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# THE DISAPPEARANCE OF BRADYKININ AND ELEDOISIN IN THE CIRCULATION AND VASCULAR BEDS OF THE CAT

BY

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Bradykinin is short-lived in the circulation and has a half life of about 20 sec in man (Saameli & Eskes, 1962), and 16 sec in dog's blood (McCarthy, Potter & Nicolaides, 1965). Although little is known about its inactivation in the various vascular beds, Oates, Melmon, Sjoerdsma, Gillespie & Mason (1964) suggested that it was partially destroyed during passage from the venous to the arterial side of the circulation. Bumpus, Smeby, Page & Khairallah (1964) infused tritiated bradykinin into rats and found large quantities of labelled material in kidney, liver and urine. Another hypotensive peptide, eledoisin, is relatively stable in blood (Sicuteri, Fanciullaci, Franchi and Michelacci, 1963 ; Sturmer & Berde, 1963 ; Nobili, 1965), although Nobili (1965) has shown that it can be fairly rapidly inactivated by tissue homogenates, especially those from the kidney and the liver.

We have measured the rate of inactivation of bradykinin and eledoisin in the circulating blood of cats, and have also studied their disappearance during passage through various vascular beds.

## METHODS

Cats of either sex weighing 1.5–4 kg were anaesthetized with ethyl chloride and ether ; anaesthesia was then maintained with chloralose (80 mg/kg intravenously). The trachea was cannulated to facilitate artificial respiration. Fine polyethylene catheters (external diameter 0.96 mm) were tied into various arteries and veins, as detailed later. Heparin (1,000 i.u./kg intravenously) was injected through a venous cannula.

Three blood bathed organs (Vane, 1964) were used to detect changes in concentration of circulating bradykinin and eledoisin. To superfuse the smooth muscle preparations, blood was taken from a cannulated artery, pumped by roller pump at 15 ml./min over three isolated organs, then collected in a reservoir and returned to a suitable vein either by gravity or by a second channel in the roller pump. In those experiments where the inactivation by blood was studied, enough silicone tubing of 3 mm internal diameter to contain 15 ml. of blood was included in the external circuit between the cannula supplying the blood and the assay organs. In this way infusions could be made either close to the assay organs (I.B.B.) or after incubation for known time intervals of up to 1 min with the circulating blood on its way through the extended external circuit (incubating circuit). The blood outside the animal was kept at 37° C. Mean blood pressure was measured with a mercury manometer from a side arm on the arterial cannula.

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Circulating bradykinin was assayed continuously on strips of cat jejunum (Ferreira & Vane, 1967). Both the rat duodenum (Horton, 1959) and the rat fundus strip (Vane, 1957) contract to eledoisin in the same range of concentrations as does the strip of cat jejunum. One or both of these muscles was sometimes used in conjunction with the cat jejunum when eledoisin was being estimated.

The "half life" of bradykinin in the circulating blood was measured as follows: first, a dose-response relationship was established to different infusion rates of bradykinin into the bathing blood. An infusion rate which gave a good response was then chosen. The response to this infusion was then compared with responses to double the infusion rate given at varying positions into the incubating circuit to give increasing times of contact in steps of 5 sec. In this way, the time necessary to incubate the double dose with the blood in order to reduce the response to that of a single dose was estimated; this was the time for 50% destruction, or the "half life." All the infusions were made for sufficient time to reach equilibrium conditions, as shown by the plateaus of contraction of the assay organs.

To calculate the disappearance of the peptides in particular vascular beds, infusions were made into the blood supplying the vascular bed. The plateau contractions of the assay organs (bathed in arterial blood) produced by these infusions were compared with the contractions produced by infusions of peptides into the blood leaving the particular vascular bed. Thus, the ability of a particular vascular bed to remove the peptide could be calculated—for example, in the lungs a "disappearance ratio" of 4 means that the plateau responses of the assay tissues to 4 $\mu$g/min intravenously were comparable with plateau responses to an infusion of 1 $\mu$g/min into the ascending aorta.

