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Na Absorption Across Endometrial Epithelial Cells is Stimulated by cAMP-dependent Activation of an Inwardly Rectifying K Channel

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Abstract. Previous studies in our laboratory have shown that Na absorption across the porcine endometrium is stimulated by $PGF_{2\alpha}$ and cAMP-dependent activation of a barium-sensitive K channel located in the basolateral membrane of surface epithelial cells. In this study, we identify and characterize this basolateral, barium-sensitive K conductance. Porcine uterine tissues were mounted in Ussing chambers and bathed with $KMeSO_4$ Ringer solution. Amphotericin B (70 μM) was added to the luminal solution to permeabilize the apical membrane and determine the current-voltage relationship of the basolateral K conductance after activation by 100 μM CPT-cAMP. An inwardly rectifying current was identified which possessed a reversal potential of −53 mV when standard Ringer solution was used to bathe the serosal surface. The K:Na selectivity ratio was calculated to be 12:1. Administration of 5 mM barium to the serosal solution completely inhibited the current activated by cAMP under these conditions. In addition to these experiments, amphotericin-perforated whole cell patch clamp recordings were obtained from primary cultures of porcine surface endometrial cells. The isolated cells displayed an inwardly rectifying current under basal conditions. This current was significantly stimulated by CPT-cAMP and blocked by barium. These results together with our previous studies demonstrate that cAMP increases Na absorption in porcine endometrial epithelial cells by activating an inwardly rectifying K channel present in the basolateral membrane. Similar patch clamp experiments were conducted using cells from a human endometrial epithelial cell line, RL95-2. An inwardly rectifying current was also identified in these cells which possessed a reversal potential of −56 mV when the cells were bathed in standard Ringer solution. This current was blocked by barium as well as cesium. However, the current from the human cells did not appear to be activated by cAMP, indicating that distinct subtypes of inwardly rectifying K channels are present in endometrial epithelial cells from different species.

Key words: K secretion — Na channel — Epithelial ion transport — Uterus — $PGF_{2\alpha}$

Introduction

The mammalian endometrium is composed of surface and glandular epithelial cells surrounded by a vascular interstitium containing fibroblast-like stromal cells and white blood cells. In a recent study, we showed that surface epithelial cells of the porcine endometrium absorb sodium (Na) and secrete potassium (K) [26]. These findings are consistent with data from pigs and other species which showed that K concentrations were elevated and Na concentrations were decreased in uterine fluid relative to serum [16]. Our studies also demonstrated that electrogenic Na absorption across the porcine endometrium occurs through amiloride-sensitive Na channels present in the apical membrane of surface epithelial cells. Na absorption was stimulated by $PGF_{2\alpha}$ and cAMP. This increase in transepithelial Na transport was due to activation of a barium-sensitive K conductance located in the basolateral membrane of these cells, rather than by a direct effect of cAMP on apical Na channels to increase Na absorption. We proposed that activation of a basolateral K channel hyperpolarizes the

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cell and increases the electrical driving force for Na entry across the apical membrane.

The purpose of this study was to characterize the properties and regulation of the barium-sensitive basolateral K conductance present in porcine endometrial epithelial cells. In addition to experiments involving intact and amphotericin-permeabilized tissues, whole cell patch clamp recordings were obtained from primary cultures of porcine surface epithelial cells as well as from a human endometrial epithelial tumor cell line, RL95-2, for comparison with results from porcine tissues.

Materials and Methods

Materials

Tetrodoxin and 8-chlorophenylthio-cyclic AMP (CPT-cAMP), were obtained from Research Biochemicals, Natick, MA. Prostaglandin $F_{2\alpha}$ was purchased from BIOMOL Research Laboratories, Plymouth Meeting, PA. Amiloride, indomethacin, amphotericin B, and high purity grade salts were from Sigma, St. Louis, MO. RL95-2 cells were obtained from American Type Culture Collection, Rockville, MD. D-MEM media, fetal bovine serum, collagenase, and trypsin-EDTA were from Life Technologies, Grand Island, NY.

