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The pharmacological properties of fibrinogen degradation products

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1. A method of obtaining partially purified products of plasmin degradation of fibrinogen (F.D.P.) is described. The F.D.P. passes through dialysing tubes and does not lose its activity during heating for 15 min at a temperature of 56° C.

2. In experiments on isolated organs, F.D.P. in small doses potentiates the contractile action of bradykinin, kallidin, angiotensin, histamine, 5-hydroxytryptamine, acetylcholine, adrenaline and noradrenaline. In high concentrations, F.D.P. induces an increase in initial tonus of the smooth muscles and simultaneously lowers the sensitivity of the smooth muscles to the action of the pharmacologically active substances mentioned.

3. A similarity in the effect of different concentrations of KCl and different concentrations of F.D.P. on the contractile reaction of the guinea-pig intestine to constant doses of histamine and bradykinin and on the initial tonus of the smooth muscles is observed.

4. A hypothesis is presented that F.D.P. in small doses potentiates the contractile action of amines and polypeptides on the smooth muscles and in larger doses increases the initial tonus of these muscles by changing the concentration of the cell electrolytes.

The products of fibrinogen degradation, as well as fibrin itself, potentiate the contractile effects of bradykinin, kallidin, angiotensin and histamine on the isolated guinea-pig intestine (Buluk, Małofiejew & Czokało, 1966). They also potentiate the action of oxytocin on the isolated cornu of the rat uterus (Małofiejew, 1967b) and *in vivo* they enhance the permeability of the capillaries of the depilated skin of the guinea-pig (Małofiejew & Buluk, 1968) and potentiate the hypotensive effect of bradykinin and hypertensive effect of angiotensin in the rat (Małofiejew, 1967a).

Gladner, Murtaugh, Folk & Laki (1963) described the potentiation of the contractile effect of bradykinin on the isolated cornu of the rat uterus by fibrinopeptide B. The contractile effect of bradykinin on the isolated guinea-pig intestine is also potentiated by products of proteolytic degradation of some protein fractions of the plasma (Hamberg, 1966), kutapressin (Tewksbury & Stahmann, 1965), products obtained from snake poisons (Fereira, 1966), substances with free SH groups (Doleschel & Auerwald, 1966) and chymotrypsin (Edery, 1966).

The various reports concerning the potentiation of the contractile effect of different biologically active substances on the smooth muscle *in vitro* suggest that such a potentiation of the action of these substances may occur in the organism. In particular, the products of fibrinogen degradation which are always present (Merskey, Kleiner & Johnson, 1966 ; Das, Allan, Woodfield & Cash, 1967) and the products of proteolytic degradation of other proteins of the blood plasma may take part in this phenomenon.

The aim of these investigations was to elucidate the action of products of plasmin degradation of fibrinogen on the smooth muscle *in vitro*.

Methods

The tyrosine content of the protein preparations in an incubated mixture of fibrinogen and plasmin preparations, before and after centrifugation of the sediment formed during heating, and in the dialysate of the material, was determined by the Folin–Ciocalteau method (1927). Activation of plasmin by urokinase was measured by the method of Buluk, Januszko & Olbromski (1966).

The plasmin degradation products of fibrinogen (F.D.P.) were obtained from an aqueous solution, alkalized with $NaHCO_3$ to pH 7.4, containing, in 1 ml. of the system, fibrinogen (about 1,000 μg tyrosine) and 10 fibrinolytic units of plasmin (about 70 μg tyrosine). During incubation at a temperature of 18° C the incubate produced a progressive potentiating action of bradykinin and an increase in initial tonus of the isolated guinea-pig intestine. The activity of the fibrinogen degradation products was evident in the first few minutes of incubation of the system and reached its highest level between 70 and 90 min, after which it decreased. After establishing the pharmacological activity, the incubate was heated for 15 min at a temperature of 56° C, centrifuged and the supernatant was drawn off. The supernatant was found to contain F.D.P., and while retaining on an average only 30% of the tyrosine, to have a similar activity to that of the crude material before heating. The F.D.P. preparation thus obtained was dialysed through the dialysing tubes with a double volume of distilled water (dialysis time 20–26 hr, temperature 4° C). The fluid from outside the dialysing tube, the " dialysate," contained 2–5% of the tyrosine contained in the crude material. The procedure by which F.D.P. was obtained was repeated several times and it was found that when constant amounts of fibrinogen and plasmin were used the products formed in the different preparations possessed practically the same activity, calculated in μg of tyrosine content. For experiments, or for further steps in chemical purification, after evaporation at a temperature of 45° C, the dry product with F.D.P. was dissolved in distilled water, 1/50–1/100 of the initial volume of crude material.

