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# MECHANISM OF ACTION OF CHYMOTRYPSIN ON PLAIN MUSCLE

BY

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It has been shown that chymotrypsin stimulates isolated smooth muscle segments to contract (Gaddum, 1953; Gilfoil, Hauber & Kelly, 1963; Edery, 1964). This study was made to find out if the smooth muscle stimulant activity of the enzyme is affected by the same functional group responsible for the proteinase and esterase activities (Jansen, Fellows Nutting & Balls, 1949). It was made also to learn whether the action of the enzyme on smooth muscle segments is due to an action directly on the muscle fibres or to stimulation of nervous structures or the release of chemical mediators in the muscle wall.

## METHODS

The animals providing the smooth muscle preparations described below were first stunned by a blow on the head and killed by decapitation.

### *Rat uterus*

A segment approximately 2 cm long was cut from either horn of the uterus of Sprague-Dawley rats (190–260 g), either untreated or treated (16–20 hr previously by subcutaneous injection of diethylstilboestrol, 0.2 mg in 0.5 ml. of corn oil. One end was attached to the hook of a glass tissue holder and suspended in an isolated organ bath containing 5 ml. of de Jalon solution, as modified by Gaddum, Peart & Vogt (1949), aerated with 100% oxygen and maintained at $30 \pm 0.2°$. The free end of the segment was attached with a metal hook and thread to a Grass force-displacement transducer, Ft. 03B, which measured the tension developed by the segment. The output from the transducer was amplified and recorded on a Grass Polygraph.

### *Rat fundic strip*

The fundus was cut from the stomach of male or female Sprague-Dawley rats (190–250 g), previously untreated, and cut into a strip by the method of Vane, (1957). The strip was suspended in an organ bath containing 5 ml. of de Jalon solution aerated with 100% oxygen and maintained at $35 \pm 0.2°$. The free end of the segment was attached by a thread to one end of a lever (with a magnification of 5×) arranged for frontal recording of isotonic contractions on an ink-writing Kymograph.

### *Guinea-pig ileum*

A segment approximately 2 cm long was cut from the terminal ileum of fasting male or female, Hartley, albino guinea-pigs (350–450 g) and suspended in an organ bath containing 5 ml. of Tyrode solution aerated with 100% oxygen and maintained at $35 \pm 0.2°$.

*Guinea-pig uterus*

A segment approximately 2 cm long was cut from either horn of the uterus of previously untreated Hartley, albino guinea-pigs, and suspended in 5 ml. of Tyrode solution aerated with 100% oxygen and maintained at 35±0.2°. The tissue segments were arranged for recording as described for the rat uterine segment.

After suspending a preparation in the organ bath the muscle was gradually stretched until the resting tension measured about 0.5 g. All preparations were allowed to equilibrate with the external solution for 30 to 60 min before being subjected to the experimental procedures.

The test drugs, dissolved in 0.2 ml. of the bathing solution, were injected through a 1½-in. 21 gauge hypodermic needle inserted into a 2 mm capillary side arm at the base of the muscle chamber. The point of the needle extended about 3 mm into the bath just below the place of attachment of the tissue and just above the inlet for the $O_2$. A stopcock, closing the other end of the needle, prevented leakage of chamber fluid between injections. Thus the test drug rapidly entered the muscle chamber and was aided in diffusion throughout the bath fluid by the current of $O_2$. A drug was allowed to remain in contact with the tissue until the maximal effect occurred, usually 30 to 60 sec after injection, and was then washed out. The interval between injections was 3–4 min. The enzymes remained in contact with the tissue for 4 min if no effect occurred; otherwise, they were washed out at the time of the maximal effect. Hence the interval between injection of the enzymes was 5–6 min. The guinea-pig uterine preparation often showed a long latency in response to chymotrypsin, sometimes as long as 6 min. Accordingly, the interval between injections was increased. A given antagonist, in the case of the guinea-pig ileum and uterine preparations, was allowed to remain in the bath for 10 min. It was removed from the bath at the time of washing out of the test drug and again added at once when the washing was stopped. In the case of the rat uterine segment preparation, the inlet to the muscle chamber was switched to a reservoir of de Jalon solution containing the antagonist. Thereafter, until the end of the experiment, the tissue was constantly exposed to de Jalon solution containing the antagonists.

The *p*H of de Jalon and Tyrode solutions, freshly prepared on the day of the experiment, varied from 8.03–8.10 and 7.98–8.10, respectively. In some experiments the *p*H of these solutions was adjusted to 7.78. At this *p*H there was no observable difference in response of the rat uterus or the guinea-pig ileum to chymotrypsin from that noted at the higher *p*H values.

