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SENSITIZATION OF GUINEA-PIG ILEUM TO THE ACTION OF BRADYKININ BY TRYPSIN HYDROLYSATE OF OX AND RABBIT PLASMA

BY

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Rocha e Silva, Beraldo & Rosenfeld (1949) found that bradykinin is released from ox plasma proteins by the proteolytic action of trypsin. Hamberg, Merlin Bumpus & Page (1961) and Elliott, Horton & Lewis (1961) used this method for the preparation of natural bradykinin. A micromethod for the determination of bradykinogen in plasma, described by Diniz & Carvalho (1963), is also based on the release of bradykinin by trypsin.

In an attempt to prepare relatively pure bradykinin from ox plasma, a tryptic digest was chromatographed on Amberlite CG-50. It was found that a number of the collected fractions, which in themselves showed no bradykinin activity, enhanced the effect of subsequently added synthetic bradykinin on the guinea-pig ileum. In the present paper an investigation of this sensitizing effect of ox and rabbit plasma treated with trypsin is described.

METHODS

Treatment of ox and rabbit plasma with trypsin

The procedure described by Diniz & Carvalho (1963) for the bradykinogen determination in plasma was used with minor modifications. In a vessel containing heparin 500 i.u., 250 ml. of ox blood was collected. Rabbit blood, withdrawn from the carotid artery of an animal anaesthetized with diethylether, was collected in portions of 50 ml. containing heparin 100 i.u. As soon as possible after collection the blood was centrifuged at 3,000 rev/min for 30 min. The plasma was centrifuged again, poured into nine volumes of 0.2% acetic acid and heated in a boiling water bath for 30 min. The mixture was cooled to room temperature and the pH adjusted to 7.8 by the addition of sodium hydroxide and buffered by the addition of 0.25 volume of 0.2 M Tris-(hydroxymethyl-) aminomethane. Trypsin (1 mg) was added for each ml. of plasma used and the mixture was incubated for 30 min at 37° C. The hydrolysis was stopped by pouring the fluid into twice its volume of boiling 96% ethanol which was then kept for 10 min at 70° C. All the tubes and vessels used during this preparation were made of polythene. The volume of the trypsin hydrolysate was reduced to two to four times the original volume of plasma in a rotary vacuum evaporator at 35° C. A part of the hydrolysate, referred to as stage 1, was assayed on the isolated guinea-pig ileum. Before testing, it was usually diluted with saline to ten or twenty times the original volume of plasma. The rest of the concentrated hydrolysate was adjusted to pH 5 and filtered through a No. 588 Schleicher and Schüll filter. This filtrate is referred to as stage 2.

Ion exchange chromatography

Stage 2 was applied to a column (38 or 13 × 1 cm) filled with Amberlite CG-50 (100–200 mesh), equilibrated with 0.05 M ammonium acetate buffer (pH 5). The column had previously been washed with 0.05 M ammonium acetate buffer (pH 5) and eluted with 0.2 M ammonium acetate buffer (pH 9) until the pH of the eluate was the same as that of the elution fluid. The ox plasma preparations were collected in fractions of 10 ml. The hydrolysate of rabbit plasma was collected at regular intervals. Because the flow rate did not remain constant, the fraction size decreased from 10 ml. to about 2 ml. The extinction at 260 mμ and the pH of each fraction was determined.

Estimations of bradykinin and bradykinin-sensitizing activity

Estimations were performed on the isolated guinea-pig ileum preparation. A terminal piece of ileum was suspended in a 10 ml. organ bath with Krebs-Ringer solution at 34° C and gassed with 5% carbon dioxide and oxygen unless stated otherwise. In preliminary experiments neither acetylcholine-like nor histamine-like activity could be determined in the trypsin hydrolysates so no antagonists were added to the bath fluid in most experiments.

The bradykinin activity of the hydrolysates and the fractions from the Amberlite column was estimated by comparing them with synthetic bradykinin by a 2 × 2 assay as described by Schild (1942). The preparations were left in contact with the ileum for 1 min.

The bradykinin-sensitizing activity of the hydrolysates and their fractions from the Amberlite column was demonstrated by determining, on the same piece of ileum, the log dose-response relationship of synthetic bradykinin alone and in the presence of a constant dose of the preparation under test. With the crude hydrolysates only a relatively low dose could be used because higher doses by themselves produced a contraction of the ileum.

In order to test quantitatively the bradykinin-sensitizing activity of the fractions from the Amberlite column, the log dose-response relationship for bradykinin was first determined. A dose producing one eighth of the maximal effect was chosen as a test dose (in most experiments this was 200 ng bradykinin) and 0.2 ml. of each fraction under test was added to the bath 30 sec before this test dose, which was then left in contact with the tissue for 1 min. At regular intervals during the experiment the response to the test dose alone was determined. When this response had changed considerably and did not return to its original height after repeated additions of the test dose, a new piece of ileum was used. The increased responses to the test dose of bradykinin in the presence of the sensitizing fractions are expressed in terms of doses of bradykinin, which by themselves would produce a contraction of similar height to the contractions observed after addition of the test dose in the presence of sensitizer. These doses of bradykinin corresponding to the increased effect were read from a log dose-effect curve for bradykinin determined separately on the same piece of ileum. The sensitizing activity was expressed as the ratio: dose of bradykinin (ng) corresponding to the increased effect divided by the test dose of bradykinin (usually 200 ng).