Pharmacological experiments were carried out on the following isolated organs: guinea-pig intestine in Tyrode solution (Magnus, 1904) ; rat duodenum in Tyrode solution (Vane, 1964) ; rabbit aorta strip in Krebs solution (Furchgott & Bhadrakom, 1953).

Experiments to determine the pharmacological action of F.D.P. were carried out in an 18 ml. organ bath at a temperature of 31° C. After determining the reactivity of the isolated muscle preparation to known doses of the pharmacologically active substance, its reactivity to the same substance with F.D.P. was investigated. The potentiating action of F.D.P. was then measured after adding to the organ bath a

dose of F.D.P. expressed in μg of tyrosine. The reaction of the organs within 2 min of incubation was recorded on the kymogram after which, without washing the organ bath, a control dose—that is, the usual threshold dose of the pharmacologically active substance—was added and the amplitude of the contraction was recorded. After determination of the contraction, the F.D.P. was washed from the bath together with the substance investigated. The record of the contractions of the smooth muscles of the isolated organs was made on a kymograph with a lateral recorder 400 mm in length and a ratio of the arms of 5:1. The smoked-paper drum rotated at 7.5 mm/min. A typical experiment carried out on an isolated guinea-pig intestine is shown in Fig. 1. In all the curves illustrating the successive experiments, the ordinates denote the contraction or, depending on the organ investigated, relaxation of the smooth muscles in mm and the abscissae denote on a logarithmical scale, the doses of the investigated substance in grams. The effect of concentrations of potassium in the nutritive solution on the sensitivity and initial tonus of the guinea-pig intestine was investigated in the following way. The isolated intestine was incubated for 30 min in a nutritive solution without potassium, then in a solution containing 5, 10, 20 and 40 mM KCl. The osmolarity was maintained by an appropriate change in the glucose concentration. For these investigations the nutritive fluid used contained (mM):

KCl	—	5	10	20	40
NaCl	100	100	100	100	100
$CaCl_2$	1.8	1.8	1.8	1.8	1.8
NaH_2PO_4	0.36	0.36	0.36	0.36	0.36
$NaHCO_3$	5.8	5.8	5.8	5.8	5.8
Glucose	84	74	64	44	4

The experiments were carried out at a constant temperature of 31° C. After incubating the isolated intestine in the solution for 30 min, the amplitude of the initial tonus was measured and the sensitivity of the intestine to a constant dose of bradykinin or histamine was determined.

