THE SHARK (*SQUALUS ACANTHIAS*) RECTAL GLAND C-TYPE NATRIURETIC PEPTIDE RECEPTOR ACTIVATES CFTR IN XENOPUS OOCYTES

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C-type natriuretic peptide (CNP) receptors (NPR-B) activate Cl^- secretion in the shark (*Squalus acanthias*) rectal gland (Forrest et al., Bull. MDIBL 31:71-72, 1992; Solomon et al., Am. J. Physiol. 262:R707-11, 1992) and play important roles in vasodilation (Kid. Int. 52:202-7, 1997) and angiogenesis (Furuya et al., Biochem. Biophys. Res. Comm. 177:927-31, 1991; Furuya et al., Biochem. Biophys. Res. Comm. 193:248-53, 1993) in mammalian systems. NPR-Bs are membrane spanning proteins that bind specifically to CNP and catalyze the production of the second messenger cyclic GMP.

Cyclic GMP has been shown to activate the cystic fibrosis transmembrane conductance regulator (CFTR) in T84 (Lin et al., Am. J. Physiol. 262:C1304-12, 1992) and Caco-2 cells (Tien et al., J. Biol. Chem. 269:51-54, 1994), but this effect has received less attention than cAMP regulation of CFTR. The shark rectal gland expresses a homolog of human CFTR (Marshall et al., J. Biol. Chem. 266:22749-54, 1991) and secretes Cl^- in response to C-type natriuretic peptide (Schofield et al., Am. J. Physiol. 261:F734-9, 1991; Solomon et al., Am. J. Physiol. 262:R707-11, 1992). However, direct activation of CFTR by a cloned natriuretic peptide receptor has not yet been demonstrated.

We have recently cloned the shark rectal gland CNP receptor (NPR-B) by PCR intensive techniques using degenerate primers designed from eel NPR-B (*Anguilla japonica*, Katafuchi et al., Eur. J. Biochem. 222:835-42, 1994) and sea urchin guanylate cyclases (*Arbacia punctulata*, Singh et al., Nature 334:708-12, 1988 and *Strongylocentrotus purpuratus*, J. Biol. Chem. 264:6545-49, 1989). This fragment was used to design shark specific primers for RACE-PCR to obtain 5′ and 3′ ends and as a probe for rectal gland cDNA library screening to obtain the full length sequence. The full length shark cDNA clone was bi-directionally sequenced and the resulting deduced amino acid sequence had 67% homology to human NPR-B (see abstract by Aller et al., this volume).

We sought to determine if shark NPR-B could activate human CFTR when co-expressed in Xenopus oocytes. cRNA was prepared from plasmid DNA of shark NPR-B and human CFTR by RNA transcription using MMLV transcriptase (Ambion). 15 ng of shark NPR-B and 10 ng human CFTR were injected into defolliculated oocytes and were tested for expression two to four days after injection using two electrode voltage clamping (TEVC). Oocytes co-expressing NPR-B and human CFTR displayed a dose-dependent outward Cl^- current when exposed to synthetic shark CNP (Fig. 1). Cl^- current was identified by performing voltage ramps and measuring the potential at zero current. The reversal potential rose to the Cl^- equilibrium potential for oocytes (-30 to -28 mV) during stimulation with CNP and the current was completely blocked by glybenclamide. Similar results were obtained with oocytes co-injected with shark NPR-B and shark CFTR. CNP (50 nM) did not increase current in control oocytes expressing human CFTR only or shark NPR-B only. Rat ANP (50 nM) did not significantly elevate current in oocytes expressing NPR-B and human CFTR.

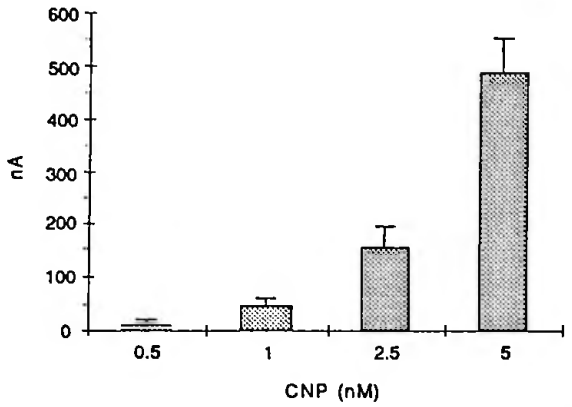

Figure 1. CNP dose-response and Cl^- current in Xenopus oocytes co-expressing shark NPR-B and human CFTR. Panel A: Current trace of a representative oocyte exposed to increasing doses of CNP. Panel B: IV plot of a representative experiment demonstrates a shift in reversal potential toward Cl^- equilibrium in a CNP stimulated oocyte.

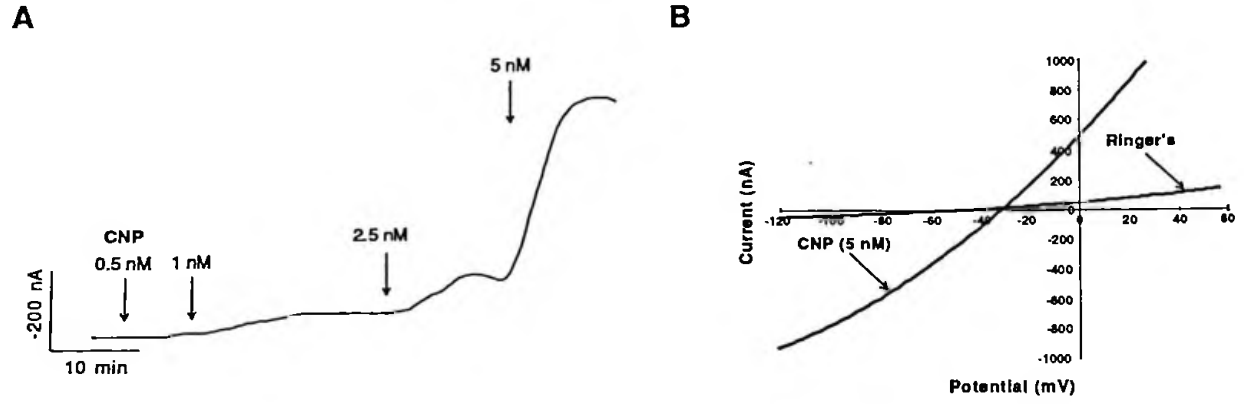

Figure 2. Mean CNP dose response and chloride current at increasing concentrations of CNP (n=4 per group).

These experiments provide the first evidence for activation of CFTR chloride channels by a cloned natriuretic hormone receptor and establish CFTR as an effector of NPR-B. These findings identify the specific receptor by which shark heart CNP activates chloride secretion in the rectal gland and suggest a pathway for CFTR regulation in mammalian tissues where both NPR-B and CFTR are expressed.

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