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Cardiovascular Changes During Focal Cerebral Ischemia in Rats

Alejandro D. Perez-Trepichio, MD; John L. Williams, PhD; Christine H. Block, PhD; and Stephen C. Jones, PhD

**Background and Purpose:** Recent studies have suggested that cerebral infarction influences autonomic activity and may contribute to sudden death. The goal of this study was to examine the effects of focal cerebral infarction on mean arterial pressure and heart rate.

**Methods:** Halothane-anesthetized rats were assigned to two groups: stroke (n=10), in which the middle cerebral artery or an adjacent vessel was embolized with a silicone cylinder, and sham (n=8), in which rats were sham embolized (saline). Arterial pressure and heart rate were measured for 90 minutes and again 24 hours after vascular occlusion. A change in electroencephalographic amplitude of −45% after embolization was used to determine if a significant degree of infarction was present.

**Results:** Vascular occlusion produced a significant increase in mean arterial pressure at 10, 60, and 90 minutes (p<0.05). Changes in heart rate were significantly greater (p<0.05) than in sham-treated rats at 10 and 30 minutes after embolization. In contrast, mean arterial pressure and heart rate measured 24 hours after embolization were similar in both groups. Anatomic analysis of the infarcted areas demonstrated that either insular cortex or amygdala was affected in all embolized rats.

**Conclusions:** This study indicates that cerebral infarction produces a transient elevation of mean arterial pressure and heart rate. However, within 24 hours both parameters returned to preinfarcted levels. Our findings are consistent with clinical reports that indicate that mean arterial pressure and heart rate of stroke patients are similar to those of other groups when they are admitted to the hospital, although other cardiovascular parameters are greatly altered. *(Stroke 1993;24:691–696)*

**Key Words** • blood pressure • cerebral infarction • heart rate • rats

Recent studies suggest that stroke in humans can produce changes in autonomic mechanisms that may contribute to sudden death. Although the mechanisms are not known, patients with cerebral infarction have a higher incidence of cardiac arrhythmias and myocardial damage than control subjects. In addition, plasma levels of norepinephrine, epinephrine, and dopamine are increased after cerebral infarction. In contrast, changes in arterial pressure and heart rate that result from stroke in humans apparently are minimal when patients are admitted to the hospital for treatment, which usually occurs not sooner than 24 hours after stroke.

Cechetto and coworkers reported that occlusion of the left middle cerebral artery (MCA) in normotensive rats produces some changes in autonomic and cardiac functions that resemble those in humans after cerebral infarction. In those experiments, arterial pressure was greater in stroke rats than in control rats for up to 3 hours after MCA occlusion. In contrast, in another study resting arterial pressure was similar in control and stroke rats 10 days after occlusion.

More recently, Hachinski et al reported results that apparently contradict earlier findings by the same laboratory in the same model under similar conditions. In this report, occlusion of the left MCA had no effect on heart rate or mean arterial pressure for up to 6 hours. In a separate and concurrent study, the same group reported increases in mean arterial pressure when the right MCA was occluded but not when the left MCA was occluded.

The primary goal of these studies was to examine arterial blood pressure and heart rate at different times after occlusion of the left MCA in rats. In this study, we measured arterial pressure and heart rate immediately after (for 90 minutes) and at a much later time (24 hours) after MCA occlusion with a silicone cylinder. This model has the advantage of a closed cranium and a less invasive approach than other models of focal cerebral infarction, thereby minimizing the influences of direct brain manipulation. In addition, the use of arterial embolization more closely resembles a significant subgroup of strokes in humans and also permits prompt recovery from surgery.

**Materials and Methods**

**Animal Preparation and Physiological Measurements**

Eighteen male Sprague-Dawley rats weighing 321±8 g (mean±SEM) were orally intubated and mechanically

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ventilated (model 683, Harvard Apparatus, South Natick, Mass.). Anesthesia was induced with 2–3% halothane in a mixture of 70% N₂O and 30% O₂. A maintenance dose of 0.5–1.0% halothane was adjusted slightly as needed when a tail pinch caused abrupt changes in heart rate, mean arterial pressure, respiratory rate, or pupillary diameter. Body temperature was maintained at 37°C with a servo-controlled heating lamp and a rectal thermistor (YSI 74, Yellow Springs Instrument Co., Yellow Springs, Ohio). Femoral arteries and veins on both sides were permanently cannulated with polyethylene tubing. The left common carotid artery was accessed by a midline neck incision, and the left pterygopalatine artery was cauterized. The left external carotid artery was cannulated retrogradely for embolization. For electroencephalographic (EEG) recording over the MCA territory, five stainless steel screws were placed in the skull and embedded in dental acrylic (Lang Dental, Chicago, Ill.). The reference electrode was placed 11 mm rostral to the bregma on the midline, and bilateral, bipolar electrodes were placed 4 mm and 9 mm lateral to the bregma, respectively.

