Effect of electrical stimulation of the cervical spinal cord on blood flow following subarachnoid hemorrhage

Laboratory investigation

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Object. Cervical spinal cord stimulation (SCS) increases global cerebral blood flow (CBF) and ameliorates cerebral ischemia according to a number of experimental models as well as some anecdotal reports in humans. Nonetheless, such stimulation has not been systematically applied for use in cerebral vasospasm. In the present study the authors examined the effect of cervical SCS on cerebral vasoconstriction in a double-hemorrhage model in rats.

Methods. Subarachnoid hemorrhage (SAH) was induced with 2 blood injections through an indwelling catheter in the cisterna magna. Spinal cord stimulation was applied 90 minutes after induction of the second SAH (Day 0) or on Day 5 post-SAH. Measurements of the basilar artery (BA) diameter and cross-sectional area and regional CBF (using laser Doppler flowmetry and 14C-radiolabeled N-isopropyl-p-iodoamphetamine hydrochloride) were obtained and compared between SAH and sham-operated control rats that did not receive SCS.

Results. At Day 0 after SAH, there were slight nonsignificant decreases in BA diameter and cross-sectional area (89 ± 3% and 81 ± 4%, respectively, of that in controls) in no-SCS rats. At this time point, BA diameter and cross-sectional area were slightly increased (116 ± 6% and 132 ± 9%, respectively, compared with controls, p < 0.001) in SCS-treated rats. On Day 5 after SAH, no-SCS rats had marked decreases in BA diameter and cross-sectional area (64 ± 3% and 39 ± 4%, respectively, compared with controls, p < 0.001) and corrugation of the vessel wall. These changes were reversed in rats that had received SCS (diameter, 110 ± 9% of controls; area, 106 ± 4% of controls; p < 0.001). Subarachnoid hemorrhage reduced CBF at Days 0 and 5 post-SAH, and SCS increased flows at both time points, particularly in regions supplied by the middle cerebral artery.

Conclusions. Data in this study showed that SCS can reverse BA constriction and improve global CBF in this SAH model. Spinal cord stimulation may represent a useful adjunct in the treatment of vasospasm.


Key Words • cerebral blood flow • cerebral vasospasm • double-hemorrhage rat model • spinal cord stimulation • subarachnoid hemorrhage

Cerebral vasospasm following aneurysmal SAH is still a major cause of morbidity and death.20,25,37 Cerebral vasospasm is an angiographically demonstrable, variable arterial constriction leading to reduced rCBF.20 Delayed cerebral vasospasm is generally recognized as the most significant cause of disability or death following the successful treatment of a ruptured aneurysm in patients.25

Abbreviations used in this paper: BA = basilar artery; CBF = cerebral blood flow; 14C-IMP = 14C-radiolabeled N-isopropyl-p-iodoamphetamine hydrochloride; LDF = laser Doppler flowmetry; MCA = middle cerebral artery; rCBF = regional CBF; SAH = subarachnoid hemorrhage; SEM = standard error of the mean; SCS = spinal cord stimulation.

It usually occurs between 3 and 14 days after SAH. The rate of morbidity and mortality is 30%, despite the administration of maximal therapy.20,25 Although intensive research has been performed over more than 5 decades, the pathogenesis of delayed cerebral vasospasm is not entirely clear, and the disorder remains a major clinical challenge. In addition to delayed vasospasm, acute vasoconstriction has been shown to occur within 48 hours after the onset of SAH in up to 13% of patients, with a significant association to an unfavorable outcome.1,28,31 Approximately 25% of patients with aneurysmal SAH die within the first 24 hours, and postmortem examinations in such cases show extensive ischemic brain injury.2
have shown that cervical SCS can increase CBF globally.1,3,6,18,24,26,30 In the last few years, there has been increasing interest in the use of SCS to treat cerebral vasospasm after experimental SAH.10,14,15,19 However, researchers have used a single-hemorrhage model that is inappropriate for the study of delayed cerebral vasospasm.26,32 Furthermore, the stimulation paradigms have differed widely.10,14,15,19 We recently developed a model of SAH involving a double hemorrhage that demonstrates delayed vasocnstriction similar to that seen in humans.21 Our aim in the current study was therefore to assess the effect of cervical SCS on vasospasm and CBF in this double-hemorrhage model.