Bradykinin and the other biologically active polypeptides were added at intervals of 4 min, whereas the time cycle for the other smooth muscle contracting substances was 2 min.

Materials

The following substances were used: trypsin (twice crystallized; Koch Light Ltd., Colnbrook, Bucks.), synthetic bradykinin (Sandoz BRS 640), synthetic eledoisin (Sandoz Eld 950), valyl[5] angiotensin II aspartyl-β-amide (Hypertensin CIBA), acetylcholine chloride, histamine acid phosphate, 5-hydroxytryptamine creatinine sulphate. The doses of the last three substances are expressed in terms of their bases and all other doses in terms of the weight of material used.

RESULTS

Bradykinin-like activity of trypsin hydrolysates of plasma

Table 1 summarizes the results of two assays against synthetic bradykinin, on the guinea-pig ileum, of material from stage 1 of the hydrolysis of ox plasma. It is clear

that the dose-response line for the hydrolysate is steeper than that for bradykinin. Calculation of the regression coefficients for the trypsin hydrolysate (b_T) and for bradykinin (b_B) gave the following values: experiments 1: $b_T = 25.5$, $b_B = 17.7$; experiment 2: $b_T = 35.0$, $b_B = 25.2$. Analysis of variance of the data of Table 1 showed a significant deviation from parallelism (experiment 1: $P < 0.005$; experiment 2: $P < 0.025$), so that the smooth muscle contracting activity of the trypsin hydrolysate could not be expressed with any amount of accuracy in doses of bradykinin.

TABLE 1

FOUR-POINT ASSAYS OF THE BRADYKININ ACTIVITY OF THE TRYPSIN HYDROLYSATE. OF OX PLASMA

Two volumes of the hydrolysate (stage 1), of which 11·9 ml. was equivalent to 1 ml. plasma, were assayed on the isolated guinea-pig ileum in comparison with two equal volumes of a solution of synthetic bradykinin 1 μg/ml.

Expt. No.		Trypsin hydrolysate		Bradykinin solution (1 μg/ml.)	
1	Volumes (ml.)	0·1	0·2	0·1	0·2
	Effect	21	46	42	59
	(% maximal	18	45	37	55
	contraction)	20	43	42	55
		16	43	36	59
	Average	18·75	44·25	39·25	57·0
	Ratio	2·4		1·5	
2	Volumes (ml.)	0·2	0·4	0·2	0·4
	Effect	38	69	48	71
	(% maximal	28	64	50	74
	contraction)	25	64	45	72
		28	62	43	70
	Average	29·75	64·75	46·5	71·75
	Ratio	2·2		1·5	

The fact that the dose-response line for the trypsin hydrolysate of ox plasma is significantly steeper than that for bradykinin may be an indication that this hydrolysate contains, in addition to bradykinin, other substances which enhance the activity of bradykinin. To investigate this, in two experiments the dose-response relationship was determined for synthetic bradykinin alone and for bradykinin in the presence of hydrolysate 0.1 ml./10 ml. of bath fluid, an amount which in itself had hardly any contractile activity. The results of these experiments are summarized in Table 2. At all doses tested the responses obtained by bradykinin in the presence of hydrolysate corresponded to responses obtained by at least twice the dose of bradykinin given without hydrolysate. So the resulting responses expressed in terms of ng of bradykinin increased progressively and were much higher than could be explained by a possible additive effect of a subliminal dose of hydrolysate.

These experiments were repeated with the trypsin hydrolysate of rabbit plasma (which, in contrast to ox plasma, was obtained from blood which had been heparinized and treated immediately after collection). Both stage 1 and stage 2 of the hydrolysate were assayed for bradykinin-like activity. Table 3 shows the results of these experiments; the activity ratios of the two volumes of the hydrolysates are about twice those of equal volumes of the pure bradykinin solution (1 μg/ml.). The calculated regression coefficients were: in experiment 1, 60 for the hydrolysate (stage 1) and 28.5 for bradykinin; in

experiment 2, 52 for the hydrolysate (stage 2) and 33.5 for bradykinin. These results show once more that the log dose-response relationship for the trypsin hydrolysate of plasma differs considerably from that for synthetic bradykinin.

TABLE 2

INCREASED EFFECT OF BRADYKININ ON THE ISOLATED GUINEA-PIG ILEUM BY ADDITION OF TRYPSIN HYDROLYSATE OF OX PLASMA

In two experiments on different pieces of ileum the dose-response relationship was determined for synthetic bradykinin with, and without, the addition of 0·1 ml. of hydrolysate (equivalent to 0·0084 ml. plasma)/10 ml. bath fluid. The hydrolysate was added to the bath 30 sec before the administration of bradykinin. The responses are expressed as a percentage of the maximal contraction obtainable with bradykinin alone.

Expt. No.	Hydrolysate added	Response to bradykinin (doses in ng/10 ml. bath fluid)					
		0	50	100	200	400	800
1	−		9	29	50	70	90
	+	19	44	61	93	95	
2	−		14	24	41	72	100
	+	0	36	50	71	100	

TABLE 3

FOUR-POINT ASSAYS OF THE BRADYKININ ACTIVITY OF THE TRYPSIN HYDROLYSATE (STAGE 1 AND 2) OF RABBIT PLASMA

Two volumes of the hydrolysate under test were assayed on the isolated guinea-pig ileum in comparison with two equal volumes of a solution of synthetic bradykinin 1 μg/ml. 10 ml. hydrolysate (stage 1) was equivalent to 1 ml. plasma.

