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FUNCTIONAL DISTINCTION BETWEEN TWO TRANSPORT MECHANISMS IN RABBIT GALL-BLADDER EPITHELIUM BY USE OF OUABAIN, ETHACRYNIC ACID AND METABOLIC INHIBITORS

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SUMMARY

1. Net fluid transport rate, transepithelial p.d. and resistance, and unidirectional Na^+-fluxes were measured in rabbit gall-bladder preparations exposed on both sides to bicarbonate–Ringer solution *in vitro*.

2. Both ouabain and ethacrynic acid (ETCA) caused dose-dependent decreases of net fluid transport rate; ouabain inhibited fluid transport predominantly from the serosal side, whereas the inhibitory effect of ETCA was elicited mainly from the mucosal (luminal) side. Applied bilaterally, the ID_{50} for ouabain was $2{\cdot}5 \times 10^{-6}$ M, and for ETCA $2{\cdot}3 \times 10^{-4}$ M. After maximal inhibition at each concentration level of the two inhibitors fluid transport could not be reversed.

3. 2,4-Dinitrophenol (2,4-DNP) (2×10^{-4} M) or substitution of O_2 by N_2 caused an 80% reversible decrease of net fluid transport.

4. The spontaneous p.d. across the rabbit gall-bladder was about 2·7 mV, mucosal side positive. 2,4-DNP, N_2 and serosal application of ouabain depressed the p.d. after an initial hyperpolarization. This decrease was reversible during recovery from 2,4-DNP and N_2, but irreversible after removal of ouabain at concentrations $\geqslant 10^{-4}$ M. Mucosal application of ETCA (10^{-3} M) caused no decrease in p.d., which actually increased slightly.

5. Calculated passive serosal-to-mucosal Na^+-fluxes changed in the same direction as did changes in conductance.

6. It is concluded that ETCA does not interfere primarily with the Na-K-ATPase or cellular oxidative metabolism. The data support the proposal that the pump responsible for isosmotic transepithelial fluid transfer is located in the luminal end of the cells. This pump is ETCA-sensitive. The ATPase-dependent Na–K pump, which can be inhibited by ouabain, is localized in the serosa-facing cell membrane. The data suggest that the inhibition of net fluid transport by ouabain is indirect and mediated by changes in intracellular ion concentrations.

7. The results support the concept that the transepithelial fluid transport mechanism is electroneutral, and suggest that the mucosa positive transepithelial p.d. is due to differences in electromotive forces arising from ion (mainly K^+) diffusion across the mucosal and serosal cell membranes.

INTRODUCTION

Over the last decade evidence has been presented from studies in several animal species, indicating that isosmotic transporting epithelia such as those of the proximal tubule and mammalian gall-bladder possess two pumps handling sodium (Kleinzeller, 1961; Kleinzeller & Knotkova, 1964; MacKnight, 1968; Whittembury, 1968; Whittembury & Fishman, 1969; Whittembury & Proverbio, 1970; Giebisch, Sullivan & Whittembury, 1973). One is the classical ATPase-dependent Na-K pump maintaining high K and low Na concentrations in the cytosol; this pump is inhibited by ouabain or absence of extracellular K. The other is a pump handling the extrusion of NaCl and water in isosmotic proportion across the baso-lateral cell border, responsible probably for both transepithelial isosmotic salt and water transport and cell volume regulation; this pump is inhibited by the diuretic ethacrynic acid (ETCA). Although the nature of this latter transport mechanism is not yet clarified some evidence has been presented supporting the view that a mechano-chemical process, spatially separated from the Na-K ion pump, may be involved (Kleinzeller & Knotkova, 1964; Rorive & Kleinzeller, 1972; Rorive, Nielsen & Kleinzeller, 1972; Frederiksen & Leyssac, 1969; Leyssac & Frederiksen, 1974). The mechano-chemical nature of the transepithelial isosmotic transport mechanism in rabbit gall-bladder was further supported by its sensitivity to cytochalasin B (Frederiksen & Leyssac, 1977).

However, the usefulness of ETCA as a specific inhibitor of the transepithelial transport mechanism in isosmotic transporting epithelia has been questioned, since it (*a*) has a direct inhibitory action on cell metabolism in kidney cortex slices (Epstein, 1972) and (*b*) may reduce the Na-K-ATPase activity of kidney (Duggan & Noll, 1965) and gall-bladder cells (van Os & Slegers, 1970).

The concept of an isosmotic transepithelial transport of fluid involving an electroneutral transport mechanism for NaCl was inferred from studies on gall-bladder (Diamond, 1968) and proximal tubules (Maude, 1970). However, this concept of a coupled non-electrogenic transfer of Na and Cl has also recently been questioned after the demonstration of a paracellular shunt pathway (Barry, Diamond & Wright, 1971; Frömter, 1972; Machen, Erlij & Wooding, 1972) which would tend to short-circuit the epithelium internally (Schultz, 1972).

Based on these criticisms and studies on gall-bladder Na-K-ATPase it has recently been proposed that the Na-K pump in the gall-bladder serves as the driving mechanism for the isosmotic salt and water transport (cf. review by van Os, 1974).

The aim of the present investigation was to explore in further detail the differences in action of ouabain and ETCA on isosmotic fluid transport in order to test whether or not such differences might clarify the points of disagreement mentioned above.

Preliminary results of part of this investigation have been reported previously (Frederiksen, 1972; Leyssac & Frederiksen, 1974).

