Effect of plasma from patients with essential hypertension on vascular resistance in the isolated perfused rat kidney

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SUMMARY
1. Isolated perfused rat kidneys were used to study the effects of plasma fractions obtained by gel filtration from essential hypertensive patients \(n = 40\) and from normotensive subjects \(n = 36\) on resistance vessels. Perfusion pressure was recorded at a constant flow.

2. Plasma fractions were obtained by gel filtration and contained substances with a molecular mass in the range 1000–1500 Da. The plasma fractions from hypertensive patients used in this study had been shown to increase blood pressure after intravenous injection in rats.

3. In the isolated rat kidneys, the hypertensive fractions increased perfusion pressure by 20±17 mmHg (mean±so, range 5–58 mmHg, \(n = 40\)). The analogous fractions from normotensive subjects did not change perfusion pressure significantly.

4. In Ca\(^{2+}\)-free medium containing 2 mmol/l ethylene-glycol bis-(aminoethyl ether)tetra-acetate, the change in perfusion pressure induced by active plasma fractions was reduced by 95.2±6.3%. Addition of nifedipine to the perfusion medium reduced, but did not abolish, the pressure response of the kidneys.

5. In solutions containing phentolamine or saralasin, vasoconstriction was not reduced.

6. Thus in the active fractions from hypertensive plasma, a vasopressor agent with direct action on resistance vessels can be demonstrated. This substance probably acts by increasing Ca\(^{2+}\) influx in vascular smooth muscle cells.

Key words: essential hypertension, humoral factor, isolated perfused kidney.

Abbreviations: ANG II, angiotensin II; ATPase, adenosine triphosphatase; EGTA, ethylene glycol bis-(aminoethyl ether)tetra-acetate; NA, noradrenaline; SHR, spontaneously hypertensive rats; WKY, Wistar–Kyoto rats.

INTRODUCTION
The role of humoral factors in the pathogenesis of primary hypertension is currently being discussed. Early evidence in hypertensive rats stems from parabiosis experiments in Dahl salt-sensitive and salt-resistant rats [1–3] and in spontaneously hypertensive rats (SHR) [4]. More recently, the humoral transmission of hypertension from SHR to normotensive rats was demonstrated by parabiosis and cross-circulation experiments [5–8]. Humoral factors may also account for structural vascular changes usually found in hypertension. As Greenberg et al. [4] demonstrated by parabiosis experiments performed between SHR and normotensive Wistar–Kyoto rats (WKY), portal veins obtained from WKY after parabiosis showed similar increased contractility, decreased extensibility and hypertrophy to the portal veins from SHR in the hypertensive state.

Several groups have assessed the activity of a circulating factor sensitizing to pressor agents such as noradrenaline (NA) and angiotensin II (ANG II) in animal models of hypertension. A vasopressor potentiator to NA was found in the serum of salt-induced hypertensive rats [9] and in the serum of SHR [10]. A similar substance could be demonstrated in plasma of dogs with renovascular hypertension [11, 12], in hypertensive Dahl salt-sensitive rats [13] and in SHR [14]. In some experiments, the detected substances were shown to increase blood pressure in a nephrectomized normotensive rat [11–13].

A substance sensitizing to pressor agents has also been described in human hypertension. Such a substance has been detected in essential hypertension [15, 16], malignant hypertension [11] and renovascular forms of hypertension [17]. In essential hypertension, recent experiments indicated the presence of a yet unidentified
circulating vasopressor agent [18]. After intravenous injection of plasma fractions from essential hypertensive patients, the blood pressure of an anaesthetized normotensive rat increased significantly. By adding [3H]ANG II to a sample of plasma before gel filtration, it was demonstrated that this effect was not due to ANG II, because ANG II was not detected in the plasma fraction which elicited the maximum rise in blood pressure [18].

In this study we examined whether the pressure substance characterized by intravenous injection in the intact animal directly acts on resistance vessels of an isolated perfused rat kidney.

