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The action of bombesin on the systemic arterial blood pressure of some experimental animals

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Summary

1. The changes in blood pressure in response to parenteral administration of bombesin, the active tetradecapeptide of the skin of the European discoglossid frogs *Bombina bombina* and *Bombina variegata variegata* have been investigated in some experimental animals.

2. In most species, the polypeptide elicited hypertension which was usually gradual in onset and slow to disappear. Blood pressure increases rarely exceeded 40–50 mmHg. At the beginning of an experiment some dose-response relationship could often be observed, but later tachyphylaxis developed. During an intravenous infusion of bombesin the rise in blood pressure could sometimes be maintained at a steady level as long as the infusion was continued, but at other times, the rise of pressure slowly subsided with continued administration of the polypeptide. In the rat and the chicken hypertension elicited by high doses of bombesin was often followed by secondary hypotension.

3. Bombesin-induced hypertension was apparently not affected by pretreatment with either α- or β-adrenergic blocking agents. Similarly secondary hypotension was not abolished by atropine. Thus, the effect of bombesin on vascular smooth muscle seems to be predominantly a direct one.

4. Angiotensin was usually more potent than bombesin, and its effect on blood pressure was more rapid and of shorter duration. Tachyphylaxis to angiotensin was lacking or moderate.

5. In sharp contrast to the other species, the monkey responded to bombesin with frank hypotension, which was usually proportional to the dose. In the monkey the hypotensive effect of bombesin was equal to, or greater than that of eledoisin or physalaemin and bombesin-induced hypotension was of longer duration than that of the other polypeptides. Tachyphylaxis was moderate for low and adequately spaced doses of the polypeptide, but prompt and intense for high doses. Long-lasting hypotension was obtained by intravenous infusion of bombesin, but repeated infusions caused tachyphylaxis. Bombesin-induced hypotension was not affected by pretreatment with atropine.

6. Bombesin may be easily distinguished from all other known peptides active on vascular and extravascular smooth muscle by its effects on blood pressure. This does not apply to bombesin-like peptides, such as alytesin and ranatensin.

Introduction

In an earlier paper the occurrence of bombesin in the skin of the European

discoglossid frogs *Bombina bombina* and *Bombina variegata variegata,* as well as the actions of the polypeptide on a number of extravascular smooth muscle preparations were reported (Erspamer, Falconieri Erspamer, Inselvini & Negri, 1972). In this paper the actions of the polypeptide on the systemic blood pressure of some laboratory animals are described.

Bombesin has a predominantly hypertensive action in experimental animals. This effect distinguishes it from all other active polypeptides hitherto found in amphibian skin.

Methods

Dogs and monkeys (*Macacus rhesus*) were anaesthetized with sodium pentobarbitone (40 mg/kg, i.v.), cats with urethane (1 g/kg, i.p.) followed by chloralose (50–70 mg/kg, i.v.), rabbits and rats with urethane (1 to 1·5 g/kg, i.p. or i.v.), and chickens with sodium phenobarbitone (250 mg/kg, i.v.). In some experiments rats were anaesthetized with ethanol: 5 ml of 15% (v/v) ethanol per 100 g body weight, given by stomach tube, followed by an intravenous infusion of 2% ethanol at a rate of 50 μl/min.

Injections were made into a femoral, jugular or wing vein. Systemic arterial blood pressure was recorded in anaesthetized animals from a carotid, femoral or ischiatic artery by means of a mercury manometer or a pressure transducer (P23D6, Statham Lab. Inc., Puerto Rico) connected to an isometric microdynamometer (7001, Basile, Milan).

Adequate oxygenation of the blood in pithed or spinal animals was maintained by a ventilatory pump.

Blood pressure in the tail of intact non-anaesthetized rats was recorded by means of the Blood Pressure Recorder of the W+W Electronic, Basel.

