K^+ CHANNELS WITH VERY SMALL CONDUCTANCE IN THE BASOLATERAL MEMBRANE OF THE RECTAL GLAND OF *SQUALUS ACANTHIAS*

Markus Bleich, Martin J. Hug, Dirk Heitzmann and Rainer Greger
Physiologisches Institut, Albert-Ludwigs-Universität, Freiburg, Germany

Recently we have reported that the basolateral membrane of colonic crypts contains two types of K^+ channels: one has a mean conductance of around 16 pS and is directly activated by cytosolic Ca^{2+}. It is this channel which is activated by acetylcholine or ATP (Bleich, M. et al. Pfluegers Arch Eur J Physiol 432:1011-1022, 1996). The other type of K^+ channel has a very small single channel conductance and is activated with some delay by cAMP. The current produced by this channel causes fluctuations but no discernible single channel events. Noise analysis revealed that its conductance is in the 1-2 pS range or even lower. This channel can be blocked reversibly by the cromanol 293B (Lohrmann, E. et al. Pfluegers Arch Eur J Physiol 429:517-530, 1995, Warth, R. et al. Pfluegers Arch Eur J Physiol 432:81-88, 1996). Shortly thereafter we have shown that K_VLQT1, which has been cloned from cardiac cells by Barhanin, J. et al. (Nature 384:78-80, 1996), is the molecular correlate of this current (Bleich, M. et al. Pfluegers Arch Eur J Physiol 434:499-50, 1997). Last year we have cloned a shark equivalent of K_VLQT1 (s-K_VLQT) (Waldegger, S. et al. MDIBL Bulletin 37:30-31, 1998) and found that this protein is expressed strongly in the rectal gland and in the heart. This prompted the question of whether K_VLQT1-type K^+ currents could also be found in isolated *in vitro* perfused rectal gland tubules of *Squalus acanthias* (RGT). Previously we had shown that the basolateral membrane of RGT possesses ROMK-type K^+ channels (Greger, R. et al. Pfluegers Arch Eur J Physiol 409:100-106, 1987). The open probability of these channels was not altered during stimulation and their density was less than expected for the macroscopic K^+ conductance of the basolateral membrane (Greger, R. et al. Pfluegers Arch Eur J Physiol 436:538-544, 1998).

RGT, pretreated by collagenase (Greger, R. et al. Pfluegers Arch Eur J Physiol 436:538-544, 1998) were perfused *in vitro* as described previously (Greger, R. and Schlatter, E., Pfluegers Arch Eur J Physiol 402:63-75, 1984). Measurements of equi-valent short circuit current (I_{sc}) and of basolateral membrane voltage (V_{bl}) were obtained as customary in this laboratory. Patch clamp recordings of the basolateral membrane were obtained in cell attached and excised mode. The patch pipette contained either a high K^+ (K^+ 278 mmol/l) or a low K^+ (4 mmol/l) shark Ringer-type solution. The bath was perfused with normal shark Ringer solution. When excised patches were studied the bath solution was changed to the high K^+ solution with various modifications (cf. below).

Transepithelial observations and impalement studies: All RGT were stimulated to secrete by 0.5 mmol/l cAMP, 0.5 mmol/l adenosine, and 10 µmol/l forskolin (Stim). Ba^{2+}, a well known K^+ channel blocker, inhibited I_{sc} and depolarized the basolateral membrane in a concentration-dependent manner. Low concentrations (53 µmol/l) reduced I_{sc} to 50 ± 6% (n = 9) and depolarized by 25 ± 2.5 mV (n = 9). Much higher Ba^{2+} concentrations of 10 mmol/l were required for more complete inhibition of I_{sc} to 11 ± 2% of control (n = 6) and for a depolarization by 43 ± 5

mV (n =5). This suggests that the K^+ conductance has two components: one with high Ba^{2+} sensitivity, probably ROMK, and one with low Ba^{2+} sensitivity, compatible with s-K_VLQT. Of the many other blockers examined (all in the recommended concentrations, n = 2 for I_{sc} and V_{bl}): veruculogen, MCD-peptide, margatoxin, tityustoxin, noxiustoxin, penitren, stichodactylotoxin, iberiotoxin, apamin, α-dendrotoxin, ß-dendrotoxin, γ-dendrotoxin, dendrotoxin I, agiotoxin and kalliotoxin none had any significant effect on I_{sc} and V_{bl}. Charybdotoxin (8 nmol/l) inhibited I_{sc} significantly by 24 ± 4% (n = 8). The cromanol 293B (0.1 mmol/l), TEA^+ (20 mmol/l), and 4-aminopyridine (0.1 mmol/l) were ineffective. However, clotrimazol (0.1 mmol/l) led to a 15 ± 6% (n = 6) inhibition.