METHODS

Gall-bladders were obtained from female albino rabbits weighing about 3 kg. The animals were sacrificed by a blow on the neck. Isolation and cannulation of the gall-bladder sac preparation for fluid transport measurements were performed as described previously (Frederiksen & Leyssac, 1969).

Fluid transport measurements. Fluid transport rate was measured gravimetrically as described

elsewhere (Diamond, 1964; Frederiksen & Leyssac, 1969). All experiments were carried out at 37 °C. Weighing periods of 10 min were used, and luminal (mucosal) content was renewed between each weighing period.

Solutions. The composition of the Ringer solution in all types of experiments was (mM): Na^+, 114·7; K^+, 7·0; Ca^{2+}, 2·0; Mg^{2+}, 1·2; Cl^-, 102·0; HCO_3^-, 17·5; SO_4^{2-}, 1·2; $H_2PO_4^-$, 1·2; monoglutamate, 5·0; glucose, 11·0. The pH was adjusted to 7·4 by equilibration with 96 % O_2 and 4 % CO_2 at 37 °C. When the effect of omission of oxygen was investigated the solution was equilibrated with 96 % N_2 and 4 % CO_2 thereby maintaining pH at 7·4.

Measurements of transepithelial potential difference (p.d.) and resistance (R) were performed with gall-bladders cut open and mounted between two half-chambers as described in detail previously (Leyssac, Bukhave & Frederiksen, 1974). Exposed gall-bladder surface area was 0·9 cm^2; half-chambers contained 10·0 ml. of Ringer solution each; and temperature was kept at 37 °C. R was calculated from the change in p.d. after a short-lasting passage of 100 μA of d.c. current (from the mucosal to the serosal side). All R and p.d. values were corrected for those measured without the gall-bladder mounted.

Measurements of unidirectional Na^+-fluxes. Mucosal-to-serosal (M-to-S) $^{22}Na^+$-flux was determined as described in detail previously (Leyssac *et al.* 1974). Briefly, the gall-bladder was filled with Ringer containing 2–4 μCi $^{22}Na^+$/ml. The bladder was then weighed and incubated in 25·0 ml. Ringer solution (serosal medium) without labelled Na^+. 100 μl. samples of serosal fluid were taken every 30 sec during the flux period (about 10 min). The preparation was then re-weighed. The M-to-S Na^+-flux was calculated from the increment in serosal $^{22}Na^+$ content and the specific activity of $^{22}Na^+$ on the mucosal side. No correction was made for serosal-to-mucosal S-to-M $^{22}Na^+$-backflux since the specific activity of the serosal medium never exceeded 1·5 % of that of the mucosal medium. Countings of radioactivity were done to at least 10^4 counts in a γ-well scintillation counter (Selektronik, Copenhagen, Denmark). The M-to-S Na^+-flux became rectilinear within 2–3 min. Net Na^+-flux was calculated from the loss of weight of the gall-bladder preparation during the flux measurement period assuming the transported fluid to be isosmotic and Na^+ to constitute 95 % (125 m-equiv/l.) of the transported cations (Diamond, 1964). Finally, the S-to-M Na^+-flux was calculated as the difference between the M-to-S and the net Na^+-fluxes.

Inhibitors. Ouabain (crystalline g-strophanthin) was obtained from Mecobenzon, Copenhagen. ETCA (batch no. E58685) was a generous gift from Merck, Sharpe & Dohme. 2,4-dinitrophenol (2,4-DNP) was obtained from Sigma Chemical Co.

Presentation of data. The data are presented as the means of data from individual gall-bladders or the mean of differences of paired observations in individual experiments ± the S.E. of mean. Student's t test was used to determine statistical significance.

RESULTS

Effects of ouabain and ETCA on net fluid transport rate

The absolute values of stable net fluid transport rates in control bladders (60–90 min after sacrifice of the animal) averaged about 0·5 $mg.min^{-1}.mg$ tissue dry $wt.^{-1}$ (or 0·05 $mg.min^{-1}.mg$ tissue wet $wt.^{-1}$) in agreement with previous observations (Frederiksen & Leyssac, 1969). Fluid transport rate decreased by 12 ± 2 % ($n = 30$) during the following 2 hr of incubation in control Ringer solution. Percentage changes in fluid transport rates induced by inhibitors were corrected for this spontaneous decrease in transfer rate.

Ouabain applied to both sides of the gall-bladder epithelium at concentrations ranging from 5×10^{-7} to 5×10^{-4} M caused a dose-dependent decrease in fluid transport rate (Fig. 1*A*, continuous line). Fig. 2 demonstrates that the time course and degree of inhibition was the same whether 5×10^{-4} M-ouabain was added to both sides or to the serosal side only, while the effect of mucosal application was less pronounced. At all concentrations used, the effect on net fluid transfer of exposure to ouabain for 1 hr or more was irreversible.

