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Necturus Gallbladder Epithelial Cell Volume Regulation and Inhibitors of Arachidonic Acid Metabolism

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Abstract. Inhibition of the metabolism of arachidonic acid by the epoxygenase (cytochrome P-450) pathway with the inhibitor ketoconazole results in excessive cell swelling upon exposure to hyposmolality instead of the rapid and complete regulatory volume decrease (RVD) normally observed. NaCl entry from bathing solutions to cell interior was shown to cause this swelling, with Na influx occurring across the basolateral membrane and electrically silent Cl influx across the apical membrane. Ion substitution experiments show that the KCl efflux mediating RVD was unimpaired by ketoconazole, but was overwhelmed by the NaCl influx. Measurements of transepithelial fluid flux, Cl concentration, osmolality and pH showed that gallbladders treated with ketoconazole transiently secreted fluid rather than the normal absorption. We conclude that inhibition of arachidonic acid metabolism does not directly affect RVD by *Necturus* gallbladder, but that blockade of the epoxygenase pathway can have a profound influence on NaCl entry into gallbladder epithelial cells.

Key words: Cell volume—Ion permeabilities—Epoxygenase inhibitors—Ketoconazole

Introduction

Metabolites of arachidonic acid are thought to be involved in the regulation of many transport processes in epithelia [1]. The three pathways by which arachidonic acid may be metabolized are: (i) cyclooxygenase (forming prostaglandins, prostacyclins, and thromboxanes), (ii) lipoxygenase (forming leukotrienes and some HETE's), (iii) epoxygenase or cytochrome P-450 (forming EET, HETE, and OHAAS metabolites). Arachidonic acid metabolites have been shown to play a significant role in activation of regulatory volume decrease (RVD) by Ehrlich ascites tumor cells. In these cells RVD was reported to involve the activation of KCl and taurine efflux by lipoxygenase metabolites and simultaneous inhibition of Na entry by cyclo-oxygenase metabolites [7–10]. Blockage of leukotriene production by the inhibitor norhydroguarietic acid (NDGA) prevented RVD in Ehrlich cells [8, 10]. Addition of the leukotriene D_4 or C_3 caused Ehrlich cell shrinkage in the absence of an osmotic challenge leading to the conclusion that these compounds directly activated the efflux pathways for K, Cl and taurine [8]. Inhibition of the production of epoxygenase metabolites prevented activation of organic osmolyte release by cultured rabbit renal papillary epithelial cells (PAP-HT25) in response to a reduction in the osmolality of the bathing medium [3]. The release of sorbitol from these cells after osmotically induced swelling was the primary solute efflux mechanism responsible for RVD [4]. Inhibitors of the cyclooxygenase and lipoxygenase pathways did not have substantive effects on activation of the sorbitol efflux. Thus, metabolites of arachidonic acid generated by all three pathways may be involved in the regulation of solute permeabilities during RVD.

Transepithelial solute and fluid transport is also affected by inhibition of the production of arachidonic acid metabolites. Prostaglandin E_2 inhibits the Na,K ATPase of renal cortical collecting duct cells [1], thereby inhibiting transepithelial Na absorption.

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Inhibitors of the production of epoxygenase metabolites activate an apical Cl conductance in cultured cystic fibrotic pancreatic cells with resultant inhibition of transepithelial fluid absorption [6]. It was proposed that epoxygenase metabolites inhibit Cl channels in the apical membrane of cystic fibrosis cells and that prevention of their production relieves the inhibition allowing activation of these quiescent channels [6].

In the present study we tested the effects of inhibitors of all three arachidonic acid metabolic pathways on RVD by *Necturus* gallbladder epithelium, a tissue in which volume regulation has been extensively characterized [2, 12]. Inhibitors of the cyclo-oxygenase and lipoxygenase pathways did not alter RVD in response to an osmotic challenge. After treatment with epoxygenase inhibitors, on the other hand, gallbladder cells underwent rapid and massive swelling which was due to Na entry across the basolateral membrane and Cl entry across the apical membrane. Transepithelial fluid and solute transport was temporarily reversed from absorption to secretion by epoxygenase inhibitors.

Materials and Methods

Experiments were performed on adult *Necturus maculosus* that had been kept in aquaria at 17°C and fed goldfish at least one month before use. Animals were anesthetized by immersion in 0.1% tricaine methane-sulfonate (Finquel, Ayerst, NY). Gallbladders were removed, drained of bile, and kept in gassed *Necturus* Ringer solution.

