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PHARMACOLOGICAL ACTIONS OF ELEDOISIN ON EXTRAVASCULAR SMOOTH MUSCLE

BY

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Some general effects and the action of eledoisin on a number of isolated smooth muscle preparations have been studied. It has been found that eledoisin possesses a potent stimulating action on all preparations of gastro-intestinal smooth muscle examined and also on the bronchial muscle of the guinea-pig *in situ*. Preparations of other smooth muscles were less sensitive. The rabbit colon, the guinea-pig ileum and, subordinately, the rabbit uterus and the frog stomach may be profitably used, owing to their sensitivity and the satisfactory dose/response relationship, for the quantitative bioassay of eledoisin in crude or pure preparations of the polypeptide. In parallel assays eledoisin could be easily distinguished from the biogenic amines and from all other known naturally occurring hypotensive polypeptides (bradykinins, wasp kinin, bradykinin-like polypeptides of the amphibian skin, physalaemin, substance P). In the unanaesthetized dog, subcutaneous doses of 25 to 100 μg/kg eledoisin caused a powerful stimulation of the motility and some secretions of the alimentary canal. This effect was much less pronounced in the rat and the rabbit.

Eledoisin is the active endecapeptide isolated from methanol extracts of the posterior salivary glands of *Eledone moschata* and *E. Aldrovandi*, two molluscan species belonging to the octopod Cephalopods (Erspamer & Anastasi, 1962 ; Anastasi & Erspamer, 1962).

In this paper some general effects of eledoisin and its action on a number of extravascular smooth muscle preparations will be described. Furthermore, some isolated smooth muscle preparations will be described, which seem to be best suited for the quantitative bioassay of eledoisin. Eledoisin may be easily distinguished, by parallel assays, from all biogenic amines and from all naturally occurring polypeptides with hypotensive action.

METHODS

Rabbit colon. A segment of terminal large intestine 5 to 6 cm long (the whole narrower smooth portion caudal to the sacculated colon is suitable) was suspended in a 10 ml. bath using Tyrode solution at 37° C. The tissue was stored at 4 to 5° C for 12 to 48 hr before use. A dose cycle of 5 to 7 min with 2 to 3 min contact was used.

Guinea-pig ileum. A segment of terminal ileum 4 to 5 cm long from starved guinea-pigs (250 to 400 g) was suspended in a 10 ml. bath of Krebs solution at 32° C. The tissue was used either immediately after its removal or stored at 4 to 5° C for 12 to 24 hr. A dose cycle of 2 to 3 min with 30 to 60 sec contact was used.

Dog duodenum and dog colon. Strips of intestinal wall, 5 to 7 cm long and 1 to 1.5 cm wide, were suspended in a 10 ml. bath of Tyrode solution at 37 to 38° C. A dose cycle of 5 to 7 min was used, the drugs being allowed to act for 2 to 3 min.

Cat ileum, cat colon, monkey (Papio hamadryas) *ileum, monkey colon, human appendix, hog colon.* The method used for these preparations was the same as for the dog intestine. The tissues were used either immediately after their removal or stored at 4 to 5° C for 3 to 5 hr. The human appendix was removed operatively. Only segments having a normal appearance were used.

Rat stomach. The method described by Vane (1957) for the estimation of 5-hydroxytryptamine was followed. The stomach was suspended in a 10 ml. bath containing Tyrode solution at 37° C. A dose cycle of 5 to 6 min, with 2 min contact, was used.

Rat duodenum. The proximal 3 to 4 cm of duodenum from rats weighing 200 to 250 g was suspended in a bath of 10 ml. of Krebs solution at 30° C. The tissue was stored at 4° C for 3 to 4 hr before use. A dose cycle of 3 to 5 min with 30 to 60 sec contact was used.

Rat colon. A 5 to 7 cm segment of colon was suspended in a 10 ml. bath of de Jalon solution at 28 to 30° C. The same dose cycle as for rat duodenum was used.

Fowl rectal caecum. The fowl rectal caecum was prepared and used as described by Cleugh, Gaddum, Holton & Leach (1961).

Frog stomach and frog intestine. The whole stomach or a 5 cm segment of intestine from frogs (*Rana esculenta*) weighing 25 to 35 g was suspended in a 10 ml. bath, using Tyrode solution for cold-blooded animals. A dose cycle of 8 to 10 min was used, the drugs being allowed to act for 3 to 4 min.

Rat uterus. Ovariectomized rats weighing 120 to 200 g were injected, on two successive days, with two doses of oestradiol dipropionate (200 μg for each dose) 3 to 6 days before use. Uteri were suspended in a 10 ml. bath of Tyrode solution at 30° C. A dose cycle of 4 to 6 min with 2 min contact was satisfactory.

Rabbit uterus, cat uterus, guinea-pig uterus and hog uterus. The whole uterus (guinea-pig) or uterine strips from non-pregnant animals were suspended and treated exactly as above.

Cat urinary bladder, dog ureter, hog ureter, dog gall bladder, rat seminal vesicles. Longitudinal strips of urinary bladder, the whole gall bladder, both seminal vesicles ligated together at their blind end, and 6 to 8 cm segments of ureter were suspended in a 10 ml. bath of Tyrode solution at 37 to 38° C. Dose cycles were of 10 to 15 min with 2 to 5 min contact.

Composition of bath fluids. The composition of the bath fluids was as follows:

(a) Tyrode solution: sodium chloride 8 g, potassium chloride 0.2 g, calcium chloride 0.2 g, magnesium chloride 0.1 g, sodium bicarbonate 1 g, sodium dihydrogen phosphate 0.05 g/l. The Tyrode solution for poikilothermic animals was prepared by adding 300 ml. of distilled water to 1,000 ml. of normal Tyrode solution.

(b) Modified Krebs solution: sodium chloride 8 g, potassium chloride 0.4 g, calcium chloride 0.3 g, magnesium sulphate 0.2 g, sodium bicarbonate 1 g, sodium dihydrogen phosphate 0.15 g, glucose 1 g/l.

(c) de Jalon solution: sodium chloride 9 g, potassium chloride 0.4 g, calcium chloride 0.06 g, sodium bicarbonate 0.5 g, glucose 0.5 g/l.

The bath was generally washed out by upward displacement and overflow. Air was bubbled through the bath fluids.

Intact unanaesthetized animal. Eledoisin in physiological saline was injected subcutaneously or intravenously into dogs, rabbits and rats to study the effects of the polypeptide on the gastro-intestinal motility, as shown by vomiting and bowel evacuation, as well as on salivary and other secretions.

Bronchial smooth muscle in vivo. Bronchoconstriction, i.e. resistance of the lungs to inflation, was measured according to Collier, Holgate, Schachter & Shorley (1960) in guinea-pigs anaesthetized with urethane, 1 to 1.3 g/kg, by intraperitoneal route.