The disappearance of the peptides was studied in six different areas of the body. The positions of the infusion catheters (which were checked at the end of each experiment) were as follows:

*Heart chambers and lungs*

The venous infusion catheter was introduced through a jugular vein so that its tip lay close to the right atrium. The arterial infusion catheter was introduced through the right carotid artery so that the tip was in the ascending aorta just above the aortic valves. The assay organs were bathed in femoral arterial blood.

*Head*

Arterial infusions were made in two different ways. In one, a catheter was pushed several centimetres up a carotid artery towards the head; infusions through this catheter were compared with infusions made into a catheter in the jugular vein. The assay organs were bathed in blood from another catheter pushed down the same carotid artery towards the heart. In the other, a carotid artery was tied and both ends were cannulated. The cannulae were connected with a loop of silicone tubing and the flow of blood was re-established. Infusions were made through a hypodermic needle into the silicone tubing. The venous catheter was pushed up a femoral vein so that the tip lay in the inferior vena cava within the thorax. The assay organs were bathed in blood from a femoral artery.

For the following experiments, the assay organs were bathed in carotid arterial blood.

*Body below diaphragm*

The arterial infusion catheter was introduced through a femoral artery and pushed up the descending aorta so that its tip was level with the diaphragm. The venous catheter was introduced to a similar position in the inferior vena cava through the femoral vein.

*Hind quarters*

The arterial catheter was introduced through a femoral artery so that its tip lay just above the bifurcation of the aorta. The venous catheter was introduced into the femoral vein on the same side and pushed up to the same level.

*Liver*

The abdomen was opened and the spleen was removed. A catheter was introduced through the splenic vein so that its tip lay in the portal vein. Infusions through this catheter were compared with infusions through a catheter introduced into a femoral vein so that its tip lay in the inferior vena cava above the diaphragm.

*Kidneys*

The abdomen was opened and the coeliac axis and superior mesenteric artery were tied. The arterial catheter was introduced through a femoral artery so that the tip lay just above the renal arteries. The aorta was occluded around the catheter just below the renal arteries. In this way the infusion was made into blood predominantly flowing to the kidneys. The venous catheter was introduced through a femoral vein so that its tip lay at the level of the renal veins.

*Drugs*

Synthetic bradykinin was kindly supplied by Parke Davis & Co. Ltd., and synthetic eledoisin by Sandoz Products Ltd.

## RESULTS

### *Inactivation of bradykinin by blood*

In five cats the incubating circuit was included in the extra-corporeal circulation and bradykinin was infused to give different contact times with the blood before reaching the