While the animals were anesthetized, mean arterial pressure and end-tidal CO₂ were continuously monitored with a pressure transducer (MP-15D, Micron, Los Angeles, Calif.) and an infrared CO₂ detector (model 223, Puritan-Bennett, Boston, Mass.), respectively, and recorded on a polygraph (RS-3800, Gould, Cleveland, Ohio). Heart rate was determined from the arterial pulse. Arterial Paco₂, Po₂, and pH were determined with a blood gas analyzer (ABL-3, Radiometer, Copenhagen, Denmark). While rats were anesthetized, physiological variables were measured before embolization of the MCA and 10, 30, 60, and 90 minutes after embolization: (Table 1). Control measurements followed embolization by 26±3 minutes.

Ninety minutes after embolization catheters were heparinized, securely positioned in a subcutaneous pouch, and all surgical wounds were sutured. As the rats recovered from anesthesia, they were returned to their cages with access to food and water ad libitum. After 24 hours, the rats were reanesthetized to a level similar to that of the previous day, and catheters were reexteriorized. Measurements of mean arterial pressure, heart rate, Paco₂, PaO₂, and pH were repeated 21±3 minutes after induction of anesthesia.

### Embolization

Animals were randomly assigned to two groups: stroke (n=10) and sham (n=8). In the stroke group, during occlusion of the left common carotid artery with an aneurysm clip, a single silicone cylinder (0.8–1.0×0.3 mm) was infused with 0.8 mL of 0.9% saline into the left external carotid artery.⁸ The protocol for animals in the sham group was analogous to that of the stroke group, except that saline without a silicone cylinder was injected into the external carotid artery cannula. Animals in the stroke and sham groups received 11 mL/kg of saline while under anesthesia.

EEG was recorded just before and for 90 minutes after embolization (or sham embolization). Fourier frequency analysis with a digitizing system (Rhythm, Stellate Systems, Westmount, Canada) was used for spectrum analysis. Postembolization EEG ratio was calculated as percent reduction in amplitude after embolization. This ratio was designed to account for side-to-side and time variations that were independent from the effects of embolization. The ratio was calculated as (100[Apost-ipsi×Apref-contral]−1), where Apost-ipsi and Apref-contral represent the amplitudes after embolization ipsilateral and contralateral to the ischemic side, respectively, and Apref-ipsi and Apref-contral represent the amplitudes before embolization ipsilateral and contralateral to the ischemic side, respectively. An EEG ratio less than or equal to −45% indicated that embolization had produced an acceptably large area of infarction.

### Infarct Assessment

After the 24-hour measurement, the anesthetized rats were decapitated. Each brain was removed from the skull, and the silicone cylinder was visually located. The extent of the cerebral infarct area was determined by staining with 2,3,5-triphenyltetrazolium chloride (TTZ) or cresyl violet. In some animals (n=10), brain slices (2 mm thick) were obtained in a matrix device (Activation Systems Inc., Warren, Mich.) and incubated with TTZ at 40°C for 15 minutes. Photographic transparencies were made of both the rostral and caudal faces of these slices. Lack of TTZ staining (white), compared with normal tissue (red), indicated infarcted area.¹⁰ In other animals (n=8), the brains were frozen immediately after decapitation in chlorodifluoromethane.
(-44°C) and later sectioned at 20 μm in a cryostat (-12°C). Every 20th section was stained with cresyl violet. The sections were examined by a blinded investigator to determine whether the insular cortex and amygdala were included in the infarcted area. Representative sections were matched with a stereotaxic atlas for comparison of selected areas.

**Statistical Analysis**

Values are expressed as mean±SEM. Dependent variables were evaluated with an analysis of variance. When an analysis of variance indicated that differences existed, sham and stroke groups were compared at each point in time with an independent samples t test. In addition, changes from control were compared between groups with an independent samples t test. All values of p<0.05 were considered to indicate statistical significance.