Tissue Preparation and Measurement of Electrical Parameters

Porcine uterine tissues used in these studies were collected from 3–4 month old (reproductively immature) Yorkshire or Pietrain cross pigs purchased from stock herds maintained by the University of Minnesota College of Agriculture. The animals were housed for 5–10 days in large holding rooms at 25°C under a 12-hr light-dark cycle with continuous access to feed and water. The pigs were anesthetized prior to euthanasia with intramuscular ketamine (10 mg/kg), then rapidly killed with intravenous euthanasia solution (2 mls/50 lb) containing 6 grs/ml pentobarbital and 3.75 mg/ml potassium chloride. The intact uterus was removed immediately and placed in ice-cold porcine Ringer solution containing (in mM): 153 Na, 6 K, 143 Cl, 3 Ca, 1 Mg, 20 HCO_3, 0.3 H_2PO_4, 1.3 HPO_4, 10 D-glucose; pH = 7.4. The serosal muscle layers were removed by careful dissection. The tissues were then mounted in Ussing chambers (0.64 cm^2), bathed on both sides with identical Ringer solution (composition as above) at 39.5°C, and bubbled with 95% O_2/5% CO_2. Transepithelial potential difference, tissue conductance and short circuit current (I_{sc}) were measured using voltage clamp circuitry from JWT Engineering Corporation, Overland Park, KS. The mean basal I_{sc} and mean tissue conductance were similar to those published previously [26]. All tissues were pretreated with tetrodotoxin (0.2 μM) added to the solution bathing the serosal surface and indomethacin (10 μM) added to both luminal and serosal solutions at least 10 min prior to the beginning of experiments. These compounds had no effect on either tissue conductance or I_{sc}.

Experiments involving measurements of apical membrane K permeability in endometrial epithelial cells have been described in detail in a previous manuscript [26]. In this study, current-voltage relationships of the basolateral K conductance were determined using amphotericin-permeabilized tissues mounted in Ussing chambers as described above. Amphotericin B was added to the luminal solution at a concentration of 70 μM to eliminate the apical membrane as a resistive barrier to ion movement. The solution used to bathe the luminal surface of the tissue was $KMeSO_4$ Ringer solution containing (in mM): 120 $KMeSO_4$, 30 mannitol, 3 CaGluconate, 1 $MgSO_4$, 20 $KHCO_3$, 0.3 KH_2PO_4, 1.3 K_2HPO_4, 10 D-glucose; pH = 7.4. Either standard porcine Ringer solution or $KMeSO_4$ Ringer solution (compositions as above) was used to bathe the serosal surface as indicated in the figure legend for each experiment in Fig. 2. Current-voltage (*I-V*) relationships were obtained by imposing voltage step commands to the tissue from −150 to +110 mV (with reference to the serosal solution) in 20 mV increments from a holding potential of 0 mV. The resulting currents before and 10 min after addition of barium to the serosal solution were then subtracted to obtain the barium-sensitive component of the current either before or 10 min after luminal addition of CPT-cAMP. Linear regression analysis was used to fit the data in the linear portion of the curves to obtain the average reversal potential of the current ($R \geq 0.99$). A World Precision Instruments (Sarasota, FL) epithelial voltage clamp and Dagan LM-12 A-D interface (Dagan Corp, Minneapolis, MN) controlled by P-CLAMP software (Axon Instruments, Foster City, CA) were used to generate the voltage step commands and record the resulting currents.