Methods

Surgical Procedures

Seventy-one male Sprague–Dawley rats weighing 280–350 g were used in this study. The University Committee on the Use and Care of Animals at the University of Michigan approved the study protocols.

Induction of SAH

Subarachnoid hemorrhage was induced in 56 rats by using a double-hemorrhage injection method described previously.21 General anesthesia was induced with 5% isoflurane (Aerrane, Baxter Healthcare Corp.). After inserting a tube for breathing and initiating mechanical ventilation, isoflurane was titrated between 2.25 and 2.75% to maintain a mean arterial pressure between 80 and 120 mm Hg. Body temperature was maintained at 37°C. The femoral artery was cannulated to monitor blood pressure and blood gas levels, and the femoral vein, to administer radioactive isotope.

After laminectomy at C-1, a PE-10 catheter was introduced into the cisterna magna through a small bur hole just rostral to the interparietal-occipital suture. Autologous blood (0.2 ml) from the femoral artery was then injected into the cisterna magna using the catheter. A second (0.1 ml) injection of blood was administered through the same catheter after 24 hours. Control rats received 2 intracisternal injections of physiological saline solution according to the same procedure.

Spinal Cord Stimulation

Immediately (90 minutes) after the second blood injection (Day 0) or on Day 5 post-SAH, the dura mater over the spinal cord was reexposed at the level of C-1, and a platinum ball electrode was placed epidurally in the midline over the dorsal surface. A ground electrode was placed in the proximal hindquarters of the rat. We used a standard simulation setup consisting of a stimulator (model S48, Grass Instruments), a constant-current unit (model CCU1-A, Grass Instruments), and an oscilloscope (model 5111-A, Tektronix). Based on previous studies,30,39 unipolar stimulation at a frequency of 50 Hz, a pulse width of 0.2 msec, and a current of 0.6 mA was used in the experiments.

Electrical stimulation was applied for 2 minutes at 3 intervals. Prior to stimulation, the motor threshold stimulus intensity (tonic contraction of the neck muscle) was tested in each animal at 50 Hz for 0.2 msec and was usually between 1.2 and 1.5 mA. The first stimulation was performed after a stable baseline LDF measurement had been obtained. After an 8-minute rest period, the second stimulation cycle followed the establishment of stable and reproducible LDF measurements. The third stimulation was then performed after another 8-minute rest period.

Histological Examination and BA Measurements

Rats in which SAH had been induced and that had or had not received SCS were examined histologically either 90 minutes after the second hemorrhage or on Day 5 (4 animal groups, 5 rats per group). Control rats were also examined (5 rats). Perfusion fixation was performed with transthoracic cannulation of the left ventricle by using a 23-gauge butterfly needle, clamping of the descending thoracic aorta, and opening of the right atrium. Perfusion was performed at 100 cm H2O pressure at room temperature, beginning with 50 ml of 0.1-ml/L phosphate-buffered solution (pH 7.4) and followed by 50 ml of 4% formaldehyde. The entire brain was removed and immersed in the same fixative overnight at 4°C, followed by immersion in 30% sucrose for 3–4 days. The brainstem was then carefully separated at the level of the superior cerebellar artery and placed in embedding optimal cutting compound (Sakura Finetek USA, Inc.).

For examination of the vascular changes in the BA, the brainstem was divided into 4 segments at an interval of 2 mm. Eight-micrometer sections were cut on a cryostat at 200 µm below the superior cerebellar artery (upper portion), and standard staining with H & E was performed. For each vessel at each level, 10 sequential sections were measured and averaged. Diameter and lumen cross-sectional areas of the BA were determined by a blinded observer using computerized image analysis software (NIH Image, National Institutes of Health).

