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

BY

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In the preceding paper the occurrence of physalaemin in the skin of *Physalaemus fuscumaculatus* and of related *Physalaemus* species as well as the actions of the polypeptide on a number of extravascular smooth muscle preparations were reported (Bertaccini, Cei & Erspamer, 1965). In this paper the actions of physalaemin on the systemic arterial blood pressure of some common laboratory animals are described.

It will be seen that physalaemin, from amphibian skin, eledoisin, from the posterior salivary glands of an octopod, and substance P, from the brain and gastrointestinal mucosa of all vertebrate classes, constitute a polypeptide group possessing a strictly similar, potent action on the blood pressure of all examined experimental animals.

Results here reported offer a basis for a more thorough study of the cardiovascular actions of physalaemin, especially of its actions on different vascular areas, and for the trial of the polypeptide in man.

METHODS

Dogs were anaesthetized with sodium pentobarbitone (30 mg/kg, intravenously), cats with urethane (1 g/kg, intraperitoneally) followed by chloralose (50 to 70 mg/kg, intravenously), rabbits, rats and chickens with urethane (1 to 1.5 g/kg, intraperitoneally or intravenously).

Chickens were decapitated by severing the spinal cord between C5 and C6 and firmly tying the neck with a 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 pithed or decapitated animals was maintained by a ventilatory pump.

The electrocardiogram of the intact anaesthetized dog was recorded by means of a direct-writing Sanborn Twin-Viso recorder. Both bipolar and unipolar limb leads were taken.

Drugs. Physalaemin was either the pure natural or the synthetic polypeptide or else a partially purified extract of the skin of *Physalaemus fuscumaculatus* containing 180 to 200 μg of physalaemin per mg of dry residue. Paper chromatography and paper electrophoresis showed that in this extract physalaemin was virtually the only substance with activity on vascular smooth muscle.

A preparation of substance P from horse serum (Ro 1-9256/7), containing 150 units/mg, was kindly supplied by Messrs Hoffmann-La Roche, Basel; samples of synthetic bradykinin and of Lys^8-vasopressin by Messrs Sandoz, Basel; samples of synthetic angiotensin by Ciba, Basel; and samples of synthetic eledoisin and 5-hydroxytryptamine creatinine sulphate by Farmitalia S.p.A., Milan.

RESULTS

Dog

In dogs anaesthetized with pentobarbitone, intravenous injections of physalaemin regularly produced a fall of blood pressure which with low doses was proportional, both in intensity and duration, to the dose. However, increasing the dose above a certain level did not increase the intensity of the depressor effect so much as the duration. For large doses, blood pressure began to return rapidly until 10 to 20 mm Hg below the pre-injection level, but then took a considerably longer time to reach this level. In no instance was either tachyphylaxis or sensitization observed. The hypotension was generally accompanied by an increase in heart rate.

The threshold dose for physalaemin given by rapid intravenous injection was 0.1 to 1 ng/kg, but a dose one million-times greater could be tolerated by the animal, with full recovery after 5 to 6 hr. The administration of still larger doses was hindered by the difficult solubility of the polypeptide. This unique example of tolerability is shown in Fig. 1 which illustrates, far better than any description, the effect of different, rapidly increasing doses of physalaemin on blood pressure.

