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POTENTIATION OF THE ACTION OF BRADYKININ ON SMOOTH MUSCLE BY CHYMOTRYPSIN, CHYMOTRYPSINOGEN AND TRYPSIN

BY

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Chymotrypsin, chymotrypsinogen and trypsin sensitized the guinea-pig isolated ileum and rat isolated uterus preparations to the action of bradykinin, whilst the responses to histamine, acetylcholine and 5-hydroxytryptamine were unaffected. Chymotrypsin caused a quick contraction of the guinea-pig ileum which was abolished by mepyramine and therefore probably mediated by histamine. Trypsin contracted the rat uterus as well as the guinea-pig ileum; the latter contraction was slow, resistant to mepyramine and gave rise to tachyphylaxis. It is suggested that isolated smooth muscle preparations should be treated with chymotrypsin for use in the estimation of minute amounts of bradykinin.

In the course of recent studies on the pharmacologically active components of *Aedes aegypti* venom (Edery & Galun, unpublished) it was found that, in addition to histamine, it contains a principle which stimulates the guinea-pig ileum preparation and was not antagonized by mepyramine. The active substance was probably a peptide, since after incubation with chymotrypsin it no longer contracted the isolated ileum. Furthermore it was observed that, after applying a mixture of venom and chymotrypsin or chymotrypsin alone to the isolated preparations, these became extremely sensitive to subsequent additions of standard doses of bradykinin. This effect of chymotrypsin has now been further investigated.

METHODS

Smooth muscle preparations

The terminal piece of ileum from guinea-pigs (300 to 450 g body weight) and the uterus from rats (180 g body weight), injected 18 hr previously with 1 mg/kg of dihydrostilboestrol, were used. The organs were suspended in a 5 ml. organ-bath of Tyrode solution at 35° C for the ileum and de Jalon solution at 29° C for the uterus. The nutrient solutions contained 0.2 μg/ml. of atropine sulphate except in those experiments in which acetylcholine was tested. A mixture of 95% oxygen and 5% carbon dioxide was bubbled through the bath fluid. Contractions were recorded with a frontal writing lever (five-times magnification).

The substances tested were in contact with the preparation for 1 min and the bath fluid was changed twice after each addition of the drugs. The time cycle was 5 min.

Materials

The following substances were used: histamine dihydrochloride (Fisher Scientific Co.), acetylcholine chloride (L. Light) and 5-hydroxytryptamine creatinine sulphate (Sigma Chemical

Co.); quantities refer to the base. Synthetic bradykinin (Sandoz Ltd.), crystallized bovine plasma albumin (Armour Laboratories), bovine thrombin (Nutritional Biochemicals Corp.), human plasmin (Marcus Memorial Blood Institute, Yafo, Israel), proteose-peptone (Difco), bacto-peptone (Difco), carboxypeptidase crystallized five-times (Nutritional Biochemicals Corp.), chymotrypsinogen crystallized six-times (Sigma Chemical Co.), trypsin crystallized twice (Nutritional Biochemicals Corp.) and three brands of crystalline chymotrypsin (Nutritional Biochemicals Corp., Mann Research Laboratories and Sigma Chemical Co.) were also used. Dog pseudoglobulin was prepared by precipitating plasma proteins with ammonium sulphate, 33 to 46% saturation. The precipitate was dialysed against running tap water first and then against Tyrode solution, and the final product was freeze-dried. Human saliva was collected immediately before use and was diluted tenfold.

All substances were diluted in 0.9% saline and concentrations of the solutions were adjusted so that 0.5 ml. was the maximum volume added to the bath. Quantities are expressed in amounts present in the bath.

RESULTS

Effects on the guinea-pig isolated ileum preparation

Chymotrypsin. Chymotrypsin (100 to 500 μg) caused after a few seconds a rapid contraction which was prevented by 0.5 to 1 μg of mepyramine (Fig. 1). Tachyphylaxis developed after four to five successive equal doses of chymotrypsin.

