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Sodium alterations in isolated rat heart during cardioplegic arrest

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Schepkin, V. D., I. O. Choy, and T. F. Budinger. Sodium alterations in isolated rat heart during cardioplegic arrest. *J. Appl. Physiol.* 81(6): 2696–2702, 1996.—Triple-quantum-filtered (TQF) Na nuclear magnetic resonance (NMR) without chemical shift reagent is used to investigate Na derangement in isolated crystalloid perfused rat hearts during St. Thomas cardioplegic (CP) arrest. The extracellular Na contribution to the NMR TQF signal of a rat heart is found to be $73 \pm 5\%$, as determined by wash-out experiments at different moments of ischemia and reperfusion. With the use of this contribution factor, the estimated intracellular Na ($[Na^+]_i$) TQF signal is $222 \pm 13\%$ of preischemic level after 40 min of CP arrest and 30 min of reperfusion, and the heart rate pressure product recovery is $71 \pm 8\%$. These parameters are significantly better than for stop-flow ischemia: $340 \pm 20\%$ and $6 \pm 3\%$, respectively. At 37°C , the initial delay of 15 min in $[Na^+]_i$ growth occurs during CP arrest along with reduced growth later ($\sim 4.0\%/min$) in comparison with stop-flow ischemia ($\sim 6.7\%/min$). The hypothermia (21°C , 40 min) for the stop-flow ischemia and CP dramatically decreases the $[Na^+]_i$ gain with the highest heart recovery for CP ($\sim 100\%$). These studies confirm the enhanced sensitivity of TQF NMR to $[Na^+]_i$ and demonstrate the potential of NMR without chemical shift reagent to monitor $[Na^+]_i$ derangements.

ischemia-reperfusion; noninvasive monitoring of sodium; nuclear magnetic resonance; triple-quantum filter

THE PRESENT STUDY was undertaken primarily to investigate the possibility of triple-quantum-filtered (TQF) nuclear magnetic resonance (NMR) to monitor intracellular sodium ($[Na^+]_i$) changes in the rat heart without chemical shift reagent and secondarily to evaluate the changes of $[Na^+]_i$ during cardioplegic (CP) arrest followed by reperfusion for different durations of ischemia and at different temperatures. The application of a CP solution with high concentrations of K^+ and Mg^{2+} is one of the preferred ways to arrest the heart during cardiac surgery. The hypothesis is that stabilization of $[Na^+]_i$ is a part of the mechanism of cardioprotection by CP and that studies of ion homeostasis will aid in developing strategies for effective myocardial protection (4, 12, 23, 41, 48).

NMR provides a unique opportunity for the measurement of $[Na^+]_i$ in a rat heart by using shift reagents (3, 47, 15, 25, 26, 31–33, 47) or by using a shift reagent in combination with multiple-quantum filtering (20, 27, 28, 36). Despite the many advantages of using a shift

reagent, limitations are inevitable. A chemical shift reagent may perturb the physiology (11, 40), and it is not practical for brain or human study due to the blood-brain barrier or toxicity. Another aspect is that the high concentration of Mg^{2+} , as in a CP solution, can decrease the frequency shift of extracellular Na ($[Na^+]_o$) and make it impossible to quantify $[Na^+]_i$.

A method is needed to monitor $[Na^+]_i$ without shift reagent. One approach is the direct use of differences in relaxation times between $[Na^+]_o$ and $[Na^+]_i$ in T_2 (11) or T_1 (14, 17). The more attractive alternative is the application of the multiple-quantum NMR technique (8, 18, 19, 29, 30, 35, 44, 45). Among multiple-quantum NMR methods, TQF is more sensitive (at least 1.5 times) than the double-quantum selection in detection of the nuclei experiencing quadrupolar interactions (6, 44).

Most studies using multiple-quantum filtration are done at high magnetic field; however, a recent study showed the possibility of using a surface coil to monitor the NMR TQF signals in human skeletal muscle and brain in vivo at magnetic field of 2 T (35).

The opportunity to monitor $[Na^+]_i$ by the NMR TQF method follows primarily from the fact that Na nuclei have nonaveraged quadrupolar interactions inside cells (40). Some of the $[Na^+]_o$ also exhibits quadrupolar interaction. Thus the total ^{23}Na NMR TQF signal has intracellular and extracellular components (20). In the present investigation, the method of TQF NMR without the use of a chemical shift agent is evaluated for physiological experiments. It is conceivable that the $[Na^+]_o$ contribution to the NMR TQF signal in the rat heart may be essentially unchanged, as in rat liver (36). This expectation follows from the fact that the TQF NMR, being more sensitive to the $[Na^+]_i$ (8), is attenuating the effect of possible changes in $[Na^+]_o$. In this case, all observable changes of the TQF signal may be regarded as $[Na^+]_i$ changes. This presumption is tested in the present study of a rat heart by evaluating the extracellular contribution in wash-out experiments (2, 22, 43).