Cell Culture and Patch Clamp Recording

Cells were isolated from the luminal surface of the porcine endometrium by the following method. One end of a 3-inch section of intact uterus was ligated, and the uterus was inverted over a glass rod to expose the luminal surface. The tissue was secured onto the rod and placed in cold calcium-free standard Ringer solution for one hour. The tissue was then placed in cold D-MEM media containing 0.2% collagenase, bubbled with 95% O_2/5% CO_2, and subjected to centrifugal force to aid in detachment of the surface epithelial cells. This procedure was carried out for one hour. The detached cells were centrifuged to remove collagenase, resuspended in fresh media with 10% fetal bovine serum, plated onto culture dishes, and incubated in 5% CO_2 at 37°C. Relatively pure cultures of surface epithelial cells were isolated from stromal cells in this manner and formed incomplete monolayers within 5–7 days of isolation. At this time, individual cells were dissociated from the culture dishes with trypsin-EDTA in preparation for patch clamp experiments. The perforated whole cell patch configuration was used in these experiments in order to retain regulatory compounds within the cells which are typically lost during standard whole cell recordings. Pipette electrodes were pulled to a resistance of 2–4 MΩ from 7052 glass (Garner Glass, Claremont, CA). The pipette tip was filled with $KMeSO_4$ Ringer solution consisting of (in mM):130 $KMeSO_4$, 5 KCl, 1 $CaCl_2$, 10 HEPES, pH 7.2. The pipette was then backfilled with the same solution containing 240μg/ml amphotericin B. High resistance seals were formed between the pipette and the cell membrane, and amphotericin was allowed to partition into the membrane to obtain the whole cell configuration prior to recording currents. The mean access resistance and whole cell capacitance for the isolated porcine cells were 7.8 ± 1.4 MΩ and 15.6 ± 1.7 picofarads, respectively. The mean access resistance and whole cell capacitance for the isolated RL95-2 cells were 9.9 ± 0.7 MΩ and 18.4 ± 1.3 picofarads, respectively. Both solutions were either $KMeSO_4$ Ringer solution or standard Ringer solution, as indicated in the figure legends for Figs. 4, 5, 6 and 7. The standard Ringer solution for patch clamp experiments consisted of (in mM): 130 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, pH 7.4. Whole cell currents were obtained in response to the voltage step protocols described in the figure legends. For tail current protocols, cells were initially held at 0 mV and then stepped to −100 mV to activate the inward rectifier. Cells were then stepped to a series of voltages from −200 up to +100 mV in 20 mV increments. An Axopatch 1D voltage clamp and TL-1 A-D interface were used (both from Axon Instruments). P-CLAMP software was used to generate the voltage step commands and record the resulting currents.

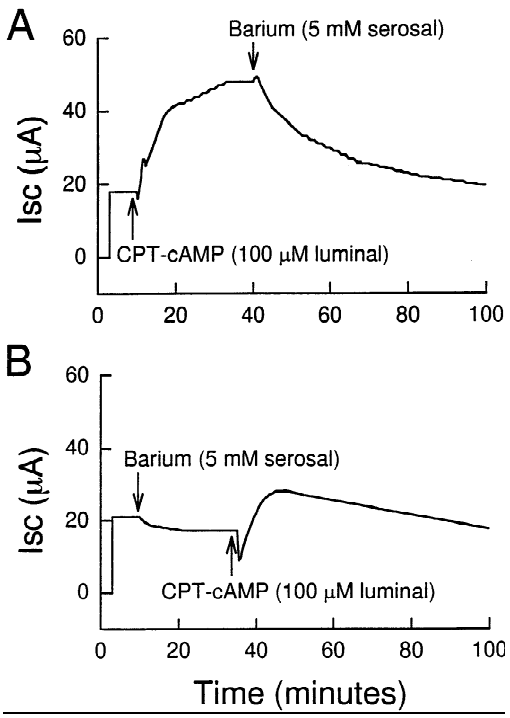

Fig. 1. Representative I_{sc} tracings from endometrial tissues mounted in Ussing chambers. (*A*) Addition of CPT-cAMP or $PGF_{2\alpha}$ (100 nM, *data not shown*) to the luminal solution produced a sustained twofold increase in I_{sc} which was completely inhibited by addition of barium to the serosal solution, as shown in the figure ($n = 8$ tissues from 4 animals for CPT-cAMP, 6 tissues from 4 animals for $PGF_{2\alpha}$. (*B*) Treatment of the tissue with serosal barium alone produced only a small decrease in I_{sc} (average = 3.4 ± 0.4 μAmps, $n = 14$ tissues from 6 animals), but significantly inhibited the I_{sc} response to subsequent addition of CPT-cAMP or 100 nM $PGF_{2\alpha}$ ($n = 6$ tissues for each compound from 4 animals).

Results

Effects of CPT-cAMP and Barium on Isc

As illustrated in Fig. 1, treatment of the serosal surface of endometrial tissues mounted in Ussing chambers with 5 mM barium produced a small decrease in I_{sc} (panel *B*), indicating a relatively small amount of basal activity of the barium-sensitive conductance when compared with the barium-sensitive current following treatment of the luminal surface of the tissues with CPT-cAMP (panel *A*). The cAMP-stimulated increase in I_{sc} following pretreatment with barium showed a slower rise to maximum levels than the current increase in response to cAMP alone, after which the current dropped back down to basal levels (panel *B*). The time course for this secondary decrease in I_{sc} closely matched that observed for the decrease in I_{sc} after administration of barium to the tissue at the peak increase in cAMP-stimulated current (panel *A*). In addition, the initial rapid decrease in I_{sc} in response to cAMP after barium (panel *B*) was potentiated when compared to this decrease in response to cAMP alone (panel *A*). Cesium and tetraethylammonium chloride (TEA) at concentrations up to 10 mM had no effect on I_{sc} in these tissues when administered to either the luminal or serosal surface.