Expt. No.		Trypsin hydrolysate (stage 1)		Bradykinin solution (1 μg/ml.)	
1	Volumes (ml.)	0·2	0·4	0·2	0·4
	Effect (% maximal contraction)	23 17	85 75	34 32	65 58
	Average	20	80	33	61·5
	Ratio	4·0		1·8	
		Trypsin hydrolysate (stage 2)		Bradykinin solution (1 μg/ml.)	
2	Volumes (ml.)	0·2	0·4	0·2	0·4
	Effect (% maximal contraction)	14 10	67 61	25 22	56 58
	Average	12	64	23·5	57
	Ratio	5·3		2·4	

Table 4 presents the responses to various doses of synthetic bradykinin alone and in the presence of hydrolysate 0.1 ml. (stage 1)/10 ml. of bath fluid. This amount of hydrolysate has no contractile activity by itself. The responses to bradykinin in the presence of the hydrolysate correspond with those of four times the dose of bradykinin when given alone. Table 4 also suggests that the maximum effect of bradykinin has been increased by the hydrolysate.

From these findings and the fact that in the same experimental conditions the hydrolysates did not increase the responses to acetylcholine and histamine, it was concluded that the trypsin hydrolysates from ox and rabbit plasma possessed both bradykinin-like activity and bradykinin-sensitizing properties. To separate these activities, the hydrolysates were passed down a column of ion-exchange resin (Amberlite CG-50).

TABLE 4

INCREASED EFFECT OF BRADYKININ ON THE ISOLATED GUINEA-PIG ILEUM BY ADDITION OF TRYPSIN HYDROLYSATE OF RABBIT PLASMA

On the same piece of ileum the dose-response relationship was determined for synthetic bradykinin without and in the presence of 0·1 ml. hydrolysate (stage 1)/10 ml. of bath fluid. This volume of hydrolysate (equivalent to 0·01 ml. plasma) was added to the bath 30 sec before the administration of bradykinin. The responses are expressed as % maximal contraction to bradykinin given alone.

Hydrolysate added	Response to bradykinin (doses in ng/10 ml. bath fluid)						
	0	50	100	200	400	800	1600
−			3	16	46	67	100
+	0	27	47	66	104	105	

Ion exchange chromatography of the trypsin hydrolysate of ox plasma.

A volume of 72 ml. of hydrolysate (stage 2) obtained from 33.3 ml. of ox plasma was passed down a column (38 × 1 cm) of Amberlite CG-50. The column was then washed with ammonium acetate buffer 60 ml. (pH 5) and eluted with ammonium acetate buffer (pH 9).

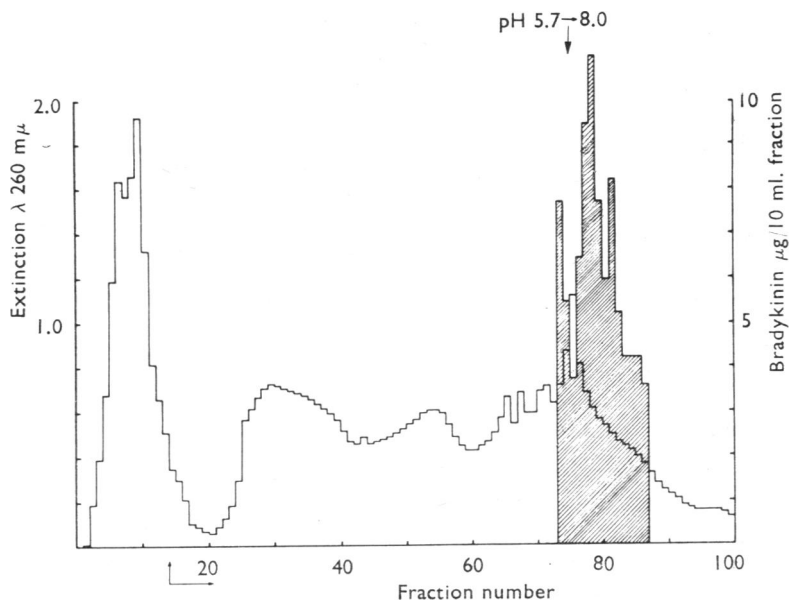

Fig. 1. Ion exchange chromatography on Amberlite CG-50 of 72 ml. trypsin hydrolysate of ox plasma. After applying this load to the column and washing with 60 ml. ammonium acetate buffer (pH 5) the elution with ammonium acetate buffer (pH 9) was started at fraction 14 (see arrow). The main extinction peak roughly coincided with the bradykinin-sensitizing activity. Bradykinin activity (shaded area) appeared in the effluent just before the moment when the pH changed from 5.7 to 8.0. Fraction size 10 ml.

Fig. 1 shows the results of a typical experiment. The main extinction peak was found in fractions 4 to 15 but a second peak was found after elution in fractions 75 and 76—that is, just after the moment that the pH of the eluate changed from 5.7 to 8.0.

