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The inactivation of bradykinin in the pulmonary circulation of isolated lungs

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Summary

1. The isolated lungs of guinea-pigs, rats and dogs, perfused with Krebs bicarbonate solution via the pulmonary artery, inactivated 95, 98 and 99% respectively of the bradykinin infused through them.

2. This inactivation process was not inhibited by 2-mercaptoethanol (1 mM) or EDTA (500 μM).

3. The inactivation was inhibited by 2:3-dimercaptopropanol (100 μM), *N*-ethylmaleimide (100 μM), and several o fthe bradykinin potentiating peptides from *Bothrops jararaca*, in both natural and synthetic forms.

4. The effect of these compounds, 2-mercaptoethanol, EDTA, 2:3-dimercaptopropanol, *N*-ethylmaleimide and *Bothrops* peptides on the pulmonary bradykininase activity is the same as their effect on pulmonary angiotensin I converting enzyme.

Introduction

Bradykinin is inactivated in blood and in the vascular beds of the systemic and pulmonary circulation. In blood, bradykinin is inactivated enzymically by kininases (Erdös & Sloane, 1962 ; Erdös, Renfrew, Sloane & Wohler, 1963), the half-life being 16–17 s in dog and cat blood (Ferreira & Vane, 1967a). Bradykinin or its metabolites may be taken up by tissues since, after an infusion of tritiated bradykinin large amounts of radioactivity were found in kidney and liver (Bumpus, Smeby, Page & Khairallah, 1964). Tissue homogenates of kidney, spleen, liver, brain and lung readily inactivate bradykinin (Trautschold, Fritz & Werle, 1966 ; Iwata, Shikimi & Oka, 1969).

The pulmonary circulation inactivates as much as 80% of the bradykinin infused intravenously in the anaesthetized cat (Ferreira & Vane, 1967a). This could not be accounted for by inactivation in blood alone. Bradykinin is also removed from the pulmonary circulation in the anaesthetized dog (Biron, 1968) and rat (Stewart & Roblero, 1967), and by perfused lungs from guinea-pigs and dogs (Pojda & Vane, 1971). Since cell-free extracts of dog lung readily inactivated bradykinin (Bakhle, 1968) it was considered likely that inactivation of bradykinin in the pulmonary circulation was due to enzymic degradation. During our experiments, Ryan, Roblero & Stewart (1968, 1969) established the enzymic nature of the removal process for bradykinin in isolated lungs.

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We have studied the inactivation of bradykinin in the pulmonary circulation of isolated lungs perfused with Krebs bicarbonate solution, in order to characterize the enzyme(s) responsible for this inactivation.

Methods

Isolated lungs

Isolated lungs perfused with oxygenated Krebs bicarbonate solution at 37° C, were prepared as described previously (Bakhle, Reynard & Vane, 1969). The perfusion rate was maintained at 8 ml/min for rat and guinea-pig lungs and at 10 ml/min for lobes of dog lung. The effluent from the lungs superfused strips of cat jejunum (Ferreira & Vane, 1967b), or terminal ileum or kitten terminal ileum. For these last two assay tissues, the distal 20 cm of the terminal ileum was removed from the animal, opened longitudinally and the contents washed out with Krebs solution. Strips of longitudinal muscle 3–5 cm long and 3–6 mm wide were cut and the mucosa trimmed off. The muscle from kittens shows less spontaneous activity than that of adult cats.

The three tissues contract in response to bradykinin concentrations of 0·1–4 ng/ml and to injections of 2–4 ng bradykinin. The isolated chick rectum (Mann & West, 1950) was used to detect prostaglandins in the lung effluent since it does not respond to these concentrations of bradykinin. All assay tissues received infusions (0·1 ml/min) of the combined antagonists methysergide, 0·56 μM, mepyramine 0·35 μM, hyoscine 0·33 μM, phenoxybenzamine 0·33 μM, propranolol 7·5 μM. Contractions were recorded with auxotonic levers attached to Harvard isotonic transducers, and displayed on six-channel Watanabe recorders (type WTR 281). Mean pressure in the cannula in the pulmonary artery was detected by a SE Laboratories pressure transducer attached to a side arm of the cannula, and displayed on the Watanabe recorder.

The inactivation of bradykinin was calculated by comparing the contractions of the assay tissues in response to infusions (or injections) of bradykinin given directly, with those in response to bradykinin infused into the cannula in the pulmonary artery. Thus, if an infusion of 40 ng bradykinin/ml through the lung caused a contraction comparable in height to that caused by 2 ng/ml infused directly, the inactivation of bradykinin would be calculated as $40-2/40 \times 100 = 95\%$.

