Ouabain-induced stimulation of sodium-hydrogen exchange in rat optic nerve astrocytes

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Mandal A, Delamere NA, Shahidullah M. Ouabain-induced stimulation of sodium-hydrogen exchange in rat optic nerve astrocytes. Am J Physiol Cell Physiol 295: C100–C110, 2008. First published April 30, 2008; doi:10.1152/ajpcell.90636.2007.—Sodium-dependent transporters are inhibited indirectly by the Na-K-ATPase inhibitor ouabain. Here we report stimulation of sodium-hydrogen exchange (NHE) in ouabain-treated cells. BCECF was used to measure cytoplasmic pH in cultured rat optic nerve astrocytes. Ammonium chloride was applied to acid load the cells. On removal of ammonium chloride, cytoplasmic pH fell abruptly, then gradually recovered toward baseline. Ouabain (1 μM) did not change cell sodium content, but the rate of pH recovery increased by 68%. Ouabain speeded pH recovery both in the presence and absence of bicarbonate. In bicarbonate-free medium, dimethylamiloride, an NHE inhibitor, eliminated the effect of 1 μM ouabain on pH recovery. Western blot analysis showed an NHE1 immunoreactive band but not NHE2, NHE3, or NHE4. Immunoprecipitation studies showed phosphorylation of NHE1 in cells treated with 1 μM ouabain. Ouabain evoked an increase of cAMP, and the effect of 1 μM ouabain on pH recovery was abolished by H-89, a protein kinase A inhibitor. 8-Bromoadenosine-cAMP increased the pH recovery rate, and this recovery was not further increased by ouabain. Although 1 μM ouabain did not alter cytoplasmic calcium concentration, it stimulated calcium entry after store depletion, a response abolished by 2-APB. Ouabain-induced stimulation of pH recovery was suppressed by inhibitors of capacitative calcium entry, SKF-96365, and 2-APB, as well as the cytoplasmic calcium chelator BAPTA. The cAMP increase in ouabain-treated cells was abolished by BAPTA and 2-APB. Taken together, the results are consistent with increased capacitative calcium entry and subsequent cAMP-PKA-dependent stimulation of NHE1 in ouabain-treated cells.

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kinase rabbit monoclonal antibody, phospho-p44/42 MAPK (Thr202/ 
Tyr204) mouse monoclonal antibody and phospho-(Ser) 14-3-3 bind-
ing motif mouse monoclonal antibody were obtained from Cell 
Signaling Technology (Danvers, MA). Goat anti-Na-K-ATPase α2 
antibody, rat brain, and kidney microsomal preparations were ob-
tained from Upstate Laboratories (Lake Placid, NY). Rat skeletal 
muscle lysate was obtained from Zygwen Laboratories (San Diego, 
CA). Alexa Fluor 680 goat anti-mouse secondary antibody was 
purchased from Invitrogen. Goat anti-rabbit IR dye 800 conjugated 
secondary antibody was obtained from Rockland (Gilbertsville, PA).

**CAMP** [125I] radioimmunoassay kit was obtained from Perkin-
Elmer Life and Analytical Sciences (Shelton, CT).

**Solutions.** Cytoplasmic pH, cAMP, sodium, and calcium measure-
ments were carried out on cells bathed in bicarbonate-containing 
Kreb's solution (in mM) NaCl, 119; KCl, 4.7; KH2PO4, 1.2; NaHCO3, 
25; dextrose, 5.5; MgCl2, 1; CaCl2, 2.5 with a pH adjusted to 7.4 and 
an osmolarity of ~300 mosM. As specified, cells were exposed to a 
modified Krebs solution containing 20 mM ammonium chloride 
added in substitution for an equimolar amount of NaCl. The compo-
state-free HEPES buffer was (in mM) 115 NaCl, 5 KCl, 2.5 CaCl2, 1.2 MgSO4, 2 NaH2PO4, 32.2 HEPES, 10 glucose, 
with a pH adjusted to 7.4 and an osmolarity of ~300 mosM. Test 
agents were dissolved either in dimethylsulfoxide (0.06% final con-
centration) or in water according to solubility and were added to the 
Krebs solution to obtain intended final concentration. Control cells 
received only the vehicle. Nigericin was dissolved in methanol.

