Sodium-23 magnetic resonance imaging of the eye and lens

(ion gradient/transport/pump-leak mechanism/sodium-shift reagent/intracellular/extracellular sodium concentrations)

WILLIAM H. GARNER†, SADEK K. HILAL‡, SANG-WOOK LEE‡, AND ABRAHAM SPECTOR*†

Departments of 1Ophthalmology and 1Radiology and 1Laboratory of Biochemistry and Molecular Biology, College of Physicians and Surgeons, Columbia University, 630 West 168th Street, New York, NY 10032

Communicated by Zacharias Dische, November 1, 1985

ABSTRACT In order to develop a better understanding of cataract and to evaluate the effectiveness of potential drugs, noninvasive techniques must be devised to detect early metabolic changes. As a prelude to these goals, sodium-23 imaging experiments operating at 29.8 MHz (2.7 teslas) were performed on the bovine eye and lens. A spatially localized transverse relaxation time (T2)-weighted spin-density map of the sodium-23 within the lens is presented, with a resolution better than 250 μm. Due to the presence of short-T2 (3 msec) components within the lens, only the use of the planar-integral projection reconstruction (PPR) imaging scheme allowed sufficiently short echo-times (1 msec) to permit sodium-23 signal detection. These noninvasive imaging results show differences in the apparent sodium concentration within the lens that are consistent with separate, invasive measurements of sodium concentration. Separate analysis (with no spatial localization) at 79.4 MHz (7.2 teslas), using a shift reagent (dysprosium) to distinguish extracellular from intracellular sodium, indicates that ~62% of the detected sodium-23 signal is intracellular. These results are consistent with observations based on invasive measurements and further support the existence of the pump-leak system and a sodium gradient within the lens.

As with many disease processes, the formation of cataract can be correlated frequently with increasing concentration of sodium (Na+) and cell hydration. In the normal transparent lens, intracellular cation concentrations (Na+, 25 mM; K+, 130 mM) are actively maintained against concentrations (Na+, 140 mM; K+, 4 mM) found in the extracellular fluids surrounding the lens. This critical ion balance is regulated by the Na,K-ATPase located mainly in the lens epithelium (1-7). Therefore, the overall intracellular lens [Na+]i is controlled primarily by a single anterior epithelial cell layer and a free communication between the lens cells by gap junctions. The structure of the normal lens (represented schematically in Fig. 1) and the distribution of Na,K-ATPase would suggest that an internal gradient of [Na+]i may exist between the anterior and posterior regions of the lens, with lower [Na+]i in the anterior region. Such a model of a lens cation pump-leak mechanism was originally proposed by Kinsey and Reddy (1).

Although the final effects of cataract are related to aggregation of the lens proteins, metabolic changes may precede lens opacification allowing sufficient time for effective therapeutic measures to be taken. Disruption in the normal Na+ gradient may precede visual opacities. The [Na+] increases dramatically in many human senile-cataract lenses (8-10). Amino acid transport and protein synthesis can also be linked to the [Na+] gradient (11-15). It seems apparent from a number of investigations that any substance that can increase Na+ permeability (e.g., H2O2) is likely to be cataractogenic.

FIG. 1. Schematic representation of the pump-leak system in the lens as visualized by Kinsey and Reddy (1). The epithelial cell layer is shown on the anterior (Ant.) side containing the active pump, the Na,K-ATPase. The leak (or passive) component is depicted on the posterior (Post.) surface.

(16). Therefore, the [Na+] in the lens may be a sensitive parameter of lens cellular dysfunction.

Magnetic resonance imaging techniques have been developed to spatially localize specific nuclei in soft tissue (17-20). These images provide evidence of both the functional and the physiological state of internal organs. Applications of magnetic resonance imaging techniques with proton maps of the eye and lens have been reported (19, 20), but the sodium-23 distribution in the eye has not been reported previously. As a prelude to detecting, noninvasively, changes in lens sodium with human cataract development, imaging techniques are described here to spatially map the sodium distribution in the lens.

