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The K^+ Channels of Colonic Crypts and Their Regulation

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INTRODUCTION

Like any other cell the colonic crypt cell possesses K^+ channels. These channels in general serve to hyperpolarize the cell. In fact, a certain hyperpolarization of the colonic crypt cell is required to serve its main transport functions, namely Na^+ absorption as well as Cl^- secretion (Greger et al, 1997). These transport functions can occur within one cell, but besides the cells along the crypt axis are specialized in the sense that absorption of Na^+ (and Cl^-) is a main function of the surface cells, whilst secretion of Cl^- (and Na^+) is a main function of the crypt base cells. In addition, the colon serves other transport functions (Conigrave et al, 1996). In the present context its ability to secrete or absorb K^+ is of specific importance (Binder & Sandle, 1994). Needless to say that all these functions must be coordinated by innervation and hormones (Conigrave et al, 1996).

One of the most intriguing regulatory changes is the inversion of vectorial transport: Na^+ absorption in the presence of aldosterone and Cl^- secretion in the presence of e.g. prostaglandin E_2 (PGE_2). This redirection requires that the Cl^- channels activated by PGE_2 in the luminal membrane shut down the Na^+ channels present and function otherwise in this membrane (also cf. Fig. 6). This type of crosstalk between membrane transport proteins has indeed been recently demonstrated in expression systems and, even more importantly, in the mid crypt cells (Ecke et al, 1996).

The present short review will deal specifically with the types of K^+ channels present in the basolateral membrane and their role in electrolyte transpost. Four very recent types of observations have attracted our attention: i.) in the presence of agonists acting via cAMP the K^+ channels present in the basolateral membrane are different from those present under control conditions or with agonists acting via Ca^{2+} and PKC/DAG; ii.) the cromanols such as 293B inhibit one of these conductances specifically; iii.) the cromanol inhibited K^+ channel appears to be identical to the K_vLQT1-I_{sK} present in cardiomyocytes and other cells; iv) the other type of K^+ conductance appears exclusively Ca^{2+} regulated in the physiological reange of Ca^{2+} activities and this conductance is shut down by cAMP and inhibited by clotrimazol.

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The different types of K^+ channels in the basolateral membrane of the colon

Up until now three major types of K^+ channels have been reported for the intact colonic crypt (Burckhardt & Gögelein, 1992; Greger et al, 1997): One is the so called big K^+ channel (BK) with approximately 300 pS single channel conductance in symmetrical 250 mmol/l K^+ solutions (Burckhardt & Gögelein, 1992). This channel is very rare in our hands. We have seen it in far more than 1000 recordings only on a few occasions and we believe that the Ca^{2+} activation curve of this channel explains our findings. Usually around >1 μmol/l of ionized Ca^{2+} is required to activate this channel.

Another K^+ channel has been seen regularly in almost every sigle cell attached patch of the basolateral membrane. This channel has a conductance of ca. 16 pS under cell attached conditions (Greger et al, 1997). A typical recording is shown in Fig. 1 (Bleich et al, 1996). This channel is usually very active immediately after the seal is formed and the open probability falls thereafter. We have explained

