Original Article

Medullopressin: A New Pressor Activity from the Renal Medulla

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The kidney contains blood pressure-lowering substances, such as prostaglandin E_2 or prostaglandin A_2, and blood pressure-raising substances such as thromboxane A_2. Most of the postulated substances, however, have not yet been isolated in a pure state and are of unknown structure. In the present study, we separated a chloroform extract from the medulla of pig kidneys using various chromatography procedures. Each fraction was tested in spontaneously hypertensive Wistar rats. One of these fractions caused a powerful blood pressure increase of 30.1±7.1 mmHg systolic and 34.7±6 mmHg diastolic (*N*=7; *p*=0.0003), reaching its maximum 55±27 s after completion of the injection and lasting for 201±59 s. A long-lasting contractile response in porcine and bovine coronary artery rings was observed. In the mouse aortic rings, the contractile response accounted for 0.38±0.13 g, *i.e.*, 31.9±10.9% of the maximum potassium response (*N*=11; *p*=0.003). Because this activity could not be attributed to any known vasoactive substance, it was considered to arise from a novel underlying active substance in the kidney medulla, which we named medullopressin. (*Hypertens Res* 2005; 28: 827–836)

Key Words: **kidney medulla, chromatography, blood pressure, pressoric substances, medullopressin**

Introduction

In 1898 an aqueous extract from the kidney was found to have a pressor effect (*1*). Many years later, this discovery led to detailed investigation and elucidation of both the components and the mode of action of the renin-angiotensin-aldosterone system, and since that time the kidney has been recognized as the principal blood pressure-regulating organ of the body. This was followed by the observations that stable hypertension can be induced by inducing ischemia (*2*) or by wrapping the kidney in cellophane (*3*).

Although *Rauwolfia serpentina* roots had been used in India for the treatment of high blood pressure since 1931 (*4*), the drug was not used in the West until the mid-fifties. The treatment of high blood pressure remained difficult long after the introduction of hydralazine, which was the first drug that allowed effective treatment of this severe condition. Therefore, great interest arose in 1940, when Page *et al*. (*5*) reported that renal extracts given to patients with malignant hypertension caused regression of the changes in their optic fundi and lowered their high blood pressure. This prompted numerous attempts to isolate vasoactive substances from the kidney (*6–10*). Experiments in dogs showed that renoprival hypertension could be alleviated by intraperitoneal administration of macerated renal medulla but not by intraperitoneal administration of macerated renal cortex (*11*), and it was clear that the blood pressure-lowering active principle must be located in the renal medulla. This ultimately led to isolation of the powerful vasodilator substances prostaglandin A_2 and E_2

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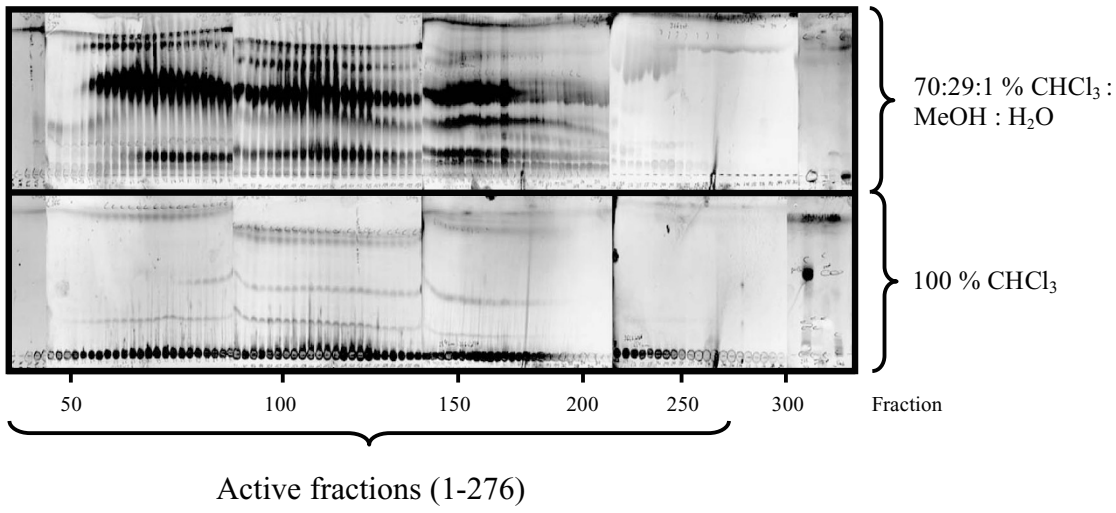

Fig. 1. *Thin layer chromatogram of the 332 fractions resulting from gel liquid chromatography of fraction E. The medullopressin activity is contained in fractions 1–276.*

from the renal medulla (*12*).

