Expression of shark (*Squalus acanthias*) TASK-1 potassium channel in *Xenopus* oocytes

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Potassium leak channels active at rest were first proposed in 1952 by Hodgkins and Huxley[1,2,3] but were a curiosity until the era of molecular cloning[4]. Study of the physiology of these channels is still in its infancy. The K2P channels are identified by their molecular structure of 4 transmembrane domains (TM1-TM4) and two P loops or pore-forming domains (P1 and P2) in each subunit[4]; the channels are active as homodimers[5] (Figure 1). A feature of this new class of channels is that in addition to being potassium selective, they are highly regulated. Because they are active at rest and stabilize membrane potentials below the threshold at which excitable cells fire, the emphasis to date has remained on excitable tissue (neurons, nerves, and muscles).

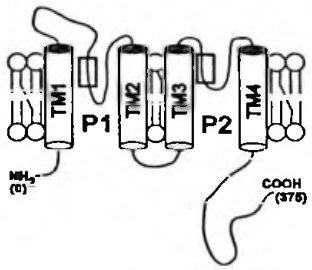

Figure 1. Model of TASK-1 channel showing four transmembrane segments, and two pore regions. P domains are outlined in squares.

There are now 14 known members of the human K2P family (Figure 2); however, the identification of physiologic functions associated with these channels lags behind their molecular identification. We believe that the highly specialized single function of the rectal gland, chloride secretion, is a useful model for defining the physiologic role of these channels in chloride secreting epithelia.

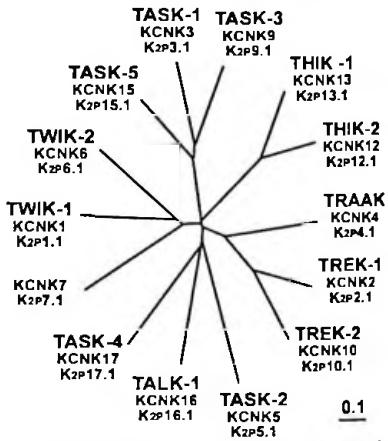

Figure 2. Dendrogram of Human K2P Channels using ClustalW and Treeview (adapted from Patel, AJ 2001).

Previous studies by our laboratory described the cloning of TASK-1, a 2 pore, 4 transmembrane domain potassium channel, from the shark rectal gland[7]. Perfusion studies using specific blockers of this TASK-1 channel indicate that chloride secretion is inhibited by TASK-1 blockade[1]. The present study sought to further characterize the cloned TASK-1 channel by expression in the *Xenopus oocyte* system.

To generate an expression clone, an 1188bp start to stop TASK-1 PCR product was cloned in a pRAT vector and used as the template for cRNA synthesis using T7 in vitro transcription (Ambion Inc., Austin,TX). Human TASK-1 plasmid cDNA in the pRAT vector was kindly provided by Dr. Steve N. Goldstein (University of Chicago). Capped hTASK-1 cRNA was synthesized using T7 RNA polymerase and *in vitro* transcription. *Xenopus* oocytes were prepared as previously descibed[8]. After 12-24 h, the oocytes were injected with 1 ng shark TASK cRNA/50 nl, or 1 ng of human TASK, or an equivalent volume of water and then stored at 18-20°C for 18-24 h in modified Barth solution holding (MBSH containing (in mM) 88 NaCl, 1 KCl, 2.4 $NaHCO_3$, 0.82 $MgSO_4$, 0.33 $Ca(NO_3)_2$*$4H_2O$, 10 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)(5 Na Salt, 5 Acid), buffered to pH 7.4, and 150 mg/liter gentamicin sulfate. Two electrode voltage clamping (TEVC) was performed 18-30 h

after injection. IV curves were obtained by clamping the voltage over a series of steps from -140 to +60 mV at 20mV increments (each increment 250 ms), using a Dagan TEV-200 amplifier and Axon 1320 digidata interface.

During a typical experiment, oocytes were perifused with Frog Ringer's solution containing (in mM) 98 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 10 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)(5 Na Salt, 5 Acid), buffered to pH 7.4 (reagents from Sigma Chemical, Co., St.Louis, MO). IV ramps were taken under basal conditions, TEVC readings were taken every 8 min, with several readings under basal conditions before adding a drug to the basal ND96 solution and then for at least 2 readings after the drug. Data were analyzed with pCLAMP software (version 9.0, Axon Instruments).

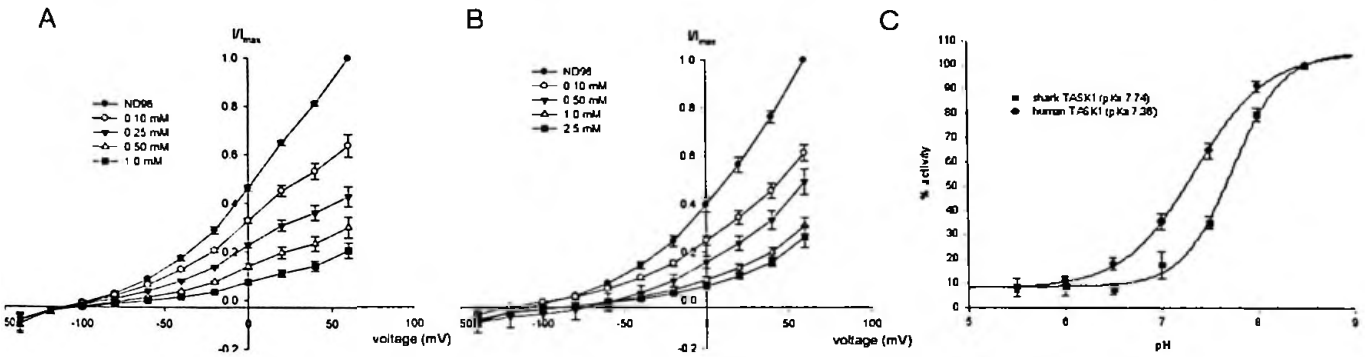

Figure 3. A: Dose dependent inhibition of shark TASK-1 by quinine. B: Dose dependent inhibition of shark TASK-1 by bupivacaine. Quinine and bupivacaine are relatively specific blockers of TASK-1 and show a similar inhibition of shark and human TASK-1. C: Different pH sensitivity in shark and human TASK-1. The pKa for shark is 7.75 and the pKa for human is 7.37. These differences are highly significant, $p<0.001$.

Heterologously expressed shark and human TASK-1 show identical current voltage relationships (outward rectifying) with a reversal potential near 90 mV compared to water injected controls. The inhibitory responses to the K2P family inhibitor quinine, and the TASK-1 inhibitor bupivacaine, were identical in shark and human TASK-1 (Figure 3, Panels A and B), and corresponded to the effects of these inhibitors in the *in vitro* perfused gland[1]. However, the shark channel was much more sensitive than human TASK-1 to inhibition with zinc (data not shown). Shark TASK-1 also differed from the human orthologue in its response to pH (Figure 3, Panel C). The pKa for shark TASK-1 was 7.75 vs. 7.37 for human TASK-1, ($p<0.01$), values that are exceedingly close to the arterial pH for each species, suggesting that TASK-1 channels are regulated closely by the ambient pH. These findings are consistent with in vitro perfusion studies which show that perfusion with acidic pH inhibits chloride secretion.

The current studies define by oocyte expression the properties of the shark rectal gland TASK-1 potassium channel. This channel appears to be the major conductive pathway for potassium ion exit across the basolateral membrane of this tissue.

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