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Page 28 - 36
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Original Article

EFFECT OF PERINATAL CAPTOPRIL TREATMENT ON RENAL FUNCTION OF YOUNG ADULT, CONSCIOUS RATS: EXACERBATION DUE TO DISCONTINUATION OF TREATMENT

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Perinatal, systemic inhibition of the renin-angiotensin system appears to improve renal function and decrease hypertension in the adult spontaneously hypertensive rats, but some studies indicate that this early treatment can damage the kidney. Further, in normotensive, salt-resistant rats perinatal inhibition of the renin-angiotensin system increases the hypertensive response of these animals to a high NaCl diet. This study tests the hypothesis that perinatal inhibition of angiotensin converting enzyme (i.e., from conception onward) decreases renal function in young adult normotensive rats, especially in rats in which the treatment is discontinued. Male Sprague-Dawley rats were treated from conception onward with oral captopril (lifetime), or treated with captopril from conception to 5 weeks of age (acute). A third group was untreated (control; n = 9/group). At 7 weeks of age, all rats were implanted with arterial, venous, and bladder catheters, and 48 hours later, arterial pressure was measured continuously in restrained, awake rats before, during, and after an intravenous infusion of isotonic saline (5% of body weight, 0.5 ml/min). Urine and blood samples were collected before and up to 90 minutes after saline infusion. The lifetime (but not the acute) captopril treatment significantly blunted body weight gain (142.4 ± 3.4 g (lifetime), 207.0 ± 5.0 g (acute), and 221.2 ± 4.0 g (control)), heart weight (0.53 ± 0.02 g (lifetime), 0.83 ± 0.02 g (acute), and 0.85 ± 0.02 g (control)), and kidney weight (1.45 0 ± 02 g (lifetime), 2.20 ± 0.15 g (acute), and 1.86 ± 0.04 g (control)). The treatment reduced mean arterial pressure in the lifetime group but not in the acute group (86.8 ± 2.4 mm Hg (lifetime), 112.6 ± 2.7 mm Hg (acute), and 114.6 ± 1.4 mm Hg (control)). Although the acute captopril treatment did not alter mean arterial pressure, it lowered basal urine flow rate, sodium excretion, and fractional water excretion. Following saline infusion, water excretion, sodium excretion, and glomerular filtration rate were similar between the lifetime and the acute captopril-treated rats, but compared to controls all three functions were impaired in the treated groups. Fractional sodium excretion was not significantly different among the three groups throughout the study. Compared to the control, only the acute perinatal captopril-treated rats displayed lower percent water and sodium excretion per gram of kidney weight. These data suggest that perinatal angiotensin converting enzyme inhibition blunts renal function in young adult rats.

Key words: captopril, renin-angiotensin system, kidney function

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The renin-angiotensin system (RAS) plays an important role in the pathogenesis of arterial hypertension (Navar et al., 2000; Stroth and Unger, 1999), through several mechanisms, including increased vasoconstriction, water and sodium retention, and sympathetic nervous system activation. Further, the RAS appears to promote growth in several organs including, kidney, heart, and vascular smooth muscle (Niimura et al., 2000). Perinatal inhibition of the RAS prevents or attenuates hypertension in spontaneously hypertensive rats (SHR) (Wu and Berecek, 1993; Wyss et al., 1994), but in rats and pigs, this treatment has been reported to damage kidneys and impair urinary concentrating ability of the adult animals (Friberg et al., 1993; Guron et al., 1999). These latter findings are supported by studies in transgenic mice that have a compromised RAS (Cvetkovic and Sigmund, 2000). Moreover, normotensive, salt resistant Wistar-Kyoto (WKY) rats treated with lifetime oral captopril display hypertensive responses to a high NaCl diet (Fang et al., 1999), suggesting the possibility that a renal dysfunction exists in these rats. The salt sensitivity of these rats appears to be related to RAS and sympathetic nervous system overactivity (Wyss et al., 1994; Wyss et al., 1995), and the blunted pressure-diuresis/natruresis (Mozaffari et al., 1991; Roman and Cowley, 1985). In contrast to these findings, lifetime treatment with oral captopril does not cause obvious renal damage and appears to improve renal water and sodium excretion in the 3- to 4-month-old SHR (Roysommuti et al., 2000). In the present study we examined the possibility that early discontinuation of perinatal RAS inhibition leads to an up-regulation of the RAS and an inhibition of renal function.