The drugs used were: bradykinin (a gift from Dr. H. O. J. Collier), prostaglandin E_2 (a gift from Dr. J. Pike, Upjohn), 2-mercaptoethanol, *N*-ethylmaleimide (Sigma); 2:3-dimercaptopropanol, disodium salt of ethylene diamine tetra acetic acid (EDTA, Analar, BDH); methysergide bimaleate (Sandoz); mepyramine hydrochloride maleate (May & Baker); hyoscine hydrobromide (BDH); phenoxybenzamine hydrochloride (Smith, Kline & French); (±)-propranolol hydrochloride (I.C.I.). The peptides from *Bothrops jararaca* venom were a gift from Dr. L. Greene (Brookhaven, N.Y.), and the synthetic peptides a gift from Dr. M. Ondetti (Squibb Institute for Medical Research; designations SQ. 20475, 20881 and 20859 for P5, P9 and P10 respectively). All drugs were dissolved in saline (0·9% NaCl w/v). Doses are given in molar concentrations or as the weight of base per ml and refer to the final concentration of the substance.

TABLE 1. *Inactivation of bradykinin in isolated perfused lungs and its modification by various substances*

Substances infused through lungs	Concentration	% Inactivation of bradykinin (BK) on single passage through isolated lungs of different species (mean ± SEM and number of experiments) Rat	Guinea-pig	Dog
None (control)	(10–100 ng/ml BK)	98·2±0·4 (22)	94·9±0·4 (27)	99·0±0·4 (5)
2-Mercaptoethanol	1 mM	97·9±0·7 (6)	93·2±1·1 (5)	99·3 (3)
2:3-Dimercaptopropanol (BAL)	100 μM	59·2±9·0* (4)	57·4±3·0* (9)	70 (1)
N-Ethylmaleimide (NEM)	100 μM	90·4±1·9* (5)	83·5±3·2* (4)	— —
EDTA	500 μM	—	93·3 (3)	—
BPF fractions V-3-A	1 μg/ml	82·6 (3)	—	—
	0·5 μg/ml	—	75 (2)	—
II-1-A	1 μg/ml	—	73 (2)	—

* Significantly different from control, $P<0·05$
Bradykinin was infused for 3–5 min, at rates of 0·05–0·2 ml/min. The other substances were infused throughout the test periods at 0·1 ml/min. The inactivation was calculated from the heights of the contractions of the assay tissues as described in the text.

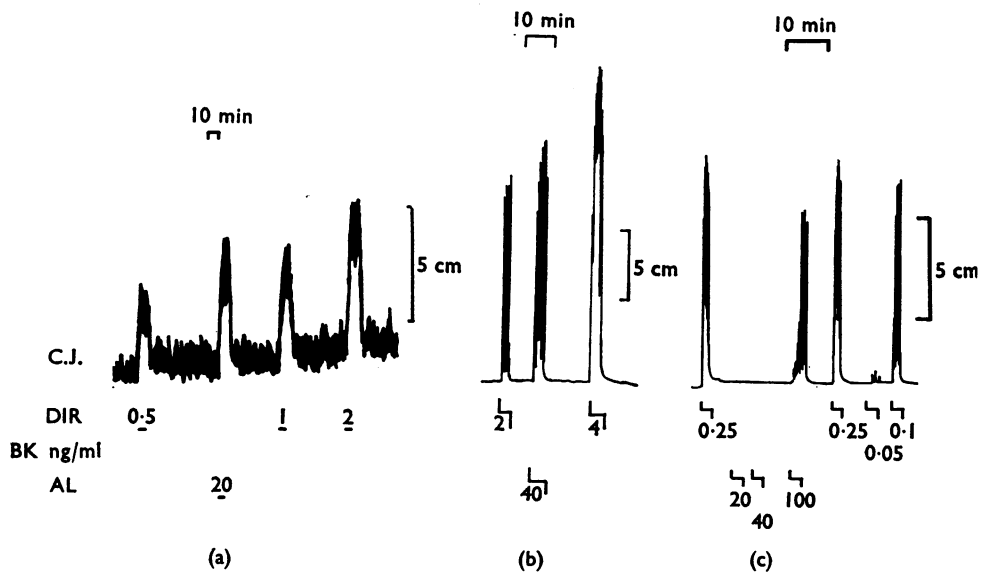

FIG. 1. Inactivation of bradykinin in isolated lungs. The records show contractions of a strip of cat jejunum (CJ) superfused with the effluent from isolated lungs perfused with Krebs solution, to infusions of bradykinin (BK) given directly (DIR) or into the pulmonary artery cannula (AL). In panel (a), isolated guinea-pig lungs were used, and an infusion of BK 20 ng/ml through the lungs is equivalent to between 1 and 2 ng/ml infused directly, corresponding to an inactivation of 93·5%. Similarly in panel (b) another pair of guinea-pig lungs inactivated 94% of an AL infusion of 40 ng BK/ml. In panel (c) an isolated lobe of dog lung inactivated about 99·9% of an infusion of BK (100 ng/ml) given through the lungs.

