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K-Induced Alkalinization in All Cell Types of Rabbit Gastric Glands: A Novel K/H Exchange Mechanism

Aldebaran M. Hofer and Terry E. Machen
Department of Molecular and Cell Biology, University of California at Berkeley, Berkeley, California 94720

Summary. Digital image processing of the pH-sensitive dye BCECF was used to examine the effects of high [K] media on cytoplasmic pH (pH_i) of individual cells within isolated rabbit gastric glands. When cells were acidified to pH_i 6.5 from the resting pH_i of 7.2–7.3 and then exposed to solution containing 77 mM K plus amiloride (to block Na/H exchange), recovery to pH_i 7.0 was observed. This K-induced alkalinization occurred in all cell types of the gland, including cells within antral glands that were devoid of parietal cells (PC). This process was independent of extracellular Na and Cl and was unaffected by: 5 mM Ba or 200 μM bumetanide, or acute treatment with either 500 μM ouabain or 100 μM cimetidine, histamine or carbachol. SCH28080, which inhibits the PC H/K-ATPase when used in the low μM range of concentrations, blocked the K effect on pH_i at 100 μM but was ineffective at 1 μM. A similar pH_i recovery was also stimulated by Li, Cs (both 72 mM), and Tl (10 mM), in the order Li > K > Cs > Tl (all in the presence of amiloride), and these alkalinizations were also blocked by 100 μM SCH28080. Parallel experiments were performed to test the effect of these ions on ^{14}[C]-aminopyrine accumulation, an index of acid secretion by the H/K-ATPase at the lumenal membrane of the PC. There was no correlation between the rates of cation-induced pH_i recovery from an acid load and H secretion as measured by the accumulation of aminopyrine. We conclude that the K- (and Cs- and Li-) dependent pH_i recovery is mediated by a novel cation/H exchange mechanism that is distinct from the PC H/K-ATPase.

Key Words gastric glands · SCH28080 · K-dependent alkalinization · intracellular pH · BCECF · aminopyrine

Introduction

The mammalian gastric gland is responsible for generating the acid and enzyme secretions found in the lumen of the stomach and consists of several cell types. An apically situated H/K-ATPase, restricted to the PC only, accounts for the evolution of low pH gastric secretions. Other residents of the gastric gland include chief cells (CC) and mucous neck cells (MNC), which produce pepsiogen and mucus, respectively. The isolated gastric gland shares several features of the intact epithelium (such as maintenance of cell-cell contacts and retention of polarity), yet is amenable to many types of manipulation that are not possible in the whole tissue. Because there is limited access to the glandular lumen by the extracellular bathing solution, the accumulation of trapped secretory products (namely HCl) can be readily monitored. At the same time, the study of basolateral transport in these cells is also simplified, since alteration of the bathing medium has immediate effects at this membrane.

The ion transport properties of the PC have been investigated extensively. These cells have a variety of pH regulatory mechanisms that maintain intracellular pH (pH_i) at a constant level irrespective of secretory state (Muallem et al., 1988; Paradiso et al., 1989), and the intracellular accumulation of base equivalents resulting from the activity of the H/K-ATPase is compensated by the basolateral $Cl/HCO_3(OH)$ exchanger, which normally serves to acidify the cell (Muallem et al., 1985). It appears that this exchanger can also operate in the nominal absence of HCO_3, exchanging OH for Cl (Paradiso et al., 1987*a*). Parietal cells also have an amiloride-sensitive Na/H exchanger, which in its normal mode of operation acts as a base loader (Muallem et al., 1985; Paradiso, Tsien & Machen, 1987*b*). In addition, these cells are also believed to possess an electrogenic H_2DIDS-sensitive Na/HCO_3 cotransporter (Townsley & Machen, 1989), which assists in the recovery from acid loads but is manifest only in HCO_3/CO_2-buffered solutions. Less information is available regarding the pH regulatory mechanisms peculiar to the CC and MNC, although it has been established that these cells also have Na/H antiport (Paradiso et al., 1987*b*).

In the present study we report that, in addition to the mechanisms mentioned above, there exists a cation/H exchange-like mechanism that affects pH_i only when the cytosol has been previously acidified and is dependent on high external [K]. This phenom-

enon was uncovered in the course of attempts to depolarize cells by placing them in high K media. K-induced alkalinizations were evident in all cell types of the gastric gland, as well as in glands derived from the antral mucosa, which were devoid of PC, and for this reason we feel that this process is likely distinct from the PC H/K-ATPase.

Materials and Methods

Gland Isolation Procedure

Gastric glands from male New Zealand white rabbits weighing 2–3 kg were prepared as described previously using retrograde perfusion of the abdominal aorta and collagenase digestion (Berglindh & Obrink, 1976). Animals were euthanized by intravenous administration of nembutal. Following perfusion and removal of the stomach, the fundic mucosa was scraped from the underlying muscle coat, minced with a razor blade, and then rinsed. A separate preparation utilized the thickened antral mucosa derived from the pyloric and cardiac regions of the stomach.

The minced tissue was incubated at 37°C in digestion media (Gibco Eagle medium with 1 mg/ml bovine serum albumin, 0.5 mg/ml type 1A collagenase, 20 mM HEPES, and 40 μM cimetidine) until glands were formed (usually about 45 min). Glands were filtered through nylon cloth, allowed to settle by gravity,and then resuspended several times in unsupplemented Eagle medium prior to loading with dye. Cimetidine, a histamine (H_2) antagonist, was present during the entire isolation procedure to prevent excessive stimulation.

Dye Loading and Calibration

Glands were loaded at room temperature immediately following isolation with 2 μM BCECF-AM, the cell-permeant acetoxymethylester derivative of the pH indicator BCECF. Endogenous esterase activity residing exclusively in the cell cytoplasm promotes cleavage of BCECF-AM to an anionic, impermeant species, effectively trapping the dye within the cell. After 30 min, glands were rinsed several times and then allowed to rest undisturbed for 1 hr to permit cells to overcome any toxic effects associated with AM ester hydrolysis (Negulescu, Reenstra & Machen, 1989).

An *in situ* calibration was performed following most runs using the high K/nigericin technique. Nigericin treatment is equivalent to introducing an H/K exchanger in the membrane; thus, when $[K]_o$ is set equal to $[K]_i$, pH_o and pH_i equilibrate. Calibration solutions were titrated to at least three different pH_o. The response of the dye has been shown to be linear between pH 6.2 and 8.0 (Negulescu & Machen, 1990).

