Biochimica et Biophysica Acta, 690 (1982) 95–105
Elsevier Biomedical Press

BBA 71310

K^+ TRANSPORT IN 'TIGHT' EPITHELIAL MONOLAYERS OF MDCK CELLS

EVIDENCE FOR A CALCIUM-ACTIVATED K^+ CHANNEL

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(Received February 11th, 1982)

Key words: K^+ transport; K^+ channel; Adrenalin stimulation; Ionophore stimulation; Ca^{2+} dependence; Monolayer; α-Adrenergic receptor; (Cultured epithelium)

Measurements of ^{86}Rb efflux across the apical and basal-lateral aspects of intact monolayers of 'high-resistance' MDCK cells mounted in Ussing chambers have been made. A transient increase in ^{86}Rb efflux across both epithelial borders upon stimulation with adrenalin or ionophore A23187 is observed. The increased ^{86}Rb across the basal cell aspects is of greatest quantitative importance. Measurements of total cellular K^+ contents by flame photometry of tissue extracts indicate a net loss of K^+ following adrenalin addition. The effects of adrenalin and ionophore A23187 upon ^{86}Rb efflux are abolished in 'Ca^{2+}-free' media. The properties of the Ca^{2+}-dependent increase in ^{86}Rb efflux show similarities to Ca^{2+}-activated K^+ conductances in other tissues, notably human red cells, including inhibition by quinine (1 mM), tetraethylammonium (25 mM) and insensitivity to bee venom toxin (apamin) (25 nM). Adrenalin is only effective when applied to the basal bathing solution suggesting that the receptors mediating adrenalin action are located upon the basal-lateral membranes. Half maximal stimulation of ^{86}Rb efflux by adrenalin is observed at $9.1 \cdot 10^{-7}$ M. The action of various adrenergic receptor agonists and antagonists are consistent with adrenalin action being mediated by an α-adrenergic receptor.

Introduction

Although it is well established that the ($Na^+ + K^+$)-ATPase is essential both for generation of intracellular K^+ accumulation within epithelial cells [1] and for the establishment of active K^+ secretion in mammalian epithelia [2,3], relatively little is known concerning the properties of dissipative K^+ fluxes across the apical and basal-lateral membranes of epithelial cells. We have chosen to investigate K^+ fluxes in the cultured renal epithelium MDCK, which in culture retains many of the differentiated features of natural epithelia (e.g. a polarised morphology; apical brush border; apical cell-cell tight junctions and lateral interspaces) [8, 30]. In a previous study of transcellular and transepithelial K^+ fluxes in high resistance MDCK epithelia [4] we reported that the apical cell membrane was relatively impermeable to K^+, and that K^+ exchanges across the basal-lateral membranes could be divided into three components consisting of an ouabain-sensitive portion mediated by the ($Na^+ + K^+$)-pump, a diuretic sensitive K-K exchange which shared many features of the $Na^+ + K^+ + 2\,Cl^-$ cotransport system in ascites cells, including anion sensitivity, and finally an ouabain- and diuretic-insensitive K^+ flux representing the ground permeability of the basal-lateral membranes to K^+. In the present paper, we show that there is, in addition to the flux components described, a Ca^{2+}-activated K^+ channel located principally at the basal-lateral

membranes which is similar to that observed in a variety of tissues including human red blood cells [5]. The Ca^{2+}-dependent K^+ flux in MDCK cells may be activated physiologically by stimulation of an α-adrenergic receptor. A preliminary report of some of the present findings has been published [6].

Methods

(i) Cell culture

Experiments were mainly performed upon MDCK dog kidney cells of 60–72 serial passages (type I) obtained from Flow Laboratories, Irvine, Scotland. A small number of experiments were made upon Type II cells of 111–116 serial passages [7]. Culture conditions and preparation of epithelial monolayers upon permeable filter supports were as previously described [8] except that 10% foetal calf serum was replaced by 5% foetal calf serum supplemented with 5% horse donor serum. This change in growth media had no discernible effect upon the properties of the cells. In addition, monolayers were also grown upon plastic petri dishes (Sterlin, 3 cm radius) after seeding at $1 \cdot 10^5$ cells/plate in 2 cm^3 of growth media. For experimental purposes (4–5 days of growth), the cell monolayers were still subconfluent, thus allowing access of solution to the basal aspects of the cell monolayer. The final cell density was $(3–4) \cdot 10^5$ cells/plate.

(ii) K^+ influx into subconfluent cell monolayers

^{86}Rb was used as an isotopic tracer to indicate K^+ movements. The use of ^{86}Rb as a tracer for K^+ influx was validated in separate control experiments [4]. ^{86}Rb influxes were measured over a 5 min incubation period at 37°C in a Krebs solution containing 0.2 $\mu Ci/cm^3$ ^{86}Rb. This ensured all measurements were made within the linear portion of the uptake curve. At the end of the influx period, the cell layer was rinsed four times with ice-cold Krebs (< 20 s). This washing protocol removed 99% of an extracellular space marker, [^{14}C]inulin in separate experiments. Cell monolayers were then treated with trypsin/EDTA (0.25%/0.1 mM) in Ca^{2+}- and Mg^{2+}-free Earles salt solution (Flow Laboratories) to form a single cell suspension. After trypsinisation, the cell number and cell volume of an aliquot from each experimental plate was determined on a Coulter Counter (ZF) with Channelyser (C1000) [9]. The ^{86}Rb content of samples were determined in a Packard liquid scintillation counter (Model 3255) by the Cerenkov effect.