Solutions

The composition of the control Ringer solution was as follows (in mM): NaCl 85.5, $NaHCO_3$ 10, NaH_2PO_4 0.5, KCl 2.5, $MgCl_2$ 1.0, $CaCl_2$ 1.8. Solutions were gassed with 99% air and 1% CO_2, pH was 7.6, and osmolality 200 ± 2.5 mOsmol/kg H_2O. Hypotonic Ringer had a NaCl concentration of 70 mM with an osmolality of ~169 mOsmol/kg H_2O, or ~16% less than that of control Ringer. Na gluconate was used to replace NaCl in Cl-free or low Cl (8 mM) Ringer solutions and the Ca concentration was increased to 8 mM to maintain normal calcium activity. Low Na Ringer contained 10 mM Na, the remaining NaCl was replaced by N-methyl-D-glucamine-Cl (NMDG). To study relative K conductance, 22.5 mM NaCl was first replaced by mannitol then the mannitol was replaced by K gluconate. To study relative Na conductance, all Na was replaced by NMDG.

Chemicals

Arachidonic acid, ETYA (5,8,11,14,-eicosatetraynoic acid), indomethacin, ketoconazole and NDGA (nordihydroguaiaretic acid) were purchased from Biomol (Plymouth Meeting, PA).

Cell Volume Measurements

Gallbladders were mounted in a miniature Ussing chamber in which the epithelial cells were visualized and analyzed with a microscope-video system as previously described [2]. Cell volume was determined by planimetry of stored video images of "optical sections" obtained at 3 μm displacements of focus through the cells. The area of each section was determined from tracings of the cell outline. Cell volume was computed from the areas and displacements of focus as previously described [2].

Electrical Measurements

Gallbladders were mounted in a rapid perfusion chamber and both surfaces were perfused with *Necturus* Ringer solution as previously described [2]. Membrane potential was measured by impaling cells from the apical side with glass microelectrodes filled with either 1 or 3 M KCl; electrode resistances ranged from 40 to 80 MΩ and from 10 to 30 MΩ, respectively. Recordings were analyzed only if they showed a sudden negative voltage deflection and an increase in overall electrode resistance after cell impalement, a stable cell membrane potential during the experiment as well as a rapid return of electrode resistance and membrane potential to control values at withdrawal. The apical solution was grounded by a 3 M KCl flowing bridge to a calomel electrode. Microelectrodes were connected through a Ag:Ag half cell to the input probe of an electrometer (Model 750, WP Instruments, Sarasota, FL). Relative ion conductances were estimated from the ratio of induced voltage deflections to the theoretical voltage deflections calculated by the Nernst equation.

Sac Preparations

Transepithelial fluid transport was calculated from the change in weight of the gallbladder tied as a sac to the end of a polyethylene tube. The sac was filled with Ringer solution (apical Ringer) and suspended in a beaker containing 10 ml Ringer solution (basolateral Ringer). After a control period of about 30 min, the sac was weighed at hourly intervals for 6 hr. For weight measurement the sac was removed from the beaker and carefully transferred to a tared empty beaker on the stage of an analytical balance. Measurements were repeated three times in rapid succession and values were averaged to get each data point. Mechanical disturbances, e.g., touching the beaker with the sacs or blotting of the sacs, resulted in nonreproducible measurements as was shown in control experiments. To rule out leaks due to damaged areas of the epithelium, only sacs with reproducible weights in repeated measurements were used. In some control experiments ouabain was added to abolish fluid transport with the resulting steady weight indicating that the sac epithelium was intact. At the end of the experimental period, usually after six hours, the volume of the apical Ringer solution was estimated from its weight. The surface of the sac was calculated from the volume of the apical fluid assuming a spherical shape. The rate of transepithelial fluid transport was calculated from measurements obtained in the first two hours of the experimental period when fluid transport was a linear function of time.

