SYNTHETIC SHARK CNP BASED ON THE AMINO ACID SEQUENCE OF CLONED PRE-PRO SHARK HEART CNP POTENTLY STIMULATES CHLORIDE SECRETION IN THE PERFUSED SHARK RECTAL GLAND

J.N. Forrest, Jr.,[1] G.G. Kelley, [1] J.K. Forrest,[1] D. Opdyke,[1] J.P. Schofield,[2] and C. Aller[1]

[1]Department of Medicine,Yale University School of Medicine New Haven, CT 06510 and [2]MRC Molecular Genetics Unit, Hills Rd, Cambridge, England

Previous studies have suggested that cardiac ANP-like peptides play an important role in sodium chloride secretion by the rectal gland. Investigation of the synthesis, regulation, sites of action and signal transduction mechanisms of ANP in the rectal gland have been limited because native cardiac peptides have not been identified in the shark.

We recently cloned and sequenced pre-pro C-type natriuretic peptide (CNP) from the shark heart and identified a primary amino acid sequence that is distinctly different from all other cardiac peptides known to date (Schofield et al., Am J. Physiol. 30:F734-F739, 1991). This was the first report of a cDNA encoding CNP specifically in cardiac tissue and indeed in any non-neuronal tissue. This study also indicated that the mRNA encoding shark heart CNP is a highly abundant message. We have now synthesized shark heart CNP based on the deduced amino acid sequence of cloned CNP and herein report a comparison of the potency of shark heart CNP vs. mammalian ANP in the stimulation of chloride secretions in the in vitro perfused shark rectal gland.

Shark heart CNP was prepared at the Yale Protein and Nucleic Acid Chemistry Facility by the solid-phase method using an Applied Biosystems 430A peptide synthesizer (Foster City, CA). Standard tBOC-amino acids were incorporated as their hydroxybenzotriazole) activated esters in dimethylformamide. The peptide was cleaved using hydrogen fluoride and the resulting crude material, containing the reduced peptide, was purified by reverse-phase HPLC on Vydac C18 columns. Elution of the peptide using an acetonitrile gradient in 0.05% trifluoroacetic acid gave purified, reduced peptide according to amino acid analysis, analytical HPLC, and FAB-mass spectroscopy. The peptide was allowed to form an intramolecular disulfide by adjusting the pH of the reduced peptide to 7.5 with potassium phosphate buffer and allowing the solution to stir, uncovered overnight. The oxidized peptide was repurified as described above, pooled, and lyophilized. 25 mg of dried, oxidized material was obtained and characterized as greater than 90% pure by amino acid analysis, analytical HPLC and FAB-mass spectroscopy.

Figure 1 demonstrates the effect of shark CNP (10 nM) added to the perfusate of the in vitro perfused rectal gland after thirty minutes of basal perfusion.

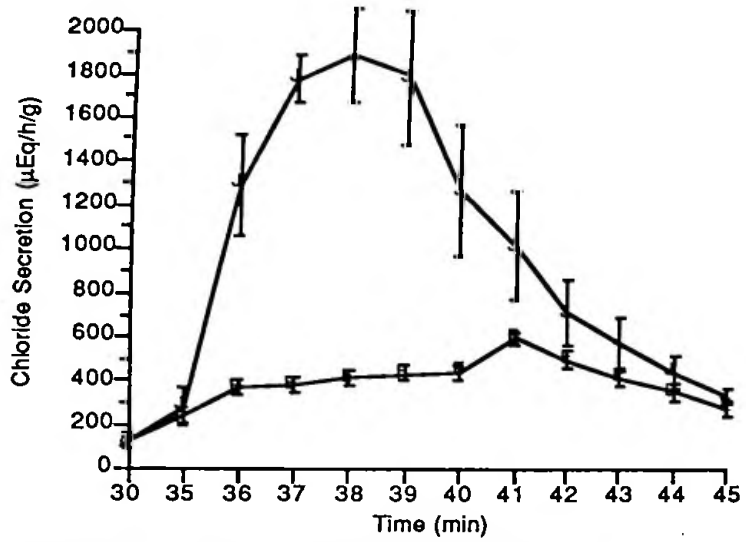
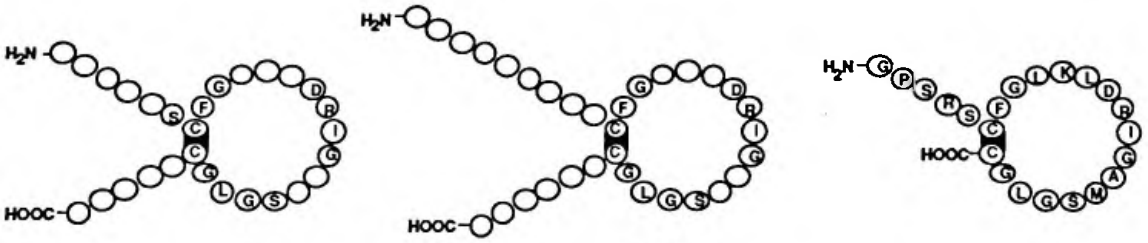


Figure 1. Response to 10 nM shark CNP (upper curve) vs 10 nM rat ANP (lower curve) on chloride secretion (µEq/h/g) in the perfused shark rectal gland.

As shown in Fig 1 (left), 10 nM shark CNP produces a profound stimulation of chloride secretion compared to 10 nM ANP. This stimulation reached a peak 8-9 min after addition and declined thereafter. In other experiments in the perfused gland shark CNP produces a marked increase in chloride secretion at each concentration studied (1-100 nM) in comparison to mammalian ANP. CNP (10 nM) produced a striking elevation in tissue cyclic GMP compared to controls. Homologous shark CNP thus has marked stimulatory effects on chloride secretion in comparison to mammalian ANP at each concentration studied. The amino acid sequence of shark heart CNP compared to ANP and BNP is given below. Identical amino acids compared to shark CNP are indicated by letters.

Shark CNP Structure Compared to ANP and BNP



Prior to our identification of CNP in the shark heart, CNP had been identified only in brain tissue of five species (pig, rat, frog, killifish and eel) (Schofield et al., Am J. Physiol. 30:F734-F739, 1991). It is intriguing that none of the brain CNPs contain a serine residue in position 2 as found in shark CNP and mammalian ANP. With the availability of this first cardiac natriuretic peptide from the shark we will be able to carry out structural-functional studies and plasma measurements to define the synthesis, regulation, sites of action and signal transduction mechanisms of CNP in the regulation of chloride transport in the shark.

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