Potassium secretion in winter flounder (*Pseudopleuronectes americanus*) intestine: Effects of K^+ channel blockers on electrogenic NaCl absorption

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Marine teleosts like the winter flounder, maintain plasma osmolarities and serum K^+ concentrations (2 to 5 mM) that are significantly lower than sea water. Water balance and regulation of plasma Na^+ and Cl^- concentrations are primarily achieved through the coordinate absorptive and secretory functions of the small intestine and gill epithelium, respectively[18]. The ingestion of sea water, containing significantly higher $[K^+]$ ($\approx$ 10 mM) relative to plasma, poses the problem of increasing extracellular fluid $[K^+]$ to levels that could seriously compromise function of excitable tissues. To limit increases in plasma $[K^+]$, the ion is actively secreted by the small intestine and urinary bladder[4,5,6].

K^+ secretion by the intestinal epithelium occurs in parallel with NaCl and fluid absorption. The mechanism of NaCl transport is similar to that present in the thick ascending limb of Henle's loop in mammalian kidney[19]. Electroneutral uptake of Na^+, K^+ and Cl^- across the apical membrane is mediated by a Na-K-2Cl cotransporter that is thought to be homologous to the isoform previously characterized in mammalian kidney[22]. Na^+ entering across the apical membrane is transported across the basolateral membrane by the Na-K-ATPase. $[Cl^-]_i$, which is maintained above electrochemical equilibrium, exits the cell across the basolateral membrane through both conductive and electroneutral pathways. K^+ entering the cell by both Na-K-2Cl cotransport and Na-K-ATPase activities exits across the apical membrane through K^+ channels that have yet to be identified. Apical K^+ efflux is critical for sustaining the electrical driving force for Cl^- exit through Cl^- channels present in the basolateral membrane[7,11]. It also ensures that $[K^+]$ within the luminal fluid does not become rate limiting with respect to the activity of the Na-K-2Cl cotransporter.

The apical K^+ channel responsible for K^+ recycling and secretion in thick ascending limb cells of mammalian kidney was identified as the ROMK (Kir1.1) K^+ channel[12,21,23]. Kir1.1 is an inwardly rectifying K^+ channel[22] that is blocked by Ba^{2+} and Cs^+. Previously, whole cell amphotericin B perforated patch clamp studies using freshly dissociated winter flounder enterocytes failed to detect inwardly rectifying K^+ channels, but instead identified a Ba^{2+}-sensitive voltage-gated (Kv) K^+ channel[17]. This Kv channel was activated by membrane depolarization above -60 mV and exhibited slow, C-type inactivation similar to Kv channels identified in mammalian alveolar epithelium[15]. Analysis of activation and steady-state inactivation curves revealed that an overlap (window current) exists between -60 and -20 mV, indicating constitutive Kv channel activity within this range of voltages. The channel was blocked by charybdotoxin (CTX) and by treatment with 8-Br-cGMP[17].

The major objective of the present study was to characterize the effects of known K^+ channel blockers and peptide toxins on K^+ transport pathways in the apical membrane of the winter flounder intestine to obtain pharmacologic data that would aid in the molecular identification of channels involved in K^+ recycling and secretion. Intestinal mucosa, stripped of submucosal muscle layers, was mounted in Ussing chambers (area = 0.64 cm^2) and bathed on both aspects with flounder saline solution containing, (in mM) 150 NaCl, 5 KCl, $1CaCl_2$, $1MgSO_4$, $3Na_2HPO_4$, 5 HEPES, pH 7.8. Dextrose (10 mM) was added to the basolateral solution and 10 mM mannitol was added to the apical solution. The tissues were gassed with air and kept at a constant temperature of 15°C. Under these conditions the tissues exhibited a serosa negative transepithelial potential difference that ranged between 2-5 mV and a mean tissue conductance of 22.7 $\pm$ 0.73 mS (n = 25). Short circuit current (Isc)

responses are presented as means ± SE and were normalized to an area of 1 cm^2. Statistically significant differences between mean current values were determined using an unpaired Student's t test with the level of significance set at $p < 0.05$.

