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THE ACTION OF ELEDOISIN ON THE SYSTEMIC ARTERIAL BLOOD PRESSURE OF SOME EXPERIMENTAL ANIMALS

BY

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The blood pressure response following parenteral administration of eledoisin varies conspicuously from one animal species to another. In the dog the polypeptide elicits a clear hypotension under all experimental conditions. Eledoisin is active at extremely low dose levels and hypotension is not appreciably affected by previous treatment with atropine, sympatholytic drugs, ganglion-blocking agents or reserpine. Eledoisin potently antagonizes the pressor effects of catechol amines and of angiotensin. Long-lasting hypotension can be obtained by intravenous infusion or subcutaneous injection of the polypeptide. The mechanism of action of eledoisin in the dog is believed to be chiefly peripheral. The rabbit, the guinea-pig and the cat behave like the dog in the type but not in the intensity of their blood-pressure response to eledoisin. The rat gives variable responses to the polypeptide. After treatment with a ganglion-blocking drug, or in the pithed rat, a rise in blood pressure is the predominant effect. In the anaesthetized chicken eledoisin generally produces a diphasic response, but in the decapitated animal only hypertension. This last effect is believed to depend largely, if not entirely, on the release of catechol amines from body stores. The dog blood pressure may be used for the assay of eledoisin ; suitable results may also be obtained from the pressor effects in decapitated chickens.

In a previous paper the actions of eledoisin on a number of extravascular smooth muscle preparations were reported (Erspamer & Erspamer, 1962). In this paper the actions of the polypeptide on the systemic arterial blood pressure of some common laboratory animals are described. The results have facilitated the assay of eledoisin and its distinction from other naturally occurring substances which stimulate plain muscle. The information should offer a basis for the trial of the polypeptide in man.

METHODS

Dogs and cats were anaesthetized with chloralose (70 mg/kg intravenously) or sodium pentobarbitone (30 mg/kg intravenously), guinea-pigs, rabbits and chickens with urethane (0.5 to 1.2 g/kg intravenously or intraperitoneally) and rats with urethane or pentobarbitone.

Spinal dogs were prepared as described by Burn (1951) for the preparation of the spinal cat, and pithed rats were prepared according to Shipley & Tilden (1947). Chickens were decapitated by severing the spinal cord between C5 and C6 and by firmly tying the neck with string immediately below the spinal section.

Injections were made into a femoral, jugular or brachial vein. Systemic arterial blood pressure was recorded from a carotid or femoral artery by means of a mercury manometer. Adequate oxygenation of the blood in spinal, pithed or decapitated animals was maintained by a respiratory pump.

The electrocardiograph of the intact anaesthetized dog was recorded by means of a Hellige multiscriptor apparatus.

Drugs. Eledoisin was either the pure natural or the synthetic polypeptide or else a partially purified extract of posterior salivary glands of *Eledone* containing 50 μg of eledoisin per mg. Paper chromatography and paper electrophoresis showed that in this extract eledoisin was virtually the sole substance with activity on vascular smooth muscle.

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

Preparations of substance P contained 75 U/mg and 12 U/mg; 1 μg of pure substance P will probably be equivalent to somewhat more than 120 "von Euler units" (Haefeli & Hürlimann, 1962).

RESULTS

Dog

Anaesthetized preparations

In dogs anaesthetized with chloralose or pentobarbitone, eledoisin regularly reduced blood pressure. Both the duration and intensity of the hypotension were proportional to the injected dose of the polypeptide (Fig. 1). In no instance was

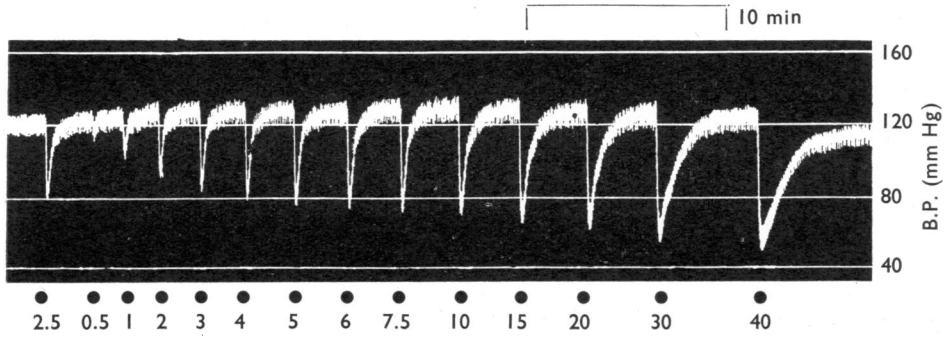

Fig. 1. Blood pressure of a dog anaesthetized with sodium pentobarbitone (30 mg/kg, intravenously) after treatment with 0.2 mg/kg of atropine sulphate, intramuscularly. Falls in pressure were produced by increasing intravenous doses of eledoisin (in ng/kg). Note the clear dose/response relationship.

