Brit. J. Pharmacol. (1965), **24**, 485–496.

FURTHER STUDIES OF THE SENSITIZATION OF SMOOTH MUSCLE TO THE ACTION OF PLASMA KININS BY PROTEOLYTIC ENZYMES

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(*Received August* 13, 1964)

Enzymes have been used extensively to induce morphological as well as functional changes in tissues and cells. For instance, sea urchin eggs become susceptible to polyspermic fertilization after treatment with trypsin and this effect is blocked by chymotrypsin (Hagström & Hagström, 1954). Hyaluronidase enhances the penetration of procaine in frog isolated sciatic nerve (Nordqvist, 1952) and some lipases, but not proteases, produce block of excitability in lobster giant axon (Tobias, 1960).

It has been reported recently (Edery, 1964) that chymotrypsin, chymotrypsinogen and trypsin sensitize isolated smooth muscle preparations to the action of bradykinin, but not to acetylcholine, histamine and 5-hydroxytryptamine. The experiments were continued in order to find out if there were other stimulating substances whose action on smooth muscle increased after the presence of chymotrypsin and if, besides this latter, there were other proteins which caused sensitization. Attempts were also made to elucidate the mechanism of this phenomenon.

A preliminary account of the experiments reported here was presented at a meeting of the Israel Physiological and Pharmacological Society (Edery, 1963).

METHODS

Smooth muscle preparations

The guinea-pig isolated ileum (thirty-six experiments) and rat isolated uterus (ten experiments) preparations were suspended in a 5-ml. organ-bath using the procedure described previously (Edery, 1964).

The longitudinal and circular muscle strips of the guinea-pig isolated ileum (five experiments each) were suspended in a 5-ml. organ-bath according to the method of Harry (1963). The circular muscle preparations were in contact with 500 μg of *N,N*-di-isopropylphosphodiamidic fluoride (mipafox) and 2.5 μg of atropine sulphate during 90 min before the application of the stimulating drugs.

Materials

The following substances were used: synthetic kallidin (Parke Davis), synthetic bradykinin (Sandoz), synthetic angiotensin (Ciba), synthetic oxytocin (Sandoz), eledoisin (kindly supplied by Professor V. Erspamer), substance P (obtained through the courtesy of Sir John Gaddum), 6-glycine bradykinin (Squibb), 7-glycine bradykinin (Squibb), methionyl-lysyl bradykinin (Schering), histamine dihydrochloride (Fisher Scientific Co., quantities refer to the base), barium chloride (Hopkins & Williams), potassium chloride (Agan Chemical Manufacturers), adenosine triphosphate (Sigma Chemical Co.), chymotrypsin (Nutritional Biochemicals Corp.), chymotrypsinogen (Sigma Chemical Co.), trypsin (Nutritional Biochemicals Corp.),

papain (Worthington; activated before use according to the method of Kimmel & Smith (1954)), *Vipera xanthina palestinae* snake venom, phospholipase A (an electrophoretically purified fraction of the same snake venom; kindly supplied by Dr E. Condrea) and lecithinase (Behring & Sons). Human urine was dialysed against running tap water during 24 hr and after tenfold dilution with distilled water was used as a source of kallikrein; fresh guinea-pig serum was obtained after spinning the blood at 2,500 revs/min for 30 min; guinea-pig coagulating gland was prepared as described by Bhoola, Yi, Morley & Schachter (1962).

All the above-mentioned substances were diluted in 0.9% saline, except guinea-pig serum which was diluted 100-fold in Tyrode solution. Dog plasma pseudoglobulin was prepared as described previously (Edery, 1964) and was diluted with phosphate buffer, 0.1 M at *p*H 6.2, in order to inhibit kininase activity during incubation (Edery & Lewis, 1962). Dyflos (a pure specimen synthetized by Dr Z. Pelah) was used immediately after dilution with phosphate buffer, 0.2 M at *p*H 7.3.

In a series of experiments, a 4-ml. sample of the organ-bath fluid was taken before, during and after 500 μg of chymotrypsin had been in contact with the guinea-pig isolated ileum for 1 min. The samples were mixed with 0.5 ml. of 10% trichloracetic acid, then spun at 2,000 revs/min for 45 min, the supernatant fluid was freeze-dried and the residue obtained kept in ampoules.