Laser Doppler Flowmetry

Cortical blood flow was examined using a laser Doppler flowmeter (Laserflo BMP2, Vasamedics). A bur hole was drilled 6 mm lateral (right) and 1.5 mm posterior to the bregma for placement of the LDF probe, as previously described.30 After obtaining the LDF baseline values, SCS was started for 2 minutes, during which time LDF was continuously monitored. Changes in LDF were recorded as the percentage change in control (prestimulation) values.

Radiotracer Studies

Quantitative measurements of cCBF were obtained with the indicator fractionation method by using the radiotracer 4C-IMP (American Radiolabeled Chemicals) as described previously.21 Fifteen microcuries of 4C-IMP was injected into the femoral vein 10 seconds after the onset of stimulation. Stimulation was applied for 2 minutes while blood was continuously sampled from the femoral artery. Once stimulation was stopped, the animal was killed and its brain was immediately removed and stripped of arach-
noid membrane. After separation of the cerebellum and brainstem, the hemispheres were sampled as follows: the cortical shell from each hemisphere was flattened and, by means of a 7- and 10-mm diameter concentric punch, divided into 3 samples representing the core of the MCA territory, an intermediate zone around the core, and a remaining outer zone. Brain samples were dissolved in methylbenzethonium hydroxide before radioactive counting. Blood withdrawn from the animal during the experiment was analyzed in a scintillation counter to allow quantification of CBF measurements according to the following equation: \[ F_b/M_b = Q_b(T) \times \frac{F_s}{Q_s(T)} \times M_b \]

where \( F_b \) represents CBF, \( M_b \) is the brain mass (g), \( Q_b(T) \) is the quantity of indicator in the tissue at time \( T \), \( F_s \) is the rate of femoral blood withdrawal from \( t = 0 \) to \( t = T \), and \( Q_s(T) \) is the quantity of indicator present in the cannula at time \( T \). Cerebral blood flow, expressed as \( \text{ml} \times 100 \text{ g}^{-1} \times \text{min}^{-1} \), was measured in SAH animals that did or did not receive SCS on Day 0 and Day 5 after the second hemorrhage. Statistical Analysis

Values are presented as the means ± SEM. Statistical comparisons between groups were performed using a one-way analysis of variance followed by either a Dunnett or a Tukey post hoc test. The Dunnett post hoc test was used for comparisons to a single control group, whereas the Tukey post hoc test was used to compare across multiple groups. A probability value < 0.05 was considered statistically significant.

Results

Basilar Artery Diameter and Cross-Sectional Area

In rats that did not receive SCS, at Day 0 after the second blood injection there were no significant changes in BA diameter or cross-sectional area compared with controls (Fig. 1). By Day 5 after the second hemorrhage, however, there was a significant reduction in BA diameter (Fig. 2 left) and cross-sectional area (Fig. 2 right) compared with controls. These changes were associated with corrugation of the internal elastic lamina, narrowing of the arterial lumen, and thickening of the vessel wall.

Spinal cord stimulation resulted in an increase in the diameter (Fig. 2 left) and consequently the cross-sectional area (Fig. 2 right) of the BA on Day 0 as well as on Day 5 after the second hemorrhage. On Day 0, BA diameter increased from 89 ± 3% to 116 ± 6% of controls treated with SCS (p < 0.001), and the cross-sectional area increased from 81 ± 4% to 132 ± 9% (p < 0.001). On Day 5, BA diameter increased from 64 ± 3% to 110 ± 5% of controls treated with SCS (p < 0.001), and the cross-sectional area increased from 39 ± 4% to 106 ± 4% (p < 0.001). Interestingly, SCS also caused visible dilation of smaller arteries in the subarachnoid space without a corresponding morphological change in the parenchymal vessels (data not shown).

