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Impact of aging on spreading depolarizations induced by focal brain ischemia in rats

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ABSTRACT

Spreading depolarization (SD) contributes to the ischemic damage of the penumbra. Although age is the largest predictor of stroke, no studies have examined age dependence of SD appearance. We characterized the electrophysiological and hemodynamic changes in young (6 weeks old, $n = 7$), middle-aged (9 months old, $n = 6$), and old (2 years old, $n = 7$) male Wistar rats during 30 minutes of middle cerebral artery occlusion (MCAO), utilizing multimodal imaging through a closed cranial window over the ischemic cortex: membrane potential changes (with a voltage-sensitive dye), cerebral blood volume (green light reflectance), and cerebral blood flow (CBF, laser-speckle imaging) were observed. The initial CBF drop was similar in all groups, with a significant further reduction during ischemia in old rats ($p < 0.01$). Age reduced the total number of SDs ($p < 0.05$) but increased the size of ischemic area displaying prolonged SD ($p < 0.01$). The growth of area undergoing prolonged SDs positively correlated with the growth of ischemic core area ($p < 0.01$) during MCAO. Prolonged SDs and associated hypoperfusion likely compromise cortical tissue exposed to even a short focal ischemia in aged rats.

1. Introduction

Ischemic stroke is a main contributor to disability worldwide and a leading cause of death (Chen et al., 2010). Stroke symptoms may appear at any age, but the vast majority of patients are aged. Sixty-five percent of patients who suffered stroke are more than 65 years, and the mean age of stroke is 71 years in the United States (Fonarow et al., 2010). Elderly patients have higher mortality and worse functional outcome because of comorbidities, lower regenerative capacity, and age-related cardiovascular dysfunction (Allen and Bayraktutan, 2008; Fonarow et al., 2010; Knoflach et al., 2012; Ungvari et al., 2010; Weimar et al., 2004). A major clinical study showed that the conversion of ischemic tissue into infarction is accelerated with age (Ay et al., 2008; Copen et al., 2001), and this observation was repeated in an experimental stroke model in rats (Petcu et al., 2008).

Spreading depolarization (SD) is a sustained dramatic loss of cellular membrane potential, which disrupts cellular and extracellular ion homeostasis, causes cellular swelling, and requires tremendous energy from the cell to restore its normal ion balance and membrane potential. Normally, the increased metabolites required to supply this energy are provided by elevated local cerebral blood flow (CBF) mediated via an intact neurovascular coupling. SD events commonly appear in regions surrounding focal ischemia (Hossmann, 1996). When SDs inundate metabolically compromised tissue, they can promote total cellular energy collapse, exacerbating ischemic damage and contributing to early brain infarct development (Dijkhuizen et al., 1999).

SDs during ischemia are associated with various types of hemodynamic responses that are largely dependent on the distance from the ischemic core and on the animal species studied (Ayata et al., 2004; Luckl et al., 2009; Shin et al., 2006; Strong et al.,

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2007). When SDs are coupled with sustained hypoperfusion (the underlying mechanism termed inverse neurovascular coupling) in areas closer to the core, this reduction of local blood flow superimposed on ischemia is suggested to contribute to SD-related injury (Shin et al., 2006). In clinical studies, the frequency of SDs and the concomitant inverse hemodynamic response highly correlated with mortality and poor neurologic outcome after ischemic brain damage, traumatic brain injury, and subarachnoid hemorrhage (Dohmen et al., 2008; Dreier et al., 2009; Fabricius et al., 2006). No clinical study has systematically investigated the relationship between depolarization events, hemodynamic responses, and age. Previously, we have shown that topical potassium (K^+) elicits fewer recurrent SDs in the cortex of healthy aged rats compared with young rats (Farkas et al., 2011). Considering the high incidence of stroke in elderly patients and the importance of SDs in the pathophysiology of stroke, we identified and described the emerging SDs and the coupled local CBF changes in a large ischemic area of young, middle-aged, and old rats during and after a short transient middle cerebral artery occlusion (tMCAO). As age broadly impacts neurovascular health, we expected that the appearance and length (transient or prolonged) of spontaneous SDs and the accompanying hemodynamic response during ischemia differ with age. We used a multimodal imaging technique combined with a unique custom-made matrix analysis of the recorded signal over a large field of the parietal cortex including parts of the ischemic core (<20% of baseline CBF) and the penumbra (20%–60% of baseline CBF).

2. Materials and methods

2.1. Surgical procedures

Procedures were approved by the Ethical Committee for Animal Care at the University of Szeged. Detailed descriptions of similar surgical preparations were previously published (Clark et al., 2012; Farkas et al., 2008), with modifications. Male Wistar rats of 3 age groups were compared: young (6 weeks, $n = 7$), middle-aged (9 months, $n = 6$), and old (2 years [23–25 months], $n = 7$). Rats were anesthetized with halothane (2.5% during surgery and 1.5% during imaging) in N_2O:O_2 (2:1), and animals were allowed to breath spontaneously throughout the experiment. To avoid the production of airway mucus, rats were pretreated with 0.05 mL atropine (0.1%) intramuscularly. Body temperature was maintained at 37.0 ± 0.2 °C with a servo-regulated heating pad, and the tail artery was cannulated for monitoring of mean arterial blood pressure.