To perform acid hydrolysis the contents of the ampoules were dissolved in 2 ml. of 6 N-hydrochloric acid and boiled for 8 hr during three consecutive days. The solution was then dried in a vacuum dessicator over sulphuric acid. The dry residue was washed with water and the 2-ml. final solution adjusted to *p*H 6.5. Amino acid analysis was performed in a Beckman amino-acid analyser (model 120 B).

RESULTS

Effect on the guinea-pig isolated ileum preparation

Chymotrypsin. The contractions elicited by synthetic kallidin, 6-glycine bradykinin and methionyl-lysyl bradykinin were slow and sustained similar to that of bradykinin but these peptides were approximately three, four and five times respectively less active than the nonapeptide. 7-Glycine bradykinin caused a rapid, histamine-like contraction of the ileum and was about 2,000-times less active than bradykinin.

Chymotrypsin (100 to 500 μg), after being present in the organ-bath for 1 min, increased the sensitivity of the preparations towards the above-mentioned peptides and also to those naturally formed by the action of plasma-kinin-forming enzymes. These latter include *Vipera xanthina palestinae* snake venom which has been found to liberate a bradykinin-like substance from human and dog plasma (Rosen, Edery & Gitter, unpublished). The sensitivity to other stimulating substances was not affected or sometimes even reduced after chymotrypsin. The results are presented in Table 1. The sensitizing effect of chymotrypsin was particularly remarkable in those preparations of low initial sensitivity.

Fig. 1 shows that after three doses of 500 μg of chymotrypsin there was such a pronounced increase in the sensitivity of the preparation that it responded to as little as 2 ng of synthetic kallidin. The contractions elicited by eledoisin and substance P after chymotrypsin were either temporarily diminished (Fig. 1) or not affected (Fig. 2). Fig. 3 shows the sensitizing effect of chymotrypsin towards the responses to natural kallidin (liberated by urinary kallikrein from plasma pseudoglobulin) and to plasma-kinin formed by action of *Vipera xanthina palestinae* venom on dog plasma pseudoglobulin.

It has been observed in previous experiments (Edery, 1964) that, after exposure to chymotrypsin, not only the height of the responses to bradykinin was enhanced but, in addition, their latent period was shortened. These effects were now studied in more detail by recording the contractions of the gut on a fast-revolving kymograph. The stimulating substances

TABLE 1

EFFECT OF CHYMOTRYPSIN ON THE SENSITIVITY OF THE GUINEA-PIG ISOLATED ILEUM TOWARDS STIMULATING SUBSTANCES OF DIVERSE ORIGIN

The height of responses to the stimulating substances was estimated before and after chymotrypsin (100 to 500 μg) had been present in the organ-bath for 1 min. + Means that chymotrypsin increased the sensitivity of the preparation to the stimulating substance and — that it did not

* Denotes the substance is referred to by the generic name as the active peptide has not yet been identified. Natural kallidin was prepared by incubating for 5 min 0·5 ml. of dialysed urine, diluted 1 : 10 with water, with 5 mg of dog plasma pseudoglobulin. Plasma kinin (serum) was prepared by incubating for 2 min 0·05 ml. of guinea-pig serum, activated by dilution with 5 ml. of Tyrode solution, and 1 ml. of the incubated mixture was tested. Plasma kinin (gland) was prepared by incubating for 2 min 0·5 mg of guinea-pig coagulating gland with 0·5 mg of dog plasma pseudoglobulin. Plasma kinin (venom) was prepared by incubating for 5 min 2 μg of snake venom with 0·5 mg of dog plasma pseudoglobulin. Natural kallidin and plasma kinin (gland) were tested in the presence of 1 μg of mepyramine

Substance	Dose in the bath	Sensitization
Synthetic kallidin	60–90 ng	+
Methionyl-lysyl bradykinin	250–300 ng	+
6-Glycine bradykinin	40–80 ng	+
7-Glycine bradykinin	20–30 μg	+
Natural kallidin		+
Plasma kinin (serum)*		+
Plasma kinin (gland)*		+
Plasma kinin (venom)*		+
Eledoisin	2·5–5 ng	—
Angiotensin	10 ng	—
Substance P	0·5 U	—
Adenosine triphosphate	50–100 μg	—
Potassium chloride	5 mg	—
Barium chloride	120–150 μg	—