MATERIALS AND METHODS

Experiments in rats

The experiments were performed in normotensive WKY weighing 300–400 g which were anaesthetized with 1.4 g/kg body weight administered intraperitoneally. Mean arterial pressure was monitored through a plastic catheter inserted into the common carotid artery using a Statham element. The plasma fractions were administered intravenously through a central venous catheter inserted through the external jugular vein. To avoid major changes in blood volume, each sample injected had a volume of 0.5 ml. Not more than six fractions were tested in one rat. Between the injections a recovery period of 10 min was allowed. In the experiments with the isolated perfused kidney, the fractions used were those which had been found to increase blood pressure when injected intravenously in the rat, and the analogous normotensive fractions.

Preparation of the isolated perfused rat kidney

Four- to six-week-old normotensive WKY were anaesthetized with urethane (1.4 g/kg body weight, intraperitoneally). The abdominal cavity was opened by a midventral incision. The aorta and the left kidney were carefully isolated from adhesive tissue by blunt dissection. Ligatures were placed around the left renal artery and the infrarenal aorta. A polyethylene catheter was placed in the distal aorta. Immediately after the insertion of the catheter, 500 units of heparin sodium were injected. Then perfusion was started. The catheter was gently advanced into the left renal artery without interruption of flow. The kidney was excised and immediately mounted in the perfusion system.

Perfusion system

The perfusion procedure generally followed the description given by Hofbauer et al. [19]. Briefly, the isolated rat kidney was perfused by means of a peristaltic pump in a single-pass system with a solution containing 115 mmol/l NaCl, 4.6 mmol/l KCl, 1 mmol/l CaCl2, 1.2 mmol/l MgSO4, 1.2 mmol/l NaH2PO4, 22 mmol/l NaHCO3, 49 mmol/l glucose and 35 g of gelatine/l (Haemaccel; Behringwerke, Marburg, F.R.G.), and equilibrated with 95% O2/5% CO2. The perfusion medium and the kidney were kept at 37°C. Perfusion flow was constant at 8–9 ml/min. Perfusion pressure was continuously monitored by a transducer (Gould P 23) connected to a bridge amplifier (Hugo Sachs, Freiburg, F.R.G.). After an equilibration period of 30 min, plasma fractions and vasoactive hormones, e.g. ANG II, were injected in bolus injections of 0.1 ml. Only plasma fractions from single patients were tested. ANG II was injected in concentrations of 0.5–10 ng/ml. In four experiments, isolated perfused kidneys were used after administration of 1 mg of reserpine 18 h previously. Tyramine (10−5 g) was injected to control the effect of chemical denervation.

Preparation of plasma fractions

Forty plasma samples from essential hypertensive patients (age 44.8±25.3 mean±SD, 24 males, 16 females) and 36 samples from normotensive subjects (age 41.2±22.6 mean±SD, 20 males, 16 females) were used for the experiments. In the isolated perfused kidney, samples equivalent to 60 ml of plasma were tested. In the hypertensive patients diagnosis of essential hypertension was established after exclusion of secondary forms of hypertension by the appropriate clinical, radiological and laboratory examinations (digital subtraction angiography of renal arteries, 24 h urinary catecholamines and cortisol, plasma aldosterone and renin activity). The patients studied had not received antihypertensive drugs previously. Their blood pressure (systolic/diastolic) was 187±17/121±10 mmHg (mean±SD). The blood pressure of the normotensive subject was 124±13/84±9 mmHg.

