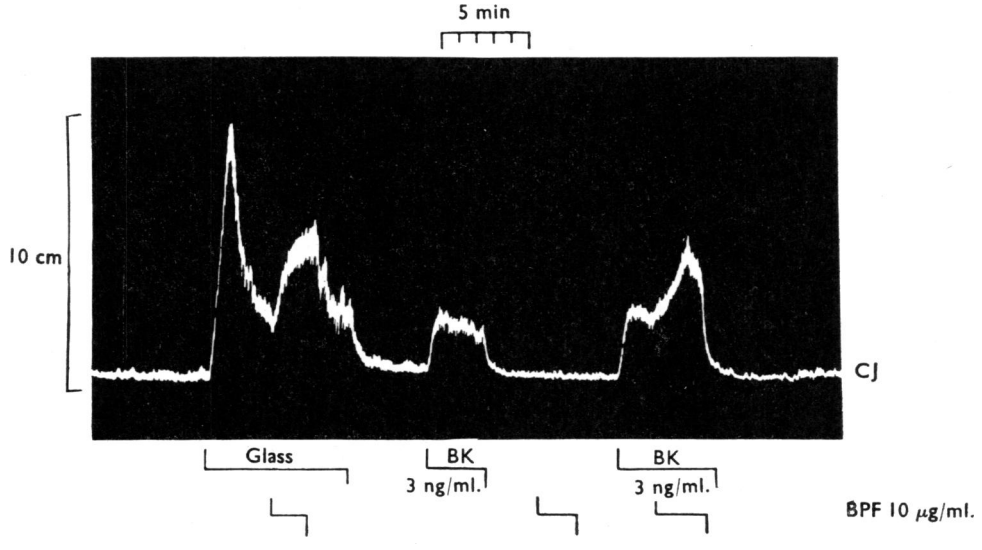

Fig. 4. Strip of cat jejunum superfused at 15 ml./min with arterial blood from a cat anaesthetized with chloralose. At the point marked, the blood was diverted through a tube containing 1 g of glass beads. There was an almost immediate generation of bradykinin which rapidly declined to a rate of 45 ng/min and a concentration of 3 ng/ml. Infusion of BPF (10 μg/ml.) into the blood increased once more the contraction of the cat jejunum. The contraction caused by an infusion of bradykinin to give a concentration of 3 ng/ml. was similarly increased by BPF. Time in min: vertical scale 10 cm.

rate of formation of bradykinin at first greatly exceeded 45 ng/min (3 ng/ml.); the rate declined over 5 min to about 45 ng/min. An infusion of BPF (10 μg/ml.) potentiated the response of the cat jejunum to about the same extent as that produced by an infusion of bradykinin (45 ng/min). When the glass beads were next included in the external circuit much less bradykinin was formed and on the fourth inclusion no bradykinin could be detected.

(b) *Release by saliva.* Human saliva was diluted 1:40 with saline and then infused at 0.2 ml./min into the incubating circuit to give a contact time with blood of 40 sec before reaching the assay tissues. Boiled diluted saliva had no effect (Fig. 5) but bradykinin was generated with normal diluted saliva. BPF (10 μg/ml.) potentiated the effects on the cat jejunum. This dilution of saliva did not contract the cat jejunum bathed in Krebs solution.