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 250–300 g were anesthetized with 75 mg/kg pentobarbital sodium and anticoagulated with 1,000 U/kg sodium heparin by intraperitoneal injection. The hearts were rapidly excised via thoracotomy

and immersed in an ice-cold perfusion buffer. Hearts were loaded in <2 min onto the nonrecirculating Langendorff system, and the aorta was retrograde perfused at a constant pressure of 80 cmH₂O. Perfusions were done by Krebs-Henseleit (KH) modified solution (pH 7.4, 37°C). The perfusate content was (in mM) 118.5 NaCl, 25.0 NaHCO₃, 4.9 KCl, 1.2 KH₂PO₄, 1.2 MgSO₄, 1.2 CaCl₂, and 11.1 D-glucose. During perfusion, the solution was continuously aerated with a gas mixture of 5% CO₂-95% O₂. The heart was hanging in the air inside the coil, and the perfusate was evacuated through the tubing connected to the bottom of the NMR probe head coil. The hearts beat spontaneously without pacing at 180–250 beats/min. A compliant fluid-filled balloon catheter was placed in the left ventricle through the mitral valve via a left atriotomy and was connected to a pressure transducer (Abbott) for continuously monitoring the contractile function of the heart by a patient monitor (Vitatek 511). Hearts were allowed to stabilize for 25 min before an intervention commenced. The hemodynamic performance of the heart was estimated by heart rate and left ventricular developed-pressure product [rate-pressure product (RPP)]. The temperature of the perfusate solution was maintained by using a water jacket around the perfusate reservoir and in-flow lines. The temperature around the hanging heart was kept at 37°C (or 21°C), using a water-jacketed NMR probe head. Cardiac arrest was achieved by stop-flow ischemia or by infusion (~2 min) of warm St. Thomas CP solution containing (in mM) 110 NaCl, 10 NaHCO₃, 16 KCl, 1.2 CaCl₂, and 16 MgCl₂ at pH 7.8 and stop-flow ischemia later. For hypothermic experiments at 21°C, the CP solution was also at 21°C.

NMR experiments were performed on the specially designed NMR-imaging system, using a 2.35 T Bruker magnet with a 25-cm clear bore. Resonance frequency for Na was 26.47 MHz. An observation of TQF signal was performed by the pulse sequence

$$90^\circ_\phi - \tau/2 - 180^\circ_{\phi+\alpha} - \tau/2 - 90^\circ_{\phi+\alpha} - \delta - 90^\circ_\epsilon \quad (1)$$

where the preparation delay $\tau = 4.4$ ms, $\delta = 40$ μ s, $\alpha = 90^\circ$, $\epsilon = 0^\circ$. Phase ϕ was incremented through 30, 90, 150, 210, 270, and 330°, with a receiver-phase alternation by 180° each step (35, 46). In addition, the exocycle phase rotation was applied giving the resulting 24-step phase cycle. The resonance offset was always set to zero. The TQF filter resulted in the complete elimination of a single-quantum signal from Na in a perfusate solution containing 145 meq/liter Na.

The probe coil had a 14-mm internal diameter. The duration of the 90° pulse was 37 μ s. The NMR signal was acquired by 2K points in a spectral width of 10 kHz. A line broadening of 15 Hz was applied to the free-induction decay before Fourier transformation. Repetition time of the whole pulse sequence was 310 ms. Each observation point of 192 acquisitions required ~1.5 min, where 68 s were used for the TQF signal and 22 s for a single-quantum-signal monitoring. The NMR TQF peak intensities were used to monitor the Na time course changes (1). The Na NMR TQF signal from each heart was normalized to the average value of the preischemic TQF signals. Summary data are presented as an average in percent \pm SE. The preischemic level of [Na⁺]_i in the rat heart, as determined by NMR and other methods, is ~15 mM (15). This value can be used for quantitative comparisons with the results of other methods.

The washout of the [Na⁺]_o from the rat heart was performed, using a commonly accepted procedure (2, 22, 43). The washout solution (350 mM sucrose, 5 mM histidine, pH 7.4) was ice-cold and perfused through the heart for 1 min. The starting moment of the washout was detected by a sharp decrease of the Na NMR signal from the heart.

