Activation of the Humoral Antihypertensive System of the Kidney Increases Diuresis

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SUMMARY Isolated kidneys taken from normotensive Wistar-Kyoto rats were cross-perfused extracorporeally by normotensive strain-matched donor rats. The extracorporeal perfusion circuit was arranged so that the perfusion pressure to the normotensive recipient kidney could be varied from 90 to 200 mm Hg without any change in total flow through this circuit. This setup avoided hemodynamic or mechanical interferences with reflexogenic circulatory control in the normotensive donor rat when the recipient kidney was manipulated. Diuresis and natriuresis were measured in the normotensive donor rat and the normotensive recipient kidney. A few minutes after normotensive recipient kidney perfusion pressure had been raised, mean arterial pressure (MAP) and heart rate started to decline rapidly in the normotensive donor rat, and circulatory collapse ensued within 15 to 100 minutes. During the control period at 90 mm Hg normotensive recipient kidney perfusion pressure, urinary flow, MAP and heart rate were stable in the normotensive donor rat and the normotensive recipient kidney. When perfusion pressure was raised to 200 mm Hg in the recipient kidney, the urinary flow in the donor rat increased 62% on average in the first 10 minutes over values recorded before the pressure rise (p<0.05) while MAP simultaneously fell by 16% and HR remained unchanged. During the subsequent period, the urinary flow of the donor rat declined together with MAP and heart rate. In the extracorporeally high-pressure perfused recipient kidneys, an eightfold to ninefold increase in diuresis and natriuresis occurred during the first 45 minutes. In conclusion, the present study lends further support to the theory that the humoral renal antihypertensive system is involved in ordinary blood pressure regulation and in the control of kidney excretory function as a possible counterpart to the renocortical renin-angiotensin system. (Hypertension 11: 597–601, 1988)

Key Words • rats • renomedullary antihypertensive system • Wistar-Kyoto rats • diuresis • natriuresis • kidney function • perfusion • renomedullary lipids • pressure-related natriuresis

During the last decade, results have accumulated showing that the medulla of the kidney harbors an important humoral antihypertensive mechanism that also may be involved in normal blood pressure regulation.1–3 Many positive feedback loops have been identified in primary (essential) hypertension that tend to increase pressure,4 and when combined, they would seem capable of raising blood pressure out of control quite rapidly. It is therefore, in a way, more surprising that some 90% of the population manages to stay normotensive than that some 10% become hypertensive. Furthermore, once primary hypertension appears, it usually stays at a moderately raised level for decades, indicating that there actually are mechanisms counteracting high pressure in a situation where most ordinary barostats seem to be fully reset.4 The antihypertensive renomedullary lipids, initially suggested and described by Muirhead and his group,1,2,5,6 may well be such a durable negative feedback mechanism, though knowledge about the physiological role of this system is only fragmentary.

Previous studies in our laboratory have shown that the rapid decrease in blood pressure after unclipping two-kidney, one clip renal hypertensive rats is associated with reductions of both heart rate (HR) and efferent renal sympathetic nerve activity.7,8 Furthermore, a
differentiated hemodynamic pattern is seen in the different systemic circuits upon unclipping; the splanchnic and renal vascular beds in particular show a markedly reduced vascular resistance while cardiac output increases modestly despite some bradycardia.9

Immediately after unclipping a renal hypertensive rat kidney that is cross-perfused from a normotensive rat, the renal venous effluent induces a marked blood pressure fall associated with bradycardia and reduced effluent renal sympathetic nerve activity in this normotensive donor rat.10,11 The same response is seen after intravenous injection of the antihypertensive neutral renomedullary lipid (recently renamed medullipin I) of Muirhead et al.12 to normotensive rats.

