Dietary NaCl and KCl do not regulate renal density of the thiazide diuretic receptor

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Dietary NaCl and KCl do not regulate renal density of the thiazide diuretic receptor. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1241–R1245, 1997.—We tested the postulate that the renal density of the thiazide-inhibitable Na-Cl cotransporter or thiazide receptor (TZR) is modulated as part of the renal homeostatic response to changes in dietary intake of NaCl or KCl. Renal excretion of NaCl or KCl varied 10- to 100-fold in response to alterations in oral intake. Renal TZR density was quantitated by binding of [3H]metolazone to renal membranes. Renal TZR density was not altered by sodium deficit (with increased plasma aldosterone concentration), by sodium surfeit (8% NaCl content of diet), by potassium deficit (with hypokalemia), or by potassium surfeit (drinking 1% KCl solution). Unexpectedly, we conclude that regulation of the renal density of TZR is not part of the renal homeostatic responses that adjust excretion of NaCl and KCl to changes in dietary intake of NaCl or KCl.

METHODS

Male animals of the Sprague-Dawley rat strain, purchased from a commercial supplier, and of the Wistar-Kyoto (WKY) rat strain, purchased from Taconic Farms, were maintained in the University of California San Diego's (UCSD) animal care facility. All protocols and procedures were approved and monitored by the UCSD Animal Care Committee. Within each study, animals were of the same strain and age and, in addition, were matched by weight in control and experimental group(s).

Sodium. Sodium intake was manipulated in two separate experiments. In the first experiment, three groups of Sprague-Dawley animals were provided ad libitum for 7 days a sodium-deficient diet (TD 90228), which the supplier (Harlan Teklad) indicated contained 0.02% Na. Three different drinking solutions were provided ad libitum: distilled water to the low-sodium group, 0.1% NaCl to the mid-sodium group, and 1.0% NaCl to the high-sodium group. In the second experiment, lasting 4 wk, the NaCl content of the food itself differed; all animals (WKY strain) had free access to distilled water. The 1% NaCl group received ad libitum diet TD 90229, which the supplier (Harlan Teklad) indicated contained 1% NaCl (0.39% sodium), and the 8% NaCl group received ad libitum diet TD 90228, which the supplier (Harlan Teklad) indicated contained 8% NaCl (3.15% sodium).

Potassium. Potassium intake was manipulated in two separate experiments, each lasting 7 days. In the first experiment, two groups of animals with free access to distilled water were provided different diets. The control group received TD 8604 diet, containing 0.006% K, 0.1% Na, and 0.04% Cl, while the low-K group received “Potassium Deficient Diet, Rat & Mouse,” which the supplier (ICN Biomedicals) indicated contained 0.004% K, 0.1% Na, and 0.15% Cl. The intake of potassium in the drinking solution was the variable in the second potassium experiment. Both groups received ad libitum diet TD 8604 diet. The control group was provided free access to distilled water while the high-K group received ad libitum 1.0% KCl drinking solution.

A CHANGE IN ORAL INTAKE of sodium or potassium is followed by a change in the renal excretion of the respective cation, with half-lives of 21 and 28 h, respectively, so that intake and output are equalized within a few days, with only minor adjustments in total body content of the cation (27). Multiple mediator and effector systems acting on multiple nephron segments have been implicated in these important control systems. Few previous studies have examined the response of the distal convoluted tubule (DCT) to changes in intake of sodium, potassium, or chloride.

The DCT is the predominant locus of the thiazide diuretic receptor (TZR) or the Na-Cl cotransporter inhibitable by thiazide-type diuretics (2, 20, 21). The TZR plays a major role in the reabsorption of sodium and chloride by the DCT (10, 13, 17). Moreover, pharmacological inhibition of TZR results not only in decreased reabsorption of sodium and chloride, but also in increased tubular secretion of potassium (25). Thus adjustments in the renal density of TZR might contribute to the overall renal response to changes in intake of sodium, chloride, and potassium. This postulate is supported by observations that dietary sodium restriction increased the rate of sodium reabsorption in the DCT that is inhibitable by chlorothiazide (12) and that the renal density of TZR is both diminished by adrenalectomy and restored by administration of mineralocorticoid hormones (9, 24). However, direct examination of the effects of changes in intake of sodium chloride or potassium chloride on renal TZR density has not been reported. In this communication we report that changing the oral intake of NaCl or KCl more than 10-fold fails to produce significant changes in rat renal TZR density.