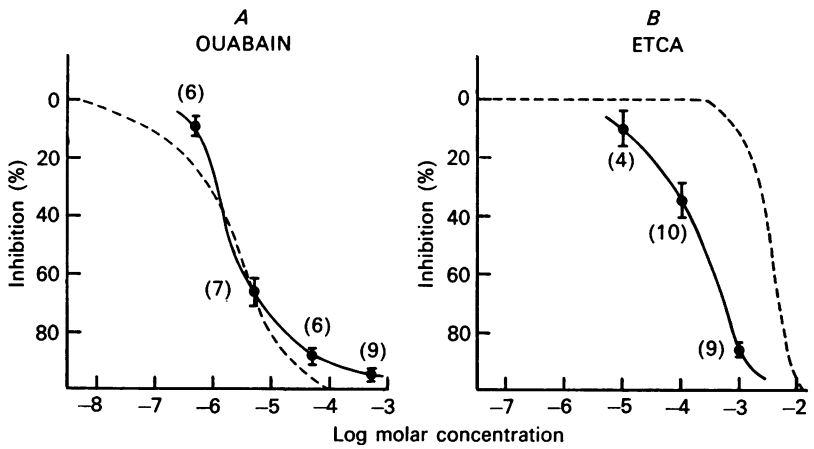

Fig. 1. Dose–response curves for the effects of ouabain (*A*) and ETCA (*B*) on net fluid transfer rate (continuous lines; present investigation) and microsomal Na-K-ATPase (dashed lines; curves obtained from van Os & Slegers (1971)). For fluid transport measurements the drugs were added to both the mucosal and serosal medium. Number of fluid transport experiments in parentheses. Means ± S.E. of means.

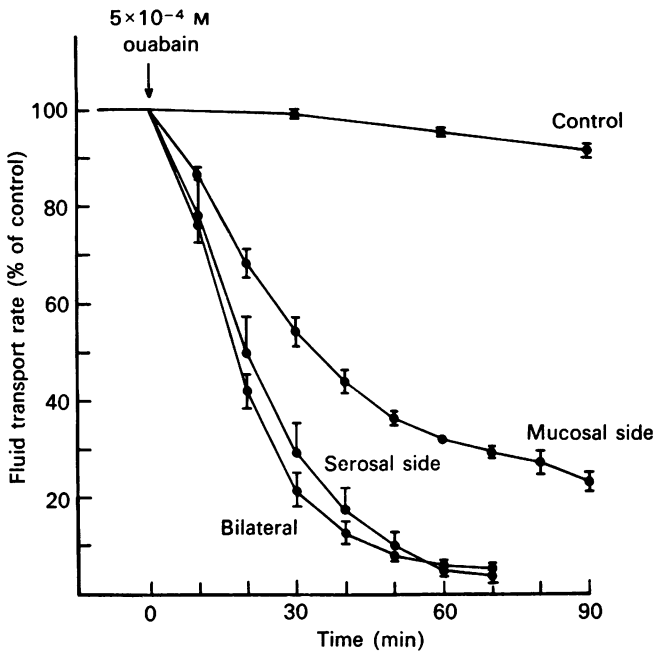

Fig. 2. Effects of 5×10^{-4} M-ouabain applied to the mucosal medium ($n = 3$), to the serosal medium ($n = 4$), or to the medium on both sides ($n = 6$) of the gall-bladder on net fluid absorption rate relative to the initial steady state control value. The upper curve represents the spontaneous change in non-treated gall-bladders ($n = 30$). Means ± S.E. of means.

Fig. 3 shows the time course of the effect on net fluid transport of serosal, mucosal and bilateral application of 10^{-3} M-ETCA. In contrast to ouabain, the inhibitory effect of ETCA was stronger when applied from the mucosal side than from the serosal side. It is seen that, although mucosal and serosal effects to some extent seem to be additive, mucosal application ultimately lowered fluid transport rate to the same degree as did bilateral application, only the effect was obtained more slowly (about 85% inhibition after 170 min and 100 min, respectively). The time course of the effect of ETCA was considerably longer than that of a concentration of ouabain of equal potency. The dose–response curve for bilateral application of ETCA is shown in Fig. 1*B* (continuous line). The effect of ETCA on fluid transport was irreversible, at least after application for more than 90 min.

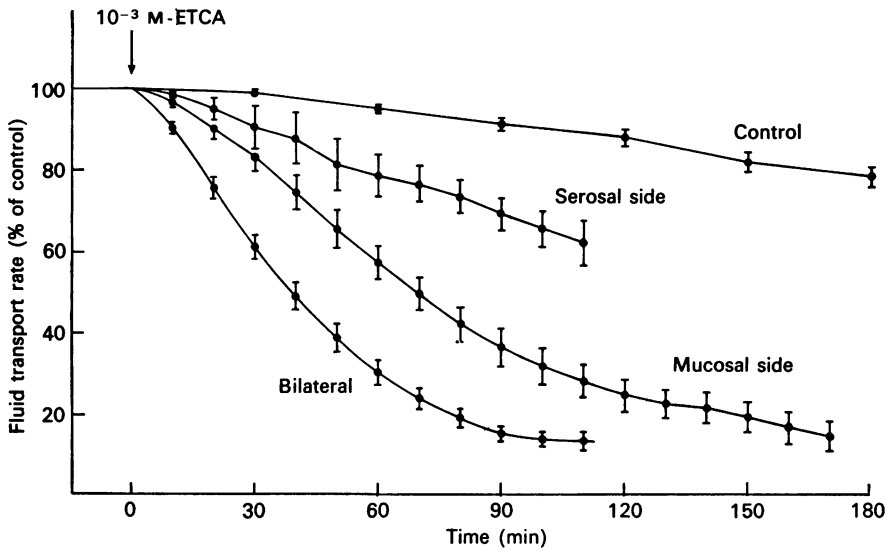

Fig. 3. Effects of 10^{-3} M-ETCA applied to the serosal medium (n = 4), to the mucosal medium (n = 4), or to the medium on both sides (n = 9) of the gall-bladder on net fluid absorption rate relative to the initial steady-state control value. The upper curve represents the spontaneous change in non-treated gall-bladders (n = 30). Means ± S.E. of means.