Results

Removal of bradykinin in isolated lungs of rat, guinea-pig and dog

Isolated lungs of rat, guinea-pig and dog inactivated bradykinin infused into the pulmonary circulation. The perfused lobes of dog lung were the most efficient, removing $99 \pm 0{\cdot}4\%$ ($n=5$) of an infusion of bradykinin while rat lungs removed $98{\cdot}2 \pm 0{\cdot}4\%$ ($n=22$), and guinea-pig lungs removed $94{\cdot}9 \pm 0{\cdot}4\%$ ($n=27$) (Table 1). The weight of blotted lobes of dog lung after perfusion was between 30–60 g, whereas rats' lungs weighed 1·5–2·5 g. However, the difference in weight alone cannot explain the less efficient removal in guinea-pigs' lungs since they weighed 3–4 g. The concentration of bradykinin infused through the lungs was usually 10–40 ng/ml and over this range of concentrations the percentage removed remained constant. In some experiments in dog and rat lungs where the removal was very efficient, 80–100 ng bradykinin/ml had to be infused before a detectable amount of bradykinin appeared in the perfusate. In Fig. 1, the first two sections show the removal of bradykinin in guinea-pig isolated lungs at two concentrations, 20 ng/ml and 40 ng/ml. The proportion of bradykinin removed is 93·5% and 94% respectively. The third section shows the results obtained with a lobe of dog lung when 100 ng bradykinin/ml had to be infused through the lungs in order to detect any bradykinin ($\approx$0·1 ng), in the perfusate. In lungs of rat and guinea-pig, bradykinin in concentrations of 10–40 ng/ml infused into the pulmonary artery produced a small increase (2–7 mmHg) in perfusion pressure in about half the experiments, while infusions of higher concentrations of bradykinin (80–100 ng/ml) consistently produced increases of 4–13 mmHg.

Bradykinin may release active substances from the lung (Piper & Vane, 1969) and the possibility had to be considered that other substances, which could interfere with the bioassay, were present in the lung perfusate. Contractions of the cat jejunum and cat and kitten terminal ileum were used to measure bradykinin (0·1–4 ng/ml), but these tissues also contract to histamine (5–50 ng/ml). Mepyramine was therefore used to increase the specificity of the bradykinin assay. The removal of bradykinin by the lung measured by contractions of a mepyramine-treated tissue, was the same as that measured by contractions of a non-mepyramine-treated tissue. The results indicated that histamine was not released from the lung by infusions of bradykinin. The cat and kitten terminal ileum responded to prostaglandin E_2 (1–2 ng/ml) in some experiments. Since the chick rectum does not contract to bradykinin but does respond to prostaglandins, this tissue was included among the assay tissues. Bradykinin in concentrations of 10–100 ng/ml infused through rat and guinea-pig lungs did not release any prostaglandin-like material as shown by the lack of response of the chick rectum.

In an experiment with rat lungs illustrated in Fig. 2, bradykinin (20 and 40 ng/ml) infused through the lungs did not produce a contraction of the chick rectum which was sensitive to prostaglandin E_2 (1 ng/ml) infused directly to the tissue. In the second half of the experiment when a prolonged contraction of the ileum was caused in response to bradykinin (2 ng/ml) infused through the lungs in the presence of 2:3-dimercaptopropanol (BAL), there was still no response from the chick rectum.

Higher concentrations of bradykinin (200–500 ng/ml) infused through rat lungs or 2–10 μg, given as a single injection, released a substance which contracted the

chick rectum and may have been a prostaglandin-like substance. These higher concentrations of bradykinin were not used to estimate pulmonary removal of bradykinin. Isolated lungs removed the same proportion of an infusion of bradykinin when the same infusion was repeated up to 4 times: thus the effect of drugs on lung inactivation of bradykinin could be investigated over a period of hours.