(iii) K^+ efflux from sub-confluent cell monolayers

Subconfluent cell monolayers in petri dishes were preloaded with ^{86}Rb (0.75 $\mu Ci/cm^3$) for 3 h [9]. The cell layers were then rinsed briefly with Krebs solution (four times) at 37°C to remove extracellular isotope. K^+ efflux was determined by successive addition and collection of aliquots of Krebs solution. The ^{86}Rb content of the cells at the end of the experimental period was determined from aliquots of a cell suspension prepared by trypsinisation (see above). ^{86}Rb was an adequate tracer for K^+ efflux, including adrenalin- or ionophore A23187-stimulated K^+ efflux (determined in a separate experiment utilising simultaneous $^{86}Rb/^{42}K$ efflux measurements).

(iv) K^+ efflux from confluent monolayers of MDCK cells

Cell monolayers, grown upon millipore supports, were mounted in Ussing type chambers (1.76 cm^2 exposed monolayer) allowing access to both the apical and basal bathing solutions. Cell monolayers were maintained in open-circuit conditions. The monolayers had been preloaded with ^{86}Rb (0.75 $\mu Ci/cm^3$) for 5 h and rinsed briefly (four times) with Krebs solution to remove isotope from the extracellular space. K^+ efflux across the apical and basal cell membranes were measured by the successive addition and collection of 5 cm^3 aliquots of Krebs simultaneously from both bathing solutions at 2-min intervals. At the end of the experiment, cell monolayers were extracted for their remaining ^{86}Rb content. Fractional apical efflux values were corrected for the decrease in ^{86}Rb content due to loss of isotope across the basal-lateral cell membrane.

(v) Estimation of intracellular K^+ content

Following incubation subconfluent cell-layers were washed four times in ice-cold isotonic sorbitol solution (< 20 s) and extracted in distilled water for 2 h at 20°C. K^+ was then measured by flame

photometry. The cell numbers and cell volumes of identical plates in the same batch were determined using a Coulter Counter and Channelyser (see above).

(vi) Experimental solutions

Experiments were carried out, except where otherwise stated, in a Krebs solution containing 137 mM NaCl; 5.4 mM KCl; 2.8 mM $CaCl_2$; 1.2 mM $MgSO_4 \cdot 7H_2O$; 0.3 mM NaH_2PO_4; 0.3 mM KH_2PO_4; 14 mM Tris base; 12 mM HCl; 10 mM glucose and 1% horse donor serum, pH 7.4. A nominally Ca^{2+}-free Krebs solution was prepared as above but $CaCl_2$ was omitted and 2 mM EGTA added. The serum used in Ca^{2+}-free solutions, was dialysed overnight in distilled water ($\times 50$ vol.).

(vii) Materials

Wherever possible inorganic salts were of Analar grade or equivalent. Adrenalin, isoprenalin and phenylphrine (all as bitartrate salts), isobutylmethylxanthine, ouabain, apamin and quinine hydrochloride were all purchased from Sigma Chemical Co. Ltd., Poole, Dorset, U.K. Phentolamine (rogitine) was from Ciba-Geigy, Manchester, U.K. and oxymetozaline from E. Merck, Darmstadt, F.R.G. ^{86}Rb as the Cl^- salt was obtained from Amersham International Ltd. Propranonol was generously donated by ICI (Pharmaceuticals), Macclesfield, U.K. Furosemide was donated by Dr. S. Dombey of Hoechst (Hounslow, U.K.)

(viii) Statistical methods

Variation in results is expressed as the standard error of the mean (S.E.). Tests for significance of difference were made by a two-tailed Student's *t*-test (unpaired means solution). $K_{1/2}$ values for dose-response curves were determined using Probit analysis on a Olivetti P6060 minicomputer.

(ix) Calculation of fractional Rb effluxes

Fractional ^{86}Rb effluxes were calculated from the following equations. For efflux across the basal-lateral membranes:

$$F_p = (C_B)_p \Big/ \left(C_t + \left[\sum_{i=p}^{p_f} (C_A)_i + \sum_{i=p}^{p_f} (C_B)_i - \tfrac{1}{2}\left((C_A)_p + (C_B)_p\right) \right] \right)$$

and for apical effluxes:

$$F_p = (C_A)_p \Big/ \left(C_T + \left[\sum_{i=p}^{p_f} (C_A)_i + \sum_{i=p}^{p_f} (C_B)_i - \tfrac{1}{2}\left((C_A)_p + (C_B)_p\right) \right] \right)$$

where for each time period (p): (C_A) and (C_B) are the ^{86}Rb activities appearing in the apical and basal bathing solutions, respectively.
C_t is the ^{86}Rb remaining in the tissue at the end of the experiment.

$$\sum_{i=p}^{p_f} (C_A)_i \quad \text{and} \quad \sum_{i=p}^{p_f} (C_B)_i$$

are the activities of ^{86}Rb appearing in the apical or basal bathing solutions, respectively, from period p to the final period p_f.