In a recent study, we examined whether this renal humoral antihypertensive mechanism was active within the physiological pressure range by using an isolated kidney from a normotensive rat that was cross-perfused from a normotensive donor rat and in which the perfusion pressure to the isolated kidney could be altered over a wide range without changing the extracorporeal flow for the donor rat.13 We found that the renal depressor system seems to be closely dependent on the renal perfusion pressure, showing pressure-dependent reductions of both mean arterial pressure (MAP) and HR in the donor when perfusion pressure to the isolated kidney was increased from 80 to 100 and then to 150 mm Hg. "Threshold" depressor responses occurred in the 90 to 100 mm Hg pressure range. These results are in good agreement with earlier findings by Muirhead et al.14 noting how minor amounts of antihypertensive neutral renomedullary lipid (medullipin I) also seemed to be present in the renal venous effluent in normotensive rat kidneys, while far higher amounts were found after renal unclipping in renal hypertensive rats. It is therefore increasingly likely that this system is an important physiological counterpart to the renin-angiotensin system, as suggested by Muirhead.1 Prostaglandins and the renal kallikrein-kinin system also have been suggested to be involved in renal regulation of blood pressure. Both Muirhead's and Swales' groups15-17 have, however, clearly shown that the fall in blood pressure after unclipping is independent of these systems.

The direct aim of this study was to further explore how normotensive kidneys of Wistar-Kyoto rats (WKY) can release this blood pressure reducing factor and whether the mechanism per se induces any changes in kidney function.

Materials and Methods

Normotensive male WKY weighing about 250 g (n = 18; Møllegaards Breeding Center, Denmark) were used for cross-circulation experiments, and strain-matched rats weighing over 400 g were used as blood donors to the perfusion circuit.

Two 250-g WKY were anesthetized with methohexitol (75 mg/kg i.p.), and the tail artery was cannulated. After recovery from anesthesia, MAP and HR were recorded in the awake rats. Thereafter, one of the rats was once again anesthetized with methohexitol (7 mg of 0.1% solution i.a.), tracheotomized, and cannulated with polyethylene catheters in the left carotid artery (PE-90) and right jugular vein (3 PE-50 catheters). An initial dose of chloralose (7.5–10 mg i.a.) was given followed by a continuous chloralose infusion (8 mg in 3.2 ml of physiological saline solution per hour). The urinary bladder was cannulated with a PE-160 cannula through a midline abdominal incision, and the rat was placed on a Plexiglas table with a slit for the bladder catheter. This normotensive donor rat (NDR) was used as blood flow donor to the isolated cross-perfused kidney and also served as a bioassay indicator of the hemodynamic and renal effects induced by the humorally mediated antihypertensive activity of the cross-perfused kidney.

The other rat was anesthetized with pentobarbital (50 mg/kg i.p. or i.a.) after awake MAP and HR recordings. Through an abdominal incision, the abdominal aorta and the left renal artery were cautiously isolated and the corresponding ureter was cannulated with a PE-10 cannula while the renal capsule was left intact. A PE-90 cannula, later used for cross-perfusion of this normotensive recipient kidney (NRK), was inserted into the lower aorta, with the tip placed just caudal to the renal artery.

Perfusion Arrangement

The NDR and NRK were joined by a perfusion circuit from the left carotid artery containing, in series, a constant flow pump and a variable Windkessel device. After them, the NRK and a pressure-sensitive shunting valve followed in parallel (all 3 devices were constructed in our department). The blood from the shunting valve was returned through one of the three catheters in the right jugular vein, while the renal venous effluent of the NRK circuit was returned through one of the other catheters. The parallel-coupled shunting valve made it possible to keep total flow through the circuit constant throughout (3–5 ml/min) and still vary the perfusion pressure to NRK over a wide range. Thus, any alterations in hemodynamics and mechanical interferences with normal reflexogenic circulatory control in NDR due to the extracorporeal perfusion circuit per se could be avoided. The cross-perfusion of NRK was started without any episode of renal ischemia as the proximal aorta was obstructed first when flow through the extracorporeal circuit was started. Then, the recipient rat was killed and the left kidney was left in situ, being perfused from NDR and thermostatically kept at 38°C, as was the NDR itself. A pressure recording cannula for NRK was introduced into the proximal aorta at the diaphragmatic level of the recipient rat with the tip placed just caudal to the renal artery.