Effect of drugs on removal of bradykinin in isolated lungs

(i) 2:3-Dimercaptopropanol (BAL). BAL inhibits inactivation of bradykinin by plasma and kidney extracts (Erdös & Yang, 1966). BAL infused at a concentration of 100 μM through the lungs inhibited inactivation of bradykinin in isolated lungs of rat, guinea-pig and dog, reducing the removal of bradykinin to about 60%. The results of these experiments are summarized in Table 1. In some experiments, BAL potentiated the height of contractions of the cat jejunum and cat and kitten terminal ileum to bradykinin infused directly but did not prolong the duration of the responses. However, the duration of the response of the tissues to infusions of bradykinin through the lungs was prolonged two to threefold by BAL. The pulmonary inactivation of bradykinin in the presence of BAL was calculated by measuring the height of contraction of the assay tissues and the duration of the response was not taken into account. However, since bradykinin appeared in the perfusate for a longer time in the presence of BAL, the actual removal of bradykinin was much less than that calculated.

The effect of BAL on removal of bradykinin by rat lungs is shown in Fig. 2. An infusion of 40 ng bradykinin/ml through the lungs was equivalent to 0·8 ng/ml

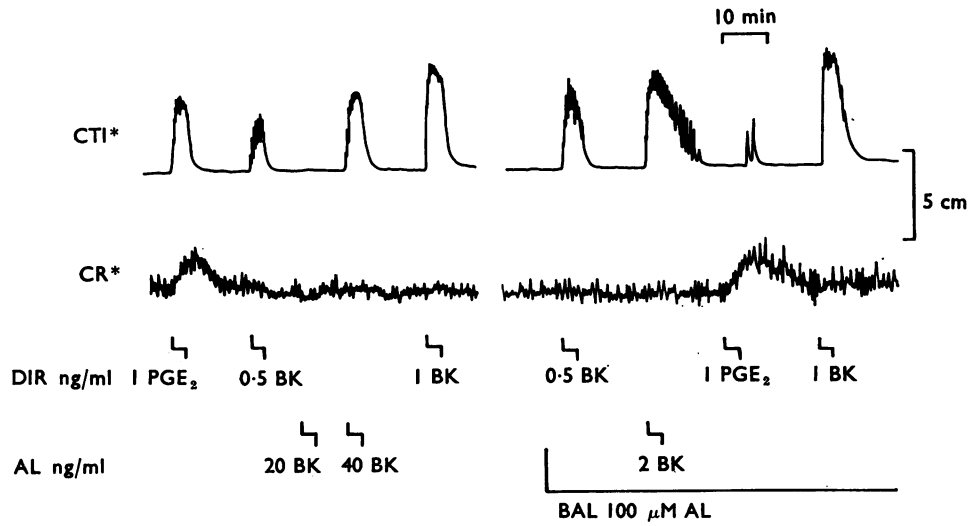

FIG. 2. Effect of 2:3-dimercaptopropanol (BAL) on removal of bradykinin in rat isolated lung. The record shows contractions of a cat terminal ileum (CTI) and a chick rectum (CR) both treated with combined antagonists, superfused in series with the effluent from a rat lung perfused with Krebs solution. Bradykinin (BK) was infused either directly to the assay tissues (DIR) or into the pulmonary artery cannula (AL). In the untreated lung, removal of BK was 98%. However, BAL infused through the same lung as indicated, reduced lung removal to 62% as measured by the height of contraction of the cat ileum, but this response was potentiated in duration. Infusions of BK through the lung did not release prostaglandins as shown by the lack of contraction of the chick rectum. The chick rectum and cat ileum responded to prostaglandin E_2 (PGE_2) 1 ng/ml infused directly. Scales, 10 min, 5 cm.

infused directly to the cat terminal ileum representing a 98% removal, but in the presence of BAL (100 μM), 2 ng bradykinin/ml through the lungs was equivalent to about 0·76 ng/ml representing 62% removal. Thus, if we had given the 40 ng/ml infusion in the presence of BAL, the concentration of bradykinin in the effluent from the lung would have risen from 0·8 ng/ml to about 15 ng/ml, an approximately 20-fold increase. The total amount of bradykinin in the effluent after BAL is probably higher than this since the contraction of the assay tissue was considerably prolonged, suggesting a delay in the washout of bradykinin from the pulmonary vessels. The inhibitory effect of BAL was readily reversible and 5 min after stopping the BAL infusion, the removal of bradykinin by the lungs had almost returned to control levels.

(ii) 2-Mercaptoethanol (2-ME). 2-ME potentiates the hypotensive effect of bradykinin in the rat (Erdös & Yang, 1966) and inhibits carboxypeptidase N of plasma (Trautschold *et al.*, 1966; Erdös & Wohler, 1963) but does not affect kininases of erythrocytes (Trautschold *et al.*, 1966).