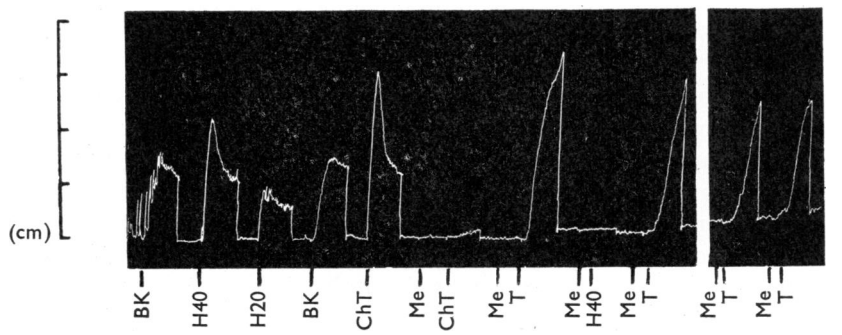

Fig. 1. Contractions of a guinea-pig isolated ileum preparation, suspended in 5 ml. of atropinized Tyrode solution, in response to 30 ng of synthetic bradykinin (BK), histamine (H, doses in ng), 200 μg of chymotrypsin (ChT) and 200 μg of trypsin (T). Time of contact 1 min ; time cycle 5 min. After each contraction the fluid of the organ-bath was changed twice. 1 μg of mepyramine (Me) prevented the response to chymotrypsin but not to trypsin. Tachyphylaxis developed after successive doses of trypsin.

When 20 to 500 μg of chymotrypsin, in the presence of mepyramine, was added 1 min before bradykinin, the response to the last drug was potentiated. The potentiation was roughly proportional to the amount of chymotrypsin added. Furthermore, after addition of chymotrypsin the preparation became more sensitive to subsequent applications of bradykinin as ascertained by the shortening of the latent period of the responses and the increase of their height. After an initial increase the contractions diminished to a steady height which in most experiments was higher than that of the control. These findings are illustrated in Fig. 2.

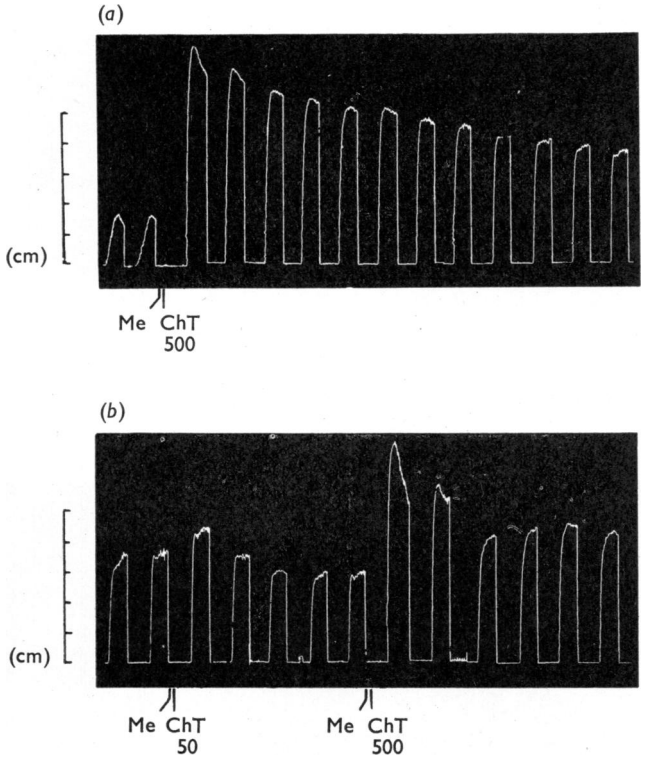

Fig. 2. Guinea-pig isolated ileum preparation, with similar conditions to Fig. 1. (*a*) and (*b*) are successive tracings of the same experiment. Contractions were in response to 20 ng of synthetic bradykinin (unmarked). Chymotrypsin (Cht, doses in μg) in the presence of 1 μg of mepyramine (Me) potentiated the response to bradykinin and increased the sensitivity of the preparation to the peptide.

Chymotrypsin not only sensitized the isolated ileum to the action of synthetic bradykinin but also to that liberated by trypsin or by salivary kallikrein from plasma pseudoglobulin. For instance the responses to a mixture of 1 mg of plasma pseudoglobulin and 0.3 ml. of diluted saliva incubated for 2 min and to a mixture of 50 μg of trypsin and 0.5 mg of plasma pseudoglobulin incubated for 1 min were considerably enhanced by the presence of chymotrypsin (Fig. 3).