Similar results to those observed with cAMP were obtained with $PGF_{2\alpha}$, as discussed in the figure legend and in a previous study [26]. Each compound (when administered alone to the tissues) produced a twofold increase in I_{sc} which remained at near-maximum levels for a prolonged period of time (1 hr or longer) as previously described (26).

Effects of CPT c-AMP and Barium on Basolateral K Permeability

Amphotericin-permeabilized endometrial tissues mounted in Ussing chambers were bathed on the luminal surface with $KMeSO_4$ Ringer solution. Current-voltage (*I-V*) relationships of the basolateral membrane were obtained by imposing voltage step commands from −150 to +110 mV in 20 mV increments. The resulting currents before and after addition of barium were subtracted to obtain the barium-sensitive component of the current. Figure 2 (lower panel) shows the *I-V* relationships for the barium-sensitive component of the current from experiments conducted in the presence of $KMeSO_4$ Ringer solution bathing both surfaces of the tissues. As shown in the figure, a relatively small barium-sensitive current was present under basal conditions. After the addition of CPT-cAMP, a substantial increase in the barium-sensitive component of the current was observed. The reversal potentials of the currents obtained before and after administration of CPT-cAMP to the tissue under symmetric K conditions were near 0 mV. In the presence of standard porcine Ringer solution bathing the serosal surface of the tissues, the reversal potential of the barium-sensitive component of the current after cAMP shifted to −53 ± 6 mV, as shown in the inset.

Whole Cell Patch Clamp Recordings from Isolated Cells

Figure 3 shows whole cell current tracings recorded from an isolated porcine endometrial surface epithelial cell bathed in $KMeSO_4$ Ringer solution. The current tracings were obtained in response to a series of voltage step commands from −160 to +80 mV in 20 mV increments from a holding potential of 0 mV. The predominant current observed under these conditions had inwardly rectifying properties, and reversed near 0 mV. Addition of CPT-cAMP to the bath stimulated an increase in inward current, and barium inhibited the inward current to levels similar to those observed prior to the addition of cAMP.

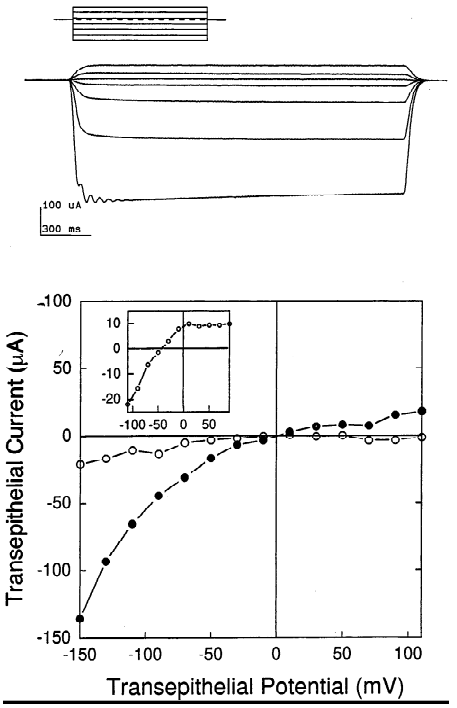

Fig. 2. Current-voltage relationship of basolateral membrane K permeability. Experiments were performed using amphotericin-permeabilized tissues mounted in Ussing chambers and bathed on the luminal surface with $KMeSO_4$ Ringer solution. Either standard Ringer solution or $KMeSO_4$ Ringer solution was used to bathe the serosal surface as indicated. *Upper panel:* Representative set of current tracings recorded in response to voltage step commands from −150 to +110 mV in 20 mV increments. The current tracings shown were obtained by subracting the current recorded at each voltage step after serosal addition of 5 mM barium from the current recorded after luminal addition of 100μM CPT-cAMP. Tissues were bathed on both sides with $KMeSO_4$ Ringer solution. *Lower panel:* The component of the current that was inhibited by barium is plotted as a function of transepithelial voltage. Tissues were bathed on both sides with $KMeSO_4$ Ringer solution. The barium-sensitive component of the current is shown before (○) and after (●) treatment of the luminal surface of the tissue with CPT-cAMP (n = at least 4 tissues from 3 animals for each condition). The mean reversal potential for the cAMP-stimulated current under these conditions was −0.8 ± 0.4 mV. The mean reversal potential for the current in standard Ringer solution (shown in the inset) was −53 ± 6 mV (the experiment is representative of 4 tissues from 3 animals).

