J. Pharm. Pharmacol., 1964, **16**, 744–750 Received May 4, 1964

Activity of eledoisin, other polypeptides and ergometrine on the uterus *in situ* of rabbit and other animal species

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The oxytocic activity of eledoisin on the uterus *in situ* of different animal species has been studied and compared with other oxytocic drugs. Eledoisin stimulated the rabbit uterus at doses as low as 0·01–0·1 μg/kg i.v. but had no significant oxytocic action on the uterus of cat, rat, and guinea-pig. It is, however, more active on the vascular than on the non-vascular smooth muscle preparations of the same animal species. The oxytocic activity of oxytocin and ergometrine, on the other hand, has been confirmed on almost all the animal species studied in doses which did not significantly alter the blood pressure (at least in the rabbit).

In the rabbit the oxytocic effect of ergometrine but not that of eledoisin and oxytocin was abolished by the adrenolytic drugs dibenzyline, piperoxan and hydergine. Hypertensin also stimulated almost all the uteri tested, whilst bradykinin was not active in the doses used.

THE endecapeptide eledoisin has a potent action on vascular and non-vascular smooth muscle organs. In the cardio-vascular system, it dilates the vascular bed to cause hypotension particularly in the dog (Erspamer & Anastasi, 1962; Olmsted & Page, 1962; Erspamer & Glässer, 1963; Bergamaschi & Glässer, 1963) and in man (Sicuteri, Fanciullacci, Franchi & Michelacci, 1962; Shapiro, Kontos, Page Manck & Patterson, 1963). A pressor effect was observed in the chicken and to a lesser extent in the rat (Erspamer & Glässer, 1963). Both *in vivo* and *in vitro*, eledoisin possesses an intense stimulating action on non-vascular smooth muscle preparations including the isolated uterus preparations from rabbit, rat, guinea-pig, cat, and dog (Erspamer & Falconieri-Erspamer, 1962). Recently, Stürmer & Berde (1963) confirmed the results described above and showed an oxytocic action of eledoisin on the rabbit uterus *in situ*.

Eledoisin (Erspamer & Glässer, 1963) and ergometrine (Konzett, 1960) produce responses on the chicken blood pressure similar to those seen with sympathomimetic drugs. Also, the oxytocic action of ergometrine on the rabbit uterus *in situ* has been shown by Konzett (1960) to resemble sympathomimetics. It therefore seemed interesting to investigate further the mechanism of action of eledoisin and other polypeptides on the uterus *in situ* of the rabbit and other animal species.

Material and methods

Drugs. Synthetic eledoisin (prepared by Sandoz and Farmitalia); synthetic oxytocin (Syntocinon); synthetic bradykinin; 1 methyl-D-lysergic acid butanolamide (UML 491); a mixture containing equal parts of dihydroergocornine, dihydroergocristine and dihydroergocryptine(Hydergine); synthetic hypertensin; (–)-adrenaline; 933 F (piperoxan); (–)-noradrenaline; ergometrine; 5-hydroxytryptamine (5-HT); acetylcholine;

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mepyramine; hexamethonium; atropine; histamine; dibenzyline. Drugs were administered intravenously. Blood pressure was recorded from the carotid artery using a mercury manometer. Uterine activity *in situ* was recorded kymographically using an isotonic gravity lever attached to one uterine horn following Rothlin's procedure (1938) modified as described below.

Rabbit, guinea-pig, and cat uterus. The ovarian and cervical ends of a uterine horn were attached to a rod, and the movements recorded from a thread attached to the centre of the uterus. With pregnant animals, the foeti were first removed by surgery before commencement of the recording. Uteri from virgin, non-virgin–non-gravid, gravid, and post-partum animals were used.

Rat uterus. The two uterine horns from ovariectomised young albino rats were dissected free from the ovarian and mesenteric attachments, without disturbing the blood supply, and the combined movements recorded by the method described above.