Drugs used. Eledoisin used in the present experiments was either the pure natural or synthetic polypeptide or a partially purified extract of posterior salivary glands of *Eledone* containing 50 μg eledoisin per mg. It was shown that in this extract eledoisin was the sole substance with activity on smooth muscles.

The available synthetic eledoisin had 75 to 80% of the activity of pure natural eledoisin.

Wasp kinin was used as the dried venom sacs of *Polistes gallica* washed with alcohol according to Holdstock, Mathias & Schachter (1957). 1 mg of the preparation showed on the rat uterus the activity of 12 μg bradykinin.

We are grateful to Professor von Euler, Stockholm, and to Dr T. B. B. Crawford, Edinburgh, for preparations of substance P containing 75 u./mg and 12 u./mg, respectively; to Dr W. Vogt, Göttingen, for a sample of "Darmstoff" containing 8,000 u./ml. butanol; and to Messrs Sandoz, Basle, for samples of synthetic eledoisin, synthetic bradykinin, (+)-lysergic acid diethylamide and 2-bromolysergic acid diethylamide.

Other drugs were obtained from the following sources: 5-hydroxytryptamine and creatinine sulphate, and acetylcholine bromide—Farmitalia, Milan; histamine dihydrochloride—Hoffman La Roche, Basle.

RESULTS

General effects in the unanaesthetized animal

Dog. Experiments were carried out on 7 mongrel dogs, weighing 7 to 15 kg.

Dog A was given by subcutaneous route, at the inguinal region, an amount of concentrated purified *Eledone* extract (5 ml.) corresponding to 100 μg eledoisin/kg. After a few minutes vomiting occurred, accompanied by profuse salivation. Vomiting was at first alimentary, then the emesis episodes, of increasing severity, resulted in the ejection of masses of mucus, sometimes spotted with blood. Shortly after commencement of vomiting there was a discharge of formed stools, soon followed by evacuation of watery stools containing mucus and blood, accompanied by violent tenesmus. The tremendous gastrointestinal stimulation, joined to profound depression of the animal, lasted unchanged for 1 hr, then gradually decreased. During the night there was complete recovery and for a few days afterwards the dog showed unusual voracity.

Dogs B and C received a subcutaneous dose of *Eledone* extract corresponding to 50 and to 25 μg/kg eledoisin, respectively. Stimulation of salivary secretion and of gastrointestinal motility, as well as general depression, was as intense as in dog A. However, full recovery was more rapid.

Doses of extract corresponding to 10 μg/kg eledoisin (dogs D and E) caused moderate salivation, lasting for 20 to 40 min, and one or two evacuations of formed stools. There was neither vomiting nor diarrhoea.

No evident gastrointestinal effects, except slight salivation in one animal, appeared in dogs F and G injected with the *Eledone* extract corresponding to 5 μg/kg eledoisin.

Dogs C and E were given, 30 min before the injection of the *Eledone* extract, a subcutaneous dose of 0.15 mg/kg atropine sulphate. The alkaloid failed to block stimulation of either salivary glands or gastrointestinal smooth muscle.

Rat. Groups of 2 to 3 rats weighing 150 to 180 g were given, by intraperitoneal route, a purified posterior salivary gland extract corresponding to 300, 600, 1,500 and 2,500 μg/kg eledoisin, respectively.

All animals presented, within 1 min, a more or less intense flushing of the ears, snout and paws, accompanied not only by general depression and some respiratory distress, but also by increased salivation and nasal secretion and, possibly, by increased lacrimal secretion. Nasal secretion was sometimes slightly haemorrhagic. There was no chromodacryorrhoea, and no diarrhoea or other signs of gastro-intestinal stimulation. Full recovery took 20 to 120 min.

Rabbit. Four rabbits, weighing 1.8 to 2.5 kg, were given into the ear vein a purified salivary extract corresponding to 50 to 150 μg/kg eledoisin. The animals presented evident dilatation of the ear vessels, myosis, and some general depression. There was no evacuation of the bowel. Full recovery took 20 to 60 min.

General effects in the anaesthetized dog

Intravenous infusion of purified *Eledone* extract at a rate of 10 to 50 ng/kg/min of eledoisin produced neither salivation nor evacuation of the bowel. Infusion rates of 100 to 300 ng/kg/min of eledoisin, on the other hand, caused salivation and evacuation of liquid stools. Stimulation of both salivary glands and gastro-intestinal smooth muscle was atropine-resistant. High infusion rates often produced a more or less evident cutaneous vasodilatation.

Action on the bronchial smooth muscle in vivo

The threshold dose of intravenous eledoisin causing an increased resistance to inflation in the guinea-pig ranged from 0.3 to 1 μg/kg. Under comparable conditions histamine was (weight for weight) approximately 20 to 25 times less active than eledoisin.

The bronchoconstrictor action of eledoisin, which will be described in detail in another paper, was not affected by pre-treatment of the animal with mepyramine (1 mg/kg, intravenously), atropine (1 mg/kg, intravenously), or acetylsalicylic acid (100 mg/kg, intraperitoneally, suspended in 5% gum acacia). Adrenaline (2–5 μg/kg intraperitoneally), given together with eledoisin, completely suppressed its action on the lungs.

Action on isolated preparations of gastro-intestinal muscle

Rabbit. Isolated loops from all sections of the rabbit intestine responded to eledoisin with contraction. The most suitable segment was the large intestine, and particularly its terminal 20 to 40 cm.

Eledoisin caused the appearance or the reinforcement of rhythmic movements and a tonus increase. The effect was immediate with high doses of the polypeptide, and preceded by a short latency period with small doses. Stimulation was never preceded by depression. High doses of eledoisin produced an intense spasm of the intestinal loop, followed, as spastic contraction slowly subsided, by rhythmic movements of increasing amplitude. Stimulation of the gut often lasted for hours and ceased immediately after washing with fresh nutrient liquid (Fig. 1). There was neither tachyphylaxis nor sensitization.

The stimulant action displayed by 0.5 ng/ml. eledoisin was unaffected by hexamethonium and morphine, in concentrations up to 100 and 200 μg/ml., respectively.