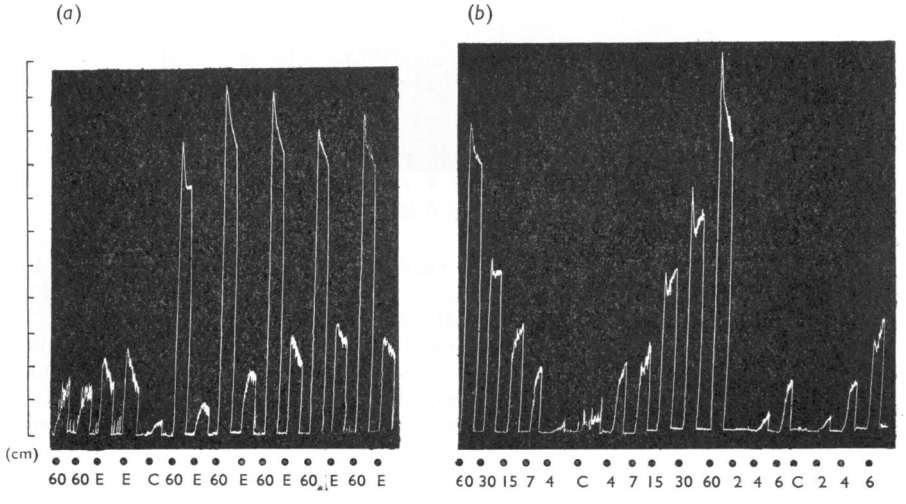

Fig. 1. Guinea-pig isolated ileum suspended in 5 ml. of atropinized Tyrode solution. Time of contact, 1 min; time cycle, 5 min. The preparation was washed twice after each application of drug. (*a*) and (*b*) are successive tracings from the same experiment. Responses to synthetic kallidin (at marks, doses in ng) and to eledoisin (E, 5 ng). Chymotrypsin (C, 500 μg) sensitized the preparation to kallidin so much that 2 ng of the peptide produced a contraction. Responses to eledoisin returned to their control height after a temporary reduction.

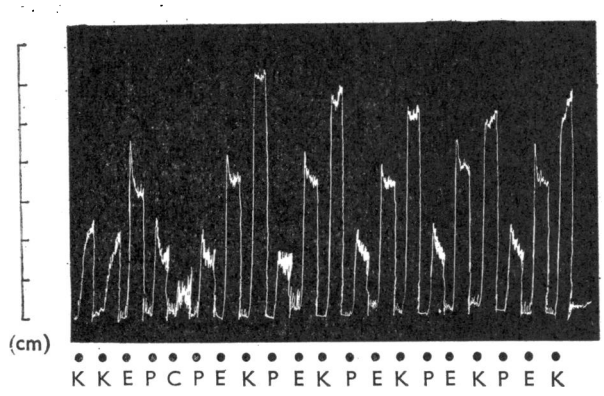

Fig. 2. Guinea-pig isolated ileum preparation with similar conditions to Fig. 1. Contractions were in response to 90 ng of synthetic kallidin (K), 5 ng of eledoisin (E) and 0.5 U of substance P (P). After 400 μg of chymotrypsin (C) the preparation became sensitized only to kallidin.

