*Proc. Natl. Acad. Sci. USA*
Vol. 86, pp. 6431–6435, August 1989
Physiological Sciences

# Coordinated regulation of intracellular $K^+$ in the proximal tubule: $Ba^{2+}$ blockade down-regulates the $Na^+,K^+$-ATPase and up-regulates two $K^+$ permeability pathways

(kidney/ion transport/quinine/furosemide/$K^+$ channels)

BRUCE C. KONE*, HUGH R. BRADY, AND STEVEN R. GULLANS

Renal Division and Department of Medicine, Brigham and Women's Hospital, and the Harvard Center for the Study of Kidney Diseases, Harvard Medical School, Boston, MA 02115

*Communicated by Gerhard Giebisch, May 12, 1989*

**ABSTRACT** **To avoid large changes in cell $K^+$ content and volume during variations in $Na^+,K^+$-ATPase activity, $Na^+$-transporting epithelia must adjust the rate of $K^+$ exit through passive permeability pathways. Recent studies have shown that a variety of passive $K^+$ transport mechanisms may coexist within a cell and may be functionally linked to the activity of the $Na^+,K^+$-ATPase. In this study, we have identified three distinct pathways for passive $K^+$ transport that act in concert with the $Na^+,K^+$-ATPase to maintain intracellular $K^+$ homeostasis in the proximal tubule. Under control conditions, the total $K^+$ leak of the tubules consisted of discrete $Ba^{2+}$-sensitive (≈65%), quinine-sensitive (≈20%), and furosemide-sensitive (≈10%) pathways. Following inhibition of the principal $K^+$ leak pathway with $Ba^{2+}$, the tubules adaptively restored cell $K^+$ content to normal levels. This recovery of cell $K^+$ content was inhibited, in an additive manner, by quinine and furosemide. Following adaptation to $Ba^{2+}$, the tubules exhibited a 30% reduction in $Na^+$-$K^+$ pump rate coupled with an increase in $K^+$ leak by means of the quinine-sensitive (≈70%) and furosemide-sensitive (≈280%) pathways. Thus, the proximal tubule maintains intracellular $K^+$ homeostasis by the coordinated modulation of multiple $K^+$ transport pathways. Furthermore, these results suggest that, like $Ba^{2+}$, other inhibitors of $K^+$ conductance will cause compensatory changes in both the $Na^+$-$K^+$ pump and alternative pathways for passive $K^+$ transport.**

As the major intracellular cation, $K^+$ plays a central role in a variety of important cellular processes (1). To preserve normal cell function, intracellular $K^+$ activity must be maintained within narrow limits through the concerted actions of pathways for $K^+$ entry and exit. In most cells, active $K^+$ uptake is mediated exclusively by the $Na^+,K^+$-ATPase and is balanced by the passive leak of $K^+$ from the cell by means of $K^+$ channels or electroneutral cotransport pathways (i.e., KCl or NaK2Cl cotransport). This "pump–leak" relationship (2) is thought to be especially complex in $Na^+$-transporting epithelia, such as the renal proximal tubule, because maintenance of intracellular $K^+$ homeostasis during large variations in active $Na^+$ reabsorption requires coordinated modulation of $Na^+,K^+$-ATPase activity and $K^+$ leak pathways (3). In support of this model, electrophysiological studies in several epithelia have shown cell $K^+$ activities to be relatively invariant despite significant fluctuations in the rate of transepithelial $Na^+$ transport and have suggested that maintenance of intracellular $K^+$ homeostasis involves parallel changes in $Na^+,K^+$-ATPase activity and $K^+$ conductance (4–8). Despite this inferential evidence, no direct measurements of ion flux have yet been reported to substantiate this hypothesis. Moreover, the identification of several distinct types of $K^+$ channels and electroneutral cotransport pathways within individual cells suggests that $K^+$ transport may be mediated by different pathways under various physiological conditions.

Previous studies of the proximal tubule indicated the presence of multiple $K^+$ transport pathways under different experimental conditions. Soltoff and Mandel (9) suggested that steady-state, passive $K^+$ transport in the rabbit proximal tubule was mediated almost exclusively by barium ($Ba^{2+}$)-sensitive pathways. Indeed, patch clamp studies of this nephron segment have directly demonstrated $Ba^{2+}$-inhibitable $K^+$ channels in the plasma membrane (10–12). In addition, $Na^+$-independent, electroneutral KCl cotransport was demonstrated in the basolateral membrane of rabbit proximal straight tubule (13) and in basolateral membrane vesicles prepared from renal cortex (14). More recently, a glucose-activated pathway for net $K^+$ efflux, sensitive to furosemide, a known inhibitor of $Cl^-$-dependent $K^+$ cotransport, but not sensitive to $Ba^{2+}$, was reported (15). Finally, the $K^+$ channel inhibitors quinine (16) and $Ba^{2+}$ (17) inhibited, but failed to prevent, the $K^+$-dependent volume regulatory decrease following hypoosmotic swelling. The relative roles of these or other $K^+$ transport pathways in regulating intracellular $K^+$, however, remain unknown.

