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THE ACTION OF OUABAIN UPON CHLORIDE SECRETION IN CULTURED MDCK EPITHELIUM

N.L.SIMMONS

Department of Physiology, University of St. Andrews, St. Andrews, Fife, KY16 9TS (U.K.)

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Net Na^+ loss from confluent monolayers of cultured epithelial cells grown on plastic petri dishes into choline chloride is consistent with loss from two separate pools ($t_{1/2}$ 2.4 and 43.7 min). Tissue K^+ is lost with a single time constant ($t_{1/2}$ 76.9 min). Since tissue equilibration of [^{14}C]inulin is also rapid ($t_{1/2}$ approx. 1 min), it is inferred that the fast component of Na^+ loss comprises loss from extracellular pools, whereas the slow component comprises intracellular loss. By washing extracellular cations from cell monolayers and directly measuring cell numbers and volumes by Coulter Counter, intracellular Na^+ and K^+ concentrations were estimated to be 16 ± 2 (S.E.) and 151 ± 2 (S.E.) mM. Ouabain at high concentrations ($1 \cdot 10^{-5}$ to $1 \cdot 10^{-3}$ M) raised intracellular Na^+, and lowered intracellular K^+. The $t_{1/2}$ for cation equilibration with the external medium was approx. 70 min (+ ouabain). Ouabain inhibited ATP-stimulated Cl^- secretion by epithelial MDCK monolayers mounted in Ussing chambers. The inhibition was time-dependent and consistent with dissipation of intracellular cation gradients. The ATP-dependent increase in monolayer conductance, observed in control tissues, was largely unaffected by ouabain.

Introduction

Cl^- secretion by various epithelia (pancreas [1], bird salt glands [2], teleost gills [3,4], shark rectal gland [5] and mammalian small intestine treated with theophylline or choleragen [6,7]) is inhibited by (Na^+, K^+)-pump blockade using ouabain [1–7]. This action of ouabain has been invoked to advance a central role for the ($Na^+ + K^+$)-ATPase in mechanisms of Cl^- secretion usually by linking Cl^- accumulation within the epithelial cell to the Na ion gradient [5,6], or to the generation of a hypertonic NaCl solution contained within the lateral space [7–9]. Alternative explanations are possible, for instance an anion pump directly linked to the ($Na^+ + K^+$)-ATPase which serves as the basic energy transducer has been envisaged for net iodide fluxes in choroid plexus [10]. Inhibition of Cl^- secretion by ouabain may, of course, simply represent a non-specific effect of Na pump inhibition.

To differentiate between the various possibilities requires that a continuous measure of Cl^- secretion be available and that this be then correlated to the dissipation of the various cation gradients. Previously [11,12], the effect of exogenous ATP upon transepithelial ion fluxes in the cultured MDCK epithelium was examined. ATP was found to stimulate a rheogenic Cl^- secretion and there was a good correlation between the short-circuit current and net Cl^- movement under various conditions including inhibition of Cl^- secretion by furosemide [11,12].

The present experiments were designed to determine the effect of ATP upon intracellular cation contents; to determine the effect of ouabain upon the dissipation of intracellular cation gradients, and to attempt to correlate such changes with Cl^- secretion.

Methods

(i) Cell culture. MDCK dog kidney cells were obtained from Flow Laboratories (Irvine, Scotland) at 60 serial passages. Growth of MDCK cells and

preparation of epithelial monolayers by high-density seeding [15] upon 0.22 μm pore-diameter millipore filters has been described previously [13,14]. Confluent cell monolayers were also prepared upon plastic petri dishes (Sterilin, Teddington, U.K.).

(ii) Electrical measurements. Cell monolayers were mounted in Ussing chambers (0.75 cm window radius, 1.76 cm^2 exposed monolayers) at 37°C for measurement of the potential difference (p.d.) and resistance as in Refs. 7 and 16. An automatic voltage clamp device was used to continuously voltage clamp the epithelial monolayers [7,16]. Conductance determinations were made by passing 2 μA hyperpolarizing current pulses across the cell monolayer, alternatively square-wave excursions (± 3 mV) of the voltage clamp were continuously made and the current monitored.

