Impaired urinary concentrating ability and cyclic AMP in K+-depleted rat kidney

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MATERIALS AND METHODS

Sprague-Dawley rats weighing 200-250 g were K+-depleted by feeding a low-K+ diet 15 g/day for 3-6 wk. The diet was composed of 64.2 g cornstarch, 30 g casein, 3.5 g butterfat, 53 meq Mg++, 16 meq Ca++, 17 meq Na+, and less then 0.2 meq K+/100 g diet. Control rats received the same diet supplemented with KCl 53 mmol/100 g diet. K+-repleted rats were prepared by feeding high-K+ diets (KCl 53 mmol/100 g) for 7 days to previously K+-depleted rats which had been prepared by feeding the same low-K+ diet for 4 wk. For acute K+ repletion, KCl 7.5 μmol/min was infused into the K+-depleted rats for 90 min immediately before the experiments.

In vivo experiments. Each rat received an intraperitoneal injection of 15 ml of a solution containing 15 meq inulin and 7 mg sodium pentobarbital, which was adjusted to 160 mosmol/kg with glucose. For the collection of urine samples, polyethylene catheters were placed in the inferior vena cava through a femoral vein for intravenous infusion, in the contralateral femoral vein for inulin clearance using the conventional technique (28) therefore, evaluated during that period.

In the previously reported experiments (3), the maximal effect was observed from 10 to 20 min after the injection of vasopressin. The effect of vasopressin was, therefore, evaluated during that period.

Glomerular filtration rate (GFR) was measured by inulin clearance using the conventional technique (28), and osmolality with a Fisk ice-freezing point osmometer. Na+ and K+ were measured with an Instrumentation Laboratory flame photometer, and Mg++ with an atomic absorption spectrophotometer. Cyclic AMP in urine

IN K+ DEPLETION, the impairment of urinary concentrating ability is a prominent and consistent finding in both man (20, 27) and experimental animals (13, 15), including the rat (1, 8, 18). Two major mechanisms have been proposed to explain the urinary concentrating defect in K+-depletion. The first is an alteration of the countercurrent mechanism induced by changes in tubular Na+ reabsorption (10, 21, 30), and the second is an impairment of water permeability in the collecting duct (1, 12, 17). These two mechanisms do not necessarily conflict. In experiments in the toad bladder (7, 11, 30), the increase in water permeability induced by vasopressin was significantly less in K+-depleted preparations, supporting the second hypothesis. From data obtained from the isolated rabbit collecting duct, Grantham and Burg (10) and Orloff and Handler (24) postulated that the increase in water permeability induced by vasopressin is mediated through cyclic AMP. It is then reasonable to postulate that the impairment of urinary concentrating ability in K+ depletion may be mediated through an alteration of vasopressin-dependent cyclic AMP in the kidney. This possibility was evaluated in the rat, and the results support this hypothesis.
specimens was measured by the method of cyclic AMP-binding protein, as described by Gilman (14), with modification (4). The validity of the cyclic AMP assay method has been evaluated (4).

K+ concentrations in muscle and kidney tissues were measured by homogenizing the preweighed tissue slices in glass-distilled water and by measuring K+ and protein concentrations in the homogenates. Protein was measured by Lowry's method (20). Tissue K+ concentration was then expressed as milliequivalents K+ per kilogram protein.

Cyclic AMP in tissue slices. Rats were prepared as described in the in vivo studies. After water diuresis had been established, kidneys were rapidly removed, and the medulla, including both outer and inner portions (9), was separated from the cortex. The medulla was sliced to a thickness of less than 0.5 mm with a Stadic-Riggs microtome. Each slice was divided in two: one half for the basal and the other half for the vasopressin group. Each slice was weighed and then incubated in Krebs-Ringer bicarbonate solution. The slices obtained from control rats were incubated in media containing K+ 5 meq/liter, and the slices obtained from K+-depleted rats were incubated in media containing K+ 2.5 meq/liter in order to make the concentration of K+ in the incubation media comparable to the respective plasma K+ concentrations in vivo. In the vasopressin group, vasopressin was added to the incubation media (2).

After 10 min incubation at 37°C, the slices were homogenized with a glass tissue grinder in 0.5 ml of ice-cold glass distilled water. The homogenization required less than 15 s. The homogenates were placed in a boiling-waterbath for 3 min to terminate the enzyme activity and to aggregate the tissue proteins, and were then centrifuged at 700 × g for 15 min. Cyclic AMP in the supernatant was assayed as described by Gilman (14), with modification (4).

To validate the method terminating the enzyme activity, the renal cortical slices were homogenized and boiled as described above in the first group. In the second group, the slices were homogenized in cold 5% trichloroacetic acid (TCA), and the TCA was extracted by water-saturated ethyl ether. In the first group, the basal cyclic AMP concentrations were 2.5 ± SE 0.2 pmol/mg tissue, and 6.5 ± 0.6 in the presence of vasopressin 5 mU/ml; and in the second group, the concentrations were 2.6 ± 0.2 and 6.7 ± 0.5, respectively, P > 0.05 between the two groups, suggesting that the two procedures are comparable.

