Cardiac Dysfunction After Left Permanent Cerebral Focal Ischemia  
The Brain and Heart Connection  
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Background and Purpose—Stroke can lead to cerebrogenic cardiac arrhythmias. We sought to investigate the effect of ischemic stroke on cardiac function in a mouse model of permanent middle cerebral artery occlusion (pMCAO).

Methods—Twenty-four hours after the induction of focal ischemia, cardiac function was measured in mice by endovascular catheterization of the heart. Immediately after hemodynamic measurements, mice were euthanized and brains were excised and sectioned to measure infarct volume and the severity of insular cortex injury. Myocardial damage was evaluated by hematoxylin-eosin staining. Serum and heart levels of norepinephrine (NE) were also determined.

Results—Cardiac dysfunction occurred in 9 out of 14 mice that underwent left pMCAO. In these 9 mice, the severity of left insular cortex lesion was greater than the mice with normal heart function. The serum and heart levels of NE were significantly higher in left pMCAO mice with heart dysfunction. Linear regression analysis indicates significant inverse correlation between the severity of left insular cortex damage and heart dysfunction. Mice that underwent right pMCAO did not exhibit cardiac dysfunction.

Conclusions—This study shows that left focal cerebral ischemia can produce cardiac dysfunction, which is associated with the extent of left insular cortex damage. Furthermore, mice exhibiting cardiac dysfunction had elevated levels of NE in the serum and heart. (Stroke. 2009;40:00-00.)

Key Words: mouse focal ischemia ■ cardiac dysfunction ■ left insular cortex lesion ■ norepinephrine

Previous studies have shown that cardiac arrhythmias can occur after cerebral ischemia. The insular cortex has attracted much attention as the possible cerebral locus for stroke-associated cardiac arrhythmias. Clinical evidence suggests that as well as arrhythmias, stroke may also cause myocardial injury. The precise pathways and mediators of stroke related cardiac arrhythmias and injury are unknown at this time. Using endovascular catheterization of the mouse heart after focal cerebral ischemia, the present study provides insights on the effect of focal cerebral ischemia on cardiac performance in mice with pMCAO. We also explored the relationship between myocardial dysfunction with the extent of left insular cortical injury and explored possible mechanisms of injury.

Materials and Methods

Focal Cerebral Ischemia

All experimental procedures were approved by the All-University Institutional Animal Care and Use and performed in accordance with the Guide for the Care and Use of Laboratory Animals. Permanent focal cerebral ischemia (pMCAO) was induced in 12-week-old male C57BL/6 mice (Charles River Laboratory) by electro-coagulation of the right or left middle cerebral artery, as previously described. A homeothermic blanket feedback system was applied to maintain the body temperature at 37°C. A perimed PF-3 laser Doppler perfusion monitor (Järfalla) was used to measure regional blood flow before and after the surgical procedure. Animals with less than 80% reduction in cerebral blood flow were excluded from the study.

Measurement of Cardiac Function

Heart function hemodynamic parameters were assessed 24 hours after induction of ischemia using endovascular cardiac catheterization as previously described. Experimental mice were anesthetized with halothane throughout the procedure. The left ventricular (LV) systolic pressure (LVSP), the LV end-diastolic pressure (LVEDP), the maximum rate of LV systolic pressure rise (+dP/dt), and the maximum rate of LV systolic pressure fall (−dP/dt) were recorded and analyzed by a PowerLab data-acquisition system (AD Instruments).

Assessment of Infarct Volume and Insular Cortex Lesion

Mice were euthanized 24 hours after pMCAO, brains were removed and cut into 1-mm-thick coronal sections, and processed for 2% 2,3,5-triphenylterazolium chloride (TTC) staining to measure infarct volume and the severity of insular cortex injury.
volume as previously described. The pallor on TTC stained sections (unstained by TTC) was considered infarcted tissue. The infarct volume of each slice was calculated by taking the average of the infarct area on both sides of the slice and multiplying it by the section thickness. The infarct volumes of individual sections were then summed to determine the total brain infarct volume and adjusted for edema. The severity of insular cortex injury was scored in TTC-stained sections by a blinded observer. The insular is an “island” of cortex that lies at a marginal position on the lateral surface of the cerebral hemisphere and runs along the bottom and lower wall of the rhinal sulcus. Histological evidence of insular cortex injury was classified in terms of the degree of insular cortex infarct and graded on a 4-point scale ranging from 0 to 3 (0 = no insular cortex damage; 1 = limited insular cortex infarct, <25%; 2 = intermediate severity with extended insular cortex infarct, 25% to 50%; 3 = extensive insular cortex infarct, >50%).

Norepinephrine Assay and Histology
Blood samples taken from the inferior vena cava at 24 hours after pMCAO were centrifuged to obtain serum. The hearts were then rapidly removed after decapitation. Experimental samples collected from left pMCAO mice were frozen on dry ice and stored at −20°C until norepinephrine (NE) assays were performed. NE content was quantitatively determined by enzyme immunoassay kits (Rocky Mountain Diagnostics). Another batch of mice (3 sham and 3 with left pMCAO) were euthanized at 24 hours after surgery, and the dissected hearts were then processed hematoxylin and eosin (H.E.) staining.