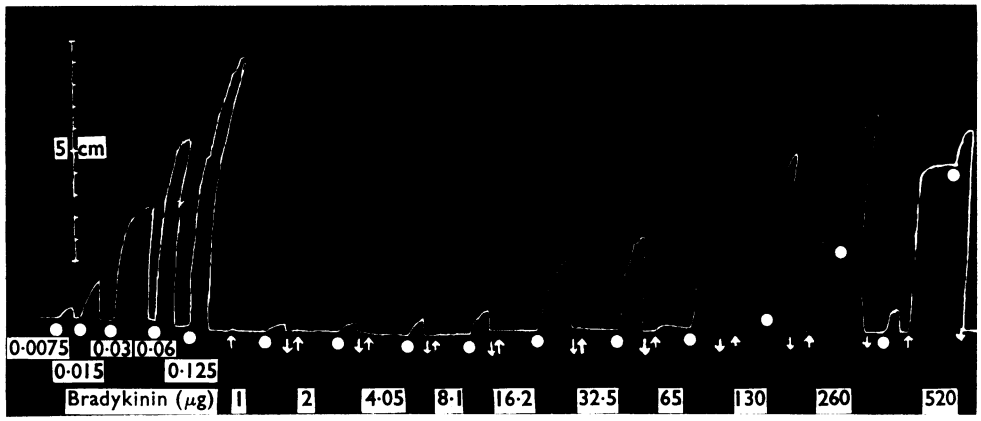
Materials

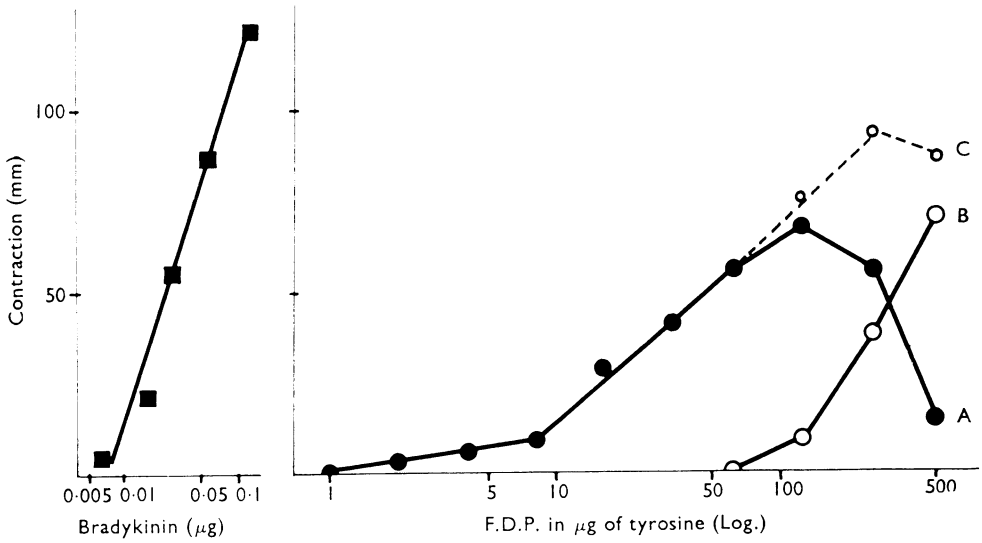
1. Fibrinogen of bovine blood (Kekwick, Mackay, Nance & Record, 1955).
2. Plasminogen from ox blood plasma (Buluk, Januszko & Olbromski, 1966).
3. Urokinase from human urine (Januszko & Dubińska, 1965).
4. Tyrode solution (Bjüro, 1963).
5. Krebs solution (Regoli & Vane, 1964).
6. Synthetic bradykinin (BRS 640, Sandoz).
7. Synthetic kallidin (KL-698, Sandoz).
8. Synthetic angiotensin (Hypertensine, Ciba).
9. Synthetic oxytocin (Richter).
10. Histamine hydrochloride (Polfa).
11. Acetylcholine (Le Roche).
12. 5-Hydroxytryptamine (serotonin, Sandoz).
13. Adrenaline (Byk).
14. Noradrenaline (Lewonor, Polfa).
15. Urethan (Xenon, Łódź).
16. Dialysing tubes (20 mm diameter, Kemikaiebolaget Kebo, Stockholm).

Results

Effect of the fibrinogen degradation products on the isolated guinea-pig intestine

The left side of Fig. 1 shows the reaction of the isolated guinea-pig intestine to increasing doses of bradykinin. The right hand side shows the behaviour of the initial tonus and the sensitivity of the intestine to bradykinin after incubation for 2 min with increasing concentrations of F.D.P. For the investigations, an F.D.P.





FIGS. 1 AND 2. Effect of plasmin degradation products of fibrinogen (F.D.P.) on the initial tonus and reactivity of the isolated guinea-pig intestine to bradykinin. Fig. 1: ↑, Flow of Tyrode solution cut off and F.D.P. with a definite tyrosine content introduced into the organ bath; ●, addition of 0.0075 μg bradykinin to the organ bath; ↓, restoration of flow of Tyrode solution and washing of F.D.P. and bradykinin out of the organ-bath. Fig. 2: ■, Intestinal contraction induced by different doses of bradykinin; A, intestinal contraction under the influence of 0.0075 μg bradykinin in the presence of increasing concentrations of F.D.P.; B, initial tonus of intestine in the presence of different concentrations of F.D.P.; C, the sum of the initial tonus and the intestine contraction under the influence of different doses of F.D.P. and 0.0075 μg bradykinin.

preparation containing from 1 to 520 μg of tyrosine and the dose of bradykinin which induced the first registrable contraction (0.0075 μg) were used. The results obtained in the experiment presented on the kymogram are shown in the form of a graph in Fig. 2. As can be seen from the curves, F.D.P. in concentrations up to 65 μg of tyrosine only potentiates the action of bradykinin, whereas a further increase in the F.D.P. concentration induces an increase in the initial tonus and lowers the sensitivity of the intestine to bradykinin. It should be noted that the initial tonus does not exceed the maximal contraction of the organ used for the investigations.