Fractional effluxes from subconfluent plates were calculated from the equation:

$$F_p = Cp_m \Big/ \left(C_t + \sum_{i=p}^{p_f} (C_p)_i - \tfrac{1}{2} Cp_m \right)$$

where for each time period (p):
Cp_m = total ^{86}Rb appearing in media during p.
C_t = total ^{86}Rb activity remaining in tissue at the end of the experiment.

$$\sum_{i=p}^{p_f} (C_p)_i$$

is the ^{86}Rb activity appearing in the media from period p to the final period p_f.

Results

(a) Characteristics of ^{86}Rb efflux across the apical and basal-lateral boundaries: effects of adrenalin and ionophore A23187

Fig. 1 shows that the efflux of ^{86}Rb from con-

fluent cell monolayers of Strain I MDCK cells in open-circuit conditions into the apical and basal bathing solutions is time independent after an initial period of 4 min in control conditions. The fractional steady-state basal efflux ($(4.23 \pm 0.43) \cdot 10^{-2}$, mean ± S.E., $n = 14$) exceeds fractional apical efflux ($(0.25 \pm 0.03) \cdot 10^{-2}$, $n = 14$) by 16-fold, in agreement with previous data [4]. Addition of 100 μM adrenalin to the basal solution (Fig. 1, Table VI) stimulates a transient increase in ^{86}Rb efflux across both apical and basal cell borders. Addition of 100 μM adrenalin to the apical bathing solution has no effect on either apical (Fig. 1) or basal ^{86}Rb loss (after 2 min, control ^{86}Rb fractional efflux across basal aspects = $(3.0 \pm 0.27) \cdot 10^{-2}$ ($n = 4$) and efflux plus apical adrenalin = $(3.39 \pm 0.25) \cdot 10^{-2}$ ($n = 4$)), suggesting that the receptors mediating the adrenalin response are localised exclusively upon the basal-lateral cell membranes. The mean increase in fractional ^{86}Rb efflux across the basal cell border 4 min following basal adrenalin addition ($(8.9 \pm 2.1) \cdot 10^{-2}$, $n = 6$) exceeds that across the apical border ($(0.30 \pm 0.09) \cdot 10^{-2}$, $n = 6$) by a factor of 30-fold. The increased apical efflux is statistically significant ($p < 0.01$). These results indicate that in both control and stimulated conditions cellular ^{86}Rb effluxes are dominated by flux across the basal-lateral membranes, similar to natural epithelia such as rabbit colon [10].

20 μM A23187, a Ca^{2+} ionophore [11], stimulates a similar increase in the apical and basal ^{86}Rb effluxes compared with adrenalin (Fig. 2). In contrast to the action of adrenalin, however, A23187 is only effective when applied to the apical bathing solution.

(b) Characterisation of the adrenalin-stimulated ^{86}Rb efflux across the basal cell aspects

(i) Comparison of epithelial and subconfluent preparations. Since ^{86}Rb efflux across the basal cell aspects in all conditions is of greatest quantitative importance, measurements of total cellular ^{86}Rb in subconfluent layers grown upon plastic petri dis-

