HOW DOES C-TYPE NATRIURETIC PEPTIDE (CNP) ACTIVATE NaCl SECRETION IN ISOLATED *IN VITRO* PERFUSED RECTAL GLAND TUBULES OF *SQUALUS ACANTHIAS*?

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In previous studies in the intact gland and in cultured rectal gland tubule cells, it has been shown that CNP activates NaCl secretion (Forrest, J.N., *Kidney Int.* 49:1557-1562,1996; Forrest, J.N., et al., *Bull. Mt. Desert Isl. Biol .Lab.* 31:71-72, 1992; Gunning, M., et al., *Am. J. Physiol.* 264:F300-F305, 1993; Karnaky, K.J., et al., *Bull. Mt. Desert Isl. Biol. Lab.* 32:67-68, 1993; Schofield, J.P., et al., *Am. J. Physiol.* 261:F734-F739, 1991). We wanted to examine the mechanism of action by *in vitro* perfusion of isolated rectal gland tubules (Greger, R., et al., *Pfluegers Arch. Eur. J. Physiol.* 402:376-384, 1984) and measuring transepithelial voltage (V_{te}), transepithelial resistance (R_{te}) equivalent short circuit current ($I_{sc} = V_{te}/R_{te}$), basolateral and luminal membrane voltage (V_{bl},V_l), voltage divider ratios (R_l/R_{bl}), and by measuring cytosolic Ca^{2+} activity using Fura-2 indicator in the isolated *in vitro* perfused tubule (Nitschke, R., et al., *Pfluegers Arch. Eur. J. Physiol.* 417:622-632, 1991).

CNP added to the bath solution increased I_{sc} in a concentration-dependent manner, with a maximum effect at 40 nmol/l added to the bath solution. CNP had no effect from the lumen side (n = 6). I_{sc} was -20.5 ± 2.7 μA/cm^2 under control conditions and was increased to -470 ± 111 μA in the presence of 40 nmol/l CNP (n=9). CNP had no effect on basolateral membrane voltage in nine impalements (-88.7 ± 1.14 mV versus -87.4 ± 2.47 mV (n=9)). However, it significantly depolarized luminal membrane voltage by 4 mV. The voltage divider ratio in the presence of CNP was 45 ± 16% (n=4) of its control value. An increase of the bath K^+ concentration from 3.6 to 30 mmol/l had a similar depolarizing effect under control conditions (46.1 ± 0.88 mV (n =7)) and in the presence of CNP (44.9 ± 1.5 mV (n = 3) which corresponded to 81% of the Nernstian slope. In the presence of CNP, the Fura-2 fluorescence ratio (340/380 nm) increased significantly from 0.96 ± 0.044 to 1.35 ± 0.086 (n=5). These data indicate that CNP increases cytosolic Ca^{2+}. In additional studies, CNP (10 nmol/l) did not act additively with VIP (10 nmol/l, n = 6) nor was the effect of CNP or VIP inhibited by somatostatin (0.1 μmol/l, n = 6).

The data confirm that CNP acts as a secretagogue. It increases I_{sc}, corresponding to the rheogenic secretion of Cl^-. This effect is paralleled by a slight but significant depolarization of the luminal membrane but no change in basolateral membrane voltage. Also, the fractional conductance of the basolateral membrane to K^+ stays almost unaltered in the presence of CNP. CNP reduces transepithelial resistance. This is probably caused by the synchronous opening of a luminal Cl^- conductance and a basolateral K^+ conductance. The increase in cytosolic Ca^{2+} may be responsible for later effects. In comparison to agonists acting via cAMP (Forrest, J.N., *Kidney Int.* 49:1557-1562,1996; Greger, R., et al., *Pfluegers Arch. Eur. J. Physiol.* 402:376-384, 1984), CNP has a very similar mode of action (also cf. accompanying report). Supported by DFG Gr480/12, and NIH grant to JNF.

observed by ourselves as a slight fall in fluorescence with 360nm excitation (isosbestic point) in Fura-2 fluorescence studies (data not shown). This cell swelling and unfolding of the plasma membrane might then be responsible for the small capacitance increase. Along the same lines, addition of furosemide by shrinking the cells reduces membrane capacitance in a similar way.