(iii) Solutions. The standard experimental medium was a modified Krebs' solution (pH 7.4) containing 137 mM NaCl, 5.4 mM KCl, 2.8 mM $CaCl_2$, 1.2 mM $MgSO_4$, 0.3 mM NaH_2PO_4, 0.4 mM KH_2PO_4, 12 mM HCl, 14 mM Tris base, 10 mM glucose, 2 mM glutamine, 2 mM sodium pyruvate, 2% v/v foetal bovine serum and amino acids for Eagles medium (Flow Laboratories).

The wash solution used for determining the net loss of Na^+, K^+ from cells grown upon plastic petri dishes contained 150 mM choline chloride, 14 mM Tris base and 12 mM HCl (pH 7.4).

(iv) Unidirectional Cl^- ion flux measurements. ^{36}Cl-tracer (Radiochemical Centre, Amersham, U.K.) was used to determine the unidirectional trans-epithelial Cl^- fluxes. Measurements were made on the same cell monolayer using a randomised order to avoid error due to monolayer deterioration [12]. For ouabain-treated tissue flux determinations were made upon adjacent cell monolayers after a 20-min preincubation in ouabain-containing solutions as a progressive increase in monolayer conductance plus ouabain is observed [17].

(v) Procedure for wash-out experiments. Confluent cell monolayers grown upon petri dishes were washed quickly (three times with ice-cold choline chloride wash solution (total time approx. 5 s). 5 cm^3 of choline wash-solution at room temperature 21–22°C) was then placed in each petri dish for various time periods. At the end of the time period the choline wash solution was sucked off the plate and 3 cm^3 of double-glass distilled water added to extract the remaining cellular cations. Extraction was for 3 h at room temperature. The $[Na^+]$ and $[K^+]$ of aliquots were determined by flame photometry.

(vi) Use of extracellular space marker. The extracellular space was determined from the distribution ratio of ^{14}C-labelled inulin. Inulin was used only in tracer quantities. Following incubation in [^{14}C]-inulin containing Krebs' solution cell monolayers upon petri dishes were washed quickly (three times) in ice-cold choline solution prior to extraction in distilled water for 3 h.

(vii) Radioisotope counting. ^{36}Cl and [^{14}C]-inulin activities were determined using a β-counter (Packard Instruments). Aqueous samples were counted in 10 cm^3 of scintillation cocktail (500 cm^3 toluene, 500 cm^3 Triton X-100, 50 cm^3 Scintol 2 (Koch-Light, Colnbrook, U.K.)).

(viii) Measurement of cell size/number. Electronic counting of cell number and cell sizing was performed on suspensions of MDCK cells using a Coulter Counter (model ZF) with Channelyser attachment [20]. Cell suspensions were made from monolayers of MDCK cells by treating them for 10–15 min in a ($Ca^{2+} + Mg^{2+}$)-free Earles balanced salt solution with 0.1% v/v trypsin solution (Flow Laboratories) with 0.1 mM EDTA, to detach the cells, then neutralising trypsin with complete growth media containing foetal serum. Gentle syringeing was used to finally disperse small cell clumps.

The method of preparing a cell suspension is thought not to affect cell volume compared to substrate-attached cells [21]. This was checked for MDCK cells by comparison of morphometric measurements of the major cell dimensions in living-substrate attached cells viewed with Normaski optics, with Coulter-measured cell volumes of the same cell cultures after trypsinisation. Accurate measurements of cell depth were not possible by light microscopy, so cell depth was measured on fixed araldite-embedded monolayers grown upon petri-perm dishes under identical growth conditions. Cell shrinkage in fixation and dehydration will most probably result in a 10–20% underestimate of cell depth. The cell volume measured by morphometry in three separate cell cultures was 2280, 2940 and 1572 μm^3 (assuming a cuboidal cell shape) whereas the Coulter cell volume was 2636, 2360 and 1717 μm^3, respectively. Within limits (of ±20%) cell volume is not affected by trypsinisation.

