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URINARY AND PROXIMAL TUBULE ACIDIFICATION DURING REDUCTION OF RENAL BLOOD FLOW IN THE RAT

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SUMMARY

1. The effects of reduction in renal blood flow (RBF) on urinary acidification and proximal tubule H^+ ion secretion were studied after partial aortic clamping in rats.

2. Acute reduction of the renal perfusion pressure (from $109 \pm 3{\cdot}88$ to $77{\cdot}4 \pm 1{\cdot}05$ mmHg) decreased both inulin and PAH (*p*-aminohippurate) clearances to about one-third of their control values. Absolute levels of urinary sodium excretion also decreased markedly, but fractional sodium excretion did not change significantly.

3. Urine pH and bicarbonate levels were not affected, but titratable acidity increased significantly from $0{\cdot}12 \pm 0{\cdot}011$ to $0{\cdot}25 \pm 0{\cdot}042$ μequiv min^{-1} ml^{-1} glomerular filtration rate (GFR). During aortic clamping, cortical P_{CO_2} as determined by means of Severinghaus microelectrodes was reduced by a mean value of $7{\cdot}0 \pm 1{\cdot}5$ mmHg.

4. Proximal tubule acidification kinetics were studied by stationary microperfusion techniques in which the time course of pH changes was monitored by pH microelectrodes. Steady-state pH fell from a mean control value of $6{\cdot}77 \pm 0{\cdot}03$ to $6{\cdot}65 \pm 0{\cdot}02$, and stationary bicarbonate concentrations from $4{\cdot}70 \pm 0{\cdot}27$ to $2{\cdot}84 \pm 0{\cdot}18$ mM. Acidification half-time decreased from $5{\cdot}07 \pm 0{\cdot}30$ to $4{\cdot}39 \pm 0{\cdot}19$ s, and net bicarbonate reabsorption increased from $1{\cdot}63 \pm 0{\cdot}14$ to $1{\cdot}99 \pm 0{\cdot}12$ nmol cm^{-2} s^{-1}, these changes being statistically significant.

5. The experiments demonstrate that both overall acid excretion and proximal acid secretion are not compromised by a large decrease of RBF to about one-third of the control value; titratable acid excretion and proximal net bicarbonate reabsorption were even moderately increased under these conditions.

INTRODUCTION

An adequate level of renal blood perfusion is essential for all functions of the kidney. It is well known that periods of renal ischaemia produce significant functional alterations that take considerable time for recovery (Burke, Arnold & Schrier, 1983; Fried, Hishida, Barnes & Stein, 1984). Partial clamping of the aorta or renal artery has also been used to analyse the effect of variations in glomerular filtration rate (GFR) and renal blood flow (RBF) on the reabsorption of salt and

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water and on autoregulation of RBF in rat and dog (McNay & Abe, 1970; Arendhorst, Finn & Gottschalk, 1975; Johnston, Perrin, Bernard & Levinsky, 1981). Epithelial transport mechanisms depend on the nutritional blood supply, which includes the supply of oxygen. Thus, it has been reported that absolute proximal reabsorption of fluid was reduced, in the rat, by approximately 50% when RBF and GFR were reduced by 50% (Buentig & Earley, 1971). This effect was independent of filtered load, since it was observed during pump perfusion of proximal tubules. It is interesting to note, however, that in the isolated perfused rat kidney preparation, proximal tubule acidification is maintained to a considerable extent, leading to transepithelial pH gradients near those of normal kidney (lumen pH of 6·8) when perfusion is performed with artificial solutions which do not contain haemoglobin, and even at the P_{O_2} of air, that is, at an oxygen supply which is markedly lower than that found in the 'in vivo' kidney (Rubio, de Mello, Mangili & Malnic, 1982). This is consistent with the well-known observation that RBF exceeds the nutritional needs of the organ. Thus, RBF, of the order of 4 ml min^{-1} g^{-1} renal cortex, is much larger than that of other organs, e.g. muscle at rest (0·04 ml min^{-1} g^{-1}) (Mellander & Johannson, 1968).

To define the functional changes following renal haemodynamic alterations in the present study we investigated the effects of partial clamping of the aorta on overall renal acidification and, more specifically, on H^+ ion transport kinetics of the proximal tubule.

