Sodium Pump Inhibition and Regional Expression of Sodium Pump α-Isoforms in Lens

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Abstract—Both hypertension and cataract formation have been associated with reductions in sodium pump activity, possibly as a result of an endogenous inhibitor. The objective of the present study was to answer 4 closely related questions: (1) Is the lens sodium pump effectively inhibited by a labile, digitalis-like factor we have identified in the peritoneal dialysate from hypertensive patients in end-stage renal failure? (2) How does that inhibition compare to that induced by ouabain? (3) Does sodium pump isoform distribution determine the degree of lens sodium pump inhibition? (This question was precipitated by the unanticipated finding that the labile DLF was more effective in inhibiting lens sodium pump than was anticipated.) (4) Is sodium pump activity altered in lens in response to increased salt intake, a maneuver known to increase endogenous digitalis-like factor? We found that whereas ouabain produced equivalent or significantly less inhibition of lens Na⁺,K⁺-ATPase from calf or rabbit, respectively, compared with brain, labile digitalis-like factor preferentially inhibited lens compared with brain. Analysis of whole-lens preparations from rabbit, calf, and normal human lens revealed substantial α2- and α3-isofoms of the sodium pump but little α1-isofom. Ouabain inhibition of whole-lens Na⁺,K⁺-ATPase from rabbit and calf were comparable: for rabbit lens, \( K_i = 5.2 \times 10^{-7} \) mol/L; for calf lens, \( K_i = 1.0 \times 10^{-6} \) mol/L. Limited quantities of labile digitalis-like factor prohibited similar determinations; however, its concentration-activity profile paralleled that of ouabain. Na⁺,K⁺-ATPase activity, measured in the 3 major anatomic regions of lens and normalized to nucleus, was greatest in epithelium (56.9±17.9) compared with cortex (5.8±1.4) and nucleus (1.0±0.0; \( P = 0.01 \)). Immunohistochemistry of rabbit lens found abundant α2- and α3-isofoms in epithelium and limited α3 but undetectable α1 in cortex and nucleus. Finally, rats randomized to a high Na diet showed significantly reduced lens Na⁺,K⁺-ATPase activity compared with those on a low Na diet, consistent with the effects of a sodium pump inhibitor. In conclusion, the present study suggests that digitalis-like factor may provide a link between hypertension and cataract formation. (Hypertension. 1999;34:1168-1174.)

Key Words: lens ■ sodium pump ■ sodium ■ potassium ■ ATPase ■ isofoms ■ cataracts

An association exists between hypertension and human cataract formation,1 and this same relationship occurs in some animal models of hypertension.2 Rodrigez-Sargent et al2 recently reported that development of cataracts in Dahl S rats in response to a high salt diet can be predicted by a reduction in ouabain-sensitive Na⁺,K⁺-ATPase activity several weeks before cataract formation. Likewise, a reduction in lens Na⁺,K⁺-ATPase (sodium pump) activity occurs in several other animal models of cataract2-5 and in human cataract formation.3,4-8 A similar reduction in sodium pump activity is also a common feature of hypertension and is thought to be mediated in part by a circulating sodium pump inhibitor9 called either digitalis-like factor (DLF) or ouabain-like factor. These observations led us to address a series of closely related questions. The first was whether the labile DLF isolated from the peritoneal dialysate of volume-expanded, hypertensive renal-failure patients, treated with that modality of dialysis,10 inhibited the sodium pump in lens and how this response compared with the inhibition produced by ouabain in this and a reference tissue. When labile DLF was applied to lens, we found it to have an unanticipated preferential effect on lens compared with brain, a difference not found with ouabain. This led us to question whether this differential inhibition could be accounted for by clear differences in sodium pump α-isofom distribution in these tissues. To date, 3 unique isoenzymes of the catalytically active α-subunits of the sodium pump have been conclusively identified, each coded by a different gene.11-13 A substantial amount of variation in sensitivity of the sodium pump in different tissues to cardiac glycosides such as ouabain can be accounted for on the basis of the isofoms present in that particular tissue. For example, it is now well recognized that the α1-isofom in rat kidney is highly “resistant” to ouabain, whereas other sodium pump isofoms present in rat brain, which has relatively equal amounts of all 3 isofoms, are substantially more sensitive to ouabain.11
Sodium pump α-isof orm in lens has previously been studied only in the rat, and those studies were limited to the epithelium. We recognized that differences in sodium pump sensitivity to labile DLF might be accounted for by isoform distribution within regions of the lens and might be coupled with in vivo sensitivity to ouabain and labile DLF. Consequently, we undertook a systematic assessment of the sodium pump α-isof orm presence and distribution in whole lens and within the major structural regions of lens. We performed immunohistochemistry to determine unambiguously the location of the various sodium pump isoforms in rabbit lens. Because of potential species differences, we performed several of our studies in lens obtained from rabbit, calf, and, where possible, normal humans. Finally, we also raised the question of whether increased sodium intake, a maneuver known to increase levels of the endogenous sodium pump inhibitor, could lead to changes in lens Na⁺,K⁺-ATPase activity in vivo. The results are reported in the present article.

