Perivascular nerves contribute to cortical spreading depression-associated hyperemia in rats

UWE REUTER, JOERG R. WEBER, LORENZ GOLD, GUY ARNOLD, TILO WOLF, JENS DREIER, UTE LINDAUER, AND ULRICH DIRNAGL
Department of Neurology, Charité Hospital, Humboldt University, 10098 Berlin, Germany

Perivascular nerves contribute to cortical spreading depression-associated hyperemia in rats. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H1979–H1987, 1998.—We investigated the contribution of perivascular nerves and neurotransmitters to cortical spreading depression (CSD)-associated hyperperfusion in the rat. Chronic transection of the nasociliary nerve (NCN, 2 wk before) decreased ipsilateral CSD-associated hyperperfusion by 23 ± 13% (mean ± SD; n = 5, P < 0.05), whereas acute transection of the NCN or sham surgery had no effect (n = 8). When the NCN and parasympathetic nerve fibers (PSN) were both chronically transected, CSD hyperperfusion was attenuated by 55 ± 19% (n = 5, P < 0.05). Cerebrovascular reactivity to hypercapnia was not significantly affected. Brain topical superfusion of the muscarinic receptor antagonist atropine (10⁻⁴ M) caused a reduction of CSD hyperperfusion by 41 ± 13% (n = 5, P < 0.05). The competitive blockade of calcitonin gene-related peptide (CGRP) receptors by CGRP-(8–37) (5 × 10⁻⁷ M) afforded a decrease by 49 ± 19% (n = 5, P < 0.05), without affecting CO₂ reactivity (n = 4). The combined application of both CGRP-(8–37) and atropine further attenuated CSD hyperperfusion by 69 ± 17% (n = 5, P < 0.05). After chronic NCN and PSN transection brain topical superfusion of CGRP-(8–37) (5 × 10⁻⁷ M) reduced CSD hyperperfusion slightly by 9.5 ± 5% (n = 3). Atropine (10⁻⁴ M) afforded a decrease by 17 ± 6% (n = 3). These reductions were not statistically significant. We conclude that CSD-associated hyperperfusion is mediated in part by a depolarization of trigeminal sensory and parasympathetic nerve fibers, resulting in a release of vasoactive trigeminal and parasympathetic neurotransmitters. Acetylcholine; atropine; brain; calcitonin gene-related peptide; hypercapnia; cerebral blood flow.

There is strong evidence that cortical spreading depression (CSD) is the underlying cause of the migraine aura (13). CSD, first described by Leao (14) in 1944, is a transient suppression of cortical activity with a speed of ~3 mm/min. It is associated with the dilation of pial arterioles, resulting in a cerebral blood flow (CBF) hyperperfusion and followed by a long-lasting hypoperfusion of several hours. The mechanisms and physiological role underlying these reactive blood flow changes seen with CSD are not fully understood. Several vasoactive parenchymal metabolites, such as K⁺, CO₂, adenosine, NO, and glutamate, are known to be released during CSD and may contribute to pial vasodilation (4, 10, 12, 31). In addition, neurotransmitters released from perivascular nerve fibers that surround the circle of Willis and cortical pial vessels may also participate in CSD-associated vasodilation (32). These neurotransmitters belong mainly to the trigeminal, sympathetic, and parasympathetic nervous systems (17, 29, 30). Calcitonin gene-related peptide (CGRP), substance P, and neurokinin A have been demonstrated immunohistochemically as transmitters of perivascular trigeminal nerves (29), which originate in the ipsilateral division of the trigeminal ganglia cells and continue in the nasociliary nerve. The nasociliary nerve runs together with postganglionic parasympathetic nerve fibers originating in the ipsilateral sphenopalatine ganglia cells through the ethmoidal foramen. The postganglionic parasympathetic nerves contain the vasodilators acetylcholine (ACh), vasoactive intestinal peptide (VIP), neurokinin Y, and NO synthase (NOS) (24, 28, 30).