*Proteinase activity.* The proteinase activity of untreated and DFP-treated chymotrypsin was determined by the following modification of the haemoglobin method of Anson (1938).

To separate test tubes each containing 5 ml. 3% denatured haemoglobin solution, buffered at *p*H 7.5, was added either 1 ml. of trypsin reference standard solution containing the equivalent of 16, 48 or 80 proteolytic units or 1 ml. of untreated or DFP-treated chymotrypsin solution containing 5, 10, 50 or 100 μg per ml. After incubating the tubes at 37° for 30 min, the contents were precipitated by the addition of 10 ml. of 5% trichloroacetic acid and filtered. Nonincubated control samples, in which enzyme and acid solutions were added in reverse order, were prepared concurrently. The tyrosine content in 2 ml. aliquots of the filtrate was estimated colorimetrically by adding 1 ml. of Folin-Ciocolteu reagent, 5 ml. of $Na_2CO_3$ solution and diluting to volume with distilled water. After mixing and standing for 30 min the optical density of the solutions was recorded with a Lumetron colorimeter at 660 mμ. The results are expressed in proteolytic units per ml. by multipling the value representing the difference between the incubated and nonincubated samples by the dilution factor.

The drugs used were: Acetylcholine chloride (Merck, Sharpe & Dohme); synthetic bradykinin (Sandoz Pharmaceuticals); cyproheptadine hydrochloride (Merck, Sharpe & Dohme); alpha, beta and gamma chymotrypsins (Calif. Biochemicals) (Nutritional Biochemicals) (Sigma Chemicals) and (Worthington Biochemicals); delta chymotrypsin (Nutritional Biochemicals) and (Worthington Biochemicals); chymotrypsinogen (Nutritional Biochemicals) and (Sigma Chemicals); Dyflos (DFP) (Mann Chemicals); histamine $PO_4$ (Burroughs Wellcome); hexamethonium (Delta Chemical Works, Inc.); 5-hydroxytryptamine creatinine $SO_4$ (Nutritional Biochemicals); nicotine $SO_4$ (Merck, Sharpe & Dohme) and soybean trypsin inhibitor (Nutritional Biochemicals). Drugs were dissolved in de Jalon or Tyrode solution.

## RESULTS

### *Delayed, slow contraction induced in the uterine segment of the rat by varieties of chymotrypsin*

Several varieties of chymotrypsin, alpha, beta, gamma and delta, obtained from different sources, induced a marked contraction in the uterine segment of the rat (Fig. 1A). The contractions were slower in onset than those induced by acetylcholine, 5-hydroxytryptamine or synthetic bradykinin, the latent period for the chymotrypsins varying from 40 sec to $2\frac{1}{2}$ min. Delta chymotrypsin was the most potent of the enzymes regardless of the order in which they were tested. The effects of the enzymes were enhanced (see Fig. 1B) by atropine and methysergide in concentrations which abolished the responses to large doses of ACh and 5-HT. Chymotrypsinogen (up to 1 mg) failed to induce a contraction.

