

Aus dem Institut für Experimentelle Neurologie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

A possible role of the Na⁺/K⁺-ATPase in the pathomechanism
of spreading ischemia.

zur Erlangung des akademischen Grades
Doctor medicinae (Dr. med.)

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Table of contents

| | |
|--|----|
| Abstract..... | 1 |
| Abstract (German)..... | 2 |
| Affidavit | 3 |
| Excerpt of the Journal Summary List (ISI Web of Knowledge SM)..... | 4 |
| “A role of the sodium pump in spreading ischemia in rats” | 6 |
| Supplemental material..... | 25 |
| Curriculum vitae..... | 34 |
| List of publications | 35 |
| Acknowledgements | 38 |

Abstract

Spreading depolarization (SD) is characterized by a sustained neuronal depolarization and near-complete breakdown of the ion gradients across the cellular membranes. The recovery from SD is energy-dependent. Accordingly the local parenchymal ATP concentration falls by 45%. In healthy tissue the increased energy demand is satisfied by a marked rise in cerebral blood flow (CBF), but this hemodynamic response can become inverted under pathologic conditions like subarachnoid hemorrhage, cerebral ischemia or traumatic brain injury. Under this condition SD induces long-lasting vasoconstriction, which spreads together with the depolarization wave in the tissue. In an experimental animal model the resulting spreading ischemia (SI) led to cortical infarcts. SI occurs when SD runs in tissue with increased baseline extracellular potassium ($[K^+]_o$) and depletion of nitric oxide (NO).

In cell culture chronically increased $[K^+]_o$ reduces the activity of the sodium-potassium ATPase (NaKA). Therefore, we tested *in vivo* if direct inhibition of NaKA with ouabain induces SI in rats when the NO concentration is simultaneously decreased.

First we confirmed that chronically increased $[K^+]_o$ also reduces NaKA activity *in vivo*. Notably the α_2/α_3 isoforms were selectively affected. We then found that ouabain, in a concentration selectively inhibiting the α_2/α_3 isoforms, induced SI when NO was simultaneously depleted.

What could be the mechanism underlying this effect of α_2/α_3 NaKA inhibition? The α_2/α_3 isoforms are colocalized with the Na^+/Ca^{2+} -exchanger (NCX) at cell membrane sites adjacent to the (sarco-)endoplasmic reticulum (SER). Inhibition of the α_2/α_3 isoforms decreases the local sodium gradient across the cell membrane, which consequently reduces the amount of Ca^{2+} transported out of the cell by the NCX. The surplus Ca^{2+} is then stored in the SER and might directly increase calcium dependent contractility of vascular smooth muscle cells and pericytes. Their contractility is additionally enhanced by increased Ca^{2+} release from astrocytic SER and consequent release of vasoconstrictive substances from astrocytes. These processes appear to play a specific role for the duration of SI because thapsigargin, depleting Ca^{2+} from the SER, significantly shortened SI.

These results are clinically relevant because we and others showed that SD and SI occur in patients with the above-mentioned diseases and are associated with unfavorable outcome. Better understanding of the pathomechanisms underlying SI may lead to the development of new diagnostic and therapeutic strategies for clinical conditions associated with SD and SI.

Abstract (German)

Spreading Depolarization (SD) zeichnet sich durch einen fast vollständigen Zusammenbruch des neuronalen Membranpotentials aus, der durch massive Ionenverschiebungen zwischen Intra- und Extrazellulärraum verursacht wird. Die Wiederherstellung der physiologischen Ionenkonzentrationen führt bereits nach einer einzigen SD zur Reduktion der ATP-Konzentration im betroffenen Hirngewebe auf etwa 55% des Ausgangsniveaus. Dieser erhöhte Energiebedarf wird im gesunden Hirngewebe durch einen Blutflussanstieg ausgeglichen.

Unter pathologischen Umständen, z.B. nach einer Subarachnoidalblutung, einem Hirninfarkt, oder -trauma, kann es zur Umkehr dieser Blutflussantwort kommen. Dann induziert SD eine anhaltende Vasokonstriktion, die zu einer wandernden Mangeldurchblutung führt (englisch = Spreading Ischemia (SI)) und im Tiermodell kortikale Infarkte verursacht. Experimentell wird SI beobachtet, wenn die extrazelluläre Basis- K^+ -Konzentration ($[K^+]_o$) vor der SD erhöht und gleichzeitig die NO-Konzentration ($[NO]$) erniedrigt ist.

