Effect of changes in osmolarity on sodium transport across isolated toad bladder

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Lipton, Peter. Effect of changes in osmolarity on sodium transport across isolated toad bladder. Am. J. Physiol. 222(4): 821-828. 1972.—Reducing the osmolarity of the solution bathing the serosal surface of the isolated toad bladder by one-third (hypotonicity) doubled short-circuit current (SCC) across the tissue (osmotic response). 22Na tracer studies showed that in the steady state this was due to an increase in active transepithelial Na transport. Increasing serosal osmolarity by 20% halved transepithelial Na transport. Hypotonically induced alterations in cell volume and electrolyte concentration were complete well before the SCC was maximal, indicating that the increase did not result from a direct effect of altered environment upon the transport mechanism itself. Hypotonicity reduced epithelial cell Na concentration by ~25%. If this reduction occurs in the Na pool participating in the osmotic response, then, assuming the two-barrier transport model, hypotonicity must lead to a stimulation of the basally located Na pump which is not a result of increased availability of Na. Hypotonicity caused an ouabain inhibitable increase in respiration which rose faster than the SCC. Insolubly as the early rise in SCC represents a rise in active Na transport, the data suggest that hypotonicity acts by increasing energy metabolism with a resultant activation of the transport mechanism via increased supply of high-energy substrate.

THE ISOLATED URINARY BLADDER OF THE TOAD, Bufo marinus, actively transports Na from the mucosal or lumen-facing surface to the serosal or blood-facing surface (15). This transport is very probably generated by the epithelial layer of cells lining the mucosal border of the bladder (8).

In this communication, I report the effect of altering bathing solution osmolarity on the Na transport. A reduction in the osmolarity of the serosal bathing medium by one-third leads to a sustained (6 hr) doubling of active Na transport. Increasing the osmolarity by 20% halves active transport. Because the bladder is very probably involved in blood osmoregulation, this direct interaction between blood osmolarity and the rate of salt reabsorption has important osmoregulatory potential for the organism.

An increased SCC across the bladder in response to hypotonicity was first noted by Maffly and Edelman (18) in 1963, and a similar phenomenon was more fully explored in frog skin by Ussing (22), who correlated osmotically induced alterations in cell size, observed microscopically, with alterations in short-circuit current (SCC). Insofar as they overlap, the data presented below are in qualitative agreement with those of the above workers.

According to the most widely held model for transepithelial Na movement, the two limiting membranes of the epithelial cell are the rate-limiting barriers (8, 14). Any increase in Na transport must involve an increased transport at both membranes and there are, in principle, two ways that this may be achieved. In the first, the properties of one barrier may be altered to give a greater intrinsic transport capacity, and the flux across the second barrier would then be increased through an alteration in the intracellular electrochemical potential of Na (E\(\text{Na}^+\)). In the second, the properties of both membranes would be directly affected, leading to greater intrinsic transport capacities. In such a case, intracellular E\(\text{Na}^+\) would not have to alter to couple the effects at the two boundaries. In order to assess the barriers affected by hyposmolarity, we analyzed its effect upon the electrolyte contents of the isolated cells. The analysis indicated that hyposmolarity leads to an activation of the basally located Na pump. It does not answer the question as to whether the increased apical transport results from a coupling to a reduction in intracellular E\(\text{Na}^+\) or a direct effect upon apical conductance. Further evidence is presented which suggests that hyposmolarity increases transport through an activation of cellular energy metabolism.

METHODS AND MATERIALS

Four basic experimental procedures were used. In all cases the tissue investigated was the urinary bladder of the female toad, Bufo marinus (Tarpon Zoo, Fla.). Toads were maintained on tap water and without food for 3 days to 3 weeks prior to their use. Hemibladders were removed from the toads within 5 min after the animals were double pithed. They were put directly into frog-Ringer solution (see Solutions) and rinsed.

