Cloning and molecular identification of a TASK-1 channel cDNA and protein in the rectal gland of the spiny dogfish shark, *Squalus acanthias*

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The dominant conductive basolateral K^+ channel in the shark rectal gland (SRG) is unknown. The SRG, composed of homogenous tubules of a single cell type, is an important model for secondary active chloride transport[1]. Apical Cl^- conductance in this tissue is tightly linked to a basolateral K^+ conductance. Since the secreted fluid of the gland is essentially potassium free, K^+ entry through the basolateral Na-K-ATPase pump and Na-K-2Cl cotransporter must be accompanied by basolateral K^+ exit to maintain the driving force needed for Cl^- secretion[5]. An inwardly rectifying K^+ channel in the SRG was identified in electrophysiological studies[6] and subsequently cloned (KIR 6.1)[10] by our lab as was the shark homolog of the human KvLQT1[9]. However, based on inhibitor studies these channels account for only for a minor part of the total K^+ conductance in the SRG[2].

We carried out further experiments to identify the K^+ channel that plays a dominant role in chloride secretion in this model tissue. We first performed perfusion studies[2,3] with numerous K^+ channel inhibitors and perfusate solutions of varying pH which narrowed the search to the Two-Pore-Domain (4TM 2P/KCNK/K2P) family[7] of potassium channels. In this present study, we have used molecular biological techniques to clone this 4TM 2P channel.

Eighteen pairs of degenerate primers were designed to target regions of high amino acid homology in available mammalian and teleost 2P family potassium channel subtypes: TWIK, THIK, TASK, TREK, and TRAAK. Using degenerate PCR and shark cDNA template reverse transcribed from RNA extracted from shark tissues, one primer pair amplified a putative TASK-1 fragment (394 bp) in the shark rectal gland, brain, gill, and kidney. The forward and reverse primers that amplified TASK-1 in the shark tissues were: 5'- ATCCCCCTGACCCTGGTNATGTTYCA -3' and 5' GCACCACCAGGTTCAG GAANGCNCCDAT -3' respectively and the PCR conditions for the Touch Down PCR protocol were 94° for three minutes, 40 cycles of (95° 0:45, 65° 1:00 – 0.5°/cycle, 72° 1:30) and 72 °C for 10 min. 5' and 3' RACE PCR was used to obtain the entire 3' sequence and a partial 5' sequence of the shark gene. Genome walking (BD Biosciences GenomeWalker Universal Kit) with shark genomic DNA was necessary to obtain the remainder of the 5' sequence, including 335 bp of untranslated region sequence upstream of the methionine start codon.

Figure 1. Cloning of shark TASK-1 channel. A: Degenerate PCR fragments (394 bp) of shark TASK-1 with cDNA template from SRG (lane 7), brain (lane 8), kidney (lane 9) and gill (lane 10). Lanes 2-5 represent a degenerate primer pair that did not amplify template cDNA. cDNA was omitted in the PCR reaction in lane 6 and 11). 1 Kb plus ladder (lane 1).

B: full length clone, confirmed with nested PCR. 1 Kb plus ladder (lane 1), 1386 bp primary product (lane 2), 1282 bp nested product (lane 3). C: Cloning strategy for obtaining full-length sequence of shark TASK-1. 394 bp fragment from degenerate PCR identified as TASK-1. Using this sequence, primers were constructed for 5' and 3' RACE which yielded a 694 bp 3' product that included the stop codon, and a partial 5' product of 592 bp. To obtain the remainder of the 5' sequence Genome Walking (GW) was used to obtain a 428 bp product which included the start codon and 334 bp of upstream untranslated region. Using this complete sequence, primers were designed to amplify a 1282 bp full length clone. * Position of the start and stop sequence of shark TASK-1

With the complete sequence information we designed primers to amplify full-length shark TASK-1 cDNA (Figure 1). This full length product was then cloned in TOPO TA cloning vectors (Invitrogen) and transformed into One Shot TOP10 Chemically Competent *E. Coli* cells (Invitrogen). The full length clone (1282bp) had an 1128 bp open reading frame encoding a protein of 375 amino acids compared to the 1188 bp human TASK-1 of 394 amino acids. The sequence was then confirmed with bidirectional sequencing at the MDIBL sequencing center using vector specific primers M13 F and M13R yielding a nucleotide sequence that was 71% conserved between shark and human isoforms.