**Results**

In the stroke group, the EEG ratio was -56±2% immediately after embolization, which indicated that significant infarction was present. In contrast, in the sham group, EEG ratios were similar before and after sham embolization (p>0.05). Visual inspection at the end of each experiment demonstrated that in eight of 10 rats in the stroke group the silicone cylinder was in the MCA. In one case, the cylinder was located in the distal internal carotid artery adjacent to the MCA, and in one other case the cylinder was not located.

Arterial pH was similar between sham and stroke groups before embolization and 60 minutes and 24 hours after embolization (Table 1). Although PaCO2 was slightly increased at 60 minutes in the stroke group, the change in PaCO2 from the control level was not different between groups at 60 minutes. This analysis indicates that the magnitude of the differences in PaCO2 between groups had no significant impact on mean arterial pressure or heart rate. Similarly, although PaO2 was significantly elevated in embolized animals at 24 hours in the stroke group, the change from control values at 24 hours was not significantly different between groups (Table 1). Levels of halothane were similar in stroke (0.65±0.02%) and sham (0.68±0.02%) groups at all times of measurements (p>0.05).

Embolization produced an immediate (10 minutes) increase in mean arterial pressure. Although control values for mean arterial pressure were similar in stroke and sham groups, mean arterial pressure was significantly greater in the stroke group at 10, 60, and 90 minutes after embolization by 20%, 14%, and 16%, respectively (Table 2). In addition, changes in mean arterial pressure from control at 10, 60, and 90 minutes were greater in embolized animals than in sham-treated animals (Figure 1). In contrast, 24 hours after embolization or sham treatment, mean arterial pressure and changes in mean arterial pressure were similar in stroke and sham groups, respectively (Table 2, Figure 1).

Heart rate also increased transiently after embolization. Heart rate was significantly greater at 10 minutes after embolization and tended to be greater than in sham-treated rats at 30, 60, and 90 minutes, although the differences between groups at these latter times were not significant (Table 2). Changes in heart rate were significantly greater at 10 and 30 minutes in the stroke group after embolization (Figure 2). In contrast,

**Table 2. Mean Arterial Pressure and Heart Rate After Vascular Occlusion in Rats (Sham and Stroke Groups)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time</th>
<th>Sham</th>
<th>Stroke</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure</td>
<td>Control</td>
<td>114±4</td>
<td>112±2</td>
</tr>
<tr>
<td>(mm Hg)</td>
<td>10 minutes</td>
<td>106±5</td>
<td>127±5*</td>
</tr>
<tr>
<td></td>
<td>30 minutes</td>
<td>107±6</td>
<td>117±5</td>
</tr>
<tr>
<td></td>
<td>60 minutes</td>
<td>111±4</td>
<td>126±4*</td>
</tr>
<tr>
<td></td>
<td>90 minutes</td>
<td>109±6</td>
<td>126±4*</td>
</tr>
<tr>
<td></td>
<td>24 hours</td>
<td>116±6</td>
<td>111±8</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>Control</td>
<td>352±17</td>
<td>342±6</td>
</tr>
<tr>
<td></td>
<td>10 minutes</td>
<td>331±11</td>
<td>380±19*</td>
</tr>
<tr>
<td></td>
<td>30 minutes</td>
<td>335±14</td>
<td>398±30</td>
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<tr>
<td></td>
<td>60 minutes</td>
<td>347±22</td>
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<td></td>
<td>90 minutes</td>
<td>353±29</td>
<td>413±26</td>
</tr>
<tr>
<td></td>
<td>24 hours</td>
<td>334±39</td>
<td>358±12</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Differences in n are due to missing values. bpm, Beats per minute. *p<0.05 different from sham group.

![Figure 1](http://stroke.ahajournals.org/)

**Figure 1.** Line graph shows changes from control in mean arterial pressure (MAP) at various times after cylinder embolization (stroke group, ○) or sham embolization (sham group, □). Values are mean±SEM. **p<0.01 different from sham group.
heart rate and change in heart rate were similar in both groups at 24 hours (Table 2, Figure 2).

Embolization resulted in brain infarction in all rats in the stroke group, but the size and location of the infarcted region varied considerably (Figure 3). In three animals, the infarcted area was extensive and included portions of insular, somatosensory, and motor cortex as well as the amygdala. In five rats infarction was present in insular, somatosensory, and motor cortex but not in the amygdala. In contrast, in two animals embolization caused infarction in the amygdala, and the cerebral cortex was minimally affected. No evidence of brain infarction was apparent in sham-treated rats.