The trigeminovascular system is the anatomic substrate for the key hypothesis of migraine pathophysiology (9, 20). Trigeminal neurotransmitters (like CGRP) contribute substantially to vasodilation in several physiological and pathophysiological conditions (8, 15, 22). In CSD the brain stem nucleus caudalis becomes activated, as demonstrated by the induction of c-fos, which is blocked by meningeal deafferentation (21). Thus CSD leads to trigeminal activation and putatively to the release of neurotransmitters from this system. Recently it has been described that parasympathetic nerve fibers modulate cerebral infarct volume and seem to be neuroprotective by increasing CBF (11). Regional CBF (rCBF) can also be increased by stimulation of the sphenopalatine ganglion or other parasympathetic pathways. This increase is mediated by NO, VIP, and ACh pathways (18, 23, 27).

On the basis of evidence presented above, we reasoned that CSD-associated hyperperfusion is mediated at least in part by an activation of the trigeminal nervous system. Because of their dose functional and anatomic correlation, we also focused on the contribution of parasympathetic nerve fibers to CSD-associated hyperperfusion. To achieve these aims we selected two different strategies in anesthetized rats: a surgical approach, severing fibers of the system under study anatomic correlation, and thus depleting its neurotransmitters, and a pharmacological approach, in which vasodilators of the parasympathetic and trigeminovascular system were blocked by brain topical administration of receptor antagonists.

Methods

General experimental procedures were identical for all animals. Male Wistar rats were initially anesthetized with 100 mg/kg thiopental sodium (Trapanal, Byk Gulden, Konstanz, Germany) intraperitoneally and maintained by intermittent application of further thiopental doses (5 mg ip), aiming at the absence of a reaction to a tail pinch. The animals were

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mechanically ventilated after tracheotomy with room air and supplemental oxygen, and end-expiratory CO₂ was monitored during the experiment. One femoral vein was cannulated for the administration of saline infusion and one artery for permanent monitoring of mean arterial blood pressure (MABP), intermittent sampling of arterial blood gases, and pH. Afterward the skull was fixed in a stereotaxic frame, and the skin was opened by a medial incision and retracted laterally.

In group I (n = 22) surgical transections of the nasociliary nerve alone or together with parasympathetic nerve fibers and sham operations were performed (see below).

To elucidate whether the surgical denervation (nasociliary nerve + parasympathetic nerve fibers) affects cerebrovascular reactivity, we investigated the CBF response to CO₂ inhalation in four of these rats. Two burr holes were drilled above the frontal cortex bilaterally, and a laser-Doppler flow probe was positioned on each side above the dura as described below. The first hypercapnia (inspired gas, 5% CO₂ in air) was induced after monitoring rCBF for at least 20 min; the following ones were induced after a time interval of 30 min. The rats were exposed to arterial hypercapnia for 5 min, and arterial blood samples were taken before and during CO₂ inhalation.

All other rats of group I were equipped with three skull burr holes in a row bilaterally. One was placed over the frontal cortex, the second frontoparietally, and the third one parietally (Fig. 1). The dura was cut underneath the parietal burr hole for the elicitation of CSD. A laser-Doppler flow probe (Perimed, Periflux 4001 Master, J arfälla, Sweden) to monitor rCBF and a calomed electrode for measuring direct current (DC) potential shifts were placed in the frontal and frontoparietal burr holes on the intact dura. The laser-Doppler flow probes (Probe Periflux PF 403, fiber separation 250 µm) were positioned in an area free of large pial and dural vessels to minimize a large-vessel contribution to the signal (33). A reference electrode was fixed in the neck. CSDs were elicited bilaterally by the short (60-s) application of one drop KCl (1 M) on the parietal opening every 15–20 min. In five of these rats, CBF responses to CSD were recorded before and immediately after acute transection of the right nasociliary nerve as described below. The experimental paradigm is summarized in Fig. 2.