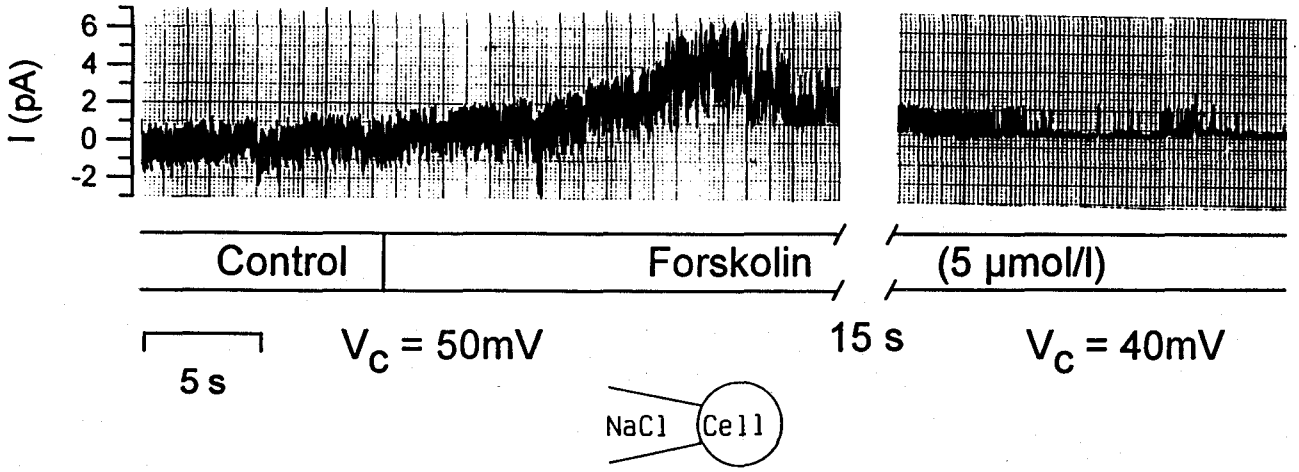

Fig. 1. Effect of increasing cytosolic cAMP (by forskolin) on the K^+ channels in a cell attached patch of the basolateral membrane of a rat colonic crypt base cell (Bleich et al, 1996). A single channel with approximately 1 pA corresponding to 15 pS single channel conductance is apparent under control conditions. After addition of forskolin the current amplitude initially increases. Thereafter the K^+ channel disappears. This type of small conductance K^+ channel is characteristic for these cells. Its open probability is increased by increases in cytosolic Ca^{2+} activity (cf. Fig. 4). V_c = clamp voltage.

this observation by a local increase in the Ca^{2+} activity caused by suction and seal formation. A few minutes after seal formation the local Ca^{2+} activity apparently falls and with it the open probability of this channel. Any agonist induced increase in cytosolic Ca^{2+} activity activates this channel (cf. below). These channels are probably identical to the recently cloned hsK-channel (human small K^+ channel).

This type of K^+ channel is downregulated when secretion is stimulated by cytosolic cAMP formation (e.g. addition of PGE_2, forskolin, dibutyryl-cAMP, IBMX). This is also shown in Fig. 1. A close inspection reveals that initially the current amplitude of the single current events increases. This can be explained by an increase in driving force for the K^+ current. This increase in driving force is caused by cell depolarization, which in turn is caused by the cAMP-dependent opening of Cl^- channels (Greger, 1996). The downregulation of open probability is also apparent in Fig. 1. This downregulation comes with a delay of several min.

With this channel closed Cl^- secretion can only go on because another type of K^+ channel is activated under these conditions. This channel cannot be seen in Fig. 1 because its single channel conductance is too small to be revealed in normal heavily filtered recordings. We have analyzed the current traces under the conditions of Fig. 1 and also of excised inside/out patches by Lorentzian analysis (Warth & Greger, 1993) and have found that they are characterized by K^+ channels of very small conductance. These channels probably possess a single channel conductance in the low pS range or even lower (Warth & Greger, 1993). Very many of these channels are present in the usual area of a cell attached patch. We have proven recently that this channel is formed by a protein named K_vLQT1, according to its role in cardiomyocytes to repolarize. A defect of this channel therefore causes a long Q-T-syndrome in the electrocardiogram (LQT) (Barhanin et al, 1996; Deal et al, 1996; Suessbrich et al, 1996). The mechanisms of activation of K_vLQT1 channels in colonocytes is unclear. It probably involves cAMP- (and hence PKA-) mediated activation.

Cromanols such as 293B inhibit the small K_vLQT1 channel

Several years ago we have designed a new type of pharmacological tool which inhibits cAMP-mediated Cl^- secretion (Lohrmann et al, 1995). The structure of 293B is shown in Fig. 2. It has some similarity to K^+ channel openers such as cromakalim, although this compound does not exert any effect on colonic Cl^- secretion. Recently we coluld show that 293 blocks Cl^- secretion by inhibiting a K^+ conductance