RESULTS

Typical Na NMR TQF signals detected in the rat heart for 68 s at 2.35 T before and after 40 min of warm (37°C) ischemia are presented on Fig. 1. The preischemia peak intensity of the TQF signal was only $2.9 \pm 0.15\%$ of the total peak intensity of the Na signal observed in the same heart ($n = 32$). To optimize the TQF pulse sequence, the dependence of the TQF signal on an interpulse delay τ was measured (Fig. 2). The temperature of the heart was set at 21°C to prevent changes in the heart during NMR measurement. The results are shown for three different conditions of the heart: normally perfused, CP arrested, and postmortem hearts (several hours of ischemia). The interpulse delay $\tau = 4.4$ ms gives the largest TQF signal for these conditions.

The contribution of [Na⁺]_o to the total TQF signal was estimated by washing out the [Na⁺]_o of the rat heart in 14 experiments. The changes in amplitude of the Na NMR TQF signal as a result of 1-min washout were detected at different moments: at preischemia and after 20- and 40-min ischemia. The washout decreased the Na TQF signal on average by $73 \pm 5\%$ relative to the total preischemic Na TQF signal.

The sensitivity of the NMR TQF method to monitor [Na⁺]_i changes at 2.35 T is demonstrated on Fig. 3. The rat heart perfusion with KH solution was switched to perfusion with KH plus 1 mM ouabain. The Na TQF signal growth commenced immediately and continued

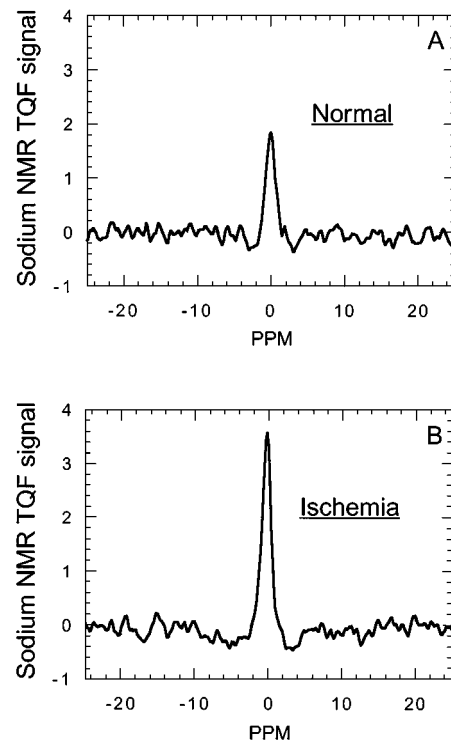


Fig. 1. Sodium (Na) nuclear magnetic resonance (NMR) triple-quantum filter (TQF) spectra obtained from rat heart at 2.35 T ($\nu_0 = 26.5$ MHz) before (A) and after (B) 40 min of stop-flow ischemia at 37°C; ppm, parts per million. Acquisition time 68 s, no. of acquisitions = 192.

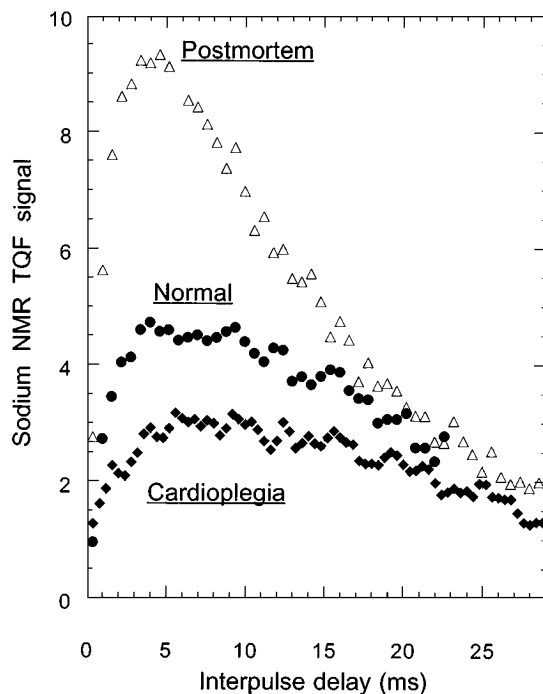


Fig. 2. Dependence of ^{23}Na NMR TQF signal as a function of interpulse preparation delay (τ) in rat heart (21°C). ●, Heart at normal perfusion; ◆ cardioplegia (CP)-arrested heart; △ postmortem heart. Each point corresponds to ~ 1 min of acquisition time.

to rise until it reached a plateau ~ 30 min after ouabain infusion at $\sim 210\%$ of the preintervention level.

