Biochimica et Biophysica Acta, 1105 (1992) 89–96

BBAMEM 75562

Inactivation of a volume-sensitive basolateral potassium conductance in turtle colon: effect of metabolic inhibitors

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(Received 18 September 1991)

Key words: Epithelium; Basolateral membrane; Potassium ion channel; Volume regulation; Ion conductance

Previous work has shown that the basolateral membrane of turtle colon epithelium contains a quinidine-sensitive potassium conductance which can be activated by osmotic cell swelling. In this work and in the present study, potassium flow across the basolateral membrane was measured as a short-circuit current across intact pieces of epithelial tissue in which amphotericin B was used to permeabilize the apical membrane. Quinidine-sensitive currents were generated when the mucosal bath contained chloride, a permeant anion. Replacement of chloride by sulfate or addition of sucrose to the bathing solutions abolished 75–90% of the current and caused the quinidine-inhibitable fraction of the current to go from over 90% to around 6% – suggesting that decreases in cell volume had brought about inactivation of the quinidine-sensitive conductance. When metabolic inhibitors were present, inactivation of the conductance by these maneuvers was prevented. Activation of the conductance by replacement of mucosal SO_4 by Cl, however, was not affected.

Introduction

Previous investigations have revealed that osmotically-induced cell swelling activates a quinidine-inhibitable potassium (K) conductance in the basolateral membrane of the turtle colon epithelium [1,2]. Thus, the turtle colon joins the growing list of epithelia in which cell volume changes have been shown to bring about alterations in the transport properties of cell membranes – presumably reflecting the presence of regulatory processes which are designed to protect cells against unduly large volume perturbations [3,4].

In the aforementioned study, the basolateral K conductance was measured in intact pieces of epithlial tissue using a modification of the method of Kirk and Dawson, in which the polyene antibiotic, amphotericin B, was employed in order to functionally eliminate the apical membrane as a barrier to cation flow [5]. Through measurements of macroscopic currents and ionic tracer fluxes, they were able to show that the volume-activated (quinidine-sensitive) conductance was physically and pharmacologically distinct from a 'resting' K conductance (quinidine-insensitive) which was present in the basolateral membrane when cells were not swollen [1,2]. The contribution of either of these two conductances to the overall conductance of the basolateral membrane was found to be highly dependent on the anionic composition of the bathing media. In particular, the volume-activated conductance was present when the mucosal bath contained anions capable of permeating amphotericin pores (Cl, NO_3 or SCN), while the resting conductance was observed when the predominant mucosal anion was impermeant (SO_4 or gluconate). Results were consistent with a scenario in which the quinidine-sensitive conductance was activated by cell swelling subsequent to a gain in cell solute content vis-a-vis co-diffusion of K and a counterion through amphotericin pores.

In the present study, we show that the quinidine-sensitive K conductance can be inactivated by maneuvers which would be expected to bring about decreases in cell volume. This finding is significant because if this conductance is activated as part of a volume-regulatory process, it would be expected that the activation would be reversible. Furthermore, we show that agents which are commonly employed as blockers of ATP production can prevent the inactivation, but not the activation, of the quinidine-sensitive conductance. This finding differentiates the volume-activated K conductance of turtle colon from volume-activated K conductances found in other epithelia and may shed some light on the mechanism by which volume changes modulate the

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conductance. (This work has been published in abstract form, [6]).