Drugs

Bombesin was the synthetic polypeptide prepared at the Farmitalia S.p.A. Laboratories for Basic Research, Milan, and it was put at our disposal in generous amounts. Synthetic eledoisin and physalaemin, synthetic Lys^8-vasopressin and synthetic Val^5-angiotensin II-Asp-β-amide were gifts of Farmitalia S.p.A. (Milan), Sandoz A.G. (Basel) and Ciba A.G. (Basel), respectively. Other drugs used were as follows: atropine sulphate, (±)-propranolol hydrochloride (Imperial Chemical Industries) and phenoxybenzamine hydrochloride (Smith, Kline & French, Philadelphia, Pa.).

Results

Dog

Anaesthetized preparations

When given by rapid intravenous injection, bombesin always caused a rise of blood pressure. The response varied from one animal to another, as did the appearance of tachyphylaxis. If tachyphylaxis were delayed, some dose-response relationship could be observed. Except at high doses, the rise in blood pressure was gradual, and so too was the return to the basal level: the rise rarely exceeded 30–40 mm mercury. The threshold dose varied between 5 and 30 ng/kg.

In more than 30 dogs, bombesin was administered by intravenous infusion. Again a rise in blood pressure was always seen. The threshold dose was of the order of (1 to 3 ng/kg)/min, and up to (25–50 ng/kg)/min the response was dose-dependent. The rise in blood pressure was gradual and the peak was reached within 2–10 minutes. Thereafter, the maximum response was sometimes maintained as long as the infusion was continued, but often lasted only for a few minutes and was followed by slow decline even with continued infusion. The time taken for blood pressure levels to return to normal after discontinuing the administration of the polypeptide varied in different experiments. Even with infusions of the order of (200–1,000 ng/kg)/min the blood pressure rise did not exceed 30–50 mmHg.

Val^5-angiotensin II given by intravenous infusion was less active or as active as bombesin at the beginning of an experiment; its effect subsided immediately or within a few minutes of discontinuing the infusion, while that of bombesin persisted. However, later on, depending on the intensity of tachyphylaxis for bombesin, which was lacking or very moderate with angiotensin, the relative potency of the two polypeptides was strikingly altered. In one experiment, for example, a first dose of (25 ng/kg)/min of bombesin had the same effect as a dose of (50 ng/kg)/min of angiotensin, a second equal dose of bombesin had 25% of the action of angiotensin and a third dose barely 5–7% (Fig. 1).

Atropine (0·2 mg/kg, i.v.), propranolol (0·5 mg/kg, i.v.) and phenoxybenzamine (0·5 mg/kg, i.v.) did not appreciably affect the response to bombesin (Fig. 2).

Spinal preparations

The spinal dog did not show any increased sensitivity to bombesin, in spite of its low blood pressure. Given by rapid intravenous injection, the polypeptide

