Sodium and lithium handling in the isolated hypertensive rat kidney

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SUMMARY

1. Isolated kidneys taken from spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY) were perfused over a range of perfusion pressures.

2. Lithium clearance was used as an index of proximal tubule sodium handling.

3. When the perfusate contained an oncotic agent (albumin, 6.7 g/dl) the SHR kidneys performed differently from the WKY kidneys with a reduction in inulin clearance, sodium excretion, fractional sodium excretion and fractional lithium excretion \[\text{at } 105 \text{ mmHg (14 kPa) perfusion pressure, SHR } 6.0 \pm 1.1\% \text{ vs WKY } 12.6 \pm 2.4\% \text{ (mean } \pm \text{SEM); at } 150 \text{ mmHg (20 kPa), SHR } 17.1 \pm 1.6\% \text{ vs WKY } 27.0 \pm 2.3\%\]. Calculated indices of distal tubular function showed no major differences between SHR and WKY.

4. When kidneys were perfused without oncotic agent in the perfusate the differences between SHR and WKY in tubular handling of sodium and lithium were largely abolished.

5. These findings are consistent with the hypothesis that increased sodium reabsorption occurs in the proximal tubules of the kidneys of SHR and suggest that this is an intrinsic property of the kidney, not immediately dependent on neural or humoral factors. Increased sodium reabsorption in the proximal tubule may contribute significantly to the existence of hypertension in the SHR.

Key words: hypertension, isolated perfused kidney, lithium, pressure-natriuresis.

Abbreviations: \(C_{\text{io}}\), inulin clearance; \(C_{\text{Li}}\), lithium clearance; FDSE, fractional distal sodium excretion; FDVE, fractional distal volume excretion; \(F_{E_{\text{K}}}\), fractional potassium excretion; \(F_{E_{\text{Li}}}\), fractional lithium excretion; \(F_{E_{\text{Na}}}\), fractional sodium excretion; GFR, glomerular filtration rate; SHR, spontaneously hypertensive rats; \(U_{\text{K}}V\), urinary potassium excretion; \(U_{\text{Na}}V\), urinary sodium excretion; WKY, Wistar-Kyoto rats.

INTRODUCTION

Guyton et al. [1] originally suggested the dominant role of the kidneys in the long-term regulation of arterial pressure and in hypertension. Their hypothesis proposed that whenever arterial pressure was elevated the pressure-natriuresis mechanism would increase urinary losses of sodium and water, causing reduction in blood volume and leading to a fall in arterial pressure. One implication of this hypothesis is that hypertension can only be sustained if renal function is altered so that the capacity of the kidney to carry out pressure-natriuresis is impaired. Many studies have demonstrated such an abnormality of renal function in experimental models of hypertension. In spontaneously hypertensive rats (SHR) [2, 3] and Dahl salt-sensitive rats (Dahl rats) [4] the ability of the kidney to excrete sodium is reduced at any given arterial pressure \(in vivo\). The isolated kidney taken from Dahl rats [5-7], SHR [8] and the unclipped kidney from two-kidney Goldblatt hypertensive rats [9, 10] also exhibit such an abnormality. Omvik et al. [11] demonstrated similar findings in hypertensive man.

The present study examines the abnormality in sodium handling in the SHR further by using the technique of lithium clearance \(C_{\text{Li}}\) estimation applied to the kidney perfused in isolation over a range of pressures and divorced from neural or humoral influences. Provided that animals are not sodium restricted, Kirchner [12] has confirmed the finding of Thomsen et al. [13] in anesthetized rats that lithium reabsorption is a good index of sodium handling in the proximal tubule. Filtration at the glomerulus and reabsorption of sodium from the nephron are dependent to some degree upon the oncotic pressure within the glomerular and peritubular capillaries [14,15]. To examine the possible role of altered oncotic forces in generating the tendency of SHR kidneys to retain sodium, experiments have been per-
formed both with and without oncotic agent in the perfusate.

METHODS

Animals

Studies were performed in male SHR aged 6–10 months and age-matched Wistar-Kyoto rats (WKY) as controls; all were purchased from Olac, Bicester, U.K., and weighed 320–410 g at the time of study. All were given free access to a standard rodent diet containing 16.7% (w/w) protein and 0.21% (w/w) sodium (91 mmol/kg) (Beekay Feeds, Hull, U.K.), and to tap water up to the time of experiment. The mean arterial pressure of such SHR determined in vivo in this laboratory [8] is considerably higher than that of WKY controls [16].

