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K⁺ dynamics in ischemic rat brain in vivo by ⁸⁷Rb MRI at 7 T

Victor E. Yushmanov^{1,*}, Alexander Kharlamov¹, Tamer S. Ibrahim^{2,3}, Tiejun Zhao⁴,
Fernando E. Boada^{2,3}, and Stephen C. Jones^{1,3,5}

¹Department of Anesthesiology, Allegheny-Singer Research Institute, Pittsburgh, Pennsylvania, USA

²Department of Bioengineering, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

³MR Research Center, Department of Radiology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

⁴Siemens Medical Solutions USA, Pittsburgh, Pennsylvania, USA

⁵Department of Neurology, Allegheny-Singer Research Institute, Pittsburgh, Pennsylvania, USA

Abstract

The aims of the present study were 1) to perform the first ⁸⁷Rb MRI in live rats with a focal ischemic stroke; and 2) to test the hypothesis that K⁺ egress from the brain in this model is quantifiable in individual animals by the high field (7 T) K/Rb substitution MRI. Rats pre-loaded with dietary Rb⁺ (resulting in replacement ratios Rb/(K+Rb) of 0.1 to 0.2 in the brain) were subjected to permanent occlusion of the middle cerebral artery, and ⁸⁷Rb MRI was implemented with 13-min temporal resolution using a dedicated radiofrequency coil and a spiral ultrashort-TE sequence (TR/TE of 3/0.07 ms). The ischemic core was localized by apparent diffusion coefficient mapping, by microtubule-associated protein-2 immunohistochemistry, and by changes in surface reflectivity. [K], [Na] and [Rb] were independently determined in the micropunched samples by post-mortem flame photometry. Both techniques were generally in agreement in the non-ischemic cortex, however, the MRI-assessed [K⁺+Rb⁺] drop in ischemic brain was less pronounced (average efflux rate of 4.8 ± 0.2 nEq/mm³/h, vs. 10 ± 1 nEq/mm³/h by flame photometry, *P* < 0.0001). The use of higher field gradients for better spatial resolution and, hence, more accurate quantitation, is suggested.

Keywords

rat brain; cerebral ischemia; tissue potassium; K/Rb substitution MRI; ⁸⁷Rb MRI; flame photometry

INTRODUCTION

Ischemia can be accompanied by dramatic disturbances in the tissue ion balance. A change in [K⁺]_{br} has been suggested as an index of progressive ischemic damage, with possible connections to the integrity of the blood-brain barrier, developing vasogenic edema and post-ischemic hemorrhagic transformation (1,2), i.e., the events which are critical for success of thrombolytic therapy of stroke.

*Correspondence to: Victor E. Yushmanov, PhD, Department of Anesthesiology, Allegheny-Singer Research Institute, 320 East North Avenue, Pittsburgh, PA 15212-4772, voice: 412-359-4362, fax: 412-359-5288 vyushman@wpahs.org.

