Br. J. Pharmac. (1973), **48**, 438–455.

The action of bombesin on the kidney of the anaesthetized dog

V. ERSPAMER, P. MELCHIORRI AND N. SOPRANZI

Institute of Medical Pharmacology I, University of Rome, Rome, Italy

Summary

1. In the anaesthetized dog bombesin had a potent antidiuretic effect, and sometimes arrested urine flow completely. Threshold doses, by i.v. infusion, were of the order of 0·5–1 (ng/kg)/minute. Antidiuresis was the result of a reduction in glomerular filtration rate provoked by a fall in intraglomerular hydrostatic pressure. This, in its turn, was due to afferent vasoconstriction.

2. The spasmogenic effect of bombesin on the smooth muscle of the afferent arterioles was directly demonstrated by the radioactive microspheres technique and indirectly by the ^{85}Kr washout method and by [^{3}H]-*p*-aminohippurate clearance. The vascular compartment most sensitive to bombesin was that of the outer cortical zone, especially in its external half.

3. Filtration fraction decreased under the influence of bombesin, indicating that the effect of the polypeptide on postglomerular arterioles was, if present, only of minor importance.

4. At high infusion rates (above 6 (ng/kg)/min), bombesin produced a decrease in [^{3}H]-*p*-aminohippurate extraction. The effect of the polypeptide on fractional distal delivery of sodium varied with the dose: at moderate infusion rates it decreased, at high infusion rates it increased. The total glucose appearing in urine following a glucose load was sharply reduced by bombesin. However, the glomerular filtration rate/maximum tubular glucose transport ratio did not show any appreciable change.

5. Afferent vasoconstriction produced by bombesin was accompanied by an intense activation of the renin-angiotensin system, as shown by a conspicuous increase in renin secretion, followed by increases in renin activity and angiotensin II concentration in arterial blood. When bombesin was infused into one renal artery only the infused kidney showed afferent vasoconstriction and increased renin secretion. The time-course of renin secretion produced by bombesin depended upon the rate of infusion of the polypeptide. At low rates an increased renin secretion was observed throughout the infusion period, at high rates two peaks of renin secretion could be seen, one at the beginning of the infusion, the other soon after the infusion had finished.

6. The mechanism of action of bombesin is discussed and the interest of the polypeptide as a possible hormonal regulator of the circulation and function of the kidney is pointed out.

Introduction

The actions of bombesin on a number of preparations of extravascular smooth muscle and on the systemic blood pressure of six common laboratory animals have

been described in preceding papers (Erspamer, Falconieri Erspamer, Inselvini & Negri, 1972a ; Erspamer, Melchiorri & Sopranzi, 1972b).

This paper describes the effects of the polypeptide on the circulation and on some functional parameters of the kidney of the anaesthetized dog including the renin-angiotensin system. Our interest in the kidney, as a possible target organ for bombesin, was first aroused by the reduction of urine flow observed in the dog following administration of the polypeptide.

Methods

Surgical preparation of dogs

Eighty-four mongrel dogs of either sex weighing from 10 to 20 kg were used. All animals received a normal chow diet until 12 h before an experiment, when food was withdrawn. The dogs were anaesthetized with sodium pentobarbitone (30 mg/kg, i.v.), or less frequently, with sodium phenobarbitone (80 mg/kg, i.v.) and were then given, by stomach tube, an oral water load of 50 ml/kg to produce a urine flow greater than 1 ml/minute. Light anaesthesia was maintained throughout the experiment by intermittent infusion of a 0·05% sodium thiamylal solution at a rate sufficient to abolish responses to painful stimuli but to maintain the corneal reflex. By the use of fluoroscopy, fine radiopaque catheters (0·2–0·6 mm o.d.) were introduced into the aorta from the femoral arteries and into one or both renal arteries. Injection of 1–3 ml of a 20% iothalamate solution was used to confirm the position of the catheter in the main renal artery, define the location of the kidney, and ensure the absence of reflux into the aorta of liquids injected into the catheter. Preliminary experiments had indicated that renal blood flow was not reduced by the presence of these small catheters in the main renal artery. Each catheter was kept patent by an infusion of isotonic saline at a rate of 0·5 ml/minute. A radiopaque catheter was then introduced, through a small incision, into an external jugular vein, via the superior vena cava, right atrium and inferior vena cava. Isotonic saline was allowed to flow slowly (0·2 ml/min) through the catheter while the latter was kept in position. To avoid dilution by the infusion liquid, 2 or 3 ml of blood was withdrawn and discarded before a sample was taken for analysis.

In experiments where the renin secretion rate was determined, extracorporeal circuits were constructed through which blood from a systemic artery (right femoral) and from the left renal vein was continuously pumped via coiled polyethylene tubes (PE 100) into scintillation detectors (5 cm sodium iodide crystals). The blood was reinfused through a right femoral venous cannula.

The carotid artery, the femoral vein and the femoral artery were cannulated for measurement of arterial blood pressure, for drug infusion and to take samples of peripheral blood, respectively. For clearance studies, catheterization of both ureters was performed from a suprapubic incision. In ^{85}Kr washout experiments ventilation was maintained with a Palmer respirator.

Changes in ureteral pressure and ureteral peristaltic waves were recorded by the following pressure-flow method. The left ureter was infused with 0·9% w/v NaCl solution at a rate of 1 ml/min through a catheter (PE 240) and connected with a constant rate infusion pump. Pressure changes were monitored with a pressure transducer (P23D6, Statham Lab. Inc., Puerto Rico).

Creatinine, [^3H]-p-aminohippurate and [^{125}I]-iothalamate clearance

A priming dose of [^{3}H]-*p*-aminohippurate (200 μCi) plus creatinine (1 g), given by rapid intravenous injection, was followed by an infusion of the same substances dissolved in isotonic saline solution at a rate of 10 μCi/min for [^{3}H]-*p*-aminohippurate and 12 mg/min for creatinine. After a 60 min equilibration period, urine was collected for 15 min periods and blood withdrawn from the renal vein and the femoral artery half way through each 15 min period. Blood samples were immediately cooled to 0° C, centrifuged at 4° C, and the plasma decanted and frozen.

The concentration of creatinine was determined in an aliquot of protein-free filtrate with alkaline picrate reagent according to the method of Folin & Wu (1919). The radioactivity of [^{3}H]-*p*-aminohippurate was counted in blood and urine samples with a Beckman LS automatic liquid scintillation counter, after dissolving 0·5 ml samples in 15 ml of Beckman scintillation fluid containing 20% BBS3 as solubilizer.

In experiments in which the renin secretion rate was determined, continuous on line measurements of renal plasma flow and glomerular filtration rate were calculated from the clearance of [^{125}I]-iothalamate.

A loading dose of this material (20 ml of a 20 mCi/100 ml solution) was followed by an intravenous infusion of 20 mCi/100 ml sufficient to maintain a virtually constant arterial count rate of 10,000 counts per minute. The radiation measuring equipment consisted of Italelettronica RA two channel rate meter coupled with Italelettronica D 12 discriminators.