Methods

Lens Preparation

Bovine and rabbit lenses were frozen within one half hour of euthanatization and stored at −70°C (for prolonged periods) or −20°C (for short periods) until use. Rabbits were housed and used locally in compliance with the guidelines of the Committee on Animals of the Harvard Medical School. Bovine lenses were obtained from a local abattoir. Normal human lens was obtained from the New England Eye and Tissue Bank (Boston, Mass) as postmortem research materials representing multiple individuals. Whole-lens or individually dissected anatomic regions of lens were processed into microsomal Na⁺,K⁺-ATPase preparations. Animal lenses were removed atraumatically from the enucleated globe and homogenized with several strokes of a glass-Teflon homogenizer in buffer containing (in mmol/L): sucrose 250, histidine 30, and EDTA 1; pH 7.2). The homogenate was pelleted by centrifugation at 10000 × g for 30 minutes and then resuspended in the same buffer (0.7 mL for each set of 3 isoforms). Western blot analysis. Equivalent regional preparations of rabbit lens were also prepared, and Na⁺,K⁺-ATPase activity was measured for each of the 3 layers of lens tissue as described above. Participation occurred after subjects had given informed signed consent. Peritoneal dialyseate (≈2 L) was taken during the period of volume expansion for each subject was substantially purified by a previously published method that involved ultrafiltration through a 1000-Da exclusion membrane and 3 high-performance liquid chromatographic steps. Similar volumes of dialyseate purified by this protocol have only 1 active component and have yields in the 0.1- to 0.5-ng range. The factor that correlated with volume, blood pressure, and plasma DLF was the one used in these experiments; however, this factor is chymically labile and when purified has a half-life of ≈10 hours. Because of this lability, specimens could not be pooled; 1 preparation of labile DLF was divided and used the day it was processed against the tissues studied. Quantities were insufficient to both measure concentration and also study tissue inhibition; however, previously, the labile DLF was shown to be ∼50 times more potent than ouabain against rabbit kidney (a non–ouabain resistant source for α1) and ∼1000 times more potent than ouabain against rabbit vascular smooth muscle (predominantly α2). This factor has been extensively characterized in previous publications that show that it acts on the Na⁺,K⁺-ATPase in a manner analogous to known cardiac glycosides.

Measurement of Na⁺,K⁺-ATPase Activity

The activity of Na⁺,K⁺-ATPase in animal lens cell membrane preparations was determined by measuring hydrolysis of ATP. Lens membrane preparation (20 μL) was incubated for 3 hours in a buffer containing (in mmol/L) Na 100, K 5, Mg 3, EGTA 1, and Tris 80; pH 7.5, 3°C in the absence or presence of ouabain in graded concentrations. The reaction was started by adding 10 μL of 40 mmol/L [γ-32P]ATP (Amersham) and was ended after 90 minutes by addition of 40 μL charcoal in 0.1 mmol/L HCl solution. Na⁺,K⁺-ATPase activity was defined as that portion of ATPase activity inhibited by 10 mmol/L ouabain. For comparison, microsomal Na⁺,K⁺-ATPase from rabbit brain was similarly prepared and ouabain and labile DLF were also applied to it.

