Inhibitors of 4TM 2P potassium channels inhibit chloride secretion in the perfused rectal gland of the spiny dogfish (*Squalus acanthias*)

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The shark rectal gland is a sodium chloride secretory organ comprised of polarized epithelial cells that was first described by Wendell Burger[2] over 40 years ago. The Na-K-ATPase pump and the Na-K-2Cl cotransporter on the basolateral membrane are required to drive basolateral chloride entry and basolateral recycling is required to maintain a favorable electrochemical gradient driving luminal chloride exit through CFTR channels. In the perfused gland, when basolateral K^+ exit is inhibited by the non-specific K channel blocker barium, chloride secretion ceases[5]. By cloning studies we have identified both a KIR 6.1 channel[8] and a KVLQT channel[6,7] in this tissue. However, in perfusion studies inhibitors of these subtypes had no effect on chloride secretion suggesting that they are not the dominant channels for basolateral K^+ exit[3]. Only one K^+ channel inhibitor, quinidine, had a significant effect on chloride secretion[3]. Quinidine is an inhibitor of several families of K^+ channels, including rapid delayed rectifier K^+ channels, the K_A channel, cell-volume sensitive K^+ channels, and the 4 transmembrane, 2 pore (4TM-2P) family of K^+ channels.

We sought to further classify the basolateral K^+ channel in shark rectal gland epithelial cells by examining the effects of other putative inhibitors of quinidine sensitive K^+ channel subtypes on maximally stimulated chloride secretion. These effects were examined in *in vitro* perfused rectal glands and in cultured monolayers of shark rectal gland epithelial cells. Inhibitors tested were sotalol, a blocker of rapid delayed rectifier K^+ channels; TEA, and 3,4 DAP, blockers of the K_A channel; lidocaine, a blocker of cell volume sensitive K^+ channels and 4TM, 4P K^+ channels; and quinine, bupivicaine, anandamide and acidic pH, also blockers of the 4TM, 2P subtype. The effects of these inhibitors were compared to those of $BaCl_2$, a potent, non-specific K^+ channel inhibitor.

Table 1. Effect of K^+ channel inhibitors on chloride secretion in *in vitro* perfusion studies

DRUG	Max Dose	N =	Inhibition of Cl- Secretion
BaCl2	5 mM	6	++++
Chromanol	200 µM	6	+
Tolbutamide	100 µM	15	0
Glybenclamide	1 µM	4	0
Clotrimazole	10µM	3	0
Charybdotoxin	50nM	3	0
Phrixotoxin	12 nM	1	0
Phentolamine	200µM	4	0
Quinidine	200 µM	6	++++
Sotalol	100µM	2	0
TEA	10 µM	8	0
3,4 DAP	1 µM	9	0
Lidocaine	1 mM	6	++
Quinine	1 mM	6	+++
Bupivicaine	2.5 mM	6	++++
Anandamide	150 µM	9	+++
Acidosis	PH 5	6	++++

0 no inhibition
+ 0-20 % inhibition
++ 20-40% inhibition
+++ 40-80% inhibition
++++ 80-100% inhibition

Freshly excised rectal glands were perfused *in vitro* using methods previously described[4]. 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. 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 Ba^{2+} completely blocked Cl^- secretion in the perfused gland, inhibitors of calcium sensitive, delayed rectifier, and ATP sensitive K^+ channels (chromanol, tolbutamide, glybenclamide, clotrimazole, charybdotoxin, phrixotoxin, phentolamine) had no effect on secretion. Likewise, the K^+ channel inhibitors sotalol, TEA, and 3,4 DAP had no effect on stimulated chloride secretion (Table 1). In contrast, the potassium channel blockers quinine and bupivicaine dramatically inhibited chloride secretion. Acidic perfusate also resulted in dramatic inhibition of Cl^- secretion. Addition of the K^+ channel blockers anandamide and lidocaine inhibited chloride secretion to a lesser extent (Table 1). The effects of quinine, bupivicaine, acidic pH, and lidocaine were reversible. Figure 1 depicts the time course for inhibition and reversal by barium and acidic pH. Both barium and acidic pH (6.0) abruptly decrease Cl^- secretion in the perfused gland with a similar time course. With both, the half maximal effect is seen within 1-2 min, maximal inhibition is 80-90% and the effects are promptly and completely reversed within minutes.

