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CATECHOLAMINE-STIMULATION OF Cl^- SECRETION IN MDCK CELL EPITHELIUM

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Cultured epithelial monolayers of MDCK cells grown upon Millipore filter supports and mounted in Ussing chambers for transport studies respond to addition of $5 \cdot 10^{-7}$ M adrenalin from only the basal bathing solution by an increased short-circuit current, due both to an increased transmonolayer potential difference (basal solution electropositive) and an increased transmonolayer conductance. Measurement of tracer Na^+, K^+ and Cl^- fluxes demonstrate that the adrenalin-stimulated short-circuit current results primarily from basal to apical net Cl^- secretion. Half-maximal stimulation of the short-circuit current was observed at $(3.1 \pm 0.3) \cdot 10^{-8}$ M adrenalin; the order of potency of adrenergic agonists for short-circuit current stimulation was isoprenalin > adrenalin > noradrenalin, consistent with adrenalin action being mediated by a β-adrenergic receptor. The adrenalin-stimulated short-circuit current was sensitive to inhibition (75%) by basal additions of furosemide ($1 \cdot 10^{-4}$ M); phloretin inhibition (54%, 57%) was observed from both epithelial surfaces. Amiloride (10^{-4} M) and 4-acetamido-4-isothiocyanostilbene-2,2′-disulphonic acid (SITS) (10 μM) were ineffective as inhibitors of the adrenalin response. The increased short-circuit current was sensitive to replacement of medium Na^+ by choline (87%) and Tris (93%). Li^+ was a partially effective substitute cation for Na^+. NO_3^-, and isethionate were ineffective substitutes for Cl^- whereas Br^- was partially effective. Partial replacement of medium Na^+ by choline gave an upward-curving non-saturable dependence of the adrenalin-stimulated short-circuit current upon [Na]; partial replacement of Cl^- by NO_3^- in contrast gave a saturable increase with a $K_{1/2}$ of approx. 65 mM Cl^-.

Introduction

The actions of catecholamines upon epithelial tissue has attracted considerable interest. In frog [1,2] and rabbit cornea [3] adrenalin stimulates a net chloride secretion which is associated with an increased transepithelial conductance. Similar findings are reported for frog skin [4,5]. In renal tubules, the actions of catecholamines are less clear, it would appear that adrenergic stimulation results in changes in renal sodium handling, which are independent of changes in renal hemodynamics, although the exact site and mode of action remain unclear [6]. Morel et al. [7] have demonstrated a catecholamine sensitive adenylate cyclase located solely in the late distal convoluted tubule and the cortical collecting tubule of rabbit kidney which may mediate an (as yet) unclear physiological role in control of tubular transport parameters.

This paper describes the action of catecholamines upon the transepithelial ion transport properties of cultured monolayers of MDCK cells. MDCK cells are an established cell-line of renal origin [8] which, when grown upon permeable filter supports form confluent epithelial monolayers of differentiated morphology (apical brush border, apical cell-cell tight junctions and lateral spaces) [8,9,10]. They also retain a pattern of adenylate cyclase activity (vasopressin, prostaglandin E_1 and prostaglandin E_2 [11] and isoprenalin [12]) similar to distal/collecting tubules [7,13].

Abbreviations: s.c.c., short-circuit current, SITS, 4-acetamido-4′-isothiocyanostilbene-2,2′-disulphonic acid.

Our major finding is that the adrenalin-stimulated short-circuit current observed in MDCK cell epithelium results from a rheogenic chloride secretion mediated by a β-adrenergic receptor.

Some of the present results have appeared in abstract form [14].

Materials and Methods

Cell culture. MDCK cells were 60–72 serial passages (Strain I) [15]. Culture conditions and preparation of epithelial monolayers upon Millipore filters were as previously described [10] except that in some cases 10% foetal calf serum was replaced by 5% horse donor serum supplemented with 5% foetal calf serum. This change in growth media did not affect the properties of the epithelial monolayers.

Solutions and electrical measurements. Cell monolayers were mounted in Ussing chambers (0.75 cm window radius, 1.76 cm^2 exposed monolayer area), thermostatically controlled at 37°C for the measurement of potential difference (p.d.) and resistance.

An automatic voltage clamp [16] was connected to the chambers via matched calomel half cells (for potential measurement), Ag/AgCl half cells (for current passage) and saturated KCl salt bridges. The use of KCl salt bridges minimised error due to liquid junction potentials and prevented contamination of experimental solutions with AgCl. All potential differences (p.d.'s) are expressed as basal bathing solution positive. Resistance measurements under open circuit conditions were made by passing 2 μA hyperpolarizing current pulses across the cell monolayer and measuring the subsequent voltage deflection [10].

