J. Membrane Biol. 151, 11–18 (1996)

The Journal of
Membrane
Biology

Regulation of Ca^{2+}-activated K^+ Channel from Rabbit Distal Colon Epithelium by Phosphorylation and Dephosphorylation

D.A. Klærke,[1] H. Wiener,[2] T. Zeuthen,[3], P.L. Jørgensen[1]

[1]Biomembrane Research Centre, The August Krogh Institute, Copenhagen University, 2100 Copenhagen Ø, Denmark
[2]Department of Pharmacology, University of Vienna, 1090 Vienna, Austria
[3]The Panum Institute, Department of Medical Physiology, Copenhagen University, 2200 Copenhagen N, Denmark

Received: 28 February 1995/Revised: 22 December 1995

Abstract. The Ca^{2+}-activated maxi K^+ channel is predominant in the basolateral membrane of the surface cells in the distal colon. It may play a role in the regulation of the aldosterone-stimulated Na^+ reabsorption from the intestinal lumen. Previous measurements of these basolateral K^+ channels in planar lipid bilayers and in plasma membrane vesicles have shown a very high sensitivity to Ca^{2+} with a $K_{0.5}$ ranging from 20 nM to 300 nM, whereas other studies have a much lower sensitivity to Ca^{2+}. To investigate whether this difference could be due to modulation by second messenger systems, the effect of phosphorylation and dephosphorylation was examined. After addition of phosphatase, the K^+ channels lost their high sensitivity to Ca^{2+}, yet they could still be activated by high concentrations of Ca^{2+} (10 μM). Furthermore, the high sensitivity to Ca^{2+} could be restored after phosphorylation catalyzed by a cAMP dependent protein kinase. There was no effect of addition of protein kinase C. In agreement with the involvement of enzymatic processes, lag periods of 30–120 sec for dephosphorylation and of 10–280 sec for phosphorylation were observed. The phosphorylation state of the channel did not influence the single channel conductance. The results demonstrate that the high sensitivity to Ca^{2+} of the maxi K^+ channel from rabbit distal colon is a property of the phosphorylated form of the channel protein, and that the difference in Ca^{2+} sensitivity between the dephosphorylated and phosphorylated forms of the channel protein is more than one order of magnitude. The variety in Ca^{2+} sensitivities for maxi K^+ channels from tissue to tissue and from different studies on the same tissue could be due to modification by second messenger systems.

Correspondence to: D.A. Klærke

Key words: Maxi K^+ channel — Calcium — Phosphorylation — Dephosphorylation — cAMP dependent protein kinase — Rabbit distal colon

Introduction

Na^+ and water homeostasis of the organism is maintained in part by the colon. The Na^+ transport is regulated by aldosterone and involves the cooperation of several membrane transport systems (Schultz, 1984; Smith & McCabe, 1984). The Na,K-pump in the basolateral membrane drives the Na^+ reabsorption via amiloride-sensitive channels in the luminal membrane. The K^+ ions recycle via K^+ channels across the basolateral membrane to keep intracellular K^+ homeostasis and therefore transepithelial Na^+ transport requires strict regulation of the basolateral K^+ channels, e.g., during hormonal stimulation of the tissue.

In the basolateral membranes of the surface cells in the distal colon, Ca^{2+}-activated maxi K^+ channels are a dominant channel type (Turnheim et al., 1989). It has been shown previously that these channels are regulated by several factors, such as membrane potential, pH and Ca^{2+} in intracellular concentrations (Wiener, Klærke & Jørgensen 1990; Klærke et al., 1993). In single channel studies, it has been observed that the Ca^{2+}-activated K^+ channels from the surface cells of distal colon may exist in either of two states, one with a high sensitivity to Ca^{2+} and another with a low sensitivity to Ca^{2+} (Klærke et al., 1993). Transition from high to low sensitivity to Ca^{2+} following incubation with Mg^{2+} has suggested that the loss of Ca^{2+} sensitivity could be associated with dephosphorylation of the channel protein (Klærke et al., 1993). Furthermore, these channels are particularly interesting because they may provide a link between second messenger systems and membrane conductance.

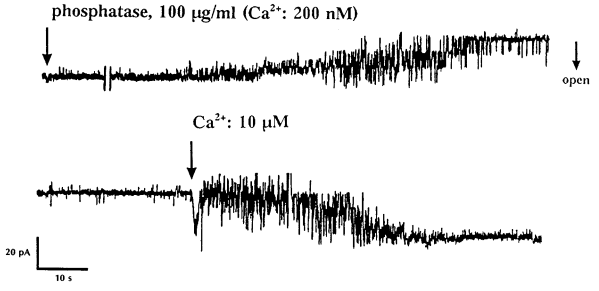

Fig. 1. Effect of dephosphorylation. Ca^{2+}-activated maxi K^+ channels were incorporated in the bilayer at a free Ca^{2+} concentration of 200 nM in 2 mM $MgCl_2$, 10 mM Hepes-Tris (pH 7.2) and 300 mM KCl (*cis* chamber) or 50 mM KCl (*trans* chamber). At the time indicated by the arrow, phosphatase was added and after app. 2 min the channels had lost their activity (upper trace). The measurements continue in the lower trace where it is seen that addition of a high concentration of free Ca^{2+} (10 μM) reactivated the channels to full activity.