2-ME infused through the pulmonary artery cannula in concentrations of up to 1 mM, had no significant effect on removal of bradykinin by lungs of rat, guinea-pig and dog (see Table 1). These concentrations of 2-ME did not affect perfusion pressure in the lungs or affect responses of the assay tissues to bradykinin.

(iii) EDTA. EDTA inhibits carboxypeptidase *N* of plasma (Erdös *et al.*, 1963), kininase II of plasma (Erdös & Yang, 1967) and the endopeptidase of kidney (Erdös & Yang, 1966). EDTA (500 μM) had no effect on removal of bradykinin in isolated lungs of the guinea-pig. The results are given in Table 1.

(iv) N-Ethylmaleimide (NEM). NEM inhibits the kininase of erythrocytes (Erdös & Yang, 1966) and rat brain kininase, but it has no effect on kininase of rat plasma (Iwata *et al.*, 1969).

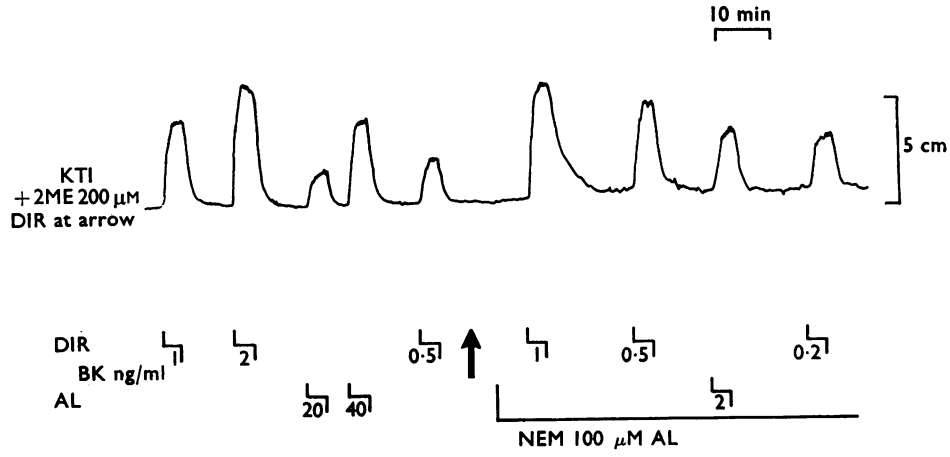

FIG. 3. Effect of *N*-ethylmaleimide on removal of bradykinin in rat isolated lung. The record shows contractions of a kitten terminal ileum (KTI) superfused with effluent from rat lungs perfused with Krebs solution to infusions of bradykinin (BK) given directly (DIR) or through the lungs (AL). *N*-Ethylmaleimide (NEM) was infused through the lung as indicated, and 2-mercaptoethanol (2-ME) was infused over the assay tissues as shown at the arrow to remove excess NEM from lung perfusate. NEM reduced removal of bradykinin by the lung from 97·6% to 86·5%. Scales 10 min, 5 cm.

Infusions of NEM (100 μM) through the lungs produced contractions of the cat jejunum and cat terminal ileum which made bioassay of bradykinin impossible. However, 2-ME (in twofold molar excess) infused directly over the tissues prevented this action of NEM. We do not know if the protective action of 2-ME in this situation was due to reaction with unchanged NEM in the lung effluent (our reason for using it) or to some other reaction. Responses of the assay tissues to bradykinin were slightly potentiated by the combination of NEM infused through the lungs and 2-ME infused directly over the tissues.

Removal of bradykinin in isolated lungs was reduced by NEM (100 μM) from 98% to 90% in the rat and from 95% to 83% in the guinea-pig. The results are given in Table 1. Figure 3 shows the effect of NEM in a preparation of rat isolated lungs. An infusion of 40 ng bradykinin/ml through the lungs was equivalent to 0·96 ng bradykinin/ml given directly to the kitten terminal ileum representing a 97·6% removal. In the presence of NEM, 2 ng bradykinin/ml through the lungs was equivalent to 0·27 ng bradykinin/ml representing an 86·5% removal.

