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

BY

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The occurrence of caerulein in Australian hylids has been described in the preceding paper, together with some characteristics of the polypeptide (De Caro, Endean, Erspamer & Roseghini, 1968).

In this paper the actions of caerulein on the systemic blood pressure of some common laboratory animals are reported. The information obtained should be useful for the trial of the polypeptide in man.

Subsequent papers will describe the actions of caerulein on extravascular smooth muscle and on external secretions of the gut.

METHODS

Dogs were anaesthetized with sodium pentobarbitone (30 mg/kg, intravenously), cats with urethane (1 g/kg, intraperitoneally) followed by chloralose (50–70 mg/kg, intravenously), rabbits and chickens with urethane (1 to 1.5 g/kg, intravenously), rats with urethane (1.2 g/kg, intraperitoneally) or with amylobarbitone (150 mg/kg in 2% acacia solution, intraperitoneally).

Pithed rats were prepared according to Shipley & Tilden (1947). Chickens were decapitated by severing the spinal cord between C_5 and C_6 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 anaesthetized dog was recorded by means of a direct-writing Twin-Viso recorder. Bipolar and unipolar limb leads as well as precordial leads were taken.

Isolated rabbit heart. The rabbit heart was suspended in a modified Langendorff apparatus at 37° C and perfused under constant pressure with an oxygenated Ringer-Locke solution. Ventricular contraction was recorded by means of a light frontal lever (×12 magnification) connected to the left ventricle. Electrocardiographic tracings were recorded according to the technique described by Venturi (1955).

Capillary permeability. Guinea-pigs were injected intravenously with a 5% solution of pontamine sky blue 6 BX in saline (1.2 ml./kg) using the saphenous vein, and the injection of the test substances was then made intradermally (0.1 ml.) into the previously depilated dorsal skin. The extent of the coloured area was measured 60 min after the injection of the test substances.

In man the injections of the test substances were made intradermally (0.1 ml.) on the flexor surface of the forearm. Attention was paid to the onset, duration, extension and characteristics of the cutaneous reaction represented by a typical weal.

Drugs. Caerulein was either the pure natural or the synthetic polypeptide, both prepared at the Farmitalia S.p.A. Laboratories for Basic Research, Milan. Synthetic bradykinin was kindly supplied by Messrs. Sandoz, Basel, synthetic human gastrin-I and des-glutamyl human gastrin-I by R. C. Sheppard, Robert Robinson Laboratories, University of Liverpool, and synthetic eledoisin and synthetic physalaemin by Farmitalia S.p.A., Milan.

RESULTS

Dog

In dogs anaesthetized with pentobarbitone caerulein consistently reduced blood pressure. Only in a few exceptional cases was a biphasic response, consisting of moderate hypertension followed by a pressure fall, observed. Both the intensity and duration of the hypotension were proportional to the dose of polypeptide injected (Fig. 1). Above a certain level, however, increase of dosage usually had a greater effect on the duration of the depressor response than on its intensity.

Tachyphylaxis was generally lacking but, not infrequently, a moderate, progressively increasing desensitisation could be observed.

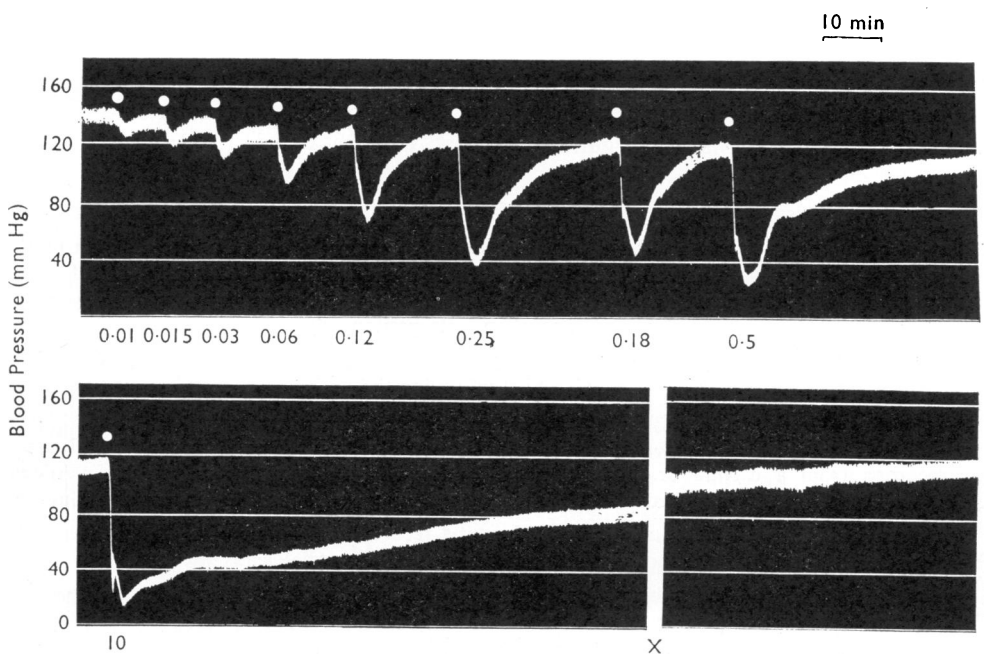

Fig. 1. Blood pressure of a dog weighing 15 kg, anaesthetized with sodium pentobarbitone (30 mg/kg, intravenously) after treatment with atropine sulphate (0.2 mg/kg, intramuscularly). Time, 10 min. At × the drum was stopped for 75 min. The hypotensive effect of increasing doses of caerulein (in μg/kg) is shown. Whereas the threshold dose was 0.01 μg/kg, a dose of 10 μg/kg could be tolerated with full recovery after 3 hr and 30 min.