The present study was designed to identify the major $K^+$ transport pathways of the proximal tubule and to study their respective contributions to the regulation of cellular $K^+$ balance. In addition, the adaptation of the proximal tubule to inhibition of its principal $K^+$ leak pathway with $Ba^{2+}$ was examined. Collectively, our results indicate that the proximal tubule possesses at least three distinct pathways for passive $K^+$ transport that act in concert with the $Na^+,K^+$-ATPase to preserve intracellular $K^+$ homeostasis. Portions of this work were presented at the annual symposium of the Society of General Physiologists† and at the annual meeting of the American Society of Nephrology.‡

## MATERIALS AND METHODS

**Preparation of Proximal Tubules.** Suspensions enriched in proximal tubules were prepared from female New Zealand White rabbits by *in situ* collagenase perfusion as previously described (18). This preparation yielded a nearly homoge-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

*Present address: Nephrology Division, The Johns Hopkins University School of Medicine, 1830 East Monument Street, Baltimore, MD 21205.

†Kone, B. C., Brady, H. R. & Gullans, S. R., 42nd Annual Symposium of the Society of General Physiologists, Sept. 7–10, 1988, Woods Hole, MA.

‡Kone, B. C., Brady, H. R. & Gullans, S. R., 21st Annual Meeting of the American Society of Nephrology, Dec. 11–14, 1988, San Antonio, TX.

neous population of tubule fragments with open lumina, which share morphologic, metabolic, and ion transport properties of proximal tubules *in situ*. The final pellet of tubules was suspended at a density of 6–12 mg of tubular protein per ml in a bicarbonate Ringer's solution (115 mM NaCl/25 mM $NaHCO_3$/5 mM KCl/1 mM $MgCl_2$/0.4 mM $NaH_2PO_4$/1.6 mM $Na_2HPO_4$/1.2 mM $CaCl_2$/4 mM sodium lactate/5 mM D-glucose/1 mM L-alanine). Aliquots (2.4 ml) of the tubule suspension were incubated under an atmosphere of 95% $O_2$/5% $CO_2$ at 37°C for 30 min before each experiment to restore normal cellular contents of $Na^+$, $K^+$, and ATP (19, 20).

**Net $K^+$ and $Na^+$ Fluxes.** Net fluxes of $K^+$ and $Na^+$ were measured as described (18, 21) by using a thermoregulated, 2-ml glass chamber and computer-linked extracellular electrode system. The $K^+$ electrode (POT-1, W-P Instruments, New Haven, CT) had a slope of 54–56 mV per decade of $K^+$ concentration, whereas the $Na^+$ electrode (MI-420, Microelectrodes, Londonderry, NH) exhibited a slope of 55–57 mV per decade of $Na^+$ concentration. An ultrawick glass electrode (MERE-1, W-P Instruments) filled with 1 M *N*-methyl-D-glucamine chloride served as the reference. The voltage output was amplified (10 times for the $K^+$ electrode and 60 times for the $Na^+$ electrode), filtered, and converted to a digital signal for computer analysis of initial transport rates. The $Na^+$ electrode could resolve changes of 0.4–0.6 mM $Na^+$ in the 146 mM $Na^+$ Ringer's solution (21). The typical peak-to-peak noise of the amplified $K^+$ electrode signal was ≈400 μV, which, given the amplified electrode slope (560 mV per decade of $K^+$), corresponded to 8.0 μM $K^+$ in the 5 mM $K^+$ buffer. Therefore, with signal averaging, changes of 16–24 μM $K^+$ could be reliably resolved. Initial rates were determined over the initial linear portion (10–15 sec) of the digital tracing following experimental additions. Increases or decreases in the extracellular ion concentrations were taken to represent the net release or net uptake, respectively, of ions from the tubules. Given the high resolution of the extracellular electrodes and the low cytocrits (≈4%) used, errors in the measurement of net ion flux caused by cell volume changes were estimated to be <4% (22). Cytosolic $K^+$ content was estimated from the increase in medium $K^+$ concentration resulting from digitonin (20 μg/mg of tubule protein) permeabilization of the tubule plasma membrane.

**Chemicals.** Analytical grade chemicals obtained from standard commercial sources were used. Experimental reagents, prepared as concentrated stock solutions, were added in microliter volumes such that the volume of the suspension was increased by not more than 0.2%. Aside from minor electrode offsets, neither the reagents nor the solvents altered electrode performance or cell function. The final concentrations of ouabain (21), $Ba^{2+}$, quinine, and furosemide used in these studies represent maximal inhibitory concentrations, as determined by dose–response experiments.