Although differences were not statistically significant, rats with lesions involving primarily the insular cortex tended to have a greater increase in heart rate compared with rats with lesions involving the amygdala alone. The largest increases tended to occur when both insular cortex and amygdala were involved. No differences between subgroups for mean arterial pressure were observed.

![Figure 3](http://stroke.ahajournals.org/)

**Figure 3.** Drawings of coronal brain sections from three representative animals demonstrating the extent of infarction (shaded areas) and anatomic locations. Top panel: Both insular cortex (IC) and amygdala (AM) are infarcted. Middle panel: IC but not AM is affected. Bottom panel: AM but not IC is involved. Drawings are modified from Paxinos and Watson.11

Discussion

The results of this study indicate that occlusion of the MCA in rats produces immediate increases in mean arterial pressure that are sustained for at least 90 minutes after occlusion. In addition, heart rate increased immediately after MCA occlusion. By 24 hours after MCA occlusion, however, mean arterial pressure and heart rate returned to control values.

Consideration of Methods

Stroke from embolic origin accounts for approximately 30% of the stroke cases in humans.12 In contrast to most previous experimental studies, our model used an injected embolus. In most other models, the MCA is occluded directly, after surgical exposure of the MCA. In our study, the MCA was occluded by a single silicone cylinder that was injected retrogradely into the external carotid artery. The model of MCA occlusion with an injected silicone cylinder minimizes surgery and, therefore, tissue manipulation. This approach also avoids the craniotomy required in other, more invasive models.13 By maintaining a closed cranium, embolization does not interfere with the normal development of infarction, edema, and changes in intracranial pressure that accompany MCA occlusion. In addition, the stability of the silicone cylinder as an insoluble embolus permits permanent occlusion of the MCA. Although the exact location that the embolus lodges cannot be controlled in this model, the injected cylinder lodged within the MCA in 80% of the cases in the present study.

Interpretation of Findings

Previous experiments in animals have indicated that occlusion of the MCA causes immediate increases in mean arterial pressure. Cechetto et al15 reported that arterial pressure was greater in rats with left MCA occlusion than in sham control animals. Similarly, in cats MCA occlusion produced elevations in mean arterial pressure that were sustained for 6 hours.14 In contrast, in a different study arterial pressure in rats has been reported to be normal 9–10 days after MCA occlusion.6

To clarify this apparent discrepancy we examined effects of MCA embolization on mean arterial pressure both acutely and chronically in the same animals. Our studies indicate that occlusion of the MCA increases arterial pressure acutely. By 24 hours after MCA occlusion, however, mean arterial pressure returned to control levels.

Our findings on response of heart rate to MCA occlusion in rats are also consistent with some previous reports. In one study, heart rate at 90 minutes after MCA occlusion tended to be elevated, but differences were not significant.5 Although changes in heart rate were significantly elevated for 30 minutes in stroke rats in the present study, differences were not significant after that time. By 24 hours, heart rate apparently had completely recovered. This latter finding is consistent with a long-term study (10 days) in rats that indicates that heart rate is similar in MCA-occluded and sham-treated animals.6

A recent study reported that in most cases left MCA occlusion had no effect on heart rate or mean arterial pressure for 6 hours after stroke onset.7 In a concurrent
study, the same group also reported a significant increase in mean arterial pressure when the right MCA was occluded but not when the left MCA was occluded. These recent reports are in contrast to a previous study by the same investigators, who reported that left MCA occlusion produced elevation in mean arterial pressure. We embolized the left MCA and observed increases in both mean arterial pressure and heart rate acutely.

The reason for these discrepancies in the response of heart rate and mean arterial pressure to MCA occlusion between studies in rats is not known but may be attributed to the different types of anesthetic used. In our study, rats were maintained at similar levels of halothane throughout the experiment. This anesthesia provided relatively normal and stable levels of baseline mean arterial pressure and heart rate. In contrast, in another acute rat study urethane was used, which apparently caused lower baseline and progressively decreasing levels of mean arterial pressure.