Materials and Methods

1. Preparation and mounting of epithelial tissues

Colons were dissected from turtles (*Pseudomys scripta*) and stripped of musculature, as described previously [7]. Isolated pieces of tissue, (consisting of the mucosal epithelium, underlying connective tissue, and the muscularis mucosae) were mounted in Ussing chambers (5.2 cm^2 area). Standard amphibian Ringer's solution (112 mM NaCl, 2.5 mM $KHCO_3$, 1 mM $CaCl_2$) containing 10 mM glucose was added to both sides of the chamber (volume of mucosal or serosal bath was 10 ml) and the baths were stirred and aerated with room air. The pH of this and all other solutions was approximately 8.3 at room temperature. The chambers were connected to a four-electrode voltage clamp (Physiologic Instruments, San Diego, CA) by means of agar bridges containing 3 M KCl. In all experiments, transepithelial electrical potential was held at zero, and the current required to maintain this condition (the 'short-circuit' current, I_{sc}) was displayed on a strip-chart recorder. In addition, the 'small-signal' or total tissue conductance was monitored at 60-s intervals by measuring the change in current which resulted from the imposition of a brief (1 s) step of voltage (usually 10 mV) across the tissue. After mounting the tissues, sufficient time was allowed for I_{sc} and the small-signal conductance to attain steady values (typically 1 hour) before any further experimental maneuvers were performed.

2. Measurement of basolateral K conductance

K gradients were imposed across the tissues by replacing the mucosal bath with an iso-osmotic Ringer's solution in which all of the Na had been replaced with an equal amount of K (mucosal K = 114.5 mM). The ionic composition of the serosal bath was kept constant so that transmural K gradients were 114.5 : 2.5 mM. Replacement of the mucosal bath with Na-free solution typically reduced I_{sc} to around 10% of its initial value. Following replacement of the mucosal bath, 10^{-4} M ouabain was added to the serosal bath in order to inhibit the basolateral Na/K-ATPase [8]. 1 μM atropine was added to the serosal bath in order to prevent modulation of the basolateral K conductance by acetylcholine which might be released from nerve terminals in the submucosa [9]. Tissues were then incubated for at least one hour before any further manipulations were performed. During this time, I_{sc} typically declined to near zero.

Following incubation, amphotericin B ($9 \cdot 10^{-6}$ M) was added to the mucosal bath. This typically caused an increase in I_{sc} consistent with the flow of positive charge from mucosa to serosa. This current usually reached a steady state within 10–20 min. Comparison of the I_{sc} with simultaneously-measured tracer K fluxes have shown that under these conditions, the amphotericin-induced current is entirely due to the flow of K down its concentration gradient and that the rate-limiting barrier for conductive K flow is the basolateral membrane [1]. Hence, the amphotericin-induced I_{sc} is a direct indicator of the K conductance of the basolateral membrane.

K transport through the volume-activated K conductance was identified as that part of the amphotericin-induced I_{sc} which was inhibited by quinidine. Quinidine was added to the mucosal bath at a final concentration of 0.2 mM – a dose which was found to be capable of inhibiting the volume-activated conductance completely [1]. This was then routinely followed by a second application in order to insure that inhibition was complete.

3. Mucosal anion substitutions

In the present study, only two major mucosal anions were employed – Cl and SO_4. Anion substitutions were carried out isoosmotically by adding either KCl Ringer's solution (112 mM KCl, 2.5 mM $KHCO_3$, 1 mM $CaCl_2$) or K_2SO_4 Ringer's solution (56 mM K_2SO_4, 2.5 mM $KHCO_3$, 1 mM $CaCl_2$, 56 mM sucrose) to the mucosal bath. Exchange of Cl for SO_4 or vice-versa was accomplished using a sample-and-replace paradigm in which the total volume of replacement solution was 50 ml (5-times the volume of the mucosal bath). If amphotericin or any other drugs were present in the mucosal bath prior to exchange, they were present in the replacement solution at the same concentration.

4. Metabolic inhibition

In experiments involving metabolic inhibitors, iodoacetate (as the sodium salt) was added to the mucosal and serosal baths in combination with either cyanide (as potassium salt) or carbonyl cyanide *p*-trifluoromethoxyphenyl hydrazone (FCCP). All were present at 1 mM concentration. Iodoacetate was added in order to inhibit glycolysis, while cyanide (a blocker of electron transport) or FCCP (an uncoupling agent) was employed to inhibit oxidative phosphorylation. These were added after ouabain and were present for at least one hour prior to any further manipulations.