Therefore, the purpose of the present study has been to determine if the Ca^{2+}-activated K^+ channels from this epithelium are a target for second messenger systems through phosphorylation catalyzed by protein kinases. Phosphorylation and dephosphorylation of the Ca^{2+}-activated K^+ channels from rabbit distal colon were examined in single channels after incorporation into planar lipid bilayers. The cytoplasmic aspects of the ion channels were exposed to reactions with phosphatases and protein kinases. This allowed for a detailed characterization of the Ca^{2+}-activation and the conductance of K^+ channels before and after dephosphorylation and phosphorylation. In addition, the time dependence of the enzymatic processes was studied.

Materials and Methods

Vesicle Preparation

Basolateral plasma membrane vesicles from rabbit distal colon epithelium were isolated from mucosal scrapings as described earlier (Wiener et al., 1989) and resuspended in 250 mM Sucrose, 10 mM Hepes-Tris, pH 7.2 to a protein concentration of 10–15 mg/ml (Bradford, 1976). Vesicles were prepared from several animals, frozen in liquid nitrogen, stored at −80°C, and thawed immediately prior to use. For incorporation of ion channels into planar lipid bilayer, the highly enriched basolateral membrane fraction from surface cells (Wiener, Turnheim & Os, 1989) was used.

Incorporation of Channel Protein into Planar Lipid Bilayers

Fusion of plasma membrane vesicles from the surface cells was done essentially as described before (Klærke et al., 1993). In short, planar lipid bilayers consisting of phosphatidylethanolamine (10 mg/ml) and phosphatidylserine (10 mg/ml) in decane were painted over a 0.2 mm drilled aperture in a polystyrene cup (Sarstedt, Germany) placed in a teflon block (Alvarez, 1986). Initially both bath compartments (*cis* and *trans*) contained about 2 ml 50 mM KCl, 0.2 mM $CaCl_2$, 10 mM Hepes-Tris, pH 7.2. To promote fusion of plasma membrane vesicles with the bilayer, the salt concentration in the *cis* compartment was raised to 450 mM KCl before addition of 5 μl aliquots of vesicles containing app. 15 μg protein. The *cis* compartment was then stirred using a magnetic stirring bar until gated currents across the bilayer were detected.

The single channel currents were measured using a home built patch clamp amplifier and the data were analyzed on an IBM AT personal computer using the ''Patch and Voltage Clamp Analysis Program'' from Cambridge Electronic Design, Cambridge, UK.

The voltages were related to the direction of the incorporated channel: a negative voltage was negative with respect to the intracellular face of the channel as it is the case under physiological conditions in the cell. Movement of the K^+ from the *cis* to the *trans* chamber was indicated by a negative current and appeared as a downward deflection in the current traces.

Phosphorylation and Dephosphorylation Experiments

Phosphorylation and dephosphorylation experiments were done in the presence of 2 mM $MgCl_2$ and protein kinase A, Sigma P-5511 (50 μg/ml), phosphatase, Sigma P-3752 (100 μg/ml), cAMP (50 μM) and ATP (0.5 mM) were added directly to the chambers. For heat inactivation, enzymes were boiled 15 min. During the experiments, solutions were changed by additions directly to the chambers. EGTA was added to control the free Ca^{2+} concentration; equilibrium constants for the EGTA-ATP-Mg^{2+}-Ca^{2+}-buffer system were calculated according to Pershadsingh and McDonald, 1980.

Materials

Phosphatidylethanolamine and phosphatidylserine from bovine brain were from Avanti Polar Lipds, Alabaster, Alabama; Pentane and Decane were from Aldrich, Steinheim, Germany. ATP, cAMP, protein kinase A, phosphatase and 1-oleoyl-2 acetyl-glycerol were from Sigma, St. Louis, MO. Protein kinase C was from Boehringer. All other chemicals were analytical grade.

Results

Effect of Dephosphorylation

After fusion of basolateral plasma membrane vesicles from the surface cells of rabbit distal colon epithelium with the lipid bilayer, the ion channel type incorporated with highest frequency is a Ca^{2+}-activated maxi K^+ channel (Turnheim et al., 1989; Klærke et al., 1993). If the vesicles from the distal colon are incubated at room temperature in the presence of Mg^{2+} prior to fusion with the bilayer, about half of the incorporated channels display a low sensitivity to Ca^{2+} (Klærke et al., 1993). Since Mg^{2+} is known to activate endogenous phosphatases in vesicle preparations (Wen, Famulski & Carnfoli, 1984), this observation could be ascribed to dephosphorylation. Therefore, we examined the effect of treatment of Ca^{2+}-activated maxi K^+ channels incorporated into the lipid bilayer with phosphatase. A typical experiment is shown in Fig. 1. Initially, the channels had a high open prob-