Procedure 1—Short-Circuit Experiments

Active transport of Na across the isolated bladder was measured by the short-circuit technique of Ussing and Zerahn (23). Hemibladders were mounted as diaphragms across plastic chambers and bathed with appropriate solutions. Electric contact between the bathing media and the external electrodes was made with polyethylene tubing filled with 3% agar in 0.1 M KCl. The fluid resistance between electrodes (in the absence of tissue) was about 5% of the bladder resistance in isotonic medium and about 7% in hypotonic medium. It was taken into account when com-
computing SCC. The chambers were such that each hemi-
bladder was divided into two electrically and chemically
insulated quarter-bladders. Unless otherwise noted, one
of the quarter-bladders was used as the experimental and
the other as the control tissue in a given experiment. After
mounting (support was given the tissue by a nylon stocking
stretched across its serosal surface), hemibladders were
rinsed with frog-Ringer solution for ~10 min and then
incubated overnight (~15 hr) in frog-Ringer solution con-
taining 10 mM glucose and antibiotic (2,000 U/ml of
penicillin and 1.6 mg/ml streptomycinsulfate). Following
this, solutions were changed to fresh solutions, which
varied according to the particular experiment. Readings
were taken from this point. In the experiments in which
QO₂ and SCC were measured on paired hemibladders (see
RESULTS), the overnight incubation was carried out in a
beaker shaking in a metabolic shaker rather than in the
SCC chamber. Short-circuit current was recorded on a
Weston d-c microammeter, and the transepithelial elec-
trical potential difference (PD) was recorded on a Hewlett-
Packard d-c vacuum voltmeter.

Procedure 2—Measurement of Na Fluxes

Hemibladders were mounted as diaphragms in glass
chambers, as described by Porter and Edelman (20). Mucosal-serosal flux (ϕNa_mw) was measured in one hemi-
bladder and serosal-mucosal flux (ϕNa_mw) in the other
hemibladder from the same toad. Bladders were continu-
ously short circuited throughout the flux measurements.
Tracer amounts of 22Na were added to the mucosal solution (1 μc/ml) of one hemibladder and to the serosal solution (3 μc/ml) of the other. After allowing 1 hr to attain a steady
state with respect to tissue accumulation of the tracer
(others have shown this is more than adequate time (5)),
samples were taken from the low-radioactivity side of the
hemibladder at 0.5-hr intervals. After a change in the
osmolarity of the bathing medium, 1 hr was allowed for the
fluxes to reach their steady state before samples were again
taken at 0.5-hr intervals. 22Na was “counted” in a Packard γ-ray spectrometer. Unidirectional fluxes were calculated as total Na moved in 1 hr. Total charge moved was calcu-
lated by measuring SCC every 15 min, drawing the “curve”
of SCC vs. time, and computing the area under that
curve. This involved some approximation as straight lines
were drawn between the SCC readings. To calculate osmotically induced change in net flux (ϕNa_net) from these
data, two assumptions were made: 1) that SCC = ϕNa_net
in standard solutions, as shown by Leaf et al. (15), and 2)
that the fractional change in ϕNa_mw was the same in paired
hemibladders subjected to the same treatment. The latter
assumption is based on the general observation that SCC
in paired hemibladders responds proportionally to identic-
ally experimental manipulations. Assumption 1 allowed ϕ_m
in isotonic solution to be calculated in the hemibladders in
which ϕ_m was being measured (= ϕ_m - SCC). Then
assumption 2 allowed ϕ_m in this same hemibladder to be
calculated after the change in osmolarity (= ϕ_m computed
before the change X (the measured ratio of ϕ_m in the
paired hemibladder after and before the change in osmolarity)). Then with SCC and ϕ_m measured in this
hemibladder, SCC and ϕ_m could be compared.

Procedure 3—Isolation of Epithelial Cells; Analysis of
Water and Electrolyte Content