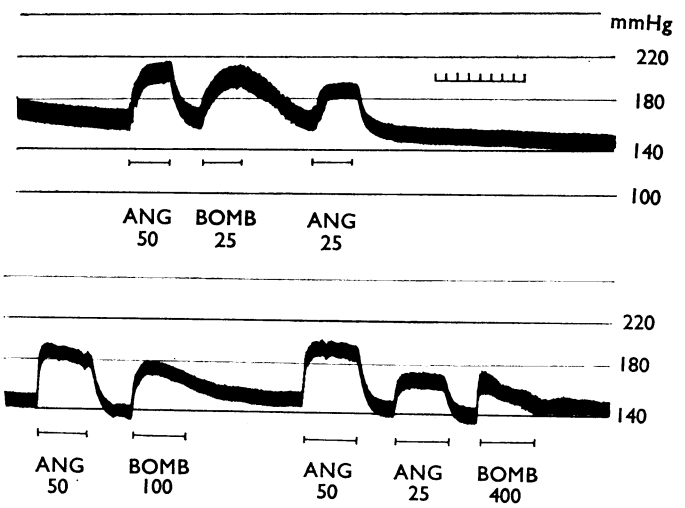

FIG. 1. Blood pressure of a dog anaesthetized with sodium pentobarbitone (40 mg/kg, i.v.). Time marks, 2 minutes. The hypertensive effects of different doses of bombesin (BOMB) and angiotensin (ANG), in (ng/kg)/min, infused for 7 to 10 min periods are shown. At the beginning of the experiment (25 ng/kg)/min of bombesin had the same effect as (50 ng/kg)/min of angiotensin; later on, because of tachyphylaxis, as much as (400 ng/kg)/min of bombesin was required to produce the same effect as (25 ng/kg)/min of angiotensin. Moreover, the blood pressure declined in spite of continuing the infusion of bombesin.

caused a hypertensive response which was very similar to that caused by angiotensin, but of shorter duration than that evoked by vasopressin.

Cat

Anaesthetized preparations

Bombesin, given by rapid intravenous injection, caused a rise in blood pressure in the anaesthetized cat. The threshold dose ranged between 10 and 50 ng/kg and there was occasionally some dose-response relationship in regard to both intensity and duration of pressure rise (Fig. 3). The rise was always moderate and never exceeded 40 mmHg, with doses up to 2 μg/kg.

In contrast to bombesin, angiotensin produced hypertension that was abrupt and short-lasting. In one experiment the rise in blood pressure produced by 1 μg/kg of bombesin and by 0·2 μg/kg of angiotensin was the same; however, hypertension produced by 1 μg/kg of bombesin persisted for 40 min, whereas that caused by the same dose of angiotensin lasted only 10 minutes.

Spinal preparations

The cat spinal preparation was relatively insensitive to bombesin (threshold

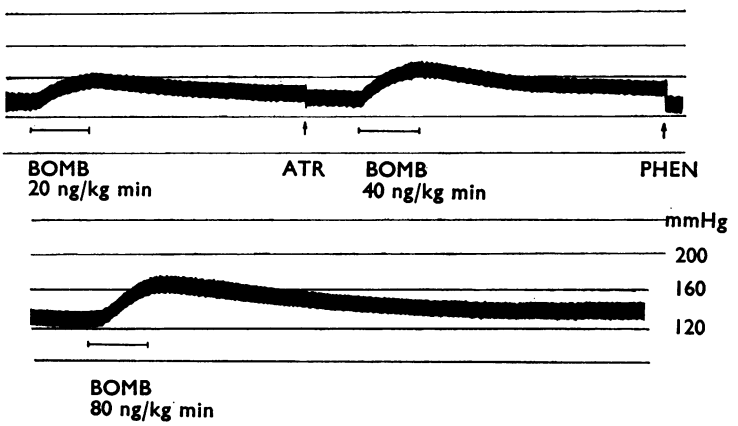

FIG. 2. Blood pressure of a dog anaesthetized with sodium pentobarbitone (40 mg/kg, i.v.). The effects of three different doses of bombesin, each infused over a 5 min period, are shown. At the first arrow the drum was stopped for 10 min and 0·2 mg/kg of atropine (ATR) was injected intravenously; at the second arrow the drum was stopped again for 15 min and 0·5 mg/kg of phenoxybenzamine (PHEN) was injected intravenously. Note that bombesin hypertension was not appreciably affected by the autonomic blocking drugs.

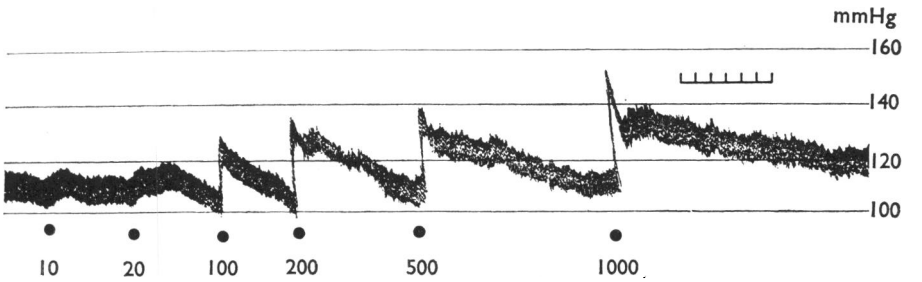

FIG. 3. Blood pressure of a cat anaesthetized with urethane (1 g/kg, i.p.) and chloralose (70 mg/kg, i.v.). Time marks, 1 minute. The effects of different intravenous doses of bombesin, in ng/kg, are shown. In this experiment some dose-response relationship was seen.