either tachyphylaxis or sensitization observed. The hypotension was generally accompanied by elevation of heart rate. The threshold dose for eledoisin given by rapid intravenous injection was 0.2 to 0.5 ng/kg. The blood pressure falls obtained in six experiments with different intravenous doses of eledoisin are shown in Table 1.

Given by intravenous infusion, eledoisin caused hypotension which lasted as long as the infusion was continued. The threshold dose was 2 to 5 ng/kg/min. With 5 ng/kg/min hypotension started promptly, then increased until a maximum drop of 20 to 30 mm Hg was attained after 40 to 60 min. Later, in spite of the infusion continuing at the same rate, the blood pressure slowly increased and after 3 hr it

TABLE 1

BLOOD PRESSURE FALL PRODUCED IN THE DOG BY DIFFERENT INTRAVENOUS DOSES OF ELEDOISIN

Ranges of values for groups of six experiments

Dose of eledoisin (ng/kg)	Blood pressure fall	
	Size (mm Hg)	Duration (min)
1	10–20	0·25–0·75
2	15–30	0·75–1·5
5	40–50	1–2
10	40–60	1–3
20	50–70	2–6
50	60–80	4–8
100	50–90	7–10

was only 10 mm Hg below the basal level. Infusion of higher doses of eledoisin (15 and 150 ng/kg/min) produced a more rapid and conspicuous fall, which persisted until the infusion was discontinued; the hypotension was accompanied by copious salivation and by repeated defaecation.

The threshold subcutaneous dose of eledoisin which lowered the blood pressure was between 1 and 2 μg/kg. With 2.5 μg/kg the drop in pressure was 8 to 15 mm Hg and lasted 90 to 120 min; 10 μg/kg reduced the pressure for more than 3 hr with a maximum decrease from 150 to 80 mm Hg at 60 min; 80 to 100 μg/kg produced a hypotension lasting up to 8 hr with a maximum fall of 70 to 80 mm Hg.

With 150 to 300 μg/kg of eledoisin only one of three dogs survived. The other two died after 7 to 9 hr. Generally the initial rapid fall in pressure (from 170 to 130 down to 60 to 45 mm Hg) was succeeded by a slight rise of 10 to 15 mm Hg lasting 3 to 4 hr and then, with lethal doses, by a progressively increasing fall in pressure. Respiration ceased a few min after cardiac arrest (Fig. 2). Sometimes cutaneous vasodilatation was observed in the groin, snout and conjunctiva.

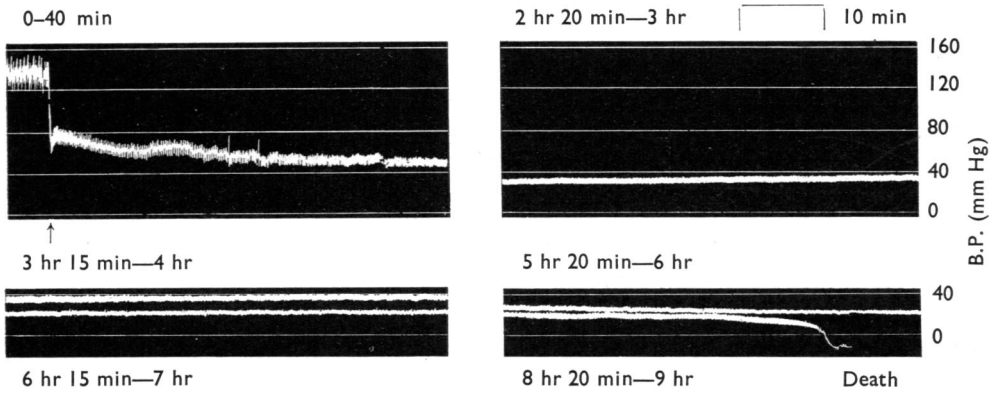

Fig. 2. Blood pressure of a dog anaesthetized with pentobarbitone after treatment with atropine. The fall in pressure was produced by a subcutaneous injection of 200 μg/kg of eledoisin at the arrow. Records at six times after the injection are shown. Note the extreme hypotension, lasting over 8 hr and leading to death.