5. Morphological studies

In order to assess whether or not cell volume changes had taken place, tissues were examined using light microscopy. Tissues were fixed by addition of a small volume of concentrated formaldehyde to both sides of the chamber to give a final concentration of 3%. This was done in the presence of amphotericin under three

conditions: (1) in the presence of mucosal Cl (with and without metabolic inhibitors), (2) following the replacement of mucosal Cl by SO_4, and (3) following addition of 200 mM sucrose to both sides in the presence of mucosal Cl (with and without metabolic inhibitors). Fixation was begun only after stable electrical responses had been attained following each maneuver. Tissues were fixed in situ for 5 min and then were removed and stored in 10% formalin. Following fixation, tissues were dehydrated and embedded in paraffin. Sections 5 μm thick were cut using a microtome (American Optical Co.) and were stained with hematoxylin and eosin. In order to minimize variations from section to section, care was taken to orient tissue blocks so that the plane of the section was perpendicular to the plane of the epithelium, and all sections were subjected to the same staining protocol. Tissues from at least two separate experiments for each condition were examined under the light microscope. Since previous studies have shown that cell swelling is reflected clearly by a change in height of the epithelial cells [2], apparent cell heights were measured at regular intervals along the length of tissue sections. Because cell height varied from location to location within a particular section, several (at least twenty) measurements were made for each condition and then were averaged. Results are expressed as ratios of averages obtained under two conditions. Photographs were taken of tissues fixed before and after replacement of mucosal Cl by SO_4 (without metabolic inhibitors) and are presented at the same final magnification.

6. Reagents

Atropine, ouabain, quinidine, iodoacetate, cyanide and sucrose were added to chambers as concentrated aqueous solutions. Amphotericin was dissolved in DMSO prior to addition, while FCCP was dissolved in ethanol. Quinidine and atropine solutions were prepared daily and were stored in light-proof bottles prior to use. All drugs were obtained from Sigma Chemical Co. (St. Louis, MO).

Results

1. Inactivation of quinidine-sensitive currents by sucrose or mucosal SO_4

Fig. 1 shows representative experiments in which KCl Ringer's solution was present in the mucosal bath. Note that prior to the addition of amphotericin, I_{sc} was near zero despite the presence of a 114.5:2.5 mM K gradient. Following amphotericin, there was an abrupt increase in the current, which in most instances reached steady state within 15–20 min. The mean amphotericin-induced I_{sc} was 297 ± 20 (mean $\pm$ S.E.; $\mu A/cm^2$, $n = 11$). The control experiment shows that most (92.8 $\pm$ 0.2%, $n = 2$) of this current was inhibited by quinidine, which is in agreement with earlier results [2]. The other records show that the quinidine-sensitive current was dramatically reduced by one of two maneuvers: (1) addition of 200 mM sucrose to the bathing solutions, or (2) replacement of mucosal KCl with K_2SO_4 Ringer's solution. Sucrose reduced I_{sc} by a factor of 92.3 $\pm$ 2.3%, ($n = 3$), while SO_4 reduced the current by 75.1 $\pm$ 2.5% ($n = 6$). Following these maneuvers a small current usually remained, and a fraction of this current