Bradykinin activity was found in fractions 74 up to and including 87. The activity of these fractions on the guinea-pig ileum showed a log dose-response relationship similar to that of synthetic bradykinin ; the regression coefficient for the pooled fractions was 18 and for synthetic bradykinin, 17.5. It is therefore possible to express the bradykinin-like activity per 10 ml. fraction in terms of doses of synthetic bradykinin (see Fig. 1). The total activity corresponded with that of synthetic bradykinin 87.6 μg, that is, 2.6 μg/ml. of original plasma.

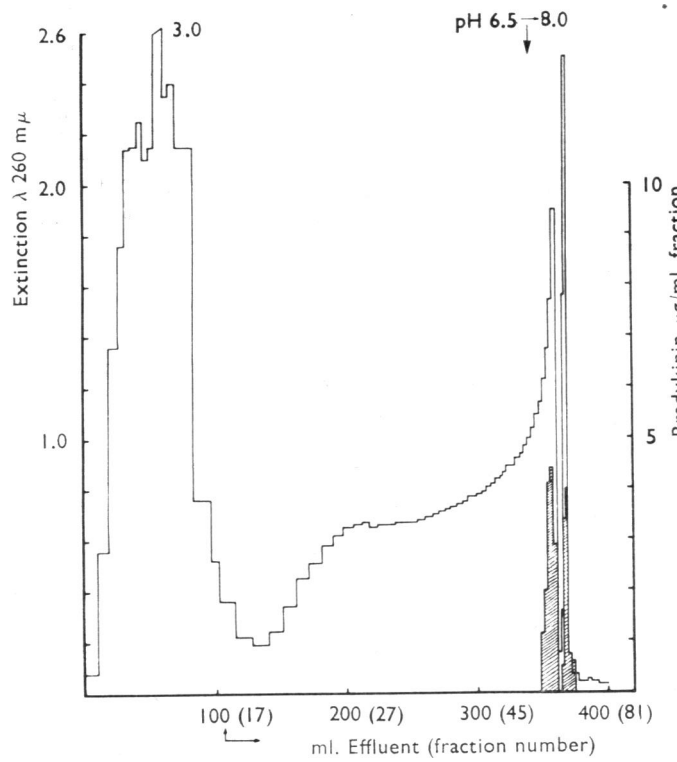

Fig. 2. Ion exchange chromatography on Amberlite CG-50 of 71.6 ml. trypsin hydrolysate of rabbit plasma. After applying this load to the column and washing it with 33 ml. ammonium acetate buffer (pH 5) the elution with ammonium acetate buffer (pH 9) started at arrow. The main extinction peak in the beginning of the curve roughly coincided with the bradykinin response sensitizing activity. Just after the change of pH from 6.5 to 8.0 a second peak was detected, which coincided approximately with the biological bradykinin activity (shaded area). Fraction size varied from 10 to 2.3 ml.

Ion exchange chromatography of the trypsin hydrolysate of rabbit plasma

In one experiment trypsin hydrolysate 71.6 ml. (stage 2), obtained from 20 ml. rabbit plasma was passed down a column (13 × 1 cm) of Amberlite CG-50 at 4° C. After the addition of the hydrolysate and 33 ml. of washing fluid to the column, elution was started. In spite of the varying conditions the elution curve shown in Fig. 2 shows essentially the same picture as that of the hydrolysate of ox plasma. The main extinction

peak was again found in the fractions containing the effluent before the elution was started. A second peak was observed just after the change in pH of the eluate—the moment at which bradykinin can be detected. In this case the total calculated bradykinin activity in the active fractions corresponded with 59.6 μg synthetic bradykinin—that is, 2.9 μg/ml. of original plasma.

Bradykinin-sensitizing activity of trypsin hydrolysed plasma, partly purified on Amberlite CG-50

When fractions of the Amberlite CG-50 effluent were tested for bradykinin-like activity on the guinea-pig ileum, it was found that the fractions collected before the start of the elution did not contract the ileum by themselves, but most of them enhanced the responses to bradykinin added 30 sec later. Sensitizing activity was chiefly found in the fractions forming the first extinction peaks in Figs. 1 and 2. The bradykinin-sensitizing activities of 0.2 ml. of these fractions/10 ml. of bath fluid are given in Table 5. The chromatographed fractions of trypsin hydrolysed rabbit plasma were considerably more active in enhancing the actions of bradykinin than were the fractions from trypsin hydrolysed ox plasma.

TABLE 5

INCREASE IN THE RESPONSE OF THE ISOLATED GUINEA-PIG ILEUM TO BRADYKININ BY AMBERLITE FRACTIONS OF TRYPSIN HYDROLYSED PLASMAS

0·2 ml. of each fraction under test was added to the bath 30 sec before the administration of synthetic bradykinin 200 ng. The responses are expressed in terms of doses of synthetic bradykinin estimated by reference to the relating log dose-effect curves for bradykinin. As a measure for the sensitizing activity the following ratio is used : dose of bradykinin (ng) corresponding to the increased effect/test dose of bradykinin (ng).