The intra-aortic pressures of the rats used in this study were measured under anaesthesia before cannulation of the renal artery: for SHR the mean pressure was 146.5±3 mmHg (19.5±0.4 kPa), for WKY 97±2 mmHg (12.9±0.3 kPa).

Isolated kidney perfusion

Rats were anaesthetized with pentobarbital (100 mg/kg body weight) given by intraperitoneal injection. The right kidney was perfused without ischaemia using the operative technique described by Nishitsutsuji-Uwo et al. [16]. The perfusion cannula introduced into the renal artery was double-barrelled: the perfusion medium passed through the outer cannula (external diameter, 1.20 mm; internal diameter, 1.04 mm) which was cut with a bevelled end to ease cannulation; the tip of the inner cannula (external diameter 0.41 mm, inner diameter 0.28 mm) lay in the plane of the bevel and was used to record perfusion pressure within the renal artery directly via a pressure transducer (PDCR 75; Ormed Limited, Herts., U.K.), preamplifier (4820, Ormed Limited) and chart recorder (MX 216, Ormed Limited). Prior experiments performed with an apparatus similar to that described by Loutzenhiser et al. [17] showed that the pressure recorded through the internal cannula was a valid measure of perfusion pressure over a range from 50 to 200 mmHg (6.7 to 26.7 kPa) with flows from 5 to 120 ml/min, values encompassing those encountered in these studies. The ureteric catheters used were similar to those of Schurek et al. [18] and consisted of 12 mm lengths of PP-10 tubing connected to 10 cm lengths of PP-50 tubing. These catheters could sustain a flow rate of 0.5 ml/min of water with a pressure drop of less than 10 mmHg (1.3 kPa) across them. Perfusate flow was recorded by an in-line flow meter proximal to the arterial cannula (L16X; Glass Precision Engineering Limited, Herts., U.K.). Perfusate temperature was controlled at 37°C. The perfusate was continually gassed with a pre-warmed and moistened gas mixture of 95% O2/5% CO2. Lithium chloride (100 μmol/l) was added to the perfusate.

Kidneys were perfused for a short period on open circuit to remove all traces of blood before being transferred to a recirculating unit with a capacity of approximately 120 ml. [14C]Inulin (Radioamersham, Bucks., U.K.) was then added to the perfusate to permit estimation of glomerular filtration rate (GFR) by inulin clearance (C\text{in}).

The left kidney was removed, decapsulated, blotted dry and weighed.

Group I: experiments with 6.7 g/dl albumin

In these experiments the perfusion medium consisted of 6.7% (w/v) dialysed bovine serum albumin fraction V (Miles Laboratories, Slough, U.K.) in Krebs–Henseleit buffer supplement with 5 mmol/l glucose and 20 amino acids in concentrations previously reported by Epstein et al. [19].

Immediately after recirculation of medium had been established, the renal artery perfusion pressure was adjusted to a baseline value of 90 mmHg (12 kPa) in the case of six WKY kidneys by means of a manually operated valve. In five SHR kidneys the baseline pressure was 105 mmHg (14 kPa). The pressure was maintained constant at these values for a 30 min period of stabilization followed by two 10 min baseline urine collection periods. The perfusion pressure was then increased by 15 mmHg (2 kPa) by rapid adjustment of the valve and maintained at the new level. After a 10 min washout period (adequate to clear the measured urinary dead space, urine was collected for analysis over 10 min. A further 15 mmHg (2 kPa) increment in pressure was then made and the washout and collection periods were repeated. Further increments of 15 mmHg (2 kPa) were made up to final pressures of 150 mmHg (20 kPa) and SHR at 180 mmHg (24 kPa). At 120 mmHg (16 kPa) and above in the WKY and 135 mmHg (18 kPa) and above in the SHR, the washout periods were reduced to 5 min; all collection periods remained 10 min in duration. Total experimental time in the WKY was 115 min, and in the SHR 130 min. Perfusate volume was maintained constant throughout by the addition of a mixture of Krebs–Henseleit buffer/water (5:50, v/v) as necessary, which replaced urinary and evaporative losses and served to maintain a constant level of sodium in the perfusate (133.5±0.9 mmol/l initial, 132.7±1.3 mmol/l final) and to prevent an increase in perfusate albumin concentration which would otherwise occur during the course of the experiment.

