NICKEL INHIBITS THE EFFECTS OF VIP BUT NOT C-TYPE NATRIURETIC PEPTIDE IN THE SHARK (SQUALUS ACANTHIAS) RECTAL GLAND

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Nickel is a toxic heavy metal that inhibits calcium channels and binds to divalent metal sites on proteins. In the shark rectal gland, we have previously shown that nickel inhibits VIP stimulated increases in intracellular calcium, as measured by the photoprotein aequorin (Tse et al., CMTS Progress Report 3: 24-25, 1988). In the present studies, we examined the effects of nickel on chloride secretion stimulated by Vasoactive Intestinal Peptide (VIP), a secretagogue whose intracellular action is mediated by the cAMP-protein kinase A pathway. We also determined the effects of nickel on the action of shark C-type natriuretic peptide (CNP), a shark heart hormone whose intracellular effects are mediated by cGMP. Our results indicate a striking difference in the effects of nickel on the actions of VIP compared to CNP.

Chloride secretion and tissue cyclic nucleotide content were measured as previously described (Kelley et al., J. Clin. Invest. 88: 1933-1939, 1991). All data are mean ± SEM.

Figure 1 illustrates the dose response to nickel (1 to 100 μM) on VIP stimulated chloride secretion in the isolated perfused rectal gland. VIP (10 nM), added to elasmobranch Ringer's perfusing the rectal gland, promptly increased chloride secretion from basal levels of 143±22 to 2076±167 μEq/h/g (Figure 1). Nickel, at 1 nM, only slightly reduced VIP stimulated chloride secretion. However, 10 μM and 100 μM nickel completly inhibited VIP simulated chloride secretion to basal values or values observed in the presence of bumetanide (100 μM).

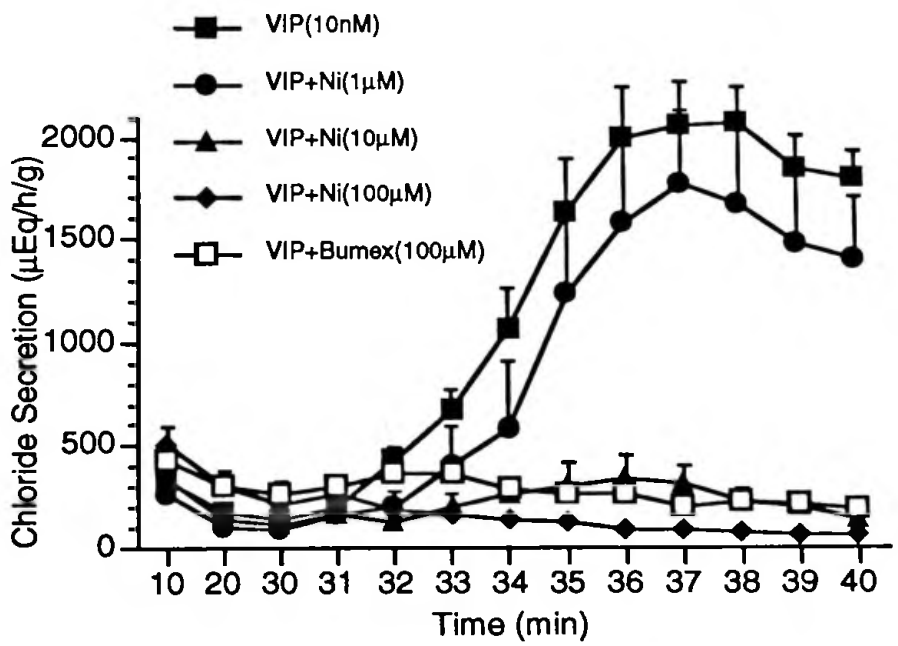

Figure 1. Chloride secretion stimulated by VIP (10nM) in the presence of nickel (1 to 100 μM) or bumetanide (100 μM). Substances were added following 30 min of basal perfusion and chloride secretion was measured at one minute intervals for an additional 10 min (n=4-7 per group).

In contrast to the effects of nickel on chloride secretion stimulated by VIP, concentrations of nickel up to 300 μM were entirely without effect on chloride secretion stimulated by CNP

(Figure 2). When perfused with 10 nM CNP alone, chloride secretion rose from basal values of 216±67 to 1689±256 μEq/h/g. At 1 and 300 μM, nickel was entirely without effect on the action of CNP. A modest reduction of chloride secretion was observed at 1mM nickel (Figure 2).