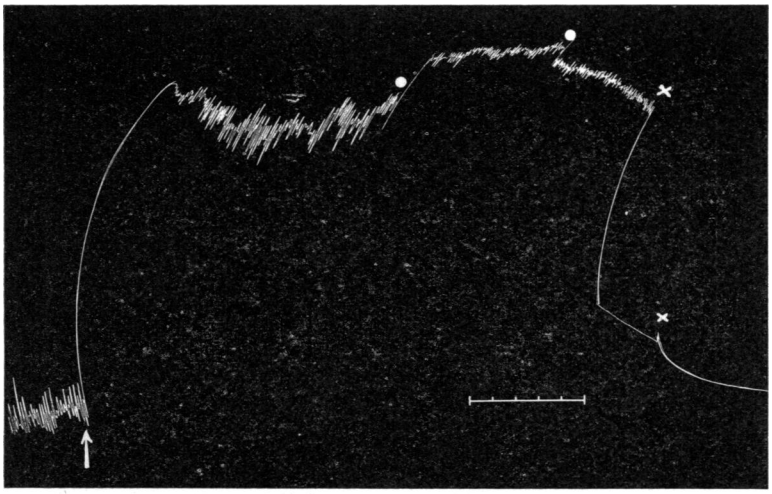

Fig. 1. Rabbit large intestine suspended in 10 ml. Tyrode solution at 37° C. At arrow, 40 μg pure natural eledoisin; at ●, arrest of the drum for 2 hr; at x, washing. Time, 3 min. Eledoisin produced a tremendously intense increase in tone which lasted as long as 5 hr, until washing.

Mepyramine had no action at concentrations of 0.1 to 1 μg/ml.; concentrations of 10 μg/ml. antagonized only moderately the spasmogenic effect of eledoisin. Chlorpromazine and papaverine were ineffective up to 1 μg/ml., but at higher concentrations (3 to 10 μg/ml.) they reduced or even abolished the stimulation due to eledoisin.

Atropine did not affect the response of the gut to eledoisin at concentrations of 0.01 to 1 μg/ml., but reinforced it at higher concentrations (10 μg/ml. and more). Potentiation, which could be promptly abolished by washing with fresh nutrient liquid, was 50% at atropine concentrations of 20 μg/ml. and 100% at concentrations of 100 μg/ml. (Fig. 2).

Antagonism between eledoisin and catecholamines was not very strong: 1 μg/ml. (−)-noradrenaline reduced by 50%, and 1 μg/ml. (−)-adrenaline by 90%, the stimulation elicited by 5 ng/ml. eledoisin.

Nicotine (1 to 100 μg/ml.) and lysergic acid diethylamide and its bromo derivative (10^{-7} to 10^{-5}) failed to produce any change in the response to either eledoisin or substance P.

It has been reported in the preceding paper (Anastasi & Erspamer, 1962) that chymotrypsin and trypsin caused an inactivation of eledoisin, but that there was no reduction of the spasmogenic activity of the polypeptide after incubation with carboxypeptidase.

The rabbit large intestine is one of the best preparations for the qualitative detection and quantitative estimation of eledoisin in tissue extracts. It is extremely sensitive to the polypeptide (threshold concentration 0.3 to 1 ng/ml.) and has a very satisfactory dose/response relationship (Fig. 3). In addition the preparation is rather insensitive to most biogenic substances known to stimulate smooth muscle.

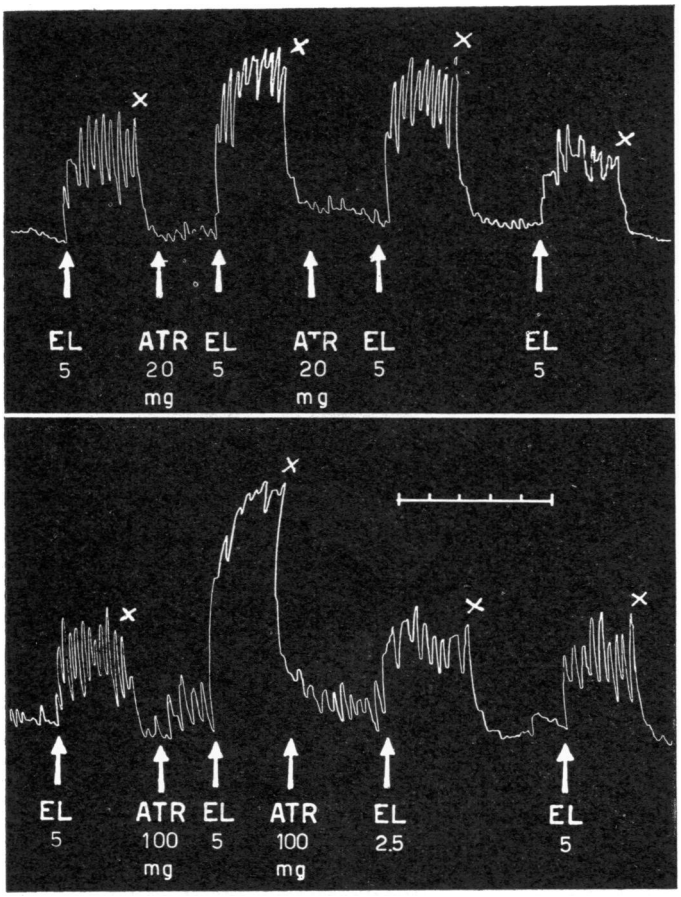

Fig. 2. Rabbit large intestine suspended in 10 ml. Tyrode solution at 37° C. At *EL*, pure natural eledoisin (in ng); at *ATR*, atropine sulphate (in mg); at x, washing. Time, 1 min. Atropine reversibly potentiated the stimulant action of eledoisin. With 100 mg atropine the response to eledoisin was doubled.

5-Hydroxytryptamine, for example, is practically inactive, and histamine is, on a weight basis, more than 1,000 times less active than eledoisin. Bradykinin, with less than one-hundredth of the activity of eledoisin, produces a contraction which is poorly proportional to the dose of the polypeptide and is often preceded by brief depression ; hypertensin has less than one-tenth of the activity of eledoisin ; finally, the contraction produced by Darmstoff (1,000 Vogt units = 50 ng eledoisin) is less prompt than that elicited by eledoisin and is considerably reduced by atropine. The natural polypeptide most similar to eledoisin in its action on the rabbit intestine is substance P. An amount of 1 μg eledoisin corresponds to approximately 200 to 400 v. Euler units, or to 5 to 10 μg pure substance P (Stürmer & Franz, 1961).