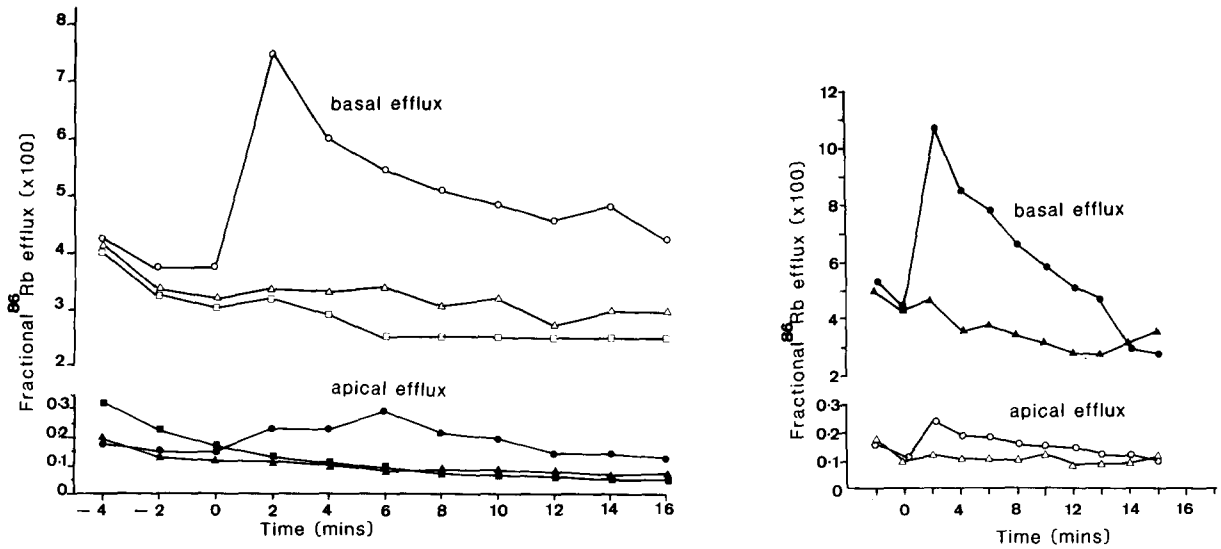

Fig. 1. Action of 100 μM adrenalin upon fractional ^{86}Rb efflux across the apical and basal-lateral aspects of intact epithelial preparations of MDCK cells mounted in Ussing chambers in open-circuit conditions. Adrenalin was added at $t = 0$ min. Data are from single epithelial layers from a single representative experiment. (○/●) 100 μM adrenalin addition to basal-bathing solution, (△/▲) 100 μM adrenalin addition to apical-bathing solution. (○,△,□) efflux across basal aspects, (●,▲,■) efflux across apical aspects, (□,■) control fluxes minus adrenalin.

Fig. 2. Effect of 20 μM A23187 upon fractional ^{86}Rb efflux across the basal-lateral and apical aspects of intact MDCK epithelia. Data are from a single representative experiment. (●,○) apical A23187 addition, (▲,△) basal A23187 addition. (●,▲) ^{86}Rb efflux across the basal cell aspects, (○,△) ^{86}Rb efflux across the apical cell aspects. Ionophore A23187 was added at $t = 0$ min.

hes will consist primarily of basal ^{86}Rb flux [4]. Fig. 3 shows that 100 μM adrenalin has quantitatively similar effects upon ^{86}Rb efflux from subconfluent MDCK layers grown upon plastic petri dishes as is observed in intact epithelial preparations (Fig. 1).

(ii) Pharmacological characterisation of adrenergic receptor sub-type. Fig. 4 shows that half-maximal stimulation of fractional ^{86}Rb loss is observed at $(9.1 \pm 0.2) \cdot 10^{-7}$ M adrenalin. Stimulation of fractional K^+ efflux is observed with the α-adrenergic receptor agonist phenylephrine (Table I) but not with the β-adrenergic receptor agonist isoprenalin. The relatively specific α_2-adrenergic receptor agonist oxymetozaline [12] is also without effect upon ^{86}Rb efflux (Table I). Adrenalin stimulation of fractional ^{86}Rb efflux is abolished by phentolamine but not by propananol which paradoxically potentiates adrenalin action. This action of propananol may be mediated by β-adrenergic receptor antagonism of the response (but see effect of IBMX below). A direct effect of propananol upon adrenalin-stimulated ^{86}Rb efflux is possible [5] but is considered unlikely since propananol has no effect upon unstimulated ^{86}Rb efflux at equivalent molar doses (unpublished results). The present results imply that catecholamine stimulation of ^{86}Rb efflux is mediated by an α-adrenergic receptor of the α_1 sub-type. Similar activations of K^+ permeability by α-adrenergic receptors are observed in a variety of tissues, including epithelia [13–16].

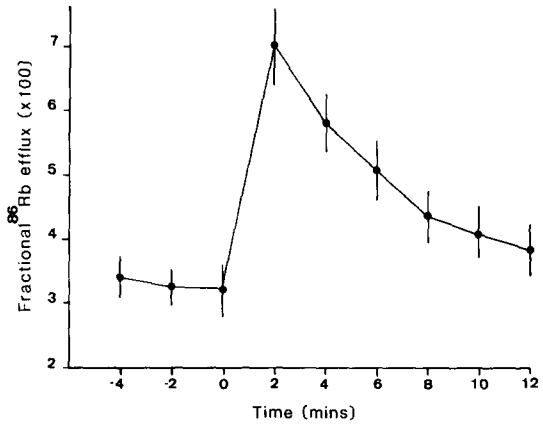

Fig. 3. Effect of 100 μM adrenalin upon fraction ^{86}Rb efflux from sub-confluent MDCK cell monolayers grown upon plastic petri dishes. Each datum is the mean ± S.E. of twenty observations. Adrenalin is added at $t = 0$ min.

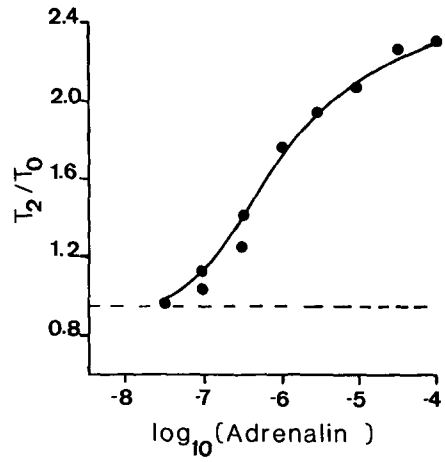

Fig. 4. Log-dose response curve for adrenalin action upon fraction ^{86}Rb from subconfluent cell layers grown upon petridishes. The increment in fractional ^{86}Rb loss plus adrenalin is expressed as the ratio of fraction ^{86}Rb losses immediately prior to ($T = 0$) and 2 min subsequent to adrenalin stimulation ($t = 2$ min). Each datum is a single determination.

