EFFECTS OF PROCAINE ON SECRETAGOGUE STIMULATED CHLORIDE SECRETION IN THE RECTAL GLAND OF THE DOGFISH SHARK, *SQUALUS ACANTHIAS*

William Motley[1], Suzanne J. Forrest[3], Raquel Williams[2], Sarah Decker[1,3], John N. Forrest Jr.[3]
[1] Middlebury College, Middlebury VT, 05753
[2]Georgia Southern University, Statesboro, GA 30460
[3]Dept. of Medicine, Yale University School of Medicine, New Haven, CT 06510

C-type natriuretic peptide (CNP) stimulates chloride secretion in the shark rectal gland (Schofield et al., Am. J. Physiol., 261: F734, 1991; Solomon et al., Am. J. Physiol., 262: R707, 1992). There is evidence that CNP activates chloride secretion directly via a CNP receptor located on the basolateral membrane of the rectal gland epithelial cell (Aller et al; Am. J. Physiol., 276:C442-C449, 1999). It has been proposed that CNP also acts indirectly, by releasing vasoactive intestinal peptide (VIP) from nerves, leading to activation of CFTR via the basolateral VIP receptor. This hypothesis was supported in part by the observation that procaine, a local anesthetic, significantly inhibited CNP stimulated chloride secretion in the perfused shark rectal gland (Silva et al; Am. J. of Physiol., 277: R1725-R1732, 1999) whereas procaine appeared to be without effect on VIP stimulated secretion in earlier studies (Stoff et al; Am. J. Physiol., 255: R212-R216, 1988). In these previous experiments, the secretagogues (VIP and CNP) were given as 1 min bolus infusions and secretion was measured at 10 min intervals.

We reasoned that if procaine selectively inhibited putative CNP-stimulated nerve release of VIP, then procaine should have no effect on either VIP or forskolin infused at constant concentrations. Therefore, we perfused rectal glands *in vitro* in paired experiments conducted by

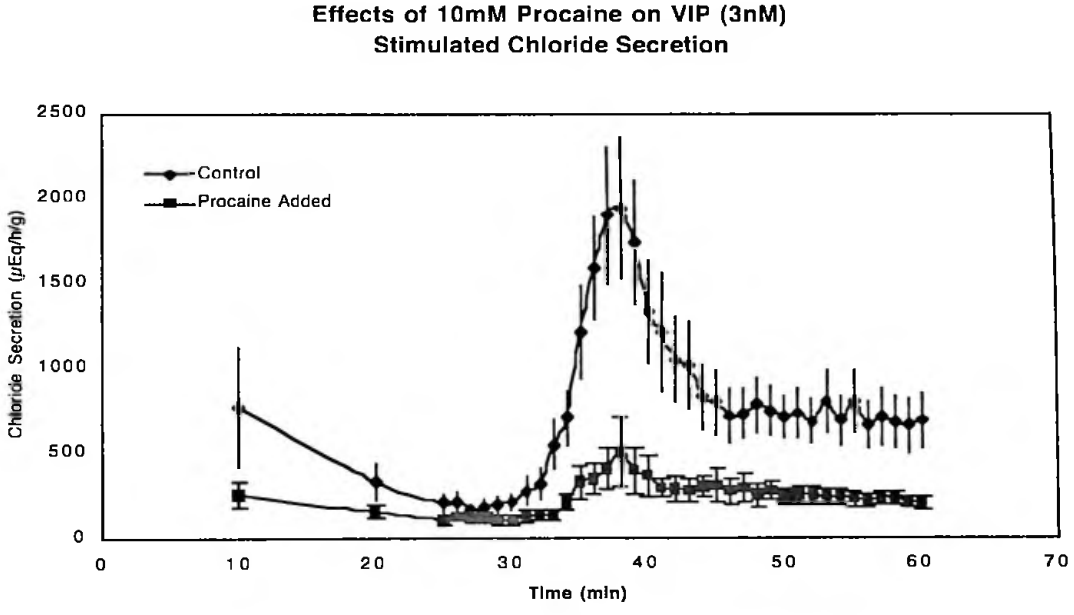

Figure 1. Effects of procaine (10mM) added at t=0 on VIP (3nM) stimulated chloride secretion. VIP was added to both groups at t=30. $P<0.01$ at 36, 37 and 50 min and $P<0.05$ at other time points. Data are means± standard error for n=6 paired experiments.

blinded observers with methods described previously (Lehrich et al., J. Clin. Invest. 101:737-745, 1998). The effects of procaine on constant infusions of two known secretagogues, VIP and

forskolin, were examined. In control experiments, after thirty minutes of basal perfusion, the secretagogue was perfused continuously for 30 min, with 1 min measurements from minutes 25-60. In the experimental perfusions, procaine was added in both the basal state and during the secretagogue infusions. The solution containing secretagogue was divided into two aliquots prior to the addition of procaine to ensure identical concentrations of secretagogue in the paired experiments.