Spinal preparations

Eledoisin manifested its hypotensive action even at very low pressure levels. In a spinal dog in which the basal pressure was 40 mm Hg, 2 ng/kg of eledoisin reduced blood pressure by 15 mm Hg for 4 min, and 20 ng/kg reduced the pressure by 20 mm Hg for more than 20 min.

Effects of atropine, dihydroergotamine, ganglion-blocking agents and reserpine

Atropine (0.1 to 0.2 mg/kg, subcutaneously) or dihydroergotamine (0.15 mg/kg, intravenously) did not appreciably affect either the intensity or the duration of the hypotension. Hexamethonium bromide (5 mg/kg, intravenously) or azamethonium bromide (2 to 3 mg/kg, intravenously) did not prevent the depressor action of eledoisin.

Two dogs were treated, 24 hr before recording blood pressure, with 1 mg/kg of reserpine, giving intraperitoneally. The responses to different doses of eledoisin were similar to those seen with dogs not given reserpine, and there was no appreciable lowering of the threshold dose of the polypeptide.

Effects of catechol amines and of angiotensin

Eledoisin was very effective in antagonizing the pressor effects of noradrenaline and of angiotensin in the dog. In the experiments illustrated in Figs. 3 and 4, 0.05 and 0.1 μg/kg of eledoisin completely abolished the hypertensive effect of 2 and 10 μg/kg of noradrenaline respectively, and 0.2 μg abolished the effect of 5 μg/kg of angiotensin, when given in the same intravenous injection. Surprisingly, the hypotension elicited by 0.2 μg of eledoisin plus 10 μg of angiotensin was greater than that caused by 0.2 μg of eledoisin plus 5 μg of angiotensin. In other experiments the antagonistic effect of eledoisin towards angiotensin was less pronounced. When eledoisin was given by intravenous infusion at a rate of 0.2 μg/kg/min it prevented the appearance of the usual hypertensive response to 1 μg/kg/min of angiotensin.

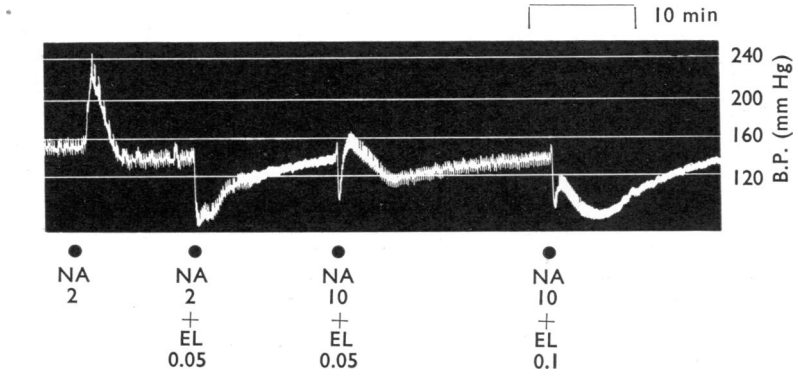

Fig. 3. Blood pressure of a dog anaesthetized with pentobarbitone after treatment with atropine. The antagonism of eledoisin (EL) towards noradrenaline (NA) is shown. Doses are in μg/kg, intravenously. The hypertensive effects of 2 and 10 μg/kg of noradrenaline were completely abolished by the simultaneous injections of 0·05 and 0.1 μg/kg of eledoisin, respectively.

