NATRIURETIC PEPTIDE EXPRESSION IN ILYANASSA OBSOLETA

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Mammals synthesize a family of highly conserved small natriuretic peptides that modulate fluid and electrolyte homeostasis and that are expressed in tissue-specific patterns: (a) ANP: a 28 aa polypeptide originally detected primarily in cardiac atria and ventricles during embryonic development and in cardiac atria in adults (Zeller, R. et al., 1987, GenesDev.1:693-698); also detected in very low amounts in the central nervous system, thyroid, lung, kidney, spleen, thymus, ovary and uterus; but not in striated muscle cells (Vollmar, A.M. 1990, Klin.Wochenschr.68:699-708); (b) BNP: a 26-45 aa polypeptide expressed mainly in the heart, but also detected in the central nervous system, lung, thyroid, adrenal, kidney, spleen, small intestine, striated muscle, ovary, and uterus (Gerbes, A.L. et al., 1994, J.Clin.Endocrin.Met.78:1307-1311); and (c) CNP: a 22 aa polypeptide detected only in the central nervous system (Komatsu, Y. et al., 1991, Endocrinologyl 29:1104-1106), endothelium (Suga, S. et al., 1992, J.Clin.Invest.90:1145-1149), and monocytic cells (Ishizaka, Y., et al., 1992, Biochem.Biophsy.Res. Commun.189:697-704). In other organisms, the presence and tissue distribution of natriuretic peptides are less well understood. In frogs, the principal vasorelaxant peptide is CNP, present in various isoforms (Yoshihara, A. et al., 1990, Biochem.Biophys. Res.Commun.173:591-598). The dogfish shark heart expresses CNP (Schofield, J.P. et al., 1991, Am.J.Physiol. 261:F734-F739), and some fish express BNP-like and/or CNP-like, but no ANP-like, immunoreactivity in heart and brain tissue (Donald, J.A. et al., 1992, MDIBL Bull.31:120-121). Eels have ANP, CNP, and a ventricle-specific VNP, but no BNP (Takei, Y. et al., 1991, FEBSLett.282:317-320). Using a polyclonal antibody that is ANP-specific in mammalian natriuretic peptide cross-reactions, ANP has been detected immunochemically in the eggs of both veterbrates(Kim, S.H. et al., 1993, Comp. Biochem.Physiol.104A:219-223) and invertebrates (Kim, S.H. et al., 1994, Gen.Comp.Endocrinol.94:151-156), as well as in hearts of the oyster (Vesely, D.L. et al., 1993, Comp.Biochem.Physiol.106B:535-546) and the earthworm (Vesely, D.L. et al., 1992, Comp.Biochem.Physiol.101C:325-329), in paramecium (Vesely, D.L. et al., 1992, Peptides13:177-182), and in the roots, stems, leaves, and flower petals of higher plants (Vesely D.L. et al., 1993, Am.J.Physiol.265:E465-E477). The adult marine mollusc Ilyanassa obsoleta contains a two chambered heart, but if the third polar lobe cytoplasm is removed from the egg at first cleavage, the heart and several other tissues fail to form. We have used immunocytochemical and PCR methods to examine the tissue specificity of ANP and CNP expression in adult snails and in eggs, third polar lobes, and Day 5 and Day 6 (D5 & D6) lobed and lobeless Ilyanassa embryos.

The precleavage eggs from 10 capsules and samples of adult heart auricle, heart ventricle, gill, kidney, intestine, mantle, and ovary were collected separately into TBSA (Tris buffered saline, pH 6.8, with 0.1% NaN_3 [Sodium Azide]) containing 10 mM EDTA (Etylenediaminetetraacetic Acid) and 25 μM PMSF (Phenylmethylsulfonyl Fluoride) on ice, homogenized, microfuged to remove insoluble cellular material, boiled for 5 min to inactivate endogenous alkaline phosphatase and other proteases, and spotted onto nitrocellulose paper. Dot blots were blocked with Blotto, developed with antibody to human/canine ANP that does not react with human BNP or porcine CNP (Peninsula Laboratories, Inc.), and visualized with alkaline-phosphatase-labeled secondary antibody. All tissues

showed some reactivity, but the auricles, gills, mantle and ovary were the most responsive on a per unit soluble protein basis. Control blots developed without primary antibody showed no staining at all, while those developed with anti-alpha tubulin showed a tissue distribution different from that of the natriuretic peptide distribution. These results suggest that marine snails express natriuretic peptide(s) in many tissues.

However, the isoform specificity of the antibody has not been confirmed in nonmammalian systems, so RT-3' RACE (Rapid Amplification of cDNA Ends; Gibco-BRL)-PCR using nested degenerate 5' primers specific for ANP and CNP, respectively, was carried out in order to determine whether multiple natriuretic peptide isoforms are present in marine snail tissues. Tissues were isolated in Millipore-filtered (0.22 μM) sea water, mRNA was isolated by the Micro-Fastrack procedure (Invitrogen), cDNA was synthesized using 3'-RACE procedures, and the cDNA was amplified using one of the 5' natriuretic primers at 52°C. Auricles, ventricles, gills, and mantle, but not the ovary, precleavage eggs, or D5 or D6 lobed or lobeless embryos, yielded a band of ~ 680 bp with the most N-terminal ANP primer, and ventricles gave a band of ~ 550 bp both with the more C-terminal ANP primer and with the more C-terminal CNP primer that contained some overlapping sequence with the C-terminal ANP primer. Auricles, ventricles, kidney, mantle, intestine, eggs before first cleavage, and isolated third polar lobes yielded a 920 bp band with the most N-terminal CNP primer. With the more C-terminal CNP primer (derived from the very highly conserved, CNP-specific, KLDRIG sequence) adult tissues plus the ovary, D5 lobed and lobeless embryos, and D6 lobed embryos yielded an 840 bp band and a 470 bp band, whereas the D6 lobeless embryos yielded only the 470 bp band. The auricle CNP-N-terminal 920 bp band yielded an 840 bp band when re-expanded with the nested CNP-C-terminal primer. The ubiquitous KLDRIG 470 bp band has been sequenced and found to be the widely conserved ribosomal protein L27a. Sequencing of the ventricular ANP/CNP 550 band and the more wide-spread CNP 920/840 bands are in progress. These preliminary studies suggest that Ilyanassa obsoleta may express more than one isoform of natriuretic peptide, and that one of them may be ventricle-specific. This work was supported by NASA-BioServe NAGW-1197 AND NASA-NSCORT NAGW-2328.