METHODS

Male Wistar rats weighing 225–350 g were anaesthetized with Inactin (100 mg kg^{-1} intraperitoneally). The jugular vein, the femoral artery and the trachea were cannulated by means of polyethylene tubing. The bladder was cannulated by a double catheter allowing for air injection to expedite emptying. A lateral abdominal incision was made for the introduction of a Blalock clamp around the aorta above the renal arteries (McNay & Abe, 1970; Lynch, Schneider, Willis & Knox, 1972).

For 'in vivo' micropuncture experiments, the kidney was isolated by a lumbar approach, and immobilized in a lucite cup by a Ringer–agar layer (Malnic & Mello Aires, 1971; Rebouças Fernandes, Elias, Mello Aires & Malnic, 1984). After the surgical preparation, blood plasma separated from blood collected from other rats was injected (1·4 ml 100 g^{-1} body weight) at a rate of 0·1 ml min^{-1} to compensate for blood loss during surgery. Inulin and *p*-aminohippurate (PAH) prime and sustaining infusions were given via the jugular vein and 3% mannitol in saline was infused at a rate of 0·1 ml min^{-1} during the experiments. Arterial blood pressure (BP) was measured by connecting the femoral artery catheter to a Statham P-23AA transducer, BP being recorded on an RP Beckman Dynograph. In all experiments, several control measurements were performed during a first ('control') period before the clamp was adjusted to reduce blood pressure to a lower level. Measurements were then continued during the experimental period, for 30–60 min, after which the clamp was removed and blood pressure in the femoral artery ascertained to return to within 10 mmHg of the control value.

Proximal tubules were perfused by means of double-barrelled micropipettes made out of theta glass tubing, using a perfusion solution containing (in mM): 90 NaCl, 25 $NaHCO_3$, 4 KCl, 1 $CaCl_2$ and raffinose to reach 300 mosM. Antimony microelectrodes were prepared as previously described (Vieira & Malnic, 1968). Their tips were ground to a diameter of the order of 5 μm. Voltages between these electrodes and a Cu^{2+}–$CuSO_4$ reference electrode connected to the rat's tail by an agar bridge were measured by a Keithley 615 digital electrometer and recorded on an R511A Beckman Dynograph. Data were digitized and processed on a Hewlett–Packard model 9603 data acquisition system. The electrodes were calibrated by means of buffers of composition similar to the perfusion fluid, but containing phosphate instead of bicarbonate. An appropriate pH correction

related to the anion composition was made as described previously (Lopes, Mello Aires & Malnic, 1981).

P_{CO_2} in renal cortical proximal tubules was measured using Severinghaus microelectrodes (Sohtell & Karlmark, 1976; Pucacco & Carter, 1978) containing antimony microelectrodes as the pH sensing device (M. Mello Aires, A. G. Lopes and G. Malnic, unpublished observations). The electrodes had a silicone rubber membrane of approximately 10 μm thickness near their tips, and the pH electrode was within 10–30 μm from the membrane. The electrodes were filled with a solution containing 100 mM-NaCl and 25 mM-$NaHCO_3$ plus 0·5 mg ml^{-1} bovine erythrocyte carbonic anhydrase (Sigma, St Louis, MO, USA). They were calibrated in bicarbonate buffer solutions kept at 37 °C by a water jacket and bubbled by two gas mixtures of different CO_2 concentrations in air. P_{CO_2} was measured 'in vivo' before and during partial aortic clamping above the renal arteries, while arterial BP was measured from a femoral artery.

Blood samples were obtained from the femoral artery at the mid-point of every urine collection period. Sodium, inulin and PAH were measured by flame photometry, the anthrone method and spectrophotometry, respectively (Smith, Finkelstein, Aliminosa, Crawford & Graber, 1945; Fuehr, Kaczmarczyk & Kruettgen, 1955). pH and P_{CO_2} were measured in blood and urine using a Radiometer BMS3–MK2 blood micro-system.

Data are presented as means ± S.E.M. Comparisons were made by the *t* test or, when more than two samples were compared, by analysis of variance and the Scheffé contrast test (Snedecor & Cochran, 1967).

RESULTS

Data on blood and urine composition obtained in rats used in the present series of experiments are summarized in Table 1. Mean arterial blood pressure, measured before and after aortic clamping, fell from 109 to 77·4 mmHg in the femoral artery, confirming a marked reduction in renal perfusion pressure. Blood pH fell by 0·05 units in these conditions, but remained within the normal range. We observed no changes of blood and urine P_{CO_2}. Urine pH showed a tendency to decrease but this change did not reach statistical significance. In contrast, titratable acidity (per millilitre GFR) increased significantly in rats during aortic clamping.

