ELSEVIER

European Journal of Pharmacology 349 (1998) 89–95

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Inhibition of a K^+ conductance by the phosphatase inhibitor calyculin A in rat distal colon

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Received 18 February 1998; accepted 24 February 1998

Abstract

Basal membrane permeability of epithelial cells from the lower third and the middle of rat colonic crypts is dominated by a K^+ conductance as shown by ion replacement experiments. Calyculin A, an inhibitor of protein phosphatases, induced a depolarization of these cells. The depolarization was concomitant with an inhibition of membrane current. The current inhibited by calyculin A had a reversal potential identical with the theoretical K^+ equilibrium potential indicating that the drug inhibits a basal K^+ conductance. The efficiency of calyculin A was comparable with that of other well-known K^+ channel blockers such as Ba^{2+}, tetraethylammonium or quinine. In the intact tissue, calyculin A exerted an inhibitory action on forskolin-induced anion secretion, an effect which may be explained by the decrease in the driving force for Cl^- exit after inhibition of cellular K^+ conductance. Together with previous results, these data suggest an inhibition of epithelial K^+ conductance by phosphorylation.

Keywords: Calyculin A; cAMP; Electrolyte transport; K^+ channel; Okadaic acid; Protein phosphatase; Rat colon

1. Introduction

The epithelium of the rat colon is characterised by the presence of three different ionic conductances, i.e., a K^+ conductance (Böhme et al., 1991), a Cl^- conductance (Böhme et al., 1991; Diener, 1994; Warth et al., 1996) and a nonselective cation conductance (Siemer and Gögelein, 1993). The cellular Cl^- conductance is increased by cAMP (Böhme et al., 1991; Warth et al., 1996) and by cell swelling (Diener et al., 1992; Diener and Scharrer, 1995). At the fundus of the crypt, based on the Na^+-dependency of forskolin-induced depolarization, it has been concluded that the nonselective cation conductance is regulated by cAMP in these cells (Siemer and Gögelein, 1993), although this report has been reinterpreted recently as indirectly mediated via a basolateral Na^+–K^+–Cl^- cotransporter (Ecke et al., 1996).

The K^+ conductance of the crypt cells increases during cell swelling (Diener and Scharrer, 1995) and after elevation of intracellular Ca^{2+} (Böhme et al., 1991). Controversy exists, however, regarding the effect of cAMP on the conductance for this cation. Warth et al. (1996) observed the activation of small conductance K^+ channel in cell-attached patches by forskolin, an activator of the adenylate cyclase. In contrast, whole-cell data indicate rather a decrease of total K^+ conductance in the presence of forskolin, vasoactive intestinal peptide or a membrane-permeable analogue of cAMP (Diener et al., 1996).

Recently, we tested the effect of genistein, an inhibitor of protein tyrosine kinases, on electrolyte transport and ionic currents in the rat distal colon. This drug potentiated cAMP-mediated activation of apical Cl^- conductance and inhibited at the same time a basal K^+ conductance (Diener and Hug, 1996). From experiments in colonic tumour cells, it has been suggested that genistein causes the inhibition of protein phosphatases, thereby the drug would be able to augment cAMP-mediated effects in the cell (Illek et al., 1996). Therefore, in the present study the effect of genistein was compared with that of calyculin A, a well-known inhibitor of protein phosphatases (Ishihara et al., 1989) in order to elucidate the controversial effect of cAMP on K^+ conductance in the colonic epithelium.

2. Materials and methods

2.1. Solutions

For the experiments with isolated crypts the following buffers were used. The EDTA solution for the crypt isola-