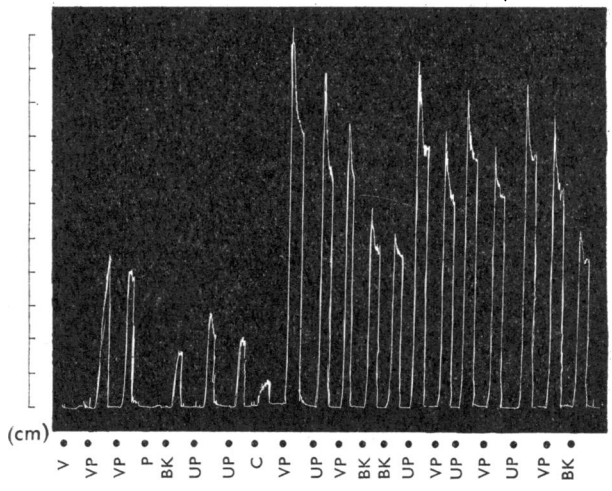

Fig. 3. Guinea-pig isolated ileum preparation with similar conditions to Fig. 1. Responses to 2 μg of venom of *Vipera xanthina palestinae* (V); to a mixture of 2 μg of venom and 0.5 mg of dog plasma pseudoglobulin in 0.2 ml. of phosphate buffer, *p*H 6.2, incubated for 5 min at 34° C (VP); to 0.5 mg of dog plasma pseudoglobulin and 0.1 ml. of phosphate buffer, *p*H 6.2, incubated for 5 min (P); to 20 ng of synthetic bradykinin (BK); to a mixture of 0.5 ml. of dialysed human urine (diluted 1 : 10) and 5 mg of dog pseudoglobulin incubated for 5 min (UP). After 500 μg of chymotrypsin (C) the preparation became sensitized to the plasma kinin liberated by the snake venom, to natural kallidin and to synthetic bradykinin.

were 20 ng of synthetic bradykinin, 20 ng of histamine and 60 ng of synthetic kallidin. After control responses had been obtained, 100 μg of chymotrypsin was left in the organ-bath for 1 min, the preparation was then washed and the stimulating substances were again tested. Three additional doses of 100 μg of chymotrypsin and the contracting drugs were alternately applied. The results of three separate experiments are presented in Table 2.

TABLE 2

EFFECTS OF FOUR SEPARATE DOSES OF CHYMOTRYPSIN (100 μg) ON THE SENSITIVITY OF THE GUINEA-PIG ISOLATED ILEUM PREPARATION TOWARDS BRADYKININ, KALLIDIN AND HISTAMINE

The gut was stimulated with synthetic bradykinin (20 ng), histamine (20 ng) and synthetic kallidin (60 ng) before and after exposure to chymotrypsin (100 μg). The procedure was repeated four times, chymotrypsin being washed out each time. The number of the experiment is indicated in roman figures

Condition	Substance	Latency of the response (sec)			Height of the contraction (mm)		
		I	II	III	I	II	III
Control	Bradykinin	13	24	26	10	6	4
	Histamine	3	1	2	35	19	24
	Kallidin	16	25	24	15	5	5
After 1st dose of chymotrypsin	Bradykinin	7	11	12	45	14	13
	Histamine	3	1	3	32	17	22
	Kallidin	9	11	12	52	20	14
After 2nd dose of chymotrypsin	Bradykinin	7	8	10	69	32	18
	Histamine	4	1	2	36	26	17
	Kallidin	7	8	5	57	30	23
After 3rd dose of chymotrypsin	Bradykinin	5	7	9	67	46	22
	Histamine	3	1	3	34	22	20
	Kallidin	5	6	6	57	30	23
After 4th dose of chymotrypsin	Bradykinin	4	6	6	70	53	30
	Histamine	3	1	2	38	29	23
	Kallidin	5	4	4	62	50	40

The experiment of Fig. 4 (experiment II, Table 2) shows the three main effects elicited by chymotrypsin on bradykinin and kallidin responses, namely a gradual shortening of the latent period, a concomitant increase of the height of contractions and a change in the shape of contractions in a way that they became similar to that elicited by histamine.

The sensitization of the isolated preparations to plasma kinins by chymotrypsin appears to be remarkably specific and therefore may be useful for detecting minute amounts of these peptides in mixtures containing other stimulating substances. To test such a hypothesis the preparations were made to contract with: (1) a mixture containing 2.5 ng of eledoisin, 0.5 U of substance P and 10 ng of angiotensin; and (2) 20 ng of histamine. In order to simulate " traces " of plasma kinins, 5 ng of bradykinin or 15 ng of kallidin were added to mixtures (1) and (2). These amounts of bradykinin and kallidin by themselves caused no contraction, or at most a barely perceptible response, in the five experiments performed. When steady control responses had been obtained, the preparations were treated with 500 μg of chymotrypsin for 1 min and washed twice afterwards. When the stimulating mixtures were again applied it was observed that those carrying the plasma kinins elicited a contraction of the gut which was twice, or even more than twice, as high as that of their controls.

