CELL OSMOLARITY AND CHLORIDE FLUXES IN THE DOGFISH (SQUALUS ACANTHIAS) RECTAL GLAND

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We have previously suggested that in the dogfish shark rectal gland the presence of non-dissociated, (effectively) non-diffusible osmolytes, e.g. trimethylamine oxide, decreases the levels of intracellular cations (Kleinzeller & Fluk, Bull. MDIBL 23:36-38, 1983). The advanced theoretical considerations were based on two assumptions: 1) The lack of a (major) osmotic gradient across the cell membrane; and 2) free permeability for Cl^-. The validity of these assumptions has now been tested.

1. Tissue osmolarity: The osmolarity of tissue and plasma solutes was determined as described by B. Schmidt-Nielsen et al. (Am. J. Physiol. 244:F472-F482, 1983), using a vapor pressure osmometer. The results are given in mosmoles/kg tissue water, i.e. in mosM.

Results: plasma osmolarity: 942 mosM ± 10 S.E. (5 fish); fresh tissue: 987 ± 13 S.E., (4 fish, 15 analyses); incubation medium: 895 mosM; incubated tissue: 941 ± 16 (4 fish, 16 analyses). The differences between the respective media (plasma or Ringer's) and the tissue were not significant. Hence, it is concluded that no major osmotic pressure gradient is maintained across the membrane of the rectal gland cells, both in vivo (at the plasma side), and in vitro (at both cell faces)..

2. $^{36}Cl^-$ fluxes: Previous data suggested a free permeability of the rectal gland cells for Cl^- : replacement of medium Cl^- by NO_3^- or isethionate produced a rapid loss of cell Cl^- and after 60 min incubation in Cl^- free media intracellular Cl^- concentration was practically nil (Kleinzeller et. al., J. Comp. Physiol. B. 155: in Press, 1984). Such observations are consistent with existing models of the rectal gland cells (Greger et. al., Bull. MDIBL 23:8-10, 1983), postulating a conductive pathway for Cl^- at the luminal side, and the Na-K-2 Cl^- cotransport system for entry (and exit?) at the plasma cell face. $^{36}Cl^-$ was measured in order to ascertain that the Cl^- permeability of the cells is not limiting the rate of exprimentally-induced volume changes. The tissue was first loaded with $^{36}Cl^-$ by preincubation (air + 1% CO_2) at 15°C for 30 min. Under these conditions, practically all cell Cl^- exchanges with $^{36}Cl^-$. The efflux of the label was then followed (cf. Kleinzeller & Goldstein, J. Comp. Physiol. B 154:561-572, 1984).

Results: Fig. 1 shows a representative efflux curve. Two cellular components of $^{36}Cl^-$ efflux were ascertained (means of 4 experiments, ± S.E.): A slow component, with a rate constant $k' = 0.72 \pm 0.06$ min^{-1}, and a space of 18 ± 1.6% of the wet tissue; and a fast component, with rate constant $k'' = 0.60 \pm 0.12$ min^{-1}, and a space of 32 ± 7%. The rate constant for the diffusion component from the extracellular space could not be ascertained. From the mean distribution of the extracellular marker polyethylene glycol, the space occupied by extracellular Cl^- is about 54% of the tissue. This value compares well with the kinetically determined extracellular space (total tissue [= 100%] minus sum of cellular spaces).

It should be noted that the fast component of Cl^- efflux exceeds that of K^+ threefold, and approximates the rate of H_2O efflux from the cells (Kleinzeller and Goldstein, 1984). Hence, the rectal gland cells are indeed highly permeable to Cl^-. Preliminary experiments indicate that $^{36}Cl^-$ efflux was not inhibited by absence of external Cl^-, by acetazolamide, but was somewhat inhibited by 10 μM diphenylamine carboxylate, suggesting that the measured $^{36}Cl^-$ efflux corresponds predominantly to the conductive pathway.

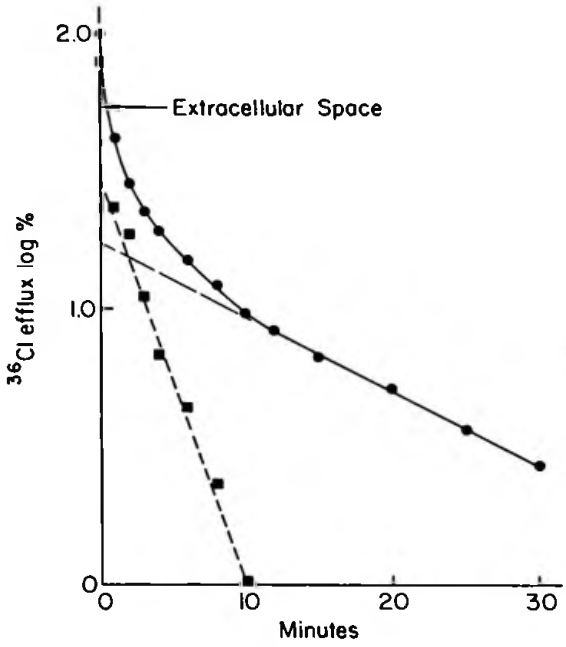

Fig. 1 Steady-state $^{36}Cl^-$ efflux from slices of the dogfish rectal gland

The measured parameters are consistent with the proposed model for the role of TMAO in cell volume maintenance.

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