C-TYPE ATRIAL NATRIURETIC PEPTIDES ARE POTENT DILATORS OF SHARK (SQUALUS ACANTHIAS) VASCULAR SMOOTH MUSCLE.

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A significant component of the array of physiological actions of members of the atrial natriuretic peptide (ANP) family of hormones is dilation of vascular smooth muscle, both in vivo and in vitro (e.g., Winquist and Hintze. Pharmacol Ther 48: 417-426, 1990). We have recently shown that the endothelium-free, vascular smooth muscle (VSM) from the ventral aorta of the dogfish shark, Squalus acanthias, is sensitive to synthetic, rat ANP (rANP; Evans, J Exp Biol 157: 551-555, 1991). The EC_{50} of the vasodilation is 7 nM, in the same range as that of ANP on isolated, mammalian VSM rings (e.g.,Winquist and Hintze, Op. Cit., 1990). Despite this rather high sensitivity of shark VSM to rANP, one might suggest that fish peptides might be even more effective. In fact, Takei et al. (Biochem. Biophys. Res. Comm. 164: 537-543, 1989; Biochem. Biophys. Res. Comm. 170: 883-891, 1990) have shown that both eel ANP and eel CNP (C-type natriuretic peptide) are 100 times as potent as rANP in production of hypotension in the eel in vivo. To date, Takei's studies on the eel are the only published data on the physiological effects of an homologous ANP-like peptide on a fish species. Recently, Schofield et al. (Am J Physiol 261: F734-F739, 1991) have described CNP from the heart of the spiny dogfish, Squalus acanthias which has 91% homology with CNP isolated from the heart of the European dogfish, Scyliorhinus canicula, (Suzuki et al. FEBS Lett 282: 321-325, 1991), and 82% homology with either porcine CNP (Sudoh et al., Biochem. Biophys. Res. Commun. 168: 863-870, 1990) or killifish CNP (Price et al., Biol. Bull. 178: 279-285, 1990). This recent sequencings of shark CNP's are especially interesting because they are the first to show that a CNP is present in cardiac tissue and, therefore, is presumably released and circulates as a hormone. The present study was undertaken to investigate the putative role of the homologous S. acanthias CNP in the control of gill hemodynamics in that species by examining the effect of the peptide on VSM rings from the ventral aorta. In order to test the specificity of the response, we also examined the efficacy of both killifish and porcine CNP on this system.

Endothelium-free, VSM rings were prepared and mounted in 10 ml of shark Ringer's solution as previously described (Evans and Weingarten, Toxicology 61: 275-281, 1990) The initial tension was approximately 500 mg. Rings were not preconstricted before the cumulative addition of shark, killifish or porcine CNP[1] to produce a concentration range of approximately 10 pM to 100 nM (depending on the agonist). Spiny dogfish shark CNP (sCNP) was diluted in phosphate buffered saline and added in small volumes directly to the experimental baths. Killifish CNP (kCNP; in HPLC solvent; 30% acetonitrile, 70% water, 0.1% trifluoroacetic acid) was aliquoted into polyethylene microfuge tubes, lyophilized in a Speedvac (Savant, Farmingdale, NY), and stored at -70 °C until used. Porcine CNP (pCNP; Peninsula Laboratories) was dissolved in 0.2 M acetic acid, aliquoted, lyophilized, and stored at -70 °C. Concentrated solutions were made up in small quantities of distilled water before addition to the shark Ringer's to produce the specific concentration. Maximal decreases in tension produced were: 16 ± 4.6 % (4) (mean ± S.E., (Number of rings)), 8.1 ± 1.2% (9), and 15 ± 4.6% (7) for sCNP, kCNP, and pCNP, respectively, all somewhat below the 24% recorded in an earlier study using rANP (Evans, Op. Cit., 1991). Curve fitting and apparent EC_{50} values were estimated using Cricket Graph (1.3; Cricket Software, Inc., Malvern, PA) and SuperPaint (2.0; Silicon Beach Software, San Diego, CA) on a Macintosh II microcomputer.