Materials and Methods

All experiments were performed in male Sprague-Dawley (SD) rats. The animal unit of the Faculty of Medicine, Khon Kaen University, supplied all rats. They were maintained at constant humidity (60 ± 5%), temperature (24 ± 1 °C), and light cycle (0600-1800 h). The rats were divided into three groups: lifetime captopril treatment (n = 9), acute, perinatal captopril treatment (n = 9), and control treatment (n = 9). Food and water (containing 400 mg/ml captopril, Sigma; for captopril treated rats) were available *ad libitum* throughout the experiment. Female SD rats were continuously treated with oral captopril (or remained on water alone, control) from conception and throughout lactation, and the male offspring were treated throughout life (lifetime) or from weaning to 5 weeks of age (acute) with captopril. At seven weeks of age, under ether pentobarbital anesthesia, each rat was instrumented with femoral arterial, venous, and bladder catheters. Forty-eight hours later, each rat was placed in an environmental conditioning unit (Braintree, Braintree, MA, USA), to which it was acclimated for 7 days (3 h/day) prior to surgery. On the day of an experiment, arterial and venous catheters were flushed with 0.25 ml of isotonic saline containing 20 Units/ml heparin. The arterial catheter was connected to a pressure transducer for continuous monitoring of arterial pressure and heart rate using a computer-based amplifier and recorder (Biopac system, CA; mean arterial pressure (MAP) and heart rate were measured offline using AcqKnowledge software version 3.5.5, Biopac, CA, USA). Using an infusion pump (Harvard apparatus), isotonic saline containing 0.5% inulin (solution A) was intravenously infused (20 µl/min after a bolus of 0.5 ml of solution A) for 45 minutes to determine baseline cardiovascular and renal function. After baseline determinations (30 minute), the infusion rate was increased to 0.5 ml/min to volume load the rat with isotonic saline (solution A) equal to 5% of the animal's body weight. The infusion rate was then decreased to the basal infusion rate (20 µl/min) to maintain plasma inulin concentration throughout the remainder of the experiment. Urine samples were collected at 0, 15, 30, 60, and 90-minute intervals following the initiation of volume expansion, and blood samples were collected at mid points of these intervals. Blood was replaced by an equal volume of

saline. At the end of the experiment, all animals were killed by ether and their heart (HW) and kidney (KW) weights were recorded.

Urine volume was measured gravimetrically, and urine and plasma sodium and potassium were measured by frame photometry (Hitachi, Tokyo). Urine and plasma inulin concentrations were measured by a standard colorimetric method, and glomerular filtration rate (GFR) was calculated as the ratio of the urine/plasma concentration of inulin multiplied by the urine flow rate. All values were expressed as mean ± SEM. Statistical analyses of data were performed using one-way ANOVA with post-hoc tests (Duncan's multi-range) to test for significant ($p < 0.05$) effects. All studies were conducted in compliance with NIH and American Physiology Society standards for the care and use of research animals.

Results

Lifetime captopril-treated rats displayed lower body, heart and kidney weight than control and acute captopril-treated rats (Table 1). The body weight of the acute captopril-treated compared to control rats was slightly lower ($p < 0.05$), but their kidney weight (and kidney weight/body weight ratio) was significantly higher than the control ($p < 0.05$). Heart weight to body weight ratios were not significantly different among groups.

Table 1. Body, kidney, and heart weights in control, lifetime and acute captopril-treated rats.

Treatment	BW (g)	KW (g)	HW (g)	KW/BW (%)	HW/BW (%)
Control (n = 9)	221.2 ± 4.0	1.86 ± 0.04	0.85 ± 0.02	0.84 ± 0.01	0.38 ± 0.01
Lifetime (n = 9)	142.4 ± 3.4*	1.45 ± 0.02*	0.53 ± 0.02*	1.02 ± 0.03*	0.37 ± 0.01
Acute (n = 9)	207.0 ± 5.0*,#	2.20 ± 0.15*,#	0.83 ± 0.02#	1.06 ± 0.06*	0.40 ± 0.01

BW, body weight; KW, kidney weight; HW, heart weight; *, $p < 0.05$ compared to control; #, $p < 0.05$ compared between lifetime and acute treatments.