Effects of ouabain and ETCA on p.d. and R

The rabbit gall-bladder generated a significant spontaneous mucosa positive p.d. when incubated at 37 °C between identical bicarbonate-Ringer solutions in agreement with previous observations (Machen & Diamond, 1969; Frederiksen & Leyssac, 1977). In fourteen control experiments the average initial stable p.d. was 2·72 ± 0·07 mV and R was 46·5 ± 1·5 Ω/cm². These values were not significantly changed even after 4 hr of incubation.

Ouabain

Ouabain applied from both sides of the epithelium at concentrations of 5×10^{-4} M (n = 3; Fig. 4) or 10^{-4} M (n = 5; Fig. 5*A*) caused a short-lasting variable mucosal hyperpolarization (max. after 7–8 min) followed by a decrease of the p.d. to values

not significantly different from zero. These treatments caused an initial 15–30% decrease in R (Figs. 4, 5A); this was followed by an increase in R to values in the order of 150–250% of the control values (Fig. 4) with a maximum reached after about 150 min. Bilateral application of 10^{-5} M-ouabain ($n = 3$; Fig. 5B) decreased p.d. to about half of the control value without any significant effect on R. 10^{-6} M-ouabain from both sides ($n = 2$) affected neither p.d. nor R consistently. Mucosal application of 10^{-4} M-ouabain ($n = 3$) did not change p.d. and R significantly.

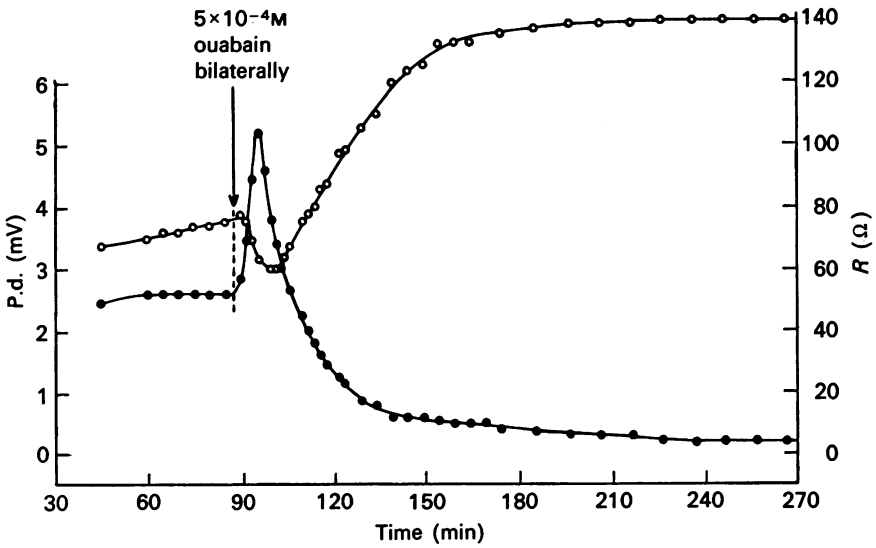

Fig. 4. Record of an experiment demonstrating the effects of bilateral application of 5×10^{-4} M-ouabain on potential difference (p.d.; ●) and resistance (R; ○).

Fig. 5 demonstrates the reversibility of ouabain effects on the electrical parameters. After 45 min of exposure to 10^{-4} M-ouabain bilaterally ($n = 2$) the effect on p.d. was irreversible but the increase of R to values above control level was completely prevented (Fig. 5A). The effects of 10^{-5} M-ouabain for 90 min ($n = 3$) were completely reversible both regarding p.d. and R (Fig. 5B). This result should be compared with the lack of reversibility of net fluid transport after exposure to 10^{-5} M-ouabain for a similar period of time.

The increase in R caused by high concentrations of ouabain (Fig. 4) could be a consequence of cell swelling and concomitant compression of lateral intercellular spaces (cf. Tormey & Diamond, 1967). Such an effect should be counteracted by an osmotically induced M-to-S water flow (Smulders, Tormey & Wright, 1972). However, serosal application of 25 mM- ($n = 2$) or 50 mM- ($n = 2$) sucrose, which induces such a water flow, did not reduce the increased R induced by 10^{-4} M-ouabain.

ETCA

10^{-3} M-ETCA applied to the mucosal side caused a small and gradual *increase* in the mucose positive p.d. ($+25 \pm 4{\cdot}8$% after 80 min, $n = 5$; Fig. 6A) which was maintained for at least 2 hr. R was not significantly changed. The lack of a decrease in p.d. should be compared with the marked inhibitory effect on fluid transport rate of

10^{-3} M-ETCA applied from the mucosal side (cf. Fig. 3). 10^{-3} M-ETCA from both sides (n = 9; Fig. 6*C*) or from the serosal side only (n = 3; Fig. 6*B*) did not cause any significant change in p.d. or R for the first 40–60 min; thereafter an often rather abrupt decrease in both parameters was observed. On average R decreased to 50% of the control value and p.d. to 13% (bilateral application) and 40% (serosal application) of control values. 10^{-4} M-ETCA from both sides (n = 6) had no significant effect on either p.d. or R.