Cl concentrations were determined by colorimetry (Chloride Color Reagent from Sigma) on a UV/VIS spectrophotometer (Perkin Elmer, Oak Brook, IL). Osmolality was measured using the freezing point method (Micro Osmette, Precision Systems, NY). pH was measured with pH microelectrodes (response time:

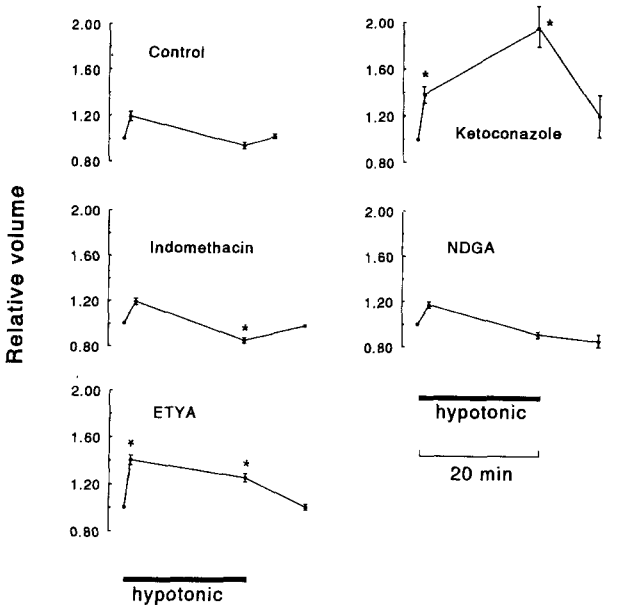

Fig. 1. Cell volume measured at time zero (isotonic), 1 min after switching to a 16% hypotonic apical perfusate, 20 and 30 min later. All inhibitors were added at a concentration of 0.1 mM 30 min prior to time zero and were present in both perfusion solutions. Mean values with standard errors are indicated; (*) significant differences from control ($P < 0.05$).

5 sec, minimal sample volume 5 μl; Micro-Combination pH Probe, Microelectrodes, Londonderry, NH) in 1 ml vials, after equilibration with 5% CO_2.

Data Analysis

Data are expressed as a mean ± SEM. Significances ($P < 0.05$) were calculated with the two-sample independent-group t-test or with the paired-group t-test, as indicated.

Results

Inhibition of Arachidonic Acid Metabolism and RVD

Necturus gallbladder cell volume was constant at 8,422 ± 357 μm^3 ($n = 65$) prior to any osmotic perturbation or drug addition. When the epithelium was exposed to a hypotonic apical perfusate the cells swelled rapidly as nearly perfect osmometers. As shown in Fig. 1 (control), an apical perfusate solution which was 16% hypotonic resulted in a swelling of 19 ± 5% within 1 min. Cell volume 20 min after the osmotic challenge returned to values indistinguishable from initial volume (94 ± 3% of control). Treatment of the tissues with indomethacin (0.1 mM), the cyclo-oxygenase inhibitor, for 30 min prior to time zero had no effect on initial cell volume, the magnitude of the swelling at 1 min after the osmotic challenge or the rate of RVD (Fig. 1, indomethacin), but the final volume achieved was significantly reduced (85 ± 3% of initial) compared to untreated tissues. The lipoxygenase inhibitor, NDGA (0.1 mM for 30 min), had no significant effects on any of the experimental results (Fig. 1, NDGA). Inhibition of the production of all metabolites of arachidonic acid by the inhibitor ETYA (0.1 mM for 30 min) did not alter initial cell volume but had significant effects on both the initial osmotically induced cell swelling and the subsequent RVD (Fig. 1, ETYA). The initial swelling was 40 ± 4%, more than twice as large as the theoretically expected osmometric response, and RVD was incomplete at 20 min. This initial overswelling can only be explained by rapid solute influx into the cells during the time of the osmotically induced water entry with the resulting increase in cell solute content causing additional swelling. The epoxygenase inhibitor, ketoconazole (0.1 mM for 30 min) had even more dramatic effects than ETYA (Fig. 1, ketoconazole) although initial cell volume was not significantly different from untreated, control tissues. The osmotically induced swelling (38 ± 7%) again was far larger than theoretically expected and was followed by continual increase at a rate of about 3%/min such that the cells reached a volume of 195 ± 16% of control 20 min after the osmotic challenge. The results of the ketoconazole experiments indicated that the effects of ETYA primarily resulted from inhibition of the production of epoxygenase metabolites of arachidonic acid. It should be noted that ketoconazole treatment in the absence of any change in the osmolality of the apical bathing solution did not result in any detectable change in cell volume or in cell membrane potential (*see below*). In the experiments which followed we analyzed the sites and mechanisms by which ketoconazole caused the initial osmotically induced overswelling as well as the subsequent massive volume increase.