(ix) Chemicals. All chemicals were of Analar grade. ATP was the synthetic sodium salt from B.D.H. Chemicals, Poole, U.K. Ouabain was obtained from the Sigma Chemical Co., Poole, Dorset, U.K.

(x) Statistics. Variation in experimental results expressed as the mean ± S.E. Significance of differences between mean values was tested using Student's t-test (two-tailed for independent means).

Results

(i) Compartmental analysis of cellular cation content

Fig. 1 shows the wash out of cellular Na^+ and K^+ from confluent MDCK cell monolayers grown upon plastic petri dishes. Percentage loss is plotted semi-logarithmically against time. Net K^+ loss is best-fitted by a single exponential function with a $t_{1/2}$ of 76.9 ± 12.4 (S.D.) min. This result is consistent with the localisation of K^+ within a single intracellular pool. In contrast to net K^+ loss, net Na^+ loss is best-fitted by a double exponential function of time; the $t_{1/2}$ for the fast component is 2.7 ± 1.4 (S.D.) min, whilst the $t_{1/2}$ for the slow component is 43.7 ± 12.9 (S.D.) min. Since the $t_{1/2}$ for the slow Na^+ component is similar to that for K^+, this pool of Na is likely to represent intracellular Na. Extrapolation to zero time gives the original Na content of this pool; this is 12.6 ± 2.8 (S.E.) % of the original Na content. The rapidly washing component of Na^+ loss most probably represents loss from extracellular compartments. With confluent layers of MDCK cells these will comprise the lateral spaces, basal fluid, blister structures [14,15] and adherent apical fluid. Since it is likely that Na^+ loss from those compartments will be dissimilar, the $t_{1/2}$ for the fast component of Na^+ loss is most probably a lumped parameter. The bi-exponential nature of Na^+ loss is similar to that observed for intact in vitro epithelial tissue such as mammalian small intestine [18].

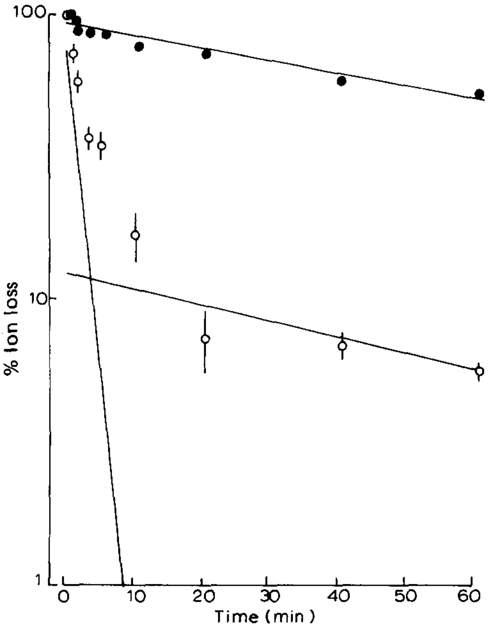

Fig. 1. Loss of Na (○) and K (●) from confluent monolayers of MDCK cells grown upon plastic petri dishes into Tris-buffered choline chloride at 20–22°C. Percentage loss is plotted semi-logarithmically against time in the wash media. Each point is the mean ± S.E. of three separate determinations. Solid lines indicate the least-square regression lines for the two components of net Na^+ loss, and the single component of net K^+ loss.

(ii) Estimation of the extracellular space

Uptake of tracer [^{14}C]inulin by confluent monolayers of MDCK cells is shown in Fig. 2. Equilibration of [^{14}C]inulin is observed by 10 min, and the half-time for equilibration is 1–2 min. Although the use of extracellular space markers is subject to ambiguities relating to molecular size and cellular uptake [19], this present result supports the contention that the rapidly washing Na pool (Fig. 1) is loss of Na from the extracellular space.

The extracellular space calculated from the equilibrium inulin values (at 60 min) is 2.3 μl per petri dish. By using trypsin-EDTA containing buffers it is possible to detach cells from the plastic plate into suspension (Methods). Counting cell numbers and cell volume using a Coulter Counter/Channelyser is then possible (Methods); for four plates identical to those used for [^{14}C]inulin determinations, the total cell volume is 5.2 μl, assuming that cell-volume is unaffected by trypsin-treatment (see Methods). The inulin space therefore represents 31% of the total volume of the intact monolayer, similar to natural epithelia such as mammalian small intestine [18].

