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Sodium and anion transport across the avian uterine (shell gland) epithelium

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Summary

The uterine (shell gland) epithelium from the domestic chicken was mounted in Ussing chambers, bathed in symmetric avian saline solution on both apical and basolateral aspects and voltage clamped at 0 mV. The epithelium exhibited a basal short circuit current (I_{sc}) that was partially inhibited by the epithelial Na^+ channel (ENaC) blockers, amiloride and benzamil (IC_{50} values of 0.8 and 0.12 μmol l^{-1}, respectively). Inhibition of basal Na^+ absorption by 10 μmol l^{-1} amiloride was confirmed by measurements of transepithelial Na^+ and Cl^- fluxes, where inhibition of the apical-to-basolateral and net Na^+ flux occurred, but no significant effects on Cl^- fluxes were detected. The amiloride-insensitive portion of the basal I_{sc} was both Cl^- and HCO_3^- dependent and was inhibited by the Cl^- channel blocker, diphenyl-2-carboxylate (DPC; 100 μmol l^{-1}). Stimulation with 8-(4-chlorophenylthio)-cyclic 3′-5′, adenosine monophosphate (8-cpt cAMP) produced a sustained increase in I_{sc} that was dependent on both Cl^- and HCO_3^-. The magnitude of the amiloride-sensitive I_{sc} was approximately twofold greater in birds where shell formation was complete, but oviposition had not yet occurred. In addition, the amiloride-sensitive I_{sc} was greater in hens over the age of 55 weeks and in molting birds. The anion-dependent component of the basal I_{sc} was reduced in older birds, and electrogenic HCO_3^- transport was nearly absent in molting birds. These results demonstrated that electrogenic Na^+ transport in avian shell gland was similar to the mammalian uterine epithelium and increased with age and during molting. Electrogenic Cl^- and HCO_3^- transport were coupled under basal and cAMP stimulated conditions and basal anion transport decreased with age and during molting.

Key words: eggshell formation, ENaC, bicarbonate secretion, acetazolamide, carbonic anhydrase, chicken, *Gallus domesticus*.

Introduction

The eggshell plays an important role in the process of respiratory gas exchange in developing avian embryos. Its strength and porosity are essential for embryonic metabolism, development and resistance to bacterial infection (Bennett, 1992). Significant defects in shell integrity often result in death of the developing embryo (Bennett, 1992). Factors that influence the structure and composition of the eggshell include: calcium (Ca^{2+}) secretion, bicarbonate (HCO_3^-) secretion, protein content, vitamin D, vitamin C, phosphorus and various fatty acids (Eastin and Spaziani, 1978a). The major chemical component of the eggshell is calcium carbonate (97% by mass). The avian shell gland is responsible for the secretion of Ca^{2+} and HCO_3^- that combine within the shell gland lumen to form calcium carbonate or calcite (Eastin and Spaziani, 1978b). Presently, the mechanisms and regulation of monovalent ion absorption and secretion by the shell gland epithelium have not been characterized.

Previous studies of Na^+ transport *in vivo* have demonstrated net absorption when the shell gland was perfused with plasma-like saline solution (Eastin and Spaziani, 1978b). Addition of ouabain, an inhibitor of Na^+-K^+ ATPase activity, inhibited basal Na^+ transport. *In vivo* studies also demonstrated net HCO_3^- secretion during shell formation that was inhibited by acetazolamide (Eastin and Spaziani, 1978a,b). This result suggested that HCO_3^- transport was dependent on carbonic anhydrase activity and that the mechanism for HCO_3^- uptake presumably involved CO_2 diffusion across the plasma membrane. These findings also indicated that HCO_3^- secretion was transcellular rather than paracellular and involved transport pathways located within the apical membrane of the shell gland epithelium. Measurements of Cl^- transport *in vivo* showed net absorption under conditions where the shell gland was perfused with plasma-like saline solution. Cl^- absorption was inhibited when the gland was treated with ouabain or with acetazolamide (Eastin and Spaziani, 1978a,b). Thus Cl^- transport also appeared to be dependent on carbonic anhydrase activity and coupled to HCO_3^- secretion.

Earlier Ca^{2+} flux experiments demonstrated net Ca^{2+} secretion into the lumen of the shell gland that was dependent on the presence of HCO_3^- in the bathing solutions (Pearson and

Goldner, 1973, 1974; Pearson et al., 1977). Biochemical and histochemical studies suggested that a Ca^{2+}-ATPase, associated primarily with tubular gland epithelial cells, was involved in active Ca^{2+} secretion across the epithelium (Yamamoto et al., 1985; Coty, 1982; Gay and Schraer, 1971; Gay and Mueller, 1973). In addition, vitamin D-dependent calcium binding proteins were also localized to tubular gland epithelial cells, using immunohistochemical localization techniques (Lippielo and Wasserman, 1975). These results supported the hypothesis that the glandular epithelium was the site for Ca^{2+} secretion and that the mechanism of secretion involved an electrogenic Ca^{2+}-ATPase located in the apical membrane of these cells. The basis for the HCO_3^- dependency of Ca^{2+} secretion was not determined.