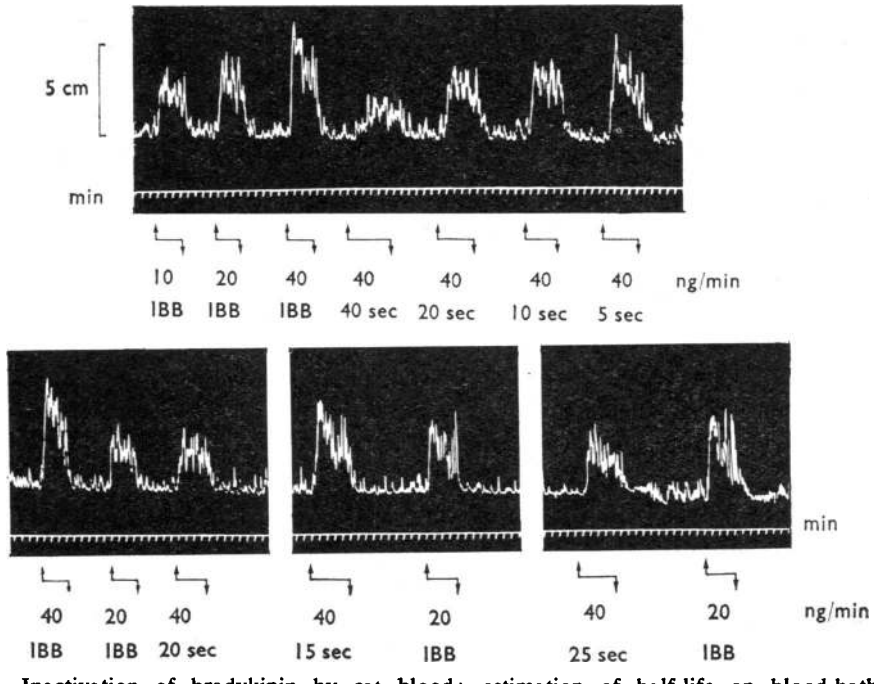


Fig. 1. Inactivation of bradykinin by cat blood: estimation of half-life on blood-bathed cat jejunum. Upper panel: Infusion of 10, 20 and 40 ng/min into the bathing blood close to the assay muscles (IBB). Bradykinin (40 ng/min) was exposed to blood in the incubating circuit, for an extra 40, 20, 10 and 5 sec. The response to 40 ng/min incubated for 20 sec almost matched that induced by 20 ng/min given IBB. Lower panel: It was confirmed that 40 ng/min exposed for 20 sec in the incubating circuit gave a response which matched that elicited by 20 ng/min IBB. The responses of 40 ng/min incubated 15 and 25 sec gave responses greater and smaller respectively than that to 20 ng/min infused IBB. Time in min: vertical scale 5 cm.

assay organs. One of these experiments is illustrated in Fig. 1. First (upper panel) bradykinin was infused into the bathing blood (I.B.B.) at 10, 20 and 40 ng/min. Since the rate of blood flow was 15 ml./min this gave concentrations of 0.67, 1.4 and 2.8 ng bradykinin/ml. These concentrations gave graded contractions of the cat jejunum. When bradykinin (40 ng/min) was infused into the incubating circuit to increase the exposure time by 40 sec there was a much smaller contraction of the cat jejunum. As the exposure time was reduced the contraction of the cat jejunum increased, showing that more and more bradykinin was preserved. Approximately half the bradykinin infusion at 40 ng/min was being destroyed by incubation with blood for 20 sec. This was confirmed in the lower panel. Less bradykinin was destroyed when the incubation was for 15 sec and more bradykinin was destroyed when the incubation was for 25 sec. Thus the half life of bradykinin in the circulating blood of this cat was 20 sec. In four

TABLE 1

DISAPPEARANCE OF BRADYKININ AND ELEDOISIN IN SOME VASCULAR BEDS