During severe cerebral ischemia, ischemic depolarization is associated with elevated extracellular $[K^+]$ (3) and a gradual fall in brain tissue total potassium concentration ($[K^+]_{br}$) (4-6), although several investigators have documented the stability of extracellular $[K^+]$ (3) and $[K^+]_{br}$ (1) for 3 h after arterial occlusion. If an equalization of intra- and extracellular K^+ (and Na^+) concentrations occurs during ischemic depolarization, similarly to that during spreading depression (7), then extracellular and total brain $[K^+]$ would be expected to track one another. The discrepancy between $[K^+]_{br}$ stability or its gradual fall could be resolved by performing repeated, regionally specific $[K^+]_{br}$ measurements on a single animal to define its precise time course in rat cerebral ischemia. Although MRI is a noninvasive experimental technique suitable to study concentration dynamics in vivo in a single animal, the very low sensitivity of ^{39}K MR makes monitoring potassium with MRI highly problematic or possible only with low spatial resolution and excessive scan times (8). To overcome this limitation, it was proposed to partially replace K with its more MR-sensitive congeners, such as ^{87}Rb (9) or ^{133}Cs (10) in spectroscopic studies in vivo. Cs^+ is a probe of K^+ in the intracellular space only (10), whereas Rb^+ has a tissue distribution more closely resembling that of K^+ (11). The ^{87}Rb NMR methods for inferential studies of K^+ in cardiac metabolism and injury were significantly refined, and ^{87}Rb MRI was originally developed and used for detection of changes of K^+ balance in cardiac ischemia in the model of isolated perfused pig heart (12). To monitor K^+ in the rat brain in vivo, K/Rb substitution MRI was recently introduced (13). Partial K replacement with Rb was shown not to interfere with ion dynamics in ischemic brain over time and can be detected by ^{87}Rb MRI in vivo with custom-built radiofrequency (RF) coils (13). MRI quantification of ^{87}Rb in the brain still poses a significant challenge, however, because of its very short T_2 (~0.35 ms (9)), natural abundance of only 28%, and, therefore, relatively low tissue concentration.

The aims of the present study were 1) to perform the first ^{87}Rb MRI in vivo in a rat model of focal ischemic stroke; and 2) to test the hypothesis that K^+ egress from the brain in this model is quantifiable in individual animals by the high field (7 T) K/Rb substitution MRI.

METHODS

Animal preparation

Approval for animal use was obtained from the appropriate institutional committee and was consistent with the "Principles of laboratory animal care" (NIH publication No. 86-23, revised 1985). Seven normally fed male Sprague-Dawley rats weighing 300 ± 40 g were given 40 mM RbCl in the drinking water for 14 days (13).

Anesthesia was induced with 3% isoflurane, and maintained with 1.0% to 2.5% isoflurane, 30% oxygen, and balance nitrous oxide, administered via endotracheal tube and artificial respiration before (Model 681, Harvard Apparatus, South Natick, MA, USA) and during (MRI-1, CWE, Ardmore, PA, USA) MRI. Femoral arterial and venous catheters were inserted. Arterial blood pressure was continuously monitored from a femoral artery using a strain gauge transducer, and recorded using WinDaq software (DataQ Instruments Inc., Akron, OH, USA). An appropriate maintenance level of isoflurane was determined by monitoring the blood pressure response to tail pinch. Body temperature was maintained at 37°C by means of a servocontrolled system consisting of a rectal temperature probe and a heating pad outside the magnet or a thermostated water jacket inside the magnet. Immobilization was implemented with 0.7 mg/kg pancuronium bromide injected at 60 min intervals (on the bench) or continuously infused at 0.7 mg/kg/h (delivered at 1 mL/h) inside the magnet. Arterial blood gases (p_aCO_2 , p_aO_2) and pH were measured using a portable analyzer i-STAT (Heska Corp., Fort Collins, CO, USA) before middle cerebral artery (MCA) occlusion, after occlusion, and at the end of experiment.

Middle cerebral artery transection and bilateral common carotid artery occlusion

Permanent focal cerebral ischemia was produced by MCA transection and bilateral common carotid artery occlusion (MCAT) (14,15). The MCA was accessed using microsurgical techniques through a subtemporal route, coagulated with a bipolar device at the level of midway between olfactory tract and inferior cerebral vein, and transected. Both common carotid arteries were permanently ligated using '0' silk suture.