Recordings

MAP, HR, and pulse pressure in NDR were recorded continuously through the tail artery, and central venous pressure was recorded through the third cath-
eter in the right jugular vein using a Microswitch pressure transducer (Model 163PC01D36, Honeywell Microswitch, Freeport, IL, USA). Urinary flows in NRK and NDR were collected and continuously assessed using Grass force-displacement transducers (Model FT03D, Grass, Quincy, MA, USA). All recordings were performed on two Grass Model 7B polygraphs. The sodium content of urinary samples was measured on a flame spectrophotometer (Model FLM3, Radiometer, Copenhagen, Denmark). Hemoglobin, arterial bicarbonate, and O₂ and CO₂ tensions were analyzed every 30 minutes in NDR in a 160-μl blood sample drawn from the left carotid artery using an Osm 2 hemoxometer and an ABL 30 acid-base analyzer (both from Radiometer). The small blood loss was immediately replaced by the same amount of stored, heparinized blood. For the cross-perfused NRK, arterial perfusion pressure, urinary flow, and urinary sodium content were measured using the same arrangements as described for NDR.

Experimental Protocol

Control experiments were performed first for 3 hours using the extracorporeal circuit arrangements and flow rate as just described but without any NRK included in the circuit. In the main series, a cross-perfused NRK was included and, after 30 to 60 minutes of equilibration, the experiment started with a 30-minute recording period, during which the NRK perfusion pressure was kept at 90 mm Hg while urine samples were taken from the NRK and NDR kidneys. Then, the shunting valve was changed to give a 200 mm Hg NRK perfusion pressure to produce a drastic release of depressor material to reveal whether it also had direct diuretic effects. As in the control experiments, urine was then collected for 30 minutes in NDR and every 15 minutes in NRK. Hemodynamic and metabolic events were followed as already described.

Calculated and Statistics

MAP and HR in NDR, which were plotted every 5 minutes, were normalized as 100% at the beginning of each experiment. The area below the curve was thereafter calculated for each experiment from the start of the 200 mm Hg perfusion pressure period until circulatory collapse or until the experiment was finished at 180 minutes. The control rats were observed for 180 minutes, and the area below these curves was also calculated. All results are presented as means ± SE. Means were compared using one-way analysis of variance; in the case of repeated urinary analyses, the analysis of variance was specially adapted for repeated measurements. Duncan’s test was used as a test of statistical significance. A p level below 0.05 was considered statistically significant.

Results

Awake MAP and HR were 110 ± 8 mm Hg and 358 ± 9 beats/min for NDR (n = 9) and 114 ± 9 mm Hg and 367 ± 10 beats/min, respectively, for the animals from which the NRK was taken (n = 9). MAP and HR of NDR remained stable for 3 hours of extracorporeal perfusion when no kidney was inserted in the circuit. In the experiments in which NRK were included, MAP and HR remained constant during the equilibrium period and the subsequent 30-minute period at 90 mm Hg NRK perfusion pressure. Earlier pilot experiments at 80 mm Hg NRK perfusion pressure showed stable hemodynamic conditions for at least 2 hours.

One or 2 minutes after NRK perfusion pressure had been raised to 200 mm Hg, MAP in NDR started to decline rapidly, and after a while bradycardia ensued. No signs of reflex tachycardia were seen even when severe hypotension ensued. In fact, at this high NRK perfusion pressure all NDR died in hypotensive circulatory collapse in 15 to 100 minutes. Pressure and HR were plotted as described in Materials and Methods. The areas below the curves for the first 180 minutes or until circulatory collapse were compared and showed a highly significant decrease for both MAP and HR at 200 mm Hg NRK perfusion pressure when compared with the control experiments (3440 ± 647 mm Hg × min and 5003 ± 932 beats × min vs 19,676 ± 533 mm Hg × min and 19,803 ± 431 beats × min; p < 0.001).

