Acute Carotid Occlusion Alters the Activation Flow Coupling Response to Forepaw Stimulation in a Rat Model

Beau M. Ances, MSc; Joel H. Greenberg, PhD; John A. Detre, MD

Background and Purpose—To determine whether the hemodynamic response to functional stimulation is sensitive to proximal arterial occlusion, we measured the activation flow coupling response in a rat model of acute reversible vascular occlusion.

Methods—In α-chloralose–anesthetized rats (n=18), laser Doppler measurements were made through a thinned skull over the somatosensory cortex in response to electrical forepaw stimulation. Signal-averaged responses to 4 and 8 seconds of electrical forepaw stimulation were obtained before, during, and shortly after acute unilateral or bilateral carotid occlusion produced with the use of a surgically placed snare.

Results—Baseline cerebral blood flow was significantly decreased over the forepaw region of the somatosensory cortex after both occlusion of the carotid contralateral to the stimulated forepaw and bilateral occlusion compared with preocclusion (P<0.05). Postocclusion and ipsilateral occlusion led to a nonsignificant increase in baseline cerebral blood flow compared with preocclusion. Contralateral carotid occlusion and bilateral occlusion significantly prolonged the temporal characteristics of the flow response, especially the delay to peak (P<0.05), compared with preocclusion, whereas ipsilateral carotid occlusion significantly shortened the delay to peak (P<0.05). Only contralateral carotid occlusion produced a significant reduction in the peak amplitude of the flow response compared with preocclusion (P<0.05).

Conclusions—These findings suggest that temporal characteristics of functional activation responses are sensitive to alterations in the proximal arterial supply and, conversely, that functional activation studies must be interpreted with consideration of proximal arterial disease. (Stroke. 2000;31:955-960.)

Key Words: carotid artery occlusion ■ cerebral blood flow ■ cerebrovascular circulation ■ rats

Activation flow coupling (AFC), which is the coupling of cerebral blood flow (CBF) with neuronal activity, is the basis for many functional neuroimaging techniques. A variety of techniques, including optical imaging,1,2 near-infrared spectroscopy,3 video microscopy,4,5 and laser Doppler (LD) flowmetry,6–11 have been used to determine the spatial and temporal characteristics of the AFC response in animal models under normal conditions. However, the influence of pathophysiological alterations on AFC remains poorly understood.

Previous studies that have examined the effects of proximal carotid occlusion on resting CBF have demonstrated that acute carotid occlusion leads to a decrease in blood flow in the hemisphere of the occluded side.12,13 However, with chronic occlusion, baseline flow returns to preoccluded values within a few days.12 Two studies investigated the effects of transient severe global ischemia (4-vessel occlusion) and reperfusion on the AFC response. Dietrich et al14 reported that functional activation due to whisker stimulation in the rat was reduced for up to 5 days after 4-vessel occlusion, whereas Ueki et al15 demonstrated that 30 minutes after severe forebrain ischemia, the functional activation response was almost completely suppressed, although evoked potentials were present. No studies in animals have investigated the AFC response during mild ischemia induced by acute unilateral or bilateral carotid occlusion.

Previously, we characterized the AFC response in normal rats with the use of LD flowmetry.6,7 The aim of the present study was to determine whether the AFC responses due to functional stimulation would be affected by acute carotid occlusion in our well-characterized rat model using both short and long stimulus durations.

Materials and Methods

General Preparation

Eighteen male Sprague-Dawley rats (320 to 370 g) obtained from Charles River were anesthetized with 2% to 4% halothane in 70% N2O/30% O2 by face mask. Subcutaneous 2% lidocaine was used to elevate the tail dermis away from the tail artery before incision and to prevent vasospasm during catheter insertion. A polyethylene
catheter (PE-50) was used to cannulate the tail artery to measure arterial blood pressure and to monitor arterial blood gasses. Rats were tracheostomized, mechanically ventilated (Harvard Rodent Ventilator; Harvard Apparatus), and maintained on 1% halothane in 70% N₂O/30% O₂. Through a median sternotomy, the common carotid arteries were isolated, and remote snare occluders were placed around both common carotid arteries to produce unilateral or bilateral carotid occlusion. The occluders were tested to ensure the carotid arteries were adequately occluded. Subsequently, the head was placed in a stereotactic frame, a midline incision was performed, and the scalp of the frontoparietal cortex was retracted. An area of 3×4 mm that overlaid the forepaw area of the somatosensory cortex was thinned with the use of a saline-cooled dental drill to ≈0.5 second.