Egg production rates and eggshell thickness both decrease with age (Bahr and Palmer, 1989; Joyner et al., 1987). Associated with these changes are decreases in the levels of vitamin $1\alpha,25(OH)_2D_3$ and the mass of medullary bone in older hens (Bahr and Palmer, 1989). Vitamin $1\alpha,25(OH)_2D_3$ is synthesized from cholecalciferol through hydroxylation by the liver and kidney (Soares and Ottinger, 1988). The active metabolite ($1\alpha,25(OH)_2D_3$) regulates calcium metabolism by increasing intestinal Ca^{2+} absorption, and by increasing the mobilization of Ca^{2+} from bone (Bahr and Palmer, 1989). Its synthesis by the kidney is regulated by estrogen (Bar and Hurwitz, 1987; Baksi and Kenny, 1977). It is interesting to note that $1\alpha,25(OH)_2D_3$ concentration decreases with age even though plasma levels of progesterone and estradiol-17β are similar in young and older hens (Bar and Hurwitz, 1987). Moreover, plasma levels of ionized Ca^{2+} do not significantly change in hens between 33 and 122 weeks of age (Bahr and Palmer, 1989). Thus, the reasons for decreased eggshell thickness may involve changes in shell gland function rather than availability of Ca^{2+} for secretion. Such changes could involve a decrease in the ability of the epithelium to transport Ca^{2+} directly or perhaps changes in the coupling of Ca^{2+} transport with HCO_3^- secretion across the epithelium.

The objective of the present study was to investigate the Na^+ and Cl^- transport properties of the shell gland epithelium from the domestic chicken *Gallus domesticus* under *in vitro* conditions where transepithelial voltage and ion concentration gradients could be controlled. Transepithelial Na^+ and Cl^- flux measurements and ion substitution experiments were performed to determine the ionic basis of the basal short circuit current I_{sc}. The effects of epithelial Na^+ and Cl^- channel blockers were tested to identify apical membrane conductance pathways for these ions. The effects of cAMP on ion transport was determined using a cell-permeant cAMP analog and the effects of age and molting on both basal Na^+ and anion transport were examined. The results showed that electrogenic transepithelial Na^+ absorption was dependent on amiloride-sensitive Na^+ channels present in the apical membrane and that a significant portion of the basal I_{sc} was dependent on Cl^- and HCO_3^-. The data also indicated that rates of Na^+ and anion transport were altered with age and during molting, which could potentially contribute to the decrease in shell thickness often associated with eggs from older birds.

Materials and methods

Materials

Benzamil was purchased from Molecular Probes, Eugene, OR, USA. Tetrodotoxin and 8-chloro-phenyl-thio-cAMP (8-cpt cAMP) were purchased from Research Biochemicals International, Natick, MA, USA. Amiloride, indomethacin and 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) were obtained from Sigma-Aldrich, St Louis MO, USA. Diphenyl-2-carboxylate (DPC) was purchased from Fluka, Milwaukee, WI, USA.

Histology

Freshly isolated, intact shell gland mucosa was cut into 1 cm^2 pieces and fixed in 10% buffered formalin prior to paraffin embedding. The embedded tissue was cut into 3–5 μm sections and stained with Hematoxalin and Eosin. Slides were examined using a compound microscope (Nikon diaphot, Niles, IL, USA) and images acquired with a digital camera (Coolsnap, Millersville, MD, USA) at 100× (Fig. 1A) and 400× (Fig. 1B).

