Electrical Forepaw Stimulation During Reversible Forebrain Ischemia Decreases Infarct Volume

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Background and Purpose—Functional stimulation is accompanied by increases in regional cerebral blood flow which exceed metabolic demands under normal circumstances, but it is unknown whether functional stimulation is beneficial or detrimental in the setting of acute ischemia. The aim of this study was to determine the effect of forepaw stimulation during temporary focal ischemia on neurological and tissue outcome in a rat model of reversible focal forebrain ischemia.

Methods—Sprague-Dawley rats were prepared for temporary occlusion of the right middle cerebral artery (MCA) using the filament model. Cerebral blood flow in the MCA territory was continuously monitored with a laser-Doppler flowmeter. Subdermal electrodes were inserted into the dorsal forepaw to stimulate either the forepaw ipsilateral or contralateral to the occlusion starting 1 minute into ischemia and continuing throughout the ischemic period. A neurological evaluation was undertaken after 24 hours of reperfusion, and animals were then euthanized and brain slices stained with 2,3,5-triphenyltetrazolium chloride. Cortical and striatal damage was measured separately.

Results—The cortical and striatal infarct volumes were both significantly reduced in the contralateral stimulated group compared with the ipsilateral stimulated group (48% total reduction). There were no statistically significant differences in the neurobehavioral scores between the 2 groups, or in the laser-Doppler flow measurements from the MCA core.

Conclusions—Functional stimulation of ischemic tissue may decrease tissue damage and improve outcome from stroke. Although the precise mechanism of this effect remains to be determined, functional stimulation could readily be translated to clinical practice. (Stroke. 2006;37:1327-1331.)

Key Words: cerebral ischemia, focal ■ electrical stimulation therapy ■ middle cerebral artery occlusion

There remains intense interest in discovering novel neuroprotective therapies for use in acute cerebral ischemia. Most recent research efforts have focused on compounds designed to interfere with the cascade of deleterious events that occur in tissue during and in the minutes and hours after cerebral ischemia. Although many compounds have been investigated, to-date none have proven clinically useful in humans. Nonpharmacological treatment techniques, such as hypothermia and hyperoxemia, have also been proposed, but it remains to be seen how they transfer from laboratory to bedside. There are even studies suggesting that electrical stimulation of the cerebellar fastigial nucleus before ischemia, or the spinal cord 9 weeks and months after the ischemic insult can reduce damage.

Functional stimulation is accompanied by an increase in regional cerebral blood flow (CBF) in activated brain regions. We have shown previously in a rat model of graded cerebral ischemia that when CBF is reduced by as much as 90%, forepaw stimulation is still able to elicit an increase in blood flow in the somatosensory cortex, and studies in stroke patients demonstrate that CBF can be activated in hemodynamically compromised tissue during functional stimulation. It is therefore possible that blood flow may be increased in the ischemic region in excess of the increase in metabolism leading to some salvage of the ischemic tissue. There is, however, a paucity of data examining the effect of functional stimulation or activity during cerebral ischemia, particularly during the acute phase. Most investigators have assumed that stimulation during ischemia would be detrimental to tissue. By its very nature, in ischemic tissue there is a critical mismatch between the available substrate delivered to the tissue by the blood and the metabolic needs of the tissue. Because functional stimulation puts additional metabolic demand on cells by increasing neuronal activity, there is a potential for exacerbating damage. This would occur if the tissue was unable to increase blood flow commensurate with the increase in metabolic demand. In the present study we sought to investigate this possibility by performing electrical forepaw stimulation during acute reversible forebrain ischemia in a rodent model.
Materials and Methods

Surgical Preparation
All procedures performed were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania. Adult male Sprague-Dawley rats (300 to 325 g) from Charles River Laboratory (Wilmington, Mass) were anesthetized with halothane (4.0% for induction and 0.6% to 1.2% subsequently) in a mixture of 70% nitrous oxide and 30% oxygen. Anesthetic was carefully titrated and increased in response to pain as evidenced by movement (body, extremity, whisker). The body temperature was monitored by a rectal probe and maintained at 37.0±0.5°C with a heating blanket. The tail artery was cannulated with a polyethylene catheter (PE-50) to measure arterial blood pressure and arterial blood gases. The head was then placed in a stereotaxic frame, a midline scalp incision was made and the frontoparietal region of the skull was exposed. A 1-mm diameter circular region of skull overlying the core of the middle cerebral artery (MCA) territory was thinned using a saline-cooled dental drill (Star Dental).