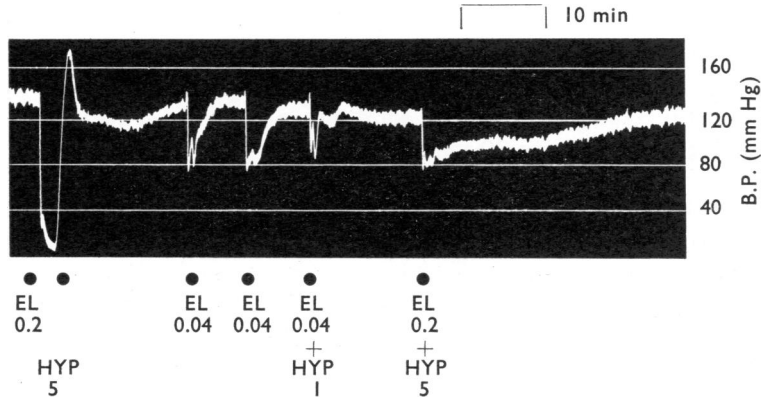

Fig. 4. Blood pressure of a dog anaesthetized with pentobarbitone after treatment with atropine. The antagonism of eledoisin (EL) towards angiotensin (HYP) is shown. Doses are in μg/kg, intravenously. The hypertensive effect of 5 μg/kg of angiotensin was abolished by the simultaneous administration of 0.2 μg/kg of eledoisin.

Potencies relative to bradykinin, histamine and substance P

In a comparative experiment (Fig. 5) eledoisin was about 100-times more active than bradykinin and about 300-times more active than histamine, on a weight basis ; 30 ng of eledoisin were equivalent to 2.5 U of substance P.

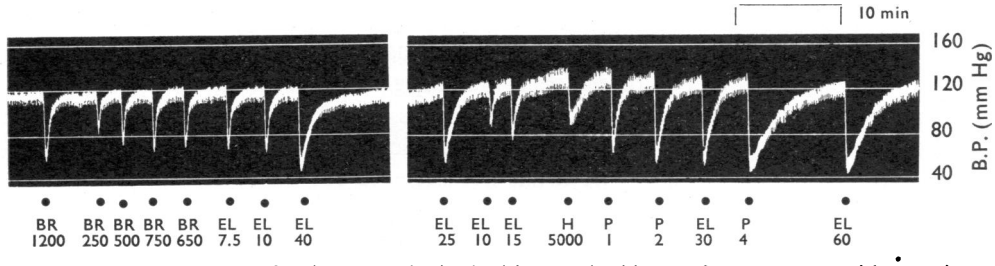

Fig. 5. Blood pressure of a dog anaesthetized with pentobarbitone after treatment with atropine. The effects on blood pressure of different intravenous doses of bradykinin (BR), eledoisin (EL), histamine (H) and crude substance P (P) are shown. All doses are in ng/kg, except for substance P, for which doses are in U/kg.

Effects on the electrocardiograph

The electrical activity of the heart was recorded from two dogs, anaesthetized with pentobarbitone, during hypotension produced by the subcutaneous injection of 200 μg/kg of eledoisin, which is 100- to 200-times larger than the minimum effective dose. In the first dog the blood pressure fell from 120 down to 45 to 50 mm Hg within 10 min. At 15 min it was 65 mm Hg, at 45 min 70 mm Hg and at 2.5 hr 80 mm Hg. The animal survived. At the time of maximum hypotension there was a 20% decrease in heart rate, the appearance of a diphasic T wave (type $-+$) in lead II, and a 40% reduction in the amplitude of the R wave. In the second dog the pressure fell rapidly from 130 to 50 mm Hg within 12 min and

then to 35 to 40 mm Hg within 30 min. The pressure then rose to 60 mm Hg and, beginning at the 4th hour, there was a progressive fall in pressure resulting in death of the dog at 8 hr. During the initial period of rapid fall in pressure there was a 30% increase in heart rate and an inversion of the T wave. After 4 hr the heart rate was 40% above the control and the T wave was positive in leads II and III. Later the heart rate further increased up to 50%, the amplitude of the R wave diminished and, before death, the T wave became diphasic (− +) in leads I and II. Therefore even when the blood pressure was very low there was no electrocardiographic evidence of any direct damage to the heart by the polypeptide.

Cat

The fall in blood pressure produced by eledoisin in the cat (anaesthetized with chloralose or pentobarbitone) varied conspicuously from one animal to another and was, on the whole, moderate. Moreover, striking tachyphylaxis developed when intravenous doses of eledoisin were repeated at short intervals. Often the sensitivity to the polypeptide decreased by 20- to 50-times, and 30 to 60 min were required until the original sensitivity was restored (Fig. 6).

