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DIFFERENTIAL CITRATE CLEARANCE IN 2-KIDNEY, 1-CLIP RENOVASCULAR HYPERTENSION

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SUMMARY The systemic state of acid base balance exerts a dominant influence on renal citrate handling and rats with 2K-1C renovascular hypertension have been reported to exhibit a marked derangement in acid-base balance status. Citrate clearance was therefore investigated in the clipped and contralateral kidneys of 2-kidney, 1-Clip (2K-1C) renovascular hypertensive rats, in order to determine to what extent deranged acid-base balance affect renal citrate handling.

2K-1C renovascular hypertension was induced in male SD rats (100±5g) by clipping the left renal artery using a 0.2mm silver clip under ether anesthesia. Control rats were sham-operated.

2 weeks post renal artery clamping, the contralateral kidney of 2K-1C rats exhibited elevated fractional excretion of citrate (16.7±6.30% vs. 1.70±0.04%; P<0.01), which was sustained into the established stages of 2K-1C hypertension (10.2±4.8% vs. 6.2±1.30%; p<0.05 at 14 weeks). The fractional excretion of citrate from the clipped kidney was not significantly different from those of controls.

Since the excretion of a citrate ion is equivalent to loss of 3 bicarbonate ions, the enhanced citrate clearance of the contralateral kidney contributes to the metabolic acidosis associated with 2K-1C renovascular hypertension.

Key words: *Citrate Clearance-Goldblatt 2K-1C-Renovascular Hypertension-Acid- Base Balance*

Introduction

Urinary citrate excretion is important for acid-base balance regulation. Metabolism of a citrate ion generates bicarbonate ions (Sato et al, 1997 (a); Levi et al, 1991) and the excretion of a citrate ion is equivalent to the loss of 3 bicarbonate ions (Levi et al, 1991). Previous observations reveal that the major influence on renal citrate handling is the systemic state of acid-base balance rather than the pH or bicarbonate concentration of the tubular fluid (Simpson, 1983). The effect of acid-base changes on the rate of metabolism of labelled citrate observed in the intact renal tubules persisted after disruption of the cell membrane by digitoxin pre-treatment and was reproducible in isolated tubules (Simpson et al, 1992). These effects are due to alteration in citrate levels in the cytoplasm without corresponding changes within mitochondria (Simpson et al, 1992).

In the rat, the percentage of filtered citrate that appears in the urine is usually between 3% and 7% (Adler et al 1981) and citrate is actively taken up by the brush border membrane Na^+/citrate co-transporter (Levi et al, 1991) so that the tissue levels of citrate in the renal cortex exceeds plasma levels (Neith & Schollmeyer, 1965).

The effect of acid-base changes on renal citrate excretion is entirely due to intrarenal alteration of citrate handling (Crawford et al, 1959). These effects are mediated via alteration in the pH gradient of the inner mitochondrial membrane (Simpson, 1983). Consequently, metabolic acidosis causes acute decrease in citrate excretion while metabolic alkalosis causes a dramatic increase in citrate excretion (Simpson, 1983; Jenkins et al, 1985). In salt sensitive subjects in whom reduced extracellular fluid pH and bicarbonate levels have been reported, cumulative urinary bicarbonate excretion was lower in the salt sensitive subjects than salt resistant controls after sodium citrate administration and after acute oral alkali loading (Sharma et al, 1993). Dietary alkali and acid loading also produces the same effect on citrate excretion in the laboratory animal (Packer et al, 1995).

Like in other hypertensive states, Sprague Dawley (SD) rats with 2K-1C renovascular hypertension exhibits marked derangement in acid base balance status (Odigie and Marin-Grez, 2000) and the contralateral kidney of rats with 2K-1C renovascular hypertension is associated with significant bicarbonate loss in urine while the bicarbonate excretion of the clipped kidney is comparable to that of controls (Odigie and Marin-Grez, 2000). Renovascular hypertension may be associated with derangement of renal citrate handling which may in turn result in acid-base disturbance. Differential citrate clearance was therefore investigated in 2K-1C hypertensive rats in order to ascertain to what extent disturbed acid-base balance affect renal citrate handling.

Materials and Methods:

Animal Preparation:

2K-1C renovascular hypertension was induced in male Sprague Dawley (SD) rats weighing 100±5.0g by clipping the left renal artery using a 0.2mm silver clip (inner diameter) under ether anaesthesia as previously reported (Marin-Grez et al, 1994). Control rats were sham-operated.

Blood pressure was measured weekly using rat's tail sphymomanometry as previously reported (Marin-Grez et al, 1994). The animals received standard institutional care throughout the period of observation.