The effects of 20 and 40 min of stop-flow ischemia in rat hearts at 37°C are shown in Fig. 4. The average growth of the Na TQF signal during the first 20 min was $\sim 1.8\%/min$. Reperfusion after 20 min of stop-flow

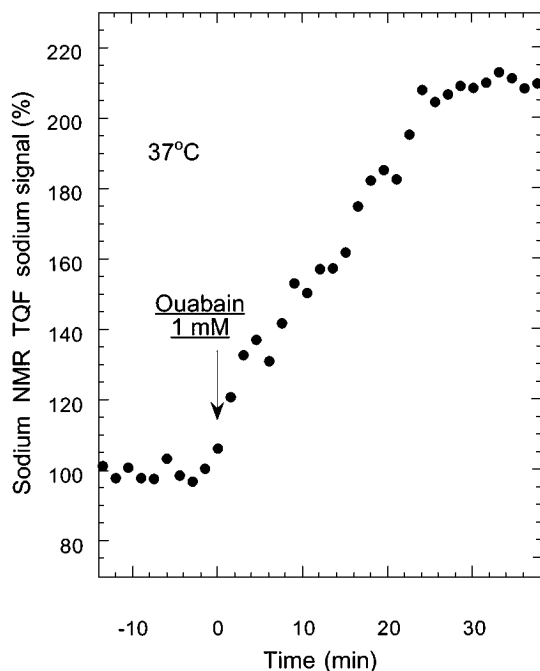


Fig. 3. Time course of Na NMR TQF signal in rat heart (37°C) during perfusion with Krebs-Henseleit modified solution plus 1 mM ouabain at 2.35 T. Rat heart contraction stopped after ~ 5 min of ouabain infusion.

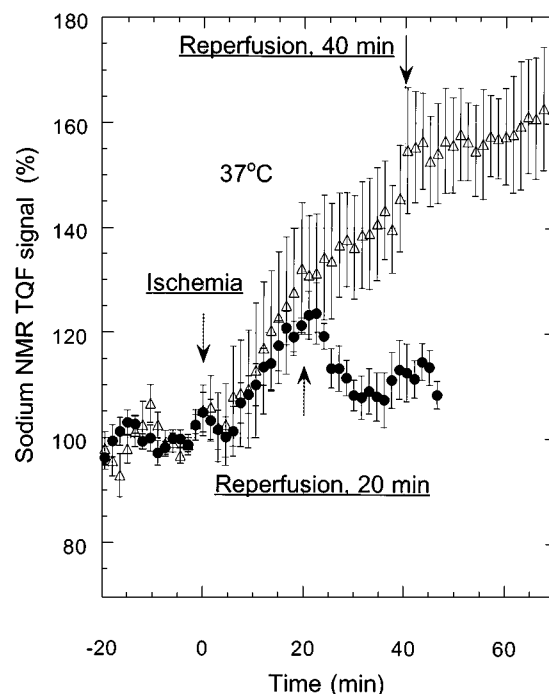


Fig. 4. Time course of ^{23}Na NMR TQF signal in isolated rat hearts during 20 min (●; $n = 6$) and 40 min (△; $n = 6$) of stop-flow ischemia at 37°C . Reperfusion after 20 min of ischemia gave partial restoration of Na-TQF signal, whereas reperfusion after 40 min showed no recovery.

ischemia reversed the Na TQF signal change with a recovery of the signal to $110 \pm 5\%$ of preischemic level. The rat heart recovery determined by the ratio of the average RPP before and after ischemia was $70 \pm 8\%$. The 40 min of stop-flow ischemia resulted in further growth of the ^{23}Na TQF signal. Immediately on reperfusion, a small increase was noted, and the signal remained elevated at $165 \pm 12\%$ of preischemic level. The recovery of the rat heart estimated by RPP was $6 \pm 3\%$. There was a 4-min delay in the growth of the Na TQF signal which was reproducible in both cases at the beginning of 20- and 40-min warm stop-flow ischemia.

In contrast to stop-flow ischemia, the application of CP produced an immediate (< 2 min) fall $\sim 27\%$ in the Na NMR TQF signal and a 15-min interval of no change in the signal (Fig. 5). After this delay, a growth of the TQF signal at a rate $\sim 1.1\%/min$ was observed. Reperfusion after 40 min was accompanied by a fast rise of $\sim 25\%$ in the TQF signal. The final level of the Na TQF signal after 40 min of CP arrest ($133 \pm 10\%$) was significantly less than for stop-flow ischemia. The corresponding RPP recovery of the rat heart was $71 \pm 8\%$.