Digital Image Processing of Gastric Glands

Simultaneous fluorescence measurements from multiple cells within a gland were made using digital processing of video images. A low light level Dage 66 SIT television camera collects separately (via a 510-nm long-pass filter) emitted light generated from the alternate excitation of the sample at 440 and 490 nm. Because BCECF fluorescence at 440 nm is relatively insensitive to pH, the ratio of the two intensities (490/440 nm) is a direct reflection of pH_i that is independent of photobleaching, dye leakage, and cellular volume changes, since emitted light at both wavelengths experiences these factors equally. Intensities were typically averaged over 16 video frames at each wavelength by a Gould FD5000 image processor and converted to a ratio. The interval between successive measurements (usually about 10 sec), as well as other parameters of data collection, were controlled by a PDP 11/73 DEC clone computer, utilizing software written by R.Y. Tsien (Department of Pharmacology, University of California at San Diego).

The ratio, calculated for each pixel, was displayed as a pseudocolor on a television monitor, providing a color representation of pH_i within the specimen. While such images could be recorded and stored, data analysis was simplified by conversion at a remote emulation terminal (Smarterm 240) to ratio *vs.* time plots for each cell examined. Circular or polygonal shapes approximating the area of single cells within a gland were placed over identified PC or CC/MNC, thus defining the spatial boundary of data acquisition for these types of plots. PC were readily distinguished from other cell types because of their distinctive morphology and location. Ratios were converted to pH_i using the calibration technique described above.

Microspectrofluorimetry of Single Cells Using a Photomultiplier

On a few occasions microspectrofluorimetry of single cells within BCECF-loaded glands was also carried out using a photomultiplier tube and an image plane pinhole to restrict the collection of emitted fluorescence to a single cell. Experiments with the imaging system have demonstrated that the pH response of individual cells of a given cell type are remarkably similar throughout the entire gland. Thus, single-cell measurements provide a reasonable reflection of the behavior of all the cells of that type in the gland. A computer-controlled filter wheel provided alternate excitation at 440 and 490 nm. BCECF fluorescence was collected via a 510-nm bandpass filter using a photomultiplier tube and amplifier, and the data were relayed to an IBM/AT computer. The ratio of the two intensities was calculated and displayed on-line each second. Data acquisition, display, and storage were accomplished with UMANS software. Additional analysis of single-cell measurements employed a custom program (by William Weintraub, Department of Molecular and Cell Biology and Joe Bonanno, School of Optometry, University of California at Berkeley) to facilitate the conversion of ratios to pH_i.

Extracellular Perfusion of Glands

Glands were allowed to settle onto glass coverslips, where they were immobilized by the interaction between the cells and the glass, and mounted into a metal flow-through perfusion chamber that has been described elsewhere in more detail (Negulescu & Machen, 1990). Solutions, equilibrated with room air and maintained at 37°C, were allowed to flow continuously across the gland preparation, thereby eliminating artifacts due to dye leakage and collection in the extracellular medium. Use of a Hamilton 8-way valve (Reno, Nevada) permitted rapid manipulation of the external bathing solution with complete turnover of the chamber contents in 1 to 2 sec.

Table 1. Solutions

Solution	Composition (mM)
NaCl Ringer	145 NaCl, 2.5 K_2HPO_4, 1 $CaCl_2$
Na-free	145 NMG Cl, 2.5 K_2HPO_4, 1 $CaCl_2$
Cl-free	145 Na gluconate, 2.5 K_2HPO_4, 1 Ca gluconate
Cl-free/72Na	72.5 NMG gluconate, 72.5 Na gluconate, 2.5 K_2HPO_4, 1 Ca gluconate
Cl-free/77K	72.5 Na gluconate, 72.5 K gluconate, 2.5 K_2HPO_4, 1 Ca gluconate
Cl-free NH4	15 $(NH_4)_2SO_4$, 100 NaCl, 2.5 K_2HPO_4, 1 Ca gluconate
NH4	30 NH_4Cl, 115 NaCl, 2.5 K_2HPO_4, 1 $CaCl_2$
77K/72Na	72.5 NaCl, 72.5 KCl, 2.5 K_2HPO_4, 1 $CaCl_2$
72Li,Cs/72Na	72.5 NaCl, 72.5 Cs/LiCl, 2.5 K_2HPO_4, 1 $CaCl_2$
77K/72NMG	72.5 NMG Cl, 72.5 KCl, 2.5 K_2HPO_4, 1 $CaCl_2$
72Na,Li,Cs/72NMG	72.5 NMG Cl, 72.5 Na/Cs/LiCl, 2.5 K_2HPO_4, 1 $CaCl_2$
10TlCl	135 NMG Cl, 10 TlCl, 2.5 K_2HPO_4, 1 $CaCl_2$

In addition to the listed constituents, all buffers also contained (in mM): 10 HEPES, 10 glucose, 1 $MgSO_4$. All solutions were pH 7.4 unless otherwise noted in the text. In experiments where Ba was added to the bath, solutions were PO_4- and SO_4-free (Cl replacement).

Solutions and Chemicals

All chemicals were obtained from Sigma unless otherwise noted. BCECF-AM (from Molecular Probes, Eugene, Oregon), was dissolved in DMSO to make a 10-mM stock solution. The composition of the various Ringer solutions used in this study are named and summarized in Table 1. All solutions were adjusted to pH 7.40, with the exception of the low-pH, Na-free solution, which was adjusted to pH 6.70 for use in acidifying the cells in one particular protocol.

Solutions containing both H_2DIDS and amiloride were prepared as follows: amiloride (0.5–1.0 mM final concentration) was completely dissolved in Ringer solution by heating the solution gently (40°C). H_2DIDS was added to the warmed buffer and stirred until dissolved. This method prevented coprecipitation of the two compounds, as commonly occurs in the absence of heating.

SCH28080, a kind gift of Dr. George Sachs, was prepared in DMSO as a 100-mM stock solution. SCH28080 fluorescence did not interfere with measurements made at the 440- and 490-nm excitation wavelengths.

Calculation of Transmembrane H Fluxes

The proton fluxes shown in Table 2 were computed using the following method: An initial rate (in pH units/min) of K- (or Cs- or Li- or Tl-) dependent alkalinization was calculated using a computer-generated linear fit (Kaleidagraph 2.0.2, Abelbeck Software) to the pH *vs.* time plots such as the ones shown in Figs. 2–9. The rate of alkalinization or acidification prior to K addition, if any, was subtracted from this value, and then the net recovery rate was converted to a proton flux (in mM/min) using previously published buffering capacities (Wenzl & Machen, 1989). Since buffering capacity is a function of pH_i, the inflection point pH marking the transition between K Ringer and the previous perfusate was used in calculating proton flux. We assumed that the pH_i dependence of buffering capacity of the other cell types was the same as values published for the PC.