Forepaw Stimulation

Electrical forepaw stimulation was performed with 2 needle electrodes inserted subdermally into the forepaw contralateral to the LD probe. Constant-current 1.0-ms rectangular pulses of 5 Hz and 1 mA were applied. A function generator (Global Specialties) was used to control the stimulus frequency, which was fixed at 5 Hz. Stimulus amplitude was maintained at 1.0 mA with a constant-current stimulus isolation device (A-36V; World Precision Instruments). These stimulus parameters were chosen because previous experiments with this model system demonstrated maximal AFC response without systemic blood pressure changes. The stimulus duration was under software control. Based on our previous results, which demonstrated a peak response for 4-second stimuli and a peak-and-plateau response for stimuli of ≈4 seconds,6,7 we obtained data for both 4- and 8-second stimuli. All stimuli were administered 8 seconds into a 24-second-long iteration.

Changes in CBF were measured with an LD probe (Vasamedics) that was positioned normal to the thinned skull with a micromanipulator mounted onto a stereotactic coordinate system (Stoelting). The LD probe was positioned ≈5 mm lateral to bregma for all experiments based on previous experiments that used the same model system and that demonstrated maximal peak flow response occurred at this position.6 Care was taken to ensure that the LD probe was positioned away from large pial vessels so that readings represented parenchymal flow.8,9 For all LD measurements, the time constant was 0.5 second.

Data Acquisition

Signal averaging of LD data was accomplished as previously described.6,7 Measurements of signal-averaged LD_CBF were made for repetitive periodic stimulation (4 seconds every 24 seconds and 8 seconds every 24 seconds) for the various states, including preclosure (n=18), unilateral occlusion (occlusion of the carotid artery either ipsilateral [n=9] or contralateral [n=9] to the stimulated forepaw), bilateral occlusion (n=18), and postocclusion (n=18) (Figure 1A). LD measurements of changes in CBF due to forepaw stimulation were obtained within 5 minutes of tightening of the carotid snares for each of the occlusion states or on removal of both carotid snares for the postocclusion state. A single iteration was composed of 240 data points acquired at 10 Hz with a single trial consisting of 10 of these signal-averaged iterations. A minimum of 2 LD trials were performed for each state for each of the rats. The arterial blood pressure tracing was also recorded at all times to ensure that changes in local CBF were not the result of systemic blood pressure changes.

Data Analysis

Signal-averaged LD data were converted from voltages to corrected flow and expressed as percent change from baseline through normalization of the corrected flow to the average baseline flow value obtained before application of the stimulus. Data were averaged from all trials for a particular state for each rat. Data from multiple rats with the same experimental stimulation protocol and state were collated, and an overall average for all rats was determined along with intrasubject SDs. The shape of the AFC response was characterized with the use of previously described measures. The delay to response, peak amplitude, delay to peak, and half-width/half-maximum measures were determined for the AFC responses (Figure 1B). The delay to response was defined as the first point in the series of sustained values that exceeded 2 SDs above the average prestimulus baseline. As such, the delay to response tends to be dependent on the peak amplitude, with shorter delay to response values occurring in association with larger peak amplitude responses. The peak
amplitude was calculated for each rat by averaging 1 second of data surrounding the determined group average peak. This procedure allowed for averaging across identical time points in all rats and avoided errors in peak picking due to noise within individual rat data. The delay to peak was defined as the latency from the stimulus onset to the peak response.

Statistical Analysis
All data are expressed as mean ± SEM. A 1-way ANOVA with repeated measures was performed on delay to response, delay to peak, half-width/half-maximum, and peak amplitude values obtained for the different carotid states (SigmaStat; SPSS Inc). Subsequent analysis was performed with Tukey’s test when a significant difference was found; differences were considered to be significant at the P < 0.05 level.

Results
Physiological variables that were measured during the stimulation paradigms for the different carotid states (mean ± SEM, n = 18) are shown in the Table. Physiological values did not change significantly during the various states.

Figure 2 shows variations in the baseline LDCBF for the different carotid states normalized to the preocclusion values. For both 4- and 8-second stimulus durations, the baseline LDCBF tended to increase after occlusion of the carotid artery contralateral to the stimulated forepaw, although this increase was not significant compared with preocclusion. Both occlusion of the carotid artery contralateral to the stimulated forepaw and bilateral carotid occlusion significantly decreased the baseline LDCBF compared with preocclusion (P < 0.05, Tukey’s test). After occlusion, there was a hyperemic response as the baseline LDCBF increased, but this increase was not significant compared with preocclusion.

The temporal characteristics of the AFC responses, the delay to peak and the half-width/half-maximum, for the different states for 4-second stimuli are summarized in Figure 3. Nearly identical results for both variables were obtained for 8-second stimuli (data not shown). The delay to peak was significantly prolonged for contralateral occlusion and bilateral carotid occlusion compared with the preocclusion (P < 0.05, Tukey’s test). The delay to peak was also significantly shortened for occlusion of the carotid artery ipsilateral to the stimulated forepaw compared with preocclusion (P < 0.05, Tukey’s test) (Figure 3A). The half-width/half-maximum results were similar to those seen for delay to peak (Figure 3B). The half-width/half-maximum was significantly prolonged for contralateral occlusion and bilateral carotid occlusion compared with preocclusion (P < 0.05, Tukey’s test). There also was a nonsignificant shortening of the half-width/half-maximum values for ipsilateral occlusion compared with preocclusion.

