Continuous Measurements of Na, Li, and Cl in the Perfused Salivary Gland by Use of NMR

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Summary The present study was undertaken to measure tissue contents of Na, Li, and Cl non-invasively in the isolated perfused organ by nuclear magnetic resonance (NMR) using a broadband tunable probe. The NMR signals of $^{23}$Na, $^7$Li, and $^{35}$Cl from the isolated perfused rat mandibular gland were collected continuously and each spectrum was obtained for every 15 or 20 s. The Na concentration in the perfusate was varied by replacement with Li, and the resulting changes were monitored by measuring the signal intensities of the electrolytes. The time constant for Na exchange was slower following complete removal of extracellular Na than following its half replacement, suggesting that the Na extrusion by Na$^+/K^+$ ATPase was reduced by lowering the extracellular Na level. The time constant for Li exchange was slower than that for Na exchange. The level of Cl was nearly constant during experiment, except for a very slow increase in Cl, possibly resulting from increasing edema and/or intracellular Li storage.

Key words: multinuclear NMR, electrolyte measurement, perfused organ.

Na$^+$, K$^+$, and Cl$^-$ play important roles (Na$^+/K^+$ ATPase, Na$^+/Cl^-$ cotransport, etc.) in the process of epithelial transport. In order to determine the tissue amounts of electrolytes, the flame photometry and electron probe microanalysis have been applied. But these methods require destruction of the tissue, and the continuous measurement of the same organ is impossible or difficult. Nuclear magnetic resonance (NMR) spectroscopy is a non-invasive method which enables us to measure phosphorus energy metabolites continuously in the isolated perfused salivary gland (Murakami et al., 1983, 1984; Nakahari et al., 1985). In principle,
NMR spectroscopy permits us to observe many nuclides which have nuclear spin (e.g. $^{31}$P, $^{23}$Na, $^7$Li, $^{35}$Cl, etc.). Because the natural abundances of $^{23}$Na, $^7$Li, and $^{35}$Cl are 100, 92.6, and 75.53%, respectively, these ions are observable by NMR spectroscopy without adding or using isotope. Among these, $^{23}$Na-NMR has been applied to many kinds of biological materials (CIVAN and SHPORER, 1978). The present study demonstrates the utility of NMR spectroscopy to allow a kinetic and non-invasive measurement of electrolytes such as Na$^+$, Li$^+$, and Cl$^-$ in the perfused mandibular gland using a broadband tunable probe.

Male rats (Std: Wistar, Shizuoka Laboratory Animal Center, 250–350 g body weight) were anesthetized by intraperitoneal injection of pentobarbital sodium (50–70 mg/kg, Nembutal®). The mandibular gland, weighing 150–250 mg, was placed in a 10 mm diameter NMR tube (24°C), and perfused at the rate of 2 ml/min with the peristaltic pump (Cole-Palmer). The composition of the control perfusate (in mM) was; Na 146, K 4.3, Ca 1, Mg 1, Cl 148.3, H$_2$PO$_4$– 1, and glucose 5. The perfusate was buffered at pH 7.4 by 10 mM HEPES and gassed with 100% O$_2$. To avoid contamination from the electrolytes in the venous effluent, a space outside of the gland was washed by an isotonic solution of sucrose (300 mM) at a rate of 10 ml/min. The mandibular vein was cut, and the venous effluent was removed with a washing solution by aspiration.

NMR spectra were collected using a WM-360wb NMR spectrometer (Bruker, West Germany) with a broadband probe tuned to $^{23}$Na, $^7$Li, and $^{35}$Cl. The $T_1$ values of $^{23}$Na, $^7$Li, and $^{35}$Cl were measured as 61, 18, and 40 ms, respectively, in the NaCl or LiCl solution (150 mM). According to these $T_1$ values, the recycling time was chosen for NMR measurement of each nuclide. The $^{23}$Na-NMR spectra were obtained at 95.24 MHz every 15 or 20 s. Accumulations of 12 or 17 transients were collected with a recycling time of 1 s and a 45° RF pulse; an additional 3 s was necessary for computer processing and data storing. The $^7$Li-NMR spectra were obtained at 139.9 MHz every 15 s. The recycling time was 1 s, the tip angle of the RF pulse was 5° and either 12 or 16 scans were accumulated. The $^{35}$Cl-NMR spectra were obtained at 35.28 MHz every 15 s, using a recycling time of 0.5 s, a 60° RF pulse and accumulations of 24 transients.

The Na concentration of the perfusate was varied by a replacement with Li, either completely or in half. During these experiments, the tissue contents of Na, Li, and Cl were estimated by measuring the signal intensities of NMR resonance (peak height or area). To estimate the solution exchange in the perfusion system, the Na$^+$ in the NMR tube was measured by $^{23}$Na-NMR spectroscopy, and the Na in the effluent of the perfusion line was measured by flame photometer (Corning 480). Both results coincided and the time constant of Na exchange in the perfusion system without the gland was 0.3–0.5 min.