In humans stroke may contribute to sudden death by producing changes in autonomic mechanisms, with detrimental consequences in cardiac function. Plasma levels of norepinephrine, epinephrine, and dopamine are elevated, which suggests that sympathetic activity is increased by stroke. Other consequences of stroke that may result from altered autonomic activity include increases in electrocardiographic abnormalities with elevated creatine phosphokinase, incidence of serious and potentially lethal arrhythmias, and myocardial damage. Many of these changes that are associated with stroke in humans also occur in animal models with MCA occlusion, including increases in plasma catecholamines, cardiac arrhythmias, and myocardial damage. In addition, a recent study in rats indicates that stroke impairs the baroreceptor reflex.

As a group, differences in arterial pressure and heart rate in stroke patients compared with control subjects are reportedly small or not statistically significant. Both stroke and control patients may exhibit elevated mean arterial pressure caused by mental stress on admission. Subgroups of patients with a history of hypertension or intracerebral hemorrhage exhibit significantly higher levels of arterial pressure and heart rate than other stroke patients. In most instances, arterial pressure and heart rate in stroke patients have not been measured acutely after stroke onset, but rather at 24 hours or longer. The findings of the present and previous studies in rats suggest that arterial pressure and heart rate may also be elevated acutely in stroke in humans but have recovered to normal levels by the time of measurement.

**Anatomic Correlates**

In the present study, MCA occlusion was consistently associated with lesions that included either insular regions of the cerebral cortex or the amygdala. Our findings are in agreement with other studies in animals that suggested that the insular cortex is an important cardiovascular control area that becomes dysfunctional after MCA occlusion. Although our results are not definitive evidence of causality, they strongly support the involvement of the insular cortex, amygdala, or both regions in the transient elevation in mean arterial pressure and heart rate. The participation of the insular cortex in cardiovascular control also has been demonstrated by experiments with chemical or electrical stimulation of regions within the insula, which altered mean arterial pressure and heart rate. Moreover, firm evidence exists from anatomic transport studies for connections between the insula and other centers that modulate cardiovascular responses, such as the nucleus tractus solitarius, amygdala, lateral hypothalamic area, and thalamus. The amygdala also modulates cardiovascular activity. Electrical stimulation of the central amygdaloid nucleus produces an increase in heart rate and mean arterial pressure in awake rats but has the opposite effect in animals under anesthesia. The possibility that the observed responses were mediated by other structures in the ischemic area or outside the ischemic area cannot be excluded. Because the ischemic areas in this study were extensive, a cautious interpretation of the anatomic correlates is warranted.

An analysis of the three subgroups (insular cortex alone, amygdala alone, or both) within the stroke group suggested that heart rate can increase with ischemia of either insular cortex or amygdala. Moreover, the largest increases in heart rate were observed in rats with lesions involving both the insular cortex and the amygdala. Mean arterial pressure between subgroups was not apparently different.

The recent development of new diagnostic techniques allows very early anatomic localization of stroke in humans. Experimental evidence suggests that it may be important to consider the contribution of specific areas of the brain (e.g., insular cortex, amygdala, or others) in the autonomic imbalance that results from cerebral infarction. Early recognition of the infarct location could aid in the treatment of autonomic changes that are associated with some types of strokes.

In summary, the findings of the present study indicate that occlusion of the MCA in rats increases mean arterial pressure and heart rate acutely. Within 24 hours, however, mean arterial pressure and heart rate returned to control levels. Although mean arterial pressure and heart rate compensate, other studies in animal models and in humans indicate that an autonomic imbalance persists chronically after stroke that may have serious cardiovascular consequences and lead to sudden death.

**Acknowledgments**

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**References**

This investigation demonstrated that rats whose middle cerebral artery was embolized with a silicone cylinder demonstrated a transient increase in mean arterial blood pressure that was no longer apparent at 24 hours after the vascular occlusion. This acute increase in mean arterial blood pressure is consistent with previous results in the rat showing an acute increase in plasma catecholamines and sympathetic nerve activity after middle cerebral artery occlusion. However, the human data suggest that the vulnerable period for cardiac complications after stroke persists for up to 30 days. Thus, it may be that the initial increase in sympathoadrenal activity may be the critical factor in predisposing individuals to longer-term cardiac complications.

In addition, the results of this investigation indicated that involvement of the insular cortex is necessary for the acute increase in mean arterial blood pressure. We have recently confirmed this finding by making specific lesions of the insular cortex in acutely anesthetized rats. This suggests that it would be important in clinical stroke to assess the involvement of the insular cortex in hemispheric stroke for the identification of patients who may be predisposed to a cardiac vulnerable period following stroke.

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