**Cell culture.** Optic nerve astrocytes were isolated and cultured 
during a modification of a previous method (24). Eyes from 1-
to 5-day-old rat pups were obtained from Hilltop Laboratories (Scottdale, 
PA) and washed in DMEM/F12 containing penicillin (100 U/ml) 
and streptomycin (0.1 mg/ml). The optic nerve was dissected, plated 
onto 35 mm culture dishes, and maintained at 37°C in a humidified 
350 mosM. As specified, cells were exposed to a 

**Measurement of cell sodium.** Sodium was measured by atomic 
sorption spectrophotometry following a published method (26). 
Briefly, cells grown on culture dishes were washed with ice-cold 
insoluble magnesium chloride solution (100 mM MgCl2, pH adjusted 
to 7.4 with Tris base). The magnesium chloride solution was then 
removed, and the cells were digested in 30% nitric acid. The acid 
digest was diluted with deionized water, and the sodium content of 
the diluted cell lysate was measured by using an atomic absorption 
spectrophotometer (Analyst 100; Perkin-Elmer, Norwalk, CT) at 

**Measurement of cAMP.** The effect of ouabain on cAMP concen-
tration was measured in cells incubated in normal Krebs solution (pH 
7.4) for 12.5 min. In a different experiment, ouabain-treated cells 
were subjected to same perturbation as in the case of pH measurement, i.e., 
5-min exposure to modified Krebs solution containing 20 mM amm-
ion chloride, followed by a 2.5-min recovery in control Krebs 
solution. Preparation of sample from cultured cells and measurement 
of cAMP in the sample was carried out as described previously (47) 
using a cAMP [125I] radioimmunoassay kit (Perkin-Elmer Life and 
Analytical Sciences). Briefly, the Krebs solution was removed, and 
700 μl chilled trichloroacetic acid (TCA; 6%) was added to each 
culture dish. The cells were scraped from the culture dish, and the 
cell/TCA mixture was frozen at ~20°C. The samples were thawed 
rapidly at 37°C and sonicated for 2 min in a Misonix 3000 sonicator 
(power setting 2). The freeze, thaw, sonication cycle was repeated 
four times, and the mixture was centrifuged at 5,000 g for 20 min at
RESULTS

Effect of ouabain and 5-(N,N-dimethyl)amiloride hydrochloride on pH recovery. Exposure of the cells to bicarbonate buffer containing 20 mM ammonium chloride caused a sharp increase in pH from the baseline value of 7.24 ± 0.01 (n = 37) to 8.25 ± 0.03. Removal of the ammonium chloride-containing test solution caused a sharp decrease in cytoplasmic pH to 5.88 ± 0.01, followed by a period of gradual recovery toward baseline (Fig. 1A). In control cells, the recovery was roughly linear during the initial 2.5 min. The recovery rate was 2.81 ± 0.15 uH11003 per min. Exposure of the cells to bicarbonate-free HEPES buffer. In the absence of bicarbonate, 1 μM ouabain still caused a marked increase in the pH recovery rate (Fig. 2).

To dissect the contribution of NHE and bicarbonate transporters to the ouabain response, studies were carried out in bicarbonate-free HEPES buffer. In the absence of bicarbonate, 1 μM ouabain still caused a marked increase in the pH recovery rate (Fig. 2). Baseline pH was not changed in bicarbonate-free buffer. Because the ouabain effect on pH recovery persisted in bicarbonate-free buffer, studies were conducted using DMA to examine the contribution of NHE. DMA (100 μM) did not change the baseline pH, but the pH recovery rate was reduced to 0.5 ± 0.1 × 10⁻³ pH U/s (n = 3). DMA eliminated the ability of 1 μM ouabain to increase the pH recovery rate (Fig. 2).