Comparison of the Effects of Labile DLF and Ouabain on Lens and Brain

Ouabain (10⁻⁶ mol/L) was applied to microsomal preparations of lens Na⁺,K⁺-ATPase from both calf and rabbit and also rat fetal brain Na⁺,K⁺-ATPase, and inhibition was measured. A single pool of labile DLF was simultaneously applied to the same preparation of Na⁺,K⁺-ATPase microsomes.

Western Blot Analysis

Equal amounts of individual lens membrane preparations from all 3 species (30 μL) were simultaneously run on 7% SDS-PAGE and transferred to nitrocellulose membrane. For immunodetection of the isoforms of the sodium pump, the monoclonal antibodies McK1 (specific for α1-isof orm; a gift from Dr Kathleen J. Sweadner, Massachusetts General Hospital, Harvard Medical School, Boston), McB2 (specific for α2-isof orm; also a gift from Dr Sweadner), and MA3-915 (specific for α3-isof orm; Affinity BioReagents Inc) were used to bind the individual isoforms. A second antibody coupled to an enzyme that converts inactive substrate into chemiluminescent product allowed for detection (enzyme-linked chemiluminescence; Amersham) and was used to register the α-isof orm;first-antibody complex. This procedure was performed according to manufacturer’s protocol. Each gel was probed only once for a specific α-isof orm (ie, we did not reprobe gels), but the same lens preparations were used for each set of 3 isoforms.

Measurement of α-Isof orm Distribution and Activity in Regions of Rabbit Lens

To remove rabbit lens with the capsule intact, the posterior of the rabbit eyeball was dissected open and the suspensory ligaments of the lens were cut with fine scissors. The intact lens was then carefully removed and placed in sterile normal saline solution at room temperature. Individual lenses were divided into 3 segments as follows: (1) the superficial (thin) layer, peeled off gently with fine forcesps, which contains the capsule and epithelium (and also a few fiber cells, because the lens epithelium lies beneath and is tightly attached to the anterior and equatorial capsule); (2) the median layer, which represents the soft portion of lens adjoining the superficial layer containing the cortex and some nucleus; and (3) the center layer, which represents the residual hard, sticky core and comprises most of the nucleus of the lens. Each layer was individually homogenized and centrifuged as described above. The expression of isoforms in these fractions of rabbit lens was evaluated by Western immunoblot analysis. Equivalent regional preparations of rabbit lens were also prepared, and Na⁺,K⁺-ATPase activity was measured for each of the 3 layers of lens tissue as described above.

Immunohistochemical Detection of the Individual α-Subunit of the Sodium Pump

Immunohistochemistry was performed following a published method, with some modifications. Briefly, cryostat-produced thin (7-μm) sections of rabbit lens were mounted on glass slides and fixed.
with 4% formaldehyde for 5 minutes. The sections were pretreated with protein block serum-free solution (Dako Corp) for 30 minutes at room temperature and then incubated with primary monoclonal α-isoform antibody McK1, McB2, or MA3-915 overnight in the same blocking solution in a humidified chamber at 4°C. Next, sections were incubated for 1 hour with a peroxidase-coupled anti-mouse IgG (Amersham) after these sections were washed with PBS containing (in mmol/L) NaCl 145 and Na₂HPO₄/NaH₂PO₄ 10; pH 7.4). Finally, peroxidase staining was developed for 20 minutes by use of AEC reagent (Dako AEC substrate system, Dako Corp). The slides were subsequently mounted with coverslips, and the specimens were examined by light microscopy. Photomicrographs were taken (microscope, Carl Zeiss). Frozen sections were used the same day as prepared or on the next day, after storage at −70°C. Longer storage periods were found to reduce the staining.