Ionic Dependence of Ketoconazole Effects on Cell Volume

Entry of NaCl into the cells of ketoconazole-treated tissues seemed the most probable mechanism for both the initial and subsequent swelling observed in hypotonic media. As shown in Fig. 2 and Table 1, substitution of 90% of the Na by NMDG in the apical perfusate only slightly reduced the initial overswelling and did not prevent subsequent further volume increase. However, when 90% of the Na in the basolateral perfusate was substituted by NMDG, both

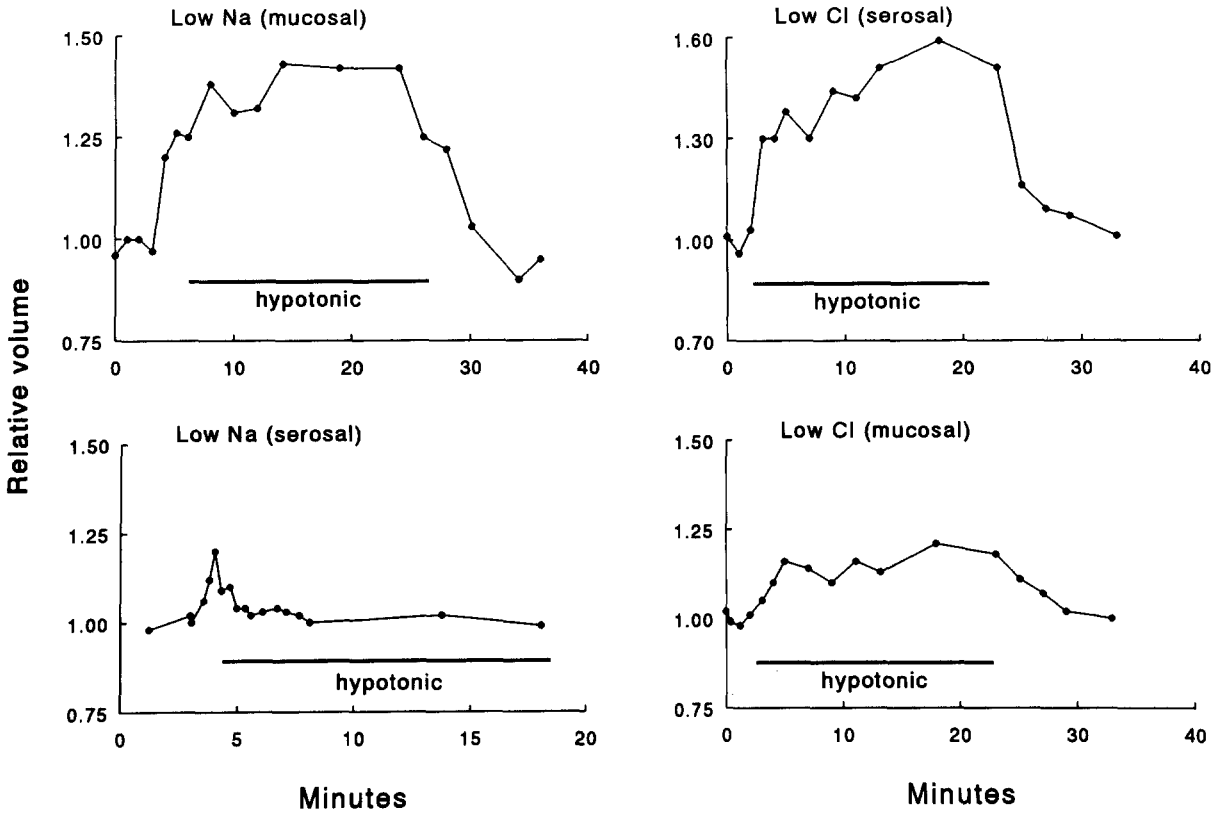

Fig. 2. Individual experimental records for four cells showing the time course of response to ion substitutions of tissues treated with ketoconazole for 30 min prior to time zero. Na was reduced to 10 mM and replaced by N-methyl-D-glucamine; Cl was reduced to 8 mM and replaced by gluconate. Mean data are in Table 1.