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0014-2999/98/$19.00

tion contained (mmol l^{-1}): NaCl 107, KCl 4.5, NaH_2PO_4 0.2, Na_2HPO_4 1.8, $NaHCO_3$ 25, EDTA (ethylene diamino tetraacetic acid) 10, glucose 12, with 1 g l^{-1} bovine serum albumin. The pH was adjusted to 7.4 by Tris-base (tris(hydroxymethyl)aminomethane). The high K^+ Tyrode for the storage of the crypts consisted of (mmol l^{-1}): K gluconate 100, KCl 30, NaCl 20, $CaCl_2$ 1.25, $MgCl_2$ 1, HEPES (*N*-(2-hydroxyethyl)piperazine-*N*′-2-ethansulfonic acid) 10, glucose 12, Na pyruvate 5 and 1 g l^{-1} bovine serum albumin. The solution was adjusted with KOH to a pH of 7.4. The medium for the superfusion of the crypts was a Ringer solution containing (mmol l^{-1}): NaCl 140, KCl 5.4, $CaCl_2$ 1.25, $MgCl_2$ 1, HEPES 10. For cation substitution, a KCl–Ringer solution was used consisting of (mmol l^{-1}): KCl 140, $CaCl_2$ 1.25, $MgCl_2$ 1, HEPES 10. The pipette solution was a K gluconate/KCl solution, which contained (mmol l^{-1}): K gluconate 100, KCl 30, NaCl 10, $MgCl_2$ 2, EGTA (ethyleneglycol bis-(β-aminoethylether) *N*,*N*,*N*′,*N*′-tetraacetic acid) 0.1, Tris 10, ATP (adenosine 5′-triphosphate disodium salt) 5; pH was 7.2. The Ussing chamber experiments were carried out in a bathing solution containing (mmol l^{-1}): NaCl 107, KCl 4.5, $NaHCO_3$ 25, Na_2HPO_4 1.8, NaH_2PO_4 0.2, $CaCl_2$ 1.25, $MgSO_4$ 1 and glucose 12. The solution was gassed with carbogen (5% CO_2/95% O_2) and kept at a temperature of 37°C; pH was 7.4.

2.2. Tissue preparation

Female Wistar rats were used with a weight of 180–220 g. The animals had free access to water and food until the day of the experiment. Animals were stunned by a blow on the head and killed by exsanguination (approved by Regierungspräsidium Gießen, Gießen, Germany). The serosa and muscularis propria were stripped away by hand to obtain the mucosa–submucosa preparation of the distal part of the colon descendens.

2.3. Short-circuit current measurement

The tissue was fixed in a modified Ussing chamber, bathed with a volume of 3.5 ml on each side of the mucosa and short-circuited by a voltage clamp (Aachen Microclamp, AC Copy Datentechnik, Aachen, Germany) with correction for solution resistance. The exposed surface of the tissue was 1 cm^2. Short-circuit current (I_{sc}) was continuously recorded and tissue conductance (G_t) was measured every min. The baseline in electrical parameters was determined as mean over 3 min just before administration of a drug.

2.4. Crypt isolation

The mucosa–submucosa was fixed on a plastic holder with tissue adhesive and transferred for 8 min in the EDTA solution. The mucosa was vibrated once for 30 s in order to isolate intact crypts. They were collected in an intracellular-like high K^+ Tyrode buffer (Böhme et al., 1991). The mucosa was kept at 37°C during the isolation procedure. All further steps including the patch-clamp experiments were carried out at room temperature.

2.5. Patch-clamp experiments

The crypts were pipetted into the experimental chamber (volume of the chamber about 0.5 ml). The crypts were fixed to the glass bottom of the chamber with the aid of poly-L-lysine (0.1 g l^{-1}). The preparation was superfused hydrostatically throughout the experiment (perfusion rate about 1 ml min^{-1}). The chamber was mounted on the stage of an inverted microscope (Olympus IMT2-FIX-70).

Patch pipettes were pulled from thick-walled borosilicate glass capillaries (Jencons, Bedfordshire, UK; outer diameter 2 mm, inner diameter 1 to 1.25 mm) on a two-stage puller (H. Ochotzki, Homburg/Saar, Germany). After fire-polishing, the tips had resistances of 5 to 10 MΩ when filled with the standard pipette solution. To obtain a whole-cell recording, the membrane patch under the tip of the pipette was broken by a stronger suction pulse after formation of the seal. Seal resistances were 52 to 10 GΩ. Membrane capacitance was corrected for by cancellation of the capacitance transient (subtraction) using a 50 mV pulse.

Patch-clamp currents were recorded on a RK-400 amplifier (Biologics, Meylan, France). Current and voltage signals were digitized at 48 kHz and stored on a modified digital audio recorder (DTR-1200, Biologics). The reference point for the patch potentials was the extracellular side of the membrane assumed to have zero potential. Current–voltage (*I*–*V*) curves were obtained by clamping the cell to a holding potential of −80 mV and stepwise depolarization for 30 ms. After each depolarization, the cell was clamped again to the holding potential for 1 s before the following voltage step (incremented by 10 mV) was applied. For statistical comparison of membrane currents, outward current was measured at the end of a pulse depolarizing the cell for 30 ms from −80 to +60 mV, and inward current was measured at the holding potential of −80 mV. All whole-cell recordings were performed at cells located at the lower third and the middle of the crypts.