**Data Analysis.** Ion fluxes and contents were normalized to tubular protein as determined by the method of Lowry *et al.* (23) with bovine serum albumin as the standard. Data are presented as the mean ± SEM of *n* observations and were analyzed for significance by the paired or unpaired *t* test as appropriate. A statistically significant difference between group means was concluded when $P < 0.05$.

## RESULTS

To investigate the mechanisms by which the proximal tubule maintains intracellular $K^+$ homeostasis, we examined the time course of net $K^+$ transport following inhibition of the major $K^+$ leak pathway with 5 mM $Ba^{2+}$. By blocking passive $K^+$ permeability pathways, $Ba^{2+}$ promoted an initial net $K^+$ influx (Fig. 1), mediated by the $Na^+$,$K^+$-ATPase, that gradually slowed until a transient steady state (point B, Fig. 1) was achieved. Thereafter, the tubules spontaneously released the accumulated $K^+$ into the medium until the cytosolic $K^+$ content had been restored to a final steady-state value (point C, Fig. 1) not significantly different (2 ± 4%, $n = 6$) from the original, pretreatment value (point A, Fig. 1). Additional $Ba^{2+}$, given during the final adapted state (point C, Fig. 1), caused no further net $K^+$ flux, indicating that all $Ba^{2+}$-sensitive pathways were completely inhibited. The fact that cytosolic $K^+$ content was restored to its original value despite the continued inhibition of the principal $K^+$ leak pathway suggested that a new pump–leak relationship had been established.

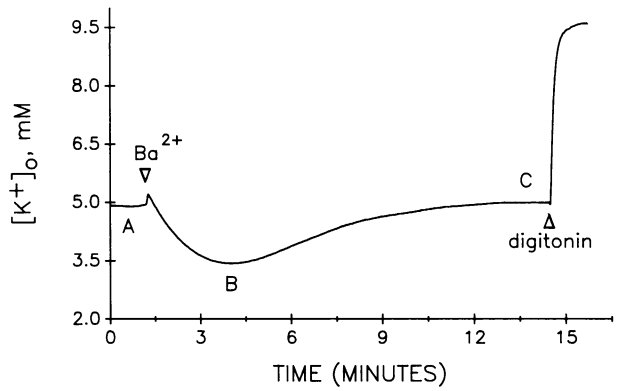


FIG. 1. Time-dependent effects of $Ba^{2+}$ on proximal tubule $K^+$ transport. Digital output of the extracellular $K^+$ electrode from a representative experiment ($n = 6$) in which $Ba^{2+}$ (5 mM, final concentration) was added to the tubule suspension. Note that, despite the continued presence of $Ba^{2+}$, extracellular $K^+$ concentration (and, by inference, intracellular $K^+$ content) returned to the original, pretreatment value. Points A, B, and C denote the control, transition, and $Ba^{2+}$-adapted states of the tubules. The $K^+$ release that occurred between points B and C was termed the recovery phase.

The restoration of cytosolic $K^+$ content to the control level following treatment with $Ba^{2+}$ required the cells to increase $K^+$ leak through $Ba^{2+}$-insensitive pathways and/or reduce the $Na^+$-$K^+$ pump rate. To determine whether $Na^+$ and $K^+$ transport rates were diminished or restored to control levels in the $Ba^{2+}$-treated cells, we measured the initial rates of net $Na^+$ influx and net $K^+$ efflux promoted by 0.1 mM ouabain in control tubules and in tubules treated for 12 min with 5 mM $Ba^{2+}$ ($Ba^{2+}$-adapted). Ouabain, by inhibiting the active translocation of $Na^+$ and $K^+$ by the $Na^+$,$K^+$-ATPase, allowed the unopposed leaks of $Na^+$ and $K^+$ through passive permeability pathways to be measured. Since, at steady state, the leaks of $Na^+$ and $K^+$ are exactly matched by the cation exchanges of the $Na^+$-$K^+$ pump (i.e., leak = pump), these initial rates of ouabain-induced $Na^+$ and $K^+$ leak also provided a measurement of the apparent transport rate and ionic stoichiometry of the $Na^+$-$K^+$ pump. In control tubules (Table 1), ouabain promoted a net $Na^+$ influx (229 ± 7 nmol $Na^+$/min

Table 1. Effect of $Ba^{2+}$ on ouabain-induced cation fluxes of the proximal tubule

| | Control | $Ba^{2+}$-adapted |
|---|---|---|
| Net $Na^+$ influx | 229 ± 7 | 161 ± 12* |
| Net $K^+$ efflux | 154 ± 5 | 115 ± 3* |
| $Na^+$:$K^+$ flux ratio | 1.49 | 1.40 |