Urine samples were collected under light paraffin oil in preweighed tubes and volume was determined by weight. Perfusate samples were withdrawn at the mid-point of each urine collection period. If necessary, samples were stored at −20°C before analysis.

Group II: experiments with crystalloid

In these studies the perfusate consisted of Krebs–Henseleit buffer supplemented with 5 mmol/l glucose. In both five WKY and six SHR experiments, renal artery perfusion pressure was initially adjusted to 90 mmHg (12 kPa) and, after a 20 min stabilization period, two 7.5 min baseline urine collections were made. The
pressure was then increased by 10 mmHg (1.3 kPa) and a 5 min urine collection performed after a 5 min washout period. This protocol was repeated until final collections were made at a pressure of 140 mmHg (18.7 kPa). Perfusate volume was maintained constant by addition of a mixture of Krebs–Henseleit buffer/water (85:15, v/v) as necessary (perfusate sodium concentration: initial 133.6 ± 0.9 mmol/l, final 133.1 ± 1.0 mmol/l). Total experimental time was 85 min in both groups.

**Analyses**

Sodium and potassium were analysed by flame photometry (IL543, Instrumentation Laboratory, Warrington, Cheshire, U.K.). $^{14}$C-$\text{[C]}	ext{inulin activity was assayed using a flat plate liquid scintillation counter. Lithium was measured by atomic absorption spectrophotometry (2380; Perkin Elmer, Beaconsfield, Bucks., U.K.). All analyses were performed in duplicate and the mean value was used in calculations.

**Calculations and statistics**

The average of the two baseline collection periods was used for analysis. All values were factored per g left kidney wet weight.

$C_{\text{in}}$, sodium excretion ($U_{\text{Na}}V$), sodium clearance ($C_{\text{Na}}$), potassium excretion ($U_{\text{K}}V$), potassium clearance ($C_{\text{K}}$) and $C_{\text{Li}}$ were calculated using standard formulae. Fractional sodium excretion (FE$_{\text{Na}}$) was calculated as $C_{\text{Na}}/C_{\text{in}}$, fractional potassium excretion (FE$_{\text{K}}$) as $C_{\text{K}}/C_{\text{in}}$ and fractional lithium excretion (FE$_{\text{Li}}$) as $C_{\text{Li}}/C_{\text{in}}$.

Based on the assumption that lithium was exclusively reabsorbed in the proximal tubule, that its concentration in the proximal tubule was equal to that in the perfusate, and that it was neither absorbed from nor secreted into the distal nephron [12, 13], the following calculations were made:

Fractional distal sodium excretion (FDSE) $= \frac{C_{\text{Na}}}{C_{\text{Li}}}$

Fractional distal volume excretion (FDVE) $= \frac{V}{C_{\text{Li}}}$

where $V$ is urinary volume.

All results are expressed as means ± SEM. The significance of difference between values in experimental and control kidneys at any given pressure was determined using a modified t-test assuming inequality of variance [20]. $P<0.05$ (two-tailed) was taken as indicating statistical significance.

**RESULTS**

**Group I: experiments with 6.7 g/dl albumin**

The mean weight of the six WKY was 382.5 ± 5.7 g compared with 361.3 ± 14.7 g for the five SHR. The mean kidney weight for the WKY was 1.355 ± 0.017 g and for the SHR was 1.446 ± 0.095 g.

Fig. 1 shows the relationships between perfusate flow, $C_{\text{in}}$, $U_{\text{Na}}V$ and FE$_{\text{Na}}$ and perfusion pressure in WKY and SHR. At any given pressure all values were lower in SHR than WKY. In Fig. 2 it can be seen that FE$_{\text{Li}}$ was markedly reduced in SHR at any given pressure, whereas FDSE and FDVE were very similar over the range of pressures studied. Fig. 3 depicts the relationship between FE$_{\text{Na}}$ and FE$_{\text{Li}}$; for any particular value of FE$_{\text{Li}}$ the value of FE$_{\text{Na}}$ was greater in the SHR.

