Retention of Cerebrovascular Dilation After Cortical Spreading Depression in Anesthetized Rabbits

David W. Busija, PhD, Wei Meng, MD

Background and Purpose: We examined responses of rabbit pial arterioles to three different stimuli before and after induction of cortical spreading depression.

Methods: In urethane-anesthetized rabbits equipped with a closed cranial window, we measured pial arteriolar diameter during baseline conditions, topical application of calcitonin gene–related peptide (CGRP), topical application of acetylcholine, and inhalation of 10% CO₂ in air (arterial hypercapnia) before cortical spreading depression and 30, 60, and 120 minutes after cortical spreading depression. Cortical spreading depression was induced by localized application of a 5% KCl solution anterior to the arteriole being measured.

Results: Average baseline diameter was approximately 90 μm. During cortical spreading depression, arteriolar diameter increased to a peak value that was 50±4% above baseline (n=32). Before cortical spreading depression, arteriolar diameter changed 47±7% (n=9) during hypercapnia, 17±3% (n=4) during 10⁻⁹ mol/L CGRP, 42±10% (n=7) during 10⁻⁷ mol/L CGRP, 29±6% (n=4) during 10⁻⁶ mol/L acetylcholine, and 61±13% (n=6) during 10⁻⁵ mol/L acetylcholine. Arteriolar responsiveness to any of these stimuli was not changed significantly by prior cortical spreading depression.

Conclusions: Dilator capacity of pial arterioles is still intact in urethane-anesthetized rabbits after cortical spreading depression. (Stroke. 1993;24:1740-1745.)

Key Words: cerebral circulation • hypercapnia • neurotransmitters • rabbits

Cortical spreading depression (CSD) can be initiated by mechanical, electrical, or chemical stimulation of the brain or by ischemic foci and results in major perturbations of cerebrovascular tone. Despite a rapid recovery of cortical function and cerebrovascular tone, it has been reported that cerebrovascular responses to many different stimuli are attenuated or abolished after CSD whereas responses to yet other stimuli are intact. Therefore, we decided to examine post-CSD cerebrovascular responses at several different time periods using three stimuli that act via different mechanisms. These include (1) topical acetylcholine, which dilates via a mechanism involving generation of a nitric oxide–like substance by endothelium, which then acts on vascular smooth muscle; (2) topical calcitonin gene–related peptide (CGRP), which dilates because of activation of receptors on vascular smooth muscle cells and increased intracellular levels of cyclic adenosine monophosphate; and (3) arterial hypercapnia, which dilates via changes in extracellular fluid pH and activation of ion channels in vascular smooth muscle.

Materials and Methods

Adult male rabbits (2.7 to 3.4 kg) were initially anesthetized with pentothal sodium (20 to 30 mg/kg IV) and maintained on urethane (1.6 to 1.8 g/kg IP). Catheters were placed into a femoral artery for monitoring of blood pressure and sampling of blood for gases and pH, and into a femoral vein for infusion of fluids or drugs. After intubation, animals were either ventilated with a small animal respirator or allowed to breathe on their own. After fixation of the head in a stereotaxic instrument, the scalp was cut and reflected, and an opening 20 mm in diameter was made in the skull as previously described. The dura was cut and reflected, and a closed cranial window was inserted into the opening in the skull and cemented into place with dental acrylic. A small opening (1.0 mm) was made using a drill bit in the skull over the frontal cortex 1 to 5 mm in front of the cranial window, and a length of PE-50 tubing was inserted so that the bottom of the tubing touched the top of the dura. This opening was used for injection of 5 to 10 μL of a 5% KCl solution via a needle to initiate CSD. The tubing was cemented into place with dental acrylic. In some animals, another opening of approximately 1.0 mm was made between the first opening and the cranial window, and a glass semimicropipette (2.0 mm outer diameter, 10 to 15 mm long, tip diameter 100 to 150 μm) filled with 0.9% saline solution was implanted approximately 1 mm below the...
cortical surface and fixed into place with dental acrylic. The open end of the pipette was then connected with a cotton wick to a recording calomel electrode (impedance, 3 to 6 Ω) for monitoring slow potential changes (SPC, a DC shift) accompanying CSD. A reference electrode of the same type was placed directly on the exposed skull along the midline over the olfactory bulb. We chose SPC to monitor CSD because the negative shift of SPC closely follows the CSD event and the time course of ionic movements across the membranes of neuronal and glial cells. In some other animals, initiation of CSD was documented by ipsilateral narrowing of the electroencephalograph. Rectal temperature was maintained at 38 to 39°C with a heating pad.