In rats of group II (n = 14), in which the contribution of CGRP and ACh to CSD-induced hyperperfusion was studied in a pharmacological approach, the cranial preparation was as follows. After rats were fixed in a stereotaxic frame, a burr hole was drilled over the right frontoparietal cortex. The cortical surface was exposed by removing the dura mater, and a closed cranial window was implanted. The window contained an Ag-AgCl silver ball electrode for measuring DC potential changes and an inflow and outflow for the superfusion of artificial cerebrospinal fluid (aCSF). A reference electrode was fixed in the neck and a Doppler flow probe was positioned on top of the cranial window, monitoring rCBF in an area of the window free of larger pial vessels (~20 µm). In this setup the distance from laser-Doppler flow probe tip to brain surface is ~1 mm. Additionally, a small opening over the frontal cortex was made and the dura mater underneath was cut for the elicitation of CSD. In all rats four CSDs were elicited while normal aCSF was superfused at 1–2 ml/h. A superfusion of the muscarinic antagonist atropine (10⁻⁴ M in aCSF) was then started (n = 5). In five additional rats, the CGRP-receptor blocker CGRP-(8—37) (5 × 10⁻⁷ M in aCSF) was superfused for ~2 h, and then the superfusion was switched to atropine (10⁻⁴ M). CSDs were elicited every 20 min, starting 30 min after onset of drug superfusion (Fig. 2).

In four of the rats of group II the contribution of the perivascular neurotransmitter CGRP to the CBF increase caused by hypercapnia was investigated. Hypercapnia was induced three times as described above for group I while aCSF was superfused. Brain topical superfusion of CGRP-(8—37) (5 × 10⁻⁷ M, 1.5 ml/h) was then started. After 1 h of superfusion several periods of hypercapnia (every 30 min) were induced.

In rats of group III (n = 3) the effect of CGRP-(8—37) and atropine in chronically denervated rats (nasociliary nerve + parasympathetic nerve fibers) was studied. The nerve transections were performed unilaterally as described below. After 2 wk the rats were equipped with a closed cranial window above the chronically denervated hemisphere. The experimental procedure was identical to that described for the rats of group II.

DC potential changes were registered in all animals (except the ones in the hypercapnia experiments) with an electrometer (WPI Instruments, FD 223, Sarasota, FL). CBF was measured in arbitrary units and expressed as percentage of baseline. Baseline was defined as mean CBF over a period of 180 s under resting conditions (100%), immediately before CSD was elicited or hypercapnia was induced. Data were converted to digital form and fed into a personal computer running ASYST (MacMillan Software, New York) data-acquisition software (sample interval 3 s).

Nasociliary nerve and parasympathetic nerve fiber surgery. Seventeen male Wistar rats of group I (300–350 g) were anesthetized with 5% chloral hydrate (200 mg/kg body wt ip). Unilateral nasociliary nerve sections were performed as modified from Suzuki et al. (28). In brief, the scalp was incised sagittally and medially of the right eye. The skin and perios- teum were reflected and the intraorbital structures were retracted laterally so that the medial orbital wall, the ethmoidal foramen, and its contents (nasociliary nerve, anterior ethmoidal artery and vein, and postganglionic parasympathetic nerve fibers) were visible. In five of the animals the nasociliary nerve was cut, whereas in nine other rats the nasociliary nerve and parasympathetic nerve fibers were interrupted ipsilaterally. Sham operations were performed in three rats to rule out effects on CSD-associated hyperperfusion due to the surgical procedure. In the sham experiments the structures of the ethmoidal foramen were gently exposed as described above, and the situs were closed afterward without any lesion performed. After the skin was sutured, the animals were kept in their cages for 12–16 days until the transected axons and nerve terminals were degenerated (29). General surgery was performed as described above. To study the effects of acute denervation in five additional animals of...
group I, we transected the nasociliary nerve during, rather than before, the experiment. The general experimental paradigm is summarized in Fig. 2 for all groups.