Table 1. Effect of Na and Cl substitutions on RVD in ketoconazole-treated tissues

Condition	Relative volume (Exp/Cont)	
	~1 min	~20 min
Control	1.19 ± 0.05(7)	0.94 ± 0.03 (7)
Ketoconazole		
Low Na, apical	1.28 ± 0.03 (8)	1.53 ± 0.07*(10)
Low Na, basolateral	1.20 ± 0.01 (8)	0.99 ± 0.01 (8)
Low Cl, apical	1.11 ± 0.01 (6)	1.21 ± 0.02*(6)
Low Cl, basolateral	1.29 ± 0.03 (7)	1.50 ± 0.04*(7)

The apical perfusate (control Ringer) was switched to hypotonic Ringer at time zero and cell volumes were measured after ~1 and ~20 min. Ketoconazole-treated gallbladders were incubated with the drug (10^{-4} M) for 30 min prior to time zero and the same concentration of the drug was added to the perfusion solutions. Low Na (10 mM Na) and low Cl (8 mM) Ringer solutions were switched to hypotonic at time zero. Number of experiments are shown in parentheses. Significances were calculated using the two-sample independent *t*-test; $^*P < 0.01$ in comparison to corresponding control value.

the initial swelling and the RVD which followed were indistinguishable from results in the untreated controls (Fig. 2 and Table 1). Thus, ketoconazole treatment activated an influx of Na across the basolateral membrane when the tissue was exposed to a hypotonic solution but did not directly block RVD. Additional information about the nature of Na entry across the basolateral membrane came from exposure of the basolateral surface of the tissue to amiloride (1 mM), known to inhibit the Na/H exchanger in the apical membrane of *Necturus* gallbladder [12]. Cell volume of tissues treated for 30 min with ketoconazole (10^{-4} M) in both bathing solutions and amiloride (1 mM) in the basolateral bath was 9,283 ± 1,040 μm^3, not significantly different from control. Perfusion of a hypotonic solution in the apical bath resulted in an initial swelling after 1 min of 17 ± 5% (n = 7), not significantly different from control. After 20 min of hypotonic stress, volume regulation was incomplete as cell volume was still 14 ± 4% (n = 7) larger than the initial value. Although the cell volume after 20 min of exposure to hypotonicity was significantly larger compared to control tissues, amiloride prevented the initial overswelling and dra-

matic subsequent volume increase seen when ketoconazole-treated tissues were exposed to hypotonicity (Fig. 1). When all but 8 mM of the Cl was removed from the basolateral perfusate and substituted by gluconate, the initial overswelling and subsequent increase was unaffected (Fig. 2 and Table 1). On the other hand, reduction of the Cl of the apical perfusate to 8 mM prevented the initial overswelling as well as RVD. Blockage of the initial and subsequent excessive swelling by low Cl indicated that Cl entered the cell across the apical membrane. Blockage of RVD was expected because previous studies [2, 11] had shown that Cl removal from the apical bathing solution lowered intracellular Cl activity thereby preventing RVD. Additional support for this conclusion came from the observation that the volume of cells exposed to a low Cl apical perfusate was reduced to 81 ± 3% (n = 7) of that in control Ringer, consistent with the loss of virtually all intracellular Cl.

Ketoconazole treatment, therefore, did not directly inhibit RVD but effectively overshadowed it because of the simultaneous activation of Cl entry across the apical membrane as well as amiloride-sensitive Na entry across the basolateral membrane triggered by a reduction in the perfusate osmolality.

Ketoconazole-Induced Effects on Apical Membrane Conductances

Because the cell volume experiments showed that Cl entry occurred across the apical membrane in ketoconazole-treated tissues when the perfusate osmolality was reduced, we estimated the apical membrane Cl conductance from the voltage deflections caused by brief pulses of a Cl-free apical perfusate during exposure to a hypotonic apical bathing solution. Apical membrane potential did not differ significantly between the control tissues (64.9 ± 1.7 mV, n = 27) and those treated with ketoconazole (64.9 ± 3.7 mV, n = 14). As shown in Table 2, ketoconazole significantly reduced the magnitude of the Cl conductance of the apical membrane normally activated during perfusion of a hypotonic solution. Thus, activation of a Cl conductance in the apical membrane cannot be the mechanism of the Cl influx associated with cell swelling.

The effect of ketoconazole on the Na, K, and Cl conductances of the apical membrane in the absence of a change in osmolality was also evaluated by determination of the deflections of the apical membrane potential in response to pulses of a Na-free, high K, or Cl-free solution, respectively (Table 3). Voltage deflections in the presence of ketoconazole were unchanged during the conductance measurements, consistent with the conclusion that the apical membrane conductance was not altered by the drug.