MATERIALS AND METHODS

Bovine Eyes and Lenses. Fresh bovine eyes were obtained within 4 hr of death from an abattoir (donated by Great American Veal, Newark, NJ). Lenses (1.5 g wet weight) were removed and cultured for 2 hr in a TC199 medium at 37°C under 5% CO2 (21). In order to lower the extracellular sodium concentration ([Na+]o), the lens was equilibrated with a Tyrode's medium (containing ~5 mM Na+) by perfusion at a flow rate of 30 ml/hr for 3 hr (22). The osmolarity in the Tyrode's buffer was maintained at 290 mosM by substituting choline chloride for sodium. Subsequent to imaging experiments, invasive measurements with Na+-specific microcombinator electrode (MI-425, Microelectrodes, Londonderry, NH) were used to measure total [Na+] in the buffers and

Abbreviations: PPR, planar-integral projection reconstruction; [Na+], and [Na+]i, intra- and extracellular Na+ concentration; TE, echo-time; T2, transverse relaxation time.

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lenses to correlate with the images and spectra obtained. The lens value of \([Na^+]\) represents the sum of both \([Na^+]_{\text{a}}\) and \([Na^+]_{\text{e}}\).

**Magnetic Resonance Imaging Measurements of Sodium-23.**

The electronics and instrumentation for the magnetic resonance imaging setup have been described (23). Modifications with higher power gradient coil driving amplifiers were needed for fast rising pulses to decrease echo-time \((T_2)\). A solenoidal radiofrequency coil (9-turn, 24-mm i.d.) (29.764 MHz) design was used with the \(B_1\) field aligned perpendicular to the static magnetic \((B_0)\) field (2.7 teslas). Two Lucite cylindrical flow chambers were designed to minimize the cross-sectional area. A 35-mm cell and an 18-mm cell for the enucleated eye and lens, respectively, were used to optimize the filling factor and to maximize volume element (voxel) resolution. The transverse relaxation \((T_2)\) times were measured according to the procedure of Carr and Purcell (24).

**NMR Sodium-23 Measurements.** NMR analysis without spatial localization of the sodium-23 nuclei was performed in a fashion similar to that described by Pettigrew et al. (25), on a Bruker (Billerica, MA) 300WM wide-bore spectrometer using a broad-band 20-mm probe operating at 79.384 MHz for sodium-23. The experimental parameters used were a 90° pulse (110 \(\mu\)sec), an acquisition time of 0.41 sec, 8192 data points per free induction decay, 400 acquisitions, and a sweep width of \(\pm 5000\) Hz. A solution of 0.1 M NaCl in deionized water containing 50% \(^{2}H_{2}O\) (for the deuterium "lock") was used as the external chemical-shift reference (0.0 ppm). Approximately 3 hr prior to the experiment, the dysprosium-tripolyphosphate complex (1:2) was added (8.2 mM final concentration) to the lens contained in 10 ml of the low-sodium Tyrode's buffer. Vortex plugs were used to minimize buffer volume.

**RESULTS AND DISCUSSION**

Although the sodium-23 nucleus is less sensitive to NMR detection than is the proton, the quadrupolar relaxation mechanism shortens the sodium longitudinal \((T_1)\) and transverse \((T_2)\) relaxation times (where \(T_1 \approx T_2)\) to allow more data to be collected per unit time (26–29). The sodium-23 image of the bovine eye is shown in Fig. 2A. The bovine eye is composed of a number of physical compartments that can be seen readily from the two-dimensional sodium-23 image (30). Both the anterior and posterior aqueous and the vitreous chambers contain \(\approx 140\) mM sodium and appear as light regions in Fig. 2. The sclera, cornea, zonulur fibers, ciliary body, and lens, however, appear as dark regions in Fig. 2, since they contain sodium at low concentration.