2.6. Drugs

Calyculin A (Calbiochem, Bad Soden, Germany) was dissolved in dimethylsulfoxide (DMSO; final maximal concentration 2.5 μl ml^{-1}). For the patch-clamp experiments, calyculin A was administered by a micropipette to the experimental chamber after the superfusion was stopped. Membrane current and membrane voltage after termination of the perfusion served as control to measure the effect of the phosphatase inhibitor. Forskolin and qui-

nine were added from an ethanolic stock solutions (final maximal concentration 2.5 μl ml^{-1}); okadaic acid (Calbiochem) was dissolved in DMSO (final concentration 2.5 μl ml^{-1}). $BaCl_2$ and tetraethylammonium chloride (TEA) were dissolved in aqueous stock solutions diluted in salt buffer just before use. If not indicated differently, drugs were from Sigma, Deisenhofen, Germany.

2.7. Statistics

Results are given as mean ± S.E.M. The significance of differences was tested by paired or unpaired two-tailed Student's *t*-test or an *U*-test. A *F*-test was applied to decide which test method was to be used.

3. Results

3.1. Characterisation of the basal conductance of crypt cells

Under basal conditions, conductances for K^+-ions, Cl^- and a nonselective cation conductance have been described in rat colonic crypts. In order to reveal the contribution of the K^+ conductance to total cellular conductance, ion substitution experiments were performed. Basal membrane potential from cells pooled from different locations along the crypt–surface axis in standard, i.e., 140 mmol l^{-1} NaCl–Ringer solution, amounted to -59.7 ± 1.6 mV ($n = 9$). Substituting Na^+ with equimolar K^+ led to a depolarization by 61.6 ± 3 mV ($P < 0.05$; $n = 9$). This depolarization was completely reversible, when changing again to standard buffer, and could be repetitively evoked at the same cell (Fig. 1a). There was no difference in the response of the cells along the crypt–surface axis to the high K^+ solution. Therefore, data from cells located at different positions along the crypt axis were pooled.

The membrane potential in the 140 mmol l^{-1} KCl–Ringer solution reached a final value of $+1.9 \pm 2.0$ mV. This potential was not significantly different from the theoretical K^+ reversal potential of +2.0 mV with 130 mmol l^{-1} K^+ in the pipette and 140 mmol l^{-1} in the superfusion solution, indicating that the cells behaved as perfect K^+ electrodes. This was confirmed by measuring *I–V* relationships (Fig. 1b). Changing from the 140 mmol l^{-1} NaCl to the 140 mmol l^{-1} KCl–Ringer solution changed the outwardly rectifying *I–V* relation to a linear one. As expected, membrane inward current measured at −80 mV increased from -38.0 ± 5.3 pA to -282.8 ± 70.5 pA ($P < 0.05$; $n = 9$), whereas the outward current measured at +60 mV did not change significantly. From the slopes of the individual *I–V* curves a cellular conductance near the zero-current potential of 3.7 ± 0.9 nS ($n = 9$) was calculated. The data could be adequately fitted to the Goldman–Hodgkin–Katz equation (Hille, 1992), revealing a K^+ permeability of 6.8×10^{-12} cm^3 s^{-1}. Thus these data provide evidence that the basal conductance of rat colonic crypt cells is dominated by a K^+ conductance.