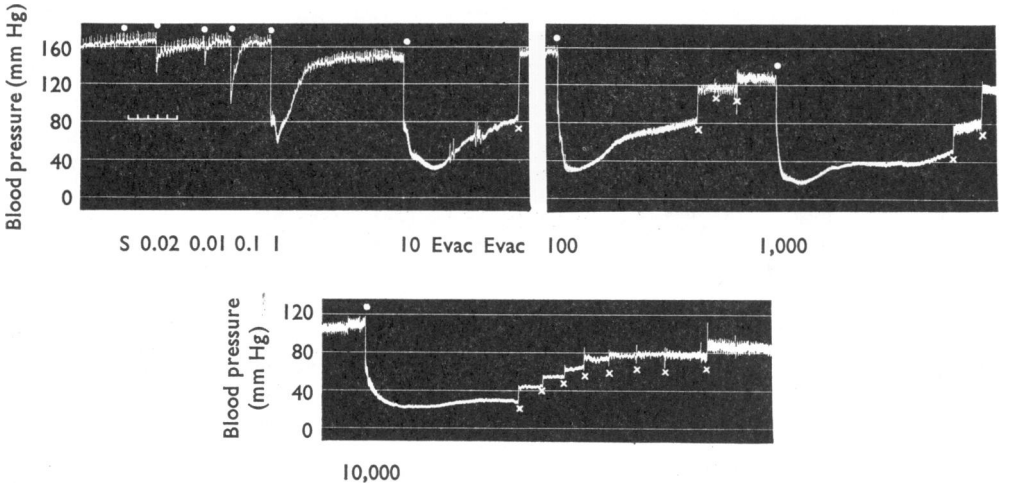

Fig. 1. Blood pressure of a dog weighing 12 kg, anaesthetized with sodium pentobarbitone (30 mg/kg, intravenously) after treatment with 0.2 mg/kg atropine sulphate, intramuscularly. S, Control physiological solution; Evac, evacuation of the bowel. Time marks, 1 min. At × the drum was stopped for 15 to 30 min. The hypotensive effect of increasing doses of physalaemin, in μg, is shown. Whereas the threshold dose was 0.01 μg, a dose of 10,000 μg could be tolerated, with recovery of the animal.

Intravenous doses of 1 μg/kg of physalaemin produced moderate salivation, borborygmi and evacuation of formed stools, doses of 10 μg/kg intense salivation, again borborygmi and respiratory stimulation, and doses of 100 to 1,000 μg/kg salivation and considerable respiratory stimulation. Authentic diarrhoea was never seen in anaesthetized animals.

Given by intravenous infusion, physalaemin caused hypotension which again was proportional to the dose and lasted as long as the infusion was continued. However, whereas in some experiments it was possible to keep blood pressure at a fairly constant

level during infusion, in other instances the pressure after an initial rapid fall showed large oscillations, sometimes with a tendency to rise slowly, in spite of continuous infusion of the polypeptide at a constant rate.

Fig. 2 shows the effect of intravenous infusion of different doses of physalaemin for 20 to 30 min. It may be seen that after the infusion had been discontinued there was a rapid rise of blood pressure which, however, never attained the original level. The threshold dose was generally of the order of 2 to 5 ng/kg/min, but in the experiment illustrated in Fig. 2 a dose of 0.6 ng/kg/min produced an appreciable effect. With a dose

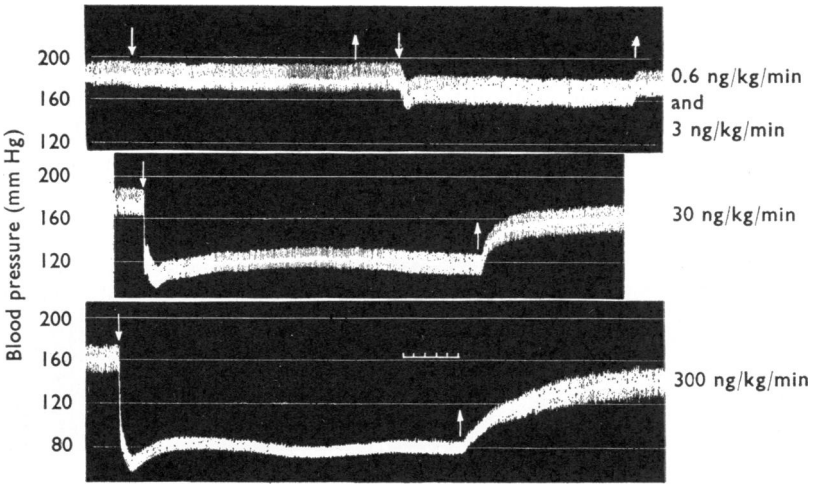

Fig. 2. Blood pressure of a dog anaesthetized with pentobarbitone after treatment with atropine. Time marks, 1 min. The hypotensive effect of intravenous infusion of different doses (at right) of physalaemin is shown. ↓ Infusion started; ↑ infusion stopped. The pressure fall was proportional to the dose of physalaemin, and lasted as long as the infusion was continued.

of 30 ng/kg/min the fall in pressure was 50 to 55 mm Hg, with 300 ng/kg/min 80 mm Hg. The infusion of 16 μg/kg/min (3,000- to 10,000-times the threshold dose) for a 30-min period (total 0.5 mg/kg) was followed by prompt, partial recovery. Hypotension was sometimes accompanied by evacuation of formed stools and salivation.