The experiments were carried out, unless otherwise stated, in a modified Krebs' solution 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 and 2% v/v donor horse serum.

An Na-free solution was prepared by replacing NaCl by either choline chloride, LiCl or Tris chloride, NaH_2PO_4 by KH_2PO_4 and normal serum by serum which had been dialysed against distilled water (50 X volume) at 4°C for 24 h.

A Cl-free solution was prepared in a similar manner, NaCl was replaced with either NaBr, NaSCN, sodium isethionate, NaI or $NaNO_3$, KCl by K_2SO_4, $CaCl_2$ by $CaSO_4$ and HCl by H_2SO_4, dialysed serum was used and isosmolarity was maintained by the addition of mannitol. In all experiments solutions were left ungassed due to the inclusion of serum [10].

Na^+-flux measurements. Bidirectional Na^+ fluxes were determined simultaneously upon the same cell monolayer voltage clamped to zero p.d. using ^{22}Na and ^{24}Na as tracers as previously described [10,16].

K^+-flux measurements. Bidirectional K^+ fluxes were determined upon the same voltage clamped monolayers simultaneously using ^{42}K and ^{86}Rb as tracers in a similar manner to the Na^+ fluxes [10]. The use of ^{86}Rb as a tracer for K^+ was validated in control experiments (Fig. 1) in which both ^{86}Rb and ^{42}K were used simultaneously to measure a single trans-monolayer K^+-flux. ^{42}K activity was determined by its emissions using a Packard Auto-gamma counter. ^{86}Rb activity was determined by its Cerenkow radiation after ^{42}K decay (10 half-lives).

Cl^--flux measurements Cl^--fluxes were measured using ^{36}Cl as tracer. Bidirectional fluxes were determined upon the same monolayer sequentially, in a randomised order to avoid error due to deterioration in the condition of the monolayers with time. Results were rejected if monolayer resistance fell by more

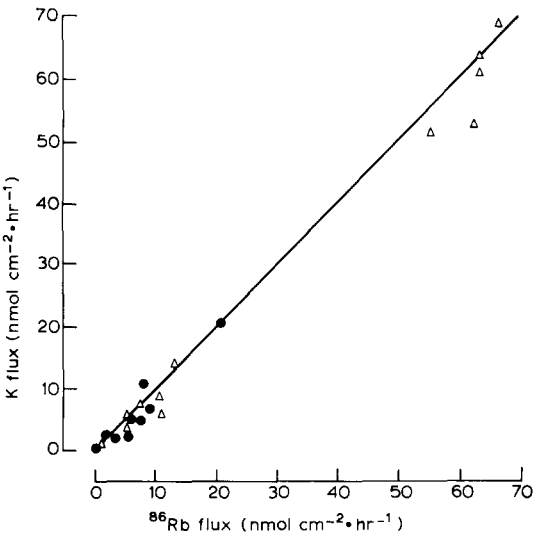

Fig 1. Relationship between the bidirectional K^+ fluxes simultaneously measured using either ^{42}K or ^{86}Rb. The solid line represents a 1 1 relationship. •, apical to basal K^+ flux, △, basal to apical K^+ flux.

than 5% over the experimental period.

Chemicals. All chemicals were of Analar grade or equivalent. Radiochemicals were obtained from the Radiochemical Centre, Amersham, U.K. ^{22}Na, ^{24}Na, ^{42}K and ^{86}Rb were obtained as aqueous solutions of the chloride salt, ^{36}Cl was obtained as the sodium salt. Adrenalin, noradrenalin and isoprenalin, as bitartrate salts, were obtained from Sigma, Poole, U.K. Salbutamol was from Allen and Hanburys, Macclesfield, U.K. SITS (4-acetimide-4′-isothiocyano-2,2′-stilbenedisulphonic acid) was from B.D.H. Chemicals, Poole, U.K. Amiloride was donated by Merck Sharp and Dohme, Hoddesdon, Herts., U.K. Furosemide was a gift of Dr. Dombey of the Hoechst Chemical Company, Milton Keynes, U.K. Phloretin was purchased from Sigma, Poole, U.K. Furosemide and phloretin were dissolved in 10^{-2} M Tris base.