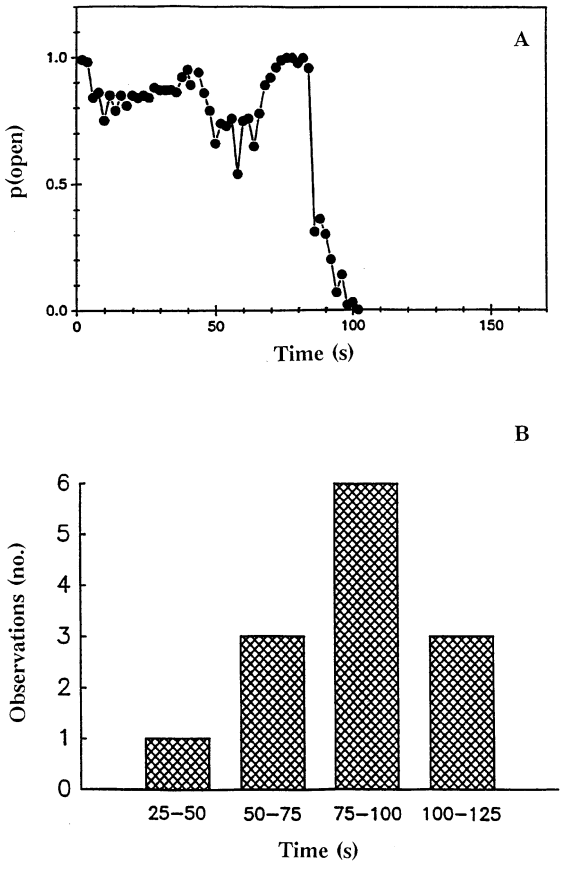

Fig. 2. Time course of the decrease in K^+-channel activity after addition of phosphatase. (*A*) One K^+ channel was incorporated into the bilayer and at zero time phosphatase was added as in Fig. 1. In the time following the addition, the channel open probability was continuously calculated and each point represents the value for a 2-sec interval. The channel protein is dephosphorylated when a sudden large decrease in open probability is observed. (*B*) The histogram summarizes the dephosphorylation times for 13 K^+ channels incorporated into the bilayer at the conditions described above. The dephosphorylation time is defined as the time from addition of the phosphatase until dephosphorylation of the channel protein is observed.

ability (p(open) > 0.95; $V = 0$ mV) at a free Ca^{2+} concentration of 200 nM as expected from their known high sensitivity to Ca^{2+} (Klærke et al., 1993). After addition of phosphatase, the channels closed (upper trace), but the experiment shows that the K^+ channels could still be reactivated by addition of high concentrations of Ca^{2+} (10 μM) about 5 min. after the addition of phosphatase (lower trace). There was no effect of addition of heat inactivated enzyme ($n = 4$).

To study the nature of the phosphatase effect in more detail, we examined the time dependence of the reaction. Figure 2*A* shows the open probability as a function of time for a single channel where phosphatase was added at time zero. The effect of the phosphatase addition appeared as a dramatic decrease in open probability after about 85 sec. Similar effects of the phosphatase addition were obtained in 6 out of 7 experiments. The abrupt decrease in open probability was characteristic for the effect of the phosphatase addition and it was therefore possible to determine the lag period from addition of phosphatase until the effect was seen. The histogram in Fig. 2*B* summarizes the time dependence of the phosphatase effect on 13 channels in 6 experiments. The effect occurred with a mean time of 82 sec after addition. This lag time of the phosphatase effect is consistent with the elapse of an enzymatic reaction.

The phosphatase experiments demonstrate that the phosphatase catalyzed dephosphorylation results in a decrease in the Ca^{2+} sensitivity of the maxi K^+ channels, and it seems probable that the decrease in Ca^{2+} sensitivity seen after incubation of the vesicles with Mg^{2+} is due to dephosphorylation of the channel protein by endogenous phosphatase activity.

Effect of Phosphorylation

Next, we examined if dephosphorylated K^+ channels could regain their high sensitivity to Ca^{2+} after phosphorylation catalyzed by a protein kinase. In these experiments, dephosphorylated channels were defined as channels which were closed at a free Ca^{2+} concentration of 200 nM and a potential of 0 mV.

Figure 3 shows a typical experiment, where dephosphorylated K^+ channels from vesicles preincubated with Mg^{2+} were incorporated into the bilayer, and as expected there was no K^+ channel activity at a free Ca^{2+} concentration of 200 nM. After a lag period of 40–80 sec, the addition of ATP, cAMP and protein kinase resulted in activation of two K^+ channels incorporated into the bilayer. We observed two typical patterns of K^+ channel activation after phosphorylation, either there was a short flicker period before activation of the channel (Fig. 3*A*) or the channel was immediately activated (Fig. 3*B*). We never saw any effect when cAMP, ATP or protein kinase was added separately, consistent with the notion that all three components are necessary for kinase function. The activation by phosphorylation was seen for 9 channels in 7 out of 13 experiments.

The time dependence of the protein kinase A phosphorylation is summarized in the experiments in Fig. 4*A*. It is seen that phosphorylation opened the channel at a well-defined time after addition of the protein kinase. In Fig. 4*B*, we have shown the time distribution for phosphorylation of 9 channels and estimated the mean phosphorylation time to be 85 sec.

The amplitude histograms for the single channel traces before and after addition of the protein kinase A show that the amplitude of the single channel traces was not altered by phosphorylation (Fig. 5). This means that the single channel conductance is not dependent on the phosphorylation state of the channel protein.