Table 2. Apical membrane voltage deflections during Cl replacement in the presence of a hypotonic apical perfusate

Condition	Voltage deflections (mV)		
	1 min	5 min	10 min
Control	3 ± 1 (5)	7.5 ± 1(7)	7.8 ± 1(45)
Ketoconazole	1.6 (1)	2.6 ± 0.3*(5)	1.9 ± 0.4*(22)

Apical isotonic Ringer solution was switched to hypotonic Ringer solution at time zero. Voltage deflections of the apical membrane potential difference induced by apical, hypotonic, Cl-free Ringer solution were measured after 1, 5 and 10 min. Ketoconazole-treated gallbladders were preincubated for 30 min with ketoconazole (10^{-4} M) and ketoconazole was added to superfusion solutions. Number of experiments are shown in parentheses. Significances were calculated using the two-sample independent t-test; $^*P < 0.01$ in comparison to controls.

Transepithelial Fluid Transport Is Transiently Reversed by Ketoconazole

Control transepithelial fluid absorption rate, measured from the rate of weight change of sacs in the first two hours was 4.8 ± 1.9 μl/hr cm^2 (Fig. 3). Ketoconazole-treated tissues showed a dramatically different pattern. Transepithelial fluid transport during the first two hours was reversed to secretion (Fig. 3) at a rate of −3.7 ± 2.9 μl/hr cm^2. Fluid secretion did not occur at a constant rate and essentially stopped in the 4–6 hr period.

The fluid collected from the sac lumen of control tissues after six hours was slightly hypotonic to the basolateral bath. Table 4 shows that the luminal fluid osmolality was 11.8 mOsm less than that of the basolateral bath. Such an osmolality difference corresponds to a NaCl concentration difference of about 6 mM. Ketoconazole treatment reduced the transepithelial osmolality gradient after 6 hr to 4 mOsm. Ketoconazole-treated tissues did not exhibit significant fluid transport at the time of collection. The small osmolality differences under control conditions are consistent with the conclusion that the fluid absorbed must be slightly hypertonic to the bathing solutions.

Transepithelial gradients for Cl were assessed by measurements of the Cl concentration in the luminal fluid of the sac after a 6-hr experimental period. As shown in Table 4, sac Cl concentration was lower than that of the bathing solution by 17.8 mM. Cl absorption of this magnitude would reduce luminal

Table 3. Effect of ketoconazole of apical membrane conductances

Condition	Voltage deflections caused by ion concentration change		
	K	Na	Cl
	mV	mV	mV
Control	35.3 ± 1.9 [60%] (15)	−8.6 ± 0.5 [15%] (9)	0.8 ± 0.4 [1%] (10)
Ketoconazole	33.9 ± 1.2 [57%] (11)	−8.3 ± 0.4 [15%] (11)	2.4 ± 0.9 [2.5%] (7)

Voltage deflections of the apical membrane potential difference were induced by ion substitutions in the apical Ringer superfusate. Positive values indicate depolarization and negative values indicate hyperpolarization of the membrane potential. Estimations of relative ion conductances are shown in square brackets. Number of experiments are shown in parentheses (n). Gallbladders were preincubated 30 min with ketoconazole (10^{-4} M), and ketoconazole was added to superfusion solutions as indicated. No significant differences in the magnitude of the voltage deflections were found between the ketoconazole-treated and control measurements.

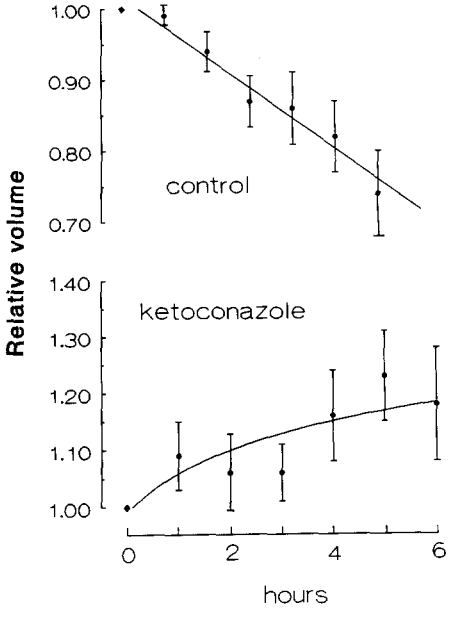

Fig. 3. Fluid transport by gallbladder sac preparations under control conditions (top) and during ketoconazole treatment (bottom). Mean values with standard errors are shown. Number of experiments equals eight for control and nine for ketoconazole.