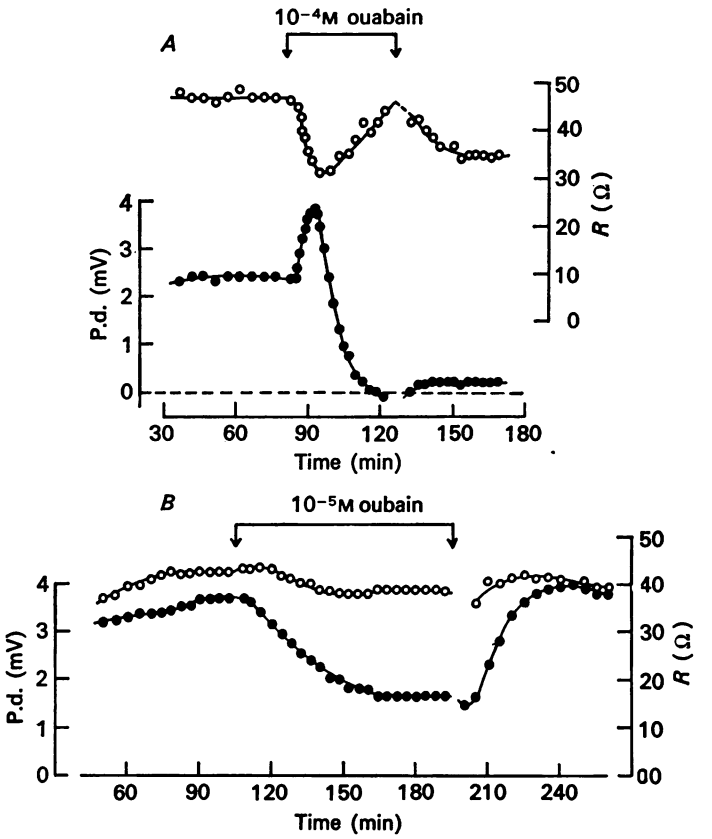

Fig. 5. Records of two experiments demonstrating the effects of bilateral application of 10^{-4} M-ouabain for 45 min (*A*) and 10^{-5} M-ouabain for 90 min (*B*) on potential difference (●) and resistance (○).

Effects of ouabain and ETCA on Na-fluxes

In twelve control experiments M-to-S and S-to-M Na^+-fluxes were $7{\cdot}29 \pm 0{\cdot}45$ and $3{\cdot}46 \pm 0{\cdot}31$ μequiv.hr^{-1}.mg dry wt.$^{-1}$, respectively, when measured after net fluid transport rate had reached a stable level (60–80 min after preparation). In seven of these experiments subsequent Na^+-flux determinations were carried out for at least 3 hr, at which time changes compared to initial values were: Δ(M-to-S flux) = $-0{\cdot}31 \pm 0{\cdot}18$ μequiv.hr^{-1}.mg dry wt.$^{-1}$ ($P > 0{\cdot}05$) and Δ(S-to-M flux) = $+0{\cdot}01 \pm 0{\cdot}15$ μequiv.hr^{-1}.mg dry wt.$^{-1}$ ($P > 0{\cdot}9$). Thus, no significant changes in the fluxes were seen, although the M-to-S flux tended to decrease as did the net flux. The absolute values of the present calculated S-to-M control Na^+-fluxes are in agreement

with later direct measurements of S-to-M flux (O. Frederiksen, unpublished observation).

Bilateral application both of ouabain (10^{-4} M) and of ETCA (10^{-4} or 10^{-3} M) caused the M-to-S Na^+-flux to decrease.

