Social Stress Exacerbates Focal Cerebral Ischemia in Mice

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Background and Purpose—The purpose of the present study was to determine whether exposure to stress or elevated corticosterone concentrations in the days preceding cerebral ischemia exacerbates ischemic injury as assessed by histological and behavioral outcomes.

Methods—For 7 consecutive days, male C57/BL6 mice were exposed to social stress for 45 minutes or injected with 1 mg/kg corticosterone or vehicle. The animals exposed to social stress were injected with either 1 mg/kg mifepristone, a glucocorticoid receptor antagonist, or the vehicle 30 minutes before stress. On the seventh day, all animals were trained in a passive avoidance task. Twenty-four hours after training, the animals were subjected to 60 minutes of intraluminal middle cerebral artery occlusion (MCAO) or sham surgery. At 72 hours of reperfusion, the animals were tested for retention of the passive avoidance task, and infarction size was determined.

Results—Animals subjected to chronic social stress or treated with exogenous corticosterone before MCAO exhibited larger infarcts and reduced retention of passive avoidance compared with the nonstressed MCAO control. The effects of social stress on infarct volume and passive avoidance were reversed by pretreatment with mifepristone. There was no difference between stressed and control groups in physiological parameters or reduction of laser-Doppler flow signal during MCAO or reperfusion.

Conclusions—Prior exposure to social stress increases infarction volume and exacerbates cognitive deficits associated with transient cerebral ischemia. The mechanism underlying the effects of stress on stroke outcome likely involves corticosterone acting through glucocorticoid receptors to increase subsequent ischemia-induced neuronal death. (Stroke. 2002;33:1660-1664.)

Key Words: behavior ■ glucocorticoids ■ stress ■ stroke ■ mice

Exposure to stress is a universal human experience, but the magnitude, duration, frequency, and nature of stressors vary greatly among individuals. Although there are many circumstances under which acute activation of the hypothalamic-pituitary-adrenal (HPA) axis during stress may be adaptive, chronic activation of the HPA axis often has deleterious consequences (for review, see McEwen1). In particular, chronic psychological stress has been associated with an increased incidence or exacerbation of several neurological disorders, including multiple sclerosis, Parkinson’s disease, depression, and age-related dementia.2,3 Anecdotal evidence suggests that stress can precipitate stroke; however, only a few clinical studies and case reports have provided support of a relationship between severe emotional stress and the onset of stroke.4–6 Other reports have concluded that there is no effect of psychosocial factors on stroke.7–10 The discrepancy in conclusions among these clinical studies may be due to many factors, including small sample size in some of the studies, differences in mean age of subjects, and the methods associated with reporting and rating of stressful events. Clearly, additional studies are needed to assess the impact of stressful life experiences on stroke outcome.

Stroke is itself a stressor. Activation of the HPA axis is among the first measurable physiological responses to cerebral ischemia, which leads to sustained increases in blood concentrations of glucocorticoids.11–15 The predominant glucocorticoid released from the adrenal cortex during stress is cortisol in humans and corticosterone in most rodents. There is strong evidence to suggest that intraischemic and postischemic serum glucocorticoid concentrations alter stroke outcome in both humans and rodents. Elevated cortisol concentrations after stroke are significantly correlated with increased morbidity and mortality in humans13,16,17 and increased infarct size in mice.18 Furthermore, a causal link between corticosteroids and stroke outcome is suggested by animal studies in which manipulating blood corticosteroids concentrations during or after an ischemic event alters stroke outcome in both humans and rodents. Elevated cortisol concentrations after stroke are significantly correlated with increased morbidity and mortality in humans13,16,17 and increased infarct size in mice.18 Furthermore, a causal link between corticosteroids and stroke outcome is suggested by animal studies in which manipulating blood corticosteroids concentrations during or after an ischemic event alters infarct size.19–22 It has been suggested that exposure to elevated glucocorticoid concentrations typically does not kill neurons directly but instead increases stroke-induced neuronal death.
by creating a sublethal state of catabolic crisis that renders neurons less likely to survive a subsequent ischemic injury.\(^2\)

In the present study male mice were repeatedly exposed to social stress or treated with exogenous corticosterone for several days before induction of transient focal ischemia via middle cerebral artery occlusion (MCAO). The role of glucocorticoid receptors in mediating stress-induced changes in ischemic outcome was determined by treating a subset of experimentally stressed animals with a specific receptor antagonist. Both histological damage and cognitive function were assessed because these 2 outcome measures are rarely correlated in experimental stroke (for review, see DeVries et al\(^2\)).

