Endogenous vasopressin regulates Na-K-ATPase and Na\(^+\)-K\(^+\)-Cl\(^-\) cotransporter \textit{rbsc-1} in rat outer medulla

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Bertuccio, Claudia A., Fernando R. Ibarra, Jorge E. Toledo, Elvira E. Arrizurieta, and Rodolfo S. Martin. Endogenous vasopressin regulates Na-K-ATPase and Na\(^+\)-K\(^+\)-Cl\(^-\) cotransporter \textit{rbsc-1} in rat outer medulla. \textit{Am J Physiol Renal Physiol} 282: F265–F270, 2002; 10.1152/ajprenal.00354.2000.—Previous reports have shown a stimulatory effect of vasopressin (VP) on Na-K-ATPase and \textit{rbSC-1} expression and activity. Whether these VP-dependent mechanisms are operating in vivo in physiological conditions as well as in chronic renal failure (CRF) has been less well studied. We measured ATPase expression and activity and \textit{rbSC-1} expression in the outer medulla of controls and moderate CRF rats both before and under in vivo inhibition of VP by OPC-31260, a selective V\(_2\)-receptor antagonist. OPC-31260 decreased Na-K-ATPase activity from 11.2 \pm 1.5 to 3.7 \pm 0.8 in controls \((P < 0.05)\) and from 19.0 \pm 0.8 to 2.9 \pm 0.5 \text{nmol P\(_1\)/mg protein}\(^{-1}\)h\(^{-1}\) in moderate CRF rats \((P < 0.05)\). CRF was associated with a significant increase in Na-K-ATPase activity \((P < 0.05)\). Similarly, CRF was also associated with a significant increase in Na-K-ATPase expression to 164.4 \pm 21.5\% compared with controls \((P < 0.05)\), and OPC-31260 decreased Na-K-ATPase expression in both controls and CRF rats to 57.6 \pm 9.5 and 105.3 \pm 10.9\%, respectively \((P < 0.05)\). On the other hand, OPC-31260 decreased \textit{rbSC-1} expression in both controls and CRF rats to 60.8 \pm 6.5 and 30.0 \pm 6.9\%, respectively \((P < 0.05)\), and was not influenced by CRF \((95.7 \pm 5.2\%)\). We conclude that 1) endogenous VP modulated Na-K-ATPase and \textit{rbSC-1} in both controls and CRF; and 2) CRF was associated with increased activity and expression of the Na-K-ATPase in the outer medulla, in contrast to the unaltered expression of the \textit{rbSC-1}. The data suggest that endogenous VP could participate in the regulation of electrolyte transport at the level of the outer medulla.

vasopressin; \textit{rbsc-1}; sodium-potassium-adenosine 5’-triphosphatase; chronic renal failure

There is strong evidence that vasopressin (VP) stimulates Na-K-ATPase and Na\(^+\)-K\(^+\)-Cl\(^-\) cotransporter in the kidney outer medulla and, more precisely, in the medullary thick ascending limb of Henle (mTAL) (15). However, the experimental designs so far have been mostly related to the administration of exogenous VP, to either intact animals or in vitro preparations.

The question of whether the level of endogenous VP activity plays a homeostatic role at the kidney outer medulla has not yet been examined. The introduction of powerful VP inhibitors has made it possible to design experiments looking at that question. OPC-31260 is a nonpeptide V\(_2\)-receptor inhibitor developed by Yamamura et al. (21), and its actions are limited to states where VP activity is present. This approach was recently used by Ohara et al. (18), who were able to show a VP-mediated upregulation of the aquaporin-2 water channel in pregnant rats by using OPC-31260.

In addition, previous experiments from our laboratory and those of others have suggested that in chronic renal failure the remaining nephrons suffer diverse adaptive mechanisms. Thus Bertuccio et al. (1, 2) showed an increase in intracellular AMP levels in microdissected mTAL segments in chronic renal failure (CRF) rats, which were unresponsive to VP in vitro, opposed to baseline lower levels and a dose-response stimulation in control animals (1, 2). Alternatively, Kwon et al. (16) demonstrated compensatory increases in Na\(^+\)-K\(^+\)-Cl\(^-\) cotransporter expression per nephron in distal segments of the remnant kidney model (16). These results are compatible with the notion of an augmented delivery of sodium and water to distal segments in CRF, caused by an increase in filtered load and a decrease in the fractional reabsorption in proximal tubule (4). Therefore, the question of whether endogenous VP also regulates sodium transporters at the outer medullary level in CRF, and if so, to what extent, is of interest.