Immunohistochemistry. In rats of group I (nasociliary nerve + parasympathetic nerve fiber transection) in which the response of cerebral blood vessels to hypercapnia had been investigated, immunohistochemistry was done immediately after the experiment to demonstrate successful denervation and neurotransmitter depletion. Immunohistochemistry was performed as described by Suzuki et al. (29). In brief, the brain was removed, together with the major cerebral arteries, immediately after decapitation and was immersed in an ice-cold mixture of 2% formaldehyde and 15% saturated picric acid dissolved in 0.2 M phosphate buffer (pH 7.4). The cerebral arteries were dissected out carefully under a microscope (×40) and fixed for 2 h by immersion in the same...
fixative. After the specimens were rinsed in a Tyrode solution containing 10% sucrose for 24 h at 4°C and immersed for 2 h in buffer without sucrose, they were placed on glass slides and air-dried for 0.5 h. The specimens were incubated in a moist chamber for 18 h at 4°C and for 3 h at room temperature with a CGRP antiserum, which was raised in the rabbit (Sigma C8198) and diluted 1:640. To label the primary antibody, we applied fluorescent isothiocyanate pig anti-rabbit immunoglobulin G in a dilution of 1:20 on the arteries as described for the primary antibody. The glass slides were covered with a coverslip, examined under a fluorescence microscope fitted with the appropriate filter settings, and photographed.

RESULTS

In all groups the physiological parameters were kept in normal ranges throughout the experiments (Table 1). To prove the success of the nasociliary nerve transection, we performed immunohistochemistry. A rich staining of CGRP-positive nerve fibers was found along the medial cerebral artery, the anterior and medial part of the circle of Willis, and the anterior cerebral artery on the nontransected side. When the nasociliary nerve was chronically transected, no or only a few CGRP-positive stained perivascular nerve fibers were found on these vessels. Figure 3 shows the typical findings 2 wk after right nasociliary nerve transection in comparison to the nontransected side.

The application of one drop KCl (1 M) on the cortex caused the typical CSD response with a negative deflection of the DC potential and a monophasic rCBF increase in the rat as shown in Fig. 4. No hypoperfusion was seen preceding or after the hyperperfusion. Note that the first CSD in each animal was excluded from analysis.

Effect of time on CBF responses. In group I statistical analysis revealed no significant difference of CSD-associated hyperperfusion either on the transected side or on the control side over time for at least 3 h (Table 2). In a previous study, using an identical experimental setup, we have shown that there are no time-dependent effects on CSD hyperperfusion in closed cranial window preparations (6a).

Table 1. Physiological data

<table>
<thead>
<tr>
<th>Group 1</th>
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<tbody>
<tr>
<td></td>
<td>NCN (Chronic)</td>
<td>NCN + PSN</td>
</tr>
<tr>
<td></td>
<td>Sham surgery</td>
<td>NCN (acute transection)</td>
</tr>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>pH</td>
<td>7.38 ± 0.02</td>
<td>7.39 ± 0.03</td>
</tr>
<tr>
<td>MABP, mmHg</td>
<td>98 ± 13.5</td>
<td>105 ± 13.5</td>
</tr>
<tr>
<td>PaCO₂, mmHg</td>
<td>29 ± 2</td>
<td>31 ± 5</td>
</tr>
<tr>
<td>PaO₂, mmHg</td>
<td>140 ± 37</td>
<td>142 ± 32</td>
</tr>
</tbody>
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|                         |                  |                  |
| Group 2                 | Atropine         | CGRP-(8—37)      |
|                         | Before           | After            | Before           | After            |
| pH                      | 7.4 ± 0.02       | 7.37 ± 0.02      | 7.38 ± 0.11      | 7.39 ± 0.02      |
| MABP, mmHg              | 113 ± 10         | 113 ± 21         | 128 ± 7          | 128 ± 22         |
| PaCO₂, mmHg             | 32 ± 8           | 27 ± 5           | 31 ± 4           | 29 ± 5           |
| PaO₂, mmHg              | 147 ± 29         | 148 ± 28         | 122 ± 13         | 135 ± 14         |

Values are means ± SD. NCN, nasociliary nerve; PSN, parasympathetic nerve fibers; MABP, mean arterial blood pressure; PaCO₂, arterial PCO₂; PaO₂, arterial PO₂; CGRP-(8—37), calcitonin gene-related peptide-(8—37).
sham control experiments. There was no significant difference between CSD-associated hyperperfusion on the control side compared with the sham-operated side (141 ± 19 vs. 134 ± 21%; \( P = 0.28 \); Fig. 5).