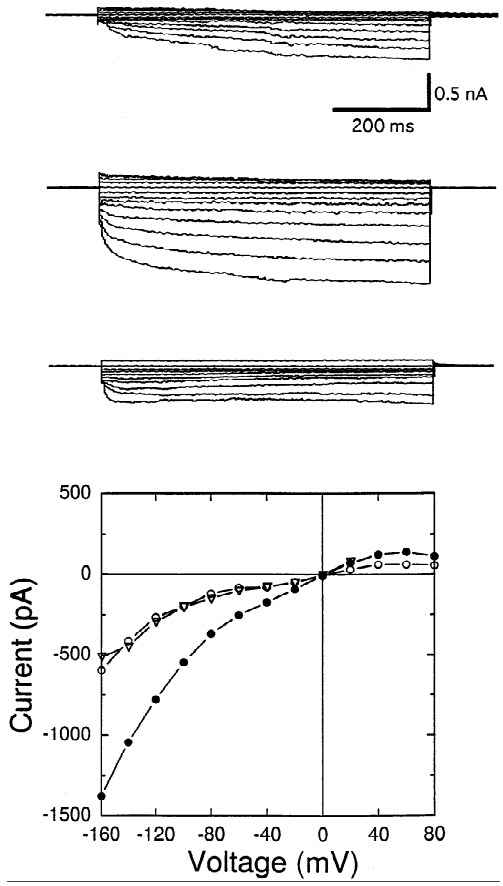

Fig. 3. Whole cell current tracings recorded from a surface epithelial cell isolated from porcine endometrium and bathed in $KMeSO_4$ Ringer solution. Currents were obtained in response to a series of voltage step commands from −160 to +80 mV in 20 mV increments from a holding potential of 0 mV. Top tracing, control currents; middle tracing, currents after addition of 100 μM CPT-cAMP to the bath; bottom tracing, currents remaining after addition of 1 mM barium to the bath. The experiment is representative of 4 cells. *Lower panel:* Peak current-voltage relationships for the above tracings: (○), control current; (●), current after CPT-cAMP; (△), current remaining after barium.

I-V relationships from the current tracings at steady-state conditions are shown in the lower panel of Fig. 3. We were unable to determine the reversal potential for the inward rectifier under conditions where cells were bathed in standard Ringer solution due to the presence of an outwardly rectifying current observed under these conditions. Figure 4 shows the near-instantaneous current-voltage relationship recorded from a porcine surface epithelial cell in response to a tail current protocol following the addition of CPT-cAMP to the bath ($KMeSO_4$ Ringer solution). The magnitude of the outward currents recorded at positive voltage steps during tail current protocols and standard voltage step protocols were similar.

Whole cell current tracings similar to those observed from porcine cells were obtained from isolated human endometrial epithelial cells (RL95-2) in response to standard voltage step (Fig. 5) and tail current (Fig. 6) protocols. The data shown in Figs. 5 and 6 are representative of inwardly rectifying currents obtained under basal conditions. In contrast to the porcine cells, no further activation of current was observed after addition of CPT-cAMP to the bath. The peak current-voltage relationships from current tracings obtained in the presence of $KMeSO_4$ Ringer solution and in the presence of standard Ringer solution in the bath are shown in the lower panel in Fig. 5. The reversal potential of the current in the presence of high K in the bath was 0 mV, and shifted to a negative reversal potential (−56 mV) in the presence of standard Ringer solution, indicating that K was the pre-

