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Research report

Activation–flow coupling during graded cerebral ischemia

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Abstract

Most functional neuroimaging techniques rely on activation–flow coupling (AFC) to detect changes in regional brain function, but AFC responses may also be altered during pathophysiological conditions such as ischemia. To define the relationship between progressive ischemia and the AFC response, graded levels of cerebral blood flow reduction were produced using a rat compression ischemia model, and the cerebral hemodynamic response to forepaw stimulation was measured. Graded levels of cortical ischemia of the somatosensory cortex were induced in male Sprague–Dawley rats (n = 16) by compressing the intact dura with a 4-mm-diameter cylinder equipped with a laser-Doppler probe, combined with ipsilateral common carotid artery occlusion. At each level of CBF reduction, electric forepaw stimulation was conducted, and signal-averaged laser Doppler and evoked potential responses were recorded. A visible AFC response was present at all levels of CBF reduction (0–90% reduction from baseline), and the temporal characteristics of the response appeared largely preserved. However, the amplitude of the AFC response began to decline at levels of mild ischemia (10% flow reduction) and progressively decreased with further CBF reduction. The amplitude of the evoked response appeared to decrease in concert with the AFC amplitude and appeared to be equally sensitive to ischemia. AFC appears to be a sensitive marker for cerebral ischemia, and alterations in the AFC response occur at CBF reductions above the accepted thresholds for infarction. However, the AFC response is also preserved when flow is reduced below ischemic thresholds.

Theme: Other systems of the CNS
Topic: Brain metabolism and blood flow

Keywords: Activation–flow coupling; Cerebral ischemia; Graded ischemia; Somatosensory cortex; Evoked potentials; Laser Doppler; Rat

1. Introduction

A close connection between neuronal activity and cerebral blood flow (CBF), termed activation–flow coupling (AFC), has been shown to exist under physiologic conditions and forms the physiologic basis for most current functional imaging techniques. There is evidence, however, that pathological states such as cerebral ischemia will alter the AFC response. In animal models of cerebral ischemia, an uncoupling of AFC has been observed with cortical electrical potentials being recorded in response to somatosensory activation in the absence of changes in CBF or cerebral glucose metabolism [23]. Although reactivity to CO_2 is also impaired acutely following an ischemic episode, recovery of the AFC is more gradual, indicating that abnormalities in AFC following ischemia cannot be inferred from changes in CO_2 reactivity but reflect a disruption of functional brain integrity [18].

In a study comparing the recovery of ion homeostasis as determined by diffusion MRI, and AFC as assessed by perfusion MRI, it was shown and that perfusion changes recovered more slowly following an ischemic event [19]. This suggests that the AFC response is a sensitive indicator of impaired brain function, but the dynamics of the relationship between ischemia and AFC remain obscure. In order to more

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accurately describe this relationship, it is necessary to use a model which allows for graded levels of CBF reduction to be achieved without significantly altering systemic physiology.

Recently, Gröhn and colleagues attempted to produce graded ischemia in the rat using a modification of the four-vessel occlusion model to study MRI findings during hypoperfusion [12]. The vertebral arteries were ligated, and silicon-coated snares were placed around both carotid arteries. The snares were connected to a controllable screw system that was tightened to progressively occlude the carotids. This method, originally described for a similar study in gerbils [1], is able to produce gross CBF reductions but only across a limited range of CBF levels. Another study attempted graded ischemia by varying the duration of MCA occlusion in association with graded hypotension [26]. Hypotension was induced by titration of inhaled halothane. As with the snare technique, this method did not allow for the fine control of CBF needed to study AFC and neural activity across a broad range of CBF reductions. Moreover, drugs such as halothane are known to reduce or abolish the AFC response [24].