Hemibladders were incubated for 15 hr in standard
frog-Ringer solution in a metabolic shaker and then trans-
ferred to fresh standard frog-Ringer solution which con-
tained 0.02% collagenase. They were then incubated for
an additional 2 hr at room temperature and subsequently
rinsed and transferred either into hypotonic (155 mOsm/
liter) solutions (experimental tissue) or into standard
solutions (control tissue). (Four toads were used per ex-
periment: experimental tissue was four hemibladders, one
from each toad, and control tissue was the remaining four
hemibladders). During the ensuing incubation, the tissues
were exposed to inulin-3H. In the case of the 90-min and
4-hr exposures to hypotonicity, inulin (1 μc/ml) was
added 1 hr prior to the end of incubation. For the 10-min
exposure, the inulin was added at the beginning of the
incubation and the solutions and tissue shaken vigorously
for 30 sec to facilitate uniform distribution of the label.
After the appropriate incubation time, a hemibladder was
removed and stretched across a plastic block, and the
mucosal surface was gently scraped with a glass slide. The
epithelial cells collected on the slide were rapidly removed
with tweezers, blotted on a Millipore filter, and placed in a
tared, closed-top vial. The above procedure took approxi-
mately 30 sec and was repeated for all four hemibladders.
The scrapings were weighed and dried for 24–36 hr at 88 C.
They were then extracted for 24 hr in 1 N HCl. They
were then extracted for 24 hr in 1 N HCl. The nitric acid
extracts were analyzed for Na, K, Cl, and radio-
activity. (Maximum extraction of these ions and inulin was
obtained in 24 hr.) Na and K were analyzed on an atomic
absorption spectrometer (Perkin Elmer), Cl on an Amino-
Codlove chloride titrator. 3H was analyzed in a Packard
scintillation counter. The bathing media were also ana-
lyzed for these constituents, and intracellular Na, K,
Cl, and water were calculated after correcting for the
electrolyte and water content in the inulin space. In the
calculations it was assumed that the inulin and electrolyc
concentrations in the extracellular fluid of the epithelial
cell sample were the same as those in the bulk medium. Cell
water was taken as noninulin wet weight. Cell dry weight
was taken as the weight of the residue after drying in the
oven minus the weight of the extracellular electrolyte.

Procedure 4—Tissue Respiration

Respiration of segments of whole bladder was measured
using a Yellow Springs oxygen monitor, modified to accept
the Beckman oxygen macroelectrode, in a closed system.
Oxygen tension was recorded by eye from a Radiometer pH
meter with a gas monitor attachment. Rates of respiration
were calculated as the differences in oxygen tension be-
tween two successive recordings divided by the time
interval. Temperature was maintained constant (23 C)
using a Haake circulator. Pieces of tissue were cut as sym-
metrically as possible from one hemibladder, and the two
portions were used as control and experimental tissue for a
given experiment. After equilibration—about 20 min—
readings of oxygen tension were made simultaneously for
control and experimental tissues.
OSMOLARITY ON NA TRANSPORT

Composition of solutions

a) Frog-Ringer. Na = 113 mM; K = 3.4 mM; Ca = 2.7 mM; Cl = 119 mM; HCO₃⁻ = 2.4 mM; pH = 8.1; mOsm/liter = 225.

b) H₂O-Ringer. Na = 2.4 mM; K = 3.4 mM; Ca = 2.7 mM; Cl = 9 mM; HCO₃⁻ = 2.4 mM; pH = 8.1; mOsm/liter = ~20.

c) Sucrose-Ringer. Na = 2.4 mM; K = 3.4 mM; Ca = 2.7 mM; Cl = 9 mM; HCO₃⁻ = 2.4 mM; sucrose = 200 mM; pH = 8.1; mOsm/liter = ~220.

d) Low Na-Ringer. Na = 2.4 mM; K = 3.6 mM; Ca = 2.7 mM; choline = 105 mM; Cl = 119 mM; HCO₃⁻ = 2.4 mM; pH = 8.1; mOsm/liter = ~225.

All solutions contained, in addition, 10 mM glucose.

Chemicals. The conventional reagents were all of reagent grade quality (Baker and Adamson). Sodium pyruvate was obtained from Sigma Chemical Company; methoxy-inulin-³H from New England Nuclear Corp.

RESULTS

Figure 1 shows the effect on SCC of reducing the osmolarities of the bathing media to 155 mOsm/liter by dilution with H₂O-Ringer. Equal quantities of sucrose-Ringer were added to the mucosal and serosal solutions of the control quarter-bladder to allow for the effects of the reduction in ionic strength and specific ion concentrations. The reduction of the osmolarities of the bathing media resulted in a twofold increase in SCC which, in this series, was maximal by 90 min and persisted relative to the control for 6 hr. The response is reversible for at least 4 hr (16). I shall term this increased SCC an “osmotic response.” Whereas there is very little water movement across the apical surface of the bladder epithelial cells in response to an osmotic gradient, such a gradient causes a rapid water flow across the basal membrane (19). To investigate whether the osmotic response might be a result of water movement into the cells, the effect of altering the osmolarity on each side of the epithelium separately was tested. There was no effect on SCC of lowering the osmolarity of the mucosal solution alone. In contrast, lowering the osmolarity of only the serosal solution produced a maximal (100% increase) osmotic response. These results are consistent with the inference that osmotic equilibration across the serosal surface of the epithelial cells leads to the osmotic response.