The large intestine was most suitable for the bioassay of eledoisin after it had been kept for 12 to 24 hr in Tyrode solution at 3 to 5° C. However, intestines

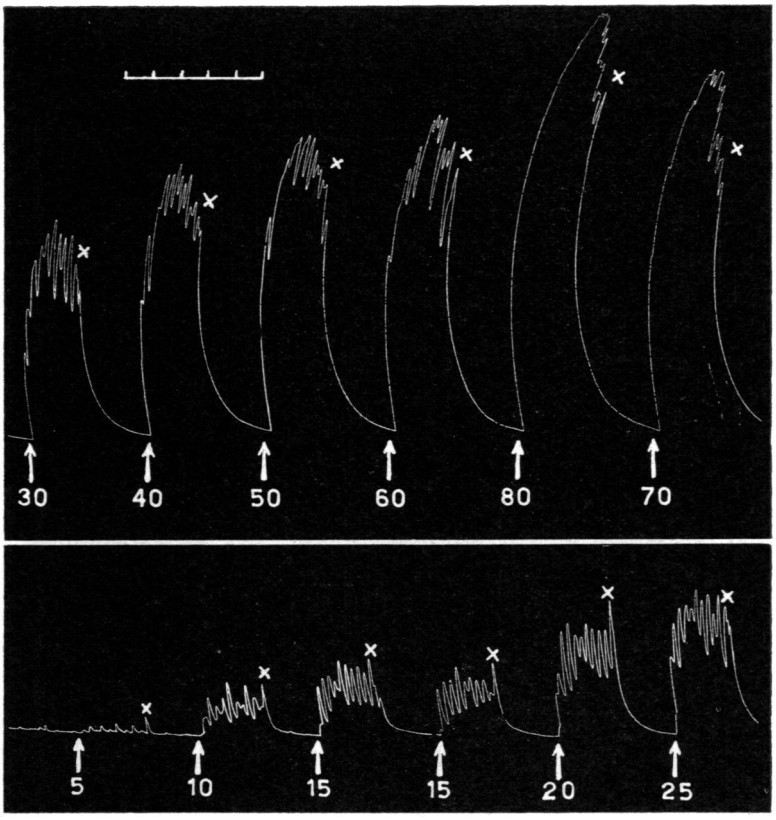

Fig. 3. Rabbit large intestine suspended in 10 ml. Tyrode solution at 37° C. Contractions produced by increasing doses of eledoisin (in ng). At x, washing. Time, 1 min. Note excellent dose/response relationship.

stored in cold Tyrode for a considerably longer time also gave excellent responses. Generally the sensitivity remained unchanged for 3 to 4 days, then it slowly declined, but even nine- to ten-days-old segments were contracted by doses of eledoisin as low as 2 to 3 ng/ml. Responses to substance P and to bradykinin were similar to those seen for eledoisin.

The response of the longitudinal smooth muscle of the rabbit intestine to eledoisin was reduced by lowering the temperature of the bath. A similar reduction was observed with substance P but not with acetylcholine. In fact, at 38° C 1 μg eledoisin was equiactive to 50 μg acetylcholine bromide and to 200 to 260 u. of substance P ; at 19° C again to 200 to 230 u. of substance P but only to 16 to 18 μg acetylcholine. Such temperature sensitivity of the response has been taken as evidence of an indirect effect on intestinal musculature, through stimulation of nervous structures (Pernow, 1960).

Guinea-pig. Like the rabbit intestine, that of guinea-pig, especially the ileum, responded to eledoisin with a pure contraction, the effect produced by the

polypeptide closely resembling that elicited by substance P. The threshold concentration was 0.3 to 1.5 ng/ml. and there was again a good dose/response relationship. Atropine and mepyramine in doses that completely abolished the action of acetylcholine and histamine (0.1 to 0.2 μg/ml.) did not affect the spasmogenic action of eledoisin. The same was true for lysergic acid diethylamide in concentrations up to 1 μg/ml.

Rat. Both rat intestines (duodenum and large intestine) and stomach were contracted by eledoisin, and contraction was never preceded by depression. However, neither duodenum nor large intestine were very sensitive to the polypeptide (threshold concentration 2 to 3 ng/ml.) and, what is more important, the dose/response relationship was not satisfactory. It is well known that bradykinin produces a depression of the tonus of the rat duodenum. When given at the peak of the eledoisin contraction, bradykinin still potently lowered the tonus, but the antagonism towards eledoisin was short-lasting and eledoisin stimulation predominated (Fig. 4).

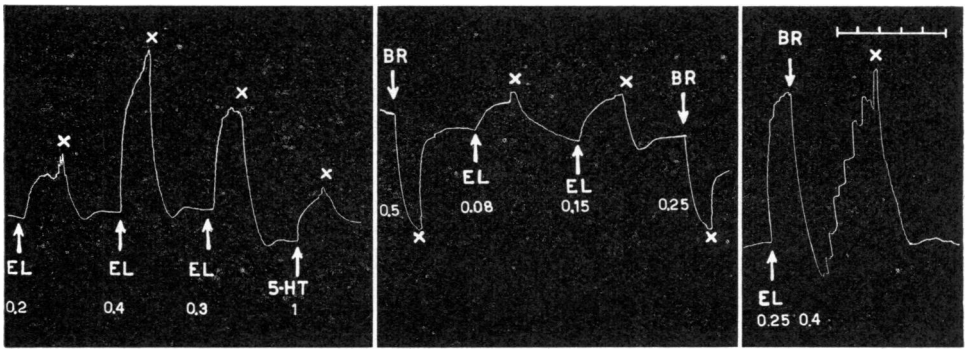

Fig. 4. Rat duodenum suspended in 10 ml. Krebs solution at 30° C. At *EL*, synthetic eledoisin; at *5-HT*, 5-hydroxytryptamine (base); at *BR*, synthetic bradykinin; at x, washing. Time, 1 min. All doses in μg. Like 5-HT, but unlike bradykinin, eledoisin displayed in this preparation a stimulant action. Bradykinin relaxed both the normal gut and that contracted by eledoisin.

Responses to eledoisin more proportional to the dose were obtained with the rat fundus preparation. Threshold concentration was 2 to 4 ng/ml. 1 μg eledoisin was equiactive to 10 to 20 μg bradykinin, 100 to 150 u. substance P, and 0.02 to 0.04 μg 5-hydroxytryptamine.

Dog. Eledoisin produced, both in the duodenum and in the large intestine, an increase in tone which was proportional to the dose. There was also a reinforcement of the rhythmic activity. The threshold concentration was about 1 to 2 ng/ml. Atropine and mepyramine at the usual doses did not affect the eledoisin action. Relaxation consequent to washing with fresh nutrient liquid was gradual.

Bradykinin, like eledoisin, stimulated the dog duodenum. However, it may be seen from Fig. 5 that there was a considerable difference in the shape of the curves produced by the two polypeptides. In fact, unlike that produced by eledoisin, the

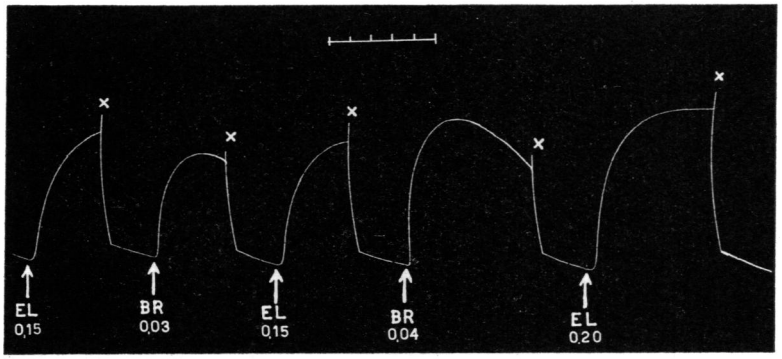

Fig. 5. Dog duodenum suspended in 10 ml. Tyrode solution at 37° C. At *EL*, synthetic eledoisin; at *BR*, synthetic bradykinin; at x, washing. Time, 1 min. Doses in μg. The shape of the curve produced by bradykinin was different from that produced by eledoisin.

contraction elicited by bradykinin declined spontaneously, without washing, after having reached its maximum. Stimulation of the dog intestine produced by substance P could not be distinguished from that caused by eledoisin (Fig. 6).

