EXPRESSION OF AN EPITHELIAL K^+ CHANNEL FROM WINTER FLOUNDER INTESTINE IN XENOPUS OOCYTES

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K^+ secretion or NaCl absorption across the intestinal epithelium of the winter flounder Pseudopleuronectes americanus occurs through the concerted actions of both apical and basolateral membrane ion transporting events. The apical membranes of these enterocytes contain both a Na/K/2Cl co-transporter and a K^+ conductance. The basolateral membrane has been shown to contain both a Na^+/K^+-ATPase and a Cl^- channel (Musch et al., Nature 300:351-353, 1982; O'Grady et al., Am. J. Physiol. 253:C177-C192, 1987). When intact flounder intestinal epithelium is placed in an Ussing chamber, transepithelial K^+ current, conductance and net K^+ transport can be inhibited by the addition of mM concentrations of Ba^{2+} to the mucosal, but not serosal bathing solution (Frizzell et al., Am. J. Physiol. 246:F9946-F951, 1984). This apical K^+ conductance, therefore, appears to be inhibited by Ba^{2+}. Other inhibitors of K^+ channels, for instance TEA (tetraethylammonium) (Musch et al., Bull. MDI Biol. Lab. 21:5-99; Sullivan et al., PNAS 87:4553-4556, 1990) have demonstrated at best partial blockade of this K^+ conductance at concentrations below 10 mM. Inhibitors such as charybdotoxin, apamin, capsaicin and 4-aminopyridine are unable to block this channel even at doses 2-fold higher than their effective inhibitory concentration on other K^+ channels (Sullivan et al., PNAS 87:4553-4556, 1990). Thus, we have utilized the selective Ba^{2+} sensitivity of this apical membrane K^+ conductance to initiate the cloning of this channel from winter flounder intestine using the frog oocyte expression system.

Total polyA^+ mRNA was obtained from freshly scraped flounder intestinal mucosa using a commercially available kit (In Vitrogen, FastTrack™), according to the manufacturer's instructions. Stage V and VI Xenopus laevis oocytes were isolated and their capacity to translate message assessed by injecting a cRNA encoding a secreted form of alkaline phosphatase as previously described (Cunningham et al., Am. J. Physiol. 262:C783-788, 1991). Oocytes injected with total mRNA were assayed for the expression of new conductances within 1-4 days by dual electrode voltage clamp. Total membrane current in control and mRNA-injected oocytes was recorded using standard Ringer's solutions as previously described (see Cunningham et al.).

1-2 days following the injection of 5-25 ng of flounder intestinal mRNA ($mRNA_{Fl}$) into oocytes, a tonically active conductance was observed. This new conductance was associated with a pronounced hyperpolarization of the oocyte's membrane potential (V_m) under standard NaCl bath gradients. The mean resting potential of control (H_2O-injected) oocytes was -51 ± 9 mV (S.D., n=23); in $mRNA_{Fl}$-injected oocytes, V_m hyperpolarized to -93 ± 15 mV (n=23). The reversal potential of this current remained at or near the predicted equilibrium potential for K^+ (E_K) when either external Cl^- was replaced with gluconate$^-$ (n=2) or when external Na^+ was replaced with N-methyl-D-glucamine$^+$ (n=2). Replacing external Na^+ for K^+ led to a depolarization of the membrane potential to 0 mV, indicating that the exogenous conductance was predominantly K^+-selective. The current-voltage relationship for this exogenous conductance was slightly inwardly-rectified (Fig. 1, control). No time-dependent activation was

observed for the current during the onset of voltage pulses. Thus, channel opening was voltage-insensitive and the expressed conductance was tonically active at all holding potentials.