Statistical methods. Variation in results is routinely expressed as the standard error of the mean (±S.E.). Tests for significances were made using a two-tailed Student's *t*-test (un-paired means solution). One-tailed tests were employed where appropriate. Affinity constants for dose response curves were determined by Probit analysis.

Results

(a) Actions of adrenalin upon the electrophysiological parameters of MDCK cell monolayers

The properties of epithelial monolayers of 60–72 serial passages has been described in previous publications [10,15]. Fig. 2 shows that addition of $5 \cdot 10^{-7}$ M adrenalin to the apical bathing solution elicits no significant change in the small basal short-circuit current (mean results: control = 2.66 ± 1.1 ($n = 6$), and plus adrenalin = 1.58 ± 0.7 ($n = 6$) $\mu A \cdot cm^{-2}$, $P > 0.5$). Addition of the same concentrations to the basal bathing solution results in a prompt increase in short circuit current to an initial peak of 28.2 ± 3.6 $\mu A \cdot cm^{-2}$ ($n = 6$) a lower maintained level of stimulation is then reached. The adrenalin-dependent short-circuit current is fully reversed by repeated washing (Fig. 2). Re-application of an equi-molar concentration after 15 min results in an identical response (data not included). The asymmetric nature of the response to adrenalin suggests an asymmetric distribution of adrenergic receptors to the basal-lateral membranes of MDCK cells, access to these sites from the apical bathing solution being limited by the high trans-epithelial resistance.

Adrenalin stimulation of short circuit current is dose dependent, half-maximal stimulation occurring at $(3.11 \pm 0.3) \cdot 10^{8}$ M (Fig. 3) similar to that reported for in vitro tissue preparations such as chick rectal smooth muscle [17] and human lung [18]. Half-maximal stimulation of the short-circuit current by isoprenalin and noradrenalin was observed at $(1.5 \pm 0.2) \cdot 10^{-8}$ M and $(6.0 \pm 0.2) \cdot 10^{-7}$ M, respectively; this gives a potency series for agonists of isoprenalin > adrenalin > noradrenalin, consistent with

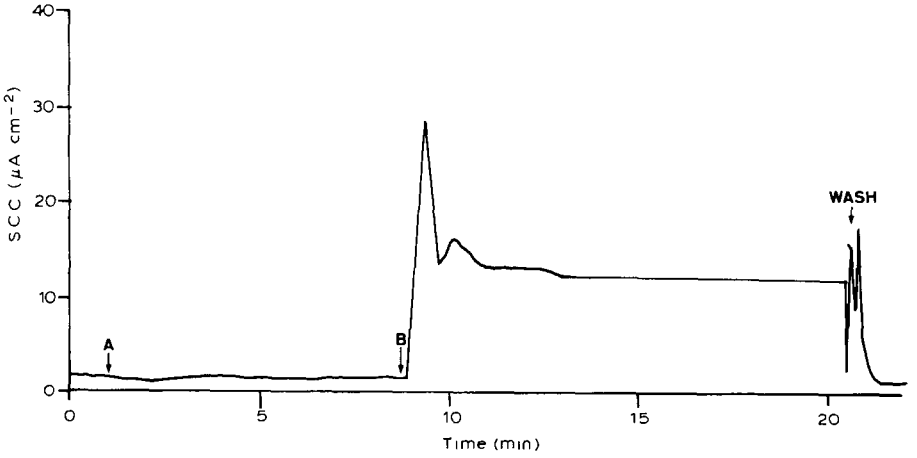

Fig. 2. Effect of addition of $5 \cdot 10^{-7}$ M adrenalin to the apical (A) and to the basal (B) bathing solutions upon the short-circuit current maintained by the epithelial monolayer. The increased short-circuit current is rapidly reversed by repeated washing. The basal short-circuit current is 1 $\mu A \cdot cm^{-2}$. Epithelial resistance prior to adrenalin stimulation was 3.2 kΩ cm^2.

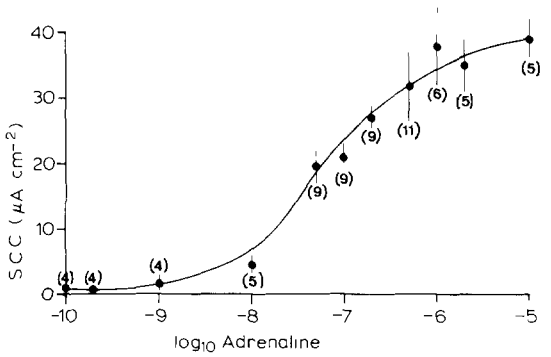

Fig. 3 Log-dose response curve for the action of adrenalin upon the short-circuit current response of MDCK cell monolayers Figures in parentheses are the number of observations at each concentration of adrenalin. Errors are expressed as ±S E The half maximal stimulation of short-circuit current was (3 1 ± 0 3) · 10^{-8} M

adrenalin stimulation of the short-circuit current resulting from interaction with a β-adrenergic receptor [19].