It has been reported (Edery, 1964) that after heating chymotrypsin at 90° C for 10 min it no longer sensitized the preparations to bradykinin. However, heating is a rather crude procedure to inhibit the enzyme because it also causes denaturation; therefore, a milder procedure was sought. It is known that dyflos inhibits both the esterase and proteinase activity of chymotrypsin without producing configurational changes (Jansen, Nutting, Jang & Balls, 1949) by binding phosphorus to the serine present in the active centre of the

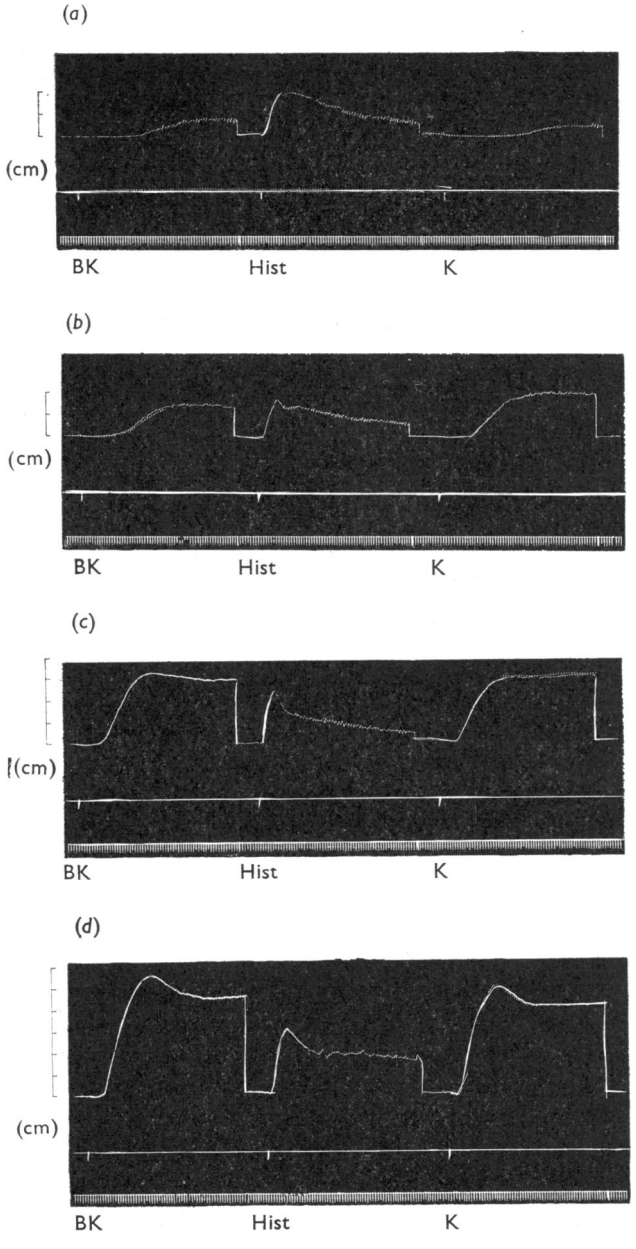

Fig. 4. Guinea-pig isolated ileum preparation with similar conditions to Fig. 1. (*a*), (*b*), (*c*) and (*d*) are tracings of the same experiment. In each panel records from above downwards: contraction of the gut, signal of injection and time marker (sec). Contractions were in response to synthetic bradykinin (20 ng, BK), histamine (20 ng, Hist) and to synthetic kallidin (60 ng, K). Between (*a*) and (*b*) and between (*b*) and (*c*) chymotrypsin (100 μg) was present in the bath for 1 min. Between (*c*) and (*d*) two separate doses of chymotrypsin (100 μg) were given. After chymotrypsin there was a progressive increase in height and a shortening of the latent period of the responses to bradykinin and kallidin. The shape of the contractions became similar to that of histamine.

enzyme. Accordingly chymotrypsin was incubated with dyflos (final concentration 10^{-4} M) for 1 hr and at times aliquots (200 to 500 μg) of the enzyme were tested on the preparations for its sensitizing effect to bradykinin, kallidin and methionyl-lysyl bradykinin. A typical experiment is shown in Fig. 5. It shows that chymotrypsin treated with dyflos was ineffective and, in contrast, the enzyme incubated with phosphate buffer only increased the sensitivity of the preparation to the peptides.