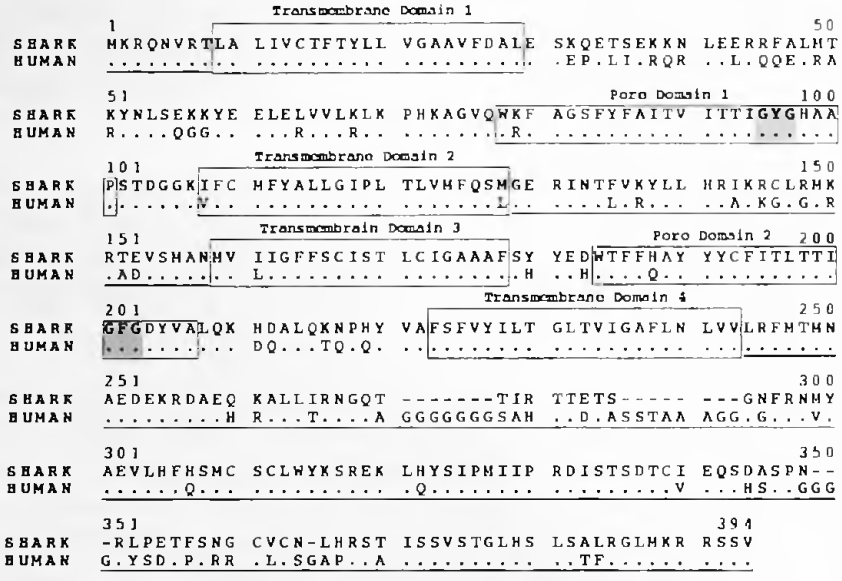

Figure 2. Amino acid alignment of shark and human TASK-1 proteins. Transmembrane domains (TM 1-4) and pore domains (P 1-2) are identified with boxes. Cytoplasmic domains are underlined. The GYG/GFG K^+ selectivity motif conserved in each pore region is shaded with a gray box. Human amino acid residues that are identical to shark TASK-1 are represented as dots. Dashes indicate gaps introduced when necessary for proper alignment between the two sequences.

PROTEIN DOMAIN	AA CONSERVATION	
Whole Protein	316/394	80%
Cytoplasmic 1	8/8	100%
TM1	21/21	100%
P1	23/24	95.8%
TM2	19/21	90.5%
Cytoplasmic 2	21/30	70%
TM3	19/20	95%
P2	23/24	95.8%
TM 4	21/21	100%
Cytoplasmic 3	101/151	66.9%

TABLE 1. Percent conservation of amino acid sequence comparing shark TASK-1 sequence to the human TASK-1 isoform[10].

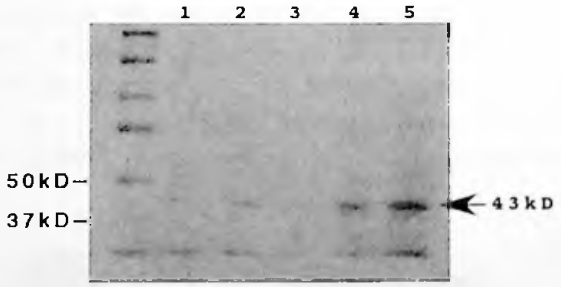

Figure 3. Western blot demonstrating that a TASK-1 antibody recognizes a ~43kD protein in crude SRG lysate. Protein concentration was incrementally increased in lanes 1-5 (~200μg maximum).

Shark TASK-1 was 80% identical at the amino acid level to the human TASK-1 protein (Table 1).

Major structural features of the human protein were conserved in the shark homolog, including the four transmembrane segments (M1-M4), the 2P domains (P1 and P2), short NH2-terminal and long COOH-terminal cytoplasmic parts, and an extended extracellular loop between M1 and P1[7] when analyzed via Clustal W alignment (Figure 2 and Table 1). The presence of a TASK-1 protein in the rectal gland was confirmed with Western Blot analysis using commercially available (Sigma) antibody raised against mammalian TASK-1 revealing a 43kD protein (Figure 3). The target epitope of the Sigma antibody differed in 3 amino acid residues from shark sequence. The mammalian epitope was EDEKRDAEHRALLTRNGQ (TASK-$1_{252-269}$) and shark sequence was EDEKRDAEQKALLIRNGQ (differences underlined).

With more than 70 types, K^+ channels are the most diverse group of ion channels. These channels are classified by their number of transmembrane and pore forming domains. The two-pore, four transmembrane domain family of potassium channels includes the TWIK, THIK, TASK, TREK, and TRESK subfamilies[7]. The first of these cloned from human tissue was TWIK-1 (Tandem of P-domains in a Weakly Inward rectifying K^+ channel). The TASK (TWIK-related acid-sensitive K^+ channel) gene family encompasses 5 members, which encode background K^+ channels that help set the resting membrane potential. These channels are characterized by their sensitivity to changes in extracellular pH. The TASK-1 subtype has been identified in human pancreas, placenta, brain, heart, lung, and kidney[6]. TASK-1 has also been shown to have segment specific expression in the human nephron, being present in the glomerulus and distal nephron segments[8].

Shark rectal gland TASK-1 is the oldest family member identified to date, and the first TASK orthologue found in lower marine vertebrates. These studies suggest that TASK-1 channels play a major role in basolateral K^+ conductance in the shark rectal gland model and provide the first evidence that TASK-1 channels are coupled to chloride secretion.

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