Transcorneal stimulation of trigeminal nerve afferents to increase cerebral blood flow in rats with cerebral vasospasm: a noninvasive method to activate the trigeminovascular reflex

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Object. The goal of this study was to investigate whether stimulation of trigeminal afferents in the cornea could enhance cerebral blood flow (CBF) in rats after they have been subjected to experimental subarachnoid hemorrhage (SAH). Cerebral vasospasm following SAH may compromise CBF and increase the risks of morbidity and mortality. Currently, there is no effective treatment for SAH-induced vasospasm. Direct stimulation of the trigeminal nerve has been shown to dilate constricted cerebral arteries after SAH; however, a noninvasive method to activate this nerve would be preferable for human applications. The authors hypothesized that stimulation of free nerve endings of trigeminal sensory fibers in the face might be as effective as direct stimulation of the trigeminal nerve.

Methods. Autologous blood obtained from the tail artery was injected into the cisterna magna of 10 rats. Forty-eight and 96 hours later (five rats each) trigeminal afferents were stimulated selectively by applying transcorneal biphasic pulses (1 msec, 3 mA, and 30 Hz), and CBF enhancements were detected using laser Doppler flowmetry in the territory of the middle cerebral artery. Stimulation-induced changes in cerebrovascular parameters were compared with similar parameters in sham-operated controls (six rats). Development of vasospasm was histologically verified in every rat with SAH. Corneal stimulation caused an increase in CBF and blood pressure and a net decrease in cerebrovascular resistance. There were no significant differences between groups for these changes.

Conclusions. Data from the present study demonstrate that transcorneal stimulation of trigeminal nerve endings induces vasodilation and a robust increase in CBF. The vasodilatory response of cerebral vessels to trigeminal activation is retained after SAH-induced vasospasm.

Key Words • subarachnoid hemorrhage • cerebral vasospasm • cerebral ischemia • cerebral blood flow • trigeminovascular reflex • rat

Flushing of the face during trigeminal rhizotomy is a well-known phenomenon. Similarly, electrical stimulation of the trigeminal ganglion causes a decrease in the vascular resistance and induces vasodilation of branches of the internal carotid artery.11 Several authors have investigated the mechanism of trigeminal nerve-mediated vasodilation.4,7,12,13,17,20

Cerebral blood vessels are innervated by sympathetic, parasympathetic, and trigeminal sensory nerve fibers, all of which play an important role in cerebrovascular regulation.12 The ophthalmic division of the trigeminal nerve, which is the origin of the nasociliary nerve, is the primary sensory nerve to supply cerebral blood vessels.20,21 Major vasodilator innervation of the MCA comes from the nasociliary nerve.21 Stimulation of this nerve causes the antidromic release of vasoactive neuropeptides, such as calcitonin gene–related peptide and substance P, from perivascular nerve fibers,3,13–15,20,21 resulting in an increase in CBF as well as an orthodromic transmission of impulses to activate brainstem parasympathetic nuclei in a reflexive manner. The afferent arm of the trigeminoparasympathetic reflex consists of the peripheral (innervating the cerebral vasculature) and central (projecting to the brainstem) axons of the trigeminal ganglionic neurons. Central axons form synapses with each facial nucleus on both sides of the brainstem.8,10,18,20,21 After forming synapses within the sphenopalatine and otic ganglia, the parasympathetic efferents from the facial nuclei innervate cerebral blood vessels through postganglionic fibers. The postganglionic parasympathetic fibers release the following potent vasodilators: acetylcholine, vasoactive intestinal peptide, and nitric oxide.4,12,20,22

Cerebral vasospasm following SAH may compromise CBF and increase the risks of morbidity and mortality.5,9,16,18,19 Currently, there is no effective treatment against posthemorrhagic cerebral vasospasm. Salar, et al.,37 found that stimulation of the trigeminal nerve in pigs may dilate constricted cerebral arteries after experimental SAH, suggesting that activation of trigeminal afferents in patients with SAH may help improve the compromised CBF. Clearly, a noninvasive method is preferable for human applications. We thought that stimulation of the free nerve endings of the trigeminal sensory fibers in the face might be as effective as
direct electrical stimulation of the trigeminal ganglion. In the present study, we tested this hypothesis in a rat model of cerebral vasospasm induced by an intracisternal autologous blood injection. To be able to induce a reproducible trigeminal activation in every animal under study, we stimulated the cornea of the rats instead of their facial skin. Corneal electrodes provided a more reliable electrical contact than skin electrodes, and allowed us to activate a fairly consistent number of trigeminal fibers with each stimulation. The stimulation pattern that we used evoked robust and highly reproducible increases in CBF in healthy rats. In line with the report by Salar, et al., increases in CBF were also observed in rats suffering from cerebral vasospasm, suggesting that the vasodilatory response of cerebral vessels to trigeminal activation is retained after SAH-induced vasospasm, which may be instrumental in clinical practice, if a safe and efficient procedure applicable to humans can be developed in future studies.

