The effect of ouabain on volume regulation in the rat lens

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In an anisotonic medium the tendency of the lens to swell or shrink in response to the osmotic gradient is countered by a change in the content of lens solute, so that the change in the volume of lens water is minimal. This phenomenon is known as volume regulation. The rapid phase of volume regulation occurs in less than 1 hr and is followed by a slower phase that may require about 36 hr. In the present study the changes in the content of water, potassium, and sodium were measured in the presence and absence of ouabain during 1 hr of incubation in anisotonic media. The effects of the ouabain-insensitive components of fluxes along electrochemical gradients are indicated by changes in the presence of ouabain, and the effects attributable to an active pump by the difference in changes in the presence and absence of ouabain. The results indicate that the volume of the lens after the initial phase of volume regulation is the same in the presence and absence of ouabain, that sodium influx varies with osmotic change, that the degree of sodium change cannot be accounted for by changes in the electrochemical driving force, that the sodium permeability is apparently decreased in hypotonic media and increased in an hypertonic medium, and that the changes in sodium influx can account for other changes associated with volume regulation.

Key words: rat lens, volume regulation, cation fluxes, ouabain, chloride ratios

In the past 10 years evidence has been accumulating which demonstrates that cells tend to maintain the same volume in spite of changes in the osmolarity of the surrounding medium.1–3 This phenomenon has been called "volume regulation" and is beneficial to cells in two important ways. First, it maintains the relative spacial and functional relationships among intracellular organelles, and second, in multicellular organisms it prevents disproportionate swelling that may occlude capillaries and prevent the normal exchange of extracellular fluid around a cell.4 Volume regulation is of particular importance in marine organisms5 that live in waters of different salinity, and in the course of evolution the phenomenon seems to have been retained as a general characteristic that is also found in mammalian cells.4, 6–8 Although the osmotic environment of the internal organs in man is held relatively constant by the action of the kidney, it may change under extremes of hydration and dehydration or under certain therapeutic regimens.4, 9 Volume regulation has been demonstrated in various mammalian cells1 and in rat lens.10

The initial phase (60% to 80%) of volume regulation is rapid, with the rest of the process, or second phase, taking more time.10 In a hypotonic medium the initial osmotic swelling is followed by a loss of solute that is termed a volume regulatory decrease (VRD), and in a hypertonic medium the initial osmot-
onic shrinking is followed by a volume regulatory increase (VRI) in solute content. The change in solute content involves sodium, potassium, accompanying anions, and amino acids. Reports of changes in the last-named have been limited to marine organism, isolated tumor cells, and the lens.

The role of the Na,K pump in volume regulation may vary among cells. The initial phase of volume regulation in flounder and avian red cells is not affected by the presence of ouabain in the medium. However, ouabain inhibits VRD in lymphoma cells. From the present study of the effect of ouabain on volume regulation in rat lenses, it was concluded that the volume attained in the initial phase of volume regulation was independent of the action of the Na,K pump and that the final steady state achieved after volume regulation is complete may be the result of a change in sodium permeability with osmolarity.

The estimation of the ouabain-insensitive and ouabain-sensitive components of the fluxes from changes in cation content in the presence and absence of ouabain as employed by Cala and in the present study has the advantage of providing results for different fluxes on the same series of lenses. It allows for estimates of sodium fluxes which are not as readily obtained with isotopes.

Methods

Lenses were obtained with a posterior approach from the eyes of decapitated male Sprague-Dawley strain rats weighing 100 ± 20 gm. The enucleated eye was washed in normal saline containing 1% Wescodyne for at least 1 min, the excess solution was removed on a sterile towel, and the dissection was carried out under sterile conditions in the medium required for the experiment.