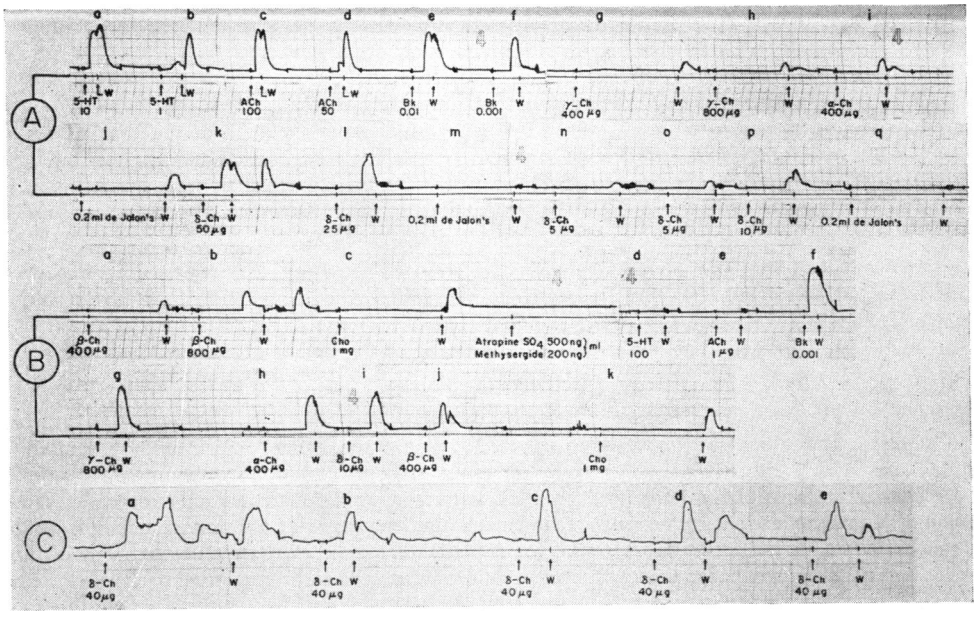


Fig. 1. Effects of chymotrypsin on the uterine segment from the rat suspended in 5 ml. de Jalon solution at 30°. The dose of a given drug (5-HT = 5-hydroxytryptamine, ACh = acetylcholine, Bk = bradykinin, Ch = chymotrypsin, Cho = chymotrypsinogen), dissolved in 0.2 ml. of de Jalon solution, was injected into the bath at the times indicated by the arrows. Doses are in nanograms unless otherwise stated. The response is designated by a lower case letter and its magnitude is in mm at a setting of 0.2 mv/cm. The time of washing out (W) of a drug is also indicated by an arrow. At the chart speed of 0.25/mm/sec the distance (5 mm) between adjacent vertical lines was traversed in 20 sec. Parts A and B are continuous but C was recorded from a different segment. During the recording of tracings d to k, part B, the tissue was constantly exposed to de Jalon solution containing atropine ($5 \times 10^{-7}$) and methysergide ($2 \times 10^{-7}$). As a control, at tracings j, m and g, part A, 0.2 ml. of de Jalon solution was injected; this did not induce a contraction. These sections also show that the preparation was free of spontaneous contractions.

The uterine segment did not show tachyphylaxis to chymotrypsin, for the response of the preparation was similar to delta chymotrypsin (40 $\mu$g) repeated five times in succession (Fig. 1C).

The results illustrated in Fig. 1 are representative of those obtained with 27 out of 34 uterine segments. The others failed to respond to doses of delta chymotrypsin (up to 500 $\mu$g).

*Inhibition by DFP of the effects of chymotrypsin and trypsin on segments of the rat uterus and guinea-pig ileum*

Jansen, Fellows Nutting, Jang & Balls (1949) showed that both the proteinase and esterase activities of chymotrypsin and trypsin were inhibited by treatment with DFP in 1:1 molar ratio. The effects of delta chymotrypsin and trypsin were also inhibited by DFP (Fig. 2). In seven experiments DFP-treated chymotrypsin failed to elicit a contraction even when tested at two and four times the dose of the untreated enzyme needed to

