Spinal cord stimulation reducing infarct volume in a model of focal cerebral ischemia in rats

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Object. The authors previously showed that spinal cord stimulation (SCS) increases cerebral blood flow in rats, indicating that this technique may be useful in the treatment of focal cerebral ischemia. In the present study, the neuroprotective potential of SCS in the setting of middle cerebral artery occlusion (MCAO) was investigated.

Methods. The authors induced permanent, focal cerebral ischemia by using either suture-induced occlusion or direct division of the MCA in Sprague–Dawley rats. Electrical stimulation of the cervical spinal cord was performed during cerebral ischemia. Cerebral blood flow was assessed using both laser Doppler flowmetry (LDF) and quantitative radio-tracer analysis. Stroke volumes were analyzed after 6 hours of ischemia.

Spinal cord stimulation resulted in a 52.7 ± 13.3% increase in LDF values (nine animals). Following MCAO, LDF values decreased by 64.1 ± 3.6% from baseline values (10 animals). Spinal cord stimulation subsequently increased LDF values to 30.9 ± 13.5% below original baseline values. These findings were corroborated using radiotracer studies. Spinal cord stimulation in the setting of transcranial MCAO significantly reduced stroke volumes as well (from 203 ± 33 mm³ [control] to 32 ± 8 mm³ [MCAO plus SCS], seven animals in each group, p < 0.001). Similarly, after suture-induced MCAO, SCS reduced stroke volumes (from 307 ± 29 mm³ [control] to 78 ± 22 mm³ [MCAO plus SCS], 10 animals in each group, p < 0.001).

Conclusions. A strategy of performing SCS for the prevention of critical ischemia is feasible and may have the potential for the treatment and prevention of stroke.

KEY WORDS • spinal cord stimulation • electrical stimulation • cerebral blood flow • middle cerebral artery • stroke • rat

Because the brain is relatively intolerant of reductions in blood flow, a number of mechanisms exist to minimize such reductions. There is evidence that when a major artery is occluded, arterioles in the ischemic region dilate in an attempt to mitigate the injury caused by the occluded artery. Moreover, there is evidence that these intrinsic control mechanisms are functioning at their limit during ischemic stress, leaving no room for further flow augmentation. Indeed, vessels within nonischemic regions could potentially exacerbate the extent of ischemia by dilating and “stealing” blood from ischemic zones. It is therefore widely held that interventions to reduce vulnerability to stroke cannot take advantage of intrinsic blood flow regulation mechanisms.

Despite the evidence that manipulating intrinsic blood flow mechanisms may not be beneficial in the treatment of stroke, there is emerging evidence that it may be beneficial in the treatment of other disease processes marked by ischemia, such as peripheral vascular disease and angina. For example, electrical stimulation of the spinal cord has been shown to augment blood flow in the extremities by a sympatholytic mechanism. More recently, study data have shown that ischemic tissue damage associated with peripheral vascular disease can be reduced by SCS. In the case of myocardial ischemia, SCS initially was purported to lessen the pain of angina; now there is mounting evidence that it may also improve myocardial blood flow and performance.

The effect of SCS on CBF was initially investigated by Hosobuchi, who noted that global CBF increased in a small series of patients in whom SCS systems had been implanted for pain. Several investigators have subsequently examined CBF changes in response to SCS in experimental models. In goats and dogs, Garcia-March, et al., found that electrical stimulation at C-2 increased CBF 55% when observed using LDF, and 35% when observed with iodoantipyrine studies. Visocchi and associates constructed a model of cervical SCS and sympathetic trunk stimulation in rabbits to demonstrate that increases in CBF induced by SCS can be attenuated by a concurrent sympathetic stimulation. In cats, Isono and colleagues found that changes in CBF occurred only when SCS was applied to the cervical spine. Moreover, these authors revealed that sectioning of dorsal columns at the cervicomедullary junction abolished these changes in CBF. These lines of evidence suggest that cervical SCS produces a significant cerebrovascular effect and that this effect may involve alterations in sympathetic tone as well as in indirect activation of brainstem or cere-
bicellar vasomotor centers. Such evidence indicates that one may increase CBF by applying electrical stimulation to the cervical spinal cord.