The effect of increasing the osmolarity of the medium was also investigated. The addition of concentrated sucrose solution to the serosal bathing medium, sufficient to give an increase of osmolarity of about 20%, caused a decrease in SCC to about one-half its base-line value (Fig. 2). A similar increase in the osmolarity of the mucosal bathing medium had no effect on the SCC. The decrease in SCC shown in Fig. 2 cannot be attributed to sucrose toxicity, as a far higher concentration of sucrose (70 mM) used on the control tissue in the experiments summarized in Fig. 1 had no effect on SCC.

By analogy to a d-c circuit, the total transepithelial electrical “resistance” (Rₑ) is defined as:

\[ Rₑ = \frac{V}{SCC} \]

where V denotes the open-circuit electrical PD and SCC denotes the short-circuit current (corrected for the resistance of the solution between electrodes and tissue). In contrast to frog skin (22), hypertonicity caused no significant change in bladder resistance relative to control after 90 min. (An increase of 27 ± 4% compared with one of 34 ± 4%, n = 9 experiments.) The increases in both sets very probably resulted from the decreases in the conductivities of the media permeating the bladder caused by the 1:2 dilutions with the H₂O and sucrose-Ringer, respectively. Hypertonicity caused a small (15 ± 4%) increase in resistance.

The osmotically induced change in SCC is indicative of a change in the current carried by actively transported ions, but does not indicate which ions were affected. From previous studies on the toad bladder, it seemed likely that the
TABLE 1. Effects of changes in osmolarity of bathing media on SCC and unidirectional flux of sodium

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Effect of Decreased Osmolarity (225-125 mOsm/liter) (n = 11 Exp)</th>
<th>Effect of Increased Osmolarity (225-265 mOsm/liter) (n = 6 Exp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCC before change</td>
<td>2.13 ± 0.25</td>
<td>1.77 ± 0.31</td>
</tr>
<tr>
<td>$\phi_{ms}$ before change</td>
<td>3.15 ± 0.25</td>
<td>2.18 ± 0.31</td>
</tr>
<tr>
<td>$\phi_{sm}$ before change</td>
<td>1.02</td>
<td>0.41</td>
</tr>
<tr>
<td>SCC after change</td>
<td>3.50 ± 0.27</td>
<td>0.93 ± 0.24</td>
</tr>
<tr>
<td>$\phi_{ms}$ after change</td>
<td>4.06 ± 0.26</td>
<td>1.36 ± 0.29</td>
</tr>
<tr>
<td>$\phi_{sm}$ after change</td>
<td>0.65</td>
<td>0.44</td>
</tr>
<tr>
<td>$\Delta \phi_{sm}$</td>
<td>0.93 ± 0.12</td>
<td>-0.78 ± 0.15</td>
</tr>
<tr>
<td>$\Delta \phi_{ms}$</td>
<td>-0.37</td>
<td>0.03</td>
</tr>
<tr>
<td>$\Delta \phi_{net}$</td>
<td>1.30 ± 0.29</td>
<td>-0.82 ± 0.09</td>
</tr>
<tr>
<td>$\Delta$SCC</td>
<td>1.37 ± 0.25</td>
<td>-0.84 ± 0.11</td>
</tr>
</tbody>
</table>

Results are means ± 1 SE. For $\Delta$ values, SEM are calculated for the paired differences in the values before and after the osmotic changes. All values are total microequivalents transported during a 1-hr interval. The values before change of osmolarity are for the 1-hr period immediately preceding the change. $\Delta$ values are the differences between the values for the 1-hr periods between 90 and 150 min and 150 min and 210 min after the osmotic changes and the values for before change of osmolarity. $\phi_{ms}$ is calculated as $\phi_{ms} - SCC$ (see Methods). $\phi_{sm}$ after change is calculated as $\phi_{sm}$ before change $\times$ $\phi_{sm}$ measured after change in paired hemibladder/$\phi_{sm}$ measured before change in paired hemibladder (see Methods).