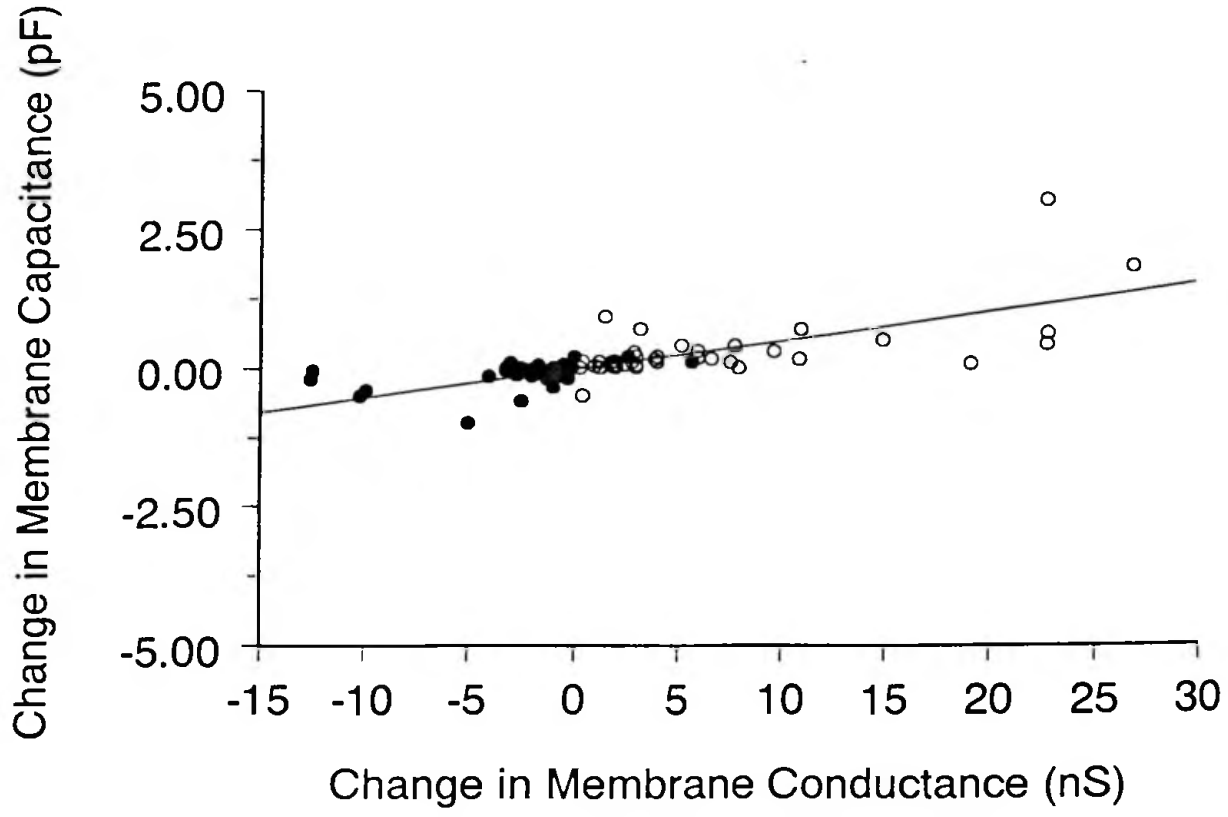

Fig. 1: Correlation of membrane capacitance changes (ΔC_m) and membrane conductance changes (ΔG_m) seen after addition of Stim (closed circles, forskolin (10 µmol/l); dibutyryl-cAMP (0.5 mmol/l); and adenosine (0.5 mmol/l)) or furosemide (open circles, 0.5 mmol/l) in the presences of Stim in whole cell patch clamp experiments in rectal gland tubule cells. The correlation was positive: $\Delta C_m = -0.03 + 0.51\ \Delta G_m$, $r = 0.65$.

To further examine the possible involvement of vesicular traffic in the activation of Cl^- conductance cytoskeletal inhibitors were added to the patch pipette solution and measurements of C_m, G_m and V_m were performed. Phalloidin (10 µmol/l), colchicine (10 µmol/l) and cytochalasin D (10 µmol/l) were all without effect on C_m nor did they prevent the activation of the Cl^- conductance ($n = 4$ to $n = 14$). These data indicate that cAMP activates Cl^- secretion with little increase in membrane capacitance and renders an activation of Cl^- conductance by exocytosis in these cells unlikely. This study has been supported by DFG Gr 480/12.