Calculations of renal plasma flow (RPF) and glomerular filtration rate (GFR) were performed in accordance with the following equations: $RPF=RBF(1-H)$, where RBF is renal blood flow and H is the systemic haematocrit; $RBF=\frac{V(U-R)}{A-R}$ according to the Wolf equation (1941), where U, R, and A represent the concentrations of [^{125}I]-iothalamate in the urine, venous blood and arterial blood, respectively; $GFR=RPF\times E$, where E is the extraction rate of [^{125}I]-iothalamate, $E=\frac{A-R}{A}$.

Measurement of sodium concentration in blood and urine

Sodium in blood and urine was determined by flame photometry with a Beckman DU flame accessory. Distal fractional sodium delivery was expressed by the ratio

$$\frac{C_{Na}\text{ (sodium clearance)}}{C_{Cr}\text{ (creatinine clearance).}}$$

Clearance of sodium was calculated by the usual formula $C_{Na}=\frac{UV\times UNa}{PNa}$, where UV is urine volume, and UNa and PNa the concentrations of sodium in urine and plasma, respectively.

Estimation of maximum rate of tubular glucose transport (TmG)

TmG was estimated according to Handley, Sigafoos & La Forge (1949). A 20% glucose solution (4 ml/kg) was given intravenously as a priming dose, and then infused at a rate of 6–8 ml/minutes. Plasma glucose concentrations ranged from 400 to 900 mg/100 ml. Glucose in blood and urine was measured by standard

methods (Johnson, Nash & Fusaro, 1963). Glucose reabsorption rates were calculated as the difference between filtered load (creatinine clearance × plasma glucose concentration) and urinary excretion of glucose (urine volume × urine glucose concentration).

Since the ratio of filtered load to reabsorption rate exceeded 1·5 in most of the clearance periods, we assumed that the reabsorption rate was maximal for the conditions used.

Measurement of intrarenal blood flow

^{85}Kr washout curve Intrarenal capillary blood flow distribution was measured from the renal washout of ^{85}Kr, according to the method of Thorburn, Kopald, Herd, Hollenberg, O'Morchoe & Barger (1963).

Radioautographs In some experiments the renal vessels were exposed from a flank incision without disturbing the kidney and a bolus of 1 mCi of ^{85}Kr was then injected into the renal artery. At specified times after injection the renal pedicle was cross-clamped and the kidney was rapidly removed and frozen in a dry ice-acetone mixture. Coronal and sagittal sections of frozen kidney were prepared and then exposed to Kodak 'no screen' medical X-ray film between ferrotype plates for 1 to 72 h at −20° C.

Radioactive microspheres method In other experiments the distribution of cortical blood flow was determined with radioactive microspheres according to the technique described by McNay & Abe (1970). A suspension of plastic microspheres, 15 μm in diameter, in 20% dextran, mixed with an ultrasonic probe 3 min before injection, was injected into the left ventricle through a catheter introduced via the left carotid artery. Each injection contained 0·3–0·5 mg bead mass, representing approximately 65,000–95,000 microspheres. Sequential injections were performed using microspheres labelled with two different gamma emitting isotopes. The first injection (^{85}Sr microspheres) was given 1 min before bombesin infusion, the second (^{169}Yb microspheres) after 20 min of bombesin infusion. The amounts of the two isotopes yielding equivalent counting rates in our experimental conditions were 0·6 mCi of ^{169}Yb and 1 mCi of ^{85}Sr. To obtain equivalent cpm per g of tissue in the case of uniform distribution of the two isotopes (i.e. no change in blood flow), the first injection contained 5 Ci of ^{85}Sr and the second 3 Ci of ^{169}Yb labelled microspheres. Different cortical zones of equal thickness were analysed for individual isotope content, as described by McNay & Abe (1970).

In this work with the radioactive microsphere method, no attention was paid to the absolute values of blood flow in the different zones of the renal parenchyma. We were only interested in percentage changes in zonal flow rates provoked by vasoactive stimuli. These changes were established through measurements of the activity ratio of the two different isotopes in the same tissue sample; one isotope was administered before, and the other after the administration of vasoactive drugs. It is evident that per cent variations in flow rates during control and drug injection periods are independent of the number of the glomeruli per gramme of tissue.

Radioimmunoassay of plasma renin activity and angiotensin II

Blood was collected in pre-chilled tubes containing approximately 1 mg of disodium edetate (EDTA) per ml of blood. The samples were kept cold and

centrifuged in the cold. Packed red cells were resuspended in isotonic saline solution and reinjected into the animal. Plasma samples were stored frozen until assayed. When thawing plasma samples before assay the temperature did not exceed 4° C. Plasma samples were adjusted to pH 5·5–5·7 with 0·1 N HCl and incubated for 2 h at 37° C in the presence of EDTA 15 mmol, dimercaprol 1·6 mmol, and 8-hydroxyquinoline 3·4 mmol. Aliquots taken before and after incubation were diluted 1:1 with distilled water and boiled for 2 minutes. After centrifugation the supernatant was assayed for angiotensin I in 0·02–0·1 ml aliquots by the method of Haber, Koerner, Page, Kliman & Purnode (1969). Recovery of added standard Asp^1-Ile^5-angiotensin I from pooled plasma carried out through the 2 h incubation period was 98–100%, indicating effective inhibition of angiotensinase and converting enzyme activities. Since serial increments of the material formed during incubation of the dog plasma and the standard Asp^1-Ile^5-angiotensin I produced parallel displacements of [^{125}I]-angiotensin I from antibody-antigen complex, renin activity was expressed in terms of nanograms of Asp^1-Ile^5-angiotensin I produced per ml dog plasma and per hour of incubation.

In preliminary experiments it was found that addition of the same quantities of renin (0·0008 dog unit/ml) to plasma samples from different dogs led to generation of different amounts of angiotensin I. To overcome this difficulty we decided to add to each plasma sample three different concentrations of hog renin (0·0002, 0·0004 and 0·0008 dog units/ml) and to plot the quantity of angiotensin I against the renin concentration. In the same plasma sample the formation of angiotensin was linearly related to the quantity of renin added, but differences in slopes between different plasma samples were observed. Thus values of renin activity were corrected for different slopes.

The radioimmunoassay of angiotensin II in the plasma was based essentially on the procedure described by Gocke, Gerten, Sherwood & Laragh (1969). Because this method was directly applied to dog arterial plasma without previous extraction of angiotensin II the assay of the polypeptide below 10–15 pg/ml was unreliable because of interference by unknown plasma components. For the same reason direct radioimmunoassay of angiotensin II could not be used for venous plasma, since highly reactive fragments of angiotensin II deprived of biological activity were found in venous blood samples (Cain & Catt, 1969).

Renin secretion per min and g kidney, expressed in ng of Asp^1-Ile^5-angiotensin I, was calculated by the formula:

$$\frac{\left((\text{RPF}-\text{V}) \times \text{Vra}\right)-\left(\text{RPF} \times \text{Ara}\right)}{\text{Kidney weight}}$$

where RPF is renal plasma flow (in ml/min), calculated by the [^{125}I]-iothalamate method, Vra renin activity in renal venous blood, and Ara renin activity in systemic arterial blood.