Trypsin hydrolysed ox plasma			Trypsin hydrolysed rabbit plasma		
Fraction No.	Dose of bradykinin (ng), corresponding to effect	Sensitizing activity	Fraction No.	Dose of bradykinin (ng), corresponding to effect	Sensitizing activity
1	200	1	1	200	1
2	200	1	2	205	1·02
3	230	1·15	3	470	2·35
4	230	1·15	4	470	2·35
5	290	1·45	5	252	1·26
6	490	2·45	6	540	2·70
7	470	2·35	7	740	3·70
8	360	1·80	8	760	3·80
9	530	2·65	9	1200	6·00
10	330	1·65	10	800	4·00
11	260	1·30	11	1180	5·90
12	280	1·40	12	720	3·60
13	260	1·30	13	1450	7·25
14	250	1·25	14	840	4·20
			15	800	4·00
			16	390	1·95

Portions (8 ml.) of fractions 6 to 9, inclusive, of the chromatographed hydrolysate of ox plasma weighed respectively 312.2, 302.1, 76.4 and 264.9 mg after freeze-drying. Thin-layer electrophoresis, at 1,000 V on Avicel microcrystalline cellulose in a phosphate buffer of pH 7 and an ionic strength of 0.1, demonstrated that these fractions contained many ninhydrin-positive components, which all moved to the anode.

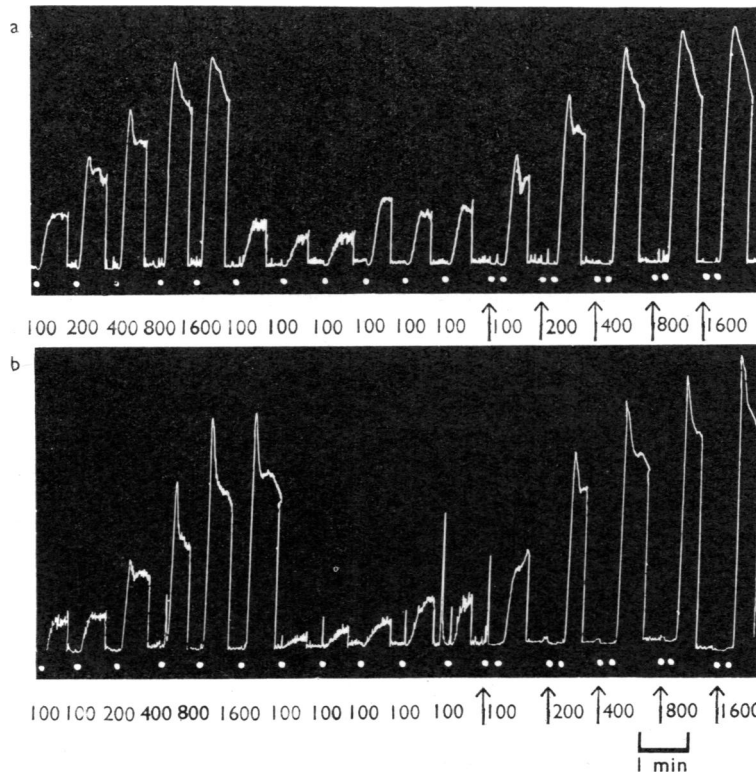

Fig. 3. Isolated guinea-pig ileum preparations suspended in 10 ml. Krebs-Ringer solution. Responses are shown to various doses of synthetic bradykinin (indicated in ng by the numbers), with and without the presence of an Amberlite effluent of trypsin hydrolysed ox plasma: 0.2 ml. fraction 7 at arrows in (a); 0.2 ml. fraction 9 at arrows in (b).

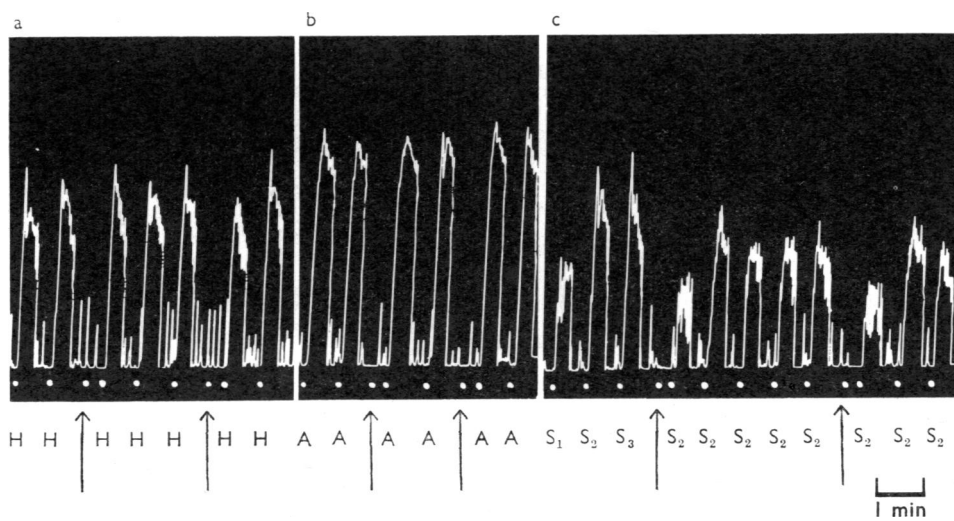

Fig. 4. Isolated guinea-pig ileum preparation with conditions as in Fig. 3. Responses to (a) histamine (H, 150 ng); (b) acetylcholine (A, 150 ng); and (c) 5-hydroxytryptamine (S_1, 200 ng; S_2, 400 ng and S_3, 800 ng). Fraction 9 of the Amberlite effluent of trypsin hydrolysed ox plasma (0.2 ml., at the arrows) did not increase the responses to histamine, nor those to acetylcholine; and decreased the responses to 5-hydroxytryptamine.