The threshold dose for caerulein given by rapid intravenous injection was 10–100 ng/kg. Doses of 100–1,000 μg/kg could be tolerated, with full recovery of the animal after 3 to 9 hours.

The shape of the response to caerulein varied conspicuously from one animal to another. With low or medium doses of the polypeptide the pressure fall was generally gradual, and the descent was eventually interrupted by a short-lived moderate pressure rise. With large doses, pressure fall was always abrupt and the inscription in the hypotensive curve of a hypertensive return was more frequent.

Blood pressure usually returned to basal levels within 2–15 min if small or medium doses of caerulein were used. After large doses of the polypeptide, however, blood pressure began to return gradually until 10–20 mm Hg below the pre-injection level, but then took a considerably longer time to reach this level.

In about half the number of dogs injected with caerulein hypotension was accompanied by bradycardia.

When administered by intravenous infusion, caerulein again produced hypotension, which was proportional to the dose received and lasted as long as the infusion was continued. The same effect was produced if infusion was repeated, after a period of interruption, at the same rate as before the interruption (Fig. 2). In some experiments pressure level was fairly constant throughout the experiment ; in others is showed moderate oscillations ; in still others it showed a tendency to rise, despite infusion of the polypeptide at a constant rate.

The threshold dose was generally of the order of 5–15 ng/kg/min. With a dose of 15–20 ng/kg/min the fall in pressure was 10–20 mm Hg, with 250–300 ng/kg/min it was 40–80 mm Hg. Hypotension was sometimes accompanied by borborygmi and occasionally by evacuation of formed or liquid stools.

A dog given caerulein at a rate of 15 μg/kg/min presented a fall of pressure from 170 to 30 mm Hg. As the infusion continued, the pressure slowly returned to 50 mm Hg and then fell again to 40 mm Hg. Pulse pressure became progressively smaller. The dog died after 3.5 hr, having received in the course of the experiment as much caerulein as 3 mg/kg. A short interruption of the infusion after 1 hr was followed by a prompt rise of pressure, indicating the possibility of recovery. Atropine (0.3 mg/kg) injected intramuscularly after 1.5 hr did not affect hypotension but diminished bradycardia.

The threshold subcutaneous dose effective in lowering the blood pressure of the dog was approximately 5–10 μg/ml.: 10 μg/ml. produced a 10 mm Hg fall lasting 60–90 min ; 25 μg/kg reduced the pressure for 3 hr with a maximum decrease from 140 to 110 mm Hg between 20 and 70 min after injection ; 60 μg/kg produced hypotension lasting 12 hr, with a maximum decrease from 120 to 70 mm Hg during the first 2 hr ; finally 750 μg/kg caused a drop of pressure lasting more than 10 hr, with a maximum decrease from 150 to 60 mm Hg during the first hour.

Effects of atropine and sympatholytic agents

Atropine (0.1–0.2 mg/kg, intramuscularly or intravenously) produced a 25–40% reduction of the intensity of the hypotensive response to small doses of caerulein, with no appreciable effect on the duration of hypotension.

