NICKEL INHIBITS VIP STIMULATED CHLORIDE TRANSPORT IN THE SHARK RECTAL GLAND BY BINDING TO THE PEPTIDE HORMONE

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Ni^{2+} potently inhibits vasoactive intestinal peptide (VIP)-stimulated chloride secretion in the rectal gland of *Squalus acanthias* and this inhibition is accompanied by an inhibition of cAMP accumulation in vivo (Kelley, G.G. et al., Bull MDIBL. 27:129-131, 1988). In contrast, other secretagogues, including adenosine receptor agonists, C-type natriuretic peptide (CNP) and forskolin are not inhibited by micromolar concentrations of Ni^{2+} (Kelley, G.G. et al., Bull. MDIBL, 27:129-131, 1988; Kelley et al., Bull. MDIBL. 33:87-89, 1994). Although Ni^{2+}, at low micromolar concentrations is a potent inhibitor of calcium influx pathways, and is a specific inhibitor of T-type calcium channels, the effects of Ni^{2} in the shark rectal gland do not appear to be mediated by inhibition of calcium influx (see abstract by Motley W. et al, this Bulletin).

Nickel has been shown to bind certain proteins and peptides with a high affinity. (Bal, W. et al., Arch Biochem Biophys. 364:161-166, 1999; Bal, W., et al., Acta Biochim Pol. 44(3): 467-476, 1997; Predki, P.F., et al., Biochem J. 287: 211-215, 1992). Nickel and Cu2+ complex with certain peptide hormones including luteinizing hormone-releasing hormone and angiotensin II fagments (Gerga, K., et al., J Inorg Biochem 33:11-18, 1988: Decock-Le R. B., et al., J Chem Soc Dalton Trans 887-894,1988; Pettit, L., et al, J Chem Soc Dalton Trans 1471-1475,1989.)

To evaluate the possibility that binding of Ni^{2+} to VIP might account for the inhibitory effect observed in perfusion studies, we examined the interaction between nickel and VIP spectrophotometrically using a Beckman DU-640 scanning spectrophotometer. Difference absorption spectra over wavelengths 300-1100 nm were determined for $NiCl_2$, 0.6 and 1.2 mM in Ringer's, in the absence and presence of VIP at 21 °C. Solutions were incubated for 120 min prior to measurements. The reference blank was VIP in elasmobranch Ringer's.solution.

To detect Ni^{2+} binding to VIP, we first determined if VIP shifted the absorption spectra of Ni^{2+}. Ni^{2+} displayed a typical maximal absorbance at 396 nm of 0.0030 at 0.6 mM and 0.0065 at 1.2 mM. In the presence of VIP, the maximal absorbance was shifted to 438 nm and markedly enhanced to 0.0181 at 0.6 mM and 0.0247 at 1.2 mM. The effects of 0.6 mM Ni^{2+} are shown in figure 1. The first five amino acids are required for this interaction since the antagonist peptide that has these amino acids substituted for amino acids KPRRY did not bind to Ni^{2+} (see Fig. 1). This demonstrates that Ni^{2+} binds to VIP and suggests that Ni^{2+} inhibits VIP-stimulated chloride transport by inducing a conformational change that inhibits the interaction of VIP with its receptor.

Pituitary adenylate cyclase activating peptide (PACAP) and peptide histidine isoleucine (PHI) also stimulate chloride transport in the shark rectal gland. Because of the conserved amino acid sequence (see Fig. 2), especially the N-terminal six amino acids which appear critical for binding to Ni^{2+}, the effects of Ni^{2+} on PACAP and PHI stimulated chloride secretion were

determined in perfusion studies. Similar to VIP, Ni^{2+} at 10 μM completely inhibited chloride secretion stimulated by these related hormones (data not shown). Cu2+ also potently inhibited the secretory response to VIP by 89%. In the absence of Cu2+, 1nM VIP stimulated chloride secretion 2235 ± 301 μEqCl-/h/g (n=5) above basal values compared to 255±161 in the presence of 10 μM Cu2+ (n=3) ($p < 0.0004$). Cu2+, 10 μM, was equally effective in inhibiting 10nM VIP.

