DUAL MECHANISM OF ACTION OF C-TYPE NATRIURETIC PEPTIDE IN THE RECTAL GLAND OF SQUALUS ACANTHIAS: THE ROLE OF PROTEIN KINASE C

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C-type natriuretic peptide (CNP), from both the shark and mammalian species (human), is capable of stimulating chloride secretion by the shark rectal gland (Solomon et al; Am. J. Physiol, 262: R707, 1992). It is thought that most of the biologic activity of this peptide results from generation of a second messenger cGMP. Using the perfused rectal gland model, we provide support for a dual mechanism of action of natriuretic peptides; an indirect effect mediated by release of VIP and a direct effect on the epithelial cells. With regard to the direct effect of CNP, we describe the effects of both agonists and antagonists of protein kinase C activity and postulate a necessary role for activation of protein kinase C in the stimulatory action of CNP on chloride secretion.

Isolated shark rectal glands were perfused and oxygen consumption measured as previously described (Solomon et al., Ibid. and Silva et al., Miner. Electrol. Metab. 12:286-292, 1986 respectively). All data are means±SEM. The effect of CNP (both shark C-type natriuretic peptide [sCNP] and human C-type natriuretic peptide [hCNP] is inhibited approximately 50% by simultaneous perfusion with 10^{-2}M procaine. In the presence of procaine, the stimulation of chloride secretion by hCNP was lower at all the concentrations examined (Figure 1). Procaine reduced the stimulatory effect of hCNP by an average of 44.5±3.3 %, n=5, $p< 0.01$. A similar effect was observed with sCNP (Figure 2). With this latter peptide, procaine reduced the stimulatory effect by 57±9.2 %, n=4, $p< 0.01$. There was no difference between hCNP or sCNP in the effects of procaine on either absolute or percent reduction of stimulated chloride secretion.

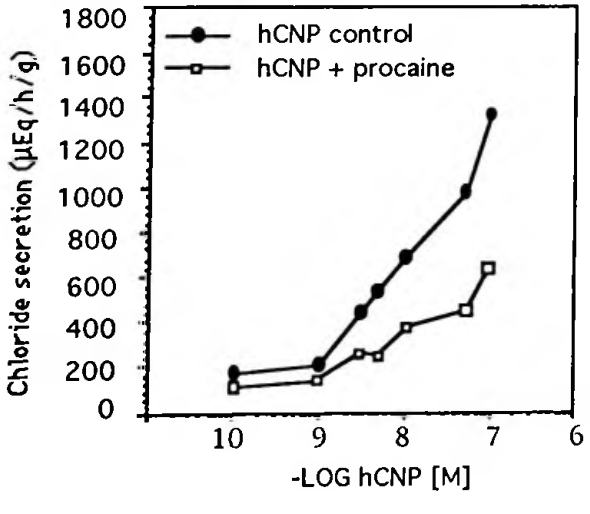


FIGURE 1.

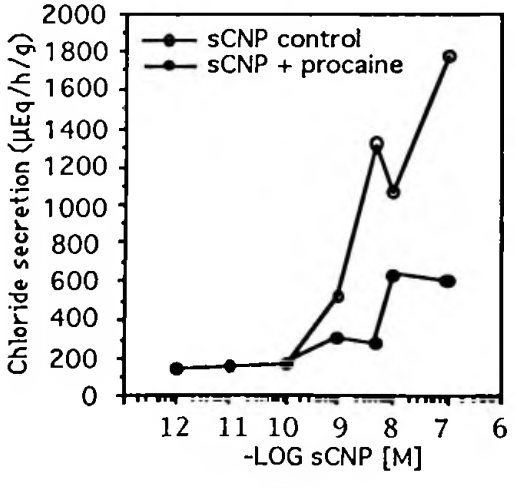
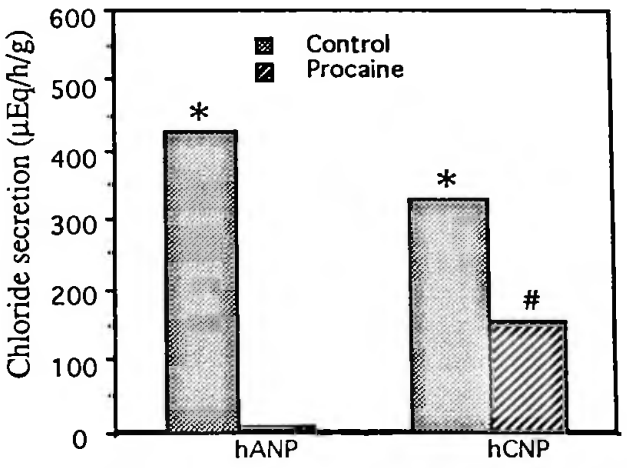