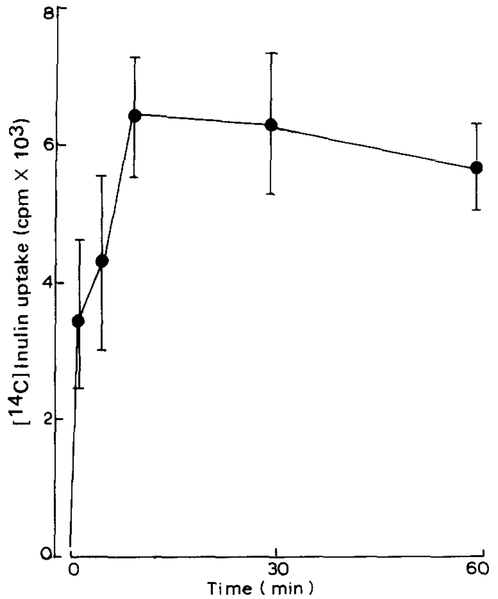

Fig. 2. Uptake of tracer [^{14}C]inulin by confluent monolayers of MDCK cells grown upon plastic petri dishes incubated in standard Krebs' medium at 37°C. Tissue was extracted for [^{14}C]inulin content following three rapid washes in ice-cold choline chloride medium (see Methods). Each point is the mean ± S.E. of three separate petri dishes. Solid line drawn by eye.

(iii) The effects of ouabain and exogenous ATP upon intracellular ion contents

Since the total Na^+ content of MDCK layers exists in two kinetically identified pools and since measurement of tissue mass is not possible by weighing of intact monolayers of cells, the following procedure was adopted for estimation of intracellular ion contents. Cell monolayers on petri dishes were pretreated with drug for appropriate times, rapidly washed (three times) in ice-cold choline medium, then incubated in 5 cm^3 of choline medium at room temperature for 10 min, after which period, the choline medium was discarded and the remaining Na^+ and K^+ content was extracted in distilled water in the usual manner. Though intracellular Na^+ and K^+ contents will, therefore, be underestimated by 14.6 and 8.7%, respectively, Na^+ estimations will not be subject to error from extracellular Na^+ contamination.

Cell numbers/volume were determined in parallel experiments by using the Coulter Counter following trypsin-EDTA treatment [20]; since this procedure involves incubations in non-experimental media (– drugs) for 10–15 min, ion contents are usually expressed relative to cell number (Fig. 3, Table I).

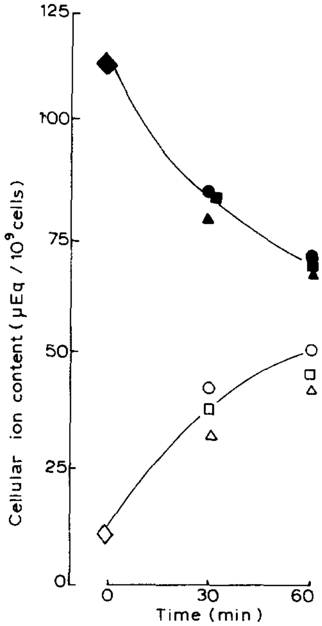

Fig. 3. Effect of three concentrations of ouabain (○, ● $1 \cdot 10^{-3}$ M; □, ■ $1 \cdot 10^{-4}$ M; △, ▲ $1 \cdot 10^{-5}$ M) upon the estimated intracellular Na (○, □, △) and K (●, ■, ▲) ion contents of confluent MDCK monolayers grown upon plastic petri dishes. Each point is the mean of three separate determinations except for control cells minus ouabain where $n = 5$. ◇, control Na^+ contents; ◆, control K^+ contents.

In control cells the estimated intracellular Na and K concentrations were 16 ± 2 ($n = 5$) and 151 ± 2 ($n = 5$) mM assuming that 20% of the measured Coulter cellular volume comprises non-osmotically active space (unpublished observations).