The adrenalin-dependent short circuit is a result of both an increased trans-monolayer potential difference and a decreased trans-monolayer resistance. The peak change in resistance occurred 1 min after addition of adrenalin to the basal bathing solution trans-monolayer potential rose from a resting value of 1.1 ± 0.5 ($n = 6$) to 24.4 ± 1.8 ($n = 6$) mV ($P < 0.001$), whilst trans-monolayer resistance fell from 1.8 ± 0.3 ($n = 6$) to 1.3 ± 0.2 ($n = 6$) kΩ · cm^2 ($P < 0.005$, for paired values, Student's one-tailed t-test).

(b) The effect of adrenalin upon the transepithelial Na^+, K^+ and Cl^- fluxes

As reported previously [10,20] the net fluxes of Na^+ and Cl^- do not differ significantly from zero ($P > 0.5$, $P > 0.5$, respectively) in control epithelial layers, in accord with the small current flux equivalent recorded over the flux measurement period (Table I). Additionally, there is no significant net K^+ flux ($P > 0.5$, Table I).

2 μM adrenalin causes a significant increase in the short-circuit current, averaged over the flux-measurement period for each of the three experimental series (Table I). Net Na^+ flux was slightly increased by adrenalin, but this was not significant compared to control data and was not of sufficient magnitude to explain the increased short-circuit current (Table I). No change in net K^+ influx was observed with adrenalin compared to controls (Table I) In contrast, adrenalin stimulates a significant net secretion of Cl^- from the basal to apical bathing solution which is of sufficient magnitude to account for the observed increase in short-circuit current in the presence of adrenalin (Table I). A similar adrenalin-stimulated

TABLE I

SUMMARY OF THE TRANSEPITHELIAL Na^+, K^+ AND Cl^- FLUXES ACROSS MDCK CELL MONOLAYERS VOLTAGE CLAMPED TO ZERO p.d

$J_{A\text{-}B}$ denotes the flux of an ion from the apical to basal surfaces of the cell layer. $J_{B\text{-}A}$ denotes the reverse flux. Values of short-circuit current are mean values recorded continually throughout the flux measurement period at 5-min intervals.

Ion	Adrenalin (μM)	N	Flux (μmol cm^{-2} h^{-1})			scc (μmol cm^{-2} h^{-1})
			$J_{A\text{-}B}$	$J_{B\text{-}A}$	J_{NET}	
Na^+, 137 mM	0	11	1.28 ± 0 07	1 16 ± 0.03	0 14 ± 0.14	0 03 ± 0 01
	2	11	0 69 ± 0.14 [c]	0 54 ± 0 10 [c]	0.18 ± 0 07 [a]	0 38 ± 0 06 [c]
K^+, 5 4 mM	0	13	0 21 ± 0.09	0.16 ± 0.06	0.04 ± 0.04	0 04 ± 0 01
	2	11	0 14 ± 0.05 [a]	0.11 ± 0.04 [a]	0 03 ± 0 02 [a]	0.53 ± 0.11 [c]
Cl^-, 160 mM	0	7	0 82 ± 0.24	0.74 ± 0.34	0 08 ± 0.16	0 04 ± 0 01
	2	11	1.29 ± 0.15	1.75 ± 0 24 [b]	−0.46 ± 0 06 [b]	0 44 ± 0.05 [c]

[a] Non significant
[b] $P < 0.02$
[c] $P < 0$ 01

chloride secretion has been reported in cornea [1–3], trachea [21] and frog skin [4,5]. The reduction in the bidirectional Na^+ fluxes plus adrenalin (Table I) suggests that the net Cl^- secretion is transcellular rather than paracellular, since an increased paracellular permeability would be reflected in increased exchange fluxes of both Na^+ and Cl^-.