In further investigations into the effect of F.D.P. on the potentiation of the action of other active substances, a preparation of F.D.P. containing 65 μg tyrosine was used. This is the F.D.P. dose, which, as can be seen from Fig. 1 and Fig. 2, does not induce an initial tonus measurable in the conditions of these experiments but gives the greatest potentiation.

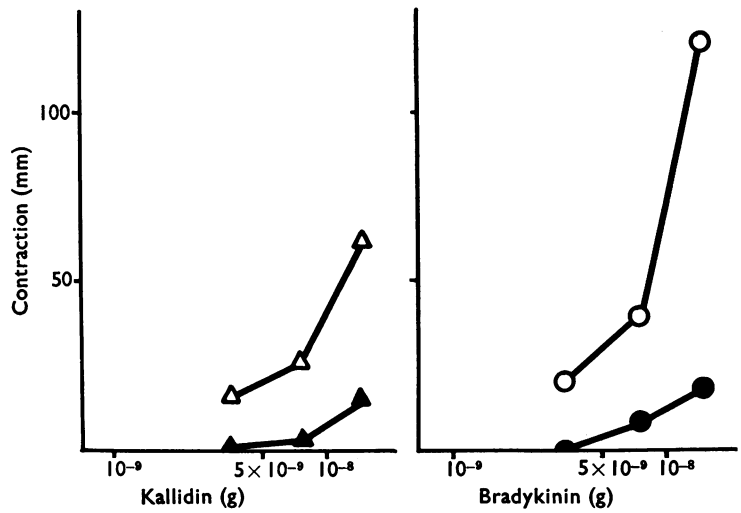


FIG. 3. Potentiation of the action of increasing concentrations of bradykinin and kallidin under the influence of a constant dose of F.D.P. containing 65 μg tyrosine on the isolated guinea-pig intestine in 18 ml. organ bath. ▲, Kallidin ; △, kallidin + F.D.P. ; ●, bradykinin ; ○, bradykinin + F.D.P.

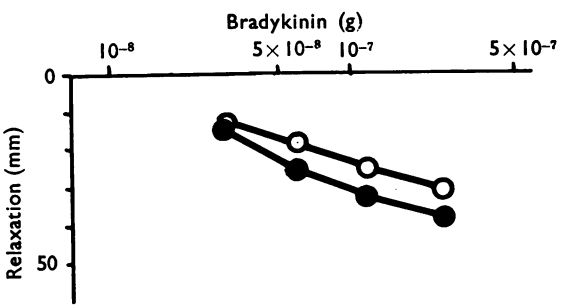


FIG. 4. Behaviour of the isolated rat duodenum under the influence of increasing doses of bradykinin (●) and bradykinin + F.D.P. containing 100 μg tyrosine (○).

Potentiation of the action of polypeptides

The contractile effects of bradykinin and kallidin were potentiated by F.D.P. (Figs. 3 and 4). After action for 2 min on the intestine, F.D.P. caused a 3.5×10^{-9} g dose of bradykinin or kallidin to induce a reaction of the intestine corresponding to 1.6×10^{-8} g of these polypeptides without the presence of F.D.P.

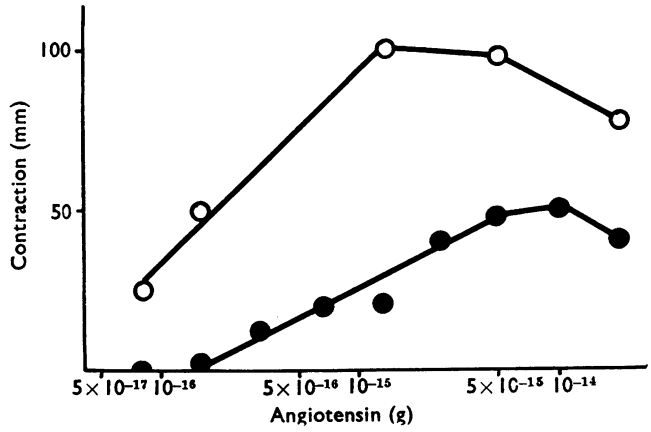


FIG. 5. Effect of angiotensin (●) and angiotensin + F.D.P. containing 65 μg tyrosine (○) on the isolated guinea-pig intestine.