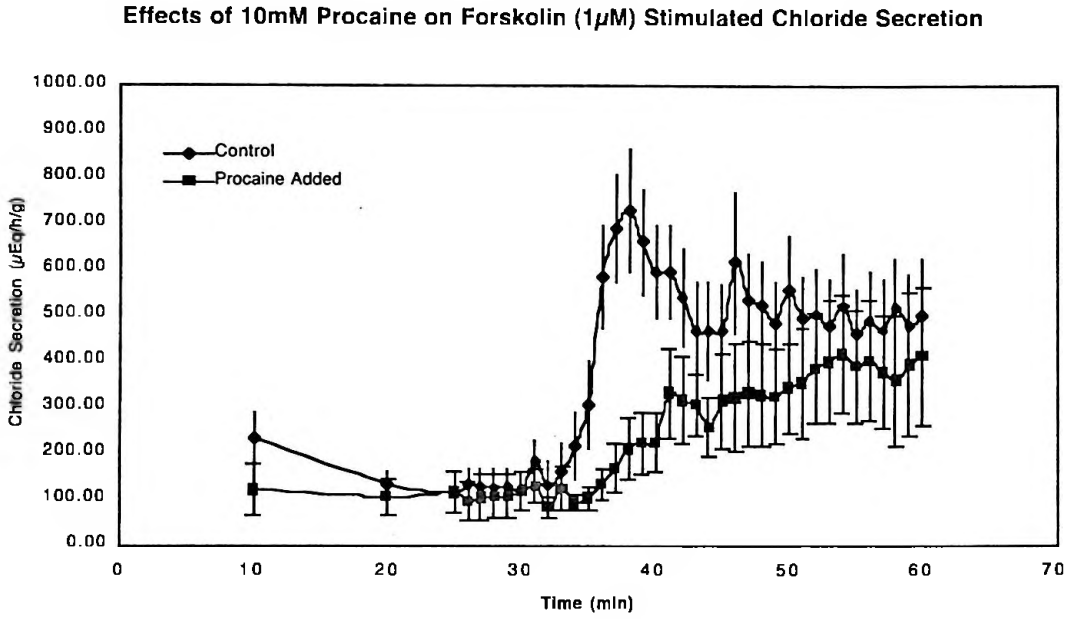

Figure 2. Effects of procaine (10mM) added at t=0 on forskolin (1μM) stimulated chloride secretion. Forskolin was added to both groups at t=30. P<0.001 at points 36 and 37 min and P<0.01 from 38-40 min. Data are means ± standard error for.n=10 paired experiment

VIP and forskolin stimulated chloride secretion in the shark rectal gland were significantly inhibited by 10^{-2}M procaine. The chloride concentration of the secreted fluid was also significantly reduced by the addition of procaine (data not shown). VIP acts on extracellular G-protein coupled VIP receptors, while forskolin acts at an intracellular site on the catalytic unit of adenylate cyclase. The effects of procaine to block significantly both a receptor agonist (VIP) and a secretagogue acting downstream at an intracellular second messenger site (forskolin) suggest that procaine is a non-specific inhibitor acting at another site(s) or that the anesthetic has a toxic metabolic effect on the shark rectal gland cell.

Our experimental results differ from those reported previously by Stoff et al and Silva et al who observed that identical concentrations of procaine were without effect on VIP but inhibited CNP stimulated secretion. Our methods differed from the earlier reports in three regards: use of paired blinded experiments, constant infusion of secretagogues, and measurements at one minute intervals. In other systems, procaine has been shown to inhibit Na-K-ATPase (at 17 mM) (Kutchai et al., Pharmacol Res 43:399-403, 2001). voltage gated sodium and potassium channels (at 60μM) (Brau et al., Anesth Analg 87:885, 1998), calcium stores (Leppanen et al., J Neurophysiol, 78(4):2095-107, 1997), and calcium channels (Yoshino et al; Naunyn-Shmiedebergs Archives of Pharmacology, 353(3):334-41, 1996).

These data suggest that procaine cannot be used to specifically inhibit neurotransmitter release in the perfused shark rectal gland. The results are also consistent with the view that CNP acts primarily, and possibly solely, on basolateral guanylate cyclase mediated CNP receptors in the shark rectal gland.

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