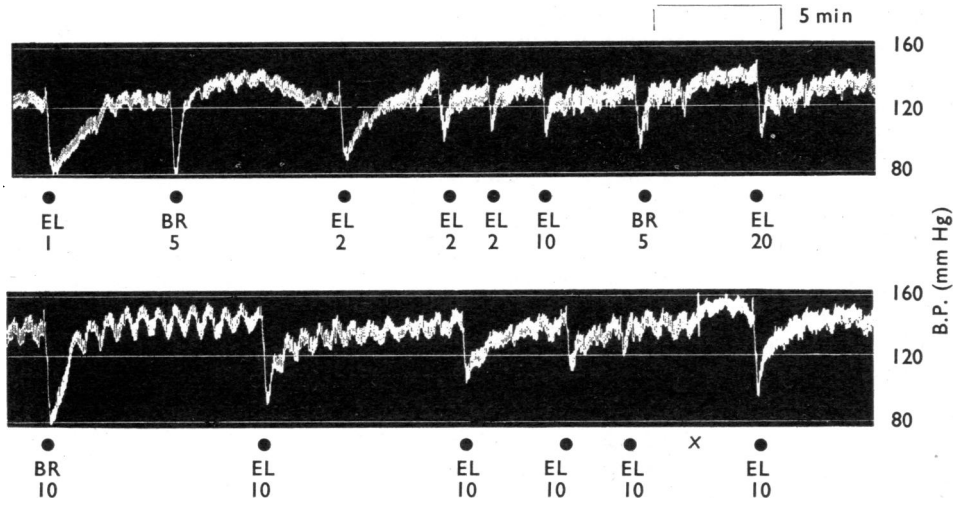

Fig. 6. Blood pressure of a cat anaesthetized with chloralose (70 mg/kg, intravenously) after treatment with atropine (1 mg/kg, intramuscularly). The effects of different intravenous doses (in μg/kg) of bradykinin (BR) and of eledoisin (EL) are shown. At X the drum was stopped for 30 min. Note the tachyphylaxis towards eledoisin and, to a lesser degree, towards bradykinin.

Eledoisin produced a fall in pressure, which was unaffected by previous medication with atropine (1 mg/kg, intramuscularly). The threshold intravenous dose varied between 0.05 and 2 μg/kg. The antagonistic action of eledoisin towards noradrenaline was negligible ; 20 μg of the polypeptide did not appreciably affect the rise in pressure caused by 10 μg of noradrenaline.

Comparison with other hypotensive substances was very difficult owing to tachyphylaxis. In one experiment, for example, 50 μg of eledoisin was equiactive to 2 to 3 μg of bradykinin, in another to 150 μg, and in a third to 500 μg. Histamine was, on a weight basis, from 2- to 20-times more active than eledoisin.

Rabbit

Rabbits, anaesthetized with urethane, responded to intravenous eledoisin by a fall in blood pressure; the threshold dose was 1 to 5 ng/kg. The size of the fall was related to the dose, and the response included an increase in respiration. Atropine did not affect the response. 1 μg of eledoisin was equivalent, with respect to the size of the pressure fall, to 50 to 100 U of kallikrein and to 10 μg of bradykinin. However, the duration of hypotension was different for the substances producing the same fall in pressure.

Guinea-pig

In guinea-pigs (anaesthetized with urethane) eledoisin produced a primary hypotension, sometimes followed by a hypertensive phase. The threshold dose was 5 to 10 ng/kg. In one experiment, 5, 15 and 100 ng/kg of eledoisin given intravenously elicited pressure falls of 14, 22 and 30 mm Hg, respectively, which lasted 30 to 60 sec. 1 μg/kg caused a pressure drop of 40 mm Hg, lasting over 10 min. Secondary hypertension was more evident and sustained following larger doses of eledoisin (0.1 to 1 μg/kg).

Rat

The pressure response to eledoisin (threshold dose, 0.1 to 0.2 μg/kg, intravenously) of the intact rat, anaesthetized with chloralose or urethane, was variable. Sometimes moderate and short-lasting hypotension was predominant, more rarely hypertension.

In rats previously treated with hexamethonium (5 to 30 mg/kg, intravenously), eledoisin (0.5 to 5 μg/kg) produced only hypertension (up to 60 mm Hg) which, however, was not correlated with the dose since there was tachyphylaxis. In some experiments 1 μg of eledoisin was equiactive to 0.1 to 0.4 μg of adrenaline and to 0.2 to 0.5 μg of angiotensin. The pithed rat responded, like the ganglion-blocked animal, with a rise in blood pressure which was potentiated by hexamethonium (Fig. 7). Repeated administration of either adrenaline or angiotensin did not appreciably change the response to eledoisin.