The threshold subcutaneous dose of physalaemin which lowered the blood pressure was approximately 1 μg/kg. Successive doses of 1 μg/kg given at an interval of 30 to 40 min, before the effect of the preceding dose had completely subsided, produced approximately the same pressure drop, starting from a progressively lower basal level. 150 μg/kg of physalaemin reduced the pressure for more than 5 hr with a maximum decrease from 160 to 90–95 mm Hg between 20 and 90 min after injection; 800 μg/kg produced hypotension lasting more than 12 hr, with a maximum decrease from 130 to 55–70 mm Hg during the first 3 hr. After 12 hr blood pressure was still 40 mm Hg below the control level, but the animal had definitely overcome the hypotensive crisis (Fig. 3).

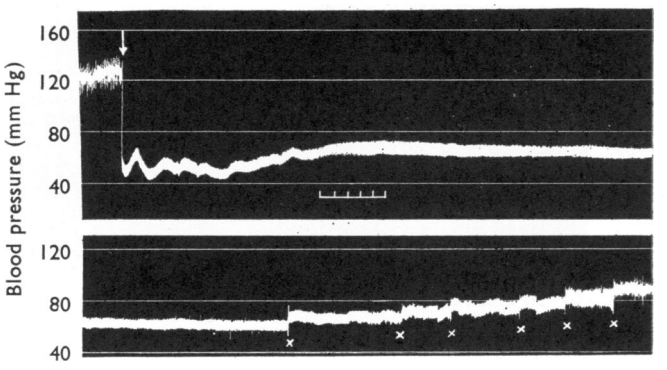

Fig. 3. Blood pressure of a dog anaesthetized with pentobarbitone after treatment with atropine. Time marks, 5 min. The fall of pressure was produced by a subcutaneous injection of 0.8 mg/kg of physalaemin, given at the arrow. At × the drum was stopped for 1 to 4 hr. Hypotension lasted for more than 12 hr. The records are continuous.

Effects of atropine, ganglion-blocking agents, sympatholytic agents and chlorpromazine

Atropine (0.1 to 0.2 mg/kg, intramuscularly) did not appreciably affect either the intensity or the duration of the hypotension produced by physalaemin (Figs. 1, 2 and 3).

Hexamethonium bromide (5 mg/kg, intravenously) or azamethonium bromide (2 to 3 mg/kg, intravenously) enhanced both duration and intensity of the hypotensive effect of physalaemin. In some experiments prolongation of duration was more evident (Fig. 4,*b*), in others enhancement of intensity. As the blocking action of the ganglion-blocking agent subsided, potentiation of the depressor effect of the polypeptide disappeared.

The effect of 2-diethylaminomethylbenzo-1,4-dioxan (Prosympal, 3 to 5 mg/kg intravenously) was similar in that it caused a clear increase of the hypotensive effect of physalaemin and apparently also a slight prolongation of this effect. The depressor action of 7.5 to 30 ng/kg of physalaemin was nearly doubled by Prosympal (Fig. 4,*a*).

Physalaemin and adrenaline administered in the same injection after Prosympal produced a hypotensive response which was approximately the sum of the responses given by single drugs.

Chlorpromazine (1+2 mg/kg, intravenously) caused only a slight prolongation of the pressure fall induced by 25 to 50 ng/kg of physalaemin. Even after doses of the polypeptide as large as 250 ng/kg blood pressure promptly returned, as usual, to the control level. Thus, whereas, according to Rocha e Silva, Corrado & Ramos (1960), chlorpromazine displayed in the cat an intense potentiating effect on the bradykinin-induced hypotension, nothing similar could be observed in the dog for physalaemin.

Effect of catechol amines and of angiotensin

Physalaemin, like eledoisin, was very effective in antagonizing the pressor effects of catechol amines, nicotine and angiotensin in the dog. When given in the same quick intravenous injection, 0.5 μg physalaemin completely abolished the hypertensive effect of 50 to 75 μg of (−)-noradrenaline, 50 μg of (−)-adrenaline, or 2 mg of nicotine bitartrate.