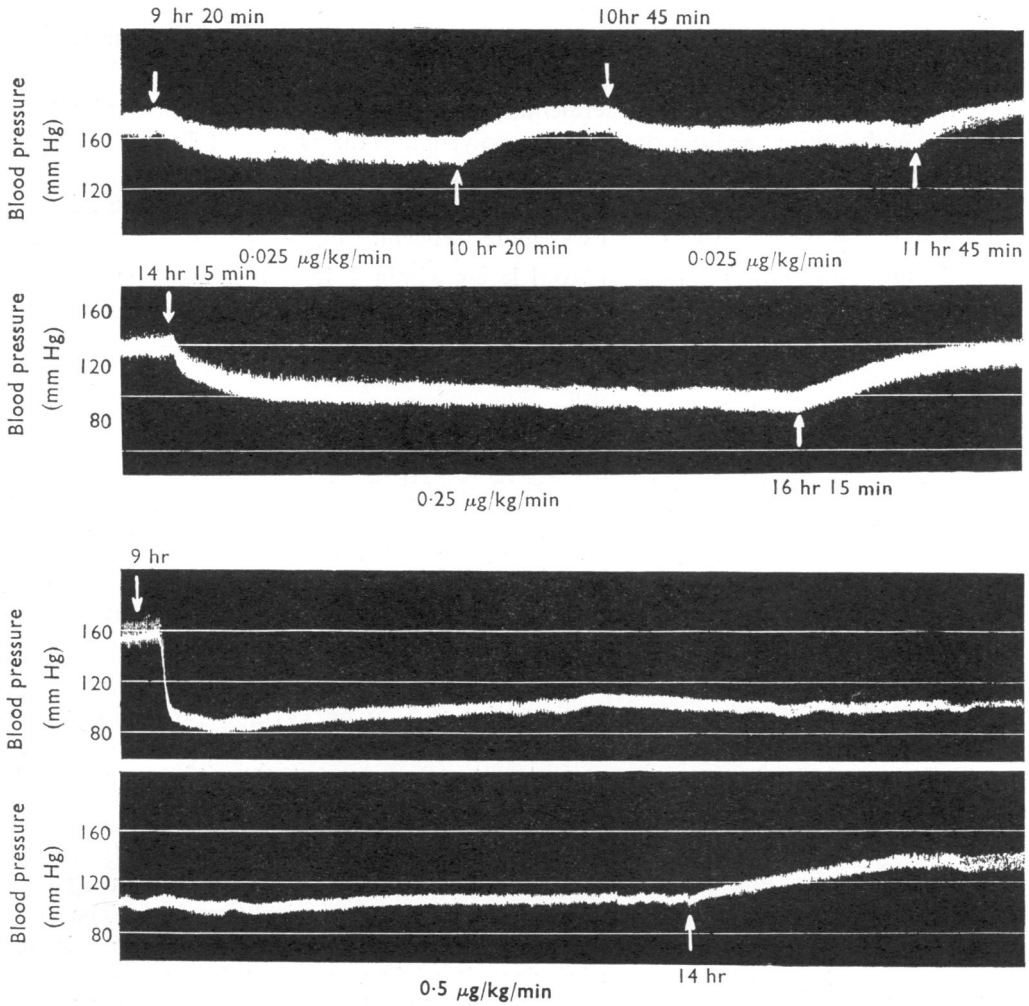

Fig. 2. Blood pressure of two dogs anaesthetized with pentobarbitone after treatment with atropine. First dog, first and second tracings, second dog, third and fourth tracings. The hypotensive effect of intravenous infusion of different doses of caerulein is shown. ↓, infusion started; ↑, infusion stopped. The pressure fall was proportional to the dose of caerulein, and lasted as long as the infusion was continued. Repeating the infusion at the same rate, after an interuption produced similar effects (first tracing).

On the other hand, *N,N*-dibenzyl-β-chloroethylamine (dibenamine, 10 mg/kg, intravenously) caused an evident prolongation of the hypotensive effect of caerulein, with obvious effect also on the intensity of hypotension (Fig. 4). The action of 2-diethylaminomethylbenzo-1,4-dioxan (Prosympal, 2–4 mg/kg, intravenously) was similar.

The β-adrenergic blocking agent propranolol (1.5 mg/kg, intravenously) did not affect the hypotensive action of caerulein.

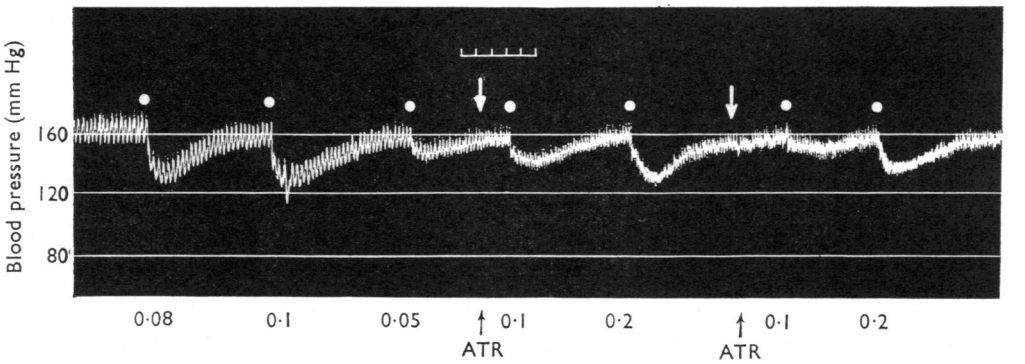

Fig. 3. Blood pressure of a dog anaesthetized with pentobarbitone. Time marks, 1 min in this and subsequent figures. The action of different doses of caerulein (in μg/kg) before and after intravenous administration of atropine sulphate (ATR) 0.05 mg/kg (first arrow) and 0.2 mg/kg (second arrow). The hypotensive action of caerulein was reduced, especially in its intensity, by atropine.