Additional experiments were performed to investigate the role of Mg^{2+} on the Na NMR TQF signal. A freshly thawed rat heart was homogenized with 10 ml of KH perfusate solution. ^{23}Na NMR TQF signal was observed from a sample of 2 ml (number of acquisitions = 192) at room temperature, using successive 1.0-ms increments in the interpulse delay. Immediately after this, concentrated MgCl_2 (1 M) was added to obtain a final concentration of 16 mM, and the TQF NMR experiment was repeated. The addition of Mg^{2+} produced a 30% drop in Na TQF signal (Fig. 6).

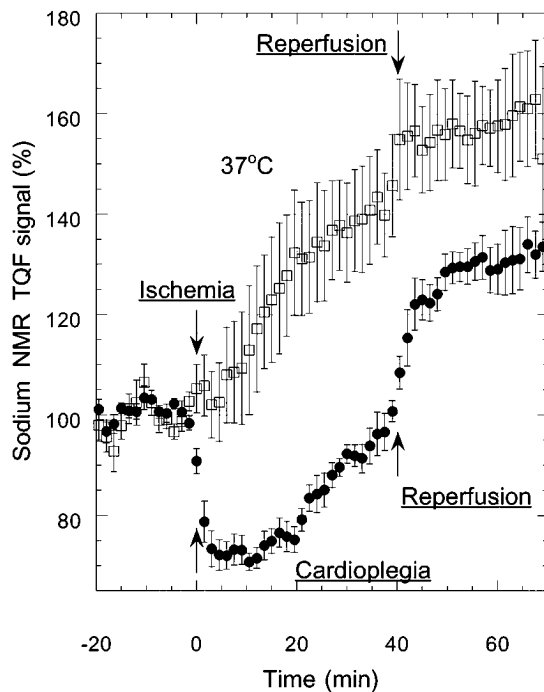


Fig. 5. Time course of ^{23}Na NMR TQF signal for CP arrest (37°C) in rat heart (\bullet ; $n = 6$) and for stop-flow ischemia (\square ; $n = 6$) during 40 min of ischemia and reperfusion. Note ~ 15 -min delay for beginning of Na NMR TQF signal growth after infusion of CP compared with stop-flow ischemia.

The hypothermic CP arrest is known as an effective myocardial protective measure (4, 12) and is very often used in heart surgery. These facts initiated the study of CP arrest and stop-flow ischemia at 21°C (Fig. 7). The

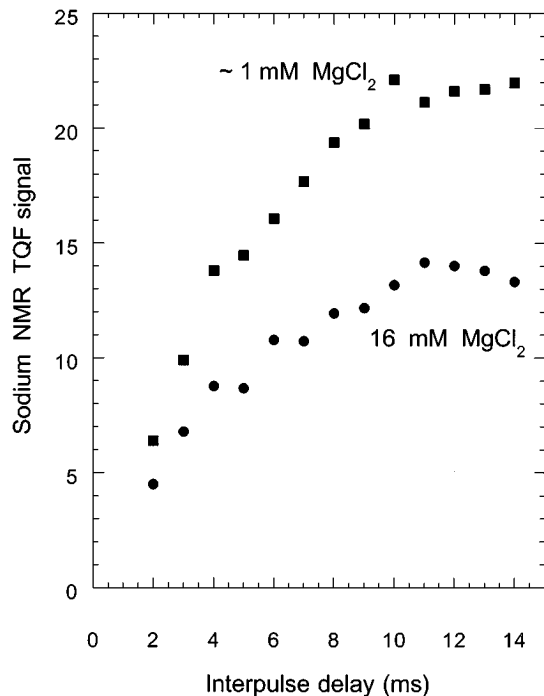


Fig. 6. Effect of high MgCl_2 (16 mM; \bullet) on Na NMR TQF signal from homogenized rat heart muscle at 21°C ; \blacksquare , ~ 1 mM MgCl_2 . Successive 1.0-ms increments in preparation interpulse delay demonstrate similar τ dependencies in both solutions. Mg^{2+} produced $\sim 30\%$ decrease of Na NMR TQF signal.