MRI

For $^{87}\text{Rb}/^1\text{H}$ MRI, a dedicated dual-tuned, 33-mm-diameter transmit/receive coil was designed and built. The animal's head was positioned inside the coil in the animal cradle with a recirculating water bed and fittings for respiratory and anesthesia gas supply. Calibration standards (10, 20 and 50 mM RbCl in 10% agarose gel with ^{87}Rb T_1 (< 1 ms) and T_2 (0.49 ms estimated from the line width) approximating those in brain tissue) were placed next to the animal's head. Images were obtained on a whole-body Magnetom 7 T scanner (Siemens Medical Solutions, Erlangen, Germany) within a field-of-view of $50 \times 50 \times 50$ mm. For ^{87}Rb , a rotated spiral isotropic three-dimensional (3D) ultrashort-TE sequence (16) with a voxel size of 3.8 mm^3 ($32 \times 32 \times 32$ matrix) and a typical acquisition time of 13.1 min was used. The sequence employed 256 spiral interleaves and 1024 rotations of 2D spiral trajectory along the x-axis, the RF flip angle 90° (pulse duration of 100 μs), and a maximum gradient amplitude and slew rate of 24 mT/m and 120 mT/m/ms, respectively. An ultra-short TE of 0.07 ms (calculated as a sum of one-half of the pulse duration, 0.05 ms, plus the pre-acquisition delay, 0.02 ms) and a relatively long TR of 3 ms were used to ensure maximum MRI visibility of quadrupolar ^{87}Rb and to eliminate a quantitation bias resulting from possible changes in relaxation times in ischemic brain. For anatomic reference, the ^1H multislice gradient echo sequence was used (in-plane resolution of 0.39 mm, 128 0.39-mm-thick slices). The infarct size and location were assessed by apparent diffusion coefficient (ADC) trace maps reconstructed from ^1H single-scan trace (17) diffusion-weighted multislice spin-echo images (TR/TE of 1300/107 ms, in-plane resolution of 0.39 mm, ten 2.5-mm-thick slices, diffusion weighting b -factors of 0, 200, 500, and 800 s/mm^2 , scan time per b -factor 4.2 min). The ^{87}Rb MRI series typically spanned the period from 1 to 6 h after ischemic onset. For every animal, MRI acquisition started as early as was possible; therefore, the number and particular timing of ^{87}Rb images varied in different animals.

Brain processing

After the end of MRI scanning (approximately 6 h after MCAT), rats were decapitated, and their heads were immediately frozen in dry ice and stored at -80°C in order to preserve the spatial characteristics of the brain for further superposition and comparison with MR images. The brain was chipped out of the skull in a -20°C cold box and mounted into the cryostat (-8°C). The 35- μm thick coronal sections of the brain at different levels from bregma were mounted on glass slides and digitized. Ischemic damage was identified by the change in surface reflectivity of ischemic tissue and further verified by immunohistochemistry with microtubule-associated protein-2 (MAP2) antibody in slide-mounted brain sections (18), as shown in Fig. 1c,d. Twenty-four samples of approximately 0.5 mg wet weight were punched from the ipsilateral and contralateral brain (13,19) at two coronal levels, approximately +1.5 and -0.5 mm from bregma. The micro-puncher inner diameter was 0.53 mm. The sampling depth was determined by the number of 35- μm coronal brain sections over the extent of the punch hole. To ensure that the samples were taken from the ischemic core and contralateral area, the sampling location was guided by the reflective changes (RC) in ischemic tissue (18). Brain K, Rb and Na content was determined by emission flame photometry of punched samples at 766, 791 and 589 nm for K, Rb and Na, respectively, using an IL943 flame photometer (Instrumentation Laboratory, Lexington,

MA, USA) with a Rb interference filter (Omega Optical, Brattleboro, VT, USA). Experimental readings were corrected for the K/Rb mutual interference effects (20).