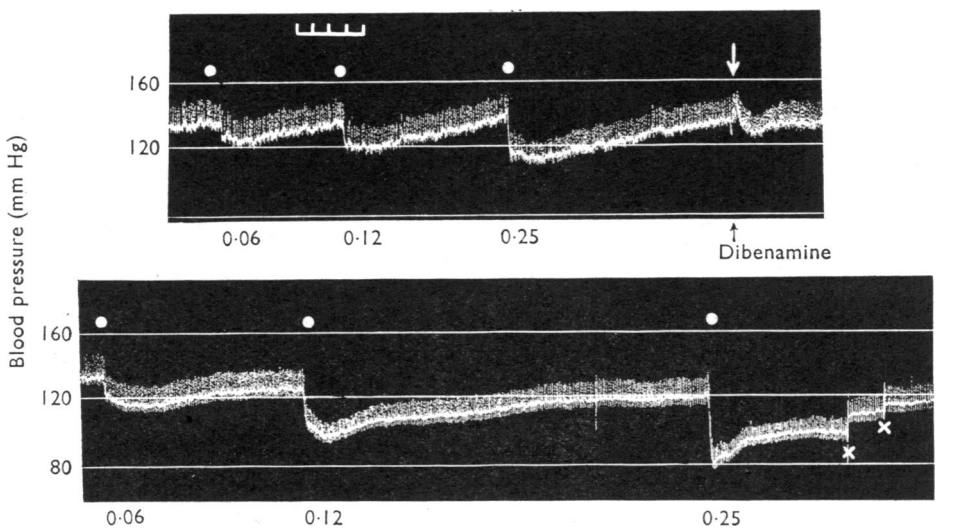

Fig. 4. Blood pressure of a dog anaesthetized with pentobarbitone. The action of different doses of caerulein (in μg/kg) before and after the intravenous administration of dibenamine 10 mg/kg (at arrow). At ×, the drum was stopped for 10 min. The hypotensive action of caerulein was reinforced and prolonged by the α-adrenergic blocking agent.

Effect of noradrenaline

Unlike eledoisin and physalaemin, caerulein was poorly effective in antagonizing the pressor effect of noradrenaline. In fact, when given in the same quick intravenous injection, caerulein 5 μg left virtually unchanged the effect of noradrenaline 15 and 30 μg, except for a slight shortening of the hypertensive peak. Caerulein 10 μg injected

simultaneously with noradrenaline 6 μg produced the same hypertensive peak as that caused by noradrenaline alone, but there was a secondary conspicuous pressure fall, of the same intensity as that produced by caerulein 7 μg alone.

Potencies relative to physalaemin, bradykinin and gastrin

The shape of the hypotensive response elicited by caerulein administered by rapid intravenous injection differed considerably from that produced by either physalaemin or bradykinin. In particular, the response elicited by caerulein had a slower onset and a greater duration than that elicited by the other two polypeptides. Hence comparison of the hypotensive effects of these polypeptides presented difficulties. Consideration was given only to the effects caused by threshold doses or small doses of the polypeptides. It was found that caerulein was 2 to 10 times more active than bradykinin but 100 to 500 times less active than physalaemin with respect to the intensity of the pressure fall produced. With respect to the duration of the effect, however, it was found that caerulein 0.1 μg and 1 μg/kg produced a hypotensive effect lasting as long as that produced by bradykinin at least 3 μg and 50 μg/kg and by physalaemin 0.01 μg and 0.15 μg/kg, respectively.

The potency of caerulein relative to physalaemin was more favourable to caerulein if the polypeptides were given by intravenous infusion or by subcutaneous route. Indeed, caerulein administered by intravenous infusion was only 3 to 20 times less active than physalaemin, and by the subcutaneous route was only 5 to 10 times less active. These results seem to stem from the fact that hypotension elicited by caerulein is more prolonged than that elicited by physalaemin.

The action of pure gastrins on blood pressure was, as already noted by Gregory & Tracy (1964), small and inconstant. In our experiments the pressure response of the dog to intravenous human gastrin-I and to des-glutamyl human gastrin-I consisted of slight hypotension or biphasic response (slight hypertension followed by slight hypotension), which was less than 1% of the response produced by caerulein.

Effects on the electrocardiogram

Electrocardiogram tracings were recorded from four anaesthetized dogs, immediately before and at regular intervals after administration of caerulein. One dog received caerulein by a single intravenous injection of 150 μg/kg; the second by intravenous infusion at a rate of 15 μg/kg/min; the third received repeated intravenous injections from 0.1 to 300 μg/kg, and the fourth received 750 μg/kg by the subcutaneous route.

In the first dog, blood pressure following the administration of caerulein fell to 20 to 30 mm Hg, then, after 10–15 min, it rose to 60–70 mm Hg and slowly returned to normal levels within approximately 3 hr. During the period of maximum hypotension electrocardiogram tracings revealed signs of severe myocardial anoxia represented by profound depression of the *Q-T* segment and of the *T* wave. Moreover, a marked bradycardia and a lengthening of the *P-Q* segment were present. All the above changes persisted, though less markedly, even after blood pressure had returned to approximately normal levels.

In the second dog hypotension (30–50 mm Hg) lasted as long as the infusion of caerulein (3.5 hr). Electrocardiogram changes were practically identical with those described above. Bradycardia was even more pronounced, the heart rate being 50% of the pre-infusion values during the first 15 min after infusion commenced.

The third dog was particularly resistant to the hypotensive effect of caerulein, and blood pressure never fell below 60–70 mm Hg even after the administration of an enormous amount (300 μg/kg) of the polypeptide. Electrocardiogram changes were scarcely evident and rapidly disappeared.

In the fourth dog the pressure fell from 150 to 60 mm Hg for 1 hr and then remained at 80 mm Hg for 3 hr. Pre-injection levels were not attained in the course of the experiment. No obvious electrocardiogram changes were observed, apart from bradycardia during the first hour. On the whole, it seems probable that observed electrocardiogram changes following administration of caerulein are caused by the hypotension produced by the polypeptide and not by any direct damage to the heart.