Effects of chronic nasociliary nerve and parasympathetic nerve fiber transection on CBF during CSD. CSD-associated hyperperfusion was significantly lower on the transected side in all experiments.

Hyperperfusion was 190 ± 27% above baseline on the control side compared with the nasociliary nerve-cut side, where the blood flow increase was decreased by 22 ± 13% (\( P < 0.05 \); Fig. 5).

In the group with nasociliary nerve and parasympathetic nerve fibers cut, a peak flow increase of 77 ± 40% above baseline was obtained ipsilaterally, which was significantly lower than on the contralateral side, where CBF increased to 177 ± 54% above baseline (\( P < 0.05 \); reduction of 55 ± 19%). The reduction by combined nasociliary nerve-parasympathetic nerve fiber transection was significantly different from the group in which only the nasociliary nerve was transected (55 ± 19 vs. 22 ± 13%; Fig. 5). Neither chronic nasociliary nerve transection alone nor combined nasociliary nerve + parasympathetic nerve fiber transection affected the pattern and amplitude of the DC potential changes associated with CSD (Fig. 4).

Effects of chronic nasociliary nerve and parasympathetic nerve fiber transection on CBF response to hypercapnia. The blood flow increase to hypercapnia was slightly, but not significantly, reduced on the side where the nasociliary nerve and parasympathetic nerve fibers were both cut. The \( CO_2 \) response was 1.78 ± 0.5% per mmHg arterial \( P_{CO_2} \) increase on the transected side compared with 2.23 ± 0.3% per mmHg arterial \( P_{CO_2} \) increase on the intact side (\( n = 4 \)).

Effects of acute nasociliary nerve transection on CBF in CSD. To investigate whether central neurotransmission is mediating hyperperfusion in CSD, we acutely interrupted the nasociliary nerve. This acute interruption allowed us to distinguish between a local cause of neurotransmitter release and a neurotransmitter release due to signal transmission from the perikaryon. If it were the case that CSD causes neurotransmitter release by local stimulation of nerve terminals resulting in vasodilation and hyperperfusion, then no changes in CSD hyperperfusion after acute nasociliary nerve transection were to be expected.

There were no rCBF changes observed immediately after the acute nasociliary nerve transection as reported before (3, 33). The peak flow during CSD hyperemia was nearly equal on both sides with a hyperperfusion of 151 ± 79% ipsilaterally compared with 149 ± 93% above baseline contralaterally (\( P = 0.71 \); Fig. 5).

Effects of atropine and CGRP-(8–37) on CBF during CSD. Because our chronic transection experiments indicated the involvement of trigeminal and parasympathetic neuromodulators, we conducted pharmacological experiments to investigate the identity of these vasoactive transmitters.

Atropine (10\(^{-4}\) M) attenuated the CSD hyperperfusion by 41 ± 13% (241 ± 89 vs. 138 ± 39%; \( P < 0.05 \); Fig. 6). The topical application of CGRP-(8–37) reduced hyperperfusion in CSD by 49 ± 19% (236 ± 104 vs. 111 ± 47%; \( P < 0.05 \)). When atropine was superimposed after CGRP-(8–37), CSD-associated hyperperfusion was attenuated by 69 ± 17% (236 ± 104 vs. 58 ± 40% above baseline).
21%, \( P < 0.05 \); Fig. 6). Neither atropine nor CGRP-(8—37) affected baseline CBF.

