EFFECTS OF MERCURIAL COMPOUNDS ON THE TRANSMEMBRANE POTENTIAL OF CULTURED RECTAL GLAND CELLS FROM SQUALUS ACANTHIAS

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Studies of the function of the elasmobranch rectal gland center on an epithelium whose cell secretory functions, particularly those concerning transepithelial chloride movement, are similar to the mechanisms of chloride absorption by cells in the thick ascending limb of Henle's loop. Consequently, apparent effects of mercurial compounds on membrane function of the rectal gland may have clinical significance by elucidating mechanisms of their nephrotoxity and of their diuretic action. A current model for rectal gland secretory function proposes that transmembrane potential, V_m, provides the electromotive force for apical membrane Cl^- secretion through regulated Cl^- channels. Accordingly, intracellular Cl^- activity is maintained above electrochemical equilibrium by the action of a basolateral, bumetanide-sensitive Na-K-Cl cotransporter. Thus, any substance that blocks membrane K^+ channels, decreasing gK and thereby reducing V_m, has the capability to inhibit cell Cl^- secretion. Here we examine whether such action can be attributed to mercurial compounds.

Standard electrophysiological techniques were used to measure V_m in cultured rectal gland cells obtained from Squalus acanthias. These methods have been described previously (Wondergem and Amsler, Bull. MDIBL 26:105, 1986). During the course of continuous recording of V_m in a single cell, elasmobranch Ringer's solution was replaced with an identical solution plus an added mercurial compound. Effects on V_m were recorded continuously on chart paper and on a digital voltmeter. Cell conductance (g_{cell}) was measured throughout by passing 0.25 nA of intermittent current (300 ms duration) through the recording microelectrode.

The average V_m for cultured rectal gland cells from Squalus acanthias was -90 ± 1.6 mV (n = 53). Table 1 shows the effect of various concentrations of added $HgCl_2$ on the cell V_m, and Table 2 shows the effect on g_{cell}. These effects of Hg^{2+} were irreversible. The effect of Hg^{2+} at 10^{-4} M occurred immediately. At 10^{-5} M the depolarization was protracted over 20 min, beginning on average 18 min after addition of Hg^{2+}. Also, at these concentrations g_{cell} increased during Hg^{2+}-induced depolarization. In contrast, at 10^{-6} M Hg^{2+} caused a smaller decrease in V_m, which was accompanied by a decrease in g_{cell}. This response occurred rapidly but began on average 52 min after addition of Hg^{2+}. Ba^{2+} at 10^{-4} M had similar but immediate effects on V_m and g_{cell}.

Table 1. Effect of added concentrations of $HgCl_2$ on the V_m of cultured rectal gland cells.

Hg^{2+} (M)	Control V_m (mV)	Experimental V_m (mV)	ΔV_m (mV)
10^{-4}	-94 ± 6.6 (3)	-20 ± 0.9 (3)	74 ± 7.5 (3)
10^{-5}	-91 ± 4.7 (3)	-24 ± 6.6 (3)	67 ± 10.1(3)
10^{-6}	-98 ± 3.3 (4)	-58 ± 3.2 (4)	41 ± 1.4 (4)

numbers in parentheses indicate number of measurements

Table 2. Effect of added concentrations of $HgCl_2$ on the cell conductance (g_{cell}) of cultured rectal gland cells.

Hg^{2+} (M)	Control g_{cell} (nS)	Experimental g_{cell} (nS)	Δg_{cell} (nS)
10^{-4}	24 ± 2.5 (3)	31 ± 4.5 (3)	7 ± 6.7 (3)
10^{-5}	25 ± 4.7 (3)	41 ± 8.3 (3)	17 ± 4.8 (3)
10^{-6}	29 ± 7.2 (4)	16 ± 2.4 (4)	-13 ± 4.9 (4)

numbers in parentheses indicate number of measurements

V_m also was measured and plotted as a function of the $\log_{10}$ $[K^+]_o$ at 5, 50, 140, and 230 mM (where external KCl was substituted for equimolar concentrations of NaCl). This was done first under control conditions and then in the same group of cells 1 hr after addition of Hg^{2+} at 10^{-6} M. Slopes of the linear portions of these plots comprising the three highest $[K^+]_o$ were -89 ($r = 0.99$) and -65 ($r = 0.99$) mV/decade for control and 10^{-6} M Hg^{2+}, respectively. Superfusing cells with 10^{-4} M p-chloromercuribenzene sulfonate (PCMBS) resulted in an immediate increase in V_m from -88 ± 2.8 to -111 ± 1.8 mV ($n = 5$); however, V_m decreased to -49 ± 5.0 mV ($n = 13$) 60-90 min after addition of PCMBS. In contrast, superfusing cells with 10^{-4} M mersalyl for 90 min had no effect on V_m. Also, Hg^{2+} at 10^{-4} M had no effect on V_m if dithiothreitol was present at 1 mM.

Our results show that Hg^{2+} decreases V_m of cultured epithelial cells obtained from shark rectal gland. The rapid, complete depolarization accompanied by increases in g_{cell} at high concentrations indicates a nonspecific, toxic effect at these doses. However, at 10^{-6} M Hg^{2+} caused a delayed, moderate depolarization of V_m accompanied by an decrease in g_{cell}, and it reduced the slope of V_m vs $\log_{10}$ $[K^+]_o$. This is consistent with an effect that reduces membrane K^+ conductance, perhaps by blocking K channels (Venglarik and Dawson, MDIBL Bull. 26: 1, 1986). The delay in response at 10^{-6} M indicates block of K channels either through an intracellular site or by some more complex mechanism of action involving other cell mediators. Regardless of the mechanism of action of Hg^{2+}, its resultant depolarization of V_m is sufficient to account for its dose-dependent effect to reduce Cl^- secretion by the perfused rectal gland (Silva, Solomon, and Epstein, MDIBL CMTS 2nd Ann. Prog. Rept.: 60, 1987).

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