(c) The effect of ion replacements upon the adrenalin-dependent short-circuit current

Fig. 4 shows that there is a saturable relationship between the magnitude of the adrenalin-dependent short-circuit current and bathing solution Cl^- concentration (Cl^- replaced isosmotically by nitrate) over the range 20–180 mM, K_m 65 mM Cl. In addition, a series of anion substitutions was tested for their ability to support the adrenalin-dependent short-circuit current (Table II). SCN^-, isethionate, and NO_3^- were ineffective replacements for Cl^- whereas Br^- was able to act as a partial replacement for Cl^-. The relative potency of anions for their ability to support adrenalin-stimulated chloride secretion was therefore $Cl^- > Br^- \gg SCN^- >$ isethionate$^- > NO_3^-$. The dependence of the adrenalin-stimulated short-circuit current upon medium Cl^- is similar to that previously reported for

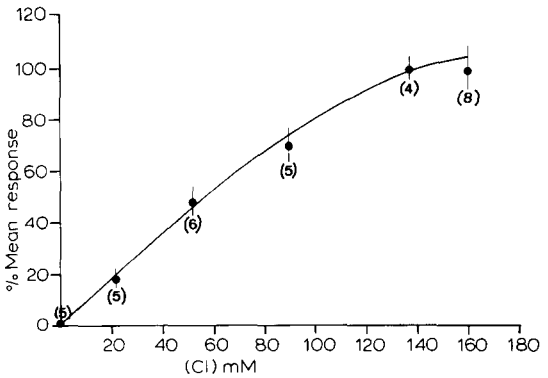

Fig. 4. The effect of varying the Cl^- concentration of the media upon the short-circuit current response to 5 10^{-7} M adrenalin. Figures in parentheses are the number of separate determinations. Errors are expressed as ±S.E. Epithelial monolayers were preincubated for 10 min in the appropriate Krebs' solution before addition of adrenalin.

TABLE II

THE EFFECT OF ION REPLACEMENT UPON THE SHORT–CIRCUIT CURRENT RESPONSE TO ADRENALIN

In all cases the monolayers were preincubated for 10 min prior to the addition of adrenalin Unless otherwise stated, both bathing solutions were replaced and the adrenalin concentration was 2 10^{-7} M. In the experiments where either the apical or basal bathing solutions were replaced the response is to 5 · 10^{-7} M adrenalin The adrenalin-dependent increase in short-circuit current was measured 1 min following adrenalin addition. Figures in parentheses are the number of separate observations Errors are expressed as ± S.E N.S., non significant

Ion replacement	s.c.c. ($\mu A \cdot cm^{-2}$)		*P* value of difference
	Control response	Test response	
Br^-	23.2 ± 1.6 (4)	13.2 ± 1.9 (4)	N S.
SCN^-	20 6 ± 2.6 (5)	2.9 ± 0.4 (5)	<0 01
I^-	23 2 ± 1.7 (15)	2 2 ± 0.4 (9)	<0.01
Isethionate	21 4 ± 1 7 (6)	0 8 ± 0 03 (6)	<0.01
NO_3^-	22 2 ± 3 8 (5)	No response (5)	<0 001
Li^+	28.5 ± 2 9 (6)	14.9 ± 2 1 (6)	<0 01
Tris	23.6 ± 3.8 (5)	1.8 ± 1 0 (4)	<0 01
Choline	28.5 ± 4.5 (9)	3 7 ± 1 1 (8)	<0 01
Choline (apical)	41.4 ± 6.8 (6)	39.4 ± 3.8 (6)	N.S.
Choline (basal)	35.8 ± 5.2 (7)	6.4 ± 1 6 (7)	<0.01
Isethionate (apical)	28 2 ± 3.5 (7)	36.6 ± 4 5 (6)	N S
Isethionate (basal)	28.2 ± 3 5 (6)	6.5 ± 0.9 (6)	<0.01

ATP-stimulated Cl^- secretion [20]. Replacement of medium Na^+ by Li^+, Tris or choline reduces the adrenalin-dependent short-circuit current. Li^+, however, is a partial substitute for Na^+ (Table II). The marked reduction in short-circuit current in choline medium observed here for adrenalin, is dissimilar to the effect of Na^+ replacement by choline for ATP-stimulated Cl^- secretion in MDCK cell epithelia [20]. 13–18% of the adrenalin-stimulated short-circuit current is Na^+ independent with both apical and basal choline replacements (Fig. 5, Table II).

Partial replacement of medium Na^+ by choline on the adrenalin-dependent short-circuit current is shown in Fig. 5, there is a non-saturating upwards curving dependence upon medium Na^+.