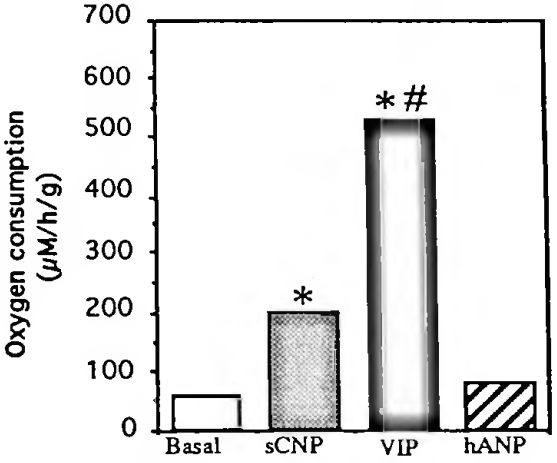
FIGURE 2.

The inhibition by procaine of both sCNP and pCNP-induced stimulation of chloride secretion is to be contrasted with the almost complete inhibition by procaine of hANP stimulated chloride secretion (Figure 3). To determine whether the failure of procaine to completely inhibit the effect of hCNP or sCNP was due to the greater rate of stimulation of chloride secretion observed with these peptides than with hANP, we injected sequential boluses of hANP and hCNP into the arteries of glands perfused with and without procaine. The doses of hCNP were lowered so as to increase the secretion of chloride to approximately the same rate achieved with hANP. Figure 3 shows that procaine completely suppresses the stimulatory effect of hANP while reducing but not preventing the stimulatory effect of a dose of hCNP that stimulates chloride secretion to the same rate as hANP.



* p<.05 compared to basal secretory rate
# p<.05 compared to hANP + procaine
FIGURE 3.

To isolate the direct effects of natriuretic peptides on the epithelial cells from possible indirect effects on other elements present in the intact gland (e.g. neural and vascular tissue), we determined ouabain-sensitive oxygen consumption in freshly isolated rectal gland tubules. Oxygen consumption directly correlates with active chloride transport in this preparation (Silva et al, J. Memb. Biol. 53:215, 1980). Vasoactive intestinal peptide (1 μM) increased ouabain-sensitive oxygen consumption confirming the known effects of VIP in this preparation. sCNP (1 μM) also significantly increased ouabain-sensitive oxygen consumption but the increase was significantly lower than that evoked by VIP. In contrast, hANP (1 μM) did not stimulate oxygen consumption (Figure 4).



* p<.05 compared to Basal
# p<.05 compared to sCNP
FIGURE 4.

We have interpreted these observations to reflect a dual mechanism of action of hCNP and sCNP. Procaine inhibits that component of their action which is mediated by the release of VIP from neurons within the gland. The chloride stimulatory effect of hANP is mediated entirely through this indirect mechanism. Thus the effect of hANP is completely inhibited by procaine (Figure 3) and hANP failed to stimulate oxygen consumption in a tubule preparation devoid of neural elements (Figure 4). We have previously reported that hANP releases VIP and that this release is completely inhibited by procaine (Silva et al. Am. J. Physiol. 252:F99, 1987). hCNP and sCNP, on the other hand, stimulate oxygen consumption in the tubule preparation and only 50% of the stimulatory effect in the intact gland is inhibitable by procaine. The residual chloride

stimulatory activity in the intact organ (in the presence of procaine) reflects the direct effect of hCNP or sCNP on the epithelial cells.

