Clusters of Spreading Depolarizations Are Associated With Disturbed Cerebral Metabolism in Patients With Aneurysmal Subarachnoid Hemorrhage

Oliver W. Sakowitz, MD*; Edgar Santos, MD*; Alexandra Nagel, MD; Kara L. Krajewski, MD; Daniel N. Hertle, MD; Peter Vajkoczy, MD; Jens P. Dreier, MD; Andreas W. Unterberg, MD; Asita S. Sarrafzadeh, MD

Background and Purpose—We studied the dynamics of extracellular brain tissue concentrations of glucose, lactate, pyruvate, and glutamate during the occurrence of spreading depolarizations (SDs) in patients with aneurysmal subarachnoid hemorrhage.

Methods—In this prospective observational study, patients with aneurysmal subarachnoid hemorrhage received multimodal cerebral monitoring, including intracranial pressure, cerebral microdialysis, and subdural electrocorticography.

Results—Seven of the 17 recruited patients had intracerebral hemorrhage, acute ischemia and severe brain oedema leading to acute ischemic neurological deficits associated with early disturbance of metabolism at the recording site. They displayed a total of 130 SDs. The remaining 10 patients without acute ischemic neurological deficits exhibited 138 single SDs and 68 SDs in clusters. In patients without acute ischemic neurological deficits, clustered SDs were associated with a significant transient decrease in glucose and increase in lactate compared with baseline during the first 140 minutes after SDs. Moreover, the number of clustered SDs correlated with the outcome (R=–0.659; P<0.01).

Conclusion—SDs can propagate in nonischemic human brain tissue. Clusters of SDs are related to metabolic changes suggestive of ongoing secondary damage in primarily nonischemic brain tissue. (Stroke. 2013;44:220-223.)

Key Words: aneurysms • cerebrovascular disease • delayed cerebral ischemia • energy metabolism • focal ischemia • glucose • spreading depolarization

Spreading depolarizations (SDs) have been found in abundance in patients with aneurysmal subarachnoid hemorrhage (aSAH) and may play an important role in the development of the neuronal injury both in the early and the delayed phase. Further clinical evidence suggests that SDs may be particularly harmful when they occur in a cluster of recurrent events, which often leads to a persistent depression of activity between the SDs.

We studied the dynamics of glucose, lactate, pyruvate, and glutamate using microdialysis during the occurrence of SDs in patients with spontaneous aSAH. This study was carried out on the basis of the Cooperative Study on Brain Injury depolarization study protocol (www.cosbid.org).

Materials and Methods
Multimodal cerebral monitoring, including cerebral microdialysis (CMA70/71, CMA, Solna, Sweden) and electrocorticography, was performed in a prospective observational study in patients with aSAH from 2004 to 2008. Inclusion criteria were age ≥16 years and a Glasgow Coma Score ≥4. Exclusion criteria were bilaterally fixed and dilated pupils or other signs of imminent death, as well as a history of trauma/bleeding ≥5 days before admission. For the analysis, only the 17 patients who presented with SDs were considered. All research procedures were approved by the Ethics Committee for the University of Heidelberg Medical School and Charité University Medicine Berlin. Surrogate informed consent was obtained for all patients.

Operative Procedures
The patients underwent craniotomy for early (<72 hours) aneurysm clipping and received an invasive intracranial pressure probe and a flexible cerebral microdialysis probe, which was inserted in the vascular territory of the aneurysm-bearing vessel. Care was taken to avoid insertion into macroscopically lesioned brain tissue or intra-cerebral hemorrhage. In addition to routine monitoring, patients had either a subdural 6 or 8 contact linear electrode strip (Wyler, 5/10 mm platinum; Ad-Tech Medical Instrument Corp, Racine, WI) placed on cortex accessible through the craniotomy or a burr hole.

Monitoring
A postoperative computer tomography scan confirmed the location of the probes. Neurological, Glasgow Coma Score, and pupil

Received August 01, 2012; final revision received September 11, 2012; accepted September 20, 2012.

From the Department of Neurosurgery, University Hospital Heidelberg, Germany (O.W.S., E.S., K.L.K., D.N.H., A.W.U.); and Departments of Neurosurgery (A.N., P.V., A.S.S.); Department of Neurology (J.P.D.), Charité University Medicine Berlin, Germany of Neurosurgery, Geneva University Hospitals, Geneva Neuroscience Center, Faculty of Medicine, University of Geneva, Switzerland (A.S.); and Department of Neurosurgery, Charité-Universitätsmedizin Berlin, Berlin, Germany (A.S.).

*Drs Sakowitz and Santos authors contributed equally to this article.

Correspondence to Edgar Santos, MD, University of Heidelberg, Department of Neurosurgery, Im Neuenheimer Feld 400, D-69120 Heidelberg, Germany. E-mail edgar.santos@med.uni-heidelberg.de

© 2012 American Heart Association, Inc.