100–200 ng/kg, by rapid intravenous injection) and tachyphylaxis was prompt. After a few injections a 100-fold dose was required to produce the same effect as the first dose. It is evident that under these conditions it was impossible to evaluate the potency of bombesin relative to angiotensin.

Rabbit

In the anaesthetized rabbit bombesin always caused a rise in blood pressure. The threshold dose ranged between 1 and 15 ng/kg, by rapid intravenous injection, and some dose-response relationship could be observed for low and medium doses of the polypeptide, especially at the beginning of an experiment. Later on, intense and persistent tachyphylaxis was evident. The rise in pressure never exceeded 30–40 mm Hg.

Angiotensin produced a prompt, but short-lasting hypertensive response. Considering only the peak of this response, angiotensin was 10 to 50 times more potent than bombesin, depending on the dose.

Monkey

In this species bombesin always displayed a purely hypertensive action which, for low or moderate doses, given by rapid intravenous injection, showed a good proportionality to the dose, at least at the beginning of an experiment. Tachyphylaxis appeared gradually, but once established was sometimes very intense and required a long time to disappear. In 4 monkeys the threshold dose was between 2 and 10 ng/kg; however, when tachyphylaxis had fully developed even doses of 1–3 μg/kg were ineffective. At the beginning of an experiment the hypotensive effect of bombesin was similar to that of eledoisin. Later on, because of tachyphylaxis to bombesin, the relative potency of the two polypeptides changed. In the experiment shown in Fig. 4 bombesin was 6 to 10 times more effective than eledoisin, twice as effective as physalaemin, and more than 50 times as potent

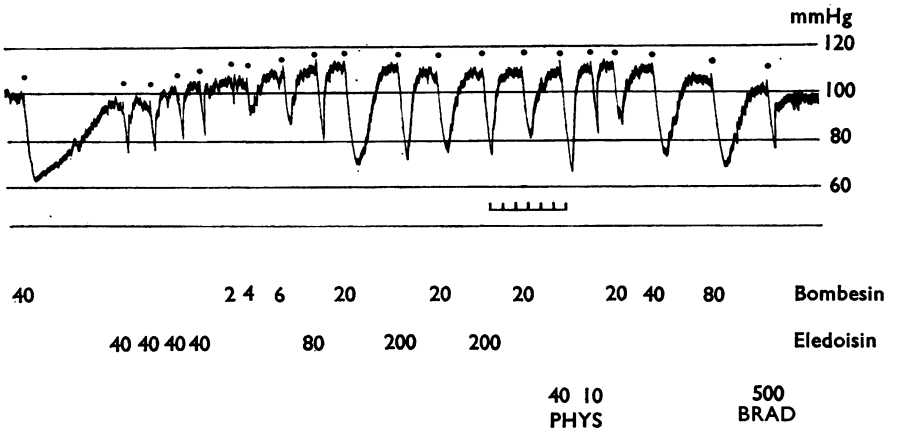

FIG. 4. Blood pressure of a monkey anaesthetized with sodium pentobarbitone (40 mg/kg, i.v.). Time marks, 1 minute. The effects of different intravenous doses, in ng/kg, of bombesin, eledoisin, physalaemin (PHYS) and bradykinin (BRAD) are shown. It may be seen that the hypotensive effect of bombesin was proportional to the dose. Tachyphylaxis, however, was obvious. In this experiment bombesin was 6 to 10 times as potent as eledoisin.

as bradykinin. In another experiment in which intense tachyphylaxis had developed bombesin showed barely 1–2% of the activity of eledoisin. For doses that produced a fall in pressure of the same intensity, hypotension caused by bombesin was of longer duration than that caused by either eledoisin or physalaemin.