Effects of Dietary Sodium Intake on Rat Lens Na⁺,K⁺-ATPase Activity

Adult Sprague-Dawley rats were randomized to either a high (1.6% NaCl) or low (0.04% NaCl) salt diet for 5 to 7 days. The animals were euthanatized. The lens was then rapidly excised and homogenized in cold homogenization buffer (see above) using 3 strokes of a glass-Teflon homogenizer and centrifuged at 15 000g for 30 minutes at 4°C. The protein pellet was resuspended in 140 mM assay buffer and a 20 µL aliquot was placed in each assay tube. After a 30-minute equilibration at 37°C, the reaction was initiated by adding 10 µL of 40-mmol/L ³²P-ATP, allowed to run for 60 minutes, and ended as described above. Ouabain 10⁻³ mol/L was added to other tubes to determine ouabain-sensitive Na⁺,K⁺-ATPase activity, expressed in micromoles of ATP hydrolyzed per hour per lens.

Statistics

Data are expressed as mean±SE. Comparisons of responses of 2 matched aliquots of inhibitor were performed by paired, 2-tailed Student’s t test. The effects of 2 different sodium diets were analyzed statistically by unpaired, 2-tailed Student’s t test. Comparisons among the 3 regions of lens were performed by ANOVA with post hoc Newman-Keul’s analysis. A value of P<0.05 was considered statistically significant.

Results

Effects of Ouabain and Labile DLF on Preparations of Lens and Brain

Ouabain at 1×10⁻⁴ mol/L was presented to microsomal preparations of calf and rabbit lens and also to rat fetal brain Na⁺,K⁺-ATPase. Against calf lens, ouabain produced somewhat greater but statistically insignificant inhibition than against brain (lens, n=6, 60.8±12.9% versus brain, n=6, 46.6±7.0% inhibition; P=0.42; Figure 1A). Against rabbit brain lens, ouabain caused significantly less inhibition than against brain (lens, n=7, 42.4±2.4% versus brain, n=6, 55.8±2.6% inhibition; P=0.01; Figure 1B). In contrast, when equal aliquots of individual preparations of the labile DLF were applied to these same microsomal Na⁺,K⁺-ATPase preparations, the labile DLF produced significantly greater inhibition of calf lens than of brain Na⁺,K⁺-ATPase activity (lens, n=6, 82.2±9.5% versus brain, n=6, 37.5±4.3% inhibition; P=0.01). Also, in contrast to ouabain, when equal aliquots of individual preparations of the labile DLF were applied to rabbit lens Na⁺,K⁺-ATPase, there was also greater, but not statistically significant, inhibition compared with brain Na⁺,K⁺-ATPase activity (lens, n=7, 55.4±7.8% versus brain, n=7, 42.8±6.5% inhibition; P=0.11). However, for both calf and rabbit, the differences between lens and brain were greater for DLF than for ouabain whether against calf (DLF, 44.6±11.0% versus ouabain, 12.9±14.7%; P=0.047) or rabbit lens (DLF, 11.2±6.4% versus ouabain, −11.2±3.0; P=0.025). These results might be explained by tissue-specific distribution of sodium pump α-isoforms.

Na⁺,K⁺-ATPase α-Subunit Expression in Lens

The distribution of Na⁺,K⁺-ATPase α-isoforms was examined by immunoblot analysis using monoclonal antibodies specific for the α₁-, α₂-, or α₃-isoform of the sodium pump after PAGE protein separation. Representative Western blots are shown in Figure 2. Lens from each of the 3 species studied showed all 3 α-isoforms of the sodium pump. The intensity of the blot is dependent on both the density of sodium pump units and on the affinity of the antibody for each isoform in the lens of each of these 3 species. Nevertheless, abundant α₃- and α₂-signal was seen but only a faint immunoreactive band was seen for α₁. This pattern was similar in rabbit, calf, and human lens.
Ouabain-Induced Inhibition of Na\(^{+}\),K\(^{+}\)-ATPase Activity

The concentration-dependent inhibition of Na\(^{+}\),K\(^{+}\)-ATPase by ouabain in lens preparations from rabbit and calf was examined. Na\(^{+}\),K\(^{+}\)-ATPase activity decreased with increased ouabain concentration, with half-maximal inhibition achieved in rabbit (n=4; 5.2×10\(^{-7}\) mol/L) or calf lens (n=4; 1.0×10\(^{-6}\) mol/L) at similar concentrations (data not shown). Although no significant difference was seen in the affinity of Na\(^{+}\),K\(^{+}\)-ATPase for ouabain between calf and rabbit lens, the absolute level of enzyme activity in rabbit lens was significantly higher than in calf lens (0.50±0.07 versus 0.20±0.02 mmol inorganic phosphate per lens per hour; P<0.02).