After three to five successive applications of 100 to 300 μg of chymotrypsin the preparations became strongly sensitized to bradykinin and most of them responded to as little as 1 ng of the peptide, that is to say to a concentration in the bath of 200 pg/ml. Such an effect is illustrated in Fig. 4.

To ascertain whether chymotrypsin sensitized the ileum to other stimulating substances besides bradykinin, the gut was made to contract with 20 ng of bradykinin, 10 to 20 ng of acetylcholine and 20 to 40 ng of histamine, and sometimes also with 100 to 200 ng of 5-hydroxytryptamine. When steady responses had been obtained, 200 to 500 μg of chymotrypsin were left in contact with the gut for 1 min, the

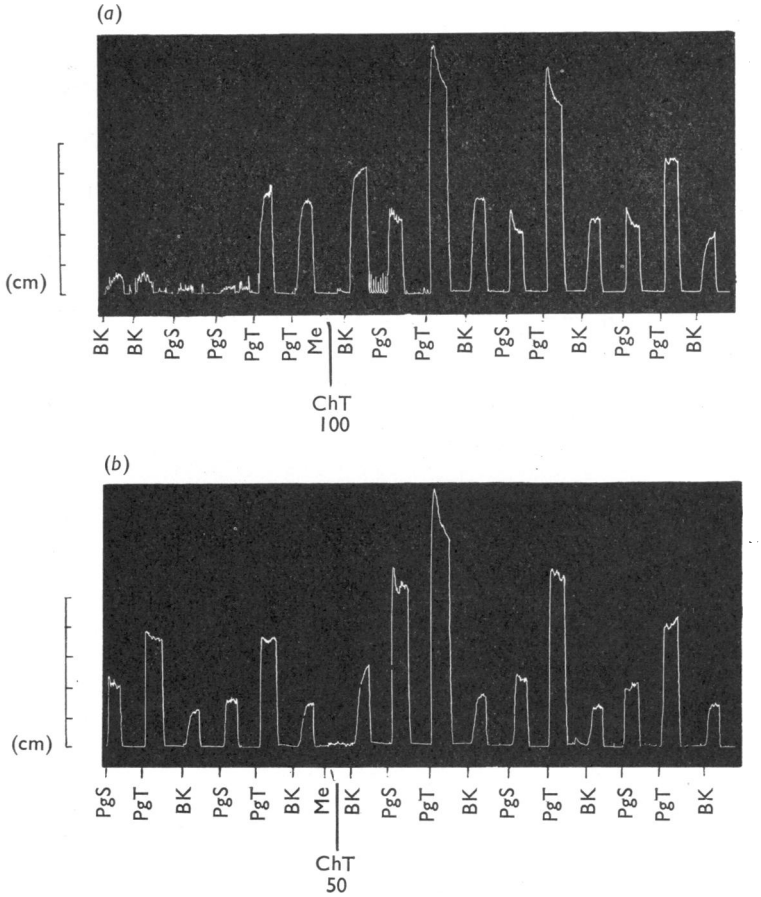

Fig. 3. Guinea-pig isolated ileum preparation, with similar conditions to Fig. 1. (*a*) and (*b*) are successive tracings of the same experiment, showing responses of the ileum to 20 ng of synthetic bradykinin (BK), to a mixture of 1 mg of dog plasma pseudoglobulin and 0.3 ml. of human saliva (diluted 1:10) incubated during 2 min (PgS), and to a mixture of 0·5 mg of dog plasma pseudoglobulin and 50 μg of trypsin incubated for 1 min (PgT). Chymotrypsin (ChT, doses in μg) in the presence of 1 μg of mepyramine (Me) sensitized the ileum to both synthetic and natural bradykinin.

preparation washed and the stimulating substances were again applied. Chymotrypsin sensitized the isolated ileum only to bradykinin (Fig. 5). In most experiments, after 500 μg of chymotrypsin, responses to acetylcholine and histamine were reduced.

After chymotrypsin had been heated at 90 to 100° C for 10 min it did not cause any of the effects described above.