Cat. As for the dog, eledoisin caused both in the small and large intestine a tonus increase and the appearance or reinforcement of rhythmic movements. The

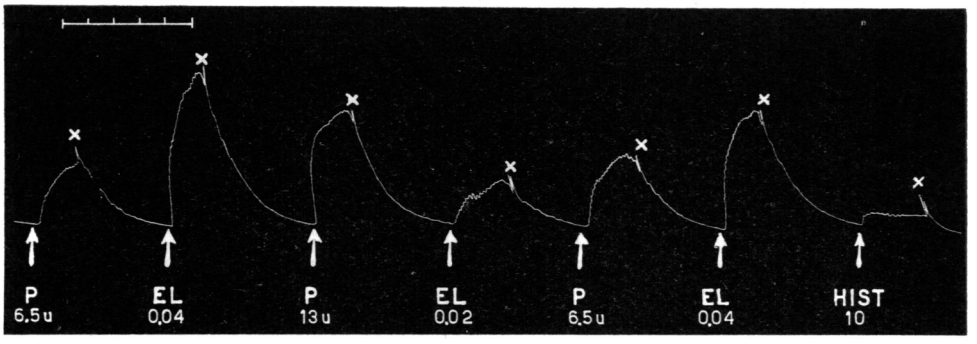

Fig. 6. Dog large intestine suspended in 10 ml. Tyrode solution at 37° C. At *EL*, synthetic eledoisin; at *P*, substance P; at *HIST*, histamine dihydrochloride; at x, washing. Time, 1 min. Doses in μg, or units. The response to eledoisin was indistinguishable from that to substance P. A dose of 30 ng eledoisin was of approximately the same activity as 6.5 u. substance P, and was much more potent than 10 μg histamine.

sensitivity was fairly elevated (threshold concentration 1 to 2 ng/ml.) but generally there was no satisfactory dose/response relationship. The cat small intestine is apparently an excellent preparation for the bioassay of the kinins (Fig. 7).

Monkey. Doses of 1 to 2 ng/ml. eledoisin were sufficient to stimulate both the small and the large intestine of *Papio hamadryas*. Larger concentrations produced more marked effects. However, once again, the response was not proportional to the dose. Bradykinin displayed a predominantly depressive effect on tonus and

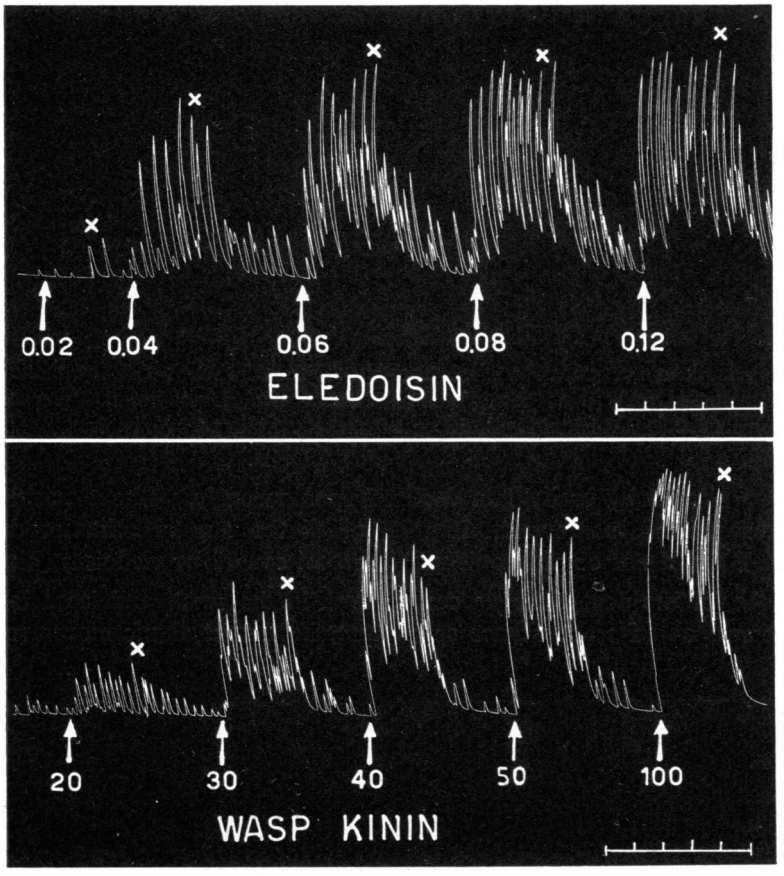

Fig. 7. Cat large intestine suspended in 10 ml. Tyrode solution at 37° C, containing atropine (0·1 μg/ml.) and mepyramine (0·2 μg/ml.). At x, washing. Time, 1 min. Doses in μg of eledoisin and of wasp kinin (dried venom sacs). There was a good dose/response relationship for wasp kinin, but not for eledoisin.

movements. When given prior to eledoisin it reduced the response to the endecapeptide, when given at the peak of eledoisin contraction it produced a conspicuous decrease in tone. In the presence of both eledoisin and bradykinin, washing with fresh nutrient liquid was followed by a prompt but short-lasting contraction (Fig. 8). This is probably due to a more tenacious binding of eledoisin to the receptor sites.

Man. Eledoisin (threshold 5 to 10 ng/ml.) reinforced spontaneous movements and increased tonus of the human appendix, removed by operation. The response was proportional to the dose.

Fowl. The fowl rectal caecum responded to eledoisin with a contraction roughly proportional to the dose. Threshold concentration was about 10 ng/ml. The preparation is not recommendable for the routine bioassay of eledoisin.