In all instances the horns were kept in a humid environment. Some virgin guinea-pigs and all of the rats were injected subcutaneously with 1 mg/kg of oestradiol 3–4 days before the experiment. Rabbits and guinea-pigs were anaesthetised with urethane (1 g/kg intravenously to rabbits and intraperitoneally to guinea-pigs); cats were anaesthetised with a mixture of chloralose (60 mg/kg) and urethane (250 mg/kg) intravenously after pre-anaesthesia with ether. Some rats were anaesthetised with amylobarbitone sodium (90 mg/kg i.p.) others were anaethestised with ether, pithed and then maintained by artificial respiration.

Results

RABBIT BLOOD PRESSURE

Eledoisin lowered the blood pressure at doses ranging from 0·001–0·01 μg/kg. Oxytocin did not significantly affect the blood pressure at least in the doses tested (0·01–0·5 IU/kg). Ergometrine, at 100 μg/kg and higher, had an irregular action, either lowering or increasing the blood pressure.

RABBIT UTERUS

Most experiments were on the non-virgin–non-gravid uterus which either had a rhythmic activity of low intensity or was completely quiescent.

Threshold doses of eledoisin, 0·01–0·1 μg/kg, elicited spontaneous activity in quiescent uterine muscle and/or induced a small increase in frequency and amplitude of spontaneous rhythmic contractions. Larger doses, 0·2–1 μg/kg, produced powerful contractions of greater amplitude accompanied by a progressive increase in tone and lasting up to 20 min in some instances. This phase of contraction was followed by a quiescent period. The response of the uterus increased with increasing dose (Figs 1 and 2) and no evidence of tachyphylaxis was observed. The effect of eledoisin did not seem to depend on the hormonal state of the uterus because it was also present in virgin, pregnant, and post-partum rabbits.

Threshold doses of oxytocin, 0·01–0·02 IU/kg, produced similar responses on the rabbit uterus and within certain limits the activity increased with the dose (Fig. 1). The responses of the uterus to oxytocin resembled in some ways those seen with eledoisin, but with increasing doses of oxytocin the period of latency decreased and the effect was more evident on the tone than on the amplitude of the contractions. The effect of 0·5 IU/kg of oxytocin lasted for 30 min or more.

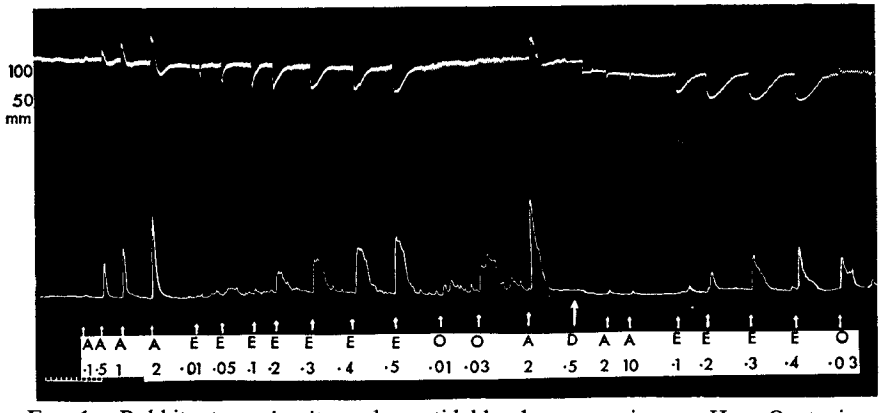

FIG. 1. Rabbit uterus *in situ* and carotid blood pressure in mm Hg. Oxytocic activity of adrenaline (A), eledoisin (E), and oxytocin (O) before and after treatment with 0·5 mg/kg of dibenzyline (D). The numbers denote doses in μg/kg for A and E, IU/kg for O. Time in min.

Hypertensin, 0·1–0·5 μg/kg, also produced contractions of the uterus but was less potent than either eledoisin or oxytocin; larger doses, 5–10 μg/kg, caused an immediate onset of strong contractions of short duration (4–5 min).

