cDNA CLONING OF A CYCLOOXYGENASE GENE AND EFFECTS OF CYCLOOXYGENASE INHIBITION ON CHLORIDE SECRETION IN THE RECTAL GLAND OF THE DOGFISH SHARK, *SQUALUS ACANTHIAS*

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A large body of evidence accumulated in mammals suggests that prostaglandins regulate salt excretion directly by inhibition of renal tubular transport or indirectly by regulation of renin release or renal hemodynamics. Recent molecular approaches have identified two mammalian isoforms of cyclooxygenase (COX), a constitutive form, COX-1, and an inducible form, COX-2 (DeWitt, D. et al., Cell 83:345-348, 1995). We have previously shown that chronic salt loading or water deprivation augmented COX-2 expression in the renal medulla of rats in vivo, and that hypertonic NaCl induced the expression of COX-2 in collecting duct cell lines in vitro (Yang, T. et al., Am. J. Physiol. 274:F481-F489, 1998; Yang, T. et al., Am. J. Physiol. 277:F1-F9, 1999). In contrast, the expression of COX-1 was not affected in the same circumstances. These findings suggest a potential role of COX-2 in the regulation of salt and water homeostasis in mammals. Rectal glands of the dogfish shark *Squalus Acanthias* have been used extensively as a model to study the process of salt excretion. The present studies were carried out with the aims to identify the existence of cyclooxygenase in the shark rectal gland by molecular approaches and to study the effect of COX inhibition on chloride secretion in perfused rectal glands.

Using long primers (45 bp) derived from the zebrafish COX-2 sequence, a 600 bp product was amplified from shark rectal glands under low stringency conditions and was found to be almost equally homologous to mammalian COX-1 and COX-2 (about 65%). The remaining 5' sequence was obtained by 5' Race whereas the 3' sequence was obtained by analyzing an EST clone generated by the EST Project of the MDIBL Marine DNA Sequencing Center. The gene, designated as shark COX (sCOX) contains a 237 bp 5'-untranslated region (UTR), an about 1800 bp reading frame (with a gap of currently about 200 bp), and a 322 bp 3'UTR. The deduced amino acid sequence is 68% and 65 % identical to mammalian COX-1 and COX-2 respectively. The key residues in the active site of cyclooxygenase (S530, Y385, H386, and H388) are conserved between the shark and mammalian COX. sCOX contains V509 that has been shown to be a key residue determining the sensitivity to COX-2-specific inhibitors including NS-398 (Gierse, J.K. et al., J. Biol. Chem. 271:15810-15814, 1996). RT-PCR was performed to study the tissue distribution of sCOX. The signal was detected abundantly in rectal gland, kidney, and spleen, and to a lesser extent in gill, liver, brain, and heart, but not in fin (Fig. 1).

To assess a potential functional role of sCOX we determined the effect of the COX-2 specific inhibitor NS-398 on VIP-stimulated chloride secretion in rectal glands from *squalus acanthias*. Rectal glands were perfused in vitro with shark Ringer's solution for 30 min to reach basal levels of chloride secretion (<250 μEq/h/g). Vasoactive intestinal peptide (VIP) at a concentration of 5 nM was then added in the presence or absence of the COX-2 inhibitor NS-398

(50 μM) and chloride secretion was measured at 1 min intervals for additional 60 minutes. NS-398 was removed from the perfusate at 60 min to determine reversibility. The diluent (DMSO) was present in all experiments. As can be seen in Fig. 2, NS-398 inhibited both the peak response (3108 ± 479 μEq/h/g vs 2131 ± 307) and the sustained response to VIP (50-60 min). When NS-398 was removed at 60 min, there was a prompt recovery of chloride secretion to control values.