Figure 1 provides data on renal haemodynamic changes. The inulin clearance, representing GFR, fell from 6·20 ± 0·72 ml min^{-1} kg^{-1} in nine control rats, each with one clearance period, to 2·41 ± 0·42 ml min^{-1} kg^{-1} (fifteen clearance periods in nine rats) after aortic clamping. Renal plasma flow, measured by the PAH clearance, fell from 21·5 ± 2·71 (nine periods) to 8·27 ± 1·21 (fifteen periods) ml min^{-1} kg^{-1}. The filtration fraction remained unchanged.

Figure 2 compares the absolute and fractional sodium excretion rates in control and experimental conditions. Control rats had a mean excretion rate of 2·10 ± 0·57 ($n = 9$) μmol min^{-1}, a value that fell after aortic clamping to 0·93 ± 0·14 ($n = 15$) μmol min^{-1}, significantly less than the control values. On the other hand, fractional excretion rates of sodium (0·74 ± 0·18 % in controls *vs.* 1·15 ± 0·08 % after clamping) were not significantly different. Absolute rates of sodium reabsorption were markedly lower in clamped kidneys (336·9 ± 59·3 μequiv min^{-1}) than in control kidneys (866·2 ± 100·8 μequiv min^{-1}) due to the reduced filtered load of sodium.

In an additional series of experiments, several kinetic parameters of acidification were studied in perfused proximal tubules during an initial control period and during periods of aortic clamping. Table 2 summarizes relevant data. Renal cortical P_{CO_2}, as measured by Severinghaus microelectrodes, fell by a mean value of 7 mmHg after aortic clamping. These values were used to calculate bicarbonate concentrations

from measured pH values in blocked proximal tubule segments. Mean values of these data are given in Table 3. It is of interest that the final stationary pH in the proximal tubule lumen was moderately but significantly reduced after aortic clamping. Similarly, stationary bicarbonate concentrations were reduced. Acidification half-

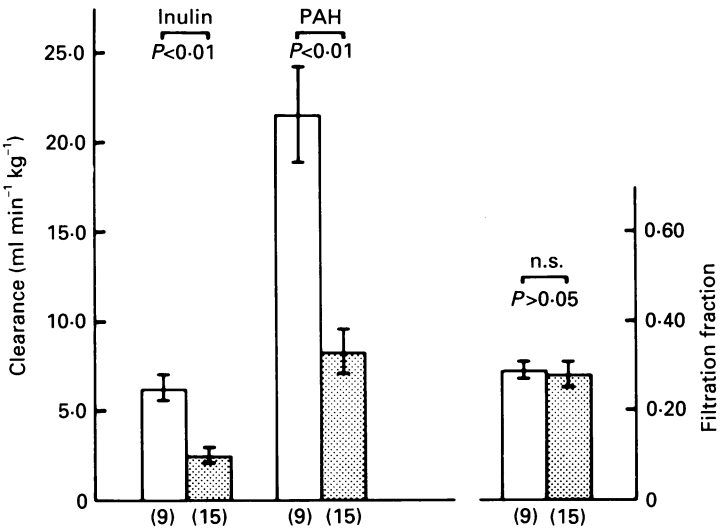

Fig. 1. Inulin and PAH clearances and filtration fraction in control (open bars) and aortic clamped (stippled bars) rats. Number of measurements in parentheses; probabilities refer to significance of differences between control rats and rats with aortic clamping.

TABLE 1. General data from rats before and during aortic clamping

	Control	Clamped
Femoral BP (mmHg)	109·0 ± 3·88 (9)	77·4 ± 1·05 (15)**
Arterial blood pH	7·40 ± 0·015 (9)	7·35 ± 0·014 (15)*
Arterial P_{CO_2} (mmHg)	33·8 ± 1·99 (9)	34·2 ± 1·72 (15)
Urine pH	6·41 ± 0·10 (9)	6·24 ± 0·07 (15)
Urine P_{CO_2} (mmHg)	28·6 ± 1·07 (9)	31·4 ± 1·40 (15)
Titratable acidity (μequiv min^{-1} ml^{-1} GFR)	0·12 ± 0·011 (9)	0·25 ± 0·042 (15)*

BP and blood samples taken from femoral artery.