Laser Doppler Flowmetry

On Days 0 and 5 after SAH, cervical SCS caused an immediate (within 10 seconds) increase in the LDF values to 199 ± 10% and 174 ± 16% above baseline, respectively. Thereafter, LDF values progressively decreased, reaching values ~ 50% above baseline at 40 seconds and remaining constant during stimulation (Fig. 3). There were no significant differences in the percentage increase in LDF values with SCS between the first, second, and third cycles at Days 0 (184 ± 13%, 209 ± 19%, and 199 ± 10%, respectively) and 5 (150 ± 8%, 167 ± 14%, and 174 ± 16%, respectively).

Regional CBF

Quantitative measurement of rCBF showed a global reduction in flow on Days 0 (~ 32% reduction) and 5 (~ 25% reduction) after SAH as compared with controls (Table 1 and Fig. 4). After SCS, there was a significant global increase in CBF at Days 0 (~ 58% above no-SCS rats) and 5 (~ 46% above no-SCS rats). The CBF increase was more pronounced in cerebral regions supplied by the MCA compared with that seen in regions supplied by the BA. Interestingly, the most remarkable effect of SCS was observed in the core MCA region; in that region, CBF increased from 69 ± 7% to 118 ± 10% of controls (p < 0.01) on Day
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Discussion

In humans, reductions in CBF after SAH seem to occur in 2 phases, immediately following hemorrhage (early) and late in the 1st week after (delayed). Although the phenomenon is well described in animal models of SAH, much less is known about reductions in blood flow at the time of, or shortly after, patient admission. Nevertheless, early and delayed reductions in blood flow might lead to significant disability or death, and there still exists a need for novel therapeutic strategies to prevent or treat these incapacitating conditions. The current management of delayed cerebral vasospasm is limited to hemodynamic augmentation of circulating volume and arterial blood pressure (hemodynamic therapy), the use of the calcium channel blocker nimodipine, and intraarterial interventions such as balloon angioplasty. These interventions are costly, complex to administer, and associated with significant morbidity, even death from arterial rupture.

Since the first report by Hosobuchi in 1985, the ability of SCS to augment CBF has been demonstrated in several clinical and experimental studies. A number of clinical reports in which authors describe the use of SCS in the treatment of patients with cerebral ischemia have been published. In animals, the effect of SCS on CBF is immediate and lasts for at least 5 minutes after ceasing stimulation. Recently, Sagher and colleagues have reported the use of SCS in rats to mitigate the ischemic injury associated with focal occlusion of the MCA. The neuroprotection associated with SCS was robust and occurred even when stimulation was started up to 6 hours after the ischemic insult. In the last few years, a few authors have investigated the use of SCS to treat cerebral vasospasm after experimental SAH. These authors used a single-hemorrhage model in either rats or rabbits. They demonstrated that SCS could increase CBF.

Fig. 2. Bar graphs demonstrating diameter (left) and cross-sectional area (right) of the BA in SAH rats (Days 0 and 5) that did or did not receive SCS and control rats. An analysis of variance demonstrated significant differences between the groups in terms of BA diameter (F = 15.35, p < 0.001) and cross-sectional area (F = 33.39, p < 0.001). A Tukey “honestly significant difference” post hoc test indicated that there was a significant reduction in artery diameter and cross-sectional area at Day 5 but not Day 0 and that SCS increased vessel diameter and cross-sectional area at Day 0 and 5. Values are presented as the means ± SEM. Five animals per group. **p < 0.001, compared with controls. ***p < 0.001, compared with controls.

Fig. 3. A: Schematic demonstrating overall experimental design. B: Graph showing changes in LDF during cervical SCS at Days 0 and 5 after SAH. Spinal cord stimulation caused an immediate increase in LDF within 10 seconds in both groups. Thereafter, LDF values progressively decreased, reaching values ~ 50% over baseline at 40 seconds and then remained constant during stimulation (shaded area). The results shown were obtained from the third cycle of SCS. There was no significant difference in the percentage increase in LDF with SCS between the first, second, and third cycles at Day 0 (184 ± 13%, 209 ± 19%, and 199 ± 10%, respectively) and Day 5 (150 ± 8%, 167 ± 14%, and 174 ± 16%, respectively). Values represent the means ± SEM.