The acute captopril treatment did not alter mean arterial pressure (MAP) in the young adult rats from that displayed by controls, but lifetime captopril treatment reduced arterial pressure in these SD rats (MAP at time 0: control 114.6 ± 1.4 mm Hg, lifetime 86.8 ± 2.4 mm Hg, and acute 112.6 ± 2.7 mm Hg, $p < 0.05$; Figure 1). The isotonic saline loading had no significant effect on MAP in any group. Basal water and sodium excretion were significantly lower in the acute (11.8 ± 2.7 μl/min/gKW [water], 1.4 ± 0.3 μmol/min/gKW [sodium]) compared to the lifetime (35.1 ± 3.5 μl/min/gKW [water], 2.8 ± 0.3 μmol/min/gKW [sodium]) and the control rats (44.1 ± 5.7 μl/min/gKW [water], 4.0 ± 0.7 μmol/min/gKW [sodium]; Figure 2). Compared to the control

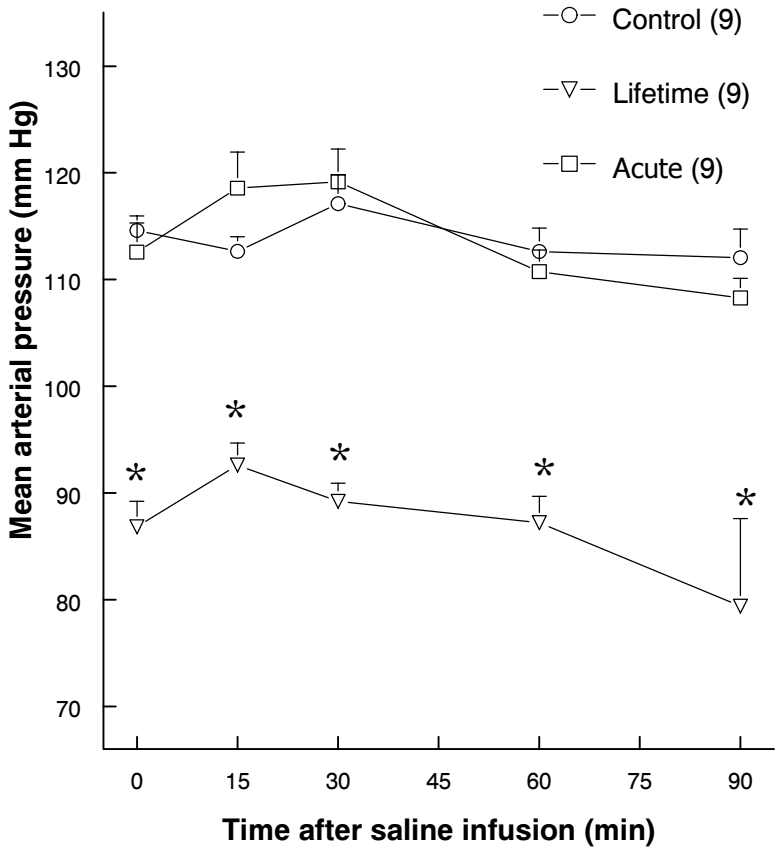

Figure 1. Lifetime but not acute perinatal captopril treatment significantly decreased mean arterial pressure in the SD rats when compared to the control (* denotes $p < 0.05$ compared to the control).

group, both the acute and the lifetime captopril-treated groups displayed lower urine flow and sodium excretion rates in response to the isotonic loading. When these values were normalized as the percent water and sodium excretions per kidney weights, the acute captopril-treated rats remained lower than control or lifetime-treated rats (water: control 49.1 9.4 %, lifetime 36.2 ± 8.7 %, acute 15.6 ± 3.4 %, $p < 0.05$; sodium: control 45.5 ± 7.7 %, lifetime 36.7 ± 8.1 %, acute 24.2 ± 5.7 %, $p < 0.05$; Figure 3). Further, fractional water but not sodium excretion was lower in acute compared to control or lifetime-treated rats. Plasma sodium and potassium were not significantly different among the three groups and were steady throughout the experiment.

Basal glomerular filtration rates were almost identical among the three groups, but after saline load, both the acute and the lifetime captopril-treated rats displayed similar GFR responses that were significantly lower than those of control rats (Figure 4).