Cat

The blood pressure response elicited by caerulein varied considerably from one animal to another and, on the whole, was not very intense.

The threshold intravenous dose was of the order of 0.1–0.2 μg/kg. Increasing the dose led to a moderate increase in response, but there was no good dose-response relationship and some degree of tachyphylaxis was common. Usually, caerulein produced a pressure fall, frequently preceded by a slight, short-lived pressure rise. When large doses were administered the pressure fall was rather abrupt, accompanied by intense bradycardia, and return to control level was biphasic, the first phase being rapid, the second considerably slower. Partial, rapid pressure recovery coincided with attenuation or cessation of bradycardia (Fig. 5).

After atropine (0.5 mg/kg, intravenously), hypotension was completely abolished and the only response to caerulein (up to 30 μg/kg) was a short-lasting, moderate (20–30 mm Hg) pressure rise, showing considerable tachyphylaxis. The pressure rise persisted after dibenamine (10 mg/kg, intravenously). In one cat in which the control level of pressure was 100 mm Hg, small doses of caerulein produced only an increase in blood pressure. Large doses, however, again produced a pressure fall with bradycardia. Comparison of the effects of caerulein on the blood pressure of the cat with those produced by other hypotensive peptides was virtually impossible and was not attempted.

Rabbit

Rabbits, anaesthetized with urethane, regularly responded to intravenous caerulein by a fall of blood pressure which was satisfactorily correlated with the dose, both with respect to its intensity and even more with respect to its duration (Fig. 6). The threshold dose was 10–50 ng/kg. Doses 100 to 500 times larger were well tolerated. Tachyphylaxis was completely lacking.

The shape of the pressure fall differed somewhat from one experiment to another. Often, especially for medium and large doses, the initial abrupt pressure fall was followed by partial but prompt pressure recovery, followed in turn by a plateau or even by a secondary fall preceding the definitive return to the pre-injection level. In other cases recovery was less eventful and the hypotensive curve was smooth.

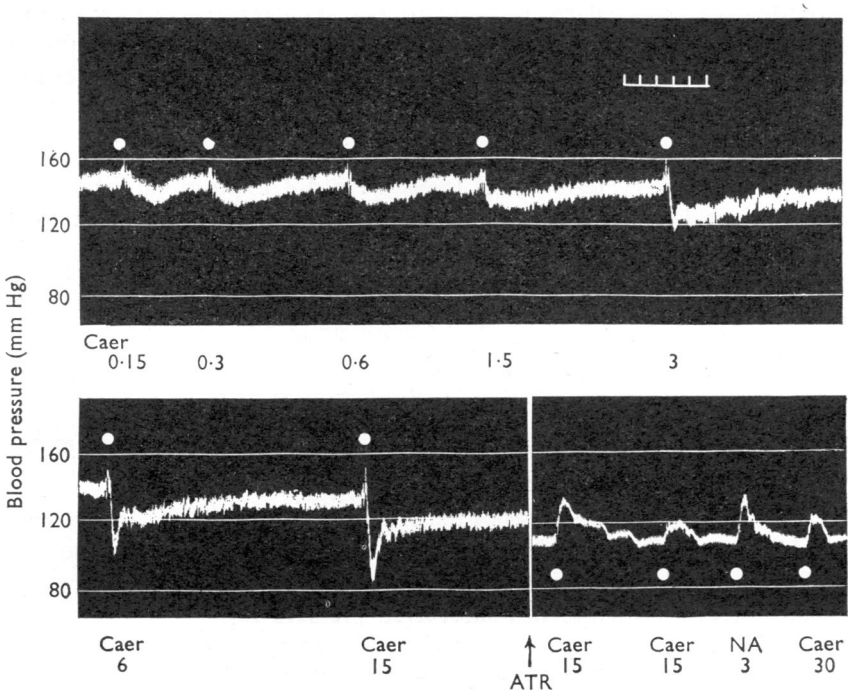

Fig. 5. Blood pressure of a cat anaesthetized with urethane and chloralose (1 g/kg and 50 mg/kg, intravenously). In the first part of the tracing the effects of different intravenous doses of caerulein (Caer, in μg/kg) are shown ; in the second part the action of atropine sulphate (ATR, 0.5 mg/kg, intravenously) on caerulein hypotension is shown ; NA, noradrenaline (in μg/kg). It can be seen that there was only a rough proportionality between dose and effect of caerulein, and that after atropine the hypotensive effect of caerulein was replaced by a moderate hypertensive effect.

