DIRECT EFFECT OF ATRIAL NATRIURETIC PEPTIDE ON THE MEMBRANE POTENTIAL OF CULTURED RECTAL GLAND CELLS FROM SQUALUS ACANTHIAS

Robert Wondergem[1] and John D. Valentich[2]
[1]Department of Physiology, James H. Quillen College of Medicine
East Tennessee State University, Johnson City, Tennessee 37614
[2]Department of Physiology and Cell Biology, University of Texas
Medical School, Houston, Texas 77030

Atriopeptin II (ANP) at 10^{-7} M stimulates Cl^- secretion in the isolated, perfused shark rectal gland (Solomon, et al., Am. J. Physiol. 249: R348-R354, 1985; Silva, et al., Am. J. Physiol. 252: F99-F103, 1987). Atriopeptin also increases rectal gland blood flow in vivo (Solomon, et al., ibid.). However, it fails to stimulate oxygen consumption when added directly to rectal gland slices or to dispersed cells (Solomon, et al., Am. J. Physiol. 15: R63-R66, 1984; Silva, et al., ibid.), and it has no direct effect on Cl^- secretion when added to isolated, perfused rectal gland tubules (Silva, et al., ibid.). Vasoactive intestinal polypeptide (VIP) also stimulates Cl^- secretion in the isolated perfused rectal gland (Solomon, et al., Am. J. Physiol. 249: R348-R354, 1985b; Silva, et al., ibid.); however, either the organotin neurotoxin bis (tributyltin) oxide or procaine inhibits ANP-stimulated Cl^- secretion in perfused glands but not that stimulated by VIP (Solomon, et al., Bull. MDIBL 26: 37-39, 1986; Silva, et al., ibid.). Thus, blocking local neurosecretion inhibits ANP stimulation of rectal gland Cl^- secretion. These investigators have concluded that ANP stimulation of rectal gland Cl^- secretion is mediated by a secondary, enteric neurotransmitter, which is probably VIP (Silva, et al., ibid.). However, direct stimulation of transepithelial Cl^- secretion by ANP I and III in confluent, monolayer cultures of shark rectal gland epithelial cells recently has been reported (Karnaky, et al., J. Cell Biol. 109: 131a, 1989). These results are substantiated here by demonstrating direct depolarizing effects of ANP on the transmembrane potential (V_m) of rectal gland epithelial cells in monolayer culture.

Standard electrophysiologic techniques were used to measure V_m in cultured rectal gland cells obtained from Squalus acanthias. These methods have been described (Wondergem and Amsler, Bull. MDIBL 26:105, 1986; Moran and Valentich, Bull. MDIBL, 27:14, 1987/88). V_m was recorded continously in single cells that were superfused at 2 ml/min with Ringer solution plus vehicle (control). The elasmobranch Ringer solution then was switched to an identical solution plus an added experimental agent. V_m was recorded on a digital voltmeter and on chart paper. Cell conductance (g_{cell}) was measured throughout by passing 0.25 nA of intermittent current (300 msec duration) through the recording microelectrode.

Rat ANP III (Sigma) at 10^{-7} M decreased V_m over 5-8 min from -93 ± 3.0 mV to -73 ± 3.7 mV, $p<0.001$ (SE; $n = 10$ paired measurements). This depolarization of V_m reversed immediately when ANP was washed from the cells. Forskolin at 10^{-4} M also depolarized these cells to -57 ± 2.9 mV ($n = 9$), and this too was reversible. These effects of forskolin, although qualitatively similar to the effects of ANP on V_m of rectal gland cells, occurred faster and were larger. Constant perfusion of these cells dilute cell secretions, and this argues against conceivable secondary activation of the epithelial cells by autocrine or neural secretions, the latter of which originate from possible cocultured, enteric neurons.

Increases in g_{cell} accompanied depolarization of V_m due to added ANP or forskolin. When Ba^{2+} (1 mM) was added to unstimulated cells, V_m decreased from -93 ± 1.4 mV to -65 ± 2.2 mV ($n = 5$), but here g_{cell} decreased. Ba^{2+} is a broad-spectrum blocker of membrane K^+ channels, and the decrease in V_m accompanied by decreases in g_{cell} are consistent with this action. This also shows that ANP- and forskolin-induced depolarization, which are accompanied by increases in g_{cell}, occur by a mechanism different from a block of membrane K^+ conductance.

Intracellular Cl^- activity in rectal gland cells is higher than equilibrium with the apical transmembrane potential (Greger and Schlatter, Pflugers Arch. 402: 63-75, 1984). This results from a basolateral Na-K-Cl cotransporter, which actively accumulates cell Cl^- by secondary active transport that is coupled to the transmembrane electrochemical Na^+ gradient. Thus, any agent that selectively activates apical membrane Cl^- channels will increase membrane Cl^- conductance and depolarize V_m. A subsequent decrease in external Cl^- concentration by substituting impermeant anions will further decrease V_m, and the magnitude and time course of this additional depolarization will depend on membrane Cl^- conductance and how rapidly cell Cl^- is depleted. Gluconate for Cl^- substitutions in cells stimulated for 10 min by forskolin (10^{-4} M) or ANP (10^{-7} M) decreased V_m to -35 ± 6.9 mV and -26 ± 9.1 mV, respectively. Identical substitutions in unstimulated cells had no effect on V_m. With gluconate still present, V_m of the forskolin-stimulated cells repolarized to -82 ± 8.9 mV, and this repolarization was inhibited by Ba^{2+} (1 mM). In contrast, V_m of ANP-stimulated cells did not repolarize in the presence of gluconate.

These results demonstrate a direct effect of ANP on cultured cells from shark rectal gland. The gluconate/Cl substitutions suggest that both forskolin and ANP activate membrane Cl^- conductance in rectal gland cells. The subsequent repolarization-hyperpolarization in forskolin-treated cells shows that cAMP stimulates other events in the rectal gland cells, perhaps in addition the membrane K^+ conductance and the Na-K-Cl cotransport. The absence of this hyperpolarization in ANP-stimulated rectal gland cells suggests that only Cl^- conductance is activated here.

Supported by a grant to R.W. from the Eagles D.D. Dunlap Kidney Fund and by a grant to J.D.V. from the Cystic Fibrosis Foundation.