Ouabain at high concentrations (Fig. 3) causes a reciprocal fall in cellular K^+ content and gain of Na^+ which is consistent with the well known action of ouabain in inhibiting the (Na^+, K^+)-pump and supports the intracellular locations for cations inferred from the wash-out kinetics. The half-time for Na^+ and K^+ equilibration, plus ouabain is approx. 70 min, which reflects a low passive membrane permeability to these ions compared to other cultured cell-types such as HeLa and primary chick heart cell cultures [22]. By monitoring

TABLE I

Effect of 0.1 mM exogenous ATP upon the estimated intracellular Na and K cellular ion contents at times up to 20 min following ATP addition. Each time is the mean ± S.E. of five separate determinations.

Condition	Na^+ content (μM/10^9 cells)	K^+ content (μM/10^9 cells)
Control (t = 0)	25.4 ± 7.7	108.5 ± 2.3
+0.1 mM ATP		
t = 1 min	13.8 ± 1.5	86.9 ± 3.1
t = 2 min	11.5 ± 1.5	87.7 ± 1.5
t = 3 min	14.6 ± 2.3	84.6 ± 2.3
t = 6 min	13.8 ± 0.8	86.1 ± 1.5
t = 10 min	19.2 ± 1.5	93.8 ± 1.5
t = 20 min	15.3 ± 3.0	96.1 ± 0.8

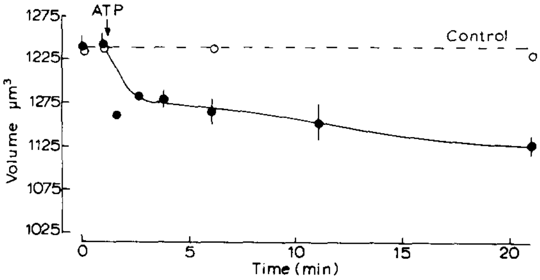

Fig. 4. Effect of 0.1 mM exogenous ATP upon cellular volume measured directly in cell suspensions (see Methods) in the standard Krebs' media using a Coulter Counter and Channelyser, at room temperature. Determinations were made at room tempeature. Each point is the mean of three separate determinations. Error bars are ±S.E. (where not shown, they lie within the points).

cell volume directly using the Coulter counter in an MDCK cell suspension obtained by trypsinisation, $1 \cdot 10^{-4}$ M ouabain was found to have no significant effect upon cell volume with incubations of up to 1 h (after 1 h incubation the control cell volume was 1229 ± 42 μm^3 compared to 1261 ± 26 μm^3 (n = 5) for ouabain-treated cells). The lack of cellular swelling plus ouabain is most likely to result from the presence of serum in the Krebs' solution [23], alternatively a ouabain-insensitive volume-regulating system may be present in MDCK cells.

Addition of 10^{-4} M exogenous ATP to cell monolayers results in a loss of intracellular K^+ content (Table I) in the first minute. This corresponds to the peak short circuit current (Fig. 5A). Intracellular Na content also falls but this effect is not significant ($P < 0.2$). The net loss of intracellular cation content is associated with a reduction in cell volume (Fig. 4) monitored in a separate experiment using a cell suspension obtained by trypsinisation.

(iv) The action of ouabain upon the ATP-stimulated Cl^- secretion

Addition of 0.1 mM exogenous ATP to the basal bathing solution of epithelial monolayers of MDCK cells mounted in Ussing chambers results in a prompt stimulation of the short circuit current (from 0.5 $\mu A \cdot cm^{-2}$ to a peak value of 19.8 $\mu A \cdot cm^{-2}$) and to an increased transmonolayer conductance (from 0.13 $mmho \cdot cm^{-2}$) to 0.78 $mmho \cdot cm^{-2}$) (Fig. 5A). These values are similar to those previously reported [11,12]. The increased short-circuit current plus ATP results from a net Cl^- secretion from basal to apical cell surfaces (Table II) [11,12]. Net Cl^- secretion plus ATP is primarily due to elevation of J^{Cl}_{b-a}, though both unidirectional Cl^- fluxes are elevated compared to controls (Table II) [11,12].