Fractions 7 and 9 were dissolved in distilled water and tested in more detail for their sensitizing effects. Fig. 3a shows that the responses of the guinea-pig ileum to doses of bradykinin ranging between 100 and 400 ng increased proportionally in about the same order in the presence of 0.2 ml. of fraction 7/10 ml. of bath fluid; this means that the effects of bradykinin 100, 200 and 400 ng in the presence of fraction 7 corresponded approximately with those of bradykinin 200, 400 and 800 ng respectively, without fraction 7. Figure 3b shows the activating effect of 0.2 ml. of fraction 9. Furthermore, these figures suggest that the maximal effect of bradykinin was also increased. Figure 4 shows that the same dose of fraction 9 in the same experimental conditions increased neither the effect of histamine, nor that of acetylcholine; the responses to 5-hydroxytryptamine were even decreased.

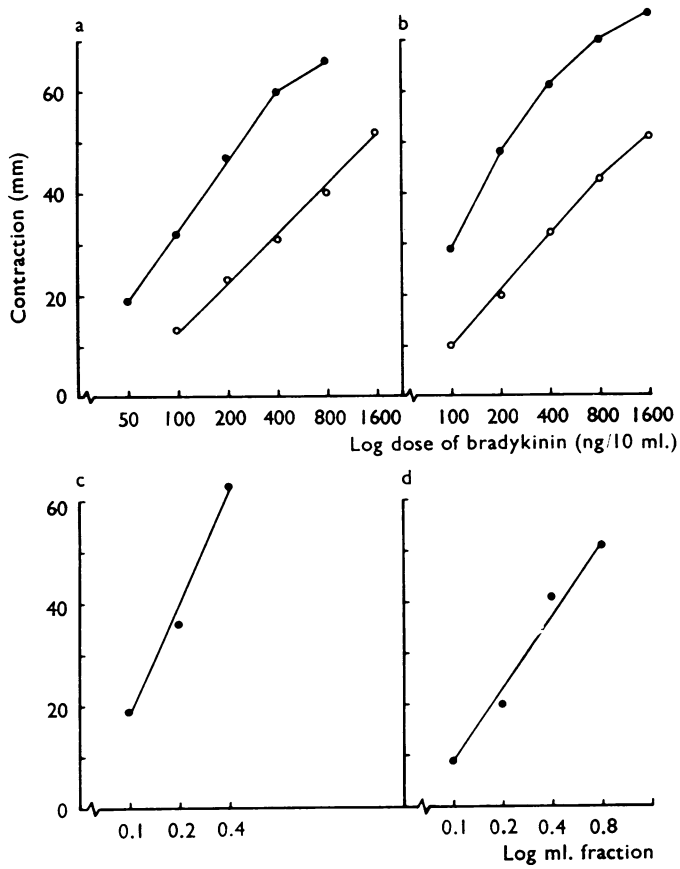

Fig. 5. Relationships between effect and dose (plotted logarithmically) for synthetic bradykinin alone (○——○) and in the presence of Amberlite effluent of trypsin hydrolysed rabbit plasma (●——●). (a) 0.2 ml. fraction 9, (b) 0.2 ml. fraction 11. Relationship between volume of fraction 9 (c) and of fraction 11 (d) (both plotted logarithmically) and their enhancement of the response to synthetic bradykinin 100 ng. The curves in (a) and (c) on the one side and in (b) and (d) on the other are plotted from experiments done on the same piece of ileum suspended in 10 ml. of Krebs-Ringer solution.

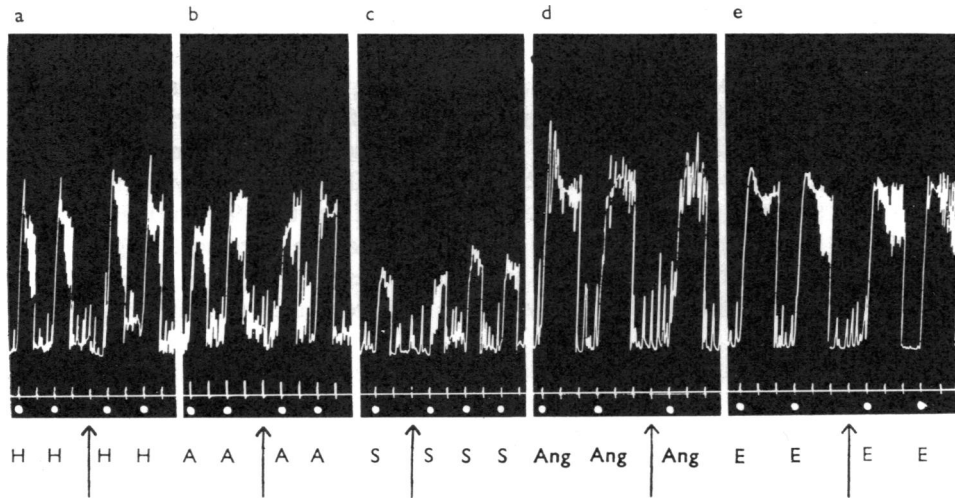

Fig. 6. Isolated guinea-pig ileum preparation suspended in 10 ml. Krebs-Ringer solution. Responses to (a) histamine (H, 100 ng); (b) acetylcholine (A, 200 ng); (c) 5-hydroxytryptamine (S, 400 ng); (d) angiotensin (Ang, 100 ng); and (e) eledoisin (E, 100 ng). Fraction 9 of trypsin hydrolysed rabbit plasma (0.2 ml., at the arrows) did not enhance the responses to histamine, acetylcholine, 5-hydroxytryptamine or to the polypeptides angiotensin and eledoisin. Time scale 30 sec.