The observed spin-density map (Fig. 2) is not, however, strictly a function of sodium-23 nuclei concentration per se. The final image observed is sensitive to a number of parameters. The echo formation, from which the final image is ultimately made, depends upon \(T_2\), or the exponential decay rate of the signal. Thus, the quadrupole relaxation can impose a serious restriction on signal observation if the \(T_2\) is very short. The two-dimensional image shown in Fig. 2 was obtained with a \(T_E\) of 30 msec. The lens appears as a dark region with no apparent sodium (invasively measured lens \([Na^+] = 29\) mM). Bovine lens \(T_2\) measurements (Fig. 3) indicate that \(\approx 34\%\) of the total observed sodium signal yielded a \(T_2\) value of 26.5 msec, and \(66\%\), a \(T_2\) value of 2.9 msec. The former lens \(T_2\) value is similar to the \(T_2\) value of 28.2 \(\pm 1.6\) msec previously measured for the bovine lens (31). The \(T_2\) value of 14 \(\pm 3\) msec measured for sodium-23 in the erythrocyte (25) is intermediate between the shorter and the longer lens \(T_2\) values. The shorter, 3-msec \(T_2\) value reported here for the lens is closer to the \(T_2\) value \(< 1\) msec) of Chelex-100-bound sodium (25). If the sodium-exchange rate were further shortened in a bound state in a given region, the \(T_2\) value would be further decreased. The term bound is used here to designate partial or total immobilization of sodium. However, Paterson (32) reported that only 15% of the total lens sodium does not readily exchange and could therefore be termed bound.

With a \(T_E\) of 30 msec and a major \(T_2\) component of 3 msec, the lens sodium-23 signal would be only 14% based on the sodium-23 signal decay (neglecting field inhomogeneity factors). It is therefore apparent that the \(T_E\) must be short in order to observe lens sodium-23. Typically, any Fourier imaging technique requires a spatial encoding gradient period. The use of the limited angular view planar-integral

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**Fig. 2.** (A) Two-dimensional Fourier sodium-23 magnetic resonance image of the enucleated bovine eye, at an operating field strength of 2.7 teslas. The image was obtained with the Carr–Purcell–Meiboom–Gill pulse sequence (42). We used a 90° selective radiofrequency pulse (8.0 msec) with a \(G_x\) selection gradient \((\approx 0.5\) cm), followed by a gradient encoding period \((G_z)\) of 7.8 msec and a 0.3-msec 180° nonselective pulse. The echo formation \((T_E)\) was at 30 msec. The data-acquisition period was 20.46 msec \((80\mu\text{sec} \times 256\text{ samples})\) with a repetition rate of 110 msec. (B) Diagram showing the corresponding anatomical eye compartments.
projection reconstruction (PPR) method is considered to be the best-suited for the imaging of short-$T_2$ sodium (33–35), since neither gradient-encoding nor slice-selection processing is involved. A representative example of the PPR lens-imaging experiments ($T_E = 1$ msec) is shown in Fig. 4A with an external [Na$^+$] of 2 mM. Visual examination of this image suggests the existence of a gradient of sodium in the lens similar to that expected for the pump–leak-type model depicted in Fig. 1. There is some inherent degree of image blurring and loss of boundary (anterior and posterior) definition due to the low signal-to-noise ratio and data reprocessing. Computer analysis of the pixel (picture element) intensity (Fig. 4B) along the mid-sector of the image (shown by the line in Fig. 4A) shows the quantitative change in the sodium gradient.

There appears to be an overall good correlation between the invasively measured [Na$^+$] and the NMR sodium-23 image pixel-intensity level. Invasive sodium measurements indicated that the superficial anterior cortex [Na$^+$] = 22.6 mM, whereas the posterior region [Na$^+$] = 27.0 mM. The lens image suggests that the central nuclear region has the lowest sodium-23 signal. The corresponding inner or nuclear region invasive sodium measurement is ~18 mM. These [Na$^+$] values correspond to previously reported results. Amoore et al. (36) reported a concentric sodium gradient within the calf lens with lower [Na$^+$] in the nuclear region (inner zone) compared to the anterior/posterior cortical region (outer zone), with values ranging from 11.0 to 15.2 mM and from 18.3 to 32.0 mM, respectively, for these two compartments. Paterson (37) examined the [Na$^+$] across five sections of the lens from anterior to posterior with the following results: 14.45, 16.28, 15.00, 21.48, and 25.41 mM, respectively. The histogram plot of these data (37) shows a similar trend to the pixel plot shown in Fig. 4B.