Effects of atropine and CGRP-(8—37) in chronically denervated rats on CBF during CSD. To elucidate whether the effects of CGRP-(8—37) and atropine on CSD hyperperfusion are selective effects on perivascular nerves, we combined chronic trigeminal and parasympathetic denervation with brain topical superfusion of atropine and CGRP-(8—37).

CSD hyperperfusion on the chronically denervated hemisphere was attenuated by 9.5 ± 5% after superfusion of CGRP-(8—37) \( (5 \times 10^{-7} \text{ M}) \). When the muscarinic receptor antagonist atropine \( (10^{-4} \text{ M}) \) was superfused after CGRP-(8—37) \( (5 \times 10^{-7} \text{ M}) \), CSD-associated hyperperfusion was further reduced to 114 ± 38% (further reduction of 17 ± 6%). However, these reductions were not statistically significant. The results are summarized in Table 3.

Effects of CGRP-(8—37) on CBF response to hypercapnia. When aCSF was superfused a blood flow increase above baseline of 3.22 ± 1.4% per mmHg increase of arterial \( \text{PCO}_2 \) was measured. After CGRP-(8—37) superfusion the blood flow increase amounted to 2.9 ± 1.9% per mmHg arterial \( \text{PCO}_2 \) increase \( (P = 0.71) \).

### DISCUSSION

The key finding of the present study was that chronic transection of nasociliary nerve and parasympathetic nerve fibers crossing the ethmoidal foramen led to a significant reduction of ipsilateral cortical blood flow during CSD. Acute transection or sham operation did not influence cortical hyperperfusion. Brain topical application of the muscarinic receptor antagonist atropine or the CGRP receptor antagonist CGRP-(8—37) also decreased CSD hyperperfusion. These findings suggest that CSD-associated cerebral hyperperfusion is mediated, at least in part, by an activation of trigeminal sensory and parasympathetic nerve fibers.

Role of the trigeminal nervous system. The trigeminal nervous system has already been shown to modulate CBF in other physiological and pathophysiological conditions of the central nervous system. Electrical stimulation of the nasociliary nerve leads to pial perivascular neurotransmitter release, resulting in vasodilation. Chronic ganglionicectomy or capsaicin treatment, but not chronic rhizotomy of the trigeminal nerve, reduces the short-lasting hyperemia after transient global cerebral ischemia. Because dendrites and axons degenerate after ganglionicectomy and topical capsaicin treatment but not after rhizotomy, it has been postulated that postischemic hyperperfusion is linked to the activation of unmyelinated c-fibers of the trigeminal nerve. In contrast, functional inhibition of the nasociliary nerve by nerve cooling did not attenuate postischemic hyperemia after common carotid occlusion and reperfusion. These results suggest that peripheral trigeminal fibers contribute to the blood flow responses elicited by acute severe hypertension or seizures. We have recently demonstrated that chronic, but not acute, transection attenuates the hyperemia in the early phase of bacterial meningitis (33). These results suggest that perivascular nerves play an important role in CBF control under pathophysiological conditions. Thus it is likely that cerebrovasodilation due to trigeminal activation is mediated at least in part by the release of neurotransmitters from local nerve terminals. This is consistent with the finding that trigeminal nerve stimulation in the cat increases CBF in a frequency-dependent manner, which can be reduced after CGRP receptor blockade. In addition, the histological finding of lamina-specific c-fos expression after recurrent CSD in the brain stem trigeminal nucleus, which is blocked after meningeal deafferentation, revealed an activation of the trigeminal nerve in CSD. When CGRP-(8—37) was superfused brain topically, Colonna et al. (5) found a markedly reduced hyperemia in CSD in the rabbit, as did Wahl et al. (32) in the cat. In accordance with these findings, our results of a reduced hyperperfusion in CSD after ipsilateral chronic, but not acute, transection of the nasociliary nerve, as well as after pharmacological blockade of vascular CGRP receptors, indicate a neurogenic component of the cerebrovascular response in CSD. Because peripheral axons are destroyed after chronic nasociliary nerve and parasympathetic nerve fiber transection, we propose that local changes due to CSD, e.g., elevated \( K^+ \) or glutamate concentrations, may cause a neurotransmitter release of perivascular nerve terminals that is, at least partially, mediating CSD hyperperfusion.