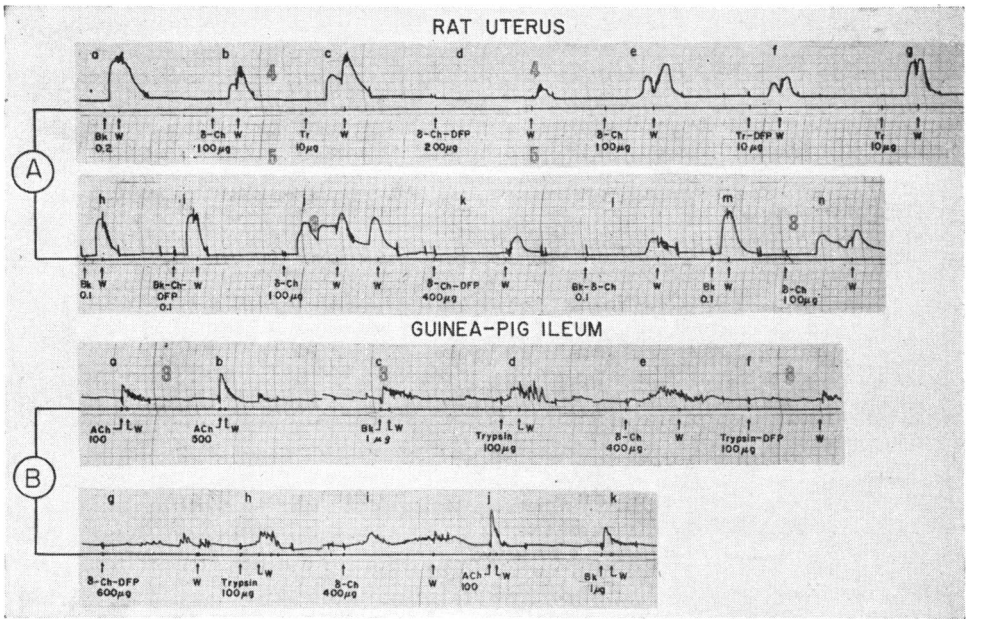


Fig. 2. Abbreviations and conditions of recording as in Fig. 1. Inhibition by DFP of the effect of chromotrypsin on the uterine segment of the rat, suspended in 5 ml. de Jalon solution at 30° (A) and the guinea-pig ileal segment, suspended in 5 ml. of Tyrode solution at 35° (B). Part A (b) and (c) represent responses to delta chymotrypsin (100 $\mu$g) and trypsin (10 $\mu$g). There was no response to DFP-treated chymotrypsin (200 $\mu$g at d and 400 $\mu$g at k) and the response to DFP-treated trypsin (10 $\mu$g at f) was smaller than the control (c). The effects of Bk (0.1 ng) reacted with delta chymotrypsin (100 $\mu$g) were virtually absent (compare l and m); whereas the response to Bk (0.1 ng) reacted with 100 $\mu$g DFP-treated chymotrypsin was unaffected (compare i and h). Part B shows that the responses of the guinea-pig ileum to DFP-treated chymotrypsin (compare e and i with g) and DFP-treated trypsin (compare d and h with f) were also inhibited.

produce a response (see Fig. 2A b, e, j, n). DFP was somewhat less effective in blocking the smooth muscle contractions induced by trypsin (Fig. 2A, f), for 10 $\mu$g of DFP-treated trypsin still elicited a response, though only about 50% of that induced by 10 $\mu$g of untreated enzyme (Fig. 2A c and g). The bradykininolytic activity of chymotrypsin, previously described by Boissonnas, Guttmann & Jaquenoud, (1960) and by Elliott, Lewis & Horton, (1960) was also blocked by DFP (Fig. 2A, h, l and m). The effect of bradykinin (0.1 ng) was abolished when the polypeptide was tested after treatment with delta chymotrypsin (100 $\mu$g) at 24° for 15 minutes, whereas the effect of bradykinin (0.1 ng) similarly treated with DFP-inhibited delta chymotrypsin (100 $\mu$g) was unchanged. Fig. 2B illustrates the inhibition by DFP of the effects of trypsin (f) and chymotrypsin (g), on the guinea-pig ileum segment.

Inhibition by DFP of the activity of samples of delta chymotrypsin, as illustrated by their effects on smooth muscle, was verified in three experiments by showing that their proteolytic activity, measured in a modification of the haemoglobin method of Anson 1938) was blocked. The results are presented in Table 1.

TABLE 1

INHIBITION BY DFP OF THE PROTEINASE (MODIFIED ANSON HAEMOGLOBIN METHOD) AND PLAIN MUSCLE STIMULANT ACTIVITY OF CHYMOTRYPSIN

Activity is expressed in proteolytic units (N.F. XII) per ml. (mean of 3 experiments).
* Inactive means <0·5 units per ml.

| Delta chymotrypsin, $\mu$g/ml. added to Hb substrate solution | Activity in proteolytic units per ml. | Dose delta chymotrypsin ($\mu$g) added to 5 ml. bath to induce contraction in rat isolated uterine segment |
|---|---|---|
| 5 | 7 | 5–10 |
| 10 | 12 | |
| 50 | 15 | |
| 100 | 15 | |
| Delta chymotrypsin, $\mu$g/ml. DFP, 100 $\mu$g/ml. added to Hb substrate solution | | |
| 5 | Inactive* | |
| 10 | Inactive | Inactive at 200 and 400 $\mu$g |
| 50 | Inactive | |
| 100 | Inactive | |

*Inhibition by soybean trypsin inhibitor (SBTI) of the effect of chymotrypsin and trypsin on the uterine segment of the rat*

The effects on segments of rat uterus of beta chymotrypsin and trypsin before and after reaction with SBTI are illustrated by the tracings in Fig. 3 which is typical of five similar experiments. The effects of both enzymes were abolished when they were tested after reaction with soybean trypsin inhibitor in a 1 to 1 weight ratio. Unlike the DFP inhibited chymotrypsin, the samples treated with the soy inhibitor retained their ability to inactivate bradykinin. The soy inhibitor did not, however, exert a nonspecific blocking effect on the smooth muscle because the effects of 5-HT and bradykinin similarly treated with the same dose of soy inhibitor that inhibited chymotrypsin were not reduced (Fig. 3 a, p, m and o).