For all ionic replacement (Na^+, Cl^-) the adrenalin-stimulated short-circuit current is recorded after 1 min, since the short-circuit current then declines to lower values it was important to establish that similar data could be obtained for plateau values of s.c.c. at 5 min after adrenalin stimulation. For Na^+-free solutions the s.c.c. at 1 min was reduced from 35.8 ± 5.2 $\mu A \cdot cm^{-2}$ to 6.4 ± 1.6 $\mu A \cdot cm^{-2}$ ($n = 7$), an 83% reduction, whereas at 5 min after adrenalin stimulation the s.c.c. was reduced from 9.7 $\mu A \cdot cm^{-2}$ to 1.2 ± 0 3 $\mu A \cdot cm^{-2}$ ($n = 7$) an 86% reduction. Similarly for Cl^- substitutions with isethionate, the s.c.c.

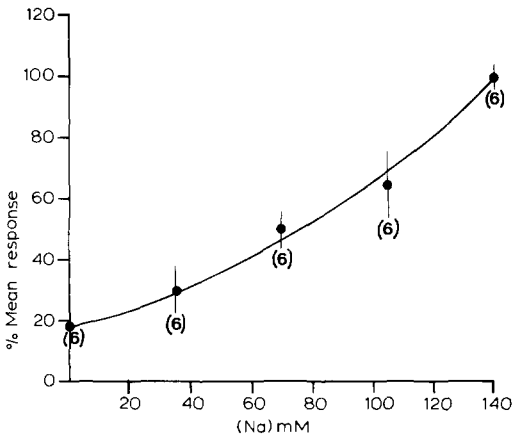

Fig 5. The effect of varying the Na^+ concentration obtained by isosmotic substitution of NaCl with choline chloride upon the short-circuit current response to $5 \cdot 10^{-7}$ M adrenalin. Figures in parentheses are the number of separate determinations. Errors bars are ±S.E Epithelial monolayers were incubated for 10 min in the appropriate Krebs' solution prior to the addition of adrenalin.

is reduced from 28.9 ± 4.1 ($n = 5$) $\mu A \cdot cm^{-2}$ to 6.8 ± 1.2 $\mu A \cdot cm^{-2}$ at 1 min after adrenalin stimulation, compared to a reduction from 7.9 ± 1.5 $\mu A \cdot cm^{-2}$ ($n = 5$) to 0.9 ± 0.3 $\mu A \cdot cm^{-2}$, 5 min after adrenalin stimulation.

In frog skin it has been suggested that a component of the adrenalin-dependent short-circuit current is due to an Na^+ reabsorption across the apical membrane [5]. To investigate whether a small component of the s.c.c. plus adrenalin is due to Na^+ absorption in MDCK cell monolayers we investigated the effect of replacing either the apical or basal bathing solution Na^+ by choline. This experiment is possible since the bidirectional Na^+ permeabilities are low (Table I) so that Na^+ diffusion across the monolayer is negligible over the experimental period. This was confirmed by flame photometry subsequent to the experimental period. Preincubation in experimental solutions was for 10 min, followed by a final solution change to ensure equilibration of extracellular space. An Na^+-free apical bathing solution reduced the short-circuit current reponse to adrenalin only slightly (Table II) and this reduction was not significant. An Na^+-free basal bathing solution in contrast results in a large (>80%) reduction in the adrenalin-stimulated short-circuit current (Table III), a significant adrenalin-stimulated short-circuit current, is, however, still observed. Similarly replacement of the apical bathing solution Cl^- by isethionate (Table III) was without effect whereas replacement of the basal bathing solution Cl^- resulted in a 76% reduction in the adrenalin-stimulated short-circuit current. Together with the bilateral replacements these results imply the existence of an Na^+-dependent component of Cl^- secretion. Providing that Cl^- diffusion across the thickness of the epithelial cell layer is negligible the existence of a small component of adrenalin-stimulated Na absorption dependent upon apical Cl^- cannot, however, be excluded.

(d) The action of certain pharmacological agents upon the adrenalin-stimulated short circuit current

Previously it was demonstrated that the ATP-stimulated Cl^- secretion in MDCK cell epithelium was abolished by furosemide, partially inhibited by phloretin and was unaffected by incubation with amiloride and SITS containing media [20]. Table III shows that amiloride and SITS are without effect

TABLE III

THE EFFECT OF INHIBITORS OF ION TRANSPORT UPON THE ADRENALIN-STIMULATED ($5 \cdot 10^{-7}$ M) SHORT-CIRCUIT CURRENT

Amiloride, phloretin and SITS were added for a 10-min preincubation period. The adrenalin-dependent increase in short-circuit current was measured 1 min following adrenalin stimulation Furosemide was added 6 min subsequent to adrenalin stimulation and the short-circuit current recorded after a further 5 min; control values are obtained 11 min after adrenalin stimulation (a) and (b) denote apical or basal additions, respectively. Figures in parentheses are the number of observations. Errors are expressed as ± S.E n.s., non significant.