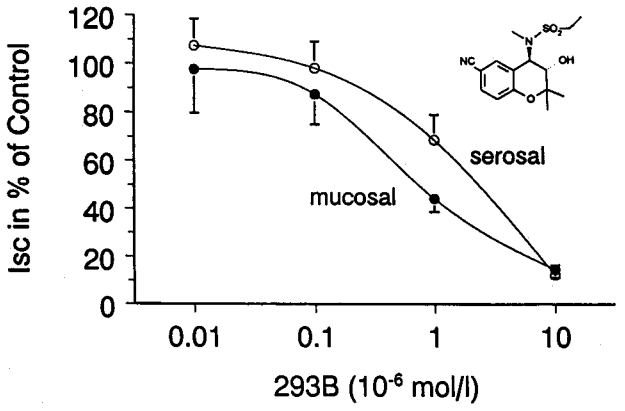

Fig. 2. The cromanol 293B inhibits the Cl^- secretory current in rabbit distal colon. The equivalent short circuit current in percent of control is plotted semilogarithmically versus the concentration of 293B added to the mucosal or serosal side. 293B blocks Cl^- secretion concentration dependently with an IC_{50} of approximately 0.3~0.5 μmol/l. Its inhibition occurs not via Cl^- channels but by a specific type of basolateral K^+ conductance (Lohrmann et al, 1995). This channel has now been identified as K_vLQT1 (Bleich et al, 1997; Busch et al, 1997).

in the basolateral membrane (Ecke et al, 1995). This seems perplexing at first glance but it is easily explainable by the fact the K^+ recycling across the basolateral membrane is an absolute requirement for Cl^- secretion to occur: the hyperpolarization caused by this K^+ conductance is required to extrude Cl^- into the lumen inasmuch as it provides the driving force. Stated differently: for each anion (Cl^-) leaving the cell a cation (K^+) must also leave. Hence, it does not matter whether Cl^- secretion is blocked by an inhibitor of Cl^- or of K^+ channels. Anyhow Cl^- secretion must cease.

The target of 293B is a K^+ conductance, but it was unclear for some time which K^+ channel it corresponds to. An important clue came from experiments in which the I_{sK} proten (also called min-K) was expressed in oocytes. It was shown that 293B blocked the K^+ currents produced by I_{sK} expression, although these currents have otherwise largely differing biophysical properties (Suessbrich et al, 1996). A little more than a year ago a new type of K^+ channel has been identified on a molecular basis (Barhanin et al, 1996; Sanguinetti et al, 1996). This channel was named K_vLQT1 (cf. above). It binds to the IsK protein to form a K^+ current, which resembles the delayed outward rectifier (I_{Ks}), e.g. in the heart. This data is interesting in two respects: it is clear now that I_{sK} does not form a channel by itself but that is dimerizes (?) with K_vLQT1 to form the respective K^+ channel; secondly these findings open the possibility that K_vLQT1 or the K_vLQT1-I_{sK} complex is the target for 293B. This has been examined directly by injecting cRNA for K_vLQT1 into oocytes (Bleich et al, 1997; Busch et al, 1997). It was found that K_vLQT1 induces a K^+ conductance which was reversibly inhibited by 293B. The co-expression of K_vLQT1 and I_{sK} shifted the concentration response curve to the left (Busch et al, 1997), suggesting that both components may be required for 293B inhibition. This class of substances appears to be rather specific inasmuch as it does not inhibit many other K^+ channels (Busch & Suessbrich, 1997).

The cromanol inhibited K^+ channel appears to be identical to the K_vLQT1 present in cardiomyocytes and other cells and it resembles a very small conductance K^+ channel

The above experiments indicate that the target of 293B is the K_vLQT1-I_{sK} channel. This channel has peculiar biophysical properties. In the heart they show delayed outwardly rectifying behavior ((Deal, 1996). Surprisingly, no slow channel activation was apparent in colonic crypt cells (unpublished from the author's laboratory). We have no ready explanation for this discrepancy. Thus far we have been unable to demonstrate significant amounts of I_{sK}-mRNA in colonocytes which raises the possibility that another I_{sK}-type module interacts with K_vLQT1 in colonocytes and that this heterodimer has distinct biophysical properties.