Reagents and drugs

Analytical grade reagents and solvents were used throughout the investigation. ^{85}Kr, [^{3}H]-*p*-aminohippuric acid and [^{125}I]-iothalamate were obtained from the Radiochemical Centre, Amersham, England; hog renin from Nutritional Biochemical Corp., Cleveland, Ohio; renin activity radioimmunoassay kit and Ile^5-angiotensin II

radioimmunoassay kit from Sorin, Saluggia, Italy; TLA fluor (Fluoralloy) and BBS-3 (B10-Solv) from Beckman Instruments Inc., Fullerton, Canada. A toluene/BBS-3/fluor mixture was batch-prepared in advance. Synthetic bombesin and synthetic Val^5-angiotensin II-Asp-β-amide were gifts from Farmitalia S.p.A., Milano, and Ciba Ltd., Basel, respectively.

Results

Urine volume

Bombesin caused a striking reduction in urine volume in all dogs examined. The threshold dose of the polypeptide was about 10–20 ng/kg by rapid i.v. injection, and 50–100 ng/kg by s.c. injection. However, the i.v. infusion was the most effective route of administering the polypeptide, and was used in most of our experiments. The threshold dose was approximately 0·5 (ng/kg)/min and the response was related to the dose.

Table 1 shows the reduction of urine volume caused by doses of bombesin ranging from 1 to 12 (ng/kg)/min, infused over a 30 min period. It may be seen that 4 (ng/kg)/min was capable of causing a 70% reduction of urine volume and 8 (ng/kg)/min a virtually complete block of diuresis (<10%). The effect was always rapid.

TABLE 1. *The effect of graded doses of bombesin, given in 5 dogs by i.v. infusion over a 30 min period, on some renal functional parameters*

	Bombesin infusion (ng/kg)/min					
	1	2	4	6	8	12
UV	76·6±6·4*	46·8±9·9†	29·7±3·7†	14·7±1·7†	6·4±1·4†	2·5±0·4†
GFR	82·3±7·8*	69·7±6·1*	35·0±3·8†	19·3±2·4†	6·6±2·1†	0·9±0·3†
RPF	81·9±4·4*	71·3±5·3*	43·2±4·8†	34·8±4·1†	—	—
FF	99·4±6·4	93·0±4·8	79·4±4·6	65·0±3·0*	—	—
E_{PAH} %	97·9±4·3	88·9±4·8	81·7±3·4	66·2±3·2*	—	—
$U_{Na}V$	88·1±5·3	45·8±8·1†	16·5±3·6†	13·0±4·2†	—	—
C_{Na}/C_{Cr}	108·7±4·1	80·4±3·8	50·2±4·8*	65·5±3·8*	—	—
TmG	—	75·8±6·2	46·3±5·2†	13·7±4·1†	—	—
GFR/TmG	—	84·0±6·7	84·4±7·1	103·0±7·3	—	—

All values are % of controls ± S.E.M;—, not estimated; *t* test for paired observations: *$P<0·05$, †$P<0·01$. Absolute basal values (±S.E.M.) obtained in 30 dogs were as follows: urine volume (UV, ml/min) 1·3±0·1; glomerular filtration rate (GFR, ml/min) 26·6±1·9; renal plasma flow (RPF, ml/min) 109±6; filtration fraction (FF) 0·24±0·01; % *p*-aminohippurate extraction (E_{PAH}%) 79±1; total urinary excretion of sodium ($U_{Na}V$, μEq/min) 115±20; fractional distal sodium delivery (C_{Na}/C_{Cr}) 0·031±0·004; maximum tubular glucose transport (TmG, mg/min) 81±6; GFR/TmG 0·30±0·02.

Antidiuresis was generally accompanied by a slight or moderate rise of blood pressure, ranging from 5 to 40 mmHg. Both phenomena rapidly subsided after the infusion had been stopped, although urine volume often took a little longer to return to normal than blood pressure. In a few experiments in which the infusion of bombesin was continued for 4 h a progressive increase of the effect of the polypeptide was seen. For example, with 4 (ng/kg)/min a complete blockade of urine flow was attained after 90–120 minutes. At an infusion rate of 10 (ng/kg)/min urine flow ceased within a few minutes and thereafter no diuresis was observed as long as the infusion was continued. However, blood pressure returned to normal within 1 hour. With 200 and 500 (ng/kg)/min of bombesin administered for 10 min antidiuresis outlasted the infusion period for 50 to 80 min, respectively, whereas the rise in blood pressure disappeared 5–10 min after the infusion had been stopped. Repeated infusions of bombesin generally produced tachyphylaxis.

Val^5-angiotensin II infused i.v. for 30 min at a rate of 200 (ng/kg)/min produced a rise of blood pressure of 40–60 mmHg but did not reduce diuresis.

In 4 dogs bombesin was infused into one renal artery for 30 min at a rate of 5 ng/min and urine was collected from the homolateral ureter. The other kidney acted as control. A 50–75% reduction of urine flow could be seen only in the infused kidney, whereas urine flow in the control kidney was slightly increased. Val^5-angiotensin II infused into a renal artery at a rate of 250 ng/min caused a 30% reduction of urine volume, again limited to the infused kidney.

Glomerular filtration rate

The behaviour of the glomerular filtration rate (GFR), as estimated from the creatinine clearance, during an intravenous infusion of graded doses of bombesin is shown in Table 1. Bombesin greatly reduced GFR and the reduction of the creatinine clearance at various infusion rates paralleled reductions in urine volume. At an infusion rate of 12 (ng/kg)/min GF was virtually abolished. When given by close infusion into a renal artery of 4 dogs for 30 min, 5 ng/min of bombesin caused a 70% reduction in GFR, a figure which compares well with the 50–75% reduction of urine volume. Under the same experimental conditions and in the same dogs, 250 ng/kg of Val^5-angiotensin II reduced GFR by only 13 per cent.

Renal plasma flow

Bombesin caused an evident reduction in renal plasma flow (RPF), as indicated by the [^{3}H]-*p*-aminohippurate clearance. Results obtained with graded doses of bombesin, given by intravenous infusion for 30 min are shown in Table 1. It may be observed that reduction of RPF was less pronounced than reduction of GFR.

When infused into the renal artery in 5 dogs, for 30 min, 5 ng/min of bombesin reduced RPF by 37% in the infused kidney. Under the same conditions, 250 ng/min of Val^5-angiotensin II reduced RPF by 50 per cent.

Filtration fraction

The influence of bombesin on filtration fraction (FF), i.e. on the percentage fraction of plasma flowing through the kidney which is filtered through the glomeruli (GFR/RPF$\times$100), is shown in Table 1. With infusion rates of bombesin up to 2 (ng/kg)/min there was no change in FF whereas at higher rates this was reduced. With 6 (ng/kg)/min the decrease averaged 35 per cent.

