Role of Citrate Synthase in Aldosterone-Mediated Sodium Reabsorption

Michael E. Ullian, Christopher J. Robinson, Claudia T.B. Evans, Joel Z. Melnick, Wayne R. Fitzgibbon

Abstract—Aldosterone and other mineralocorticoids increase citrate synthase activity in the kidney and enhance renal sodium reabsorption, but it is unclear whether the increased citrate synthase activity is involved in renal sodium transport. We used the Wistar-Furth rat, an inbred strain found to be deficient in renal citrate synthase activity, as an experimental model to investigate this issue. We confirmed that renal citrate synthase activity from adrenalectomized Wistar-Furth rats was decreased compared with that from control Wistar rats (by 28%). Similarly, urinary citrate excretion was 23% lower in Wistar-Furth rats. Subnormal citrate formation in Wistar-Furth rats could not be accounted for by differences in systemic pH or circulating potassium levels. Because renal citrate synthase activity was reduced in Wistar-Furth rats, we hypothesized that renal sodium excretory responses to mineralocorticoids would be reduced as well. Four-hour sodium excretion after intraperitoneal injection of 5 μg of aldosterone was reduced by 56% in adrenalectomized Wistar rats and by 52% in adrenalectomized Wistar-Furth rats (both P<0.01 compared with vehicle injection). Similarly, the pattern of urinary sodium excretion in response to subcutaneous injections of deoxycorticosterone acetate over a 2-week period was similar in adrenalectomized Wistar and Wistar-Furth rats. In summary, acute and chronic antinatriuretic responses to mineralocorticoids are maintained in Wistar-Furth rats at the level of Wistar rats, despite the marked reduction in citrate synthase activity. These findings are not consistent with an important role for citrate synthase activity in mineralocorticoid-mediated renal sodium transport. (Hypertension. 2000;35:875-879.)

Key Words: rats, Wistar-Furth ■ rats, Wistar ■ aldosterone ■ mineralocorticoids ■ sodium ■ deoxycorticosterone acetate

The mineralocorticoid aldosterone stimulates activity of certain key enzymes in the tricarboxylic acid cycle (eg, citrate synthase) in the rat kidney.1–5 Adrenalectomy reduces renal citrate synthase activity, and the administration of aldosterone to adrenalectomized animals restores citrate synthase activity.4 These findings arose from an ongoing effort to identify aldosterone-induced proteins (ie, proteins synthesized in response to the transcription factor activity of the occupied mineralocorticoid receptor) and to determine how these proteins mediate the classic action of mineralocorticoids: renal sodium reabsorption. It has been hypothesized that enhancement of citrate synthase activity increases ATP formation and, subsequently, activity of Na⁺,K⁺-ATPase and sodium reabsorption in the distal nephron. This schema is consistent with the “energy hypothesis” of aldosterone action.

However, the correlation of aldosterone-induced enhancement of renal citrate synthase activity with aldosterone-induced stimulation of sodium reabsorption has been investigated in only a few studies, and the results have been inconsistent. At 3 to 4 hours after aldosterone was injected into rats, both a maximum increase in renal citrate synthase activity and a maximum decrease in urinary sodium-to-potassium ratio were observed.3 Other studies have been performed in cultured cell lines. In toad bladder and toad kidney cells, aldosterone stimulated sodium transport without an increase in citrate synthase activity.6 In another study, aldosterone stimulated both vectorial sodium transport and citrate synthase activity in toad bladder cells, whereas aldosterone stimulated sodium transport in the absence of an increase in citrate synthase activity in A6 cells.7

In the present study, we used Wistar-Furth rats (WF) as an experimental model to investigate the role of citrate synthase activity in aldosterone-mediated sodium reabsorption. WF, which were bred from normal Wistar rats (W), have been shown to be resistant to the development of mineralocorticoid-excess hypertension.8–13 During the biochemical evaluation of the mechanisms that underlie this resistance to mineralocorticoids, we observed that basal and aldosterone-stimulated citrate synthase activities in whole kidney were markedly reduced in WF compared with W.13

Received August 19, 1999; first decision September 21, 1999; revision accepted November 21, 1999.