The gland became edematous during the perfusion, but the development of edema stopped and the size became constant about 30 min after start of perfusion. In order to evaluate the degree of edema, the same gland was weighed as followings: 1) 60 min after start of perfusion (weight of the perfused-gland), 2) 30 min after stop
of perfusion (weight of the perfusion-stopped-gland), 3) after removal of the adherent connective tissue and the edema fluid (the wet weight of the gland), and 4) dried for 24 h at 180°C (dry weight of the gland). Using these weights, the water contents (g/kg gland weight) of the perfused-gland, the perfusion-stopped-gland and the wet-gland, were calculated as 910±5, 877±8, and 769±7 (± S.D., n=8) and the solid mass was 23.1% of the wet weight of the gland. Since the solid mass is constant during the perfusion, the water content of the perfused-gland increases up

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\frac{\text{23Na-NMR}}{A. \ Na \rightarrow Li} \quad \frac{\text{10}}{\text{4.0 min}}
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Fig. 1. \textsuperscript{23}Na-NMR spectra of the isolated perfused mandibular gland of rat. Actual traces of \textsuperscript{23}Na-NMR spectra were lined along the experimental time course. The height of spectrum is identical to the amount of Na. \textsuperscript{23}Na-NMR spectra were obtained at 95.24 MHz every 20 s (with a recycling time of 1 s and a 45° RF pulse, 17 transients were accumulated, and additional 3 s for computer processing and data storing). A: complete replacement of Na with Li. B: complete replacement of Li with Na. The values of the time constant during the replacement were entered (in min) above the graph.
to 304% compared with the wet-gland and decreases to 214% by stopping the perfusion. This edema is due to a high rate of perfusion (about 10 ml/g-min) which is required for adequate oxygen supply to the cell. Fortunately, this edema had no influence on the secretory rate of saliva, and the cellular levels of ATP and creatine phosphate kept their resting levels during the perfusion for longer than 4 h.

The H2O extracts of the dried glands were analysed for Na, K, and Li using a flame photometer (Corning) and for Cl using a chloride titrator (Radiometer). For the glands perfused with the control solution, tissue contents (mmol/kg wet weight) of Na, K, and Cl were 37.0 ± 2.0, 102.0 ± 3.5, and 64.3 ± 3.0 (± S.E., n = 8). For one gland perfused with the Li-perfusate, Na, K, Cl, and Li were 4.4, 56.8, 61.6, and 65.7 mmol/kg wet weight.

23Na, 7Li, and 35Cl-NMR resonances of the perfused salivary gland showed single peaks, precluding discrimination of the intracellular and extracellular components of 23Na, 7Li, and 35Cl in those spectra. As shown in Fig. 1A, when Na was replaced completely with Li, the 23Na signal of the perfused gland decreased exponentially to 10% of the original level in 3 min. During this exchange of Na with Li, two time constant values were calculated as 1.0 and 4.0 min. These rate constant values were averaged as 0.89 ± 0.04 and 3.05 ± 0.28 min (± S.E., n = 5). Since the time constant for the solution exchange in the perfusion system was 0.3-0.5 min, the time constants as 1-4 min in this experiment could reflect the Na exchange in the gland. However, we cannot assign these time constants to the exchange sites among vascular, interstitial and intracellular spaces only from these values. After removal of Na, 23Na signal decreased to 2% of the original level in 10 min, and then decreased to the noise level in 15-20 min. This suggests that the intracellular Na concentration of the salivary gland could be very low, and/or that the intracellular Na could be removed quickly (within 15 min) by the depletion of extracellular Na.

Figure 1B shows the restitution of Na to the perfusion. The time constant for restoring Na was calculated as a single value of 1.6 min (averaged as: 0.99 ± 0.21 min, ± S.E., n = 4). The 90% recovery required about 3.7 min. The Na+ gradient across plasma membrane is believed to be established by the balance between a Na pumping-out by Na+/K+ ATPase and a leak-in by passive process. The exchange rate of Na during the Na-replacement reflected the exchange in the perfusion system and the intra- and extracellular exchange. If the rate of inward leak and outward extrusion decreased, a slower component of Na exchange would
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a. 23Na-NMR

b. 7Li-NMR

c. 35Cl-NMR
be expected to appear. As shown in Fig. 1A, when the extracellular Na was decreased, a slower component of Na exchange appeared, suggesting that the extrusion of Na by Na\(^+\)/K\(^+\) ATPase was reduced by lowering the extracellular Na level. On the other hand, as in Fig. 1B, when the extracellular Na was restored, the time constant of Na exchange was faster than that observed during the depletion of extracellular Na (Fig. 1A). These findings indicate that the rate of inward leakage and outward extrusion of Na were increased by the recovery of the extracellular Na level.