The mechanism of this direct effect of hCNP and sCNP is presently unknown. We hypothesized a role of the phosphoinositol pathway based upon studies of the effect of ANP in the rectal gland (Ecay and Valentich, J. Cell Physiol. 146: 407-416, 1990). To explore this possibility further, we perfused the rectal gland with staurosporin, an inhibitor of protein kinase C. Glands were perfused for 30 minutes with staurosporin, procaine, or the combination of staurosporin and procaine before the addition of hCNP 10^{-8}M or VIP $1.5x10^{-9}$M (Figure 5). Staurosporin, 10^{-8} M, reduces hCNP stimulated chloride secretion by approximately 50% (hCNP-ST vs hCNP). Staurosporin does not interfere with VIP stimulated chloride secretion (VIP-ST). When perfused with procaine, staurosporin reduces the hCNP stimulated chloride secretion by >90% (hCNP-ST/P vs hCNP). We interpret these observations to indicate that protein kinase C activation is necessary for the direct effect of hCNP in the rectal gland.

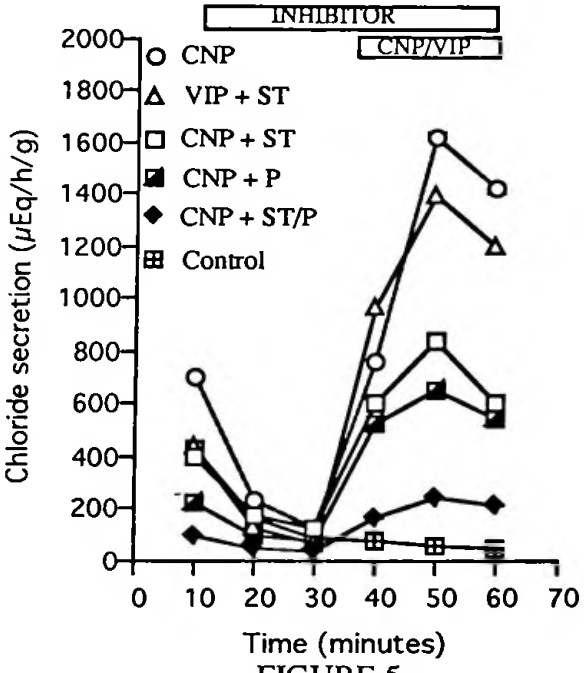
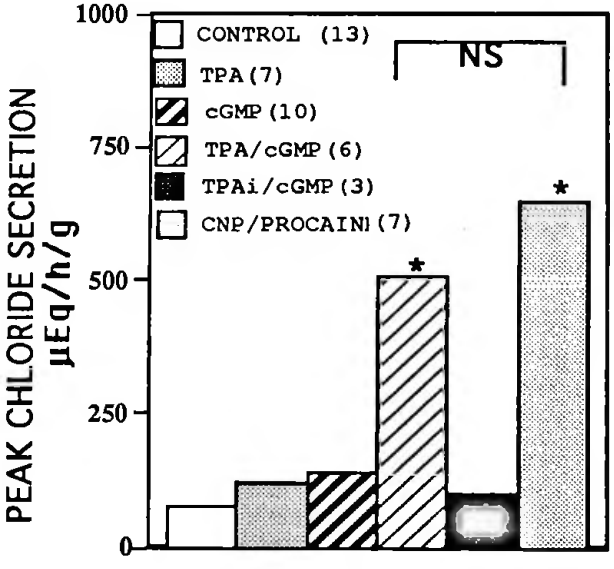


FIGURE 5.

To further evaluate the role of protein kinase C, we perfused rectal glands with phorbol 12-myristate 13-acetate (TPA), an agonist for the catalytic subunit of protein kinase C (Figure 6). Following a 30 minute basal period, TPA, 10^{-6}M, alone did not stimulate rectal gland chloride transport. Infusion of 10^{-4}M 8Br-cyclic-GMP also failed to stimulate chloride secretion (See also Silva et al; Bull MDIBL 1993). However, combined perfusion with TPA and 8Br-cyclic-GMP leads to stimulation of chloride secretion. The magnitude of stimulation is approximately 40% of that seen with CNP alone (compare to peak values at 50 minutes in Figure 4) and similar to that observed during perfusion with procaine. Perfusion with 8Br-cyclic-GMP and the inactive form of the phorbol ester, TPAi, failed to stimulate chloride secretion.