Materials and Methods
Experiments were performed in accordance with institutional guidelines for the use of experimental animals and after approval was granted by the ethics committee of Hacettepe University Medical School. Male albino Wistar rats, each weighing between 220 and 250 g, were used in this study. The rats were housed in separate cages in an insecticide-free environment at standard temperature to avoid inhibition of acetylcholinesterase and, hence, potentiation of the parasympathetic nerve-mediated vasodilation. Fasting was imposed on the animals overnight before the experiment.

Induction of SAH
For induction of experimental SAH, intraperitoneal chloral hydrate (375 mg/kg) was used to anesthetize the rats. Autologous blood obtained from the animal’s tail artery was injected intracisternally while the rat was immobilized in the prone position by using a stereotactic frame. A midline occipitocervical incision was created and the suboccipital muscles were dissected to reveal the atlantooccipital membrane. The membrane was punctured with a 27-gauge needle and 0.4 ml/kg of cerebrospinal fluid was replaced with the same amount of autologous blood. After the injection, the rat was placed in the Trendelenburg position for 30 minutes to allow the blood to reach all basal cisterns while the wound was being closed. The rat was then returned to its cage.

Measurement of Blood Flow
For blood flow experiments, urethane (1.2 g/kg) was used as the anesthetic agent. A catheter was placed into the left femoral artery for continuous blood pressure monitoring. A rectal temperature probe was inserted to monitor body temperature, which was maintained at 37˚ ± 0.1˚C by using a homeothermic blanket unit. The rat was again immobilized in the prone position by using a stereotactic frame and a midline skin incision was created over the skull. Using a dental drill under continuous irrigation, we performed a craniotomy 2 mm posterior and 5 mm lateral to the bregma with the aid of stereoscopic magnification (magnification × 4–8; OPMI surgical microscope; Carl Zeiss, Göttingen, Germany). A nonvascular area between two arteries was selected for placement of the needle probe (0.5 mm thick) of the LD flowmeter (PF 2B or PF 302; Perimed, Järfalla, Sweden). The baseline CBF value was calibrated as 100% and changes in CBF were recorded relative to this value.

In the initial experiments, CBF measurements were obtained after destruction of the sphenopalatine ganglion or after a local anesthetic agent (lidocaine %1) had been applied to the rat’s cornea to establish the role of trigemino-parasympathetic reflex in corneal stimulation–induced increases in CBF. For destruction of the sphenopalatine ganglion, the rat was placed in a stereotactic frame, after which a sagittal scalp incision was made near the right orbit. The intraorbital structures were retracted laterally, and structures in the ethmoidal foramen (nasociliary nerve, ethmoidal artery, and postganglionic parasympathetic nerve fibers from the sphenopalatine ganglion) were exposed.

Animal Groups and Corneal Stimulation
After completion of initial control experiments, which demonstrated that the observed flow changes were mediated by the trigemino-vascular reflex, 16 rats were separated into three groups: six rats were subjected to a sham operation; five rats received an intracisternal blood injection. To be able to induce a reproducible trigeminal activation in every animal under study, we stimulated the cornea of the rats instead of their facial skin. Corneal electrodes provided a more reliable electrical contact than skin electrodes, and allowed us to activate a fairly consistent number of trigeminal fibers with each stimulation. The stimulation pattern that we used evoked robust and highly reproducible increases in CBF in healthy rats. In line with the report by Salar, et al., increases in CBF were also observed in rats suffering from cerebral vasospasm, suggesting that the vasodilatory response of cerebral vessels to trigeminal activation is retained after SAH-induced vasospasm, which may be instrumental in clinical practice, if a safe and efficient procedure applicable to humans can be developed in future studies.

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Histological Study

After the experiment, the rats were killed with a high dose of intravenous urethane and were transthoracally perfused initially with 50 ml of 0.03-M phosphate-buffered saline and later with 100 ml of 4% paraformaldehyde. Specimens were postfixed in 4% paraformaldehyde and examined with the aid of a light microscope. Hematoxylin and eosin were used to detect erythrocytes and meningeal reaction as indicators of SAH. Verhoeff elastic tissue stain was used to identify changes in the IEL and those in the vessel wall due to vascular spasm. The wall thicknesses of MCAs were measured at the level just posterior to the optic chiasm, and trabeculations of the IEL, vac- uolization in the tunica media, and thickening of the arterial wall were detected as indicators of vasospasm.