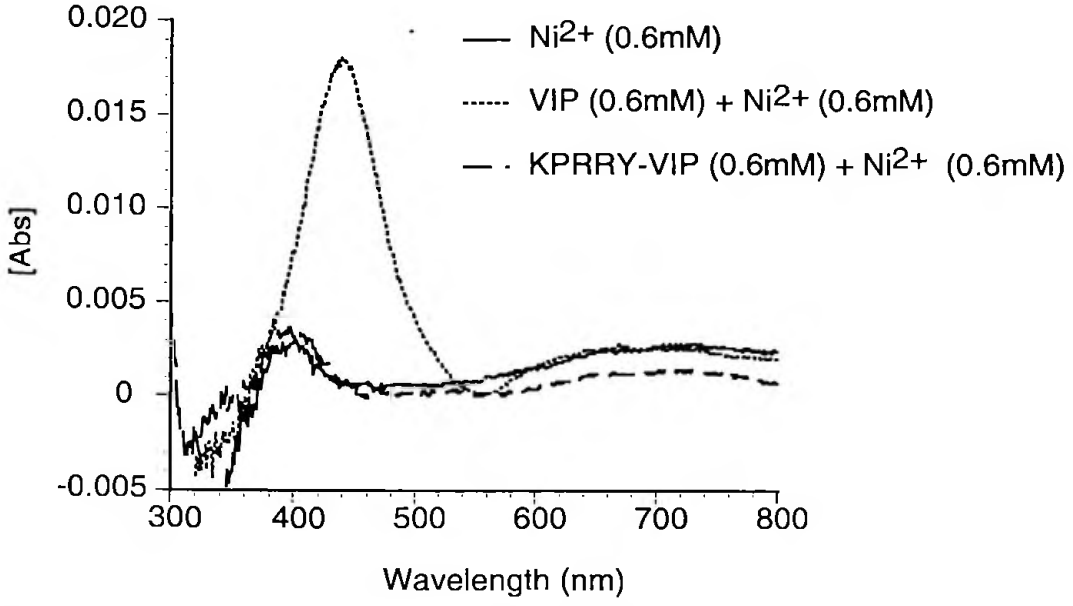

Figure 1. Effect of VIP and the VIP analogue KPRRY-VIP on the absorption spectra of Ni^{2+}. An interaction between nickel and VIP was determined spectrophotometrically using a Beckman DU-640 scanning spectrophotometer. Difference absorption spectrum over wavelengths 300-1100 nm was determined for $NiCl_2$, 0.6 mM in Ringer's at 21 °C, in the absence and presence of VIP or the VIP receptor antagonist KPRRY-VIP. Solutions were incubated at 21 °C for two hr to allow binding. The reference blank was VIP or KPRRY-VIP in Ringer's. Data are representative of three similar experiments.

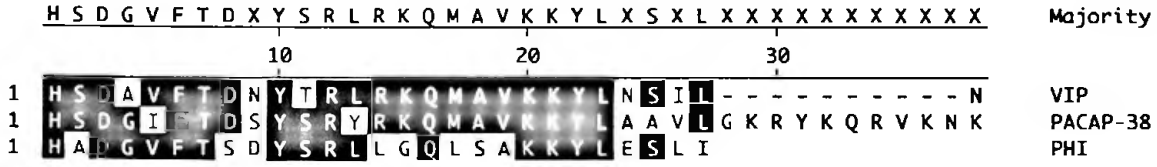

Figure 2. Alignment of VIP, PACAP and PHI. Alignment was generated using DNAStar Megalign software.

Taken together with work reported elsewhere in this Bulletin (See Motley et al.), these studies suggest that the effects of Ni^{2+} on VIP-stimulated chloride transport are not mediated by an inhibition of a Ca^{2+} channel but by a direct interaction with the peptide hormone. This interaction requires the first five amino acid residues of the peptide, since the VIP antagonist KPRRY-VIP did not bind to Ni^{2+}. A direct interaction with VIP is consistent with lack of an effect of Ni^{2+} on adenosine analog- or forskolin-stimulated chloride transport. Furthermore, the effects of Ni^{2+} on VIP can be extended to other members of the VIP/GHRH/PHI/PACAP family that have a similar structure since Ni^{2+} blocked PACAP-27- and PHI-stimulated chloride transport. This work was supported by NIH grants DK 34208, DK56294, and NIEHS P30-ES 3828 (Center for Membrane Toxicology Studies).