Inhibitor	s.c.c. ($\mu A \cdot cm^{-2}$)		*P* value of difference
	Control response	Test response	
Amiloride (a) $1 \cdot 10^{-4}$ M	34.6 ± 4.6 (4)	26.8 ± 3.3 (4)	n.s.
Phloretin (a) $1 \cdot 10^{-4}$ M	30.2 ± 3 7 (7)	16.4 ± 2 6 (7)	<0 01
Phloretin (b) $1 \cdot 10^{-4}$ M	20.0 ± 4 0 (5)	11.4 ± 1.8 (5)	<0.01
SITS (a) 10 μM	27.7 ± 2.2 (9)	30.5 ± 1.4 (6)	n.s.
SITS (b) 10 μM	27 7 ± 2.2 (9)	32 6 ± 1 2 (6)	n.s.
Furosemide (a) 1 10^{-4} M	8.9 ± 0.9 (5)	7.9 ± 0.8 (5)	n.s.
Furosemide (b) 1 10^{-4} M	6.8 ± 0.6 (5)	2.4 ± 0.4 (5)	<0 001

upon the greater part of the adrenalin-stimulated short circuit current. Phloretin is a partially effective inhibitor of the adrenalin-stimulated short-circuit current applied from either epithelial surface (Table III), whereas furosemide is effective only when applied from the basal bathing solution. In contrast to the action of furosemide upon ATP-stimulated Cl^- secretion where all of the short-circuit current is furosemide sensitive [20], a proportion (35%) of the adrenalin-stimulated short-circuit current is furosemide insensitive. As with ionic replacements, a similar action of furosemide was observed 1, 6, and 16 min following adrenalin-stimulation.

Discussion

The data presented in this paper show that MDCK cell epithelium possesses functional β-adrenergic receptors located upon the basal-lateral aspects of the epithelial cell layer, stimulation of which results in increased transepithelial ion transport, most likely via increased intracellular cyclic AMP levels [2]. On the basis of the measurements of net Cl^- flux plus adrenalin and of the effect of bilateral and unilateral Cl^- replacements of the bathing solutions upon the adrenalin-stimulated short-circuit current, the greater proportion of the increased short-circuit current may be concluded to comprise basal to apical Cl^- secretion.

Certain features of the MDCK cell epithelium response to adrenalin are similar to the ATP-dependent Cl^- secretion reported previously [20] and to Cl^- secretion in other epithelia [1–5,21–25]. Thus adrenalin-stimulated Cl^- secretion is accompanied by an increased tissue conductance, is inhibited by the diuretic furosemide and by phloretin, but is unaffected by amiloride or SITS. The ability of various anions to be able to support the adrenalin-stimulated short-circuit current is similar to that for ATP-stimulated Cl^- secretion in MDCK cell epithelium [20] and is similar to a selectivity isotherm for anions of type 4 of Wright and Diamond [26] suggesting that the rate limiting transport pathway for Cl^- is hydrophobic similar to mammalian small intestine [27]. The dependence of the adrenalin-stimulated short-circuit current upon medium Na^+ though dissimilar to that for ATP-stimulated Cl^- secretion, is similar to that reported for other Cl^- secreting epithelia such as shark rectal gland [24], fish gills [23] and theophylline-treated mammalian small intestine [28].

Since transepithelial Cl^- secretion comprises Cl^- transport across two cellular membranes in series, an attempt has been made to vary solution composition on either epithelial surface. This experiment requires

that transepithelial ion diffusion is negligible within the experimental time-course; for bulk solution Na^+ concentrations, this has been experimentally demonstrated Ionic equilibration within small intra-epithelial compartments cannot, however be excluded. Notwithstanding this possible difficulty, the Na^+ dependence of the adrenalin-stimulated short-circuit current has been demonstrated to be due to an effect of Na^+ upon the basal-lateral membranes. Similarly, the sensitivity of the adrenalin-dependent short-circuit current upon partial Cl^- replacements is due primarily to effects upon the basal-lateral membranes.

The use of the Cl^- transport inhibitors phloretin and furosemide suggests the existence of two pharmacologically separate Cl^- transport mechanisms at the apical and basal-lateral membranes, thus, whereas phloretin inhibits the adrenalin-stimulated short-circuit current from either epithelial surface, furosemide acts only from the basal lateral surfaces A similar result has been previously obtained for ATP-stimulated Cl^- secretion in MDCK cell epithelium [20].