* $0{\cdot}05 > P > 0{\cdot}01$; ** $P < 0{\cdot}01$.

times were also significantly shortened after clamping. As a consequence of these changes in acidification kinetics, net bicarbonate reabsorption rates (J_H) were significantly increased after aortic clamping, compared to control periods.

DISCUSSION

The effects of changes of renal blood flow on proximal tubule salt and water transport have been studied extensively. For instance, a large number of investigations on the nature of glomerulo-tubular balance have clarified the

relationship between proximal salt and water reabsorption and variations in their filtered load (Falchuk, Brenner, Tadokoro & Berliner, 1971; Ichikawa & Brenner, 1980; Kon, Hughes & Ichikawa, 1983). This relationship has been shown to depend, in part, on modifications of peritubular Starling forces, including changes of

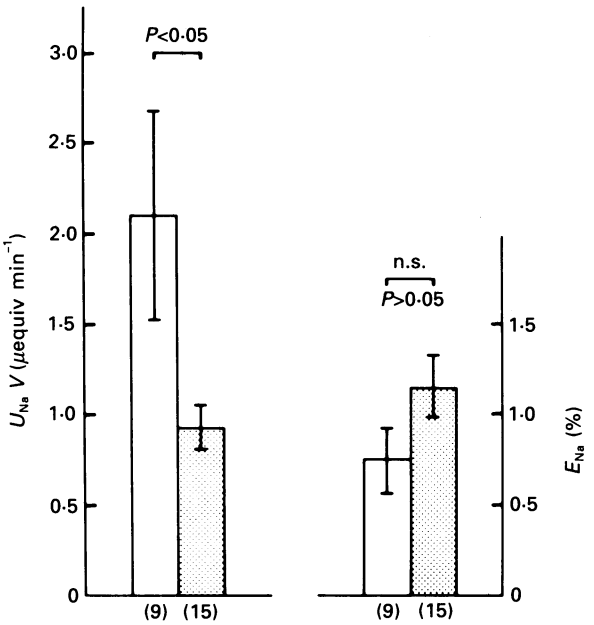

Fig. 2. Urinary excretion of sodium ($U_{Na}V$) and fractional excretion of sodium (E_{Na}) in percentage in control (open bars) and aortic clamped (stippled bars) rats. Numbers of measurements in parentheses; probabilities refer to significance of differences between control rats and rats with aortic clamping.

TABLE. 2. Proximal tubule P_{CO_2} in control rats before and during aortic clamping, measured by Severinghaus microelectrodes

	Control	Clamped	n
Femoral BP (mmHg)	115·1±4·90	75·9±7·62	9
Proximal tubule P_{CO_2} (mmHg)	35·1±4·45	28·0±3·41	9
ΔP_{CO_2} (mmHg)	—	7·0±1·48*	9

* $P < 0·01$.

hydrostatic and oncotic pressure gradients across the capillary wall (Falchuk *et al.* 1971; Ichikawa & Brenner, 1980). As a consequence of glomerulo-tubular balance, fractional salt reabsorption is kept approximately constant when GFR is reduced by e.g. aortic constriction, but the absolute proximal reabsorption rate (APR) of salt and water decreases since the same fraction of a smaller load is now reabsorbed along the same surface of epithelium. In addition, it has been shown that there is also a marked luminal load dependence of APR. Thus, Holzgreve & Schrier (1975) have

demonstrated that APR is reduced in rats with aortic constriction even when peritubular capillaries are perfused at a constant rate with an artificial solution both in controls and in the period with aortic constriction.

In accordance with the role of peritubular Starling forces in modulating proximal

TABLE 3. Kinetics of acidification in proximal tubules of rats before and during aortic clamping

	Control	Clamped
pH_s	6·77 ± 0·025	6·65 ± 0·022**
$[HCO_3^-]_s$ (mM)	4·70 ± 0·27	2·84 ± 0·18**
$t/2$ (s)	5·07 ± 0·30	4·39 ± 0·19*
Tubule diameter (μm)	23·5 ± 0·55	22·7 ± 0·47
J_H (nmol cm^{-2} s^{-1})	1·63 ± 0·14	1·99 ± 0·12*
n	21	40

* $0{\cdot}05 > P > 0{\cdot}01$; ** $P < 0{\cdot}01$; index s, stationary levels.