Both common carotid arteries were delicately separated from the surrounding tissue, including the vagal nerve, through a midline incision in the neck. A long silk suture was placed around each artery. Rats were transferred to a stereotactic frame and fixed in the prone position. A cranial window (~4 × 4 mm) was prepared over the right parietal cortex. A second smaller window (2 × 2 mm) was drilled on the temporal bone over the distal MCA. In both cases, the bone was carefully thinned using a water-cooled drill (Technobox, Bien Air 810) and gently peeled away to reveal the dural surface. A ring of dental cement was built around the edge of the parietal craniotomy, incorporating a sealed inlet and outlet tubes. The resultant chamber was filled with artificial cerebrospinal fluid (aCSF, 126.6 NaCl, 3KCl, 1.5 $CaCl_2$, 1.2 $MgCl_2$, 24.5 $NaHCO_3$, 6.7 urea, and 3.7 glucose [with concentrations in millimolar], bubbled with 95% O_2 and 5% CO_2 to achieve a constant pH of 7.45), and the dura was gently dissected. The window was then sealed with a fitted glass coverslip, using 2-component glue (UHU GmbH & Co, KG, Bühl, Germany). The closed window was continuously perfused with aCSF at a rate of 25 μL per minute, unless otherwise specified. In the lateral window, only a small slice of dura was gently removed over the trunk of the distal MCA, to allow access for clipping of the vessel. The temporal window was kept moist with warmed aCSF.

2.2. Experimental paradigm and ischemia induction

The cortical tissue underlying the closed cranial window was loaded with a voltage-sensitive (VS) dye (RH-1838; Optical Imaging Ltd, Rehovot, Israel), as described in detail elsewhere (Farkas et al., 2010). This dye binds to the cell membrane and increases in fluorescence in response to reduced cellular transmembrane potential (Grinvald and Hildesheim, 2004). The VS-dye concentration was adjusted in such a way that, after 20× dilution, the optical density measured at 580 nm with a spectrophotometer was between 0.110 and 0.130 (Farkas et al., 2008). Diluted VS dye in aCSF was circulated over the exposed cortex (80 μL per minute) for 1 hour, followed by an aCSF rinsing for 1 hour. A laser diode, a green light-emitting diode (LED) light source, and 2 charge coupled device cameras were set up for multimodal imaging. The experimental paradigm is shown in Fig. 1A. Image acquisition was initiated, and a 10-minute baseline period was recorded. To induce focal cerebral ischemia of the cortex, a small microaneurysm clip was placed (Sundt AVM Microclip, Codman) gently around the most proximal branch of the distal MCA visible through the temporal craniotomy. Immediately after the application of the clip, both common carotid arteries were transiently occluded by gently pulling on the long silk sutures looped around them until a small tension was detected. The sutures were secured in place. After a period of 30 minutes, the microclip was removed and both carotid sutures were released to allow for reperfusion. Images were captured for an additional hour, after which rats were euthanized by an injection of a 5-mL bolus of air into the tail artery that stopped the heart. Ten minutes of biologic zero were recorded for all parameters after death.

2.3. Multimodal imaging of the cortex

A multimodal imaging system, developed in our laboratory and described in detail in the earlier publications (Farkas et al., 2008, 2010), was used to investigate changes in cellular membrane potential, using a VS dye, and associated hemodynamic responses in the cortical area of interest. To capture VS fluorescence images, the cortical surface was illuminated with a flashing (1-Hz, 100-ms pulse length) red high-power LED (625 nm peak wavelength, SLS-0307_A; Mightex Systems, Pleasanton, CA, USA) fitted with a 620- to 640-nm excitation filter (3RD620-640; Omega Optical Inc, Brattleboro, VT, USA). Regional changes in CBF were assessed with laser-speckle contrast imaging: the cortex was intermittently illuminated with a laser diode (Sanyo DL7140-201S, 70 mW, 736 nm emission wavelength), and CBF maps were calculated from the obtained raw speckle images as previously described (Obrenovitch et al., 2009). Intrinsic optical signal (IOS) was investigated by recording the reflected light evoked under 540–550 nm green high-power LED illumination (pulse length: 100 ms). At this wavelength, the molar extinction coefficients of both oxyhemoglobin and deoxyhemoglobin are similar; so, the IOS primarily indicates changes in local cerebral blood volume, independent of oxygen saturation (Farkas et al., 2008). However, green IOS is also altered by changes in extracellular volume associated with SD (Ba et al., 2002; Dahlem and Hanke, 2005). The different LEDs and the laser diode were arranged around the cranial window in such a way as to ensure homogeneous illumination across the field of view with each light source.

Images of the cortex were captured at an effective frame rate of 1 Hz using 2 monochrome charge coupled device cameras (1024 × 1024 pixel max resolution; Pantera 1M30, DALSA, Gröbenzell, Germany), as described in Farkas et al. (2010). Both cameras were