Table 2. H flux rates

Protocol	H fluxes (mM/min) ± SEM	
	PC	CC/MNC
72Na/72NMG		
+ amiloride	1.9 ± 1.4	0.5 ± 0.3
raw flux	(n = 96, 21, 8)	(n = 57, 17, 8)
72Na/77K		
+ amiloride	4.4 ± 1.8	2.5 ± 0.5
net flux	(n = 96, 21, 8)	(n = 57, 17, 8)
145NMG		
(Na free)	−1.5 ± 0.3	−1.3 ± 0.6
raw flux	(n = 64, 18, 6)	(n = 41, 13, 5)
72NMG/77K		
(Na free)	5.3 ± 0.9	5.9 ± 1.1
net flux	(n = 64, 18, 6)	(n = 41, 13, 5)

H flux rates for parietal cells (PC) and chief/mucous neck cells (CC/MNC) in mM/min. ± SEM; n = number of cells, glands, and rabbits. "Raw flux" refers to absolute rate of recovery prior to high K treatment, while the "net flux" takes into account rates of acidification or alkalinization prior to K addition, as described in Materials and Methods.

^{14}[C]-Aminopyrine Uptake

Experiments to determine the accumulation of ^{14}[C]-aminopyrine (AP) were performed using methods described previously by Berglindh, Helander and Obrink (1976). AP is a permeant weak base that becomes positively charged upon protonation, and as such, the compound accumulates in acidic compartments, particularly in the enclosed canalicular/tubulovesicular space of the PC. AP accumulation (expressed as a ratio of sequestered molecule to that in the bath), then, can be used as an indirect estimate of HCl formation via the H/K-ATPase. We compared the effects of K, Cs, Li, and Tl on AP uptake under conditions similar to those used

to monitor pH_i changes. Details of the experimental protocols are mentioned in the text.

One-ml aliquots of glands suspended in various test buffers (four replicates per test condition per rabbit) were incubated with ^{14}[C]-aminopyrine (0.04 mCi/ml, 117.9 mCi/mmol, New England Nuclear) at 37°C in a shaking water bath for 20 min. Samples were centrifuged and an aliquot of supernatant retained for counting in a scintillation counter before aspirating the residual supernatant. Pellets were dried, weighed, and solubilized overnight at 90°C in 1 N KOH. The pellet suspension was neutralized with 1 N HCl, and a sample was delivered to a scintillation vial for counting. The aminopyrine ratio (AP ratio) was calculated by dividing the pellet cpm (the counts per ml of intraglandular water) by the cpm/ml in the supernatant. A value of 2ml/mg was used to approximate the volume of cell water in the pellet.

Results

K Accelerates pH_i Recovery of Parietal and Chief/Mucous Neck Cells, Blocked by 100 μM SCH28080

The chance observation that prompted this study was that exposure to higher than normal extracellular [K] caused intracellular pH (pH_i) of both PC and CC/MNC[1] in isolated rabbit gastric glands to recover from acid loads. A light micrograph of a typical fundic gland is shown in Fig. 1*A*, and the PC and CC/MNC are both readily visible.

A representative experiment showing the effect of K on average pH_i of seven PC and three CC/MNC cells within a single gland is shown in Fig. 2. In this case the gland was acidified using the ammonium prepulse technique. After a 9-min treatment with 30 mM NH_4Cl Ringer, the perfusate was switched to 72Na/72NMG plus amiloride, and this resulted in a large acidification of the cytoplasm and a slow recovery of pH_i. This gradual, but finite, rate of pH_i recovery occurred frequently (52% of both PC and CC/MNC), in spite of the presence of amiloride (to prevent Na/H exchange) and the nominal absence of HCO_3 in the bathing solution. When this pH_i recovery was converted to an H flux, it averaged 1.9 ± 1.4 mM/min in PC, and 0.5 ± 0.3 mM/min in CC/MNC (*see* Table 2).

Subsequent addition of 77K/72Na solution plus amiloride resulted in an enhanced recovery rate of pH_i in both cell types to approximately pH_i 7.0 (Fig. 2; *see* Table 2 for averages). Note that this protocol ensured the maintenance of constant external [Na] during the normal-to-high K transition. In the 77% of the cells that responded to the K treatment, average H fluxes were 4.4 ± 1.8 mM/min, which is a modest rate compared to that exhibited by the other well-known ion exchangers in the PC. For example, the Na/H exchanger extrudes H at a rate of 12 mM/min at pH 6.5 (M.C. Townsley and T.E. Machen, *unpublished*), and the Cl/HCO_3 exchanger has an even larger capacity to transport base equivalents: 13 mM/min at pH 8.1 (Wenzl & Machen, 1989).

K-induced recovery was inhibited by addition of 100 μM SCH28080 to the bath (Fig. 2). In contrast, when 1 μM SCH28080 [a concentration which is known to inhibit acid secretion as assessed by aminopyrine uptake in normal Ringer's (Wallmark et al., 1987)] was applied to glands recovering in normal Na-containing Ringer, there was no effect on the rate of alkalinization, either in the absence of amiloride (when Na/H exchange is operational) or in the presence of amiloride. This last result shows that SCH28080 does not affect the pH_i recovery generated by the Na/H exchanger or the slow recovery process observed in Na- and amiloride-containing solutions.

Since all cell types of the gland responded to K treatment, we considered the possibility that H-transporting mechanisms residing in one cell type might somehow influence the pH_i response in neighboring cells. In particular, the H/K-ATPase in PC might be stimulated by K Ringer, and this alkalinization might be communicated to neighboring CC/MNC through gap junctions. One way to test this possiblity would be to perform experiments on isolated cells prepared from intact glands. Unfortunately, in our hands, isolated cells had poor viability. We were, though, able to perform experiments on gastric glands isolated from the antral region of the stomach, which have fewer PC and increased numbers MNC. While the majority of the glands derived from this preparation had an appearance typical of those obtained from the fundus or body of the stomach (Fig. 1*A*), some glands were comprised entirely of small, nongranular cells and appeared to be devoid of PC (Fig. 1*B*).

As seen in Fig. 3, when these antral glandular cells were acidified by ammonium prepulse into a Na-free solution, K treatment elicited an alkalinization that was very similar to that observed in both PC and CC/MNC in fundic glands. As further confirmation of the absence of PC in these glands, the pH_i response to Cl-free solutions was tested. Because PC possess vigorous Cl/HCO_3 activity, while non-PC do not (Paradiso et al., 1987*b*), switching to Cl-free Ringer's invariably results in a rapid increase in pH_i in PC due to reversal of the anion exchanger

[1] Parietal cells are easily identified in the glands by their large size and bulging appearance. The other cells in the fundic glands are both chief cells and mucous neck cells, but it is impossible at the light microscopic level to tell the difference between these latter two. We therefore refer in the text to PC and CC/MNC.