### Materials and Methods

#### Animals

This study was conducted in accordance with National Institutes of Health guidelines for the use of experimental animals, and the protocols were approved by the local institutional animal care and use committees. Adult male C57Bl/6 mice (weight, 22 to 30 g; Charles River, Wilmington, Mass) were individually housed, allowed ad libitum access to food and water, and maintained on a 14:10 light/dark cycle. The experimental groups consisted of animals (1) injected with vehicle for 7 days, then subjected to sham MCAO surgery (sham group; n = 8); (2) injected with vehicle for 7 days, then subjected to MCAO (nonstressed group; n = 7); (3) injected with vehicle and experimentally stressed for 7 days, then subjected to MCAO (stressed group; n = 6); (4) injected with mifepristone, a glucocorticoid receptor antagonist, and experimentally stressed for 7 days, then subjected to MCAO (mifepristone group; n = 6); or (5) injected with corticosterone for 7 days, then subjected to MCAO (corticosterone group; n = 6).

#### Social Stress

Experimental animals were placed in the home cage of a large aggressive male mouse (>30 g). The animals were allowed to interact freely until they engaged in 5 antagonistic “boots.” Then a screen barrier was used to divide the cage in half and separate the 2 animals. The screen prevented additional physical interaction but allowed the animals to continue to make visual and auditory threats. The experimental animal remained in the cage for an additional 45 minutes. On the following 6 days, the experimental animal interacted freely with the aggressive male until 1 aggressive bout occurred, then the 2 animals were separated by the screen for an additional 45 minutes. At the end of each stress session, experimental and stimulus animals were closely inspected before being returned to their home cages. None of the animals sustained wounds that required medical intervention. Control animals were handled and inspected daily in a fashion similar to that used with the animals in the social stress group but otherwise remained undisturbed in their home cages. The last stress session occurred 24 hours before induction of ischemia.

#### Injections

All animals received an intraperitoneal injection of vehicle (final concentration: 0.001% ethanol in sterile isotonic saline), corticosterone (1 mg/kg; Sigma Chemicals), or mifepristone (100 mg/kg; Sigma Chemicals). The chosen corticosterone dose produces serum corticosterone concentrations in male mice that approximate those resulting from social stress induced through the aforementioned methods. The animals in the experimentally stressed groups received the injections 30 minutes before being placed in the aggressive male’s cage. The corticosterone dose was chosen on the basis of a small dose-response study in which treatment with 1 mg of corticosterone produced an increase in blood corticosterone concentrations that was similar to that produced by social stress (644 ± 78 ng/mL; A.C. DeVries, PhD, unpublished data, 1999).

### Cognitive Function

Twenty-four hours before ischemia, animals were tested for baseline motor ability (approximately 6 hours into the light phase of the circadian cycle). Latency to initiate walking was used as an index of motor ability. The animals were placed on a flat surface in the center of a circle with a radius of approximately 1 body length. Latency to move all 4 feet completely outside of the circle was recorded. Each animal was tested twice, and a mean latency was calculated. After the assessment of motor ability, each mouse was then trained in a step-through passive avoidance task. The apparatus (Accuscan Instruments, Inc) consisted of a small chamber illuminated by two 60-W light bulbs (22 × 11 × 12 cm) and connected by an automatic sliding door to a large, dark chamber (32 × 20 × 16 cm). The animal was placed into the light chamber, then after 5 seconds the door connecting the light and dark chambers was opened. Latency to cross into the dark chamber was recorded. Once the animal crossed into the dark chamber, the door closed, and the animal received a 1-mA electric shock for 3 seconds. The animal was then removed and returned to its home cage.