Tissue preparation and measurement of electrical parameters

Domestic chickens *Gallus domesticus* L. were maintained at the Animal Care Facility in the College of Veterinary Medicine at the University of Minnesota under a 12 h:12 h light:dark cycle. Hens were pre-anesthetized by an intramuscular injection of ketamine (10 mg kg^{-1}) followed by intravenous administration of Beuthanasia solution (4 ml kg^{-1}) containing 50 mg ml^{-1} pentobarbital. The shell gland was subsequently removed and bathed in ice-cold avian saline solution containing (in mmol l^{-1}) 150 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 25 $NaHCO_3$, 1 NaH_2PO_4, pH 7.4. The serosal muscle layers were removed by blunt dissection. The mucosa was mounted in Ussing chambers and bathed with avian saline solution on both sides of the tissue. The solutions were gassed with 95% O_2/5% CO_2 and maintained at 40°C (avian core temperature). Transepithelial potential difference (3.0±0.22 mV, N=30), tissue conductance (17.3±0.62 mS cm^2, N=30) and short circuit current (I_{sc}; 71.7±5.1 μA cm^{-2}, N=30) were measured with the ground electrode placed in the luminal solution, using voltage clamp circuitry from JWT Engineering Corporation (Kansas City, KS, USA). The chamber area was 0.64 cm^2. For experiments that involved anion substitutions, Cl^- was replaced with methane sulfonate and HCO_3^- with Hepes buffer to maintain a constant pH of 7.4. Tissue conductance was calculated from voltage and current measurements obtained at various intervals throughout the experiment. Glucose (10 mmol l^{-1}) was added to the basolateral solution to sustain the metabolic activity of the tissue, and mannitol (10 mmol l^{-1}) was added to the apical solution to balance osmotic pressure without stimulating Na^+-dependent glucose transport mechanisms that might exist within the apical membrane. In

all experiments, 100 nmol l^{-1} tetrodotoxin and 10 μmol l^{-1} indomethacin were added to the basolateral solution to block any spontaneous activity of submucosal nerves and to inhibit basal prostaglandin secretion from the epithelium and stromal cells.

Transepithelial Na^+ and Cl^- flux measurements

Tissues were prepared as described above. All flux measurements were performed on tissues under short circuit conditions. After allowing 30 min for the tissues to stabilize, 5.5×10^4 Bq $^{22}Na^+$ and 1.1×10^5 Bq $^{36}Cl^-$ were added to either the luminal or basolateral side of the epithelium and allowed to equilibrate for 30 min. The first flux period (30 min duration) served as a control to establish basal rates of Na^+ and Cl^- transport across the epithelium. Unidirectional fluxes were determined from measurements of isotope (0.1 ml) recovered from the reservoir where isotope was initially added and from 1.0 ml of saline solution from the opposite reservoir. Subsequently, the epithelium was treated with 10 μmol l^{-1} amiloride for 5 min, and a second 30 min flux measurement was performed. $^{22}Na^+$ was measured using an LKB gamma counter and $^{36}Cl^-$ was detected using a Beckman liquid scintillation counter. The net flux across the epithelium was determined by subtracting the basolateral-to-apical unidirectional flux from the apical-to-basolateral flux using paired tissues from the same animal. Tissues were paired on the basis of transepithelial conductance within 10%.

Statistics

Results are presented as the mean ± standard error (S.E.M.). For statistical comparisons of flux data and I_{sc} responses between tissues obtained before and after ion replacement or drug treatment (where the same tissue was used as control), a paired *t*-test was used. An unpaired *t*-test was used for comparisons between I_{sc} responses from animals in different age groups or during molting. The level of significance was set at $P<0.05$.

Results

Figure 1 shows a cross section of shell gland mucosa and associated smooth muscle layers. The mucosa is composed of a pseudostratified surface epithelium and glandular epithelium. The interstitum is composed of various matrix elements and stromal cells, which are specialized fibroblasts that closely associate with the surface and glandular epithelium. Circular and longitudinal smooth muscle layers are visible at the bottom of the section. These muscle layers were removed prior to mounting in Ussing chambers.

The shell gland epithelium exhibited a basal I_{sc} that ranged from 40–100 μA depending on the stage of egg production. Addition of the Na^+ channel blockers amiloride or benzamil to the apical bathing solution produced inhibition of the basal current by approximately 60–70%. Inhibition of I_{sc} was concentration dependent for both blockers with IC_{50} values and a rank order of potency consistent with inhibition of ENaC Na^+ channels (Fig. 2). Interestingly, the magnitude of amiloride-sensitive I_{sc} was nearly twofold greater in birds following completion of shell formation compared to hens where shell deposition was incomplete.

To confirm that the decrease in I_{sc} produced by apical addition of amiloride was a consequence of inhibition of Na^+ absorption, transepithelial Na^+ and Cl^- fluxes were measured (Fig. 3). These experiments were performed under short circuit conditions where the transepithelial voltage was clamped at 0 mV and identical saline solution was used on both sides of the tissue. Amiloride (10 μmol l^{-1}) produced a significant decrease in the apical-to-basolateral unidirectional flux and in