We have attempted to use induced hypotension, pharmacologically and via phlebotomy, to produced graded ischemia but have found that these methods significantly alter systemic physiology. Our laboratory as well as others has observed that decreased systemic blood pressure (i.e., below 80 mm Hg) is associated with spontaneous regular oscillations in CBF spanning up to 100% of baseline levels. The amplitude of these oscillations is negatively related to blood pressure levels, and oscillations subside when blood pressure returns to normal levels or the level of inspired CO_2 is increased to dilate the cerebral vasculature [14]. These oscillations are thought to primarily represent cerebral arterial pressure fluctuations, and their presence confounds AFC measures as does the use of hypercapnia to eliminate them [17].

Our laboratory has recently described the cortical compression model for the production of ischemia in the whisker-barrel cortex [25]. In this model, a 4-mm cylinder is used to create temporary ischemia by measured compression of the brain over intact dura. Cortical compression is able to focally reduce CBF to below 10% of baseline to create a focal cerebral infarction without tissue trauma. We have found that, by controlling the level of cortical compression, the level of CBF reduction can be easily adjusted, making it suitable for studying cerebral physiology during graded ischemia. In the present study, we have adapted the cortical compression model to investigate the effects of CBF reduction on neuronal activity and AFC during forepaw stimulation in the rat.

2. Materials and methods

2.1. Animal preparation

All procedures performed on animals were approved by the University Institutional Animal Care and Use Committee of the University of Pennsylvania. A total of 16 adult male Sprague–Dawley rats (weight, 270 to 450 g) obtained from Charles River Laboratory (Wilmington, MA) were used in this study. They were anesthetized with halothane (4.0% for induction and 0.6% to 1.0% subsequently) in a mixture of 70% nitrous oxide and 30% oxygen by facemask. The body temperature was monitored by a rectal probe and maintained at 37.0 ± 0.5 °C with a heating blanket regulated by a homeothermic blanket control unit (Harvard Apparatus Limited, Boston, MA). The tail artery was cannulated with a polyethylene catheter (PE-50) for the measurement of arterial blood pressure and arterial blood gases. The rats were tracheotomized, mechanically ventilated, and maintained on 0.6% to 1.0% halothane in 70% nitrous oxide and 30% oxygen. Mean arterial blood pressure (MABP) was monitored continuously, and a systemic pressure between 90 and 110 mm Hg was maintained at all times throughout the animal preparation by titrating halothane concentration. 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 6 × 6 mm-wide square area of skull overlying the forepaw portion of the somatosensory cortex was thinned using a saline-cooled dental drill (Star Dental, Lancaster, PA) and then carefully removed from the underlying dura. Halothane was discontinued after surgery and for at least 45 min before data acquisition. After surgery and during all stimulation studies, anesthesia was maintained with 60 mg/kg of α-chlorolose given intraperitoneally followed by supplemental doses of 30 mg/kg 45–60 min after the initial dose and then immediately after the data collection following the 4-mm cortical compression (see below). A tail pinch was administered periodically to ensure adequate depth of anesthesia, but in none of the animals was additional α-chlorolose needed during the cortical compression sequence. Arterial blood gas measurements were made prior to data acquisition, and ventilation parameters were adjusted to maintain the $PaCO_2$ in the range of 35 mm Hg. The right common carotid artery (CCA) was isolated, and loops made from a polyethylene catheter (PE-10) were carefully passed around it for later remote occlusion.

2.2. Forepaw stimulation, laser Doppler flowmetry, and evoked potentials

Electrical forepaw stimulation was performed using two subdermal needle electrodes inserted into the left dorsal forepaw. The microvascular blood flow in the right primary somatosensory cortex was continuously monitored using a laser Doppler flowmeter (LDF) (Vasomedics, St. Paul, MN) with a 0.75-mm tipped probe that was imbedded within a 4-mm-diameter plastic compression cylinder. The compression cylinder was mounted on a micromanipulator and placed perpendicular to the exposed dura at the craniectomy site to allow for simultaneous cortical compression and LDF measurements as described by Watanabe et al. [25]. Somatosensory evoked potentials (SSEP) were measured