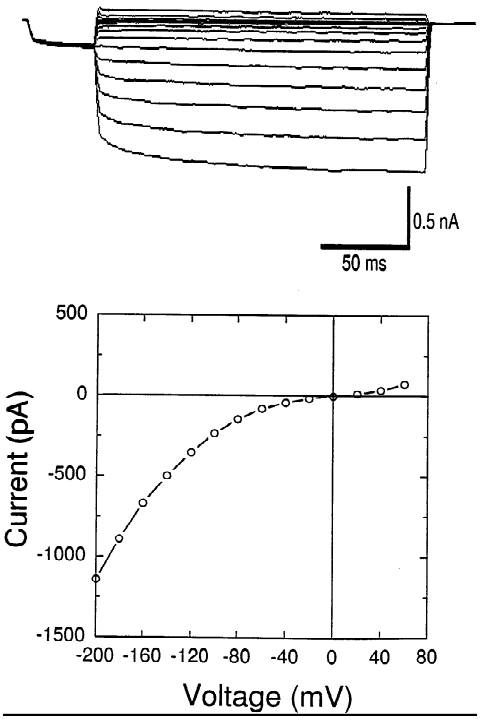

Fig. 4. Whole cell current tracings recorded from a porcine endometrial surface epithelial cell in response to a tail current protocol in the presence of high external K solution after addition of CPT-cAMP to the bath. The cell was initially held at 0 mV and then stepped to −100 mV to activate the inward rectifier. The cell was then stepped to a series of voltages from −200 to +60 mV in 20 mV increments. The experiment is representative of 6 cells. *Lower panel:* Peak current-voltage relationship for the above tracings.

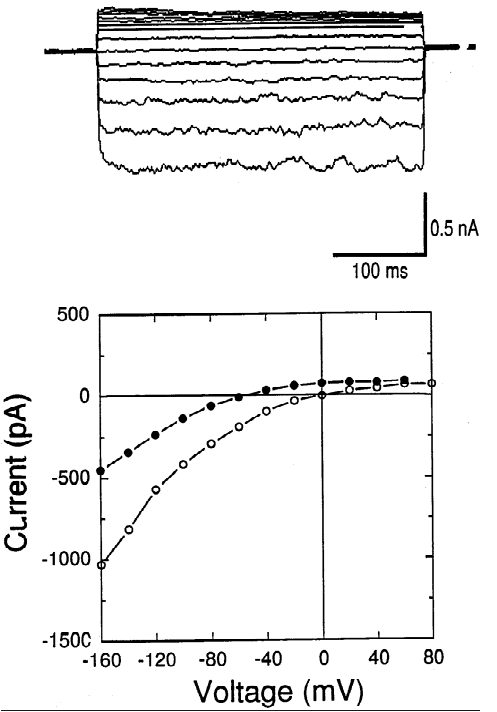

Fig. 5. Whole cell current tracings recorded from a human endometrial epithelial cell (RL95-2) bathed in $KMeSO_4$ Ringer solution. Currents were obtained in response to a series of voltage steps from −160 to +80 in 20 mV increments from a holding potential of −60 mV. The experiment is representative of 6 cells. *Lower panel:* Peak current-voltage relationships for the above tracings (○), and for current tracings recorded from a cell bathed in standard Ringer solution (●), $n = 5$ cells). The reversal potential for the current in standard Ringer solution was −56 mV.

dominant current-carrying ion through the inward rectifier channel in these cells.

Figure 7 shows a comparison of results obtained after addition of barium and cesium to porcine and human endometrial epithelial cells bathed in $KMeSO_4$ Ringer solution. Addition of 1 mM barium to the bath inhibited 54 ± 7% of the current present after cAMP stimulation for isolated porcine surface epithelial cells, whereas cesium at concentrations up to 5 mM had no significant effect on the current recorded from these cells. Barium and cesium at concentrations of 1 mM inhibited 79 ± 6% and 84 ± 4%, respectively, of the current recorded from RL95-2 cells.