In der Zellkultur reduziert chronische Erhöhung der $[K^+]_o$ die Aktivität der Natrium-Kalium-ATPase (NaKA). Daher haben wir in der vorliegenden Arbeit untersucht, ob eine direkte Hemmung der NaKA mit Ouabain in Kombination mit $[NO]$ -Erniedrigung bei Ratten zu SI führt.

Zunächst konnten wir auch *in-vivo* bestätigen, dass chronisch erhöhte $[K^+]_o$ die Aktivität der NaKA reduziert, und zwar vorrangig die der α_2/α_3 Isoformen. Dass dieser Mechanismus zur SI beitragen könnte, belegten wir dadurch, dass wir SI auch durch direkte Inhibition der α_2/α_3 Isoformen mit Ouabain bei gleichzeitiger $[NO]$ -Verminderung induzieren konnten.

Auf welche Weise führt eine Hemmung der α_2/α_3 NaKA zu verstärkter SD-induzierter Vasokonstriktion während SI? Die α_2/α_3 Isoformen der NaKA werden gemeinsam mit dem Na^+/Ca^{2+} -Austauscher (NCX) in Bereichen der Plasmamembran exprimiert, die in unmittelbarer Nachbarschaft zum (sarco-)endoplasmatischen Reticulum (SER) liegen. Inhibition der α_2/α_3 Isoformen führt zum Abfall des lokalen Na^+ -Gradienten über die Zellmembran. Dadurch kann der NCX weniger Ca^{2+} aus der Zelle heraustransportieren, welches stattdessen vermehrt in das SER gepumpt wird. Daraus resultiert eine direkt verstärkte Ca^{2+} -abhängige Kontraktilität von glatten Gefäßmuskelzellen und Perizyten. Außerdem nimmt ihre Kontraktilität auch indirekt als Folge verstärkter Ca^{2+} -Ausschüttung aus dem astrozytären SER mit nachfolgender Freisetzung vasokonstriktiver Substanzen aus Astrozyten zu. Diese Prozesse scheinen in besonderer Weise eine Rolle für die Dauer der SI zu spielen. So konnten wir SI deutlich verkürzen, indem wir mit Thapsigargin die Ca^{2+} -Konzentration im SER vor der SI-Induktion reduziert haben.

Die Ergebnisse dieser Arbeit sind klinisch relevant, da wir und andere zeigen konnten, dass SD und SI bei den oben erwähnten neurologischen Krankheitsbildern im Patienten auftreten und mit schlechterem Outcome assoziiert sind. Die Entschlüsselung der zugrundeliegenden Pathomechanismen kann zum besseren Verständnis dieser Krankheiten und zur Entwicklung neuer diagnostischer und therapeutischer Strategien beitragen.

Affidavit

I, Stoigniew Sebastian Major certify under penalty of perjury by my own signature that I have submitted the thesis on the topic “A possible role of the Na⁺/K⁺-ATPase in the pathomechanism of spreading ischemia”. I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The section on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) corresponds to the URM (s.o) and are answered by me. My contribution in the selected publication for this dissertation corresponds to those that are specified in the following joint declaration with the responsible person and supervisor.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

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Detailed Declaration of Contribution

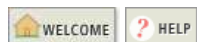
Sebastian Major had the following share in the following publication:

Publication: Sebastian Major, Gabor C. Petzold, Clemens Reiffurth, Olaf Windmüller, Marco Foddis, Ute Lindauer, Eun-Jeung Kang and Jens P Dreier, **A role of the sodium pump in spreading ischemia in rats**, Journal of Cerebral Blood Flow & Metabolism, 2016. <http://dx.doi.org/10.1177/0271678X16639059>.

Contribution in detail: Sebastian Major worked on design, performed and analyzed all experiments in groups 4-10, 12-15, 23 and 24 (all together 89 *in-vivo* experiments). Further, he analysed the experiments in groups 21 and 22, interpreted the data, wrote the initial version of the manuscript, prepared the manuscript for submission and took part in revising the study according to the reviewer's comments.

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Page 1 of 13

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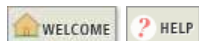
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Page 1 of 13

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Page 2 of 13

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