The closed cranial window was filled with warmed artificial cerebrospinal fluid (aCSF) gassed with 6% CO₂, 6.5% O₂, and balance N₂. The composition of rabbit aCSF was as follows (in mmol/L): KCl 2.9, MgCl₂ 0.6, CaCl₂ 1.5, NaCl 131.9, urea 6.7, dextrose 3.7, and NaHCO₃ 20.2. Images of an appropriate arteriole were visualized with a microscope, television camera, and monitor, and diameter was measured with a dimensional analysis system (VPA 1000, FOR-A-Corp).

At least 20 minutes after filling the cranial window with aCSF, baseline vascular responsiveness was determined. Diameter was determined during control conditions, during which arterial blood gases and pH were within normal limits. Then, animals were exposed to arterial hypercapnia (inspired gas = 10% CO₂ in air); or topical application (infusion of drug in aCSF) of 10⁻⁹ mol/L, 10⁻⁸ mol/L, and 10⁻⁷ mol/L CGRP; or topical application of 10⁻⁶ mol/L, 10⁻⁵ mol/L, and 10⁻⁴ mol/L acetylcholine. For the topical application experiments, the corresponding control period involved infusion of aCSF containing no drug. Peak arteriolar diameter was recorded. After diameter returned to baseline, CSD was initiated by microinjection of 5% KCl. Using the PE-50 tubing as a guide, a 26-gauge needle was inserted through the dura into the superficial cortex, and 5 to 10 μL of the KCl solution was injected. The needle was removed promptly. Peak diameter and time to reach this value were recorded. Time from injection to arteriolar dilation corresponded to a CSD speed of 2 to 3 mm/min. Arteriolar responses to arterial hypercapnia, topical CGRP, and/or topical acetylcholine were determined starting at approximately 30, 60, and 120 minutes from the time of peak arteriolar dilation to CSD. For the experiments involving 10⁻⁹ mol/L CGRP and 10⁻⁶ mol/L acetylcholine, the 120-minute determination was not done. For each experiment, dilator stimuli might be given at 1, 2, or 3 of these times. For the control period before arterial hypercapnia, the CSF in the window was not replaced with fresh aCSF. However, for the drug application experiments, aCSF was infused into the window during the control periods. In some experiments more than one type of dilator stimulus was given. Arterial blood pressure did not change during application of any stimulus.

Values are presented as mean±SEM. Data for arterial hypercapnia, 10⁻⁵ mol/L and 10⁻⁴ mol/L acetylcholine, and 10⁻⁶ mol/L and 10⁻⁷ mol/L CGRP were analyzed using a double repeated-measures analysis of variance, followed by appropriate pairwise comparisons using a Bonferroni adjustment for multiple comparisons. Data for 10⁻⁹ mol/L CGRP and 10⁻⁴ mol/L acetylcholine were analyzed using paired t tests (control versus treatment) and analysis of variance (percent change in data).

Results

In all of the experiments used, microapplication of 5% KCl resulted in pial arteriolar dilation lasting approximately 1 to 1.5 minutes (Fig 1). Baseline diameter was 90±4 μm, and peak diameter was 133±6 μm (50±4% dilation; n=32). When recorded, the SPC showed a negative deflection of approximately 10 to 15 mV, and this deflection always preceded arteriolar dilation (Fig 1).

Before CSD, acetylcholine diluted pial arterioles at all three doses administered (Table 1). Percent changes were 29±6% at 10⁻⁶ mol/L (n=4), 55±8% at 10⁻⁵ mol/L (n=6), and 61±13% at 10⁻⁴ mol/L (n=6). After CSD, acetylcholine diluted pial arterioles at roughly similar amounts 30, 60, and 120 minutes after CSD (Table 1). Fig 1 demonstrates that pial arterioles are still responsive to acetylcholine after CSD.