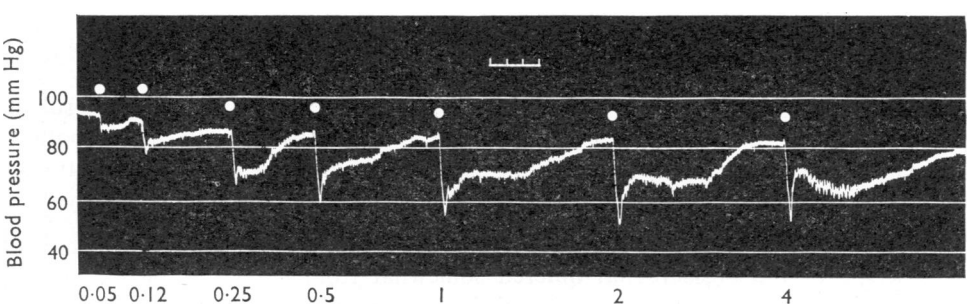

Fig. 6. Blood pressure of a rabbit anaesthetized with urethane (1.2 g/kg, intravenously). The effects of different intravenous doses of caerulein (in μg/kg) are shown. The pressure fall was fairly proportional to the dose of caerulein administered.

Atropine given by the intravenous route (1–2 mg/kg) usually caused an enhancement of the hypotensive response which became either more intense or more prolonged or both (Fig. 7). In other cases, atropine produced the opposite effect—that is, a reduction in the hypotensive response.

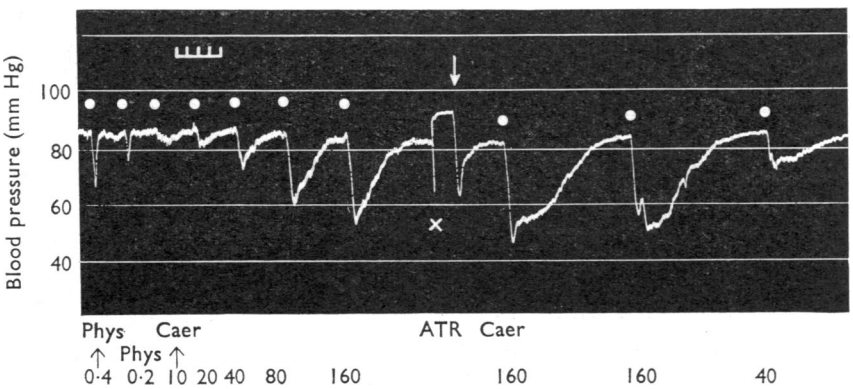

Fig. 7. Blood pressure of a rabbit anaesthetized with urethane. Phys, Physalaemin (in ng/kg); Caer, caerulein (in ng/kg). At × the drum was stopped for 20 min. The effects of different doses of caerulein before and after the intravenous injection of atropine sulphate (ATR, 1 mg/kg, intravenously) are shown. In this experiment atropine conspicuously reinforced both intensity and duration of the hypotensive effect of caerulein.

If the intensity of the pressure fall only is considered, caerulein was 100 to 400 times less active than physalaemin.

The effects of caerulein on the contractile force and frequency of the isolated rabbit heart were variable and not dose-dependent. In different experiments the threshold dose ranged from 5 to 20 ng/ml. A reduction of the contractile force (up to 60% less than basal value) was the most frequent response elicited. Only in two out of ten experiments was there an increase which, however, did not exceed 30% of the control values. The effects on heart rate were less noticeable, and variations ranged between 5 and 10%. Bradycardia was the most frequent change. Electrocardiographic tracings showed no significant departures from the normal picture. Only in one instance was *A-V* conduction moderately altered. This change, however, was completely reversible.

Rat

The pressure response of the normotensive rat to intravenous caerulein was rather erratic and unpredictable. Small and medium doses (from the threshold dose, 10–100 ng/kg, to 2–5 μg/kg) usually caused a pure hypertensive response, barely related to the dose. With larger doses hypertension was preceded by a short-lived hypotensive peak and followed by a more sustained hypotension (Fig. 8). In some rats the administration of doses as high as 10,000 times the threshold dose (up to 300 μg/kg) was followed by recovery, and in some rats intravenous doses of 20 μg/kg apparently caused death.

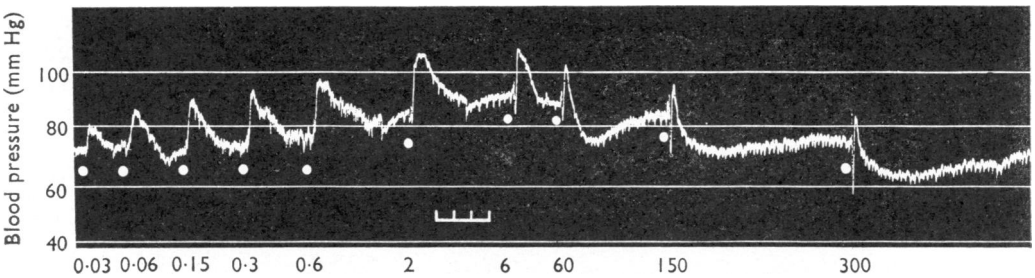

Fig. 8. Blood pressure of a rat, anaesthetized with amylobarbitone (150 mg/kg in 2% acacia solution, intraperitoneally). The effects of different doses of caerulein (in μg/kg) are shown. Whereas small doses (from 0.03 to 6 μg/kg) produced a frank hypertensive effect, barely correlated with the dose, large doses caused a triphasic response consisting of fleeting hypotensive peak, short-lasting hypertensive peak and long-lasting hypotension.