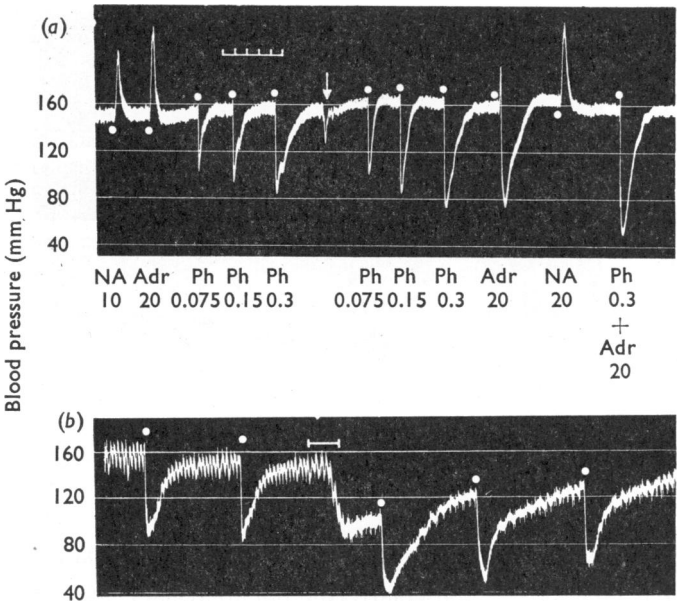

Fig. 4. Blood pressure of two dogs anaesthetized with pentobarbitone after treatment with atropine. Time marks, 1 min. (*a*) The actions of physalaemin (Ph), adrenaline (Adr) and noradrenaline (NA) before and after the intravenous administration, at the arrow, of 5 mg/kg of prosympal. Doses in μg. Prosympal potentiated only the intensity of the hypotensive response to physalaemin. (*b*) The action of 0.5 μg of physalaemin (at white dots) before and after the intravenous administration (during white bar) of 3 mg/kg of azamethonium bromide. The hypotensive action of physalaemin was somewhat prolonged by the ganglion-blocking agent.

10 μg/kg of noradrenaline injected during an intravenous infusion of 50 ng/kg/min of physalaemin had no effect, 100 μg/kg produced a hypertensive response similar to that elicited by 5 to 10 μg/kg noradrenaline in animals not receiving infusion of physalaemin.

As far as angiotensin is concerned, approximately 10 μg of this polypeptide was necessary to antagonize the hypotensive effect of 1 μg of physalaemin.

Potencies relative to eledoisin, bradykinin, substance P and histamine

In comparative experiments physalaemin was about three- to four-times more active, as a hypotensive agent, than eledoisin, 200- to 1,000-times more active than bradykinin, and 200- to 800-times more active than histamine, on a weight basis. 1 μg of physalaemin was equivalent to 100 to 140 units of substance P (Fig. 5).

The hypotensive curve produced by small and medium doses of physalaemin was indistinguishable from that produced by equivalent doses of either eledoisin or substance P. However, when the effects of larger doses of physalaemin and eledoisin were compared, it clearly appeared that the hypotension produced by eledoisin lasted longer than that due to physalaemin. Fig. 6 shows that return to the basal level was more prompt for 0.5 and 5 μg/kg of physalaemin than for 0.5 and 5 μg/kg of eledoisin, respectively. This occurred in spite of the fact that, as stated above and shown in Fig. 6, physalaemin was considerably more potent than eledoisin as far as the size of the pressure fall is concerned.

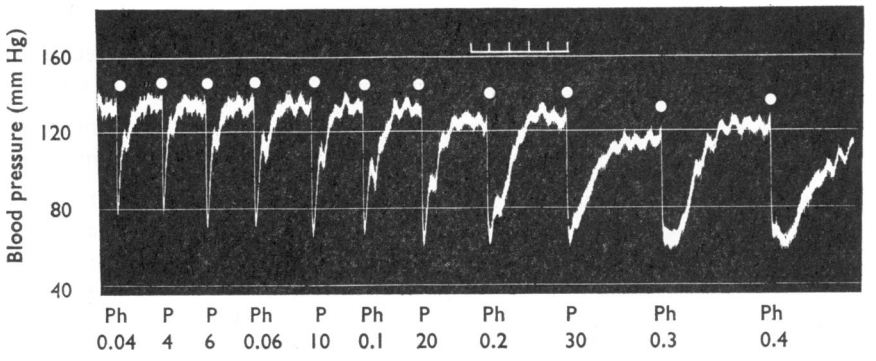

Fig. 5. Blood pressure of a dog anaesthetized with pentobarbitone after treatment with atropine. Time marks, 1 min. The effects on blood pressure of different intravenous doses of physalaemin (Ph, in μg) and crude substance P (P, in units) are shown. The hypotensive effect of substance P is indistinguishable from that of physalaemin.