During the period of 90 mm Hg NRK, perfusion pressure urinary flow in NDR was 5.90 ± 1.08 μl/min while MAP and HR were 113 ± 3 mm Hg and 434 ± 4 beats/min. When the NRK perfusion pressure was raised to 200 mm Hg, the urinary flow in NDR increased to a peak of 13.5 ± 4.0 μl/min at 5 minutes and to 9.55 ± 3.00 μl/min (+ 62%) as an average over the first 10 minutes (p < 0.05) while MAP simultaneously fell to 95 ± 7 mm Hg (- 16%) and HR remained unaltered at 438 ± 8 beats/min. During the subsequent period urinary flow declined more or less in parallel with MAP. When the whole 30-minute period of 200 mm Hg NRK perfusion pressure was used for calculation of average values, MAP was as low as 72 ± 13 mm Hg (- 37%) and HR was 371 ± 49 beats/min while mean urinary flow was 4.13 ± 1.49 μl/min, being thus decreased about 30% compared with control, but at a greatly reduced average MAP. The sequence of events is shown in Figure 1. Central venous pressure remained about the same in the control experiments and in the NRK experiments during the 90 mm Hg NRK perfusion, but when MAP in NDR fell during the 200 mm Hg period central venous pressure also fell in NDR.

The sodium excretion in NDR largely followed the diuresis, being 0.385 ± 0.112 μmol/min at the 90 mm Hg NRK perfusion pressure. Unfortunately, however, the urine volume during the first 10 minutes of increased diuresis (as described) was too small for reliable sodium estimation with our technique. Therefore, only the mean sodium excretion for the entire 30 minutes of 200 mm Hg NRK perfusion pressure is available (0.204 ± 0.113 μmol/min), during which period average MAP was reduced to about 70 mm Hg. If the sodium concentration of this portion is used to calculate sodium excretion during the first 10 minutes of
FIGURE 1. The upper two panels (-----) show mean values ± SE of the perfusion pressure and diuresis in extracorporeally cross-perfused isolated normotensive recipient kidneys (NRK; n = 9). The lower three panels (——) show mean values ± SE for the MAP, diuresis, and HR of the bioassay normotensive donor rats (NDR; n = 9). Note that when kidney perfusion pressure suddenly was increased to 200 mm Hg, MAP in the NDR immediately started to decline. A significantly increased diuresis (p < 0.05) was seen in NDR during the first 10 minutes in spite of the fall in blood pressure, while the HR was still largely unaltered (see text for further details).

Discussion

The present results confirm that a humorally mediated blood pressure–reducing mechanism can be activated in normotensive rat kidneys. It also indicates that the renal excretory function per se may be altered by the released renal depressor agents. The experimental situation was rigorously controlled: The NDR were under controlled light anesthesia, with maintenance of acid-base balance, fluid status, body temperature, and blood volume, and changes of pressure and flow to the cross-perfused kidney were made without any alterations of the extracorporeal flow volume. Therefore, the initial changes in renal function and the increased urine production in NDR must be related to the presence of humoral renal depressor agents in the venous effluent from the cross-circulated NRK. If anything, the 15% MAP reduction during the initial 10-minute period ought to have reduced urine production, if no other influence had affected kidney function. It cannot, however, be settled whether the 60% average increase of diuresis during this 10-minute period in the NDR kidneys was due to a direct humoral action on the kidneys or if it was mainly secondary to, for example, the reduced efferent sympathetic nerve activity to the kidneys that is known to be elicited by the renal depressor agent.7-8-•12 However, from these latter studies it is also known that HR is closely related to the efferent renal sympathetic activity. Hence, the finding that HR remained largely the same (434 ± 4 and 438 ± 8 beats/min) during the first 10 minutes after increasing the NRK pressure to 200 mm Hg speaks against a reduced sympathetic nerve activity as the only explanation of the increased diuresis during this early period.