At all pressures $U_{\text{K}}V$ was lower in SHR than WKY [at 105 mmHg (14 kPa), 0.25 ± 0.06 vs 1.28 ± 0.20 μmol min$^{-1}$ g$^{-1}$ kidney weight ($P<0.01$); at 150 mmHg (20

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**Fig. 1.** Relationship in kidneys perfused with 6.7 g/dl albumin between perfusion pressure and perfusate flow rate (a), $C_{\text{in}}$ (b), $U_{\text{Na}}V$ (c) and FE$_{\text{Na}}$ (d). Values are means ± SEM. ● SHR (n = 5); ○, WKY (n = 6). Statistical significance: *$P<0.05$, †$P<0.01$ compared with WKY. To convert mmHg to kPa, divide by 7.5.
Fig. 2. Relationship in kidneys perfused with 6.7 g/dl albumin between perfusion pressure and 
$\text{FE}_\text{Lj}$ (a), $\text{FDSE}$ (b) and $\text{FDVE}$ (c). Values are means $\pm \text{SEM}$. ■, SHR ($n=5$); □, WKY ($n=6$). 
Statistical significance: *$P<0.05$, †$P<0.01$ compared with WKY. To convert mmHg to kPa divide 
by 7.5.

The mean kidney weight for the WKY was 1.379 ± 0.047 
g and for the SHR 1.337 ± 0.041 g.

Fig. 4 shows the relationship between perfusate flow, 
$C_{\text{in}}$, $U_{\text{Na}}$, and $\text{FE}_{\text{Na}}$ and perfusion pressure in WKY and 
SHR. Perfusion flow was virtually identical at all pres-
sures. In contrast to the situation with 6.7 g/dl albumin, 
perfusate $C_{\text{in}}$ was higher in SHR than WKY. $U_{\text{Na}}$ was 
also higher in SHR than WKY, and $\text{FE}_{\text{Na}}$ was similar at all 
pressures. In Fig. 5 it can be seen that $\text{FE}_{\text{Lj}}$, $\text{FDSE}$ and 
$\text{FDVE}$ are broadly similar at all perfusion pressures. Fig. 
6 illustrates the relationship between $\text{FE}_{\text{Na}}$ and $\text{FE}_{\text{Lj}}$; a 
similar pattern to that seen with 6.7 g/dl albumin perfus-
ate is observed: for any particular value of $\text{FE}_{\text{Lj}}$ the value 
of $\text{FE}_{\text{Na}}$ is greater in the SHR.

SHR excreted more potassium than WKY: $U_k$ was 
2.81 ± 0.25 vs 2.00 ± 0.23 $\mu$mol min$^{-1}$ g$^{-1}$ kidney 
weight ($P<0.05$) at 90 mmHg (12 kPa) perfusion pressure; at 
140 mmHg (18.7 kPa) the difference had increased 
4.52 ± 0.27 $\mu$mol min$^{-1}$ vs 3.37 ± 0.07 $\mu$mol min$^{-1}$ g$^{-1}$ 
kidney weight ($P<0.01$). $\text{FE}_k$ was not significantly dif-
ferent at any pressure.

**DISCUSSION**

In this study, reduced $\text{FE}_{\text{Lj}}$, together with reduced $U_{\text{Na}}$ 
and $\text{FE}_{\text{Na}}$, has been demonstrated in the isolated albumin-
perfused SHR kidney when compared with the WKY 
kidney perfused at the same pressure. The finding of a 
reduction in $U_{\text{Na}}$ and $\text{FE}_{\text{Na}}$ confirms that demonstrated 
in this model by Raine et al. [8] and is concordant with 
demonstrations of altered pressure-natriuresis in SHR *in 
vivo* by Roman & co-workers [2, 3]. The observed reduc-
tion in $U_{\text{Na}}$ may have reflected a lower GFR to some
degree, but tubular factors must also be invoked as FE\textsubscript{Li} was reduced in addition. The decrease in FE\textsubscript{Li} (Fig. 2a) in the isolated SHR kidney is a new finding which suggests that the abnormality of sodium handling is predominantly a feature of the proximal nephron. At any given perfusion pressure the calculated indices of distal handling of sodium and water (FDSE and FDVE) show no major differences between SHR and WKY (Fig. 2b and 2c); the plot of FE\textsubscript{Li} against FE\textsubscript{Na} (Fig. 3) would suggest that for a given fractional load delivered to the distal nephron the hypertensive kidney tends to reabsorb less than its normotensive counterpart.