In this study, we performed experiments in homogenates of outer medulla of control and moderate CRF rats to answer the following questions: 1) does endogenous VP regulate the Na-K-ATPase and Na\(^+\)-K\(^+\)-Cl\(^-\) cotransporters in rats with normal renal function as well as in CRF? and 2) are Na-K-ATPase and Na\(^+\)-K\(^+\)-Cl\(^-\) cotransporters modified by CRF? The results primarily showed that endogenous VP modulates Na-K-ATPase protein expression and activity and \textit{rbSC-1} expression in the outer medulla, both in health as well as in a model of CRF.

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METHODS

Materials. Male Wistar rats (Animal Care Laboratory, Alfredo Lanari Institute of Medical Research, Buenos Aires, Argentina), weighing 250–350 g, with either normal renal function or moderate CRF, on a free diet (Ganave, Buenos Aires, Argentina) containing 24% protein, 0.5% NaCl, and 1.25% potassium, tap water ad libitum, and under controlled room temperature and light conditions, were used. The protocol was approved by the local Committee for Animal Research. Renal insufficiency was produced by suppressing a major portion of renal tissue, as described (2). Briefly, the left kidney was dipped in hot water for 14 s to burn the outer cortex. After a 7-day recovery, the right kidney was removed and the rat was studied 1 wk later. Previous studies in our laboratory (19) have demonstrated that this procedure reduces overall inulin clearance to roughly 50% of control levels, resulting in the loss, predominantly, of cortical nephrons.

Experimental protocol. The experimental protocol is depicted in Fig. 1. Control and CRF rats were housed individually in metabolic cages on the day of reduction of renal mass of the left kidney and 7 days before the right nephrectomy (see Materials). Four days after the right nephrectomy, the animals received saline or the nonpeptide V₂-receptor antagonist 5-dimethylamino-1-[4-(2-methylbenzoylamino)-benzoyl]-2,3,4,5-tetra-hydro-1H-benzazepine hydrochloride (OPC-31260; kindly provided by Otsuka Pharmaceutical, Tokyo, Japan). OPC-31260 was given by subcutaneous injection (15 mg/kg twice a day for 3 days for a total of 6 injections). This dose has been proven to inhibit VP effects in normal rats (18). Body weight was measured every 12 h for the mean of all observations. The urine was collected, the volume maintained on ice during dissection. Trunk blood was collected, and kidneys were harvested and experiment and 4 h before being killed by decapitation. The outer medulla, containing both outer and inner stripe, was isolated and homogenized with 300 μl of buffer containing (in mM) 20 Tris, 2 EGTA, 2 EDTA, 1 phenylmethylsulfonyl fluoride (PMSF), and 10 β-mercaptoethanol as well as 100 KIE/ml aprotinin (pH 7.4). The sample was stored at −70°C until assay. The protein content for each sample was measured by Lowry et al. (17). The proteins were separated on denaturing SDS-PAGE 8% polyacrylamide minigels (Bio-Rad Mini Protean II), loading an equal amount of protein per lane (≈ 80 μg for Na-K-ATPase and rBSC-1). Prestained protein markers were used for molecular weight determinations. The proteins were then transferred from the gels to nitrocellulose membranes. After being blocked with 5 g/dl nonfat dry milk for 1 h, the blots were probed for 1 h at room temperature with previously characterized antibodies: the Na-K-ATPase α₁-subunit (anti-Na-K-ATPase rabbit antibody, diluted 1:1,000), the affinity-purified polyclonal antibody against a COOH-terminal fragment of the Na⁺-K⁺-Cl⁻ cotransporter protein rBSC-1 (rabbit anti-rat kidney, diluted 1:2,000) and the affinity-purified polyclonal antibody against Tamm-Horsfall protein (rabbit anti-rat kidney, diluted 1:2,000). After being washed in Tris-buffered saline in Tween 20 (TTBS), the membranes were exposed to secondary antibodies (donkey anti-goat immunoglobulin G conjugated with horseradish peroxidase, diluted 1:3,000, for Na-K-ATPase; goat anti-rabbit immunoglobulin G conjugated with horseradish peroxidase, diluted 1:2,000, for rBSC-1; and goat anti-rabbit immunoglobulin G conjugated with horseradish peroxidase, diluted 1:5,000, for Tamm-Horsfall). After being washed in TTBS, antigen-antibody complexes were detected with an enhanced chemiluminescence detection kit (Western blot chemiluminescence reagent, NEL 100, NEN Life Science Products). After being developed with NEL 100, nitrocellulose membranes were stained with Amido black and showed a variation of <10% in protein loading. Densitometric analyses of films and membranes were performed on a PC computer using the Bio-Rad Laboratories Molecular Analyst Software (model GS-670 Imaging Densitometer). Labeling density was always determined on blots, where samples from CRF and OPC-31260 treated animals were simultaneously run on each gel with samples of controls and nontreated animals. The magnitude of the immunosignal is given as a percentage of control rats without treatment with OPC-31260.