Sprague-Dawley rats were supplied by Zivic-Miller Laboratory (Allison Park, Pa.), [3H]cAMP 25 Ci/mmol by New England Nuclear Corp. (Boston), arginine-vasopressin by Parke-Davis Co. (Detroit), and the low-K+ diet by Nutritional Biochemicals Corp. (Cleveland).

For statistical analysis, the Student two-tailed t test was used, for paired or unpaired samples, as dictated by the experimental design.

RESULTS

In vivo experiments. In rats fed a low-K+ diet, K+ concentration in plasma, muscle, and renal medullary homogenates were significantly lower than those in control rats, P < 0.01 for each (Table 1). In contrast, plasma Na+ and osmolality were significantly higher in rats fed a low-K+-diet, both P < 0.05. Plasma Mg++, an important cofactor for cyclic AMP synthesis, was not measurably different between the two groups of rats, P > 0.05.

Glycemic filtration rate measured by inulin clearance was not measurably different between the two groups of rats, and GFR did not change during the experiments, P > 0.05 (Table 2). Before the vasopressin injection, both urine osmolality (Uosm) and free water clearance (C(w)free) were not measurably different between the two groups of rats, P > 0.05 (Table 2). However, 10–20 min after the injection of vasopressin (1 mU/rat), both Uosm and negative C(w)free, and the changes in those values by vasopressin (Δ by VP) were significantly less in K+-depleted rats than in control rats, P < 0.05 for each (Table 2).

In contrast to a significant increase in urinary cyclic AMP excretion in response to a small dose of vasopressin (1 mU/rat) in control rats, the same dose of vasopressin failed to increase measurably urinary cyclic AMP excretion in K+-depleted rats, P > 0.05 (Table 3). These results suggest that both the antidiuretic response and the increase in urinary cyclic AMP excretion in response to vasopressin (1 mU/rat) were impaired in K+-depleted rats.

TABLE 1. Effects of low-K+ diet on plasma and tissue electrolytes

<table>
<thead>
<tr>
<th></th>
<th>Control Diet</th>
<th>Low-K+ Diet</th>
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<tbody>
<tr>
<td></td>
<td>(8)</td>
<td>(8)</td>
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<tr>
<td>Plasma K+, meq/liter</td>
<td>5.1 ± 0.2</td>
<td>2.6 ± 0.2*</td>
</tr>
<tr>
<td>Muscle K+, meq/kg protein</td>
<td>438 ± 24</td>
<td>246 ± 18*</td>
</tr>
<tr>
<td>Renal medullary tissue K+, meq/kg protein</td>
<td>322 ± 24</td>
<td>261 ± 3*</td>
</tr>
<tr>
<td>Plasma Na+, meq/liter</td>
<td>138 ± 1</td>
<td>141 ± 1*</td>
</tr>
<tr>
<td>Plasma osmolality, mosmol/kg</td>
<td>294 ± 4</td>
<td>311 ± 6*</td>
</tr>
<tr>
<td>Plasma Mg++, meq/liter</td>
<td>1.60 ± 0.16</td>
<td>1.72 ± 0.08</td>
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</table>

Values are means ± SE. Number in parentheses are numbers of rats studied. *P < 0.01 compared to the corresponding values in the rats fed the control diet. tP < 0.05 compared to the corresponding values in the rats fed the control diet.

TABLE 2. Effects of K+ depletion on urinary concentrating ability in rats

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Before VP</th>
<th>After VP</th>
<th>Changes by VP</th>
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<tbody>
<tr>
<td>Control rats</td>
<td>8</td>
<td>1.62 ± 0.12</td>
<td>1.63 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>GFR, ml/min</td>
<td></td>
<td>1.52 ± 0.15</td>
<td>1.62 ± 0.14</td>
<td></td>
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<tr>
<td>Urine osmolality, mosmol/kg</td>
<td>122 ± 16</td>
<td>496 ± 48</td>
<td>374 ± 51</td>
<td></td>
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<tr>
<td>C(w)free, ml/min</td>
<td></td>
<td>523 ± 84</td>
<td>626 ± 145</td>
<td>Δ-1.149 ± 129</td>
</tr>
<tr>
<td>K+-depleted rats</td>
<td>8</td>
<td>1.54 ± 0.15</td>
<td>1.62 ± 0.14</td>
<td></td>
</tr>
<tr>
<td>GFR, ml/min</td>
<td></td>
<td>1.54 ± 0.15</td>
<td>1.62 ± 0.14</td>
<td></td>
</tr>
<tr>
<td>Urine osmolality, mosmol/kg</td>
<td>153 ± 15</td>
<td>385 ± 35*</td>
<td>Δ339 ± 31*</td>
<td></td>
</tr>
</tbody>
</table>