Columns A and B represent the infusion rates (μg/min/cat) into the blood supplying or returning from a particular vascular bed, in order to produce matched contractions of the blood-bathed assay preparations. They therefore represent the infusion rates to give similar arterial blood concentrations. Two head experiments, 6 and 15, were made with the first type of arterial infusion; the remaining were performed with the second kind of infusion (see Methods for details)

| Vascular bed | Bradykinin | | | | | | Eledoisin | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cat No. | Wt. | A | B | Ratio A/B | % Disappearance | A | B | Ratio A/B | % Disappearance |
| Lungs | 1 | 2·3 | 4·0 | 1·0 | 4·0 | 75 | — | — | — | — |
| | 2 | 3·6 | 4·0 | 1·0 | 4·0 | 75 | 0·6 | 0·6 | 1·0 | 0 |
| | 3 | 3·0 | 9·0 | 2·0 | 4·5 | 78 | 0·4 | 0·35 | 1·1 | 13 |
| | 4 | 2·5 | 4·0 | 0·7 | 5·7 | 83 | — | — | — | — |
| | | Average | | | 4·5 | 78 | | | 1·05 | 7 |
| Body below diaphragm | 5 | 2·0 | 8·0 | 3·0 | 2·7 | 62 | 0·5 | 0·25 | 2·0 | 50 |
| | 6 | 2·7 | 8·0 | 4·0 | 2·0 | 50 | 1·0 | 0·5 | 2·0 | 50 |
| | | Average | | | 2·3 | 56 | | | 2·0 | 50 |
| Hind quarters | 7 | 2·0 | 4·0 | 2·0 | 2·0 | 50 | 0·2 | 0·13 | 1·5 | 35 |
| | 8 | 1·9 | 3·0 | 2·0 | 1·5 | 34 | — | — | — | — |
| | 9 | 2·6 | 6·0 | 4·0 | 1·5 | 34 | 0·5 | 0·35 | 1·4 | 30 |
| | 10 | 2·5 | 2·0 | 1·4 | 1·4 | 30 | 1·0 | 0·75 | 1·3 | 25 |
| | | Average | | | 1·6 | 37 | | | 1·4 | 30 |
| Liver | 11 | 2·0 | 2·0 | 1·0 | 2·0 | 50 | — | — | — | — |
| | 12 | 2·4 | — | — | — | — | 0·8 | 0·7 | 1·1 | 15 |
| Kidneys | 13 | 2·3 | 8·0 | 2·0 | 4·0 | 75 | 1·6 | 0·8 | 2·0 | 50 |
| | 14 | 3·0 | 8·0 | 2·5 | 3·2 | 69 | 0·6 | 0·3 | 2·0 | 50 |
| | | Average | | | 3·6 | 72 | | | 2·0 | 50 |
| Head | 6 | 2·7 | 4·0 | 2·0 | 2·0 | 50 | 0·5 | 0·4 | 1·2 | 20 |
| | 15 | 2·0 | 3·0 | 0·75 | 4·0 | 75 | 0·25 | 0·2 | 1·2 | 20 |
| | 16 | 1·8 | — | — | — | — | 0·8 | 0·6 | 1·3 | 25 |
| | 17 | 2·7 | 6·0 | 2·0 | 3·0 | 67 | 0·4 | 0·3 | 1·3 | 25 |
| | 18 | 3·0 | 6·0 | 2·0 | 3·0 | 67 | — | — | — | — |
| | | Average | | | 3·0 | 65 | | | 1·25 | 23 |