[^{3}H]-p-Aminohippurate extraction

The per cent extraction of [^{3}H]-*p*-aminohippurate ($E_{PAH}\% = A-V/A\times 100$, where A and V are concentrations of *p*-aminohippurate in arterial and venous blood, respectively) under the influence of graded doses of bombesin given by intravenous infusion is shown in Table 1. Low infusion rates of the polypeptide did not appreciably change E_{PAH} but at infusion rates of 4 (ng/kg)/min and above, a reduction of E_{PAH} was obvious. In contrast to bombesin, angiotensin is reported to cause an increase in E_{PAH} (Carrière & Friborg, 1969).

Washout curve of ^{85}Kr

The effects of i.v. infusions of bombesin on the ^{85}Kr washout curve, i.e. on

intrarenal capillary blood flow, are illustrated in Table 2. Per cent distribution of injected ^{85}Kr was measured between 20–26 min of a bombesin infusion lasting 30 min and data concerning regional blood flow are valid for the same time interval. For infusion rates of 6 and 20 (ng/kg)/min, per cent distribution of injected ^{85}Kr, half time of ^{85}Kr radioactivity and regional blood flow are given for the total cortical zone (compartments I+II), because methodological limits do not allow a clear distinction between outer cortex (compartment I) and juxtamedullary cortex (compartment II). Similarly, for compartments III and IV only ^{85}Kr half time and per cent distribution of the radioactive gas are given, since the identification of these compartments with well-defined renal zones (medullary zone and hilar zone, respectively) is still controversial (Slotkoff, Logan, Jose, D'Avella & Eisner, 1971).

TABLE 2. *Effects of intravenous infusions of bombesin on ^{85}Kr washout curve and regional blood flow*

		Controls (11)	Bombesin (ng/kg)/min 2 (6)	6 (6)	20 (6)
% ^{85}Kr	Compartment I	72·90±2·6	58·30±3·9	69·00±4·1	70·3± 3·9
	,, II	17·80±1·8	27·00±3·4		
	,, III	5·90±0·8	9·30±1·1	15·7±1·1	17·7±1·6
	,, IV	3·50±0·6	5·30±0·6	8·0±0·7	8·7± 0·8
^{85}Kr $t_{\frac{1}{2}}$	Compartment I	0·09±0·02	0·18±0·04	0·76±0·09	1·9± 0·1
	,, II	0·40±0·06	0·42±0·10		
	,, III	1·70±0·3	1·50±0·5	2·4±0·8	4·4± 0·7
	,, IV	26·80±4·3	45·00±7·1	53·4±9·3	90·2±11·2
% RCM	Compartment I	51·30±3·9	47·00±2·4		
	,, II	48·70±3·6	53·20±5·7		
RBF	Compartment I	385±34	200±19*	100±11†	37±5†
	,, II	89± 8	90±11		

% ^{85}Kr = % distribution of administered ^{85}Kr; ^{85}Kr $t_{\frac{1}{2}}$ = half time of ^{85}Kr radioactivity (min); % RCM = % of renal cortical mass; RBF = regional blood flow ((ml/min)/100 g kidney). The values given are the means ± S.E.; *t* test for paired observations: * $P<0·05$; † $P<0·01$. The number of dogs is given in parentheses.

It may be seen that the vascular compartment first affected by bombesin was that corresponding to the outer cortex. Bombesin, 2 (ng/kg)/min reduced outer cortical flow by 48% leaving juxtamedullary flow unaffected. Similarly, in the outer cortex ^{85}Kr half time was increased from 0·09 to 0·17 minutes. At infusion rates of 6 and 20 (ng/kg)/min the total cortical flow was reduced by 79 and 92%, respectively.

Radioautograms of the kidney after injection of ^{85}Kr confirmed that washout of the gas was strikingly retarded following infusion of bombesin at a rate of 20 (ng/kg)/minute. In fact, a similar pattern of distribution of radioactivity in external cortex was seen after 15 s in the kidney of control dogs, and after 4 min in the kidney of bombesin-treated animals, whereas full medullary and hilar phases were observed after 2 and 15 min, respectively, in the control animals, and after 20 and 50 min, respectively, in the bombesin-infused animal (Figure 1).

Thus, radioautograms confirmed that bombesin exerted a potent vasoconstrictor effect in the renal cortex and showed that the cortical vasoconstriction was uniform and not patchy as is that produced by angiotensin II (Carrière & Friborg, 1969).

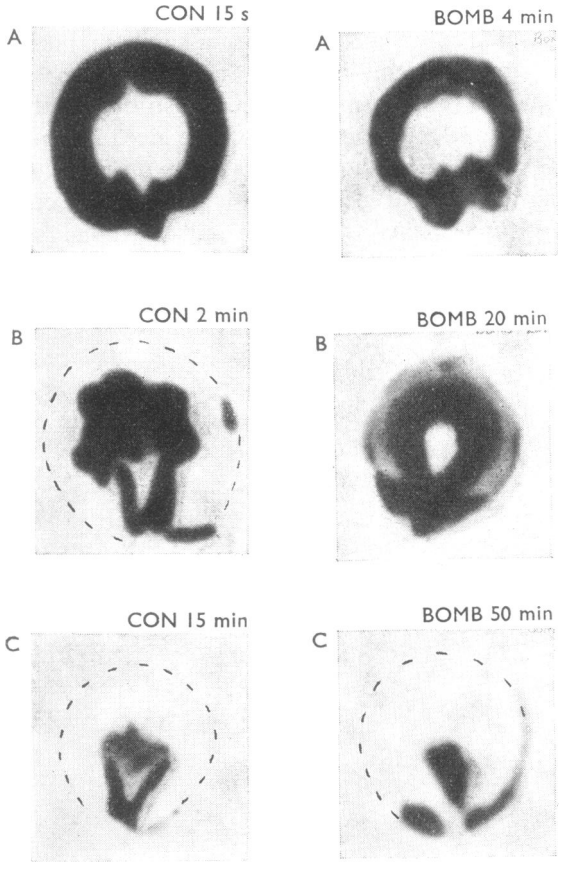

FIG. 1. Radioautograms of sagittal kidney slices prepared at different times (min) after the injection of ^{85}Kr. After administration of bombesin (20 (ng/kg)/min) all phases of ^{85}Kr disappearance were greatly retarded. Similar patterns of distribution of radioactivity could be seen after 15 s (A, cortical phase), 2 min (B, medullary phase) and 15 min (C, hilar phase), respectively, in the control kidney (CON), as after 4 min (A, cortical phase), 20 min (B, medullary phase with residual cortical phase) and 50 min (hilar phase), respectively, in the bombesin-treated kidney (BOMB).

Intrarenal blood-flow distribution measured by the radioactive microspheres technique

The effect of bombesin on intrarenal blood-flow distribution as measured by the plastic radioactive microspheres technique is shown in Fig. 2. It can be seen that the outer cortex was affected by the polypeptide at lower infusion rates than the juxtamedullary cortex. At 6 (ng/kg)/min average blood flow reduction in the first zone was 52% whereas it was only 18% in the second zone, and at 12 (ng/kg)/min average reductions of flow were 68 and 57%, respectively.

A kidney was removed from three dogs at the end of a 30 min infusion of 6 (ng/kg)/min of bombesin and following the injection of the two isotopes (the first given before, and the second 20 min after starting the bombesin infusion). A cubic block of renal tissue comprising the full thickness of the organ from the cortex corticis to the renal pelvis was removed with a lancet and then cut in slices 6 mm thick, tangential to the kidney surface, with the aid of a free hand microtome.