Initial rates of net $Na^+$ influx and net $K^+$ efflux were measured following the addition of 0.1 mM ouabain to untreated tubules (control) and to tubules exposed to 5 mM $Ba^{2+}$ for 12 min ($Ba^{2+}$-adapted). Flux rates are expressed in nmol/min per mg of protein and represent the mean ± SEM ($n = 5$).
*$P < 0.005$ versus control.

per mg of protein) and a net $K^+$ efflux (154 ± 5 nmol $K^+$/min per mg) in a ratio of approximately 3 $Na^+$ to 2 $K^+$. The $Ba^{2+}$-adapted tubules exhibited significantly lower rates of net $Na^+$ influx (161 ± 12 nmol/min per mg) and net $K^+$ efflux (115 ± 3 nmol/min per mg) in response to ouabain, but the initial $Na^+$:$K^+$ flux ratio was virtually unchanged. Thus, in the new pump–leak equilibrium of the $Ba^{2+}$-adapted tubules, $K^+$ flux through both passive transport pathways and (by inference) the $Na^+$-$K^+$ pump was reduced by ≈30%. Notably, however, since $Ba^{2+}$ failed to abolish the net $K^+$ efflux induced by ouabain, $Ba^{2+}$-insensitive pathways for $K^+$ leak were clearly operative in the adapted tubules.

Next, we designed a protocol to identify the distinct, inhibitor-sensitive pathways for $K^+$ leak in both control and $Ba^+$-adapted tubules. As shown in Fig. 2, selected inhibitors of $K^+$ channels or cotransport pathways were added (at maximal inhibitory concentrations) simultaneously with ouabain, and the resultant net efflux of $K^+$ was measured. In this manner, a specific inhibitor-sensitive pathway for $K^+$ transport could be distinguished by the reduction in the rate of ouabain-induced net $K^+$ efflux. In control tubules, $Ba^{2+}$ (5 mM) reduced the ouabain-induced $K^+$ leak of control tubules by ≈65% from 154 ± 5 nmol of $K^+$ per min per mg of protein (Table 1) to 56 ± 3 nmol of $K^+$ per min per mg of protein (Table 2), indicating that the $Ba^{2+}$-sensitive pathways mediated ≈65% of the total $K^+$ leak. The inability of $Ba^{2+}$ to prevent the ouabain-induced $K^+$ efflux clearly indicates the presence of $Ba^{2+}$-insensitive $K^+$ leak pathways. In an additive manner (Fig. 2 and Table 2), quinine (1 mM) and furosemide (1 mM) further inhibited (by ≈20% and ≈10%, respectively) all but a small "residual" component of the $Ba^{2+}$-insensitive $K^+$ leak. Taken together, these data suggest the constitutive operation of at least three distinct $K^+$ permeability pathways in the control tubules. In comparison, the $Ba^{2+}$-adapted tubules exhibited 2-fold greater rates of $K^+$ leak through $Ba^{2+}$-insensitive pathways than controls (115 ± 3 nmol/min per mg vs. 56 ± 3 nmol/min per mg; Table 2 and Fig. 3). Furthermore, the quinine-sensitive, the furosemide-sensitive, and the residual components of the $K^+$ efflux were greater by ≈70%, ≈280%, and ≈65%, respectively, in the $Ba^{2+}$-adapted tubules (Table 2 and Fig. 3). Thus, the enhanced, $Ba^{2+}$-insensitive $K^+$ efflux of the $Ba^{2+}$-adapted cells was primarily the result of specific increases in $K^+$ leak by means of the quinine-sensitive, the furosemide-sensitive, and the residual pathways.

Like $Ba^{2+}$, quinine (1 mM) alone promoted an initial net $K^+$ uptake followed by a recovery of cytosolic $K^+$ content to pretreatment values. The maximum $K^+$ accumulated by quinine-treated tubules (113 ± 6 nmol of $K^+$ per mg of protein, $n$ = 5) was approximately half that accumulated by

Table 2. Inhibitor sensitivity of ouabain-induced $K^+$ leak

| Inhibitor(s) | $K^+$ efflux, nmol/min per mg of protein | |
|---|---|---|
| | Control | $Ba^{2+}$-adapted |
| Ouabain + $Ba^{2+}$ | 56 ± 3 | 115 ± 4* |
| Ouabain + $Ba^{2+}$ + quinine | 26 ± 4 | 66 ± 6** |
| Ouabain + $Ba^{2+}$ + quinine + furosemide | 14 ± 4 | 23 ± 3** |

Initial rates of net $K^+$ efflux were measured at steady state following exposure of control tubules to ouabain simultaneously with $Ba^{2+}$, quinine, and/or furosemide. In the $Ba^{2+}$-adapted tubules (5 mM $Ba^{2+}$ for 12 min), ouabain was added (in the continued presence of $Ba^{2+}$) either alone or in combination with quinine and/or furosemide. Each value is representative of five observations. Final concentrations of inhibitors were ouabain, 0.1 mM; $Ba^{2+}$, 5 mM; quinine, 1 mM; furosemide, 1 mM.
*$P < 0.001$ versus control.
**$P < 0.05$ versus control.