The K_vLQT1 channel appears to have a very small single channel conductance. We have analyzed the respective K^+ currents in cell attached and cell-free patches of the basolateral membrane of colonocytes by noise analysis. Our data (Fig. 3) suggests that the single channel conductance is in the low probably 1~2 pS range (Warth et al, 1996). Preliminary studies by other investigators support this view. This data explains why this type of channel has been overlooked in previous studies searching for single K^+ channels.

The mechanism of K_vLQT1 activation in colonocytes and in oocytes over-expressing this protein is not defintively settled. It appears likely that the cAMP cascade activates this current. In the intact

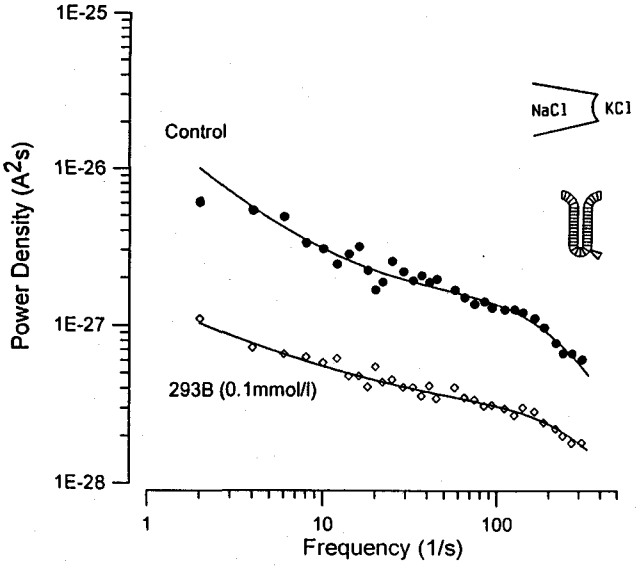

Fig. 3. Lorentzian analysis of the K^+ channel noise induced in an excised patch of a previously cAMP-stimulated rat colonic crypt base cell (Warth et al, 1996). The noise is caused by very small K^+ channels with very low sigle channel conductance (closed circles). These channels are blocked reversibly by 293B (open squares).

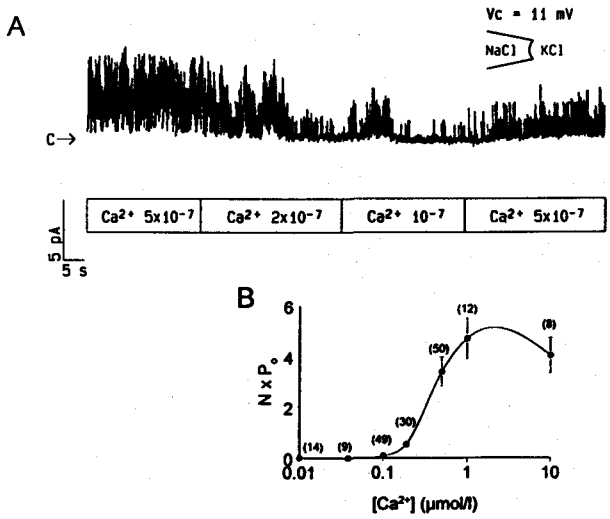

Fig. 4. Ca^{2+} dependence of small K^+ channels in excised patches of the basolateral membrane of rat colonic crypt base cells (Bleich et al, 1996). A. Typical cell excised recording V_c=clamp voltage. Note that the open probability (Po) of these channels is reduced with falling Ca^{2+} activity on the cytosolic side. B. The channel activity ($M \times Po$) is plotted versus the Ca^{2+} activity on the cytosolic side (mean values ± SEM (n)). $N \times Po$ of these channels is up-regulated by Ca^{2+} in the physiological range of Ca^{2+} activities. Half maximal activation occurs at 300 ~ 500 nmol/l.

colon agonists acting through elevation of cytosolic Ca^{2+} do not induce a 293B inhibitable K^+ conductance, yet agonists acting through cAMP activate this conductance. cAMP-activation of the 293B inhibitable K^+ conductance has originally been reported for a cell line derived from the pancreatic duct of a patient with CF and pancreatic carcinoma (Warth & Greger, 1993). Whether this activation is directly on the respective proteins or whether it is mediated by some other regulatory protein is unclear at present.