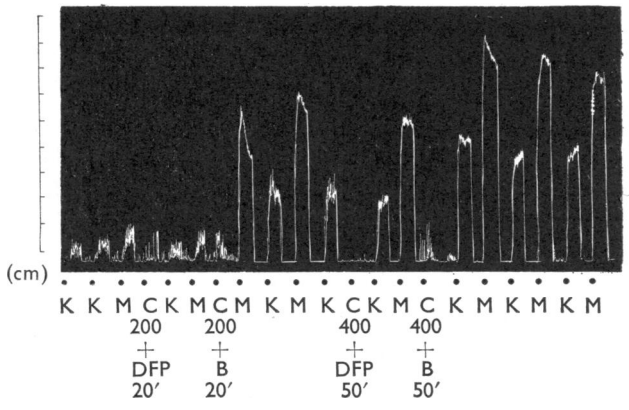

Fig. 5. Guinea-pig isolated ileum preparation with similar conditions to Fig. 1. Contractions were in response to kallidin (60 ng, K) and methionyl-lysyl bradykinin (200 ng, M). Chymotrypsin (C, doses in μg) incubated with 10^{-4} M of dyflos (DFP) in phosphate buffer during 20 and 50 min did not sensitize the preparation to kallidin or methionyl-lysyl bradykinin. In contrast, chymotrypsin incubated with phosphate buffer only (B) for the same periods of time strongly sensitized the isolated ileum to these peptides.

To ascertain whether or not peptides might be set free after chymotrypsin has been in contact with the guinea-pig isolated ileum preparation, experiments were conducted to test such a hypothesis. No amino acids were found in the organ-bath fluid taken before, during and after the presence of chymotrypsin.

Chymotrypsinogen, trypsin and papain

Chymotrypsinogen (200 μg) or trypsin (200 μg) left in contact with the preparations for 1 min increased their sensitivity to subsequent additions of standard doses of synthetic kallidin.

The effect of papain (220 to 500 μg) was examined on the responses to synthetic kallidin (60 to 90 ng), bradykinin (20 to 30 ng), methionyl-lysyl bradykinin (150 to 300 ng), histamine (20 ng) and acetylcholine (10 ng). In three out of four experiments the enzyme sensitized the preparations to all the stimulating substances.

Lipases and casein

Tobias (1960) has reported that treatment of lobster isolated giant axon with phospholipases blocked the excitability due to changes in the membrane. Furthermore, Ramwell (1964) has stated that casein sensitizes frog rectus abdominis preparation to acetylcholine.

Therefore, it seemed of interest to examine the influence of lipases and casein on the sensitivity of the guinea-pig isolated ileum to plasma kinins. Phospholipase A (200 to 500 μg), lecithinase (300 μg) and casein (400 μg) neither potentiated the responses to synthetic bradykinin and synthetic kallidin nor sensitized the preparations to these peptides.

Effect on isolated longitudinal and circular muscle preparations

Synthetic bradykinin (50 to 80 ng), synthetic kallidin (100 to 150 ng) and methionyl-lysyl bradykinin (200 to 300 ng) all contracted the longitudinal muscle preparations. The responses were potentiated by chymotrypsin (500 μg) and subsequently the preparations became sensitized to further applications of the peptides. However, the increase in sensitivity was of short duration.

The circular muscle responded with a contraction only after applying large doses of plasma kinin, namely 1 to 5 μg of synthetic bradykinin or 8 to 10 μg of synthetic kallidin. Chymotrypsin (500 to 800 μg) did not increase the sensitivity of the preparations towards these substances.

Effects on the rat isolated uterus preparation

The responses of the isolated rat uterus to synthetic kallidin (3 to 5 ng), oxytocin (5 to 15 ng) or eledoisin (250 to 500 ng) were potentiated by chymotrypsin (200 to 500 μg). When chymotrypsin was washed out, the preparations remained sensitized to the contracting peptides but for a shorter time than in the case of guinea-pig isolated ileum.

DISCUSSION

The specificity of the sensitization caused by chymotrypsin on the guinea-pig isolated ileum towards plasma kinins is remarkable. Indeed, it is surprising that chymotrypsin did not sensitize the guinea-pig isolated ileum even to peptides such as angiotensin, eledoisin and substance P, especially as the two latter have so many pharmacological properties in common with bradykinin and kallidin. It should be noted that the guinea-pig isolated ileum has been found to be the most suitable preparation for estimation of substance P (Cleugh, Gaddum, Mitchell, Smith & Whittaker, 1964). Therefore, a simple pharmacological test to distinguish between substance P and plasma kinins can be accomplished just by comparing the responses of the preparation after the presence of chymotrypsin. Only the contractions elicited by plasma kinins will be enhanced.

