Edge location of sodium accumulation in focal cerebral ischemia in the rat: ADC and ^{23}Na MRI

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INTRODUCTION

The apparent diffusion coefficient (ADC) is a marker for the extent but not severity of ischemic stroke. Recently, we proposed ^{23}Na MRI as a means to determine precisely the stroke onset time for establishing patient eligibility for thrombolytic therapy (1). To validate brain sodium concentration ($[Na^+]_{br}$) as a potential biomarker in stroke, the study of concentration dynamics in a single animal in vivo is a suitable approach. Herein, we hypothesize that 1) regional $[Na^+]_{br}$ estimated by ^{23}Na MRI provides additional 'functional' parameters for assessing ischemic damage, which are not available from ADC data; and 2) the presence of collateral circulation in the cortex and its absence in the caudate putamen (CP) are not determinants of the rate of $[Na^+]_{br}$ increase ('slope') in the rat model of focal ischemic stroke.

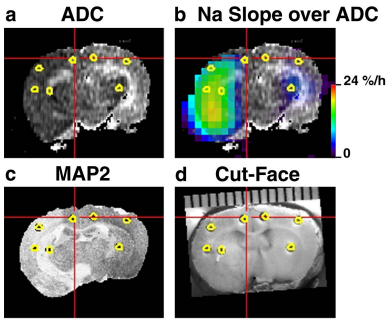

Fig. 1. ROI analysis of Na^+ accumulation and ADC deficit in a rat brain after MCAO. (a) ADC map with ischemic regions in the ipsilateral hemisphere (left-hand side of the image) appearing darker (ADC < 550 $\mu m^2/s$). (b) Parametric image of the rate of ^{23}Na signal increase ('slope') superimposed over the ADC map. (c) Cross-section of the 3D reconstruction of the brain from MAP2-stained brain slices. (d) Cut-face photograph of the brain in the cryostat showing punch holes after taking sampling for emission flame photometry. A millimeter scale is shown at the top. Cylindrical ROIs (shown in yellow) were placed over the punch holes. Calibration standards external to the rat head are not shown.

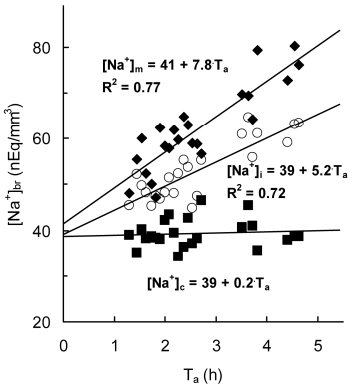

Fig. 2. $[Na^+]_{br}$ in the ROIs in ischemic cortex ($[Na^+]_i$, circles), homotopic normal cortex ($[Na^+]_c$, squares), and ROI of the maximum slope ($[Na^+]_m$, diamonds). T_a, time after MCAO.

METHODS

Ten normally fed male Sprague-Dawley rats weighing 320 ± 36 g underwent middle cerebral artery occlusion (MCAO). For ^{23}Na/^{1}H MRI, the animal's head was positioned inside a 5-cm-diameter, 5-cm-long dual-tuned dual-quadrature birdcage transmit/receive RF coil (2). Calibration standards (0-154 mM NaCl) were placed next to animal's head. Images were obtained on a 3 T GEMS scanner. ADC maps were reconstructed from ^{1}H diffusion-weighted multislice spin-echo images (*b*-factors of 0, 79, 314, and 707 s/mm^2). ^{23}Na MRI was performed using 3D twisted projection imaging (TPI) (3) with 398 projections, TR/TE of 100/0.4 ms, a voxel size of 0.48 mm^3, imaging time of 5.3 min, and the inhomogeneity correction of the B_1 field by RF mapping (4). After MRI (typically, 4.2 to 7.3 hours after MCAO), Na and K brain content was determined by emission flame photometry at 589 and 766 nm, respectively, in 12-18 0.5-mg brain samples, and by histochemical K^+ staining (5,6). The infarct size and location were verified by the change in surface reflectivity of ischemic tissue and MAP2 immunohistochemistry (7). The 40-µm thick coronal brain sections taken every 400 µm at different levels from bregma were digitized and registered to render volumetric reconstructions of the brain. MR and histological 3D images were aligned and analyzed in AMIDE (8). ROIs in the MR images were placed at the positions of punch holes (Fig. 1).

