CHLORIDE SECRETION IN THE RECTAL GLAND OF SQUALUS ACANTHIAS: THE ROLE OF C-TYPE NATRIURETIC PEPTIDE (CNP)

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The rectal gland of Squalus acanthias can be stimulated by a variety of agonists including atrial natriuretic peptide (ANP) and vasoactive intestinal peptide (VIP). The present studies were conducted to evaluate the effects of other members of the family of natriuretic peptides. Using the homologous C-type natriuretic peptide (sCNP) for Squalus acanthias, we performed studies in the isolated perfused rectal gland to establish a physiologic role for this peptide. In addition, we [illegible]'y perfused the glands with human C-type natriuretic peptide (hCNP), killifish CNP porcine BNP (pBNP) and human BNP (hBNP), human pre-pro ANP (hANP 31-67), and fragments and substitutions of the CNP peptide (hCNP 6-22, hCNP 7-21) to evaluate the requirements for ligand-receptor interaction.

Isolated shark rectal glands were perfused using a technique developed in our laboratory [Solomon, et al, Am. J. Physiol. 1985, 249; R348-R354]. The peptides hANP, hANP 31-67, and BNP were obtained from Peninsula Labs , killifish CNP (kCNP) was kindly supplied by Dr. D. Evans, and sCNP, hCNP, hCNP 6-22, hCNP 7-21, hCNP gly-9 were supplied by California Biotechnology Inc [Table 1]. Peptides were kept frozen until immediately prior to use when they were diluted into 1 ml of shark Ringer's solution. Following three baseline collection periods of 10 minutes each, the peptides were infused over 60 seconds into the perfusate line. Three additional 10 minute collections were then performed.

Table 1. Aminoacid structure of peptides studied (ring structure is outlined)

HUMAN ANP	S-L-R-R-S-S-	C-F-G-G-R-M-D-R-I-G-A-Q-S-G-L-G-C-	N-S-F-R-Y-COOH
PIG BNP	S-P-K-T-M-R-D-S-G-	C-F-G-R-R-L-D-R-I-G-S-L-S-G-L-G-C-	N-V-L-R-R-Y-COOH
HUMAN BNP	S-P-K-M-V-Q-G-S-G-	C-F-C-R-K-M-D-R-I-S-S-S-S-G-L-G-C-	K-V-L-R-R-H-COOH
HUMAN CNP	G-L-S-K-G-	C-F-G-L-K-L-D-R-I-G-S-M-S-G-L-G-C-	COOH
HUMAN CNP 6-2		C-F-G-L-K-L-D-R-I-G-S-M-S-G-L-G-C-	COOH
HUMAN CNP 7-21		F-G-L-K-L-D-R-I-G-S-M-S-G-L-G-	COOH
HUMAN CNP gly-9	G-L-S-K-G-	C-F-G-G-K-L-D-R-I-G-S-M-S-G-L-G-C-	COOH
KILLIFISH CNP	G-W-N-R-G-	C-F-G-L-K-L-D-R-I-G-S-M-S-G-L-G-C-	COOH
DOGFISH CNP	G-P-S-R-S-	C-F-G-L-K-L-D-R-I-G-A-M-S-G-L-G-C-	COOH

The effect of sCNP on chloride secretion is shown in Figure 1. The stimulatory effect seen was similar to that seen with VIP. A small stimulatory effect was first seen at a concentration of 10^{-9}M with maximal stimulation seen at a concentration of 10^{-6}M. Within the limits of the concentrations of sCNP examined half-maximal stimulation appears to be at 10^{-8}M. Figure 2 compares the effect of CNP's from different species on chloride secretion by the rectal gland. The effect of sCNP is similar to that of kCNP and an order of magnitude greater than that of hCNP. Figure 3 shows the effect of various modifications of the CNP molecule. Removal of the aminoterminal end (hCNP 6-22) causes a significant reduction in the stimulatory effect. Substitution of the leucine in position 9 by a glycine did not significantly alter the functional effect of the molecule (hCNP gly-9 vs. hCNP). Surprisingly the ringless structure (hCNP 7-21) made up of aminoacids 7 to 21 had a functional effect that was only an order of magnitude lower than that of the ring alone. Figure 4 compares the effect of sCNP with that of hANP, pork and human BNP and hANP 31-67. As can be seen the effect of hANP is considerably smaller than that of sCNP and that of pBNP is even more so. hANP 31-67 had virtually no effect.