However, there is evidence that not all the substances present in or secreted by the kidney have yet been identified. For example, renal vein effluate from an isolated perfused kidney has been shown to lower the blood pressure of spontaneously hypertensive rats, and the blood pressure reduction is directly proportional to the perfusion pressure (*13*). Because of its unique attributes, such as its abolition by excluding the liver from the circulation (*14*), by previous administration of proadifen (*15*), or by mixing the extract with Tween 20 or butyl boric acid (*16*), this blood pressure-lowering activity cannot be ascribed to any previously known substance.

Recently, tremendous progress has been made regarding the role of vasoactive hormonal peptides such as adrenomedullin (*17*), angiotensin (*18*), endothelin (*19*), or thrombomodulin (*20*). In contrast, over the same time period progress has been very slow in the characterization and isolation of non-peptidic vasoactive substances such as medullipin or the endogenous digitalis-like factor (*21*), and accordingly, in the determination of their role in cardiovascular physiology. In the course of investigating vasoactive, nonpeptidic substances from the kidney medulla, a strong blood pressure-increasing activity was unexpectedly detected. Since a similar activity has not been described before, the aim of the present research was to further characterize this previously unknown active pressor principle from the kidney.

Methods

Drugs and Chemicals Applied

Potassium chloride, sodium chloride, calcium chloride, potassium dihydrogenphosphate, magnesium sulfate, sodium hydrogencarbonate, edetate calcium disodium, acetylcholine chloride, serotonin, norepinephrine chloride, and sodium nitroprusside dihydrate were obtained from Sigma Chemicals Inc. (Deisenhofen, Germany). All organic solvents used were of analytical grade and were obtained from Riedel De Haen (Seelze, Germany). For preparation of aqueous solutions, aqua bidestillata was used. In organ chambers, dissection baths and laboratory equipment Duran® glass and laboratory equipment made of Duran® glass (Schott, Mainz, Germany) were employed throughout.

The Raw Material

The source material consisted of pig kidneys. About 15 min after slaughtering and immediately after the meat inspection, the kidneys were excised with a knife from the lung-liver-kidney block as it passed along the conveyor belt, and were immediately placed in crushed ice. Not more than 45 min later they were snap-frozen in liquid nitrogen and stored at −40°C until dissection. The kidneys were longitudinally sectioned at 2–3 mm thickness using an electric slicing machine (Graef, Germany). The kidney slices were carefully dissected with a scalpel so as to separate the medulla from the cortex and renal pelvis. The medulla was immediately placed in liquid nitrogen. This material was again stored at −40°C.

Further Treatment of the Renal Medulla

The renal medulla was then pulverized in the liquid nitrogen-cooled steel bowl of a kitchen mixer (Vorwerk Thermomix 3300, Wuppertal, Germany). Subsequent freeze-drying of the powder was carried out by Suwelack Inc. (Billerbeck, Germany).

Extraction

The lyophilizate was transferred to a 20-l beaker (Schott, Mainz, Germany). Chloroform was added and the material was mixed for about 10 min with a mixing machine (Ultra-Turrax T50; Janke and Kunkel, Staufen, Germany) at maximum speed. The solid material was exhaustively extracted with nine successive portions of chloroform until it became colorless. The obtained solution was evaporated to dryness *in vacuo* at room temperature, leaving a dark yellowish viscous oil that was free of peptides and proteins. For biological test purposes, an aliquot portion of the solution was set aside for the animal experiments. After adding a small amount of chloroform, the remaining extract was applied to 259.9 g of silica gel (ICN, Eschwege, Germany) having an irregular grain of size 32–64 μm and a pore size of 60 Å, and transferred to a desiccator to remove the chloroform *in vacuo*. The yield was 464 g of dry powder.

Silica Gel Chromatography

A customized vacuum liquid chromatography (VLC) column with a height of 400 mm and an internal diameter of 160 mm was constructed and filled with 2,204 g of irregular silica gel (ICN; 32–64 μm, 60 Å pore size). Elution was carried out in 73 stages using a stepwise gradient beginning with hexane, progressing through hexane–diethylether, hexane–diethylether–ethanol, diethylether–ethanol, ethanol–methanol and terminating with pure methanol. Fraction 73 consisted of straight chloroform. Specimens were taken from each fraction for the animal experiments. After monitoring by thin-layer chromatography (Merck KG60 GF; 20×10 cm plates, *n*-hexanes–EtOAc mixtures of different polarity for development, anisaldehyde–H_2SO_4 detection), the 73 fractions were recombined to form five combined fractions as follows: A=1–16, B=17–28, C=29–35, D=36–46, and E=47–73.