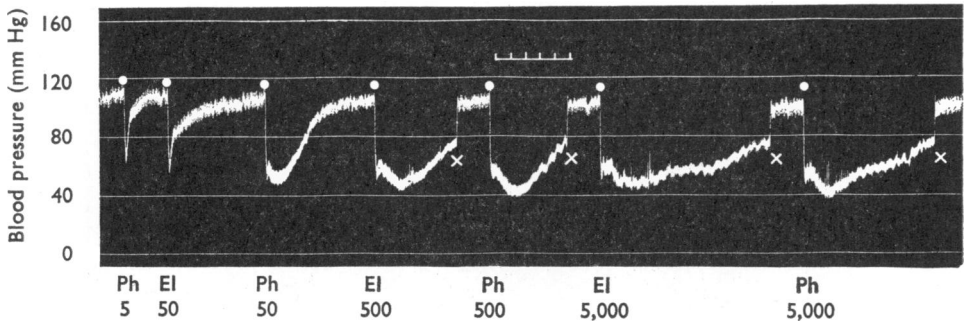

Fig. 6. Blood pressure of a dog anaesthetized with pentobarbitone after treatment with atropine. Time marks, 1 min. At the first and third × the drum was stopped for 10 min, and at the second × for 7 min. The effect of different intravenous doses (in ng) of physalaemin (Ph) and eledoisin (El) is shown. Note that the hypotensive effect of the 500- and 5,000-ng doses of physalaemin was of shorter duration than that of the corresponding doses of eledoisin, in spite of the more intense effect of the former polypeptide.

Effects on the electrocardiogram

Electrocardiogram tracings were recorded from five anaesthetized dogs immediately before and at regular intervals, up to 96 hr, after administration of physalaemin. Two dogs received physalaemin by intravenous infusion (5 μg/kg/min for 60 min) and three by subcutaneous injection (0.6 mg/kg). In both instances the dose of physalaemin was approximately 1,000-times the threshold dose.

Changes in the electrocardiogram were on the whole rather moderate. They consisted essentially of modifications of the P and T waves and the QRS complex dependent on tachycardia. Changes in the T wave were observed mainly at the time of maximum hypotension. The Q-T segment : frequency ratio was constantly shortened and signs of damage never occurred. All these changes may therefore be related to modifications of heart rate, which give rise to a higher electrical homogeneity of myocardial layers, or to changes in

myocardial tension caused by hypotension. Moreover, the T wave showed a clear tendency to return to normal. This appeared clearly in experiments in which electrocardiogram tracings were recorded up to 24 hr after subcutaneous administration of physalaemin.

All these results, together with the constant absence of marked S-T segment depression, suggest that there was no serious damage caused to the heart by physalaemin.

Only one dog presented rather marked changes in the P wave indicating a possible overload of the pulmonary circulation. In this connexion it may be noted that pulmonary hypertension was observed by Nakano (1964) in dogs following administration of eledoisin.

Cat

The fall of blood pressure produced by physalaemin varied considerably from one animal to another and was, on the whole, moderate as for eledoisin. In one cat, in which the control level of blood pressure was low (80 mm Hg), physalaemin increased blood pressure. Tachyphylaxis was a constant feature; when intravenous doses of physalaemin were repeated at short intervals there was a striking decrease in the sensitivity to the polypeptide, up to thirty-times or more (Fig. 7). At the beginning of the experiment the threshold intravenous dose of physalaemin varied between 10 and 200 ng/kg.

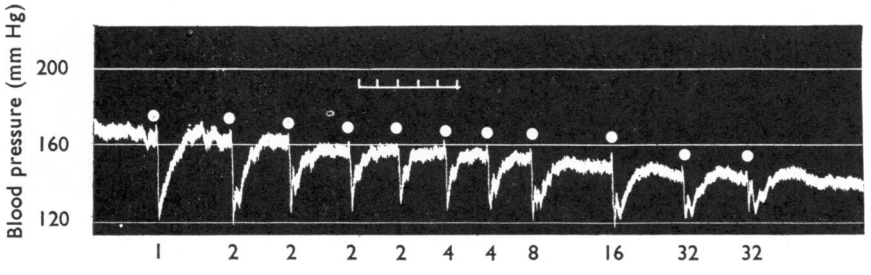

Fig. 7. Blood pressure of a cat anaesthetized with chloralose (90 mg/kg, intravenously). Time marks, 1 min. The effects of different intravenous doses of physalaemin, in μg, are shown. Note the evident tachyphylaxis.