The contribution of active Na transport to the osmotic response was tested directly by measuring the effect of osmotic changes on unidirectional fluxes ($\phi_{Na}$) of Na in continuously short-circuited hemibladders. The results in Table 1 show that the primary effect of hyposmolarity is to increase $\phi_{Na}$ and that the changes in SCC and $\phi_{Na}$ are equal in magnitude. They show also that hyperosmolarity causes equal decreases in SCC and net Na flux. The results thus indicate that the osmotic response is entirely attributable to changes in active Na transport. In hypotonic media, $\phi_{Na}$ decreased by 37%. This was about equal to the decrease in Na concentration of the bathing medium, and it may be assumed that it resulted from this decrease as the s → m flux is considered to be a passive one.

Effect of Ouabain and Ion Na-Ringer Solution on Osmotic Response

Ouabain is a specific inhibitor of active Na transport in a great variety of tissues, including the toad bladder (11). The effect of ouabain on the osmotic response is presented in Fig. 3. The glycoside ($5 \times 10^{-4}$ M) reduced the SCC to identical low levels in control and hypotonically treated tissue, completely abolishing the increment which had been induced by the hyposmolarity. This is consistent with the hypothesis that the osmotic response results from an increase in active sodium transport.

FIG. 3. Effect of ouabain on hypotonically induced increase in SCC across isolated toad bladder. Solid curve shows effect on SCC of decreasing bathing media osmolarities (first arrow) and of subsequent addition of $5 \times 10^{-4}$ M ouabain (second arrow). Dashed curve is control tissue which was treated with isotonic sucrose-Ringer (first arrow) and $5 \times 10^{-4}$ M ouabain (second arrow). Bars are ±1 SE; n = 8 experiments.

FIG. 4. Effect of different mucosal medium sodium concentrations on hypotonic stimulation of SCC across isolated toad bladder. At $t = 60$ min low Na-Ringer (see Solutions) was substituted for standard solution in both experimental (solid curve) and control (dashed curve) systems. Solid curve shows effect of next reducing osmolarity of serosal bathing medium (second arrow) and then restoring concentration of the mucosal bathing medium to its normal Na level (third arrow). Control tissue was treated similarly, except that isotonic sucrose-Ringer was added rather than H2O-Ringer at second arrow. Bars are ±1 SE; n = 11 experiments.

If the sodium concentration of the bathing media is lowered sufficiently by substitution of choline for sodium, the rate of transepithelial sodium transport falls significantly (9). In the experiment described by Fig. 4, the effect...
of reducing the SCC by this maneuver on the subsequent osmotic response was tested. Lowering Na to 2.5 mm reduced SCC to about 40% of the baseline. When the osmolarity of the serosal medium bathing one quarter-bladder was then lowered, there was an approximate doubling of its (reduced) SCC. Upon replacement of the low Na-Ringer mucosally by normal Ringer in both quarter-bladders, there was a rise in SCC. This rise was far larger, in absolute magnitude, in the hyposmotically treated tissue. The dependence of the magnitude of the osmotic response on medium sodium concentration (the concentration of all other ions except choline remained constant throughout the experiment) provides additional support for the inference that the response is a result of increased transepithelial sodium transport.

Effect of Hypotonicity on Water and Electrolyte Contents of Epithelial Cells

The epithelial cell preparation from the bladder which was used in these analyses is described in Methods; as reported elsewhere it consisted almost exclusively of sheets of the epithelial cells (17) bounded basally by the basement membrane of the epithelium (16).