Preincubation of epithelial monolayers with ouabain applied to the basal bathing solution results in an increased transmonolayer conductance compared to controls (Table II, Fig. 5B and C) [17]. This increase in monolayer conductance is also evident in the bidirectional Cl^- fluxes which are elevated compared to controls minus ouabain (Table II). The isotopically measured Cl^- secretion plus ATP is abolished by ouabain (Table II). Though a small increase in the ATP dependent short circuit current plus ouabain is observed (Table II), this value is significantly reduced compared to values minus ouabain ($P < 0.001$). The action of ouabain on Cl^- secretion is, therefore, similar to that observed in epithelia such as mammalian small intestine [7].

The action of ATP was also tested electrically upon monolayers preincubated in ouabain for various times (Figs. 5 and 6). With $1 \cdot 10^{-4}$ M ouabain added to the basal bathing solution for 20 min monolayer conductance is raised to 0.75 $mmho \cdot cm^{-2}$ (Fig. 5B); the ATP-stimulated initial increase in the short-circuit current is similar to controls, thereafter there is a rapid decline towards prestimulation levels (Fig.

5B). No sustained increase in the short-circuit current similar to controls is observed. The ATP-dependent increase in monolayer conductance is maintained, however (Fig. 5B). With 60-min preincubation, monolayer conductance had increased to 0.9 mmho $\cdot$ cm^{-2} from an initial value of 0.28 mmho $\cdot$ cm^{-2}; the ATP-dependent increase in short-circuit current is markedly reduced, although an increased ATP-dependent transmonolayer conductance is still observed (Fig. 5C). The increased conductance plus ATP is not seen in grouped data (Table II) due to between monolayer variation in conductance. The magnitude of the conductance increase plus ATP in Fig. 5C is most probably reduced due to an increased paracellular conductance plus ouabain, although this is not certain.

The time-course of the effect of ouabain preincubation upon the ATP-dependent short-circuit current is shown in Fig. 6; a small though significant response remains up to 1 h. This result contrasts with the virtually instantaneous inactivation of the $(Na^+ + K^+)$-ATPase at these ouabain concentrations [24] (see also Discussion).