Chicken

Anaesthetized preparations

Eledoisin usually produced, in the intact chicken anaesthetized with urethane, a diphasic response consisting of a brief hypotension followed by a more sustained rise in pressure (Fig. 8). More rarely pure hypo- or hypertension was seen. The effects differed from one animal to another and they were not always correlated with the dose of the polypeptide.

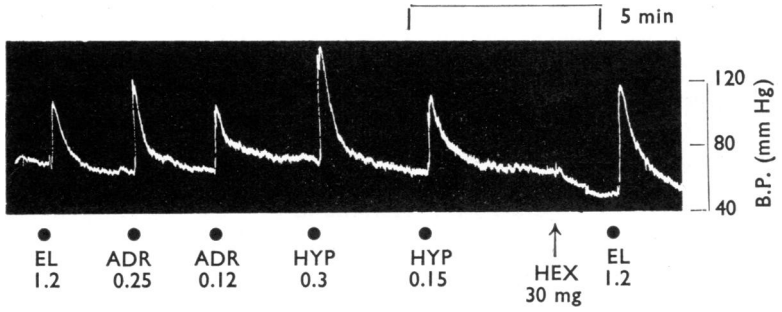

Fig. 7. Blood pressure of a pithed rat. The pressor responses to intravenous doses (in μg/kg) of eledoisin (EL), adrenaline (ADR), and angiotensin (HYP). Hexamethonium (HEX, 30 mg/kg, intravenously) enhanced the pressor effect of eledoisin.

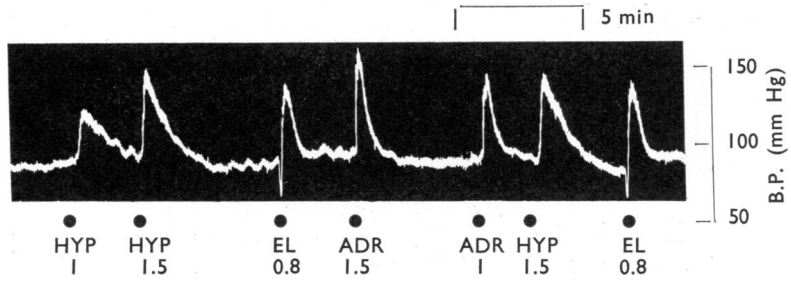

Fig. 8. Blood pressure of a chicken anaesthetized with urethane (0.9 g/kg, intraperitoneally). The blood pressure responses to eledoisin (EL), angiotensin (HYP) and adrenaline (ADR) are shown. Doses are in μg/kg, intravenously. 0.8 μg of eledoisin was equivalent, in this experiment, to approximately 1.2 μg of angiotensin and 1 μg of adrenaline.

TABLE 2

BLOOD PRESSURE CHANGES ELICITED BY INTRAVENOUS DOSES OF ELEDOISIN IN THE INTACT ANAESTHETIZED CHICKEN

Values in parentheses give the numbers of chickens for each dose. Blood pressure responses are means for each group of chickens

Dose of eledoisin (μg/kg)	Blood pressure response (mm Hg)	
	Hypotension	Hypertension
0·1 (9)	−10	+18
0·5 (15)	−20	+28
0·8 (5)	−25	+36
1·0 (4)	−30	+35

The average results obtained with thirty-three chickens are summarized in Table 2.

Atropine (0.5 to 1.5 mg/kg, intramuscularly) and ganglion-blocking agents (hexamethonium, 2 to 20 mg/kg, or azamethonium, 15 to 30 mg/kg, each intravenously) usually enhanced the hypertension due to eledoisin. Local anaesthetics also potentiated the rise in blood pressure. In one experiment successive intravenous doses of procaine (2+8+40 mg/kg) increased the pressor effect of 0.3 μg/kg of eledoisin from 28 to 36, 64 and 160 mm Hg, respectively. Cocaine acted similarly at intravenous doses of 1 to 10 mg/kg.

Sympatholytic drugs and previous treatment with reserpine reduced or abolished the pressor response to eledoisin. A complete inhibition was obtained with 150 to 200 μg/kg of "hydergin" and 4 to 6 mg/kg of either 2-diethylaminomethylbenzo-1,4-dioxane (prosympal) or piperoxan; a partial inhibition followed 300 μg/kg of phentolamine, 5 mg/kg of phenoxybenzamine and 10 mg/kg of yohimbine. All drugs were given intravenously.