The pithed rat responded with a rise of blood pressure (threshold intravenous dose 100–200 ng/kg), again scarcely proportional to the dose. As shown in Fig. 9 the shape of the pressure curve produced by caerulein was completely different from that produced by either physalaemin or noradrenaline.

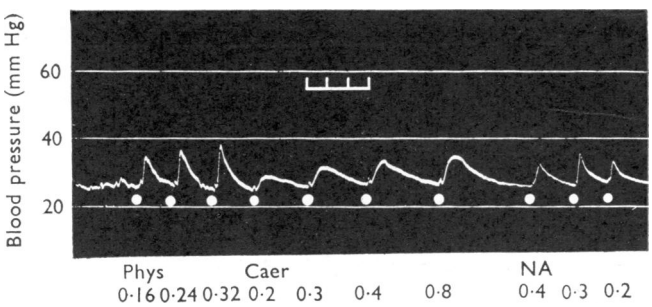

Fig. 9. Blood pressure of a pithed rat. Phys, Physalaemin ; Caer, caerulein ; NA, noradrenaline. All doses (in μg/kg) intravenously. The response to caerulein was hypertension which differed sharply, in its shape, from that caused by either physalaemin or noradrenaline.

Chicken

In the intact chicken anaesthetized with urethane, caerulein usually produced a diphasic response consisting of a brief hypertension followed by a more sustained pressure fall. The threshold intravenous dose was 0.3–1 μg/kg. Only a rough proportionality between dose and effect was observed (Fig. 10).

Atropine (2 mg/kg, intravenously) did not appreciably affect the response to caerulin.

In the decapitated chicken the polypeptide produced similar effects—a slight rise in blood pressure often, but not necessarily, followed by a moderate sustained fall of blood pressure. Tachyphylaxis frequently developed towards the hypotensive phase. If adrenaline, noradrenaline or physalaemin were administered during the caerulein hypotension, their hypertensive effect was strongly reduced.

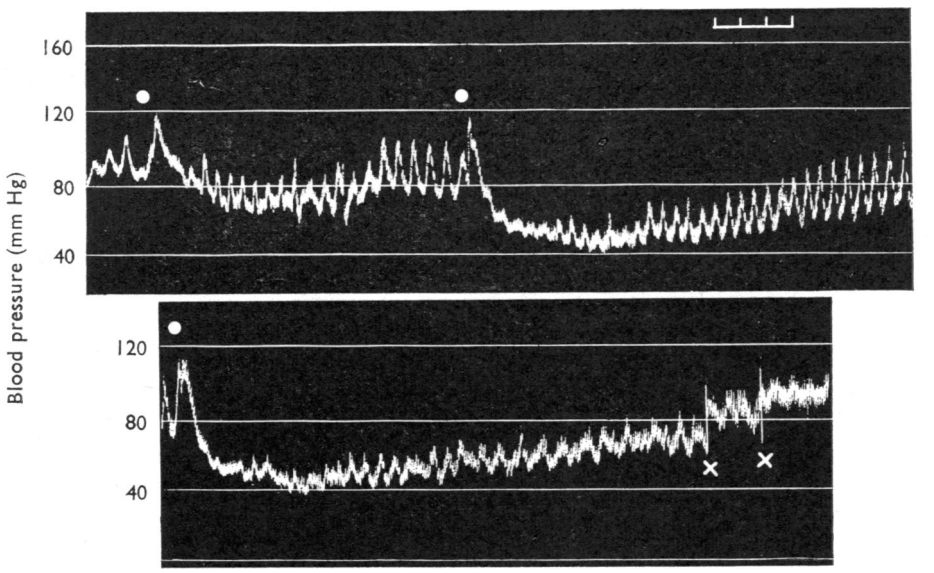

Fig. 10. Blood pressure of a chicken anaesthetized with urethane (1.5 g/kg, intravenously). At × the drum was stopped for 20 min. The hypotensive response to three successive increasing doses of caerulein (4, 10 and 20 μg/kg) is shown. Hypotension was preceded by a short-lasting hypertensive peak.

Capillary permeability

Man

On intradermal injection into the skin of the forearm, caerulein caused the appearance of a reaction very similar to that produced by bradykinin. Caerulein weal appeared within 5 min, reached its maximum in 30 min and disappeared within 45–90 min. The threshold dose was 20–50 ng. At this dose the wealing action of caerulein was approximately as intense as that of bradykinin ; at larger doses (for example, 300 ng) it was about two times less intense.

Guinea-pig

In contrast with the results obtained in man, caerulein was found to be poorly active in increasing capillary permeability to circulating dye in guinea-pigs. The threshold dose was 5–10 μg, and at dose levels of 10 and 100 μg caerulein was approximately 3,000 times less active than bradykinin.

DISCUSSION

The chief pharmacological effects of caerulein are displayed on some extravascular smooth muscles and on external secretions of the gastrointestinal tract, and these effects will be discussed in subsequent papers. The action of the polypeptide on blood vessels and on blood pressure is, however, worthy of attention. It should be pointed out that

this paper represents merely an introduction to the study of the circulatory effects of caerulein. A number of problems concerning the effects of caerulein on the different vascular areas (especially on gastrointestinal vascular districts), on the capillary permeability in different species, and on the mechanism of action of caerulein on vascular smooth muscle are simply opened by the present investigation.