Image processing

Parametric ^1H ADC maps were generated pixel-wise by exponential fitting of the diffusion-weighted image intensity vs. the b value (21) in MatLab (MathWorks, Natick, MA, USA). Selected coronal brain slices (taken every 350 μm) and histological MAP2 stained sections (taken every 700 μm) were digitized, stacked and registered using ImageJ (22) (available from: Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, MD, USA; <http://rsb.info.nih.gov/ij/>, 1997-2009) to render volumetric reconstructions of the brain. MR images stacked in four dimensions (including the time dimension) were aligned with histological 3D images and cut-face photographs and analyzed using AMIDE software (23). The brain Rb concentration ($[\text{Rb}^+]_{\text{br}}$) values were obtained from average ^{87}Rb MR image intensities over selected regions of interest (ROI) in the brain using calibration standards after correction for the non-zero noise baseline in the magnitude mode reconstruction, as has been validated previously for ^{23}Na MRI (19). The image intensities were assumed to represent the total $[\text{Rb}^+]_{\text{br}}$, because TE of 0.07 ms was much shorter than typical reported ^{87}Rb T_2 values in biological tissues (~ 0.35 ms (9)) and T_2 in calibration standards (0.49 ms), and TR of 3 ms was relatively long compared with our estimates of $T_1 < 1$ ms in tissues and agarose gel standards. To account for partial volume effects, the ROIs over the reference tubes extended beyond the tube diameter in the transverse plane, thus ensuring that the entire signal coming from solution in the tube was integrated, with subsequent normalization of the integrated signal to the actual cross section of the tube, so that no underestimation of the mean occurs (19,24). Image convolution simulations were performed using OriginPro 8.1 (OriginLab, Northampton, MA, USA).

RESULTS

Physiological monitoring and K/Rb replacement

In the rat #3, the MCA occlusion was not successful based on the post-experiment observation of two MCAs; therefore, this animal was excluded from further analysis. Physiological variables at different phases of the experimental protocol were stable within normal ranges for all RbCl-fed animals (Table 1). Although one-way repeated measures analysis of variance (with a post hoc Bonferroni correction for multiple comparisons) reveals small but statistically significant fluctuations in pH and $p_a\text{O}_2$ ($P < 0.05$), they were not considered relevant in the context of this work. As has been found by post-mortem flame-photometry, the RbCl feeding routine ensures a sufficiently high replacement ratio $\text{Rb}/(\text{K}+\text{Rb})$ between 0.1 and 0.2 in individual brains (Table 2).

^{87}Rb MRI

Figure 1b shows the first ^{87}Rb MRI of ischemic rat brain, which demonstrates a drop in $[\text{Rb}^+]_{\text{br}}$ in ischemic rat cortex after MCAT. The location of ischemic cortex was visualized in vivo using trace ADC mapping by ^1H MRI (Fig. 1a) and determined post mortem by RC (Fig. 1d) and histological MAP2 staining (Fig. 1c). ^{87}Rb MRI intensities were averaged over ROIs encompassing the ischemic cortex and the cortex in the contralateral brain hemisphere. The $[\text{Rb}^+]_{\text{br}}$ data obtained from ^{87}Rb MRI using calibration standards showed a $[\text{Rb}^+]_{\text{br}}$ decrease after MCAT in ischemic cortex, whereas no statistically significant changes in homotopic cortex ROIs over time were observed (Fig. 2a).

Cations in ischemic brain by ^{87}Rb MRI and flame photometry

To independently analyze the ion content in particular ischemic and uninvolved areas of the rat brain, directed micro-punching, validated in our previous works (13,25), was utilized. Absolute concentrations of Na, K, Rb, and the replacement ratio $\text{Rb}/(\text{K}+\text{Rb})$ in ischemic and contralateral brain of each animal were determined by flame photometry (Table 2). In ischemic brain, flame photometry showed an increase in the brain sodium concentration ($[\text{Na}^+]_{\text{br}}$) and a decrease in $[\text{K}^+]_{\text{br}}$ after MCAT, while $[\text{Rb}^+]_{\text{br}}$ mirrored the $[\text{K}^+]_{\text{br}}$ drop. In order to assess the systems of potassium homeostasis in the brain, the total concentrations of both potassium, $[\text{K}^+]_{\text{br}}$, and its congener rubidium, $[\text{Rb}^+]_{\text{br}}$, were combined. The total $[\text{K}^++\text{Rb}^+]_{\text{br}}$ concentration in ischemic cortex declined in average to about 30% of control by approximately 6 h after MCAT, $P = 0.0001$, as shown in Table 2.