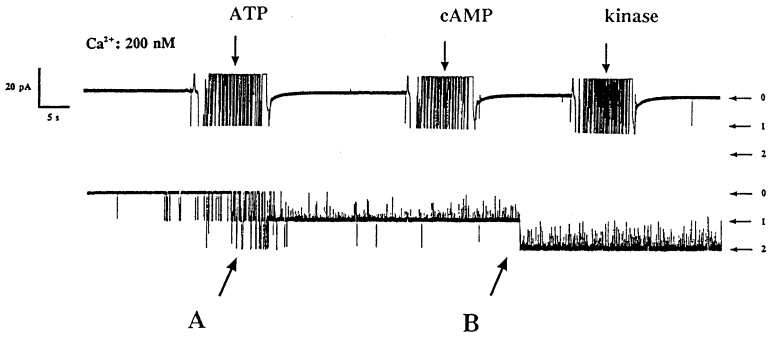

Fig. 3. Activation of a maxi K^+ channel by phosphorylation. Dephosphorylated Ca^{2+}-activated K^+ channels are incorporated into the bilayer, i.e., there is no channel activity at a free Ca^{2+} concentration of 200 nM. Addition of 0.5 mM ATP, 50 μM cAMP and 50 μg/ml protein kinase (upper trace) results in activation of two channels in the bilayer after 30–60 sec (lower trace).

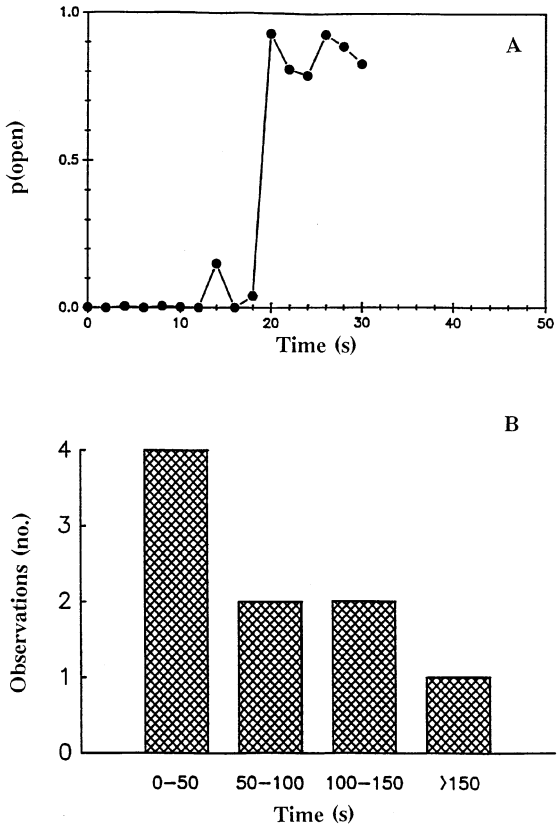

Fig. 4. Time course for phosphorylation of the K^+ channel protein. In (*A*) a dephosphorylated K^+ channel was incorporated into the bilayer as described in Fig. 3, and at time 0 protein kinase was added. The *p* (open) of the channel was then monitored in 2-sec sweeps, and phosphorylation occurred when an abrupt rise in channel open probability was observed. (*B*) shows the distribution of phosphorylation times (time lag from addition of kinase to observation of increase in *p* (open)) for 9 channels.

In 4 experiments, whether or not dephosphorylated channels could be reactivated by protein kinase C was examined, but the addition of protein kinase C never resulted in activation of the channels (*data not shown*).

Change in Ca^{2+} Sensitivity

The results of the experiments in Fig. 1 and Fig. 3 show, that the high Ca^{2+} sensitivity of the K^+ channels depends on the channel protein being in a phosphorylated state, and that a dephosphorylated channel could regain its sensitivity to Ca^{2+} by phosphorylation from a cAMP-dependent protein kinase. To examine this possibility further, we have in the experiment in Fig. 6 tried to determine the sensitivity to Ca^{2+} of a maxi K^+ channel before and after rephosphorylation by the protein kinase A. Before phosphorylation, this channel was inactive at 200 nM free Ca^{2+}, and it is seen that phosphorylation apparently increased the sensitivity of the K^+ channel to Ca^{2+} by about one order of magnitude.

Discussion

Phosphorylation and Dephosphorylation

Ca^{2+}-activated maxi K^+ channels from rabbit distal colon are activated by Ca^{2+} in the intracellular concentration range (Klærke et al., 1993). Our results show that the high sensitivity to Ca^{2+} is dependent on the channel proteins being in a phosphorylated state. If vesicles are prepared in the absence of Mg^{2+} and kept in the cold, most channels appear in a phosphorylated state and reveal a high sensitivity to Ca^{2+} after incorporation into the lipid bilayer. However, if the channels are dephosphorylated, either by activation of an endogenous phosphatase or by addition of an unspecific exogenous phosphatase, they loose their high Ca^{2+} sensitivity. The channels regain their high sensitivity to Ca^{2+} after rephosphorylation catalyzed by protein kinase A, while there is no effect of protein kinase C.