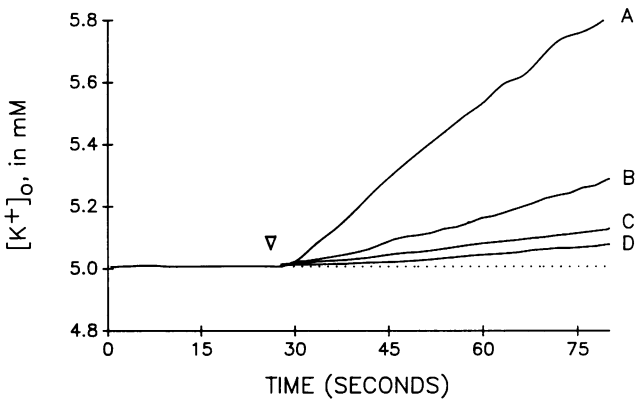


FIG. 2. Inhibitor sensitivity of the total $K^+$ leak induced by ouabain. Composite of representative $K^+$ electrode tracings from experiments in which ouabain (trace A) was added alone or simultaneously with maximal inhibitory concentrations of $Ba^{2+}$ (trace B), $Ba^{2+}$ and quinine (trace C), or $Ba^{2+}$, quinine, and furosemide (trace D) to control tubule suspensions. Final concentrations were ouabain, 0.1 mM; $Ba^{2+}$, 5 mM; furosemide, 1 mM; and quinine, 1 mM.

$Ba^{2+}$-treated tubules (206 ± 9 nmol of $K^+$ per mg of protein, $n$ = 5). In the presence of both quinine and $Ba^{2+}$, the tubules accumulated a maximum of 260 ± 9 nmol of $K^+$ per mg of protein ($n$ = 5), indicating additive inhibition of $K^+$ leak by these two agents. In contrast, furosemide (1 mM) caused no net $K^+$ flux when added alone, but it promoted a transient net $K^+$ influx (24 ± 4 nmol/min per mg, $n$ = 5) when given after $Ba^{2+}$ and quinine.

To determine whether the increased $K^+$ transport by means of the quinine-sensitive and the furosemide-sensitive pathways participated in the recovery phase of net $K^+$ release in the $Ba^{2+}$-treated tubules, the rate of net $K^+$ efflux during this recovery phase was measured in the presence of quinine and/or furosemide. In these experiments, quinine and/or furosemide were added ≈3 min after $Ba^{2+}$, during the transition period (point B, Fig. 1) preceding the recovery phase of net $K^+$ release. As seen in Table 3, both quinine and furosemide partially inhibited the rate of $K^+$ recovery; however, neither inhibitor prevented the eventual restoration of cytosolic $K^+$ content. In contrast, the combined addition of quinine and furosemide (Table 3) prevented the recovery of cytosolic $K^+$ content during the observation period (30 min). These data suggest that either the quinine-sensitive or the furosemide-sensitive pathway was sufficient to restore cytosolic $K^+$ content in the presence of $Ba^{2+}$ but that both

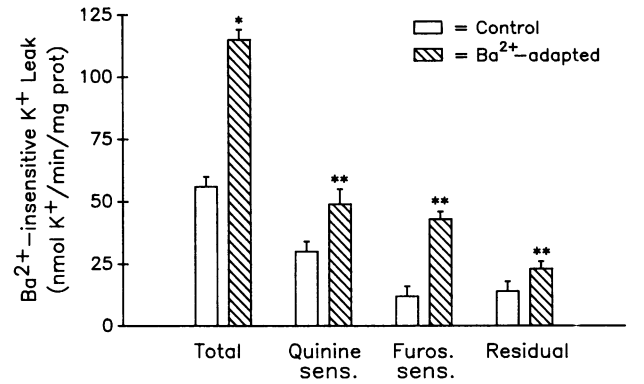


FIG. 3. Induction of $Ba^{2+}$-insensitive $K^+$ leak pathways. The inhibitor-sensitive components of the $Ba^{2+}$-insensitive $K^+$ leak (calculated from the data displayed in Table 2) are presented for both control tubules and $Ba^{2+}$-adapted tubules. Each bar represents the mean ± SEM of five experiments. *, $P < 0.001$; **, $P < 0.05$ versus control. Sens., sensitive; furos., furosemide.