Bradykinin, kallidin and methionyl-lysyl bradykinin are the only plasma kinins chemically characterized and synthetized so far (Elliott, Lewis & Horton, 1960; Nicolaides, Dewald & Craft, 1963; Elliott, Lewis & Smyth, 1963; Schröder, 1964), and the fact that chymotrypsin sensitized the guinea-pig isolated ileum not only to these synthetic peptides but also to those kinins liberated by guinea-pig coagulating gland (Bhoola *et al.*, 1962) by dilution of serum (Schachter, 1956) and by *Vipera xanthina palestinae* snake venom (Rosen, Edery & Gitter, unpublished) provides additional evidence for the view that they are similar or identical with some of the already defined plasma kinins.

In addition, it might well be that the peptide derived from vagus nerve (Euler, 1963), whose action on the guinea-pig isolated ileum was potentiated by a previous addition of trypsin, was in fact a plasma kinin. Trypsin does sensitize this preparation both to bradykinin (Edery 1964) and to kallidin.

On the other hand, it would be interesting to know whether or not chymotrypsin sensitizes the smooth muscle isolated preparations to arthropod kinins such as those present in the venom of *Vespa vulgaris* and *V. crabro* (Schachter, 1963) and in spider venom (Diniz, 1963). In this connection, for example, we have found in the venom of *V. orientalis* a kinin whose action on the smooth muscle preparations is not potentiated by chymotrypsin in contrast with plasma kinins. Furthermore, the enzyme did not sensitize the preparation to the kinin. If a similar effect occurred towards other arthropod kinins, the guinea-pig isolated ileum treated with chymotrypsin could again be a useful pharmacological method to differentiate plasma kinin from other kinins. Bradykinin and kallidin caused after a relatively long latent period the characteristic slow sustained contraction of the guinea-pig ileum. After chymotrypsin, however, the nature of the responses was transformed and they became similar to those elicited by a substance like histamine which causes rapid contractions.

The fundamental difference between the sensitization of the guinea-pig isolated ileum caused by chymotrypsin and trypsin and that elicited by papain should be emphasized. The former sensitized the preparation only to plasma kinins whilst papain increased the sensitivity to all the stimulating substances tested.

A previous report (Brownlee & Harry, 1963) that the longitudinal muscle strip is more sensitive to bradykinin than the circular one has been confirmed. In addition, it has been found that this also holds true for kallidin and methionyl-lysyl bradykinin. The fact that the circular muscle strip was not sensitized by chymotrypsin to these peptides, in contrast to the longitudinal muscle strip, would indicate that their receptors must be located in the latter.

The contraction of circular muscle by high doses of plasma kinins could be due to indirect stimulation through the nervous plexuses.

In comparing the relative potency of the bradykinin analogues, 6-glycine bradykinin and 7-glycine bradykinin, it appeared that their effect on the guinea-pig isolated ileum is considerably weaker than that on guinea-pig blood pressure and rat isolated uterus preparation. In the latter, both peptides showed an activity of the same order of magnitude as bradykinin (Rubin, Waugh, Laffan, O'Keefe & Craver, 1963), whilst on guinea-pig blood pressure 6-glycine bradykin has been reported to be even more effective than bradykinin (Erdös, Wohler & Levine, 1963).

It should be pointed out that the sensitization elicited by chymotrypsin on the guinea-pig isolated ileum appeared to be more specific than on the rat isolated uterus preparation. The latter became sensitized by the enzyme to kallidin and also to oxytocin and eledoisin. This finding is consistent with that reported by Bisset & Lewis (1962) who, after studying the effects of several peptides on smooth muscle isolated preparations, concluded that the rat isolated uterus was equally sensitive to bradykinin, oxytocin and angiotensin.