Gel Chromatography

The last two combined fractions, D and E, were further separated by gel chromatography on a Sephadex LH-20 column (Fluka, Buchs, Switzerland). For combined fraction D (=36–46) a column of 143 cm height and an internal diameter of 4.2 cm was filled with about 500 g of the gel; for fraction E (=47–73) a column of 95 cm height and an internal diameter of 6.3 cm was filled with about 700 g of Sephadex LH-20. Each column was eluted with a 1:1 mixture of methanol and dichloromethane. The elution front was discarded. The activity was located in the combined fraction E/1–276 (of 340 fractions), obtained from fraction E of the silica gel column. The combined fraction E/1–276 was free of arachidonic acid metabolites and 20-hydroxyeicosatetraenoic acid, as shown by GC-MS. No peptide traces were detected. As inactive comparison material for the biological testing the corresponding early eluting subfractions of fraction D, namely subfractions D/1–48, were used. Figure 1 shows the thin-layer chromatograms of the fractions resulting from gel chromatography (chloroform/methanol/water; 70:29:1; v/v/v for development; anisaldehyde–H_2SO_4 detection).

Further Separations

In the course of preliminary feasibility studies pursuant to the isolation of medullopressin, countercurrent chromatography, gradient VLC, and medium pressure liquid chromatograpy were performed. In all cases, the pressor activity continued to be observed after the chromatography.

Preparation of Samples for Animal Testing

Aqueous suspensions of the fat-like residues were made up in *Aqua ad injectabilia* (Braun, Melsungen, Germany). For this purpose, the fatty residue was first moistened with an aliquot of ethanol and then water was added at a ratio of 1:19 ethanol/water (v/v) to yield a test solution with a final concentration of 2.5 mg/ml. All samples were homogenized with an ultrasound microsuspension apparatus (Sonifier W-250; Branson, USA), operated 1–3 times for 15–30 s at 5 to 30 W output, until a homogenous sample was obtained. A mixture of ethanol/water 5:95 (v/v) was used as a solvent control.

Mixtures Employed and Dosages

The combined fraction E/1–276, containing the activity, and for comparison the combined fraction D/1–48, also as described above, were each tested in seven rats, together with the solvent control. The volume was 1.5 ml, and the injection was given over about 10 s. In each instance the dose was 10 mg/kg body weight.

Tissue Preparation

Bovine and porcine hearts were obtained from the slaughterhouse. They were excised immediately after withdrawal of the organ package, rinsed with cold (4°C) Krebs-solution, then filled with this solution and cooled down to 1–4°C. Dissection of the coronary arteries was carried out in a Krebs-Ringer bicarbonate solution (4°C; mmol/l: $CaCl_2$, 2.5; KCl, 4.7; KH_2HPO_4, 1.2; $MgSO_4$, 1.2, NaCl, 118,6; $NaHCO_3$, 25.1; calcium disodium EDTA, 0.026; glucose, 11.1). Aortas were excised from slain laboratory mice (CRL:CFW®[SW] BR) after pentobarbital anesthesia (50 mg/kg body weight pentobarbital intraperitoneally) with the aid of a magnifier. The vessel was filled with Krebs-Ringer bicarbonate solution (4°C) as soon as possible, isolated, removed and placed into a 4°C tempered Krebs-Ringer bath (pH 7.3), and dissected under a microscope (Wild-Heerbrugg, Switzerland). Vessel rings were suspended in the organ chambers from fine tungsten stirrups of different diameters as appropriate. The study design and protocols were in accordance with the institutional

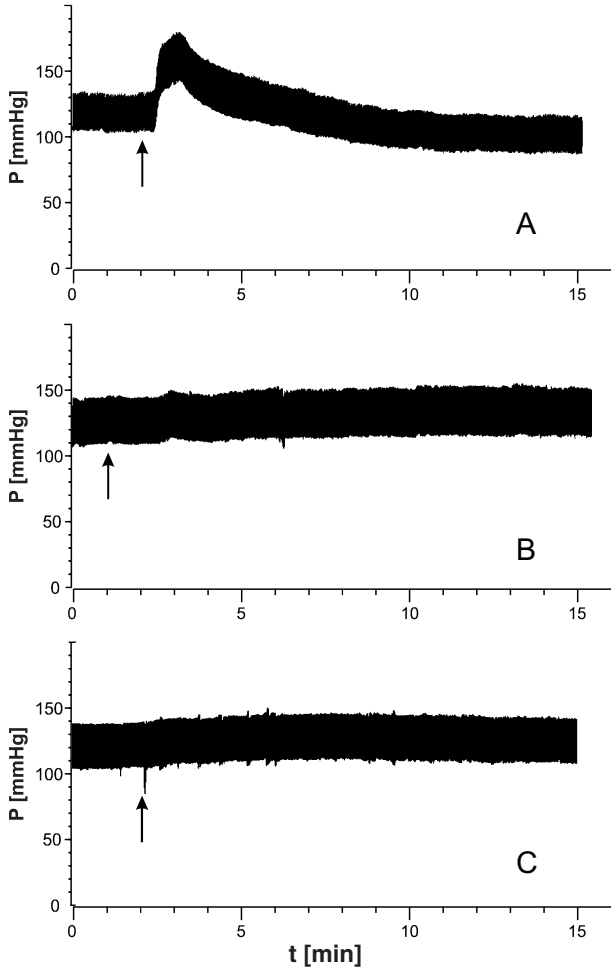

Fig. 2. *A: Effect of fraction E/1–276. The substance causing this blood pressure elevating activity has been designated medullopressin. B: Fraction D/1–48 (comparison fraction). The injection of this fraction caused no substantial change in the blood pressure. Therefore, the blood pressure response shown in Fig. 1 cannot be a nonspecific effect due to injection of a fat emulsion. C: The solvent control (a mixture of ethanol–water, 1.5 ml) used for suspending the fractions. The injection of this mixture again caused no material change in blood pressure.*