The threshold dose of bombesin given by intravenous infusion was (1–2 ng/kg)/min. The fall in blood pressure was always gradual. During the first infusion period the pressure remained generally at the same low level as long as the infusion was continued. However, when infusion was repeated, even with larger doses of bombesin, blood pressure invariably returned towards pre-infusion levels, in spite of continuing the infusion (Fig. 5).

The blood pressure fall produced by bombesin given by intravenous infusion ((2–100 ng/kg)/min) was never very intense, ranging between 5 and 35 mmHg. In a perfusion experiment (10 ng/kg)/min of bombesin caused a hypotensive response which was more intense than that produced by (100 ng/kg)/min of eledoisin. Unlike bombesin-induced hypotension, eledoisin-induced hypotension persisted, at the same level, throughout the 40-min infusion period.

Rat

Non-anaesthetized animals

Nine rats were injected subcutaneously with 100 μg/kg of bombesin and the tail artery blood pressure was recorded at regular intervals for 3 hours. A mean maximum increase of 27% in blood pressure was observed; the hypotensive effect lasted more than two hours. With 200 μg/kg of bombesin the pressure increase was also long lasting but less intense (up to 10%); with 50 μg/kg there was either moderate hypotension (−3 to −12 mmHg) or moderate hypertension (+2 to +7 mmHg).

On the whole, hypertensive responses of varying, but always moderate, intensity were predominant. Hypertension was regularly accompanied by slight tachycardia (10–15% increase in beat frequency).

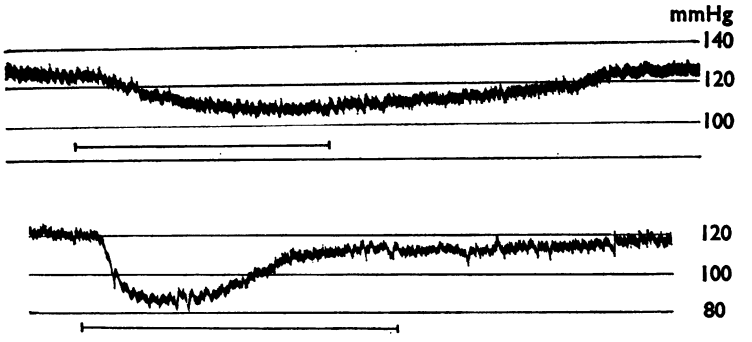

FIG. 5. Blood pressure of a monkey anaesthetized with sodium pentobarbitone (40 mg/kg, i.v.). The effects of the intravenous infusion of two doses of bombesin are shown, upper tracing (5 ng/kg)/min for 24 min; lower tracing (100 ng/kg)/min for 30 minutes. It may be seen that hypotension produced by the first small dose of bombesin lasted as long as the infusion was continued, whereas hypotension produced by the second large dose subsided rapidly, in spite of the continuing infusion.

Anaesthetized preparations

When given by rapid intravenous injection bombesin caused a rise in pressure, starting from threshold doses as low as 1–5 ng/kg. Increasing the dose produced at first a moderate increase in the response (maximum 30–40 mmHg), but intense tachyphylaxis developed rapidly (Fig. 6). The rise in pressure caused by high doses of bombesin was occasionally followed by a hypotensive phase of long duration. Phenoxybenzamine given 24 h before the experiment (10 mg/kg, i.v.) did not appreciably affect the bombesin-induced hypertension.

Val^5-angiotensin II was considerably more potent than bombesin. Since there was no tachyphylaxis to angiotensin, its relative potency to bombesin varied considerably in the course of an experiment, for example from 50 to 500.

The intravenous infusion of (0·1–1 μg/kg)/min of bombesin caused a slow, sustained pressure rise followed by spontaneous fall, even though the infusion was maintained.