other similar experiments the half life was 10, 15, 15 and 25 sec, giving a mean value and standard error of $17 \pm 2.5$ sec. In one cat, the determination of half life was also made in venous blood: no difference from the half life in arterial blood could be detected. In one dog, a half life of 15 sec was obtained; this agreed well with value of 16 sec found by McCarthy *et al.* (1965). During all these experiments, concentrations of bradykinin varying from 0.5 to 6 ng/ml. were used. There was no indication that the higher concentrations saturated the inactivation system.

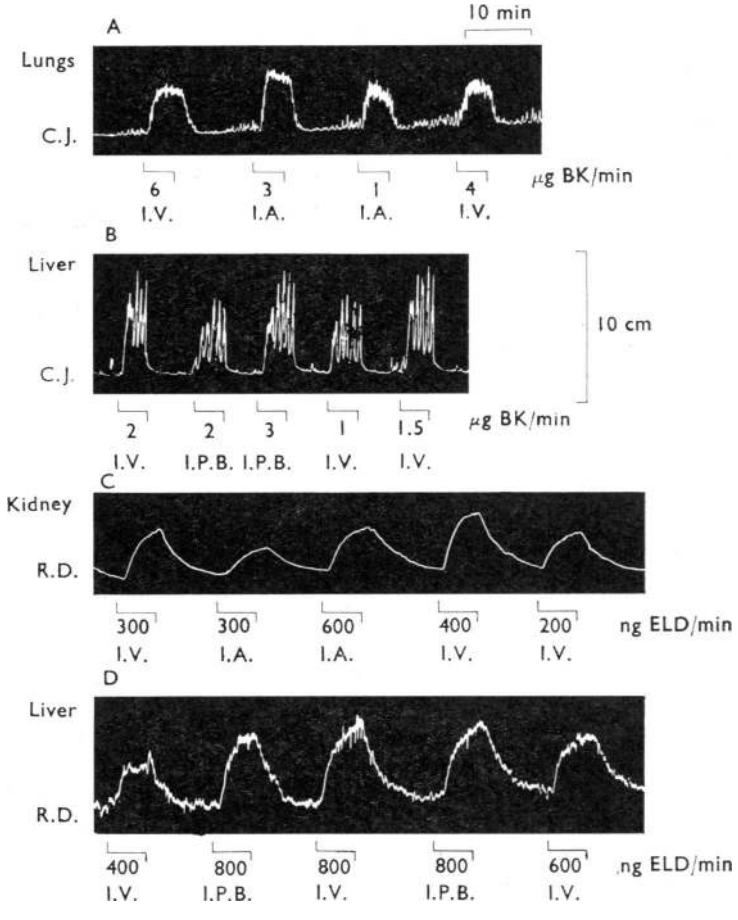


Fig. 2. Disappearance of bradykinin and eledoisin in some vascular beds. Each tracing is from a different experiment. In A and B the assay organ was a cat jejunum (CJ); in C and D, a rat duodenum (RD). Time scale 10 min, vertical scale 10 cm. (*A*) *Lungs* (*Expt. No. 2*) bradykinin infused I.V. (=right atrium) and intra-arterially (IA=ascending aorta). The response to an infusion of 1 μg/min IA matched 4 μg/min I.V. (*B*) *Liver* (*Expt. No. 11*) bradykinin infused I.V. (=inferior vena cava) and into the portal blood (IPB). The infusions of 1 and 1.5 μg/min I.V. matched, respectively, 2 and 3 μg/min IPB. (*C*) *Kidneys* (*Expt. No. 14*) eledoisin infused I.V. (=inferior vena cava) and intra-arterially (IA=kidney arteries). 600 ng/min IA gave a response similar to 300 ng/min IV. (*D*) *Liver* (*Expt. No. 12*) eledoisin infused I.V. (=inferior vena cava) and into the portal blood (IPB); 800 ng/min IPB matched 700 ng/min I.V.

In three experiments with eledoisin a similar technique was applied, except that eledoisin was infused to give an increase in exposure time of 1 min. No destruction of eledoisin in blood could be detected.

Table 1 shows the disappearance of bradykinin and eledoisin during passage through various vascular beds. For bradykinin, the greatest loss was in the lungs (a ratio (R) of 4.5 or a disappearance of 78%) and this was strikingly different from the removal of eledoisin (R = 1.1). More bradykinin (R = 3) also disappeared in the circulation to the head than did eledosin (R = 1.2). For both peptides, about half of the infusion into the body below the diaphragm disappeared (R = 2.3 and 2.0), and less than half from the infusion into the hind quarters (R = 1.6 and 1.4). Both liver and kidneys removed bradykinin to a greater extent than eledoisin. In the liver, 50% of the bradykinin infusion was lost (R = 2.0), but only 15% of the eledoisin (R = 1.1). In the kidneys 72% of the bradykinin was lost (R = 3.6), but only 50% of the eledoisin (R = 2.0).

Some of the experiments from which these figures were obtained are illustrated in Fig. 2. Each tracing is from a different experiment. Figure 2A shows that an intravenous infusion of bradykinin (4 $\mu$g/min) resulted in equivalent femoral arterial blood concentrations to an infusion into the ascending aorta of 1 $\mu$g/min, giving a ratio of 4.0. From Fig. 2B the ratio for bradykinin removal by the liver was found to be 2.0 (50% removal). Figures 2C and 2D show that more eledoisin disappeared in the kidneys (R = 2.0) than in the liver (R = 1.1).

These tracings also illustrate the difference in duration of action of bradykinin and eledoisin when given intravenously. After an infusion of bradykinin into the bathing blood, the cat jejunum returned to baseline within a few seconds, showing that the tissue response time was short. The relaxation after infusions of bradykinin into the cats was also very fast and indistinguishable from that after infusion into the bathing blood. However, after infusion of eledoisin into the cat, the relaxation of the assay organs (see Fig. 2C and 2D) was slow, taking 5–6 min to reach the original baseline. This contrasted with the recovery time of the tissues (2–3 min) after a direct infusion into the bathing blood.