Table 3. Inhibition of $Ba^{2+}$-insensitive recovery of cytosolic $K^+$ content by quinine and furosemide

| Addition(s) | Rate of recovery, nmol of $K^+$ per min per mg of protein |
|---|---|
| $Ba^{2+}$ (control) | 34 ± 4 |
| $Ba^{2+}$ + quinine | 17 ± 3* |
| $Ba^{2+}$ + furosemide | 9 ± 3† |
| $Ba^{2+}$ + quinine + furosemide | 0‡ |

Net $K^+$ transport was continuously monitored following treatment of tubule suspensions with 5 mM $Ba^{2+}$. After the initial net influx of $K^+$, the maximal rate of $K^+$ release (recovery phase) was measured over ≈30 sec in the absence (control) or presence of quinine (1 mM) and/or furosemide (1 mM). Quinine and/or furosemide were added during the transition phase (point B, Fig. 1) that preceded the net $K^+$ release. For each addition, $n = 5$.
*$P < 0.01$ versus control.
†$P < 0.002$ versus control.
‡$P < 0.001$ versus control.

pathways were required for the optimal recovery rate of cytosolic $K^+$ content.

## DISCUSSION

This study provides direct evidence for the coordinated regulation of intracellular $K^+$ content by the $Na^+,K^+$-ATPase and three distinct pathways for passive $K^+$ transport in the proximal tubule. By monitoring the ouabain-induced net $K^+$ efflux, we determined the relative importance of each $K^+$ leak pathway in mediating passive $K^+$ transport. Furthermore, we demonstrated the ability of the proximal tubule to restore its cellular $K^+$ content, by down-regulating the $Na^+,K^+$-ATPase and up-regulating $K^+$ leak by means of $Ba^{2+}$-insensitive permeability pathways, following $Ba^{2+}$ blockade of its principal $K^+$ leak pathway. Thus, the orchestrated regulation of four separate $K^+$ transport pathways—the $Na^+,K^+$-ATPase, and the $Ba^{2+}$-sensitive, the quinine-sensitive, and the furosemide-sensitive leak pathways—serves to maintain intracellular $K^+$ homeostasis in this epithelium.

In keeping with previous investigations (9, 13–15), we observed the presence of both $Ba^{2+}$-sensitive and $Ba^{2+}$-insensitive pathways for $K^+$ leak in the proximal tubule. The $Ba^{2+}$-sensitive pathway was the dominant mechanism for $K^+$ leak in the control tubules, comprising ≈65% of the total $K^+$ leak flux; it presumably reflects the $Ba^{2+}$-sensitive $K^+$ conductance observed by others (24, 25). The $Ba^{2+}$-insensitive $K^+$ leak was inhibited in an additive manner by quinine and furosemide, suggesting that it was composed of at least two different pathways. Thus, passive $K^+$ transport in the proximal tubule appears to be mediated by at least three distinct pathways.

Quinine and its stereoisomer quinidine are known to inhibit the $K^+$ conductance of the proximal tubule (25) and to block $K^+$ channels in other tissues (26, 27). Thus, the effect of quinine to inhibit $K^+$ leak, observed in the present study, probably reflects its ability to inhibit $K^+$ channels in the proximal tubule. Although quinine-sensitive $K^+$ channels have generally been regarded to be $Ba^{2+}$-sensitive, the additive inhibition of $K^+$ leak by $Ba^{2+}$ and quinine suggests the presence of separate, pharmacologically distinct populations of $K^+$ channels in the proximal tubule. Preliminary evidence for a class of $K^+$ channels sensitive to quinine, but not to $Ba^{2+}$, has recently been demonstrated in patch clamp studies of the human macrophage (28).

Given the recent demonstration of $Na^+$-independent, electroneutral KCl cotransport in the basolateral membrane of the rabbit proximal tubule (13) and the known sensitivity of KCl cotransport to the loop diuretics (14, 29), the furosemide-sensitive pathway for $K^+$ efflux reported here probably represents KCl cotransport. Alternatively, furosemide could inhibit other $Cl^-$-dependent $K^+$ transport mechanisms, such as parallel $K^+$ and $Cl^-$ channels. However, since the furosemide-sensitive $K^+$ loss was evident even in the presence of both $Ba^{2+}$ and quinine and since the $Cl^-$ conductance of the proximal tubule is extremely low (30), such an indirect coupling of $K^+$ and $Cl^-$ conductances seems unlikely. The fact that furosemide produced, comparatively, the greatest inhibition of both the rate of recovery of cell $K^+$ content following $Ba^{2+}$ treatment (Table 3) and of the steady-state $K^+$ leak of the $Ba^{2+}$-adapted tubules (Fig. 3) suggests a special role for this pathway in the regulation of intracellular $K^+$ content. Interestingly, in studies of the frog skin, Cox and Helman (31) observed furosemide-sensitive KCl cotransport in the presence, but not the absence, of ouabain and concluded that ouabain induced KCl cotransport in this epithelium. Similarly, recent studies of the proximal tubule have suggested the operation of a furosemide-sensitive $K^+$ exit pathway activated by intracellular glucose accumulation (15) or cell swelling (32). As proposed for other tissues, then, furosemide-inhibitable KCl contransport in the proximal tubule might be activated by altered cellular $K^+$ activity or volume.

