Effects of quinidine and other K^+ channel inhibitors on chloride secretion in the rectal gland of the spiny dogfish, *Squalus acanthias*

Sarah Decker[1], Carolina Klein[1], Martha Ratner[2], Catherine Kelley[3], Max Epstein[2], Kentrell Burks[4], William Motley[5], Alex Peters[2], and John N. Forrest Jr.[1]
[1]Department of Internal Medicine, Yale University School of Medicine, New Haven, CT 06510
[2]Mount Desert Island Biological Laboratory, Salisbury Cove, ME 04672
[3]Skidmore College, Saratoga Springs, NY 12866
[4]Morehouse College, Atlanta, GA 30314
[5]Middlebury College, Middlebury, VT 05753

Wendell Burger[2] first described the secretory function of the rectal gland of the spiny dogfish, *Squalus acanthias* in 1960. In rectal gland epithelial cells, apical CFTR chloride conductance is tightly coupled to basolateral K^+ conductance. Fluid secreted by the rectal gland contains negligible potassium, so the K^+ transported into the cell via the Na-K-2Cl cotransporter and the Na-K ATPase pump must exit the cell through basolateral K^+ channels to maintain cell polarization and the electrochemical gradient driving chloride secretion. Our laboratory has cloned two potassium channels from the shark rectal gland, K_vLQT[4,5] and KIR 6.1[6] but the specific identity of functional basolateral K^+ channels in this tissue remains unclear.

We sought to characterize further basolateral K^+ channels in shark rectal gland epithelial cells by examining responses to a variety of K^+ channel blockers during maximal stimulation of chloride secretion. Inhibitors were chosen based on their putative specificity in blocking specific types of K^+ channels. Effects were examined both in the *in vitro* perfused gland and in cultured monolayers of shark rectal gland epithelial cells. The channel inhibitors examined included clotrimazole and charybdotoxin, blockers of Ca^{2+}-activated K^+ channels, glybenclamide, tolbutamide, and phentolamine, blockers of ATP-sensitive K^+ channels, and quinidine, an inhibitor of voltage dependent Kv channels, KvLQT, HERG K^+ channels, the K_A channel, and volume sensitive K_{vol} channels. We compared these inhibitors to the effects of barium, a non-specific K^+ channel inhibitor known to block chloride secretion in the rectal gland.

Intact rectal glands were excised and perfused using methods described by Kelley et al[3]. Glands were perfused to basal levels with shark Ringer's only, and then chloride secretion was activated by continuous perfusion of forskolin (1μM) and IBMX (100μM) from t=30 min to the end of the experiment. Individual K^+ channel blockers were perfused continuously from t=50 to t=70 min.

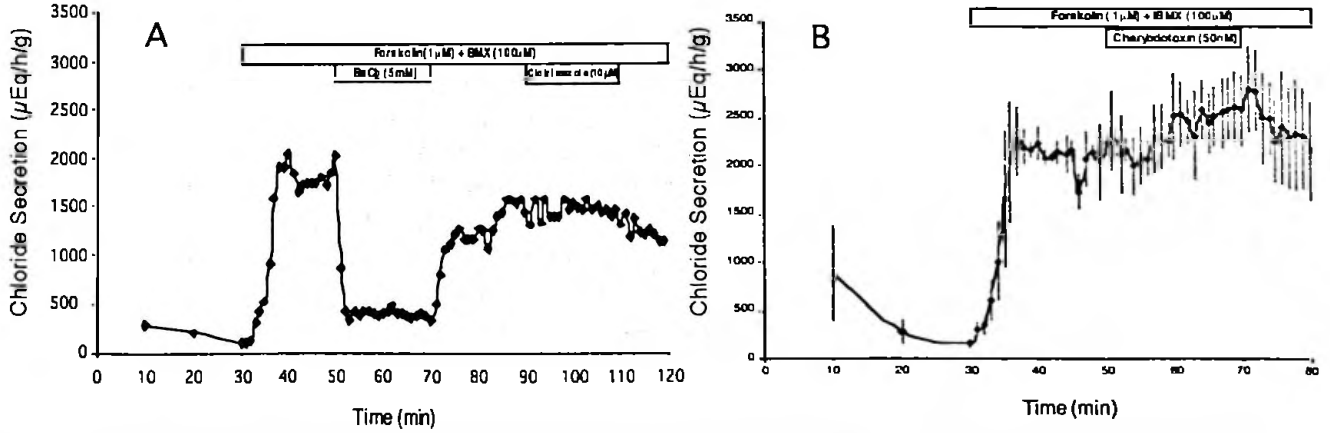

Figure 1. A) Effects of $BaCl_2$ (5mM) and clotrimazole (10μM) on forskolin +IBMX stimulated Cl^- secretion (representative of 3 experiments). B) Effects of charybdotoxin (50nM) on forskolin +IBMX stimulated Cl^- secretion (values are mean± SEM, n=3).