Characteristic AFC response shapes for 4- and 8-second stimuli were observed. For all 4-second stimuli, regardless of the state, a peak response was seen, whereas all 8-second stimuli led to a peak-and-plateau response. The time to reach the plateau phase of the AFC response tended to be prolonged for contralateral and bilateral occlusion compared with preocclusion. This prolongation was due to lengthening of the temporal dynamic parameters, delay to peak and half-width/half-maximum, seen for these occlusion states.

Figure 4 shows the peak amplitude and the delay to response values obtained for the AFC responses for 4-second stimuli during the various conditions. Very similar results were seen for 8-second stimuli (data not shown). In the present study, the peak amplitude of the AFC responses was reduced, even in the preocclusion state, compared with experiments we performed previously in normal rats, in which the carotid arteries were not manipulated.6,7 As seen in Figure 4A, neither occlusion of the carotid artery ipsilateral to the stimulated forepaw nor subsequent bilateral carotid occlusion decreased the baseline LDCBF compared with preocclusion.

<table>
<thead>
<tr>
<th>Physiological Variables for Carotid Occluded Rats (n=18)</th>
<th>Mean Arterial Blood Pressure, mm Hg</th>
<th>pH</th>
<th>Pco2, mm Hg</th>
<th>Po2, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preocclusion</td>
<td>113 ± 2</td>
<td>7.39 ± 0.01</td>
<td>33.7 ± 1.3</td>
<td>118 ± 3</td>
</tr>
<tr>
<td>Ipsilateral occlusion</td>
<td>117 ± 5</td>
<td>7.40 ± 0.02</td>
<td>33.2 ± 1.3</td>
<td>115 ± 5</td>
</tr>
<tr>
<td>Contralateral occlusion</td>
<td>116 ± 3</td>
<td>7.39 ± 0.02</td>
<td>33.9 ± 2.5</td>
<td>117 ± 3</td>
</tr>
<tr>
<td>Bilateral occlusion</td>
<td>131 ± 2</td>
<td>7.38 ± 0.03</td>
<td>32.4 ± 2.8</td>
<td>117 ± 4</td>
</tr>
<tr>
<td>Postocclusion</td>
<td>112 ± 2</td>
<td>7.38 ± 0.01</td>
<td>30.5 ± 0.4</td>
<td>115 ± 5</td>
</tr>
</tbody>
</table>
clusion significantly altered the peak amplitude of the AFC response compared with preocclusion \( (P>0.94) \). The peak amplitude was significantly reduced only for contralateral occlusion compared with preocclusion \( (P<0.05, \text{ Tukey's test}) \). The peak amplitude of the flow responses after release of the carotid snares (postocclusion) for 4-second stimuli tended to increase compared with preocclusion, but this increase was not significant. Figure 4B shows that the delay to response was significantly increased only during contralateral carotid occlusion compared with preocclusion \( (P<0.05, \text{ Tukey's test}) \). Neither bilateral carotid occlusion nor ipsilateral occlusion significantly altered the delay to response for 4-second stimuli.

**Discussion**

The major finding of the present report is that although the general shape of the AFC response to forepaw stimulation was preserved after acute carotid occlusion, the temporal dynamics of the AFC response were significantly altered. The temporal dynamic measures of delay to peak and half-width/half-maximum were the most sensitive indicators of the pathology of acute carotid occlusion on the flow response. Although the delay to peak and half-width/half-maximum values for the preocclusion state were similar to those seen for rats in which the carotid arteries were not manipulated,\(^6\) a significant prolongation of these parameters was seen with contralateral and bilateral occlusion compared with preocclusion. These results are due, most likely, to an increase in the transit time of blood flow though proximal collateral sources to meet the increasing metabolic needs of the activated area required during functional stimulation.\(^1^2,1^3\)

It is also interesting to note that there was a significant reduction in the delay to peak and a trend to a decrease in the half-width/half-maximum with occlusion of the ipsilateral carotid artery (contralateral to the recorded hemisphere) compared with preocclusion. This reduction in the temporal characteristics with occlusion of the carotid artery ipsilateral to the stimulated forepaw suggests that increased flow within the proximal vasculature that directly supplies the activated region can more rapidly meet the increased metabolic needs of that activated area. Overall, our results suggest that the temporal characteristics of the AFC response may be sensitive indicators of the “remote” proximal vasculature that supplies a given activated region.\(^1^9\) Our results during acute carotid occlusion in rats are also comparable to those previously observed in humans with chronic occlusion.\(^2^0–2^2\)

Because a peak-and-plateau response was obtained for all 8-second stimuli regardless of the state, this nonlinearity in
the AFC response is preserved during occlusion. These findings are comparable to nonlinearity previously seen in normal rats and require consideration in the modeling and quantification of AFC responses.

