Estrogen-induced recovery of autonomic function after middle cerebral artery occlusion in male rats

TAREK M. SALEH,1,2 ALASTAIR E. CRIBB,1,2,3 AND BARRY J. CONNELL1
1Department of Anatomy and Physiology, 2Laboratory of Comparative Pharmacogenetics, Atlantic Veterinary College, 3Department of Comparative Pharmacogenetics, Atlantic Veterinary College, Prince Edward Island Health Research Institute, University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada C1A 4P3
Received 23 April 2001; accepted in final form 3 July 2001

Estrogen-induced recovery of autonomic function after middle cerebral artery occlusion in male rats. Am J Physiol Regulatory Integrative Comp Physiol 281: R1531–R1539, 2001.—Several studies have provided evidence to suggest that estrogen results in a significant reduction (~50%) in the size of the ischemic zone in the middle cerebral artery occlusion (MCAO) model of stroke in a rat. The current study was done to demonstrate whether this estrogen-induced reduction in infarct size is associated with normalization of the autonomic dysfunction observed in an acute model of stroke in male rats. Experiments were done in anesthetized (thiobutabarbital sodium; 100 mg/kg) male Sprague-Dawley rats instrumented to record baseline and reflex changes in cardiovascular and autonomic parameters. Estrogen was intravenously administered 30 min before, immediately before, or 30 min after MCAO. Estrogen administration resulted in a recovery of autonomic function and prevented the detrimental changes in autonomic tone observed following a stroke. In addition, infarct size was significantly increased in the presence of the estrogen antagonistICI-182,780. These results suggest that both pre- or poststroke estrogen administration prevents or reverses acute stroke-induced autonomic dysfunction and that endogenous estrogen levels in males can contribute to this neuroprotection.

sympathetic; parasympathetic; baroreflex sensitivity; ICI-182,780

Clinically, elevated plasma norepinephrine levels (sympathoexcitation) resulting in cardiac myocyte lysis and abnormal electrocardiogram changes (S-T depression, prolongation of the Q-T interval, T-wave inversion, and premature ventricular beats and ventricular arrhythmias) have been observed within 1–2 h following thrombotic or hemorrhagic stroke (24, 26), but typically return to normal after 4–6 h. The risk of the autonomic dysfunction leading to sudden cardiac death in these patients is only significant for the first 1–2 h after a stroke and decreases almost exponentially with time (24, 26). In the majority of stroke patients, in which autonomic and cardiac changes are observed, they are usually the result of an occlusion of the middle cerebral artery (MCA; 24, 26). The autonomic dysfunction seen within 1–2 h of a stroke in the clinical scenario is mimicked by MCA occlusion in the rat and provides an acute stroke model for studying autonomic dysfunction (5). The MCAO model of acute stroke results in a focal ischemia primarily involving the insular cortex (5). The insular cortex is a region of the prefrontal cortex that is involved in the integration of cardiovascular information and autonomic responses (33). Acute cerebral infarction involving the right insular cortex in both humans and experimental animals has been associated with the greatest autonomic disturbances (sympathoexcitation) leading to arrhythmogenesis and possibly sudden cardiac death (24, 26). Such evidence implicates the insula in mediating the sympathoexcitation leading to cardiac arrhythmias in the acute phase following MCAO.

Recent work in vivo has demonstrated that estrogen reduces the size of an ischemia-induced infarct following MCAO in both female (34) and male (14, 44) rats. The magnitude of the initial infarct was also observed to be gender dependent with males and ovariectomized female rats having larger lesion sizes compared with intact female rats (1). Additional evidence of a gender difference was shown following estrogen supplementation of ovariectomized female rats, which reduced the size of the MCAO-induced infarct (41).

Recent work in our laboratory has demonstrated that direct microinjection of estrogen into various autonomic regulatory nuclei in the rat brain produces profound effects on autonomic tone and cardiovascular reflexes and protects against autonomic dysfunction (sympathoexcitation; 35, 36). In addition, our laboratory demonstrated that estrogen enhanced the cardiac baroreceptor sensitivity (BRS). Testing of the BRS is a diagnostic tool that provides researchers and clinicians insight into the autonomic status of a subject (3, 4, 9). Sympathoexcitation and a depressed BRS have been closely associated with the arrhythmogenesis and sudden cardiac death that occurs following cardiovascular and cerebrovascular insults (8, 17, 29, 30, 25). Because parasympathetic tone is generally antifibrillatory and