To assess the known anatomic distribution of rBSC-1 in our experiments, we measured rBSC-1 expression in cortex, outer medulla, and inner medulla of kidney homogenates. A ~160-kDa band was detected predominantly in the outer medulla. Conversely, no signal was found in the inner medulla and weak expression was observed in the renal cortex, findings consistent with previous results (7, 13).

Na-K-ATPase activity. Membranes of outer medulla were prepared by homogenizing slices in 25 mM phosphate buffer (pH 7.4) as previously described (11). Homogenates were centrifuged at 2,000 rpm for 10 min at 4°C. Then, supernatants were centrifuged at 12,000 rpm for 30 min at 4°C, and the pellets were resuspended in the original buffer to the same volume. The membranes so obtained were incubated during 15 min at 37°C in the absence or presence of 4 mM ouabain. Na-K-ATPase activity was measured in a buffer containing (in mM) 50 NaCl, 5 KCl, 10 MgCl₂, 1 EDTA, 100 Tris-HCl, and 10 Na₂ATP as well as 2–5 Ci/mmol in tracer amounts (5 nCi/μl) [γ-32P]ATP. When ouabain was present, NaCl and KCl were omitted from the incubation medium. The phosphate liberated by hydrolysis of [γ-32P]ATP was separated by centrifugation after adsorption of the unhydrolyzed nucleotide on 15% activated charcoal. Radioactivity of the supernatant was measured in a liquid scintillation spectrometer. Total and ouabain-insensitive ATPase activity were measured, and the difference between them was ex-
pressed in micromoles of $^{32}$P hydrolyzed per milligram protein and per hour.

**Chemicals.** Anti-Na-K-ATPase (rabbit kidney (goat)) was purchased from Calbiochem, Calbiochem-Novabiochem (La Jolla, CA); rBSC-1 was a gift of Dr. Steven Hebert, Yale University; Tamm-Horsfall antibodies were a gift of Dr. John R. Hoyer (University of Pennsylvania); OPC-31260 was a gift of Otsuka Pharmaceutical, Tokyo, Japan; and secondary antibodies for Na-K-ATPase and rBSC-1 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and Vector Laboratories (Burlingame, CA), respectively.

**RESULTS**

**Effect of OPC-31260 in controls and CRF rats.** Body weight is expressed as the mean of the last 3 days of the study, and no differences were observed among groups (Table 1). Values of inulin clearance of separated groups of animals are given, showing a reduction in glomerular filtration rate of $\sim$64%. OPC-31260 in control rats increased urine volume by about three and one-half times and nearly doubled the decrease in urine osmolality. A similar effect was found in CRF rats. Water intake increased with OPC-31260 in both control and CRF rats in response to changes in urine volume. As expected, plasma osmolality was increased in CRF rats. Last, sodium excretion increased significantly by about one and one-quarter times in control rats when they were treated with OPC-31260, but no changes were observed in CRF rats.

Taken together, the data show inhibition of VP activity on $V_2$ receptors in both controls and CRF rats.

**Kidney levels of Na-K-ATPase and influence of VP blockade.** Figure 2 depicts Na-K-ATPase activity in membrane preparations of the outer medulla of controls and moderate CRF rats, both without and under OPC-31260 treatment. When controls and CRF groups were analyzed, OPC-31260 treatment decreased Na-K-ATPase activity from $11.2 \pm 1.5$ (n = 4) to $3.7 \pm 0.8$ (n = 5) in controls ($P < 0.05$) and from $19.0 \pm 0.8$ (n = 6) to $2.9 \pm 0.5 \mu$mol P$_i$-mg protein$^{-1}$-h$^{-1}$ in CRF rats (n = 10) ($P < 0.05$). Figure 2 also shows that CRF was associated with a significant increase in Na-K-ATPase activity (11.2 $\pm$ 1.5 in controls vs. 19.0 $\pm$ 0.8 $\mu$mol P$_i$-mg protein$^{-1}$-h$^{-1}$ in CRF) ($P < 0.05$).

