NEUROPEPTIDE INHIBITION OF ACTIVE TRANSPORT IN CULTURED RECTAL GLAND CELLS OF SQUALUS ACANTHIAS

F.H. Epstein[1], K. Spokes[1], P. Silva[2], R. Solomon[2], S. Hornung, J.D. Stidham, A.S. McCraw, D.S. Nelson, K. Karnaky, Jr.[3]

Beth Israel[1] and Deaconess[2] Hospitals, Joslin Diabetes Center and Harvard Medical School[1,2], Boston, MA 02215 and Medical University of South Carolina[3], Charleston, SC 29425

The rectal gland of Squalus acanthias is innervated by nerves immunostaining for peptide inhibitors of secretion, including neuropeptide Y (NPY), bombesin and somatostatin. Somatostatin is thought to have a direct inhibitory effect on rectal gland cells, while bombesin's inhibitory action is at least partly indirect, since it releases somatostatin from the isolated perfused gland. Whether NPY inhibition is direct or indirect has been uncertain. We therefore tested the inhibitory actions of NPY, bombesin and somatostatin in primary monolayer cultures of shark rectal gland, in which nerve cells are thought to be absent, by measuring short-circuit current (I_{sc}), a direct index of chloride secretion in this preparation.

Monolayer cultures of rectal gland cells were prepared as previously described (Am J Physiol 260:C813-C823, 1991). Tubules prepared from minced rectal glands digested with collagenase were inoculated into 35 mm culture dishes containing type 1 collagen gels supported with nylon mesh (Small Parts, Inc., Miami, FL) 18 mm in diameter. Cultures were grown to confluence in approximately 10 days, mounted in Ussing chambers, bathed on apical and basal sides with shark Ringer's solution containing 5 mM glucose at pH 7.5 and studied using standard electrophysiological methods. Reagents were added to the basolateral side of the membrane in all experiments described.

Addition of forskolin 10^{-6}M to the basolateral surface of cultured rectal gland cells regularly increased I_{sc} by about 10 times, from 11.2± 7.6 uamp/cm^2 to 112.5± 25 (mean S.E.; n=6. $p<0.001$) without a consistent change in transepithelial resistance. NPY 10^{-6}M, applied after forskolin-induced stimulation had reached a plateau, consistently induced a fall in I_{sc} apparent within 2 minutes and maximum after 20 minutes, that averaged 29 ±4% ($n=6$, $p<0.01$), without a significant change in membrane resistance. The inhibition produced by NPY was not reversed by 10^{-6}M glyburide, an inhibitor of the ATP-sensitive K^+ channel.

Somatostatin, 10^{-6}M, also inhibited I_{sc} in rectal gland culture that had previously been stimulated by forskolin. The percentage inhibition induced by somatostatin in I_{sc} averaged 52 ±10% (n=4), with a parallel decrease in transmembrane voltage and no change in transepithelial resistance. By contrast, bombesin, 10^{-6}M, did not affect I_{sc} when applied to stimualted rectal gland cells in culture.

These results supply strong evidence that NPY inhibits rectal gland secretion of chloride by a direct action upon rectal gland cells, as does somatostatin. An inhibitory action of bombesin, on the other hand, cannot be demonstrated in cultured rectal gland cells, in accord with indirect evidence that bombesin's inhibition of whole perfused glands is secondary to the release of other neuroinhibitors including somatostatin.

Assisted by grants from the Burroughs-Wellcome Foundation (Stidham, McCraw, Nelson), the W.R. Hearst Foundation, and the Pew Trusts (Hornung) to MDIBL, from the NIH (DK18078) to F.H. Epstein, and from the Cystic Fibrosis Foundation to K. Karnaky, Jr.