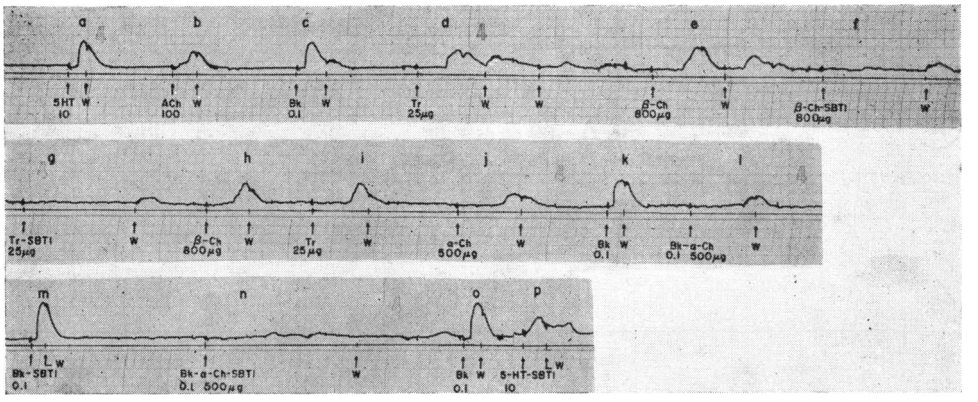


Fig. 3. Abbreviations and conditions of recording as in Fig. 1. Inhibition by soybean trypsin inhibitor (SBTI) of the effects of chymotrypsin and trypsin on the uterine segment of the rat in 5 ml. of de Jalon solution. (a), (b) and (c) are responses to 5-HT, ACh and Bk. The responses to trypsin (d) and beta chymotrypsin (e) were abolished when the enzymes were tested after reaction with an equal weight of SBTI (f and g). (h) and (i) again show responses to the untreated enzymes. Unlike DFP-inhibited chymotrypsin, the soy inhibited enzyme inactivated bradykinin (n) as readily as the untreated enzyme (l). The inactivation was specific in that 5-HT (p) similarly treated with SBTI still produced a response.

*Effect on the guinea-pig ileum segment and the rat fundic strip preparations*

The doses of chymotrypsin and trypsin inducing contractions in the guinea-pig ileum and the doses of chymotrypsin inducing contractions in the rat fundic strip preparations were usually 5 to 10-fold larger than those needed to induce contractions in the uterine segment of the rat. Unlike the latter, the guinea-pig ileum and rat fundic strip preparations rapidly developed tachyphylaxis to the enzyme (Fig. 4). Thus our results on the guinea-pig ileum agree with those of Edery (1964). In our experience, however, the ileum usually either failed to respond or responded to a lesser degree to a repetition of the same dose of chymotrypsin. Frequently, upon tripling or quadrupling the dose of the enzymes, a response nearly equal to that of the initial response was elicited, though when repeated the larger dose either failed to elicit a response or elicited a much smaller response.

After tachyphylaxis to trypsin had developed in the guinea-pig ileum segment, the preparation was capable of responding to the usual dose of chymotrypsin; or, on the other hand, if the enzymes were tested in the reverse order and tachyphylaxis to chymotrypsin had developed, the preparation was capable of responding to the usual dose of trypsin (Fig. 4).

*Effects of atropine on the response of segments of the guinea-pig ileum to chymotrypsin*

Because most guinea-pig ileum preparations showed tachyphylaxis to chymotrypsins, a preparation already exposed to the enzyme was not used for testing the effectiveness of an antagonist. Instead, the effect of chymotrypsin on preparations treated with a given antagonist were compared with those obtained from preparations not exposed to an