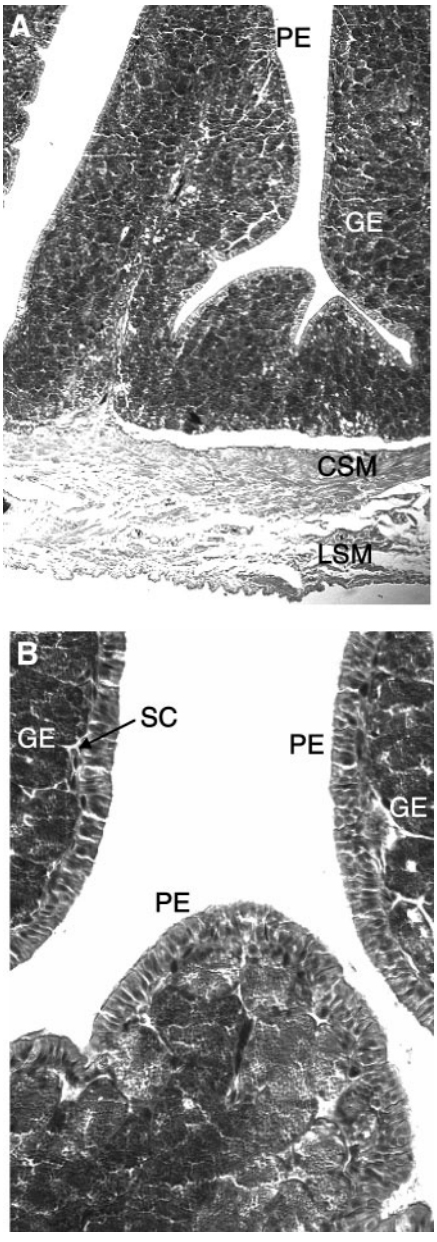

Fig. 1. Histology of the shell gland mucosa. (A) 100× magnification, (B) 400× magnification. PE, pseudostratified surface epithelium; GE, glandular epithelium; SC, stromal cells; CSM, circular smooth muscle; LSM, longitudinal smooth muscle.

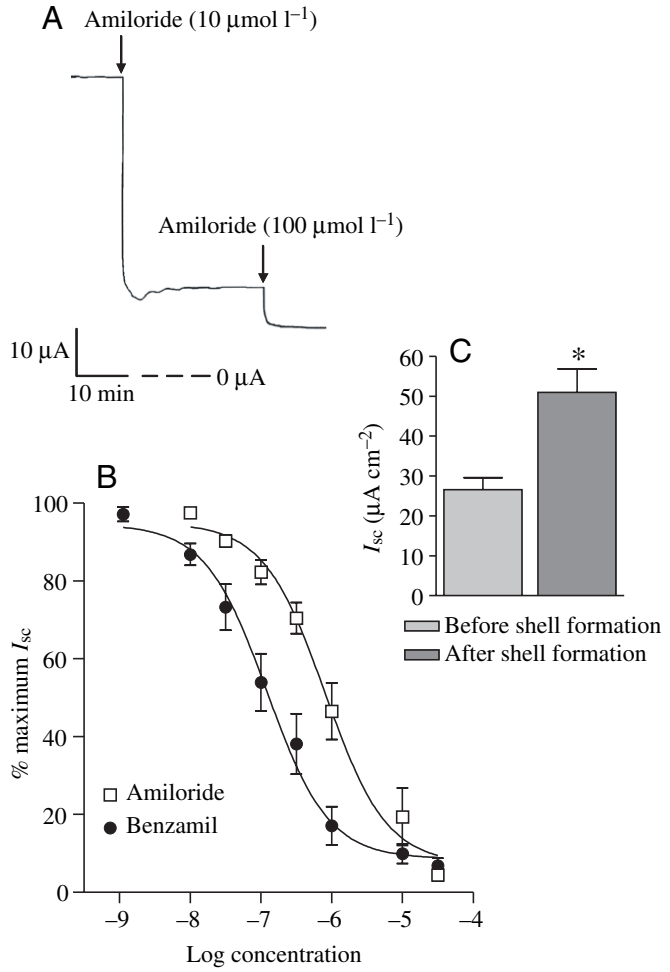

Fig. 2. Effects of apical Na^+ channel blockers on basal I_{sc}. (A) Time course of I_{sc} inhibition following sequential treatment with 10 and 100 µmol l^{-1} amiloride. (B) Concentration–response relationships for amiloride and benzamil on basal I_{sc}. The data was fitted using a four-parameter logistic function and the IC_{50} values were 120 nmol l^{-1} (N=5) and 810 nmol l^{-1} (N=5) for benzamil and amiloride, respectively. (C) Magnitudes of amiloride-sensitive (10 µmol l^{-1}) I_{sc} in tissues from hens where shell formation was complete (N=6) compared to tissues where the egg had not entered the shell gland (N=5). *Significantly different from control value (P<0.05).