Recently, it has been described that tetrodotoxin (TTX), a blocker of voltage-sensitive Na\(^+\) channels,
does not influence CSD hyperperfusion, although the preceding hyperperfusion seen in these experiments in halothane-anesthetized rats was increased after TTX (7). These findings indicate that action potentials in perivascular nerves do not contribute to CSD hyperperfusion. However, this does not exclude a role for neurotransmitters from perivascular nerves in CSD hyperperfusion. During CSD the concentration of metabolites and ions changes dramatically in the extra- and intracellular space (10). The elevated concentration of extracellular K⁺ during CSD may cause depolarization of perivascular nerve fibers, inducing the release of neurotransmitter. These mediators could thus reach their target cells by so-called volume transmission (1) via extracellular fluid pathways, inducing vasodilation and hyperperfusion.

Role of the parasympathetic nervous system. Postganglionic parasympathetic nerve fibers originate mainly from the sphenopalatine ganglion. They run together with the nasociliary nerve through the ethmoidal foramen and contain VIP, ACh, and NOS as their main neurotransmitters. Other origins for ACh-positive perivascular parasympathetic nerve fibers are the internal carotid ganglion and the otic ganglion (30). Chronic transection of postganglionic parasympathetic nerve fibers led to enlarged infarct volumes in focal cerebral ischemia (11). Electrical stimulation of parasympathetic postganglionic nerve fibers causes frequency-dependent cerebral vasodilation (18, 27). We found that these fibers and their neurotransmitter ACh also contribute to the hyperemia in CSD.

To elucidate whether the cholinergic neurotransmitter ACh is released by perivascular parasympathetic nerves or parenchymal cholinergic neurons during CSD, we investigated CSD hyperperfusion in chronically denervated rats while atropine was topically brain superfused. The superfusion of atropine in chronically denervated rats afforded a reduction of CSD hyperperfusion by 17 ± 6%, which was not statistically significant. In view of the pronounced reduction of CSD hyperperfusion after atropine in nondenervated rats (41 ± 13%), we conclude that the cerebrovascular response to CSD is mediated at least in part by the activation of parasympathetic nerve fibers and a release of their vasodilator ACh. However, a contribution of intraparenchymal cholinergic neurons to CSD hyperperfusion cannot be ruled out.

The sensitivity of vascular muscarinic receptors to atropine has been shown in vivo by the blockade of cerebral vasodilation induced by electrical stimulation of the fastigial nucleus and the basal forebrain (2, 34). In isolated intracerebral arterioles atropine blocked ACh-induced vasodilation but did not attenuate vessel diameter when administered alone, indicating an effect of ACh via atropine-sensitive receptors (6). Interestingly, the vasodilation induced by electrical stimulation of the parasympathetic nerve was not sensitive to atropine (27). One potential implication of this finding is that the ACh release by CSD may originate from perivascular nerve terminals, which do not originate from parasympathetic nerves. Furthermore, it is possible that other cholinergic neurons that do not belong to the parasympathetic nervous system, e.g., basal forebrain neurons with projection to the cortex, may contribute to hyperperfusion in CSD by a release of ACh. Therefore, from our pharmacological study alone concerning the muscarinic receptor antagonist atro- pine, we cannot conclude which cholinergic system participates in CSD hyperperfusion. However, our nerve transection experiments and the combination of nerve transection and pharmacological studies indicate that parasympathetic nerve fibers are indeed involved in this response.