Addition of NH_4^+/NH_3 (20 mmol/l) to the bath, which produces a marked intracellular acidification (Bleich et al. Pfluegers Arch Eur J Physiol 436:248-254, 1998), reduced I_{sc} to 10 ± 4% (n = 9) and led to a marked depolarization by 29 ± 3 mV (n = 9). Akalinization by the addition of HCO_3^-/CO_2 (20 mmol/l/2%, pH 7.5) increased I_{sc} significantly to 145 ± 14%. A reduction in bath Ca^{2+} to 0.01 mmol/l reduced I_{sc} by 35 ± 6% (n = 10). These data give a fingerprint of a complex K^+ conductance which is cromanol-insensitive, not blocked by any of the common channel blockers except Ba^{2+}, but regulated by Ca^{2+} and cytosolic pH.

Patch clamp data: With high K^+ in the pipette and in cell attached mode two types of K^+ channels were found: One was an inward rectifier with 27 ± 1.1 pS, reversal voltage 89.5 mV (n = 26), and the other was the ROMK-type with 89.5 ± 9.9 pS (n = 11), which we have seen in our previous studies (Greger et al. Pfluegers Arch Eur J Physiol 409:100-106, 1987).

With low K^+ in the pipette and in cell attached mode the ROMK-type K^+ channel was observed: 47 ± 5 pS (n = 5). In addition current noise was seen in most of the patches, which, on the basis of its current-voltage relation, could be identified as K^+ channel noise. Lorentzian analysis revealed that the K^+ channels producing this noise have a single channel conductance (with the assumption that the channels stay open for half of the time) of 2.2 ± 0.2 pS (n = 31, corner frequency 48 ± 7 Hz, power 38 ± 11 x 10^{-28} A^2s). This K^+ channel noise was activated by Stim in 4 experiments and was decreased by the reduction of bath Ca^{2+} to 0.01 mmol/l in 4 experiments. Ba^{2+} (10 mmol/l) inhibited this current (n = 4). In excised patches (n = 6) the mean conductance was 1.9 ± 0.5 pS. In these experiments the reduction of K^+ concentration on the cytosolic side from 278 to 4 mmol/l revealed a dramatic reduction of the K^+ channel noise. An example is shown in Fig. 1.

The present data indicate that the basolateral membrane of the rectal gland contains at least two types of K^+ conductances. One corresponds to the previously documented ROMK-type channel and is present under control and stimulated conditions. The other is made up of many K^+ channels of very small conductance (up to 10-50 per patch). This latter conductance is probably increased with stimulation. It is Ca^{2+} controlled and poorly inhibited by Ba^{2+}. It is tempting to speculate that this conductance corresponds to s-K_VLQT. In this case it is unclear why 293B acts so poorly on the in vitro perfused rectal gland tubule.

Supported by Deutsche Forschungsgemeinschaft: grant Gr 480/13 to RFG.

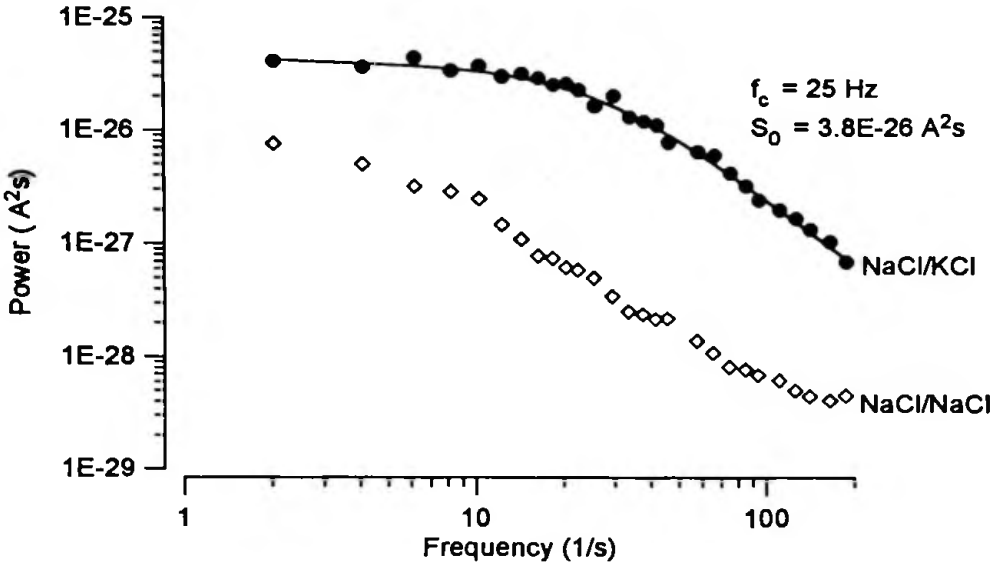

Figure 1: Lorenztian analysis of K^+ channel noise in an excised inside out patch of the basolateral membrane of a RGT. The pipette contained Na^+ -Ringer and the bath was changed from Na^+- to K^+-Ringer. Note the corner frequency in the presence of K^+ and the increase in the power of the noise.