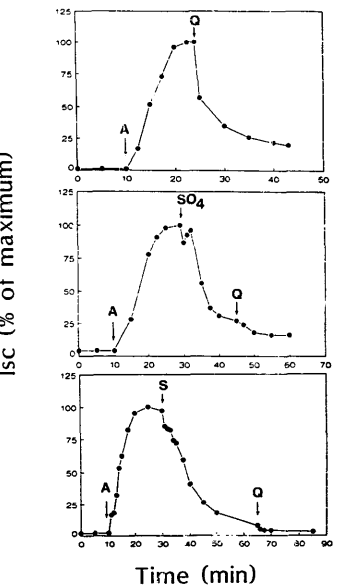

Fig. 1. Inactivation of quinidine-sensitive currents. Tissues were initially bathed on the mucosal side by KCl Ringer's solution and on the serosal side by NaCl Ringer's solution. 9 μM amphotericin B was added to the mucosal bath, and this resulted in the induction of currents which were due to the flow of K from mucosa to serosa. The upper panel (control) shows that addition of 0.2 mM quinidine (mucosal bath) inhibited most of the amphotericin-induced current. The next two panels show the effect of replacement of mucosal KCl with K_2SO_4 Ringer's solution (middle) or addition of 200 mM sucrose (lower) to mucosal and serosal bathing solutions. Symbols: A, amphotericin; SO_4, replacement of mucosal Cl by SO_4; Q, quinidine; S, sucrose.

was found to be inhibited by quinidine. However, when this quinidine-sensitive current was compared to the total amphotericin-induced current, it comprised only $5.7 \pm 0.6\%$ ($n = 3$) of the total in the case of sucrose and $6.4 \pm 1.8\%$ ($n = 6$) in the case of SO_4. This contrasts with the control experiments, in which over 90% of the amphotericin-induced I_{sc} was found to be quinidine-sensitive.

2. Effect of metabolic inhibitors

Incubation of tissues with metabolic inhibitors abolished the effect of sucrose or mucosal SO_4, as can be seen in the representative experiments shown in Fig. 2. The results show that when tissues were incubated with iodoacetate in conjunction with either cyanide or FCCP, replacement of mucosal Cl by SO_4 caused a transient dip in I_{sc} (as it did in the absence of metabolic inhibitors), but it did not induce inactivation of the current. This was true even when 200 mM sucrose was added subsequently. Because amphotericin was added in the presence of mucosal Cl, one would expect on the basis of previous results that most of the amphotericin-induced I_{sc} would be quinidine-sensitive. This was confirmed by addition of quinidine following mucosal anion exchange, showing that metabolic inhibitors did not alter the pharmacological sensitivity of the I_{sc}. In the presence of iodoacetate and cyanide, $68.0 \pm 3.1\%$ ($n = 5$) of I_{sc} was quinidine-sensitive, while $77.5 \pm 2.5\%$ ($n = 2$) was quinidine-sensitive when FCCP was used instead of cyanide. Similar results were obtained when 1 mM sodium fluoride was used in place of iodoacetate (not shown).

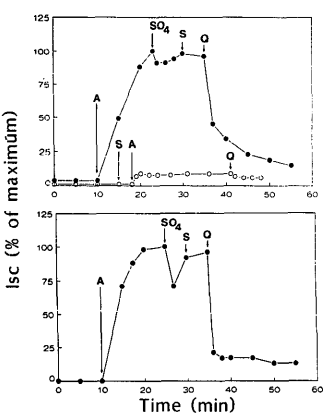

Fig. 2. Effect of metabolic inhibitors. (filled circles) Protocol was essentialy the same as in Fig. 1, except tissues were preincubated with either 1 mM iodoacetate plus 1 mM of either cyanide (upper panel) or FCCP (lower panel) in the mucosal and serosal baths. (open circles) Same as closed circles except that 200 mM sucrose was added to the mucosal and serosal bathing solutions prior to amphotericin. For meaning of symbols, see legend of Fig. 1.

