The impact of aging on spreading depolarization in the intact and ischemic rat brain

Ph.D. Thesis

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Introduction

Aging emerges as a major independent risk factor for the incidence and prevalence of ischemic stroke and predicts poor patient outcomes. In our aging population, much of the primary injury in the acute phase of ischemic stroke may prove irreversible, yet the management of secondary pathophysiological processes is of fundamental importance to improve the prospect of successful recovery. Spontaneously occurring recurrent spreading depolarizations (SDs) were recognized as contributors of the expansion of cerebral tissue damage following subarachnoid hemorrhage, stroke or traumatic brain injury patients. Furthermore, waves of SD were shown to evolve minutes after the onset of focal ischemia in the rat brain. The observation that SD appearance for days after the acute infarction was associated with delayed neurological deficits has led to promote SDs as causal biomarkers for the estimation of tissue metabolic failure in neurocritical care.

Spreading depolarization is a slowly propagating wave of near complete sustained neural and glial depolarization followed by a temporary suppression of brain electrical activity. The main electrophysiological features of SD are the transient negative shift of the slow cortical or direct current (DC) potential and the silencing of neuronal activity after the passage of depolarization, the latter known as cortical spreading depression (Fig. 1).

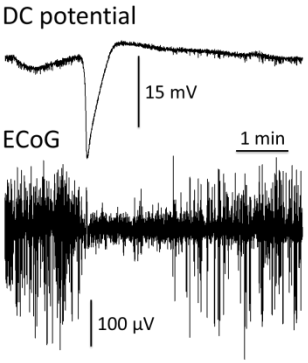

Figure 1. Electrophysiological features of spreading depolarization. Upper trace, negative shift of direct current (DC) potential, termed spreading depolarization (SD). Lower trace, transient suppression of the electrocorticogram (ECoG) called spreading depression of activity.

SDs are currently believed to exacerbate ischemic brain injury via related atypical hemodynamic responses. The cerebral blood flow (CBF) response to SD consists of at least four sequential elements, which are achieved by a finely regulated balance between vasoconstriction and vasodilation. In the intact brain, of the four separate CBF response components identified, the dominant hyperemic element, which is prominent vasodilation, evolves most reliably. In the injured brain, the CBF response to SD may undergo a gradual transformation to uncover ruling vasoconstrictive elements. This transformation represents diminishing hyperemia synchronous with hypoemia becoming increasingly more obvious. In the most severe form, the hypoemic element completely overrules hyperemia, which has become known as spreading ischemia. Prolonged SDs with spreading ischemia were recognized to produce the expansion of ischemic injury and induce widespread necrosis in animal models.

Extracellular pH changes with SD contain a transient shift from 7.35 to 6.95 in the intact rodent cortex. This relatively mild, brief acidosis by itself will not harm neurons, but could be crucial for neuronal loss or survival when repeatedly superimposed (i.e. recurrent SDs) on ischemia-induced acidosis characterized by pH values around 6.2-6.8. It is, therefore, reasonable to propose that there is indeed such an additive acid load, which would be an indicator of or, more importantly, a contributor to the SD-related metabolic crisis and related neurodegeneration in ischemic tissue.

The impact of age on stroke pathophysiology has been the target of intensive research in order to understand the reason for the increased susceptibility of the aged brain to stroke-related injury. Aging was associated with the increased conversion of penumbra into infarction in patients, more severe ischemia-related neurological impairment in old mice, and accelerated infarct development and neuronal degeneration in old rats. Although SD may be implicated in all these events, the impact of aging on SD evolution, and the potential role SD might play in the age-related worsening of stroke outcome have remained largely unexplored.

Based on the above, we hypothesized, that;

(I) SDs are more injurious in the aged brain because the associated CBF response is impaired;

(II) The occurrence of recurrent SDs in the ischemic cortex is building up acid load to a level which has been recognized to cause tissue damage;

(III) The SD –related tissue acidosis is graver in the aged ischemic brain, which could contribute to more extensive ischemic lesions.