Sevenfold to eightfold increases in diuresis and natriuresis occurred in the isolated, high-pressure perfused (200 mm Hg) kidneys, showing a clear, rapid onset and then a continuous successive rise during the next 30 minutes of constant high-pressure perfusion (see Figure 1). This finding suggests that the increased diuresis may not altogether be a consequence of the increased renal perfusion pressure per se. It may, at least to some extent, be connected to more direct effects of the humoral renal antihypertensive agents on, for example, glomerular or tubular functions (or both), resulting in increased diuresis and natriuresis.

Actually, the mechanisms behind the pressure-related diuresis and natriuresis are still not clear, as the processes do not seem to be fully explained by hydrostatic factors alone and they still function after complete inhibition of the renin-angiotensin and prostaglandin systems.18-19 It seems likely that some as yet unknown intrarenal messenger mechanism is involved in the regulation of glomerular filtration rate and tubular sodium-water absorption when renal perfusion pressure increased diuresis, it would range between 0.332 and 0.570 μmol/min, suggesting at least an unaltered natriuresis despite the MAP reduction (see Figure 1).

Hemoglobin, arterial O₂ tension, CO₂ tension, and bicarbonate concentration remained stable during the whole 3-hour period of the control experiments, and there were no changes in the NRK cross-circulation experiments until MAP declined to about 50 mm Hg, when metabolic acidosis developed rapidly.

Diuresis in NRK was stable at 90 mm Hg perfusion pressure (7.27 ± 4.01 μl/min), but when perfusion pressure was raised to 200 mm Hg, diuresis increased gradually, being 27.9 ± 8.51 μl/min after 15 minutes, 62.19 ± 17.96 μl/min after 30 minutes, and 62.57 ± 10.47 μl/min after 45 minutes (i.e., an eightfold to ninefold increase). Sodium excretion in NRK showed a marked pressure natriuresis, being 1.26 ± 0.76 μmol/min at 90 mm Hg and 3.96 ± 1.19, 8.87 ± 2.61, 8.55 ± 3.40 μmol/min at 200 mm Hg for the first, second, and third 15-minute period, respectively (about a sevenfold increase).
pressure is raised, where the humoral renomedullary system may come into focus. This is also in line with the finding that pressure-related natriuresis is markedly blunted when the renal medulla is selectively destroyed.30

Other interesting findings in this context are the results by Gerkens et al.21 that furosemide inhibited splanchnic neurogenic vasoconstriction in rats as long as the renal medulla was intact but failed to do so after selective chemical destruction of the renal medulla. These findings may indicate that the action of loop diuretics is related to the diuretic and natriuretic events seen in this study and thus be at least partly dependent on the renomedullary antihypertensive system.

In summary, this and our earlier study12 show that an isolated normotensive kidney, perfused extracorporeally by a normotensive strain-matched donor rat, releases humoral factors that lower blood pressure and heart rate in NDR when the perfusion pressure of the isolated kidney is increased above normal. This humoral activation, presumably involving the humoral renomedullary antihypertensive system described by Muirhead and Pitcock,1-2 was associated with an increased diuresis and at least unaltered natriuresis. It is not settled whether this is a direct humoral effect on the kidneys or mediated by the suppression of sympathetic activation, presumably involving the humoral renomedullary antihypertensive system described by Muirhead and Pitcock,1-2 may contribute to the process, in addition to the pressure rise per se. Thus, the present study gives further support to the opinion that the human renomedullary antihypertensive system and kidney function/Karlström et al. may also be involved in ordinary blood pressure regulation as a counterpart to the renocortical renin-angiotensin system, exerting suppressor effects on sympathetic activity, causing direct vascular relaxation, and on the kidney itself, promoting increased excretion of salt and water.

References
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