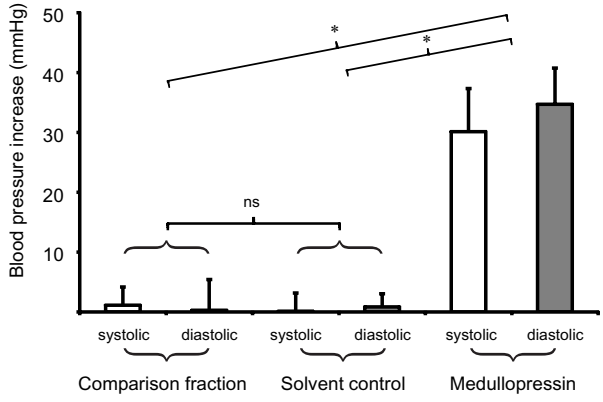

Fig. 3. *Blood pressure response upon administration of medullopressin, the comparison fraction and the control solvent in spontaneously hypertensive rats (*n*= 7 in each group). *Statistical significance at* p*< 0.05.*

animal care committee and the American Heart Association Guidelines for Research Animal Use.

Vascular Function

Aortic and coronary artery rings were suspended from the tungsten holders in organ chambers containing 10 ml of Krebs-bicarbonate solution (37°C, pH 7.4, 95% O_2 and 5% CO_2). They were equilibrated for 1 h. The resting tension was gradually increased. Rings were repeatedly exposed to 100 mmol/l KCl until the optimal tension for generation of force during isometric contraction was reached. Precontraction was not different between groups and contractions upon KCl exposure did not differ significantly between the groups. After equilibration for 30 min, rings were precontracted with 100 mmol/l KCl again, until a stable plateau was reached. Doses of 50 μg/ml medullopressin or control solution, respectively, were applied. Constriction experiments with norepinephrine and serotonin, respectively, and relaxation experiments with acetylcholine (1×10^{-10} – 3×10^{-5} mol/l) and sodium nitroprusside (1×10^{-10} – 3×10^{-5} mol/l) were subsequently performed, in order to demonstrate that the rings were vital. The endothelium was proven to be functional after the experiments. The dimensions of the arterial rings used in this study did not differ significantly. Due to the small amounts of fractions, blocking experiments with pretreatment of vascular rings by specific blocking agents, *i.e.*, 20-hydroxyeicosatetraenoic acid blockers, or different angiotensin blockers and endothelin blockers, respectively, could not be performed.

Animal Experiments

For the animal experiments, 21 adult spontaneously hypertensive rats were used. These were Wistar rats (SHR/N Crl BR) with an average weight of about 400 g obtained from Charles River (Sulzbach, Germany). Under ketanest–diazepam anesthesia (ketanest 50 mg/kg body weight; diazepam 3 mg/kg body weight), each of the extracts was injected over approximately 10 s *via* the jugular vein. The dose was set to 50 mg/kg body weight, so as to conform with the procedure of Lee *et al*. (*10*). Blood pressure was measured intra-arterially, access being gained *via* the right femoral artery. We used a pressure converter (Becton Dickinson, Lincoln Park, USA) in conjunction with a Siemens Sirecust 404 monitor (Siemens,

Table 1. Murine Aortic Rings: Mean Precontraction Values in the Mice Belonging to the Medullopressin and Control Groups, Respectively; Vascular Responses to 100 mmol/l Potassium Chloride (Potassium), Control Solution, Medullopressin, Norepinephrine and Acetylcholine

	Controls ($n=8$)	Medullopressin ($n=11$)
Precontraction	0.85±0.15	0.84±0.16
Potassium 100 mmol/l, 1st time	0.79±0.28	0.82±0.3
Potassium 100 mmol/l, 2nd time	0.88±0.23	0.92±0.23
Potassium 100 mmol/l, 3rd time	1.04±0.37	1.19±0.41
Control	0.01±0.06	
Medullopressin		0.38±0.13
Medullopressin + 100 mmol/l potassium		1.58±0.45
Norepinephrine 10^{-6} mmol/l	0.83±0.55	0.91±0.55
Acetylcholine 10^{-6} mmol/l	−0.81±0.48	−0.89±0.46

Data are given in g (tension) as absolute values.