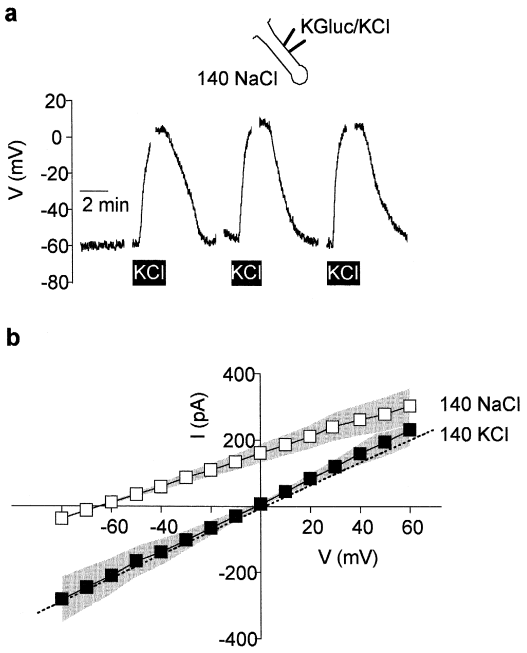

Fig. 1. (a) Effect of a high K^+ solution (140 mmol l^{-1} KCl–Ringer solution instead of a 140 mmol l^{-1} NaCl–Ringer solution; black bars) on membrane potential of a crypt cell. The line interruptions in the voltage tracing are caused by the measurements of *I–V* relations in the voltage-clamp mode. The cell was located at the middle of an isolated crypt as indicated by the schematic drawing. (b) The *I–V* relation of several cells from different locations along the crypt axis during superfusion with the 140 mmol l^{-1} NaCl–Ringer solution (open squares) and with the 140 mmol l^{-1} KCl–Ringer solution (filled squares). Values are means (symbols) ± S.E.M. (shaded area), $n = 9$. The dotted line was obtained by fitting the data to the Goldman–Hodgkin–Katz equation assuming a K^+ permeability of 6.8×10^{-12} cm^3 s^{-1}.

3.2. Effect of calyculin A on the basal K^+ conductance

Administration of calyculin A (10^{-7} mol l^{-1}), a selective blocker of type 1 and type 2A phosphatases (Ishihara et al., 1989), depolarised the colonic crypt cells from -51.3 ± 3.1 mV to -35.3 ± 3.0 mV ($P < 0.05$; $n = 10$; Fig. 2a). There was no difference in the response of cells along the crypt–surface axis to calyculin A. The solvent for calyculin A, DMSO, applied with the same protocol as that for calyculin A, had no significant effect on membrane potential. Membrane potential amounted to -46.1 ± 4.3 mV in the absence and -44.3 ± 4.5 mV in the presence of DMSO ($n = 8$; difference not significant).

Measurement of *I–V* relationship revealed a decrease in cellular conductance by the phosphatase inhibitor (Fig. 2b,c). Membrane outward current, measured at +60 mV, decreased from 397.7 ± 67.5 pA under basal conditions to 249.5 ± 59.6 pA in the presence of calyculin A ($P < 0.05$; $n = 10$), whereas membrane inward current, measured at −80 mV, was unaltered (-74.3 ± 17.7 pA in the absence

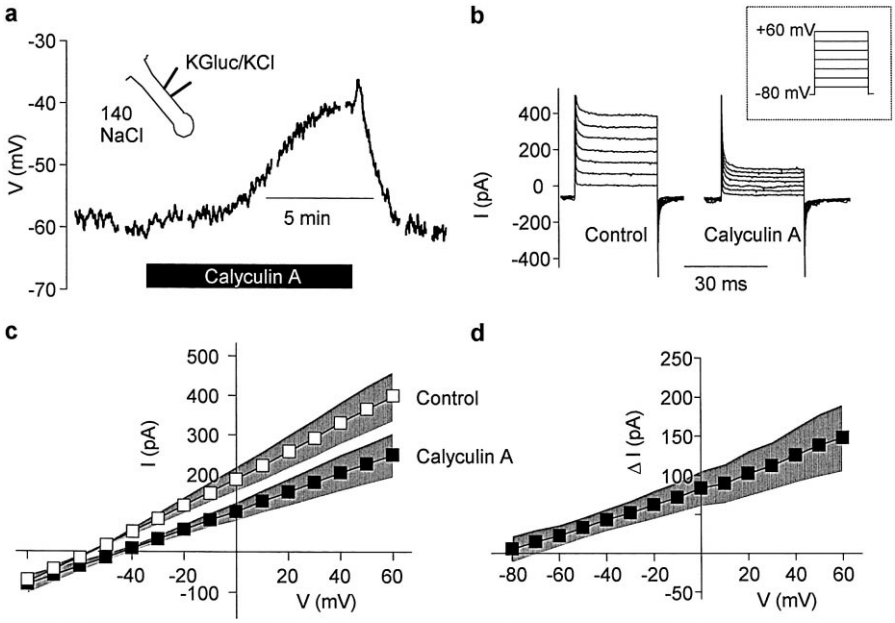

Fig. 2. (a) Effect of calyculin A (10^{-7} mol l^{-1}; black bar) on membrane potential of a crypt cell. The line interruptions in the voltage tracing are caused by the measurements of I–V relations in the voltage-clamp mode. The cell was located at the middle of an isolated crypt as indicated by the schematic drawing. (b) Original record of the membrane current of a crypt cell during depolarizing pulses of 30 ms duration (see Section 2) in the absence (control) and presence of calyculin A. For graphical clarity, only each second pulse of the 14 pulse protocol clamping the cell from −80 to +60 mV is plotted. (c) Membrane current pooled from 10 cells in the absence (control; open squares) and presence of calyculin A (filled squares). (d) Current inhibited by calyculin A obtained after subtraction of current during the control period (ΔI). Values are means (symbols) ± S.E.M. (shaded area), $n = 10$.