Measurement of Laser-Doppler Flow Response
A Laser-Doppler flow (LDF) probe (tip diameter 1 mm; fiber separation 0.25 mm) attached to a flowmeter (PeriFlux 4001; Perimed) was affixed over the area of thinned skull (4 mm lateral to bregma) using dental cement so as to obtain a continuous measure of relative CBF during the experiment.

Transient MCA Occlusion
The animal was placed prone in a custom stereotaxic holder and prepared for MCA occlusion (MCAO) via the intraluminal filament model. Briefly, the right common carotid artery was isolated from the surrounding fascia. The right external carotid artery was ligated, and a silicone coated nylon filament was inserted through the common carotid artery into the internal carotid artery and advanced until resistance was noted (18 to 20 mm).

Forepaw Stimulation
Two subdermal needle electrodes were inserted into the dorsal left (contralateral group; n = 18) or right (ipsilateral group; n = 16) forepaw. One minute after occlusion of the MCA, electrical forepaw stimulation was begun and continued for 89 minutes. One-millisecond electrical forepaw stimuli were delivered at an amplitude of 2 mA using a constant current stimulus isolation device (World Precision Instruments). Stimuli were delivered at a frequency of 5 Hz in a continuous loop consisting of 4 seconds of stimulation followed by 3 seconds of rest. After 90 minutes of MCAO, the filament was removed, and all incisions were sutured closed. Anesthesia was removed, and the animals were allowed to recover for 24 hours.

Neurological Evaluation
Neurological evaluation was performed before euthanasia 24 hours after MCAO according to the protocol of De Ryck et al. Briefly, postural reflex, visual placing in the forward and sideways directions, tactile placing of the dorsal and lateral paw surfaces, and proprioceptive placing were tested. These 6 tests were each scored from 0 to 2, and the behavioral deficit was calculated as the sum of the scores of the individual tests ranging from 0 (no deficit) to 12 (maximum deficit).

Infarct Volume Measurement
Rats were euthanized with pentobarbital sodium (150 mg/kg) 24 hours after MCAO. Brains were removed from the skull and cooled in ice-cold PBS for 15 minutes. They were sectioned in the coronal plane at 1-mm intervals using a rodent brain matrix (RBM-4000C; ASI Instruments), and the brain slices were incubated in PBS containing 2% 2,3,5-triphenyltetrazolium chloride (TTC; Sigma) at 37°C for 10 minutes. The TTC-stained sections were photographed with a digital camera, and the damaged area determined using a computer-based image analyzer (AIS 6.0; Imaging Research). To avoid artifacts attributable to edema, the damaged area was calculated by subtracting the area of the normal tissue in the hemisphere ipsilateral to the stroke from the area of the hemisphere contralateral to the stroke. Total lesion volumes in cortex and striatum were calculated by summation of the infarct areas of 10 brain slices and integrated by the thickness.

Statistical Analysis
Results are expressed as mean±SE. Significant differences between groups were determined with Student t test for infarct volume and physiological parameters. Differences between groups were determined with 2-way repeated-measures ANOVA for mean arterial blood pressure, LDF, and infarction areas. When significant differences were found, the Tukey test was used to find at which time-point or slice these differences occurred. Significant differences of neurological scores between groups were determined with the Mann–Whitney test.

Results

Physiological Parameters
Blood gas parameters were within normal physiological range both before and during ischemia. There were no differences in pH (7.35±0.015; 7.36±0.013), pCO2 (46.0±2.9 mm Hg; 47.3±2.6 mm Hg), or pO2 (99±4 mm Hg; 99±4 mm Hg) between the animals receiving contralateral forepaw stimulation and those receiving ipsilateral stimulation. Systemic arterial blood pressure increased (~20 mm Hg) during the period of stimulation (P<0.001), but the rise was almost identical in the 2 groups (Figure 1).