K^+ currents in several types of cells have been shown to be affected by changes in the activity of protein kinase A (DePeyer et al., 1982; Ewald, Williams & Levi-

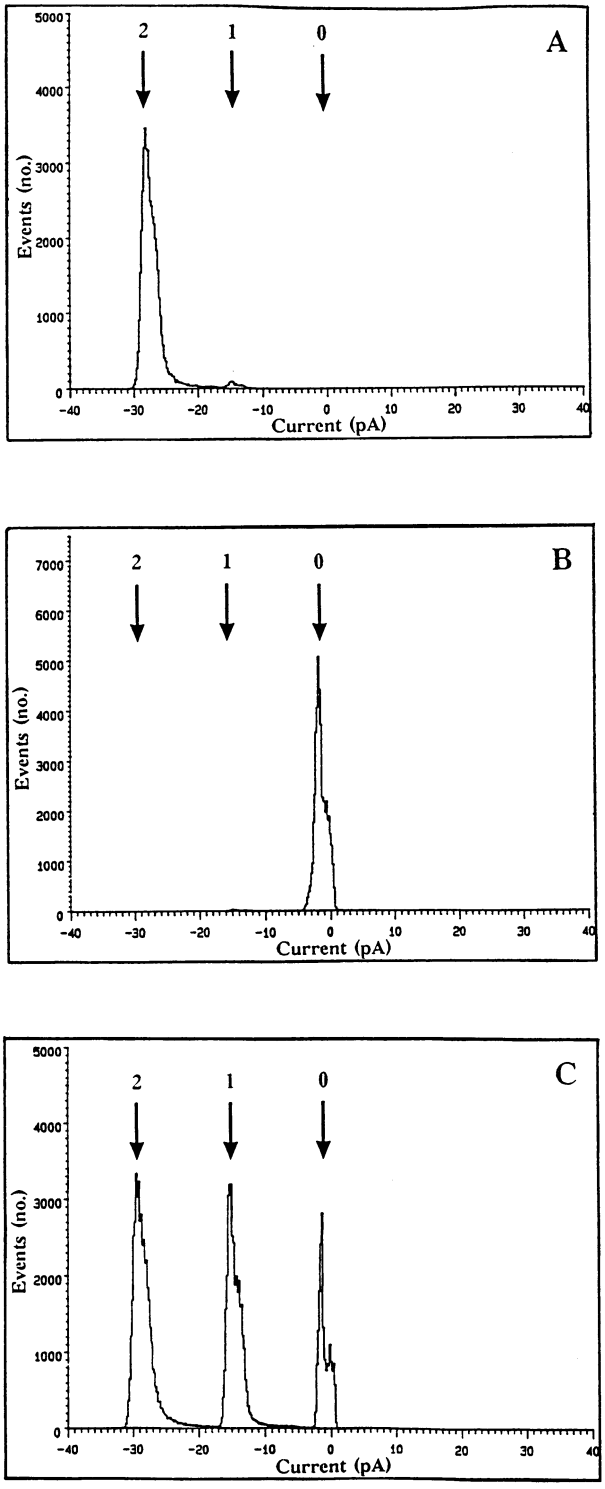

Fig. 5. Frequency histograms for maxi K^+ channels before and after phosphorylation. The figure shows a frequency histogram for the two channels in the experiment shown in Fig. 3. At 200 μM free Ca^{2+}, both channels were open (*A*), but when the free Ca^{2+} is taken down to 200 nM, the channels were inactive (*B*). After phosphorylation, the channels could be reactivated (*C*). The current levels for closed channels, for one open channel and for two open channels are shown with arrows.

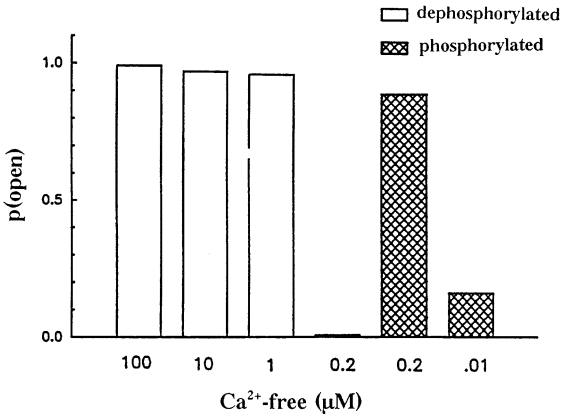

Fig. 6. Ca^{2+} dependence of a maxi K^+ channel before and after phosphorylation. A maxi K^+ channel was incorporated into the bilayer as in Fig. 1 and the free Ca^{2+} concentration was decreased to 200 nM (open bars). After phosphorylation as in Fig. 3, the channel regained its activity at 200 nM, but the channel activity was reduced when the free Ca^{2+} concentration was decreased to 10 nM (crossed bars).