The inability of sucrose or mucosal SO_4 to reduce the quinidine-sensitive I_{sc} in the presence of metabolic inhibitors suggests that the inhibitors somehow rendered inactive a mechanism by which the cells inactivate the K conductance following a reduction in cell volume. However, it could be argued that channels did not become inactivated because the inhibitors prevented sufficient cell shrinkage from occurring – perhaps by rendering the apical membrane permeable to SO_4 or sucrose. If such were the case, neither SO_4 nor sucrose would be able to exert their normal osmotic effects. Nevertheless, results shown in Fig. 2 show that this was not the case. When sucrose was added prior to amphotericin in the presence of iodoacetate and cyanide, currents induced by amphotericin were smaller and were largely insensitive to quinidine despite the presence of Cl in the mucosal bath. The quinidine-inhibitable portion of the I_{sc} was 7.05 ± 8.89 $\mu A/cm^2$ ($n = 3$) when sucrose was added prior to amphotericin, as compared to 116 ± 38 $\mu A/cm^2$ ($n = 5$) in the absence of sucrose. (The remainder of the amphotericin-induced I_{sc} was almost completely blocked by 5 mM $BaCl_2$ on the serosal side (not shown), which has previously been demonstrated to be a blocker of both quinidine-sensitive and -insensitive K conductances in this epithelium [1].) This suggests that the osmotic effect of KCl diffusion into cells was counteracted by the extracellular sucrose. Similar results were obtained in the absence of metabolic inhibitors (not shown).

3. Activation of quinidine-sensitive currents in the presence of metabolic inhibitors

In order to give rise to a steady current, K must enter cells through amphotericin pores in the apical membrane before exiting across the basolateral membrane. For this reason, it is not possible to determine whether the time course of the current increase in experiments such as those in Figs. 1 and 2 represented activation of the quinidine-sensitive basolateral conductance or whether it simply reflected the kinetics of amphotericin insertion into the apical membrane. Fig. 3 shows representative experiments in which quinidine-sensitive currents were activated after amphotericin had been in the mucosal bath for several minutes. Tissues were first incubated with K_2SO_4 Ringer's solution in the mucosal bath, and currents were induced by adding amphotericin. Previous studies have shown that this current is not sensitive to quinidine and that it

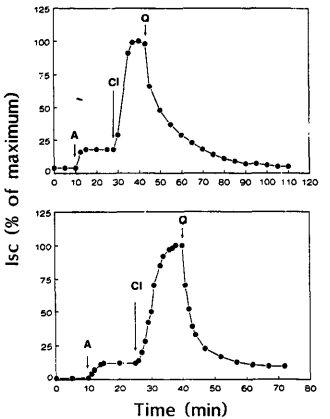

Fig. 3. Activation of quinidine-sensitive currents by mucosal chloride. Tissues were initially bathed on the mucosal side by K_2SO_4 Ringer's solution and on the serosal side by NaCl Ringer's solution. Following addition of 9 μM amphotericin to the mucosal bath, K_2SO_4 was replaced by KCl Ringer's solution. This was done in the presence (upper curve) and absence (lower curve) of metabolic inhibitors (1 mM iodoacetate + 1 mM cyanide, mucosal and serosal baths). Symbols: Cl, replacement of mucosal SO_4 by Cl, other symbols are the same as in Figs. 1 and 2.

represents K flow through a conductance which is distinct from the volume-activated conductance [2]. Following attainment of steady currents, SO_4 was replaced by Cl in the mucosal bath. This was abruptly followed by an increase in I_{sc} regardless of whether or not metabolic inhibitors were present. Other experiments (not shown) revealed that the increase in I_{sc} could be prevented by adding quinidine prior to mucosal anion replacement. Replacement of mucosal K_2SO_4 with an identical solution had no effect on the I_{sc} (not shown), demonstrating that the induction of the I_{sc} was not an artifact of the sample-and-replace procedure. Addition of quinidine following activation of the I_{sc} by mucosal Cl revealed that most of the current was quinidine-sensitive.

Amphotericin-induced (mucosal SO_4) and Cl-induced currents are given in Table I. Note that both Cl-induced currents and amphotericin-induced currents were reduced in magnitude when metabolic inhibitors were present – possibly reflecting a generalized effect of these agents on the basolateral membrane. However, the fraction of the total I_{sc} which was

TABLE I

Short-circuit currents induced

Quinidine-sensitive currents activated in the presence and absence of metabolic inhibitors (iodoacetate and cyanide). Tissues were initially incubated with K_2SO_4 Ringer's solution on the mucosal side. ΔI^{ampho} is the amphotericin-induced current, ΔI^{Cl} is the change in the current caused by replacement of mucosal SO_4 by Cl. ΔI^{total} is the total current in the presence of mucosal Cl. $\Delta I^{total} = \Delta I^{ampho} + \Delta I^{Cl}$. $\Delta I^{quin}/\Delta I^{total}$ is the fraction of the total current which was inhibited by quinidine. Values are presented as means ± S.E.