Discussion

To determine the ionic basis for the I_{sc} change produced by barium in endometrial tissues, amphotericin B was used to permeabilize the apical membrane of surface epithelial cells. This technique enabled us to investigate the *I-V* relationship and ion selectivity of the barium-sensitive conductance present in the basolateral membrane. The results of experiments using standard porcine Ringer solution to bathe the serosal surface of the tissue showed that the reversal potential of the cAMP-activated, barium-sensitive current was −53 ± 6 mV. Under conditions where $KMeSO_4$ Ringer solution was used to bathe both the luminal and serosal surfaces of the tissue, the reversal potential shifted to near 0 mV, indicating that the channel was selective for K. In addition, significant inward rectification was present under symmetric K conditions, and the relative magnitude of the inward component of the current was increased in the presence of high serosal K concentrations. These findings demonstrated that the barium-sensitive conductance present in the basolateral membrane of the epithelium was an inwardly rectifying K channel. Using the Goldman-Hodgkin-Katz equation and a mean reversal potential of −53 mV in standard Ringer solution, the K:Na selectivity ratio for this channel was calculated to be 12:1. Results of patch clamp experiments demonstrated the presence of a cAMP-activated, barium-sensitive, inwardly rectifying K channel in primary isolates of surface epithelial cells from the porcine endometrium. We

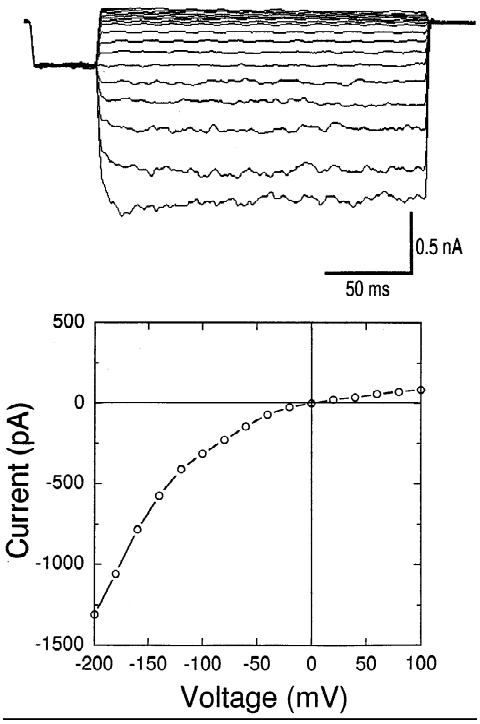

Fig. 6. Whole cell current tracings recorded from an RL95-2 cell in response to a tail current protocol in the presence of high external K. The cell was initially held at 0 mV, stepped to −100 mV to activate the inward rectifier, and then stepped to a series of voltages from −200 to +100 mV in 20 mV increments. The experiment is representative of 5 cells. *Lower panel:* Peak current-voltage relationship for the above tracings.

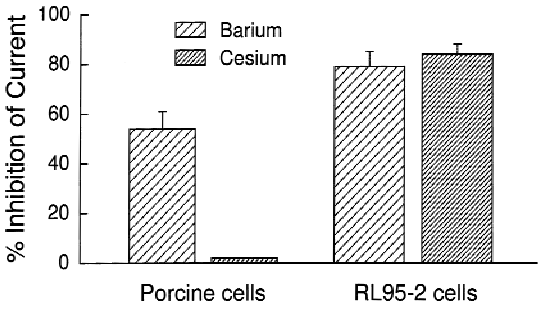

Fig. 7. Histogram illustrating the effects of 1 mM barium and 1 mM cesium on peak inward currents obtained in response to a standard voltage step protocol from isolated porcine and human endometrial epithelial cells bathed in $KMeSO_4$ Ringer solution. For these experiments, the average peak inward control currents were 1480 ± 510 pAmps for porcine cells after addition of CPT-cAMP to the bath, and 378 ± 69 pAmps for RL95-2 cells. Barium inhibited 54 ± 7% of the peak inward current for the porcine cells ($n = 4$ cells), and cesium had no significant effect on peak inward current in these cells ($n = 3$ cells). Barium and cesium inhibited 79 ± 6% ($n = 4$ cells) and 84 ± 4% ($n = 6$ cells) of the peak inward current, respectively, for the RL95-2 cells.

have also shown that an inwardly rectifying K channel was present in a human endometrial epithelial cell line, RL95-2. This channel had similar properties to the inward rectifier present in porcine cells, but did not appear to be regulated by cAMP, and was inhibited by cesium as well as barium. Thus, distinct subtypes of inwardly rectifying K channels appear to be present in endometrial epithelial cells from these species. Because RL95-2 cells do not form a polarized monolayer with any measurable transepithelial resistance, it was not possible to further characterize mechanisms and regulation of transepithelial ion transport in these cells.