To compare the time course of the osmotic response to that of the cell electrolyte and water changes, the latter were measured after three different durations of exposure to hypotonicity (155 mOsm/liter). The results are shown in Table 2. In the experiments the bladder remained intact throughout the exposure to hypotonicity. Cells were removed at the appropriate times and transferred directly to tared vials for analysis (see Methods). Although different batches of toads were used for the different intervals so that control levels vary, it is apparent that the measured physical changes (in terms of percent of control) were essentially complete by 10 min. This compared with the SCC response which, throughout a large number of experimental sets, was never complete before 50 min. There were falls in the intracellular concentrations of all three electrolytes—Na falling by between 20 and 30%, K falling by between 16 and 26%, and Cl falling by between 21 and 32%. The volume increases (H2O/dry cell wt) of ~15% were far less than those which would have occurred (45%)

### Table 2. Effect of exposure of bladders to hyposmotic solutions on electrolyte and water contents of isolated epithelial cells

<table>
<thead>
<tr>
<th>Parameter</th>
<th>10 Mln</th>
<th>90 Mln</th>
<th>4 Hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2O/dry cell wt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>4.33 ±0.15</td>
<td>4.98 ±0.17</td>
<td>4.35 ±0.12</td>
</tr>
<tr>
<td>Hypo*</td>
<td>4.98 ±0.17</td>
<td>5.30 ±0.12</td>
<td>4.35 ±0.12</td>
</tr>
<tr>
<td>Na/dry cell wt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>291 ±24</td>
<td>220 ±17</td>
<td>202 ±16</td>
</tr>
<tr>
<td>Hypo*</td>
<td>258 ±27</td>
<td>190 ±16</td>
<td>202 ±16</td>
</tr>
<tr>
<td>K/dry cell wt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>429±11</td>
<td>449±20</td>
<td>374±24</td>
</tr>
<tr>
<td>Hypo*</td>
<td>412±19</td>
<td>66±12</td>
<td>374±24</td>
</tr>
<tr>
<td>Cl/dry cell wt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>309±22</td>
<td>234±16</td>
<td>178±24</td>
</tr>
<tr>
<td>Hypo*</td>
<td>282±16</td>
<td>56±24</td>
<td>178±24</td>
</tr>
<tr>
<td>[Na + K + Cl] b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>242</td>
<td>193</td>
<td>178</td>
</tr>
<tr>
<td>Hypo*</td>
<td>242</td>
<td>193</td>
<td>178</td>
</tr>
<tr>
<td>Total medium b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>240</td>
<td>167</td>
<td>163</td>
</tr>
<tr>
<td>Hypo*</td>
<td>240</td>
<td>167</td>
<td>163</td>
</tr>
</tbody>
</table>

Values are all given ± SE; n = 10 experiments in all cases. One experiment was performed using 4 sets of paired hemibladders which yielded about 6 mg dry wt of isolated cells. The cells were prepared as described in Methods, and the experimental set of hemibladders was exposed to hypotonic medium 10 min, 90 min, and 4 hr before the bladders were scraped. * mg cell water/mg dry cell wt. b mEq/kg cell water. ± mEq/kg cell dry wt. Average difference for individual experimental and control pairs. Composition of this solution (mOsm/liter = 155), in millimoles per liter, was: Na = 79, K = 3.4, Ca = 2.7, Cl = 84, and HCO3⁻ = 2.4.
had the cells behaved as perfect osmometers.\(^1\) There was a net solute loss from the cells in all cases which included a larger loss of positively charged ions than of negatively charged ions. The excess varied from 22 to 59 mEq/kg dry wt—about 5% of the cell cation contents. As indicated in lines \(8\) and \(9\), the solute loss was such that, coupled with the small gain in cell water, the loss of intracellular Na + K + Cl concentration was almost equal to the reduction in medium electrolyte concentration. This expulsion of electrolyte and the resultant small volume change required to maintain approximate cellular isosmolarity with the medium represent a considerable self-regulation of cell volume. The mechanism of this phenomenon was not further studied.

Effects on Respiration

In showing that the physical changes were complete well in advance of the SCC, Table 2 indicates that the change in the latter might result from alteration of another cell process which was more directly affected by the physical changes. It was thought that this process might be cell energy metabolism. Thus, the kinetics of the cell respiration were compared with the kinetics of the SCC after exposure to hypotonicity in a series of carefully paired experiments.