Bradykinin, on the other hand did not show any activity even with the highest doses used (10 μg/kg).

Adrenaline and noradrenaline produced a single, prompt, strong contraction on the non-virgin–non-gravid rabbit uterus. The sensitivity in the range of doses used, 0·5–10 μg/kg, appears to be similar for both amines, although a period of relaxation and reduced frequency of spontaneous contractions was sometimes seen after adrenaline. Histamine and 5-HT showed little contractile activity even with the largest doses used (50–100 μg/kg) (Fig. 2).

With ergometrine, our results confirmed the experimental data obtained by Brown & Dale (1935) and Rothlin (1938). Ergometrine, 100 μg/kg evoked rhythmic and powerful contractions; in some experiments the threshold dose was as little as 10 μg/kg.

Antagonism by adrenolytic and other drugs. Adrenolytic drugs, such as hydergine, piperoxan and dibenzyline, administered intravenously in doses able to fully antagonise the responses to adrenaline and nonadrenaline on the uterus and on the blood pressure, also inhibited the oxytocic effect of ergometrine, but the responses to eledoisin, oxytocin, and hypertensin

were unaltered. Hydergine, 1 mg/kg, and piperoxan, 10 mg/kg, completely antagonised the uterine contactions to doses of adrenaline, noradrenaline and ergometrine in doses at least five times greater than their

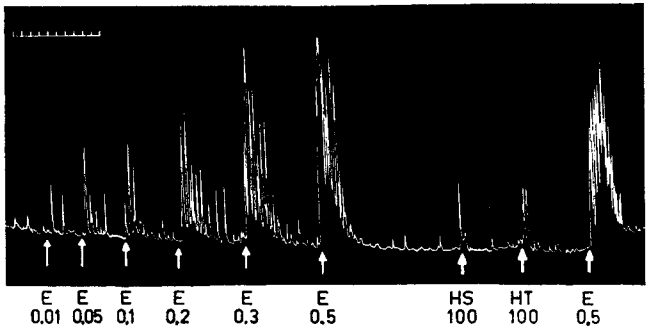

FIG. 2. Rabbit uterus *in situ*. Oxytocic activity of eledoisin (E), histamine (HS), and 5-hydroxytryptamine (HT). The numbers denote doses in μg/kg. Time in min.

minimal threshold doses. Neither adrenolytic drug impaired the responses to eledoisin, oxytocin and hypertensin (Fig. 3). Dibenzyline, 0·5–2 mg/kg, infused intravenously over at least 1 hr, completely abolished the oxytocic response to adrenaline, noradrenaline and ergometrine in doses 10–20 times greater than their minimal threshold doses (Fig. 1), and the responses

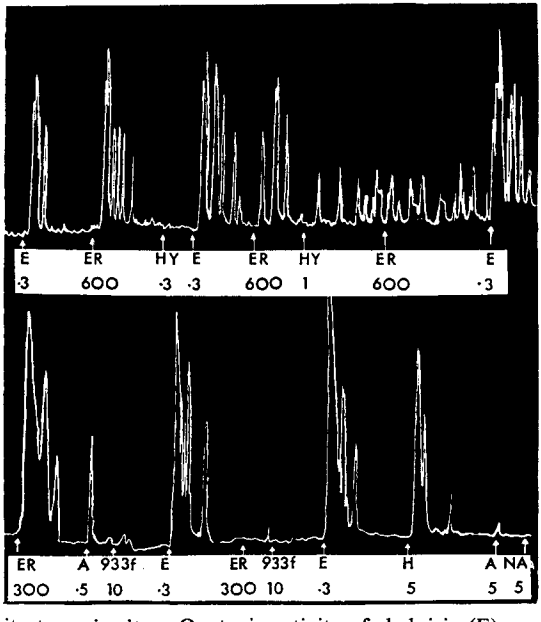

FIG. 3. Rabbit uterus *in situ*. Oxytocic activity of eledoisin (E), ergometrine (ER), hypertensin (H), adrenaline (A), and noradrenaline (NA). Hydergine (HY) in the dose range 0·1–1 mg/kg (mg = ml) and piperoxan (933 f) (10mg/kg) were able to antagonize the oxytocic responses to E, H, and oxytocin (which does not appear on the figure). The figures denote doses in μg/kg. Time in min.