The precursor of chymotrypsin, chymotrypsinogen, also sensitized the guinea-pig ileum preparation to bradykinin. 100 to 500 μg of chymotrypsinogen did not contract the gut but, after being in the organ-bath for 1 min, potentiated the response to bradykinin and sensitized the preparation to further additions of the peptide, whereas responses to acetylcholine and to histamine were not affected.

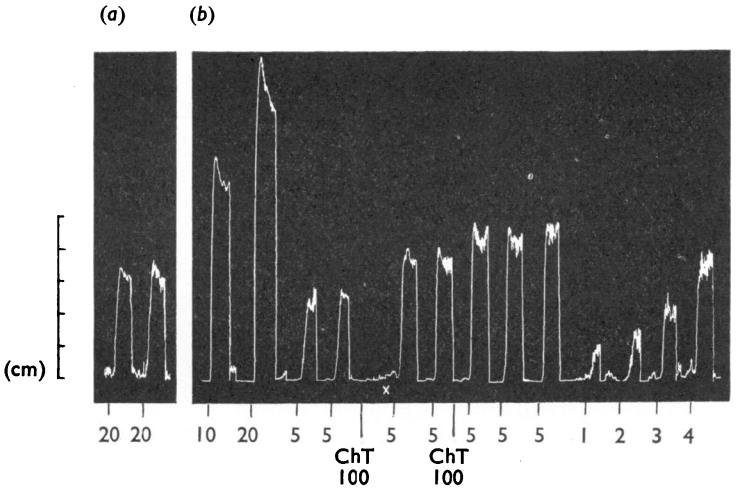

Fig. 4. Guinea-pig isolated ileum preparation, with similar conditions to Fig. 1. Responses to synthetic bradykinin (at marks, doses in ng). Between (*a*) and (*b*) there were three separate doses of 100 μg of chymotrypsin (ChT, doses in μg) in the presence of 1 μg of mepyramine, the preparation being washed after each addition. At X and after each contraction the organ-bath fluid was changed twice. After chymotrypsin the sensitivity of the preparation increased so much that 1 ng of bradykinin caused a contraction.

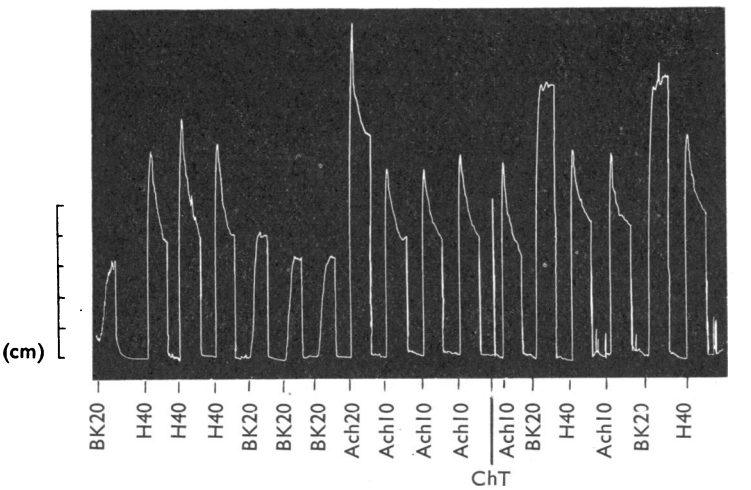

Fig. 5. Guinea-pig isolated ileum preparation, suspended in 5 ml. of Tyrode solution, with time of contact, time cycle and washing as in Fig. 1. Contractions were in response to synthetic bradykinin (BK), histamine (H) and acetylcholine (Ach). Doses in ng. At ChT, 200 μg of chymotrypsin were present in the bath for 1 min (kymograph stopped) ; the preparation was then washed. Chymotrypsin sensitized the preparation to bradykinin but not to acetylcholine or histamine.

Chymotrypsinogen was considerably less active, weight for weight, than chymotrypsin.

Trypsin. 100 to 500 μg of trypsin elicited after 15 to 25 sec a slow contraction of the ileum; on washing the preparation relaxed slowly. Mepyramine did not prevent the response to trypsin and after repeated doses tachyphylaxis developed (Fig. 1). In addition, trypsin augmented the sensitivity of the preparation to bradykinin (Fig. 6), but not to acetylcholine or histamine.