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Aldosterone. Sprague-Dawley male animals (8 wk of age) were provided adequate dietary sodium by ad libitum availability of water and diet (Teklad 8604). Osmotic minipumps (Alzet) implanted in an interscapular area via a dorsal incision under pentobarbital sodium anesthesia (50 mg/kg ip), as described previously (9), delivered aldosterone (or diluent) continuously at a rate of 1.05 µg·kg⁻¹·h⁻¹. Studies were conducted after aldosterone or diluent had been administered for 6 days.

At the end of each treatment period, animals were encouraged to void by gentle massage of the suprapubic area before being placed in individual metabolic cages without access to water or food. All urine was collected for a 2-h period, including any urine remaining in the urinary bladder at the end of the 2-h period, when the animals received pentobarbital sodium (50 mg/kg ip) anesthesia. At this time blood was also drawn from the aorta or heart into a heparinized syringe.
and analyzed within 30 s for the plasma concentrations of sodium and potassium (NOVA) and, in the exogenous aldosterone experiment, ionized calcium. The blood was centrifuged, and plasma was separated within 30 min of the time of collection of the blood. The plasma was later analyzed for chloride using a commercially available colorimetric assay (Sigma). Urine sodium and potassium were determined by ion-selective electrode (NOVA) and total calcium, creatinine, and chloride by commercially available colorimetric assays (Sigma). Urinary excretion rates are expressed as moles of ion per mole of creatinine. However, in the second, or high-potassium, experiment urine samples were lost in a laboratory accident before analysis for creatinine and chloride; therefore, only urinary concentrations of Na and K were available in this experiment. Plasma aldosterone concentration was determined in two experiments using a radioimmunoassay purchased from Diagnostic Products, Los Angeles, CA.

Renal thiazide receptor density was determined by saturation analysis of the binding of \([3H]\text{metolazone}\) to renal membranes. \([3H]\text{metolazone}\) was custom synthesized by Amersham. The binding assay for \([3H]\text{metolazone}\) was conducted as previously described in detail (1); whole kidneys were homogenized in 10 ml ice-cold 50 mM tris(hydroxymethyl)aminomethane (Tris)-PO4 buffer, pH 7.4, and membranes were prepared by centrifuging for 5 min at 600 g and the resulting supernatant twice at 45,000 g for 20 min. The final pellet was suspended at a protein concentration of 0.8–1.0 mg protein/ml in 50 mM Tris-PO4 buffer containing, in addition, 10^{-5} M acetazolamide and 10^{-5} M nicardipine (1). Binding of \([3H]\text{metolazone}\) to each membrane preparation was quantified in duplicate at six concentrations of \([3H]\text{metolazone}\), ranging from 0.313 to 10 nM. Specific binding of \([3H]\text{metolazone}\), as defined by displacement with 10^{-5} M hydroflumethiazone, was analyzed by the method of Scatchard to calculate the density and the dissociation constant of the binding using the EBDA program of McPherson (18). Protein was determined by the Bradford Coomassie blue method (6) with bovine gamma globulin as the standard.

Statistical analyses were conducted with the StatView program (Abacus Concepts, Berkeley, CA) using the unpaired t-test or analysis of variance followed by Fisher’s protected least-difference test. Statistical significance was assumed when the P value was 0.05 or less.