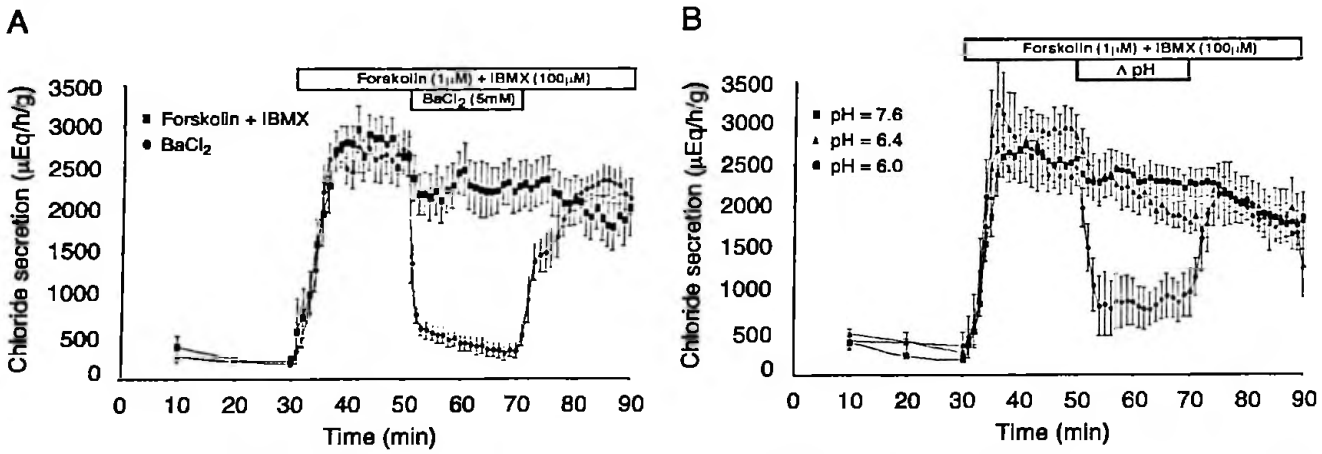

Figure 1. A) Effects of $BaCl_2$ (5mM) on forskolin + IBMX stimulated Cl^- secretion (n=6 barium, n= 10 controls. B) Effects of acidic pH on forskolin + IBMX stimulated Cl^- secretion (n= 23 pH=7.6, n=5 pH=6.4, n=6 pH=6.0).

The effects of quinine were further examined in cultured SRG monolayers (Figure 2). The inhibitory effect was more potent when added to the basolateral solution versus the apical solution. Inhibition of I_{sc} was reversible following washout of the inhibitor (Figure 2, Panels A and B).

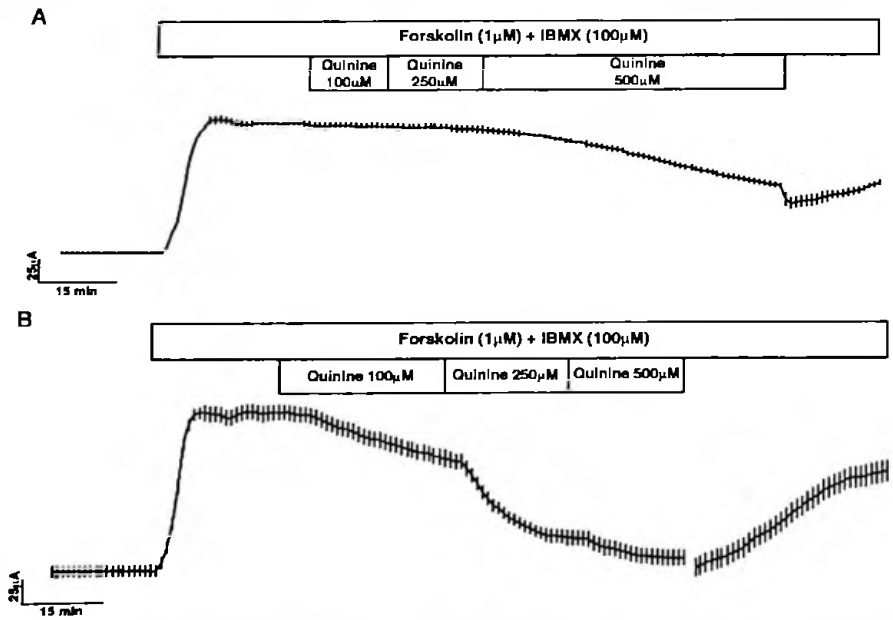

Figure 2. Effects of increasing concentrations of quinine added apically (Panel A) on I_{sc} of SRG epithelial cells, representative of 5

experiments compared to quinine added basolaterally (Panel B) on I_{sc} of SRG epithelial cells, representative of 9 experiments.

Previous studies by this laboratory have suggested that Ca^{++} sensitive K^+ channels, voltage sensitive K^+ channels, and ATP sensitive K^+ channels are not the dominant subtypes regulating chloride secretion[3]. The lack of inhibition by sotalol, TEA, and 3,4 DAP suggests that rapid delayed rectifier K^+ channels, and K_A channels also do not play important roles in the regulation of chloride secretion.

The inhibition of chloride secretion by the K^+ channel blockers quinidine[3], quinine, bupivicaine, anandamide, and lidocaine, as well as by acidic perfusate, all of which are inhibitors of the 4TM, 2P subtype of potassium channels, suggests that this family of K^+ channels is dominant in the regulation of chloride secretion. Specifically, each of these inhibitors has effects on the TASK subfamily of 4TM 2P channels. We propose that a TASK channel is present on the basolateral membrane of the shark rectal gland cell, and that this channel plays a dominant role in the regulation of chloride secretion.

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