0 and from 70 ± 6% to 114 ± 11% of controls (p < 0.01) on Day 5.
ever, because such models have not been shown to induce chronic vasospasm, the results of such studies are not easily translated to the clinical realm.\textsuperscript{23,33}

Recently, we developed a novel SAH model in rats by inducing a double hemorrhage through an indwelling catheter in the cisterna magna, which causes a biphasic CBF reduction immediately after the second hemorrhage and on Day 5.\textsuperscript{5} The changes at Day 5 post-SAH were associated with narrowing of the BA. Utilizing this model and by applying SCS, we have obtained data showing a significant increase in global CBF early and late after SAH. In both cases, SCS causes a rapid rise in LDF values over baseline. The quantitative measurement of rCBF resulted in a pronounced flow increase in the cerebellum and cortex from 62–76% to 91–115% and from 69–70% to 115–118% of controls, respectively. In accordance with data from experimental ischemic studies,\textsuperscript{18} the CBF increase was more pronounced in cerebral regions supplied by the MCA compared with that in regions supplied by the BA during vasospasm. Similarly, Ebel et al.\textsuperscript{10} reported a CBF increase in the cerebellum and cerebrum from 62 to 94% and from 75 to 97% of controls, respectively, following SCS in a SPECT study with technetium-99m hexamethylpropyleneamine oxime, whereas Karadaq et al.\textsuperscript{19} observed a gradual increase in CBF by using LDF, with the highest value ~ 30% over controls in the 10th minute of SCS.

In an attempt to reverse cerebral vasospasm, several pharmacological agents have been investigated in terms of their antispastic effect. Recently, a multicenter, placebo-controlled, double-blind randomized study showed that the novel endothelin receptor antagonist clazosentan significantly decreases the incidence and severity of angiographically confirmed cerebral vasospasm following severe aneurysmal SAH, which was associated with a clear reduction in the number of new cerebral infarcts observed on CT scans.\textsuperscript{35} Concomitantly, many antiinflammatory substances have been applied in experimental settings to prevent cerebral vasospasm in recent years. The substances were injected intracerebrally through the cisterna magna, intraperitoneally, or intravenously before or after SAH induction. The caspase inhibitor N-benzyl-oxcarbonyl-Val-Ala-Asp-fluoromethylketone could be angiographically demonstrated to cause a significant BA dilation from ~ 67 to ~83% of controls in a single-SAH model in rabbits.\textsuperscript{17} Using the same animal model, repeated treatments with endothelin-converting enzyme inhibitor (S)-2-biphenyl-4-y1-1-(1H-tetrazol-5-yl)-ethylinomethyl phosphonic acid led to significantly attenuated vasoconstriction with rising cross-sectional areas of the BA from 28 to 85% of controls.\textsuperscript{23} Moreover, adenosine A\textsubscript{2A} receptor agonist 2(4-[2-carboxyethyl]phenyl)ethylamino-5'-N-ethylcarboxamidoadenosine caused a significant increase in the cross-sectional area of the BA from 83 to 105% of controls in a single-hemorrhage model in rats.\textsuperscript{22} Lastly, Takanashi et al.\textsuperscript{44} reported an increase in BA diameter to a similar degree (from ~ 66 to ~ 88% of controls) after intrathecal application of liposome-entrapped fasudil, a Rho-kinase inhibitor, in a histological study using a double-hemorrhage model in rats. In the present study, we observed more pronounced vasodilation, with an increase in the cross-sectional area of the BA from 81 to 132% early after SAH and from 39 to 106% of controls in the delayed phase of vasospasm following SCS. However, the lesser degree of vasodilation after SCS dur-