	Control (n = 4)	With metabolic inhibition (n = 3)
ΔI^{ampho} ($\mu A/cm^2$)	62 ± 18	32 ± 7
ΔI^{Cl} ($\mu A/cm^2$)	249 ± 37	156 ± 47
ΔI^{total} ($\mu A/cm^2$)	311 ± 54	188 ± 45
$\Delta I^{quin}/\Delta I^{total}$	0.88 ± 0.04	0.79 ± 0.07

quinidine-sensitive was not dependent on the presence or absence of metabolic inhibitors – indicating that the nature of the current induced by Cl was the same in both cases.

Inactivation of the quinidine-sensitive conductance was found to be reversible, as is evident in the representative experiment shown in Fig. 4. Here, amphotericin was added in the presence of K_2SO_4 Ringer's solution in the mucosal bath. As expected, replacement of mucosal SO_4 by Cl induced an increase in I_{sc}, while subsequent replacement of Cl by SO_4 caused the I_{sc} to return to where it had been prior to the initial anion substitution. (According to Fig. 3, the current induced by substituting Cl for SO_4 should have been sensitive to quinidine.) When SO_4 was replaced by Cl a second time, an increase in I_{sc} was again induced, and this

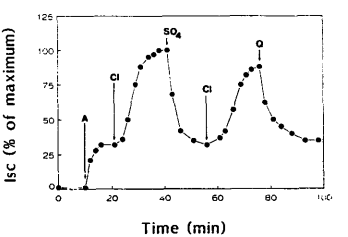

Fig. 4. Re-activation of quinidine-sensitive currents following inactivation by mucosal sulfate. Tissues were initially bathed by mucosal K_2SO_4 Ringer's solution and serosal NaCl Ringers's solution. Following addition of 9 μM amphotericin to the mucosal bath, K_2SO_4 was replaced by KCl Ringer's solution. After I_{sc} had increased to steady levels, the mucosal bath was returned to K_2SO_4 Ringer's solution. After decline of the I_{sc} to a constant value, KCl was again substituted for K_2SO_4. For meaning of symbols, see Fig. 3.

current was abolished by quinidine. Results thus show that both activation and inactivation of the quinidine-sensitive conductance are reversible processes, as would be expected of a physiological volume-regulatory mechanism.

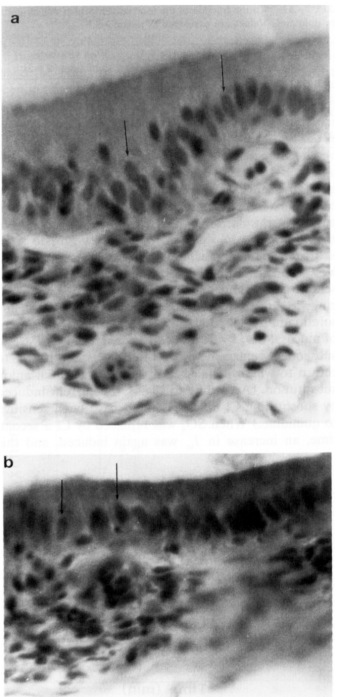

Fig. 5. Epithelia in the presence of mucosal chloride or sulfate. Tissues were fixed in the presence of mucosal Cl (a) or following the replacement of Cl by SO_4(b). Amphotericin was present under both conditions. Photomicrographs were taken using a light microscope equipped with an oil immersion objective. Magnification: 800×. Epithelial cells are located at the upper border of the tissue. Some nuclei are denoted by arrows.