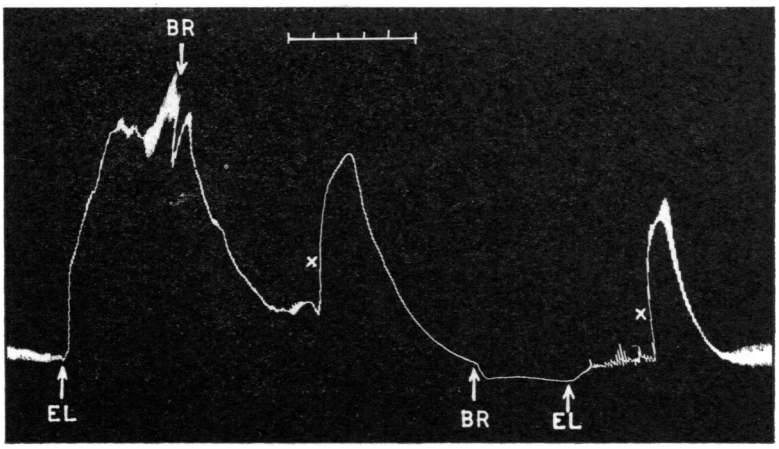

Fig. 8. Monkey ileum suspended in 10 ml. Tyrode solution at 37° C. At *EL*, 0.2 μg synthetic eledoisin; at *BR*, 1 μg synthetic bradykinin; at x, washing. Time, 1 min. Bradykinin displayed *per se* a slight inhibitory action on the gut and antagonized the stimulant action of eledoisin. After washing there was a conspicuous but short-lasting increase in tone.

Frog. Both the stomach and the intestine were stimulated (reinforcement of movements and tonus increase) by concentrations of eledoisin as low as 0.5 to 2 ng/ml. (Fig. 9). Frog stomach may serve as a useful subsidiary preparation in parallel assays.

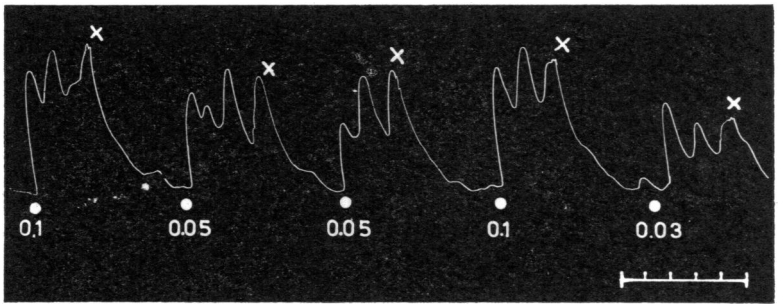

Fig. 9. Frog stomach suspended in 10 ml. Tyrode solution for cold-blooded animals at room temperature. At ●, different doses of synthetic eledoisin, in μg; at x, washing. Time, 1 min. There was a good dose/response relationship.

Action on isolated preparations of uterine muscle

Rabbit. Eledoisin stimulated the rabbit uterus preparation in concentrations as low as 1 to 2 ng/ml., and tonus increase was satisfactorily proportional to the dose. Since rabbit uterus is poorly sensitive to 5-hydroxytryptamine and histamine and is inhibited by bradykinin, it may be used with advantage in the bioassay of eledoisin (Fig. 10).

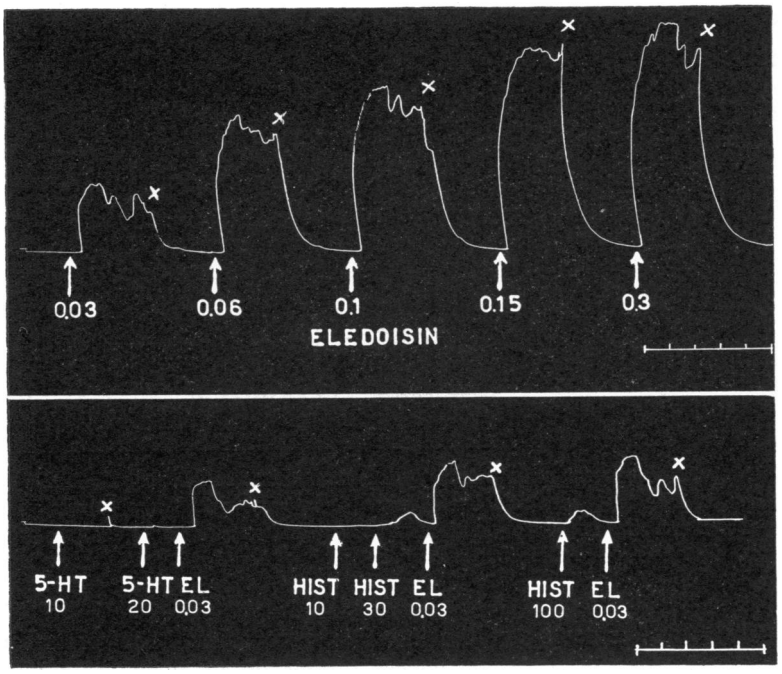

Fig. 10. Rabbit uterus suspended in 10 ml. Tyrode solution at 30° C. Upper tracing, effect of different doses of synthetic eledoisin. Lower tracing, effect of eledoisin (*EL*), 5-hydroxytryptamine (*5-HT*), and histamine dihydrochloride (*HIST*). Doses in μg. At x, washing. Time, 1 min. There was a satisfactory dose/response relationship for eledoisin. 5-Hydroxytryptamine and histamine were practically inactive.

Exactly as in monkey intestine, bradykinin given first reduced the tonus increase produced by eledoisin ; given at the peak of the eledoisin-induced contraction it produced a sharp fall of tone. Removal of both eledoisin and bradykinin was immediately followed by a fleeting contraction (Fig. 11).

Cat and rat. In sharp contrast to the stimulant action of the kinins on uterus preparations of these species, that of eledoisin was very weak. For the rat uterus the threshold concentration varied between 100 and 500 ng/ml.

Guinea-pig and hog. Uterine strips from these two species were poorly sensitive to eledoisin, and the response was irregular.

At the usual doses, atropine did not affect the action of eledoisin on any of the uterus preparations examined.

Action on other smooth muscle preparations

Urinary bladder. The dog urinary bladder was contracted by eledoisin (threshold 5 to 10 ng/ml.). Relaxation after washing was slow.

Ureter. The dog ureter responded to eledoisin (threshold 5 to 10 ng/ml.) with reinforcement of spontaneous activity and increase of tone (Fig. 12). Hog ureter was practically insensitive to the polypeptide.

