The impact of aging on pial arteriolar reactions and cerebrocortical blood flow variations with spreading depolarization

Doctoral Thesis

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Introduction

Spreading depolarization (SD) was described by Leão in 1944 but the mechanisms and its related effects form the focus of intensive research to this day. The role of SDs is fundamental in the pathophysiology of different neurovascular diseases like migraine with aura, ischemic stroke or haemorrhagic stroke. SD corresponds to the near complete breakdown of the resting membrane potential of a critical mass of neurons, which propagates over the cerebral gray matter at a slow rate (2-8 mm/min). This is coupled with a robust but transient suppression of the electrocorticographic activity known as spreading depression of activity. The restoration of the membrane potential requires the mobilization of metabolic resources for the operation of ATP-dependent ion pumps (e.g. the Na+/K+ ATP-ase), which need is met by a profound hyperemic cerebral blood flow (CBF) response coupled to SD in otherwise non-compromised tissue. The coupling between SD and local CBF becomes impaired under ischemia, which may create a metabolic supply-demand mismatch, a subsequent energy crisis superimposed on prevailing ischemia, and facilitated tissue injury.

There are four characteristic components of SD-related hyperemia: first an initial brief hypoperfusion, second a marked, transient peak hyperemia, third a less obvious late hyperemia, and fourth a sustained hypoperfusion (it is also called post-SD oligemia). In physiological

conditions the hyperemic component is the most pronounced in the young brain, while it becomes shorter and negligible during ischemia, where the hypoperfusion is the dominating component. In addition to the SD-related CBF variation, the hemodynamic response propagating with SD is obvious at the level of the pial and penetrating arterioles.

With aging, SDs propagating over the ischemic cerebral cortex appear to be increasingly more harmful. Repolarization may be delayed in the aging cortex because recovery from SD-related acidosis is considerably hampered by age and the SD-related hyperemia becomes insufficient and often seriously impaired (i.e., spreading ischemia). All of these together have been considered to indicate or promote the conversion of the ischemic penumbra to the irreversibly damaged core region, a pathophysiological process accelerated in the aged brain. Despite the age-related weakening of hyperemia in response to SD, there is no evidence that the intensity of the underlying depolarization would proportionally be smaller. This raises the assumption that age weakens the coupling between SD and the associated hyperemia.

Traditionally, SD events are recorded with a single or an array of microelectrodes. In addition, ion translocations or the metabolic condition of the tissue may be followed with ion-sensitive microelectrodes. Although the use of electrodes offers excellent temporal resolution of the signal, the investigation of the spatial

evolution of SD and the associated metabolic changes requires the application of imaging techniques that offer high spatial resolution.

In the service of our research efforts to tackle the coupling between SD and the associated CBF response, we have developed an experimental, multi-modal wide-field optical imaging system that offers an appropriate spatio-temporal resolution for studying SD. The combination of two of the synchronous modalities, laser-speckle contrast analysis (LASCA) and intrinsic optical signal (IOS) imaging at green light illumination offers a unique opportunity to examine CBF changes synchronous with variations of pial arteriolar caliber, and pial arteriolar architecture. Finally, we established tissue pH imaging in our preparations with the use Neutral Red (NR), a vital dye that indicates changes of intracellular pH (pHi). We adapted NR imaging to monitor pHi changes with SD in the rat cerebral cortex, and combined it with LASCA-based CBF imaging to directly relate tissue pH changes to CBF variations.

Based on the above, we sought to evaluate how ischemia or age would influence the perceived association between SD, CBF and tissue acidosis.

Accordingly, our goals were:

- to evaluate whether the dilation of pial arterioles in response to SD is impaired by aging;

- to investigate whether the density and resting diameter of the pial vascular network might be subject to aging;
- to determine whether the spatial pattern of flow distribution during SD propagation in the cerebral cortex is altered by aging;
- to systematically explore meaningful associations between the kinetics of the typical direct current (DC) potential signature of SD, the related variations in tissue pH, and the hyperemic component of the CBF response.