to eledoisin, oxytocin and hypertensin were unaffected. In a few experiments (Fig. 1), after dibenzyline there was a transient reduction (no more than 50%) in the contractions elicited by eledoisin, oxytocin and hypertensin, but the responses to the three polypeptides were restored to the initial value within 2 hr, whilst adrenaline, 10 μg/kg, had no action on the uterus.

Hexamethonium, 15 mg/kg, and atropine, 6 mg/kg, did not impair the responses to threshold and larger doses of eledoisin, oxytocin, hypertensin, ergometrine and catecholamines. Moreover eledoisin was not influenced by pretreatment of animals with mepyramine, 1 mg/kg, and with 1 methyl-D-lysergic acid butanolamide, 1 mg/kg.

RAT UTERUS

The uteri of ovariectomized and oestradiol-pretreated rats were either quiescent or showed small rhythmic contractions. The preparation was not very sensitive to eledoisin; and large doses of the drug, 0·5–1 μg/kg, caused a single and transient contraction. Larger doses did not produce an appreciable increase in the response and tachyphylaxis was sometimes observed.

Oxytocin at the threshold dose, 0·005 IU/kg, produced either persistent rhythmic and powerful contractions in a normally quiescent uterus or else greatly reinforced the spontaneously occurring movements. Large doses, 0·01–0·02 IU/kg, gave a definite rise of the tone which lasted for several hours.

Hypertensin, 0·5–10 μg/kg, produced strong contractions of rat uterine muscle; whilst bradykinin in very large doses, 20–100 μg/kg, gave small and evanescent responses.

Ergometrine, 100–500 μg/kg, caused the prompt appearance of powerful contractions with a rise in tone which lasted for 30–40 min.

GUINEA-PIG UTERUS

Non-virgin–non-pregnant, pregnant, post-partum and oestradiol-pretreated uteri were used. They were either quiescent or showed some spontaneous rhythm.

Eledoisin, 0·01–2 μg/kg, did not elicit any contraction in most experiments. In a few instances, particularly with oestradiol pretreated animals, small and transient contractions of the uterus were seen (Fig. 4).

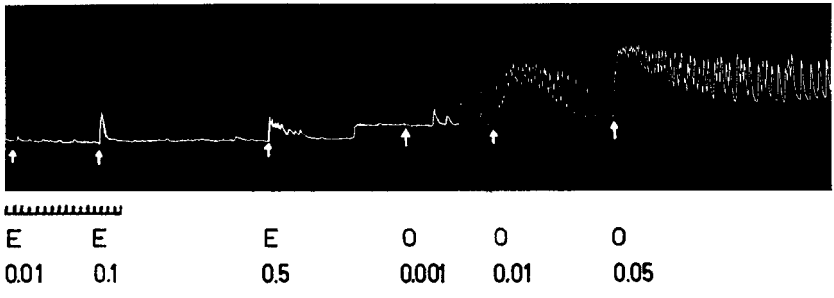

FIG. 4. Guinea-pig uterus *in situ*. Oxytocic activity of eledoisin (E) and oxytocin (O). The numbers denote doses in μg/kg for E and IU for O. Time in min.

The guinea-pig uterus in all the physiological conditions tested was extremely sensitive to oxytocin; the threshold dose being as low as 0·001–0·005 IU/kg. Oxytocin increased either the spontaneous movements, or caused the appearance of numerous powerful rhythmic contractions with an increase of the basal tone between contractions. These lasted for several hours when doses as large as 0·1–0·4 IU/kg were injected.