To restore cytosolic $K^+$ content following inhibition of the $Ba^{2+}$-sensitive $K^+$ leak, the proximal tubule adaptively reduced its apparent $Na^+$-$K^+$ pump rate (equivalent to the steady-state leaks of $Na^+$ and $K^+$ induced by ouabain) by ≈30% (Table 2). Despite this down-regulation of active transport, the ionic stoichiometry of the $Na^+$-$K^+$ pump remained invariant. The apparent $Na^+$:$K^+$ coupling ratio of the $Na^+,K^+$-ATPase observed in both control and $Ba^{2+}$-adapted tubules was approximately 3:2, in good agreement with previous determinations in this epithelium (21, 22). A number of factors, including intracellular $Ca^{2+}$ activity, ATP, membrane potential, and the $Na^+,K^+$-ATPase itself (see ref. 33 for review), have been proposed as signals that link pump and leak. Although, in the present study, the apparent $Na^+$-$K^+$ pump rate decreased in parallel with the $Ba^{2+}$-*sensitive* $K^+$ leak, it varied reciprocally with the $Ba^{2+}$-*insensitive* $K^+$ leak. These observations contradict the notion of a direct functional link between the pump and the total $K^+$ leak [i.e., "pump-leak units" (3)] and suggest some other mechanism(s) by which the $Na^+$-$K^+$ pump and the specific, $Ba^{2+}$-insensitive pathways for $K^+$ leak are matched to maintain constancy of intracellular $K^+$.

In addition to down-regulating $Na^+,K^+$-ATPase activity, the $Ba^{2+}$-adapted tubules increased $K^+$ efflux by means of $Ba^{2+}$-insensitive pathways. The $Ba^{2+}$-insensitive $K^+$ leak of the adapted tubules was nearly twice that observed in the control tubules, the result of an apparent up-regulation of the quinine-sensitive, the furosemide-sensitive, and the "residual" leak pathways (Fig. 3). The mechanism(s) by which $K^+$ leak is augmented through these pathways remains unknown, but it must involve either an increase in the permeability of the pathways to $K^+$ or an increase in the electrochemical driving force for $K^+$ exit. Given the comparable chemical gradients for $K^+$ efflux (i.e., equivalent cytosolic $K^+$ contents) in the control and $Ba^{2+}$-adapted tubules, the increased $K^+$ leak mediated by the quinine-sensitive and the furosemide-sensitive pathways in the adapted tubules would require either an increase in the $K^+$ permeability of these pathways or a depolarization of the membrane potential. This adaptation could represent an increase in $K^+$ flux through existing pathways or the activation or recruitment of quiescent pathways. Further studies will be needed to elucidate the specific mechanisms that signal and effect the increase in transport by the various $K^+$ leak pathways.

Given the limitations of tubule suspensions, the precise locations of the various $K^+$ leak pathways within the plasma

membrane remain to be determined. Patch clamp studies have indicated the presence of both apical and basolateral $K^+$ channels in the proximal tubule (10–12). However, since the basolateral membrane of the proximal tubule possesses the much greater $K^+$ conductance (24) as well as KCl cotransport (13, 14), it seems likely that the $K^+$ leak pathways we observed would reside primarily in the basolateral membrane.

In summary, cytosolic $K^+$ content of the proximal tubule appears to be regulated predominantly by balanced changes in $K^+$ transport mediated by the $Na^+$-$K^+$ pump and three passive $K^+$ permeability pathways, sensitive to $Ba^{2+}$, to quinine, and to furosemide. In addition to down-modulation of the $Na^+$-$K^+$ pump, $K^+$ leak by means of quinine-sensitive and furosemide-sensitive pathways can be adaptively increased in the presence of $Ba^{2+}$ to restore normal cytosolic $K^+$ content. The existence of multiple, regulated $K^+$ leak pathways offers the possibility that distinct pathways may perform specialized functions or may be triggered by specific intrinsic (e.g., osmolality, $K^+$ activity, $Ca^{2+}$ activity, pH, ATP, or membrane potential) or extrinsic (hormone) signals. From these observations, one would predict that other agents, that, like $Ba^{2+}$, reduce $K^+$ conductance might cause compensatory changes in both the $Na^+$-$K^+$ pump and alternative pathways for passive $K^+$ transport. Furthermore, this model suggests a mechanism by which the proximal tubule may maintain intracellular $K^+$ homeostasis during hormonally-induced reductions in $Na^+$ or $K^+$ transport.