Materials and methods

Series 1: Young (n=20, 2 month-old) and old (n=18, 18-20 month-old), male, isoflurane-anesthetized, spontaneously breathing Sprague-Dawley rats were used in the imaging in vivo project. After a cervical incision, silicone-coated fishing lines were carefully looped around both common carotid arteries as an occluder for the later induction of acute, incomplete, global forebrain ischemia (2-vessel occlusion, 2VO). A closed cranial window (4.5x4.5 mm) was built over the parietal cortex. After the surgical procedures, we have collected data over three subsequent phases of the experiments: During the baseline period (50 min), the cerebral perfusion was intact. Next, acute global forebrain ischemia was induced by 2VO (1 hour). Finally, a reperfusion period (1 hour) followed, initiated by the release of the

common carotid arteries. 3 SD-s were elicited with an interval of 15 min (1 µl, 1M KCl) during each phase of the experiments. Local changes of signal intensity (IOS and LASCA) were extracted as time series from each modality by placing regions of interests (ROI) of ~70x70 µm on the selected blood vessel-free cerebrocortical parenchyma. The vascular density of the pial surface in the field of view and the diameter changes of pial arterioles as time series were calculated by using the green IOS images. The CBF maps were used for a deeper analysis of spatial CBF distribution, too.

Series 2: A set of additional animals (n=17) were examined under the same experimental protocol using electrophysiological data acquisition. Two craniotomies were drilled on the parietal bone. The caudal window served the elicitation site for SDs with the topical application of 1 M KCl. A laser doppler probe, a pH-sensitive microelectrode and a reference DC potential electrode were positioned in the rostral cranial window. Experiments were selected for data analysis based on the quality of the recordings: experiments in which all synchronous variables (i.e., DC potential, tissue pH, and CBF) were of high quality to allow reliable quantitation were processed. The duration and relative amplitude of the negative DC potential shift indicative of SD, of the related acidosis, and of hyperaemia were measured.

Results

Series 1

Vascular architecture: Approximately half of the cortical surface was covered by pial vessels equally in both young and old rats.

Changes of pial arteriolar diameter and CBF: Event-free segments of the recording: Resting pial arteriolar diameter taken before the elicitation of the first SD obviously decreased along the branching order significantly (83±16, 51±18 and 31±9 μm, 1st, 2nd and 3rd order vessels in the young group *F=20,951, $p<0.0001$), but was similar in the two age groups. The induction of ischemia in the young group was followed by a prompt diameter reduction in 1st and 2nd order arterioles (to 89±12 and 93±15 %, respectively), while the caliber of 3rd order arterioles did not change (101±16 %), and subsequently increased their caliber above the baseline tone (107±15 and 112±19 %, 10 min after ischemia onset and shortly before reperfusion). During reperfusion, pial arteriolar diameter settled over baseline at all three levels of the pial arteriolar tree (111±8, 106±9 and 113±9 %, 1st, 2nd and 3rd order vessels). In the old animals, diameter changes of 1st and 2nd order arterioles were similar to that observed in the young group. However, 3rd order arterioles appeared less able to dilate under ischemia.

Variations of local CBF were estimated at a ROI most distant from the site of SD elicitation. The occlusion of the common carotid arteries caused a sudden drop of CBF (to 16±8 and 15±9 % of baseline, young and old). Next, flow distribution within the field of view at selected sampling times was evaluated. The flow ranges displayed a normal distribution at rest. The flow distribution was clearly skewed to low perfusion ranges shortly after ischemia onset. Finally, reperfusion resulted in the widest flow distribution, and the most remarkable difference in the spatial pattern of CBF between the young and old groups. In the young animals, nearly half of the field of view was occupied by perfusion ranges between 31-70 % CBF, the 41-50 % CBF range being just about the most represented (13±11 % of the total area). In contrast, in the old rats, approximately half of the visible cortex was involved in the perfusion ranges of 71-110 % CBF, and the 91-110 % CBF range engaged the largest area (13±6 % of the total area).