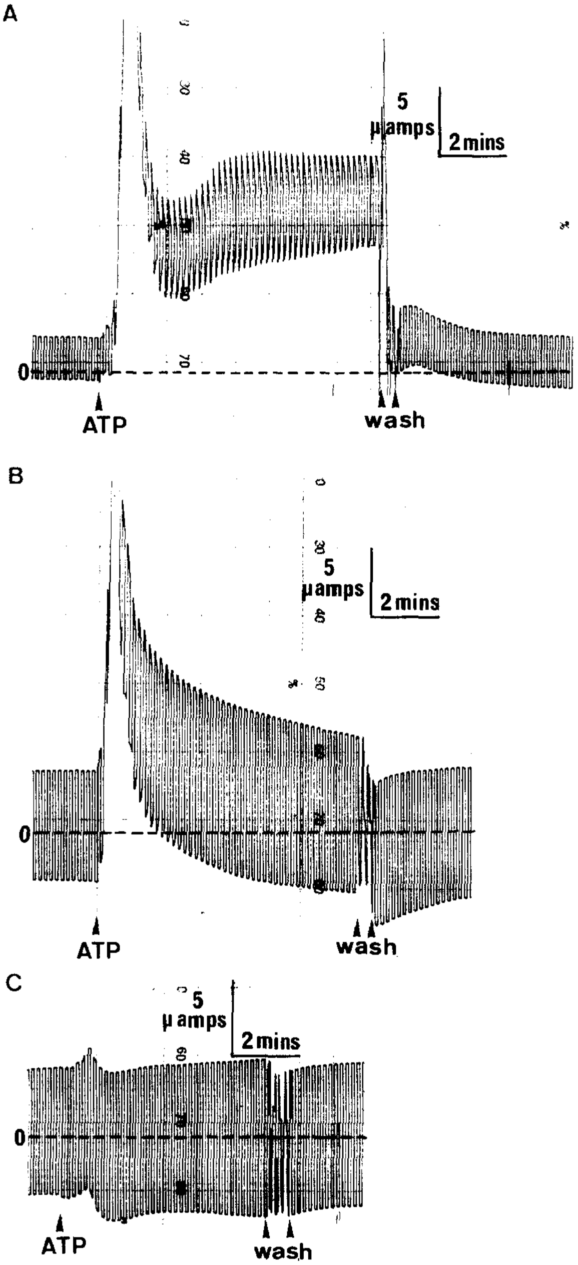

Fig. 5. Original records showing the effect of exogenous 0.1 mM ATP added to the basal bathing solution upon the short-circuit current maintained by MDCK monolayers clamped in Ussing chambers at 37°C in the standard Krebs' solution. The voltage clamp is pulsed by ±3 mV to provide a measure of tissue conductance. (A) Control media. (B) The effect of 0.1 mM ATP following a 20-min preincubation with basal ouabain ($5 \cdot 10^{-4}$ M). (C) The effect of 0.1 mM ATP following 60 min preincubation with basal ($5 \cdot 10^{-4}$ M ouabain (control data not shown).

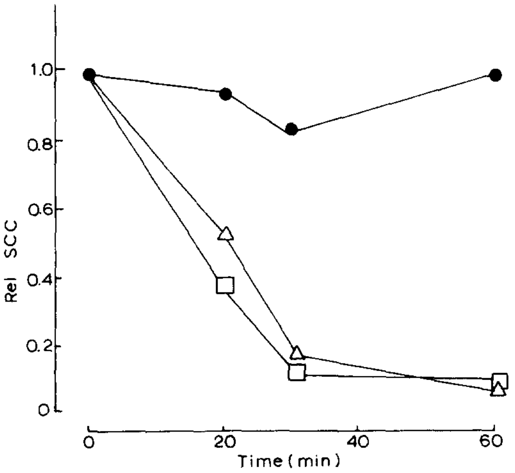

Fig. 6. The effect of ouabain incubations at $5 \cdot 10^{-4}$ M (□) and $5 \cdot 10^{-5}$ (△) for various times upon the relative increment in short-circuit (rel SSC) measured at 4 min following addition of exogenous ATP. Each point is the mean of two determinations, eighteen monolayers were used in total. ●, controls.

TABLE II

Bidirectional Cl^- fluxes across confluent monolayers of MDCK cells clamped to zero p.d. J_{a-b} denotes the flux of an ion from the apical to basal surface of the cell layer. J_{b-a} denotes the reverse flux. Values of short-circuit current and conductance are mean values recorded continually throughout the flux measurement period at 5-min intervals. Ouabain was added to the basal bathing solution 20 min prior to the flux measurement period and is present throughout the experiment; ATP was also added to the basal bathing solution.

	J (μmol · cm^{-2} · h^{-1}) (n = 6)			Short-circuit current (μmol · cm^{-2} · h^{-1}) (n = 12)	Conductance (mho · cm^{-2}) × 10^3 (n = 12)
	J_{a-b}	J_{b-a}	J_{net}		
Control	0.51 ± 0.15	0.59 ± 0.14	-0.08 ± 0.12	0.015 ± 0.003	0.44 ± 0.05
+1 · 10^{-4} M ATP	0.93 ± 0.07^{a}	1.39 ± 0.16^{b}	-0.46 ± 0.11^{a}	0.403 ± 0.041^{c}	1.05 ± 0.04^{c}
+1 · 10^{-4} M ouabain	2.00 ± 0.34	2.26 ± 0.43^{b}	-0.26 ± 0.39	0.006 ± 0.007	0.89 ± 0.09^{a}
+1 · 10^{-4} M ouabain +1 · 10^{-4} M ATP	1.90 ± 0.68	1.63 ± 0.43^{a}	$+0.27 \pm 0.64$	0.044 ± 0.011^{a}	1.03 ± 0.12^{b}

Significance differences from the control values:
$^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$.

Discussion

The cardiac glycoside, ouabain, is used in the present series of experiments as a specific inhibitor of the (Na^+, K^+)-pump. This has been established for MDCK cells by measurements of [^{3}H]ouabain binding to intact cells, transmembrane ^{86}Rb influxes [25,26] and by inhibition of ATP hydrolysis [13]. The action of ouabain upon intracellular cation contents (Results) is entirely consistent with a normal action of ouabain.