Inhalation of 10% CO₂ in air also diluted pial arterioles, and this response was largely intact at 30, 60, and 120 minutes after CSD (Table 2; Fig 2). Peak arteriolar dilation during arterial hypercapnia was 47±7% (n=9) during baseline and 36±7% (n=8) at 30 minutes, 44±9% (n=6) at 60 minutes, and 61±10% (n=6) at 120 minutes after CSD. Although peak arteriolar dilation to CO₂ inspiration was intact after CSD (Table 2), there was a tendency for dilation to wane after 6 to 7 minutes of CO₂ inspiration (Fig 2).

Before CSD, CGRP diluted pial arterioles at all three doses given (Table 3; Fig 3). After CSD, there were no
significant differences in responses to CGRP compared with the initial responses at 30, 60 (Fig 3), or 120 minutes.

Discussion

The major new finding of the present study is that dilator responses of pial arterioles largely are retained after CSD. Thus, arterial hypercapnia, topical application of CGRP, and topical application of acetylcholine dilated pial arterioles for up to 2 hours after CSD. Retention of cerebrovascular dilator responses to three stimuli acting through entirely different mechanisms indicates that a single CSD does not necessarily lead to vascular dysfunction.

Several laboratories have reported that after CSD, cerebrovascular dilation to arterial hypercapnia, local application of acidic aCSF, topical adenosine, or topical bradykinin are attenuated or abolished.5,9,14 The reported period of reduced or absent responsiveness ranges from 30 minutes to several hours. However, altered cerebrovascular dilator ability is not universal. For example, in the post-CSD period, normal responsiveness is present to reduced arterial blood pressure5 and to additional waves of CSD.13 The results of our experiments support the concept that cerebrovascular responsiveness is largely intact after CSD. Thus, fairly normal pial arteriolar responsiveness is present to three stimuli operating via different mechanisms for up to 2 hours after CSD. It is unclear why our results differ so much from those of other investigators.5,9,14 It is possible that choice of anesthetic agents or species studied or other experimental aspects compromised cortical and vascular function during and after CSD. However, there were several subtle changes in responsiveness that should be discussed. First, the arteriolar responses to acetylcholine and CGRP tended to fall slightly in some but not all cases with repeated applications. It seems likely that these relatively minor changes were due to factors such as receptor desensitization22 or other nonspecific vascular changes associated with repeated application of agents to the cortical surface. Second, while the maximal arteriolar change during arterial hypercapnia did not decrease, we tended to see a return of arteriolar diameter to control levels after approximately 6 to 8 minutes in most but not all animals despite continued inhalation of 10% CO2 (Fig 2). This response is unusual because normally arteriolar dilation to this stimulus is sustained for at least 15 minutes11 (Fig 2, top panel). It is possible that with the presence of the cortical wounds associated with the injection site for KCl and the glass micropipette, the blood-brain barrier is disrupted and circulating constrictor agents now have access to the perivascular CSF and thus to pial arterioles. It has been shown that levels of several vasoconstrictor agents increase in plasma during arterial hypercapnia.23,24 Thus, arterial hypercapnia initially dilates pial arterioles, but as plasma levels of pressor agents increase, more of these agents are able to penetrate into the CSF and exert constrictor effects on pial arterioles. We have proposed a similar mechanism to explain reduced cerebrovascular responsiveness to arterial hypercapnia several days after injection of blood into the subarachnoid space.25 However, it is possible that another mechanism, as yet undefined, is causing this change. Return of normal responsiveness to arterial hypercapnia over time might be explained by repair of the blood-brain barrier.