Figure 3 shows the expression of Na-K-ATPase, using homogenates from the kidney outer medulla of control and CRF rats with both saline and under OPC-31260 treatment. The $\alpha_1$-subunit, affinity-purified polyclonal antibody recognized a band migrating at $\sim$110 kDa (Fig. 3A). Tamm-Horsfall protein expression did not change across the groups. When groups with OPC-31260 treatment were analyzed, Na-K-ATPase expression in the outer medulla decreased significantly in both controls and CRF rats to 57.6 $\pm$ 9.5 (n = 5) and 105.3 $\pm$ 10.9% (n = 10), respectively ($P < 0.05$). Figure 3B also shows that CRF was associated with a significant increase in Na-K-ATPase expression to 164.4 $\pm$ 21.5%, n = 10, compared with controls ($P < 0.05$).

The data shown in Figs. 2 and 3 would therefore suggest that 1) exposure to endogenous levels of VP induce adaptive changes in Na-K-ATPase activity and expression, independently of renal function status and 2) moderate CRF induces an increase in both activity and expression of the Na-K-ATPase at the level of the outer medulla.

**Outer medullary levels of rBSC-1 and influence of VP blockade.** Figure 4A shows the expression of rBSC-1 in controls and CRF rats both without and under OPC-31260 administration. Tamm-Horsfall protein expres-

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**Table 1. Effect of OPC-31260 in controls and CRF rats**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls No OPC (n = 12)</th>
<th>OPC (n = 14)</th>
<th>CRF No OPC (n = 10)</th>
<th>OPC (n = 11)</th>
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</thead>
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<tr>
<td>Body weight, g</td>
<td>300.4 $\pm$ 11.6</td>
<td>312.2 $\pm$ 12.2</td>
<td>287.7 $\pm$ 12.7</td>
<td>291.9 $\pm$ 10.7</td>
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<tr>
<td>Inulin clearance, ml$\cdot$min$^{-1}$$\cdot$100 g BW$^{-1}$</td>
<td>1.1 $\pm$ 0.07</td>
<td>0.4 $\pm$ 0.03</td>
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<td></td>
</tr>
<tr>
<td>Urine volume, $\mu$L/min</td>
<td>6.4 $\pm$ 0.8</td>
<td>24.2 $\pm$ 2.8*</td>
<td>31.0 $\pm$ 6.0*</td>
<td>47.6 $\pm$ 3.3*†</td>
</tr>
<tr>
<td>Urine osmolality, mosmol/kgH$_2$O</td>
<td>2,601 $\pm$ 178.4</td>
<td>1,259.8 $\pm$ 92.9*</td>
<td>899.9 $\pm$ 55.6*</td>
<td>458.9 $\pm$ 27.1†</td>
</tr>
<tr>
<td>Water intake, ml/day</td>
<td>18.2 $\pm$ 2.1</td>
<td>40.4 $\pm$ 5.5*</td>
<td>36.4 $\pm$ 7.0*</td>
<td>54.5 $\pm$ 3.9†</td>
</tr>
<tr>
<td>Plasma osmolality, mosmol/kgH$_2$O</td>
<td>292.1 $\pm$ 4.0</td>
<td>296.4 $\pm$ 2.8</td>
<td>312.6 $\pm$ 3.6*</td>
<td>312.4 $\pm$ 3.8*</td>
</tr>
<tr>
<td>Plasma sodium, mM</td>
<td>139.3 $\pm$ 1.3</td>
<td>140.3 $\pm$ 1.9</td>
<td>139.9 $\pm$ 2.3</td>
<td>141.9 $\pm$ 1.5</td>
</tr>
<tr>
<td>UNaV, $\mu$mol$\cdot$min$^{-1}$$\cdot$100 g BW$^{-1}$</td>
<td>0.31 $\pm$ 0.03</td>
<td>0.43 $\pm$ 0.04*</td>
<td>0.26 $\pm$ 0.05</td>
<td>0.25 $\pm$ 0.02</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SE. n, No. of rats; OPC-31260, 5-dimethylamino-1-[4-2(2-methylbenzoylamino)-benzoyl]-2,3,4,5-tetra-hydro-1H-benzazepine hydrochloride; CRF, chronic renal failure; BW, body wt. *$P < 0.05$ compared with controls without OPC-31260. †$P < 0.05$ compared with chronic renal failure (CRF) without OPC-31260.
sion did not change across the groups. Densitometric analysis (Fig. 4B) shows that inhibition of the VP effect by OPC-31260 resulted in a marked decrease in rBSC-1 expression in both controls (60.8 ± 6.5%, n = 7) and CRF rats (30.0 ± 6.9%, n = 10). Last, no effect of chronic renal insufficiency (95.7 ± 5.2%, n = 7) on rBSC-1 expression was observed compared with controls (n = 20).