To monitor the K^+ dynamics in ischemic brain by MRI, the ^{87}Rb MRI data were converted into physiologically meaningful $[\text{K}^++\text{Rb}^+]_{\text{br}}$ values. For this purpose, the $[\text{Rb}^+]_{\text{br}}$ MRI data were expressed relative to the corresponding contralateral cortex average and normalized to the average value of $[\text{K}^++\text{Rb}^+]_{\text{br}}$ in the non-ischemic brain obtained by flame photometry of the brain samples. This automatically takes into account different $\text{Rb}/(\text{K}+\text{Rb})$ replacement ratios in different animals. The net $[\text{K}^++\text{Rb}^+]_{\text{br}}$ efflux values in individual rats based on the ^{87}Rb MRI data after appropriate calibration by flame photometry are presented in Table 2, and the data for all six animals pooled together and averaged over narrow time intervals (~ 10 min) are shown in Fig. 2b. The average MRI dynamics of $[\text{K}^++\text{Rb}^+]_{\text{br}}$ in animals subjected to MCAT displays the net K^+ efflux from the ischemic brain, $P < 0.0001$ for the ischemic but not contralateral cortex. In comparison with the flame photometry estimates, the $[\text{K}^++\text{Rb}^+]_{\text{br}}$ drop assessed by MRI was less pronounced (approximately to 65% of control, average efflux rate $4.8 \pm 0.2 \text{ nEq/mm}^3/\text{h}$, vs. 30% and $10 \pm 1 \text{ nEq/mm}^3/\text{h}$ by flame photometry over the same time scale), $P < 0.0001$ by paired t -test.

DISCUSSION

This study reports: 1) the first ^{87}Rb MRI co-registered with anatomic images in a live animal (Fig. 1), and 2) both the linear Rb^+ efflux from the ischemic brain and the stability of $[\text{Rb}^+]_{\text{br}}$ in the non-ischemic cortex (Fig. 2a). Previous studies using micropunch flame photometry established K/Rb substitution MRI as a safe method to monitor K^+ changes based on the fact that Rb^+ itself does not significantly change the pattern of $[\text{K}^+]_{\text{br}}$ and $[\text{Na}^+]_{\text{br}}$ time course in ischemic cortex (13). This allowed us to put forward a new animal model to monitor K^+ dynamics in the ischemic brain using K/Rb substitution MRI. In this model, despite the intricacies of the transmembrane transport of Na^+ and K^+ , which occurs through many different channels/transporters besides $\text{Na}^+/\text{K}^+-\text{ATPase}$, and where K^+ channels may possess slightly different Rb^+/K^+ ion selectivity, the general trends were clear: a $[\text{Na}^+]_{\text{br}}$ increase together with a significant $[\text{K}^+]_{\text{br}}$ and $[\text{Rb}^+]_{\text{br}}$ decrease occurred in ischemic brain after 6 h (Table 2), in agreement with previous studies (13,15).

The quantitative methodology, validated previously for ^{23}Na MRI of ischemic rat brain (19) was applied to obtain $[\text{Rb}^+]_{\text{br}}$ dynamics in the brain from the ^{87}Rb MRI data. In the contralateral brain, as the example of the rat #1 shows, the $[\text{Rb}^+]_{\text{br}}$ values obtained by MRI and flame photometry, although statistically different ($P < 0.0001$), are in reasonable agreement: $11.9 \pm 0.4 \text{ nEq/mm}^3$ by MRI (Fig. 2a) vs. $9.8 \pm 0.6 \text{ nEq/mm}^3$ by flame photometry (Table 2). In some animals, however, where the standard quantitation was significantly compromised by the partial volume effects, MRI $[\text{Rb}^+]_{\text{br}}$ values were underestimated by almost 40%. Partial volume interaction was due to the very short ^{87}Rb T_2 in tissues (~ 0.35 ms (9)), which limits the spatial resolution to 1.9 mm at the maximum gradient strength of the 7 T magnet (40 mT/m). When reference tubes were placed next to the rat facial muscles with high $[\text{Rb}^+]$ (13), the integration of the signal of the calibration