Incubations were carried out in CO₂-air incubators at 37°C, pH 7.4, with the lenses supported on a perforated Cellophane bed in individual incubation dishes containing 5 ml of medium. A TC-199 medium was used with the final concentrations (mM) of salts being 1.4 CaCl₂, 1.0 MgSO₄, 0.4 KH₂PO₄, 0.5 Na₂HPO₄, 30 NaHCO₃, 4.3 KCl, and NaCl as required to obtain the desired osmolarity (113.4 mM in 305 mOsm medium and ±0.54 mM/mOsm for other osmolarities). The medium contained 6.4 mM glucose, 1% fetal calf serum, and the organic constituents of TC-199 (Gibco) at 50% of the usual concentration.

Lens weights were measured after incubation and ranged from 20 to 30 mg. Dry weights were determined after drying to constant weight (36 hr or more) in a 96°C oven. The dry weights were 10 mg dry weight, and all results were adjusted to a 10 mg dry weight to permit easy comparison of results. The difference between the wet and dry lens weights was taken as the weight of lens water. A correction for extracellular water of 6% lens water was made for water and electrolyte levels on the assumption that the composition of the extracellular fluid was the same as the bathing medium. The 6% value is based on determinations of ³H-

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**Fig. 1.** A, Net ouabain-insensitive fluxes of sodium and potassium determined from the difference in lens content at each osmolarity in the presence of ouabain from the content in 305 mOsm medium in the absence of ouabain. B, Ouabain-sensitive fluxes determined from the differences in content in the presence and absence of ouabain.
Table I. Water, potassium, and sodium levels in eight pairs of rat lenses with and without 10⁻³M ouabain (mean ± S.E.) at each osmolarity

<table>
<thead>
<tr>
<th>Ouabain:</th>
<th>185 mOsm</th>
<th>245 mOsm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Lens water (mg)</td>
<td>18.5 ± 0.4</td>
<td>18.0 ± 0.4</td>
</tr>
<tr>
<td>Concentration (mEq/kg of lens water):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>79 ± 4</td>
<td>74 ± 2</td>
</tr>
<tr>
<td>Na</td>
<td>14 ± 2</td>
<td>32 ± 3</td>
</tr>
<tr>
<td>Content (mEq/kg of dry wt):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>145 ± 7</td>
<td>134 ± 5</td>
</tr>
<tr>
<td>Na</td>
<td>26 ± 4</td>
<td>57 ± 5</td>
</tr>
<tr>
<td>Total</td>
<td>171 ± 9</td>
<td>191 ± 6</td>
</tr>
</tbody>
</table>

Table II. Calculated values for driving force before and after 1 hr of volume regulation (mV)

<table>
<thead>
<tr>
<th>Control</th>
<th>VRD</th>
<th>VRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>305 mOsm</td>
<td>185 mOsm</td>
<td>245 mOsm</td>
</tr>
<tr>
<td>PD</td>
<td>-47</td>
<td>-48</td>
</tr>
<tr>
<td>E_EK</td>
<td>+49</td>
<td>+48</td>
</tr>
<tr>
<td>ΔV_EK</td>
<td>+96</td>
<td>+96</td>
</tr>
<tr>
<td>E_K</td>
<td>-88</td>
<td>-78</td>
</tr>
<tr>
<td>ΔV_K</td>
<td>-41</td>
<td>-30</td>
</tr>
</tbody>
</table>

E_EK, E_K = cation potential for sodium and potassium, respectively. ΔV_EK, ΔV_K = driving force for sodium and potassium, respectively.

Results

Water and cation levels. The levels of water, potassium, and sodium in the lenses after the hour of experimental incubation are shown in Table I. The results in the absence of ouabain were consistent with previous observations. In the presence of ouabain the volume of lens water was the same as in the absence of ouabain. The levels of sodium, however, were elevated, and those of potassium depressed.

Ouabain-insensitive fluxes. In isotonic 305 mOsm medium a steady state existed between the net ouabain-insensitive fluxes of...
sodium and potassium and the ouabain-sensitive effects associated with the pump. The difference in the content of cations in presence and absence of ouabain indicates the amount pumped by the Na,K pump in 1 hr under basal or isotonic conditions. Since this is balanced by the ouabain-insensitive fluxes, the quantitative value for these fluxes will be the same but in the opposite direction. Thus the net ouabain-insensitive influx of sodium under basal conditions and the net amount of sodium pumped out of the lens by the Na,K pump equaled 47 mEq/kg of dry weight. The comparable value for potassium was 25 mEq/kg of dry weight.

