FUNCTIONAL AND BIOCHEMICAL EFFECTS OF C-TYPE NATRIURETIC PEPTIDE (CNP) IN THE RECTAL GLAND OF SQUALUS ACANTHIAS

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The secretion of chloride by the rectal gland of the dogfish is under humoral control. Natriuretic peptides, released from the heart in response to a volume stimuli, stimulate the secretion of chloride by the gland. Until recently atrial natriuretic peptide (ANP) was thought to mediate this effect, but ANP has no direct effect on the secretion of chloride by the intact rectal gland, rather it causes the release of vasoactive intestinal peptide (VIP) from nerves within the gland (Silva P, et al., Am. J. Physiol. 252:F99-103, 1987). VIP in turn activates adenylate cyclase. Schofield, et al. (Am. J. Physiol. 261:F734-9, 1991) recently reported that the natriuretic peptide in shark heart is a C-type natriuretic peptide (CNP) rather than ANP. Moreover, Solomon, et al. (Am. J. Physiol. 262:R707-11, 1992) have recently shown that in isolated perfused shark rectal glands CNP is ~100 fold more potent than ANP in stimulating the secretion of chloride. We have recently characterized the binding of natriuretic peptides to rectal gland plasma membranes and find that CNP binds with high affinity to a guanylate cyclase linked receptor while ANP binds to what appears to be the so-called clearance receptor (Gunning et al. Am. J. Physiol. in press). These observations led us to examine the functional and biochemical responses of the rectal gland of the shark to natriuretic peptides.

The effect of natriuretic peptides on guanylate cyclase activity in plasma membranes of rectal gland is shown in Figure 1. CNP, both shark and human, stimulates guanylate cyclase with maximal activity at ~0.1 μM CNP, and half maximal effect ~10 nM. pBNP did not stimulate guanylate cyclase activity even at μM concentrations, and rANP stimulated activity only minimally at μM concentrations, panel A. In contrast, rANP is a potent guanylate cyclase stimulator in the rabbit renal papilla, whereas sCNP is without effect in that tissue, panel B.

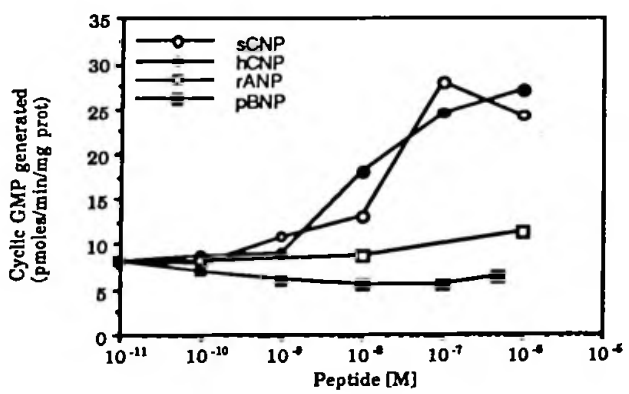

Figure 1A.

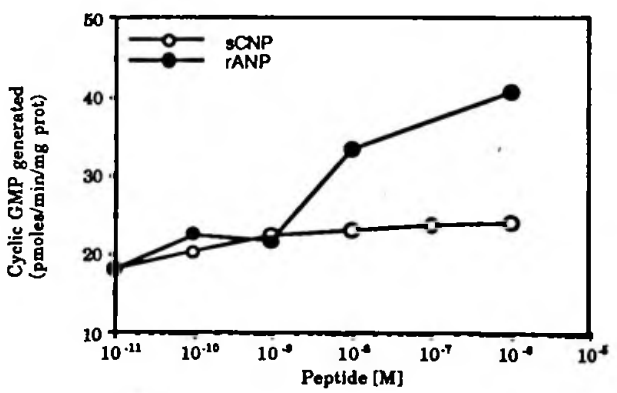

Figure 1B.

The effect of pCNP 10^{-8} M on the release of cGMP into the venous effluent is correlated with its effect on chloride secretion in Figure 2. There is an initial rapid rise in the release of cGMP into the venous effluent during the first ten minutes of perfusion with pCNP that is

followed by an equally rapid decline with levels returning to or slightly above control values ten and twenty minutes later. The secretion of chloride did not peak until after twenty minutes of perfusion with pCNP, in a pattern different from that of the release of cGMP.