osmolality more than was observed and indicates that secretion of another solute may be occurring with the most likely candidate being HCO_3. Ketoconazole reduced the basolateral-luminal Cl concentration difference to 7.9 mM. The luminal pH under control conditions differed only very slightly from that of the basolateral bath; ketoconazole treatment resulted in a slight acidification (0.11 units) of the sac lumen (Table 4).

Discussion

Previous observations, confirmed in the present study, were that *Necturus* gallbladder epithelium exhibited rapid and complete RVD [2, 11]. RVD in response to osmotic swelling was previously shown to be accomplished by the conductive efflux of KCl across the basolateral membrane [2]. Prompted by reports that arachidonic acid metabolites play prominent roles in RVD by Ehrlich ascites tumor cells [5, 7–10], we investigated the effects of various inhibitors of arachidonic acid metabolism on *Necturus* gallbladder epithelium. The present study shows that RVD in this epithelium was not affected by inhibitors of the production of arachidonic acid metabolites but was obscured by NaCl influx in the presence of epoxygenase inhibitors.

Response to Hypotonic Perfusates

Dramatic effects on transmembrane Na and Cl fluxes were observed when tissues, treated to inhibit production of the epoxygenase metabolites, were exposed to a hypotonic apical bath. The rates of Na and Cl entry during exposure to hypotonicity were of sufficient magnitude to completely overwhelm the efflux of KCl which normally results in RVD. The strongest evidence for this conclusion came from the experiments in which the Na concentration of the basolateral bathing solution was reduced (Fig. 2 and Table 1). Under this condition, RVD was indistinguishable from control observations in the absence of ketoconazole. The ion substitution experiments (Table 1) showed that NaCl influx occurred

Table 4. Ketoconazole effects on transepithelial gradients for Cl, osmolality and pH

Condition	Δ Cl (mM)	Δ Osmolality (mOsmol/kg H_2O)	Δ pH
Control	17.8 ± 4.6 (9)	11.8 ± 2.7 (9)	−0.09 ± 0.02 (8)
Ketoconazole	7.9 ± 2.7*(11)	4 ± 1.1*(9)	+0.11 ± 0.04*(8)

Cl concentrations and osmolality were measured in apical and basolateral Ringer solutions of gallbladder sacs after six hours of incubation. Data show basolateral bath values minus apical bath values. Sac preparations were preincubated for 30 min with ketoconazole (10^{-4} M) and ketoconazole was added to Ringer solutions as indicated. Number of experiments is given in parentheses. Significances were calculated using the paired-group *t*-test (*$P < 0.01$).

because Na entered from the basolateral bath and Cl from the apical bathing solution. Na entry across the basolateral membarne was almost completely inhibited by amiloride, consistent with the conclusion that Na/H exchange was the primary mechanism of entry. On the basis of the electrical measurements, Cl entry appeared to be an electrically silent mechanism whose exact nature was not ascertained. Previous studies [12] have demonstrated the existence of Cl/HCO_3 exchange in the apical membrane of *Necturus* gallbladder epithelium, making it a likely candidate for the Cl entry step activated by ketoconazole.

When tissues treated with the cyclo-oxygenase inhibitor indomethacin were exposed to hypotonicity there was no effect on the baseline cell volume or magnitude of the initial swelling (Fig. 1). However, the final cell volume after RVD was only 85% of the control value, significantly lower than that of untreated tissues. The observed shrinkage is of the magnitude expected from the loss of virtually all intracellular Cl [11]. This is most likely due to the action of cyclo-oxygenase inhibitors to increase intracellular cAMP, which activates a Cl conductance in the apical membrane of *Necturus* gallbladder [12]. Alternatively, inhibition of cyclo-oxygenase metabolites could lead to excessive shrinkage during RVD if these products were involved in the inactivation of KCl exit during RVD. Little is known about the influence of cyclo-oxygenase metabolites on RVD by epithelia [5]. It has been shown that the stimulation of prostaglandin production, which occurs during exposure of Ehrlich cells to hypotonicity, inhibits Na entry thereby accelerating RVD by these cells [5, 10].