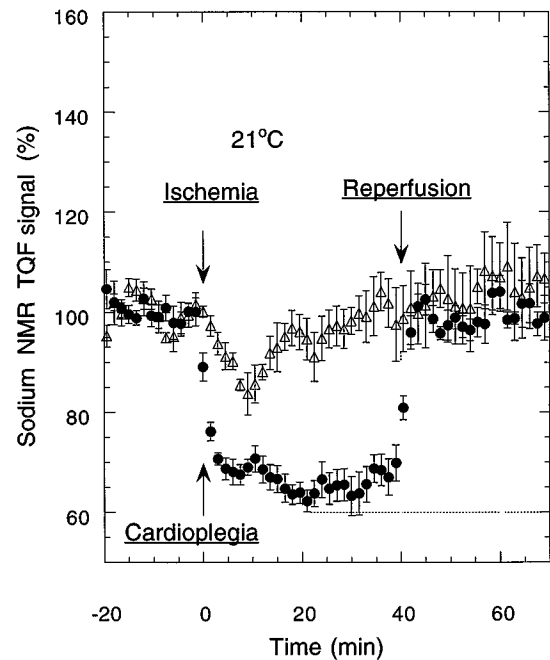


Fig. 7. Time course of Na NMR TQF signal in rat heart (21°C) during 40 min of CP arrest (\bullet ; $n = 6$) and 40 min of stop-flow ischemia (\triangle ; $n = 5$).

TQF NMR signal showed again fast decrease $\sim 30\%$ and later even further $\sim 7.5\%$ decline. The reperfusion completely reversed the TQF signal to preischemic level ($100 \pm 5\%$). The final level of the Na TQF signal was markedly lower than in corresponding experiments at 37°C . Recovery of the heart, using RPP monitoring, was $100 \pm 10\%$. For stop-flow ischemia at 21°C , a distinctive dip ($\sim 16\%$) in the Na TQF signal was observed ~ 10 min after the onset of ischemia, with almost no final increase of TQF signal at the end of reperfusion (Fig. 7). RPP recovery of the rat heart during the hypothermic stop-flow ischemia was $78 \pm 9\%$ (Table 1).

DISCUSSION

The signal-to-noise ratio for ^{23}Na NMR TQF signal from the rat heart (Fig. 1) demonstrates that Na TQF signals can be reliably studied at 2.35 T with acquisition periods < 2 min. In the experiment with ouabain (Fig. 3), the Na TQF signal acquired with the step of 1.5 min per point permitted monitoring the signal growth from which we infer a growth in $[\text{Na}^+]_i$ (10). This experiment gave evidence that the physiological mechanism for the TQF signal change is a gain of the $[\text{Na}^+]_i$ and also showed the possible range of this change. The results of the wash-out experiments gave a factor of 0.73 for the $[\text{Na}^+]_o$ contribution to the TQF signal. Thus, the observed plateau of the TQF signal (Fig. 3) corresponds to ~ 5 -fold growth of $[\text{Na}^+]_i$, which is near the maximum achievable for 30 min of ischemia (16) or $\text{Na}^+\text{-K}^+$ inhibition (13, 19). The plateau level is most likely determined by Na uptake activation of the reversal mode of the $\text{Na}^+\text{-Ca}^{2+}$ exchanger (7, 38, 43) which may decrease the Na TQF signal growth until it is equilibrated at $\sim 210\%$ of the preischemic level. For comparison, the highest possible NMR TQF signal rise

Table 1. *Postischemic sodium NMR TQF signal and recovery of a rat heart at end of reperfusion*

Parameters	Ischemia, 20 min at 37°C	Ischemia, 40 min at 37°C	CP, 40 min at 37°C	CP, 40 min at 21°C	Ischemia, 40 min at 21°C
Total sodium NMR TQF signal	110 ± 5	165 ± 12	133 ± 7	100 ± 5	104 ± 10
Intracellular sodium NMR TQF signal	137 ± 12	340 ± 20	222 ± 13	100 ± 15	115 ± 25
Rat heart recovery RPP	70 ± 8	6 ± 3	71 ± 8	100 ± 10	78 ± 9

Values are %preischemic average level ± SE. Ischemia, clamp global ischemia; CP, cardioplegic arrest; NMR, nuclear magnetic resonance; TQF, triple-quantum filtered; RPP, heart rate pressure product.

observed in our experiments in a rat heart was ~500%, when cell membranes were highly permeable to Na.

The dependence of the ^{23}Na TQF signal on interpulse delay is different for a postmortem heart than for normally perfused and CP-arrested hearts (Fig. 2). For living rat hearts, this dependence indicates more mobility and a larger range of correlation times for Na than in the postmortem rat heart. It was noted earlier that the interpulse delay τ associated with maximum intensity of the TQF signal can be different depending on the heart's condition (28). From this fact and our own experiments for normal perfusion and CP arrest, the value $\tau = 4.4$ ms was chosen for all TQF NMR experiments as the value having the lowest potential systematic error.