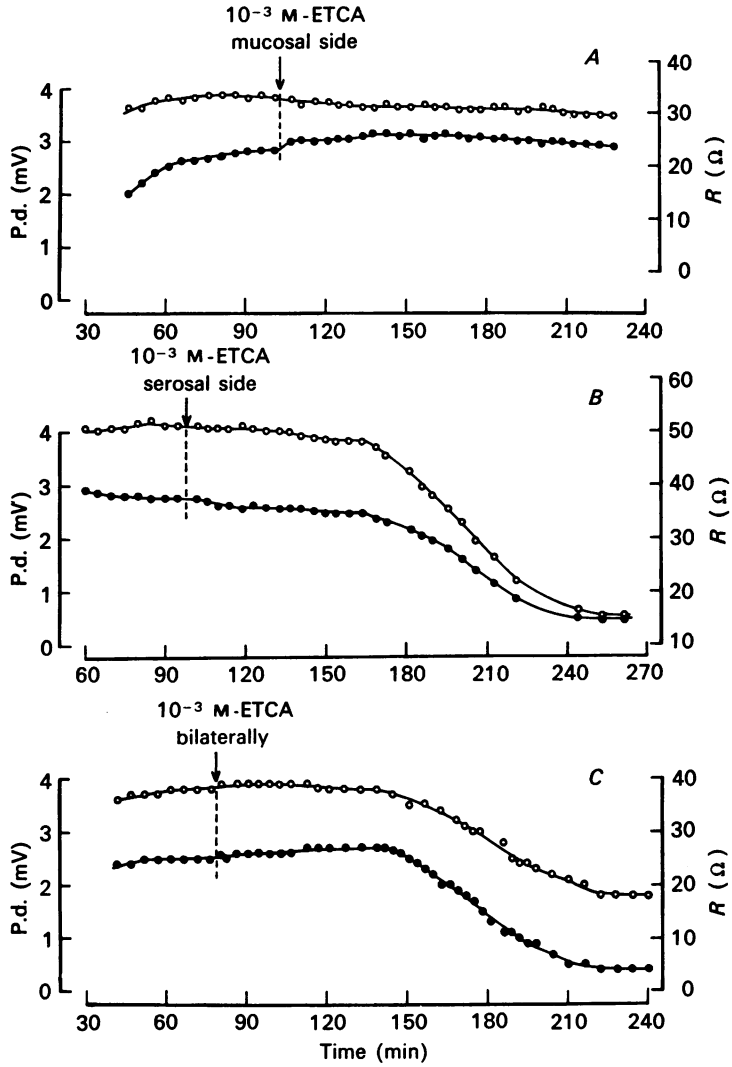

Fig. 6. *Records of three experiments demonstrating the effects of mucosal (A), serosal (B), and bilateral (C) application of 10^{-3} M-ETCA on potential difference (●) and resistance (○).*

The simultaneous changes in the calculated S-to-M Na^+-flux obtained after bilateral application of 10^{-4} M-ouabain are shown in Fig. 7*A*. The S-to-M Na^+-flux decreased markedly, but the final level (about 15% of the control) was reached already after 40 min, i.e. a long time before *R* had increased to its maximum (see Fig. 4).

As shown in Fig. 7*B* bilateral application of ETCA caused a variable and small

initial decrease in the S-to-M Na^+-flux followed by an increase of the flux after exposure to the drug for more than 1 hr. The 40% increase in the S-to-M Na^+-flux observed in the two gall-bladders after treatment with 10^{-4} M-ETCA for 115 min contrasts with the lack of change in tissue R observed at this dose level.

Effects of metabolic inhibition

2×10^{-4} M-2,4-DNP applied from either the mucosal or serosal side depressed net fluid transport by approximately 80% ($n = 3$). A 55% inhibition was obtained by 5×10^{-5} M-2,4-DNP ($n = 3$). Replacement of 96% O_2+4% CO_2 by 96% N_2+4% CO_2 reduced fluid transport rate by 80% ($n = 2$). These effects were almost completely reversible.

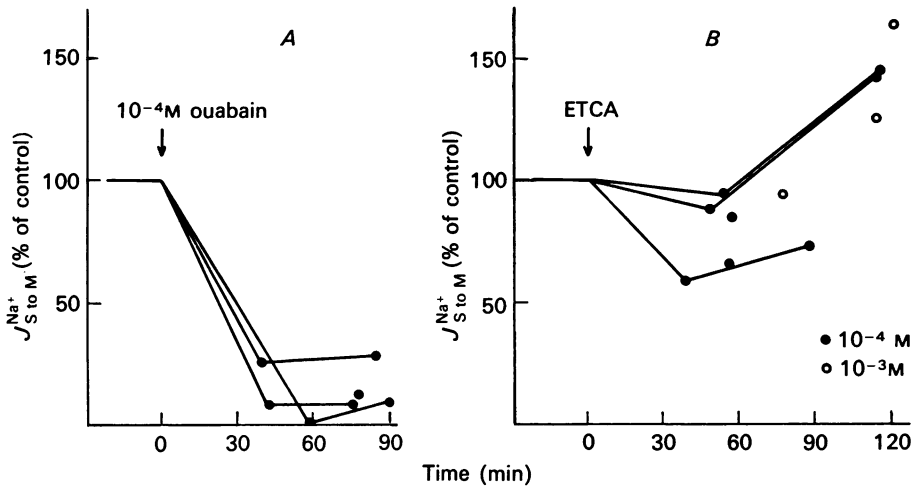

Fig. 7. Effects of ouabain (*A*) and ETCA (*B*) on serosal-to-mucosal Na^+-flux relative to initial control values. Each point represents a single flux measurement. Connected points represent flux determinations in one preparation.

Fig. 8 shows that both 2,4-DNP and N_2 treatment affected p.d. in a way very similar to that of serosal application of ouabain at high concentrations. Thus, an initial mucosal hyperpolarization (max. after 2–4 min), followed by a rapid depolarization, was observed. Also, a 25–50% decrease in R was observed, similar to the initial decrease in R observed after 10^{-4} M-ouabain. Furthermore, it appears from Fig. 8 that these effects were completely reversible; however, the p.d. showed a transient further depolarization immediately after return to control conditions and might even become serosa positive before it returned to the control level.

DISCUSSION

The present investigation confirms results from other isosmotic transporting epithelia in showing that net fluid transport by rabbit gall-bladder can be inhibited by ETCA. It further extends the evidence of marked differences in the mode of action of ouabain and ETCA with new observations pertinent to current attempts to distinguish between the various models of transepithelial transport mechanisms and to clarify the origin of the transepithelial p.d.