Stroke is available at http://stroke.ahajournals.org

DOI: 10.1161/STROKEAHA.112.672352

220
exams were carried out hourly. The 4 metabolites were assayed hourly using a bedside automatic, photometric, enzyme-kinetic analyzer (CMA600, Solna, Sweden). Values were not corrected for recovery. Recording of the subdural electrodes was done in 4 to 7 active channels using the Powerlab 16/SP analog/digital converter, coupled with the Chart-5 software (ADInstruments, New South Wales, Australia).

Data Analysis and Statistics

Patients were classified as having an acute ischemic neurological deficits (AIND) based on their initial clinical presentation, imaging, and microdialysis. A Mann-Whitney U test was performed to test for differences in the distributions of baseline metabolite concentrations between the groups (AIND and no AIND). Presence of delayed cerebral ischemia (DCI) was clinically defined as a new or progressive neurological deficit as explained above or radiological evidence of a delayed ischemic stroke. Outcome was assessed at 6 months, using the extended Glasgow Outcome Scale (eGOS).

The SDs were classified as single SD or clustered SD (SD with an interval<2 hours); no distinction was made between isoelectric SDs and true SDs (only few isoelectric SDs were seen). The hourly measurements of microdialysis metabolites were used to explore the relative changes after each SD. One measurement before each SD was used as a reference (basal value named value 0) for the next 2 hourly measurements (named value 1 and value 2). The SPSS software package was used for statistical analyses and graphical presentation of the results (SPSS v17.0, Chicago, IL). Relative values were calculated as a percentage relative to the basal value (ie, value 1×100/value 0 and value 2×100/value 0). The corresponding values were pooled and grouped in 20-minute bins relative to the SD start time. Both the mean differences and SE between the basal values and the mean relative values are presented graphically. When Friedman test showed significant changes over time, comparisons between means were done using the Wilcoxon matched pairs test. A Spearman Rho correlation with the following variables was performed: number of total SDs, single SDs, clustered SDs, mean and maximum of each basal metabolite, Glasgow Coma Score, and 6-month eGOS. P values <0.05 were regarded as significant.

Results

We recruited 17 patients in whom electrocorticography recordings revealed 336 SDs for which simultaneous MD measures were available. Patient’s details are given in Table. The mean age was 52±10. Ten of the 17 patients were women (59%). Seven patients presented with AIND, and DCI was documented in 10 patients. Four patients had both AIND and DCI.

Seven patients had AIND and displayed 118 (90%) single SDs, and 12 (10%) clustered SDs. Ten patients without AIND exhibited 138 (67%) single SDs and 68 (33%) clustered SDs. Ten out of the 17 patients showed evidence of DCI during the monitoring period. Six of those were among the patients without AIND. Number of SDs per day and SD type in relationship to DCI are presented in Figure 1.

There were no differences in glucose and pyruvate baseline concentrations between patients with AIND and those without, but a significant difference was found in glutamate (2.84±0.44 versus 8.4±2.14 µmol/L; P<0.001) and lactate (4.37±0.8 versus 6.89±0.2 mmol/L; P=0.026). An increase in lactate/pyruvate and glutamate during the monitoring period was observed in 7 of 10 patients who developed DCI.

In patients without AIND, a significant transient decrease in glucose and a transient increase in lactate (Figure 2) and in the lactate/pyruvate ratio were found in the clustered SD group but not in the single SD group. Pyruvate and glutamate were not significantly affected by either type of SD.

The only significant correlations were found between the number of clustered SDs and 6-month eGOS (n=16;...
Discussion

The role of SDs and SD-related metabolic changes in patients without preexisting neurological deficit was of particular interest because, in these patients, healthy tissue is at risk for secondary damage that could be prevented if the underlying mechanisms were known.

Experimentally, SDs lead to massive release of glutamate, decrease of glucose, and increase of lactate.6,7 The more prominent and prolonged these changes, the more the tissue is energy-depleted. Custom-made rapid sampling microdialysis in brain-injured patients has consistently shown rapid transient decreases in glucose and increases in lactate in the injured human brain in patients with traumatic brain injury.8,9 In the present study on aSAH, the assessment of SD-associated metabolite changes was limited because of the lower time resolution of the commercially available clinical monitor. The lack of an effect of SDs on glutamate, may be because of the long collection time (1 hour) of the system used here. However, despite the limitations of our setup, we detected a significant increase in lactate and decrease in glucose associated with clustered SDs in patients without AIND. This is consistent with the notion that clustered SDs rather than single SDs can lead to metabolic compromise. Correspondingly, it was shown previously in aSAH patients that clustered SDs rather than single SDs occurred in temporal association with DCI.1 In the present study, patients were grouped into those with and without AIND because the microdialysis probe was possibly positioned in already damaged tissue in patients with AIND. In such tissue, the signal–to-noise ratio is smaller and results are, thus, more likely confounded because fewer cells are available that can contribute to any metabolite changes. Therefore, whether qualitatively similar changes also occur during clustered SDs in patients with AIND has to be determined in a larger study. The tissue where recordings and samples were obtained in patients with AIND was still viable because it was capable of developing SDs, and baseline glucose levels did not differ from patients that did not have AIND. From our data, we cannot explain the lack of change in the net glucose concentrations. These are, after all, a result of glucose supply, its uptake

Figure 1. Spreading depolarizations (SDs) of 7 patients with delayed cerebral ischemia (DCI). The number of clustered SDs was higher during the day when DCI was diagnosed. Two of the reasons that explain why the number of SDs ceased were termination of the monitoring because of poor prognosis and withdrawal of support or death of the patient.

\[ R = -0.659; \ P = 0.005 \] and a strong correlation \([ R = -0.814; \ P = 0.008 \]) between the number of clustered SDs and the 6-month eGOS in the patients without AIND (n=9).