In chickens previously treated with reserpine (2 to 5 mg/kg subcutaneously 24 hr before the experiment) the hypotensive phase produced by 0.2 to 2 μg/kg of eledoisin was enhanced and the hypertensive phase reduced or abolished. Sometimes the fall in pressure was irreversible and resulted in death.

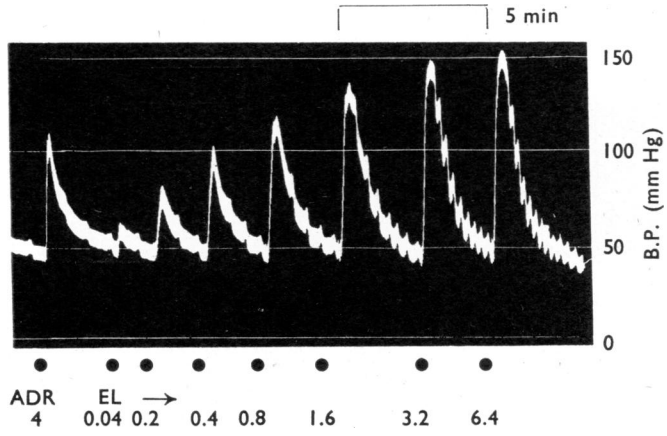

Fig. 9. Blood pressure of a decapitated chicken. The pressor effects of different intravenous doses of eledoisin (EL), in μg/kg, are shown. 0.6 μg of eledoisin was equivalent to approximately 4 μg of adrenaline (ADR). Note the clear dose/response relationship.

Decapitated preparations

In the decapitated chicken eledoisin regularly elicited a pure hypertension which satisfactorily correlated with the dose (Fig. 9). There was no appreciable tachyphylaxis. The threshold intravenous dose varied between 20 and 40 ng/kg. The response was very similar to that caused by adrenaline, the only differences being that eledoisin caused a more irregular descending segment of the pressure curve and a more prolonged latency between injection and onset of hypertension. One μg of eledoisin was equivalent to 4 to 8 μg of adrenaline, 1 to 2 μg of angiotensin and 120 to 360 μg of 5-hydroxytryptamine. Histamine and bradykinin possessed a negligible action on the chicken blood pressure.

As in the intact chicken, in the decapitated animal either dihydroergotamine (50 to 300 μg/kg, intravenously) or phentolamine reduced the hypertension due to eledoisin to the same extent as that due to adrenaline (Fig. 10). Hexamethonium increased the pressor responses to eledoisin and to adrenaline (Fig. 11). Previous treatment with reserpine (three intramuscular doses of 2 mg/kg each, on three successive

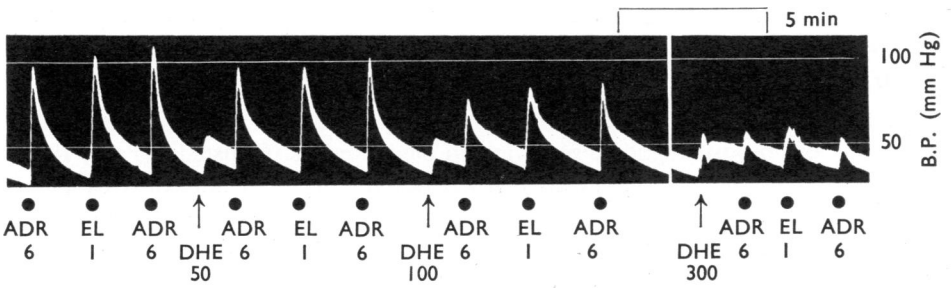

Fig. 10. Blood pressure of a decapitated chicken. The hypertensive effects of various intravenous injections (doses in μg/kg) are shown. The effects of eledoisin (EL) and of adrenaline (ADR) were lessened to the same extent by increasing doses of dihydroergotamine (DHE).

