INHIBITORY EFFECTS OF NICKEL ON VIP AND FORSKOLIN STIMULATED CHLORIDE SECRETION IN THE RECTAL GLAND OF SQUALUS ACANTHIAS

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We have previously demonstrated that vasoactive intestinal peptide (VIP) and forskolin stimulated chloride transport in the perfused rectal gland is inhibited by Co^{2+} (Kelley et al, Bull MDIBL 27:129-131, 1988). Further, we have shown that both VIP and forskolin increase intracellular free $[Ca^{2+}]$ in slices of the rectal gland as measured by aequorin (Kelley et al., Bull MDIBL 27:129-131, 1988). In other tissues, nickel, similar to cobalt, has also been shown to block calcium influx through a variety of calcium channels (Tsien et al., TINS 11:431-438, 1988). In the present study we determined the effects of Ni^{2+} on VIP and forskolin stimulated secretion in the perfused rectal gland. In addition, we determined the effects of nickel on VIP stimulated increases in intracellular free $[Ca^{2+}]$ in tissue slices.

Rectal glands were perfused as described previously (Forrest et al., Bull. MDIBL 20, 1980). Intracellular free $[Ca^{2+}]$ was determined using the photoprotein aequorin as previously described (Kelley et al., Bull MDIBL 27:129-131, 1988).

Nickel potently inhibited the secretory response to VIP. Ni^{2+}, at 1 μM and below did not significantly inhibit the response to 1 nM VIP. However, concentrations of 3 μM and greater completely blocked VIP stimulated chloride secretion. Figure 1 illustrates the inhibitory effect of 10 μM Ni^{2+} on 1 nM VIP stimulated chloride secretion.

Nickel also inhibited forskolin stimulated chloride secretion; however, this effect was much less potent than its effect on VIP. Ni^{2+} at 10 μM did not significantly inhibit 1 μM forskolin stimulated chloride secretion. At 100 μM, however, Ni^{2+} reversibly inhibited forskolin stimulated secretion by 75±26.9 percent ($p<0.001$) (Figure 2). At 1 mM, Ni^{2+} inhibited the effects of forskolin by 82±8.2 percent ($p<0.001$).

To determine if the inhibitory effect of Ni^{2+} was mediated by its well known effect on Ca^{2+} channels, we examined the effect of Ni^{2+} on the VIP induced increase in intracellular free $[Ca^{2+}]$. Ni^{2+} at 300 μM had no effect on basal intracellular free $[Ca^{2+}]$ levels as measured by aequorin luminescence. However, it inhibited the increase in aequorin luminescence induced by 300 nM VIP to basal levels (n=4). When slices were perifused with 300 μM Ni^{2+} prior to the addition of 300 nM VIP, the increase in the aequorin luminescence was completely blocked (n=4).

In summary, Ni^{2+} potently inhibited VIP and forskolin stimulated chloride secretion. Although the mechanism of this inhibition is not fully characterized, one likely explanation is that Ni^{2+} interferes with Ca^{2+} influx stimulated by VIP and forskolin. Consistent with this hypothesis is the effect of Ni^{2+} to inhibit VIP stimulated increases in intracellular free Ca^{2+}.

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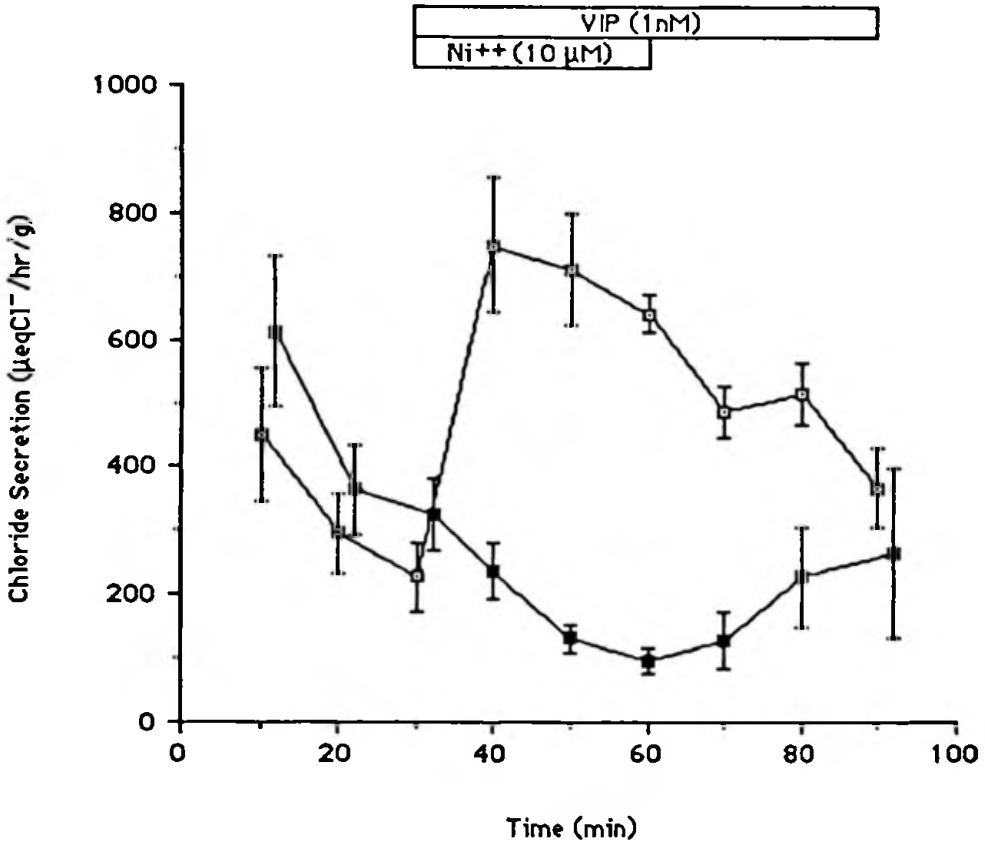