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This finding provided us with a unique opportunity to correlate renal citrate synthase activity with stimulated renal sodium transport. Therefore, we tested the hypothesis that in WF, a rat strain deficient in citrate synthase activity, renal sodium reabsorptive responses to mineralocorticoids are impaired.

Methods

Protocols

Study protocols were approved by the Medical University of South Carolina Institutional Animal Care and Utilization Committee and are consistent with NIH guidelines. Materials were obtained from Sigma Chemical except as specified. Rats (100 to 150 g) were obtained from Harlan Sprague-Dawley. A surgical plane of anesthesia was achieved with 50 mg/kg body wt pentobarbital IP. Rats were adrenalectomized via bilateral flank incisions and maintained on normal chow and 1% saline drinking water. Studies were performed at least 7 days after adrenalectomy. Conscious systolic blood pressure measurements were obtained from awake, warmed rats with an Natsume KN-2120-1 tail manometer-tachometer. Ten blood pressure measurements were obtained from each rat; the first 5 were discarded, and the remainder were averaged. Urine volume was determined gravimetrically. Protein content was determined according to Lowry’s method.14 Urinary sodium and potassium concentrations were determined with a flame photometer (Instrumentation Laboratories). Citrate concentration in the urine was measured enzymatically with a kit from Boehringer-Mannheim Biochemicals. Plasma pH was determined with a Chiron 865 blood gas analyzer.

Renal Citrate Synthase Activity

The formation of citrate from acetyl coenzyme A and oxaloacetate is catalyzed by citrate synthase, with coenzyme A-SH as a byproduct. Citrate synthase activity was quantified, as described previously,13 through measurement of the rate of generation of coenzyme A-SH as it reacted with 5,5'-dithio-bis(2-nitrobenzoic acid) (Ellman’s reagent) to form a yellow mercaptide ion, which was quantified spectrophotometrically. Then, 950 μL of substrate (0.25 mmol/L Ellman’s reagent, 0.2 mmol/L sodium oxaloacetate, 0.1 mmol/L acetyl coenzyme A, 100 mmol/L Tris-Cl buffer, pH 8.0) was added to 50 μL (50 μg protein) of enzyme source in a 1-cm-thick cuvette at room temperature. Absorbance at 412 nm was measured every 15 seconds for total of 7 to 10 minutes. Absorbance was converted to mg protein

\[ \text{mg protein} = \frac{A_{412} \times \text{velocity}}{\text{fluid volume} \times \text{thickness of reaction container}} \]

is molar absorbancy index (13,600 for the

\[ \text{m} \text{L} \text{mg mL}^{-1} \text{min}^{-1} \]

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The plot of concentration of the mineralocorticoid deoxycorticosterone acetate every third day. During the entire 21-day metabolic study period, urine output was collected daily.

Statistical Considerations

Group mean values were compared with the use of a 2-tailed, unpaired t test. Significant difference was assigned at the 0.05 level.

Results

Renal Citrate Synthase Activity

In our original study of WF, we observed that basal and aldosterone-stimulated citrate synthase activities measured in homogenates from whole kidney were significantly reduced in WF compared with W.13 This result has provided us with the opportunity to correlate citrate synthase activity with mineralocorticoid-stimulated sodium reabsorption. Before an evaluation of renal sodium reabsorptive responses to mineralocorticoids in WF in the present study, we further investigated renal citrate synthase biology in WF. Our original studies were repeated in a collaborating investigator’s laboratory (C.T.B.E.) such that the investigator was blinded to the strain being used. Three adrenalectomized rats from each strain were used as the source of renal tissue. Similar to our results published previously,13 whole kidney citrate synthase activity was found to be significantly decreased in WF (0.111, 0.084, and 0.076 μmol · mg protein⁻¹ · min⁻¹) compared with W (0.127, 0.123, and 0.125 μmol · mg protein⁻¹ · min⁻¹; P<0.05). To determine whether reduced citrate synthase activity resulted in reduced formation of citrate in the intact animal, urinary citrate excretion was measured in 6 rats from each strain. Figure 1 demonstrates that urinary citrate excretion was 23% lower in WF compared with W (P<0.05). The
reduced urinary citrate excretion is consistent with reduced renal parenchymal citrate synthase activity. Finally, we measured plasma pH and potassium concentration in W and WF, because acidemia or hypokalemia increases renal citrate metabolism and, consequently, reduces urinary citrate excretion. Figure 2 demonstrates that plasma pH and plasma potassium concentration did not differ between W and WF.