This interpretation of the lithium data is based on the assumption that no net transport of lithium occurs beyond the proximal tubules. The micropuncture studies of Kirchner [12] and Thomsen et al. [13] would suggest that this is a reasonable assumption provided that animals are not sodium restricted. Hayslett & Kashgarian [21] were also unable to detect any net transport of lithium by micropuncture technique along the distal tubule. There have, however, been no studies directly comparing C\textsubscript{Li} with micropuncture in the isolated perfused rat kidney or in SHR and it is theoretically possible that distal tubular uptake of lithium occurring in SHR but not WKY could explain the data. Nevertheless, the values of FE\textsubscript{Li} obtained in this study are broadly comparable with those reported by Biollaz et al. [22] in SHR and WKY in vivo: in SHR with a mean blood pressure of 173 mmHg (23.1 kPa) they found an FE\textsubscript{Li} of 13.7%; in WKY the mean blood pressure was 115 mmHg (15.3 kPa) and the FE\textsubscript{Li} 18.9%. We would have expected an FE\textsubscript{Li} of 13.7% to be obtained by the isolated SHR kidney at a perfusion pressure of between 135 and 150 mmHg (18 and 20 kPa), and an FE\textsubscript{Li} of 18.9% in the WKY at around 120 mmHg (16 kPa). The range of values of FE\textsubscript{Li} that we observed with variation in perfusion pressure is similar to that provoked in Dahl rats by saline infusion in vivo (7.6–23.0%) [23]. The data presented here suggest that the reduction in FE\textsubscript{Li} is an intrinsic property of the kidney taken from hypertensive SHR which is not immediately dependent on neural or humoral factors present in the hypertensive animal. Increased fractional sodium reabsorption in the proximal tubule may contribute significantly to the existence of hypertension in SHR.

The findings related to perfusion of SHR and WKY kidneys with albumin-free solution are novel and difficult to explain. Perfusate flow was virtually identical at all perfusion pressures studied; GFR, as measured by \( C\text{cr} \), was higher in the SHR than the WKY at all pressures, rather than reduced, as was the case in the experiments performed with 6.7 g/dl albumin in the perfusate; \( U\text{Na}V \) was likewise higher, rather than lower, at all pressures. Tubular handling of sodium and lithium was very similar in both groups at all pressures. Omission of oncotic agent seem, therefore, to have had two major effects: first, to have markedly increased filtered load in the SHR and yet not done so in WKY; secondly, to have largely abolished the differences in tubular handling of sodium and lithium seen when the perfusate contained albumin.

Changes in GFR must be due to alterations in either the ultrafiltration coefficient, the net driving force for filtration, or both. Changes in the net driving force for filtration may occur due to alterations in the mean transcapillary hydraulic pressure gradient, the transcapillary oncotic pressure gradient and/or the glomerular plasma flow rate. It seems unlikely that the variable

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**Fig. 4.** Relationship in kidneys perfused without oncotic agent between perfusion pressure and perfusate flow rate (a), \( C\text{in} \) (b), \( U\text{Na}V \) (c) and \( FE\text{Na} \) (d). Values are means ± SEM. □, SHR \((n = 5)\); ○, WKY \((n = 5)\). Statistical significance: *\( P < 0.05 \) compared with WKY. To convert mmHg to kPa, divide by 7.5.
response of the GFR to omission of oncotic agent in SHR and WKY could be due to differences in glomerular plasma flow rate between SHR and WKY kidneys because glomerular plasma flow rate is always grossly elevated in the cell-free perfusion system. A variation in the change in transcapillary oncotic pressure clearly cannot be the explanation either, because in the albumin-perfused kidneys albumin concentration was equal in the two groups and in the crystalloid perfusions oncotic agent was absent in both. This leaves differences in the response of mean transcapillary hydraulic pressure gradient or the ultrafiltration coefficient to omission of oncotic agent as possibilities.

Alteration in peritubular physical factors are known to have large effects on function of the proximal tubule and loop of Henle but have not been demonstrated to alter distal tubule or collecting duct function [15], although a small effect cannot be excluded. Thus, the fact that differences in tubular function were seen when SHR and WKY kidneys were perfused with albumin, but not when oncotic agent was omitted from the perfusate, is perhaps also consistent with the notion that the difference between the two resides in the proximal tubule.

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