Changes of pial arteriolar diameter and CBF: Response to SD: Because diameter changes of pial arterioles were reproducible in response to baseline SDs only, the detailed analysis focused on this first phase of the experiments. The first SD was coupled with obvious, transient vasodilation (to 124±15, 125±26 and 122±18 %, 1st, 2nd and 3rd order arterioles in young) and subsequent vasoconstriction (to 93±6, 94±5 and 95±3 %, 1st, 2nd and 3rd order arterioles in young)

corresponding to considerable transient hyperemia (209±30 % CBF in young) and successive oligemia (65±22 % in young). Likewise, recurrent SD events (elicited at a time when oligemia caused by the first SD was still persistent) were associated with an increase of pial arteriolar diameter (to 123±11, 125±23 and 129±27 %, 1st, 2nd and 3rd order arterioles in young) and marked hyperemia (198±44 % CBF in young). Age exerted a significant impact on cerebrovascular reactivity to SD. Pial arteriolar dilation proved to be less remarkable with the first SD (1st, 2nd and 3rd order arterioles: 114±10 vs. 124±15, 107±9 vs. 125±26 and 109±9 vs. 122±18 %, old vs. young,) and recurrent SDs (1st, 2nd and 3rd order arterioles: 113±7 vs. 123±11, 109±9 vs. 125±23 and 111±6 vs. 129±27 %, old vs. young). Accordingly, in the old animals, the peak of hyperemia in response to SD fell behind (first SD: 174±33 vs. 203±33 %, old vs. young; recurrent SDs: 182±57 vs. 198±44 %, old vs. young), as reported earlier. While the analysis of perfusion ranges offered only trends (e.g. the most represented perfusion ranges for recurrent SDs were 141-160 % in young and 121-140 % in the old), the histograms provided more refined information. As such, the peak of the histogram fell at a significantly lower CBF value in the old than in the young group (137±35 vs. 167±28 %, old vs. young), indicating a shift to the lower perfusion ranges on the CBF axis.

Series 2

The amplitude of the negative DC potential shift corresponding to SDs fell on a continuum, ranging between 0.99 and 19.44 mV. For the evaluation of the metabolic consequences of SDs, the association between the amplitude and duration of the DC potential shift, related acidosis, and hyperemia was analyzed in detail. During baseline, in young animals, the amplitude of all three variables was tightly coupled (e.g., DC potential shift and hyperemia: $r = 0.819$, $^{*}p < 0.05$), whereas their duration appeared to be unrelated to each other (e.g., DC potential shift and hyperemia: $r = 0.176$).

Under ischemia, the amplitude of hyperemia dissociated from the amplitude of both the DC potential shift and acidosis ($r = 0.236$ and $r = 0.429$, respectively) and remained uncoupled during reperfusion as well. The durations of the three variables independent under baseline became interrelated over the ischemic phase (e.g., DC potential shift and acidosis: $r = 0.935$, $^{**}p < 0.01$) and lost correlation again during reperfusion. Old age uncoupled the amplitude of hyperemia from that of the DC potential shift and acidosis ($r = 0.221$ and $r = 0.249$, respectively), whereas it left the amplitude of the DC potential shift and acidosis strongly correlating ($r = 0.998$, $^{**}p < 0.01$) comparable to young age. Finally, aging dissociated the duration of acidosis from the duration of the DC potential shift and hyperemia under ischemia ($r = 0.482$ and $r = 0.286$, respectively), whereas it did not alter the

baseline association between the length of the DC potential shift and hyperemia ($r = 0.657$, *$p < 0.05$). Remarkably, the duration of acidosis was relatively shorter than the duration of hyperemia in young animals but exceeded the duration of hyperemia in old animals. Finally, it is noteworthy that the relative duration of hyperemia (compared to the length of DC potential shift in the same experimental phase) was gradually decreasing (195% to 172% to 167%, young baseline to young ischemia to old ischemia, respectively), whereas the relative duration of acidosis was increasing with ischemia and age (127% to 140% to 192%, young baseline to young ischemia to old ischemia, respectively).

When SD events evoked during baseline were sorted on the basis of DC potential shift amplitude (i.e., <5 mV and >5 mV), tissue pH proved to be significantly more acidic before SDs with small DC potential shift amplitude (pH 7.20 ± 0.04 vs. 7.31 ± 0.03). In further support, a strong positive correlation was established between tissue pH before SD and the DC potential shift amplitude with SD ($r = 0.909$, **$p < 0.01$), which was abolished by ischemia ($r = 0.244$) and reestablished during reperfusion ($r = 0.739$, *$p < 0.05$).