A representative Isc tracing showing the effects of apical Ba^{2+} (1 mM at each addition) and bumentanide (100 μM) is shown in Figure 1. Note that treatment with Ba^{2+} produced only a partial inhibition of the Isc and that at a concentration of 2 mM, maximal inhibition was achieved. Subsequent addition of the Na-K-2Cl cortansport inhibitor bumetanide produced a slow, but continuous inhibition of the Isc and reduced the total current by approximately 95%.

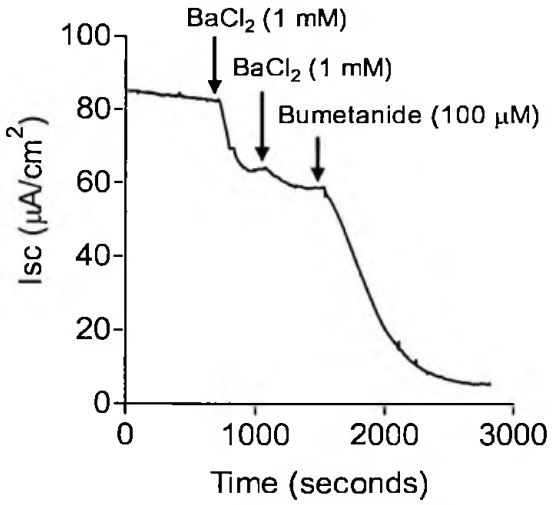

Figure 1: Representative Isc trace showing effects of apical Ba^{2+} and bumetanide.

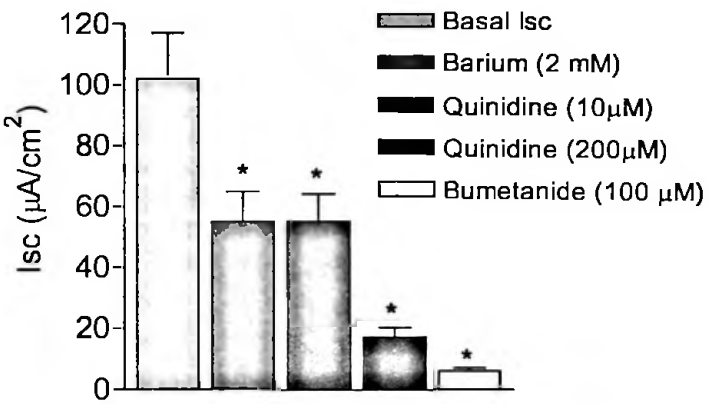

Figure 2: Bar graph summarizing the effects of Ba^{2+}, quinidine and bumetanide on Isc. (*) significantly different from the basal Isc.

A summary comparing the mean basal Isc with the effects of Ba^{2+}, quinidine and bumetanide is shown in Figure 2. Ba^{2+} at a concentration of 2 mM and quinidine at 10 μM produced approximately 50% inhibition of the Isc. Treatment with 200 μM quinidine or 100 μM bumetanide inhibited the basal Isc by more than 80% and 95%, respectively.

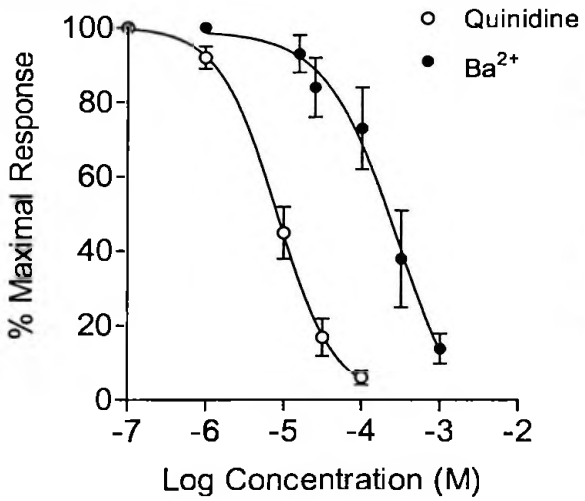

Figure 3: Concentration-response curves for Ba^{2+} and quinidine.