standard in the tube was therefore compromised by the muscle proximity. Unfortunately, the options as to reference tube positioning were limited because of the very narrow RF coil (33 mm diameter). This coil design was chosen in order to maximize the coil sensitivity and achieve the best temporal resolution possible. Therefore, to quantify the absolute $[K^+]_{br}$ and $[Rb^+]_{br}$ dynamics in different animals for the proof-of-principle purpose of the present work, the comparison of $[Rb^+]$ in the non-ischemic brain between the flame photometry and K/Rb substitution MRI was utilized.

After calibration of the ^{87}Rb MRI intensities in the non-ischemic cortex by flame photometry of the contralateral brain samples, the summary MRI dynamics of $[K^+ + Rb^+]_{br}$ after MCAT (Fig. 2b) showed the net K^+ efflux from the ischemic brain, which is in agreement with $[K^+]_{br}$ dynamics studied earlier by elemental analysis studies using groups of animals with different durations of ischemia (1,4-6). Quantitatively, however, $[K^+ + Rb^+]_{br}$ decreased at 6 h after MCAT to approximately 65% of control by MRI and 30% by flame photometry. The average rate of efflux in this study from micropunch/flame photometry analysis was 10 nEq/mm³/h, in line with the previous observations (combining results from different times after onset) in the range from 8.0 to 9.7 nEq/mm³/h (4-6). In this work, possible variations in ^{87}Rb T_1 and T_2 values in ischemia were not part of the study; however, given the relatively long TR of 3 ms and ultra-short TE of 0.07 ms in our experimental setup, any conceivable T_1 and T_2 changes between or within intra- and extracellular compartments are unlikely to explain the difference. Rather, the discrepancy is attributable to the quantitation bias in ischemic cortex resulting from the partial volume interactions with the signals from adjacent non-ischemic brain and especially muscles with $[Rb^+]$ approximately 2.7 that of the brain (13).

To explore this hypothesis, the point spread function of ^{87}Rb MRI was estimated based on a one-dimensional image profile of two 4.3-mm-inner diameter standard tubes with 50 mM RbCl in 10% agarose gel. The experimental image profile was best simulated by the convolution of the actual concentration profile with a Lorentzian point spread function of 3-mm width at half-maximum (Fig. 3). Assuming that the cortex strip is approximately 2 mm wide, such a point spread function would result in a contamination of the cortex signal by about 25% of the signal from adjacent tissues at each border. Considering a simplified one-dimensional model where the cortex is flanked by the nonischemic brain and muscle, taking the typical $[Rb^+]$ in normal brain and muscle as 25 and 69 mEq/kg, respectively, from the previous work (13), and assuming the $[Rb^+]_{br}$ drop after 6 h of ischemia to 30% of control (i.e., to 7.5 mEq/kg) from the flame photometry data, the apparent (i.e., biased by the partial volume effects) ratio of $[Rb^+]$ in the ischemic and contralateral brain would be $(7.5 \times 0.5 + 25 \times 0.25 + 69 \times 0.25) / (25 \times 0.75 + 69 \times 0.25) = 0.76$ instead of 0.3 in the absence of partial volume effects, i.e., the correction factor is $0.3 / 0.76 \approx 0.4$. Applying this correction factor to the MRI estimate of $[K^+ + Rb^+]_{br} \approx 65$ nEq/mm³ after 6 h of ischemia (Fig. 2b) yields the corrected value of 26 nEq/mm³, which is comparable with 24-36 nEq/mm³ by flame photometry (Table 2). This consideration shows that partial volume interaction between the brain and muscle in ^{87}Rb MRI can result in significant overestimation of $[Rb^+]_{br}$ in ischemic cortex and, hence, underestimation of the $[Rb^+]_{br}$ drop in ischemia. On the other hand, partial volume interaction between the calibration standards and muscle can result in underestimation of the $[Rb^+]_{br}$. Therefore, future studies are planned at narrow bore research 7 T magnets with gradient strength of up to 630 mT/m (as opposed to 40 mT/m at the whole-body magnet in this study), which would significantly improve spatial resolution and diminish partial volume effects. Correction of residual partial volume effects would be performed using high resolution segmented anatomic 1H images convolved with the 3D point spread function, the technique developed previously for positron emission tomography (26).