**Renal Sodium Handling**

Having confirmed that renal citrate synthase activity is reduced in WF compared with W, we assayed the classic mineralocorticoid action, sodium retention, in W and WF. In this way, we hoped to determine whether reduced renal citrate synthase activity correlates with reduced ability of aldosterone to stimulate sodium reabsorption in WF. Figure 3A demonstrates that 4-hour exposure to aldosterone elicited >50% reductions in urinary sodium excretion in both W and WF. Figure 3C similarly demonstrates that urinary sodium-to-potassium ratio was greatly reduced by aldosterone in both rat strains. Urine output was also reduced significantly by aldosterone exposure in W and WF (Figure 3D). In contrast, aldosterone treatment increased potassium excretion by 38% in W but did not stimulate kaliuresis in WF (Figure 3B).

Despite the fact that acute responses to aldosterone in renal sodium handling were similar in W and WF, we considered the possibility that more chronic natriuretic responses to mineralocorticoids might differ between W and WF. Therefore, we assessed sodium excretory responses to deoxycorticosterone acetate, administered subcutaneously every 3 days for 14 days, after a 7-day control period in pair-fed, adrenalectomized W and WF. Figure 4A demonstrates that daily sodium excretion (mg·g body wt⁻¹·day⁻¹) was greater in WF than in W on each of the 21 days of the study. The greater sodium excretion in WF follows from their reduced body weights (start of the study: W 244±5 g, W F1 271±4 g; end of the study: W 231±3 g, WF 230±3 g). In both strains, each injection of deoxycorticosterone acetate caused antinatriuresis after a 1-day lag period and then a rebound natriuresis (escape). The rebound prevented weekly cumulative sodium excretion from being reduced by mineralocorticoid exposure in either strain (Figure 4B). The degree of antinatriuresis was not less in WF compared with W. Conscious systolic blood pressure at the end of the study was similar between the strains (W 118±5 mm Hg, WF 118±3 mm Hg), arguing against differences in pressure natriuresis between strains. These results suggest that acute and chronic renal sodium transport responses to mineralocorticoids are not blunted in WF and do not support a role for enhanced citrate synthase activity in aldosterone-stimulated sodium reabsorption.

**Discussion**

The mineralocorticoid aldosterone has been shown to increase citrate synthase activity and transepithelial sodium transport, but it is unclear from the existing literature whether increased citrate synthase activity enhances sodium transport. It is possible that enhanced citrate synthesis activity results in increased ATP formation (substrate for N₅,K⁺-ATPase) and, consequently, increased activity of N₅,K⁺-ATPase and increased vectorial sodium transport. In an early study, citrate synthase activity was increased and urinary sodium excretion was decreased 3 to 4 hours after adrenalectomized rats were injected intraperitoneally with aldosterone. The similar time frames of the citrate synthase and sodium excretory responses suggest that a cause-and-effect relationship might exist between citrate synthase and sodium transport. However, several studies in aldosterone-responsive amphibian kidney and bladder cell lines have revealed enhanced sodium transport.
without increases in citrate synthase activity in response to aldosterone.⁶,⁷ The present study was performed in an attempt to clarify this conflicting literature. In this study, we used WF, a strain of rat that is deficient in basal and aldosterone-stimulated renal citrate synthase activity,¹³ to assess the role of citrate synthase in mineralocorticoid-stimulated sodium reabsorption. In contrast to prior experimental approaches in which aldosterone was administered and citrate synthase and sodium reabsorptions were measured, WF is an experimental model in which citrate synthase activity is reduced, both at baseline (50% less than in W) and after aldosterone administration (negligible responses to aldosterone compared with baseline (50% less than in W) and after aldosterone administration (negligible responses to aldosterone compared with baseline (50% less than in W)). Therefore, our results, both in this study and in the previous study,¹³ clearly demonstrate that renal citrate synthase is reduced in WF. Sodium reabsorptive responses to aldosterone are expressed in WF to the level of those in W but are not accompanied by significant increases in citrate synthase activity as in W. These results suggest that enhanced citrate synthase activity may not be necessary for the stimulation of sodium transport.