**RESULTS**

Sodium intake. Animals receiving three different intakes of sodium chloride excreted different quantities of sodium and chloride. The excretion of sodium was more than fivefold greater and the excretion of chloride more than tenfold greater in the high-sodium group (1% NaCl drinking solution) than in the low-sodium group (Table 1). The excretion of potassium was not altered by the changes in sodium intake. Urinary excretion of calcium was greater in the high-sodium group (Table 1). Plasma concentrations of sodium, potassium, and chloride were not affected by the salt intake (data not shown). Two indexes of plasma volume differed between the low-sodium group and the other two groups: plasma aldosterone was more than five times greater and body weight was significantly less in the low-sodium group (Table 1). The renal density of the TZR was not significantly altered by the variations in intake of sodium chloride lasting 7 days (Table 1).

Increasing the intake of NaCl content in food for 4 wk also caused greater than fivefold changes in the urinary excretion of sodium and chloride (Table 2) and increased urinary excretion of calcium. There was no effect on body weight or on the renal density of the TZR (Table 2).

**Aldosterone**. The plasma concentration of aldosterone was elevated over fivefold by administration of exogenous aldosterone (Table 3) to a level intermediate between the levels observed in the sodium-deplete and sodium-replete animals in the prior experiment (Table 1). The increase in body weight during the experimental period was greater in the aldosterone group than in the controls, although the absolute body weights were not different. Aldosterone administration decreased the plasma concentration of potassium and increased the plasma concentration of ionized calcium (Table 3). Urinary excretion of sodium and potassium were not significantly different, but urinary excretion of chloride and calcium were greater after treatment with aldosterone in this experiment. In this experiment in which dietary sodium and chloride were adequate, the renal density of the TZR was increased 40% by aldosterone (Table 3).

Potassium intake. Plasma concentrations of sodium and chloride were not altered by 7 days of potassium-deficient diet, but plasma potassium concentration was lowered (Table 4). In addition, the body weight of the

**Table 1. Effect of drinking NaCl**

<table>
<thead>
<tr>
<th>Body wt, g</th>
<th>Low (NaCl)</th>
<th>Mid (NaCl)</th>
<th>High (NaCl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>343 ± 3.1</td>
<td>359 ± 5.0*</td>
<td>369 ± 6.1*</td>
</tr>
<tr>
<td>Potassium</td>
<td>9.62 ± 1.53</td>
<td>15.7 ± 2.43</td>
<td>52.3 ± 5.37*</td>
</tr>
<tr>
<td>Chloride</td>
<td>25.9 ± 1.14</td>
<td>27.3 ± 2.90</td>
<td>21.0 ± 1.67</td>
</tr>
<tr>
<td>Calcium</td>
<td>4.13 ± 0.67</td>
<td>8.92 ± 1.72</td>
<td>54.5 ± 7.32*</td>
</tr>
<tr>
<td>Plasma aldosterone, nmol/l</td>
<td>6.2 ± 0.419</td>
<td>1.05 ± 0.155*</td>
<td>0.73 ± 0.110*</td>
</tr>
<tr>
<td>Thiazide receptor density, pmol/mg protein</td>
<td>0.567 ± 0.041</td>
<td>0.588 ± 0.058</td>
<td>0.470 ± 0.035</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 7 Sprague-Dawley rats/group). *Statistically different from low-sodium group at P ≤ 0.05 by analysis of variance and protected least-difference test.

**Table 2. Effect of dietary NaCl**

<table>
<thead>
<tr>
<th>Diet Intake</th>
<th>1% NaCl</th>
<th>8% NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>303 ± 14.2</td>
<td>304 ± 13.3</td>
</tr>
<tr>
<td>Sodium</td>
<td>28.4 ± 2.28</td>
<td>156 ± 4.57*</td>
</tr>
<tr>
<td>Potassium</td>
<td>18.7 ± 1.45</td>
<td>17.5 ± 0.814</td>
</tr>
<tr>
<td>Chloride</td>
<td>22.9 ± 2.38</td>
<td>154 ± 6.89*</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.505 ± 0.087</td>
<td>2.31 ± 0.208*</td>
</tr>
<tr>
<td>Thiazide receptor density, pmol/mg protein</td>
<td>0.857 ± 0.035</td>
<td>0.774 ± 0.031</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 12 Wistar-Kyoto rats/intake group). *Statistically different from 1% NaCl group at P ≤ 0.05.
K-deficient group did not change during the 7-day dietary period, whereas, the body weight of the control group increased by >20% (Table 4). Urinary excretion of potassium was nearly 20-fold greater in the control group, whereas urinary excretion of sodium and chloride was approximately two- and threefold greater, respectively. Neither the plasma concentration of aldosterone nor the renal density of the TZR differed significantly between the K-deficient and control groups (Table 4).