Hypertensin, 0·05–5 μg/kg, on the pregnant uterus, contracted the uterus in the late stages of pregnancy, whilst at 20 μg/kg it was unable to contract the uterus in the early stages of pregnancy.

Bradykinin, 0·1–5 μg/kg, was inactive in the doses tested; whilst ergometrine was active at the same doses used by Brown & Dale (1935).

CAT UTERUS

Most experiments were made on uteri of cats during the last stage of gestation or few days post-partum. The organ had a naturally high tone with irregular movements and for this reason, the test was more useful in demonstrating the activity of drugs which cause relaxation (i.e., adrenaline) than for oxytocic drugs. In these conditions eledoisin, 1–5 μg/kg, oxytocin, 0·1–0·3 IU/kg, and ergometrine, 200–500 μg/kg, produced an increase in the basal tone but had little effect on the already maximal contractions. Neither bradykinin, 1–10 μg/kg, nor noradrenaline, 1–10 μg/kg, had any action on the uterine smooth muscle; adrenaline 1–40 μg/kg, relaxed the uterus. Hypertensin was not tested.

A few experiments were made on cats in the first period of gestation, in which phase the spontaneous contractions were small and rapid. Neither eledoisin, 0·4–2 μg/kg, nor oxytocin, 0·01–0·1 IU/kg, were very effective on these organs. On the other hand, the responsiveness of the uteri to hypertensin, adrenaline and noradrenaline was good, 1–2 μg/kg of each drug caused the prompt appearance of a single strong contraction.

Discussion

Eledoisin stimulates particularly the rabbit uterus *in situ*, and the response of the uterus is satsifactorily proportional to the dose, 0·01–1 μg/kg (Fig. 1). The uterus *in situ* of rat, guinea-pig and cat is almost insensitive to eledoisin. These results are in accordance with those obtained *in vitro* by Erspamer & Falconieri-Erspamer (1962) and those obtained *in vivo* on the rabbit and cat uterus by Stürmer & Berde (1963). Adrenolytic drugs (dibenzyline, piperoxan, hydergine), in doses which completely abolish the activity of adrenaline and noradrenaline, do not significantly reduce the oxytocic responses of the rabbit uterus to eledoisin. Neither hexamethonium, mepyramine, 1-methyl-D-lysergic acid butanolamide, and atropine modify the responses of eledoisin on the rabbit uterus.

Oxytocin behaves like eledoisin, in that its oxytocic action is unaffected by the antagonists described, but differs from eledoisin in that it contracted the uteri tested from all species.

The mechanism of the responses to ergometrine seems to be different

from that of eledoisin and oxytocin. The three adrenolytic drugs used abolished the contractions of the uterus to ergometrine, as well as to sympathomimetic drugs. This is in accordance with results of Konzett (1960) showing that dibenamine and phentolamine (as well as hydrogenated ergot alkaloids) specifically inhibit the action of ergometrine.

Bradykinin, 5-HT, and histamine appear to be almost inactive on the rabbit uterus. Stürmer & Berde (1963) also found that only very large doses of bradykinin, 44 μg/kg, caused contractions. Hypertensin, on the contrary, is active in almost all the uteri of species tested, on the rabbit however the drug is less active than eledoisin. The adrenolytic drugs and the ganglionic-blocking agents tested do not alter the oxytocic responses of hypertensin on rabbit uterus.

In conclusion, these findings suggest that ergometrine acts at least on the rabbit uterus through an adrenergic mechanism whilst eledoisin and oxytocin (as well hypertensin) act independently. Studies are in progress to elucidate further the mechanism of the oxytocic action of ergometrine in other animal species. Erspamer & Falconieri-Erspamer (1962) and Erspamer & Glässer (1963) and the present results clearly show that eledoisin is much more active on the vascular beds than on the extravascular smooth muscles of the same animal species.

Acknowledgements. We are grateful to Sandoz Ltd., Basel, Switzerland for supplying UML 491 and bradykinin.

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