The samples were prepared as described by Zidek et al. [20]. Briefly, plasma samples were first precipitated with methanol. The supernatant was dialysed for 24 h to remove substances with a molecular mass greater than 3500 Da, using dialysis tubes (Spectra Por 6 MWCO 3500; Fa. Reichelt, Münster, F.R.G.). Thereafter salt was removed by ultrafiltration with membranes permeable to substances with a molecular mass lower than 500 Da (Amicon YC, Amicon, Witten, F.R.G.). Remaining substances were separated roughly according to their molecular mass by gel chromatography using Biogel P2. Several peaks could be identified by u.v. spectrometry at 280 nm. U.v. indicated that several fractions were formed from the eluate. These fractions were then concentrated to 0.5 ml by freeze-drying. Furthermore, the Na+ and K+ content, pH and osmolality of the final samples were measured in order to avoid unspecific effects on blood pressure or perfusion pressure due to deviations of Na+ and K+ content and osmolality from the physiological range. Osmolality was maintained between 280 and 320 mosmol/l with Na+ concentration of 100–120 mmol/l and K+ concentration below 6 mmol/l. pH was between 7.55 and 7.2. The fractions were administered to a normotensive rat as described previously [18]. The fraction which induced the maximum rise in blood pres-
sion and the analogous fraction from normotensive blood were used for further experiments in the isolated perfused rat kidney. In 10 experiments, nifedipine was added to the perfusion medium ($10^{-8}$ mol/l, $n = 5$; $10^{-7}$ mol/l, $n = 5$). In five experiments the active fraction from hypertensive plasma was incubated with trypsin (2 units/ml, bovine pancreas, type XIII, Sigma) for 12 h at 25°C. Thereafter the enzyme was separated from the compounds of the plasma fraction by ultrafiltration of substances <10000 Da with Centricon tubes (Amicon), and the trypsin-treated fraction was tested in the isolated perfused kidney together with an untreated sample. In 12 experiments, saralasin was present in concentrations of $10^{-7}$, $10^{-6}$ and $10^{-5}$ mol/l, and in 11 other experiments, the perfusion medium contained $10^{-7}$, $10^{-6}$ and $10^{-5}$ mol/l phentolamine. Four experiments were performed in Ca$^{2+}$-free solution containing 2 mmol/l ethyleneglycol bis-(aminoethyl ether)tetra-acetate (EGTA). To test the effect of adenosine triphosphatase (ATPase) inhibition, ouabain was added to the perfusion medium in concentrations of 0.1, 0.5 and 1 mmol/l.

For statistical evaluation of the data, Student's $t$-test for paired data and Wilcoxon's test were used; $P$ values of less than 0.05 were considered to be significant.

Measurement of Na$^+$/K$^+$ ATPase activity

Na$^+$/K$^+$-ATPase inhibition was measured by the procedure of Hamlyn et al. [21]. Principally, in this assay the regeneration of enzymatically hydrolysed adenosine 5'-triphosphate is coupled to the oxidation of nicotinamide–adenine dinucleotide (reduced), so that the rate of adenosine 5'-triphosphate turnover can be monitored by recording the absorbance of nicotinamide–adenine di-nucleotide (reduced) at 340 nm. Inhibition of Na$^+$/K$^+$-ATPase activity after addition of a plasma fraction was expressed as the percentage of the enzyme activity obtained after addition of saline (150 mmol/l NaCl). Na$^+$/K$^+$-ATPase from canine kidney was obtained from Sigma and had an activity of 5–6 mmol/l min$^{-1}$ mg$^{-1}$ of protein. The ouabain-insensitive Mg$^{2+}$ ATPase activity was less than 2% of the total ATPase activity.

RESULTS

Experiments in rats

Fig. 1 shows the effect of intravenous injection of plasma fractions obtained by gel chromatography on the mean arterial pressure of a normotensive rat. One fraction of hypertensive plasma containing substances of 1–1.5 kDa caused a pronounced increase in blood pressure, whereas the analogous normotensive fraction elicited either no increase or a modest increase in blood pressure (Fig. 1). The increase in blood pressure after intravenous injection of an active hypertensive plasma fraction lasted about 10–15 min. Maximum responses were obtained after administration of fractions equivalent to 80 ml of plasma. By this procedure the hypertensive plasma fractions used for experiments in the isolated perfused kidney were shown to be active.

Experiments with the isolated perfused kidney

Properties of the renal vasculature. The baseline perfusion pressure of the isolated perfused rat kidneys decreased by 15–20 mmHg during the first and by 10 mmHg during the second hour of perfusion. Vascular reactivity to vasoactive agents did not diminish during this time. After the equilibration period, the baseline pressure was 85–107 mmHg. Addition of nifedipine caused a further mean decline in perfusion pressure by 15 ± 10 mmHg, whereas the addition of phentolamine did not.