Comparison with other hypotensive substances was virtually impossible. It is of interest that our crude preparation of substance P, towards which tachyphylaxis was apparently less intense, caused death in all the three tested cats when given in doses of 25 to 50 units/kg. Yet, half the lethal dose produced a pressure fall of only 40 to 60 mm Hg.

From the above it should again be concluded that the cat blood pressure is completely unsuitable for the bioassay of physalaemin, eledoisin and related substances.

Rabbit

Rabbits, anaesthetized with urethane, regularly responded to intravenous physalaemin by a fall of blood pressure. The threshold dose was 0.3 to 3 ng/kg. The size of the pressure fall was related to the dose (Fig. 8), and the response was accompanied by a stimulation of respiration. Tachyphylaxis was completely lacking. Doses 500- to 1,000-times larger than the threshold dose were well tolerated, in spite of an initial fall of pressure down to 5 to 10 mm Hg.

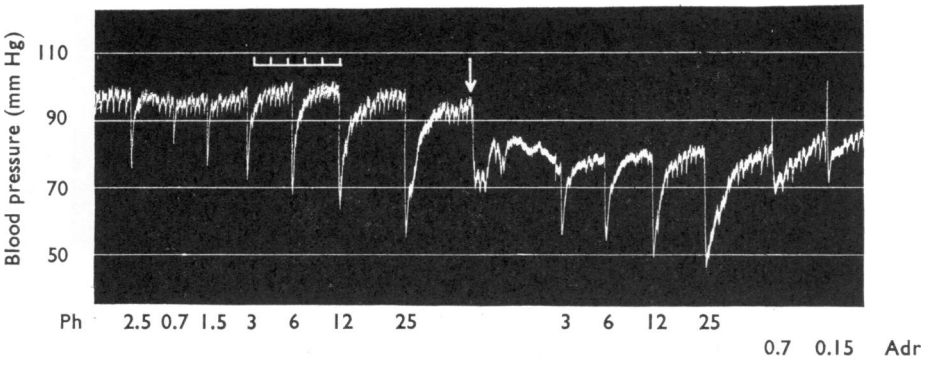

Fig. 8. Blood pressure of a rabbit anaesthetized with urethane (1.2 g/kg, intravenously). Time marks, 1 min. The effects of different intravenous doses of physalaemin (Ph, in ng/kg) and of adrenaline (Adr, in μg/kg) are shown. At the arrow an intravenous injection of 4 mg/kg of prosympal was given. Note the complete absence of tachyphylaxis and the clear dose/response relationship. Treatment with prosympal produced only slight, if any, prolongation of the hypotensive effect of physalaemin.

Atropine (3 to 4 mg/kg, subcutaneously) did not affect the response to physalaemin; Prosympal (4 mg/kg, intravenously) similarly produced little, if any, change.

Considering the intensity of the pressure fall, 1 μg of physalaemin was equivalent to 2.5 to 3 μg of eledoisin, 300 to 350 units of substance P, and 15 to 25 μg of bradykinin.

Rat

The pressure response to intravenous physalaemin was variable, mainly depending on the control level. At levels above 100 mm Hg the polypeptide generally produced a depressor response (threshold 10 to 20 ng/kg, intravenously) which had a clear correlation with the dose; 1 μg of physalaemin was equivalent to approximately 2.5 to 3 μg of eledoisin and to 300 units of substance P.

In animals with control pressure level less than 70 mm Hg (either rats not premedicated or animals previously treated with 30 mg/kg of hexamethonium, intravenously) physalaemin regularly caused hypertension. The pithed rat responded, like the animal which had received a ganglion-blocking agent, with a rise of blood pressure (threshold 0.05 to 1 μg/kg); 1 μg of physalaemin was approximately as active as 2 μg of eledoisin or 0.5 μg of adrenaline.

Chicken

The bulk of the experiments were carried out on the decapitated chicken. In this preparation physalaemin regularly elicited a diphasic response consisting of a brief hypotension followed by a more intense and sustained rise of blood pressure (Fig. 9). The response had a clear correlation with the dose and there was no appreciable tachyphylaxis. In the present series of experiments a pure hypertensive response was never seen either with physalaemin or with eledoisin. The threshold intravenous dose varied between 10 and 50 ng/kg. The response was very similar to that caused by catechol amines, leaving aside, of course, the initial pressure fall. 1 μg of physalaemin was equivalent to 3 μg of eledoisin, 8 to 10 μg of adrenaline, and 300 units of substance P. However, the initial pressure fall seemed to be somewhat more pronounced for substance P. Histamine and bradykinin possessed a negligible action on this preparation.