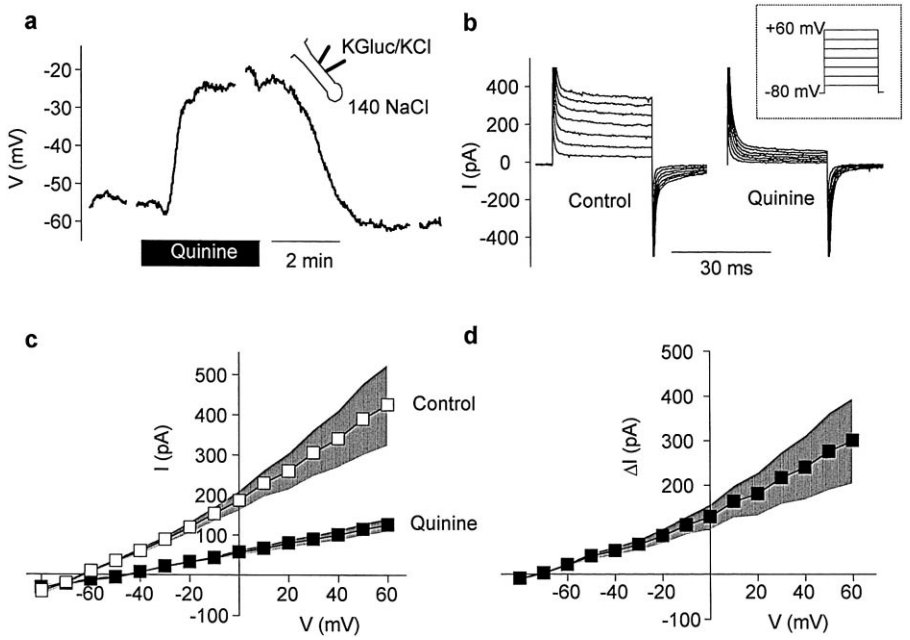

Fig. 3. (a) Effect of a quinine (10^{-3} mol l^{-1}; black bar) on membrane potential of a crypt cell. The line interruptions in the voltage tracing are caused by the measurements of I–V relations in the voltage-clamp mode. The cell was located at the middle of an isolated crypt as indicated by the schematic drawing. (b) Original record of the membrane current of a crypt cell during depolarizing pulses of 30 ms duration (see Section 2) in the absence (control) and presence of quinine. For graphical clarity, only each second pulse of the 14 pulse protocol clamping the cell from −80 to +60 mV is plotted. (c) Membrane current pooled from eight cells in the absence (control; open squares) and presence of quinine (filled squares). (d) Current inhibited by quinine obtained after subtraction of current during the control period (ΔI). Values are means (symbols) ± S.E.M. (shaded area), $n = 8$.

and -79.9 ± 26.3 pA in the presence of the drug, difference not significant). The interpolated reversal potential of the current inhibited by calyculin A amounted to -74.9 ± 8.7 mV ($n = 10$; Fig. 2d). This value was not significantly different from the calculated K^+ equilibrium of -84.3 mV for 130 mmol l^{-1} K^+ in the pipette and 5.4 mmol l^{-1} K^+ in the perfusing solution. Taken together, these data indicate an inhibition of the basal K^+ current by calyculin A.

A similar response was observed, when the crypts were pretreated with a low concentration of forskolin (2×10^{-7} mol l^{-1}). Also after this pretreatment, calyculin A inhibited membrane outward current (measured at $+60$ mV) from 342.1 ± 90.2 pA to 205.6 ± 55.4 pA ($P < 0.05$; $n = 7$), whereas membrane inward current (measured at -80 mV) was not changed significantly.

3.3. Comparison of the effect of calyculin a with other K^+ channel blockers

Various blockers of K^+-channels were applied to compare their effects with the inhibitory action of calyculin A. Quinine (10^{-3} mol l^{-1}) induced a depolarization from -62.2 ± 2.1 mV to -41.1 ± 3.1 mV ($P < 0.05$; $n = 8$; Fig. 3a) concomitant with a decrease in cellular conductance (Fig. 3b,c). Membrane outward current at $+60$ mV decreased from 425.1 ± 102.5 pA to 125.3 ± 17.4 pA ($P < 0.05$; $n = 8$). The reversal potential of the current inhibited by quinine amounted to -74.7 ± 3.6 mV ($n = 8$; Fig. 3d).