Perfusate concentrations of these blockers were chosen based on known K_i values. Rectal gland tubular cells were cultured and grown on collagen coated nylon membranes and Cl^- secretion measured as I_{sc} in intact monolayers as described previously[1].

Whereas barium completely blocked Cl^- secretion in the perfused gland (Figure 1, panel A), the K^+ channel inhibitors clotrimazole (Figure 1, Panel A) and charybdotoxin (Figure 1, Panel B) had no effect on forskolin +IBMX stimulated Cl^- secretion. Glybenclamide, an inhibitor of ATP sensitive K^+ channels at low concentrations (1μM) also showed no effect on Cl^- secretion. Secretion was inhibited by a higher concentration of glybenclamide (100μM) known to inhibit CFTR chloride channels (Figure 2, Panel A). Tolbutamide (100μM), and phentolamine (200μM) two additional inhibitors of ATP sensitive K^+ channels, also had no effect on Cl^- secretion in perfusion experiments (Figure 2, panels B and C).

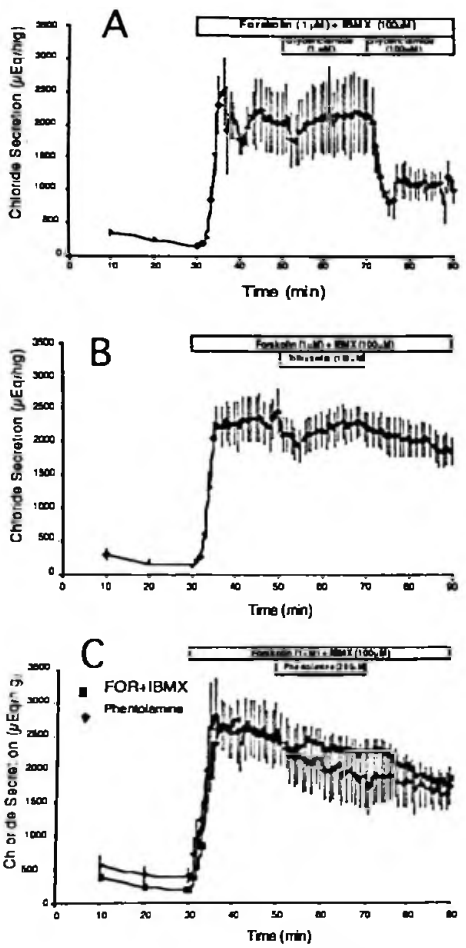

Figure 2. A) Effects of glybenclamide, 1 and 100μM (n=4); B) Effects of tolbutamide (100μM) (n=15); C) Effects of phentolamine (200μM) on forskolin (1μM) +IBMX (100μM) stimulated Cl^- secretion. (n=4 phentolamine, n=24 controls). Values are mean ± SEM.

In contrast, the potassium channel blocker quinidine (200μM) dramatically inhibited forskolin +IBMX stimulated Cl^- secretion in the perfused rectal gland and this effect was reversible following removal of the inhibitor (Figure 3, Panel A). When the effect of quinidine was examined in cultured SRG cell monolayers, the addition of quinidine (400μM) to the basolateral solution markedly inhibited Cl^- secretion measured as I_{sc} by > 80%. This effect was reversible following washout of quinidine (Figure 3, Panel B). The effect of quinidine on I_{sc} was similar to that observed with barium (5mM) in cultured monolayers of rectal gland tubular cells (Figure 3, Panel C).

The absence of effects of clotrimazole and charybdotoxin, known blockers of intermediate conductance Ca^{2+}-activated K^+ channels in several species, suggests that such channels do not play a major role in regulating secretion in the rectal gland. Similarly, the lack of inhibitory effects observed with multiple ATP- dependent K^+ channel blockers including glybenclamide (at 1μM), tolbutamide, and phentolamine, suggests that this subtype of potassium channel is not present on the basolateral membrane of rectal gland cells. Specifically, the KIR 6.1 channel that we have cloned from shark rectal gland is unlikely to be a major regulator of basolateral K^+ conductance in this tissue since this channel is sensitive to glybenclamide.

