CNP-53 STIMULATES THE SECRETION OF CHLORIDE BY THE RECTAL GLAND OF *SQUALUS ACANTHIAS* WITH THE SAME POTENCY AS CNP-22

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The secretion of chloride by the rectal gland is stimulated by C-type natriuretic peptide CNP. CNP is encoded as a 126-amino acid precursor (prepro-CNP), that generates a 22 and a 53 amino acid peptide, CNP-22 and CNP-53 (Tawaragi Y. et al. Biochem Biophys Res Commun 1990 172:627-32). They differ in the elongated amino terminal in CNP-53. In the present experiments we tested the activity of CNP-53 to ascertain whether it had similar activity to that of CNP-22.

Rectal glands were perfused using a technique previously described (Silva P, et al. Methods Enzymol. Vol 192. 1990: 754-66). The dogfish were killed by pithing and the rectal glands were removed via an abdominal incision. The rectal gland artery, vein and duct were catheterized with PE90 tubing. The glands were placed in an all glass perfusion chamber maintained at 15°C with running sea water. The glands were perfused by gravity at a pressure of 40 mm of Hg. The composition of the perfusion medium was (in mM) Na, 280; Cl, 280; K, 5; bicarbonate 8; phosphate, 1; Ca, 2.5; Mg, 1; sulfate, 0.5; urea, 350; glucose 5; pH 7.6 when gassed with 99% O2/1% CO2. Rectal gland secretion was collected in tared 1.5 ml conical centrifuge tubes over periods of ten minutes and the volume determined by weight. When secretion volume was less than 100 µl/10 minutes it was collected directly into 200 µl calibrated glass pipettes. Chloride were measured in the rectal gland secretion by amperometric titration to determine the chloride secretion rate and was expressed as µEq secreted per hour per gram of rectal gland weight. All glands were perfused with procaine 10^{-2} M to prevent the release of vasoactive intestinal peptide (VIP) from the nerves within the gland.

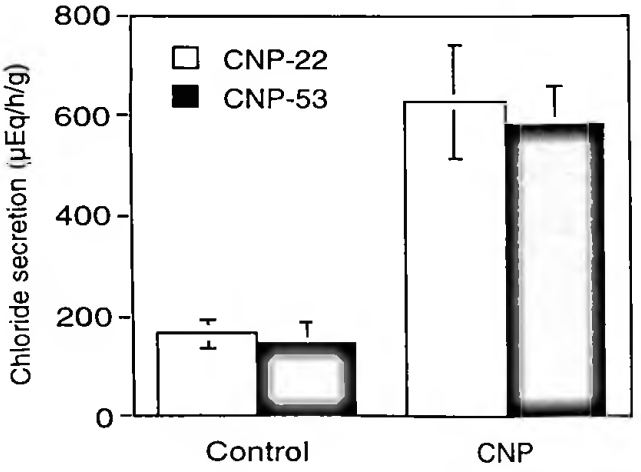

Figure 1. Effect of CNP-22 and CNP-53 on the secretion of chloride by the rectal gland. The rectal glands were perfused with procaine 10^{-2} M throughout the experiment to prevent the release of VIP and examine only the direct effect of CNP. Both peptides increased the secretion of chloride by similar amount, $p < 0.001$, by paired "t" test. Values are mean ± SEM, n=5 and 8 for CNP-22 and CNP-53, respectively.

The results are shown in Figure 1. A bolus injection of either CNP-22 or CNP-53, in an amount calculated to result in a final concentration of 5×10^{-7} M, stimulated the secretion of chloride by the rectal gland, $p < 0.001$ for both peptides, by paired "t" test. The secretion of chloride after the injection of the bolus was compared with the basal (control) secretion of chloride prior to the bolus in the same gland. The stimulation was of the same magnitude with either peptide. We conclude that the elongation of the amino terminal end of the peptide chain does not change the stimulatory capacity of CNP.