A similar result was obtained with tetraethylammonium (5×10^{-3} mol l^{-1}), which induced a depolarization of 10.4 ± 3.7 mV ($P < 0.05$; $n = 7$). The depolarization was concomitant with a decrease of cellular conductance. Tetraethylammonium reduced membrane outward current (measured at $+60$ mV) from 564.0 ± 127.3 pA under control conditions to 315.0 ± 103.7 pA ($P < 0.05$; $n = 7$) in the presence of the inhibitor, whereas membrane inward current remained unchanged. A similar result was obtained with Ba^{2+} (10^{-2} mol l^{-1}), which induced a depolarization of 21.1 ± 2.9 mV ($P < 0.05$; $n = 7$) concomitant with an inhibition of membrane outward current from 431.7 ± 100.5 to 306.0 ± 94.4 ($P < 0.05$; $n = 7$).

3.4. Effect of calyculin A on intact tissue

To test the influence effect of calyculin A on intact mucosa, Ussing chamber experiments were performed. Tissues were pretreated with a low concentration of forskolin (5×10^{-7} mol^{-1}) in order to induce a submaximal Cl^- secretion and to directly compare the data with those obtained for genistein (Diener and Hug, 1996).

Basal I_{sc} just prior administration of forskolin amounted to 67.0 ± 8.0 μA cm^{-2} at a G_t of 8.3 ± 0.5 mS cm^{-2} ($n = 7$). Forskolin induced an increase in I_{sc} of 115.2 ± 18.8 μA cm^{-2} ($P < 0.05$; $n = 7$; Fig. 4a) to a stable plateau, which was associated with an increase in G_t by 4.1 ± 0.6 mS cm^{-2} ($P < 0.05$; $n = 7$). Subsequent administration of increasing concentrations of calyculin A had no effect on I_{sc}, when the drug was administered at concentrations below 10^{-7} mol l^{-1}. At the highest concentration tested (10^{-7} mol l^{-1}) calyculin A caused a strong inhibition of the anion secretion induced by forskolin (Fig. 4a,c). Calyculin A at this concentration induced a fall in I_{sc} to 61.6 ± 13.4 μA cm^{-2} ($n = 7$), a value, which was not significantly different from the baseline prior to addition of forskolin.

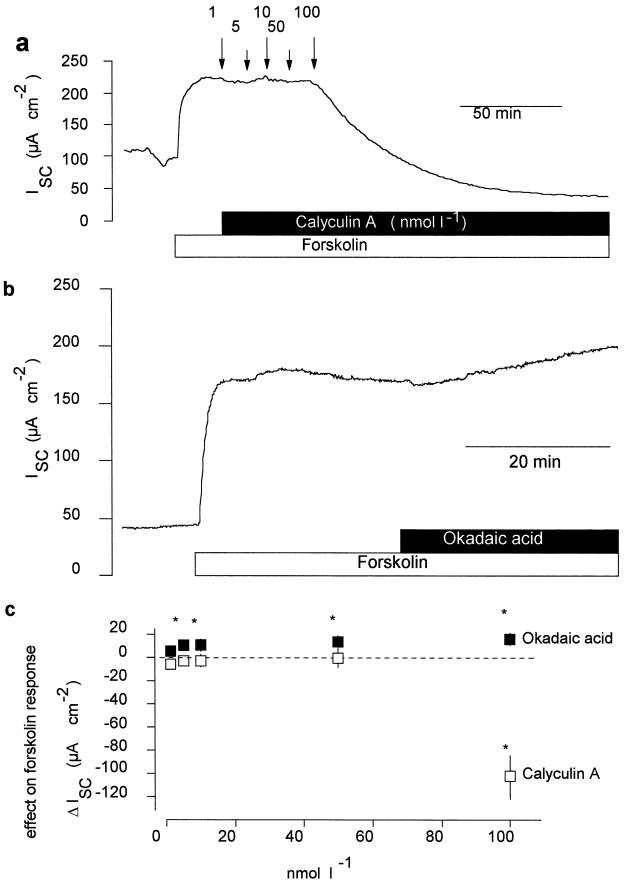

Fig. 4. (a) Original tracing demonstrating the effect of calyculin A, applied cumulatively in increasing concentrations from 10^{-9} to 10^{-7} mol l^{-1} at the serosal side on anion secretion induced by forskolin (5×10^{-7} mol l^{-1} at the serosal and mucosal side). (b) Original tracing demonstrating the effect of okadaic acid (10^{-7} mol l^{-1} at the serosal side) on anion secretion induced by forskolin (5×10^{-7} mol l^{-1} at the serosal and mucosal side). (c) Concentration-dependence of the effect of calyculin A (open squares) and okadaic acid (closed squares) on forskolin-induced anion secretion. The data are represented as decrease or increase of the forskolin-induced I_{sc} (ΔI_{sc}). Values are means ± S.E.M., $n = 7$–8, $* P < 0.05$ vs. forskolin alone.