tan, 1985; Lechleiter, Dartt & Brehm, 1988; Kume, Tokuno & Tomita, 1989; Sikdar, McIntosh & Mason, 1989; Suzuki, Ono & Takahira, 1992) or protein kinase C (Baraban, Snyder & Alger, 1985; Doerner, Pitler & Alger, 1988; Kaczmarek, 1988; Shearman, Sekiguchi & Nishizaha, 1989). In the case of delayed rectifier K^+ channels and Ca^{2+}-activated K^+ channels, protein-kinase A-mediated changes in K^+ channel activity have been suggested to result from direct phosphorylation of the channel proteins (*for review, see* Levitan 1994). In kidney tubule cells (Klærke, Karlish & Jørgensen, 1987; Reeves et al., 1989), pancreatic duct cells (Gray et al., 1990) and heart sarcolemma vesicles (Wen et al., 1984) Ca^{2+}-activated K^+ channels are upregulated by phosphorylation catalyzed by protein kinase A. However, in the central nervous system, it has been shown that Ca^{2+}-activated K^+ channels can be either upregulated (Ewald et al., 1985; de Peyer et al., 1982; Bielefeldt & Jackson, 1994) or downregulated (Reinhart et al., 1991) by phosphorylation catalyzed by protein kinase A. Several types of maxi K^+ channels may exist in the same tissue; Reinhart et al. (1991) have recently identified two populations of maxi K^+ channels in plasma membrane vesicles from rat brain; one population (type 1) is upregulated by protein kinase A whereas the other is downregulated (type 2). Type 1 shows fast opening kinetics and is inhibited by charybdotoxin, while type 2 shows slow opening kinetics and is insensitive to charybdotoxin. The Ca^{2+}-activated K^+ channels from the rabbit distal colon epithelium are upregulated by phosphorylation, and we have previously shown that they are very sensitive to charybdotoxin (Klærke et al., 1993). With the terminology suggested by Reinhart et al. (1991) the colon Ca^{2+}-activated K^+ channels can therefore be characterized as type 1 maxi K^+ channels.

Regulation of ion channels by phosphorylation is usually catalyzed by just one type of protein kinase, and only rarely the same ion channel type can be phosphorylated by different types of protein kinases (Shearman et al., 1989; Levitan, 1994). In the present study, only protein kinase A activates the Ca^{2+}-activated K^+ channels whereas protein kinase C does not modulate the channel activity. This indicates that in the rabbit distal colon epithelium, regulatory pathways involving protein kinase A are important for regulation of the basolateral K^+ conductance.

Comparison with Cloned Channels

The Ca^{2+}-activated maxi K^+ channels are predicted to consist of 4 subunits each having 6 transmembrane segments (S1–S6) with the pore-forming region between S5 and S6, and a long cytoplasmic C-terminus (Adelman et al., 1992; Atkinson, Robertson & Ganetzky, 1991; Butler et al., 1993). This C-terminal part of the protein contain the putative Ca^{2+} binding sites and several possible phosphorylation sites (Adelman et al., 1992; Butler et al., 1993). The fact that the phosphorylation sites are situated close to the Ca^{2+} binding sites but far away from the conductive pore corresponds well with our finding that phosphorylation or dephosphorylation of the channel protein actually affect the Ca^{2+}-activation without altering the channel conductance.

Activation of closed channels by phosphorylation can occur in either of two ways: the channel can be activated instantly or the activation can take place after a short flicker period. Analysis for consensus amino acid sequences reveals several possible phosphorylation sites. This opens the possibility that instant activation of the channels is seen after phosphorylation of all target sites, while the transient flicker period could reflect phosphorylation of a fraction of the phosphorylation sites.

A recent report has shown that the slowpoke channel is downregulated by phosphorylation by protein kinase A and keeping in mind that this channel is insensitive to charybdotoxin, the slowpoke channel must be a type 2 maxi K^+ channel (Perez et al., 1994). The mSlo channel, in contrast, is sensitive to charybdotoxin (Butler et al., 1993), and could therefore be a type 1 channel. However, the effect of protein kinase A catalyzed phosphorylation on the mSlo channel remains to be examined, and it is therefore unknown whether this channel resembles the maxi K^+ channel from epithelia described in the present paper.

Effects of Ca^{2+} Sensitivity

Our results show a clear change in Ca^{2+} sensitivity of the maxi K^+ channels with a shift in the Ca^{2+} sensitivity of about one order of magnitude after phosphorylation. This means that at certain Ca^{2+} concentrations and voltages (e.g., 200 nM free Ca^{2+} and a voltage of 0 mV), phosphorylation of the channel protein changes the channel from being closed to being almost totally open. This is a large effect compared to the effect on Ca^{2+}-activated maxi K^+ channels from other tissues where the effect of phosphorylation is limited to a 2–4 fold change in open probability (*for review, see* Levitan 1994). It will be interesting to learn if the different responses to phosphorylation in a similar way reflect structural differences in amino acid sequence e.g., resulting from alternative splicing of RNA. Recently, it has been shown that alternative spliced variants of the *slowpoke* channel show considerable differences regarding single channels conductance, Ca^{2+} sensitivity and gating (Lagrutta et al., 1994).

Physiological Role of Phosphorylation and Dephosphorylation

From studies on intact epithelia, it is known that K^+ channels are responsible for a significant part of the basolateral K^+ conductance (Dawson & Richards, 1990). Since recycling of K^+ across the basolateral membrane is important for the transepithelial transport of Na^+, it is necessary that the basolateral K^+ channels are under strict control, e.g., by hormones. Studies on intact colon have shown that the basolateral K^+ conductance is increased during aldosterone stimulation (Dawson, 1991) and Ca^{2+} is an obvious mediator of second messenger signals after hormonal stimulation. However, our results show that the channel protein must be in a phosphorylated state to respond to intracellular levels of free Ca^{2+}, and it is therefore obvious to propose that the cAMP-dependent protein kinase through modulation of the Ca^{2+} sensitivity of the K^+ channels plays an important role in regulation of the transcellular transport. Measurements of cAMP concentrations in the distal colon surface cells are not available, but it has been shown that K^+ channel cells isolated from distal colon crypt cells can be stimulated by a rise in intracellular cAMP (Loo et al., 1989). In other epithelia such as thick ascending limb of Henle's loop in the kidney, the intracellular levels of cAMP are increased after hormonal stimulation (Morel Imbert-Teboul & Chabardes, 1981; Rasmussen et al., 1986).