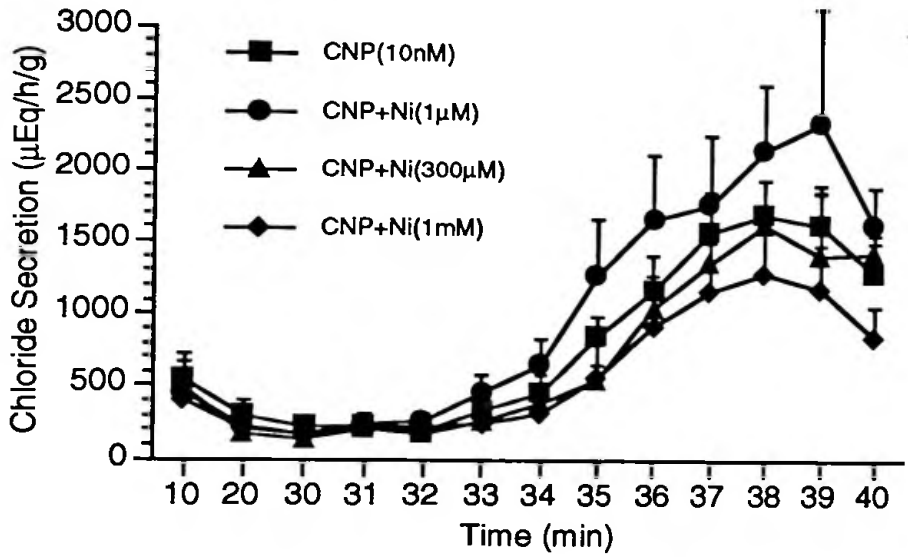

Figure 2. Chloride secretion stimulated by CNP in the presence of nickel (1 to 300 μM). Substances were added following 30 min of basal perfusion and chloride secretion was measured at one minute intervals for an additional 10 min (n=3-6 per group).

To determine if nickel had different effects on the intracellular second messengers for VIP and CNP, we measured tissue nucleotide content from sections of the glands snap frozen in liquid nitrogen at 40 min of perfusion in the previous experiments.

Figure 3 illustrates the effect of nickel (1 to 100 μM) on VIP stimulated increases in chloride secretion and tissue cAMP and cGMP content. Values of chloride secretion are the final minute readings before freezing of tissue. VIP (10 nM) alone increased chloride secretion to 1788±145 μEq/h/g and increased tissue cAMP content from basal values of 7±1 pmol/mg protein (data not shown) to 29±7 pmol/mg protein (Figure 3). At 1 μM nickel, chloride secretion was modestly reduced and was accompanied by a substantial reduction of tissue cAMP. At 10 and 100 μM nickel, chloride secretion was inhibited to basal values and cAMP content was completely inhibited to values that were not different from basal.

To demonstrate that the reduction of cyclic nucleotide content was a direct effect of nickel and not a non-specific response to reduced secretion, four glands were perfused with VIP in the presence of bumetanide, an inhibitor of the Na-K-2Cl cotransporter. As shown in Figure 3, bumetanide (100 μM) completely inhibited VIP stimulated chloride secretion but was without effect on tissue cAMP content.

In contrast, the effects of nickel on CNP stimulated increases in chloride secretion and cGMP content are shown in figure 4. CNP (10 nM) increased chloride secretion to 1574±170 μEq/h/g at the 40 min time period. Simultaneous cGMP tissue content increased from basal levels (less than 2 pmol/mg protein) to 69.5±9 pmol/mg protein. Concentrations of 1 to 300 μM nickel were entirely without effect on CNP stimulated increases in cGMP. At 1 mM nickel, there was a modest reduction in chloride secretion and cGMP content.