The first fact which clearly emerged from the experiments reported here is that the action of caerulein on the systemic arterial pressure differed quantitatively and qualitatively from one animal species to another, as is the case with the actions of all other known hypotensive polypeptides. The dog and the rabbit were relatively sensitive to caerulein, which was more potent than bradykinin and produced only hypotension in all experimental conditions. In the cat and the chicken response was biphasic, with initial pressure rise and subsequent, more sustained, pressure fall. The rat gave the most erratic response, with hypertension predominating at small doses and hypotension at large doses.

The pressure fall produced by caerulein in the dog and the rabbit differed sharply from that produced by the other hypotensive polypeptides (bradykinin, eledoisin, physalaemin) in its slower onset and its longer duration. Preliminary experiments have shown (De Caro, unpublished) that dog blood inactivates caerulein very slowly.

The mechanism by which caerulein causes a fall of blood pressure is probably complex and is again different in different species. In the dog a parasympathetic, cholinergic mechanism certainly participates in the production of hypotension, as shown by the reduction of the pressure fall following atropinization. It is likely, however, that a direct peripheral effect of caerulein plays an important part in causing hypotension in the dog, as shown by the persistence of the effect after atropine and β-adrenergic blocking agents. Because α-adrenergic blocking agents prolong the action of caerulein, it may be inferred that the polypeptide causes a discharge of catecholamines, which counteract its vasodilator effects.

Preliminary experiments by Glässer (personal communication) demonstrated that there were striking differences in the sensitivity of the different vascular districts towards caerulein. The most sensitive by far seemed to be the pancreatico-duodenal region.

In the cat a cholinergic mechanism seems to play a predominant part in causing caerulein hypotension. In fact, the pressure fall elicited was completely or almost completely abolished by atropine.

The position is different in the rabbit and the chicken, where caerulein is atropine-resistant and, as a consequence, the involvement of cholinergic mechanisms can be ruled out.

It is probable that release of catecholamines plays a part in the production of hypertensive responses or phases but the problem requires further investigation using catecholamine-depleted animals.

Man seems to be rather sensitive to caerulein and to respond to the polypeptide with hypotension. It would seem that in man caerulein possesses a peripheral action on vascular smooth muscle or on postganglionic pathways to blood vessels, because the intradermal injection of small doses of the polypeptide produces a typical weal.

SUMMARY

1. The blood pressure response produced by parenteral administration of caerulein, the active decapeptide of the skin of the Australian amphibian *Hyla caerulea*, has been investigated in some experimental animals. The response varied from one animal species to another.

2. In the dog, the polypeptide elicited hypotension in all experimental conditions. Tachyphylaxis was always lacking. Caerulein was more potent than bradykinin, but considerably less potent than physalaemin. Hypotension caused by caerulein differed sharply from that caused by the other hypotensive polypeptides. The hypotension produced by caerulein had a lower onset and a much greater duration than that produced by the other polypeptides. Caerulein hypotension was reduced by atropine, but enhanced, especially in its duration, by α-adrenergic blocking agents. Doses of caerulein as large as 10 to 100 thousand times the threshold dose could be administered by rapid intravenous injection with full recovery of the animals. Long-lasting hypotension could be obtained by intravenous infusion or subcutaneous injection of caerulein.

3. The rabbit behaved like the dog in response to caerulein, in that the polypeptide always produced hypotensive responses and tachyphylaxis was lacking. The hypotension elicited, however, was atropine-resistant.

4. The cat was less sensitive and some degree of tachyphylaxis was apparent. After atropine the pressure fall was replaced by a very moderate and inconstant hypertensive reaction.

5. The chicken was even less sensitive than the cat. Response to caerulein was atropine-resistant and was biphasic, consisting of a brief, moderate pressure rise followed by a sustained pressure fall.

6. The rat gave the most erratic responses, with hypertension predominating at low doses and hypotension at large doses. In the pithed rat only a moderate, hardly repeatable, hypertensive response was observed.

7. Capillary permeability was increased by intradermal injection of caerulein. This effect was marked in man but negligible in guinea-pigs.

8. The mechanism of the action of caerulein on blood vessels is apparently complex and different in different animal species. It has not been elucidated in the present study.

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REFERENCES

DE CARO, G., ENDEAN, R., ERSPAMER, V. & ROSEGHINI, M. (1968). Occurrence of caerulein in extracts of the skin of *Hyla caerulea* and other Australian hylids. *Br. J. Pharmac. Chemother.*, **33**, 48–58.

GREGORY, R. A. & TRACY, H. J. (1964). The constitution and properties of two gastrins extracted from hog antral mucosa. *Gut*, **5**, 103–117.

SHIPLEY, R. E. & TILDEN, J. H. (1947). A pithed rat preparation suitable for assaying pressor substances. *Proc. Soc. exp. Biol. Med.*, **64**, 453–455.

VENTURI, V. (1955). Contributo allo studio della tecnica di derivazione elettrocardiografica del cuore di mammifero isolato e perfuso. *Boll. Soc. ital. Biol. sper.*, **31**, 239–241.