The channels examined in this study are already in a phosphorylated state in our plasma membrane preparation. Thus, demonstration of the effect of protein kinase A requires dephosphorylation by activation of endogenous phosphatases or by addition of phosphatase. This indicates that in the native epithelia, the channels are in a state where they may be activated by Ca^{2+} in intracellular concentrations, supporting the notion that they play an important role in regulation of the transepithelial transport. Similar phenomena have been observed for

Ca^{2+} and Na^+ channels, where biochemical studies have shown that protein kinase A often produces little or no phosphorylation of channel proteins because the target residue has already been phosphorylated (Wen et al., 1984; Rossie & Catterall, 1989; Emerick & Agnew, 1989).

In conclusion, the correlation between the phosphorylation state and the Ca^{2+} sensitivity of the channels could be one explanation for the large differences in the Ca^{2+} sensitivity observed for maxi K^+ channels from different tissues (Latorre et al., 1989) and even for channels in different studies on the same tissue. Moreover, our results show that at the values for Ca^{2+}, pH and voltage found in the resting cell, the K^+ channels may be opened and closed by phosphorylation and dephosphorylation alone.

The authors thank Tove Soland for valuable technical assistance. The work was supported by the Novo Nordisk Foundation, the Danish Medical Research Council, and the Austrian Research Council.

References

Alvarez, O. 1986. How to set up a bilayer system. *In:* Ion Channel Reconstitution. C. Miller editor. pp. 115–130. Plenum Press, New York

Adelman, J.P., Ke-Zhong, S., Kavanaugh, M.P., Warren, R.A., Wu Y-N., Lagrutta, A., Bond, C.T., North A. 1992. Calcium-activated potassium channels expressed from cloned complementary DNA's. *Neuron* **9:**209–216

Atkinson, N.S., Robertson, G.A., Ganetzky, B. 1991. A component of calcium-activated potassium channels encoded by the *Drosophila slo* locus. *Science* **253:**551–555

Baraban, J.M., Snyder, S.H., Alger, B.E. 1985. Protein kinase C regulates ionic conductance in hippocampal pyramidal neurons: electrophysiolotical effects of phorbol esters. *Proc. Natl. Acad. Sci. USA* **84:**2538–2542

Bielefeldt, K., Jackson, M.B. 1994. Phosphorylation and dephosphorylation modulate Ca^{2+}-activated Ca^{2+} channel in rat peptidergic nerve terminals. *J. Physiology* **475:**241–254

Bradford, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem* **72:**248–254

Butler, A., Tsunoda, S., Mccobb, D.P., Wei, A., Salkoff, L. 1993. mSLO, a complex mouse gene encoding ''maxi'' calcium-activated potassium channels. *Science* **261:**221–224

Dawson, D.C., Richards, N.W. 1990. Basolateral K conductance: role in regulation of NaCl absorption and secretion. *Am. J. Physiol.* **259:**C181–C195

Dawson, D.C. 1991. Ion channels and colonic salt transport. *Ann. Rev. Physiol.* **53:**321–339

DePeyer, J.E., Cachelin, A.B., Levitan, I.B., Reuter, H. 1982. Ca^{2+}-activated K^+ conductance in internally perfused snail neurons is enhanced by protein phosphorylation. *Proc. Natl. Acad. Sci. USA* **79:**4207–4211

Doerner, D., Pitler, T.A., Alger, B.A. 1988. Protein kinase C activators blocks specific calcium and potassium current components in isolated hippocampal neurons. *J. Neurosci.* **8:**4069–4078

Emerick, M.C., Agnew, W.S. 1989. Identification of phosphorylation sites for adenosine 3′,5′-cyclic phosphate dependent protein kinase on the voltage-sensitive sodium channel from *Electrophorus electricus. Biochemistry* **28:**8367–8380

Ewald, D., Williams, A., Levitan, I.B. 1985. Modulation of single Ca^{2+}-dependent K^+ channel activity by protein phosphorylation. *Nature* **315:**503–506

Gray, M.A., Greenwell, J.R., Garton, A.J., Argent, B.E. 1990. Regulation of maxi-K^+ channels on pancreatic duct cells by cyclic AMP-dependent. *J. Membrane Biol.* **115:**203–215

Kaczmarek, L.K. 1988. The regulation of neuronal calcium and potassium channels by protein phosphorylation. *Adv. Sec. Mess. Phosp. Res.* **22:**113–139

Klærke, D.A., Karlish, S.J.D., Jørgensen, P.L. 1987. Reconstitution in phospholipid vesicles of calcium-activated potassium channel from outer renal medulla. *J. Membrane Biol.* **95:**105–112