Motor behavior was again assessed at 72 hours after MCAO. Animals that required >60 seconds to move outside of the circle on any trial were removed from the study. The rationale for the exclusion was that impaired motor ability can confound subsequent assessment of cognitive function (for review, see DeVries et al\(^2\)). Our previous work demonstrated that deficits in latency to move can be observed after 90 minutes of ischemia in mice.\(^2\)\(^5\) To assess retention of the passive task, the animal was placed into the light chamber, and the door was opened after 5 seconds. The session ended when the animal crossed into the dark chamber and latency to cross was recorded. If 300 seconds elapsed without the animal crossing into the dark chamber, the session was terminated, and the animal was assigned a latency to cross of 300 seconds. Although rodents typically prefer a dark rather than illuminated environment, mice avoid a dark chamber that they associate with a previously administered electric shock. Thus, a short latency to cross into the dark chamber suggests the presence of a cognitive deficit in the postischemic animal, ie, failure to remember the association of dark chamber and electric shock.

Ischemia-independent effects of stress and exogenous corticosterone treatment on task performance were assessed in a separate cohort of animals (n = 5 per group). Experimental protocols used to stress, inject, and train these animals in the passive avoidance task were identical to those used in the ischemia experiment. In nonischemic animals, there were no group differences in latency to cross into the dark chamber among unmanipulated vehicle-treated controls, animals treated with vehicle before social stress, animals treated with mifepristone before social stress, or animals treated with exogenous corticosterone (training session: F3,19 = 0.001; P > 0.05; test session: F3,19 = 0.05; P > 0.05). In the ischemia experiments, a single sham-surgery group treated with vehicle was used instead of 4 independent sham groups.

### Experimental Stroke

Transient focal cerebral ischemia was induced in male mice by MCAO as previously described.\(^2\)\(^6\) Briefly, the mice were anesthetized (1.5% halothane), and unilateral MCAO was achieved (intraluminal filament occlusion) by introducing a 6-0 nylon monofilament into the internal carotid artery to a point 6 mm distal to the internal carotid artery–pterygopalatine artery bifurcation. Once the filament was secured, the animals were allowed to emerge from anesthesia. After 60 minutes of ischemia, the animals were reanesthetized briefly, and reperfusion was initiated through withdrawal of the filament. In sham-operated animals, the carotid artery was exposed but not disrupted. Duration of anesthesia was similar in MCAO and sham groups. Body temperature was maintained at approximately 37°C during surgery and recovery by heat lamps and water pads.

Physiological measurements were performed in a separate cohort of stressed (n = 4) and nonstressed (n = 4) animals. The femoral artery was cannulated for measurement of arterial blood gases and arterial blood pressure. Arterial blood pressure was recorded every 15 minutes beginning at baseline. Blood samples (100 μL) were...
collected at baseline and after 45 minutes of ischemia. Laser-Doppler flowmetry (LDF) was used to assess adequacy of vascular occlusion and reperfusion. A small area in the right parietal skull (2 mm posterior, 3 mm lateral to the bregma) was thinned via a low-speed drill, as previously described. LDF was measured every 15 minutes during MCAO and at 15 and 30 minutes of reperfusion. The animals used for physiological monitoring were not allowed to survive beyond 30 minutes of reperfusion because the total amount of blood drawn during the experiment was beyond the level recommended for surviving mice.

**Determination of Stroke Volume**

Brains were removed and sectioned into five 2-mm-thick coronal sections. Sections were incubated for 10 minutes on each side in 2,3,5-triphenyltetrazolium maintained at 37°C, fixed in 10% formalin, then photographed. Images were analyzed (Inquiry; Loats), and infarct size was expressed as a percentage of the contralateral hemisphere, as previously described.

**Statistical Analysis**

Latency to move, latency to cross into the dark chamber, and infarct size were analyzed with the use of 1-way ANOVA followed by post hoc analysis with Fisher’s test. The latency to move data were log transformed before analysis because they did not fit the assumptions of ANOVA. Kruskal-Wallis 1-way ANOVA on ranks was used to analyze the test day crossover data for the animals in the nonischemic passive avoidance experiment because the data were not normally distributed. Pearson product moment correlation analysis was used to assess the relationship between infarct size and passive avoidance performance. Two-way ANOVA was used to analyze blood gas variables. LDF data (% change) were compared at each time point with unpaired t test. Effects were considered statistically significant at P<0.05.

**Results**

Mean latency to move 1 body length was similar among treatment groups before surgery (F 4,32 =1.46, P>0.05) and 3 days after surgery (F 4,32 =0.77, P>0.05). However, 4 animals (1 from each MCAO group) failed to move 1 body length within 60 seconds on either motor behavior trial. Data from these animals were accordingly not included in analysis of passive avoidance retention because the task requires the animals to be able move freely about the apparatus, which these animals could not accomplish.