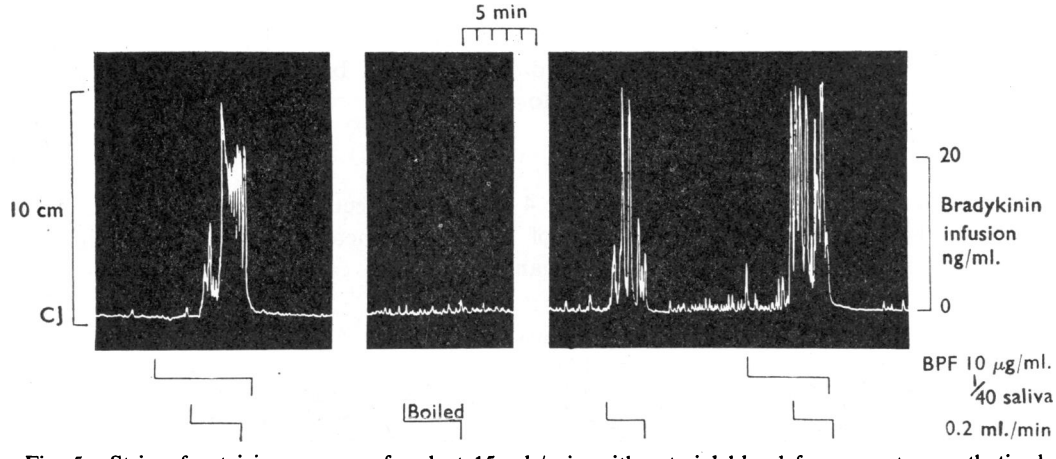

Fig. 5. Strip of cat jejunum, superfused at 15 ml./min with arterial blood from a cat anaesthetized with chloralose. An infusion of BPF to give a concentration of 10 μg/ml. had no effect by itself. When a 1/40 dilution of human saliva was also infused into the blood, to give a contact time of 40 sec before reaching the cat jejunum, bradykinin was generated. Boiled diluted saliva had no effect (2nd panel). The infusion of diluted saliva without the presence of BPF induced a smaller response of the cat jejunum, showing that less bradykinin reached the tissue. The last effect is a repetition of the saliva infusion together with BPF. Time in min: vertical scale on left hand side in 10 cm and also on right hand side to show the height of contraction produced by an infusion of bradykinin (20 ng/min) 40 sec away from the cat jejunum.

DISCUSSION

To use the blood-bathed organ technique for the estimation of circulating substances, isolated organs have to be found which are sufficiently sensitive and specific. For bradykinin, of all the tissues tested, only the rat duodenum and strips of cat jejunum showed this sensitivity and specificity. Rat duodenum proved unsatisfactory because it usually did not maintain a response. Cat jejunum bathed in blood responded by a maintained contraction to as little as 0.1 ng bradykinin/ml. and was relatively insensitive to other known circulating hormones. Although catecholamines reduced the effects of bradykinin on the cat jejunum they did not seriously interfere with its detection.

With the blood-bathed organ technique, no estimate can usually be obtained of the initial concentration of circulating hormone. For bradykinin, however, infusion of a factor (BPF) which prevented its destruction did not modify the resting baseline of the cat jejunum, indicating that in anaesthetized cats and dogs the concentration of bradykinin in arterial blood was less than 0.1 ng/ml.

Methods for estimating bradykinin indirectly which use reduction in the kininogen concentration of plasma generally require more than a 3% variation to be certain of a change. Cat plasma contains sufficient bradykininogen (Diniz & Carvalho, 1963) to liberate the equivalent of approximately 9 μg bradykinin/ml., or about 360 μg/kg body weight. A 3% reduction in kininogen over 5 min would, therefore, correspond to the release of 10 μg bradykinin/kg into the circulation. The cat jejunum easily detects this rate of release of bradykinin, even without using BPF. When BPF is used, rates of release of less than 0.2 μg/kg/min can be detected. Thus the blood bathed cat jejunum is far more sensitive for the detection of bradykinin than measurements of kininogen. The present technique is also more specific than other forms of bioassay. For instance, both the guinea-pig ileum and the rat uterus contract not only to bradykinin, but also to 5-hydroxytryptamine and angiotensin, two substances which may also be circulating under conditions in which bradykinin is liberated. Similarly, the rat duodenum which relaxes to bradykinin also does so to catecholamines.

With the blood-bathed organ technique the inclusion of other tissues in the assay circuit allows simultaneous parallel pharmacological assay. By using a rat stomach strip (Vane, 1957), a rat colon (Regoli & Vane, 1964) and a cat jejunum, the release of bradykinin can be distinguished from the release of catecholamines, 5-hydroxytryptamine and angiotensin. Further specificity can be added to the method by taking advantage of the rapid destruction of bradykinin in blood (Saameli & Eskes, 1962 ; McCarthy, Potter & Nicolaides, 1965). This inactivation is prevented by BPF so that, if free bradykinin is present in the circulating blood, inclusion of an incubating circuit should reduce the concentration leading to a smaller response of the cat jejunum, whereas infusion of BPF should potentiate the response. Thus, the criteria we have used for identification of free bradykinin in the circulating blood are: (1) contraction of the cat jejunum but not of the rat stomach strip, the rat colon or the chick rectum ; (2) that the contraction should be reduced by including an incubating circuit ; (3) that the contraction should be increased by infusing BPF into the external circuit. There is also the possibility that an active bradykinin-forming enzyme is present in blood when the second criterion may not apply.

In most of these experiments, bradykinin was assayed in carotid arterial blood. However, it is quickly destroyed not only in blood but also in the lungs (Ferreira & Vane, unpublished). For this reason, a few experiments were performed in which the cat jejunum was bathed in mixed venous blood. The response to infused bradykinin did not decrease, showing that the sensitivity of the method might be further increased by sampling mixed blood from the venous side of the circulation.

The experiment in which the generation of bradykinin in the external circuit was demonstrated by the inclusion of glass beads showed that it is better to eliminate such surfaces from the external circuit.

SUMMARY

1. A method is described for the continuous assay of bradykinin in the circulating blood, using strips of cat jejunum as the blood bathed organs.

2. The cat jejunum will detect blood concentrations of bradykinin as low as 0.1 ng/ml.

3. By using a bradykinin potentiating factor (BPF) to preserve the bradykinin in the circulating blood, the sensitivity of the method can be further increased.