![Figure 1](https://stroke.ahajournals.org/)

Figure 2. Relative changes in dialysate glucose and lactate concentrations ratios after spreading depolarizations (SDs). Significant changes were found exclusively in the SDs of patients without acute ischemic neurological deficits (AIND).

![Figure 2](https://stroke.ahajournals.org/)
and metabolism, which we have not measured and which may explain the absence of change in interstitial concentrations.

Patients without AIND showed a significant inverse correlation between the number of clustered SDs and the outcome as scored using the eGOS at 6 months. However, the present study is not sufficient to establish any causative relationship between SD clusters and outcome. The current findings support that SD has an impact on brain metabolism and homeostasis in the human brain. However, it is not known whether the healthy human brain, after aSAH, is able to compensate those transient changes in lactate and glucose and whether those changes have an impact on long-term brain tissue survival.

In conclusion, SDs do occur in nonischemic brain tissue. Clusters of SDs are related to metabolic changes suggestive of ongoing secondary damage in primarily nonischemic brain tissue.

**Sources of Funding**

Supported by a grant of the Deutsche Forschungsgemeinschaft (DFG DR 323/5-1) to Dr Sakowitz, Dr Vajkoczy, and Dr Dreier and of the Bundesministerium für Bildung und Forschung (Center for Stroke Research Berlin, 01 EO 0801) to Dr Dreier.

**Disclosures**

None.

**References**

Clusters of Spreading Depolarizations Are Associated With Disturbed Cerebral Metabolism in Patients With Aneurysmal Subarachnoid Hemorrhage

Oliver W. Sakowitz, Edgar Santos, Alexandra Nagel, Kara L. Krajewski, Daniel N. Hertle, Peter Vajkoczy, Jens P. Dreier, Andreas W. Unterberg and Asita S. Sarrafzadeh

Stroke. 2013;44:220-223; originally published online December 6, 2012; doi: 10.1161/STROKEAHA.112.672352

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/44/1/220

Data Supplement (unedited) at:
http://stroke.ahajournals.org/content/suppl/2012/12/06/STROKEAHA.112.672352.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/
SUPPLEMENTAL MATERIAL
Supplementary table 1. Demographic information from the consecutive aSAH patients enrolled in this study. Out of 48 patients, 32 patients exhibited SDs. Corresponding microdialysis measurements were available in 17 patients, and only those were used for further calculations.

Abbreviations: WFNS= World Federation of Neurological Surgeons grading system, ICA=Internal carotid artery, MCA= Middle cerebral artery, ACA=Anterior communicating artery, P.c=posterior circulation.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Count 48</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male 20</td>
</tr>
<tr>
<td></td>
<td>Female 28</td>
</tr>
<tr>
<td>Age</td>
<td>Mean 51.3</td>
</tr>
<tr>
<td></td>
<td>S.D. 10.2</td>
</tr>
<tr>
<td></td>
<td>Range 28-78</td>
</tr>
<tr>
<td></td>
<td>Mean 3.5</td>
</tr>
<tr>
<td></td>
<td>1 8</td>
</tr>
<tr>
<td></td>
<td>2 5</td>
</tr>
<tr>
<td></td>
<td>3 5</td>
</tr>
<tr>
<td></td>
<td>4 12</td>
</tr>
<tr>
<td></td>
<td>5 17</td>
</tr>
<tr>
<td>WFNS</td>
<td>ICA 7</td>
</tr>
<tr>
<td>Aneurysm location</td>
<td>MCA 23</td>
</tr>
<tr>
<td></td>
<td>ACA 16</td>
</tr>
<tr>
<td></td>
<td>P.c. 2</td>
</tr>
<tr>
<td>Aneurysm size (mm)</td>
<td>Mean 7.1</td>
</tr>
<tr>
<td></td>
<td>S.D. 4.4</td>
</tr>
<tr>
<td></td>
<td>Range 1-22</td>
</tr>
<tr>
<td>Time of ECoG</td>
<td>Mean 198.65</td>
</tr>
<tr>
<td></td>
<td>S.D. 69.2</td>
</tr>
<tr>
<td></td>
<td>Range 45-336</td>
</tr>
<tr>
<td>SD</td>
<td>Count 810</td>
</tr>
<tr>
<td></td>
<td>Mean 16.8</td>
</tr>
<tr>
<td></td>
<td>S.D. 23.2</td>
</tr>
<tr>
<td></td>
<td>Range 0-96</td>
</tr>
</tbody>
</table>