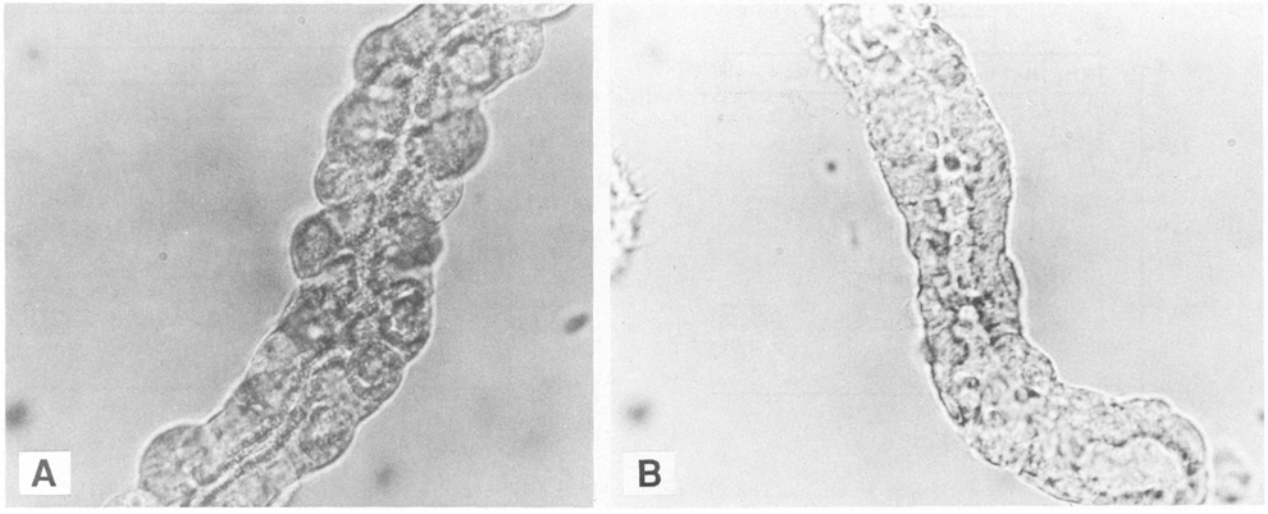

Fig. 1. Brightfield micrographs of isolated fundic (*A*) and antral (*B*) glands. The PC (easily identified by their large and bulging appearance) are interspersed with CC/MNC in the fundic gland (*A*). In some of the PC it is possible to see the connection of the intracellular canaliculus with the lumen. Note the *absence* of PC in the antral gland (*B*).

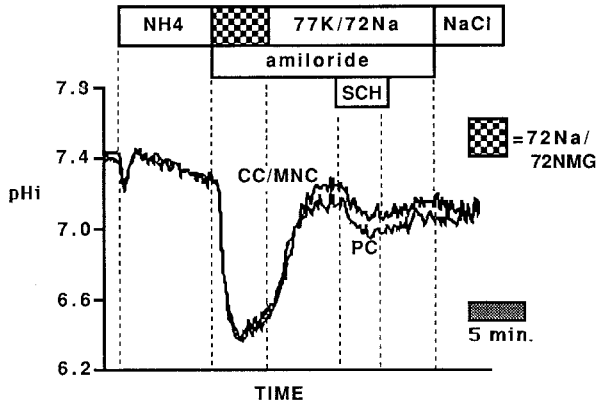

Fig. 2. Typical trace illustrating the effect of 77 mM K Ringer on the pH_i recovery of seven parietal cells (PC) and three chief/mucous neck cells (CC/MNC) in an isolated rabbit gastric gland from the fundus. Cells were acidified by the ammonium prepulse technique (30 mM NH_4Cl replacing NaCl in the buffer) with the subsequent perfusate containing 72 mM Na (NMG replacement) and 0.5 mM amiloride to inhibit Na/H exchange. Under these conditions there was often (52% of cells) a slow recovery (refer to Table 2). This recovery was accelerated by treatment with 77K/72Na Ringer plus amiloride. Application of the potent H/K-ATPase inhibitor SCH28080 (100 μM) inhibited the K-induced alkalinization, and this inhibition appeared to be only partially reversible.

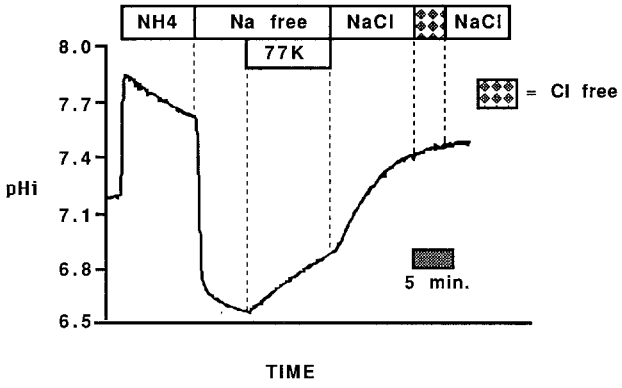

Fig. 3. K-induced alkalinization can also be elicited in antral glands that contain no PC. Cells from an antral gland were acidified using an ammonium prepulse followed by treatment with Na-free solution. Switching to 77K/72NMG caused pH_i to increase, similar to the effect noted in PC and CC/MNC in the fundic glands. Switching to a Cl-free solution, which is expected to alkalinize PC (due to reversal of the Cl/HCO_3 exchanger) had no effect on pH_i, thus confirming the absence of PC in the antral glands.

(but not in CC, which do not have an anion exchanger). As shown in Fig. 3, Cl-free solution had no effect on pH_i of cells within glands determined not to have PC. Some of these cells even acidified by 0.2 pH_i during Cl-free treatment (*not shown*).

One curious feature of K-induced alkalinization is that it was not evoked unless the cells had been previously acidified. High K treatment had no effect on pH_i when glands were at resting pH_i (*not shown*). Consistent with this finding was the fact that 100 μM SCH28080 had no effect on pH_i when it was added to control glands at resting pH_i, both with and without high K (*not shown*). The possibility that SCH28080 is only effective at reduced pH_i was also addressed by experiments described above, where cells were acidified in the presence of amiloride, and

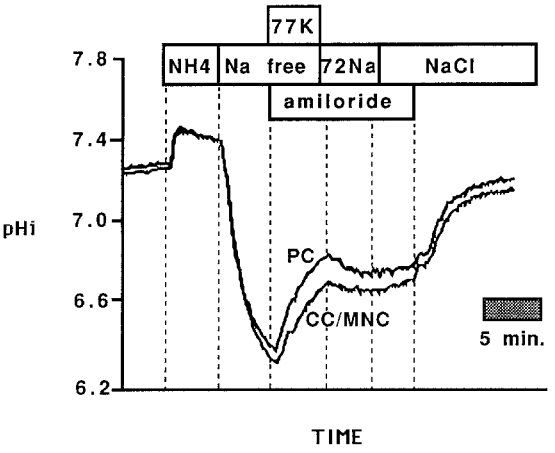

Fig. 4. K-induced recovery in Na-free solutions. The pH_i response of six PC and five CC/MNC following ammonium prepulse into Na-free solution. There was typically no pH_i recovery whatsoever in Na-free solutions, but switching to 77K/72NMG elicited a rapid alkalinization that was abruptly reversed by returning to 72Na/72NMG. As shown by this trace, changing from 72Na/72NMG to NaCl Ringer's (i.e., increasing [Na] from 72 to 145 mM) had little effect on pH_i i the presence of amiloride (0.5 mM).