* p<.05 compared to CONTROL
FIGURE 6.

These results support our hypothesis that natriuretic peptides have a dual mechanism of action in the rectal gland. Natriuretic peptides increase chloride secretion indirectly via the release of VIP (procaine-inhibitable). This accounts for the action of hANP in the intact rectal gland. Although we have not measured VIP release following hCNP, we have previously shown that shark heart extracts do so (Silva et al., Amer. J. Physiol. 1987). The fact that procaine inhibits 50% of the stimulatory action of hCNP supports this mechanism. C-type natriuretic

inhibits 50% of the stimulatory action of hCNP supports this mechanism. C-type natriuretic peptides also have a direct effect on the rectal gland epithelium. This direct effect accounts for the 50% of the stimulatory effect in the intact organ which is not procaine-inhibitable and the increase in oxygen consumption by isolated tubules. This direct effect requires both activation of guanylate cyclase and protein kinase C. 8Br-cyclic-GMP alone does not stimulate secretion. Protein kinase C activation alone also fails to stimulate secretion. However, when both protein kinase C activation and cGMP accumulation occurs, an increase in chloride secretion follows. The chloride secretory rate under these conditions is similar to that observed when the indirect effect of hCNP is inhibited by procaine. The combination of staurosporin and procaine inhibits both the direct and indirect pathways respectively, completely eliminating the stimulatory effect of hCNP.

Our present understanding of the action of natriuretic peptides is depicted in Figure 7. ANP reacts only with nervers which contain the ANPR-C receptor (not coupled to guanylate cyclase). This results in the release of VIP from neurons which binds to a VIP receptor on epithelial cells coupled to adenylate cyclase. Subsequent activation of protein kinase A (PKA) is associated with a increase in chloride conductance. CNP reacts with both the ANPR-C receptor on nerves and the ANPR-B (GC-B) receptor on epithelial cells which is coupled to guanylate cyclase. This latter receptor results in an increase in cGMP within the epithelial cell. This increase alone is not sufficient to produce an increase in chloride conductance. However, when accompanied by an increase in protein kinase C activity, an increase in chloride conductance occurs. How CNP stimulates protein kinase C is presently unknown.

DUAL MECHANISM OF ACTION MODEL
FOR NATRIURETIC PEPTIDES

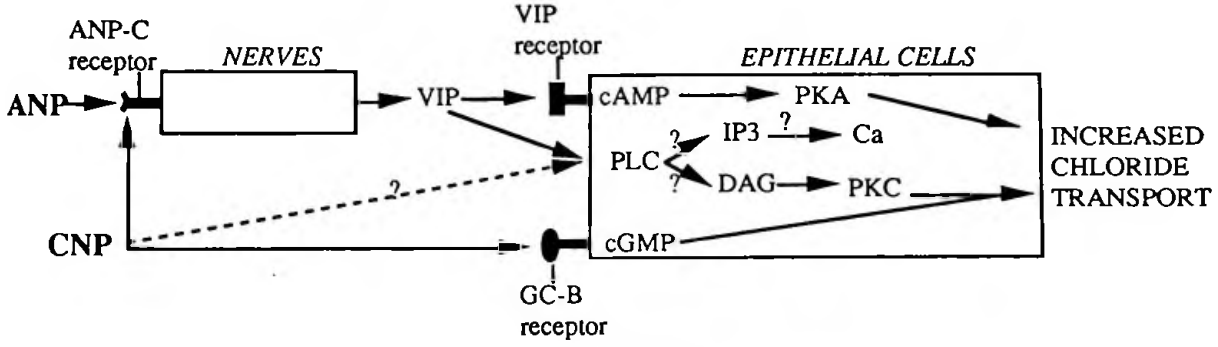


FIGURE 7.

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