With 20 min preincubation, and with Cl^- flux measurements measured over 1-h periods, net Cl^- secretion plus ATP is clearly abolished by ouabain (Results). This action of ouabain is thus similar to that observed for other Cl^- secreting epithelia [1–7]. An additional parallel concerns the cellular location of the (Na^+ + K^+)-ATPase, as with shark rectal gland [27], fish gills [28] and mammalian small intestine [29], the Na^+ pump is located exclusively at the lateral pole of the cell in MDCK monolayers [25,26] whilst net Cl^- secretion proceeds from basal to apical surfaces.

For a known affinity constant of [^{3}H]ouabain binding (1 · 10^{-7} M), with equilibrium levels of binding at 1 · 10^{-7} M being observed at 20 min (both in K^+-free media) and assuming that 5.4 mM K^+ slows ouabain-pump association by 10-fold [24] compared to K^+-free media it is possible to estimate that maximal pump inhibition is observed at 120, 12 and 1.2 s for 1 · 10^{-5} M, 1 · 10^{-4} M and 1 · 10^{-3} M ouabain, respectively. (Na^+, K^+)-pump inhibition can thus be regarded as being virtually instantaneous with regard to the time-course of ouabain action upon the ATP-dependent short-circuit current in the present experiments. Since dissipation of the estimated intracellular cation gradients occurs relatively slowly compared to Na^+-pump inhibition, with a $t^{1/2}$ of approx. 70 min, a rapid action of ouabain may clearly be dissociated from a secondary effect upon cation gradients provided that a continuous measure of Cl^- secretion is available. Under various conditions, i.e. ATP, ATP plus furosemide, or elevated basal K^+ [11,12], the short-circuit current parallels net Cl^- secretion; in the present data with prolonged ouabain incubations, net Cl^- secretion plus ATP is abolished whilst the short-circuit current is markedly reduced compared to control values. Flux measurements are time-averages and thus the possibility remains that fluxes maintained for short periods reflect ion movements other than Cl^-. In this respect, the net loss of intracellular K^+ within the first minute of ATP stimulation may indicate that the initial rise in short-circuit current may be due to Cl^- movement across the apical membranes and K^+ loss across the basal-lateral membranes.

Preincubation of MDCK monolayers with ouabain at 5 · 10^{-4} M for 20 min has little effect upon the initial short-circuit current but inhibits the maintained levels plus ATP. This effect of ouabain upon the short circuit (Cl^-) current is thus consistent with a dissipation of pre-existing cation gradients. Even at 60 min ATP stimulates a measurable current (Fig. 6 results). The ATP-dependent increase in transepithelial conductance is observed at all times. In a previous paper, the increase in conductance was attributed to an increased apical membrane conductance [12]. The present result is similar to the increased Cl^- exchange observed across the mucosal membrane in rabbit small intestine plus theophylline which is insensitive to ouabain [7]. Since net loss of Na from the extracellular space is complete within 10 min (Results) the action of ouabain upon the ATP-dependent short-circuit current is inconsistent with the dissipation of a localised Na^+ concentration within the lateral space being primarily responsible for Cl^- secretion.

Non-specific effects of (Na^+, K^+)-pump inhibition are evident in the present results. As previously noted [17] ouabain decreases transmonolayer resistance, most probaly reflecting an increased paracellular conductance. Though the short-circuit current should be independent of transmonolayer conductance [30] non-specific effects of ouabain cannot thus be entirely excluded as a cause of ouabain action.

The present data is consistent with current models of Cl^- secretion [5,6,12], where a (Na^+/K^+)-coupled furosemide-sensitive cotransport across the basal cell membranes accumulates Cl^- above its electrochemical equilibrium; the role of the ($Na^+ + K^+$)-ATPase is then to drive Na^+ extrusion from the cell, maintaining normal cation gradients. Net transmonolayer Cl^- transport is then achieved by Cl^- diffusion across the apical membrane [5,6,12].

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