Values are the means ± SE. n = number of rats studied. Before VP, mean of three 10-min samples before vasopressin injection; after VP, 10–20-min samples after vasopressin injection; 1 mU/rat; change by VP, (after VP) - (before VP). *P < 0.05 compared to the corresponding values in control rats.
After an injection of a larger dose of vasopressin (10 mU/rat), there was a small but statistically significant increase in urinary cyclic AMP excretion in K+-depleted rats, \( P < 0.01 \) by paired analysis (Table 3). However, the increase was still significantly less in K+-depleted rats than in control rats, \( P < 0.01 \). These findings suggest that, in these K+-depleted rats, there is a partial rather than a complete impairment of the vasopressin-dependent cyclic AMP system.

In K+-repleted rats, prepared by feeding high-K+ diets (KCl 53 mmol/100 g diet, approx. 0.8 mmol/day) for 1 wk to K+-depleted rats, plasma K+ (5.3 ± 0.2 meq/liter) was not measurably different from that in control rats (5.1 ± 0.2) \( P > 0.05 \). Also, the antidiuretic response and the increase in urinary cyclic AMP excretion in response to vasopressin (10 mU/rat) were no longer measurably different from those in control rats, \( P > 0.05 \) (Table 4).

In contrast, an acute infusion of KCl 7.5 \( \mu \)mol/min for 90 min into the previously K+-depleted rats elevated plasma K+ to 6.4 ± 0.4 meq/liter (\( P < 0.01 \) compared to control rats). However, the KCl infusion failed to correct both the impaired antidiuretic response and the impaired increase in urinary cyclic AMP excretion in response to vasopressin (10 mU/rat). In addition, these responses to vasopressin were still significantly less in acutely K+-repleted rats than in control rats, \( P < 0.01 \) (Table 4). These results suggest that there is a reversibly inhibited the vasopressin-dependent cyclic AMP system in K+-depleted rat kidneys.

**Cyclic AMP in tissue slices.** The possible impairment of the vasopressin-dependent cyclic AMP system in K+-depleted rats, suggested by the data on urinary cyclic AMP excretion, was further investigated in vitro. Both intracellular and extracellular K+ concentrations were kept similar to those in the in vivo experiments by obtaining renal medullary slices from control and K+-depleted rats and by incubating these slices in media containing K+ 5 and 2.5 meq/liter, respectively, to be comparable to the corresponding plasma K+ concentrations in vivo. In this in vitro experiment, the basal cyclic AMP concentration in renal medulla was not measurably different between the slices obtained from control rats and those obtained from K+-depleted rats, \( P > 0.05 \). However, the increase in cyclic AMP concentration induced by vasopressin (1 mU/ml) was significantly less in the slices obtained from K+-depleted rats than in those obtained from control rats, \( P < 0.01 \) (Fig. 1). These results are consistent with the findings of urinary cyclic AMP excretion in vivo and in addition suggest an impairment of the vasopressin-dependent cyclic AMP system in the K+-depleted kidney.

**DISCUSSION**

Plasma K+ concentration was significantly lower, but the plasma Na+ and osmolality were significantly higher in rats fed a low-K+ diet than in control rats. This fact suggests that the lower plasma K+ concentration in rats fed a low-K+ diet is due to K+ depletion rather than hemodilution. Higher plasma Na+ concentration and plasma osmolality in K+-depleted rats suggest that there was an inappropriate loss of free water due to the urinary concentrating defect in K+ depletion (26, 27).

An antidiuretic response to exogenous vasopressin measured by an increase in urinary osmolality and by negative free water clearance was significantly less in...
K⁺-depleted rats than in control rats, a fact that suggests an impairment of the urinary concentrating ability in K⁺-depleted rats. The increase in urinary cyclic AMP excretion in response to vasopressin injection was also significantly less in K⁺-depleted rats. These findings suggest that, in K⁺-depleted rats, there is an impairment of the vasopressin-dependent cyclic AMP system in the kidney.

Urinary cyclic AMP excretion rate may, however, be increased by various mechanisms independent of the activation of vasopressin-dependent cyclic AMP generation in the kidney (4-6, 19, 23). Therefore, the basal urinary cyclic AMP excretion may not exclusively reflect the status of vasopressin-dependent cyclic AMP in the kidney. Consequently, the reduction in basal cyclic AMP excretion in K⁺-depleted rats in the present experiments does not necessarily indicate an inhibition of vasopressin-dependent cyclic AMP generation in the kidney. The increase in urinary cyclic AMP excretion in response to exogenous vasopressin, however, specifically represents an increase in vasopressin-dependent cyclic AMP. In measuring that increase in cyclic AMP excretion, a small dose of vasopressin (1 mU/rat) significantly increased urinary cyclic AMP excretion in control rats, but the same dose of vasopressin failed to increase measurably urinary cyclic AMP excretion in K⁺-depleted rats. These findings are consistent with the hypothesis that there is an impairment of vasopressin-dependent cyclic AMP generation in K⁺-depleted rats, and with the in vivo findings (26, 27) that the urinary concentrating defect in K⁺-depletion is resistant to vasopressin.