Expression of NHE1 and Na-K-ATPase α2. Cultured astrocytes were subjected to Western blot analysis for NHE1, NHE2, NHE3, and NHE4. NHE1 was detected as an immunoreactive band at ~110 kDa (Fig. 3A). Immunoreactive bands were not detected for NHE2, -3, or -4 (data not shown). In addition to the housekeeping Na-K-ATPase α1 isofrom, glial cells express Na-K-ATPase α2. A dense Na-K-ATPase α2 immunoreactive band was detected in cultured rat optic nerve astrocytes (Fig. 3B).

Effect of ouabain on cell sodium. Studies were conducted to examine the sodium content of cells subjected to an experimental protocol like that used in pH studies: a 5-min period of exposure to 20 mM ammonium chloride followed by a 2.5-min recovery period in control Krebs solution. The sodium content detected in control cells was similar to the sodium content detected in cells exposed to 1 μM ouabain throughout the course of the experiment. This agrees with an earlier report that 1 μM ouabain caused no detectable increase of sodium concentration in astrocytes loaded with the sodium-sensitive dye SBFI (24). At concentrations of 1 mM and 10 mM, ouabain increased cell sodium content significantly (Fig. 4).

Effect of 8-bromoadenosine-cAMP and H-89. 8-Bromoadenosine-cAMP (8-Br-cAMP) is a cell-permeable analog of cAMP and a recognized activator of protein kinase A. When cells were exposed to 8-Br-cAMP (1 mM) throughout the experiment, the pH recovery rate following a 5-min exposure to ammonium chloride increased significantly. The rate of pH recovery observed in the presence of 8-Br-cAMP was similar to the rate of recovery observed in cells exposed to 1 μM ouabain. When 1 mM 8-Br-cAMP was added together with 1 μM ouabain, the rate of pH recovery was not significantly different from the rate of recovery measured in cells exposed to 1 μM ouabain or 1 mM 8-Br-cAMP alone (Fig. 5).

Some cells were exposed to 10 μM H-89, an inhibitor of cAMP-dependent protein kinase, protein kinase A. When
Table 1. Baseline cytoplasmic pH

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>Baseline pH</th>
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<tbody>
<tr>
<td>Control</td>
<td>7.24±0.01 (n = 37)</td>
</tr>
<tr>
<td>100 nM ouabain</td>
<td>7.22±0.01 (n = 6)</td>
</tr>
<tr>
<td>1 μM ouabain</td>
<td>7.23±0.02 (n = 10)</td>
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<tr>
<td>10 μM ouabain</td>
<td>7.32±0.01 (n = 6)</td>
</tr>
<tr>
<td>1 mM ouabain</td>
<td>7.31±0.02 (n = 9)</td>
</tr>
<tr>
<td>1 mM 8-Br-cAMP</td>
<td>7.21±0.02 (n = 10)</td>
</tr>
<tr>
<td>100 μM DMA</td>
<td>7.22±0.02 (n = 10)</td>
</tr>
<tr>
<td>10 μM H-89</td>
<td>7.21±0.01 (n = 10)</td>
</tr>
<tr>
<td>50 μM SKF-96365</td>
<td>7.22±0.01 (n = 4)</td>
</tr>
<tr>
<td>50 μM 2-APB</td>
<td>7.23±0.01 (n = 4)</td>
</tr>
<tr>
<td>50 μM BAPTA-AM</td>
<td>7.21±0.01 (n = 5)</td>
</tr>
<tr>
<td>10 μM KB-R7943</td>
<td>7.20±0.01 (n = 6)</td>
</tr>
<tr>
<td>50 μM PD-98059</td>
<td>7.21±0.01 (n = 6)</td>
</tr>
</tbody>
</table>

Values are means ± SE of data from 4 to 37 independent experiments; in each experiment, data from 15–30 individual cells were averaged and considered as n = 1. Baseline pH was measured in bicarbonate-containing buffer. 8-Br-cAMP, 8-bromoadenosine-cAMP; DMA, 5-(N,N-dimethyl)amiloride hydrochloride. *P < 0.01, †P < 0.001, significant difference from control.

added together with 1 μM ouabain, 10 μM H-89 abolished the ouabain-induced increase in the rate of pH recovery (Fig. 6). In cells exposed to H-89 alone, baseline pH was unchanged (Table 1), but the rate of pH recovery was lower than in control cells.