The small Ca^{2+}-activated K^+ conductance

Under resting conditions and in the presence of agonists such as carbachol or ATP most of the basolateral K^+ conductance is caused by small Ca^{2+} regulated K^+ channels (Bleich et al, 1996; Greger et al, 1997). The open probability of these channels appears to be directly regulated by cytosolic Ca^{2+} because the Ca^{2+} dependence can be demonstrated in excised membrane pathches (Fig. 4). Half maximal activation is seen at approximately 0.3 µmol/1 Ca^{2+}. This property explains why agonists mobilizing Ca^{2+} from cytosolic stores and increasing Ca^{2+} influx across the plasma membrane activate these channels. Conversely, reduction of cytosolic Ca^{2+} activity inactivates these channels. In colonocytes such as HT_{29} cells and in the colonic crypt cytosolic Ca^{2+} activity is reduced by depolarization (Fischer et al, 1996; Greger et al, 1997; Leipziger et al, 1994). We have explained this fall in Ca^{2+} activity by the voltage dependence of the Ca^{2+} influx channel (Fischer et al, 1996). Fig. 5 shows measurements of cytosolic Ca^{2+} activity by the fura-2 method. An increase in bath K^+ concentration or the addition of cAMP, via the opening of the luminal Cl^- conductance, depolarizes these cells and reduces Ca^{2+} activity correspondingly. This is of specific importance when the Ca^{2+} influx channels are activated indirectly by agonisits such as ATP or carbachol.

We have shown in thus far unpublished studies that the mRNA for the human small K^+ channel (hsK) is present in colonocytes. Thus it appears attractive to hypothesize that the small Ca^{2+} activated K^+ channel is identical to hsK. This channel can be inhibited reversibly by clotrimazol (Devor et al, 1997). We have examined this channel also in response to volume regulation. When colonic crypt cells were shrunk by hypertonic solution Ca^{2+}

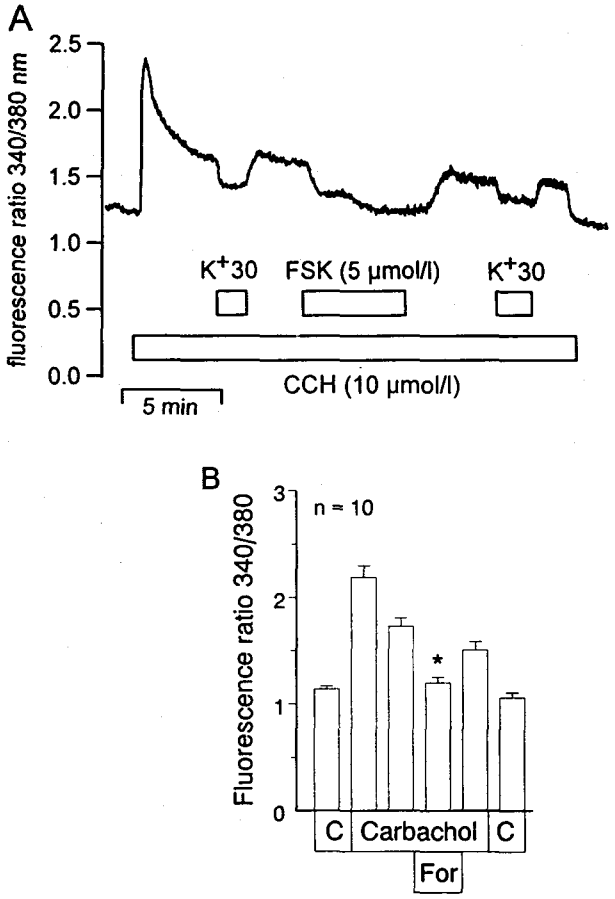

Fig. 5. Measurements of Ca^{2+} activity (via fura-2 fluorescence) in rat colonic crypts. A. Typical experiment. The fluorescence ratio 340/360 nm is shown as a function of time. Carbachol (CCH) induces a typical Ca^{2+} transient. In the plateau phase the fluorescence ratio is reduced reversibly when the cells are depolarized by an increase in bath K^+ concentration or by the addition of forskolin (FSK) to increase cytosolic cAMP. B. Summarizes a series of similar experiments. Mean values ± SEM. C=control, For=forskolin 5 µmol/l. The effect of forskolin is statistically significant (*). Physiologically depolarization occurs by the cAMP-induced opening of Cl^- channels. Therefore, cAMP reduces cytosolic Ca^{2+} activity and attenuates the open probability of the Ca^{2+}-activated small K^+ channel.