Statistical Analysis

All CBF, CVR, and MABP data were expressed as the means ± SEMs. Groups were compared by applying the Kruskal–Wallis test followed by the Mann–Whitney U-test. Probability values lower than 0.05 were considered to be significant.

Results

Cerebrovascular Responses to Corneal Stimulation

Transcorneal stimulations were well tolerated by the rats. No incidence of death or seizure was observed. Regular cycles of corneal stimulations (30 seconds every 5 minutes) were started after CBF and ABP had been stabilized (Fig. 1). The MABPs of the three groups were not significantly different from each other: 84 ± 18 mm Hg in the control group, and 85 ± 22 mm Hg and 83 ± 14 mm Hg in animals with SAH that received corneal stimulation at 48 and 96 hours, respectively (p > 0.05, Kruskal–Wallis test).

Corneal stimulation caused a rapid increase in ABP as well as in CBF with every stimulation (Fig. 1). Both levels returned to baseline values within 5 minutes. Local anesthesia of the cornea readily abolished the CBF response. On the other hand, stimulation of the contralateral, unanesthetized cornea caused a bilateral CBF rise in these rats. Destruction of the sphenopalatine ganglion and postganglionic nerve fibers also abolished the CBF response to the corneal stimulation.

With the aid of the data acquisition and analysis software, we automatically calculated and monitored the CBF changes as an estimate of the magnitude of vasodilation (Fig. 1). Corneal stimulations consistently caused a decrease in the CBF by 24 to 39%. This abrupt decrease was sustained throughout the stimulation and readily recovered after cessation of the stimulation. There was a gradual drop in the baseline CBF by 7.4 ± 6% during successive stimulations in rats from all groups (Fig. 1). We measured the mean changes in CBF, ABP, and CVR during each 30-second stimulation period and averaged five consecutive trials so that we could represent each rat. We found no significant differences between groups in changes in the CBF as well as in changes in the CBF and ABP (p > 0.05, Kruskal–Wallis test). The gradual small drop in the CBF that occurred during successive stimulations also was not different when the groups were compared (p > 0.05, Kruskal–Wallis test). Changes in mean CBF, ABP, and CVR in the three groups are shown in Table 1 and Fig. 2.

Histopathological Observations

Histological evaluation of arterial walls showed thickening, corrugation of the IELs, vacuolization of the tunica media, and inflammation of the adventitia in rats with SAH, compared with the control group (Fig. 3). In animals in the control group the mean arterial wall thickness was 133.7 ± 7.6 μm, whereas in the SAH groups the mean arterial thicknesses were 236 ± 13.7 μm in animals receiving corneal stimulation at 48 hours and 218.3 ± 10.2 μm in animals receiving corneal stimulation at 96 hours post-SAH (Fig. 4). The Kruskal–Wallis test for the mean arterial wall thickness revealed a significant difference among the three groups (p = 0.0046). Further analysis with the Mann–Whitney U-test showed significant differences between both SAH groups and the sham-operated control group (p < 0.001).

Discussion

The results of the present study demonstrate that transcorneal stimulation of trigeminal nerve endings induces a robust increase in CBF. This CBF enhancement exceeded the accompanying increase in systemic ABP, indicating a net decrease in CVR. The increase in ABP was possibly caused by brainstem reflexes that were activated through connections among the trigeminal afferents, the facial nerves, and the vasomotor center.12

The effect of noxious mechanical stimulation of facial cutaneous areas on cortical blood flow was examined using LD flowmetry in anesthetized rats by Adachi and colleagues.1 Pinching of the face or fore- or hindpaw for 15 seconds produced significant increases in cortical blood flow and systemic blood pressure. Following spinal transection at the T-1 level, the response of ABP to forepaw pinching was suppressed, whereas the increase in cortical blood flow continued. These findings support our conclusion that the increase in CBF following noxious corneal stimulation was independent of changes in systemic ABP.