CBF
Immediately after occlusion of the MCA, CBF (measured with LDF) decreased to 26% to 27% of the control level in both groups (Figure 2). By 15 minutes after the MCAO, LDF increased to 34% to 35% of control and remained close to this level until the release of the occlusion when it increased to ~110% of control. Over the subsequent 10 minutes of reperfusion, LDF decreased to 71% to 75% of control. There was no significant difference in the time course of the LDF between the 2 groups, and at no time did the blood flow changes differ (P>0.2; 2-way ANOVA).

Infarct Volume
Temporary MCAO produced histological damage in the cortex and striatum in both groups, with animals receiving
contralateral forepaw stimulation having significantly smaller infarcts than animals receiving ipsilateral stimulation. In the cortex, infarct volume in the contralateral stimulation group was $57 \pm 13 \, \text{mm}^3$ compared with $123 \pm 21 \, \text{mm}^3$ ($P < 0.01$), whereas in the striatum infarct volume was $40 \pm 5 \, \text{mm}^3$ compared with $66 \pm 6 \, \text{mm}^3$ ($P < 0.01$). The total infarct volume exhibited a 48% decrease from the ipsilateral forepaw stimulation group ($P < 0.01$; Figure 3).

**Neurological Score**
The neurobehavioral score for the contralaterally stimulated group had a median of 8.5, not significantly smaller (better) than the score of the ipsilaterally stimulated group (10; $P = 0.06$, Mann–Whitney Rank Sum test; Figure 4).

**Discussion**
A tight linkage between neuronal activity and CBF, known as activation-flow coupling (AFC), is known to exist under physiological conditions. Studies from our laboratory and in the literature have demonstrated that the AFC response is preserved over a broad range of baseline flow values caused by a variety of pathophysiological conditions including mild and severe ischemia, as well as with pharmacological CBF modulation with acetazolamide and CO$_2$. These findings suggest that AFC may be mediated by a mechanism independent of that regulating baseline CBF. Furthermore, a delay in the AFC response during mild ischemia attributable to carotid occlusion ipsilateral to the stimulated cortex has been demonstrated, suggesting that collateral flow sources contribute to AFC under these conditions. Based on these findings, we hypothesized that functional forepaw stimulation could improve outcome from focal forebrain ischemia if the AFC response produces an incremental CBF increase through collateral flow sources that exceeds the metabolic demands of stimulation. The present data demonstrates a neuroprotective effect of functional forepaw stimulation contralateral but not ipsilateral to the ischemic hemisphere in the rat intraluminal filament model of MCA stroke.

Electrical stimulation in the chronic phase of ischemia, months to years after the stroke, can influence subsequent recovery. Cervical spinal cord stimulation (CSCS) produces appreciable increases in CBF independent of changes in systemic blood pressure, and CSCS after permanent MCAO leads to prolonged survival, significant prevention of infarct progression in surviving animals, and dramatic reduction in infarct volume. Although CSCS can augment blood flow in most brain regions, including the MCA core and regions bordering the core, other potential mechanisms must be considered. CSCS may increase activity in the brain stem and can lead to a decrease in cervical sympathetic tone, both of which are neuroprotective. In a combined ischemic and traumatic model in the rabbit, spinal cord stimulation leads to a reduction in lesions and attenuation of the CBF decrease caused by the insult. Direct electrical stimulation of the cerebellar fastigial nucleus before the ischemic insult dramatically reduces the volume of damage attributable to perma-
dent MCA occlusion.5,6 Interestingly, neuroprotection is long-lasting, with protection seen when the MCA is occluded 3 days after only 1 hour of fastigial nucleus stimulation.23 In this case, neuroprotection is not mediated by changes in blood flow in the ischemic territory or by decreases in metabolic demand in the ischemic or peri-infarct territory,5 but may be attributable to suppression of the inflammatory reaction24,25 along with suppression of apoptosis,26 probably mediated by potassium channels.