Discussion

The RAS plays an important role in arterial pressure regulation and electrolyte homeostasis (Unger, 2000), and several lines of evidence indicate that it may serve autocrine, paracrine, and intracrine growth promoting factor in several tissues including those in the kidney (Guron and

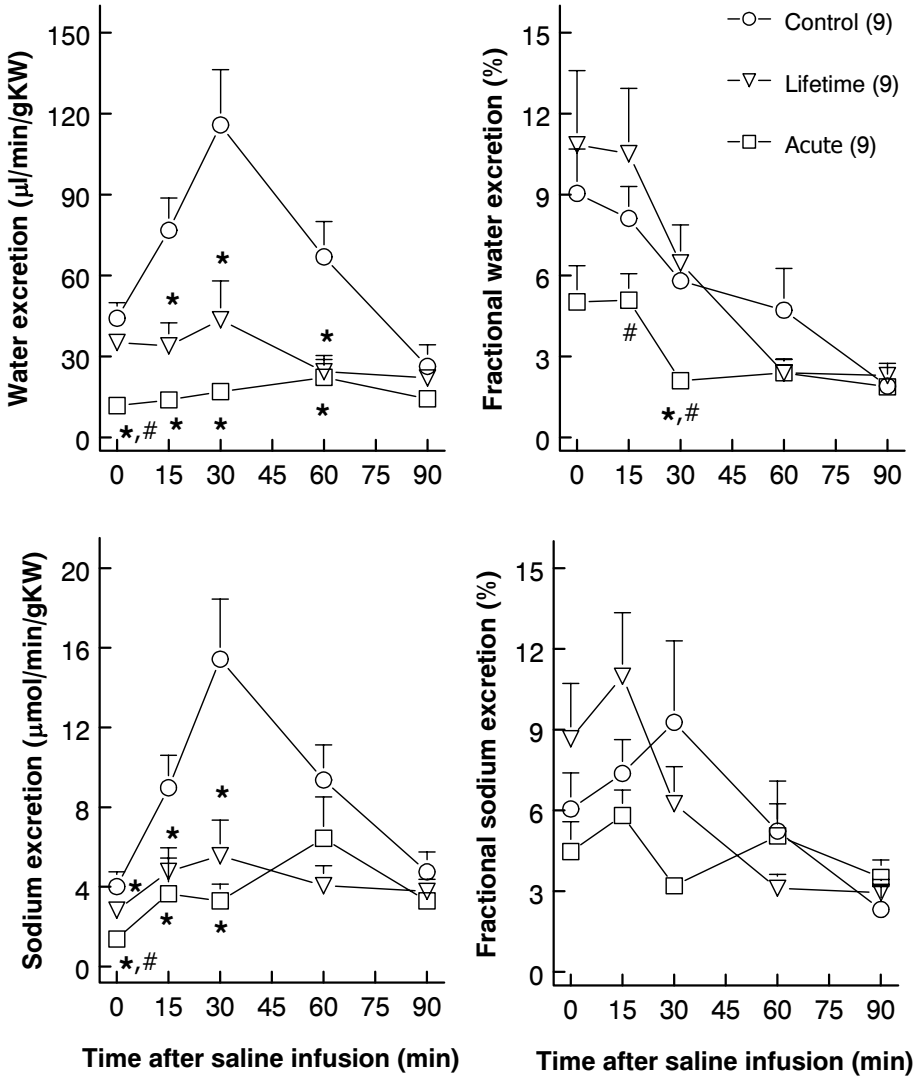

Figure 2. Both lifetime and acute perinatal captopril treatment blunted diuretic (top, right) and natriuretic (bottom, right) responses to acute isotonic saline loading, but the acutely treated group was more affected. Only the fractional water excretion of the acute group was significantly lower than the other groups (top, left) and there was no significant difference in fractional sodium excretion among groups throughout the experiment (bottom, left) (* denotes $p < 0.05$ compared to control; $^{\#}$ $p < 0.05$ compared between captopril treated groups).

Friberg, 2000). In the present study, lifetime inhibition of the RAS by oral captopril retarded body, heart, and kidney growth in adult conscious normotensive rats (Table 1). Surprisingly, acute, perinatal treatment with captopril only slightly decreased body weight and significantly increased kidney weight and kidney to body weight ratios. These and other data suggest that the rat may experience a rapid rebound overexpression of RAS actions after cessation of treatment.