The vasodilatory response that we observed was abolished by applying a local anesthetic agent to the cornea, demonstrating that the response was evoked by activation of trigeminal nerve endings in the cornea. The total loss of the CBF increase after administration of lidocaine also indicates that electrical stimulation was confined to the cornea and did not spread to neighboring areas. Destruction of the sphenopalatine ganglion abolished the CBF response, providing strong evidence that it was mediated by the trigeminosphenopalatine reflex arc. These findings conform with

### Table 1

<table>
<thead>
<tr>
<th>Factor</th>
<th>Control Group</th>
<th>48-Hr Stim</th>
<th>96-Hr Stim</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>6</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>ΔCBF (%)</td>
<td>109.9 ± 24.3</td>
<td>65.4 ± 13.9</td>
<td>118.2 ± 28.8</td>
</tr>
<tr>
<td>ΔCVR (%)</td>
<td>−39.8 ± 5.2</td>
<td>−24.6 ± 6</td>
<td>−38.7 ± 6.1</td>
</tr>
<tr>
<td>ΔABP (%)</td>
<td>15.2 ± 4.8</td>
<td>20.8 ± 7</td>
<td>25.7 ± 6.5</td>
</tr>
</tbody>
</table>

* Values are expressed as the means ± SEM. The CBF values are negative, denoting a decrease in CBF. Abbreviations: BP = blood pressure; 48-hr stim = animals that received corneal stimulation 48 hours post-SAH; 96-hr stim = animals that received corneal stimulation 96 hours post-SAH; Δ = change in.
the proposed anatomy of the vasodilatory response induced by trigeminal stimulation.

Several groups have reported resistance or flow changes of similar magnitude to those of our study. For example, Goadsby and associates\textsuperscript{7,8} noted that stimulation of the trigeminal ganglion in the cat caused a reduction in both bulk carotid artery resistance and CVR by 39%. Salar, et al.\textsuperscript{17} found that continuous stimulation of the ganglion maintained an increase in CBF of more than 100% in pigs; however, some other authors reported smaller increases in CBF, possibly due to partial and selective stimulation of the reflex arc and to differences in stimulation parameters. For instance, electrical stimulation of postganglionic fibers of the sphenopalatine ganglion induced a 32% increase in CBF in the ipsilateral parietal region in a rat study.\textsuperscript{12} Suzuki, et al.,\textsuperscript{20} found that selective stimulation of the ophthalmic branch of the trigeminal nerve in the rat increased cortical blood flow by 17%. To stimulate trigeminal nerve fibers selectively, neighboring postganglionic fibers from the sphenopalatine ganglion and proximal portion of the nasociliary nerve were transected beforehand and peripheral fibers were allowed to degenerate. In another study, stimulation of the pterygopalatine and greater superficial petrosal nerves in the dog caused 16 and 20% increases in the diameter of the MCA, respectively.\textsuperscript{22} Values of CBF were not measured in the latter study and the diameters of the arteries were determined using angiography.

Calculated CVR values are an indirect but reliable measure of vasodilation. They help eliminate the contribution of ABP to flow enhancement. If the CBF passively follows changes in ABP, the formula yields no change in CVR. We detected a net drop in CVR during the stimulations, which readily recovered after stimulation, despite the fact that the CBF remained elevated for a few more minutes due to slow recovery of the ABP increase. Although the values obtained do not indicate the absolute vascular resistance, changes relative to baseline provide a good estimate of arterial dilation.
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tion, because we can assume that the intracranial pressure remained stable during the 30-second stimulation periods. Measurements of CVR clearly showed that the vasodilatory response to trigeminal nerve activation was preserved 48 and 96 hours after onset of SAH. Histopathological examination confirmed that vasospasm developed in the proximal portion of the MCA in these rats. Nevertheless, all the animals retained the capacity to enhance blood flow in distal branches of the MCA.

The LD flow technique has the advantage of continuously monitoring CBF during the course of repeated stimulations, but it does not provide absolute flow values. Consequently, we do not know the absolute magnitude of residual cortical blood flow in groups with vasospasm or that of the changes induced by stimulation of the trigeminal nerve. Using methods designed to detect absolute CBF levels, which unfortunately do not have the same time resolution as the LD technique, may clarify these issues in future studies.

In support of our findings, 1 week after injection of autologous blood into the subarachnoid space in Pittmann–Moore pigs, a considerable decrease in CBF was noted in six of 10 animals studied. Continuous electrical stimulation of the gasserian ganglion in animals with reduced CBF produced a remarkable cerebrovascular dilation and flow increase that lasted more than 3 hours. In that study by Salar, et al.,17 however, the CBF measurements were obtained using the xenon inhalation technique and, thus, changes in CBF could not be continuously monitored as was done using the LD technique.

Conclusions

Rather than open the skull and manipulate the trigeminal nerve directly, we activated this nerve by stimulating sensory afferents in the cornea. This noninvasive method was just as effective in inducing vasodilation as other methods reported in previous studies. Hence, our data indicate that stimulation of cutaneous afferents of the trigeminal nerve may prove to be a simple method to induce cerebral vasodilation during cerebral ischemic conditions such as vasospasm, if a safe and efficient procedure can be developed for use in humans in future preclinical and clinical studies.

References


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