The only studies that used a stimulus during ischemia used pulsed electromagnetic fields (PEMF). Low-frequency PEMF stimulation starting 10 minutes after MCAO and continuing throughout the 2 hours of occlusion and 4 hours of reperfusion reduces cortical edema and histological damage27 with the degree of protection dependent on the degree of PEMF exposure. The mechanism by which PEMF provides cytoprotection is not fully understood, but a number of changes have been measured in tissue after PEMF exposure that may contribute to the protective effect, including a reduction in Ca\(^{2+}\) efflux from cerebral tissue,28 a decrease in Ca\(^{2+}\) accumulation,29 and an induction of heat shock proteins.30,31

In the present study we found that contralateral forepaw stimulation during a 90-minute period of MCAO led to a 48% decrease in total infarct volume compared with ipsilateral stimulation. The mechanism by which forepaw stimulation contralateral to the ischemia produces neuroprotection is unknown. One hypothesis is that, in spite of the imposed ischemia, stimulation produces a blood flow increase in the contralateral tissue that exceeds the increase in metabolic demand, and that this increase in blood flow is sufficient to prevent some tissue from becoming infarcted. In the present study CBF decreased to below 30% of baseline at the time of MCAO in both groups but did increase slightly during stimulation to \(\sim\)35% of control where it remained until release of the occlusion. CBF change did not differ between the contralateral and ipsilateral stimulated groups, suggesting that CBF changes do not explain the neuroprotective effect observed. However, the lack of any difference in blood flow between the 2 groups either during ischemia or the reperfusion phase may be attributable to the positioning of the LDF probe 4 mm lateral to bregma. This location might be in the core of MCA territory, whereas changes in CBF during ischemia attributable to stimulation may have occurred in the peri-infarct area peripheral to this location, and so would be missed by the LDF probe. Studies using imaging techniques to measure regional CBF in the entire cortex fed by the MCA would address this possibility.

The greatest histological difference between ipsilateral stimulated animals and animals receiving stimulation of the forepaw contralateral to the ischemia was found in the cortex, with the differences in the infarct volume in the striatum being less dramatic. As cortical regions have better collateral supply through meningeal vessels than subcortical regions, this finding is consistent with a mechanism whereby functional stimulation improved collateral blood flow beyond endogenous autoregulation. The neurological score for the contralaterally stimulated animals, although slightly lower (better), was not significantly different than for ipsilaterally treated animals. This finding also likely reflects a preferential effect of stimulation in cortical peri-infarct regions; the neurological score primarily reflects motor functions that are subserved by the cortical infarct core, and subcortical regions including the basal ganglia and descending pyramidal tracts.

Another potential mechanism for stimulus-induced neuroprotection involves the upregulation of neurotrophins. It has been shown that brain-derived neurotrophic factor (BDNF) expression, for example, increases after functional stimulation15 and seizures.33 BDNF also plays a neuroprotective role in focal cerebral ischemia,34 potentially acting through the high-affinity receptor tyrosine kinase35 or through BDNF-induced tissue type plasminogen activator secretion.36 These potential mechanisms need to be pursued in future studies. Studies designed to more fully elucidate the biological mechanism underlying the neuroprotective effect of stimulation are currently underway in our laboratory.

The results of this study may have significant clinical implications for acute stroke treatment. Physical therapy for acute stroke is typically deferred until after a diagnostic evaluation for stroke etiology is completed, and patients presenting with deficits, such as hemiparesis attributable to acute stroke, are not specifically encouraged to use their affected limbs in the acute setting. However, if a beneficial effect of functional stimulation could be demonstrated, this type of therapy could be rapidly and safely translated to clinical trials through the use of mechanical or electrical devices, and could readily be combined with pharmacological therapy including thrombolytic therapy. This report demonstrated a beneficial effect of forepaw stimulation starting soon after 90-minute MCAO in the rat. Future studies are needed to specifically elucidate the timing, duration, and stimulation parameters necessary to maximize this observed protective effect and to determine whether the neuroprotection seen at 24 hours is long-lasting.

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