The difference in the content of cations between the level in the basal 305 mOsm medium and the level in the ouabain-containing experimental media at different osmolarities indicates the net ouabain-insensitive fluxes for the cations during VRD and VRI. The results are shown in Fig. 1, A. Although the results for the net potassium effluxes were not statistically different from the basal value, the slight increase in efflux with osmolarity was consistent with measurements of 86Rb efflux under similar circumstances. The net influx of sodium during VRI in 365 mOsm medium was 189% of the basal level (p < 0.05) and during VRD in 185 mOsm medium was 51% of the basal level (p < 0.05).

Effect of the Na,K pump. The difference in cation content in the presence and absence of ouabain at each osmolarity indicates the effect of the pump in VRI and VRD. The results are shown in Fig. 1, B. The effects of osmotic change on sodium efflux and potassium influx were similar, with approximately one potassium being pumped in for every two sodiums leaving the lens. The action of the pump was decreased during VRD and increased during VRI. During the first hour of VRD the decrease in potassium influx associated with the pump was greater than the decrease in passive efflux; therefore there was a net loss of potassium content. During VRI the increase in influx associated with the pump was greater than the increase in passive efflux; therefore there was a small net gain in potassium content. The changes for sodium were in the opposite direction, with a small net loss during VRD and a net gain during VRI.

Chloride ratios. Chloride ions are reported to be distributed passively in the lens. Therefore the ratio [Cl]o/[Cl]i is useful as a means of calculating, with the Nernst equation, the potential difference (PD) across the lens membranes. The ratios were determined after 24 hr of equilibration at a given osmolarity. The volumes of lens water were determined on separate groups of at least six lenses incubated under similar conditions. The [Cl]o/[Cl]i ratios (mean ± S.E.) in 185, 295, 305 and 365 mOsm media (number of lenses in parentheses) were 6.0 ± 0.2 (17), 6.4 ± 0.2 (18), 5.8 ± 0.2 (58) and 4.1 ± 0.1 (18). The calculated values for PD are shown in Table II.
Discussion

Ouabain is recognized as a specific inhibitor of the Na, K pump. Rat lenses placed in a medium containing $10^{-3}$M ouabain for 24 hr deteriorate—gaining sodium and water and losing potassium. Although other sodium pumps have been suggested as being active in other tissues, ouabain-insensitive sodium pumps have not been found in the lens. An active ouabain-insensitive calcium pump has been demonstrated in the lens, but such a pump acting by itself must not be capable of maintaining lens water and electrolyte balance. Thus the estimation of ouabain-sensitive and ouabain-insensitive fluxes during the first hour of VRI and VRD can provide important clues regarding the factors involved in the early changes of volume regulation. Although the exact quantitation of changes is not possible because of probable variations in effects during 1 hr of exposure to ouabain, the activity of the Na,K pump is effectively diminished, and the results are useful in that they show relative changes in fluxes and trends during volume regulation. The implications of the results are discussed in terms of lens water, ouabain-insensitive fluxes, possible changes in permeability, the Na,K pump, and a possible model of volume regulation arising from the results.

**Lens water.** When changed to 185 mOsm medium, the lens gains 4.2 mg of water in 1 hr. This is 45% of the theoretical maximum swelling if the lens behaved as a perfect osmometer. When changed to a 365 mOsm medium, the lens loses 1.1 mg of water in 1 hr. This is 47% of the potential shrinkage. The modulation of these volume changes is brought about by a change in the content of lens solute. Since the change in lens water is the same in the presence and absence of ouabain, the change in the total solute content must be the same at each osmolarity. The change in the content of sodium plus potassium and accompanying anion is not the same in the absence and presence of ouabain. Therefore some other solute must make up the difference, and an interdependent relationship must exist between electrolytes and other solutes—presumably amino acids.