(iii) Nature of the channel mediating adrenalin-stimulated ^{86}Rb efflux. The action of adrenalin upon fractional ^{86}Rb efflux is abolished in nominally Ca^{2+}-free media prepared by omission of $CaCl_2$ in the presence of 2 mM EGTA (Table II); this suggests that adrenalin action requires an influx of extracellular Ca^{2+}, and is perhaps mediated by an increase in cytosolic free Ca^{2+}, as in other cells [15]. This view is consistent with α-adrenergic receptor activation and is substantiated in experiments using A23187 [17] in the presence and absence of extracellular Ca^{2+} (Fig. 2, Table VI) see also Ref. 11.

In human red cells and other tissues the Ca^{2+}-induced K^+ permeability is inhibited by quinine [18–20]; 1 mM quinine blocks the increase in ^{86}Rb efflux seen with adrenalin stimulation (Table II). That this action of quinine is consistent with blockade of the K^+ permeability mechanism rather than α-adrenergic receptor antagonism [21] is suggested by the blockade of the A23187 mediated increase in ^{86}Rb efflux (Table II). Apamin, a bee-venom polypeptide inhibitor of the Ca^{2+}-induced K^+ transport in hepatocytes, but not in human red cells [20], is without effect upon the

TABLE I

THE EFFECT OF VARIOUS ADRENERGIC RECEPTOR AGONISTS AND ANTAGONISTS UPON FRACTIONAL ^{86}Rb LOSS

The results are expressed as the ratio of fractional ^{86}Rb effluxes 2 min following adrenalin stimulation compared to Rb efflux immediately prior to adrenalin addition (t_2/t_0). Results are expressed as the mean ± S.E. The number of separate determinations is indicated in parentheses. n.s., not significant.

Condition	Response t_2/t_0	P vs. control
Control	0.984 ± 0.02 (9)	–
0.1 mM adrenalin	2.24 ± 0.25 (10)	<0.001
0.1 mM isoprenalin	1.05 ± 0.04 (6)	n.s.
0.1 mM oxymetazoline	0.98 ± 0.06 (6)	n.s.
0.1 mM phenylephrine	1.44 ± 0.04 (4)	<0.001
0.1 mM adrenalin + 1 μM propranolol	3.75 ± 0.42 (5) [a]	<0.001
0.1 mM adrenalin + 5 μM phentolamine	0.96 ± 0.09 (5)	n.s.

[a] Significantly different from 0.1 mM adrenalin, $P < 0.01$.

adrenalin-stimulated ^{86}Rb-efflux in MDCK cells (Table II).

Furosemide, though reducing control ^{86}Rb efflux (Table II) by inhibition of passive $Na^+ + K^+ + Cl^-$ cotransport [4] is without effect upon the adrenalin-stimulated ^{86}Rb efflux in MDCK cells in contrast to human red cells [22]. $(Na^+ + K^+)$-pump inhibition with ouabain (1 mM) has a significant inhibitory action upon the adrenalin-stimulated ^{86}Rb efflux (Table II) [23] though this is most likely to arise by secondary effects [24], possibly by changes in intracellular ATP/ADP

TABLE II

THE EFFECT OF A Ca^{2+}-FREE BATHING SOLUTION AND OF VARIOUS PHARMACOLOGICAL AGENTS UPON THE ADRENALIN-STIMULATED INCREASE IN TOTAL FRACTIONAL ^{86}Rb EFFLUX FROM SUBCONFLUENT MONOLAYERS OF MDCK CELLS GROWN UPON PLASTIC PETRI DISHES

The fractional ^{86}Rb loss is expressed as a ratio of the efflux rate at $t = 2$ min against $t = 0$ min (t_2/t_0). The effect of the pharmacological agents was tested, in normal Krebs solution, by including the appropriate drug in the adrenalin-containing Krebs solution. The effects of various conditions upon non-stimulated ^{86}Rb effluxes, was also examined, in separate control experiments, where the ratio (t_2/t_0) of fractional ^{86}Rb effluxes refer to each experimental condition. The figures in parentheses refer to the number of observations for each condition. Adrenalin or the various drugs were added at $t = 0$ min.

Experimental condition	Effect upon basal ^{86}Rb efflux t_2/t_0	Control response to 100 μM adrenalin t_2/t_0	Test response to 100 μM adrenalin t_2/t_0	P vs. control
Ca^{2+}-free + 2 mM EGTA	0.88 ± 0.01 (3)	2.07 ± 0.20 (12)	1.17 ± 0.11 (7)	<0.01
1 mM quinine	1.14 ± 0.30 (6) [a]	2.39 ± 0.05 (3)	1.05 ± 0.08 (3)	<0.001
1 mM ouabain	1.13 ± 0.05 (6)	2.41 ± 0.27 (9)	1.68 ± 0.16 (11)	<0.05
25 mM tetraethylammonium	0.73 ± 0.12 (4) [a]	1.68 ± 0.04 (4)	1.21 ± 0.09 (4)	<0.01
1 mM isomethyl butylxanthine (IBMX)	1.07 ± 0.06 (3)	2.10 ± 0.21 (4)	1.99 ± 0.08 (4)	n.s.
25 nM apamin	0.92 ± 0.04 (2)	1.76 ± 0.42 (4)	1.72 ± 0.08 (4)	n.s.
0.1 mM furosemide	0.64 ± 0.04 (8)	2.41 ± 0.27 (9)	2.63 ± 0.11 (5)	n.s.
		Response to 20 μM A23187	Test response to 20 μM A23187	
1 mM quinine	–	5.18 ± 0.24 (4)	1.36 ± 0.11 (4)	<0.01