Distribution of α-Isomers in Rabbit Lens

The α-isomers were assessed in rabbit lens by use of the same 3 regions of lens. Of these, only 1 region had detectable expression of all 3 isoforms, and this area was the surface layer or epithelium. As shown in Figure 4, the α2-isoform was predominantly, if not exclusively, found in lens epithelium. Likewise, the α1-isoform was also detectable only in epithelium, although its presence was faint. In contrast to α1 and α2, the α3-isoform was present in all 3 layers of the rabbit lens but with a reduced density in the cortex and with other layers (56.9±17.0 for the superficial layer, 5.8±1.4 for the medial layer, and 1.0±0.0 for the central layer; P=0.01). The median layer also had somewhat greater activity than the core (P=0.07; Figure 3).

Regional Differences in Na\(^{+}\),K\(^{+}\)-ATPase Activity in Rabbit Lens

When Na\(^{+}\),K\(^{+}\)-ATPase activity was determined by region in the rabbit lens, marked differences were found. Three regions were examined: the superficial layer containing primarily lens epithelium, a median layer containing cortex and supranucleus, and a central layer containing the nucleus. Na\(^{+}\),K\(^{+}\)-ATPase activity measured in each region (expressed per mg tissue) was normalized to the nuclear Na\(^{+}\),K\(^{+}\)-ATPase activity measured in the same lens. Sodium pump activity was significantly higher in the superficial layer than in the

![Figure 2. Western blot analysis of lens cell plasma membranes using monoclonal antibodies against specific α-isomers of Na\(^{+}\),K\(^{+}\)-ATPase.](http://hyper.ahajournals.org/)

![Figure 3. Comparison of Na\(^{+}\),K\(^{+}\)-ATPase activity in different regions of rabbit lens.](http://hyper.ahajournals.org/)

![Figure 4. Western blot analysis of rabbit lens fractions using monoclonal antibodies against each of the 3 α-isomers of Na\(^{+}\),K\(^{+}\)-ATPase.](http://hyper.ahajournals.org/)
Surface when thin sections were probed for A distinct pattern of intense staining was seen along the lens lens was accomplished by immunohistochemistry (Figure 5). to the lens epithelium, consistent with data obtained by Western blot analysis. Although high densities of the isoform were found in the epithelial layer, it was also present in the superficial layer and different nonspecific bands in the gel.

Immunohistochemistry of the α-Subunits Within Rabbit Lens
Precise localization of the α-isoforms throughout the rabbit lens was accomplished by immunohistochemistry (Figure 5). A distinct pattern of intense staining was seen along the lens surface when thin sections were probed for α2 and α3. However, α1 was not detected. Staining for α2 was confined to the lens epithelium, consistent with data obtained by Western blot analysis. Although high densities of the α3 isofrom were found in the epithelial layer, it was also present in the cortex and nucleus in a diminished, diffuse, uneven distribution (data not shown). Lens incubated with secondary antibody alone revealed no staining (Figure 5).

Effect of Sodium Diet on Na⁺,K⁺-ATPase Activity in Lens
Microsomal preparations of whole lens were rapidly prepared from rats randomized to 5 to 7 days of a high salt (n=5) or low salt (n=4) diet. Those that received the high salt diet showed reductions in both total and ouabain-sensitive Na⁺,K⁺-ATPase activity compared with lens preparations from animals that received a low salt diet (total ATPase activity, high salt 0.97±0.16 versus low salt 1.50±0.11 μmol/h per lens; P=0.03; Na⁺,K⁺-ATPase activity, high salt 0.53±0.10 versus low salt 0.79±0.04 μmol/h per lens; P<0.05; Figure 6). No difference occurred in the degree of further reductions in the nucleus. The pattern observed was easily reproducible. To ensure that the α2-isofrom found in the superficial layer was not from ciliary contamination, a preparation of the ciliary from the same lens was included on the same gel and blotted. This showed a much weaker signal for α2 than that seen in the superficial layer and different nspecifc bands in the gel. 