FIGURE 1

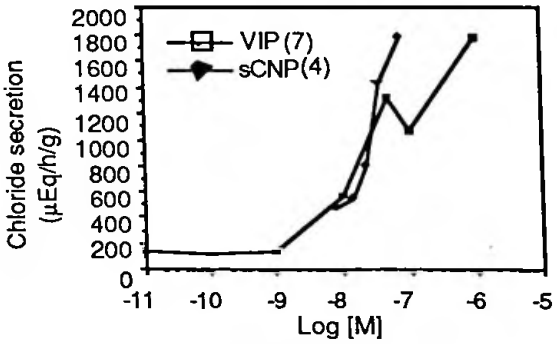


FIGURE 2

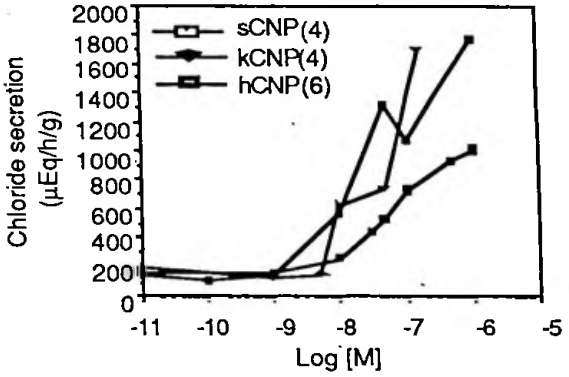


FIGURE 3

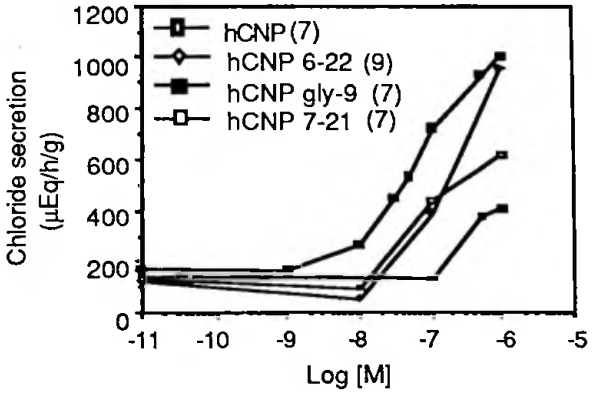
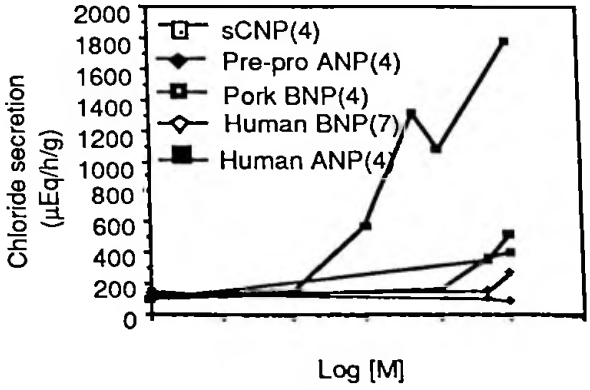


FIGURE 4



These results support a physiologic effect of sCNP to stimulate salt secretion by the shark rectal gland. sCNP is a 22 aminoacid peptide containing a 17 member ring on the COOH-terminal end. kCNP and sCNP differ in three aminoacids in the NH_2-terminal end, positions 2,3, and 5, and in position 16 in the ring structure. Both peptides had similar dose response curves suggesting that these amino acid differences were not important. On the other hand, hCNP differs from sCNP at positions 2,4,5 and 16 and from kCNP at positions 2,3,4 and is one order of magnitude less potent than sCNP or kCNP. This suggests that position 4 in the NH_2-terminal

end of the molecule is of functional significance. Further support for the importance of the NH_2-terminal end of the molecule is suggested by the marked reduction in response to hCNP 6-22 containing the ring structure alone compared to intact hCNP. In addition to the importance of the NH_2-terminal end of the molecule, the ring may also be of some functional significance. The linear fragment, hCNP 7-21, and the intact ring, hCNP 6-22, were still capable of stimulating chloride secretion albeit at a markedly blunted maximal levels and only at very high concentrations ($>5 \times 10^{-7}$M and $>10^{-7}$M) of peptides respectively. In bovine aorta smooth muscle cells which contain the ANPR-B receptor subtype, hCNP and hCNP 6-22 have similar dose response curves for the generation of cGMP. The linear fragment, hCNP 7-21, on the other hand, is inactive (A. Protter, California Biotechnology, Inc, personal communication). The substitution of glycine for leucine at position 9 in the ring also reduced the stimulatory effect (hCNP gly-9 vs hCNP) suggesting a functional role for this site as well. In the bovine aorta smooth muscle preparation, hCNP gly-9 is also inactive (Ibid.).

BNP interacts primarily with the ANPR-A receptor. In the isolated perfused rectal gland it has a dose response curve shifted well to the right of that of hANP. Taken together with the results obtained with the various CNP's, we suggest that the biologically active receptor for sCNP in shark rectal gland resembles the ANPR-B receptor. In addition, these studies suggest that sCNP may be a physiologic regulator of rectal gland function.

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