As shown above for Na-K-ATPase, rBSC-1 expression was also influenced in the same way by VP inhibition, suggesting an interrelationship between both transporters under our experimental conditions.

**DISCUSSION**

Our results clearly show that both Na-K-ATPase and rBSC-1 in the outer medulla of the rat kidney are regulated by endogenous levels of VP activity. In effect, chronic treatment with a V2-receptor antagonist (OPC-31260) decreased both Na-K-ATPase activity and expression and rBSC-1 expression in membranes and homogenates, respectively, of the outer medulla in rats with normal renal function as well as in a model of moderate CRF (Figs. 2–4). As shown in METHODS, outer medullary homogenates contained both inner and outer stripe zones. Therefore, measured ATPase expression and activity cannot be solely assigned to the mTAL, because Na-K-ATPase is also expressed in S3 proximal tubules. We feel, however, that the decay in Na-K-ATPase expression under OPC-31260 should have originated at the mTAL level, because S3 proximal tubules do not express vasopressin receptors. Whether the described effect of OPC-31260 on Na-K-ATPase is mediated through rBSC-1 inhibition cannot be answered by this study. No changes were observed in the expression of the Tamm-Horsfall protein, indicating a specific effect of VP inhibition on the Na-K-ATPase and rBSC-1 levels.

The dose of OPC-31260 used in our study was able to inhibit VP activity, because both urine volume and urine osmolality were significantly changed in both groups of animals (Table 1), consistent with previous data by Ohara et al. (18).

Previous studies looking at whether VP regulates Na-K-ATPase have been mainly performed either in vitro or in vivo, where VP was exogenously administered. Results using in vitro preparations have demonstrated, alternatively, stimulation and inhibition by VP. Thus Charlton and Baylis (5) have reported that Na-K-ATPase activity is increased by exogenous VP in the rat renal medullary thick ascending limb and that this effect is mediated by the V2 receptor. Conversely, Fryckstedt and Aperia (8) have found a decrease in Na-K-ATPase activity in the mTAL in response to VP. On the other hand, in vivo studies in Brattleboro rats

![Fig. 3. Na-K-ATPase and Tamm-Horsfall immunoblots of kidney homogenates from outer medulla in controls and CRF rats both without and with OPC-31260. Values are means ± SE expressed as the percentage of controls treated with saline. A: immunoblot was alternatively reacted with affinity-purified polyclonal antibodies against the Na-K-ATPase α1-subunit and the Tamm-Horsfall protein. B: densitometric analysis of all samples revealed an increase in Na-K-ATPase expression in CRF compared with controls (***P < 0.05). Na-K-ATPase expression decreased under OPC-31260 in both controls and CRF rats compared with their respective conditions without OPC-31260 (*P < 0.05).]
where exogenous VP was administered on a chronic basis showed, alternatively, an increase in Na-K-ATPase activity in single microdissected mTAL (20) or no change in Na-K-ATPase expression in outer medullary (inner stripe) homogenates (14). The discrepancy in the effect of VP on Na-K-ATPase could be partly ascribed to differences in either short- and long-term actions of VP in thick ascending limb transport or those in the experimental designs. In this regard, there is compelling evidence that chronic VP treatment induces a changing event characterized by 1) downregulation of V2 receptors with an escape to the action of the hormone and 2) a transient increase in NKCC2 expression at day 2 with a return to baseline at day 7 after VP administration (6). Whether these adaptive phenomena preclude the observation of Na-K-ATPase regulation by exogenous VP and magnify the effect when VP action is inhibited as in our model needs further exploration. Last, we believe that the fact that a time concordance was found in our experiments between the decrease in both rBSC-1 and Na-K-ATPase expression adds further support to our findings and would be a speculative argument for the interdependence of the two transporters at the time of the study.