There was no difference among treatment groups in latency to cross into the dark chamber during the preischemic passive avoidance training session (F 4,32 =0.15, P>0.05). However, 72 hours after surgery, there were significant differences in latency to cross among groups (F 4,32 =5.15, P<0.05; Figure 1). Post hoc analysis revealed that latency to cross was significantly longer in the nonstressed MCAO animals than in the animals that were subjected to social stress or treated with exogenous corticosterone before MCAO. However, there was no difference in latency to cross between the nonstressed group and the animals treated with the glucocorticoid antagonist mifepristone before stress.

Exposure to social stress or exogenous corticosterone also had a significant effect on infarct size (F 4,32 =4.15, P<0.05; Figure 2). Infarct volumes in the STRESS and CORT groups were more than twice as large as in the MCAO control (nonstressed) group. However, infarct volumes were of similar size in the MCAO without social stress and GR-ANT groups. As expected, the sham surgery did not result in tissue injury. There was no correlation between infarct volume and performance in the passive avoidance task among animals subjected to MCAO (correlation coefficient, −0.270; P>0.05; n=21).

During MCAO, LDF decreased to <20% of preischemia baseline in both the stressed and nonstressed animals (Table). After withdrawal of the occluding filament, blood flow was restored to >85% in each experimental animal. There were no differences between stressed and nonstressed animals in LDF analyzed at any measurement time point of MCAO (P>0.05). Mean arterial blood pressure remained steady throughout the experiment and was not affected by either time (F 1,55 =0.4, P>0.05) or treatment (F 1,55 =1.2, P>0.05; Table). Arterial Pco 2 increased significantly after 45 minutes of ischemia compared with preschismic levels (F 1,15 =42.7, P<0.05), but there were no differences between treatment
groups (F\(_{1,15}=0.4, P>0.05;\) Table). Arterial Po\(_2\) (F\(_{1,15}=6.1, P<0.05\)) and pH (F\(_{1,15}=38.9, P<0.05\)) decreased significantly after 45 minutes of ischemia compared with preischemic levels, but there was no effect of treatment (Table). There were no significant interactions between treatment and time for mean arterial blood pressure, Pco\(_2\), Po\(_2\), or pH (P>0.05).

**Discussion**

Exposure of animals to social stress for several days before induction of transient focal cerebral ischemia exacerbates histological and functional injury. As in a previous study,\(^\text{18}\) animals exposed to social stress exhibited substantially larger infarcts than did the nonstressed ischemic control cohort. We now show that stress-induced increase in infarct size is associated with a significant decline in cognitive function, ie, that stressed animals were more likely to cross into a chamber in which they had previously received an electric shock compared with animals treated with MCAO uncomplicated by stress. This decreased latency in the passive avoidance test indicates a cognitive deficit.\(^\text{24}\) Stress-induced deficits in histological and behavioral outcomes were reproduced by chronic treatment with exogenous corticosterone. Furthermore, glucocorticoid receptor antagonist treatment 30 minutes before social stress ameliorated the stress effect on infarct size and performance of the passive avoidance task. The mifepristone and nonstressed MCAO groups did not differ significantly in recovery from experimental stroke. Taken together, these data suggest that prior exposure to chronic stress exacerbates histological and behavioral stroke outcome likely involving glucocorticoid receptor–dependent corticosterone mechanisms.

The observation that prior exposure to stress increases cell death in cortex and striatum extends previous studies in which glucocorticoids have been shown to potentiate neurodegenerative processes during focal or global cerebral ischemia. Intraischemic treatment of rats with metyrapone, a drug that attenuates stress-induced corticosterone production during MCAO, reduces infarction volume in the cortex and striatum by approximately 50%.\(^\text{21}\) In contrast, treatment with exogenous corticosterone daily, beginning at reperfusion, results in increased infarction volume in hippocampus, neocortex, and striatum after global cerebral ischemia.\(^\text{19,22}\) Taken together, these studies suggest that there is a potentially wide temporal window during which elevated serum glucocorticoid concentrations can affect ischemia-induced neuronal death.