We thank Drs. Steven C. Hebert and Barry M. Brenner for their helpful comments during the preparation of the manuscript. Support was provided to S.R.G. by National Institutes of Health Grant DK 36031. B.C.K. was the recipient of an individual National Research Service Award DK 07862. H.R.B. was a recipient of a Research Fellowship from the Massachusetts Affiliate of the American Heart Association.

1. Wright, F. S. & Giebisch, G. (1985) in *The Kidney: Physiology and Pathophysiology*, eds. Seldin, D. & Giebisch, G. (Raven, New York), pp. 1223–1249.
2. Tosteson, D. C. & Hoffman, L. F. (1960) *J. Gen. Physiol.* **44,** 169–194.
3. Schultz, S. G. (1981) *Am. J. Physiol.* **241,** F579–F590.
4. White, J. F. (1976) *Am. J. Physiol.* **231,** 1214–1219.
5. Gunter-Smith, P. J. & Schultz, S. G. (1982) *J. Membr. Biol.* **66,** 25–39.
6. Grasset, E., Gunter-Smith, P. & Schultz, S. G. (1983) *J. Membr. Biol.* **71,** 89–94.
7. Messner, G., Wang, W., Paulmichl, M., Oberleithner, H. & Lang, F. (1985) *Pflügers Arch.* **404,** 131–137.
8. Matsumura, Y., Cohen, B., Guggino, W. B. & Giebisch, G. (1984) *J. Membr. Biol.* **79,** 153–161.
9. Soltoff, S. P. & Mandel, L. J. (1986) *J. Membr. Biol.* **94,** 153–161.
10. Gögelein, H. & Greger, R. (1987) *Pflügers Arch.* **410,** 288–295.
11. Parent, L., Cardinal, J. & Sauve, R. (1988) *Am. J. Physiol.* **254,** F105–F113.
12. Gögelein, H. & Greger, R. (1984) *Pflügers Arch.* **401,** 424–426.
13. Sasaki, S., Ishibashi, K., Yoshiyama, N. & Shiigai, T. (1988) *J. Clin. Invest.* **81,** 194–199.
14. Eveloff, J. & Warnock, D. C. (1987) *Am. J. Physiol.* **252,** F883–F889.
15. Avison, M. J., Gullans, S. R., Ogino, T., Shulman, R. G. & Giebisch, G. (1988) *J. Membr. Biol.* **105,** 197–205.
16. Kirk, K. L., DiBona, D. R. & Schafer, J. A. (1987) *Am. J. Physiol.* **252,** F933–F942.
17. Welling, P. A., Linshaw, M. A. & Sullivan, L. P. (1985) *Am. J. Physiol.* **249,** F20–F27.
18. Kone, B. C., Kaleta, M. L. & Gullans, S. R. (1988) *J. Membr. Biol.* **102,** 11–19.
19. Soltoff, S. & Mandel, L. (1984) *J. Gen. Physiol.* **84,** 601–622.
20. Gullans, S. R., Avison, M. J., Ogino, T., Giebisch, G. & Shulman, R. G. (1985) *Am. J. Physiol.* **249,** F160–F168.
21. Brady, H. R., Kone, B. C. & Gullans, S. R. (1989) *Am. J. Physiol.* **256,** C1105–C1110.
22. Avison, M. J., Gullans, S. R., Ogino, T., Giebisch, G. & Shulman, R. G. (1987) *Am. J. Physiol.* **253,** C126–C136.
23. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. L. (1951) *J. Biol. Chem.* **193,** 265–275.
24. Biagi, B., Sohtell, M. & Giebisch, G. (1981) *Am. J. Physiol.* **241,** F677–F686.
25. Völkl, H., Greger, R. & Lang, F. (1987) *Biochim. Biophys. Acta* **900,** 275–281.
26. Hoffman, E. K., Simonsen, L. O. & Lambert, I. H. (1984) *J. Membr. Biol.* **78,** 211–222.
27. Richards, N. W. & Dawson, D. C. (1986) *Am. J. Physiol.* **251,** C85–C89.
28. Gallin, E. K. (1988) *J. Gen. Physiol.* **92,** 40 (abstr.).
29. Larson, M. & Spring, K. (1984) *J. Membr. Biol.* **81,** 219–232.
30. Bello-Reuss, E. (1982) *J. Physiol. (London)* **326,** 49–63.
31. Cox, T. C. & Helman, S. I. (1986) *J. Gen. Physiol.* **87,** 467–502.
32. Welling, P. A. & Linshaw, M. A. (1988) *Am. J. Physiol.* **255,** F853–F860.
33. Lang, F., Messner, G. & Rehwald, W. (1986) *Am. J. Physiol.* **250,** F953–F962.