Figure 2. Effect of pCNP on the release of cGMP into the venous effluent and chloride secretion in isolated perfused rectal glands. The duration of each collection period was ten minutes. The first collection period is the control without pCNP. pCNP 10^{-8} M was infused during periods 2, 3 and 4. The initial rapid rise in the release of cGMP is followed by an equally rapid decline with levels returning to or slightly above control values, a pattern different from that of chloride secretion that peaks ten minutes later.

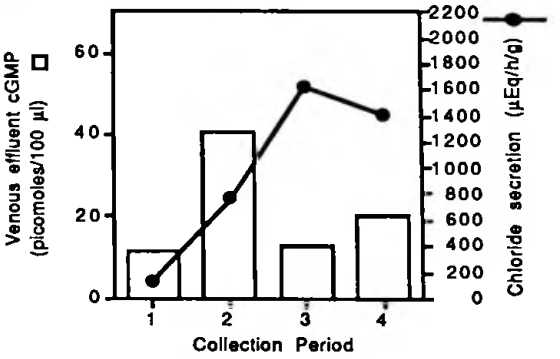

Figure 2.

Figure 3. A phosphodiesterase resistant analog of cGMP, 8-Br-cGMP does not stimulate the secretion of chloride. After thirty minutes of perfusion 10 isolated rectal glands received 8-Br-cGMP 10^{-4} M. The perfusion with the analog was continued for the remainder of the experiment, closed circles. 8-Br-cGMP did not stimulate chloride secretion. Control perfusions are shown in the open circles. The effect of pCNP 10^{-8} is shown in the closed squares for comparison.

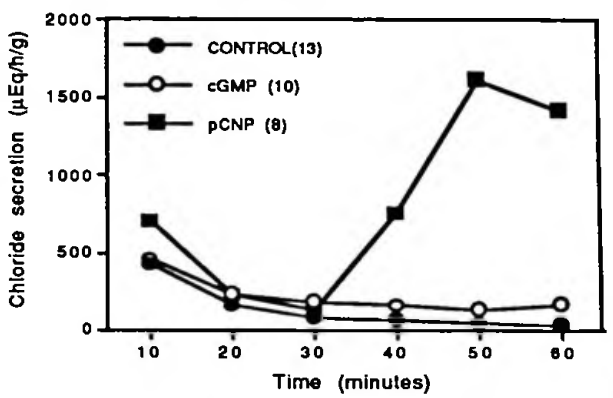

Figure 3.

Figure 4. Effect of 8-Br-cGMP and pCNP on intracellular levels of cGMP. Tissue levels of cGMP were measured by radioimmunoassay in isolated rectal glands perfused with either 8-Br-cGMP 10^{-4} M or pCNP 10^{-8} M. Perfusions with 8-Br-cGMP and pCNP lasted 30 minutes. Ten minutes after the infusions of 8-Br-cGMP and pCNP were stopped the perfusion was ended and the glands frozen. Both 8-Br-cGMP and pCNP increased dramatically the intracellular levels of cGMP; the control values of 99±40 are not appreciated in the Figure. 8-Br-cGMP caused a four-fold larger increase in tissue levels than pCNP.

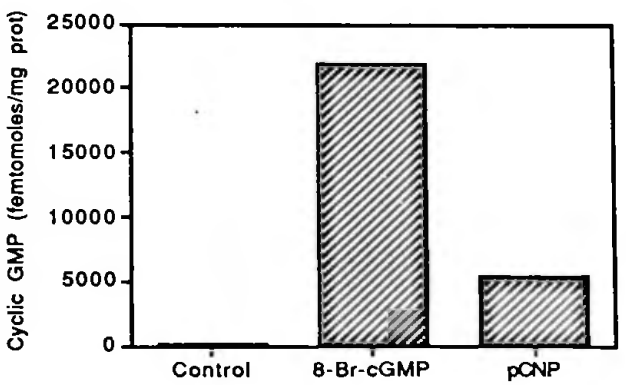

Figure 4.