After overnight incubation in a beaker, one hemibladder of a pair was mounted in an SCC chamber; the other was split into quarter-bladders and incubated in the respiration chamber. Both systems were maintained at 23 ± 1°C. After diluting the medium of the experimental quarter-bladder in each system with H2O-Ringer (equal volumes of frog Ringer were added to the control tissue), readings were taken every 2 min. The mixing in both systems was good (rapid bubbling of air in the SCC system and rapid magnetic stirring in the respiration system) so that a comparison of the results should be devoid of mixing artifacts. Also, the pH’s of the solution in the SCC and respiration chambers did not differ by more than 0.1 unit at the end of the experiment. Figure 5 summarizes the data from these experiments. The ordinates are the ratios of the normalized parameter in the experimental tissue to the normalized parameter in the control tissue, the normalization being with respect to the value at \(t = 20\) min (i.e., SCC(20) or \(QO_2(20) = 1.00\)) in each experiment. There is an early depression in \(QO_2\) and SCC in the experimental tissues. In spite of the larger percentage depression in \(QO_2\), its rise (half-time = 7 min) is more rapid than that of SCC (half-time = 11 min). Also, \(QO_2\) reaches a plateau far earlier than SCC. In another set of experiments (Fig. 6), it was found that if ouabain (5 \times 10^{-4} M) was added 50 min after the exposure to hypotonicity, the \(QO_2\) in both experimental

\(1\) In comparing “volumes” of control and treated cells, it is assumed that there is no difference between the inulin penetration into the two cell populations. The lack of any resistance change in the experimental population coupled with its ability to maintain ion gradients over at least a 4.5-hr period argues that no membrane changes have occurred which would lead to a significant leakage of inulin relative to the control population. In addition, it is assumed that hypotonicity leads to no loss of cellular constituents which would alter the dry weight per cell relative to control. Again here: the lack of any indication of large membrane changes and the reversibility of the osmotic response argues against significant changes of this sort.

DISCUSSION

Active Na transport across the toad bladder has been found to be profoundly affected by the osmolarity of the solution bathing the water-permeable serosal surface of the tissue. A similar effect was found in frogskin by Ussing (22). Although he did not specifically show an increase in net Na flux, ion substitution experiments indicated that it was Na whose transport was increased by hypotonic solutions. In
frogskin there is decreased resistance associated with the osmotic response which does not occur in bladder. Singer et al. (21) have found that isosmotic substitution of organic ions for Cl in the serosal medium leads to an increased SCC. The effect was not due to the entrance of these ions into pathways of energy metabolism, and it may well have resulted from an increased cell volume due to isosmotic entry of the organic ions into the cells. This conclusion is supported by results I have obtained (unpublished data) showing that urea and glycerol—two highly permeant organic species—cause large (maximally about 100%) increases in SCC when added isosmotically to the serosal bathing medium.

Although there was variability in the absolute values of the changes in electrolyte and water concentrations at the different times of analysis, when considered as percentages of control the changes were maximal by 10 min. The SCC, on the other hand, did not plateau until 50 min or longer after the osmotic change. If the physical changes are leading to the increased SCC, then these results eliminate a direct effect of cell volume or solute concentration upon the transport system itself as a mechanism for the response. Rather they indicate that there is mediation by some intermediate step(s). The nature of these intermediate steps was investigated by comparing the kinetics of the osmotically induced increases in SCC and QO2. The rise in QO2 preceded the rise in SCC. Although there was an initial depression in both parameters so that the times of onset could not be compared, the ensuing increase in QO2 was more rapid than that in SCC and plateaued far earlier.

Providing that the SCC is a valid measure of active ion transport during the early phase of the osmotic response, these data suggest strongly that the increased energy metabolism is leading to the increased transport. It would not be consistent with the possibility that increased transport is causing an activation of oxidative energy metabolism; at a time when the latter is maximal, SCC is still rising and is only about two-thirds maximal.

The possibility that the hypoosmotically induced transport is uncoupled from respiration is rendered small by the fact that the increased respiration is all inhibited by ouabain and that both ouabain-inhibitable respiration and transport are approximately doubled by hypomolarity.

The possibility must, however, be considered that the SCC in the early part of the osmotic response is different from the rate of active Na transport. (The equality was only demonstrated for the steady state.) There are significant ion movements from the cell (Table 2), and if these act to oppose the direction of the active Na transport, then they might lead to an underestimate of the rate of increase of the latter.