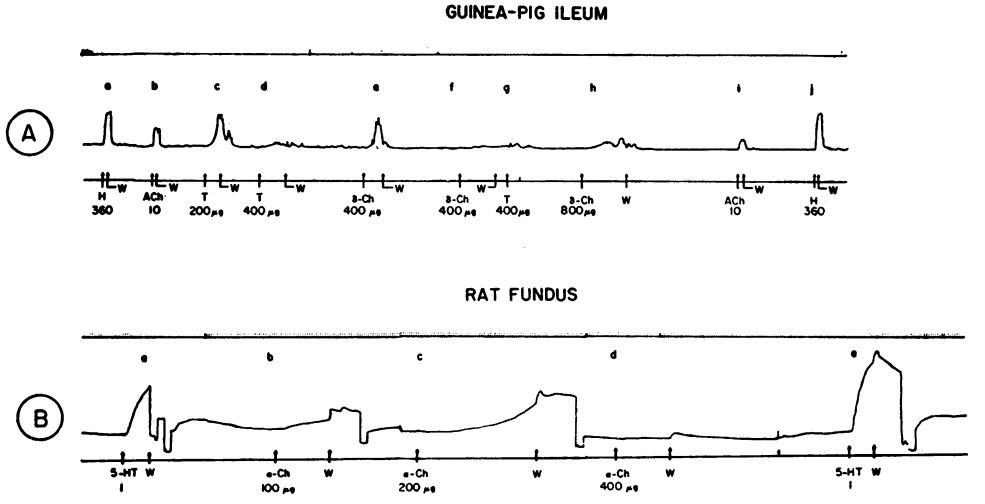


Fig. 4. Abbreviations as in Fig. 1. Time, 10 sec. Tachyphylaxis to chymotrypsin in the guinea-pig ileum segment, part A, suspended in 5 ml. of Tyrode solution at 35° and the rat fundic strip preparation, part B, suspended in 5 ml. de Jalon solution at 35°. The recordings were made on a Kymograph with a frontal ink-writing lever having a 5×magnification. Part A: Tachyphylaxis developed in the ileum after the first dose of trypsin (200 μg at c, and 400 μg at e). Tachyphylaxis also developed to chymotrypsin (400 μg at e and f); double the dose (h) gave only a minimal response. ACh and histamine gave similar responses initially (a and b) and at the end (i and j). The typical response of the rat fundic strip to chymotrypsin is shown in part B. The preparation gave a good response to α-chymotrypsin (200 μg at c) but failed to respond to double the dose (400 μg at d). At the conclusion of the test the response to 1 ng 5-HT (e) was greater than that obtained initially (a).

antagonist. The effect of delta chymotrypsin (500 μg) on a tissue exposed to atropine (0.2 μg/ml. for 10 min) was slightly inhibited when compared to the effects of an equal dose of the enzyme on an untreated preparation (Fig. 5). Atropine produced about the same degree of inhibition on a total of 6 similar preparations.

*Effect of ganglionic blockade on the response of the guinea-pig ileum segment preparation to chymotrypsin*

Hexamethonium ($C_6$) blocks autonomic ganglia in the intestinal wall (Paton & Zaimis, 1949). The effects of chymotrypsin were tested in the presence of sufficient hexamethonium to block the effects of five times the dose of nicotine previously giving a response (Fig. 5B, g). Comparison of this response with that evoked by the same dose of chymotrypsin in an untreated preparation (Fig. 5A, d and 5C, g) indicated that stimulation of the ganglion cells did not contribute to the contractions produced by chymotrypsin. Similar results were obtained on a total of eight preparations.

*Effect of the antihistamine cyproheptadine on the response of the guinea-pig ileum segment preparation to chymotrypsin*

The ability of the ileum preparation to respond to chymotrypsin appears to be related to its ability to respond to histamine. Twelve of 16 preparations failed to respond to

chymotrypsin (500 $\mu$g) in the presence of sufficient cyproheptadine ($2 \times 10^{-7}$) to block the effects of a dose of histamine 10 times that previously giving a response. The other four preparations gave a minimal response to an equal dose of the enzyme. In 14 of 15 untreated preparations chymotrypsin (500 $\mu$g) induced a marked contraction (Fig. 5C, g). However, to a second dose of chymotrypsin, twice the size of the first, seven preparations failed to respond, two gave a slightly larger response and five gave a smaller response.