Munich, Germany). To calibrate the instrument, we used a mercury sphygmomanometer as described by Gauer. The blood pressure was recorded in analogue mode and also digitally with a personal computer. All animal experiments were assessed by the Animal Protection Commission of the Westfälische Wilhelms–University of Münster, and approved by the District Government Office Münster (G42/99 RP MS).

Data Analysis, Statistics and Protocol Design

All data are given as the means±SD. Relaxations are expressed as absolute values in g, and as a percentage of precontractions. Contractions to medullopressin were normalized to the maximal contractile response to 100 mmol/l KCl. Due to the very limited quantity of medullopressin, the determination of EC_{50} could not be achieved in the present study. The maximum blood pressure readings at the peak point were compared with the blood pressure readings immediately before injection of the mixtures. Blood pressure-measuring protocols and vascular ring tension-measuring protocols were primarily designed and approved for detection of blood pressure-lowering activities. This was the rationale for testing the "medullopressin" extract after precontraction with KCl. The Mann-Whitney *U*-test was used where appropriate, with the aid of the program Graph Pad Prism (Graph Pad, San Diego, USA). A p value <0.05 was considered to indicate statistical significance.

Results

Preliminary Physicochemical Characterization of Medullopressin

From the extraction conditions and chromatographic parameters, the following can be concluded regarding the chemical and physicochemical characteristics of medullopressin. It is extractable from the renal medulla, but not from the renal cortex, when using chloroform. Within the polarity range covered by chloroform extracts, medullopressin is rather polar and behaves like a neutral lipid. Its molecular weight is less than 2,000 D and its chromatographic behavior indicates that it is a small molecule. Medullopressin is stable at neutral to moderately acidic pH, as well as within a temperature range from −196 to at least 50°C. Because presently medullopressin is not a single chemical entity amenable to MS or NMR analysis, the average dosage was estimated for a single constituent based on HPLC and TLC profiling to be in the few μg/kg body weight range or below, which corresponds to typical physiological concentrations of hormonal substances.

Blood Pressure Characteristics

By means of the spontaneously hypertensive Wistar rat model, the activities of the active fraction, an inactive comparison fraction and a control solvent were evaluated as follows (Fig. 2). The combined fraction E/1–276 (active fraction) showed powerful blood pressure-elevating activity, reaching its peak 55±27 s after ending the injection and lasting for 201±59 s. An example of this short lasting blood pressure rise is shown in Fig. 2a. As can be seen, the blood pressure rises almost immediately after starting the injection, continues to rise, reaches a peak, and finally falls back somewhat more slowly to reach its original level after ca. 10 min. The rise amounted to 30.1±7.1 mmHg in systolic blood pressure (SBP) and 34.7±6 mmHg in diastolic blood pressure (DBP) ($p=0.0003$). After injection of a inactive comparison fraction D/1–48 there was no substantial change in the blood pressure. A typical blood pressure curve is shown in Fig. 2b. At the time point at which the blood pressure peak was produced by injection of the active fraction E/1–276, *i.e.*, 55 s after the end of the injection, there was only a slight rise in SBP of 1.1±3 mmHg, and a slight rise in DBP of 0.3±5.1 mmHg. None of these increases were statistically significant. After injection of the solvent control, *i.e.*, the mixture of ethanol–water used for suspending the fractions, there was again no significant change in blood pressure. A typical example of

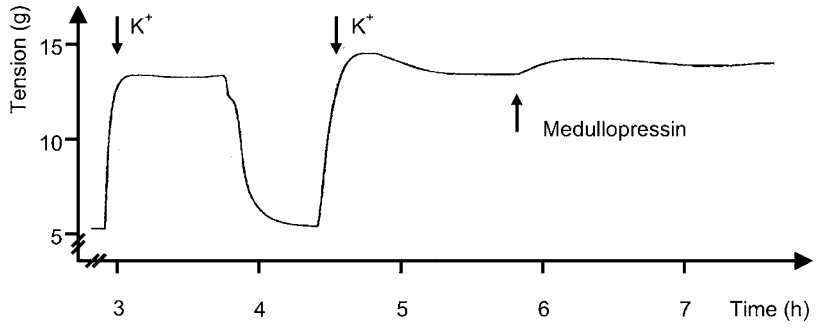

Fig. 4. *Vascular response of a bovine coronary artery ring upon administration of medullopressin. Potassium precontraction and wash out are shown.*