![Fig. 1. Mean arterial pressure (MAP) after intravenous injection of hypertensive (HF) and normotensive (NF) plasma fractions.](image)

![Fig. 2. Changes in perfusion pressure in the isolated perfused kidney at constant flow after administration of various doses of ANG II and NA to the isolated perfused kidney.](image)

<table>
<thead>
<tr>
<th>Concentration (mol/l)</th>
<th>ANG II</th>
<th>NA</th>
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<tbody>
<tr>
<td>$10^{-14}$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$10^{-13}$</td>
<td>3 ± 1</td>
<td>0</td>
</tr>
<tr>
<td>$10^{-12}$</td>
<td>19 ± 4</td>
<td>0</td>
</tr>
<tr>
<td>$10^{-11}$</td>
<td>44 ± 9</td>
<td>1</td>
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<tr>
<td>$10^{-10}$</td>
<td>65 ± 15</td>
<td>9 ± 3</td>
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<tr>
<td>$10^{-9}$</td>
<td>71 ± 17</td>
<td>38 ± 8</td>
</tr>
<tr>
<td>$10^{-8}$</td>
<td>73 ± 17</td>
<td>173 ± 24</td>
</tr>
<tr>
<td>$10^{-7}$</td>
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<td>193 ± 27</td>
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significantly change it. Saralasin induced a rise in perfusion pressure of 13±2 mmHg. Perfusion of the kidneys with a Ca\(^{2+}\)-free, EGTA-containing solution caused a decrease in perfusion pressure by 19±4 mmHg. Table 1 shows the responses of the isolated perfused kidney to ANG II and NA. The increase in perfusion pressure induced by 80 mmol/KCl could be prevented by the addition of nifedipine (10\(^{-7}\) mol/l).

**Effects of plasma fractions.** When plasma fractions from essential hypertensive patients which had been shown to induce a blood pressure rise in the intact rat were injected into the perfusion system, a transient increase in perfusion pressure of 20±17 mmHg (range 5–58 mmHg, \(n=40\), \(P<0.0001\), Fig. 2) was induced. Maximum contraction was reached after about 1 min. Subsequently, the perfusion pressure declined to baseline values after 4–22 min. Fig. 3 shows the effect of various concentrations of the active fraction on perfusion pressure. The changes in perfusion pressure were dependent on the concentration of the active fraction. Tachyphylaxis was not observed during repetitive applications of this fraction. Heating the fractions to 100°C for 10 min did not affect the vasoconstrictor activity. The analogous normotensive fraction caused an increase in perfusion pressure of 3±3 mmHg, which is significantly less than the response to the hypertensive fraction (\(P<0.01\)). For comparison, the effects of NA and ANG II in various concentrations are shown in Table 1. In 23 of 40 samples, equal amounts of the active plasma fractions were tested in both the isolated perfused kidney and in the intact rat. There was a correlation coefficient of \(r=0.79\) between the effects in both preparations (Fig. 4).

Fig. 5 shows the profile of the response to the fractions obtained by chromatography in relation to the chromatographic pattern. Fig. 5(a) displays the changes in perfusion pressure induced by the fractions labelled on the abscissa. In Fig. 5(b) the absorbance at 280 nm and the conductivity are shown. Active fractions from hypertensive plasma pretreated with trypsin showed similar effects to the untreated fractions, with a mean increase in perfusion pressure of 87.4±12.6% of the untreated

![Fig. 3. Changes in perfusion pressure in the isolated perfused kidney at constant flow after administration of 0.1 ml of an active plasma fraction from two essential hypertensive patients (HF\(_1\), HF\(_2\)) in various concentrations equivalent to 30, 3 and 0.3 ml of plasma, of saline (K) and of ANG II in various amounts.](image)

![Fig. 4. Correlation between changes in perfusion pressure in the isolated perfused kidney and changes in mean arterial pressure (MAP) in the intact rat after administration of active hypertensive fractions from the same patient.](image)