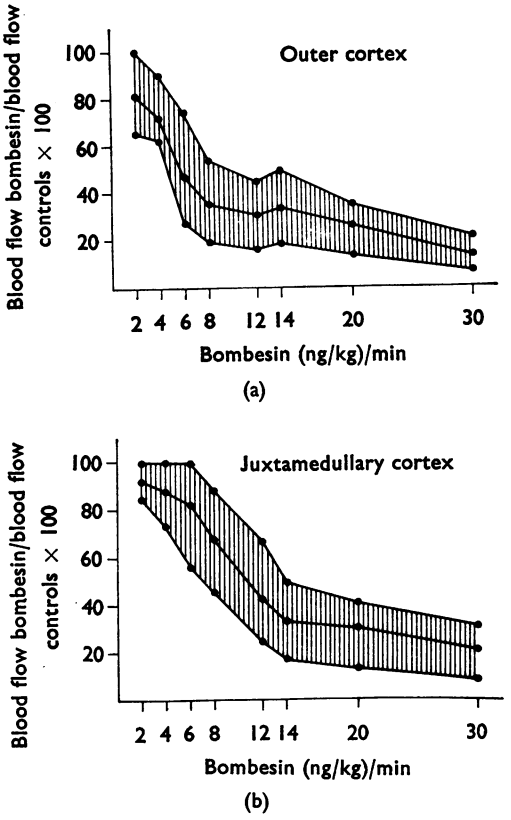

FIG. 2. Effect of graded doses of bombesin given by i.v. infusion, over a 30 min period, on blood flow in the outer cortex and in the juxtamedullary cortex, as estimated by the radioactive microspheres method. Control blood flow=100. Hatched areas, range; middle line, mean (5 dogs). It can be seen that blood flow in outer cortex was influenced by bombesin at lower infusion rates than blood flow in juxtamedullary cortex.

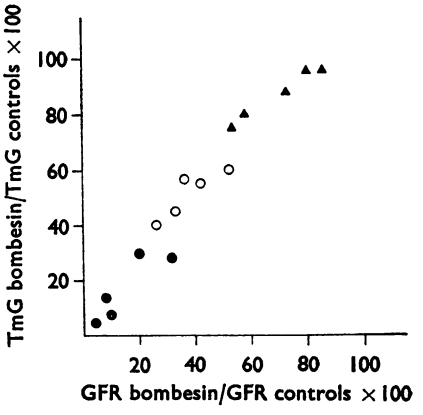

FIG. 3. Correlation between tubular glucose transport (TmG) and glomerular filtration rate (GFR) during i.v. infusions of bombesin at rates of 2 (ng/kg/min (▲), 4 (ng/kg)/min (○) and 6 (ng/kg)/min (●). Each symbol shows result from one dog. Changes in TmG paralleled changes in GFR.

Slice 1 corresponded to the cortex corticis, slices 2 to 8 to the outer cortex, slices 9 to 11 to the juxtamedullary cortex and transition zone, and finally slices 12 to 14 to the medullary and hilar zones.

Tissue slices in which specific activity was most intensely reduced (by 55–70%) were slices 2 to 5, corresponding to the external half of the outer cortex. In the inner half of the outer cortex and in the juxtamedullary cortex reductions of radioactivity were between 25 and 46%.

Maximum tubular glucose transport

Maximum glucose transport by the tubular cells (TmG), in ng/min, was sharply reduced by bombesin, as shown in Table 1. With 4 (ng/kg)/min of the polypeptide TmG was approximately halved and with 6 (ng/kg)/min it was only 3 to 28% of the control (average 13·7%).

Table 1 and Fig. 3 demonstrate that GFR/TmG, i.e., ratio between glomerular filtration rate and maximum glucose transport, remained almost constant up to 6 (ng/kg)/min of bombesin; this was the maximum infusion rate at which the parameter could be studied. This means that the total amount of glucose removed from the filtrate was directly proportional to the glomerular filtration rate.

Total and fractional distal delivery of sodium

Table 1 shows that total urinary excretion of sodium ($U_{Na}V$, in μEq/min) decreased concomitantly with the decrease in GFR. At 1 (ng/kg)/min of bombesin the average decrease was only 12%, but at 4 (ng/kg)/min the decrease averaged 84% and at 6 (ng/kg)/min it was 87%.

No appreciable changes could be observed in distal fractional delivery of sodium, i.e. in the ratio between sodium and creatinine clearances (C_{Na}/C_{Cr}), at an infusion rate of 1 (ng/kg)/min of bombesin, maintained for 30 minutes. However, at higher infusion rates reduction in C_{Na}/C_{Cr} was evident, with a maximum of 50% at 4 (ng/kg)/minute.

The renin-angiotensin system

It is generally assumed that a reduction of flow in the afferent vascular bed of the glomerulus is accompanied by a release of renin. Since bombesin elicited a potent vasoconstrictor effect on the afferent arterioles, the action of the polypeptide on the renin-angiotensin system was investigated.

Intravenous infusion of bombesin

Bombesin was infused intravenously at 3, 6 and 10 (ng/kg)/min for 30 min in 5 dogs and the following parameters were considered: (a) renin activity of arterial blood, (b) angiotensin II levels in arterial blood, and finally (c) renin secretion by the kidney.

Moreover, the effects on the renin secretion produced by 4 and 10 (ng/kg)/min of bombesin, infused over a 4 h period, were studied in two dogs for each dose.

Results of bombesin infusions lasting 30 min are shown in Table 3.

It may be seen that at infusion rates of 3 and 6 (ng/kg)/min, bombesin first caused

TABLE 3. *The action, in five dogs, of three different doses of bombesin, given by i.v. infusion over a 30 min period, on renin secretion (RS, (ng/g)/min), renin activity in arterial blood (RA, (ng/ml)/h) and angiotensin II concentration in arterial blood (ANG, pg/ml)*