In conclusion, the results presented here demonstrate the first ^{87}Rb MRI co-registered with anatomic images in a live animal. These data demonstrate the feasibility to quantitatively assess the dynamics of K^+ efflux from the ischemic brain in a single animal with 13-min temporal resolution by 7 T K/Rb substitution MRI. This technique may become a potentially unique tool to approach the mechanisms of the progression of ischemic damage, as well as to address a need for novel early biomarkers of tissue viability and for therapy monitoring.

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Abbreviations used

3D	three-dimensional
ADC	apparent diffusion coefficient
$[\text{K}^+]_{\text{br}}$, $[\text{Na}^+]_{\text{br}}$ and $[\text{Rb}^+]_{\text{br}}$	brain potassium, sodium and rubidium concentrations, respectively
MABP	mean arterial blood pressure
MAP2	microtubule-associated protein-2
MCA	middle cerebral artery
MCAT	middle cerebral artery transection and bilateral common carotid artery occlusion
p_aCO_2 and p_aO_2	partial CO_2 and O_2 pressure in arterial blood, respectively
RC	reflective changes
RF	radiofrequency
ROI	region of interest.

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FIG. 1.

Region-of-interest (ROI) analysis of Rb^+ in ischemic rat brain. Coronal images of the brain at approximately bregma -3 mm of the rat #2 are shown. (a) ADC map featuring an ischemic ROI ($\text{ADC} < 550 \mu\text{m}^2/\text{s}$, outlined). (b) An ^{87}Rb MRI in the same plane obtained in 13.1 min at 5.8 h after MCAT and showing lower $[\text{Rb}^+]_{\text{br}}$ in the ischemic ROI. (c) The absence of staining in the MAP2-stained brain section at 6.4 h after MCAT indicates the ischemic lesion. (d) The infarct location (outlined) identified by the surface reflective changes (RC) at 6.4 h after MCAT. Reference tubes with RbCl in 10% agarose gel were external to the rat head in the magnet and are not shown in MR images. The images were aligned and analyzed using AMIDE software.

FIG. 2.

(a) $[Rb^+]_{br}$ in the ischemic cortex ROI ($[Rb^+]_i$, squares) and homotopic (nonischemic) cortex ROI ($[Rb^+]_c$, circles) in the rat #1. Dashed lines are linear regressions to the data. (b) Net $[K^++Rb^+]_{br}$ efflux from the ischemic cortex ($[K^++Rb^+]_i$, squares) after MCAT assessed by ^{87}Rb MRI in vivo. $[K^++Rb^+]_c$, circles, shows $[K^++Rb^+]_{br}$ in the homotopic cortex. The ^{87}Rb MRI data were converted into $[K^++Rb^+]_{br}$ values by normalization to the average value of $[K^++Rb^+]_{br}$ in the non-ischemic brain obtained by flame photometry of the brain samples. The data are averaged over all 6 animals. Error bars are SEM. Dashed lines are linear regressions to the data. Average efflux rate and 95% confidence limits are 4.8 ± 0.2 nEq/mm³/h, $P < 0.0001$. For contralateral cortex, $P = 0.87$. T_a , time after MCAT.

FIG. 3.