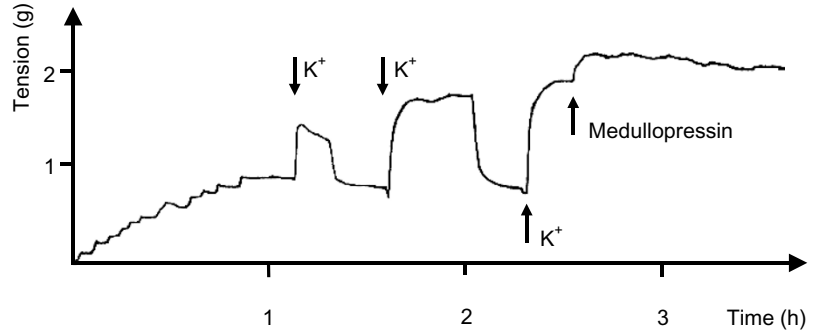

Fig. 5. *Vascular response of a mouse aortic ring upon administration of medullopressin. Two potassium precontractions and wash outs are shown.*

Table 2. Murine Aortic Rings: Vascular Responses to 100 mmol/l Potassium Chloride (Potassium), Control Solution, Medullopressin, Norepinephrine and Acetylcholine

	Controls ($n=8$; % of K^+ contraction)	Medullopressin ($n=11$; % of K^+ contraction)
Control	0.96±5.77% (n.s.)	
Medullopressin		31.94±10.92%
Medullopressin + 100 mmol/l potassium		132.77±37.81%*
Norepinephrine 10^{-6} mmol/l	79.80±52.99%*	76.47±46.22%*
Acetylcholine 10^{-6} mmol/l	−85.58±46.15%*	−74.79±38.66%*

Data is given in percent referring to maximal potassium contraction and norepinephrine contraction, respectively. Asterisks indicate statistical significance.

the blood pressure curve is shown in Fig. 2c. At the corresponding time point of the blood pressure peak produced by injection of the active combined fraction E/1–276, *i.e.*, 55 s after the end of the injection, there was a slight rise in SBP of 0.1±3 mmHg, and a slight rise in DBP of 0.9±2.2 mmHg. These differences were again of no statistical significance. The maximum blood pressure increase caused by the active combined fraction E/1–276 (Fig. 3) was 30.1±7.1 mmHg systolic and 34.7±6 mmHg diastolic, respectively, and was significantly different compared to both the fraction D/1–48 ($p=0.0003$) and the solvent control ($p=0.0003$). After injection of an analogous extract and fractions from renal cortex, no significant change in blood pressure was observed.

Vascular Function

In bovine coronary artery vessels ($n=15$), cumulative contraction upon exposure to 10^{-6} mol/l serotonin was 2.1±0.8 g. Contractions did not differ significantly between the control ($n=8$) and the medullopressin group ($n=7$, data not shown). The relaxation in response to 10^{-6} mol/l acetylcholine was −1.8±0.6 g, and did not differ significantly between the

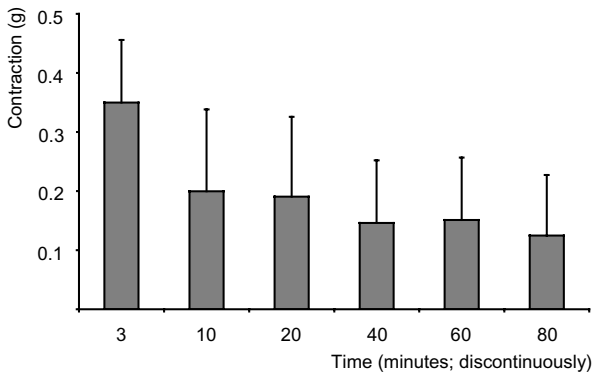

Fig. 6. *Time course of vascular response of the mouse aortic rings upon administration of medullopressin.*

groups. In porcine coronary artery vessels, the results resembled those for the bovine vessels, with strong contractions and relaxations, respectively, upon exposure to acetylcholine, norepinephrine and sodium nitroprusside. Data were not analyzed statistically ($n=4$).

Table 1 shows the extent of precontraction in the aorta of mice in the control and medullopressin group, the contractile responses to 100 mmol/l potassium chloride and norepinephrine, and the relaxing response upon exposure to acetylcholine (data are given in g as absolute values). Acetylcholine relaxation almost completely abolished the contractile response to norepinephrine after the medullopressin and the control experiments. There was no statistical difference between the two groups, showing that the aortic rings were fully vital at the end of the experimental protocol.

Vascular Response to Medullopressin

Figure 4 shows a typical vascular response to medullopressin in a bovine coronary artery; the response consisted of an additional contraction lasting for more than 1 h. The mean contractile response was 0.9±0.3, which was significantly different from that in the control group ($p=0.016$; $n=7$). Similar results were obtained in porcine coronary arteries. Figure 5 depicts the typical vascular response to medullopressin in the aortic rings of mice. The contractile response was much more pronounced compared to the effects in bovine and porcine coronary arteries. It accounted for 0.38±0.13 g, *i.e.*, 31.9±10.9% of the maximum potassium contraction (Table 2; data are given as a percentage of the maximal potassium contraction and norepinephrine contraction, respectively). This value was markedly different from that of the control group (0.01±0.06; $p=0.008$). Because all experiments were carried out using a stable potassium contraction, the maximum contraction upon injection of medullopressin was 1.58±0.45 g. This value differed markedly from the control value (1.04±0.37; $p=0.001$), as well as from the maximum potassium contraction in the medullopressin group (1.19±0.41; $p=0.008$). In contrast to the blood pressure response characteristics, the contractile response of blood vessels in both the coronary artery vessels and the aorta of mice lasted for more than 1 h, with the blood pressure peak being reflected in an early constriction response peak. Figure 6 shows the constriction response characteristics within the first 80 min after application of medullopressin to the aortic rings of mice ($n=11$).