Studies using transgenic mice have revealed that long-term, continuous RAS inhibition causes local up-regulation of some growth promoting factors in the kidney (including clusterin, TGF-1β, and PDGF-B (Niimura et al., 1995)) and up-regulation of the RAS (Nagata, Marukami, and Watanabe, 1997). Discontinuation of RAS inhibition appears to partly restore normal RAS

activity. Together these effects may result in the apparent renal hypertrophy in the acute group in the present study (Table 1). However, the present data cannot exclude the possibility that the observed increase in kidney weight and kidney-to-body weight ratio is due to water content. Tubular dilation and renal edema have been reported in mice deficient in the RAS (Friberg et al., 1994; McCausland et al., 1997). WKY rats are normotensive and salt-resistant but become salt-sensitive and hypertensive when treated with lifetime captopril from conception onward, and this effect persists even after the termination of RAS inhibition treatment (Fang et al., 1999). One explanation for this effect could be that as reported in other studies inhibition of the RAS in the early life results in adult structural damage and impairs the urinary concentrating function of the kidney (Guron and Friberg, 2000). Moreover, knockout mice in which the RAS is functionally deleted, display renal damage and diminish renal function (Carpenter et al., 1996). These effects appear to be mediated by AT_{1B} receptors rather than AT_{1A} (Ito et al., 1995) or AT_2 receptors (Oliverio et al., 1998). In contrast, in SHR lifetime inhibition of the RAS by oral captopril does not cause any apparent renal structural damage and improves renal function (Roysommuti et al., 2000).

It is interesting that in the present study both acute perinatal and chronic lifetime treatment with captopril blunted diuretic and natriuretic responses to an acute saline load in SD rats (Figure 2). Compared to the other groups, the lifetime-treated rats displayed significantly lower MAP and thus, their decreased renal function could be dependent on that change (Figure 1). In contrast, acute perinatal treatment resulted in no reduction in arterial pressure in off-captopril SD rats, suggesting that their impaired pressure-diuretic/natriuretic function in mature animals may be due to alterations in renal structure. Consistent with this interpretation, compared to the other two groups, the acute animals excreted less of the volume and sodium load during the initial 90 minutes (Figure 3). This suggests that the salt sensitivity induced in normotensive WKY rats by early inhibition of the RAS

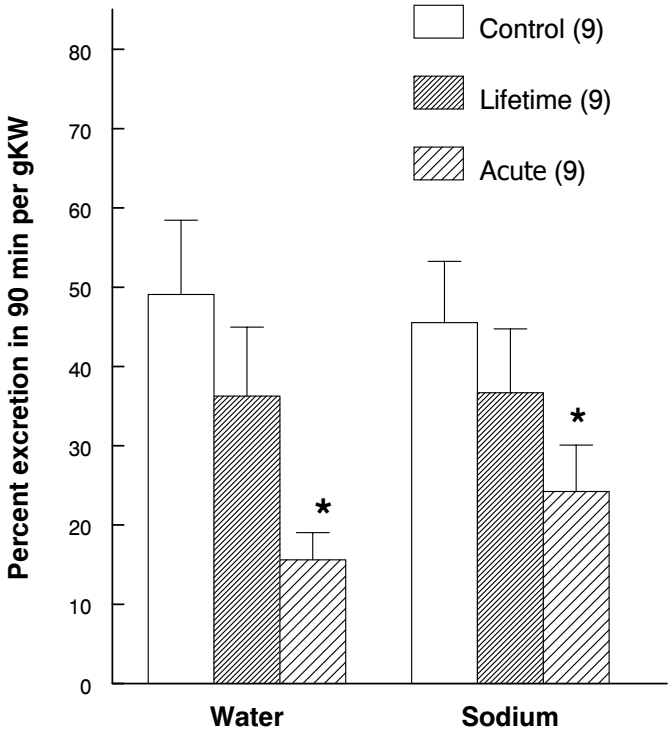

Figure 3. Water and sodium excretion (as percent of the isotonic saline load per gram of kidney weight) during the 90 minutes following acute isotonic saline infusion was significantly lower only in the acute perinatal captopril-treated group when compared to the control (*denotes $p < 0.05$ compared to control).