Because the augmentation of CBF in normal conditions does not necessarily predict salvage of tissue during ischemia, we examined the ability of SCS to reduce infarct volumes in an experimental model of ischemia. We have previously shown that SCS increases global CBF in the rat. In the present study we have extended our results and used an experimental model of permanent, focal cerebral ischemia described in the text. The percentage of change (% change) in LDF values from baseline for each stimulation epoch are depicted. Values represent the means ± SE. *p < 0.05 compared with control baseline; †p < 0.05 compared with MCAO baseline.

Materials and Methods

Animal Preparation

Adult male Sprague-Dawley rats, each weighing between 300 and 350 g, were selected for the experimental series described. Between five and 10 animals were used for each experimental group. All procedures were reviewed and approved by the University Committee on the Use and Care of Animals at the University of Michigan.

Spinal Cord Stimulation

The rats were anesthetized with a mixture of isoflurane (1.5–2.25%), which had been titrated to maintain arterial blood pressures between 80 and 120 mm Hg before experimental manipulation. They were also given gallamine (10 mg/kg) as a muscle relaxant and intubation of mechanical ventilation. The dura mater over the upper cervical cord was exposed and a platinum-ball electrode was placed onto the dorsal surface of the spinal cord at C-1. A ground electrode was placed in the proximal hind-quaters of the rat. A constant-current unit (model CCU1-A; Grass Instruments), and an oscilloscope (model 5111-A; Tektronix, Gaithersburg, MD). Unipolar stimulation with a frequency of 50 Hz, a pulse width of 100 μsec, and a current of 0.6 mA was used in the experiments.

Measurement of CBF

Cortical blood flow was measured using a laser Doppler flowmeter (Laserflo BMP2; Vasamedics, St. Paul, MN). A point 4 mm lateral to the midline was chosen for all measurements. This point was chosen because it lies outside the core region of expected infarction in this focal ischemia model. Quantitative measurements of CBF were obtained by the indicator fractionation method performed using the radiotracer 14C-IMP (American Radiolabeled Chemicals, St. Louis, MO). Fifteen microliters of 14C-IMP was injected into the femoral artery 50 seconds after the onset of stimulation. Stimulation was applied for 2 minutes while we continuously sampled blood from the femoral artery. Following the cessation of stimulation, the animal was killed and the brain was immediately removed for radioactive analysis. 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: Fb/Mb = Qb(T)/Fs/Qs(T)Mb × 100, where Fb represents CBF; Mb, brain mass; Qb(T), quantity of indicator present in the tissue at Time T; Fs, the rate of blood withdrawal into the syringe from Time 0 to Time T; and Qs(T), the quantity of indicator present in the cannula at Time T. The brain was divided into regions of interest and processed for analysis by using the scintillation counter.

Occlusion of the MCA

We used the transcranial MCA model described by Tamura, et al., to induce permanent focal ischemia in the distribution of the MCA. An incision was made over the temporal aspect of the skull and a craniectomy was fashioned with a burr drill. The zygomatic arch was also divided to provide adequate visualization. The dura mater was opened directly over the MCA, and the artery was coagulated with the aid of a bipolar electrocoagulator down to 1 mm below the level of the olfactory tract. The artery was then divided. Animals assigned to a sham surgery group underwent the entire surgical procedure except for coagulation and division of the MCA. Suture occlusion of the MCA was performed by inserting a 2-0 Prolene suture 3 cm through the cervical carotid artery according to the method described by Longa, et al. Successful MCAO was verified using LDF measurements.

Analysis of Stroke Size

Because it is not possible to use histological analysis to demonstrate stroke size after 6 hours of ischemia, we chose to assess infarct size by staining the brain with the mitochondrial stain TTC (Sigma Chemical Co., St. Louis, MO). The brain was cut coronally at 2-mm intervals, and individual slices were soaked for 10 minutes in a solution of 2% TTC in 0.1 M phosphate-buffered saline (pH 7.4) in a 37°C bath. Excess TTC was drained and the slices were refrigerated in a 10% formalin solution. Images were acquired by placing the brain sections on a color flatbed scanner that was connected to a computer running with image-analysis software (version 1.61; National Institutes of Health, Rockville, MD). Unstained regions were measured, and the percentage of the total slice area was calculated for each slice to attain a percentage of stroke volume.