Pithed preparations

The threshold dose of bombesin, by rapid intravenous injection, was approximately the same as in anaesthetized animals (5 ng/kg) and the pithed preparation had no advantage in the assay of bombesin over the anaesthetized animal with normal blood pressure.

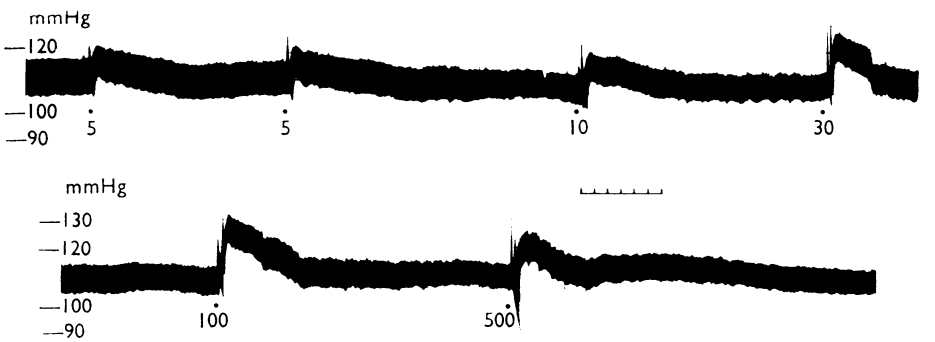

FIG. 6. Blood pressure of a rat anaesthetized with urethane (1·5 g/kg, i.p.). Time marks, 1 minute. The effects of different doses of bombesin (ng/kg) are shown. For small doses some dose-response relationship could be seen; with high doses tachyphylaxis was evident.

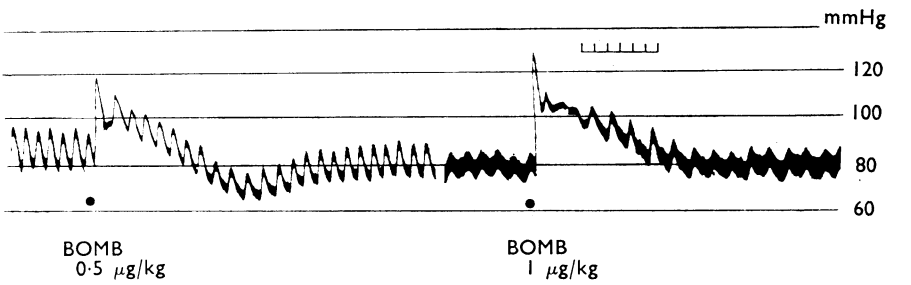

FIG. 7. Blood pressure of a chicken anaesthetized with sodium phenobarbitone (250 mg/kg, i.v.). Time marks, 1 minute. The effects of two doses of bombesin are shown. Note that hypertension may be followed by a hypotensive phase.

Chicken

Bombesin given by rapid intravenous injection produced a variable response in the chicken. With low doses (threshold 30–100 ng/kg) hypertension was predominant; with large doses (from 0·5 μg/kg) a prompt rise was often followed by a secondary fall (Fig. 7). There was rarely an acceptable dose-response relationship because of the regular appearance of tachyphylaxis.

Val^5-angiotension II was 2–3 times more potent than bombesin and its effect was often biphasic, like that of bombesin.

The hypertensive response to bombesin was apparently not affected by phenoxybenzamine (0·5 mg/kg, i.v.). Owing to tachyphylaxis it was not easy to decide whether atropine (0·5 mg/kg, i.v.) reduced the secondary hypotension but it did not abolish it.

Discussion

The pressure response elicited by bombesin in the animals examined in this study was variable. In the dog, the cat and the rabbit the polypeptide always caused a rise of blood pressure; in the rat and the chicken the effect of bombesin was similarly hypertensive, except for large doses of the polypeptide which occasionally provoked a biphasic response, a pressure rise followed by hypotension of variable duration; however, in the monkey bombesin always caused frank hypotension.

