EXPRESSION OF CRUSTACEAN HYPERGLYCEMIC HORMONE (CHH) mRNA IN NEUROENDOCRINE ORGANS OF THE SHORE CRAB *CARCINUS MAENAS*

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Neurosecretory cells that produce crustacean hyperglycemic hormones (CHHs) have been identified in the sinus gland/X organ (SG) complex of the eyestalk, in the pericardial organ (PO), and in the gut of crabs. Two CHHs have been characterized at the molecular level in the shore crab *Carcinus maenas*, one form that predominates in the sinus gland/X organ and a second form found in the pericardial organ (Dircksen et al., Biochem. J. 356: 159-170, 2001). SG-CHH and PO-CHH are identical in the first 40 amino acids in the N-terminal region; the C-terminal 33 or 32 amino acids differ. cDNA sequencing suggests that a segment is spliced out of the SG-CHH precursor, thus altering the recognized translation stop site. SG-CHH is thought to regulate blood glucose, enzyme secretion by the hepatopancreas, and Y-organ ecdysteroid production but PO-CHH exhibits none of these properties (Dircksen et al., 2001).

Extracts of sinus gland and pericardial organ are known to enhance Na^++K^+-ATPase activity and uptake of Na^+ in isolated perfused crab gills (Sommer and Mantel, J. Exp. Zool. 248: 272-277, 1988; Spanings-Pierrot et al., Gen. Comp. Endocrinol.119: 340-350, 2000). Strong evidence suggests that the active agent in sinus gland is a CHH-related peptide (Spanings-Pierrot et al., 2000) but it is not likely to be SG-CHH itself. Because of the potential regulatory role of CHHs with respect to gill ion transport, we have initiated a molecular study directed toward producing recombinant CHHs for *in vitro* studies with isolated gills. In this initial report, we examined the expression of CHHs in neurosecretory tissues of the shore crab *Carcinus maenas*, using reverse transcription and the polymerase chain reaction.

Table 1. Primer sequences and expected product sizes for amplification of crustacean hyperglycemic hormone cDNAs from sinus gland/X-organ (SG) and pericardial organ (PO) of the shore crab *Carcinus maenas*.

Primer	Sequence (3'-5')	Expected size: SG	Expected size: PO
XPF2	cga ccg tgc tct gtt caa tga ctt	192 bp	317 bp
XPR2	tcc tgc caa cca tct gta cct ttc		
PF3	aca ccg ata tta gga tca act gc	None	455 bp
PR3	cat tac ata agc tcg tta ccc aga		
PF4	gca gca ggg atg gac tta aag gat	None	223 bp
PR4	ttg gct tgt aga gtg atg gag ggt		

Pericardial organs were identified by vital staining with methylene blue. Forming a coarse meshwork of vertical bars and horizontal trunks, the pericardial organ lies over the openings of the branchiopericardial veins, partially attached to the wall of the pericardial cavity. Sinus gland/X-organ complexes dissected from eyestalks and isolated pericardial organs were quickly immersed in guanidine isothiocyanate denaturing solution and total RNA extracts were prepared (Chomczynski and Sacchi, Anal. Biochem. 162: 156-159, 1987). Poly-A-containing mRNAs were reverse transcribed using oligo-dT and SuperScript II reverse transcriptase. The resulting cDNA mixture served as template in polymerase chain reactions that contained forward and reverse primers based on published CHH sequences from *C. maenas* (Dircksen et al., 2001) (Table 1).

Amplification of CHH cDNA from sinus gland/X-organ and pericardial organ by RT-PCR revealed a surprising outcome. Primers XPF2 and XPR2 that were designed to amplify both SG and PO CHH sequences did so successfully, as determined by gel electrophoresis (Fig. 1). Primer pairs PF3/PR3 and PF4/PR4 produced the expected-size products with PO as template, but also amplified a PO-like CHH from sinus gland/X-organ. These results suggested that SG contains the mRNA for both forms of CHH. Direct sequencing of the PCR products confirmed these speculations.

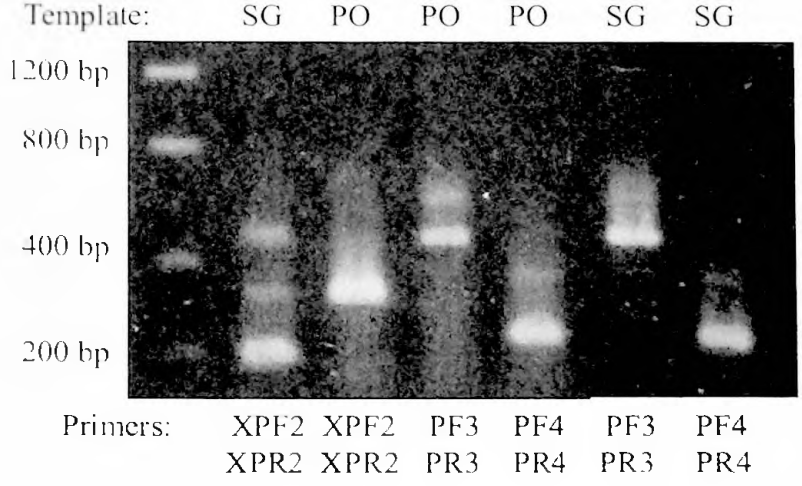

Fig. 1. Amplification of CHH cDNAs from sinus gland/X-organ (SG) and pericardial organ (PO) of the shore crab *Carcinus maenas*. Primer combinations are indicated below the photograph of the ethidium-stained electrophoretic gel and refer to the primers listed in Table 1. Size standards were electrophoresed in the left lane.

Extracts of both sinus gland/X-organ and pericardial organ are known to stimulate Na^+ uptake and Na^++K^+-ATPase activity of perfused gills (Sommer and Mantel, 1988; Spanings-Pierrot et al., 2000). Pericardial organ is shown here not to contain mRNA coding for SG-type CHH, but both the sinus gland/X-organ and pericardial organ contain mRNA coding for PO-type CHH. Our results, combined with earlier observations by other investigators, thus indicate that the ionoregulatory agent in both sinus gland and pericardial organ may be the "PO-type" CHH.
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