Bradykinin potentiating factor (BPF)

BPF is a peptide component of the venom of the snake *Bothrops jararaca*, and it potentiates some of the pharmacological actions of bradykinin *in vivo* and *in vitro* (Ferreira, 1965 ; Ferreira & Rocha e Silva, 1965). This potentiation has been attributed to inhibition of kininases since BPF partially blocks the inactivation of bradykinin by plasma (Ferreira & Rocha e Silva, 1965 ; Ferreira, 1966), by circulating blood (Ferreira & Vane, 1967a) and by cell-free extract of dog lung (Bakhle, 1968). BPF has been separated into its constituent peptides (Ferreira, Bartelt & Greene, 1970) and two of these peptides known to potentiate bradykinin on the guinea-pig ileum were tested in the perfused lung preparation. Fractions II-1-A and V-3-A at concentrations of 0·5 and 1 μg/ml reduced inactivation of bradykinin to 74% in guinea-pig lungs and to 82% in rat lungs. The results are given in Table 1.

The inhibitory effect of these BPF fractions on bradykinin inactivation was readily reversible. Responses of the cat jejunum and cat terminal ileum to bradykinin were not potentiated by the BPF fractions. An experiment showing the effect of the fraction V-3-A on bradykinin removal in guinea-pig lungs is illustrated by Fig. 4. An infusion of 20 ng bradykinin/ml through the lungs was equivalent to between 0·5 and 1 ng bradykinin/ml infused directly to the cat jejunum, but during an infusion of the BPF peptide, 4 ng bradykinin/ml through the lungs was equivalent to about 1 ng bradykinin/ml infused directly, while 20 ng bradykinin/ml through the lungs produced a response of the tissues out of the calibration range.

Recently, the structure of several of the peptides has been elucidated and their synthesis accomplished (Greene, Stewart & Ferreira, 1970 ; Ondetti, Williams, Sabo, Pluščec, Weaver & Kocy, 1970). The effect of three synthetic peptides, a pentapeptide, Pca-Lys-Trp-Ala-Pro (P5) ; a nonapeptide, Pca-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro (P9) ; and a decapeptide, Pca-Ser-Trp-Pro-Gly-Pro-Asn-Ile-Pro-Pro (P10) on the pulmonary inactivation of bradykinin was investigated in guinea-pig isolated lungs. Since the amounts of synthetic peptide were limited, the time over which they were infused had to be minimized. Bradykinin was given by injection (0·05–0·2 ml) into the stream of Krebs solution and not by infusions of

3–4 min duration. This modification enabled doses to be given every 5 min instead of every 10–12 minutes. The inactivation in guinea-pig lungs of bradykinin given by injection was 93·2±0·3% (n=35), under normal conditions.

The results of these experiments are summarized in Table 2. The nonapeptide P9 is the most potent inhibitor of bradykinin inactivation with pentapeptide (P5)

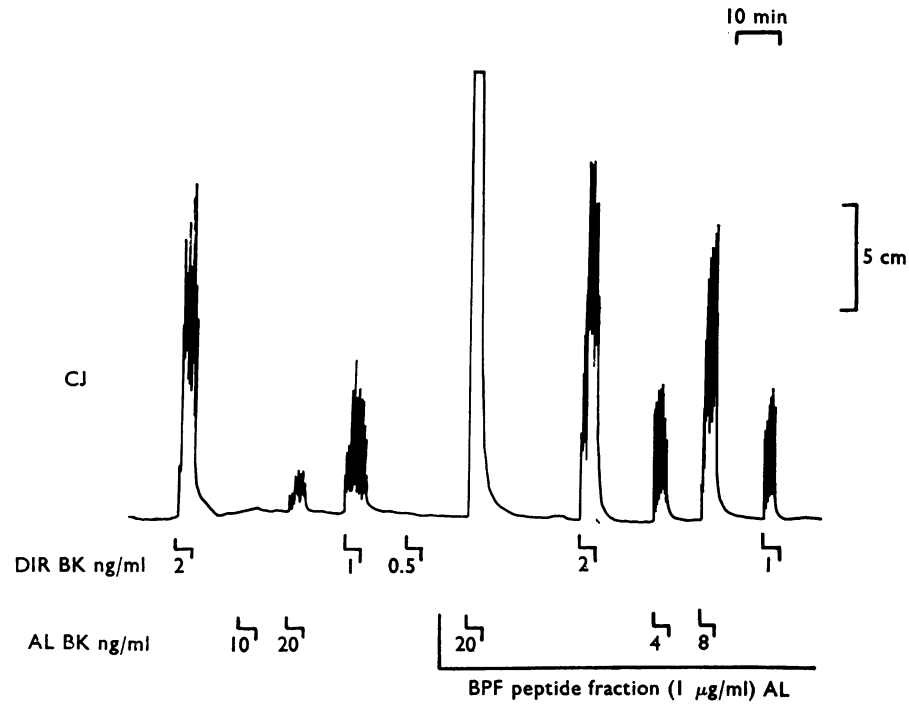

FIG. 4. Effect of bradykinin potentiating factor (BPF) on removal of bradykinin in guinea-pig isolated lungs. The record shows contractions of a cat jejunum (CJ) superfused with effluent from guinea-pig isolated lungs perfused with Krebs solution to infusions of bradykinin (BK) given either directly (DIR) or into the pulmonary artery cannula (AL). The BPF peptide fraction V-3-A infused through the lungs as indicated, reduced removal of bradykinin by the lung from approximately 96% to 75%. Scales 10 min, 5 cm.