The requirement of high concentrations of ouabain (higher than 5×10^{-4} M; see Fig. 1*A*) applied from the serosal side (see Fig. 2) in order to abolish gall-bladder net fluid transport is in agreement with previous observations (Diamond, 1962; Dietschy, 1964; Martin & Diamond, 1966; Sullivan & Berndt, 1973) and with the localization of the ouabain sensitive ATPase (Skou, 1965) in the serosa-facing cell membrane

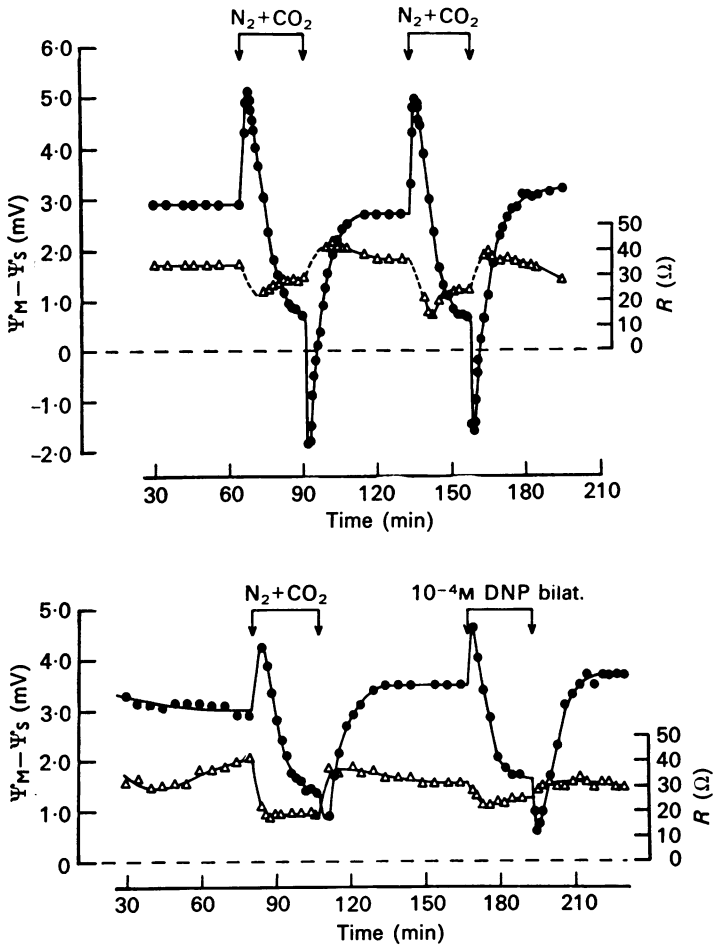

Fig. 8. Records of two experiments demonstrating the effects of substitution of 96 % O_2+4 % CO_2 by 96 % N_2+4 % CO_2 and 10^{-4} M-2,4-DNP on trans-epithelial potential difference (●) and resistance (△).

(Kaye, Wheeler, Whitlock & Lane, 1966). The full dose–response curve shown in Fig. 1*A*, however, demonstrates that the fluid transport is sensitive to ouabain down to concentrations of about 5×10^{-7} M.

In contrast to ouabain, ETCA inhibited gall-bladder fluid transport preferentially from the mucosal side (see Fig. 3), in close agreement with its action primarily from the luminal side in other salt and water transporting epithelia in which the Na-K-ATPase is also localized in the baso-lateral cell membrane (Chez, Horger & Schultz, 1969; Burg & Green, 1973). This site of action of ETCA argues against an effect

caused primarily by interference with the Na-K-ATPase. However, ETCA has been demonstrated to inhibit Na-K-ATPase activity of microsomal preparations isolated from rabbit gall-bladder (van Os & Slegers, 1970); but a comparison of the dose–response curve for the inhibition of rabbit gall-bladder fluid transport by ETCA (this investigation) with that for the inhibition by ETCA of gall-bladder microsomal Na-K-ATPase from the same animal species (van Os & Slegers, 1970) showed that the fluid transport was inhibited by concentrations ($ID_{50} = 2{\cdot}3 \times 10^{-4}$ M) much lower than those required for the inhibition of the Na-K-ATPase ($ID_{50} = 3{\cdot}5 \times 10^{-3}$ M) (cf. Fig. 1*B*). Thus, the concentrations of ETCA used in the present investigation would seem to be too low to inhibit the Na-K-ATPase. Similarly infusion of ETCA into the renal artery of dogs (Inagaki, Martinez-Maldonado & Schwartz, 1973) or intravenously in rats (Ebel, Ehrich, de Santo & Doerken, 1972) in sufficient amounts to produce profuse diuresis and natriuresis did not inhibit the Na-K-ATPase activity. Finally, the difference between the effects of ETCA and of ouabain on the p.d. and resistance indicates indirectly that the inhibition of solute and water transfer by ETCA was not mediated by interference with the Na-K-ATPase.

Inhibition of gall-bladder net fluid transport by ETCA does not either seem to be mediated by inhibition of oxidative metabolism, since mucosal application of 10^{-3} M-ETCA caused a 85% inhibition of net fluid transport rate by inhibitors of oxidative metabolism like N_2 or 2,4-DNP was accompanied by a marked depression of p.d., in agreement with previous observations (Sullivan & Berndt, 1973).