### Table 1. Effects of Acetylcholine on Pial Arterioles Before and After Cortical Spreading Depression

<table>
<thead>
<tr>
<th>Time</th>
<th>n</th>
<th>Baseline</th>
<th>$10^{-6}$ mol/L</th>
<th>$10^{-4}$ mol/L</th>
<th>$10^{-4}$ mol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before CSD</td>
<td>4</td>
<td>85±12</td>
<td>112±20*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>100±10</td>
<td>157±16*</td>
<td></td>
<td>158±14*</td>
</tr>
<tr>
<td>After CSD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td>4</td>
<td>88±13</td>
<td>111±17*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>106±9</td>
<td>164±12*</td>
<td></td>
<td>160±12*</td>
</tr>
<tr>
<td>60 min</td>
<td>4</td>
<td>86±13</td>
<td>116±18*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>109±8</td>
<td>151±13*</td>
<td></td>
<td>153±12*</td>
</tr>
<tr>
<td>120 min</td>
<td>10</td>
<td>98±12</td>
<td></td>
<td>138±14*</td>
<td>140±12*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. CSD indicates cortical spreading depression. n=9 for arteriolar diameter at $10^{-5}$ mol/L acetylcholine for the 120-minute determination. *p<.05 compared with baseline.

### Table 2. Effects of Arterial Hypercapnia on Pial Arterioles Before and After Cortical Spreading Depression

<table>
<thead>
<tr>
<th>Time</th>
<th>n</th>
<th>Baseline</th>
<th>Hypercapnia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before CSD</td>
<td>9</td>
<td>96±7</td>
<td>138±9*</td>
</tr>
<tr>
<td>After CSD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td>8</td>
<td>99±7</td>
<td>132±6*</td>
</tr>
<tr>
<td>60 min</td>
<td>6</td>
<td>99±9</td>
<td>139±11*</td>
</tr>
<tr>
<td>120 min</td>
<td>6</td>
<td>87±8</td>
<td>138±10*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. CSD indicates cortical spreading depression. *p<.05 compared with baseline.
FIG 2. Tracings show pial arteriolar diameter changes in response to arterial hypercapnia before cortical spreading depression (CSD) and 30, 60, and 120 minutes after CSD. Before CSD, arteriolar diameter increased with inhalation of 10% CO₂, and this dilation was sustained for the length of exposure. At 30 and 60 minutes after CSD, the arteriole still dilated during arterial hypercapnia, but the dilation tended to wane over time. At 120 minutes after CSD, the dilation to arterial hypercapnia was pronounced and sustained.

TABLE 3. Effects of Calcitonin Gene–Related Peptide on Pial Arterioles Before and After Cortical Spreading Depression

<table>
<thead>
<tr>
<th>Arteriolar Diameter, μm</th>
<th>CGRP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td>Before CSD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>7</td>
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<tr>
<td>After CSD</td>
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</tr>
<tr>
<td></td>
<td>8</td>
</tr>
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<td></td>
<td>5</td>
</tr>
</tbody>
</table>

Values are mean±SEM. CGRP indicates calcitonin gene–related peptide; CSD, cortical spreading depression. *P<.05 compared with baseline.

One last issue that should be discussed concerns the possibility that repeated flushings of the cortical surface for application of aCSF (for baseline determinations of diameter) or aCSF with acetylcholine or CGRP may have removed noxious agents from the vicinity of pial arterioles and thus preserved normal function. Consistent with this view, we have shown that flushing of the cortical surface with aCSF after CSD prevents subsequent arteriolar vasoconstriction but not vasodilation. However, in terms of maintained arteriolar responsiveness, we have to discount this possibility. First, in the experiments with arterial hypercapnia, the cranial window was not flushed at all after CSD. Second, in the acetylcholine and CGRP application protocols, in sev-
general cases the cranial window was not flushed until baseline determinations were made 60 minutes after CSD. In these animals, responses were similar to those obtained before CSD.

In summary, although CSD has major effects on cerebrovascular tone, we have found that dilator ability is largely present after CSD in anesthetized rabbits. Consequently, recovery from CSD-induced vascular changes is relatively rapid and complete.

Acknowledgments
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We would like to thank Mr Eugene Jensen for assistance in performing the experiments.

References

Editorial Comment

Spreading cortical depression may accompany migraine with aura and could play a role in the cerebral response to ischemia, trauma, or seizure activity. Although the evidence for its occurrence in humans is scanty, spreading cortical depression certainly occurs in rats, cats, and rabbits and may confound the interpre-
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