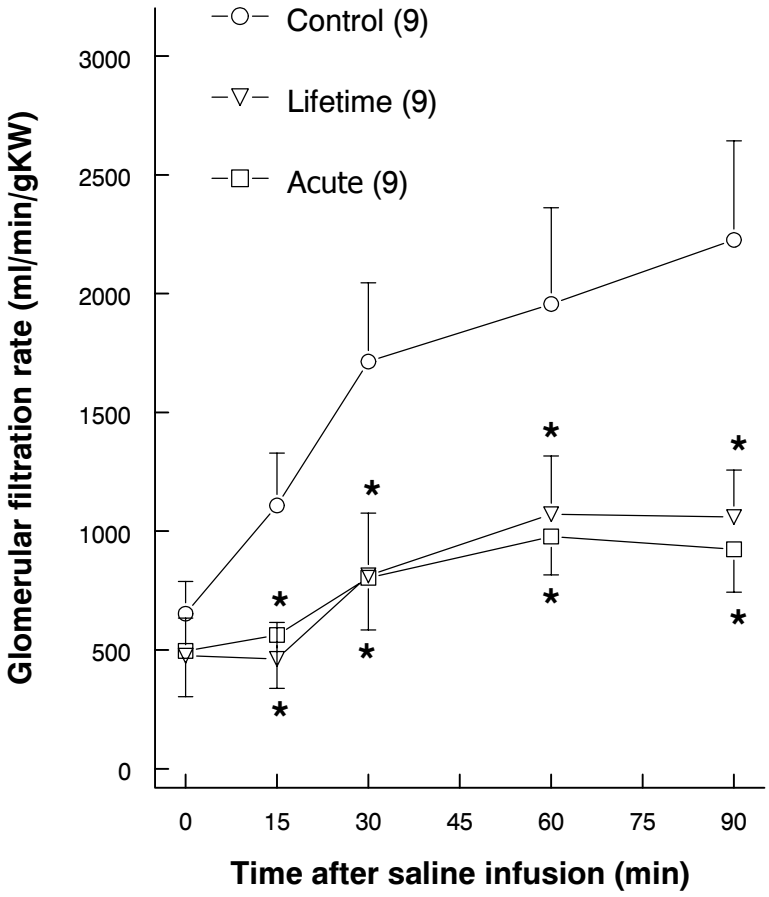

Figure 4. In both captopril-treated groups compared to controls, glomerular filtration rates following saline infusion was significantly suppressed, and there were no significant differences between the two treated groups (* denotes $p < 0.05$ compared to control).

in the early life (Fang et al., 1999) may be a consequence of blunted pressure-diuresis/natriuresis in these rats. This abnormality is consistent with salt sensitive SHR in which renal function is impaired (Mozaffari et al., 1991; Roman and Cowley, 1985), likely leading to an abnormality in plasma sodium regulation (Fang et al., 2000) and in other forms of salt-sensitive hypertension (Kimura, 1999).

In general, water and sodium excretion is modified by glomerular filtration rate and tubular reabsorption. In the present study, GFR of both captopril treated groups slowly and similarly increased and were lower than control throughout the study (Figure 4), and plasma sodium and potassium concentrations were not significantly different among the three groups. Thus, the blunted pressure-diuretic/natriuretic responses of the acute captopril-treated rats appear to have been due primarily to increases in tubular water and sodium reabsorption. This is supported by the decreased fractional water and sodium excretion in the acute treatment group (Figure 2). Withdrawal of the captopril treatment is likely followed by RAS overactivity due to a rebound increase in renin production (Kim et al., 1999) and/or the up-regulation of angiotensin receptors (Nagata, Murakami, and Watanabe, 1997) in response to the original RAS inhibition. Angiotensin II directly stimulates proximal water and sodium reabsorption and positively modulates ADH and

aldosterone secretion (Unger, 2000; Quan and Baum, 2000), both of which act primarily at the distal nephron to increase water and sodium reabsorption.

The reduced GFR responses in both treated groups of SD may have resulted from a reduction of glomerular filtration pressure (especially the glomerular hydrostatic pressure) and/or a change in glomerular surface area and/or number. Although retarded glomerular maturation is observed in mice lacking the RAS (Niimura et al., 1995), inhibition of the RAS in the early life appears to have little if any impact on glomerular maturation, but it does result in greater efferent than afferent arteriolar diameter in the mature rats (Friberg et al., 1994; McCausland et al., 1997). The RAS acts directly on renal arterioles and causes afferent and efferent arteriolar vasoconstriction. Overactivity of the RAS following cessation of acute treatment with captopril might induce greater afferent than efferent arteriolar constriction, i.e., increased afferent to efferent vascular resistant ratio, decreased glomerular hydrostatic pressure, and decreased GFR. Direct single nephron studies and measurements of glomerular hydrostatic pressure, renal blood flow, and renovascular resistance are needed to further elucidate these mechanisms.

In summary, the present data indicate that the RAS is very important to arterial pressure control and to renal growth and development in normotensive rats. Lack of the RAS in the early life appears to blunt renal pressure-diuretic/natriuretic function in young adult rats, especially after the termination of the captopril treatment. However, since the perinatal RAS treatment causes relatively long-lasting reductions in arterial pressure and longevity, irrespective of post-perinatal cessation of treatment, the functional importance of the renal changes induced by perinatal captopril treatment remains unclear.

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