IDENTIFICATION AND PARTIAL SEQUENCING OF A KIR6.1 POTASSIUM CHANNEL FROM THE SHARK RECTAL GLAND AND A ROM-K POTASSIUM CHANNEL FROM SKATE KIDNEY

Gerry Weber[1,2], Stephen G. Aller[1], Florian N. Plesch[1,3], and John N. Forrest, Jr.[1]
[1]Dept. of Medicine, Yale University School of Medicine, New Haven, CT 06510
[2]Experimentelle Nephrologie, Domaglzstr 3a, Muenster, Germany 48149
[3]Medizinische Hochschule Hannover, Carl-Neuberg-Str. 1, Hannover, Germany 30623

Secretion of NaCl by the rectal gland of the spiny dogfish, *Squalus acanthias*, was described nearly forty years ago by Burger et al. (*Science*, 131:670-1, 1960). Regulation of this function has since been a subject of intense investigation. Apical Cl^- conductance, which in the rectal gland is carried at least in part by the shark homolog of CFTR, is known to be tightly linked to a basolateral K^+ conductance. Since the secreted fluid of the rectal gland is essentially free of potassium, the entry of potassium through the basolateral NaK2Cl cotransporter and the Na-K ATPase pump must be accompanied by basolateral K^+ exit in order to maintain the driving force for apical Cl^- secretion. Although the apical CFTR chloride channel in the SRG is well characterized, little is known about the molecular identity of K^+-channels in the basolateral membrane.

Early electrophysiological studies on the rectal gland provided evidence for an inwardly rectifying K^+ channel (Greger et al., *Pflugers Arch*, 409:100-106, 1987), but its molecular basis remains unidentified. Recently, another K^+-channel was cloned from the shark rectal gland, the shark homolog of the human KCNQ1 (Waldegger et al., *Bull. MDIBL* 37:30-31, 1998; Waldegger et al., *Pflugers Arch*, 437::298-304, 1999). However, based on studies using inhibitors, this channel may account for only for a minor part of the total K^+ conductance in the shark rectal gland (Greger, personal communication). Therefore, we have used molecular biological techniques to determine if additional potassium channels are present in the rectal gland and other elasmobranch epithelia. Using degenerate PCR we have cloned and sequenced partial length transcripts of an inward rectifying KIR6.1 potassium channel expressed in the shark rectal gland and an inward rectifying ROMK channel from skate kidney.

cDNA was prepared from fresh shark rectal gland tissue and skate kidney tissue as described (Plesch et al, this issue). Degenerate primers for both KIR6 and ROMK were designed from sequence flanking the 5' region of transmembrane M1 and the 3' region end of M2. The forward and reverse primers to amplify shark rectal gland (SRG) KIR6 were: GTNGAYYTNAARTGGMSNCAYAC and GIGCIGTYTTCATRAADATRCA, respectively, and PCR conditions were for 35 cycles at 30 sec at 95°, 30 sec at 50°, and 45 sec at 68°. The forward and reverse primers to amplify the ROMK fragment from skate kidney were AARTGGCGITAYAARATG and ATIGCICCRCACATRAA, respectively, and PCR conditions were for 35 cycles at 10 sec at 95°, 1 min at 50°, and 2 min at 68°. The reaction products of KIR6 (~ 330 bp) and ROMK (~ 300 bp) were cloned (TA-cloning kit, Invitrogen), recombinants were screened utilizing blue-white selection, and plasmid preparations were sequenced at the University of Maine Sequencing Facility (Orono).

Distinct bands were obtained with PCR using KIR6 degenerate primers on SRG cDNA and ROMK degenerate primers on skate cDNA (Figure 1). Sequencing of putative KIR6 and ROMK fragments revealed open reading frames of 330 bp and 307 bp, respectively. A Genbank search revealed that the SRG band has highest homology to rat KIR6.1 with an identity of 76.5%. The skate band has highest homology to human ROMK with an identity of 86.3%.