There are several mechanisms through which stress and glucocorticoid treatment may affect stroke outcome. For example, glucocorticoids have been shown to decrease local cerebral glucose utilization in vivo\(^\text{27}\) and inhibit glucose transport in neurons in vitro.\(^\text{28}\) By impairing glucose transport, glucocorticoids cause a subsequent ATP depletion and increased neuronal vulnerability to excitotoxicity (reviewed by Sapolsky\(^\text{29}\)). Stress also may affect infarct size by suppressing endogenous expression of bcl-2, an antiapoptotic protein, induced after ischemia.\(^\text{18}\) Regardless of the down-stream mechanism, the effects of stress in the present paradigm appear to be mediated via glucocorticoid receptors. Infarct volume and passive avoidance performance were similar among animals that were pretreated with mifepristone, a glucocorticoid receptor antagonist, before stress and animals that were not experimentally stressed. Likewise, it has been shown previously that mifepristone protects hippocampal neurons in gerbils subjected to global ischemia.\(^\text{30}\)

In contrast to our previous findings with the same duration of MCAO,\(^\text{25}\) there was no difference in passive avoidance performance between sham and MCAO groups. One difference between the 2 studies was the amount of time that lapsed

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**Physiological Parameters in Stressed vs Experimentally Nonstressed Animals Before and During MCAO**

<table>
<thead>
<tr>
<th></th>
<th>Preischemic</th>
<th>Ischemic</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>MABP, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stressed</td>
<td>73.5±1.0</td>
<td>71.8±1.80</td>
<td>71.8±1.80</td>
</tr>
<tr>
<td>Nonstressed</td>
<td>70.3±0.5</td>
<td>71.8±0.63</td>
<td>69.8±1.60</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stressed</td>
<td>7.45±0.02</td>
<td>7.33±0.02*</td>
<td>...</td>
</tr>
<tr>
<td>Nonstressed</td>
<td>7.42±0.02</td>
<td>7.29±0.02*</td>
<td>...</td>
</tr>
<tr>
<td>Pco(_2), mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stressed</td>
<td>35.53±2.38</td>
<td>48.35±2.08*</td>
<td>...</td>
</tr>
<tr>
<td>Nonstressed</td>
<td>36.50±1.28</td>
<td>49.90±2.11*</td>
<td>...</td>
</tr>
<tr>
<td>Po(_2), mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stressed</td>
<td>179.0±8.91</td>
<td>146.33±14.56*</td>
<td>...</td>
</tr>
<tr>
<td>Nonstressed</td>
<td>173.63±9.98</td>
<td>144.58±15.34*</td>
<td>...</td>
</tr>
<tr>
<td>Relative LDF, % baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stressed</td>
<td>100%</td>
<td>15.93±3.04*</td>
<td>103.4±4.61</td>
</tr>
<tr>
<td>Nonstressed</td>
<td>100%</td>
<td>10.8±1.06*</td>
<td>95.8±3.00</td>
</tr>
</tbody>
</table>

MABP indicates mean arterial blood pressure. Values are mean±SEM at baseline, 45 minutes of ischemia, and 30 minutes of reperfusion.

\(^*P<0.05,\) significantly different from preischemic values.
between preischemic passive avoidance training and postischemic testing (4 days in the present study versus 15 days in the previous study). Thus, it appears that animals with mild to moderate ischemic injury retain the passive avoidance task approximately as well as animals in the sham group over short, but not long, time periods. We also have previously shown that ischemic animals with a similar level of injury were capable of learning the passive avoidance task but that they required more training than did animals in the sham group. Additional factors that may account for the discrepancy in results between our 2 studies are (1) a longer shock duration in the present study and (2) use of a step-through rather than step-down apparatus to assess passive avoidance.

In conclusion, these data with “induced stroke” provide evidence that a negative social environment can adversely affect cerebrovascular health and suggest that the underlying mechanism involves increased activation of the HPA axis. The effects of stress on behavioral and histological outcome from vascular occlusion can be reproduced by exogenous corticosterone treatment and ameliorated by pretreatment with a glucocorticoid antagonist. The deleterious consequence of eliciting a glucocorticoid-mediated stress response is present regardless of whether exposure occurs several days before, during, or immediately after injury. Lastly, the stress response in mouse produces functionally significant behavioral deficits in poststroke recovery.

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