Time of collection of blood samples	Bombesin infusion								
	3 (ng/kg)/min			6 (ng/kg)/min			10 (ng/kg)/min		
	RS	RA	ANG	RS	RA	ANG	RS	RA	ANG
Pre-infustion period (saline)									
−30 min	17·4±5·4	7·1±1·2	12·0±1·2	20·2±6·9	5·9±0·9	8·4±1·9	11·8±4·6	5·1±1·2	8·7±1·0
−20 min	16·5±5·0	7·4±1·3	11·9±1·6	17·4±5·1	6·3±0·8	7·3±1·5	13·5±7·1	5·3±1·4	8·4±1·1
−10 min	14·4±4·7	8·1±1·0	12·3±2·1	11·8±4·8	6·4±0·9	8·4±2·0	14·5±5·4	5·7±1·6	9·2±1·4
0 min	12·4±5·8	7·3±1·3	11·3±2·2	12·4±5·8	6·6±1·0	8·0±1·8	13·8±5·1	5·3±1·3	9·8±1·5
Bombesin infusion period									
2 min	30·8±6·8*	8·0±1·3	10·8±2·5	49·0±7·1*	9·1±1·4	9·9±2·1	14·4±5·4	5·8±1·5	7·8±1·2
4 min	40·2±6·0†	8·5±0·8	11·2±2·0	74·0±7·6†	11·3±1·6	12·7±2·4	16·4±6·0	6·5±1·8	10·0±1·4
8 min	33·6±8·3†	11·5±1·4	12·8±2·2	34·0±6·8*	15·3±2·1*	15·8±2·6	20·8±5·7	7·2±2·1	11·7±1·8
12 min	28·5±6·7	12·9±1·7*	16·1±2·2	23·0±6·2	19·1±2·4*	21·3±3·0	19·3±2·9	8·0±2·0	13·6±2·0
20 min	23·8±5·6	11·1±1·7	21·9±2·8*	14·6±6·0	14·3±1·9	25·9±3·1*	14·0±2·6	6·6±1·9	11·8±1·4
30 min	24·8±6·6	11·4±1·5	18·3±2·6	11·2±3·2	12·7±1·7	13·8±2·7	14·8±2·5	7·6±2·1	15·2±1·5
Post-infusion period (saline)									
60 min	18·2±5·5	10·4±1·5	11·5±2·4	18·4±4·1	11·8±1·6	11·3±2·0	42·0±4·7*	21·3±2·4*	18·3±1·1*
90 min	14·6±5·3	10·1±1·3	8·5±1·7	9·8±1·6	7·3±1·3	8·0±1·5	19·8±3·2	16·2±2·6	11·9±2·1
120 min	13·8±4·5	7·9±1·0	7·8±1·5	9·0±2·9	6·8±1·2	8·2±1·7	13·0±2·3	9·7±2·1	10·6±1·5

The values are the means ± S.E.; *t* test for paired observations: * $P<0·05$; † $P<0·01$.

an increase in renin concentration in the blood presumably arising from the kidney, followed by increase in arterial blood levels of renin activity and in turn by an increase in angiotensin II concentrations in arterial blood. At these infusion rates responses were clearly dose-dependent. The peak of renin secretion was always reached within the first few minutes and then, in spite of continuing the infusion, renin secretion declined; this was more evident and rapid at the infusion rate of 6 (ng/kg)/minute.

At an infusion rate of 10 (ng/kg)/min the effects of bombesin on the renin-angiotensin system were apparently more complex. In fact, during bombesin infusion the three parameters showed only small and short-lived changes. However, after the infusion had been stopped renin secretion increased conspicuously, lasting approximately 60 min, and the same could be observed, although less clearly, with renin activity levels and angiotensin II concentrations in systemic arterial blood.

The infusion rate of bombesin that produced the most intense response by the renin-angiotensin system was 6 (ng/kg)/min: renin secretion increased by 3 to 20 times, renin activity in arterial blood by 2 to 4 times, and finally angiotensin II concentration in arterial blood by 2 to 6 times. When the infusion rate of bombesin was increased up to 10 (ng/kg)/min it produced, as already stated, a drastic reduction in immediate renin release, which was not counterbalanced by the post-infusion renin liberation.

At an infusion rate of 4 (ng/kg)/min for 4 h two peaks of renin secretion could be observed. The first reached its maximum (400% increase) within 5 min after starting the infusion and faded within 60–80 min; the second reached a maximum (100–200% increase) 30–60 min after the infusion had been stopped and disappeared after 2 hours. Renin secretion ceased when GFR and RPF were reduced to 8 and 13% of their original values, respectively, and reappeared as soon as GFR and RPF had returned towards pre-infusion levels after stopping the bombesin infusion.

At an infusion rate of 10 (ng/kg)/min for 4 h the first peak of renin secretion was hardly appreciable but, as the infusion of bombesin was stopped, a conspicuous increase in renin secretion could be observed with a long-lasting plateau. Whereas the other renal parameters examined (GFR and RPF) had returned to normal within 45 min, a high renin secretion persisted for up to 3 h (Figure 4).

It seems conceivable that the different patterns of renin secretion observed following infusion of different doses of bombesin depend upon the rapidity and intensity of the afferent vasoconstriction caused by the polypeptide. If circulation in the renal cortex is sufficiently maintained to remove renin from its site of release, renin appears in the venous outflow of the kidney; if circulation is rapidly and greatly reduced, no renin is found in the venous blood. However, renin may appear or re-appear as soon as renal circulation is restored following interruption of bombesin infusion.

Infusion of bombesin into a renal artery

Bombesin was infused for 15 min into one renal artery of two dogs, at a rate of 10 ng/minute. The contralateral kidney was used as a control.

Figure 5 shows a representative result obtained in one dog. The kidney infused

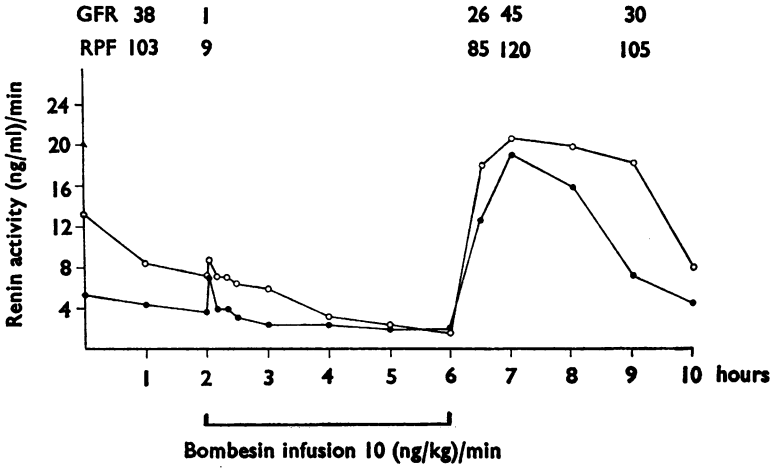

FIG. 4. Renin activity in arterial blood of two dogs given an i.v. infusion of 10 (ng/kg)/min of bombesin over a 4 h period. Start of bombesin infusion caused a short-lived, insignificant rise in renin activity. However, following the period of infusion there was a prolonged, intense rise of renin activity, that continued after the return of GFR and RPF to normal values.