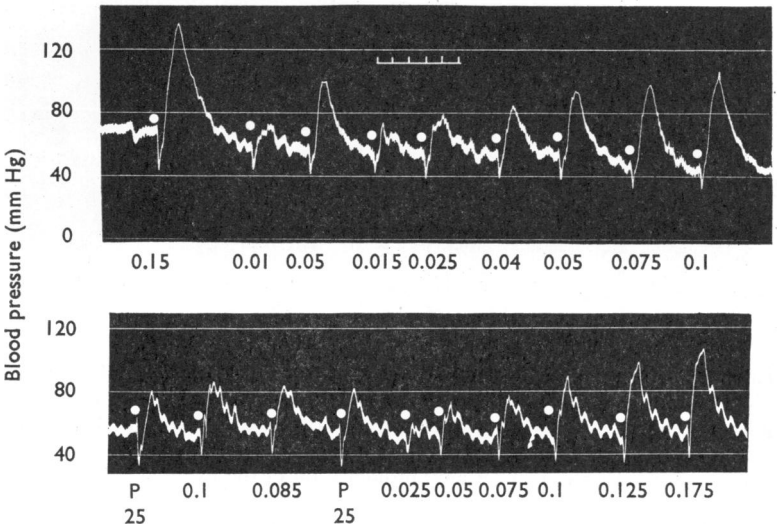

Fig. 9. Blood pressure of two decapitated chickens. The pressor response to various intravenous injections of physalaemin (doses in μg/kg) and to intravenous administration of 25 units/kg of substance P is shown. Note the clear dose/response relationship. In this experiment 1 μg of physalaemin was equiactive to approximately 300 to 350 units of substance P. Time marks, 1 min.

In decapitated chickens previously treated with reserpine (three intramuscular doses of 2 mg/kg each, on three successive days) the hypotensive phase was somewhat enhanced and the subsequent pressure rise reduced or abolished (less than 2%). However, even hypotension produced by 100 μg of physalaemin was easily antagonized by 1 μg of adrenaline.

In the intact anaesthetized chicken the response was similar, but the dose/response relationship was on the whole less satisfactory.

DISCUSSION

Our results show that, on the blood pressure of the commonest laboratory animals, physalaemin strictly resembles eledoisin in its pharmacological actions. It is generally impossible to distinguish the two endecapeptides from each other. Like eledoisin, physalaemin potently lowers the blood pressure of the dog and the rabbit, under all tested experimental conditions; like eledoisin, physalaemin shows striking tachyphylaxis in the cat; finally, exactly like eledoisin, physalaemin displays in the chicken a predominantly hypertensive effect. Thus, the action of physalaemin on the systemic arterial blood pressure, like that of eledoisin, differs sharply from one animal species to another.

In all species examined physalaemin produces a blood pressure response which is two- to four-times more intense than that elicited by equimolar doses of eledoisin. However, at least in the dog, the effect of eledoisin lasts longer than that of physalaemin. This is especially evident when comparing the effect of large doses of the two polypeptides. The different duration of the hypotensive effect is satisfactorily explained by the observations of Nobili (1965), who showed that homogenates of mammalian tissues inactivate physalaemin considerably faster than eledoisin.

It is probable that the hypotensive effect of physalaemin, like that of eledoisin, is chiefly peripheral, on vascular smooth muscle and/or on postganglionic pathways to blood vessels. In fact the hypotensive action of physalaemin is not appreciably changed by atropine and only moderately so by adrenergic neurone-blocking agents and ganglion-blocking agents; in addition, physalaemin counteracts the peripheral actions of catechol amines and of angiotensin. Since ganglion-blocking agents and adrenergic neurone-blocking agents hinder the compensatory discharge of catechol amines or block the effects of this discharge, it is quite understandable that the above agents enhance more evidently the intensity of the hypotensive response to physalaemin than its duration.