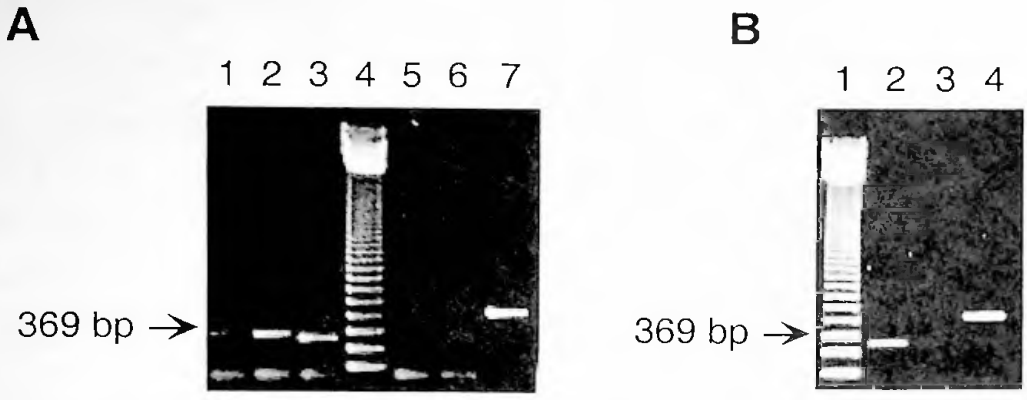

Figure 1. Degenerate PCR fragments of shark KIR6.1 and skate ROMK. Panel A: Results of PCR using KIR6 degenerate primers on cDNA from mouse brain (lane 1), mouse heart (lane 2), shark rectal gland (lane 3) and skate kidney (lane 5). 123 bp was run in lane 4. cDNA was omitted in a PCR reaction with the same conditions (lane 6). A control PCR reaction using degenerate somatostatin receptor primers on mouse brain is shown in lane 7. Panel B: Results of PCR using ROMK degenerate primers on cDNA from skate kidney (lane 2) and mouse brain (lane 4). cDNA was omitted in a PCR reaction under the same conditions (lane 3).

```
                    61                          M1                          120
human KIR6.1        FTTLVDLKWRHTLVIFTMSFLCSWLLFAIMWWLVAFAHGDIYAYMEKSGMEKSGLESTVC
mouse KIR6.1        FTTLVDLKWRHTLVIFTMSFLCSWLLFAIMWWLVAFAHGDIYAYMEKGTMEKSGLESAVC
rat KIR6.1          FTTLVDLKWRHTLVIFTMSFLCSWLLFAIMWWLVAFAHGDIYAYMEKGITEKSGLESAVC
human KIR6.2        FTTLVDLKWPHTLLIFTMSFLCSWLLFAMAWWLIAFAHGDLAP.........SEGTAEPC
rabbit KIR6.2       FTTLVDLKWTHTLLIFTMSFLCSWLLFAMVWWLIAFAHGDLAP.........GEGAAVPC
SRG KIR6.1          ....VDLKWRHTLFIFTMSFLCSWLLFAMMWWLVAFAHGDLDHYRNSVV........EPC

                    121                 H5                      M2          180
human KIR6.1        VTNVRSFTSAFLFSIEVQVTIGFGGRMMTEECPLAITVLILQNIVGLIINAVMLGCIFMK
mouse KIR6.1        VTNVRSFTSAFLFSIEVQVTIGFGGRMMTEECPLAITVLILQNIVGLIINAVMLGCIFMK
rat KIR6.1          VTNVRSFTSAFLFSIEVQVTIGFGGRMMTEECPLAITVLILQNIVGLIINAVMLGCIFMK
human KIR6.2        VTSIHSFSSAFLFSIEVQVTIGFGGRMVTEECPLAILSLIVQNIVGLMINAIMLGCIFMK
rabbit KIR6.2       VTSIHSFSSAFLFSIEVQVTIGFGGRMVTEECPLAILILIVQNIVGLMINAIMLGCIFMK
SRG KIR6.1          VTKVRSFTSAFLFSIEVQVTIGFGGRMITEECPTAITGLIVQNISGLIINAVMLGCIFMK
```

Figure 2. Amino acid sequence of shark rectal gland KIR6.1 compared to previously cloned members of this protein family from mammalian species.

```
                    61                          M1                          120
human ROMK2         IFFVDIWTTVLDLKWRYKMTIFITAFLGSWFFFGLLWYAVAYIHKDLPEFHPSANHTPCV
rat ROMK2           IFFVDIWTTVLDLKWRYKMTVFITAFLGSWFLFGLLWYVVAYVHKDLPEFYPPDNRTPCV
human ROMK3         IFFVDIWTTVLDLKWRYKMTIFITAFLGSWFFFGLLWYAVAYIHKDLPEFHPSANHTPCV
human KATP          ............KWRYKMTIFITAFLGSWFFFGLLWYAVAYIHKDLPEFHPSANHTPCV
skate kidney ROMK   ............KWRYKMAIFITAFLGSWFLFGLLWYAVAYVHRDLPEFAPSNSHIPCV

                    121                 H5                      M2          180
human ROMK2         ENINGLTSAFLFSLETQVTIGYGFRCVTEQCATAIFLLIFQSILGVIINSFMCGAILAKI
rat ROMK2           ENINGMTSAFLFSLETQVTIGYGFRFVTEQCATAIFLLIFQSILGVIINSFMCGAILAKI
human ROMK3         ENINGLTSAFLFSLETQVTIGYGFRCVTEQCATAIFLLIFQSILGVIINSFMCGAILAKI
human KATP          ENINGLTSAFLFSLETQVTIGYGFRCVTEQCATAIFLLIFQSILGVIINSFMCGA.....
skate kidney ROMK   QNINGLTSAFLFSLETQVTIGYGFRCVTEQCCEAIFLLVAQSILGVIVNSFMCGA.....
```

Figure 3. Amino acid sequence alignment of skate kidney ROMK compared to previously cloned members of this protein family from mammalian species.

In summary, we report the partial cloning of a shark rectal gland KIR6.1 potassium channel and a skate kidney ROMK-like potassium channel. We are currently determining the full length sequence and the cellular polarity and function of these proteins in the shark rectal gland and skate kidney.

This work was supported by NIH grants DK 34208 and NIEHS P30-ES 3828 (Center for Membrane Toxicology Studies), and a Grant in Aid from the American Heart Association, Maine Affiliate.