The Na^+-dependence and furosemide sensitivity of basal-lateral Cl^- transport reported here for adrenalin-stimulated Cl^- secretion is similar to that reported for shark rectal gland [24]. In this respect, therefore, the present data is consistent with the models of epithelial Cl^- secretion proposed by Field [25] and Frizzell et al. [29]; Cl^- is accumulated into the cell above its electrochemical equilibrium by a $Na^+ + Cl^-$ furosemide sensitive co-transport at the basal-lateral membranes, so utilising the Na^+ gradient established by the $(Na^+ + K^+)$-ATPase. Net downhill Cl^- movement across the apical membrane is then possible with a secretagogue induced Cl^- conductance. An identical model has been proposed for the ATP-stimulated Cl^- secretion in MDCK cell epithelium [20].

It has recently been demonstrated by Geck et al. [30] in ascites cells and by others working on human and pigeon red cells [31–34] that a variety of coupled ion transports (e.g. $Na^+ + Cl^-$, $K^+ + Cl^-$ and counter transport (e.g. $Na^+ : Li^+$) [35] may be functional expressions of a single diuretic-sensitive $Na^+ + K^+ + Cl^-$ co-transport system. Table IV shows parallels that may be drawn for the adrenalin-stimulated Cl^- secretion and known features of the $Na^+ + K^+ + Cl^-$ co-transport system. The striking parallelism is strong, albeit indirect, evidence for a central role of such an $Na^+ + K^+ + Cl^-$ co-transport system in epithelial Cl^- secretion. Recently a passive K^+ influx sensitive to diuretics and dependent upon medium Na^+ and Cl^- has been identified in MDCK cells [36,37].

TABLE IV

PARALLELS BETWEEN THE DIURETIC SENSITIVE CO-TRANSPORT ($Na^+ + K^+ + Cl^-$) IN HUMAN RED CELLS, ASCITES CELLS AND PIGEON ERYTHROCYTES AND THE ADRENALIN-STIMULATED Cl^- SECRETION IN MDCK CELL EPITHELIUM

Data for $Na^+ + K^+ + Cl^-$ co-transport are taken from Refs. 30–35 and 38. Data for MDCK cell epithelium are from the present results

	$Na^+ + K^+ + Cl^-$ cotransport	Adrenalin-stimulated Cl^- secretion in MDCK cell epithelium
Inhibition by furosemide	+	+
Inhibition by phloretin	+	+
Inhibition by SITS	None	None
Replacement of Na^+ by		
(a) Li^+	Partial	Partial
(b) choline/Tris	No	No
Replacement of Cl^- by		
(a) Br^-	Partial	Partial
(b) NO_3^-	No	No

The dependence of the $Na^+ + K^+ + Cl^-$ cotransport system upon medium Na^+, K^+ and Cl^- differs with respect to different tissues studied; thus the K_m for Na activation of K^+ influx is 34 mM in human red cells, 25 mM in HeLa cells and 10 mM in MDCK cells [34]. Recently Geck et al. [38] have demonstrated that modulation of both intracellular cyclic AMP and Ca^{2+} levels may modify the kinetics of the $Na^+ + K^+ + Cl^-$ cotransport system in ascites cells [38] providing a possible basis for the observed differences in behaviour with respect to Na^+ and Cl^- activation of the adrenaline and ATP-stimulated Cl^- secretion.

Finally, it is worthwhile to speculate upon the consequences of β-adrenergic stimulation of Cl^- transport. Under open circuit conditions modulation of transepithelial potential gradients due to Cl^- secretion will be an important driving force for both net Na^+ and K^+ passive fluxes resulting in decreased Na^+

and K^+ absorption. β-Adrenergic stimulation or blockade of intact kidneys results in varied effects upon urinary electrolyte excretion [6]. In rats and rabbits a decreased urinary Na^+ excretion is reported [6] whereas in hypophysectomised dogs an increased Na^+ and K^+ urinary excretion is observed with isoprenalin infusions [39]. Recently, Iino et al. [40] have shown that β-adrenergic stimulation of the isolated perfused rabbit cortical collecting tubule results in a decreased transtubular potential difference due to increased Cl^- reabsorption. These results, taken together, suggest that the MDCK cell epithelium retains certain features of in vivo renal tubules which augment the use of this cultured system as an in vitro model.

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