The pressure rise seen in decapitated chickens seems to be essentially due to release of catechol amines from the adrenal medulla and/or from other stores. This opinion is strengthened by results obtained in chickens previously treated with reserpine, in which the hypertensive effect of physalaemin is considerably reduced or even abolished. The capacity of physalaemin to release catechol amines is remarkable, since 1 μg of the polypeptide displays a hypertensive action equivalent to that of 8 to 10 μg of adrenaline.

Owing to the excellent dose/response relationship, the dog blood pressure and, subordinately, the pressure of the decapitated chicken may be profitably used for the assay of physalaemin and for its distinction from several other naturally occurring substances which are active on vascular smooth muscle.

Physalaemin and eledoisin represent the most potent hypotensive polypeptides so far known. The only polypeptide which can compete with them in potency is apparently substance P which, as has been repeatedly stated, shows the strictest pharmacological resemblance to physalaemin and eledoisin.

Results reported in this and in other papers (Erspamer & Falconieri Erspamer, 1962; Erspamer & Glässer, 1963; Bertaccini *et al.*, 1965) strongly suggest that substance P may be related to eledoisin and physalaemin from a chemical point of view as well, more closely than has been suspected so far.

In fact, from studies carried out in this and other laboratories (Camerino, De Caro, Boissonnas, Sandrin & Stürmer, 1963; Bernardi, Bosisio, Chillemi, De Caro, De Castiglione, Erspamer, Glässer & Goffredo, 1964; Stürmer & Franz, 1964; Schröder & Lübke, 1964) on more than 100 eledoisin- and physalaemin-like polypeptides it clearly emerged that the *conditio sine qua non* for the appearance of the most important pharmacological actions, and especially of the potent effect on the dog blood pressure, was the occurrence in the polypeptide molecule of methioninamide as C-terminal amino acid. Methioninamide may be substituted by other sulphur-containing amino acids, but these have so far not been found in nature.

According to Haefeli & Hürlimann (1962) substance P gives, upon acid hydrolysis, the following thirteen amino acids: Phe, Ser, Lys, Asp(OH), Glu, Pro, Gly, Ala, Leu, Ile, Arg, Val, Thre. It may be seen that as many as ten of them (all but the last three) are also present in the molecule of the endecapeptide eledoisin, and eight in the molecule of the endecapeptide physalaemin. The only eledoisin amino acid lacking in the molecule of substance P is apparently the tremendously important methioninamide. The suspicion seems to be justified that the labile methioninamide residue has escaped the attention of the

research workers, who have isolated and studied substance P. We would suggest that this possibility be checked.

Haefeli & Hürlimann (1962) affirm that the activity of 1 μg of absolutely pure substance P will probably be somewhat higher than 120 units. If this statement is true, the potency of physalaemin on blood pressure will be at least as intense as that of substance P.

Eledoisin, physalaemin and substance P are not the only representatives of eledoisin-like polypeptides occurring in nature; other highly active polypeptides belonging to this group are present in the skin of some *Phyllomedusae*. Their study is in progress.

SUMMARY

1. The blood pressure response produced by parenteral administration of physalaemin, the main active polypeptide of the skin of the South American amphibian *Physalaemus fuscumaculatus*, has been investigated in some experimental animals. The response varied conspicuously from one animal species to another.

2. In the dog the polypeptide elicited clear hypotension under all experimental conditions. Physalaemin was active at extremely low dose levels and, whereas hypotension was not appreciably affected by previous treatment with atropine, it was moderately enhanced by adrenergic neurone-blocking and ganglion-blocking agents. Physalaemin potently antagonized the pressor effects of catechol amines, nicotine and angiotensin.

3. Doses of physalaemin as large as one million times the threshold dose could be given by rapid intravenous injection with full recovery of the animal. Long-lasting hypotension could be obtained by intravenous infusion or subcutaneous injection of physalaemin. The mechanism of action of the polypeptide in the dog is believed to be chiefly peripheral.

4. The rabbit behaved like the dog, even in the intensity of blood pressure response to physalaemin. The cat was considerably less sensitive and showed striking tachyphylaxis towards the polypeptide. The rat gave variable responses depending on the control level of blood pressure.

5. In the decapitated chicken physalaemin produced a diphasic response with a predominant hypertensive component. Hypertension is believed to depend largely, if not entirely, on the release of catechol amines from body stores.

6. In its effects on blood pressure physalaemin showed the closest resemblance to eledoisin and substance P. The surprising similarity in the amino acid composition of the three polypeptides is emphasized.

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