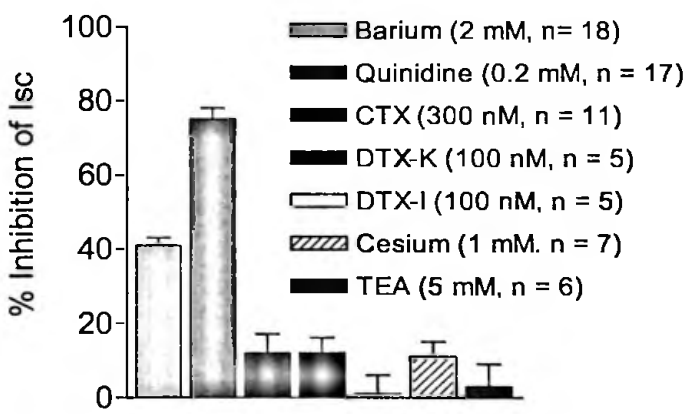

Figure 4: Effects of K^+ channel blockers on basal Isc.

Concentration response relationships for Ba^{2+} and quinidine are presented in Figure 3. Data were normalized by expressing the maximum change in Isc produced at the highest blocker concentration as 100%. The data were fit using a four parameter logistic function with Prism© software. The IC_{50} values for quinidine and Ba^{2+} were 8.2 ± 0.2 μM (n = 5) and 0.29 ± 0.03 mM (n = 5), respectively. Correlation coefficients (R^2) for both concentration-response relationships exceeded 0.99.

Figure 4 shows the effects of several K^+ channel blockers and peptide toxins on Isc. Compared to all blockers tested, quinidine (200 μM) was the most efficacious followed by Ba^{2+}. CTX and Cs^+, previously shown to inhibit Kv channel currents in dissociated winter flounder enterocytes, produced a modest 10% inhibition of Isc, similar in magnitude to the effects of dendrotoxin (DTX-K). In contrast, the effects of dendrotoxin-I (DTX-I) and TEA (5 mM) were not significantly different from zero.

The results of these experiments suggested that at least two distinct pathways for K^+ exit were present in the apical membrane of winter flounder enterocytes. One of them was blocked by Ba^{2+}, and the other appeared to be blocked by either Ba^{2+} or quinidine. Previous *in vitro* studies of winter flounder intestinal mucosa showed that Ba^{2+} significantly reduced apical membrane K^+ conductance, measured using conventional microelectrode techniques and decreased net K^+ secretion, as determined by isotopic flux measurements[7,11]. The results of the present study were consistent with these earlier findings, but indicated that 50% or more of basal NaCl absorption is sustained in the presence of 2 mM Ba^{2+}. Thus the quinidine-sensitive pathway (presumably a K^+ channel) appears to be necessary in supporting a significant portion of NaCl absorption. CTX, DTX-K and Cs^+ consistently inhibited 10% of the Isc response, suggesting the possibility that previously identified Kv channels in flounder enterocytes could be localized to the apical membrane, but if so, their role in sustaining NaCl absorption appears to be relatively minor.

The findings of this study may have significance in understanding the effects of certain heavy metals that accumulate within coastal marine sediments and benthic invertebrates adjacent to heavy industrial areas[24]. The observation that NaCl absorption by the winter flounder intestine is inhibited by cations such as Ba^{2+} suggests that certain metal cation contaminants may block intestinal salt and fluid absorption by inhibiting the activity of apical K^+ channels. Silver for example, when present as free silver ion or as silver chloride complexes, is one of the most toxic heavy metals affecting the physiology of freshwater and marine fishes[1,3,16]. Experiments with sculpin, trout, lemon sole European flounder, starry flounder and midshipmen previously showed that chronic exposure to low levels of silver resulted in significant accumulation within the intestine and that intestinal osmoregulatory function was a sensitive target for waterborne silver exposure[2,8-10,13,14]. Further studies are necessary to determine whether apical K^+ channels involved in K^+ recycling and secretion are specifically affected by heavy metal contaminants.

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