4. Morphological studies

Fig. 5 shows epithelial tissues before and after replacement of mucosal Cl by SO_4 in the presence of amphotericin. Note that cells exposed to Cl appear to be much taller than those exposed to SO_4, which is consistent with the idea that substitution of SO_4 for Cl caused cells to shrink. Perhaps the most obvious indicator of the difference in volume between the two conditions is the distance of epithelial cell nuclei from the apical border. In all cases where cell swelling (increased cell height) was observed, the apical-nuclear distance was increased, giving the impression that nuclei were crowded into the basal ends of the cells. This was also accompanied by a decreased intensity of cytoplasmic staining – a phenomenon previously reported to occur with swollen cells [2].

It is important to note that we have made no attempt here to determine what the morphology of 'normal' cells would be. It is likely that some changes in cell volume could have occurred as a result of the fixation and embedding procedures. Thus it is to be understood that cells under some conditions are reported to be 'shrunken' or 'swollen' only relative to cells fixed under other conditions.

Measurements of cell height confirmed that replacement of mucosal Cl by SO_4 or addition of sucrose (all in the presence of amphotericin) caused cells to shrink. Furthermore, this result was not affected by the presence of metabolic inhibitors (cyanide and iodoacetate). Comparison of cell heights gave ratios of 0.70 (SO_4/Cl) and 0.57 (with sucrose/without sucrose). With metabolic inhibitors, the latter ratio was 0.60. Results are consistent with the notion that reduction in the short-circuit current following sucrose addition or exchange of SO_4 for Cl (as shown in Fig. 1) were caused by cell shrinkage. Furthermore, the failure of these maneuvers to reduce the current in the presence of metabolic inhibitors (as shown in Fig. 2) cannot be attributed to an absence of cell shrinkage.

Discussion

1. Inactivation of the quinidine-sensitive conductance

That sucrose addition or replacement of mucosal Cl by SO_4 was capable of reducing the quinidine-sensitive current in the face of a constant transepithelial K gradient and mucosal amphotericin B suggests that these manipulations brought about inactivation of the volume-activated basolateral K conductance. Both maneuvers would be expected to cause loss of cell water and a concomitant reduction of cell volume – although by different mechanisms. The addition of sucrose should cause efflux of cell water via a simple increase in the osmolarity of the bathing solutions, while the exchange of mucosal Cl for SO_4 would not entail any such overt change in osmolarity. However, if mucosal

Cl causes cell swelling due to Cl influx and a subsequent increase in cell solute content (see Ref. 2), then replacement of Cl by SO_4 (an impermeant anion) should induce Cl efflux, a reduction in cell solute, and subsequent water loss. Increases in K permeability following cell-volume expansion have been observed in many different cell types and are thought to be a means whereby cells regulate their volume [3,4]. The increase in K permeability accelerates the efflux of K (along with a counterion), thus reducing cellular solute concentration. This establishes an outward driving force for water movement and allows cells to bring their volume down toward normal. If activation of the quinidine-sensitive conductance in turtle colon represents such a volume-regulatory response, it is to be expected that the activation process would be reversible upon a reduction in cell volume, as the results of the present study appear to indicate.

2. Effects of metabolic inhibition

In the present study, tissues were incubated for one hour with pairs of metabolic inhibitors – one (iodoacetate or fluoride) to inhibit glycolysis and one (cyanide or FCCP) to inhibit oxidative phosphorylation. Although we had no way of measuring cellular ATP levels to verify that the inhibitors were effective, we were able to obtain indirect evidence to that effect. When we added metabolic inhibitors (1 mM each of iodoacetate and cyanide) to tissues bathed by NaCl Ringer's solution in the absence of ouabain, active sodium absorption (measured as an amiloride-inhibitable I_{sc} [7]) was reduced to zero within 15 min (data not shown). Since the I_{sc} under these conditions is driven by a basolateral Na/K-ATPase, this result suggests that Na/K pump activity was halted as a result of ATP depletion. The fact that various combinations of inhibitors all had the same effect on the quinidine-sensitive conductance despite their widely-divergent chemical structures and modes of action is evidence that the effect of these agents on the conductance was also due to a reduction in ATP levels.