As their name implies, members of the superfamily of inwardly rectifying K channels conduct more inward than outward current. This phenomenon may be due to voltage-dependent block of the channel pore by intracellular Mg and polyamines [20], and/or an intrinsic gating mechanism as yet undefined. Since the cloning of the ROMK1 and IRK1 subfamilies in 1993 [12, 19], a considerable amount of research in this area has resulted in the description of at least 5 separate subfamilies based on the degree of amino acid sequence similarity in the transmembrane region of the channels [8]. Inwardly rectifying K channels have been found in a wide variety of epithelial cell types [4, 27], and in many cases are localized to the basolateral membrane of these cells [2, 9–11, 13–15, 17, 22, 23, 25]. Modulation of channel activity by pH, ATP, cAMP and/or calcium has been reported [1, 3, 6, 7, 18, 21, 24, 28]. Each of the cloned inwardly rectifying K channels contains consensus phosphorylation sites, making them a potential substrate for protein kinases. The function of these channels in epithelial tissues is not entirely clear. They are thought to be tightly coupled to the Na/K-ATPase, serving to recycle K brought into the cell by this mechanism. Several investigators have proposed that hyperpolarization of the cell membrane through the activity of basolateral inwardly rectifying K channels increases the electrical driving force for anion efflux across the apical membrane of T84 cells [1, 3, 6, 28]. In addition, modulation of basolateral inwardly rectifying K channels by membrane potential has been proposed by investigators who have noted that the conductance of these channels increases with hyperpolarization [5, 13]. Studies involving basolateral inwardly rectifying K channels isolated from *Necturus* enterocytes and reconstituted into lipid bilayers have suggested that the open probability of these channels is correlated to membrane potential, providing an explanation for the apparent synergy between apical Na entry, Na pump activity, and changes in basolateral K conductance observed in these cells [5].

A model summarizing the effects of cAMP on surface epithelial cells of the porcine endometrium is shown in Fig. 8. We propose that cAMP-dependent activation of an inwardly rectifying K channel present in the basolateral membrane produces hyperpolarization of the cell, which increases the driving force for Na entry through amiloride-sensitive Na channels present in the apical membrane. Evidence for the existence of amiloride-

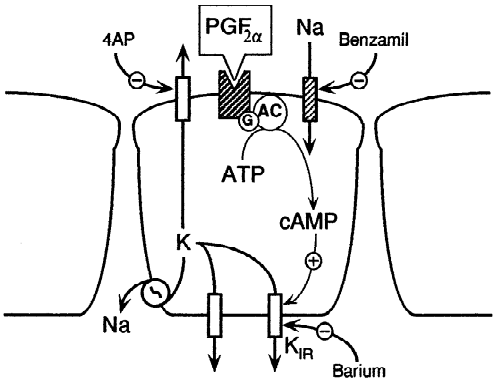

Fig. 8. Cell model and diagram illustrating the proposed mechanisms for cAMP regulation of Na and K transport across porcine endometrial surface epithelial cells (*see* Discussion for details). G, G protein; AC, adenylate cyclase

sensitive Na channels in surface epithelial cells was demonstrated in a previous publication [26]. Increasing K efflux through the inward rectifier presumably keeps the concentration of K within the unstirred fluid layer near the basolateral membrane sufficiently high enough to prevent K from becoming rate limiting with respect to Na-K ATPase activity. In addition, the increase in conductance of the inward rectifier K channel during hyperpolarization may be important in sustaining basolateral K efflux as hyperpolarization decreases the driving force for K exit from the cell.

Finally, it should be noted that a small, relatively rapid initial decrease in I_{sc} occurred in endometrial tissues in response to cAMP, which was potentiated in all cases where tissues were pretreated with serosal barium. One explanation for this decrease in I_{sc} is that, in addition to stimulating a basolateral K conductance, cAMP may act to directly stimulate K efflux across the apical membrane. In this case, redistribution of K efflux across the apical membrane following pretreatment of the serosal surface of the tissue with barium would be consistent with potentiation of the initial rapid decrease in I_{sc} seen in response to cAMP. Attempts to block this current response with 4-aminopyridine (4-AP) were unsuccessful. Thus, in addition to the constitutively active 4-AP-sensitive K conductance which we have previously identified [26], a cAMP-activated K conductance may also be present in the apical membrane of surface endometrial epithelial cells.

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