Discussion
Studies have linked hypertension and cataract formation and both vascular smooth muscle from hypertensive animals and cataractous lens have been characterized by decreased Na⁺,K⁺-ATPase activity and increased cell Na content, which may be pathogenic. The mechanisms for this reduction in Na⁺,K⁺-ATPase activity are not completely understood but could result from an endogenous inhibitor or reductions in functional sodium pump units. We have recently isolated a labile, endogenous Na⁺,K⁺-ATPase inhibitor from the peritoneal dialysate of hypertensive renal failure patients. This factor inhibits the sodium pump in a manner comparable to all known cardiac glycosides and causes vascular smooth muscle contraction but is active at much lower concentrations. Because such an inhibitor might link and mediate hypertension and cataract formation, it was important to establish that this labile DLF was active against lens Na⁺,K⁺-ATPase. As anticipated, labile DLF was an effective inhibitor of the lens sodium pump in calf and rabbit. Moreover, this inhibition was concentration dependent and had a concentration-response profile entirely comparable in pattern to that found with ouabain. Because of the intrinsic chemical instability of the labile DLF, we were unable to accumulate enough material to do the experiments described here and also determine concentrations. Previous studies have demonstrated this factor to be ~30 to 50 times more potent than ouabain against rabbit or dog kidney Na⁺,K⁺-ATPase (α1) and ~1000 times more potent than ouabain against rabbit vascular smooth muscle Na⁺,K⁺-ATPase (predominantly α2); these studies have suggested that this is caused by DLF having a preferential effect on the α2 isofrom.
Comparisons were made of ouabain-induced, sodium pump inhibition in lens versus in rat fetal brain. We used this latter tissue as a reference because it is well characterized in terms of its sodium pump isoform distribution.11,12 The brain sodium pump may also have a role in hypertension, in that it appears to have sodium-regulated increases of a “ouabain-like” factor that may contribute to hypertension in Dahl S rats.21 We found some species differences in the response of ouabain. In calf, ouabain produced a small but insignificantly greater inhibition of lens over brain, but in rabbit, ouabain actually produced significantly less inhibition of lens than brain. The labile DLF against lens from either species showed greater inhibition of lens versus brain. This was statistically significant for calf and nearly significant for rabbit. Interestingly, for either species, DLF produced significantly greater inhibition of lens than did ouabain. Given that the concentration of the labile DLF used against each tissue was identical for 1 set of experiments and that DLF produced less inhibition of brain than ouabain, these results suggest that the lens is more sensitive to labile DLF than to ouabain.

We anticipated that these findings could be explained by differences in the sodium pump α-isoform distribution among lens and brain in the species studied. We used lens from several species in these studies primarily because of the difficulty in obtaining normal human lens. The isoform distribution found for human lens was similar in pattern to that of calf and rabbit (it had abundant α2 and α3). Within the lens, the density of sodium pumps was greatest on the epithelial surface, as determined by studies of protein expression, which showed that α2 and α1 were found exclusively in the epithelial layer, whereas α3 was found in all 3 layers but was diminished in internal regions. More precise localization of the α-isoforms in rabbit lens by immunohistologic techniques confirmed these data and again revealed that the α2-isoform was found only in the epithelial layer. With this technique, the α1-isoform was not detected. The α3-isoform was distributed unevenly throughout the lens, with its highest density also in lens epithelium. These results would predict that the labile DLF would be a potent inhibitor of human lens Na⁺,K⁺-ATPase activity.