With this important proviso then, the data indicate that the osmotic response is a result of a primary stimulation of energy metabolism. The time differential between increased respiration and transport would, then, presumably reflect the buildup of high-energy substrate at the appropriate transport site(s). In this regard, it is relevant that when transport is activated by adding an energy-yielding substrate (Na pyruvate) to substrate-depleted or “starved” bladders, the increase in respiration is also more rapid than the increase in SCC. The former is maximal by 5 min; the latter is not maximal until at least 30 min after adding the substrate (16). That hypomolarity should activate energy metabolism is consistent with experiments of Atsman and Davies (1), who found that decreasing the osmolarity of the incubating medium for isolated kidney or liver mitochondria to 155 mOsm/liter caused a 50% increase in state 3 (coupled) respiration.

It is important to consider the implication of the complete ouabain inhibition of the hypomotic stimulation of respiration. If the inference of a primary stimulation of energy metabolism is correct, then the above finding implies that all of the increased energy production is consumed by the transport mechanism, i.e., that all other energy-consuming processes are “energy” saturated in normal conditions. There is no extant evidence concerning this conclusion, but it is notable that the mechanism of action of aldosterone in enhancing Na transport which has been proposed by Edelman et al. (6) (a stimulation of mitochondrial activity) also carries with it this implicit assumption, as the absence of Na from the bathing medium completely inhibits the aldosterone stimulation of bladder respiration (12). (It is interesting in regard to aldosterone action to note that the osmotic response, like the response to the steroid, is severely inhibited in substrate-depleted bladders (16). Thus, the response in these conditions is maximally only 50% of the base line and declines to 30% above base line after 2 hr.)

If the osmotic response were effected simply by a stimulation of the apical entry step for Na, then the basal step, or pump activity, would have to be increased by an increased intracellular Na. Such a change was not observed in any of the three sets of measurements. In all cases there was a 20–30% decrease in Na concentrations. Thus, if the observed ion changes in the total epithelial population qualitatively reflect those in the Na pool involved in the osmotic response, the decreased osmolarity must have led to a direct stimulation of the basally located Na pump. That this is the case is suggested by the following considerations. Recently, Finn and Rockoff (7) concluded that about 50% of the intracellular Na of the bladder is in a (kinetically) homogeneous transepithelial transport pool. Smooth muscle cells are about as numerous as epithelial cells in bladder (4, 19) and Na concentrations in smooth muscle from various sources range from 35 to 66 mM (2, 3, 10). Thus, it is likely that not less than 75% of the Na in the epithelial cell sample is in the transport pool. Furthermore, we have found (unpublished data) that increasing serosal osmolarity to 300 mOsm/liter reduces SCC to about 5% of control levels so that all of the Na transport appears to be osmotically regulated. This implies that the total Na transport pool participates in the osmotic response. The average epithelial Na concentration was 72 mM. Thus, according to the above argument, that of the transport pool is at least 72 X 3/4 ~ 54 mM and that of the nontransport pool is no greater than 18 mM. Hypomolarity induced an average decrease of 19 mM in the total Na concentration. If there were a concomitant increase in the transport pool Na, then nontransport Na would have had to become negative. Because such a large decrease is unlikely, it appears that the Na of the transport pool is, at the least, not increased by hypomolarity and is very probably decreased. Thus, a direct activation of the basal pump appears to be involved in the osmotic response. This conclusion
is consistent with that of Kleinzeller et al. (13) concerning the effect of lowered osmolarity upon the cells in kidney cortex slices and is also consistent with the notion that hyposmolality acts via a primary stimulation of energy metabolism; the pump is an energy-dependent one. In order to assess whether the apical conductivity is also enhanced by hyposmolality, the latter’s effect upon the electrochemical gradient for Na at the apical membrane would have to be estimated. Because the intracellular electrical potential was not measured, such an estimate cannot be made with any confidence.

The regulatory potential of the osmotic response for an organism should be stressed. Whenever there is an epithelium which can support an osmotic gradient, the osmotic response may act as an osmotic or ionic hemostat.

REFERENCES


16. Lipton, P. Hormonal and Osmotic Regulation of Active Sodium Transport Across the Isolated Bladder of the Toad, Bufo marinus (Ph.D. Thesis). San Francisco, Calif.: Univ. of California Medical Center, 1969.