Statistical Analysis
All data are expressed as mean values±SEM. The degree of statistical significance between groups was determined on the basis of Student t-test and 1-way ANOVA test. Linear regression modeling was used to compare the parameters of infarct volume and the LVSP, and severity of insular cortex damage and the LVSP. Statistical significance was defined at P<0.05.

Results
Effect of pMCAO on Cardiac Function
As assessed by the TTC staining, the mean infarct volume was 15.3±1.5 mm³ in the group of mice that underwent left pMCAO surgery. Neurological functional deficits were also found in these pMCAO mice (data not shown). Cardiac dysfunction was found in 9 of 14 mice that underwent left pMCAO. In these 9 mice, myocardial impairment was reflected by significantly decreased LVSP, \( dP/dt \), and \( -dP/dt \) (Figure 1). The infarct volume was 14.6±1.3 mm³ in a group of 10 mice that underwent right pMCAO. In contrast to the mice with left pMCAO, no cardiac dysfunction was found in the right pMCAO group (data not shown).

Correlation of the Severity of Left Insular Cortex Lesion and Heart Function
The severity of left insular cortical damage was significantly greater in the group of left pMCAO mice with cardiac dysfunction (Figure 2). Regression analysis revealed a significant linear inverse relationship between the LVSP and the infarct volume. However, a greater inverse correlation was found between the LVSP and the severity of insular cortex lesion.

NE Detection and Histology
Serum and heart levels of NE were significantly elevated in left pMCAO mice with heart dysfunction (n=9) compared to sham-operated group (n=5; Figure 3). However, elevation of NE levels was not significant in left pMCAO mice with normal heart function (n=5). Cardiac contraction band necrosis was detected in HE-stained heart sections from left pMCAO mice with heart dysfunction (Figure 3C).

Discussion
Cardiac arrhythmias and cardiac dysfunction have been reported after intracranial hemorrhage and brain trauma. Although cardiac arrhythmias can occur after focal cerebral ischemia, no animal data previously existed on the effect of
focal ischemia on cardiac performance. The present study demonstrates that left focal ischemia can lead to global heart dysfunction and that this dysfunction is related to the extent of left insular cortex damage. Our data also show that plasma and myocardial levels of NE were raised in mice that had cardiac dysfunction suggesting that excess NE may mediate the cardiac dysfunction. The cause of elevated serum and myocardial NE is not clear. Considerable evidence implicates the left insular cortex in regulating the vagal cardiac parasympathetic neuronal pool, and the right insular cortex in regulating sympathetic tone. It is possible that left insular injury may disturb the sympathet-

Figure 2. A linear regression study of brain damage and heart function in mice at 24 hours after surgery. A, Representative images of TTC staining indicate that mouse with heart dysfunction showing extended left insular cortex (IC) damage. B, Bar graph indicates that the score of left insular cortex damage in mice with cardiac dysfunction \( n=9 \) was significantly greater than that in the group of left pMCAO mice with normal heart function \( n=5 \). Linear regression study (C and D) demonstrated a stronger inverse correlation between the LVSP and the severity of left insular cortex damage. *\( P < 0.05 \) vs left pMCAO mice with normal heart function.

Figure 3. ELISA assay of NE. Bar graphs represent the serum levels (A) and the heart levels (B) of NE from sham and left pMCAO mice. Punctuated cardiac contractile band necrosis was found in left pMCAO mice with heart dysfunction (C) arrow. *\( P < 0.05 \) left pMCAO mice with heart dysfunction vs Sham.
ic/parasympathetic balance and results in augmented cardiac sympathetic activity and injury. Our study suggests that damage to the left insular cortex may cause a catecholamine surge. This catecholamine surge and the resulting spillover of catecholamines might provoke myocytolysis with subsequent heart dysfunction. The downstream mediators of cardiac dysfunction in our model are yet to be determined, but calcium overload is one candidate mechanism. Intracellular Ca\(^{2+}\) overload after excessive catecholamine surge has been attributed to cardiac contractile failure. Fang and colleagues reported that intracerebral hemorrhage could cause cardiomyocyte contractile dysfunction and abnormal intracellular Ca\(^{2+}\) handling, which is consistent with our previous studies indicating that abnormal intracellular Ca\(^{2+}\) modulation is a major factor in the pathogenesis of cardiac dysfunction after myocardial infarction.

Taken together, our study indicates that left focal ischemia can lead to cardiac dysfunction, which is related to the extent of insular cortical injury. Furthermore, excess NE release may mediate this cardiac dysfunction. Future studies will further explore the mechanisms and mediators of cardiac dysfunction after focal ischemia.

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Disclosures
None.

References
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