4. The cat jejunum is a relatively specific assay organ for bradykinin and similar peptides. The specificity can be increased by the simultaneous use of two other assay organs.

5. The destruction of bradykinin in the circulating blood and its protection by BPF can also be used in this technique to help identify the substance which contracts the cat jejunum as bradykinin.

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REFERENCES

ARMSTRONG, D., KEELE, C. A. & STEWART, J. W. (1960). Observations on the formation of kinins in human plasma collected during labour. *J. Physiol.*, **150**, 20*P*–21*P*.

BERALDO, W. T. (1950). Formation of bradykinin in anaphylactic and peptone shock. *Am. J. Physiol.*, **163**, 283–289.

BROCKLEHURST, W. E. & LAHIRI, S. C. (1962). The production of bradykinin in anaphylaxis. *J. Physiol.*, **160**, 15*P*–16*P*.

BROCKLEHURST, W. E. & LAHIRI, S. C. (1963). Formation and destruction of bradykinin during anaphylaxis. *J. Physiol.*, **165**, 39*P*–40*P*.

CORRADO, A. P. (1964). Considerações sobre a participação de bradicinina e peptideos analogos nos efeitos hemodinamicos induzidos por enzimas proteoliticas. *Thesis. Fac. Med.*, Ribeirao Preto.

DINIZ, C. R. & CARVALHO, I. F. (1963). A micromethod for determination of bradykininogen under several conditions. *Ann. N.Y. Acad. Sci.*, **104**, 77–89.

EDERY, H. & LEWIS, G. P. (1962). Plasma kinin-forming-enzyme activity in lymph after injury. *J. Physiol.*, **163**, 48*P*–49*P*.

ERSPAMER, V. & ERSPAMER, G. F. (1962). Pharmacological actions of eledoisin on extravascular smooth muscle. *Br. J. Pharmac. Chemother.*, **19**, 337–354.

FELDBERG, W. & LEWIS, G. P. (1964). The action of peptides on the adrenal medulla. Release of adrenaline by bradykinin and angiotensin. *J. Physiol.*, **171**, 98–108.

FERREIRA, S. H. (1965). A bradykinin-potentiating factor (BPF) present in the venom of *Bothrops jararaca*. *Br. J. Pharmac. Chemother.*, **24**, 163–169.

FOX, R. H. & HILTON, S. M. (1958). Bradykinin formation in human skin as a factor in heat vasodilatation. *J. Physiol.*, **142**, 219–232.

GADDUM, J. H. (1953). The technique of superfusion. *Br. J. Pharmac. Chemother.*, **8**, 321–326.

HILTON, S. M. & LEWIS, G. P. (1956). The relationship between glandular activity, bradykinin formation and functional vasodilatation in submandibular salivary gland. *J. Physiol.*, **134**, 471–483.

HORTON, E. W. (1959). Human urinary kinin excretion. *Br. J. Pharmac. Chemother.*, **14**, 125–132.

LECOMTE, J. (1961). Consommation des kininogènes plasmatiques au cours du choc anaphylatique du lapin. *C. r. Séanc. Soc. Biol.*, **155**, 1411–1413.

MCCARTHY, D. A., POTTER, D. E. & NICOLAIDES, E. D. (1965). An *in vivo* estimation of the potencies and half-lives of synthetic bradykinin and kallidin. *J. Pharmac. exp. Ther.*, **148**, 117–122.

OATES, J. A., MELMON, K., SJOERDSMA, A., GILLESPIE, L. & MASON, D. T. (1964). Release of a kinin peptide in the carcinoid syndrome. *Lancet*, **i**, 514–517.

PATON, W. D. M. (1957). A pendulum auxotonic lever. *J. Physiol.*, **137**, 35–36*P*.

REGOLI, D. & VANE, J. R. (1964). A sensitive method for the assay of angiotensin. *Br. J. Pharmac. Chemother.*, **23**, 351–359.

SAAMELI, K. & ESKES, T. K. A. B. (1962). Bradykinin and cardiovascular system: estimation of half-life. *Am. J. Physiol.*, **203**, 261–265.

SCHACHTER, M. (1956). A delayed slow contracting effect of serum and plasma due to the release of a substance resembling kallidin and bradykinin. *Br. J. Pharmac. Chemother.*, **11**, 111–118.

STASZEWSKA-BARCZAK, J. & VANE, J. R. (1965). The release of catecholamines from the adrenal medulla by peptides. *J. Physiol.*, **177**, 57–58*P*.

VANE, J. R. (1957). A sensitive method for the assay of 5-hydroxytryptamine. *Br. J. Pharmac. Chemother.*, **12**, 344–349.

VANE, J. R. (1964). The use of isolated organs for detecting active substances in the circulating blood. *Br. J. Pharmac. Chemother.*, **23**, 360–373.