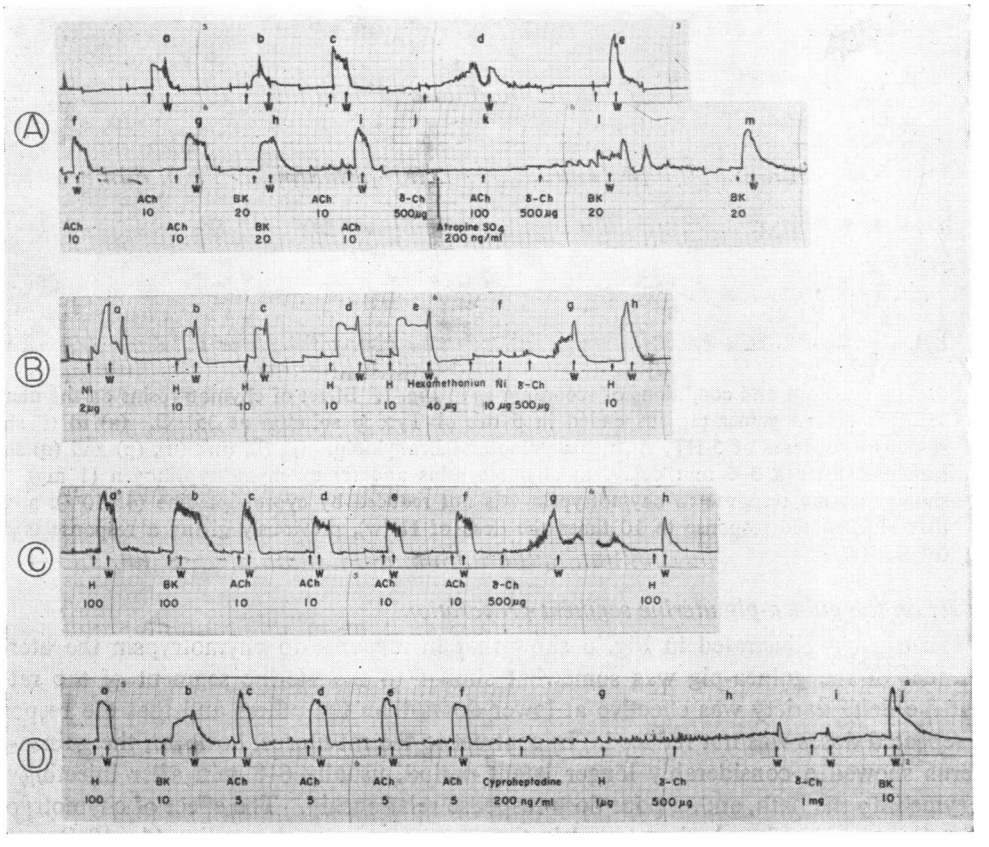


Fig. 5. Abbreviations and conditions of recording as in Fig. 1. Effects of atropine, hexamethonium and cyproheptadine on the response to chymotrypsin (Ch) of segments of guinea-pig ileum in 5 ml. of Tyrode solution at 35° C. In part A, the two records are taken from different segments. The upper row (d) illustrates responses of one to δ-Ch (500 $\mu$g at d). Responses to ACh (a and c) and Bk (b and e) are also shown. The lower row shows that δ-Ch (500 $\mu$g at k) produced a response in the presence of atropine ($2 \times 10^{-7}$) sufficient to block 10 times the dose of ACh (k), previously giving responses (f, g and i). The response to BK (compare m and h) was slightly enhanced. Part B: illustrates the response to nicotine (Ni, 2 $\mu$g at a) and to histamine (10 ng at b, c, d and e). In the presence of $C_6$ (40 $\mu$g) the ileum response to δ-Ch (500 $\mu$g at g) and to histamine (10 ng at h) but not to nicotine (10 $\mu$g at f). Part C: responses of untreated ileum to histamine (100 ng at a and h) Bk (100 ng at b) ACh (10 ng at c, d, e and f) and to δ-Ch (500 $\mu$g at g). Part D: responses of another ileum to histamine (100 ng at a) Bk (10 ng at b) ACh (5 ng at c, d, e and f). With cyproheptadine ($2 \times 10^{-7}$) responses to histamine (1 $\mu$g at g), δ-Ch (500 $\mu$g at h and 1 mg at i) were abolished. Bk (10 ng at j) still produced a response.

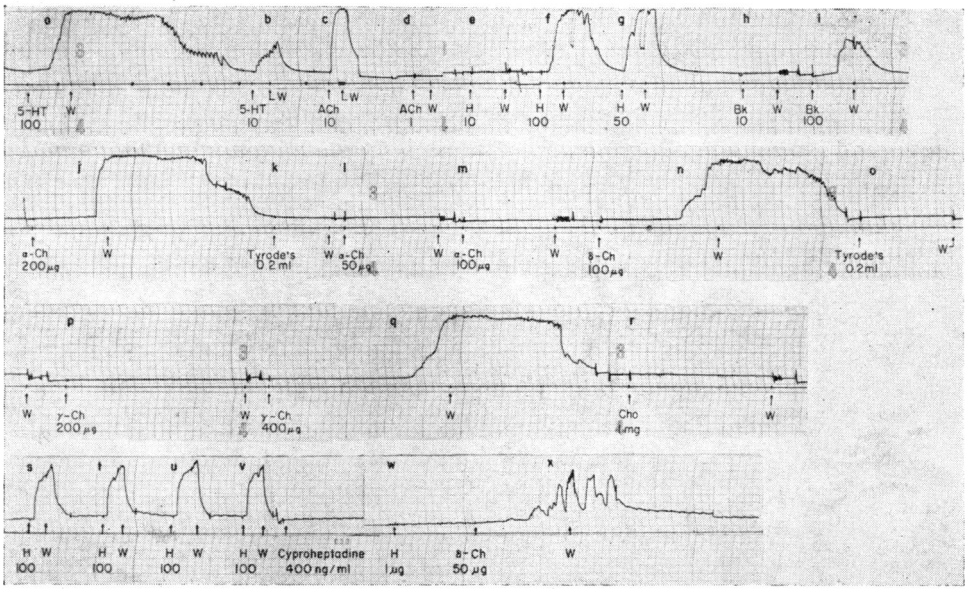


Fig. 6. Abbreviations and conditions of recording as in Fig. 1. Effect of chymotrypsins on the uterine segment of the guinea-pig suspended in 5 ml. of Tyrode solution at 35° C. (a) to (i) show responses to doses of 5-HT, ACh, histamine and Bradykinin. (j), (l), (m), (n), (p) and (q) show responses after a 3–6 min delay, to chymotrypsins and (r) to chymotrypsinogen (1 mg). (x) shows that the response to chymotrypsin was not reduced by cyproheptadine ($4 \times 10^{-7}$), a dose that blocked the response to 10 times the dose of H (w), previously giving a response (s), (t), (u) and (v).