In order to prove the presented hypotheses, the following aims were formulated;

(I) To determine the impact of age on the evolution of SD and the kinetics of the associated changes in local CBF in the intact and ischemic rat brain.

(II) To assess the degree of SD-related acidosis in the young and aged ischemic cortex of the rat, with respect to the intact condition.

Materials and methods

Experimental Project I

Male Sprague-Dawley or Wistar rats were anesthetized with halothane or isoflurane in N_2O:O_2. Transient global forebrain ischemia was induced by bilateral occlusion of the common carotid arteries (2VO) for 40 min in young (8-9 weeks old, n=8) and old (2 years old, n=6) animals. Sham-operated rats served as control (n=6). DC potential and CBF were acquired via a small craniotomy above the parietal cortex. SD was elicited by topical application of 1 or 3M KCl through a second craniotomy distal to the recording site.

Experimental Project II

Open or closed cranial windows were mounted on the parietal bone of isoflurane-anesthetized 2-months- or 18-20-month-old Sprague-Dawley rats. SD-related variations in extracellular pH together with changes in local cerebral blood flow were acquired with pH-sensitive microelectrodes and laser-Doppler flowmetry (n=17). In the imaging series of experiments, SD-coupled intracellular pH- and perfusion changes were monitored relying on the fluorescence intensity of a pH indicator dye (Neutral Red), and laser speckle contrast analysis (LASCA) (n=20). After a baseline period of 50 min, transient ischemia was achieved by 60 min of bilateral common carotid artery occlusion, followed by 50 min reperfusion. SDs were elicited by topical application of KCl to the caudal cranial window or by microinjection of 1 µl of 1M KCl into the closed cranial window at 15 min intervals prior, during, and after ischemia.

Results

Experimental Project I

The DC potential signature of SD

Ischemia elongated the duration of the SD-related negative DC shift (66.2 ± 22.8 vs. 21.4 ± 4.1 s, Young 2VO vs. Young control), which was augmented further by age as seen in the Old 2VO group (95.8 ± 46.2 s). The ischemia-related, elongated duration of SDs was also reflected in the reduced slopes of depolarization (14.5 ± 11.4 vs. 4.4

± 2.9 mV/s, young 2VO vs. young control) and repolarization (0.7 ± 0.5 vs. 2.6 ± 0.7 mV/s, young 2VO vs. young control). Old age was associated with a reduced slope of depolarization, as well (1.9 ± 0.9 vs. 2.6 ± 0.7 mV/s, old control vs. young control). Finally, the amplitude of depolarization and repolarization tended to be smaller in the ischemic groups, and older age was associated with a reduced amplitude of repolarization (2.9 ± 2.9 vs. 5.1 ± 1.2 mV, old control vs. young control).

Laser-Doppler recording of CBF response to SD

Six types of SD-coupled CBF responses were identified, ranging from dominating hyperemia to prolonged cortical spreading ischemia with intermediate forms. Spreading ischemia evolved only in the aged ischemic group (4 of the 6 animals). Quantitative analysis of the duration of early hypoperfusion indicated that this first element of the CBF response to SD was elongated during ischemia (36.8 ± 17.5 vs. 7.9 ± 6.8 sec, Young 2VO vs. Young control) and became drastically longer in the Old 2VO group (1344 ± 1047 sec), due to the prevalence of spreading ischemia.

Experimental Project II

The pH-sensitive microelectrodes applied in our study revealed three subsequent phases of pHe variations associated with SD: (i) a brief, initial acidic shift immediately followed by (ii) a rapid, short alkaline

shift, and (iii) a final, longer-lasting, dominant, transient acidosis. The latter, dominant acidosis has become the focus of our quantitative data analysis.

In all experiments, each SD in the intact and ischemic cortex was accompanied by a highly reproducible, transient elevation of NR fluorescence intensity, which was somewhat diffuse (i.e. no sharp wave front), but propagated across the field of view discernably from the site of SD elicitation in a radial fashion. The kinetics of the increase of NR fluorescence intensity with each SD was comparable to that of the dominant tissue acidosis acquired with pH-sensitive microelectrodes.