**Effect of ouabain on intracellular cAMP.** Cyclic AMP was measured in control cells and in cells subjected to an episode of acidification like that used in the pH studies, i.e., a 5-min exposure to 20 mM ammonium chloride followed by a 2.5-min recovery period, representing the midpoint of the pH recovery phase. Ammonium chloride exposure did not alter cAMP. In contrast, ouabain (1 μM) increased cAMP by ~60% (Fig. 7A). The magnitude of the increase was similar in cells exposed to 1 mM ouabain. Forskolin (10 μM), a direct adenylate cyclase stimulator, increased cAMP to a similar extent as ouabain (Fig. 7B).

**Effect of BAPTA, SKF-96365, and 2-APB on pH recovery.** The calcium chelator BAPTA abolished the ouabain-induced stimulation of pH recovery and reduced the rate to a level significantly lower than that observed in control cells. Added alone BAPTA reduced the rate of pH recovery by ~50% (Fig. 8A). When the cells were exposed to inhibitors of capacitative calcium entry, SKF-96365 or 2-APB (50 μM each), the rate of pH recovery in ouabain-treated cells was markedly reduced. The rate was not different from the rate observed in control cells (Fig. 8, B and C).

**Effect of ouabain on calcium.** In cells loaded with fura-2, cytoplasmic calcium concentration was measured continuously while the cells were exposed to 20 mM ammonium chloride for 5 min and then allowed to recover on removal of ammonium chloride. Cytoplasmic calcium concentration and pH increased at the start of the ammonium chloride exposure, cytoplasmic calcium was then declined toward the baseline even though pH remained elevated. Throughout the course of the experiment, no significant difference in cytoplasmic calcium concentration was observed between control cells and cells exposed to 1 μM calcium.
ouabain (Fig. 9A). Although 1 μM ouabain did not cause a detectable change of calcium concentration, it stimulated calcium entry when applied after store depletion. After the establishment of a stable baseline in calcium-free buffer, cells were exposed to 100 μM ATP. This caused a transient increase of cytoplasmic calcium concentration, a response we interpret as purinergic receptor-mediated emptying of calcium stores. Subsequently, 2.5 mM calcium was added to the bathing medium to allow capacitative calcium entry (Fig. 9B). Some cells were exposed to 1 μM ouabain before addition of calcium. Ouabain caused a significant increase in capacitative calcium entry (Fig. 9C). Capacitative calcium entry in ouabain-treated cells was abolished by 2-APB (Fig. 9, D and E).

To exclude the possibility that ouabain response to pH is related to calcium entry by reverse mode sodium-calcium exchange, cells were exposed to KB-R7943. Ouabain-stimulated pH recovery was unaffected in the presence of 10 μM KB-R7943 (Fig. 10).

**Effect of BAPTA and 2-APB on intracellular cAMP.** BAPTA and 2-APB both abolish the ouabain-induced stimulation of pH recovery rate. Because the pH recovery response is associated with an increase of cAMP, studies were conducted to measure cAMP in cells exposed to BAPTA or 2-APB. Both BAPTA and 2-APB abolished the increase of cAMP elicited by 1 μM ouabain (Fig. 11). Added alone, BAPTA and 2-APB each reduced the resting level of cAMP.