\begin{table}
\centering
\caption{Regional CBF values obtained using \textsuperscript{14}C-IMP\textsuperscript{*}}
\begin{tabular}{lcccc}
\hline
Region & Controls & Day 0 & Day 5 & Day 0 w/SCS & Day 5 w/SCS \\
\hline
basal ganglia cortex & 132.6 ± 12.3 & 93.9 ± 5.7\textsuperscript{†} & 105.7 ± 2.9 & 143.7 ± 9.0 & 145.8 ± 12.7 \\
core & 98.8 ± 9.7 & 72.18 ± 6.6 & 70.0 ± 5.1\textsuperscript{†} & 120.6 ± 9.0 & 120.8 ± 11.3 \\
intermediate & 96.4 ± 9.1 & 64.2 ± 5.6 & 67.2 ± 5.0\textsuperscript{†} & 110.2 ± 9.9 & 111.4 ± 11.5 \\
outer & 93.4 ± 9.7 & 63.5 ± 7.0 & 66.3 ± 4.8 & 109.1 ± 9.3 & 106.9 ± 11.0 \\
cerebellum & 129.5 ± 11.0 & 80.6 ± 8.1\textsuperscript{‡} & 98.9 ± 5.8\textsuperscript{†} & 118.4 ± 9.2 & 124.3 ± 10.8 \\
brainstem & 159.7 ± 12.0 & 112.5 ± 8.4\textsuperscript{‡} & 135.6 ± 4.8 & 152.0 ± 8.7 & 155.2 ± 10.6 \\
\hline
\end{tabular}
\textsuperscript{*} Values represent the means ± SEM in ml/100 g/min. \\
\textsuperscript{†} p < 0.05, Dunnett post hoc test. \\
\textsuperscript{‡} p < 0.01, Dunnett post hoc test.
\end{table}

![Fig. 4. Graph revealing the effect of SCS on CBF at Days 0 and 5 after SAH. Blood flow was measured using \textsuperscript{14}C-IMP. The CBF values are expressed as a % of the control value. Values represent the means ± SEM. A Tukey “honestly significant difference” post hoc test was used to compare values to controls. *p < 0.05; **p < 0.01.](image-url)
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ing delayed vasospasm compared with that early after SAH is probably due to SAH-induced pathological changes to the vessel wall and vascular remodeling, with stiffness causing decreased compliance.

The mechanisms underlying SCS-induced augmentation of CBF are not yet well understood, but it is thought that SCS acts as a vasodilator by suppressing sympathetic activity, as well as indirectly activating the brainstem or cerebellar vasomotor centers and/or causing the release of rapid, effective, potent vasoactive substances such as calcitonin gene-related peptide or nitric oxide. Recently, an antivasospastic effect of clonidine, a central α₂-blocker, has been reported in a single-hemorrhage model in rabbits. However, its use could be associated with a simultaneous decrease in systemic blood pressure that is contrary to the current treatment concept of vasospasm.

It is well known that cerebral blood vessels are densely innervated by sympathetic nerves that originate primarily from the superior cervical ganglia and trigeminal ganglia. Moreover, the density of sympathetic innervation is greatest in large arteries and in vessels of the anterior cerebral circulation. The dilatation of smaller arteries in the subarachnoid space without visible changes in intraparenchymal vessels seen in these animals may support the “neurohumoral” hypothesis. Furthermore, a significant decrease in the cerebral vasodilator effect of cervical SCS after inhibition of nitric oxide synthesis has been reported in a recent study. However, further studies focused on the physiological parameters of cervical SCS and characterizing such neurohumoral changes are required.

Conclusions

The results of this study show that in a double-hemorrhage model in rats, high cervical SCS leads to marked vasodilatation with significant improvement in rCBF, especially in the more affected MCA-supplied brain regions. Although the underlying mechanisms are unknown, SCS may represent a useful adjunct in the treatment of SAH, particularly to prevent or treat delayed vasospasm.

Disclosure

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Disclaimer

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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