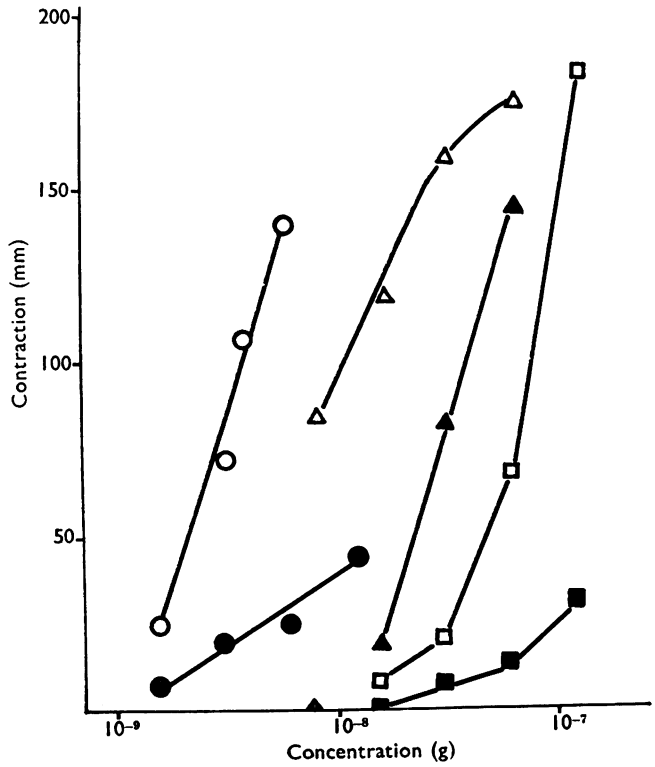


FIG. 6. Effect of 5-hydroxytryptamine (●), acetylcholine (▲), histamine (■) and 5-hydroxytryptamine + F.D.P. (○), acetylcholine + F.D.P. (△) and histamine + F.D.P. (□) on the isolated guinea-pig intestine. The doses of F.D.P. = 65 μg tyrosine.

In the experiments on the isolated rat duodenum (Fig. 4) 3×10^{-8}–1.2×10^{-7} g doses of bradykinin induced a relaxation of the intestine in proportion to the concentration of that substance. Incubation of the preparation of the rat duodenum with F.D.P. (10–200 μg tyrosine) did not alter the reaction of the organ to bradykinin. Only very high doses of F.D.P. which markedly increased the initial tonus of the duodenum, caused a lessening of the relaxation reaction to bradykinin.

The amplitude of the contraction of the isolated guinea-pig intestine is proportional to the doses of angiotensin within the limits of 10^{-16} and 5×10^{-15} g. Higher concentrations of angiotensin result in a lessening of the contractile reaction of the intestine (tachyphylaxis).

When the isolated intestine was incubated with F.D.P. containing 65 μg tyrosine, an approximately half threshold dose of angiotensin induced a contraction corresponding to that induced by 10^{-15} g of angiotensin without F.D.P. In potentiating the action of angiotensin, F.D.P. does not eliminate tachyphylaxis.

Potentiation of the action of histamine, 5-hydroxytryptamine and acetylcholine

As in the experiments described previously, F.D.P. (65 μg tyrosine) preincubated for 2 min with the isolated intestine of the guinea-pig caused a marked potentiation of the contracting action of histamine, 5-HT and acetylcholine (Fig. 6).

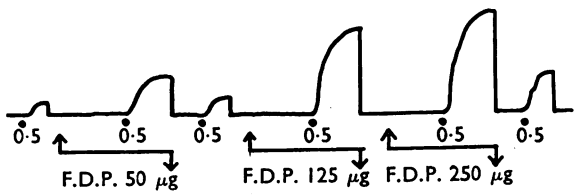


FIG. 7. Behaviour of the rabbit aorta strip under the influence of 0.5 μg noradrenaline before and during incubation with F.D.P. containing 50, 125 and 250 μg tyrosine. ●, 0.5 μg noradrenaline ; ↑, closure of flow of Krebs solution and introduction of F.D.P. into organ bath ; ↓, restoration of flow of Krebs solution and washing F.D.P. and noradrenaline from organ bath.