Only quinidine showed a barium-like effect to inhibit chloride transport in both perfused glands and cultured cells (Figure 3). Quinidine acts on multiple 6 transmembrane (TM) voltage sensitive K^+ channels, including K_vLQT1, K_A and $K_{V(r)}$, 4 transmembrane K^+ channels, and volume sensitive K^+ channels.

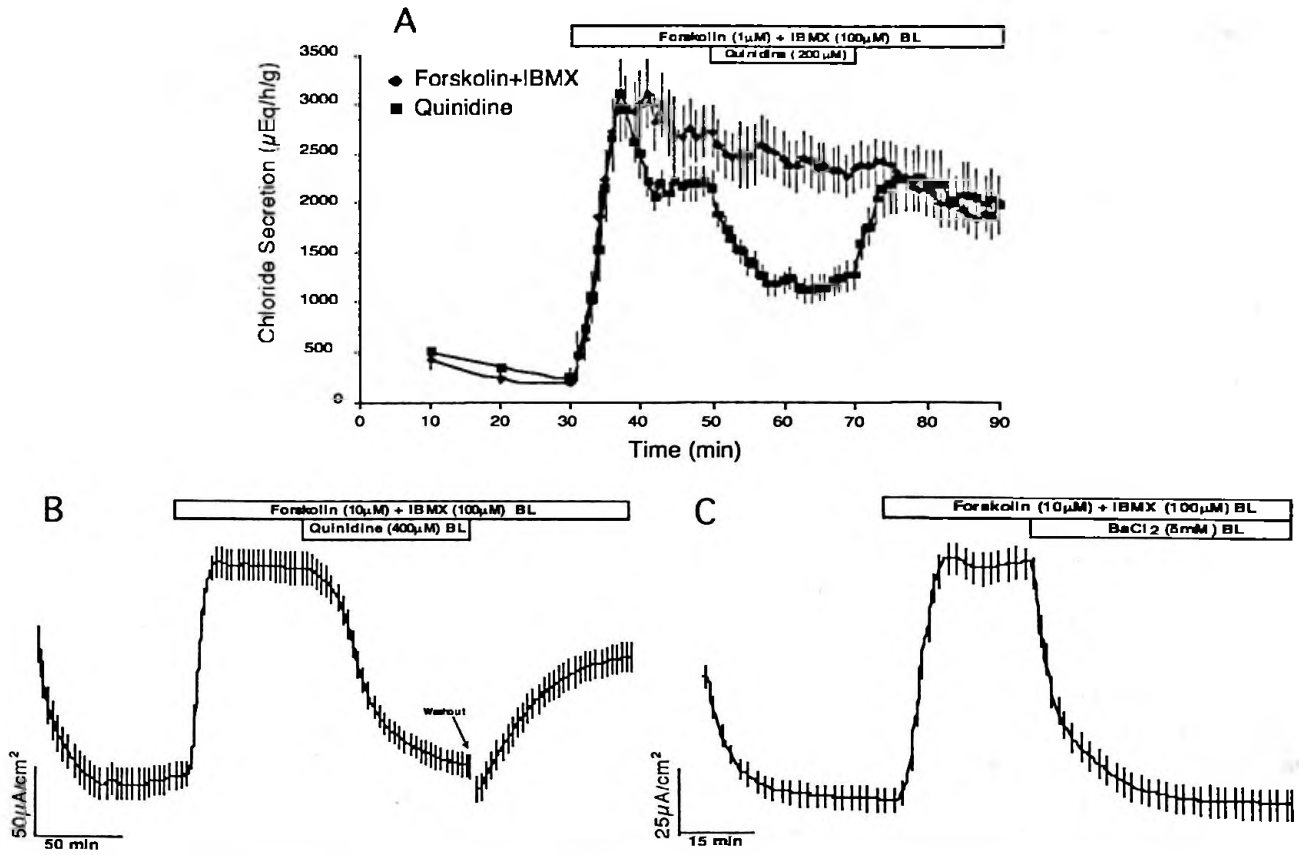

Figure 3. A) Effects of quinidine (200μM) on Forskolin(1μM)+IBMX(100μM) stimulated chloride secretion in the perfused rectal gland (values are mean ± SEM, n=6 quinidine, n=24 controls). B) Effects of quinidine (400μM) added basolaterally on I_{sc} of SRG epithelial cells, representative of 6 experiments. C) Effect of $BaCl_2$ (5mM) added basolaterally on I_{sc} of SRG epithelial cells, representative of 8 experiments.

The existence of a 6 TM voltage sensitive potassium channel such as KvLQT-1/minK or a volume sensitive K^+ channel in the basolateral membrane would explain the inhibitory effects of quinidine observed in both the perfused rectal gland and SRG cultured epithelial cells.

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