Analysis of Regional CBF

Regional CBF during focal ischemia was analyzed after division of the brain into three concentric zones around the MCA. The brain was divided into an ischemic core, an intermediate zone, and an outer zone by using a concentric punch scheme that previously has been described. Cerebral blood flow was then measured using the indicator-dilution technique with 14C-IMP, as described earlier in this paper.

Statistical Analysis

Cerebral blood flow measurements are expressed as percentages of baseline measurements and are presented as the mean values ± SE. Comparisons of values were made using the Student t-test.

Results

Spinal Cord Stimulation Increases CBF in the Setting of Direct MCAO

We performed SCS in a series of rats following either...
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duced CBF in all affected regions. Interestingly, the most remarkable effect of SCS was seen in the ischemic core, intermediate, and outer ischemic zones of the surgically treated hemisphere (Fig. 2). The addition of SCS significantly augmented CBF in all affected regions. Although these values do not represent quantitative CBF measurements, they do indicate that SCS may increase CBF to levels sufficient to reduce ischemia.

We also measured CBF values by using the radiotracer \(^{14}\)C-IMP. This technique allows a quantitative analysis of CBF as well as an analysis of the distribution of changes in CBF. Transcranial MCAO was found to reduce CBF in the core, intermediate, and outer ischemic zones of the surgically treated hemisphere (Fig. 2). The addition of SCS significantly augmented CBF in all affected regions. Interestingly, the most remarkable effect of SCS was seen in the ischemic core, intermediate, and outer ischemic zones of the surgically treated hemisphere (Fig. 2). The addition of SCS significantly augmented CBF in all affected regions. Although these values do not represent quantitative CBF measurements, they do indicate that SCS may increase CBF to levels sufficient to reduce ischemia.

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crovasculature is already maximally dilated during ischemic injury, attempts to increase CBF artificially have not met with much success. Although it is generally agreed that electrical stimulation of the subthalamic cerebroebellum (an important cerebral vasoregulatory center) confers a protective effect on the brain during ischemia. More recently, electrical stimulation of the subthalamic cerebrovasodilator area has been shown to be neuroprotective as well. Finally, there is also anecdotal evidence from studies in humans that indicates that SCS may confer some protection in cerebral ischemia. Taken together, there is significant evidence for stimulation-associated neuroprotection in ischemia. The mechanisms underlying the neuroprotection conferred by electrical stimulation are not clear, however, and may not necessarily be strictly related to changes in CBF.

Efforts to mitigate stroke risk by augmenting CBF have not met with much success. Although it is generally agreed that treating hypoxia or hypotension is helpful in reducing ischemic injury, attempts to increase CBF artificially have not resulted in dramatic reductions in the risk of stroke. One explanation offered for this finding is that cerebral microvasculature is already maximally dilated during ischemic stress and cannot respond to further vasodilatory interventions. For example, administration of the potent cerebral vasodilator acetazolamide dilates nonischemic vascular beds, resulting in a vascular steal phenomenon that is deleterious in focal ischemia.

Against this background of the futility of vasodilatory intervention is evidence indicating that it may be possible to take advantage of intrinsic blood flow control mechanisms during ischemic stress. Reis and colleagues have shown that electrical stimulation of the fastigial nucleus of the cerebellum (an important cerebral vasoregulatory center) confers a protective effect on the brain during ischemia. More recently, electrical stimulation of the subthalamic cerebrovasodilator area has been shown to be neuroprotective as well. Finally, there is also anecdotal evidence from studies in humans that indicates that SCS may confer some protection in cerebral ischemia. Taken together, there is significant evidence for stimulation-associated neuroprotection in ischemia. The mechanisms underlying the neuroprotection conferred by electrical stimulation are not clear, however, and may not necessarily be strictly related to changes in CBF.