We next examined the effect of cyclic GMP on chloride secretion by the isolated perfused rectal glands. We used 8-Br-cGMP in these experiments. In none of the experiments did 8-Br-cGMP have any effect on chloride secretion (Figure 3). In order to see whether the failure of 8-Br-cGMP to stimulate chloride secretion was due to inability of the nucleotide analog to enter rectal gland cells, or to rapid cellular degradation, we measured the intracellular content of cGMP after exposure to pCNP and 8-Br-cGMP. The results are shown in Figure 4. Both pCNP

and 8-Br-cGMP caused an increase in intracellular cGMP. In fact, the levels achieved with 8-Br-cGMP were higher than those attained with pCNP. The concentration of cGMP inside the cells was measured ten minutes after stopping the perfusion with the analog. The possibility that the high intracellular levels of cGMP in the perfusions with 8-Br-cGMP were the result of contamination with extracellular fluid was tested in the measurements reported in Figure 5. Venous effluent samples were assayed at 1, 5 and 10 minutes after stopping the perfusion with the analog. The venous effluent level of cGMP decrease rapidly after stopping its infusion and by five minutes had reached levels lower than those found in rectal gland tissue. Therefore, the tissue levels of cGMP found after perfusion with 8-Br-cGMP cannot be accounted by extracellular fluid contamination, and must approximate intracellular levels. In addition these levels represent a minimum estimate since they were measured ten minutes after stopping the perfusion.

Figure 5. Venous effluent levels of cGMP after stopping perfusions with 8-Br-cGMP. Levels of cGMP were measured by radioimmunoassay in the venous effluent of isolated rectal glands perfused with 8-Br-cGMP. The venous effluent was sampled at 1, 5, and 10 minutes after stopping the perfusion with 8-Br-cGMP 10^{-4} M. The venous effluent levels of cGMP declined rapidly after ending the infusion. By five minutes the levels were below intracellular levels and by 10 minutes they were even lower.

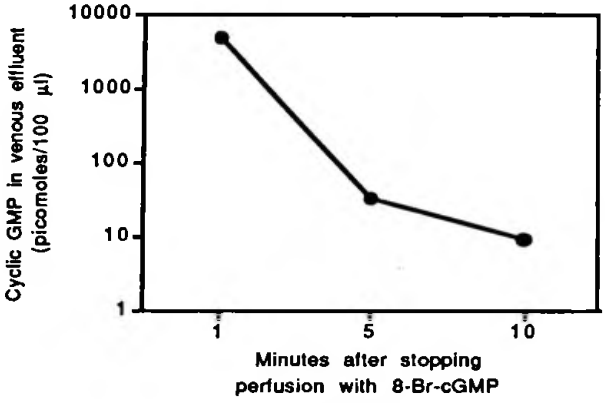

Figure 5.

Figure 6. Effect of pCNP on rectal gland adenylate cyclase. Adenylate cyclase was measured in plasma membranes of shark rectal gland. The membranes were incubated with pCNP and VIP at the concentrations indicated in the Figure. Theophylline and IBMX were both present in the incubation solution. Theophylline and IBMX by themselves doubled the enzyme activity. Aluminum fluoride, used as control, increased the activity to the same level as VIP 10^{-6} M, the highest concentration used. pCNP had no effect on adenylate cyclase in vitro while VIP increased the activity of the enzyme in a dose-dependent way.

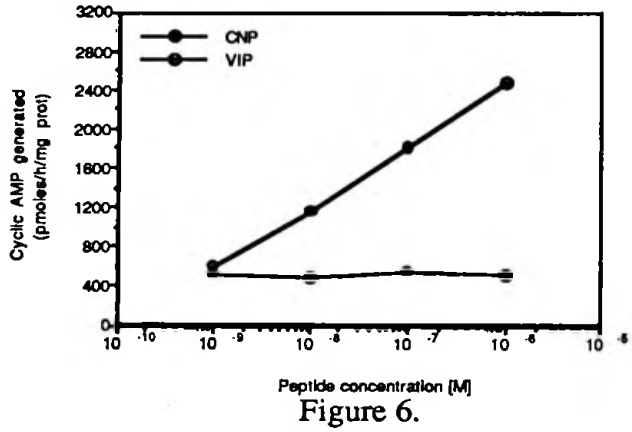

Figure 6.