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[1] Shark and killifish CNPs were synthesized by the Protein Cores at Yale and UF, respectively; procine CNP was purchased from Peninsula Labs, Belmont, CA.

Shark CNP produced a concentration-dependent reduction in tension in the isolated VSM from the shark ventral aorta, with an apparent EC_{50} of 0.5 nM. Thus, this tissue is nearly 15 times more sensitive to homologous CNP than it is to heterologous rANP (Evans, Op. Cit., 1991). The reduction in tension in the vascular rings produced by either kCNP or pCNP had approximately the same EC_{50}, suggesting that CNPs in general are more effective than ANPs in facilitating vasodilation in this species. This extreme sensitivity to CNPs by shark aortic VSM is surprising in light of the fact that pCNP is approximately 1/100th as effective as rANP in producing hypotension in intact rats (Sudoh et al., Op. Cit., 1990). In fact, recent radioimmunoassay data indicate that CNP is present only in the brain of the pig and human, not in the heart, consistent with the proposition that, in mammals, CNP is a neuropeptide, rather than a circulating hormone (Minamino et al., Biochem Biophys Res Commun 179: 535-542, 1991).

The first two fish CNPs (killifish and eel, both teleosts) also were isolated from brain tissue, but both the shark CNPs were isolated (the gene cloned in the case of S. acanthias; Schofield et al., Op. Cit., 1991) from cardiac tissue, suggesting that this might be a significant site of CNP synthesis in the Chondrichthyes. Furthermore, our immunohistochemical studies have found that antibodies which recognize rat ANP and porcine BNP show no immunoreactivity in sections of S. acanthias heart. In contrast, specific immunoreactivity is observed using an antibody that has cross-reactivity with all the known structural forms of natriuretic peptides, and this immunoreactivity is abolished by incubation of the antisera with pCNP (Donald, Vomachka, and Evans, this volume). Thus, recent data support the conclusion that, at least in the Chondrichthyes, CNP is produced in the heart and can produce relaxation of vascular smooth muscle, suggesting that it is a circulating hormone. It is unclear whether CNP is the only or even the dominant natriuretic peptide in this group, since earlier data demonstrating ANP-like immunoreactivity in the heart (Uemura et al., Cell Tiss. Res. 260: 235-247, 1990) and plasma (Evans et al., Am.J. Physiol., 257, R939-R945, 1989) of sharks did not differentiate between ANP and CNP.

The roles of CNP in shark hemodynamics and osmoregulation remain to be determined, but recent data suggest that it is 50-100 times more potent than rANP in the stimulation of Cl^- transport by the S. acanthias rectal gland (Karnaky and Forrest, this volume), again in contrast to the relatively low natriuretic ability of pCNP in the rat (1/100th that of ANP; Sudoh et al., Op. Cit., 1990). Finally, if shark branchial vessels also vasodilate in response to CNP, as is apparently the case in teleosts (e.g., Evans et al., Op. Cit., 1989), the ultimate effect of the putative release of CNP from the heart would be an increase in gill perfusion. Since the branchial epithelium is the site of diffusional gain of NaCl and osmotic gain of water from the surrounding sea water (e.g., Evans, In: Comparative Physiology of Osmoregulation in Animals, edited by G. M. O. Maloiy. Orlando: Academic Press, 1979, vol. 1, p. 305-370.), the response to CNP release would be hypervolemia and hypernatremia, directly opposite to the natriuretic and hypovolemic response to the natriuretic peptides in mammals (e.g., Brenner, et al., Physiol. Revs. 70: 665-699, 1990.). Such an apparently osmotically-inappropriate effect of CNP in sharks suggests that this peptide hormone may have evolved in this group to control other physiological systems, such as gas-exchanger hemodynamics. Our findings support the conclusion that CNP is a hormone of some hemodynamic importance in sharks, despite its apparent exclusively-neurohumoral role in mammals.

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