The $[\text{Na}^+]_o$ contribution of $73 \pm 5\%$ to the total Na TQF signal originating from the preischemic rat heart was estimated from the signal differences after the washout of extracellular space conducted at different moments of ischemia and perfusion. The contribution of the $[\text{Na}^+]_i$ to the TQF signal of 27% corresponds to the estimation by others (>25%), using a chemical shift reagent (8, 20). Because the fractional contribution of $[\text{Na}^+]_i$ to the TQF signal is 0.27, the intracellular volume is 0.33 of the extracellular space, and $[\text{Na}^+]_i = 15$ mM (15), we estimate that the TQF signal is ~10 times more sensitive to $[\text{Na}^+]_i$ than to $[\text{Na}^+]_o$. An even greater value of TQF signal sensitivity to $[\text{Na}^+]_i$ (23-fold) has been calculated by others (8). Thus, TQF NMR monitoring attenuates the possible small changes of the $[\text{Na}^+]_o$. During preischemic and reperfusion periods, the extracellular ion concentrations are determined by the perfusate solution. Under these conditions in our experiments, the $[\text{Na}^+]_o$ contribution to the TQF signal can be regarded as relatively independent of the heart's condition and can be subtracted as the base level.

The $[\text{Na}^+]_o$ contribution factor of 0.73 and the average growth of the Na TQF signal ~1.8%/min for the first 20 min of stop-flow ischemia at 37°C determine the growth of intracellular TQF signal relative to preischemic intracellular level of ~6.7%/min. This value is in the range of the linear $[\text{Na}^+]_i$ gain (4–13%/min) obtained by others for different experimental conditions, using a shift reagent (16, 26, 31, 33, 47) or washout $[\text{Na}^+]_o$ procedure (43). Consequently, especially during the first 20 min of ischemia, there is a correspondence between our results and the shift-reagent-based NMR experimental results of others. This fact supports the concept of a linear correlation between the Na multiple-quantum signal and the $[\text{Na}^+]_i$ content verified in human erythrocytes (45).

By using the factor 0.73, the level of the intracellular TQF signal after 30 min of reperfusion was $137 \pm 12\%$ of the preischemic intracellular value for 20 min of ischemia. For 40 min of stop-flow ischemia, the intracellular TQF signal was correspondingly $340 \pm 20\%$, which is substantially higher than after CP arrest at 37°C, where the final $[\text{Na}^+]_i$ TQF signal was $222 \pm 13\%$ of the preischemic value level. The rat heart RPP recovery with CP was ~71% and only ~6% for warm stop-flow ischemia. In general, the myocardial RPP recovery in all ischemic experiments was lower, the higher the final $[\text{Na}^+]_i$ (Table 1), as was observed by others (42, 43).

After 20 min of ischemia, the TQF signal showed remarkable recovery on reperfusion which is in agreement with the experiments done with chemical shift reagent (26, 28, 31). Full recovery, however, was not achieved indicating some irreversible damage in the heart (34).

In contrast to our results, a recent NMR TQF study in rat hearts without chemical shift reagent showed a decline in the Na TQF signal during stop-flow ischemia (8). This unusual result was probably due to incomplete ischemic conditions, because the heart in those experiments was submerged in the perfusate solution. Indeed, in our own TQF NMR experiments under similar conditions, there were no changes of the TQF signal. Moreover, the decreased contractile function of the rat heart was observed even after 30 min of occlusion. This fact was the main reason for suspending the heart in a temperature-controlled chamber for the present study.

A significant drop in the Na TQF signal from the rat heart was consistently observed after application of CP. The high concentration of Mg^{2+} ($[\text{Mg}^{2+}]$) in CP solution can be responsible for these changes. The following facts support this assumption: 1) Mg^{2+} can compete with Na for binding places and reduce Na NMR TQF signal (Fig. 6); 2) the variation of $[\text{Mg}^{2+}]_o$ in a range of 0.5–10 mM produces no noticeable changes of $[\text{Na}^+]_i$ in a guinea pig heart (5) or in a range of 1–16 mM only a small (~1 mM) slow decrease of $[\text{Na}^+]_i$ in sheep heart Purkinje fibers (7); 3) in conditions of no changes in $[\text{Na}^+]_i$, the CP experiments at 21°C demonstrate the amplitude and pattern of the Na TQF signal changes (Fig. 7), which are the part of the changes observed at 37°C; 4) the intracellular Mg^{2+} uptake is a slow process with a half-time of ~3 h (39); 5) the high concentration of extracellular potassium ($[\text{K}^+]_o$) produces only slow changes in $[\text{Na}^+]_i$ in rat heart (42), sheep cardiac Purkinje fibers (24), and in our preliminary experiments in a rat heart, using CP without Mg^{2+} ; 6) stop-flow