Figure 1. Inhibition of VIP stimulated chloride secretion by nickel in isolated perfused rectal glands. In the control group [-□-], rectal glands (n=6) were perfused for three 10 min periods with a basal solution containing adenosine deaminase (0.1 U/ml) and bovine serum albumin (0.1 mg/ml) in elasmobranch Ringer's at 15°C. 1 VIP (1nM) was added to the basal solution after 30 minutes and continued for six 10 min periods. In the experimental group [-■-], rectal glands (n=4) were perfused for three 10 min periods with the basal solution, three 10 min periods with 10 µM $NiCl_2$ and 1 nM VIP, and three 10 min periods with 1 nM VIP alone. $NiCl_2$ significantly inhibited VIP stimulated chloride secretion ($P<0.01$) for 30 minutes. Reversal of inhibition was observed 30 minutes after the removal of nickel.

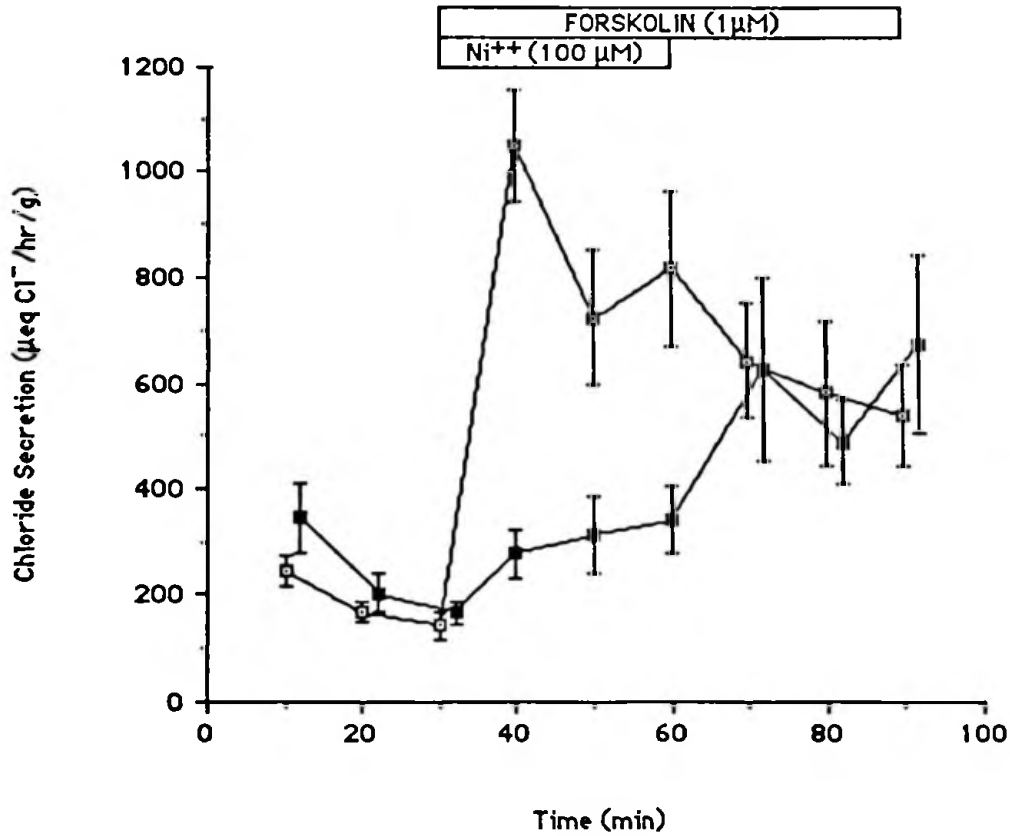

Figure 2. Inhibition of forskolin stimulated chloride secretion by nickel in isolated perfused rectal glands. In the control group [-⊡-], rectal glands (n=6) were perfused for three 10 min periods with a basal solution containing adenosine deaminase (0.1 u/ml) in elasmobranch Ringer's solution at 15°C. 1 µM forskolin was added to the basal solution after 30 min and continued for six 10 min periods. In the experimental group [-■-], rectal glands (n=7) were perfused for three 10 min periods with the basal solution, three 10 min intervals with 100 µM $NiCl_2$ and 1 µM forskolin, and three 10 min intervals with 1 µM forskolin alone. $NiCl_2$ significantly inhibited the forskolin stimulated chloride secretion response ($p<0.01$) for 30 minutes.