Klærke, D. A., Wiener, H., Zeuthen, T., Jørgensen, P.L. 1993. Ca^{2+} activation and pH dependence of a maxi K^+ channel from rabbit distal colon epithelium. *J. Membrane Biol.* **136:**9–21

Kume, H., Tokuno, H., Tomita, T. 1989. Regulation of Ca^{2+}-dependent K^+ channels in tracheal myocytes by phosphorylation. *Nature* **341:**152–154

Lagrutta, A., Shen, K.-Z., North, R.A., Adelman, J.P. 1994. Functional differences among alternatively splices variants of *slowpoke,* a *Drosophila* Calcium-activated potassium channel. *J. Biol. Chem.* **269:**20347–20351

Latorre, R., Oberhauser, A., Labarca, P., Alvarez, O. 1989. Varieties of calcium-activated potassium channels. *Ann. Rev. Physiol.* **51:**385–399

Lechleiter, J.D., Dartt, D.A., Brehm, P. 1988. Vasoactive intestinal peptide activates Ca^{2+}-activated K^+ channels through cAMP pathway in mouse lacrimal cells. *Neuron* **1:**227–235

Levitan, I. 1994. Modulation of ion channels by protein phosphorylation and dephosphorylation. *Ann. Rev. Physiol.* **56:**193–212

Loo, D.D.F., Kaunitz, J.D. 1989. Ca^{2+} and cAMP activate K^+ channels in the basolateral membrane of crypt cells isolated form rabbit distal colon. *J. Membrane Biol.* **110:**19–28

Morel, F., Imbert-Teboul M., Chabardes, D. 1981. Distribution of hormone dependent adenylate cyclase in the nephron and its physiological significance. *Am. J. Physiol.* **43:**569–581

Perez, G., Ottoha, M., Lagrutta, A., Adelman, J., Toro, L. 1994. Modulation of *slo* K_{Ca} channels by phosphorylation in lipid bilayers. *Biophys. J.* **66:**A342

Pershadsingh, H.A., McDonald, J.M. 1980. A high affinity calcium-stimulated magnesium-dependent triphosphatase in rat adipocyte plasma membrane. *J. Membrane Biol.* **105:**95–111

Rasmussen, H., Kojima, I., Apfeldorf, W., Barrett, P. 1986. Cellular mechanism of hormone action in the kidney: Messenger function of calcium and cyclic AMP. *Kidney Int.* **29:**90–97

Reeves W.B, McDonald G.A, Metha P, Andreoli T.E. 1989. Activation of K^+ channels in renal medullary vesicles by cAMP-dependent protein kinase. *J. Membrane Biol.* **109:**65–72

Reinhart, P.H., Chung, S., Martin, B.L., Brautigan, D.L., Levitan, I. 1991. Modulation of calcium-activated potassium channels from rat brain by protein kinase A and phosphatase 2A. *J. Neurosci.* **11:**1627–1635

Rossie, S., Catterall, W.A. 1989. Phosphorylation of the alfa-subunit of rat brain sodium channels by cAMP-dependent protein kinase at a new site containing Ser^{686} and Ser^{687} *J. Biol. Chem.* **264:**14220 – 14224

Shearman, M.S., Sekiguchi, K., Nishizuka, Y. 1989. Modulation of ion channel activity: a key function of the protein kinase C enzyme family. *Pharmacol. Rev.* **41:**211–237

Sikdar, S.K., McIntosh, R.P., Mason, W.T. 1989. Differential modulation of Ca^{2+}-activated K^+ channels in ovine pituitary gonadotrophs by GnRH, Ca^{2+} and cyclic AMP. *Brain Res.* **496:**113–123

Smith, P.L., McCabe, R.D. 1984. Mechanism and regulation of transcellular potassium transport by the colon. *Am. J. Physiol.* **247:**G445–G456

Schultz, S.G. 1984. A cellular model for active sodium absorption by mammalian colon. *Ann. Rev. Physiol.* **46:**435–451

Suzuki, K., Onoe, K., Takahira, H. 1992. Activation of Ca^{2+}-dependent K^+ channel and Cl^- conductance in canine pancreatic acinar cells through a cyclic AMP pathway. *Jap. J. Physiol.* **42:**267–281

Turnheim, K., Costantin, J., Chan, S., Schultz, S.G. 1989. Reconstitution of a calcium-activated potassium channel in basolateral membranes of rabbit colonocytes into planar lipid bilayer. *J. Membrane Biol.* **112:**247–254

Wen, Y., Famulski, K.S., Carafoli, E. 1984. Ca^{2+}-dependent K^+ permeability of heart sarcolemma vesicles. Modulation by cAMP-dependent protein kinase activity and by calmodulin. *Biochem. Biophys. Res. Commun.* **122:**237–243

Wiener, H., Klaerke, D.A., Jørgensen, P.L. 1990. Rabbit distal colon epithelium III. Ca^{2+}-activated K^+ channels in basolateral plasma membrane vesicles of surface and crypt cells. *J. Membrane Biol.* **117:**275–283

Wiener, H., Turnheim, K., Os, C.H., van. 1989. Rabbit distal colon epithelium: I. Isolation and characterization of basolateral plasma membrane vesicles from surface and crypt cells. *J. Membrane Biol.* **94:**147–162