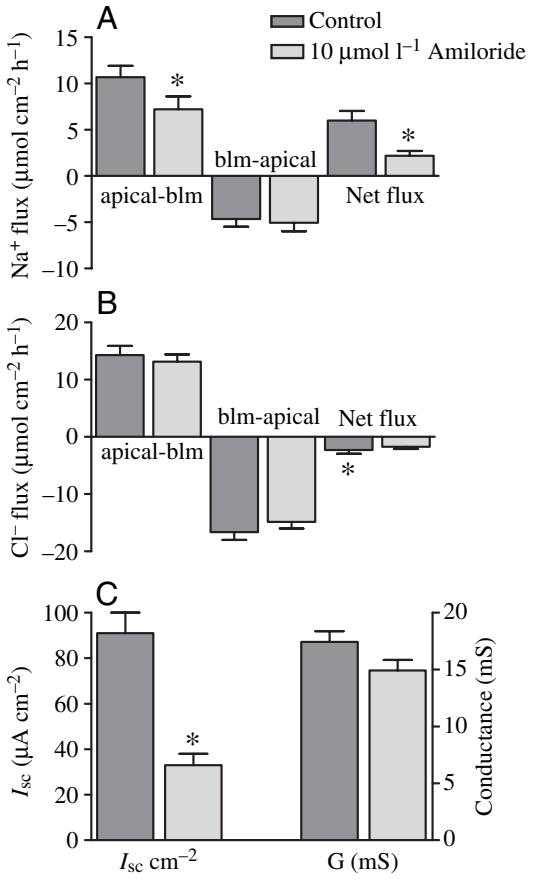

Fig. 3. Transepithelial Na^+ and Cl^- flux measurements before and after apical treatment with 10 µmol l^{-1} amiloride. (A) Unidirectional, apical-to-basolateral (apical-blm), basolateral-to-apical (blm-apical) and net ((apical-blm)-(blm-apical)) Na^+ fluxes across epithelial tissues bathed in symmetric avian saline solution and voltage clamped at 0 mV (N=6). (B) Unidirectional and net Cl^- fluxes across under the same conditions stated in A (N=5). (C) Effects of apical amiloride (10 µmol l^{-1}) on I_{sc} and conductance (G) measurements obtained from tissues used in the transepithelial flux experiments. *Significantly different from control value (P<0.05).

the net flux for Na^+. However, treatment with amiloride did not completely block all of the net Na^+ flux, suggesting that Na^+ channel activity was not completely blocked at 10 µmol l^{-1} (consistent with the additional decrease in I_{sc} observed with 100 µmol l^{-1} amiloride in Fig. 2C) or that electroneutral pathways for Na^+ transport exist in the apical membrane. No significant effects of amiloride were observed on unidirectional or net Cl^- fluxes.

Ion replacement experiments indicated that the basal, amiloride-insensitive I_{sc} was both Cl^- and HCO_3^- dependent (Fig. 4). In addition, treatment with the Cl^- channel blocker, DPC, produced a concentration dependent decrease in I_{sc} that was quantitatively similar to the Cl^- and HCO_3^- dependent current (Fig. 4B). Moreover, transepithelial Cl^- flux experiments showed a net Cl^- flux, indicating Cl^- secretion that was significantly different from zero under basal conditions. Basolateral addition of 8-cpt cAMP produced a rapid and sustained increase in I_{sc} that was dependent on Cl^- and HCO_3^- in the bathing solutions (Fig. 5). Addition of the acetazolamide (100 µmol l^{-1}) to both apical and basolateral bathing solutions reduced most of the residual cAMP-stimulated I_{sc} under Cl^- free conditions, suggesting that HCO_3^- secretion was responsible for the residual current (Fig. 5B).

The effects of age and molting on basal, electrogenic Na^+ and anion transport are shown in Fig. 6. The amiloride-sensitive I_{sc} increased with age and during molting (Fig. 6A). In contrast, the anion-dependent basal current was significantly decreased in older birds (Fig. 6B). Interestingly, molting birds exhibited a basal Cl^- dependent I_{sc} similar to that observed for hens within the 55–72 week age group, but the HCO_3^- dependency was significantly lower. This result is consistent