![Fig. 5. Relation of changes in perfusion pressure (a) in the isolated perfused kidney after injection of plasma fractions, which are labelled on the abscissa, to chromatographic patterns (b) of absorbance at 280 nm and conductivity.](image)
Effect of hypertensive plasma on vascular resistance

**Fig. 6.** Changes in perfusion pressure in the isolated kidney at constant flow after administration of samples of 0.1 ml of an active plasma fraction from essential hypertensive patients (HF) before and after addition of nifedipine (10^{-6} and 10^{-7} mol/l) to the perfusion medium, and in Ca^{2+}-free medium containing 2 mmol/l EGTA. For comparison, the effect of ANG II (1 ng) on the perfusion pressure of the isolated perfused kidney is shown.

**Fig. 7.** Changes in perfusion pressure in the isolated perfused kidney at constant flow after administration of samples of 0.1 ml of an active plasma fraction from essential hypertensive patients (HF) before and after addition of 10^{-6} mol/l phenotolamine to the perfusion medium.

control sample. The active plasma fraction did not inhibit Na^{+}-K^{+}-ATPase activity.

**Effects of drugs on the response to the hypertensive factor.** Addition of nifedipine in concentrations of 10^{-6} mol/l and 10^{-7} mol/l to the perfusion medium reduced the response of the kidney to the hypertensive fractions by 35.3 ± 12.4% and 66.5 ± 18.2% (P<0.05), respectively (Fig. 6). When Ca^{2+} in the perfusion medium was replaced by 2 mmol/l EGTA, the response to active plasma fractions was reduced by 95.2 ± 6.3% (P<0.05; Fig. 3). On perfusion with a solution containing phenotolamine (10^{-6} mol/l), the response of the kidney to active fractions was not significantly changed (Fig. 7). The same results were obtained with phenotolamine concentrations of 10^{-7} and 10^{-5} mol/l. The presence of saralasin (10^{-6} mol/l) in the perfusion medium augmented the response of the kidney to hypertensive plasma fractions of 70.3 ± 53.9% (P<0.05, Fig. 8). At lower concentrations of saralasin (10^{-8} and 10^{-7} mol/l) this effect was not significant (8.3 ± 117.5% and 41.3 ± 46.9%, respectively). Pretreatment with reserpine did not abolish the response to the active hypertensive plasma fraction (Fig. 9). Perfusion with ouabain caused a transient increase in perfusion pressure. After an initial increase, the perfusion pressure returned to baseline values within 20 min. The maximum increase in the perfusion pressure was 4 ± 1, 19 ± 7 and 42 ± 15 mmHg with 0.1, 0.5 and 1 mmol/l ouabain, respectively (Fig. 10). There was no significant correlation between vasopressor activities of the hypertensive plasma samples and casual blood pressure of the patients (r = 0.24).

**DISCUSSION**

In a previous study, a factor circulating in the blood of essential hypertensive patients which caused an increase in blood pressure of normotensive rats after intravenous injection was demonstrated [18]. There are several possibilities for the underlying mechanisms of the rise in blood pressure observed: first, a direct vasopressor action of the substance on vascular smooth muscle; secondly, indirect mechanisms such as activation of other hormones or of the sympathetic nervous system; and thirdly, positive inotropic effects and an increased cardiac output might be responsible for the pressor activity of the factor.

In this study, the isolated perfused rat kidney of a normotensive rat was used as a model to examine the effects of the aforementioned plasma fractions on vascular smooth muscle directly. This model offered the
same fraction from the plasma of essential hypertensive studied. The results obtained in this study show that the main responsible for the vascular resistance could be animal, induced a significant rise in vascular resistance of concentrations of activators of a-adrenoceptors or ANG 1