Although lipoxygenase metabolites have been shown to be important in RVD by Ehrlich cells [5, 7–10] and in some renal tubules [1], we found no effects of the most widely used inhibitor, NDGA, on RVD by *Necturus* gallbladder. Thus, we conclude that arachidonic acid metabolites play no obvious role in RVD by *Necturus* gallbladder. However, it is apparent that osmotically induced swelling of gallbladder epithelium triggers major changes in NaCl entry pathways when epoxygenase metabolism is inhibited.

Epoxygenase Metabolites and the Regulation of Transport

Inhibition of the epoxygenase pathway results in a transient reversal of the direction of fluid transport from net absorption to secretion. What is unclear is the role, if any, played by epoxygenase metabolites in the regulation of transepithelial salt and water transport by *Necturus* gallbladder and other epithelia [1]. Identifying individual metabolites and defining their role in the regulation of the rate and direction of transport is made more difficult by their unstable chemical nature. These compounds are often very short-lived, insoluble in water, and rapidly altered by membrane bound enzymes [1]. Although these characteristics make them ideal signaling compounds, they also make them difficult to isolate, characterize and quantify. The small number of cells in a *Necturus* gallbladder epithelium further limits our ability to extract and identify the metabolites involved. The information obtained from *Necturus* gallbladder epithelium may be applicable to a cultured epithelial cell line from which more detailed biochemical and physiological information could then be obtained. Although inhibition of the production of epoxygenase metabolites by ketoconazole can have profound effects on cell volume, fluid transport and the mechanisms of Na and Cl entry in *Necturus* gallbladder epithelium, the role played by these metabolites in the normal regulation of transmembrane or transepithelial transport remains to be defined.

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References

1. Bonventre, J.V., Nemenoff, R. 1991. Renal tubular arachidonic acid metabolism. *Kidney Int.* **39:**438–449
2. Furlong, T.J. and K.R. Spring. 1990. Mechanisms underlying volume regulatory decrease by Necturus gallbladder epithelium. *Am. J. Physiol.* **258:**C1016–C1024
3. Furlong, T.J., Moriyama, T., Spring, K.R. 1991. The activation of osmolyte efflux from cultured renal papillary epithelial cells. *J. Membrane Biol.* **123:**269–277
4. Garty, H., Furlong, T.J., Ellis, D.E., Spring, K.R. 1991. The sorbitol permease: an apical membrane transporter in cultured renal papillary epithelial cells. *Am. J. Physiol.* **260:**F650–F656
5. Hoffmann, E.K., Simonsen, L.O. 1989. Membrane mechanisms in volume and pH regulation in vertebrate cells. *Physiol. Rev.* **69:**315–382
6. Kersting, U., Kersting, D., Spring, K.R. 1993. Ketoconazole activates Cl^- conductance and blocks Cl^- and fluid absorption by cultured cystic fibrosis (CFPAC-1) cells. *Proc. Nat. Acad. Sci. USA* **90:**4047–4051
7. Lambert, I. H. 1987. Effect of arachidonic acid, fatty acids, prostaglandins, and leukotrienes on volume regulation in Ehrlich ascites tumor cells. *J. Membrane Biol.* **98:**207–221
8. Lambert, I.H. 1989. Leukotriene-D_4 induced cell shrinkage in Ehrlich ascites tumor cells. *J. Membrane Biol.* **108:**165–176
9. Lambert, I.H., Hoffmann, E.K. 1990. Taurine transport and cell volume regulation in a mammalian cell. *In:* Taurine: Functional Neurochemistry, Physiology and Cardiology. H. Posantes-Morales, D.L. Martin, W. Shain and R. Martin del Rio, editors. pp. 267–276. Raven, New York
10. Lambert, I.H., Hoffmann, E.K., Christensen, P. 1987. Role of prostaglandins and leukotrienes in volume regulation by Ehrlich ascites tumor cells. *J. Membrane Biol.* **98:**247–256
11. Larson, M., Spring, K.R. 1984. Volume regulation by *Necturus* gallbladder: Basolateral KCl exit. *J. Membrane Biol.* **81:**219–232
12. Reuss, L. 1989. Ion transport across gallbladder epithelium. *Physiol. Rev.* **69:**503–545