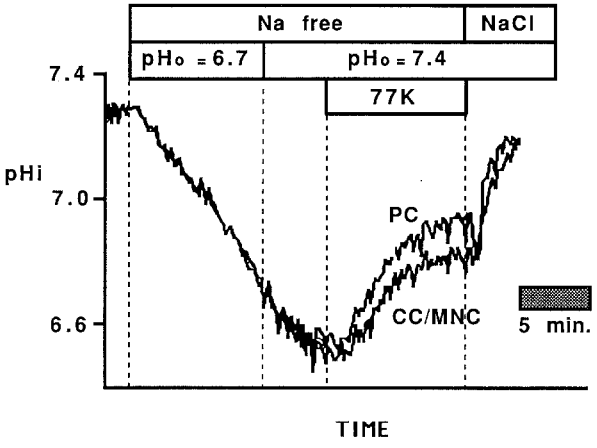

Fig. 5. K-induced alkalinization is independent of NH_4Cl prepulse. Seven PC and three CC/MNC in a single gland were acidified by treatment with a low pH (pH_o = 6.70), Na-free Ringer's. This was followed by perfusion with pH 7.4 Na-free solution. Treatment with 77K/72NMG Ringer (also at pH 7.4) resulted in a vigorous recovery of cytosolic pH.

the inhibitor was added before high K treatment while pH_i was still low (i.e., pH_i 6.7). Under these acidified conditions SCH28080 had no effect on pH_i (*not shown*).

High K Effect Is Na Independent

K-dependent recoveries were also elicited in Na-free solution, as shown in Fig. 4. Switching to Na-free solution following an ammonium prepulse resulted in a profound acidification, and, in contrast to the profile observed in NaCl or 72Na/72NMG Ringer's plus amiloride, pH_i under these conditions rarely (3% of cells) exhibited any recovery whatsoever, indicating that the recovery observed in amiloride-containing solutions is dependent in some way on the presence of Na in the solution.[2] Ringer (77K/72NMG) induced a vigorous alkalinization (for averages *see* Table 2) which was rapidly reversed by returning to Na-free (145NMG/5K) Ringer.

[2] Interestingly, switching from 72Na/72NMG to NaCl Ringer (amiloride present throughout) in cells that have been previously acidified had no effect on the recovery rate. However, when cells were acidified into Na-free media and then perfused with Na-containing solution plus amiloride, a significant alkalinization occurred. SCH28080 (100 μM) had no effect on Na-induced alkalinization following Na-free treatment (Fig. 8), and we therefore believe this process to be separate from the one described above.

K Effect Does Not Depend on NH_4 Treatment or the Presence of Cl

Additional experiments were conducted in Na-free media to establish whether the K effect was independent of the NH_4Cl prepulse (Fig. 5). Glands were acidified in a low pH (6.70) Na-free Ringer and then perfused with Na-free media at pH 7.40. High K treatment (77K/72NMG, also at pH 7.40) resulted in pH_i recovery that was very similar to that exhibited using the NH_4 prepulse treatment.

We also tested whether K-induced alkalinization was dependent on the presence of Cl in the bathing solutions. A typical experiment is shown in Fig. 6. Because it was very difficult to acidify the cytoplasm in Cl-free media using $(NH_4)_2SO_4$ prepulses (*see also* Townsley & Machen, 1989), a unique protocol was adopted for lowering pH_i under these circumstances. Glands were initially perfused with NaCl Ringer containing 30 mM NH_4Cl for approximately 5 min followed by Cl-free Ringer's containing 15 mM $(NH_4)_2SO_4$; all subsequent manipulations were then performed in the absence of Cl. We speculate that this technique promotes better acid loading (via NH_4 accumulation) for two reasons. First, because the primary acid-loading mechanism in these cells, the Cl/HCO_3 exchanger, can operate unhindered, there is increased conversion of NH_3 to NH_4 within the cell. At the same time, the entry of NH_4 through various conductive pathways is presumably facilitated because Cl can serve as counterion for NH_4 influx. Of the five PC that acidified to below pH 6.7 (out of the total of 16 that were tested),

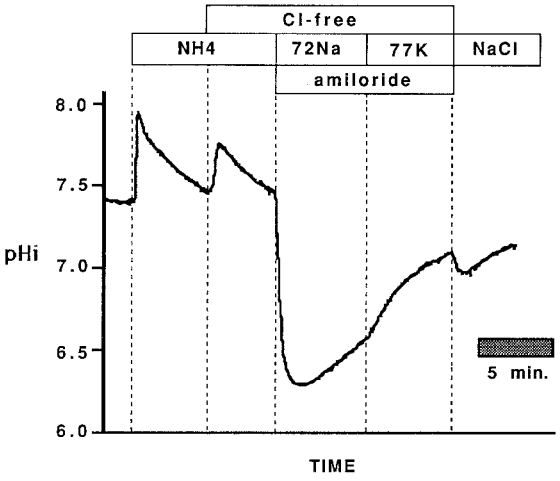

Fig. 6. High K promotes recovery of pH_i in Cl-free solutions. The trace shows the pH_i response of a single PC within a gastric gland. The cell was first treated with 30 mM NH_4Cl (Cl-containnig solution) to promote acid loading (*see* text for further explanation) followed by perfusion with Cl-free $(NH_4)_2SO_4$ (15 mM). Removal of NH_4 resulted in acidification of the cytoplasm with a slow recovery of pH_i even in the presence of 0.5 mM amiloride. Addition of Cl-free/77K Ringer yielded an increased rate of alkalinization. Subsequent perfusion with NaCl Ringer produced a small acidification, presumably due to reactivation of the Cl/HCO_3 exchanger.

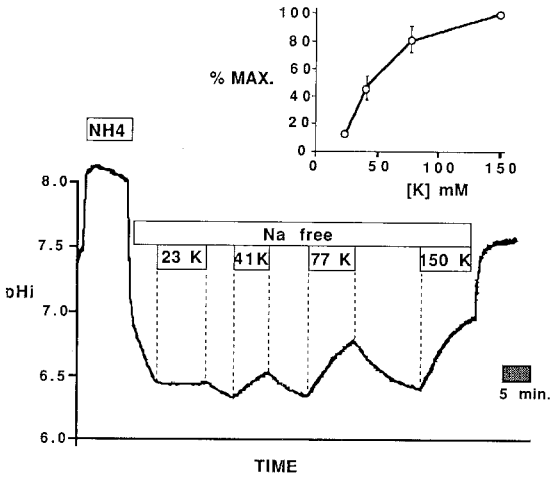

Fig. 7. The rate of pH_i recovery is dependent on external [K]. As shown for this single CC/MNC, addition of increasing [K] resulted in larger rates of alkalinizations, and these recoveries were reversed by returning to Na-free Ringer. In this way the dependence of the recovery rate on $[K]_{out}$ could be determined at a single pH_i. The inset summarizes the results of a number of such traces for both cell types (n = 6 glands, 2 rabbits); the rates have been expressed as a percenage of the maximum recovery, which always occurred at 150 mM K. The SEM for the 23-mM K data point (±2%) is obscured by the symbol. The half-maximal rate of alkalinization was achieved at approximately 45 mM K.

