RECOVERY OF CELL VOLUME AND SOLUTE COMPOSITION FOLLOWING POTASSIUM-INDUCED SWELLING IN RECTAL GLAND CELLS OF THE SHARK (SQUALUS ACANTHIAS): PROPERTIES OF THE SYSTEM

Fuad N. Ziyadeh[1], John W. Mills[3], George W. Booz[4] & Arnost Kleinzeller[2]
Departments of Medicine[1] & Physiology[2], University of Pennsylvania School of Medicine, Philadelphia, PA 19104
Department of Anatomy[3], Dartmouth Medical School, Hanover, NH 03756
Department of Pharmacology[4], Thomas Jefferson Medical College, Philadelphia, PA 19107

Rectal gland cells gain K^+, Cl^- and water, lose Na^+ and are depolarized upon incubation in isotonic high-KCl media (Kleinzeller et al., J. Comp. Physiol. B155:145, 1985). We now report on the reversibility of cell swelling, its modulation and the changes in solute composition and cellular morphology. The methods were as previously described (Kleinzeller et al., loc. cit.; Kleinzeller & J. Goldstein, ibid B154:561, 1984; Mills et al., MDIBL Bull., 26:13, 1986).

Cell water nearly doubled at 2h of incubation in KCl-media, reaching a new steady state. On subsequent transfer to standard NaCl-elasmobranch Ringers, the cell water content essentially returned to control levels in 2h. The accompanying changes in the apparent intracellular ion concentrations were as follows: In control tissues, cell $[Na^+]$ + $[K^+]$ was 203 ± 6 mM (mean ± SE, n=6), substantially below the sum of these cations in the bathing medium (285 mM). During K^+-induced swelling, the cell $[Na^+]$ + $[K^+]$ increased to 271 ± 5 mM. During the recovery phase, the sum decreased to 256 ± 10 mM, but remained significantly higher than in control tissues despite recovery of cell volume. Also upon recovery, the apparent intracellular concentration of Cl^- was higher than control tissues (cf. Kleinzeller et al., loc.cit.). This discrepancy in intracellular solute composition most likely reflected the loss of significant amounts of endogenous organic osmolytes during the swelling phase, and which was not regained during recovery as the latter media was virtually devoid of these osmolytes. We showed previously that KCl-media induced massive efflux of organic osmolytes including trimethylamine-N-oxide, from 71 to 4 mM (Kleinzeller, J. Exp. Zool. 236:11, 1985), myo-inositol, from 16 to 8 mM (Kleinzeller et al., MDIBL Bull. 25:64, 1985) and taurine (50 mM in control) (Ziyadeh et al., Biochim. Biophys. Acta 943:43, 1988).

It is noted that similar results were obtained when tissues where bathed in K^+-gluconate media, then were transferred to standard NaCl-media. Here, while cells did not swell, solute composition showed that the sum of cell $[Na^+]$ and $[K^+]$ was higher upon return to NaCl-media compared with control tissues. Again, this discrepancy in solute composition likely reflected osmolyte efflux induced by K^+-gluconate media (Ziyadeh et al., loc.cit.). Osmolyte loss which is related to K^+-induced permeabilization, irrespective of cell volume changes, correlated well with morphological disruption of fine cytoskeletal architecture including loss of cell F-actin (cf. Mills et al.,loc.cit.).

The above findings are also in keeping with the notion that cell concentrations of inorganic ionic species are maintained at relatively low levels by the "non-perturbing" high concentrations of organic osmolytes (Yancey et al., Science 217:1214, 1982). In addition, high cell K^+ concentration disrupts the cytoskeletal integrity.

Cells swollen in KCl-media shrank and reached a volume close to control in LiCl-media or in the presence of 0.5 mM ouabain in NaCl-media. Also the reversibility of the F-actin arrangement in the cells was seen after recovery in LiCl-media. Hence, an involvement of the Na,K-ATPase in the reversibility process appears to be excluded.

The KCl-induced cell swelling was partially reversible in Na^+-media in the absence of Cl^- (NO_3^- substitution) or in the presence of inhibitors of the 1Na-1K-2Cl cotransport system (0.1 mM bumetanide, 0.5 mM furosemide): While the rate of water and K^+ loss, and the uptake of Na^+, in the first hour did not differ from the control (standard NaCl-media), the subsequent net fluxes were significantly slowed. Under zero-trans conditions for recovery (transfer into isotonic K^+-free N-methyl glucamine-gluconate media) these "loop" diuretics had no effect on the rate of water and K^+ loss from the cells. Such observations are consistent with a secondary role of the 1Na-1K-2Cl system in the course of the entry of Na^+ into the K^+-swollen cells. Reversibility of KCl-induced swelling in NaCl-media or in zero-trans media was not affected by the addition of 0.1 mM DIDS to the recovery media, excluding any major role for an anion-exchange carrier (or other possible DIDS-sensitive transport systems) in the recovery process. Cell exit of K^+ and Cl^- most likely involve separate ion conductances.

While a strictly passive mechanism of the reversal of KCl-induced swelling cannot be excluded (0.1 mM 2,4-dinitrophenol had no significant inhibitory effect on the recovery process), a possible participation of the cytoskeleton, possibly involving Ca^{++} (vide infra), will also have to be considered.

The degree of cell swelling in KCl Ringers was not affected by the elimination of Ca^{++} from the media (+0.1 mM EGTA). Moreover, upon transfer to standard NaCl Ringers, cell volume recovery was similar whether cells were swollen in KCl media in the presence or absence of Ca^{++}. However, omitting Ca^{++} from the NaCl recovery media markedly retarded the return of cell volume toward control levels: At 2h recovery, cell water (corrected for ^{14}C-polyethylene glycol (PEG) extracellular space) was 2.41 ± 0.15 Kg H_2O/Kg dry weight in the presence of external Ca^{++} vs. 2.85 ± 0.05 in Ca^{++}-free media ($n=4$, $p<0.05$). This was associated with a faster loss of cell K^+, and gain of Na^+ content, with a significant increase in the fast rate constant of $^{86}Rb^+$ efflux, noted previously in Ca^{++}-free media (Kleinzeller et al. MDIBL Bull. 25:39, 1985). Reversibility of KCl-induced swelling in NaCl-media was not altered by the addition to the recovery media of 0.1 mM W-7, a calmodulin inhibitor, suggesting that the effects of Ca^{++}-free media on this recovery process are not mediated by activation of a Ca^{++}-calmodulin pathway. In addition, Ca^{++}-free media also tended to increase extracellular tissue water content as reflected by increased ^{14}C-PEG space (cf. Kleinzeller et al., MDIBL Bull. 25:39, 1985) and by widened interstitial compartments on morphological analysis. The molecular details of the involvement of Ca^{++} in cell volume regulation remain poorly understood, but its role may be related to monovalent ion conductances and/or cytoskeletal integrity (Foskett & Spring, Am. J. Physiol. 248:C27, 1985).

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