**NHE1 phosphorylation.** Studies were conducted to examine NHE1 phosphorylation in cells subjected to an experimental protocol like that used in pH studies: a 5-min period of exposure to 20 mM ammonium chloride followed by a 2.5-min recovery period in Krebs solution. Treated cells received ouabain (1 μM, 1 mM, or 10 mM) throughout the experiment. Values are means ± SE of results from 4 independent experiments. *P < 0.01, significant difference from control.

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**Fig. 4.** The effect of ouabain on the sodium content of cultured rat optic nerve astrocytes subjected to a 5-min period of exposure to 20 mM ammonium chloride followed by a 2.5-min recovery period in Krebs solution. Treated cells received ouabain (1 μM, 1 mM, or 10 mM) throughout the experiment. Values are means ± SE of results from 4 independent experiments. *P < 0.01, significant difference from control.

**Fig. 5.** The effect of 8-bromoadenosine-cAMP (8-Br-cAMP; 1 mM) and ouabain (1 μM) on the rate of cytoplasmic pH recovery after ammonium chloride removal. Ouabain was present throughout the experiment. 8-Br-cAMP was introduced after establishment of a steady baseline pH but before the addition of ammonium chloride. Values are means ± SE of results from 10–37 independent experiments. ***P < 0.001, significant difference from control.

**Fig. 6.** The effect of protein kinase A inhibitor H-89 (10 μM) and ouabain (1 μM) on the rate of cytoplasmic pH recovery after ammonium chloride removal. H-89 was introduced after establishment of a steady baseline pH but before the addition of ammonium chloride. Ouabain was added 5 min after H-89. H-89 and ouabain were present throughout the rest of the experiment. Values are means ± SE of results from 10–37 independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001, significant difference from control. ###P < 0.001, significant difference from 1 μM ouabain.

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**Fig. 7.** A: the effect of ouabain on cAMP in control cells and in cells subjected to 20 mM ammonium chloride exposure for 5 min followed by a 2.5-min recovery period in Krebs solution. Treated cells received ouabain (1 μM or 1 mM) throughout the experiment. B: the effect of forskolin on cAMP. Cells were treated with forskolin (10 μM) or ouabain (1 μM) without exposure to ammonium chloride. Values are means ± SE of results from 4 independent experiments. ***P < 0.001, significant difference from control. #P < 0.05, ##P < 0.01, significant difference from control where the cells were treated with ammonium chloride.
Fig. 8. The effect of the calcium chelator BAPTA-AM (A) and the capacitative calcium entry inhibitors SKF-96365 (B) or 2-APB (C) on the rate of cytoplasmic pH recovery after an acid load. The capacitative calcium entry inhibitors SKF-96365 and 2-APB are shown to abolish the effect of ouabain on pH recovery rate. The ability of both SKF-96365 and 2-APB to abolish the effect of ouabain on pH recovery points to the requirement for capacitative calcium entry. The findings are consistent with stimulation of capacitative calcium entry by a mechanism linked to the inhibition of the Na-K-ATPase α2 isoform (24).

The response to 1 μM ouabain was not associated with a detectable cell-wide increase in calcium concentration. Despite this, the calcium chelator BAPTA abolished the ouabain-induced increase in pH recovery rate. The ability of both SKF-96365 and 2-APB to abolish the effect of ouabain on pH recovery points to the requirement for capacitative calcium entry. The findings are consistent with stimulation of capacitative calcium entry by a mechanism linked to the inhibition of the Na-K-ATPase α2 isoform (24).

Ouabain caused an increase in the rate of pH recovery both in bicarbonate-containing and bicarbonate-free solutions. In bicarbonate-free solution, DMA abolished the effect of ouabain on the pH recovery. It is likely that the principal cellular alkalinizing mechanism is the NHE. Taken together, the findings are consistent with the idea that ouabain-induced stimulation of pH recovery is due to activation of NHE1, the only isoform detected in these cells. The experiments in bicarbonate-free solution argue against the possibility that ouabain changes the rate of pH recovery by changing the function of a bicarbonate-dependent acid extruder or bicarbonate-dependent acid loader even though it has been reported that chloride bicarbonate exchange can be modulated via PKA (10). This is noteworthy since astrocytes are known to possess a functional NHE and also sodium- and bicarbonate-coupled acid extrusion mechanism (15).