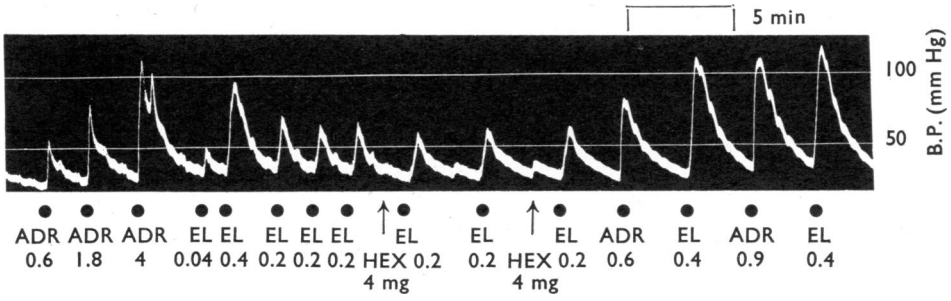

Fig. 11. Blood pressure of a decapitated chicken. The hypertensive effects of various intravenous injections (doses in μg/kg except where otherwise stated) are shown. Hexamethonium (HEX) enhanced the pressor effects of eledoisin (EL) and of adrenaline (ADR).

days) practically abolished the hypertensive effect of eledoisin but did not alter the response to adrenaline.

DISCUSSION

The first conclusion which may be drawn from the experiments described in this paper is that the action of eledoisin on the systemic arterial blood pressure differs sharply from one animal species to another. The dog is most sensitive to the polypeptide and in the anaesthetized dog eledoisin produces only a hypotension under all experimental conditions. This has been fully confirmed in unanaesthetized dogs by Olmsted & Page (1962). The response to eledoisin was opposite in the chicken to that in the dog. In the chicken the polypeptide usually caused hypertension. The responses of the rabbit and the guinea-pig were similar, leaving aside differences in sensitivity, to that of the dog; the response of the rat was variable, but there was predominantly hypertension. In the intact cat eledoisin always caused hypotension. The effect was, however, small and a marked tachyphylaxis usually developed. This made the cat unsuitable for the bioassay of eledoisin.

In the dog eledoisin was much more potent than bradykinin as a hypotensive agent. However, it is possible that other polypeptides approach, or even surpass,

eledoisin in their hypotensive effect. We refer to substance P (Stürmer & Franz, 1961; Haefeli & Hürlimann, 1962) and to some polypeptides in amphibian skin recently described (Erspamer, Bertaccini & Cei, 1962a, b).

It is likely that the effect of eledoisin is chiefly peripheral, on vascular smooth muscle and/or on postganglionic pathways to blood vessels (Olmsted & Page, 1962). In fact, the hypotensive action of eledoisin was not appreciably changed by ganglion-blocking agents, by atropine or by sympatholytic agents, and eledoisin powerfully counteracted the peripheral actions of catechol amines and of angiotensin. Moreover, injections of eledoisin into the dog femoral artery or coronary artery at extremely low doses (0.1 ng and less) produce prompt local vasodilatation without any effect on systemic blood pressure (Glässer & Bergamaschi, unpublished). Since eledoisin can antagonize enormous doses of noradrenaline in the dog it is not surprising that ganglionic blockade and prior medication with reserpine, both hindering the compensatory discharge of catechol amines, have no obvious effect on the hypotension due to eledoisin.

It has been shown in preliminary experiments (Erspamer & Anastasi, 1962) that, following subcutaneous doses of 100 to 300 μg/kg, eledoisin is detectable in the blood stream only for 10 to 15 min, whereas hypotension lasts for hours. The possibility that eledoisin is bound tenaciously to vascular smooth muscle is now being tested.

The hypertensive action of eledoisin in rats is similar to that of bradykinin (Croxatto & Belmar, 1961). The pure hypertensive response seen in decapitated chickens is probably essentially due to release of catechol amines from the adrenal medulla and/or from other stores. This opinion is strengthened by results obtained with chickens previously treated with reserpine, in which animals the hypertensive effect of eledoisin is considerably reduced or even abolished. It is evident that the capacity of eledoisin to release catechol amines is very strong, since it is manifested by doses of the polypeptide as low as 0.1 to 1 μg/kg.

Preliminary results obtained by Erspamer & Anastasi (1962) and confirmed by Glässer & Bergamaschi (unpublished) in more extensive studies, show that the hypotensive effect of eledoisin in the dog must be due chiefly to vasodilatation of the musculo-cutaneous vessels which, together with the coronary and the meningeal vessels, are particularly sensitive to the polypeptide.

Man seems to behave like the dog in his response and sensitivity to eledoisin (Sicuteri, Fanciullacci, Franchi & Michelacci, 1963). This observation represents the basis for the clinical trial of the substance in spasms of certain vascular areas or in hypertensive episodes which are attributable to excess of circulating catechol amines.

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