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EFFECTS OF SALINE TONICITY ON THE CELLULAR DISTRIBUTION OF H_2O AND ELECTROLYTES IN SLICES OF THE DOGFISH (SQUALUS ACANTHIAS) RECTAL GLAND

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We have previously shown (cf., Bulletin, MDIBL 1978; 1979; 1980) that when slices of the dogfish rectal gland are aerobically incubated (1% CO_2 in air; 15°C) in urea-free hypotonic (approx. 580 mosM) saline, the tissue swells and the efflux of urea from the tissue is associated with a net cellular uptake of K^+. This cellular uptake of K^+ occurs at a constant electrochemical gradient of this ion, i.e., no change in the Nernst potential of K^+ was seen. A working hypothesis was advanced suggesting that the influx of K^+ is essentially a passive phenomenon determined by the relative fluxes of H_2O, urea, K^+ and the cell membrane potential. This view was tested further by the following experiments:

1. The tissue fluxes of ^{14}C-urea and ^{42}K were determined at conditions approaching zero-time kinetics. The efflux of urea showed two cellular components. The fast component (determined for the 6 min period between 1 and 7 min of the efflux experiment) corresponded to 26.3±1.13 (S.E.) μmol (g^{-1} tissue wet wt. min^{-1}) (4 fish), whereas the influx of ^{42}K in the first 5 min. of incubation was 2.7±0.21 μmol^{-1} (g^{-1} tissue wet wt. min^{-1}). The urea flux thus exceeded that of K^+ by one order of magnitude. In a similar experiment, the flux of $^{86}Rb^+$ corresponded to a flux of 2.5±0.07 μmol K^+ exchanged (g^{-1} tissue min^{-1}). Such data are not consistent with the possibility of a direct coupling between the urea and K^+ fluxes.

2. As compared with controls, the swelling of the cells induced by incubation in urea-free saline in the presence of 0.5 mM ouabain, was associated with a net uptake of Na^+, rather than of K^+ (Fig. 1). The lack of cation specificity of the electrolyte influx associated with the cell swelling and urea efflux was thus demonstrated.

3. Under hypertonic conditions (addition of 253 mM urea or mannitol to standard dogfish saline) the cellular shrinking was associated with a net loss of K^+ (Fig. 1), while the Nernst ratio of K^+ remained constant.

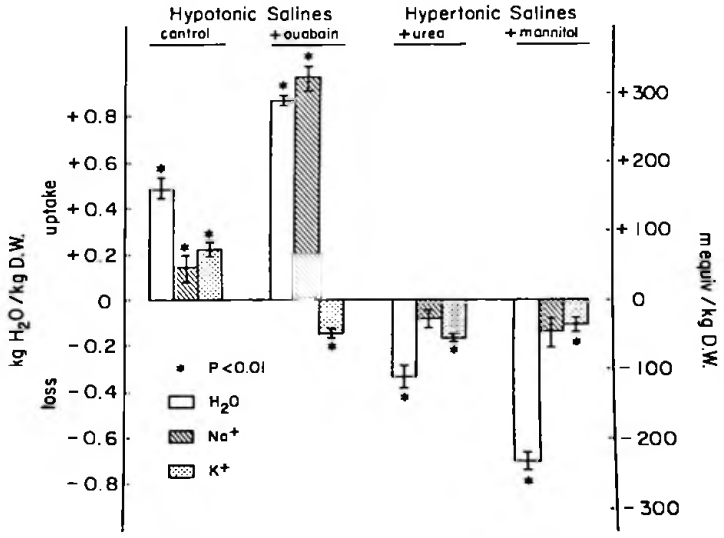

FIGURE 1

FIGURE 1. Effect of saline tonicity on changes in tissue water and electrolytes in slices of the dogfish rectal gland. Changes, i.e. differences between values in controls (isotonic media) and experimental salines are given. Mean values ± S.E. are shown for experiments on at least two fish (6 analyses per exptl. point).

4. In order to substantiate the assumption that the Nernst potential for K^+ reflects the magnitude of the cell membrane potential, the values of the Nernst K^+ potentials were compared with those of triphenylmethyl phosphonium bromide, (TPMP), a lipophilic cation. The mean values were: E_K 82±2 mV; E_{TPMP} 88±3 mV. These data are close to directly measured values of the cell membrane potential in perfused rectal glands, i.e., 78 mV (Walsh et al, Bulletin, MDIBL 20:121, 1980). E_{TPMP} was not affected by changes of external tonicity (hypotonic, i.e., urea-free saline; hypertonic salines), whereas depolarizing agents such as high external K^+ or ouabain also depressed the values of E_{TPMP}. Thus, it is justified to conclude that the cellular uptake of K^+ in urea-free salines, and the loss of K^+ in hypertonic salines (see 3 above) proceeds at a constant electrochemical gradient of tissue electrolytes.

The above data, i.e., absence of direct coupling between urea and K^+ fluxes; lack of cationic specificity; tonicity-dependent changes of cell water and electrolytes at a constant electrochemical gradient are consistent with the previously advanced hypothesis. This investigation was supported by grants from NIH (AM-12619) and the Whitehall Foundation.

CONTROL OF EXTRACELLULAR AND CELL VOLUMES IN BRAIN OF LITTLE SKATE (RAJA ERINACEA)

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A considerable literature has accumulated on the control of cell volume in the face of osmotic disturbances. In the vertebrate brain the interstitial fluid is separated from blood by a tight endothelium or epithelium (the blood-brain barrier). Hence this extracellular fluid may also be subject to control by specific transport mechanisms. Indeed, in mammals there are big shifts of sodium and chloride in and out of brain, tending to maintain extracellular volume constant in hyper- and hypo-osmolar states respectively (reviewed by Bradbury, M.W.B., The Concept of a Blood-Brain Barrier, publ. Wiley: Chichester; Patel, P. & Cserr, H.F., In preparation).

Since the little skate, Raja erinacea, will tolerate considerable osmolality changes in its external environment and since its body fluids largely conform to these, shifts in the extracellular ion content in the brain of this species have been estimated - the eventual aim being to localize the site of these net fluxes (blood-brain, extradural fluid-brain or cerebrospinal fluid-brain) with radioactive isotopes. Free swimming fish were subjected to sea-water plus 65 mM NaCl (hypertonic - these were also injected with a Na Cl i-m to raise the osmolality of their body fluids by the same proportion as the sea-water) or to dilute sea-water (hypotonic - 50% of normal osmolality). At a set time, blood was sampled by cardiac puncture and after exanguination, the brain removed, frozen on dry ice and standard pieces of telencephalon and medulla removed into tared vials. The brain pieces were dried to constant weight and extracts of the dried tissues in 0.75 N HNO_3 analyzed for chloride coulometrically and for sodium and potassium by emission flame photometry.

In Table 1, is recorded brain-water, expressed as chloride and non-chloride spaces. The predicted changes in these spaces as ideal osmometers have been calculated with the plasma concentration of sodium and chloride as a standard of reference, since the other main osmotically active solute in plasma, urea, diffuses freely in and out of brain. Whilst the non-chloride component of brain-water behaved as a near perfect osmometer with respect to plasma [$Na^+ + Cl^-$], especially in hypotonic conditions, there was considerable regulation of the chloride space, this being near perfect in the hypertonic conditions. In Table 2, it can be seen that sodium moved in the same direction and in equivalent amounts to chloride, whereas potassium changes in brain were small and not significant. Analysis of telencephalon for ninhydrin positive substances indicated little change in the amount of amino acids in this part of brain under the conditions used. In contrast to telencephalon, the medulla showed little control of water and minimal ionic shifts.