Cl-free/77K Ringer yielded an enhanced rate of alkalinization in three.

Concentration Dependence of the K Effect

The rate at which cells alkalinized in K solutions was dependent on the K concentration. Figure 7 illustrates the protocol used to assess the alkalinizing effect of different [K] in the same cell. In this case a single CC/MNC was acidified into Na-free solution (145 mM NMG/5 mM K). Sequential additions of 23, 41, 77 and 150 mM K were made with periods of intervening re-acidification produced by returning to Na-free Ringer. Using this protocol, the rates of alkalinization induced by various [K] were compared directly at a single pH_i (average inflection pH_i = approx. 6.40). This approach eliminated concerns about possible pH_i dependence of the transport mechanism or intracellular buffering capacity. The results of six such traces are summarized in the inset of Fig. 7 where K-induced recovery rates (corrected for acidification prior to K addition, as described in Materials and Methods) are normalized to the maximal recovery rate, which was always observed at 150 mM K. The estimated [K] at which the half-maximal rate of alkalinization occurred was approximately 45 mM.

Comparing the Effects of K, Li, Cs and Tl on pH_i Recovery and AP Uptake

A major concern in the experiments on PC was that the H/K-ATPase was contributing to pH_i recovery. This problem was addressed by comparing the effects of different cations on pH_i recoveries. The rationale behind this series of experiments was that Cs and Li are known to be transported by the H/K-ATPase with far less avidity than K (Sachs et al., 1976), while Tl activates the H/K-ATPase greater potency than K (Forte, Ganser & Ray, 1976). So, if the effects of high K were being mediated through the H/K-ATPase, then it might be expected that rates of pH_i recovery and AP accumulation would be directly correlated during treatment with K, Li, Cs and Tl. First, we tested the ability of these different cations to induce pH_i recovery. As shown in the traces of Fig. 8, when PC were changed from Na-free solution to either 72Na/72NMG, 72Li/72NMG, 72Cs/72NMG or 10Tl, the rate of pH_i recovery increased. When the PC were subsequently treated with 100 μM SCH28080, the recovery in 72Na/72NMG was unaffected, but the recoveries induced by Li, Cs and Tl were all blocked. Similar results were observed in CC/MNC.

Next, we compared recovery rates induced by

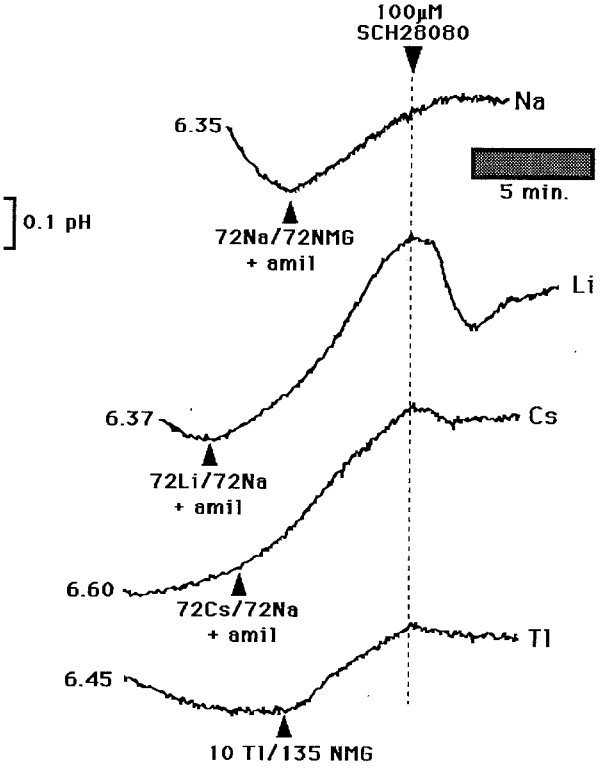

Fig. 8. SCH28080 (100 μM) blocks pH_i recovery induced by Li, Cs and Tl but not Na. Traces of pH_i *vs.* time for four different PC are shown. Cells were acidified by treatment first with NH_4 and were subsequently perfused with Na-free solution. The traces all start with the PC in the Na-free solution with the pH_i values shown at the beginning of the traces. At the arrows shown for each trace, the Na-free solution was replaced with either 72Na/72NMG, 72Li/72NMG, 72Cs/72NMG or 10Tl solution. The PC were next treated with 100 μM SCH28080 as shown by the arrow and the dotted line.

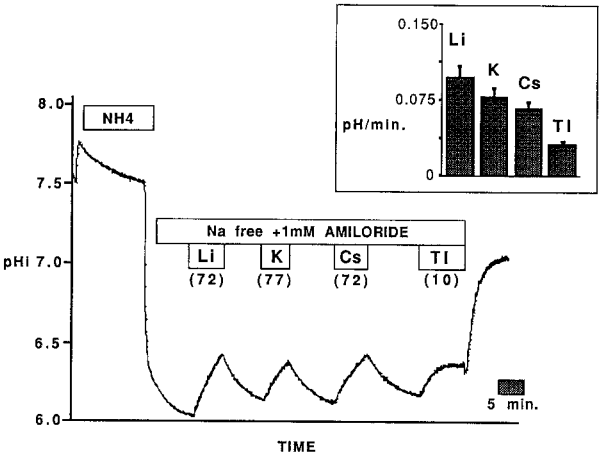

Fig. 9. Alkalinization is stimulated by Li, K, Cs, and Tl. A single CC/MNC was acidified using an ammonium prepulse into a Na-free solution containing 1 mM amiloride (to prevent Na/H exchange). Li and Cs (both 72 mM), K (77 mM), and Tl (at 10 mM, the highest concentration that will go into solution) were added sequentially, with intervals of re-acidification allowed between cation additions. Initial rates of recovery were compared for these different ions at pH_i 6.15. Results have been summarized in the inset (n = 8 cells, 8 glands, 3 rabbits). Irrespective of the order in which these cations were introduced, the order of pH_i recovery was always in the order Li > K > Cs > Tl. Note that the extent of recovery is smaller with Tl, which is likely the result of the lower concentration used with this ion.

all the cations in single PC (Fig. 9). Cells were acidified by ammonium prepulse into an amiloride-containing, Na-free solution, and Li, K, Cs, and Tl were added sequentially, again with intervening re-acidifications during removal of the test cation. Average rates of recovery with the different cations are presented in the inset of Fig. 9. Irrespective of the order in which cation additions were performed, alkalinization rates were always in the order 72 mM Li > 77 mM K > 72 mM Cs > 10 mM Tl.