For a number of active fractions from the chromatographed trypsin hydrolysate of rabbit plasma the relationship between the dose and the bradykinin-activating effect was determined. Thirty seconds before a fixed dose of bradykinin (100 ng), different volumes of a fraction under test were added. None of these produced contractions by themselves. The responses to the fixed dose of bradykinin, however, seemed to be linearly related to the logarithm of the volume of fractions 9 and 11 (both fractions with a high bradykinin-sensitizing potency) as is shown in Fig. 5c and d. Figure 5a and b shows the effects of 0.2 ml. of these fractions in enhancing the responses to bradykinin and, as Fig. 5a and c shows results obtained on one piece of ileum and Fig. 5b and d results obtained on another piece, it can be seen that 0.4 ml. of fraction 9 and 0.8 ml. of fraction 11 increased the response to 100 ng bradykinin to an extent corresponding to about 1,600 ng of bradykinin. Furthermore Fig. 5a and b suggests that the slope of the log dose-response curves for bradykinin are increased by 0.2 ml. of fraction 9 and 11 respectively. The sensitizing effect of all these fractions was specific for bradykinin. As shown in Fig. 6 which is representative of these experiments, the responses to histamine, acetylcholine, 5-hydroxytryptamine and the polypeptides angiotensin and eledoisin were not enhanced by 0.2 ml. of fraction 9.

Bradykinin-sensitizing activity of trypsin hydrolysed bovine serum albumin, of trypsin itself and of the non-hydrolysed ox plasma

Possible causes for the bradykinin-sensitizing activity of the trypsin hydrolysates of plasma are first, the tryptic digest of the blood proteins, and second, trypsin (Edery, 1964) and the blood proteins themselves.

To try to decide which was responsible, a 2.5% solution of purified bovine serum albumin was incubated with trypsin (1 mg/ml.) at 37° C for 2 hr and the hydrolysate tested for bradykinin-sensitizing activity. As a control, a trypsin solution, 1 mg/ml., without albumin was incubated for the same time at the same temperature. The influence of both the hydrolysate and the incubated trypsin solution on the responses to bradykinin, acetylcholine and histamine was tested on the guinea-pig ileum, which in contrast to all the foregoing experiments was suspended in 10 ml. of magnesium-free Tyrode solution. As can be seen from Fig. 7a, 0.1 ml. of the trypsin hydrolysate of bovine serum albumin increased the responses to bradykinin 40 ng to an extent corresponding to bradykinin about 160 ng and this effect was obtained even after the hydrolysate had been boiled for 5 min. The same volume of this hydrolysate did not increase the responses to acetylcholine and to histamine. Furthermore, Fig. 7a shows that the smaller bradykinin-sensitizing activity of 0.1 ml. of the trypsin solution disappeared after boiling.