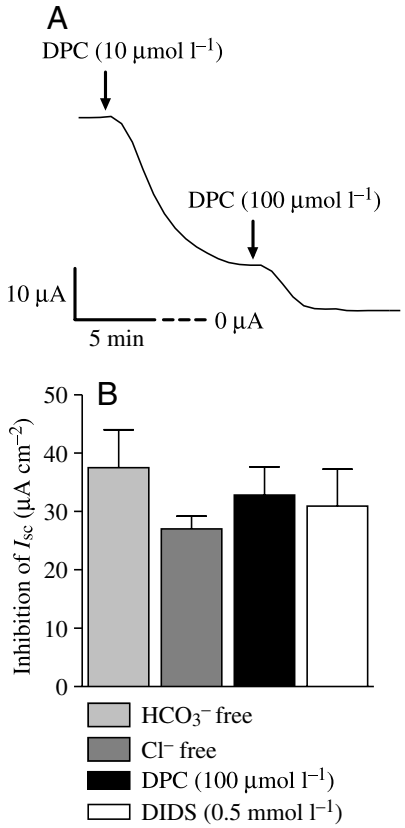

Fig. 4. Effects of symmetric anion substitution and apical addition of DPC on basal I_{sc}. (A) I_{sc} trace showing the inhibitory effects of 10 µmol l^{-1} and 100 µmol l^{-1} DPC on basal anion current in a tissue pretreated with 100 µmol l^{-1} amiloride. (B) Magnitude of basal I_{sc} inhibition produced by apical DPC, 100 µmol l^{-1} (N=5), Cl^- replacement with methane sulfonate (N=7), HCO_3^- replacement with Hepes (N=6) and 0.5 mmol l^{-1} DIDS added to the apical solution (N=5).

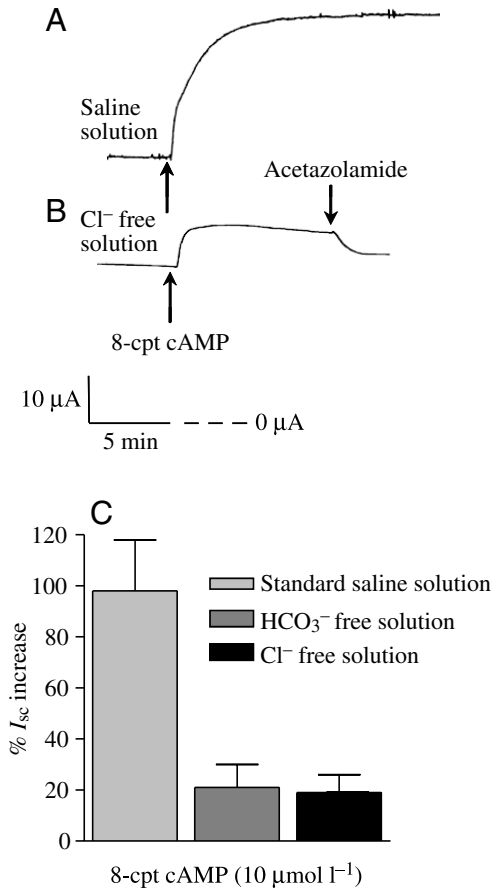

Fig. 5. Effects of 8-cpt cAMP (added to both sides) on I_{sc}. (A) Tracing showing the sustained increase in I_{sc} observed after stimulation with 10 µmol l^{-1} 8-cpt cAMP. (B) Effects of 10 µmol l^{-1} 8-cpt cAMP on I_{sc} under Cl^- free conditions. Note the inhibitory effects of 10 µmol l^{-1} acetazolamide (an inhibitor of carbonic anhydrase activity) on the residual current. (C) Effects of Cl^- (N=5) and HCO_3^- (N=5) substitution on the 8-cpt cAMP stimulated I_{sc} response.

with the interpretation that Cl^- secretion uncouples from HCO_3^- transport in molting birds.

Discussion

The results of this study demonstrated that the shell gland epithelium was capable of high rates of electrogenic Na^+ transport and that Na^+ absorption was regulated during shell formation. The observation that amiloride and benzamil produced inhibition of the basal I_{sc} and that this decrease in current was associated with inhibition of the apical-to-basolateral and net Na^+ flux strongly suggested that ENaC Na^+ channels were involved in Na^+ absorption across this epithelium. This result was consistent with previous studies of Na^+ transport across the porcine, murine and human endometrial epithelium (Vetter and O'Grady, 1996; Deachapunya et al., 1999; Palmer-Densmore et al., 2002; Chan et al., 2001; Matthews et al., 1998). In native porcine endometrial tissues mounted in Ussing chambers, the surface epithelial cells exhibited a basal I_{sc} that was blocked by amiloride with an IC_{50} value (0.8 µmol l^{-1}) identical to that reported for the shell gland epithelium in this study (Vetter and O'Grady, 1996). Similar effects of amiloride and benzamil on basal I_{sc} were reported for cultured porcine glandular endometrial epithelial cells maintained under serum-free conditions (Deachapunya et al., 1999). Analysis of tissue morphology (Fig. 1) indicated the presence of lamellae that were clearly distinct from the mucosal morphology of mammalian endometrium (Vetter and O'Grady, 1996). Whether Na^+ absorption and anion secretion are associated with separate cell types within the shell gland epithelium is unknown at this time.

