EFFECTS OF p-CHLOROMERCURIBENZENE SULFONATE (PCMBS) ON THE VOLUME MAINTENANCE OF DOGFISH (SQUALUS ACANTHIAS) RECTAL GLAND CELLS

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The relatively impermeant mercurial PCMBS interacts primarily with the outer face of cell membranes and increases its permeability for cations (Knauf and Rothstein, J. Gen. Physiol. 58:211-223, 1971). The PCMBS effect on slices of the rectal gland was studied.

Details of experimental methodologies (tissue and saline preparation: analytical procedures for the determination of cell water, cellular Na^+, K^+ Cl^-, cell membrane potential (Ψ), zero-time ^{86}Rb as an evaluation of cell Na^+-K^+-ATPase activity) were described previously (Kleinzeller and Goldstein, J. Comp. Physiol. B154:561-571, 1984). Cell Li^+ was determined in the tissue extract by flame photometry.

PCMBS (1 mM) produces swelling associated with a loss of cell K^+ and an influx of Na^+ and Cl^-; the slow swelling in the first 60 min becomes massive within 2 h. Slight effects on cell volume and electrolyte distribution can be detected at 0.05 mM. Morphologically, the PCMBS-induced swelling differs from that seen in high-K^+ media (Mills and Kleinzeller, Bull. 25:50-51, 1985) in that large swollen "lakes" are seen at the base of the cells while the apical area appears relatively normal. The role of mercurial binding to cell components was demonstrated (Fig. 1):

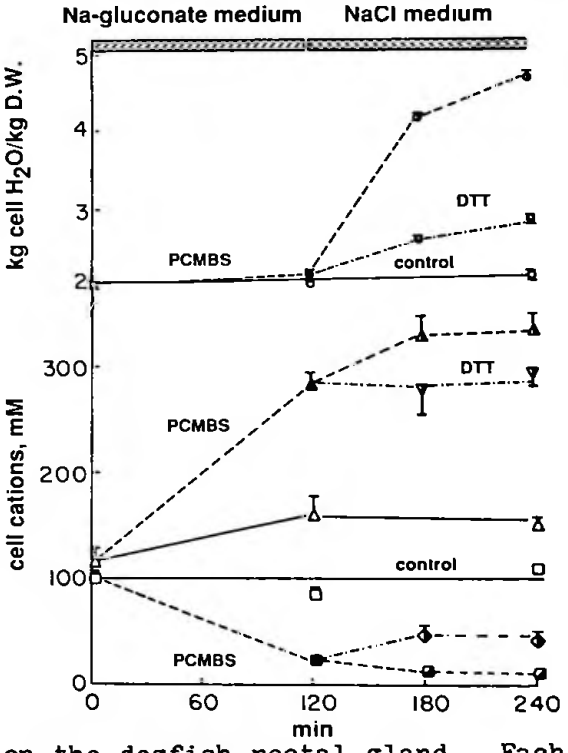

Fig. 1. PCMBS effect on the dogfish rectal gland. Each point is the mean of 4 analyses, ± S.E.

Tissue incubated with 1 mM PCMBS in a Cl^--free, gluconate medium does not swell owing to the bulkiness of the anion; however, the mercurial does produce marked changes in cation distribution (uptake of Na^+ and loss of K^+). Subsequent incubation of the mercurial-treated cells in standard (295 mM Cl^-) medium without PCMBS produced massive swelling; this swelling and cell cation distribution could in part be reversed by 2 mM dithiothreitol (DTT) (Fig. 1). The data suggested that PCMBS acts by producing a leakiness of the cell membrane to cations (but not to gluconate), and/or by an inhibition of cell metabolism (including effects on the (Na^+-K^+)-ATPase).

PCMBS (0.05 to 1 mM) does not affect cell metabolism per se (formation of $^{14}CO_2$ from (U)-^{14}C-acetate). 1 mM PCMBS does not inhibit the zero-time $^{86}Rb^+$ uptake by the cells, but produces a 50% inhibition when the tissue is pretreated with the mercurial. Thus, PCMBS slowly enters the cells and then inhibits the (Na^+-K^+)-ATPase. An inhibition of this enzyme by PCMBS was found by Dr. R. Kinne in a preparation of basal-lateral membranes of the rectal gland (personal communication). The swelling effect of PCMBS does not directly involve the (Na^+-K^+)- ATPase: This point was tested using a Na^+-free medium (Li^+ equivalently replacing Na^+). In this medium, cells depolarize (at steady-state, ψ , evaluated on the basis of the distribution of the lipophilic cation triphenylmethylphosphonium, decreased from 90.6 $\pm$ 1.5 S.E. in the control (Na^+ medium) to 64.1 $\pm$ 4.4 in Li^+ medium). The cells lose Na^+ and K^+ and take up Li^+ and Cl^-, but do not swell; no evidence for an active, ouabain-sensitive Li^+ extrusion was found. PCMBS produced a massive swelling in Li^+ medium as in the Na^+ saline. The data thus indicate that PCMBS produces cell swelling in part by increasing the leakiness of the membrane to cell cations. The sulfhydryl reagent, 1 mM N-ethylmaleinimide (NEM) did not produce cell swelling; this observation indicates that PCMBS interacts with groups differing from the -SH groups accessible to NEM (cf. gland. Rendi, Biochem. Biophys. Acta 99:564-566, 1965).

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