The absence of an effect of 8-Br-cGMP on chloride secretion led us to examine the effect of pCNP on rectal gland adenylate cyclase. Figure 6 shows that pCNP did not stimulate adenylate cyclase, while VIP, shown in the same figure, activates it in a dose-dependent way. Consistent with its lack of effect on adenylate cyclase pCNP did not increase intracellular levels of cAMP in isolated perfused rectal glands, shown in Figure 7, while VIP and theophylline increased intracellular cAMP by three and four-fold respectively. An intriguing finding was the increase in cAMP observed when pCNP was infused together with staurosporine.

These experiments show that CNP activates guanylate cyclase in isolated perfused glands and also in vitro in rectal plasma membranes. CNP was the only natriuretic peptide tested that

Figure 7. Effect of CNP, VIP and theophylline on intracellular cAMP in isolated perfused rectal glands. Cyclic AMP was measured by radioimmunassay in isolated rectal glands perfused with either pCNP 10^{-8} M, pCNP plus staurosporine (ST) 10^{-8}, VIP 1.5 x 10^{-9} M or theophylline 10^{-3} M. pCNP had no effect on intracellular levels of cAMP while VIP alone increased them three-fold and theophylline four-fold. Interestingly, pCNP together with staurosporine increased intracellular cAMP 1.5 times.

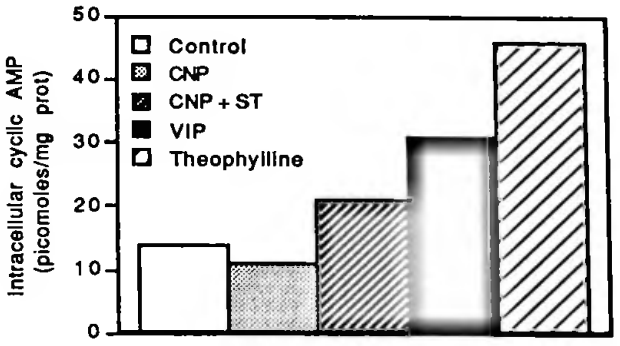

Figure 7.

stimulated guanylate cyclase in rectal gland plasma membranes. Interestingly, in another tissue with guanylate cyclase responsive to natriuretic peptides, the rabbit inner medullary collecting duct, CNP has no effect while ANP has. These findings indicate that the receptor linked to guanylate cyclase in the rectal gland is different from that in the mammalian collecting duct. The natriuretic peptide receptors linked to guanylate cyclase have been classified as GC-A and GC-B. GC-A is activated by ANP and also BNP, GC-B by CNP much more so than ANP and BNP. The rectal gland receptor does not quite correspond to any of the receptors previously described in mammalian tissue. It most closely resembles GC-B but may be a different receptor.

CNP does not increase cAMP in perfused glands or activate adenylate cyclase in plasma membranes in vitro suggesting that its mechanism of activation of chloride secretion is independent of adenylate cyclase. This finding is a surprise considering that the effect of CNP is partially blocked by procaine suggesting that VIP partially mediates its effect (see Solomon et al., Bull MDIBL, 1993) Moreover, extracts of shark heart cause the release of VIP into the venous effluent of perfused rectal glands and their effect is much more potent than that of ANP (Silva P, et al., Am. J. Physiol. 252:F99-103, 1987). Since VIP directly activates adenylate cyclase, a release of VIP caused by CNP should also activate adenylate cyclase. However, it should be noted that the concentration of VIP in the venous effluent in response to heart extracts, and hence CNP, is in the order of 10^{-13} to 10^{-12} M considerably lower than 1.5 x 10^{-9} M used in the experiments reported above. These concentrations of VIP are well below those required to measurably elevate cellular cAMP in this tissue in the absence of phosphodiesterases inhibitors to prevent the rapid degradation of cAMP (Stoff JS, et al., Am. J. Physiol., 237:F138-44, 1979). In fact, in these experiments the effect of VIP was not even as great as that of theophylline alone. Phosphodiesterase inhibitors could not be used in these experiments because, as shown in the experiments with theophylline, they cause an increase in cellular cAMP and in chloride secretion. Of interest is the observation that CNP in combination with staurosporine significantly increased cellular cAMP levels. The reason for that effect is not clear at the present time.

In summary, CNP activates guanylate cyclase in the intact rectal gland as well as in rectal gland plasma membranes. However, cGMP does not reproduce the stimulation of chloride secretion induced by CNP. CNP has no effect on adenylate cyclase.

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