Our study provides evidence for a neurogenic component correlated to perivascular nerves mediating the CSD-associated cerebrovascular response (26). Chronic denervation of trigeminal and parasympathetic nerve fibers attenuated CSD-induced hyperemia by ~65% but did not abolish it completely. This suggests that other factors contribute to hyperemia in CSD. These mediators, which could be released during CSD by neurons and glia cells, may include such diverse factors as K⁺, glutamate, and NO (10, 16, 32).

Methodological considerations. Several methodological issues of this study warrant discussion. Are the observed effects of nerve transection or pharmacological blockade specific for the CSD hyperperfusion? In accordance with previous investigations in the rabbit, cerebrovascular responsiveness to CSD, but not to hypercapnia, was reduced in our study when CGRP-(7—38) was superfused, indicating a specific effect on CSD hyperperfusion (5). No significant influence on hypercapnic vasodilation was reported after chronic parasympathetic denervation (19), trigeminal gangliectomy, and trigeminal root section (25). We thus conclude that the attenuation of CSD hyperperfusion in these experiments was not due to an unspecific reduction of cerebrovascular reactivity. In our experiments, chronic denervation of both the nasociliary nerve and parasympathetic nerve fibers slightly attenuated the CBF increase in response to hypercapnia by ~20% (albeit without statistical significance). In view of the much more pronounced attenuation of the CSD hyperperfusion (55% compared with 20%), we conclude that the effect of nasociliary nerve-parasympathetic nerve fiber transection cannot be explained by an unspecific reduction of cerebrovascular reactivity by this procedure.

Another issue concerns the stability of the CSD-CBF response over time. We found that, when the first CSD, which tends to be associated with a smaller CBF response, was excluded from analysis, there were no time-dependent changes of CSD hyperperfusion. This was demonstrated in this study in the surgical group and also applies to CSD-CBF responses in a cranial window preparation (6a). This is also in agreement with time control studies by Colonna et al. (5), who did not find any differences of CSD-associated peak arteriolar diameters over time. It can therefore be excluded that the changes in CSD-CBF responses reported in our study were affected by time-dependent effects.
The amplitudes of the CBF hyperemia induced by CSD as well as by hypercapnia were higher in the cranial window experiments (pharmacological groups) compared with the measurement made through the dura mater (surgical group). A likely explanation for this finding is the increased sample volume of laser-Doppler flow probe measurements through the cranial window. When measuring through a cranial window instead of directly on the dura mater, the laser probe tip-to-brain surface distance is greater (170-µm cover-slip + thickness of wax wall), and the top and bottom glass surfaces increase scatter. To demonstrate this effect we interposed a cranial window between dura mater and the laser-Doppler flow probe tip in the hypercapnic group. A wax wall was built around the frontal burr hole on the control side before the experiment was started, but the dura remained intact. The flow probe was positioned vertically on the dura. rCBF was recorded over a time period of 4 min under resting conditions and set as baseline (100%). The wax wall was then filled with 0.9% NaCl, and a coverslip was mounted using standard cranial window geometry. The laser-Doppler flow probe was repositioned (without changing its x-y coordinates with respect to the surface of the brain), and rCBF was monitored again, this time through the cranial window. Interestingly, rCBF now was elevated to 220% compared with baseline. This indicates a profound effect of the probe-sample volume arrangement in laser-Doppler flow probe and underscores that laser-Doppler flow probe measurements of CBF cannot be used for absolute quantitation. Because no comparisons were performed between the cranial window group and the surgical group (dura intact), the conclusions drawn from our study are not affected by the phenomenon described here.

To summarize the major conclusions of this study, we have shown for the first time that perivascular trigeminal and parasympathetic nerve fibers are involved in CSD-associated hyperemia. Our results link CSD-induced perivascular trigeminal and parasympathetic nerve activation with local release of vasodilator mediators, such as CGRP and ACh, to the acute, transient CBF hyperfusion associated with CSD.

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Address for reprint requests: U. Reuter, Dept. of Neurology, Experimental Neurology, Charité Hospital, Humboldt Univ., 10098 Berlin, Germany.

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