*Effect on the guinea-pig uterine segment preparation*

The tracings illustrated in Fig. 6 show that in response to chymotrypsin the uterine segment of the guinea-pig was somewhat similar to the uterine segment of the rat in that the delta variety was effective at lower doses than the others and that the response to repeated doses was not reduced. In contrast to the rat uterus, however, the guinea-pig uterus showed a considerably longer latent period, usually 6–8 min after injecting the enzyme into the bath, and, a poor dose-response relationship. The effect of chymotrypsin (Fig. 6) was not reduced when tested in the presence of cyproheptadine ($4 \times 10^{-7}$).

## DISCUSSION

Jansen, Fellows Nutting & Balls (1949) showed that the esterase and protease activities of chymotrypsin were equally inhibited by radioactive DFP and that the amount of radioactive phosphorous bound in the completely inhibited enzyme was 1 mole per 1 mole of enzyme. They thus provided evidence that the esterase and protease activities of chymotrypsin are referable to the same functional group. Our experiments have shown that to elicit contractions in isolated smooth muscle preparations the free functional group of the enzyme is also essential. Further evidence that the effects of chymotrypsin described on smooth muscle were mediated by the active centre is provided by the finding that large doses of chymotrypsinogen were inactive.

Since the effect of chymotrypsin on the rat uterus was not reduced by atropine and methysergide, the contractions produced by the enzyme were not due to release of Ach or 5-HT. Histamine, adrenaline and noradrenaline can also be excluded as mediators, since these agents cause relaxation rather than contraction (Kellaway, 1930; Gaddum, 1949). Since the enzyme also retained its full effect when tested repeatedly in succession, chymotrypsin probably exerted a direct effect on the smooth muscle of the rat uterus rather than indirectly through the release of a mediator.

The inhibition of the effect of chymotrypsin on the rat uterus by SBTI, like that of DFP, was specific in that the response to 5-HT or bradykinin similarly reacted with SBTI was unimpaired. But unlike DFP-inhibited chymotrypsin, the soy inhibited enzyme retained its abilty to inactivate synthetic bradykinin. This is possibly explained by the nature of the inhibition imposed by the soy inhibitor. Kunitz (1946) suggested that the reaction between soy inhibitor and chymotrypsin obeys the law of mass action. Thus bradykinin added to a mixture of chymotrypsin and SBTI previously incubated would be inactivated by the free chymotrypsin present in the equilibrum reaction. Presumably the amount of free chymotrypsin in the equilibrium mixture was not enough to stimulate the rat uterine segment to contract.

Our results with chymotrypsin on the rat uterine segment are in contrast to those reported by Edery (1964), who used enzyme preparations from the same commercial source but did not state the variety. In our experiments, alpha, beta and gamma chymotrypsins were effective only at doses of 400 $\mu$g or more, the threshold dose being nearly twice the highest dose tested by Edery on the rat uterus. We found, on the other hand, that delta chymotrypsin was effective at a dose as low as 5 $\mu$g. Edery noted, as we did, and as shown previously by Rocha e Silva (1939), that trypsin in doses of 1 to 50 $\mu$g contracted the rat uterus. We therefore considered the possibility that the effects of chymotrypsins on the rat uterine segment as described herein were due to trypsin, present as an impurity. However, assay of active samples of chymotrypsin for trypsin content showed that less than 0.001% trypsin was present.

The effects of chymotrypsin on the guinea-pig ileum were abolished by cyproheptadine and were therefore probably brought about mainly through the release of histamine. This confirms the observation of Edery (1964) who reported that mepyramine abolished the effect of chymotrypsin on the guinea-pig ileum.

SUMMARY

1. Several varieties of chymotrypsin stimulated isolated smooth muscle segments to contract. The delta variety was the most active.

2. The DFP-treated enzyme was inactive. Since DFP is known to form an irreversible bond with the functional group of chymotrypsin, thus inhibiting its proteinase and esterase activities, the smooth muscle stimulant effect was also mediated by the active centre of the enzyme.

3. The effect of chymotrypsin on the guinea-pig ileum was not due to stimulation of autonomic ganglia, for it was unaffected by hexamethonium. The effect of the enzyme on the ileum was abolished by cyproheptadine. Hence it was due mainly to the release of histamine.

4. Since the effect of chymotrypsin on the rat uterus was not reduced by atropine and methysergide and the response of the guinea-pig uterus was not reduced by cyproheptadine, the enzyme in these preparations probably acted directly on the muscle fibres.

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