The question arises as to the contribution of the separate intracellular and extracellular compartments to the observed lens sodium-23 image in Fig. 4A. Paterson (32) reported that the lens tissue extracellular space is between 4% and 9%. These values exclude the contribution of the capsular region, since in our case no sodium-23 was detected in this region (shown in Fig. 4), apparently due to the fast exchange with the low-sodium buffer. (However, sodium in the remainder of the lens changes slowly, as determined by invasive sodium measurements.) Assuming [Na$^{++}$] and [Na$^{+}$] to be 140 mM and 20 mM, respectively, and 0.65 as the fraction of the lens that is water, the expected percentage of intracellular sodium would be between 77.4% and 59.1% per lens. In order to distinguish experimentally with NMR the distribution of the observed sodium-23 signal in Fig. 4A between the intra- and extracellular compartments within the lens, separate nonspatially localized NMR experiments were performed using a membrane-impermeable sodium-shift complex, dysprosium–tripolyphosphate. Similar to the resonance positions previously observed for human erythrocytes in the presence of the shift complex (25), intracellular lens sodium was observed at ~0.097 ppm while extracellular sodium.
contributed by the lens and the buffer was observed at \(-5.645\) ppm (Fig. 5, spectrum B), with relative integrated areas of 0.55 and 0.45, respectively. Without the shift reagent (spectrum A), the intracellular and extracellular compartments overlap near \(-0.26\) ppm with a line width of 55 Hz. The negative 25% peak area in the difference spectrum C (spectrum B minus buffer blank) suggests that 75% of the area in spectrum B is contributed by the extracellular lens sodium. Therefore, using these values, it can be calculated that \(62\%\) (within the expected values described above) of the total lens sodium-23 signal measured by NMR is contributed by \([\text{Na}^+]\). These calculations also suggest that the observed percentages of the \(T_2\) values shown in Fig. 3 correspond closely to the weighted distribution of the intracellular and extracellular sodium-23 nuclei in the lens. This would further suggest that the exchange rates of sodium in these two microenvironments are sufficiently different in the intracellular \((T_2\ 3\ \text{msec})\) and extracellular \((T_2\ 27\ \text{msec})\) spaces in the bovine lens to allow discrimination between them by the \(T_2\) values alone.

According to the pump-leak model (1), membrane permeability is an important factor (in addition to the active Na,K-ATPase, or pump) in determining the type of \([\text{Na}^+]\) gradient that exists within the lens. Low posterior \([\text{Na}^+]\) is expected, from this model, to be diffusion-limited. With cataract formation, membrane permeability changes dramatically (8–10); therefore, the lens \([\text{Na}^+]\) gradient should also change. The disruption of the lens fiber cell membrane noted with cataract formation should also dramatically increase the contribution of the extracellular space (38). Further associated changes due to increased water hydration of the lens fiber cell may also be expected to dramatically alter the \(T_2\) values. The application of surface detection coils (39–41) in strong magnetic fields should eventually allow the noninvasive observation of changes in the distribution and the local microenvironment of sodium prior to lenticulopacification.

This work was supported by grants from the National Eye Institute, the National Cancer Institute, and Alcon Laboratories, Inc. W.H.G. acknowledges support from a Research to Prevent Blindness Manpower Award.


![Fig. 5. Spectrum A: Sodium-23 NMR bovine lens nonspatial measurement at 79.4 MHz in a nonspinning 20-mm sample tube at 23°C. Spectrum B: Lens under conditions otherwise identical to those for spectrum A, but equilibrated with the low sodium (8 mM) Tyrode's buffer and the shift-reagent complex 5 mM dysprosium-tripolyphosphate (1:2) for 3 hr. The intracellular and extracellular resonances are located at \(-0.097\) and \(-5.65\) ppm, respectively. The integrated areas of the resonances are superimposed on the spectrum. Spectrum C: Difference spectrum of B minus the buffer blank (data not shown).]