3.5. Comparison with the effect of okadaic acid on intact tissue

In order to characterize the type of phosphatase involved in this inhibitory action, the effect of calyculin A

was compared with that of okadaic acid in intact tissue. Okadaic acid has the same affinity for type-2A phosphatases, whereas its affinity for type-1 phosphatases is about 100-fold smaller compared to that of calyculin A Ishihara et al., 1989)

Pretreatment with forskolin (5×10^{-7} mol l^{-1}) increased the I_{sc} and G_t from 64.3 ± 5.4 to 195.6 ± 10.7 μA cm^{-2} (Fig. 4b) and 13.5 ± 0.9 to 16.4 ± 0.7 mS cm^{-2}, respectively ($P < 0.05$; $n = 8$). In contrast to calyculin A, administration of okadaic acid significantly enhanced the I_{sc} in a concentration-dependent manner (Fig. 4c). A first significant increase in I_{sc} was observed at a concentration of 5×10^{-9} mol l^{-1}. At the highest concentration tested (10^{-7} mol l^{-1}), I_{sc} increased slightly to 211.7 ± 16.1 μA cm^{-2} ($P < 0.0.5$; $n = 8$) and G_t increased to 17.2 ± 0.6 mS cm^{-2} ($P < 0.05$; $n = 8$). Prior administration of forskolin was a prerequisite for stimulation of I_{sc} and G_t by this phosphatase inhibitor as okadaic acid did not affect I_{sc} or G_t when the drug was administered under basal conditions (data not shown; $n = 6$), suggesting that okadaic acid only enhances prestimulated anion secretion.

4. Discussion

The present data demonstrate that calyculin A, a potent and efficient inhibitor of protein phosphatases type 1 and 2a (Ishihara et al., 1989), exerts an antisecretory action in the rat distal colon (Fig. 4a,c). The drug blocks the increase in I_{sc} induced by a low concentration of forskolin (5×10^{-7} mol l^{-1}), which in this concentration induces a moderate Cl^- secretion accompanied with an inhibition of Na^+ and Cl^- absorption (Diener and Hug, 1996). The inhibition of the forskolin-induced secretion is caused by an inhibition of a basal K^+ conductance as indicated by the whole-cell patch clamp recordings, which demonstrated that calyculin A induces a depolarization (Fig. 2a) and a decrease in membrane current (Fig. 2b,c). The reversal potential of the current inhibited by calyculin A was not significantly different from the theoretical K^+ reversal potential (Fig. 2d) indicating the inhibition of a conductance selective for K^+ ions. Assuming a specific action of the inhibitor, these results imply a tonic activity of protein phosphatases in the colonic epithelial cells, which is removed by calyculin A. The effect of calyculin A is not mimicked by okadaic acid (Fig. 4b,c), a phosphatase inhibitor with a divergent pattern of sensitivity for the different types of phosphatases (Ishihara et al., 1989), suggesting that the modulation of the cellular K^+ conductance is mediated by a protein phosphatase type 1. However, as direct data concerning the effect of both blockers on large intestinal phosphatases are missing, the conclusion about the enzyme subtype remains speculative.

Membrane permeability of the colonic epithelial cells is dominated by a K^+ permeability as shown by ion substitution experiments (Fig. 1). The efficiency of calyculin A to inhibit basal K^+ currents is comparable to that of other K^+ channel blockers such as tetraethylammonium, quinine or Ba^{2+} (Fig. 3). Calyculin A (10^{-7} mol l^{-1}) inhibited 37% of membrane outward current (measured at $+60$ mV) compared to an inhibition of 44% by tetraethylammonium, 71% by quinine or 29% by Ba^{2+}. With respect to the inhibitory action on a K^+ conductance the effect of calyculin A mimics the effect of genistein, a protein tyrosine kinase inhibitor (Diener and Hug, 1996). Consequently, it seems likely that this effect of genistein may be mediated by an inhibition of protein phosphatases as suggested by Illek et al. (1996). However, in contrast to calyculin A genistein exerts a quite variable effect on transepithelial Cl^- secretion. Under basal conditions this drug often inhibits spontaneous anion secretion, probably due to the inhibition of a K^+ conductance which reduces the driving force for Cl^- exit, whereas in the presence of forskolin genistein potentiates cAMP-mediated secretion. This potentiation could be shown to be due to a stimulation of a chloride conductance (Diener and Hug, 1996), which probably reflects the activation of the cystic fibrosis transmembrane regulator (CFTR) Cl^- channel as in colonic tumour cells (Illek et al., 1996). This stimulatory action on anion secretion is absent in the case of calyculin A. This may indicate that either other protein phosphatases mediate the stimulatory effect of genistein or, alternatively, that there may be a direct phosphorylation of apical Cl^- channels at a tyrosine residue, which is altered by genistein.