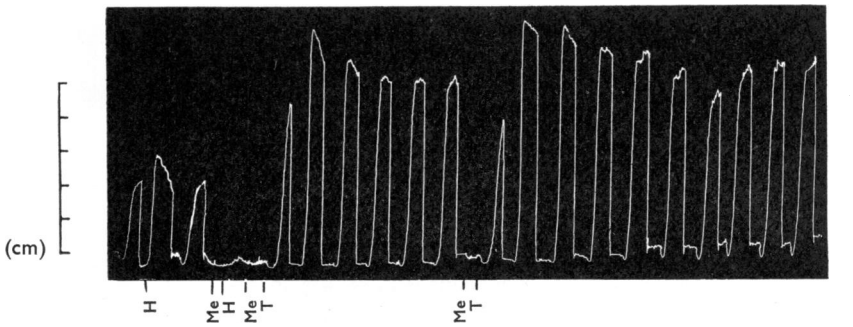

Fig. 6. Guinea-pig isolated ileum preparation, with similar conditions to Fig. 1. Contractions were in response to 20 ng of synthetic bradykinin (unmarked), 20 ng of histamine (H) and 300 μg of trypsin (T). 2 μg of mepyramine (Me) did not prevent the response to trypsin which sensitized the preparation to bradykinin.

Grant, Hood & Ramwell (1962) have reported that some proteins of unrelated structure sensitized the frog rectus preparation to acetylcholine and to other stimulating drugs. Therefore the effect of these proteins, the authors concluded, is unspecific. In order to rule out a similar possibility here, several proteins including proteolytic enzymes were tested.

Carboxypeptidase, proteose-peptone, bovine thrombin and dog plasma pseudo-globulin, in doses from 200 to 300 μg, slightly enhanced the responses to 20 ng of bradykinin, to 10 ng of acetylcholine and to 40 ng of histamine. Also human plasmin (200 μg) caused a small increase of the response to bradykinin, which agrees with a report by Lewis (1958). After washing, the sensitivity of the preparations towards the stimulating substances remained the same.

Bacto-peptone (300 μg) and bovine plasma albumin (500 μg) neither potentiated the response to bradykinin nor sensitized the gut to the drug.

Effects on the rat isolated uterus preparation

Chymotrypsin (100 to 250 μg) and chymotrypsinogen (100 to 500 μg) did not cause contraction of the rat isolated uterus but potentiated the response to bradykinin. Furthermore both substances rendered the preparation more sensitive to subsequent additions of the peptide. Fig. 7 illustrates the effect of chymotrypsinogen. After four to five successive single doses of 150 μg of chymotrypsin, most preparations became sensitized to such an extent that they responded to 0.1 ng of bradykinin.

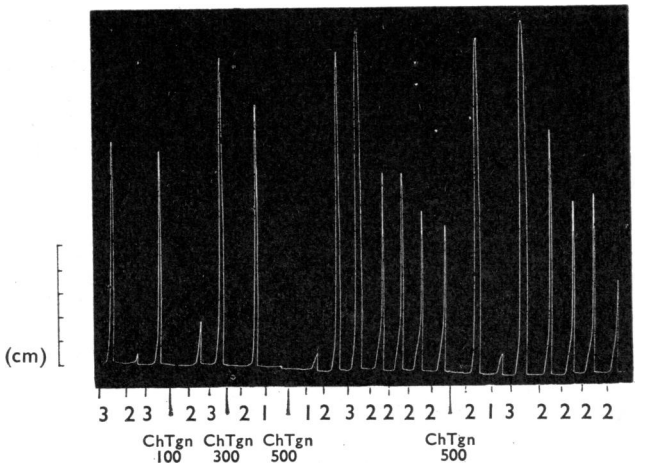

Fig. 7. Rat isolated uterus preparation suspended in 5 ml. of atropinized de Jalon solution. Time of contact, 1 min; time cycle 5 min. Responses are shown to synthetic bradykinin (at marks, doses in ng). At ChTgn, chymotrypsinogen (doses in μg) was added to the organ-bath and potentiated the response to bradykinin and sensitized the preparation to it.