Figure 2 shows the experiment of half-Na replacement and complete-Na replacement with Li. Figure 2a, b, and c show the \(^{23}\text{Na}\)-, \(^{7}\text{Li}\)-, and \(^{35}\text{Cl}\)-NMR signals, respectively. When the peristaltic pump was stopped at the beginning of experiment (Fig. 2a), the \(^{23}\text{Na}\)-signal decreased to about 65\% of the original level at 10 min from stopping the perfusion. As mentioned above, this decrement of signal is due to a shrinkage of the edema which developed during the perfusion. As shown in Fig. 2a, \(^{23}\text{Na}\)-signal was changed following the Na concentration of the perfusate. The time constant was a single value of 0.89 ± 0.04 min (± S.E., n = 6) when Na concentration of the perfusate was more than 73 mM/l. A slow component (2.4–2.7 min) appeared only with the complete replacement of Na in addition with a faster component (0.5–0.7 min). Though the extracellular low Na could inhibit Na extrusion by Na\(^+\)/K\(^+\) ATPase, the above finding that no slow rate constant appeared even during the half-Na perfusion, indicates that the 73 mM/l Na is enough for maintaining the Na exchange at resting state of the mandibular gland.

By \(^{7}\text{Li}\)-NMR spectroscopy, we could follow the kinetics of Li during the Na-Li replacement. When Na was replaced completely with Li in the same manner as shown in Fig. 1, \(^{7}\text{Li}\) signal increased exponentially with two time constants of 0.95 and 8.37 min (0.67–1.43 and 7.3–10.0 min in 3 glands), requiring 5 min to reach 90\% of the maximum. On the other hand, when Li was removed from the perfusate, Li quickly decreased with a time constant of 1.34 min (1.03–1.34 min in 3 glands). This value was in the same range as the time constant for the Na restoring in Fig. 1B. That is, the rate of Li exchange was dependent on extracellular Na, and became slower during the low-Na perfusion. Since Li cannot be extruded by Na\(^+\)/K\(^+\) ATPase (EHRICH and DIAMOND, 1980), the Na dependency of the Li exchange cannot be explained by the activation of the Na\(^+\)/K\(^+\) pump. Another possibility is a Na\(^+\)/Li\(^+\) exchange system. An amiloride-sensitive Na\(^+\)/H\(^+\) antiporter can pass Li easily (KINSELLA and ARONSON, 1981) and the Na\(^+\)/H\(^+\) antiporter was suggested in the rabbit mandibular gland (CASE et al., 1984). Accordingly, the Na\(^+\)/H\(^+\) antiporter possibly act as an Na\(^+\)/Li\(^+\) antiporter during Na replacement with Li, in the rat mandibular gland. As shown in Fig. 2b, when Na concentration of the perfusate was 0 or 73 mm, a slow time constant for Li exchange appeared as 13 or 16 min. These results support a Na-dependent Na\(^+\)/Li\(^+\) exchange system in this gland. On the other hand, comparing Fig. 2a and b, Li remained in the gland still after removal of Li from the perfusate. This is explained by the slow extrusion of Li because of absence in Li pump-out system.
Figure 2c shows the $^{35}$Cl-NMR spectra collected during Na-Li replacement. The Cl level in the gland was almost constant throughout the experiment, but only a slight increase (6%) was observed at the end of experiment. This slow increase in Cl was not affected by changing Na concentration of the perfusate. When the peristaltic pump was stopped, a signal intensity of $^{35}$Cl was quickly decreased, consistent with a shrinkage of edema. The decrement of the signal depends on the size of edema. Since the size of edema varied by several % among different glands, when one would compare the signals of the different glands, the size of the edema must be taken into account. The $^{35}$Cl signal from the shrunken gland increased slightly (3%) at the end of experiment. These may have resulted from an increased edema and/or an intracellular Li storage because of slow extrusion of Li. The Li may have remained intracellularly with Cl even after the removal of extracellular Li.

The intracellular Cl content is expected to fall following the removal of Na from the perfusate, because of a decrease in Cl entry by Na$^+$/Cl$^-$ cotransport. However, the total Cl signal was unchanged after withdrawal of Na. This suggests that the signals of $^{35}$Cl were almost all from the extracellular Cl and that the intracellular Cl could not be detected.

The present study demonstrates the advantage of the combination of the NMR and the vascular perfusion techniques. By NMR method, the tissue contents of electrolytes was measured non-invasively, continuously and in a high time resolution (several s–20 s). Since a tunable broadband probe was used in the present study, we can apply multi-nuclear magnetic resonance (Na, Li, Cl) to the same organ. This advantage has possible biological applications such as measurement of electrolyte exchange, flux, etc. However, the quantitative determination of concentration requires a value of accurate volume of the gland. The vascular perfusion provided quick exchange of the extracellular solution and an adequate oxygen supply.

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