The rise in blood pressure elicited by bombesin was never of great intensity, rarely exceeding 40 to 60 mm mercury. Larger doses usually increased the duration rather than the intensity of hypertension.

The dose-response relationship varied in different species and in different experiments. Tachyphylaxis was sometimes prompt and intense, but sometimes moderate and delayed. It was always present, especially with high doses of the polypeptide. In the course of an intravenous infusion, tachyphylaxis to bombesin was shown by the return of blood pressure towards basal levels, in spite of continuing the infusion.

It is evident that because of tachyphylaxis the blood pressure response is unsuitable both for the quantitative assay of bombesin and for its quantitative comparison with other hypertensive peptides. Similarly, tachyphylaxis made it difficult to assess the effects of autonomic blocking agents on the response of the vascular smooth muscle to bombesin. It appeared, however, that bombesin-induced hypertension was not appreciably affected either by phenoxybenzamine or propranolol and that the secondary hypotensive phase, particularly evident in the chicken, was not abolished by atropine.

There is a clear-cut difference between the effects of bombesin and angiotensin on blood pressure. The blood pressure rise produced by angiotensin was, unlike that caused by bombesin, dose-dependent up to high dose levels, prompt and of relatively short duration. Moreover, tachyphylaxis to angiotensin was negligible and less evident than for bombesin. Finally, whereas bombesin was frankly hypotensive in the monkey, angiotensin had its usual hypertensive effect in this species.

The monkey is unique (amongst the animals studied) in its response to bombesin, as the polypeptide was hypotensive under all experimental conditions. A fair

dose-response relationship could be seen when single intravenous injections were given at reasonable intervals and in small or moderate doses. In the monkey bombesin was a very potent hypotensive agent: at the beginning of an experiment its potency was similar to or even exceeded that of eledoisin. However, high doses of bombesin, given either by rapid intravenous injection or by intravenous infusion always produced tachyphylaxis.

It is obvious that the blood pressure response elicited by bombesin in the dog makes this polypeptide and bombesin-like peptides easily distinguishable from all other peptides so far isolated from the amphibian skin: physalaemin, phyllomedusin, bradykinin, phyllokinin, caerulein and phyllocaerulein were all hypotensive (Erspamer, 1971).

Our results for bombesin are in good agreement with those obtained with ranatensin by Geller, Govier, Pisano, Tanimura & Van Clineschmidt (1970) and by Louis, Tanimura & Pisano (1971). This is not surprising because of the close structural resemblance between the tetradecapeptide bombesin (I) and the endecapeptide ranatensin (II), from the skin of *Rana pipiens*.

(I) Pyr-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2

(II) Pyr————————-Val-Pro-Gln-Trp-Ala-Val-Gly-His-Phe-Met-NH_2

Some minor discrepancies in experimental results can be explained by the fact that the two polypeptides are similar but not identical: Geller *et al.* (1970) found that ranatensin did not alter blood pressure in the cat when administered intravenously in doses up to 20 μg/kg; the threshold intravenous pressor dose of bombesin in the cat ranged between 10 and 50 ng/kg.

Although the mechanism of action of bombesin on the vascular smooth muscle has not been investigated in this study, it seems unlikely that adrenergic or cholinergic mechanisms play an important role in the response of the blood vessels to the polypeptide.

Among the different vascular areas which are sensitive to bombesin, the renal one appears to occupy a special position, at least in the dog. In this species bombesin provoked an intense constriction of the afferent glomerular bed, especially in the outer cortical zone, with reduction of glomerular filtration rate and renal blood flow and sharp increase in renin liberation. Threshold doses in the dog were of the order of (0·5–1 ng/kg)/min (Melchiorri, Sopranzi & Erspamer, 1971).

The possibility that these renal effects might interfere in the changes of systemic blood pressure evoked by bombesin in the dog will be discussed in a later paper.

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