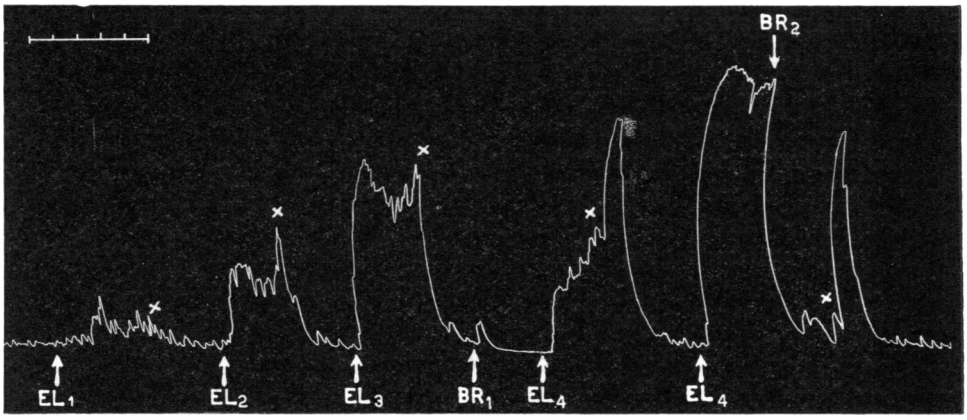

Fig. 11. Rabbit uterus suspended in 10 ml. Tyrode solution at 30° C. At EL_1, EL_2, EL_3 and EL_4, 0.1, 0.2, 0.4 and 1 μg synthetic eledoisin; at BR_1 and BR_2, 1 and 2 μg synthetic bradykinin; at x, washing. Time, 1 min. *BR* inhibited by itself the uterine preparation and antagonized the stimulant action of eledoisin. After washing, there was an intense but short-lasting contraction of the muscle.

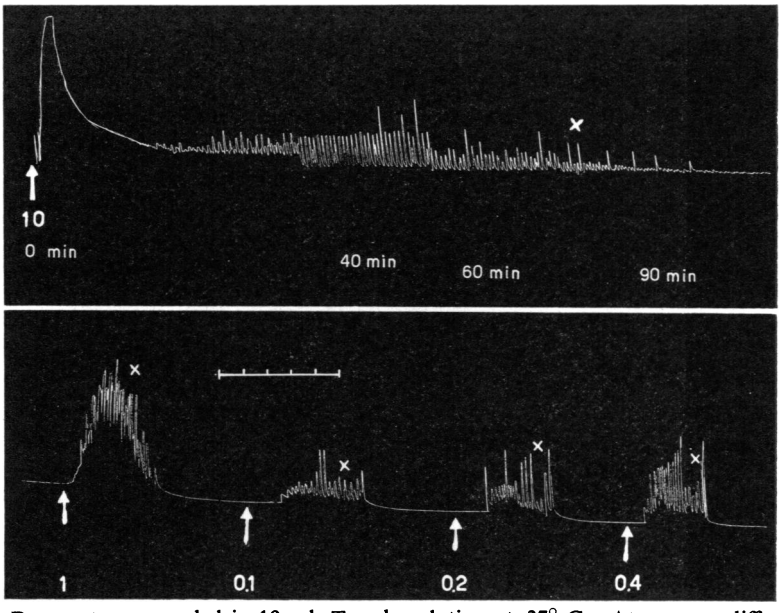

Fig. 12. Dog ureter suspended in 10 ml. Tyrode solution at 37° C. At arrows, different doses (in μg) of synthetic eledoisin; at x, washing. Time, 1 min. With 10 μg eledoisin (upper tracing) the stimulant effect persisted as long as 90 min, until washing.

Gall-bladder and seminal vesicles. The dog bladder and the rat seminal vesicles did not show any appreciable response to doses of eledoisin up to 0.5 μg/ml.

Distinction of eledoisin from other active tissue constituents

In crude tissue extracts eledoisin may be easily distinguished from all other biogenic compounds capable of stimulating extravascular smooth muscles.

Distinction from some compounds is possible on nearly every smooth muscle preparation, by the aid of specific inhibitors, for others it is necessary to have recourse to parallel assays.

Substances of non-polypeptide nature may be distinguished at once from eledoisin by their resistance towards chymotrypsin and trypsin. It should be added that in sharp contrast to eledoisin (a) acetylcholine is blocked by atropine; (b) histamine is potently antagonized by antihistaminic agents, is resistant to strong mineral acids, is inactivated by coupling with diazonium salts, and is practically inactive on the rabbit large intestine; (c) 5-hydroxytryptamine and tryptamine are antagonized by lysergic acid diethylamide and bromolysergic acid diethylamide, are inactivated by coupling with diazonium salts, and are practically inactive on the rabbit large intestine, while potently stimulating the rat uterus preparation; (d) adenosine compounds are generally resistant to alkali treatment, show a depressive action on the rabbit colon and guinea-pig ileum and, on the whole, require high dosage levels (0.1 to 1 μg/ml.) before their action on smooth muscles becomes evident (Gaddum & Szerb, 1961). In this regard it should be stressed that the threshold dose of crude salivary extract of *Eledone* capable of stimulating rabbit large intestine or the guinea-pig ileum suspended in a 10 ml. Tyrode bath leaves a dry residue of barely 2 to 5 μg.

Lipid-soluble organic acids, such as prostaglandin, vesiglandin, Darmstoff, irin, slow-reacting substance A (Vogt, 1958; Erspamer, 1961) are powerful stimulants of intestinal motility and often produce a conspicuous fall of blood pressure. Whereas the distinction of these acids from eledoisin does not present any serious difficulty (characteristic aspects of the stimulating action, parallel assays, differences in the effect on intestinal preparations which have been stored in the cold for some days, differences in the atropine antagonism, characteristics of solubility in fat solvents etc.) there is only one property which permits the distinction between the whole group of lipid-soluble organic acids and eledoisin, and this is again their resistance towards proteolytic enzymes.

More important, and sometimes more difficult, is the distinction of eledoisin from other biogenic polypeptides. Leaving aside polypeptides which are characteristically hypertensive (angiotensin, pitressin etc.), attention may be focused on the bradykinins, wasp kinin, some new hypotensive polypeptides found in amphibian skin, and particularly on substance P.

Bradykinins (ox bradykinin T, ox bradykinin B, kallidin) may be distinguished from eledoisin with great ease by parallel assays and by the use of intestinal preparations on which bradykinin displays an inhibitory and not a stimulant action. Values shown in Table 1 are more demonstrative than any calculation of indices of discrimination between bradykinin and eledoisin. In order to complete the Table some data concerning the dog blood pressure, reported elsewhere, were included.

It should be added that whereas bradykinin is resistant to trypsin but destroyed by carboxypeptidase, the opposite is true for eledoisin.

On preparations which are stimulated by both bradykinin and eledoisin, the two polypeptides given together or successively, without washing, act additively, and

sub-threshold doses of eledoisin may consistently increase the response to successive doses of bradykinin, or vice-versa. However, the action of one polypeptide is not followed, after washing, by a period of increased sensitivity to the other. On preparations which are stimulated by eledoisin, but inhibited by bradykinin (rat duodenum, rabbit uterus, monkey small intestine) it may be seen that (a) if bradykinin was given after the eledoisin stimulation had fully developed it produced a sharp tonus decrease; (b) if bradykinin was given prior to or together with eledoisin it reduced or even abolished the response to eledoisin. In both instances washing with fresh nutrient liquid was immediately followed by a short-lived but potent contraction of the smooth muscle (Figs. 8 and 11). This seems to indicate that occupation of the muscle receptors by eledoisin is more tenacious than that by bradykinin.