Discussion

In this paper we describe a powerful blood pressure increase, lasting only a few minutes and commencing immediately after the injection of a fractionated chloroform extract of renal medulla. No change in blood pressure was observed after injection of the solvent control, indicating that the aforementioned activity cannot be ascribed to a volume effect. A relatively large injection volume (1.5 ml) was needed to meet pharmaceutical requirements in terms of galenic quality. The blood pressure response cannot be a non-specific effect due to injection of a fat emulsion, because injection of the comparison fractions did not induce any such blood pressure rise. Also, such changes in blood pressure have never been reported in experiments with fractions prepared from extracts of renal medulla made by a similar (*22*, *23*) or any other processing. Furthermore, as we were able to enrich the activity in the combined fraction D/1–276, all the evidence indicates that this activity must be due to a substance present in the mixture. Even after injection of other suspensions of fatty fractions we have never found any activity of the kind described above. A slight rise in blood pressure immediately after the injection was also observed in some experiments with the crude extract and in an experiment with the fraction E (=47–73) obtained after the initial separation on the silica gel column.

The blood pressure increasing activity described here was due to an immediate contraction of the arterial blood vessels, as shown in the aorta of mice as well as in porcine and bovine coronary arteries. Surprisingly, the constricting response lasted for more than 1 h in every single vessel ring that was examined. The difference in the duration of the blood pressure and vascular response, respectively, could not be explained by the interspecies differences among rats, mice, pigs and cows. Due to the limited quantity of material, further pharmacological characterization of the compound could not be performed. This point merits further investigation.

In earlier studies by Vincent and Sheen (*24*), and Thauer (*25*), an activity similar to that of medullopressin was not observed. This matches our observation that a similar pressor activity is not present in the kidney cortex, and supports the conclusion that it is specific to the kidney medulla.

Ever since Tigerstedt and Bergmann first reported a blood pressure-elevating activity, which was eventually attributed to renin, in the kidney in 1898 (*1*), and following the observation of blood pressure-lowering activity in the kidneys and

muscle by Page *et al*. in 1940 (*5*), there have been numerous attempts to isolate and describe vasoactive substances derived from the renal medulla or cortex or from renal vein effluate. These substances and mixtures can be classified into those that lower blood pressure, and those that raise it.

Among the endogenous blood pressure-lowering substances are the following. 1) Powerful vasodilator prostaglandins, such as prostaglandin E_2 and A_2 (*12*), and diuretic prostaglandins such as prostaglandin $F_{2\alpha}$ (*26*): These prostaglandins are present in significant amounts in the renal medulla and constitute a very important blood pressure-lowering component of the medulla (*12*). The vasodilator and blood pressure-lowering actions of these substances begin immediately after injection and continue for some minutes (*27*). 2) Phospholipid renin inhibitors such as those isolated from dog kidneys (*28*), and subsequently described synthetic substances such as lysophosphatidyl ethanolamine analogues (*29*): Both are capable of lowering the blood pressure of hypertensive rats. 3) A chemically modified lipid from the renal medulla known as antihypertensive polar renomedullary lipid (APRL): APRL is an extraordinarily powerful vasodilator; its effect begins immediately after injection and lasts for 30–60 min (*23*). APRL was characterized as a mixture of alkyl ether analogues of phosphatidyl choline (*23*). 4) A presumably neutral lipid present in the renal medulla that was given the name antihypertensive neutral renomedullary lipid (ANRL) by analogy with the polar lipid (*23*). Its activity does not commence until a few minutes after injection, but may last for several hours (*23*).

On the other hand, mixtures or substances that raise blood pressure have been described as follows. 1) The previously mentioned work by Tigerstedt led to the isolation of renin (*30*), which, however, exerts no action on the blood vessels itself. 2) The blood pressure-raising peptide corticotensin was reported to be present in the pig renal cortex (*31*), but has not yet been isolated. 3) Renopressin is another protein that was found to be present in the pig renal cortex (*8*). The authors demonstrated that a preparation obtained from renin-free pig renal cortex caused a slow rise in blood pressure, and that this reaction was not antagonized by an angiotensin II blocker (*8*). 4) Several authors have claimed that urine contains a pressor substance (*32–34*). This substance is thought to be a protein with a molecular weight of 25,000 D. Upon long-term administration it causes hypertrophy of the suprarenal cortex. The blood pressure rises, as do plasma aldosterone and the urine K^+/Na^+ ratio, indicating a Na^+-retaining property of the compound (*35*, *36*). 5) The blood pressure-elevating activity of the protein nephrotensin has been identified in the venous effluate from ischemic kidneys (*37–39*). This protein has been assigned to be angiotensin I bound to α-2-globulin (*40*). However, the activity differs from the activity of angiotensin I and angiotensin II in rats (*38*).