Several aspects of our experimental design warrant discussion. The intraperitoneal aldosterone administration (5 µg) for the acute sodium handling study was adapted from a standard aldosterone bioassay.¹⁵ The results of preliminary studies in normal rats (Sprague-Dawley) validated this technique in our hands (see Methods). It is possible that subtle defects in acute sodium reabsorption would have been detected in WF if multiple doses of mineralocorticoid had been administered over a chronic time frame. Therefore, the chronic mineralocorticoid metabolic study was undertaken. In this phase of the investigation, we opted for subcutaneous injections of deoxycorticosterone acetate every third day, because we used this method of mineralocorticoid administration in a previous study¹³ to demonstrate resistance to the development of mineralocorticoid-excess hypertension in WF. In that study, hypertension did not develop in the control rats (W) until week 3 of deoxycorticosterone acetate and salt treatment.¹³ Because rats were treated with deoxycorticosterone acetate and salt for only 2 weeks in the present study, it is not surprising that blood pressure was near normal in W and WF. An alternative method of administering a mineralocorticoid would have been the administration of aldosterone via an osmotic minipump. Chronic antinatriuresis in response to deoxycorticosterone acetate was not blunted in WF compared with W. In fact, antinatriuresis may have been greater in WF than in W during the first 6 days of mineralocorticoid treatment (Figure 4A).

The results of the present study also provide information on resistance to mineralocorticoids in WF. WF are resistant to the development of mineralocorticoid-excess hypertension.⁸–¹³ Because circulating aldosterone levels are increased in WF compared with W,⁹,¹² end organ resistance to aldosterone has been suspected. Resistance to the actions of aldosterone in the vasculature of WF has been clearly documented.⁹,¹³ However, whether the kidneys (the major mineralocorticoid target organ) of WF are resistant to aldosterone is unclear. Several groups have reported that WF become as hypokalemic in response to a chronic salt and mineralocorticoid regimen as normal W.⁹,¹³ In addition, the enhancement of vasopressin-stimulated cAMP accumulation in renal cortical collecting ducts, a well known response to mineralocorticoids, was not different in WF and W.¹⁰ The present study addresses the classic action of mineralocorticoids: sodium reabsorption. Figures 3 and 4 demonstrate that acute and chronic sodium reabsorptive responses to mineralocorticoids are not blunted in WF compared with W. Therefore, a number of studies are consistent in demonstrating that the reabsorptive element of the kidney (ie, tubules) responds normally to mineralocorticoids in WF. The inability of WF to mount an acute kaliuretic response to aldosterone like normal W (Figure 3B) is the first evidence of a renal resistance to mineralocorticoids in WF. However, this result is difficult to reconcile with the 2 studies that demonstrate full hypokalemic responses to chronic mineralocorticoid treatment in WF.⁹,¹³

These studies were not designed to pinpoint the organs involved in resistance to the development of mineralocorticoid-excess hypertension in W; such studies are ongoing in our laboratory. Certainly, existing data suggest that the enhancement of vascular tone by mineralocorticoids rather than the enhancement of sodium reabsorption by mineralocorticoids is deficient in WF. The molecular mech-
anisms that mediate this resistance (possibly receptor abnormalities, postreceptor intracellular signals, or even citrate synthase effects on the vasculature) remain undefined. In addition, the performance of studies in cells or tubular segments responsive to mineralocorticoids (eg, cortical collecting duct) will be necessary to confirm the conclusions from these whole-animal studies.

**Acknowledgments**

This work was supported by funds from the American Heart Association, South Carolina Affiliate, and Dialysis Clinic Incorporated. The authors thank Jana J. Fine for technical assistance.

**References**

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Hypertension. 2000;35:875-879
doi: 10.1161/01.HYP.35.4.875
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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