The species differences for either ouabain or the labile DLF were not obviously related to α-isoform distribution (based on Western analyses) but more likely reflect the greater absolute Na⁺,K⁺-ATPase activity found in rabbit; i.e., a smaller proportion of pumps in rabbit lens will be inhibited for a given concentration of inhibitor. The differences in the effect of the labile DLF compared with ouabain on lens versus brain might reflect differences in isoform distribution combined with different affinities of isoform for each of the inhibitors. However, on the basis of distributions we found, one might have predicted that the labile DLF, which is not resistant to rat α1, would be more effective than ouabain against rat fetal brain, which contains ~30% α1 (which is ouabain resistant). The opposite was found. If the lenses were predominantly α2, this might have explained the preferential effect of labile DLF (which has been previously shown to preferentially inhibit α2) on lens over brain. However, the lens obviously has substantial α3. Unfortunately, Western blot analysis is not quantitative between species; no method currently exists to measure the concentration of individual α-isofoms, and the individual isoforms have resisted purification for affinity studies. Hence, our data do not allow us to determine the absolute quantities of the isoforms in rat as opposed to calf or rabbit tissue nor describe differences among species in α-subunit affinity for ouabain and labile DLF nor define the contribution of isoform distribution to the difference.

Our results are generally consistent with those of others,3,4,8 which have considered the location of Na⁺,K⁺-ATPase activity (but not isoforms) in human and rat lens. In human lens, ATPase reaction products were found exclusively in the epithelium.3,8 Kobatashi et al6 measured the activity of Na⁺,K⁺-ATPase in the 3 regions of normal human lens and found a third of the total activity in the epithelial monolayer, half in the cortical fibers, and the remainder in the nucleus. Our findings are also similar to those of more recent studies that have begun to explore sodium pump isoforms in lens. A study of rat lens epithelium demonstrated the presence of the 3 isoforms in the epithelium.14 Another group performed both Northern and Western analyses on rat lens epithelium and found all 3 α-isofoms, with α1 staining most intensely.15 This latter finding differs from our results and may reflect species or antibody affinity differences.

Our results may have implications for lens transparency given that it is generally accepted that Na⁺,K⁺-ATPase in the lens epithelial monolayer provides the active transport of monovalent cations for the whole lens.6 The pattern of sodium pump localization within the lens suggests that the α isoforms may be specifically regulated and have somewhat specialized functions. Recent work in other tissues provides evidence for this.26 In addition to the effects of sodium pump inhibitors on activity, the sodium pump can be transcriptionally regulated by a number of hormones.27 Additionally, other hormones can acutely affect the activity of sodium pump isoforms by initiating intracellular events that modify the phosphorylation state of the enzyme.27 Hence, any factor that reduces sodium pump activity, especially those that preferentially inhibit the α2- or α3-isoforms, may be particularly effective in promoting lens opacification, and evidence exists for such a role in cataract formation.3,6–8 Studies of human senescent cataract lens compared with age-matched normal lens demonstrated significant reductions of epithelial, cortical, and nuclear Na⁺,K⁺-ATPase activity.6 Other studies have suggested that reductions in Na⁺,K⁺-ATPase activity in cataractous lens may result from endogenous inhibitors based on their increased presence in cataractous lens, although these inhibitors appear to differ from labile DLF.28 Inhibition of the sodium pump by ouabain leads to lens opacification in parallel with reductions in sodium pump activity.19,20,28 Levels of sodium pump inhibitors increase in parallel with volume in hypertension.10 The increased incidence of cataracts in hypertensive patients raises the possibility that both disorders arise from a common mechanism. Rodriguez-Sargent et al2 found that cataract development in Dahl salt-sensitive rats was related to the hypertensive process. We also found lens Na⁺,K⁺-ATPase activity to be sensitive to dietary sodium intake. Although a sodium pump inhibitor could account for such findings, this has yet to be proven.
However, such reductions in lens sodium pump activity would predispose to pathology.

In conclusion, labile DLF is an effective inhibitor of lens sodium pump activity. Indeed, this factor appears to have substantially greater effect on lens compared with brain. Given the common finding of altered sodium pump activity in cataractous lens and the reductions in lens Na$^+$/K$^+$-ATPase activity in response to high sodium intake reported in the present study, it is possible that changes in isoform distribution, number, or susceptibility to factors that modulate sodium pump activity, including labile DLF as an effective lens inhibitor, may lead to lens opacification. Detailed studies of α-isoforms in conjunction with the labile DLF are now needed in cataractous lens.

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References

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