An interesting observation from this study was the approximate twofold increase in amiloride-sensitive I_{sc} that occurred in birds where shell formation was complete. One possible reason for this increase in Na^+ absorption may be to reduce fluid volume within the shell gland lumen and to recover the Na^+ and Cl^- that were transported across the epithelium during shell deposition. This interpretation is

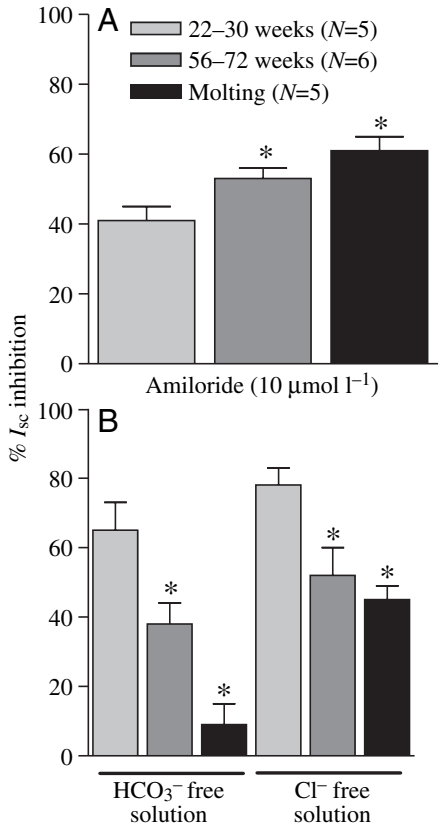

Fig. 6. Effects of age and molting on basal amiloride-sensitive and anion-dependent I_{sc}. Note that all experiments were performed in standard avian saline solution. (A) Changes in the amiloride-sensitive I_{sc} with age and molting. (B) Changes in the Cl^- and HCO_3^- dependent I_{sc} with age and molting. *Significantly different from control value (P<0.05).

consistent with earlier results from *in situ* perfused shell gland experiments where net decreases in luminal Na^+ and Cl^- concentrations were observed (Eastin and Spaziani, 1978a). Moreover, previous studies of Na^+-K^+ ATPase expression in chicken shell gland showed that mRNA levels of the α1 subunit varied depending on the stage of shell formation and exhibited different expression patterns within the surface pseudostratified epithelium and the glandular epithelium (Lavelin et al., 2001). The α1 subunit mRNA levels were relatively low prior to entry of the egg into the shell gland. Maximum increases in mRNA expression were subsequently detected during the period of peak shell deposition and rapidly decreased once shell formation was complete, 1 h prior to oviposition. Although changes in α1 subunit protein expression were not measured, it is reasonable to assume that changes in mRNA levels preceded changes in functional activity of the enzyme. Given this assumption, the increase in amiloride-sensitive I_{sc} observed in this study appeared to correlate with the enhanced expression of the Na-K ATPase α1 subunit. Whether a similar increase in Na^+ channel expression also occurred is possible, but remains to be established.

Anion substitution experiments revealed that the shell gland epithelium exhibited a basal anion dependent I_{sc} consistent with anion secretion. Apical addition of the Cl^- channel blocker DPC inhibited the Cl^- and HCO_3^- dependent current, again suggesting net anion secretion under basal conditions. It is worth noting in these experiments that tissues were pretreated with apical amiloride to block basal Na^+ channel activity and to ensure that changes in I_{sc} produced by DPC were not partially due to effects on the driving force for Na^+ uptake across the apical membrane. Although DPC is a well established inhibitor of Cl^- channel activity, it is not highly selective, thus the molecular identity of the DPC-sensitive conductance in the apical membrane could not be determined. The suggestion that anion substitution and DPC inhibition of I_{sc} represented effects on basal anion secretion was supported by measurements of transepithelial Cl^- fluxes, which demonstrated net Cl^- secretion under basal conditions. Moreover, stimulation with 8-cpt cAMP produced a sustained increase in I_{sc} that was significantly reduced if either Cl^- or HCO_3^- were replaced in the bathing solutions. Attempts to measure transepithelial Cl^- fluxes following treatment with 8-cpt cAMP were confounded by increases in both unidirectional Cl^- fluxes (ranging between 20–30%), making it difficult to measure changes in the net flux accurately. Thus we were unable to confirm cAMP stimulation of net Cl^- secretion using isotopic flux measurements. However, under symmetric saline solution conditions, I_{sc} responses to 8-cpt cAMP were stable in spite of changes in paracellular permeability, thus ion substitution experiments could be performed to investigate the anion dependence of the cAMP response. Based on these findings, we speculate that the elevated I_{sc} resulting from cAMP stimulation was due to an increase in anion secretion. Regulation of shell formation through the cAMP signaling cascade could conceivably be mediated by various autocrine factors including prostaglandins and adenosine or by certain hormones such as vasopressin and oxytocin (Saito et al., 1987; Olson et al., 1978). However, the specific signaling molecules involved in regulation of Cl^- and HCO_3^- transport by the shell gland epithelium have not been identified.