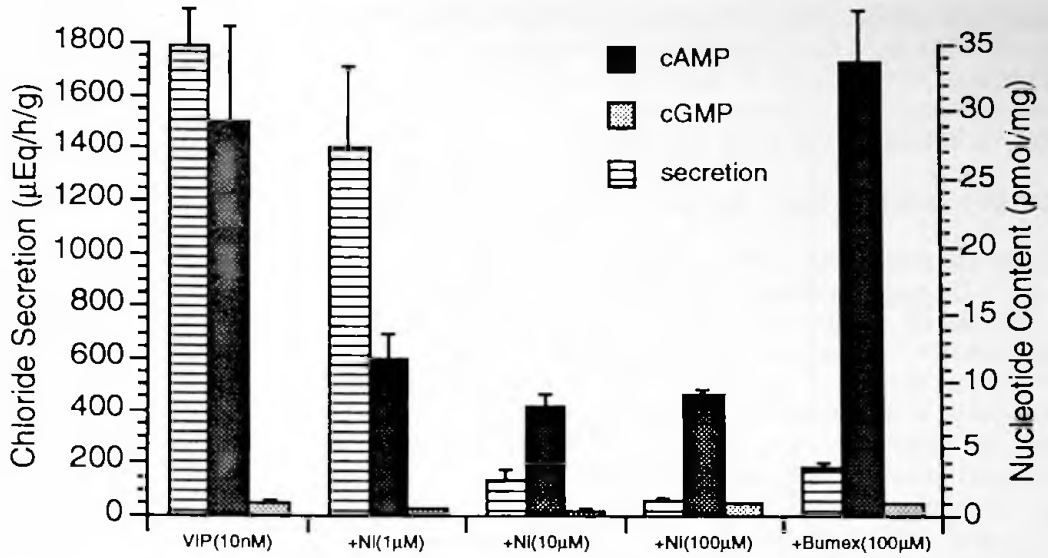

Figure 3. Effects of nickel on VIP stimulated increases in chloride secretion and cyclic nucleotide content.

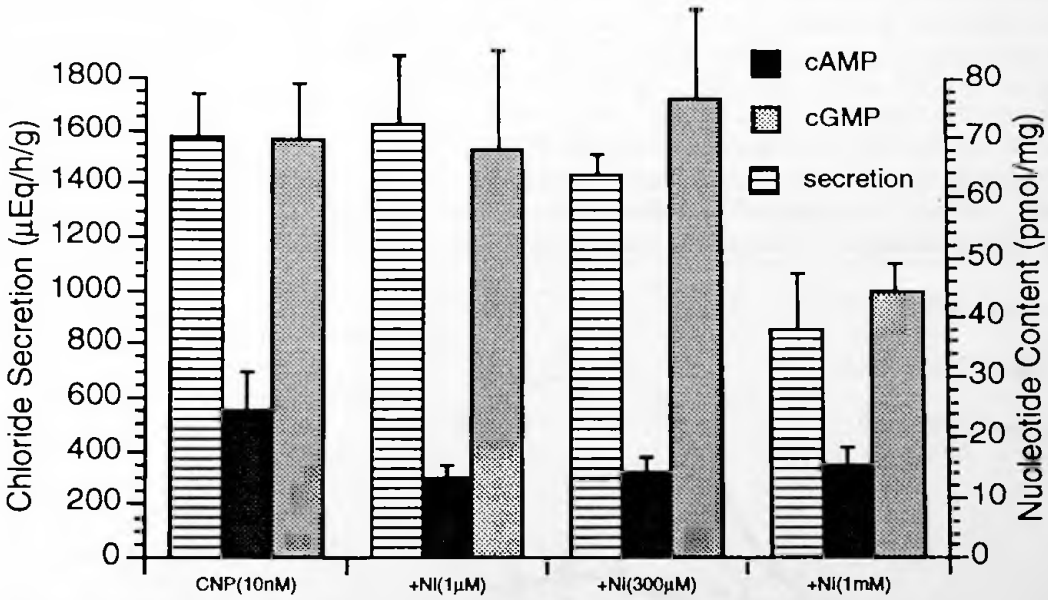

Figure 4. Effects of nickel on CNP stimulated increases in chloride secretion and cyclic nucleotide content.

In summary, these results provide the first evidence that nickel selectively blocks secretagogues in a chloride secreting epithelium. At low concentrations nickel completely inhibited VIP stimulated chloride transport and cAMP content but did not inhibit C-type natriuretic peptide stimulated secretion or cGMP content. Since nickel inhibits VIP stimulated increases in intracellular calcium, the results suggest that nickel may act at a calcium sensitive step in the signal transduction of VIP, possibly at a Gs protein. Nickel may be a useful tool in dissecting the crosstalk between cAMP and cGMP mediated pathways of secretagogues.

Work supported by NIH grants DK 34208-09 and NIEHS P30-ES 3828 (Center for Membrane Toxicology Studies), American Heart Association grant 92011310, and NSF EPSCoR grant EHR 9108766 to Dr Forrest.