Increasing potassium intake by supplementing a normal laboratory chow with drinking solution containing 1% KCl resulted in increased urinary concentration of K, whereas the urinary concentration of Na remained unchanged (Table 5). Thus the high-K group excreted almost twice as much K (relative to Na) as did the control group. Plasma sodium, potassium, and chloride concentrations were not altered by the high-potassium diet. The renal density of the TZR was not significantly altered by high intake of potassium for 7 days (Table 5).

**DISCUSSION**

The distal convoluted tubule (DCT) reabsorbs sodium, chloride, and calcium (11, 14). A major portion of this sodium and chloride absorption can be via the thiazide-inhibitable Na-Cl symporter (10, 17), the activity of which is believed to influence secondarily the driving forces for absorption of calcium (7, 16). Reported studies on the regulation of the function of the DCT (as opposed to cultured cells of DCT origin) have been infrequent, probably because of the relative inaccessibility of the DCT to micropuncture. For example, Stanton and Kaissling (22) reported that, because DCT segments of “sufficient length for continuous microperfusion were rarely observed,” they were able to microperfuse only three DCT segments. These tubule segments reabsorbed sodium but neither reabsorbed nor secreted potassium. In addition, Stanton and Kaissling (22) found that changes in dietary potassium production produced no detectable changes in the ultrastructure of DCT cells. Ellison et al. (12) found that dietary sodium intake modulated the rate of sodium reabsorption inhibitable by chlorothiazide in the microperfused distal tubule. In composite, however, little information is available concerning the role of DCT function in sodium or potassium homeostasis.

We changed intake of NaCl or KCl over large ranges in the present studies. The lowest sodium diet resulted in sodium deficiency, as reflected by a lower body weight and an increase in plasma aldosterone level to >6 nmol/l at the end of the 7-day intervention (Table 1). Renal TZR density was not significantly altered by the sodium deficit (Table 1). The largest NaCl challenge, ingesting food containing 8% NaCl for 4 wk, increased urinary excretion of sodium more than fivefold above control but failed to alter significantly renal TZR density (Table 2). With respect to potassium, the low-potassium diet resulted in potassium deficit, as reflected by a lower body serum potassium concentration and failure of the animals to grow, but renal TZR density was not altered (Table 4). Similarly, in the potassium-surfeit group excretion of potassium (relative to sodium) approximately doubled, but the renal TZR density did not change significantly (Table 5).
The failure of large changes in intake of NaCl or KCl to change renal TZR density contrasts with the results of a number of interventions known to alter TZR. Previous studies from this and other laboratories have shown that, as measured by binding of \([3H]\)metolazone, renal TZR density can be regulated. TZR density is 1) dramatically decreased by only 10 min of ischemia or hypoxia and partially restored by reperfusion or reoxygenation (3), 2) increased within 60 min by administration of furosemide (19) or a thiazide diuretic (8), 3) increased in 12 h by salmon calcitonin (5) or amylin (4), 4) increased for a few days by metabolic alkalosis and decreased by metabolic acidosis (15), and 5) decreased over several days by adrenalectomy and restored in adrenalectomized animals to or above control levels by administration of aldosterone or glucocorticoid for a few days (9, 24). The failure of TZR density to increase in the presence of excess endogenous aldosterone in the present study (Table 2) created an apparent discrepancy with these previous reports that had found that administration of aldosterone (to animals ingesting a diet with ample Na) caused an increase in TZR density (9, 24). Therefore, the ability of exogenous aldosterone to influence TZR density was retested in the experiment shown in Table 3. Exogenous excess aldosterone, unlike endogenous aldosterone excess, increased TZR density, confirming previous reports (9, 24). The results presented in Tables 2 and 3 also illustrate other differences between animals with excess endogenous aldosterone (secondary to dietary sodium deficit) versus excess exogenous aldosterone (with adequate dietary sodium). Animals administered exogenous aldosterone probably have expanded total body sodium, because their increase in body weight was greater than in controls. Animals with excess endogenous aldosterone probably have restricted total body sodium, because their increase in body weight was less than in controls. Perhaps more importantly, administration of exogenous aldosterone (with adequate dietary sodium) resulted in reduction of the plasma concentration of potassium, increased plasma concentration of ionized calcium, increased urinary excretion of chloride, and increased urinary excretion of calcium (Tables 2 and 3). Thus animals with excess exogenous aldosterone (with adequate dietary sodium) differ in many respects from animals with excess endogenous aldosterone (with dietary sodium deficit). One possible explanation for the failure of endogenous aldosterone with restricted dietary sodium intake to increase TZR density is that the effect of aldosterone (with dietary sodium) on TZR is not a direct effect of the hormone on DCT cells, but rather is an indirect effect of aldosterone, perhaps requiring the presence of an expanded plasma volume. Our data do not enable testing of this postulate.