In addition to the aforementioned five hypertensive entities there have been certain attempts to obtain pressor substances from the kidney, or to identify the activity of mixtures obtained from plasma originating from the kidney (*41–43*). However, none of these mixtures can possibly explain the extensive blood pressure rise observed in this study. All of the previously described substances are proteins, some of them with high molecular weights. Since the Sephadex LH-20 separation step employed in the present study excluded all particles above 2,000 amu, any sizeable peptide, including every endothelin, would have been eluted with the solvent front, which had been discarded.

In contrast, medullopressin was obtained using pure chloroform for extraction of the renal medulla. This corresponds to the method of lipid extraction and purification used by Bligh and Dyer (*44*), and does not yield extracts of intact proteins or peptides. The latter method starts with a monophasic mixture of homogenized tissue fluid, methanol, and chloroform, from which a biphasic mixture is produced by adding water and chloroform, so that the chloroform layer contains the lipids and the methanol–water layer contains all the other substances. Similarly, the chloroform extraction of the present study leads to isolation of the lipids and their separation from any proteins present. The method provides a reduced yield of lipids, whereas the extraction method by Bligh and Dyer (*44*) is specifically designed to optimize the total extractable lipid yield (w/w), regardless of biological activity. However, this was not the goal of the present study. In preliminary experiments, the yield of medullopressin activity when using the Bligh and Dyer method was not higher than that when using straight chloroform for extraction. Therefore, an exhaustive maceration in chloroform was chosen as the extraction method.

Also excluded from consideration as active pressor substances are the prostanoid substances present in extremely large amounts in the renal medulla (*12*). These prostaglandins isolated from the renal medulla, namely prostaglandin E_2 and A_2, are among the most potent known vasodilator agents, and therefore cannot account for the activity observed here. Additionally, they were ruled out by GC-MS. The only remaining potential candidate with known pressor activity is thromboxane A_2, an endogenous peroxide derived from arachidonic acid. Its blood pressure elevating property compares closely in extent and duration with the newly discovered agent, medullopressin. This applies especially to the rapid blood pressure rise that follows administration of thromboxane A_2 (*45*). However, because of its poor stability—thromboxane A_2 has a half life of only about 30 s at room temperature (*46*)—this prostaglandin is much too unstable to survive the chromatography, sample preparation and processing conditions of this study and, therefore, falls out of consideration.

Moreover, the method of lipid extraction that has been employed to isolate the prostaglandins from the renal medulla has to be considered in the discussion of potential candidates for the active principle (*26*). As Lee explained (*47*), the purpose of the extraction method is the exclusion of the neutral lipids, the preservation of the acidic prostaglandins, and their separation from the neutral lipids. The first step was to extract

the acidified aqueous homogenate of the renal medulla with methylene chloride. This extract was expected to contain both the acid and the neutral lipids. The methylene chloride phase was then extracted by shaking with phosphate buffer, the idea being that only the acid lipids would pass into the buffer, while the neutral lipids would remain behind. The phosphate buffer was then acidified and extracted again with methylene chloride; the organic phase now contained the activity, which later led to the discovery of the prostaglandins in the kidney (*26*). However, the original methylene chloride extract contained an activity that was later described by Muirhead (*22*) as the ANRL activity mentioned above. Because Muirhead's extract is a representative example of a prostaglandin-free extract, our extract was prepared in the same manner. If the present extract had contained any known prostanoid substances, an activity attributable to one of these substances would have been observed, in keeping with Lee's work. However, we did not observe any such activity. While the renal medulla has been shown to contain considerable amounts of prostaglandins, especially vasodilator prostaglandins, we have found no blood pressure-related activity attributable to these substances. In the case that prostaglandins had been present in the extract, it also seems very unlikely, from a physicochemical perspective, that any blood pressure-elevating prostaglandins or thromboxanes would have been present in the fraction due to their lipophilicity. Because these substances are quite non-polar organic molecules, they most likely would have been eluted with the solvent front (*i.e.*, the discarded "fraction 0") under the given chromatographic conditions, but certainly before fraction A, and not with fractions D or E, which represent the polar end of the gradient elution scheme. In summary, the results presented above demonstrate that porcine renal medulla contains a lipid, which we have designated medullopressin, that is a very potent blood pressure-raising agent. To the best of our knowledge, so far no comparable blood pressure-elevating substance from the kidney has been described in the literature.

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