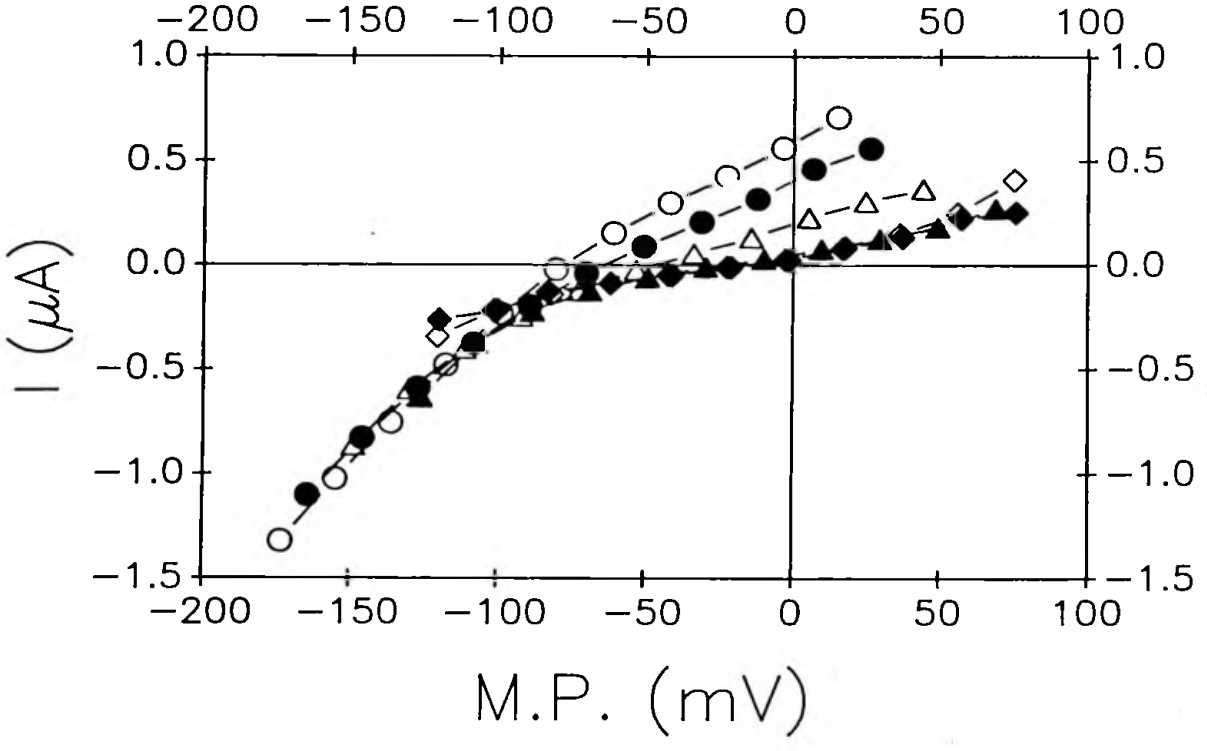

Figure 1. Current-voltage relationship for $mRNA_{Fl}$-injected oocytes. (open circles) Control, Ba^{2+}-free conditions; followed by the sequential addition of (closed circles) 20 μM Ba^{2+}, (open triangles) 200 μM Ba^{2+}, (closed triangles) 2 mM Ba^{2+}, (open diamonds) 10 mM Ba^{2+}, (closed diamonds) H_2O-injected oocytes. Currents were estimated after 5 min exposure to Ba^{2+}-containing solution.

Addition of Ba^{2+} to the external Ringer's solution resulted in a concentration-dependent blockade of the outwardly-directed $mRNA_{Fl}$ K^+ conductance and a return of the oocyte's membrane potential toward control values (Fig. 1). Ba^{2+} did not inhibit the much lower levels of endogenous current in H_2O-injected oocytes. When measured at 0 mV clamp potential, the concentration of drug required for 50% inhibition (IC_{50}) by Ba^{2+} was 70 μM. Blockade was reversible upon washout of Ba^{2+} from the bath (n=2). External TEA concentrations ranging from 0.1 to 5 mM had little effect on expressed $mRNA_{Fl}$ K^+ current (n=2). The Ba^{2+} sensitivity of this exogenous K^+ conductance was found to be slightly higher than that previously described for the flounder K^+ channel (160 μM, see Sullivan et al.).

These findings confirm that isolated flounder intestine mRNA contains a transcript or transcripts that encode for the apical membrane, Ba^{2+}-sensitive K^+ channel. We are now in the process of making a cDNA library in order to initiate the cloning of the mRNA which encodes this conductance. (Supported by NIH DK31091).