TABLE 2. *Inhibition of bradykinin inactivation in guinea-pig isolated lungs, by synthetic peptides from bradykinin potentiating factor (BPF)*

Peptide	% Control inactivation—% inactivation during infusion of peptide through lung (no. of experiments) Concentration (μg/ml)			
	0·01	0·05	0·1	0·5
P5 (M.W.=612)	—	—	8·8±3·5† (n=4)	24·6±2·6* (n=7)
P9 (M.W.=1100)	12·4±1·8* (n=13)	28·6±3·7* (n=6)	—	—
P10 (M.W.=1075)			2·8±1·2 (n=6)	12·9±3·8† (n=3)

*P<0·01 †0·1>P>0·05 Using 't' test for paired samples

In this series of experiments, bradykinin was given by injection (2–10 ng, directly; 50–200 ng into the pulmonary artery cannula). The BPF peptides were infused (0·1 ml/min) through the lung. The inactivation of bradykinin given by injection was 93·2±0·3% (n=35) under control conditions.

being about tenfold less potent. The decapeptide (P10) is the least potent inhibitor. None of these peptides potentiated the responses of the assay tissues to bradykinin given directly.

Discussion

Isolated lungs of the dog, rat and guinea-pig perfused via the pulmonary circulation removed 92–99% of an infusion of bradykinin with the rat lung being most efficient on the basis of percent removal per gramme of tissue. A disadvantage of the method of bioassay used is that biologically active materials may be liberated from the lung during an infusion of the substance under study. Bradykinin, for example, can release a rabbit aorta contracting substance (RCS) from guinea-pig lungs although the concentration required was 1–2 μg/ml (Piper & Vane, 1969). Contractions of the cat jejunum and terminal ileum to bradykinin cannot be selectively antagonized but if tissues which were not sensitive to bradykinin were used, for example, the chick rectum, there was no evidence for the release of active material by bradykinin infused at concentrations of 20–100 ng/ml. However, higher concentrations of bradykinin (500 ng/ml) released a substance from rats' lungs which contracted the chick rectum, but concentrations in this range were not used to assay pulmonary inactivation.

Ryan *et al.* (1968, 1969) showed that bradykinin was inactivated in the pulmonary circulation of rats by enzymic hydrolysis of peptide bonds. Radioactive bradykinin was infused through blood-free lungs *in situ* and the radioactive compounds in the perfusate were identified as peptide fragments of bradykinin. There was no retention of radioactivity by the lung. The circulation time was identical with that of blue dextran (av. M.W.$=2\times10^6$) indicating that bradykinin only equilibrates with the extracellular fluid on passing through the lung. Since the effluent of blood-free lungs did not contain sufficient hydrolase activity to account for this rapid and efficient degradation of bradykinin, they concluded that the enzymes responsible are bound in lung tissue, in or near the vascular endothelium.

Our measurements of biological activity are compatible with such a scheme, as the peptide fragments of bradykinin do not contract intestinal smooth muscle (Greenbaum & Yamafuji, 1966; Suzuki, Abiko, Endo, Kameyama, Sasaki & Nabeshima, 1969). Thus hydrolysis of a single peptide bond would lead to inactivation of bradykinin, and any bradykinin-like contractor activity in the lung effluent is due to unchanged bradykinin and not to a mixture of less active fragments and unchanged bradykinin. Our assay is particularly suited to detecting small amounts of bradykinin against a high background of inactive fragments, whereas in the radioactive assay, activity would be more evenly spread over all the peptides.

We have expressed our results in terms of the inactivation of bradykinin in the lung, and it may seem that to reduce inactivation from 98% to 95% is not particularly significant physiologically. However, if we consider the proportion of bradykinin appearing in the effluent, this has increased from 2% to 5% or 2·5-fold, and this is the magnitude of the change in the concentration of bradykinin entering the arterial side of the circulation.