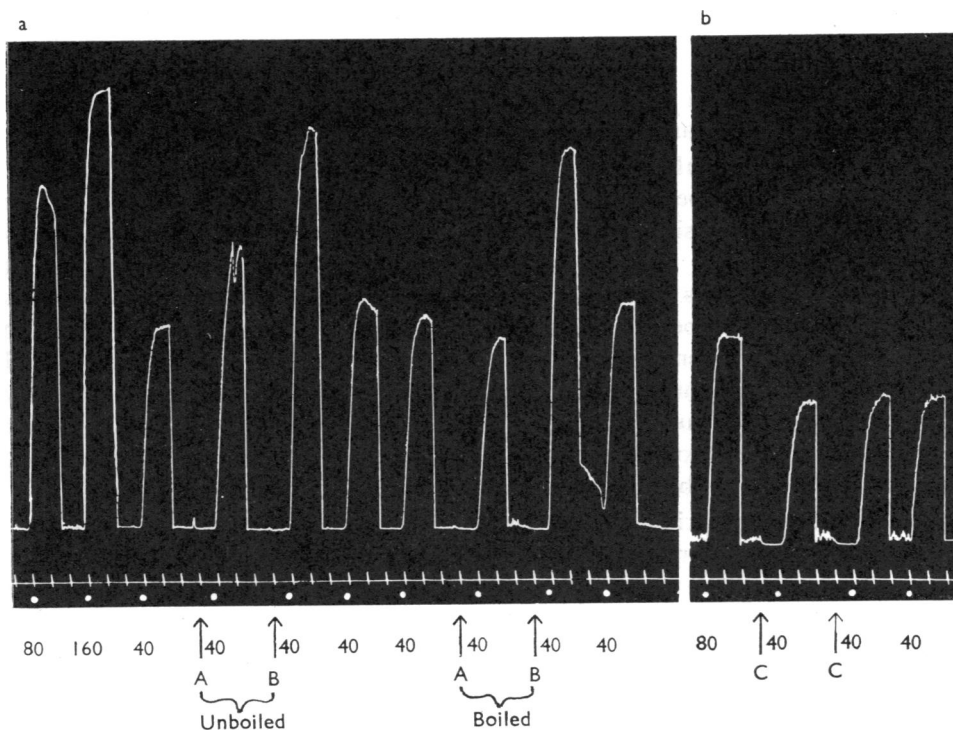

Fig. 7. Isolated guinea-pig ileum preparation suspended in 10 ml. of magnesium-free Tyrode solution with atropine sulphate (0.1 μg/ml.) and mepyramine hydrochloride (0.2 μg/ml.). Responses to doses of bradykinin indicated in ng. a: 0.1 ml. of a trypsin solution (1 mg/kg) kept at 37° C for 2 hr (A) enhances the response to bradykinin; this effect disappears after boiling for 5 min. An identical volume of a 2.5% solution of purified bovine serum albumin incubated with trypsin (1 mg/ml.) at 37° C for 2 hr (B) has a stronger effect on the response to bradykinin; this effect is resistant to boiling. b: Ox plasma (0.1 ml.), treated in the same way as the hydrolysates except for the incubation with trypsin, has no influence on the response to bradykinin (C). Time scale 30 sec.

Fig. 7b shows that 0.1 ml. of ox plasma treated in the same way as the hydrolysates, except for the incubation with trypsin, had no influence on the response to bradykinin.

From these findings it can be concluded that bradykinin-sensitizing substance(s) are released by the proteolytic action of trypsin on certain blood proteins.

DISCUSSION

The slopes of the log dose-effect curves for trypsin hydrolysates of ox and rabbit plasma containing bradykinin were significantly steeper than those for pure bradykinin. Furthermore, these hydrolysates, in doses which do not contract the guinea-pig ileum by themselves, considerably enhanced a subsequent response to pure bradykinin. Hence, the bradykinin activity in trypsin hydrolysates of plasma cannot be determined by a simple comparison with pure bradykinin on the isolated guinea-pig ileum. The method for the determination of bradykinogen in plasma described by Diniz & Carvalho (1963), which is based on the demonstration of bradykinin released by trypsin, is therefore inaccurate. When bradykinin is separated from the bradykinin-sensitizing substances by a procedure such as ion exchange chromatography on Amberlite CG-50, an apparent loss of bradykinin-activity can occur. This may explain why Hamberg *et al.* (1961) and Andrade & Rocha e Silva (1956) found a low recovery of bradykinin after treatment of trypsin hydrolysates of plasma with Amberlite CG-50.

Bradykinin-like material is retained by the column at pH 5 and is present in the fractions collected just before and just after the moment that the effluent becomes alkaline. Bradykinin-sensitizing material is not retained by the ion exchange resin at pH 5 and is found in the fractions collected before the elution with ammonium acetate buffer (pH 9) starts. This material produces larger increases in the responses to bradykinin than would be expected by the presence of a subliminal dose of bradykinin. Because the bradykinin-sensitizing potency of plasma hydrolysates is resistant to boiling it is unlikely that it is caused by the trypsin added. On the other hand no activity is produced in plasma without addition of trypsin. It is possible that bradykinin-sensitizing substances are released from blood proteins by the action of trypsin. The bradykinin-sensitizing activity of rabbit plasma was higher than that of ox plasma.

Osbahr, Gladner & Laki (1964) have isolated two acid peptides (A and B) which are released during the conversion of fibrinogen into fibrin by thrombin. Their peptide A sensitized the isolated rat uterus to bradykinin. Especially when rabbit plasma is used and the collected blood is immediately treated with heparin, clotting of fibrinogen is very unlikely to occur. It is therefore unlikely that the bradykinin-sensitizing substances described in the present paper are derived from fibrinogen by the action of thrombin. The possibility cannot be excluded, however, that an active peptide is split off from fibrinogen by the action of trypsin. In fact, it was shown that trypsin can release bradykinin-sensitizing activity from purified bovine serum albumin. It seems possible therefore that the bradykinin-sensitizing fractions of the trypsin hydrolysates contain acid peptides which may be structurally related or even identical with the fibrinopeptide A.

SUMMARY

1. Trypsin hydrolysates of ox plasma and rabbit plasma have been assayed against synthetic bradykinin in the isolated guinea-pig ileum.

2. The slopes of the log dose-effect curves for these hydrolysates were significantly steeper than those for synthetic bradykinin.

3. Small samples of these hydrolysates, themselves devoid of contracting activity, enhanced the responses of the ileum to bradykinin.

4. A trypsin hydrolysate of purified bovine serum albumin also enhanced the responses to bradykinin.

5. The bradykinin-sensitizing substances in the hydrolysates of plasma could be separated from bradykinin by ion exchange chromatography on Amberlite CG-50 ; they were found in the fractions collected before the elution with ammonium acetate buffer (pH 9) started. The activity found in the trypsin hydrolysate of rabbit plasma was much higher than that of ox plasma.

6. High voltage electrophoresis on Avicel at pH 7 of some active fractions of ox plasma showed ninhydrin-positive spots on the anodic side only.

7. A linear relationship was found between the log dose and the bradykinin-sensitizing activity in the effluents. The maximal response to bradykinin also seemed to be augmented.

8. It was shown that trypsin was not the cause of the bradykinin-sensitizing activity, nor were substances present in plasma not treated with trypsin. It was concluded that the bradykinin-sensitizing activity originates from the proteolytic action of trypsin on the plasma proteins.

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