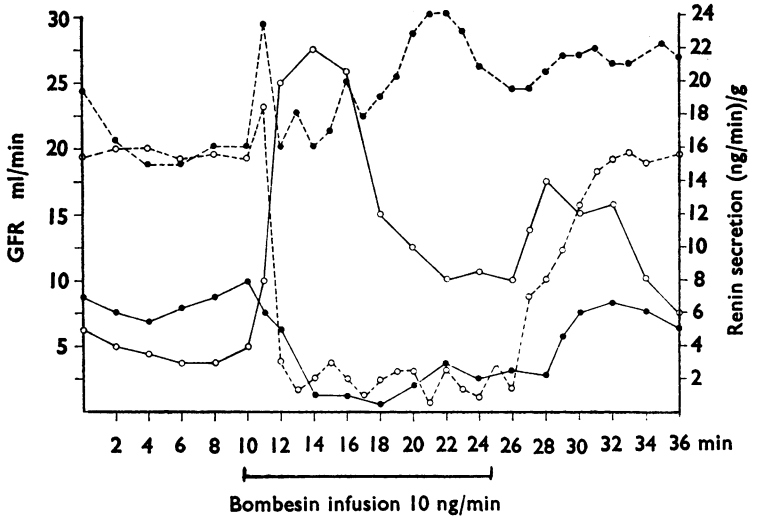

FIG. 5. Infusion of 10 ng/min of bombesin, for 15 min, into the renal artery of a kidney, with the contralateral kidney as control. Renin secretion (○——○) and glomerular filtration rate (GFR) (○- - -○) in the bombesin-infused kidney; renin secretion (●——●) and GFR (●- - -●) in the control kidney. The venous blood coming from the bombesin-infused kidney showed two peaks in renin secretion, the major at the beginning of bombesin infusion, the minor after the end of the infusion. There was a decrease in renin secretion by the control kidney. GFR showed a sharp reduction in the bombesin-infused kidney, lasting for the whole period of infusion, and a moderate, irregular increase in the control kidney.

with bombesin showed a sharp fall (80–90%) of GFR and simultaneously a conspicuous increase (more than 400%) in renin secretion. However, GFR remained at the same low level throughout the infusion period whereas renin secretion decreased. After discontinuing the bombesin infusion a second moderate peak of renin secretion could be observed, lasting 6–8 minutes. The other kidney showed a moderate, irregular increase in GFR and renin secretion was clearly reduced.

Rapid intravenous injection of bombesin

Bombesin was administered by rapid i.v. injection, in doses ranging from 10 to 100 ng/kg. At 10 ng/kg an increase in renin activity in systemic arterial blood was observed in 2 dogs out of 5, at 50 ng/kg in 3 dogs out of 4, and at 100 ng/kg in all the 3 dogs examined. Results are shown in Figure 6. Two peaks of renin activity could be seen; the first occurred within 2–3 min after the injection, the second was more delayed. The first peak was particularly evident for the 10 and 50 ng/kg doses and least evident for the 100 ng/kg dose. For the second peak the reverse was true.

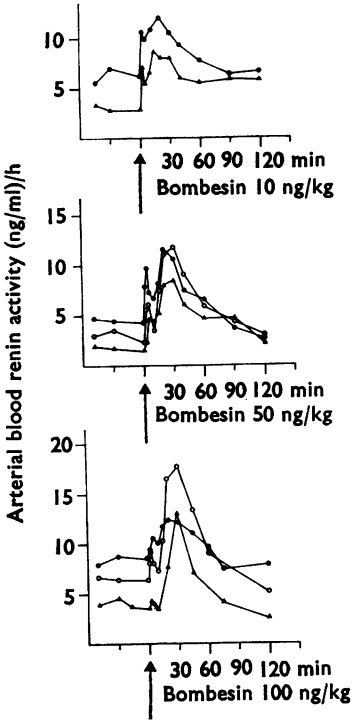

FIG. 6. The effect on arterial blood renin activity of three different doses of bombesin (10, 50 and 100 ng/kg) given by rapid i.v. injection, at arrows. Two peaks of increased renin activity may be seen: the first was most pronounced at low doses of bombesin, the second at high doses.

The smooth muscle of the urinary tract

In experiments carried out in this laboratory (Erspamer *et al.*, 1972a), it has been shown that bombesin was virtually inactive, up to concentrations of 1 μg per ml of medium, on isolated smooth muscle preparations of the dog urinary bladder, ureter and renal pelvis. Similarly bombesin infused i.v. at a rate of 50 (ng/kg)/min did not cause any change in ureteral pressure or in frequency and amplitude of ureteral peristaltic waves, registered in dogs with the pressure flow method. These results demonstrate that the renal effects of bombesin are completely independent of any change in tone of the smooth muscle of the urinary tract, and hence of any pressure change in the urinary tract.

Discussion

Bombesin, the active tetradecapeptide from the skin of the European discoglossid frogs *Bombina bombina* and *Bombina variegata variegata*, had a striking antidiuretic effect in the dog. Reduction in urine volume was accompanied by a parallel reduction in glomerular filtration rate and in renal plasma flow. Under the influence of bombesin, the ^{85}Kr washout curve from the kidney was delayed and the number of radioactive microspheres trapped in the glomerular tuft was reduced. All these data point unequivocally to an action of bombesin on the smooth muscle of the afferent arterioles of the glomeruli, which were constricted by the polypeptide. A simultaneous constriction of the efferent arterioles, with consequent increase in resistance to flow in post-glomerular vessels, cannot be excluded but is certainly of less importance because bombesin produced a decrease in filtration fraction.

The analysis of the effects of bombesin on the ^{85}Kr disappearance curve in the different renal compartments demonstrated that the first region affected by the polypeptide was the outer cortical zone. Higher infusion rates were required to influence the juxtamedullary zone. The radioactive microsphere method has confirmed the data obtained with ^{85}Kr and has permitted an even closer localization of the preferential site of action of bombesin in the kidney cortex. This is located in the external half of the outer cortical zone, beneath the cortex corticis, which is known to possess the largest number of short-looped glomeruli.

As already stated, changes in intrarenal blood flow produced by bombesin were studied by three different methods. Results were in good accordance bearing in mind that the events measured by the three methods are not exactly the same. Moreover, each method has its own limits and sources of error.

The problem of whether changes in tubular activity observed during bombesin infusion should be considered as mere consequences of the vascular effects of the polypeptide or as an expression of an independent, primary action of bombesin on the tubules must be left open and will be discussed elsewhere after the completion of additional experiments in dogs and other species. We refer to changes in PAH extraction, in fractional distal delivery of sodium and, finally in maximum glucose transport.

Concerning the last effect it may be remembered that a constancy in the GFR/TmG ratio similar to that found after bombesin administration was also observed by Handley & Moyer (1955) in dogs in which glomerular filtration rate had been reduced by a variety of drugs and procedures. These workers ascribed their results to changes in the number of functioning nephrons.

The present experiments strongly indicate that the action of bombesin on the afferent glomerular bed is a direct one. An adrenergic mediation through local liberation of catecholamines may be excluded because blockade of α-adrenoceptors did not reduce renal vasoconstriction induced by bombesin (Fregnan & Glässer, personal communication) and because it is extremely difficult to believe that an intrarenal adrenergic mechanism can maintain for hours, as in the case of bombesin, an afferent vasoconstriction. Moreover, it is probable that the amount of noradrenaline available for release in the kidney is very small (see Peters & Bonjour, 1971). Finally, whereas adrenaline produced an increase in resistance to flow both an afferent and in efferent arterioles, as shown by an increase in filtration fraction, the effect of bombesin was limited to the afferent arterioles, as shown by a decrease in filtration fraction (cf. Thurau & Levine, 1971).

It has been suggested that minute amounts of angiotensin may originate in the juxtaglomerular apparatus following release of renin and we have confirmed that following bombesin administration there was a conspicuous increase of angiotensin II in the lymph coming from the kidney (Melchiorri, Sopranzi & Erspamer, unpublished observations). However, a participation of angiotensin in the production of the afferent vasoconstriction produced by bombesin may be excluded or minimized because available experimental evidence does not substantiate the possibility that either renin or angiotensin play an appreciable role in the autoregulation of blood flow either in the whole kidney or in single nephrons (Peters & Bonjour, 1971).