Wasp kinin. Distinction of wasp kinin from eledoisin is based on the same criteria which are valid for the bradykinins, and especially on the results of parallel assays, as shown in Table 1.

TABLE 1
APPROXIMATE EQUIVALENTS TO 1 μG ELEDOISIN

	Bradykinin (μg)	Wasp kinin (mg)	Substance P (units)	Histamine (mg)	5-Hydroxy-tryptamine (mg)
Dog blood pressure	100–500	2–5	60–80	0·05–5	—
Rabbit large intestine	100–400	2	160–400	2	60
Guinea-pig ileum	5–7	0·2–0·4	200	0·005–0·01	0·2–0·5
Dog large intestine	0·5	0·02–0·03	200–300	—	—
Rat duodenum	Inhibition	Inhibition	200–300	—	—
Frog stomach	2,000	20	200	—	—
Rat uterus	0·001–0·002	0·1–0·5 μg	1–5	Inhibition	0·02–0·1 μg
Rabbit uterus	Inhibition	—	—	2	0·06

Amphibian bradykinin-like polypeptides. Erspamer, Bertaccini & Cei (1962b) have recently shown that extracts of the skin of some amphibian species belonging to the genera *Phyllomedusa* and *Rana* may contain large amounts of bradykinin-like polypeptides. So far, at least three different polypeptides have been characterized. It will be seen that all of them may be easily distinguished from eledoisin in parallel assays and, at least in part, by their behaviour towards proteolytic enzymes.

Physalaemin. This is another new polypeptide, recently found in methanol and acetone extracts of the skin of some South American amphibians, among them *Physalaemus fuscumaculatus* (Erspamer, Bertaccini & Cei, 1962a). Physalaemin has a powerful hypotensive action in the dog (threshold dose: extract equivalent to 5 μg fresh skin per kg body weight), and it also potently stimulates the rabbit large intestine and the guinea-pig ileum, the contractions being similar to those produced by eledoisin. However, once again physalaemin may be distinguished from eledoisin by the aid of parallel assays. Among other characteristics, physalaemin is relatively more potent than eledoisin on the dog blood pressure, and less potent on the rabbit and rat uterus. Moreover, the two polypeptides show some differences in their behaviour towards inactivation by trypsin.

Substance P. Among the biogenic polypeptides which have been studied so far, it is substance P which shows the closest similarity to eledoisin and, in consequence, its biological distinction from eledoisin is somewhat more difficult. In fact, (a) exactly like eledoisin, substance P is destroyed by chymotrypsin, largely inactivated by trypsin, and not attacked by carboxypeptidase ; (b) parallel assays on intestine preparations of different species do not permit a distinction between substance P and eledoisin. This is, however, possible when parallel assays are carried out on the rabbit large intestine, the dog blood pressure and the rat uterus. Whereas the stimulant action of substance P is of the same intensity on the guinea-pig ileum and the rat uterus (Gaddum & Szerb, 1961), as well as on the rabbit large intestine, this is by no means true for eledoisin, which is approximately 100 times less active on the uterus than on the gut. In other words, the index of discrimination for eledoisin against substance P using rabbit large intestine (or guinea-pig ileum) and rat uterus is approximately 100.

DISCUSSION

Eledoisin possesses a more or less intense stimulant action, which is never preceded by depression, on all examined preparations of gastrointestinal tract. Stimulation of movements and increase of tone may sometimes be obtained with doses of eledoisin as low as 0.2 ng per ml. This potency of action is of the same order as that known for other naturally occurring polypeptides (substance P, bradykinin, angiotensin, oxytocin, etc.) (Gaddum & Szerb, 1961 ; Stürmer & Franz, 1961).

On preparations of smooth muscle other than the gastro-intestinal tract, eledoisin is considerably less potent, with few exceptions, such as that of the bronchial musculature of the guinea-pig. The latter is constricted by eledoisin doses as low as the active doses of bradykinin (Konzett & Stürmer, 1960).

Owing to their high sensitivity to eledoisin and the very satisfactory dose/response relationship, the rabbit large intestine and the guinea-pig ileum may profitably be used in its quantitative estimation. The first preparation seems to be most suitable, owing to its lack of sensitivity to indolealkylamines, histamine and bradykinin, and its exceptionally long responsiveness when stored in cold Tyrode solution. It will be seen that the blood pressure of the dog is another excellent test object for the bioassay of eledoisin.

Parallel assays carried out on different smooth muscle preparations and the use of different proteolytic enzymes permit a sharp distinction of eledoisin from all other known biogenic polypeptides.

As with substance P (Pernow, 1960 ; Kosterlitz, 1960), to which eledoisin shows a close resemblance, the problem of the mechanism of action on the intestinal muscle cannot be considered solved. In the rabbit large intestine the action of eledoisin is not antagonized by atropine, hexamethonium, nicotine and morphine ; also eledoisin acts here, exactly like substance P, on preparations which have been stored in the cold for up to 10 days. These facts point to a direct stimulation of the smooth muscle fibres. However, lowering of the temperature of the bath produces a partial

depression of the response of the gut to eledoisin, exactly as happens with substance P, and in rats and fowls eledoisin may produce a hypertensive response, presumably due to liberation of catecholamines (Erspamer & Glässer, to be published); both these observations suggest that eledoisin might also act through stimulation of nervous structures.

Stimulation of gastro-intestinal motility by eledoisin can also be seen in the intact dog and, with high doses of the polypeptide, may attain dramatic proportions. However, the doses that stimulated the gut were approximately ten times higher than those that lowered the blood pressure and, after single injections, gut stimulation was of shorter duration than blood pressure fall (Erspamer & Glässer, to be published).

The effect of eledoisin on the intestinal smooth muscle was apparently insignificant in the intact unanaesthetized rat (subcutaneous doses of up to 2,500 μg/kg failed to produce diarrhoea) and in the unanaesthetized rabbit.

In the dog eledoisin potently stimulated salivary secretion, nasal secretion, and secretion of mucus by the gastro-intestinal tract, and the stimulant effect of eledoisin on salivary and nasal secretion was also seen in the rat. Owing to its resistance to atropine it is probable that the secretory effect of the polypeptide is a direct one on the secretory cells, without any important participation of the autonomic nervous system.

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Note added in proof

Since this paper was submitted it has been found that pure substance P possessed, in contrast to crude substance P preparations, a poor stimulant action on the rat uterus, which did not exceed 10% of the action displayed on the guinea-pig ileum (Haefeli, W. & Hürlimann, A., *Experientia,* **18**, 297–303 (1962); Gaddum, J. H., *Experientia,* in the press). As a consequence of these results the index of discrimination for eledoisin against pure substance P using rabbit large intestine and rat uterus (cf. Table 1) is less than 10.

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