At the end of the observation period, viz., 2 weeks and 14 weeks respectively after renal artery clipping, 2K-1C hypertensive rats and controls were subjected to standard clearance experiments under inactin^{R} anesthesia (80mg/kg intraperitonially). A tracheostomy was performed to guarantee spontaneous breathing. The body temperature of the animals was maintained at 37.0 ± 0.5 °C. throughout the experiment. The left carotid artery was cannulated for blood pressure recording using a strain-gauge blood pressure transducer (Tekmar Electronics GmBH, Munich, Germany) and for collection of blood samples. The right femoral vein was cannulated for the infusion of physiological saline (150mM Na^{+} 150 mM Cl^{-}) containing 6% polyfructosan (Inutest, Laevosan GmBH, Linz Germany) at an infusion rate of 10µl/100g.rat/min. The right and left ureters were cannulated for separate urine collection.

After 120 minutes equilibration period, urine samples were collected into pre-weighed vials for two 30 min periods. Blood samples and urine samples were collected into heparinized capillary tubes directly from the carotid artery catheter and ureter catheter respectively for determination of acid base-balance parameters using an automatic acid-base balance analyser (AVL 990, AVL Biochemical Instruments, Graz, Austria). Urine pH below 6 that could not be measured on the AVL 990, was measured using a Micro-pH meter (C-1 pH Meter, Horiba Instruments Inc, Kyoto, Japan). The bicarbonate concentration in the urine was calculated taking the ionic strength of the urine into consideration (Hastings and Sendroy, 1925; Giammarco, 1981).

Analytical Methods:

Urine volume was determined gravimetrically without correcting for specific gravity. Creatinine concentration in urine and plasma was measured using the Jaffe's reaction. GFR was determined using the clearance of polyfructosan (Führ et al, 1955). Sodium and potassium concentrations in urine and plasma were determined using a flame photometer. Chloride concentration in urine and plasma was determined electrometrically. In order to preserve the baseline acid-base balance status, the use of para-aminohippurate (PAH) for the measurement of renal blood flow (RBF) was avoided in this experiment because this substance interferes with acid-base parameters (Silbernagl, 1986).

The citrate concentration in urine and blood was measured using the citrate lyase method (Möllering & Gruber, 1966; Welschman & McCambridge, 1973).

All reagents and chemicals for citrate determination were obtained as test combination kit from Böhringer Mannheim (Böhringer Mannheim, Germany)

The American Physiological Society's guidelines for experimental animal research were adhered to in all of the experiments.

Statistical Analysis

Results are given as means ± standard error of the means. Unpaired "t" test was used for hypothesis testing of results between hypertensive and control rats. Paired "t" test was used for comparison of means of results between right and left kidneys of same animals as appropriate. Two tailed "t" test was used in all comparisons. A value of $P<0.05$ was considered statistically significant.

by a mechanism that is ouabain-sensitive (Brennan et al, 1986). Citrate reabsorption in the proximal tubule is the major determinant of the rate of renal citrate excretion (Hamm, 1990). Changes in mitochondrial pH gradient forms the basis of a very sensitive mechanism for regulating renal substrate metabolism (Wright et al, 1982; Simpson & Angelski, 1973). Thus, renal tissue acidosis promptly results in a decreased citrate clearance while alkalosis causes a dramatic increase in renal citrate excretion (Jenkins et al, 1985; Simpson, 1983). Renal citrate uptake and the effect of nephrotoxic agents on renal citrate handling has been studied extensively (Sato et al, 1997 (a); Brennan et al, 1986; Sato et al, 1995; Sato et al, 1997 (b)). Available experimental data reveals that alteration in renal citrate handling due to changes in acid-base balance status are mediated via alteration in pH gradient across the inner mitochondrial membrane (Simpson, 1983; Simpson & Hager, 1979). Thus metabolic acidosis as reported in the 2K-1C renovascular hypertensive rats leads to a decrease in cytoplasmic pH and bicarbonate levels. This change leads to an increase in mitochondrial pH gradient (Mitchell and Moyle, 1969), which in turn leads to stimulation of the tricarboxylate carrier (LaNoue & Schoolwerth, 1979; Robinson et al, 1977) leading to acceleration of citrate entry into the mitochondrial matrix compartment. This translates into increased citrate metabolism. Consequently, the cytosolic citrate concentration decreases so that tubular and peritubular citrate uptake are increased and citrate clearance decreases in the face of acidosis (Simonnet et al, 1980). The opposite effects occur in metabolic alkalosis. The differential citrate clearance of the contralateral and clipped kidneys of 2K-1C hypertensive rats may be explained by the increased bicarbonate excretion of the contralateral kidney (Odigie & Marin-Grez, 2000). The possibility of a primary impairment of renal citrate handling in 2K-1C hypertension remains to be investigated.

In conclusion, renal citrate clearance may be used as a valuable guide in seeking information on acid-base balance.

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