The changes in renal circulation and function caused by bombesin were accompanied by a conspicuous activation of the mechanism(s) which cause renin release by the kidney, and consequently by a remarkable increase of angiotensin II concentration in systemic arterial blood. Renin activity in the venous outflow from the kidney may increase up to twenty-fold, and the angiotensin II concentration in arterial blood up to six-fold. When bombesin was infused into one renal artery renin liberation was strictly homolateral.

To explain the release of renin by bombesin at least three mechanisms may be suggested: (a) direct action of the polypeptide on the granular cells of the juxtaglomerular apparatus, (b) indirect action through afferent vasoconstriction and, finally (c) indirect action, possibly at the macula densa site, through changes in the composition of the tubular fluid. The available data do not permit a choice between these possibilities.

Results described in this paper have been obtained in, and are only valid for, anaesthetized dogs. Preliminary data seem to indicate that unanaesthetized animals behave like anaesthetized preparations but are more sensitive to bombesin.

The problem of the hormonal regulation of renal circulation and function still presents a number of gaps and obscurities despite persistent studies. It is impossible to explain all events in terms of vasopressin, angiotensin, aldosterone and catecholamines. Consequently, the existence of other humoral factors has been postulated. Some workers who maintain that the macula densa is a primary sensing element in the renin release mechanism, accept the possibility that a local hormone is released by the macula densa and diffuses into the juxtaglomerular cells with the subsequent release of renin (Davis, 1971). Others have recently described a plasma-renin releasing factor which is produced and circulated in the plasma of dogs subjected to a period of hypotension by means of controlled haemorrhage (De Vito, Wilson, Shipley, Miller & Martz, 1971) or which is present in a bovine serum fraction and in a plasma fraction of nephrectomized rats (Nolly, Cabrera & Fasciolo, 1972).

Bombesin possesses a number of prerequisites for candidature as a factor intervening in the hormonal regulation of circulation and function of the kidney. However, a major obstacle for accepting this hypothesis is that the polypeptide has so far not been detected in mammals.

All active peptides found to date in the cutaneous tissue of amphibians present striking similarities to peptides occurring in the mammalian organism: amphibian bradykinin and phyllokinin have their counterpart in mammalian bradykinins, amphibian physalaemin and phyllomedusin in mammalian substance(s) P, amphibian caeruleins in mammalian cholecystokinin, and amphibian angiotensin-like peptide (Endean, Erspamer, Melchiorri & Sopranzi, unpublished observations) in mammalian

angiotensin II. The only exception is bombesin, which still lacks its counterpart in mammals. A search for bombesin-like peptides in mammalian tissues with the aid of radioimmunological methods is in progress.

This work was supported by grants from the Consiglio Nazionale delle Ricerche, Rome.

REFERENCES

CAIN, M. D. & CATT, K. Y. (1969). Immunoreactive fragments of angiotensin II in blood. *Nature, Lond.*, **233**, 617–618.

CARRIÈRE, S. & FRIBORG, J. (1969). Intrarenal blood flow and PAH extraction during angiotensin infusion. *Am. J. Physiol.*, **217**, 1708–1715.

DAVIS, J. O. (1971). What signals the kidney to release renin? *Circulation Res.*, **28**, 301–306.

DE VITO, E., WILSON, C., SHIPLEY R. E., MILLER, R. P. & MARTZ, B. L. (1971). A plasma humoral factor of extrarenal origin causing release of renin-like activity in hypotensive dogs. *Circulation Res.*, **29**, 446–451.

ERSPAMER, V., FALCONIERI ERSPAMER, G., INSELVINI, M. & NEGRI, L. (1972a). Occurrence of bombesin and alytesin in extracts of the skin of three European discoglossid frogs and pharmacological actions of bombesin on extravascular smooth muscle. *Br. J. Pharmac.*, **45**, 333–348.

ERSPAMER, V., MELCHIORRI, P. & SOPRANZI, N. (1972b). The action of bombesin on the systemic arterial blood pressure of some experimental animals. *Br. J. Pharmac.*, **45**, 333–348.

FOLIN, O. & WU, H. (1919). A system of blood analysis. *J. biol. Chem.*, **38**, 81–100.

GOCKE, D. J., GERTEN, J., SHERWOOD, L. M. & LARAGH, J. H. (1969). Physiological and pathological variations of plasma angiotensin II in man. *Circulation Res.*, Suppl. 1, **24** and **25**.

HABER, E., KOERNER, T., PAGE L. B., KLIMAN, B. & PURNODE, A. (1969). Application of radioimmunoassay for angiotensin II to the physiologic measurement of plasma renin activity in normal human subjects. *J. clin. Endocrin.*, **29**, 1349–1355.

HANDLEY, C. A., SIGAFOOS, R. B. & LA FORGE, M. (1949). Proportional changes in renal tubular reabsorption of dextrose and extraction of *p*-aminohippurate with changes of intraglomerular filtration rate. *Am. J. Physiol.*, **159**, 175–180.

HANDLEY, C. A. & MOYER, J. H. (1955). Significance of the GFR/TmG ratio. *Am. J. Physiol.*, **180**, 151–155.

JOHNSON, J. A., NASH, J. D. & FUSARO, R. M. (1963). An enzymatic method for the quantitative determination of glycogen. *Anal. Biochem.*, **5**, 379–387.

MCNAY, J. L. & ABE, J. (1970). Redistribution of cortical blood flow during renal vasodilatation in dogs. *Circulation Res.*, **27**, 1023–1032.

NOLLY, H. L., CABRERA, R. R. & FASCIOLO, J. C. (1972). Renin releasing activity of a blood plasma fraction. *Experientia, Basle*, **28**, 418–419.

PETERS, G. & BONJOUR, J. P. (1971). Renal effects of renin and angiotensin. In: *The Kidney*, ed. Rouillier, Ch. & Muller, A. F., Vol. IV, pp. 95 and 105–106. New York and London: Academic Press.

SLOTKOFF, M. L., LOGAN, A., JOSE, P., D'AVELLA, J. & EISNER, G. H. (1971). Microsphere measurement of intrarenal circulation of the dog. *Circulation Res.*, **38**, 158–166.

THORBURN, G. D., KOPALD, H. H., HERD, J. A., HOLLENBERG, M., O'MORCHOE, C. C. & BARGER, A. C. (1963). Intrarenal distribution of nutrient blood flow determined with Krypton 85 in the unanesthetized dog. *Circulation Res.*, **13**, 290–307.

THURAU, K. & LEVINE, D. Z. (1971). The renal circulation. In: *The Kidney*, ed. Rouillier, Ch. and Muller, A. F., Vol. III, pp. 35–40. New York and London: Academic Press.

WOLF, A. V. (1941). Total renal blood flow at any urine flow or extraction fraction. *Am. J. Physiol.*, **133**, 496–497.

(*Received June* 9, 1972)