As regards the mechanism of the sensitization, it might be postulated that chymotrypsin as a proteolytic enzyme would split peptide bonds present in the muscle fibre membrane of the isolated tissue preparations. Thus chymotrypsin would perform a biochemical dissection which facilitates the penetration and subsequent attachment of the plasma kinins to their receptors. Indeed, chymotrypsin did produce this latter effect. However, the fact that no amino acids were found in the organ-bath fluid would indicate that no detectable

enzymatic action took place when chymotrypsin has been in contact with the preparations. The possibility that after being split the peptides remain bound to other peptide chains could not be excluded. In close connection with our present and previous findings (Edery, 1963, 1964) are those recently reported by Osbahr, Gladner & Laki (1964). These workers have isolated two peptides (α and β) which were released during the conversion of fibrinogen into fibrin by action of thrombin. The β peptide sensitized the rat isolated uterus to bradykinin, whilst the α peptide did not. The fragments of the β peptide which produced sensitization contain the amino acid series alanine-asparagine-serine-glycine-glutamic. A similar amino acid arrangement is also present in the active centre of chymotrypsin (Schaffer, Simet, Harshman, Engle & Drisko, 1957) and trypsin (Dixon, Kauffman & Neurath, 1958); therefore, it could be postulated that the sensitization elicited by these enzymes and the β peptide has very much in common and could be caused by a similar if not identical mechanism. Supporting this view is the fact that when chymotrypsin was phosphorylated by dyflos it did not cause sensitization like the α peptide which according to Osbahr *et al.* (1964) contains a phosphate group attached to a serine residue.

Ramwell (1964) has reported that some plasma proteins and casein sensitized the frog rectus abdominis preparation to acetylcholine and potassium chloride and also that the rat isolated uterus became sensitized to acetylcholine, 5-hydroxytryptamine and bradykinin. He attributed these effects to a temporary depletion of calcium at the cell membrane and a concomitant increase in excitability. Clearly Ramwell's findings and his explanation seem to be different from those reported here. Chymotrypsin neither sensitized the rat isolated uterus to acetylcholine and 5-hydroxytryptamine (Edery, 1964) nor the guinea-pig isolated ileum to potassium chloride. Furthermore, casein did not increase the sensitivity of this latter preparation to plasma kinins. The possibility of chymotrypsin acting by binding calcium seems very remote. It is known, for instance, that even edetic acid, a most potent chelating agent, cannot withdraw bound calcium from living tissues (Shanes, 1963) though it does from extracellular fluid.

It should be pointed out, nevertheless, that the intimate mechanism of the sensitization muscle described here remains to be elucidated.

SUMMARY

1. The effects of several proteins including enzymes on the contractions of smooth muscle preparations elicited by stimulating substances has been examined.

2. Chymotrypsin strongly sensitized the guinea-pig isolated ileum to synthetic kallidin, methionyl-lysyl bradykinin, 6-glycine bradykinin, 7-glycine bradykinin and also to peptides liberated from dog plasma pseudoglobulin by plasma-kinin-forming enzymes of diverse origin.

3. The sensitizing effect of chymotrypsin was remarkably specific since there was no increase in sensitivity towards substance P, eledoisin, angiotensin, adenosine triphosphate, potassium chloride and barium chloride.

4. Chymotrypsin also sensitized the longitudinal muscle strip of guinea-pig ileum to bradykinin, kallidin and methionyl-lysyl bradykinin.

5. Papain increased the sensitivity of the guinea-pig isolated ileum not only to bradykinin, kallidin and methionyl-lysyl bradykinin but also to histamine and acetylcholine, whilst

some lipases and casein did not cause such an effect. Chymotrypsinogen and trypsin also sensitized this preparation to kallidin.

6. Chymotrypsin potentiated the responses of the rat isolated uterus preparation to kallidin, oxytocin and eledoisin and sensitized it to these peptides.

7. It is believed that the peptide which forms the active centre of chymotrypsin may be responsible for producing the sensitizing effect.

8. It is suggested that the guinea-pig isolated ileum preparation is useful as a test-object for identification of plasma kinins present in unknown mixtures, since only their responses will be enhanced after chymotrypsin.

My thanks are due to Sir John Gaddum, Professors V. Erspamer and S. Gitter, and Drs G. P. Lewis, B. Rubin, E. Nicolaides, E. Schröder and Eleanor Condrea, who kindly supplied me with some of the substances used in the present work. I am grateful to Dr A. Patchornik (Weizmann Institute of Science, Rehovoth, Israel) for the amino-acid analysis. The technical assistance of Mrs Daphna Eisenberg and of Mrs Martha Kirshenbaum is gratefully acknowledged.

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