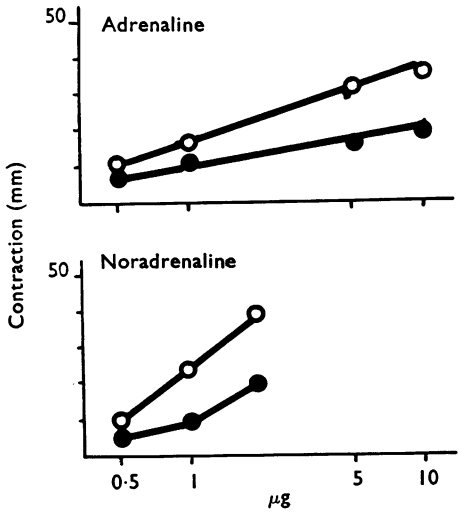


FIG. 8. Behaviour of rabbit aorta strip under the influence of increasing doses of adrenaline alone and noradrenaline alone (●) and adrenaline or noradrenaline in the presence of F.D.P. containing 125 μg tyrosine (○).

Effect of F.D.P. on the contraction of an aorta strip induced by noradrenaline and adrenaline

The slow contraction of the rabbit aorta strip under the influence of adrenaline and noradrenaline is also potentiated by F.D.P.

Figure 7 shows the reaction of the aorta strip to 5×10^{-7} g noradrenaline before and after incubation for 2 min with F.D.P. F.D.P. containing from 50 to 250 μg tyrosine cannot alone induce contraction of the aorta strip and the maximal potentiation is brought about by F.D.P. containing 250 μg of tyrosine. This dose was used in further experiments on the potentiation of the effect of noradrenaline and adrenaline.

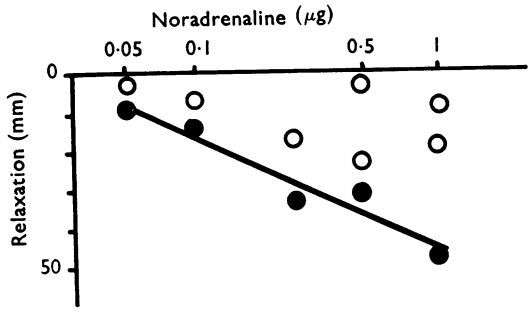


FIG. 9. Behaviour of the rat duodenum under the influence of increasing doses of noradrenaline (●) and noradrenaline+ F.D.P. containing 100 μg tyrosine (○).

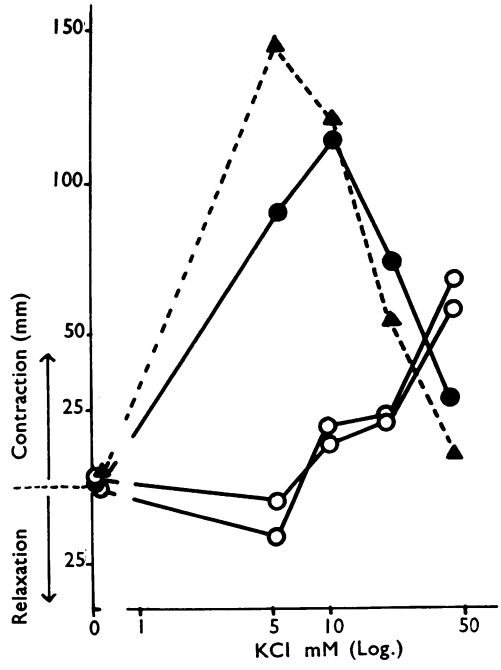


FIG. 10. Behaviour of the initial tonus (○) and the contraction of the guinea-pig intestine under the influence of a constant dose of bradykinin (0.1 μg, ●) or a constant dose of histamine (0.005 μg, ▲) in different concentrations of KCl.

Figure 8 shows the contraction of the aorta under the influence of increasing doses of adrenaline and noradrenaline before and after incubation for 2 min with F.D.P. The comparatively weak potentiating effect, observed only after the application of large doses of F.D.P. (approximately 125 μg tyrosine) may be due to the limited contractility of the aorta strip of the rabbit.