Chymotrypsin (10 to 250 μg) neither potentiated the response to 40 to 60 ng of 5-hydroxytryptamine and to 0.5 to 1 μg of acetylcholine nor affected the sensitivity of the preparation towards these drugs.

Trypsin strongly stimulated the rat isolated uterus. Contractions were elicited by 1 to 50 μg of the enzyme, though desensitization developed after repeated doses. When the preparation had become unresponsive in this way, the addition of 20 to 50 μg of the enzyme potentiated the response to 0.5 to 3 ng of bradykinin. However the potentiation was less than that produced by chymotrypsin.

DISCUSSION

The experiments show that chymotrypsin, chymotrypsinogen and trypsin potentiate the response of smooth muscle to bradykinin. This finding is somewhat surprising and difficult to explain. It is well established that bradykinin is inactivated after incubation with chymotrypsin (Boisonnas, Guttmann & Jaquenoud, 1960; Elliott, Lewis & Horton, 1960); therefore an interaction beween the two substances could hardly be expected.

Several substances which potentiate the response of smooth muscle to bradykinin have been described. Recently, Rocha e Silva & Garcia Leme (1963) reported this effect for dibenamine and phenoxybenzamine on the guinea-pig isolated ileum preparation, but stated that they had no explanation for the finding. Lewis (1960) observed potentiation by cysteine, a fact confirmed by Picarelli, Henriques & Oliveira (1962) who explained it as due to the inhibition of kininase present in the smooth muscle. Whether a similar interpretation would be valid for chymotrypsin, chymotrypsinogen and trypsin remains a matter of speculation. The fact that chymotrypsin shortened the latent period of the response to bradykinin could be interpreted as due to the enzyme somehow facilitating the access of the peptide to its receptors.

The sensitization of the isolated preparations towards bradykinin cannot be due to a nonspecific proteolytic action, since chymotrypsinogen is inactive in this respect and proteolytic enzymes such as plasmin, carboxypeptidase and thrombin did not cause sensitization. The effect appears to be rather specific ; firstly because it was produced solely by chymotrypsin, chymotrypsinogen and trypsin, which are related proteins (Northrop, Kunitz & Herriot, 1948 ; Desnuelle, 1960 ; Inagami & Sturtevant, 1960) ; and secondly, the isolated ileum became sensitized to bradykinin and not to histamine and acetylcholine despite the fact that all three substances cause contraction by a direct action on the muscle fibres (Feldberg, 1951 ; Ambache & Lessin, 1955 ; Khairallah & Page, 1963). In addition it should be pointed out that we were unable to confirm a previous report (Correale, 1958) that chymotrypsin potentiates the response of rat uterus to 5-hydroxytryptamine.

It may be worth noting that chymotrypsin, after 30 min of contact with the rabbit isolated ileum, almost abolished the response to acetylcholine (Lu, 1952). Consistent with this fact the present experiments showed that high doses of chymotrypsin reduced the response of the guinea-pig ileum to acetylcholine and histamine.

Chymotrypsin and trypsin both contracted the isolated ileum although by different mechanisms. The response to chymotrypsin appears to be mediated by histamine since mepyramine abolished the contraction which was rapid and unsustained, similar to that produced by the amine. In addition chymotrypsin did not contract the rat isolated uterus which is known to be relatively insensitive to histamine.

The response of the isolated ileum to trypsin was slow in onset, well sustained and not abolished by mepyramine ; therefore it could not be due to histamine. These findings agree with those of Rocha e Silva (1940, 1955), who also pointed out that the contraction elicited by trypsin might be due to the release of an unknown factor other than bradykinin.

Gomes (1955) reported that no tachyphylaxis developed in the superfused rat uterus after repeated applications of trypsin. In the present experiments, however, tachyphylaxis was observed. The discrepancy may be due to the difference in experimental conditions. In the superfusion method the contact time is considerably shorter than in our experiments.

Finally, as the threshold dose of the response to bradykinin (Lewis, 1961) was raised about five-times by chymotrypsin, it is suggested that the latter could be used in the estimation of minute amounts of the peptide on smooth muscle preparations.

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