Finally, we compared the effects of K, Cs, Li and Tl on the accumulation of ^{14}C-aminopyrine (AP). In performing these measurements we attempted to reproduce the conditions under which K-, Cs-, Li-, and Tl-induced alkalinizations were observed in BCECF-loaded glands, i.e., cells were first acidified either in Na-free solution or by preincubation with 1 mM amiloride. Parallel trials with control (cimetidine-treated) and stimulated (histamine + IBMX) glands were also conducted. As summarized in Fig. 10, treatment with 77K/72Na + amiloride produced a modest increase in AP uptake over control values, while 72Cs or 10Tl yielded a small diminution in the AP ratio. Incubation in 72Li/72Na + amiloride resulted in enhanced AP accumulation. As shown, the stimulatory effect of Li on AP accumulation was largely blocked by treatment with 100 μM cimetidine, indicating that Li may be causing the release of histamine from endocrine cells within the gland. However, the effects of K, Cs, and Tl were unaltered by the presence of cimetidine (*not shown*). Similar results were obtained for K, Cs, and Li in separate experiments using Na-free pretreatment to acidify the cells.

Lack of Effects of Bumetanide, Ba, Ouabain, H_2DIDS, Carbachol, and Histamine on K-induced pH_i Recovery

In order to rule out the contribution of other K-coupled transporters to the K-induced pH_i recovery, we investigated the effects of a variety of pharmacological agents on this process. The results have been summarized in Table 3. The loop diuretic bumetanide (200 μM), which blocks Na/K/Cl_2 cotransport

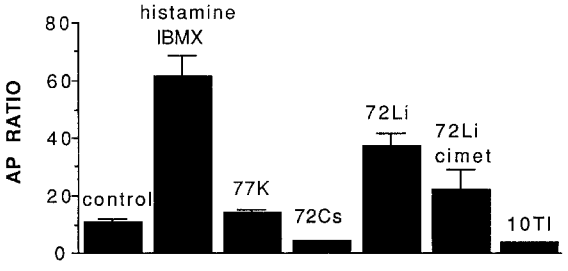

Fig. 10. Effects of Li, Cs, K, and Tl on [^{14}C]aminopyrine uptake. Glands were preincubated with 1 mM amiloride in normal Ringer to induce intracellular acidification, with subsequent additions of 72Li/72NMG, 72Cs/72NMG, 77K/72NMG or 10Tl solution. Control experiments (NaCl Ringer + cimetidine) and trials using secretagogues (histamnie + IBMX) were also performed for comparison (n = 5 rabbits). The control response was, on average, about 17% of that of the histamine + IBMX-treated glands, while glands incubated in 77K/72NMG gave AP uptakes that were only 23% of this value. The AP ratio with 72Li/72NMG was around 60% of that for stimulated glands, but the ratio declined to 36% upon addition of cimetidine. Tl and 72Cs/72NMG solutions yielded AP ratios that were below control levels. Similar results were obtained using the Na-free pretreatment protocol described in the text (n = 5 rabbits).

as well as K/Cl cotransport (O'Grady, Palfrey & Field, 1987) was without effect on K-induced recovery in either PC or CC/MNC when introduced prior to K treatment.

Barium (5 mM), an established blocker of basolateral K channels, did not alter pH_i significantly when added to acidified cells. Furthermore, K-stimulated H transport was not inhibited by Ba.

There is evidence that under certain circumstances H can be transported by the Na/K-ATPase (Polvani & Blostein, 1988). It is also known that high doses of SCH28080 antagonize the Na/K pump (Beil, Staar & Sewing, 1987). In order to exclude the Na pump as a possible pathway for H efflux, we performed experiments in the presence of ouabain. A brief (10-min) pretreatment with 1 mM ouabain, which is adequate time to inhibit the ATPase (Negulescu et al., 1990), had no inhibitory effect on K-induced alkalinization (*not shown*). These experiments utilizing acute ouabain treatments preclude an immediate contribution by the Na/K-ATPase to the K-induced alkalinization phenomenon.

Even though our experiments were carried out in nominally HCO_3-free, HEPES-buffered solutions, and the high K effect was present in Na-free solutions (Fig. 3), we wondered whether the high K solutions might be affecting the activity of the stilbene-sensitive Na/HCO_3 cotransporter in the Na-containing solutions. Because this electrogenic cotransporter operates with either two or three HCO_3 transported per Na (Boron & Boulapep, 1983; Hughes et al., 1989), depolarization by K could increase the inward movement of the cotransporter, thereby alkalinizing the cytoplasm. We tested this possibility by applying H_2DIDS during the recovery phase initiated by K. H_2DIDS did not affect the K-induced recovery.

Table 3. Agents which do not affect K-induced alkalinization

Agent	Concentration
H_2DIDS	500 μM
Amiloride	1 mM
Cimetidine	100 μM
Histamine	100 μM
Carbachol	100 μM
Bumetanide	200 μM
Barium	5 mM
Ouabain	500 μM

The cholinergic agonist carbachol is a relatively poor activator of HCl secretion by PC (Berglindh et al., 1976), but it does stimulate pepsinogen secretion by CC (Koelz et al., 1982). Histamine, on the other hand, is a potent stimulant of HCl secretion from PC. The PC-secretory response is potentiated during addition of both secretagogues (Soll, 1982). We observed that acute treatment with carbachol, histamine (100 μM each), or both agonists simultaneously did not alter the K-dependent pH_i recovery (*not shown*). Because increased turnover of the Cl/HCO_3 exchanger with histamine stimulation has been demonstrated and because this transporter normally serves as an acid-loading mechanism (Paradiso et al., 1989), the effects of secretagogues on K-induced alkalinization may be obscured by the stimulants. It was therefore necessary to perform these experiments in the presence of H_2DIDS (amiloride was present as well) to avoid misinterpretation of the results. Cimetidine (10–100 μM), a histamine (H_2) receptor antagonist, also had no effect on K-stimulated alkalinization when added during the pH_i recovery phase (*data not shown*).

Discussion

The data in this paper best support the conclusion that these cells possess a novel cation/H exchange-like mechanism, the activity of which is apparant only when pH_i is low. Other potential mechanisms which we have considered for K-induced alkalinization include the existence of parallel H and K conductances, such that treatment with K depolarizes the membrane leading to a requilibration of H within the cytosol, or, alternatively, that the phenomenon is mediated by the

H/K-ATPase in the PC and that coupling exists between the various cell types within the gland.