Previous reports have also indicated a stimulatory effect of vasopressin on rBSC-1 expression. Kim et al. (14) recently demonstrated that long-term elevations in circulating VP concentration by water restriction in normal rats or chronic deamino-8-d-arginine vasopressin (DDAVP) administration to Brattleboro rats increases rBSC-1 expression by 195 and 183%, respectively (14). Kwon et al. (16) administered DDAVP for 7 days to CRF rats and also observed a rise in total kidney Na\(^+\)-K\(^+\)-Cl\(^-\) cotransporter expression. Therefore, our results of a regulatory role of endogenous VP on Na-K-ATPase and rBSC-1 are consistent with most of the previous findings that point to a stimulatory role of VP in the expression and activity of both transport proteins. In addition, they provide strong, novel evidence for a key role of endogenous VP activity in regulating both Na-K-ATPase and rBSC-1 levels at the mTAL level of normal rats.

A second set of results in this study is related to changes in both Na-K-ATPase and rBSC-1 levels induced by a decrease in renal mass. Our data in a CRF model where renal insufficiency is moderate and medullary remnant tissue predominates showed an increase in both activity and expression of the Na-K-ATPase by using \([\gamma\text{-}^32P]\)ATP and an antibody directed to the \(\alpha_3\)-subunit of the Na-K-ATPase, respectively. In this regard, previous studies have provided different results, related mostly to the magnitude of renal tissue reduction as well as renal function status. Whereas unilateral nephrectomy has been associated with increases in Na-K-ATPase activity in the outer medulla (12), results in uremic rats have been controversial (14). Bofill et al. (3) found an increase
in the mRNA $\alpha_1$-subunit of the Na-K-ATPase in the kidney of rats with CRF, whereas a recent study by Kwon et al. (16) showed a decrease in total kidney levels of Na-K-ATPase in whole kidney homogenates of CRF rats. The differences among previous investigators and our data could be due, at least in part, to the use of different models of CRF.

The stimulus for the increase in Na-K-ATPase expression in CRF could be ascribed, at least in part, to VP. In effect, inhibition of V$_2$ receptors with OPC-31260 in this study decreased Na-K-ATPase expression in the outer medulla of CRF rats. Previous observations by Kwon et al. (16) demonstrated that chronic treatment of CRF rats with DDAVP stimulated rBSC-1 expression, consistent with mTAL sensitivity to VP in this condition. Our observation of a decrease in Na-K-ATPase expression by VP inhibition in an in vivo experimental design has not been previously reported and would suggest that VP increases Na-K-ATPase activity and expression in CRF.

In contrast to the basal increase in Na-K-ATPase activity and expression observed in the outer medulla of CRF rats, there was no significant increase in the rBSC-1 expression in this study (Fig. 4). Kwon et al. (16) have addressed this issue extensively. They found in membrane fractions of whole kidneys that the density of rBSC-1 was 134 ± 13% in CRF compared with 100 ± 13% in controls (not significant) and that total kidney levels of rBSC-1 were unchanged in rats with CRF. In this regard, our results are consistent with these findings. However, when densities of rBSC-1 per nephron were estimated, they were elevated 3.6-fold and histochemical studies also showed an increased rBSC-1 signal in mTAL. Our results are difficult to compare with those of Kwon et al. (15), because our data were not corrected for total kidney levels and our studies were performed only in outer medullary homogenates. Further studies in isolated mTAL segments are probably needed to settle this question.

In summary, the reduction in kidney outer medullary levels of Na-K-ATPase and Na$^+$-K$^+$-Cl$^-$ cotransporter in response to inhibition of endogenous VP may augment previous evidence that sodium transporters of the mTAL are regulated by VP activity. Inasmuch as these results are extended to CRF, they could give a homeostatic cellular basis for the modulation of water and electrolyte transport in disease states accompanied by a reduction of renal mass.

We are very grateful to Steven C. Hebert and Amy Hall (Yale University) and John R. Hoyer (University of Pennsylvania) for technical advice and the generous gift of affinity-purified rBSC-1 and Tamm-Horsfall antibodies, respectively. We are also indebted to Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan, for provision of OPC-31260.

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