In contrast to the absence of a measurable increase in urinary cyclic AMP excretion in response to 1 mU vasopressin/rat, there was a small but statistically significant increase in urinary cyclic AMP excretion in response to a larger dose of vasopressin (10 mU/rat) in K⁺-depleted rats. However, this increase was still significantly less in K⁺-depleted rats than in control rats. These findings suggest that in K⁺-depleted rats there is a partial, rather than a complete, impairment of both the antiidiuretic response and the vasopressin-dependent cyclic AMP generation.

In K⁺-repleted rats, fed a high-K⁺ diet for 1 wk, the impairment of both the concentrating mechanism and the increase in urinary cyclic AMP excretion in response to vasopressin (10 mU/rat) were no longer demonstrable. An acute infusion of KCle for 90 min to K⁺-depleted rats, however, failed to correct these abnormalities, although plasma K⁺ concentration was higher in KCle-infused rats than in chronically K⁺-repleted rats. These findings suggest that an acute elevation of extracellular K⁺ concentration alone does not necessarily reverse the urinary concentrating defect and the impaired vasopressin-dependent cyclic AMP generation in the K⁺-depleted rat kidney. Because K⁺ is the principal intracellular cation, it is possible that the acute infusion of KCle for 90 min did not correct the intracellular K⁺ depletion.

It has been shown (6, 22) that K⁺ depletion impairs the parathyroid hormone (PTH)-dependent cyclic AMP system in renal cortex and the phosphaturic response to PTH in the kidney and that K⁺ is an important cofactor in many enzyme systems including ATP generation (22). Therefore, the observed impairment of cyclic AMP generation in K⁺ depletion in the present experiments is probably not unique to vasopressin.

The hypothesis of an impairment of the vasopressin-dependent cyclic AMP system in the kidneys of K⁺-depleted rats was further evaluated in vitro because there are several other factors that may alter urinary cyclic AMP excretion, e.g., a) an increase in the filtered load of cyclic AMP may increase urinary cyclic AMP excretion (19); b) other hormones may also increase cyclic AMP excretion, such as PTH (5), catecholamines (4), prostaglandins (4), and thyrocalcitonin (23), and K⁺ depletion may affect these hormonal actions; and c) K⁺ depletion may affect cyclic AMP excretion through mechanisms unrelated to vasopressin-dependent cyclic AMP generation, e.g., alteration in reabsorption or diffusibility of cyclic AMP across tubular epithelium. However, in vitro, the effect of K⁺ depletion itself on the vasopressin-dependent cyclic AMP system can be directly investigated in renal medulla.

Although medullary slices were incubated in vitro, these slices obtained from the two groups of rats (control vs. K⁺-depleted rats) may retain many other endogenous hormones which affect cyclic AMP generation (3, 4, 23). Therefore, the basal cyclic AMP concentration in these slices does not exclusively reflect the status of the vasopressin-dependent cyclic AMP system in renal medulla, and these basal values should be interpreted with caution. However, the increases in cyclic AMP concentration in response to exogenous vasopressin added to the incubation media specifically represent the vasopressin-dependent cyclic AMP in renal medulla, and these increases were significantly less in the slices obtained from K⁺-depleted rats. These results are consistent with the findings of urinary cyclic AMP excretion in vivo and suggest that in K⁺ depletion there is indeed an impairment of the vasopressin-dependent cyclic AMP system in the kidney.

Pawson et al. (25) evaluated the effect of K⁺ depletion by measuring cyclic AMP concentration in renal medullary slices obtained after the injection of a large dose of vasopressin in vivo. In contrast, in the present experiments, the effect of K⁺ depletion was evaluated by measuring the increase in cyclic AMP concentration in response to a much smaller dose of vasopressin added to the incubation media. Therefore, the data in these two series of experiments are difficult to compare.

Results of both in vivo and in vitro experiments together consistently support the hypothesis that, in K⁺ depletion, there is an impairment of the vasopressin-dependent cyclic AMP system in the kidney and that this impairment plays a pathogenic role in the urinary concentrating defect observed in K⁺ depletion. These findings further support the hypothesis (1, 7, 11, 12, 17, 30) that, in K⁺ depletion, there is an impairment of water permeability across the collecting duct. This view, however, neither conflicts with, nor excludes, other possible mechanisms (10, 21, 29).
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