K-Induced Alkalinization Is Not Due to Parallel H and K Conductances

This hypothesis has been dismissed for several reasons. First, the existence of a significant proton conductance in isolated PC and in intact glands has been precluded by other investigators (Muallem et al., 1985). Furthermore, while basolateral K conductances are relatively well characterized in amphibian oxyntic cells (where they dominate the membrane potential, V_m) (Demarest & Machen, 1989; Demarest & Loo 1990), this appears not to be the case in the rabbit PC (Kafgolis, Hersey & White, 1984; Schettino, Kohler & Fromter, 1985). For example, experiments using both microelectrodes and patch-clamp techniques have shown that the membrane potential (−6 to −40 mV) in the PC within intact rabbit glands depolarized by only +1.5 mV in going from 7 mM K to 133 mM K. V_m was, though, extremely sensitive to changes in extracellular [Cl] (Sakai et al., 1989). Since the typical excursion in pH_i elicited by 77 mM K (0.5 pH units) would require a 30-mV depolarization, the membrane potential changes following K treatment, as reported in the literature, are insufficient to account for the changes in pH_i that we have observed here. In addition, Ba, a K channel antagonist, had no effect on pH_i and did not block K-induced alkalinization in either cell type (Table 3). Although we cannot exclude the possibility that high external K somehow activates previously quiescent H and K conductances, the available evidence suggests that parallel H and K conductances are not responsible for K-induced alkalinization.

K-induced Alkalinization Is Not Due to the H/K-ATPase

Experiments from other labs have shown that high K causes an SCH28080- and omeprazole-sensitive increase in AP accumulation and proton translocation into the intracellular canalicular compartment where the H/K-ATPase is localized (Gibert & Hersey, 1982; Dembinski et al., 1986; Wallmark et al., 1987). The K effect described in the present study was blocked by SCH28080 but not at 1 μM, which is an effective dose in blocking AP accumulation. However, because the drug is a K competitor, the efficacy of H/K-ATPase blockade may be compromised in the presence of elevated K (Wallmark et al., 1987). As such, the differential dose dependency cannot be used unequivocally to distinguish between the sensitive H/K-ATPase and other potential mechanisms. Thus, we carefully considered whether the H/K-ATPase mediates the SCH28080- inhibited pH_i response to high K. There are, though, three reasons why we feel the H/K-ATPase is *not* the mechanism which causes high K-induced pH_i recovery from an acid load:

(i) K caused pH_i recovery in both PC and CC/MNC. Although one might argue for the fundic glands that the pH_i recovery might have been occurring first in the PC (via the H/K-ATPase) and then was communicated through gap junctions to the adjacent cells, this argument does not apply to the antral glands, which have no PC but still exhibit K-stimulated pH_i recovery.

(ii) Incubation in 77K/72NMG had only a very small effect on AP accumulation (a measure of H secretion)[3], but a large effect on pH_i. Conversely, stimulation with histamine and IBMX caused a large increase in AP accumulation but there was no effect of histamine plus IBMX on the rate of pH_i recovery either in the presence or absence of K.

(iii) Similarly, Cs, which is poorly transported by the H/K-ATPase, caused increases in pH_i recovery but no stimulation of AP accumulation. Although Li caused an increase in AP accumulation, the effect was mostly blocked by cimetidine, indicating that Li activates the H/K-ATPase only indirectly, probably by releasing histamine from other cell types within the gland (Sernka, 1989). Li did cause a vigorous recovery from an acid load.

So while both K-induced alkalinization and the H/K-ATPase are inhibited by SCH28080, the effect of high K on pH_i cannot be accounted for entirely by the activity of the gastric proton pump. It should be noted also that the PC H/K-ATPase is not the only known target of this compound. Other transport mechanisms with ATPase-like properties have been identified in the rabbit kidney connecting tubule, collecting duct (Garg & Narang, 1988), and also in the rat distal colon (Perrone & McBride, 1988), and all of these are also inhibited by relatively high doses (>100 μM) of SCH28080.

A Novel K/H Exchanger?

The K effect on pH_i was not dependent on the presence of Na, Cl, or HCO_3, and it was also not blocked

[3] The fact that our K-induced AP ratios were much smaller than those yielded by treatment with histamine and IBMX seems to contradict others who found high K to cause an increase in AP comparable to that of histamine stimulation (Berglindh et al., 1980; Hersey et al., 1981). This apparent discrepancy may be the result of the fact that we used a lower [K] than did the previous workers (e.g., 77 mM K *vs.* 108 mM K). Also, our AP measurements utilized maneuvers which initially acidified cells.

by amiloride (inhibits the Na/H exchanger), H_2DIDS (inhibits both anion exchange and Na/HCO_3 cotransport), bumetanide (inhibits Na/K/Cl cotransport), or ouabain (inhibits the Na/K-ATPase). We therefore propose that the K-induced alkalinization observed in all cell types of the gastric gland is due to the activity of a new K/H exchanger that operates when the cells have been acidified and which is inhibited by high doses of SCH28080. Although we cannot state unambiguously that the H/K-ATPase does not contribute to the pH recovery in the PC itself, the ATPase cannot account for the pH_i recovery observed in the other cells. This new mechanism appears also to transport Li, Cs, and Tl.

While this transporter does appear to exchange extracellular K for intracellular H, the process may not be the same as those reported in other cell types, namely the cornea (Bonanno, 1991), ileum (Binder & Murer, 1986), and *Amphiuma* red cell (Cala, Mandel & Murphy, 1986). The corneal K/H exchanger imposes a continuous acid load on these cells, and its action is normally opposed by a Na/H antiporter such that a constant pH_i is maintained. Unlike the process described in the present paper, the activity of this mechanism is manifest at resting pH_i and is unaffected by SCH28080. In *Amphiuma* RBCs, a K/H exchanger functions in cooperation with other ion transporters as a volume-regulatory mechanism and is possibly activated by increases in intracellular calcium ($[Ca]_i$). In contrast, we have observed that agonists which elevate $[Ca]_i$, such as carbachol, have no effect on K-induced alkalinization in gastric cells. Unlike classical exchangers, the process described here does not seem to be reversible. For example, when cells were alkalinized and then treated with a zero K solution, there was no detectable increase in the rate of acidification (*data not shown*). That the activity of this K/H mechanism is not evident unless the cells have been previously acidified is another feature incompatible with simple antiport behavior, but this property (as well as the apparent lack of reversibility) may be a consequene of complex kinetics of the putative exchanger. It will be interesting to test whether these other previously described transporters can also carry Li and Cs and also to examine their sensitivity to SCH28080.

Because the gastric cation/H exchanger seems to operate only at low pH_i, it is unlikely that its primary physiological function is related to the regulation of intracellular pH. One plausible explanation as to why the cell might possess such a transporter is that it may be involved in volume regulation. Conceivably, cell swelling might activate this K leak pathway that is coupled to H. The protons gained at the expense of K extrusion would be readily buffered by intracellular buffers (and as such remain osmotically inert). These protons might then be ultimately disposed by the other pH_i-regulatory mechanisms residing within the cells.

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