The simplest explanation of our results would be that cell shrinkage normally brings about inactivation of the quinidine-sensitive conductance via a mechanism which utilizes ATP. However, we cannot rule out the possibility that this mechanism does not in fact utilize ATP but only requires that normal ATP levels be present. The inhibitors may have caused a change in the intracellular milieu (an increase in calcium or ADP levels, for example) which disrupted the inactivation mechanism, thus rendering the conductance unresponsive to cell shrinkage.

The above argument raises the possibility that metabolic inhibitors did not simply disrupt volume-inactivation, but rather, may have actually exerted a stimulatory effect on the conductance. Perhaps ATP itself had a direct modulatory effect on K channels, as has been shown in other cell types [10,11]. If, for example, the effect of ATP were to suppress the channels, then reducing ATP levels would have caused channels to open and perhaps remain open even in the face of cell shrinkage. Experiments shown in Fig. 3 counter this and similar arguments, however. The results show that replacement of mucosal SO_4 by Cl caused a rapid activation of quinidine-sensitive currents in both the presence and absence of metabolic inhibitors. Presumably, the mechanism was the same in both cases and involved cell swelling. Given this it is clear that metabolic inhibition by itself was not sufficient to activate the conductance.

3. Implications for the mechanism of activation / inactivation

Inhibition of ATP production is clearly a drastic maneuver and one which alters cellular function in a multitude of ways. For this reason, the results of the present study do not provide much in the way of positive insight into the mechanism whereby cell volume changes modulate the basolateral conductance. Despite the obvious difficulties, the present study clearly differentiates the mechanism of volume activation in turtle colon from mechanisms which activate basolateral K permeabilities in other epithelia. These volume-activated K permeabilities display quite a degree of heterogeneity, and both conductive and non-conductive K pathways have been found [3,12–17]. However, there is reason to believe that there may be considerable heterogeneity in the mechanism of volume-activation even among the systems employing conductive paths. For example, it has been shown that in *Necturus* small intestine, volume-activation of a basolateral conductance is blocked by metabolic inhibitors – a result which clearly distinguishes that system from ours [14].

Recent discoveries of stretch-activated channels in a wide variety of cell types (see Ref. 18) for review) has prompted speculation that such channels may be involved in the swelling-activated regulatory volume decreases which have been observed in different cell types [19–21]. In the simplest paradigm, cell swelling would induce tension in the plasma membrane, thus opening stretch-sensitive K channels and triggering an increase in K efflux. A subsequent reduction in cell volume would relieve membrane tension, allowing channels to close spontaneously. Recently, K channels exhibiting such behavior were found in the basolateral membrane of *Necturus* proximal tubule using the patch-clamp technique [22]. Although this system is attractive in its simplicity, the mechanism by which membrane stretch activates K channels in other cell types can be less direct. Stretch-activated channels have been found which trigger membrane depolariza-

tion or calcium influx, thus opening voltage-gated or calcium-activated K channels, respectively [19,20].

The results of the present study do not support a simple model in which the volume-activated K conductance of turtle colon is mediated by stretch-sensitive K channels. Besides the obvious question as to whether significant stretch actually occurs in a basolateral membrane which is characterized by extensive infolding, there is the problem that if the quinidine-sensitive conductance did in fact consist of such channels, it is expected that cell shrinkage would inactivate the conductance regardless of whether metabolic inhibitors were present or not. It is still possible that membrane stretch may directly or indirectly induce K channel opening, but the results implicate the involvement of other processes which are not simply stretch-sensitive.

Acknowledgments

This work was supported by the John B. O'Hara Chemical Sciences Institute, University of Dallas. The authors wish to thank Dr. David C. Dawson for helpful comments and suggestions.

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