Spinal cord stimulation is a frequently performed intervention for intractable pain, which has been found to cause a reduction in peripheral sympathetic tone and an improvement in peripheral ischemia. There is some anecdotal evidence that SCS may have similar benefits in situations of cerebral ischemia, but this possibility has not been adequately explored. Our results suggest that SCS increases global CBF, even in the setting of a major vascular occlusion. Although blood flow to the brain region most susceptible to ischemic damage (the core region) is still below normal, SCS does appear to restore flow to a degree sufficient to reduce damage due to infarction. This neuroprotective effect is presumably a function of increased flow from the collateral circulation. Because the microvascular bed is generally believed to be maximally dilated during severe ischemia, it is possible that SCS could actually draw vital blood flow from brain regions that need it most into other vascular beds that are being diluted by stimulation. The results of the present experimental series indicate that this is not the case.

In this study, we used two models of MCAO to study the effects of SCS in focal ischemia: direct (transcranial) and indirect (suture-induced). Although the number of animals used in each model was relatively small, the effects that we observed were robust and internally consistent. Each model of experimental focal ischemia has certain limitations and thus we have chosen to present data from both models because they indicate that the observed effects are not specific to the model. In addition, we used a 6-hour period of ischemia to test the effects of SCS. Although relatively short, this period of ischemia results in a reproducible focal infarction in rats when measured with TTC. Whether prolonged SCS would protect against longer periods of ischemia has yet to be evaluated. Because of the invasive nature of the method used for SCS in the rat, it may be better to conduct long-term studies in larger species in which SCS devices can be completely implanted. Such a long-term study in larger animals would be important to determine whether the effects of SCS on stroke size in the setting of permanent ischemia lasting 24 hours or more yields a similar degree of neuroprotection.
The application of intermittent stimulation after focal ischemia was based on data from a previous study that indicated that changes in CBF outlast the stimulus by at least 5 minutes. The off interval of 8 minutes chosen in this study is somewhat empirical and was based on our earlier study as well as preliminary findings that this interval was sufficient to provide neuroprotection. It remains to be seen whether this stimulation paradigm (2 minutes on/8 minutes off) provides optimal neuroprotection. We observed that LDF values were somewhat variable during repeated stimulation epochs, but that there was no evidence of tachyphylaxis in the LDF response over three such epochs. We did observe that the second stimulation epoch resulted in a slightly lower LDF response than either the first or third stimulation. This held true both in control animals and in animals undergoing MCAO, indicating that the off interval may be important in determining the magnitude of the CBF response to repeated stimulation epochs.

**Fig. 4.** Representative brain sections stained with TTC following suture-induced MCAO with and without SCS. Spinal cord stimulation conferred significant protection from ischemic damage. A: MCAO alone. B: MCAO and SCS.

**Conclusions**

We have offered evidence that cervical SCS offers neuroprotection in the setting of permanent focal ischemia and that this neuroprotection is related to blood flow augmentation. It is possible, of course, that the neuroprotection that we have observed may not be causally related to changes in CBF. It may, for example, be related strictly to a reduction in sympathetic tone; there is evidence that this in itself may be neuroprotective. Alternatively, it is possible that SCS indirectly modulates activity within brain centers where stimulation is known to produce neuroprotective effects (for example, the fastigial nucleus or the subthalamic cerebrovasodilator area). We do believe, however, that the differential increase in CBF in regions at the core of the ischemic zone provides evidence to support the role of CBF in the protective effect of SCS.

Data from the present study show a significant neuro-
Lower: 

vals) through the forebrain. Values are expressed as the means ± SE. *p < 0.05 compared with the no-stimulation group. 

Upper: 

Fig. 5. Upper: Bar graph showing the effects of SCS on infarction after 6 hours of MCAO (transcranial occlusion model). Rats underwent SCS (repeated stimulation with cycles of 2 minutes on/8 minutes off) starting 20 minutes after occlusion or received no stimulation. Areas of infarction were measured in serial sections (2-mm intervals) through the forebrain. Values are expressed as the means ± SE. *p < 0.05 compared with the no-stimulation group. 

References 

1. Augustinsson LE, Linderoth B, Mannheimer C, et al: Spinal cord stimulation in permanent, focal cerebral ischemia. In addition, the results indicate that stimulation instituted after the occlusive event is sufficient to provide this benefit. Although this does beg the question of just how long after the ischemic insult is it still beneficial to institute SCS, the ability to apply this therapy after the occlusive event is clinically favorable.


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