## DISCUSSION

Our estimation of the half-life of bradykinin in cat's blood to be 17 sec agrees well with that of McCarthy *et al.* (1965) of 16 sec for dog's blood using a different technique. If there were no other means of inactivation for bradykinin, it would, therefore, be reduced to about half its concentration in one circulation time. Despite this rapid disappearance, the removal of bradykinin from the circulating blood cannot be explained by its inactivation in blood alone (Trautschold, Fritz & Werle, 1966). Measurements of the inactivation rates for bradykinin in tissue homogenates (Frey, Kraut & Werle, 1950; Hamberg & Rocha e Silva, 1954; Fasciolo, 1964; Trautschold *et al.*, 1966), may bear no relationship to the *in vivo* inactivation of bradykinin by the blood-perfused whole tissue. In using the blood-bathed organ technique to measure the disappearance of bradykinin and eledoisin from the circulation in several vascular beds we have tried to overcome this objection. However, in this more physiological situation the bradykinin will be destroyed during its passage through the vascular bed, not only by the tissue enzymes, but also by those in plasma. The regional circulation times through vascular beds vary

from 2 to 10 sec (Spector, 1956). Since the half-life of bradykinin in blood is 17 sec, about 30% of bradykinin will be destroyed in 10 sec ; thus destruction of more than 30% must indicate participation by the tissues in the disappearance of the bradykinin. Taking this factor into consideration the hindquarters did not remove more bradykinin than could be accounted for by disappearance in the blood ; but the kidneys, liver and head did.

The results with eledoison raise some doubts about this interpretation, for, although eledoisin is not destroyed in blood, it disappears to about the same degree as does bradykinin in the hindquarters. Thus, the disappearance of both peptides in the hind legs may be due to tissue inactivation rather than to destruction in blood. If so, 10 sec may well be an over-estimate of the circulation time.

There is a striking contrast between the disappearance of bradykinin and eledoisin in the lungs. Whereas little or no eledoisin is removed by passage through the pulmonary circulation, only 20% of the bradykinin entering is left to reach the general circulation. The bradykinin which disappears from the circulation may be inactivated by enzymes in the interstitial spaces, on the surface of cells, or within the cells. In the kidney, it may be secreted into urine as suggested by Bumpus *et al.* (1964). In this context it is interesting that the kidneys remove more eledoisin than any other part of the body studied. However, Nobili (1965) has shown that kidney homogenates are the most potent in inactivating eledoisin.

We have not measured the half-life of bradykinin and eledoisin in the total circulation of the animal, but some approximation can be obtained by considering the blood flow to different parts of the body. Since 80% of bradykinin is removed by the lungs, at least 90% will be removed in one circulation through the animal, giving a "total half life " of less than one circulation time. The quick return of the blood-bathed cat jejunum to baseline after an intravenous infusion of bradykinin fits in well with this calculation. For eledoisin we might expect a 30–40% disappearance per circulation ; assuming a circulation time of about 15 sec, the half-life of eledoisin would then be less than 30 sec. Taking the response time of the assay organs into account, they should return to baseline within 2–3 min ; however, they took about 5–6 min (see Fig. 2). This difference might indicate that eledoisin was being bound by some constituent of the tissues, without actual destruction and after the infusion of eledoisin stopped, it was being leached into the circulation again, thereby maintaining the blood levels for a longer time than expected.

Several conclusions are possible. First, if bradykinin is liberated from a tissue into the venous circulation, only a small fraction will reach the arterial circulation to bring about general effects. Secondly, by virtue of their position, the lungs are the most important organ for the removal of bradykinin from the circulation. Thirdly, if bradykinin is liberated into the blood-stream in pathological conditions such as the carcinoid or the dumping syndrome (Oates *et al.*, 1964 ; Zeitlin & Smith, 1966) the blood levels of bradykinin are likely to be much higher on the venous side of the circulation than on the arterial side. Thus, to study release of bradykinin either by the blood-bathed organ technique or by any technique which involves measuring the concentration of bradykinin in blood samples, venous and not arterial blood should be taken.

SUMMARY

1. The blood-bathed organ technique has been used to study the disappearance of bradykinin and eledoisin from the circulating blood of cats.

2. The half life of bradykinin in blood was $17 \pm 2.5$ sec. No destruction of eledoisin could be detected within 1 min.

3. Up to 80% of bradykinin infused intravenously disappeared during passage through the pulmonary circulation. Less than 10% eledoisin was lost in the lungs.

4. Some bradykinin disappeared in other vascular beds, especially in the kidneys and the head.

5. Eledoisin was removed from the circulation mainly by the kidneys.

6. Because of the rapid removal of bradykinin by the lungs, venous, and not arterial, blood samples should be used for estimation of its possible release.

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