[a] ratio of ^{86}Rb efflux values in separate control and quinine-treated groups at $t = 0$, quinine and tetraethylammonium were added at $t = -6$ min.

levels affecting passive membrane ion permeability [25], or active Ca^{2+} extrusion. Tetraethylammonium (25 mM) is an effective inhibitor of the adrenalin-stimulated ^{86}Rb efflux as found in colon [10].

Elevation of intracellular cAMP with the phosphodiesterase inhibitor IBMX [26] is without effect upon both basal ^{86}Rb efflux and the adrenalin-stimulated ^{86}Rb-efflux (Table II).

(iv) Net K^+ movements plus adrenalin. The similarities between the adrenalin-stimulated ^{86}Rb efflux and the conductive Ca^{2+}-dependent K^+ permeability in other tissues [27] suggests that adrenalin should mediate a net loss of cellular K^+. This view is confirmed in that 100 μM adrenalin has no significant effect upon either the total ^{86}Rb influx or upon its distribution between ouabain-sensitive, furosemide-sensitive and ouabain/furosemide-insensitive components, measured over the first 5 min of adrenalin stimulation (Table III). Adrenalin also stimulates a net loss of cellular K^+ (Table IV) measured directly; moreover the time course of K^+ loss, as measured by flame photometry, follows that measured by ^{86}Rb isotope measurements (Table IV, Fig. 1).

TABLE III

THE EFFECT OF 100 μM ADRENALIN UPON THE MAGNITUDE OF THE THREE COMPONENTS OF K^+ INFLUX [4]

The results are expressed as a percentage of the total K^+ influx in the absence of 100 μM adrenalin. The results are the means ± S.E. of six separate determinations. Total control K^+ influx was 3.62 ± 0.34 (S.E., $n = 12$) mmol/l cell water per min. The ouabain-sensitive component was determined using 10^{-3} M ouabain, whilst the furosemide-sensitive component was determined in ouabain-containing solutions with 10^{-4} M furosemide.

Components	Adrenalin (μM)	% of control influx
Total influx	0	100 ± 8.3
	100	114 ± 15.6 [a]
Ouabain-sensitive component	0	45.8 ± 5.2
	100	29.1 ± 11.4 [a]
Furosemide-sensitive component	0	47.9 ± 6.2
	100	35.4 ± 8.3 [a]
Ouabain + furosemide-insensitive component	0	25 ± 5.2
	100	22.9 ± 3.1

[a] Not significantly different from control value minus adrenalin (unpaired means Students' *t*-test).

TABLE IV

THE EFFECT OF 100 μM ADRENALIN UPON INTRACELLULAR K^+ CONTENTS

Sub-confluent cell layers grown upon plastic petri dishes were used. Each datum is the mean ± S.E. of three separate determinations.

Time (min)	Intracellular K^+ content (mmol/l cell water)		Significance of difference *P*
	Control	100 μM adrenalin	
2	180.1 ± 7.6	140.7 ± 1.64	0.01
4	186.7 ± 4.4	135.9 ± 4.2	0.001
6	185.0 ± 1.6	151.3 ± 1.9	0.001
8	180.0 ± 5.0	161.8 ± 0.9	0.05

(v) Inactivation of the ^{86}Rb efflux plus adrenalin. Time-dependent decreases in A23187 or adrenalin-stimulated fractional ^{86}Rb losses are observed (Figs. 1, 2). After washing free of adrenalin fractional ^{86}Rb loss decreases towards prestimulation levels (Fig. 5); a second exposure to 100 μM adrenalin is then without effect upon fractional ^{86}Rb loss, though 20 μM A23187 increases fractional ^{86}Rb loss to a similar level in adrenalin-pre-

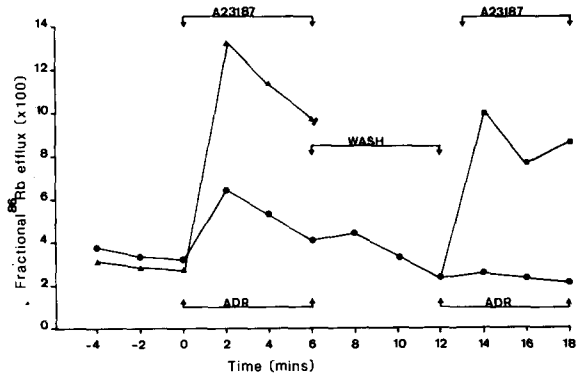

Fig. 5. Desensitisation of the increased fractional ^{86}Rb loss in the presence of 100 μM adrenalin. A 6-min exposure to 100 μM adrenalin is followed by a 6-min drug-free exposure, after which period 100 μM adrenalin (●) or 20 μM A23187 (■) is applied. A control response to 20 μM A23187 was obtained with separate epithelial layers grown on petridishes (▲). Each datum is the mean ± S.E. of at least four observations.