Conclusion

The incidence of acute ischemic stroke doubles every 5 years over 50 years of age, and the conversion of the ischemic penumbra tissue to the infarction is accelereated at old age, as well. Overall, ischemic

stroke occurs exponencially more frequently with advancing age in the elderly population, and leads to worse neurological outcome. These circumstances highly encourage investigations to explore the impact of age on the pathophysiology of ischemic stroke, to understand relevant age-related maladaptive changes in the nervous tissue or the cerebrovascular system that pose a higher risk and lead to poor outcome in elderly stroke patients. Here we have focused on the impact of age on cerebrovascular and metabolic changes associated with SD, because SD has been understood as the principal mechanism of lesion progression in acute brain injury. We have found that:

(i) The density and resting tone of the pial and cortical penetrating cerebrovascular network is preserved with aging. However, the capacity of pial arterioles to dilate in response to SD has been found weaker in the old compared to the young rat brain, suggesting an age-related impairment of neurovascular coupling.

(ii) Our experiments identified an age-related shift to a greater representation of higher flow ranges in the reperfused cortex, which carries the risk of more severe ischemia/reperfusion injury in the old brain, due to more obvious oxidative stress.

(iii) Finally, we have shown that aging disproportionately increases the duration of tissue acidosis with SD. This is of importance because the prolongation of acid exposure is

understood to lower the threshold of acidosis induced cell death, which may be thus more prominent in the aging brain.

In conclusion, we have shown that the age-related impairment of the cerebrovascular system and an increased metabolic burden imposed by SD may contribute to the worsening outcome of ischemic stroke in elderly patients.

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Articles to serve as the basis of the thesis

I. Ákos Menyhárt, Dániel Zölei-Szénási, **Tamás Puskás**, Péter Makra, Ferenc Bari, Eszter Farkas
Are or ischemia uncouples the blood flow response, tissue acidosis, and direct current potential signature of spreading depolarisation in the rat brain
American journal of physiology: heart and circulatory physiology 313: (2) pp. H328-H337. (2017)
https://doi.org/10.1152/ajpheart.00222.2017 **IF: 3.348**

II. Armand R. Bálint*, **Tamás Puskás***, Ákos Menyhárt, Gábor Kozák, Imre Szenti, Zoltán Kónya, Tamás Marek, Ferenc Bari, Eszter Farkas
* These Authors contributed equally to the work.
Aging impairs cerebrovascular reactivity at preserved resting cerebral arteriolar tone and vascular density in the laboratory rat
Frontiers in aging neuroscience 11 Paper: 301, 12 p. (2019)
https://doi.org/10.3389/fnagi.2019.00301 **IF: 4.362**

Other publications

1. Varga, DP*; Puskas, T*; Menyhart, A; Hertelendy, P; Zolei-Szenasi, D; Toth, R; Ivankovits-Kiss, O; Bari, F; Farkas, E
* These Authors contributed equally to the work.
Contribution of prostanoid signaling to the evolution of spreading depolarization and the associated cerebral blood flow response.
Scientific Reports 6 Paper: 31402 , 11 p. (2016) **IF: 5.228**

2. Menyhart, A ; Zolei-Szenasi, D ; **Puskas, T** ; Makra, P ; M., Toth O ; Szepes, BE ; Toth, R ; Ivankovits-Kiss, O ; Obrenovitch, TP ; Bari, F et al.
Spreading depolarization remarkably exacerbates ischemia-induced tissue acidosis in the young and aged rat brain
Scientific Reports 7: 1 Paper: 1154 , 13 p. (2017) **IF: 4.259**

3. Varga, DP ; Menyhart, A ; Puskas, T ; Bari, F ; Farkas, E ; Kis, Z ; Vecsei, L ; Toldi, J ; Gellert, L
Systemic administration of L-kynurenine sulfate induces cerebral hypoperfusion transients in adult C57Bl/6 mice
Microvascular Research 114 pp. 19-25. , 7 p. (2017) **IF: 2.371**

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