The observation that a portion of the basal I_{sc} and the 8-cpt cAMP stimulated I_{sc} were both Cl^- and HCO_3^- dependent suggested that transport of these anions may be coupled, as described for pancreatic duct cells that secrete HCO_3^- (Steward et al., 2005). In these cells, the apical membrane contains both cAMP activated Cl^- channels and Cl^-–HCO_3^- exchangers. Cl^- channels provide a pathway for Cl^- recycling across the apical membrane to sustain HCO_3^- efflux mediated by Cl^-–HCO_3^- exchange activity. In the shell gland epithelium, we propose that a mechanism for Cl^- uptake across the basolateral membrane must also exist to allow for transcellular Cl^- secretion. Net Cl^- secretion would presumably be necessary to maintain a luminal [Cl^-] sufficient to support HCO_3^- transport by Cl^-–HCO_3^- exchange and to compensate for paracellular absorption of Cl^- resulting from the lumen-positive transepithelial potential. To test this hypothesis, DIDS, an inhibitor of Cl^-–HCO_3^- exchange, was added to the apical solution to see if it blocked the HCO_3^- dependent component

of the basal I_{sc}. We observed a decrease in I_{sc} of 31±6.3 μA (N=5) that was abolished when tissues were bathed on both sides with HCO_3^- free saline solution. Although this result appears to be consistent with the suggested model above it is important to note that DIDS also blocks certain types of Cl^- channels, thus more direct methods will be required to establish the mechanism of HCO_3^- secretion in this epithelium.

Stimulation of Cl^- secretion by 8-cpt cAMP was previously reported in cultured mammalian endometrial epithelial cells (Deachapunya and O'Grady, 1998). However, native porcine endometrial tissues mounted in Ussing chambers and treated with 8-cpt cAMP or with PGF2α, produced a sustained increase in amiloride-sensitive Na^+ absorption (Vetter and O'Grady, 1996; Vetter et al., 1997). This increase in Na^+ transport was not associated with an increase in apical Na^+ conductance, but appeared to be the result of an increase in driving force for apical Na^+ uptake produced by activation of basolateral K^+ channels (Vetter et al., 1997).

Decreases in eggshell quality resulting from a reduction in shell thickness is a well-known problem in egg production (Bahr and Palmer, 1989; Bennett, 1992; Hughes et al., 1986; Joyner et al., 1977; Poggenpoel, 1986; Soares et al., 1988). In the present study, significant decreases in basal anion transport were observed in birds older than 55 weeks. Moreover, in molting birds there appeared to be significant downregulation of basal HCO_3^- transport that was associated with the overall regression of the reproductive tract. To date, no direct evidence for age-dependent decreases in Ca^{2+} secretion by the shell gland epithelium have been reported, but reductions in HCO_3^- secretion have been shown to significantly decrease calcite deposition. Limiting HCO_3^- secretion by reducing plasma CO_2, which can occur in heat-stressed birds, or by treating hens with carbonic anhydrase inhibitors, typically leads to reduced shell thickness (Hughes et al., 1986; Odom et al., 1986; Mashaly et al., 2004; Lavelin et al., 2001). Thus decreased rates of HCO_3^- secretion may contribute to the problem of poor shell quality often observed in older birds.

In summary, the results of this study support the following conclusions. First, the shell gland epithelium actively transports Na^+ by an electrogenic mechanism that involves apical, amiloride-sensitive Na^+ channels. We propose that this mechanism of Na^+ transport is necessary for reclaiming Na^+, Cl^- and fluid from the shell gland lumen following completion of calcite deposition. Second, the shell gland epithelium exhibits basal anion secretion, which can be stimulated by signaling molecules that increase intracellular [cAMP]. We speculate that rates of HCO_3^- secretion are regulated by changes in intracellular [cAMP] during the process of shell formation and that this is necessary for delivery of adequate amounts of HCO_3^- required for shell deposition. Finally, we conclude that anion transport decreases in older hens and speculate that this may have important consequences on shell thickness.

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