TABLE V

COMPARISON OF THE ACTIONS OF ADRENALIN AND A23187 UPON FRACTIONAL K^+ EFFLUX IN SUBCONFLUENT LAYERS OF EITHER STRAIN I, OR STRAIN II MDCK CELLS

^{86}Rb effluxes are expressed as the ratio of effluxes at $t=2$ min and $t=0$ min (t_2/t_0). Adrenalin was added at $t=0$.

Condition	t_2/t_0		P
	Strain I	Strain II	
Control	0.97±0.01 (7)	0.91±0.06 (6)	n.s.
0.1 mM Adrenaline	1.93±0.15 (8) [a]	0.82±0.03 (7) [b]	<0.001
20 μM A23187	3.58±0.14 (3) [a]	2.49±0.14 (4) [a]	<0.01
20 μM A23187 Ca^{2+}-free	1.64±0.04 (3) [a]	1.67±0.04 (4) [a]	n.s.

[a] Significantly different from control value, $P<0.001$.
[b] Not significantly different from control value.

treated tissues compared to tissues stimulated with 20 μM A23187 alone (Fig. 5). These results suggest that the decrease in fractional ^{86}Rb loss observed in the presence of adrenalin may result from either an agonist-induced desensitisation of the α-adrenergic receptor, as is observed in parotid cells [13,14], or to a Ca^{2+}-induced inactivation of Ca^{2+} channels [28] and not to a time-dependent inactivation of the Ca^{2+}-sensitive K^+ channels themselves [29]. A direct effect of A23187 upon the Ca^{2+} sensitivity of K^+ channels refractile to Ca^{2+} has been reported in red cells from patients with sickle cell anaemia [40], so that the unchanged sensitivity to A23187 after adrenalin may not be an unambigu-

TABLE VI

EFFECT OF NOMINALLY Ca^{2+}-FREE MEDIA, 1 mM QUININE AND VOLTAGE CLAMPING UPON THE ADRENALIN-STIMULATED FRACTIONAL ^{86}Rb LOSSES ACROSS THE APICAL AND BASAL ASPECTS OF INTACT EPITHELIAL PREPARATIONS EXPRESSED AS THE RATIO OF FRACTIONAL ^{86}Rb EFFLUXES AT $t=4$ AND $t=$ZERO MINUTES (t_4/t_0)

Figures in parentheses are the number of separate observations. Adrenalin was added to the basal bathing solution in each instance, at $t=0$ min.

	t_4/t_0 apical	t_4/t_0 basal
Control	0.83±0.08 (8)	0.85±0.06 (8)
100 μM adrenalin (open circuit)	1.69±0.19 (20) [c]	1.98±0.23 (20) [c]
100 μM adrenalin (voltage clamped) p.d.=0	1.11±0.09 (8) [b,g]	1.96±0.12 (8) [c,d]
100 μM adrenalin in Ca^{2+}-free media (+2 mM EGTA)	1.02±0.10 (8) [a,f]	0.94±0.11 (8) [a,e]
100 μM adrenalin +1 mM apical quinine	1.23±0.28 (8) [a,d]	0.94±0.06 (8) [a,e]
100 μM adrenalin +1 mM basal quinine	1.18±0.23 (8) [a,d]	0.66±0.07 (8) [a,e]

[a,b,c] Significantly different (unpaired means Student's t-test) from controls:
[a] not significant.
[b] $P<0.05$.
[c] $P<0.01$.
[d–g] Significantly different (unpaired means Student's t-test) from adrenalin open-circuit values:
[d] not significant.
[e] $P<0.05$.
[f] $P<0.01$.
[g] $0.1>P>0.05$.

ous result. The decrease in fractional ^{86}Rb loss observed with Ca^{2+} ionophore may result from Ca^{2+} buffering, or export of Ca^{2+} from the cell [5].

(vi) Comparison of Strain I/Strain II MDCK cells. Two separate strains of MDCK cells have been identified differing in biochemical, morphological and cell monolayer epithelial properties [7,8]. Most of the present data are from Strain I cells which form epithelial layers of high electrical resistance [30]. Table V demonstrates that although a Ca^{2+}-sensitive A23187-stimulated ^{86}Rb loss is present in both MDCK cell strains, functional activation of this channel by adrenalin is absent from Strain II cells.

(c) Properties of the adrenalin-stimulated ^{86}Rb efflux across the apical cell aspects

The small stimulation of fractional ^{86}Rb efflux across the apical cell border observed in the presence of adrenalin is abolished in Ca^{2+}-free media and is reduced by 1 mM quinine applied from either epithelial surface (Table VI); similar effects upon the basal efflux, measured simultaneously, are observed (see also above). The increased apical efflux plus adrenalin constitutes a net ^{86}Rb efflux since apical ^{86}Rb influx is reduced in adrenalin-treated tissue (control ^{86}Rb influx $= 0.021 \pm 0.002$ (S.E.) mM/l cell H_2O per min), plus 100 μM adrenalin, ^{86}Rb influx $= 0.013 \pm 0.001$ (S.E.) mM/l cell H_2O per min ($n = 4$)).