tubule fluid reabsorption, a relationship between hydrostatic pressure in peritubular capillaries and APR has been demonstrated such that APR decreased with elevated peritubular capillary pressure and increases with lower pressure (Morgan, 1970; Dresser, Lynch, Schneider & Knox, 1971; Quinn & Marsh, 1979). Thus, it has been reported that aortic constriction, which reduces hydrostatic pressure in capillaries as well as renal interstitial pressure, is responsible for reduction in renal blood flow but has only a smaller effect on APR in free-flow conditions (Brenner, Troy, Daugharty & MacInnes, 1973). This may be due to the opposing effects of aortic constriction on peritubular capillary dynamics (tending to increase reabsorption) and of the changed filtered load of sodium chloride, which is markedly reduced, thus decreasing APR. When the filtered load is kept constant, in microperfusion experiments, extracellular volume depletion causes a marked increase in APR, as expected from the changes in peritubular factors alone (Weiner, Weinman, Kashgarian & Hayslett, 1972).

Our experiments have shown that aortic constriction, although leading to a marked reduction in RPF, does not impair urinary acidification. Urinary titratable acidity was even significantly increased (see Table 1). These findings are compatible with those of Tam and co-workers (Tam, Goldstein, Richardson, Robson, Stinebaugh & Halperin, 1980), who have described increased distal H^+ ion secretion during extracellular volume contraction by the urine–blood P_{CO_2} difference technique.

Proximal tubule acidification kinetics also detected stimulation of the acidification rate. Thus, a significant reduction was observed in proximal luminal stationary pH and bicarbonate concentration as compared to controls. These changes resulted in significantly increased H^+ ion secretion rates, which may be responsible for the increased titratable acidity found in final urine via a reduced distal bicarbonate load.

What mechanisms might be evoked to explain the observed stimulation of proximal acidification? According to a model of renal tubule acidification that describes the acidification curves obtained by our experimental technique, an increase in H^+ ion secretion rates by reduction in acidification half-times may be ascribed to an increased number of transport sites (in this case, Na^+–H^+ exchangers) in the apical membrane, or to increased availability of H^+ ions for transport (Amorena, Fernandes & Malnic, 1984).

A possible mechanism for stimulation of proximal acidification is an increase of the

ion concentration gradients favouring Na^+–H^+ exchange, that is, an increase in cell H^+ or a decrease of cell Na^+ concentrations. A fall in cell pH may arise due to accumulation of acid metabolites; the decreased P_{CO_2} found after aortic clamping would act in the opposite direction (see Table 2). On the other hand, a stimulation of proximal sodium transport has been observed during extracellular volume contraction. Weiner *et al.* (1972) and Stein, Osgood, Boonjarern, Cox & Ferris (1974) found an increase in APR or proximal fractional reabsorption in conditions of pronounced extracellular volume depletion. Since proximal H^+ ion secretion is coupled to sodium transport by an ion exchange mechanism at the luminal membrane, a parallel effect of the reduction of RPF on Na^+ and H^+ (HCO_3^-) transport is expected (Murer, Hopfer & Kinne, 1976; Kinsella & Aronson, 1980). Thus, the fall in cell Na^+ expected under these conditions might constitute a factor stimulating proximal H ion secretion.

It is interesting to compare the present results with those obtained in adrenalectomized (ADX) rats. In these animals, a reduction in GFR and RPF is found (Lynch *et al.* 1972; Marver & Kokko, 1983). Inhibition of proximal sodium transport by adrenalectomy was detected by Wiederholt, Stolte, Brecht & Hierholzer (1966) and by Lynch *et al.* (1972), and attributed in part to changes in renal haemodynamics. On the other hand, ADX causes a marked reduction in proximal tubule acidification rate as well as an increase in luminal pH (Damasco & Malnic, 1987). The present results indicate that this effect of ADX, which is reversed by corticosteroid administration, is not due to the haemodynamic changes in renal cortex caused by hormone depletion, but to hormone action on the tubule epithelium itself.

In sum, the present study has shown that the reduction of renal perfusion pressure and of RBF, to about one-third of the control value, reduces absolute sodium excretion ($U_{Na}V$), absolute renal sodium reabsorption, but not fractional sodium excretion. At the same time, urine acidification is not reduced, but even stimulated. This effect is confirmed in perfused proximal tubule, where net bicarbonate reabsorption is significantly increased when RBF is reduced by aortic clamping.

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