The peak amplitudes of the AFC responses were reduced, even in the preocclusion state, compared with normal rats. This reduction in the peak amplitude may be due to manipulation of the carotid arteries that is necessary to place the remote snares and to verify their function. Although the peak amplitude of the AFC response was significantly reduced for occlusion of the carotid artery contralateral to the stimulated forepaw compared with preocclusion, subsequent bilateral occlusion did not produce a sustained reduction in the peak amplitude. This lack of a reduction in the peak amplitude of the AFC response with bilateral occlusion may be due to a temporal order of the experiment, because bilateral occlusion always followed unilateral occlusion in all rats. Similarly, the delay to response, which has previously been shown to be inversely correlated to the peak amplitude, was significantly increased only for contralateral occlusion compared with preocclusion. These findings suggest that the magnitude of the AFC response, as determined with the peak amplitude and the delay to response, is rather insensitive to the proximal cortical vasculature and may instead reflect the locally regulated increases in CBF within the activated region that occur with functional stimulation. Our results further suggest that the peak amplitude of activation is not the best characteristic for the detection of altered flow responses after acute carotid occlusion. These results are in agreement with our prior study using functional MRI with blood oxygenation level–dependent contrast (BOLD-fMRI), in which modeling of the amplitude of regional changes in metabolism was complicated by multiple factors, whereas the temporal dynamics of the hemodynamic response provided a more straightforward method for the quantification of AFC.

The observed alterations in AFC with acute carotid occlusion probably are not attributable to a reduction in neuronal activity. Although neuronal activity was not measured in the present study, previous studies have demonstrated that somatosensory evoked responses changed only after an ~50% reduction in baseline CBF compared with the control situation. In the present study, the maximal observed reduction in the baseline CBF occurred with bilateral occlusion and was <20% compared with the control values (Figure 2). However, LD measurements of blood flow are not absolute. Rather, the observed alterations with acute carotid occlusion may represent a change in the mediator or mediators involved in AFC, such as nitric oxide or adenosine.

Our findings also support the concept of using the AFC response to functional stimulation as a clinical measure of the adequacy of proximal arterial supply. Although the present results were obtained in a model of acute carotid occlusion, these findings are similar to published results in humans with chronic cerebrovascular stenosis. Hand gripping has previously been used to study the cerebrovascular response in patients with chronic carotid artery disease. Silvestrini et al used a unilateral hand-gripping stimulus and transcranial Doppler and demonstrated that the mean flow velocity, which was used as a measure of the reserve capacity, was significantly less in patients with severe carotid stenosis than in control subjects. In addition, the mean flow velocity was decreased to a greater extent on the stenotic side compared with the normal side. Stoll et al investigated the effects of bilateral hand gripping in control subjects and patients with carotid stenosis with the use of transcranial Doppler before and after acetazolamide administration. These authors demonstrated that most patients with pathological results on the acetazolamide test showed similar pathological results with the hand-gripping test. Because functional stimulation is more physiological than the administration of either acetazolamide or CO2, the response to functional stimulation may provide a better technique for examination of the response capabilities of the cerebrovasculature.

Acknowledgments
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References
The cortical hemodynamic response to functional stimulation after acute unilateral or bilateral common carotid artery occlusion was investigated. Laser-Doppler flowmetry was used to measure the activation-flow coupling (AFC) response to forepaw stimulation in rats. Carotid occlusion significantly prolonged the temporal characteristics of the AFC response and reduced the peak amplitude of the flow response compared with preocclusion values. The authors conclude that the temporal hemodynamic characteristics of a brain region after functional activation are a sensitive indicator of occlusive events in the proximal arterial supply.

In reference to potential mechanisms underlying this AFC response to carotid occlusion, it is possible that altered neuronal function in the face of reduced cortical blood flow (CBF) may play a role. Although measurements of neuronal activity were not undertaken in this study, previous investigations using somatosensory evoked responses (SEPs) have indicated that changes in SEPs occurred only after moderate reductions in baseline CBF. Because only a 20% reduction in CBF is reported after bilateral carotid artery occlusion, the authors postulate that altered neuronal activity is not primarily responsible for the AFC changes.

The authors relate their findings to the clinical stroke literature. Although acute mechanical carotid occlusion may differ from the thrombotic processes involved in chronic cerebrovascular stenosis, similarities exist between these findings and those reported in people with carotid artery disease. Studies such as these emphasize the complexity of the cerebrovascular response to carotid occlusion. The clarification of underlying mechanisms may aid in the development of novel therapeutic strategies to target people at risk for stroke.

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