Estimation of the point spread function of ^{87}Rb MRI. Solid line: one-dimensional sagittal image profile of the phantom; dotted line: actual geometry of the phantom consisting of two 4.3-mm-inner diameter cylinder tubes with 50 mM RbCl in 10% agarose gel; dashed line: convolution of the actual concentration profile (dotted line) with a Lorentzian point spread function of 3-mm full width at half-maximum.

Table 1

Physiological Variables at Three Phases of the Protocol*

	MABP (mm Hg)	pH ^a	p _a CO ₂ (mm Hg)	p _a O ₂ ^b (mm Hg)
Before MCAT	120 ± 10	7.45 ± 0.03	31 ± 4	83 ± 6
After MCAT	120 ± 8	7.45 ± 0.03	29 ± 4	100 ± 10
End of experiment	110 ± 20	7.39 ± 0.04	31 ± 6	80 ± 20

* Mean ± SD, n = 6. MABP, mean arterial blood pressure; pH, arterial blood pH; p_aCO₂ and p_aO₂, partial CO₂ and O₂ pressure in arterial blood, respectively.

^a *P* = 0.02 by repeated measures one-way ANOVA.

^b *P* = 0.001 by repeated measures one-way ANOVA.

Table 2

Rat Brain Ion Content After 14-day Administration of 40 mM RbCl and MCA*

Rat # ^a	T _d (h) ^b	Rb/(K+Rb) ^c	[Rb] (nEq/mm ³)		[K] (nEq/mm ³)		[Na] (nEq/mm ³)		[K ⁺ +Rb ⁺] _{br} efflux from IC by MRI	R ² and P ^e
			IC	CC	IC	CC	IC	CC		
1	6.3	0.120 ± 0.009	3.4 ± 0.7	9.8 ± 0.6	22 ± 4	78 ± 6	85 ± 8	36 ± 4	2.8 ± 0.4	0.74 (P < 0.0001)
2	6.4	0.117 ± 0.007	4.0 ± 0.4	10.6 ± 0.4	28 ± 2	87 ± 2	80 ± 3	31 ± 6	6 ± 2	0.53-0.73 (P < 0.001)
4	6.5	0.14 ± 0.01	6 ± 2	12.2 ± 0.3	30 ± 10	82 ± 2	80 ± 20	37 ± 1	2 ± 2	0.08-0.10 (P = 0.31-0.37)
5	6.2	0.15 ± 0.02	4 ± 1	12.6 ± 0.8	20 ± 7	83 ± 4	90 ± 20	35 ± 2	6 ± 2	0.87-0.90 (P < 0.002)
6	6.1	0.17 ± 0.02	4.6 ± 0.5	14.2 ± 0.3	22 ± 2	80 ± 3	80 ± 10	36 ± 2	4 ± 1	0.81-0.93 (P < 0.02)
7	6.3	0.19 ± 0.01	7.1 ± 0.7	17.0 ± 0.8	28 ± 3	77 ± 3	78 ± 4	37 ± 2	4 ± 1	0.69-0.83 (P < 0.002)
CC Average ^f			94 ± 3 ^g		---		35 ± 2			

* IC, ischemic cortex; CC, contralateral (non-ischemic) cortex. Errors are SD.

^a Rat #3 was excluded from analysis because of the unsuccessful MCA occlusion.

^b T_d, duration of the experiment after MCA.

^c For each brain, the value is an average over 24 samples; 8 each from the IC, CC, and subcortical regions.

^d Average over ischemic ROIs at different coronal brain levels; calculated from ⁸⁷Rb MRI data using the Rb/(K+Rb) and CC [K⁺+Rb⁺]_{br} values from flame photometry of the brain samples.

^e Range for linear fits in ischemic ROIs at different coronal brain levels.

^f An average over CC in all animals.

^g [K+Rb]; [K] and [Rb] values were combined, because the Rb/(K+Rb) ratios were different in different animals.