Effect of F.D.P. on the relaxation of the rat duodenum induced by noradrenaline

Under the influence of increasing doses of noradrenaline, the amplitude of the relaxation of the smooth muscles of the rat duodenum is proportional to the doses of noradrenaline. F.D.P. does not potentiate the relaxing action of noradrenaline and after incubation for 2 min with F.D.P. the relaxing action is decreased (Fig. 9).

Effect of the potassium concentration in the nutritive fluid on the initial tonus and sensitivity of the isolated guinea-pig intestine

An increase in the KCl concentration to 10 mM increases the contractile reaction of the intestine to a constant dose of bradykinin and histamine. With concentrations over 10 mM KCl, the initial tonus of the intestinal muscle was increased and the amplitude of the contraction under the influence of bradykinin and histamine reduced. The similarity between the behaviour of the intestine under the influence of increasing concentrations of F.D.P. and its behaviour under that of increasing concentrations of KCl is noteworthy (Fig. 10).

Discussion

In this paper a method is described by which partially purified pharmacologically active products of the plasmin degradation of fibrinogen (F.D.P.) which are thermostable and permeate the dialyser, can be obtained. In an organ-bath 18 cm^3 in volume, containing an isolated guinea-pig intestine, F.D.P. containing 16 μg tyrosine, potentiated the contractile action of bradykinin, kallidin, angiotensin, histamine, 5-hydroxytryptamine and acetylcholine, and F.D.P. containing more than 65 μg tyrosine increased the initial tonus of the organ investigated.

It was previously found that the isolated cornu of the rat uterus without spontaneous contractile action reacted in the same bath, in Jalon solution, to a dose of F.D.P. approximately 100 times smaller than the dose to which the guinea-pig intestine reacted (Małofiejew, 1967b).

F.D.P. potentiates only the contractions of the smooth muscles—that is, it potentiates the contractile action of bradykinin on the isolated guinea-pig intestine and the action of noradrenaline on a rabbit aorta strip, whereas it does not potentiate the relaxation of the rat duodenum induced by the action of bradykinin or noradrenaline.

We carried out experiments on the isolated guinea-pig intestine, similar to those of Csapo (1956, 1962) on the isolated cornu of the rat uterus with different concentrations of KCl. In these experiments, an increase in the concentration of KCl caused changes in the contraction of the intestine after constant doses of bradykinin or constant doses of histamine, and changes in the initial tonus of that muscle. It is noteworthy that, with different concentrations of KCl and of F.D.P., there is a remarkable similarity in the behaviour of the guinea-pig intestine in both its reaction to the contractile stimuli and in the increase of initial tonus.

This similarity in the behaviour of the smooth muscles in various concentrations of KCl and different concentrations of F.D.P., suggests that F.D.P. may modify the properties of the cell membrane of the smooth muscle by changing the ionic gradients inside and outside the cell.

Of all the substances so far described which potentiate smooth muscle contractions under the influence of pharmacologically active products, only F.D.P. has been found to exert such an extensive influence in potentiating the contractions induced by different amines and polypeptides of the organism. It may therefore be assumed that the effect of the fibrinogen degradation products concerns a common mechanism affecting tonus and contraction of smooth muscle whatever pharmacological stimulus is applied.

This hypothesis has also been corroborated by some recent investigations (Małofiejew, unpublished) which showed that F.D.P. in certain limited concentrations caused depolarization of the smooth muscle cells of the rat uterine cornu and enhanced the amplitude and frequency of the action potentials.

The increase in muscle tonus and fall in the reactivity to stimuli in the form of amines or polypeptides, observed when higher doses of F.D.P. were administered, not only supports the hypothesis of the influence of F.D.P. on changes in the internal and external ion concentration of the cells but also explains why F.D.P. does not potentiate the relaxing effect of bradykinin and noradrenaline on the rat duodenum and even, in the case of the action of the amines, cause a lessening of the relaxation reaction.

The results of our investigation, the short half-life of fibrinogen and the constant presence of fibrinogen degradation products in the circulating blood, justify the view that the role of fibrinogen is not limited merely to participating in the process of haemostasis and repair of damaged tissue, but that it also has an effect on the reactivity of the tissues and modifies the influence of active substances such as amines and polypeptides in the organism.

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