Enhanced tissue acidosis associated with spreading depolarization in the ischemic cortex

Under ischemia, acidosis associated with evoked SDs was substantially augmented. This was reflected by the greater relative amplitude with respect to baseline SDs (for young animals, microelectrode: 0.43 ± 0.15 vs. 0.36 ± 0.07 pH units; NR imaging: 0.37 ± 0.18 vs. 0.23 ± 0.10 ΔF/F); the longer duration (for young animals, microelectrode: 93.1 ± 26.3 vs. 40.2 ± 8.1 s; NR imaging: 127.5 ± 64.2 vs. 39.8 ± 13.8 s), the greater magnitude expressed as area under the curve (for young animals, microelectrode: 2415 ± 869 vs. 855 ± 322 pH unit x s; NR imaging: 57.4 ± 50.9 vs. 9.8 ± 5.9 ΔF/F x s), and the slower recovery from acidosis (for young animals, microelectrode: 0.44 ± 0.33 vs. 0.70 ± 0.23 pH units/s; NR imaging:

0.004 ± 0.002 vs. 0.009 ± 0.005 ΔF/F /s). During reperfusion, the quantitative measures of SD-related acidosis returned to near pre-ischemic values (e.g. for young animals, relative amplitude 0.31 ± 0.09 vs. 0.36 ± 0.07 pH units; duration: 42.0 ± 8.0 vs. 40.2 ± 8.1 s).

Aging also had noticeable impact on the pH transients with SDs. Both pH-sensitive microelectrodes and NR imaging indicated consistently that the recovery from acidosis was significantly slower in the old group as compared with the young during baseline (electrophysiology: 0.47 ± 0.24 vs. 0.70 ± 0.26 pH units/s; NR imaging: 0.006 ± 0.003 vs. 0.009 ± 0.005 ΔF/F /s) and ischemia (electrophysiology: 0.23 ± 0.12 vs. 0.44 ± 0.005 pH units/s; NR imaging: 0.002 ± 0.001 vs. 0.004 ± 0.002 ΔF/F /s).

Aggravation of ischemia-induced tissue acidosis by spontaneous spreading depolarization

The evaluation of spontaneous SDs delivered, perhaps, the most revealing findings of the present study. A single spontaneous SD event occurred within 2 min after ischemia induction in a total number of 15 out of 33 experiments. The consequences of spontaneous SDs were grave. The SD-related acidosis was superimposed on ischemia-induced acidosis thereby transiently decreasing pHe from the ischemia-related value of 6.93 ± 0.09 to as low as pH 6.48 ± 0.16 in the young group, and from the ischemia-caused pH 7.06 ± 0.10 to 6.76 ± 0.20 in the old animals. NR imaging in the old group showed that the occurrence of spontaneous SD more

than doubled NR fluorescence intensity with respect to that achieved by ischemia alone. Tissue pH in young animals measured over 10 min after spontaneous SD settled to a value more acidic than that produced by ischemia alone at a corresponding point of time (pH 7.09 ± 0.09 vs. 7.29 ± 0.16), which was further worsened by age, as pHe after spontaneous SD was maintained at an average of pH 6.94 ± 0.08 in the old animals.

Discussion

The recovery of resting membrane potential after depolarization is delayed during ischemia and in old age

Old-age aggravated the ischemia-related prolongation of SD duration: 1 of the 6 animals in the old 2VO group displayed terminal depolarization, and the duration of transient first SDs was significantly longer as compared with the young 2VO group. During ischemia, oxidative substrate supply declines, and tissue ATP availability decreases, which lead to the reduction of Na^+/K^+-ATPase activity, thereby potentially delaying the restoration of transmembrane potential. These results stand in agreement with our previous observation made in a focal forebrain ischemia model that the cortical surface involved in prolonged SDs was significantly larger in old as compared with young rats. These data together indicate that the aged ischemic brain has scarcer resources to recover

from an SD event, which reflects the increased vulnerability of the aged brain to ischemia- and/or SD-related injury.