The effects of the chelating agents EDTA and BAL on the inactivation of bradykinin are similar to their effects on angiotensin I converting enzyme (Bakhle *et al.*, 1969; Bakhle & Reynard, 1971) in the perfused lung preparation. We

consider that BAL inhibited the inactivation by virtue of its chelating ability and not merely as a sulphydryl compound, since 2-ME was not an inhibitor of the pulmonary inactivation process. However, 2-ME does inhibit some pulmonary bradykininases as Ryan *et al.* (1968, 1969) showed that the number and nature of the peptides derived from radioactive bradykinin perfused through the lung were changed after treatment with 2-ME. Instead of the fragments derived from four cleavage sites between: Arg^1-Pro^2; Pro^3-Gly^4; Gly^4-Phe^5 and Pro^7-Phe^8 under control conditions, only two fragments from a cleavage between Ser^6-Pro^7 could be detected after 2-ME treatment. This treatment did not, however, increase the amount of *unchanged* bradykinin in the effluent and, although Ryan *et al.* did not draw this conclusion, it is clear that 2-ME would not affect the overall biological inactivation process for bradykinin in the pulmonary circulation. However, Stewart & Roblero (1967) and Scholz & Biron (1969) measuring pulmonary inactivation of bradykinin by the systemic depressor response technique, found that 2-ME did potentiate the responses to bradykinin and concluded that the pulmonary inactivation of bradykinin was inhibited by this sulphydryl compound. The latter authors also contrasted the effectiveness of 2-ME against pulmonary bradykininases with its lack of effect against pulmonary angiotensin converting enzyme and stated that this difference was a point of divergence between these two pulmonary peptidase activities. In view of the results of Ryan *et al.* and those presented here, it is clear that potentiation of the effects of bradykinin *in vivo* by 2-ME must be due to inhibition of bradykininases other than those in the pulmonary circulation, and perhaps due to those in blood, which are inhibited by 2-ME (Trautschold *et al.*, 1966). Furthermore, there is no divergence between the pulmonary bradykininases and converting enzyme in their susceptibility to inhibition by 2-ME.

A further point of resemblance between these two pulmonary peptidase activities is their inhibition by peptides from ' bradykinin potentiating factor' (BPF, Ferreira, 1965) from the venom of *Bothrops jararaca*. The relative potency of these peptides as inhibitors of bradykinin inactivation is the same as that of inhibitors of the conversion of angiotensin I to angiotensin II (Bakhle, 1971) with the nonapeptide being the most potent and the decapeptide the least potent. Although we were not able to study the effects of these peptides on bradykinin inactivation as extensively as their effects on converting enzyme because of the small amounts of peptides available, it is clear that the nonapeptide and the pentapeptide have equivalent effects (inactivation reduced to 64–68%) at concentrations of 0·05 μg/ml and 0·5 μg/ml respectively. This ten-fold potency ratio may be compared with the twenty-fold potency ratio between these peptides as inhibitors of pulmonary converting enzyme (Bakhle, 1971). Using the systemic depressor response in rats, Stewart, Ferreira & Greene (1971) showed that the pentapeptide (referred to as BPP5a in their paper) would, when given as an infusion of 250 μg/min, reduce pulmonary inactivation of bradykinin from 98% to 30%.

From our study of the inactivation of bradykinin in the pulmonary circulation of isolated lungs, we must conclude that the converting enzyme activity and the bradykininase activity are closely related and there is no clear point of difference between them. However, because of the multiplicity of enzymes capable of attacking bradykinin in the pulmonary circulation we cannot be sure that the same enzyme is responsible for the first cleavage in the bradykinin molecule (which would be the rate determining step in the inactivation process) under all our experi-

mental conditions. By measuring only biological activity we lose the ability to differentiate between these enzymic activities but we are able to follow the physiologically important event of inactivation. It remains to be established whether the carboxydipeptidase activity from swine lung (Yang, Erdös & Levin, 1971) which has both converting enzyme and bradykininase activities, is the enzyme responsible for the primary step of bradykinin inactivation in the lung.

The pulmonary circulation inactivates 90–95% of the bradykinin passing through it by enzymic attack without any evidence of a preliminary uptake step, and this process may be contrasted with the combined uptake and enzymic oxidation process that inactivates a comparably high proportion of 5-hydroxytryptamine passing through the pulmonary circulation (Alabaster & Bakhle, 1970; Gruby, Rowlands, Varley & Wyllie, 1971). The ability of the pulmonary circulation to inactivate two such dissimilar substances emphasizes the multiple metabolic capabilities of this vascular bed and its potential in determining the pharmacodynamics of such substances.

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