RESULTS AND DISCUSSION

ADC deficit and $[Na^+]_{br}$ after MCAO were analyzed in the ipsilateral and homotopic contralateral frontal cortex, parietal cortex and CP (Fig. 1). In agreement with earlier studies (1,9), $[Na^+]_{br}$ showed a linear increase in ischemic brain and no statistically significant changes in contralateral ROIs over time (Fig. 2). Within the boundaries of the infarct region (as defined by the ADC deficit, changes of surface reflectivity, and MAP2 staining), sites with an elevated rate of ^{23}Na increase (slope) were observed (Fig 1b), either in the cortex (n=8) or in CP (n=2). The maximum slope was 22%/h ± 4%/h (mean±SEM averaged over all animals), significantly higher than in other ischemic ROIs in the same brain, 14%/h ± 1%/h (P<0.005). Despite the difference in collateral circulation, the slopes in the ischemic cortex and CP were not different (P>0.3), as long as ROIs did not comprise the sites of maximum slope.

The ADC ratio of ipsilateral to homotopic contralateral ROIs was uniform over time and between different ischemic regions (Fig. 1a), ADC_i/ADC_c = 0.63±0.07 (mean±SD), P>0.7. The ADC deficit ADC_i/ADC_c and ^{23}Na slope in the same ROIs did not correlate both between animals (R^2 = 0.106, P>0.4) and within the same brain (R^2 = 0.003). In individual brains, the most ADC-depressed regions did not coincide with the 'hot spots' of Na^+ accumulation. These 'hot spots' (defined as 3D isocontour ROIs at the 90% level of the maximum ^{23}Na slope) were located, typically ventrally or caudally, near the stroke periphery in 7 out of 10 animals (Fig. 3). This observation is similar to the previously published data . Histochemical K^+ staining also showed a more severe decrease of $[K^+]_{br}$ at the edges of the ischemic region. Expression of water channel aquaporin 4 in rat pups (10) was also localized in the peripheral parts of the ischemic core. Supposedly edema is more severe at the edge of the ischemic region, driven by the more available trickle flow delivering more Na^+. It may be argued that these events together with elevated Na^+ influx mark the initial position of the early blood-brain barrier abnormalities leading to vasogenic edema.

CONCLUSIONS

The rate of $[Na^+]_{br}$ increase is independent of the tissue potential for collateral arterial supply. The maximum rate of $[Na^+]_{br}$ accumulation is at the periphery of the ischemic core, possibly, due to the slightly higher trickle flow in the edge regions. The slope differences within the ischemic area suggest that ^{23}Na MRI can characterize the ischemic damage. ^{23}Na MRI is related to regional metabolic changes, in particular, the functionality of Na/K-ATPase and other ion pumps, and thus provides physiological information not available by ADC.

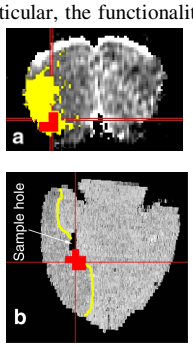

Fig. 3. Two examples of Na^+ accumulation (red ROIs) at the periphery of the focal stroke, as shown by ^{23}Na MRI. (a) Coronal image with the ischemic region (yellow mask) defined as ADC<550 $\mu m^2/s$ in the ipsilateral hemisphere. (b) Horizontal section of the 3D reconstruction from thin slice brain images, which was aligned with brain MRI. The ischemic region (outlined) was defined by the surface reflectivity changes. Red ROIs of maximum slope correspond to 13.5-15.0%/h (a) and 14.4-16.0%/h (b).

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