The reduction in adrenalin-stimulated apical ^{86}Rb efflux by voltage clamping (Table VI) the epithelium to zero p.d. (the open-circuit potential 4 min following adrenalin stimulation in controls was 11.04 ± 0.98 mV ($n = 4$)) is consistent with this net ^{86}Rb efflux being voltage sensitive. The lack of effect of voltage clamping upon the basal adrenalin-stimulated efflux is consistent with a high relative apical membrane resistance [30]; current passage across the epithelium will, therefore, predominantly effect the apical membrane potential [31].

Discussion

A K^+ conductance activated by raised intracellular Ca^{2+} levels has been described in a wide variety of tissues including red blood cells and nervous tissue [5]. In epithelial tissue Ca^{2+} activated K^+ channels are also known to exist [10,15,32–34]. For MDCK epithelium the present results demonstrate an increased ^{86}Rb efflux that is stimulated by an α-adrenergic receptor and by the Ca^{2+} ionophore A23187.

The properties of the increased ^{86}Rb efflux for MDCK cells, are similar in several respects to the Ca^{2+}-sensitive K^+ channel in human red blood cells. Activation by ionophore (or α-agonists) requires the presence of extracellular Ca^{2+} [5,11]. The pharmacological sensitivity (quinine/tetraethylammonium-sensitive, apamin-insensitive) is also similar to red cells [20]. Ouabain reduces the magnitude of the stimulated K^+ flux in both red cells and MDCK cells, presumably via a secondary effect of $(Na^+ + K^+)$-pump inhibition [24,25]. The net loss of K^+ indicated by ^{86}Rb tracer and confirmed by flame photometry in MDCK cells indicates that the relative selectivity to K^+ and Rb^+ for the Ca^{2+}-activated channel is similar, as is found in resealed red cell ghosts [35].

The K^+ efflux mechanism in MDCK cells is located principally upon the basal-lateral membrane; since activation of K^+ efflux is evident with additions to the basal bathing solution of α-agonists, or apical bathing solution additions in the case of A23187, significant compartmentalisation of raised intracellular free Ca^{2+} in MDCK cells is unlikely. MDCK cell thickness is in any case only 3–5 μm [7]. A Ca^{2+}-mediated loss of sensitivity of the K^+ channel to Ca^{2+} has been suggested in several systems [15]. In MDCK cells the reduction in ^{86}Rb flux with continued application of α-agonist is unlikely to result from this cause, since A23187 can stimulate ^{86}Rb efflux following preexposure to α-agonists. This behaviour of the MDCK cell system resembles the human red-blood cell system [29]. A direct effect of A23187 upon the configurational state of the Ca^{2+}-activated K^+ channel [40] cannot, however, be excluded.

Recently considerable attention has been focussed on the mechanism of transepithelial K^+ transport in organs involved in K^+ homeostasis such as mammalian distal nephron segments (distal convoluted tubule and cortical collecting tubule) and mammalian colon [3,10,36,37]. In principle. K^+ secretion by these epithelia may be

achieved by active K^+ uptake at the basal lateral membrane via the $(Na^+ + K^+)$-ATPase followed by downhill K^+ (or $K^+ + Cl^-$) efflux across the apical membrane [2,10,38] or alternatively the basal solution electropositive p.d. in these epithelia could drive net K^+ secretion via shunt pathways [3,37]. In both these models of K^+ secretion, net K^+ movements across the epithelium (or across the apical cell border) are sensitive to the imposed electrochemical gradient [36,37]. For MDCK epithelium, α-adrenergic agonists or A23187 stimulate an increase in ^{86}Rb loss across the apical cell border, as well as across the basal cell border, and this increase in apical ^{86}Rb loss is sensitive to the imposed transepithelial potential gradient. However, in short-circuit conditions no net K^+ secretion is observed either in control or adrenalin-stimulated conditions [4,39] similar to in vitro preparations of colon.

Previously we argued that the larger part of the transepithelial fluxes of K^+ in MDCK cells proceeded via a paracellular shunt pathway [4]; thus it is possible that the increased apical ^{86}Rb leakage following stimulation could represent leakage across a shunt pathway following leakage across the basal-lateral membranes, rather than an apical membrane K^+ conductance, per se. At the present time it is impossible to differentiate between these possibilities; a micro-electrode study of apical conductance in control and stimulated conditions in varied K^+ media is required.

The demonstration of α- and β-adrenergic receptors [26,37] in the same epithelium may be of functional significance; since activation of β-adrenergic receptors stimulates the basal bathing solution electropositive transepithelial p.d. [29], the increased cellular K^+ permeability observed via α-adrenergic receptor activation, should result in increased K^+ secretion (see above).

Acknowledgments

C.D.A. Brown is in receipt of a Maitland-Ramsay Scholarship from the University of St. Andrews. This study was supported in part by the National Kidney Research Fund.

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