Immunoprecipitation experiments indicated 1 μM ouabain caused significant phosphorylation of the NHE1 polypeptide. The findings reinforce the argument that NHE1 is activated. There was no evidence for a change in ERK activation in ouabain-treated cells, and the ERK1/2 inhibitor PD-98059 failed to suppress ouabain-induced stimulation of pH recovery. The results make it unlikely that ERK and its downstream effector p90RSK contributed to the observed NHE1 activation in ouabain-treated cells, although this mechanism of NHE activation occurs in acidic rat ventricular myocytes (25). cAMP-dependent stimulation of NHE1 in astrocytes has not been widely reported. Support for the notion that the mecha-
Fig. 9. The effect of ouabain on calcium. A: in cells loaded with fura-2, cytoplasmic calcium concentration was measured continuously while the cells were exposed to 20 mM ammonium chloride for 5 min and then allowed to recover. Cytoplasmic calcium concentration and pH increased at the start of the ammonium chloride exposure, then calcium declined toward the baseline even though pH remained elevated. Throughout the course of the experiment, no significant difference was observed between cytoplasmic calcium concentration in control and ouabain-treated cells.

B: a stable baseline was obtained in a calcium-free buffer, and the cells were then exposed to 100 μM ATP to empty the stores. Subsequently, 2.5 mM calcium was added to the bathing medium to initiate capacitative calcium entry. C: capacitative calcium entry in cells exposed to 1 μM ouabain. D: ouabain-stimulated capacitative calcium entry was abolished by 2-APB (50 μM). E: means ± SE of results from 4 independent experiments. *** P < 0.001, significant difference from control.
nism involves an increase in cAMP comes from the finding that 8-Br-cAMP and ouabain each stimulated pH recovery to a similar degree but no further increase was observed when they were added together. Moreover, H-89, an inhibitor of cAMP-dependent protein kinase (PKA), abolished the effect of 1 μM ouabain on pH recovery. The findings are consistent with a previous report that the activity of rat NHE1 was stimulated by cAMP (31, 42). Not all NHE isoforms respond similarly. The activity of rat NHE3 is inhibited by cAMP (31, 52, 54). NHE1 is the isoform present in most cells (22) and is the predominant NHE isoform in rat astrocytes (6).

Neither DMA, H-89, nor 8-Br-cAMP altered baseline cytoplasmic pH. However, reduction of the rate of cytoplasmic pH recovery by DMA points to a significant level of NHE-mediated proton export. A reduction in the rate of pH recovery also was found in cells exposed to H-89 alone. This suggests that cAMP causes a basal degree of NHE stimulation. The fact that H-89 abolished the effect of ouabain on pH recovery constituted evidence that NHE1 plays an important role in regulating pH under conditions of intracellular acidosis. Such a role for NHE1 in acidosis has been proposed under pathologic conditions such as severe ischemia (5). It is interesting to note that in the ischemic heart, the concentration of endogenous ouabain increases several fold (19).