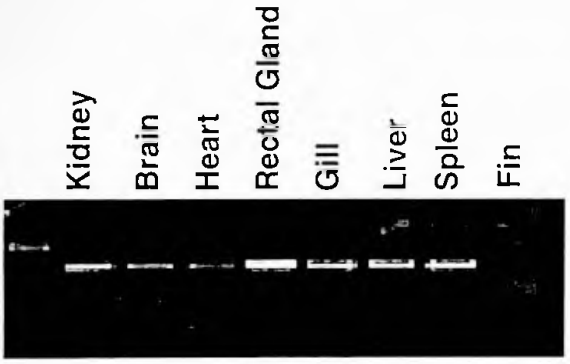

Fig. 1. Tissue distribution of sCOX. RT-PCR was performed on 1 μg total RNA isolated from various shark tissues. The PCR product was visualized on 2% ethidium bromide-stained agarose gel.

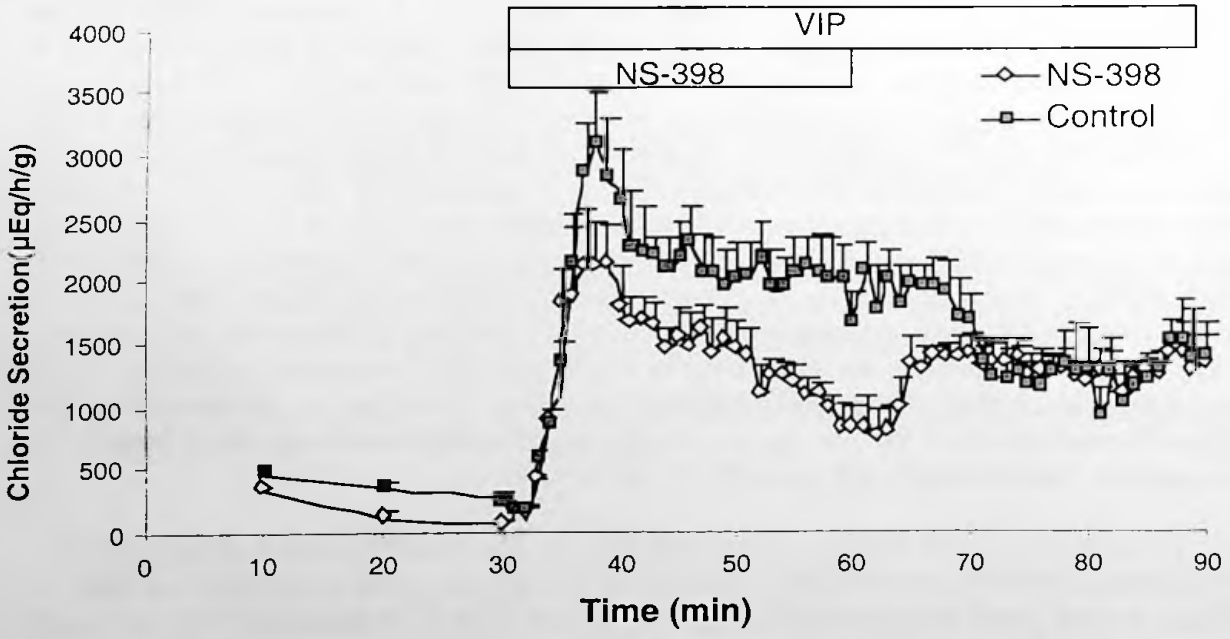

Fig. 2. Effect of NS-398 on VIP-stimulated chloride secretion in perfused shark rectal gland.

Molecular approaches used in these studies succeeded in the cloning of sCOX, the first cyclooxygenase in an elasmobranch species. The identification of a cyclooxygenase enzyme complements earlier results showing the presence of prostaglandins in the shark (Ogata H. and Nomura, T., Biochim. Biophys. Acta 388:84-91, 1975; Evans, D.H. et al., Am. J. Physiol. 274:R1050-R1057, 1998). Its high expression in the rectal gland indicates that it may play a role in the chloride secretory process. In fact, inhibition of VIP-stimulated Cl secretion by NS-398 indicates that some COX product, presumably a prostaglandin, exerts a transport-stimulatory effect in the rectal gland. Description of sCOX as COX-1 or COX-2 is not possible on the basis of sequence homology. Sensitivity of sCOX to NS-398 is consistent with the presence of V509 suggesting a COX-2 like enzyme. Further studies are needed to determine whether the expression of sCOX is induced by cytokines and inhibited by glucocorticoids.

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