Inhibition of protein phosphatases by calyculin A should lead to a hyperphosphorylation of cellular proteins due to inhibition of dephosphorylation. The consequence of this process is a decrease in total cellular K^+ conductance (Fig. 2). The same can be observed when phosphorylation of proteins is stimulated by agonists of the cAMP pathway such as forskolin, vasoactive intestinal peptide or a membrane permeable analogue of cAMP (Diener et al., 1996). The nature of the K^+ channels inhibited is currently unclear; single-channel studies to clarify this point were beyond the scope of the present study. At first glance, these data are in contrast to recent findings from Warth et al. (1996), which demonstrated by noise analysis the activation of a small conductance K^+ channel by forskolin in cell-attached patches from crypt cells. This effect could be mimicked in inside-out patches by the administration of ATP to the intracellular side of the patch. In addition, also in rabbit colonic crypts the activation of a K^+ conductance by cAMP has been clearly demonstrated (Lohrmann et al., 1995). In this tissue, the activation of a Ca^{2+}-dependent K^+ channel by cAMP has also been shown at the single channel level (Kaunitz and Loo, 1989). However, recently the same group observed the simultaneous inhibition of basolateral Ca^{2+}-activated K^+ channels by forskolin, which has been attributed to a decrease in the intracellular Ca^{2+} concentration in the presence of this drug (Bleich et al., 1996). Also in other epithelial cells an inhibition of a K^+ conductance by agents increasing the intracellular cAMP concentration has been reported. Maguire et al. (1995)

observed an inhibition of a basolateral K^+ conductance in nystatin-permeabilized human colon by forskolin. Genistein, which is assumed to act via an inhibition of a protein phosphatase, blocks a basolateral K^+ conductance in permeabilized HT29 cells (Illek et al., 1996). Del Castillo and Sepúlveda (1995) observed an inhibition of basal $^{86}Rb^+$-uptake, an indirect measurement of basal K^+ conductance, in isolated cells from guinea pig distal colon by calyculin A, an effect, which may well be explained by the inhibitory effect of calyculin A on basal K^+ current (Fig. 2).

What might be the physiological significance of an inhibitory effect of cAMP-induced phosphorylation on basal K^+ conductance? Clearly, this inhibition is contraproductive to induce Cl^- secretion, one of the most prominent effects of an increase in the intracellular concentration of this second messenger. Consequently, the decrease in the cellular K^+ conductance, which maintains the driving force for Cl^- exit across apical Cl^- channels, represents some kind of 'brake' limiting the maximal secretory capacity of the epithelium. However, stimulation of the cAMP pathway in addition leads to a stimulation of K^+ secretion together with an inhibition of K^+ absorption (for review see Binder and Sandle, 1994). This change in the net direction of transepithelial K^+ transport is associated with a change in cellular K^+ conductance, i.e. an increase in the apical and a decrease in the basolateral K^+ conductance as revealed by efflux studies (Diener et al., 1996). Consequently, the inhibition of a basolateral K^+ conductance, which is assumed to mediate the efflux of K^+ ions absorbed by the apical K^+–H^+–ATPase (Binder and Sandle, 1994), may be one mechanism underlying the inhibition of K^+ absorption, which is assumed to be localised in the more differentiated cell along the crypt–surface axis (Jaisser et al., 1993). Consequently, an inhibition of K^+ channels induced by a direct stimulation (via e.g., forskolin) or an indirect stimulation (via calyculin A or genistein) of the cAMP pathway may well play a role in the regulation of transepithelial ion transport.

Acknowledgements

The diligent care of Mrs. A. Metternich, B. Schmitt and E. Zappe is a pleasure to acknowledge. Supported by Deutsche Forschungsgemeinschaft, grant Di 388/3-1.

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