An increase in cAMP caused by ouabain is not a unique response of astrocytes. Such a cAMP rise has been reported in different tissues, including dog renal cortex (53), goldfish intestinal mucosa (2), mouse pancreatic islets (21), murine epithelioid and fibroblastic cell lines (34), rat brain (38), and rat renal collecting tubule cells in culture (28). In each of these reported studies, the concentration of ouabain was likely enough to inhibit Na-K-ATPase to a degree where cytoplasmic sodium increased. In such an instance, a rise in cAMP may be linked to the change in cytoplasmic sodium or to cell volume changes that may accompany the abnormal cytoplasmic ion composition. In the present study, however, an increase of cAMP was observed in astrocytes exposed to 1 μM ouabain, a concentration that did not change total cell sodium content determined by atomic absorption spectrophotometry and does not detectably alter cell-wide cytoplasmic sodium concentration in SBFI-loaded cells (24). The ability of both SKF-96365 and 2-APB to abolish the effect of ouabain on cAMP rise points to the involvement of capacitative calcium entry, which is stimulated by 1 μM ouabain. Capacitative calcium influx has been reported to be coupled with stimulation of a calcium-sensitive isoform of adenylate cyclase (type III) in bovine granulosa cells (12). Although changes of intracellular/extracellular pH can affect adenylate cyclase activity (43), cAMP was not
ouabain-like factors are found in a wide range of mammalian tissues and plasma, and there is growing appreciation for the notion that they can act as hormones (11, 46). A role for endogenous ouabain has been proposed in blood pressure regulation and sodium excretion (8, 46). In this respect, it is worth mentioning that ouabain-like factor has been detected in ocular tissues (35). In apparent contrast to our findings, there is a report that 100 μM ouabain failed to change the rate of pH recovery after ammonium chloride exposure in mouse astrocytes bathed in bicarbonate-containing buffer (16). Based on studies with low sodium solutions and amiloride, the authors suggested that in the presence of bicarbonate, NHE contributes minimally to pH recovery in mouse astrocytes. This differs from the present study on rat optic nerve astrocytes, where the major contribution of NHE is evidenced by ~50% inhibition of pH recovery with DMA.

Ouabain is a specific inhibitor of Na-K-ATPase, and there is no evidence to suggest a direct interaction of ouabain and NHE. On the other hand, NHE-mediated proton export uses the sodium gradient established by Na-K-ATPase, so ouabain would be expected to increase sodium in the cell and thus diminish NHE activity. Astrocytes express the Na-K-ATPase α2 isoform as well as the ubiquitous α1 isoform. The rat α1 isoform of Na-K-ATPase, which is responsible for regulating bulk cytosolic sodium, has a very low sensitivity to ouabain; the IC50 is 1,000-fold higher than α2 isoform (30, 40). Consequently, 1 μM ouabain is sufficient to inhibit the α2 isoform but not α1. Perhaps because α1 remains active, 1 μM ouabain did not cause a detectable change in cell sodium. The rise in cell sodium caused by concentrations of ouabain in the millimolar range was quite modest. This modest increase is not surprising given low plasma membrane sodium permeability and the fact that rat Na-K-ATPase α1 activity is not completely inhibited by millimolar ouabain concentrations (40). pH recovery after ammonium chloride removal was also stimulated in cells exposed to high (10 μM–1 mM) concentrations of ouabain even though cell sodium was increased. We suggest that the cAMP- and PKA-mediated stimulation of NHE activity was sufficient to overcome the inhibitory influence exerted by the modest increase in cell sodium. The fact that 1 μM and 1 mM ouabain have a similar stimulatory effect on pH recovery and on cellular cAMP content but a dissimilar effect on cell sodium suggests linkage of the ouabain-induced pH stimulation to cAMP.

Neural activity and factors such as anoxia have a tendency to cause changes of extracellular pH in the optic nerve (18, 44). Astrocytes use NHE for regulation of both extracellular pH and cytoplasmic pH (29). On this basis, altered NHE activity could impact the efficiency of pH homeostasis. Although ouabain-like factors are secreted from adrenal cortex (11, 23) and ischemia causes local release (19), the likelihood of endogenous cardiac glycosides attaining a micromolar concentration is remote. However, it is possible that NHE1 activity could be altered by other circumstances that alter Na-K-ATPase α2 activity, capacitative calcium entry, or cAMP. Both in vivo and in vitro studies of astrocytes have shown that an acidic cytoplasmic pH is cytotoxic (14).

In summary, we show that ouabain phosphorylates and stimulates NHE1 in cultured optic nerve astrocytes. The results are consistent with protein kinase A-dependent stimulation of NHE1 that occurs as the consequence of cAMP elevation.
linked to an increase of capacitative calcium entry in ouabain-treated cells.

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REFERENCES