The conclusion that ETCA seems to inhibit isosmotic fluid transport without interfering with Na-K-ATPase (or oxidative metabolism) does not itself exclude the possibility that a Na-K pump located at the baso-lateral membrane may be the primary driving mechanism for transepithelial transport of NaCl and water. In fact the similarity between ID_{50} of ouabain on net fluid transport ($2{\cdot}5 \times 10^{-6}$ M; present investigation; Fig. 1*A*, continuous line) and ID_{50} of ouabain on Na-K-ATPase in gall-bladders of albino rabbits (also about $2{\cdot}5 \times 10^{-6}$ M: van Os & Slegers, 1970; Fig. 1*A*, dashed line), and a correlation between spontaneous fluid transport rates and Na-K-ATPase activity (van Os & Slegers, 1971) would appear consistent with this proposal. However, the following observations from the present investigation conflict with this possibility. In Fig. 1*A* it is seen that concentrations of ouabain higher than 10^{-3} M are necessary for complete blocking of fluid transport, whereas only 10^{-4} M-ouabain will block the Na-K-ATPase completely (van Os & Slegers, 1970). Further, the present study demonstrates that the maintenance of the mucosa positive p.d. in rabbit gall-bladders is dependent on the activity of the Na-K- pump (or high cellular K/Na-concentration ratio) since both ouabain and metabolic inhibition decrease the p.d. However, it was observed that the drop in p.d. caused by a 90 min treatment with 10^{-5} M ouabain was completely reversible (Fig. 5*B*), suggesting complete recovery of the Na-K pump, while net fluid transport inhibition with even lower concentrations was not. These observations demonstrate a functional separation of the activity of the Na-K pump from the transepithelial fluid transport process. Thus, the present results obtained from ouabain treatment support previous evidence suggesting that the Na-K pump is not the driving mechanism for net NaCl and water transport (Frederiksen & Leyssac, 1969). Rather the Na-K pump may influence fluid transport indirectly by maintaining low cellular Na concentration and

high K concentration, which might be a prerequisite for the optimal function of a transepithelial transport process as suggested by Frederiksen & Leyssac (1969), and more directly shown by Cremaschi, Hénin & Calvi (1971). Thus, a correlation between Na-K ATPase activities and fluid transport rates would be anticipated.

It should be emphasized that, although ETCA inhibits the transepithelial isosmotic fluid transport by ways other than the Na-K-ATPase, and not by inhibition of oxidative metabolism, this by no means indicates that ETCA is a specific inhibitor of the fluid transport process. Thus, ETCA is known as an inhibitor of ubiquitous protein-bound SH-groups (Komorn & Cafruny, 1965). However, an effect of ETCA primarily from the mucosal side supports our previous conclusion that the active step of the fluid transport process is located in the luminal end of the cells (Frederiksen & Leyssac, 1969).

In gall-bladders, as in other low resistance epithelia, transepithelial resistance mainly represents the resistance of the paracellular shunt pathway (the tight junction pathway) through which the major part of passive transepithelial ion fluxes take place (Barry *et al.* 1971; Frömter, 1972; Machen *et al.* 1972). Resistances of the cell membranes are at least one order of magnitude higher (Frömter, 1972). Consequently any major change in transepithelial resistance will reflect a change in the resistance of the shunt pathway and, thus, should be accompanied by changes in passive (S-to-M) ion fluxes through the epithelium. However, the data obtained from the present flux measurements showed that, although calculated passive Na^+-fluxes changed in the same direction as the changes in conductance (reciprocal resistance), the changes in the passive Na^+-fluxes induced by ouabain and ETCA (see Fig. 7) cannot be quantitatively accounted for by the drug-induced changes in resistance. This would indicate that part of the passive S-to-M Na^+-flux may not go through the paracellular shunt pathway but rather takes a cellular route. However, further studies involving direct measurements of S-to-M Na^+-fluxes would be required to elucidate this problem.

The results indicate that high concentrations of ouabain and of ETCA when applied from the serosal side may affect the tight junction resistance. Similar effects of the drugs have been demonstrated in other epithelia (Lutz, Cardinal & Burg, 1973; Herrera, 1975). The nature of the recorded changes, however, remains unexplained.

The mechanisms involved in the generation of small transepithelial p.d.s across low resistance epithelia are not fully understood. Machen & Diamond (1969) suggested that NaCl diffusion from a hypertonic lateral intercellular space (the hypertonicity being created by a neutral NaCl pump (Diamond & Bossert, 1967)) back to the mucosal side through cation selective channels, probably in the tight junctions (Barry *et al.* 1971; Frömter, 1972), would create the mucosa positive p.d. in the rabbit gall-bladder. An electrogenic anion pump analogous to the one proposed by Frömter & Gessner (1974) for the rat proximal tubule would also create a mucosa positive p.d.

Both these models predict that NaCl and thus isosmotic net fluid transport rate and transepithelial p.d. should be directly correlated at constant resistance. However, some of the present results contradict this prediction. Thus, mucosal application of ETCA (10^{-3} M) depressed net fluid transport rate by 85% without depressing the p.d.; in fact the p.d. increased slightly without any change in resistance (Fig. 6*A*). This lack of correlation between fluid transport rate and transepithelial p.d. at the

same time argues against the concept of hypertonicity in the lateral intercellular compartment under conditions of isosmotic absorption and supports the concept of an electroneutral transport process.

A more likely explanation for the origin of the transepithelial p.d., based on recent p.d. measurements in gall-bladders by Reuss & Finn (1975*a*, *b*), Hénin & Cremaschi (1975) and van Os & Slegers (1975) would be that the transepithelial p.d. arises as a difference between ion (mainly K) diffusion p.d.s across the two opposing cell membranes, but modified by the shunt pathway. If this be the case, then any treatment which tends to eliminate ion concentration differences across the cell membranes – e.g. that with ouabain (Fig. 4) or metabolic inhibitors (Fig. 8) – would abolish the transepithelial p.d. as was observed.

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