advantage that the small peripheral arteries which are mainly responsible for the vascular resistance could be studied. The results obtained in this study show that the same fraction from the plasma of essential hypertensive patients which increased blood pressure in the intact animal, induced a significant rise in vascular resistance of the isolated perfused rat kidney. Whether additional positive inotropic effects or an activation of the sympathetic nervous system might contribute to the rise in blood pressure induced by the substance in the intact animal cannot be deduced from the results of this study. The effects of the hypertensive plasma fractions on the perfused rat kidney cannot be attributed to enhanced concentrations of activators of α-adrenoceptors or ANG II in these fractions, since the α-adrenoceptor blocker, phentolamine, or saralasin, a competitive antagonist of ANG II, did not reduce constriction of the perfused renal vasculature. Furthermore, the normotensive fractions analogous to the active hypertensive fractions did not exhibit vasopressor activity. Therefore in normotensive plasma, the agent either may not be present or may only be present in markedly reduced concentrations compared with hypertensive plasma.

The vasopressor action of the substance was shown to depend on extracellular Ca++. Among other factors, cytoplasmic free Ca++ is known to regulate the contractile activity of vascular smooth muscle [22]. Principally, an elevation in cytoplasmic Ca++ concentration can result from either a release of Ca++ from the endoplasmic reticulum and other cellular Ca++ stores or a Ca++ influx from the extracellular fluid, or from both. Since the vasopressor effect of the substance was almost abolished by Ca++-free, EGTA-containing solutions, its action seems to be mediated mainly by Ca++ influx. As the vasopressor action of the factor is not abolished, but only reduced, by addition of nifedipine to the perfusion medium, it is possible that only pressure-dependent vasoconstriction [23, 24], which may be superimposed on the action of the hypertensive factor, but not the direct effect of the factor was blocked by nifedipine. Thus a role of the nifedipine-sensitive, voltage-dependent Ca++ channels for the effect of the hypertensive factor cannot be assumed definitely. Instead, the hypertensive factor may act by opening receptor-operated or non-specific Ca++ channels.

In essential hypertension, elevated levels of intracellular Ca++ have been detected [25–28]. This may be due either to an inherited defect of intracellular Ca++ metabolism or to the action of a hormone. Several groups have presented evidence for substances in the blood of essential hypertensive patients affecting Ca++ metabolism. Lindner et al. [29] demonstrated an increase in cytosolic Ca++ in platelets from normotensive subjects when they were incubated with plasma ultrafiltrates from hypertensive subjects. Zidek et al. [6] found an increased intracellular Ca++ concentration in porcine aortic smooth muscle cells incubated in hypertensive plasma compared with those incubated in normotensive plasma. If in plasma from essential hypertensive patients, a substance was present inducing an enhanced intracellular Ca++ concentration in arteriolar smooth muscle cells, this might result in the increased vascular resistance which is the hallmark of essential hypertension.

It was postulated that the observed increase in intracellular Ca++ concentration found in essential hypertension results from an increased intracellular Na+ concentration by stimulation of Na+-Ca++ exchange [30, 31]. According to de Wardener and co-workers [32, 33], the increased intracellular Na+ concentration is caused by the so-called natriuretic hormone inhibiting the activity of Na+-K+-ATPase in a similar manner to the action of ouabain. Whether the vasoconstrictor demonstrated in this study shows any relationship to the natriuretic hormone is not clear. However, preliminary results showed that substances inhibiting Na+–K+–ATPase were
separated from the hypertensive factor by gel chromatography. Furthermore, direct vasopressor actions of the natriuretic hormone have not yet been shown.

The experiments with ouabain showed that in the isolated perfused rat kidney inhibition of Na\(^{+}-K^{+}\)-ATPase induced vasoconstriction. However, it is known that the vasoconstriction induced by higher concentrations of ouabain, e.g. mol/l, is partly due to release of Na\(^{+}\), it is likely that it acts by stimulation of Ca\(^{2+}\) influx from the extracellular space.

In summary, a substance with direct vasopressor activity was found in plasma from essential hypertensive patients. It was demonstrated that it probably does not act as an \(\alpha\)-adrenoceptor agonist or as an activator of ANG II receptors. Since its action depends on extracellular Ca\(^{2+}\), it is likely that it acts by stimulation of Ca\(^{2+}\) influx from the extracellular space.

REFERENCES