Studies of the thiazide-sensitive Na-Cl symporter (TZR), first with \([3H]\)metolazone (2) and later by in situ hybridization (20) and specific antibody (21), indicate that the TZR is predominantly localized to the luminal aspect of cells in the DCT. It should be cautioned that it is not known how accurately the renal density of TZR, as assessed by binding of \([3H]\)metolazone, reflects absolute rates of reabsorption of NaCl by the DCT. However, the fact that binding of metolazone is "competitive" (i.e., "mutually exclusive") with chloride (23) supports the concept that the number of binding sites for metolazone may reflect the number of sites available for transport of Cl (and hence Na) via the Na-Cl cotransporter. In each of the previous studies in which the renal density of TZR varied, as discussed previously, the density of the TZR correlated with renal excretion of, or with thiazide-induced changes in excretion of, sodium, chloride, or calcium. These correlations provide further support for the concept that changes in TZR density accompany changes in DCT function. Our data do not, however, provide information about potential changes either in the delivery of ions to the DCT or in the driving forces for moving ions via TZR during changes in dietary NaCl or KCl. Thus changes in the absolute rate of transport of NaCl via TZR could occur even in the absence of changes in renal density of TZR.

Urinary calcium excretion in the present studies varied directly with urinary sodium excretion (Tables 1 and 2), as expected (26). An increase in urinary calcium excretion would be expected to accompany increased reabsorption of NaCl via the TZR. Because we cannot eliminate the possibility that increased dietary intake of Na resulted in increased delivery of Na to the DCT, with resultant increased reabsorption of NaCl in the DCT in the presence of an unchanged complement of TZR, we cannot deduce whether the observed changes in calcium excretion occurred at the level of the DCT.

Perspectives

Changes in intake of NaCl and KCl are followed by prompt adjustment in the renal excretion of the ions so that intake and output are balanced within days without major changes in body content of the ions (27). The DCT’s absolute Na-Cl transport activity via the TZR is dramatically downregulated by adrenalectomy and restored to or above normal by replacement mineralo- or glucocorticoid (9, 24). One might expect, therefore, that the renal density of TZR would change in response to demands for altered excretion of NaCl or KCl. Unexpectedly, our findings are to the contrary: the density of TZR, as determined by saturation binding with \([3H]\)metolazone on renal membranes, is not altered when intake of NaCl and KCl are varied more than tenfold. These results raise the possibility that the increase in TZR produced by exogenous aldosterone (with adequate dietary sodium) is not a direct effect of the hormone on DCT cells.

In conclusion, the renal density of the DCT’s thiazide-inhibitable Na-Cl cotransporter is not normally involved in the renal homeostatic responses that adjust renal excretion of NaCl and KCl to large changes in dietary intake of NaCl or KCl.
REFERENCES