activity fell and this channel was downregulated (Weyand et al, 1998). In excised patches these channels frequently showed a run-down of activity. Their activity could be refreshed in a good fraction of the patches by addition of ATP to the bath solution. These data suggest that this channel is also up-regulated by phosphorylation (Nielson et al, 1998). The phosphorylation mechanisms are unclear at this stage.

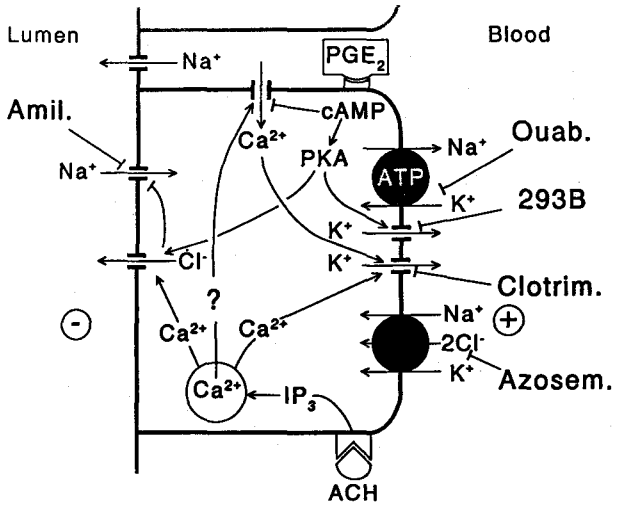

Fig. 6. Scheme of the K^+ channel regulation in rat colonic crypt cells (Greger et al, 1997). Circle with ATP=($Na^+ + K^+$)-ATPase; circle=$Na^+2Cl^-K^+$ cotransporter; arrows = ion channels. PGE_2 = prostaglandin E_2; PKA = proteinkinase A; ACH = acetylcholine; Ouab. = ouabain; Clotrim. = clotrimazole; Azosem. = azosemide (a loop diuretic corresponding to furosemide); Amil. = amiloride. Note that cAMP activates luminal Cl^- channels, reduces indirectly (depolarization) Ca^{2+} influx, and up-regulates K_vLQT1- type K^+ channels. Ca^{2+} activates the basolaterally (and luminally, not shown) localized K^+ channels.

CONCLUSION

Apart from the above K^+ channels the colonic crypt cell probably possesses other K^+ channels. In the luminal membrane we have identified a K^+ conductance which is activated by the luminal application of ATP and by increases in cytosolic Ca^{2+} (Kerstan et al, 1998). This luminal K^+ channel may serve for K^+ secretion under certain conditions (Binder & Sandle, 1994; Butterfield et al, 1997). Its nature, on the basis of single channel recordings, has thus far not been examined in our laboratory. The present report indicates that two types of K^+ channels are present in the basolateral membrane (Fig. 6). They are regulated differentially. The Ca^{2+} regulated small K^+ channel is activated by increases in cytosolic Ca^{2+}. This channel therefore assists Cl^- secretion by agonists such as ATP and carbachol (acetylcholine). This channel is closed in the presence of cAMP because the depolarization occurring then reduces cytosolic Ca^{2+} activity. Only under these conditions another K^+ channel the K_vLQT1-type

channel takes over. This channel enables Cl^- secretion to occur whenever the Ca^{2+}-dependent K^+ channel is inactivated. This channel is the target of the new cromanols. Inhibition of this channel abolishes Cl^- secretion completely (Lohrmann et al, 1995). Further studies will have to examine the following questions: whether the small K^+ channel is in fact identical to hsK; how this channel is regulated by phosphorylation; whether K_vLQT1 binds to another I_{sK} like module in the colon; how K_vLQT1 is regulated; what type of K^+ channel is present in the luminal membrane and how this channel is regulated.

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