The degree of water and total solute change varies with osmolarity, but a satisfactory explanation for the termination of the gain or loss at a level that will restore the physiological cell volume is not apparent. Even in avian red blood cells where solute changes during volume regulation are limited to electrolytes, a suitable interpretation is not available.

**Ouabain-insensitive fluxes.** The net ouabain-insensitive fluxes (Fig. 1, A) should approach unidirectional values if the Na,K pump is responsible for most of the active cation transport and this pump is inhibited. This is apparently the case for estimated potassium effluxes. The values of 0.22 to 0.34 $\mu$Eq/hr/lens found in the present study are similar to values estimated with $^{86}$Rb in the presence of ouabain of 0.32 to 0.52 $\mu$Eq/2 hr/lens. One may assume that the values for sodium influx found in the present study approach the values of unidirectional fluxes. The ouabain-insensitive fluxes tend to increase during VRI and decrease during VRD, with the changes being much more marked for sodium influx than they are for potassium efflux.

**Possible changes in permeability.** If the changes in ouabain-insensitive fluxes are greater than might be anticipated on the basis of changes in driving force, then a change in permeability may be suggested. The driving force ($\Delta \mu$) can be estimated and is equal to the cation potential ($E_Na$ or $E_K$) minus the PD or $E_m$. The cation potential is calculated with the Nernst equation for cation concentrations in the medium and the lens. The PD calculated from the $[Cl]$/[Cl] ratio is assumed to be the same after 1 hr of VRD and VRI as it is after 23 hr of equilibration. The calculated values of driving force before and after 1 hr of VRD and VRD in the absence of ouabain are shown in Table II.

During VRD the driving force for sodium changes very little, yet the sodium influx decreases 50% and 40% in 185 and 245 mOsm media. During VRI the driving force decreases, yet the sodium influx increases 90% in 365 mOsm medium. There is an apparent change in sodium permeability that parallels the change in osmolarity. This is consistent...
with the fact that changes in electrolyte content in the lens are related to changes in the sodium chloride concentration in the medium rather than to changes in the osmolarity per se. The change in sodium permeability may be related to the current concept of epithelial transport in which sodium influx is dependent on the co-transport of chloride. However, if this were the case, it would require a revision of the view that chloride is distributed passively. Furthermore, the PD values determined from the chloride ratios would be low, and microelectrode techniques would be required to measure PDs.

Changes in potassium efflux during VRI and VRD are of the same magnitude and in the same direction as the changes in the driving force during volume regulation. Therefore in the lens there is no evidence for a change in potassium permeability during volume regulation.

The Na,K pump. The activity of the pump is depressed during VRD and enhanced during VRI. This is indicated by the changes in the net influx of potassium and the net efflux of sodium.

Model for VRI and VRD. Although the initial stage of volume equilibration is independent of the activity of the pump, the eventual steady state after VRD or VRI must reflect a new pump-leak balance for lens electrolytes. The following model is consistent with the findings. With a change in the sodium chloride concentration of the medium, the influx of sodium is altered, and this is followed by a change in the lenticular concentration of sodium which affects the activity of the pump and thus the influx of potassium. The content and concentration of potassium then changes until the driving force for potassium produces an efflux that equals the potassium influx. The importance of sodium changes in establishing a new steady state after osmotic shock has also been suggested for lymphoma cells.

The chain of events during VRI and VRD are considered to be the same and secondary to a change in the sodium chloride concentration in the medium. The point of solute loss or gain, however, is different, and the mechanism for VRI and VRD could be viewed as being different. During VRI, Na influx exceeds efflux, so that sodium accumulates. During VRD, the potassium influx decreases to a greater degree than the potassium efflux, and potassium is lost. An additional difference in the two processes is related to time. Ouabain-insensitive fluxes along electrochemical gradients apparently change more rapidly than ouabain-sensitive fluxes associated with the Na,K pump. Thus, during VRD, potassium efflux keeps pace with changes in potassium efflux. During VRI, sodium efflux does not keep pace with sodium influx. With time, however, the accumulated sodium is replaced by potassium.

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REFERENCES