The hyperemic response to spreading depolarization diminishes during ischemia and becomes inverted in the aged ischemic brain

Our main observations of the SD-related CBF response are as follows: (1) the SD-coupled CBF response types, in which the hypoemic component is augmented at the expense of the hyperemic component, occur more frequently in the aged brain, (2) the combination of ischemia and old age predisposes the cortex for the evolution of spreading ischemia (inverse neurovascular coupling), and (3) the ischemia-related perfusion deficit progressively deepens in the old brain as opposed to the young. The high incidence of inverse coupling in the old ischemic rats (also seen in our previous study concerning focal cerebral ischemia) is proposed to be determined by the combination of vasoconstrictive high $[K^+]_e$ and the restricted availability of the vasodilator nitric oxide (NO). Ischemia itself imposes considerable extracellular K^+ accumulation above the dilation and/or constriction threshold (20 mM), and NO is quickly eliminated by its reaction with superoxide yielding peroxynitrite. Ischemia superimposed on aging, therefore, is thought to potentiate the impairment of NO-based vasoregulation in the face of high $[K^+]_e$, which may lead to a higher incidence of inverse neurovascular coupling in the aged ischemic brain. In turn, the resultant spreading

ischemia is suggested to deepen the perfusion deficit imposed by ischemia.

Spreading depolarization during ischemia and associated tissue pH variations

Brain pH measurement has delivered three novel observations, with neurological consequences that are anticipated to be significant: (i) The SD-related acidosis is remarkably enlarged in the ischemic as compared with the intact cortex; (ii) SD-related acidosis evolving in the ischemic cortex is additive to ischemia-induced acidosis.; (iii) In the aging brain, the recovery from SD-related acidosis is hampered, and tissue pH subsequent to SD remains more acidic with respect to the young cortex.

The intensification of SD-related acidosis under ischemia has been characterized here by the higher relative amplitude and longer duration of the acidic pH transients. Previous observations suggest, that tissue acidosis associated with SD in the intact cortex must depend on the concentration of lactate produced, because pHe was shown to decrease synchronously with the elevation of lactate. Based on the evidence listed here, we propose that the greater amplitude of SD-related acidosis in ischemic with respect to the intact cortex must be caused by the increased accumulation of lactate. Because lactate is generally the product of anaerobic metabolism, we speculate that lactate concentration, and thus the relative peak of acidosis associated with SD depends on whether the tissue can initially utilize

aerobic metabolic pathways in response to SD, or relies largely on anaerobic metabolism setting already in before SD generation, because of ischemia. The longer duration of SD-related acidosis and the underlying lactate production during ischemia, on the other hand, possibly correlate well with delayed repolarization, thus with the continuing demand for energy.

In addition to the finding that acidosis with SD is markedly enhanced in the ischemic cortex, we have also demonstrated here that the SD-related acidosis is additive to ischemia-induced acidosis, especially with spontaneous SDs. Our global forebrain ischemia model created conditions similar to the ischemic penumbra, in that CBF dropped below 40 %, and remained between 20-40 % until reperfusion was initiated. Prior to the detection of the propagating spontaneous SD, pHe ranged between pH 6.9-7.0 in the cortex, similar to pHi that was calculated for the penumbra region after middle cerebral artery occlusion (MCAO). Acidosis associated with SD here shifted pHe to as low as pH 6.48 in average, more typical of the ischemic core of focal insults. Taken that SDs occur in a recurrent fashion in ischemic zones, and that tissue pH remains acidic for at least 10 min after an SD event propagating under penumbra-like conditions, SDs are suggested to prolong tissue acidosis, thereby also increasing the risk of neuronal injury. The spontaneous generation of SD was more frequently encountered in the old animals. Here we show that SD occurs spontaneously when the perfusion deficit shortly after

ischemia onset is severe (CBF drop to between 7-23 %, albeit measured distant to the exact site of SD generation), which was encountered more frequently in old animals. In summary, our results reveal that in the aged brain the occurrence of SD is facilitated because the perfusion deficit is graver. Moreover, we propose, that the aging brain may be at higher risk for acid-induced neurodegeneration, because tissue pH after the passage of an SD remains significantly more acidic with respect to the young brain.

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