ischemia does not produce an immediate drop in the TQF signal, indicating no effects from the collapse of the rat heart vascular space. All these facts suggest that the fast TQF signal changes are due to the effect of Mg^{2+} on $[Na^+]_o$ interactions, probably, at the outer surface of membranes.

The Na TQF signal no-change period in the rat heart observed during CP arrest demonstrates that the primary process preventing $[Na^+]_i$ overload at 37°C occurs in the first 15 min (Fig. 5). One may expect this delay from CP due to its ability to decrease metabolic demands (12). The decreased O_2 consumption (12) and the ability to support the high activity of Na^+-K^+ pump (21) can be the main reasons of this effect. To our knowledge, this is the first observation of a delay in the $[Na^+]_i$ growth in a rat heart associated with prolongation of the ischemia tolerance by CP. The full advantage of this effect is achieved in multiple application of CP (4, 12). After the initial 15-min delay, the growth of the Na TQF signal was overall 1.1 or 4.0%/min relative to $[Na^+]_i$, which is much slower than 6.7%/min for the stop-flow ischemia at 37°C.

Hypothermia decreases myocardial oxygen demands and the rate of development of ischemic damage (4, 12). Thus it is not surprising that the experiments at 21°C showed a very small increase in the final Na TQF signal in conditions where metabolic and ionic transport processes are expected to be reduced in rate. The observed highest heart performance on reperfusion is associated with the smallest changes in the final TQF signal and consequently with the lowest changes of $[Na^+]_i$ (Table 1).

The small decline in the TQF signal after CP infusion at 21°C (Fig. 7) could be produced by relatively high Na^+-K^+ pump activity (21) and low activity of the Na^+/H^+ exchanger in comparison to CP arrest at 37°C. Thus the efflux of Na prevails and $[Na^+]_i$ content slowly declines. The observed slow changes in the Na^+ NMR TQF signal ~7.5% correspond to a 30% decrease in $[Na^+]_i$ content. This fact correlates with the slow $[Na^+]_i$ decline observed during 20-min exposure of sheep cardiac Purkinje fibers to histidine, tryptophan, and ketoglutarate CP, using ion-selective electrode measurements at 35°C (24). Zero $[Ca^{2+}]$ and 15 mM of $[Na^+]$ in histidine, tryptophan, and ketoglutarate CP solution were responsible for the reverse-mode activation of the Na^+/Ca^{2+} exchanger and only ~35% contribution in the decline from the ouabain-sensitive Na^+-K^+ pump activity.

The stop-flow ischemia at 21°C revealed a reproducible decrease of 16% in the Na TQF signal with the minimum at 10 min of ischemia. The mechanism for this change is unknown and could be connected to K^+ ischemic leakage and a slow transmembrane potential reduction. A corresponding decline in Na influx at a time when there is still high Na^+-K^+ pump activity could result in a $[Na^+]_i$ decrease. The decrease in $[Na^+]_i$ may activate Na^+/Ca^{2+} exchange later and generate additional influx of Na. These ion-exchange mechanisms can lead to a dip in the TQF signal observed in the experiments.

In conclusion, this study demonstrates that Na^+ TQF NMR can be used for $[Na^+]_i$ monitoring without chemical shift reagent. The ^{23}Na NMR TQF signal shows a marked effect of CP in reducing the final $[Na^+]_i$. A delay in the starting point of $[Na^+]_i$ loading was found during CP arrest along with a reduced growth later compared with stop-flow ischemia. This delay is indicative of an important protective feature of the CP that can be substantially enhanced in multidose CP application. The high level of NMR TQF signal at reperfusion corresponds to a decreased myocardial recovery. The dramatically slow growth of the Na TQF signal at 21°C indicates a very low rate of $[Na^+]_i$ change and correlates with the cardioprotective effects of hypothermia. The NMR TQF monitoring without chemical shift reagent provides a noninvasive method for the evaluation of myocardial ionic changes in the beating and ischemic hearts and has a potential for assisting in the development of cardiac preservation strategies.

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