Address for reprint requests and other correspondence: T. M. Saleh, Dept. of Anatomy and Physiology, Atlantic Veterinary College, Univ. of Prince Edward Island, Charlottetown, P.E.I., Canada C1A 4P3 (E-mail: tsaleh@upei.ca).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
sympathetic tone induces fibrillation (8), a shift in the sympathovagal balance toward the parasympathetic side (reflected by an enhanced BRS) protects against lethal arrhythmia and sudden death (25, 32). Thus the effects of estrogen at both the site of the infarct and at extracortical, autonomic regulatory nuclei may be important in determining its role in the autonomic dysfunction that occurs in acute MCAO.

Although much attention has been focused on the neuroprotective ability of estrogen to reduce stroke-induced infarct size in a variety of animal models (permanent and transient vascular occlusions, etc), no study has yet determined whether this increased cell survival correlates with a recovery of function of the cells in the infarct zone or penumbra. Therefore, the present study was designed to determine whether estrogen could prevent or reverse the autonomic dysfunction observed in the acute phase following MCAO in male rats. In addition, we tested the hypothesis that a window of opportunity exists for estrogen treatment to induce a recovery of function (autonomic regulation) of cells in the ischemic zone. This was done by administering estrogen either 30 min before, immediately before, or 30 min after MCAO while recording both baseline and reflex changes in cardiovascular and autonomic functions. In addition, infarct area was measured to correlate changes in the infarct size to the stroke-induced autonomic outcome in rats receiving estrogen before or after MCAO.

MATERIALS AND METHODS

All experiments were carried out in accordance with the guidelines of the Canadian Council on Animal Care and were approved by the University of Prince Edward Island Animal Care Committee.

General surgical procedures. Experiments were performed on 46 Sprague-Dawley male rats (Charles River; Montreal, Canada) weighing 200–250 g. For all animals, food and tap water were available ad libitum. Rats were anesthetized with thioentalorbarbitol sodium (Inactin, 100 mg/kg ip; RBI, Natick, MA), which provided a stable plane of anesthesia during the experiment (no animals required anesthetic supplementation). A polyethylene catheter (PE-50; Clay Adams; Parsippany, NJ) was inserted into the right femoral artery to monitor blood pressure and heart rate, and a second catheter (PE-10) was inserted into the right femoral vein for the intravenous administration of drugs. Arterial blood pressure was measured with a pressure transducer (Gould P23 ID; Cleveland, OH) connected to a Gould model 2200S polygraph. Heart rate was determined from the pulse pressure using a Gould tachograph (Biotach). These parameters were displayed and analyzed using PolyviewPro 32 data-acquisition software (Grass; Warwick, RI). An endotracheal tube was inserted, and animals were artificially ventilated with room air (66 strokes/min; 2.5 ml tidal volume; Harvard rodent ventilator) and paralyzed with decamethonium bromide (0.5 mg/kg iv; Sigma-Aldrich; St. Louis, MO). Body temperature was monitored with a digital rectal thermometer and maintained at 36.5 ± 0.5°C.

Autonomic nerve isolation and recording. All animals were instrumented to record changes in efferent parasympathetic and sympathetic nerve activities. To record efferent parasympathetic nerve activity, the left cervical vagus nerve was isolated through a midline cervical incision, placed on bipolar platinum recording electrodes, crushed distally, and secured in place with dental impression material (Basilix; Ash Temple, Bedford, NS). To record efferent sympathetic nerve activity, the right kidney was exposed through a retroperitoneal incision. With the aid of an operating stereomicroscope, a renal nerve branch was isolated from the surrounding tissue and a bipolar platinum recording electrode secured in place. The multunit vagus and renal nerve activities were amplified by a Grass model P55 preamplifier with a 100-Hz to 3-kHz bandpass and 60-Hz notch filter and displayed and analyzed using the PolyviewPro 32 data-acquisition and analysis software. Animals were allowed to stabilize for 30 min following nerve isolation before drug injection or nerve activity measurements.

Middle cerebral artery occlusions. All animals were placed in a David Kopf (Tujunga, CA) stereotaxic frame, and the right middle cerebral artery (MCA) was approached through a rostrocaudal incision in the skin and frontalis muscle at the level of bregma. The frontalis and temporalis muscles were then reflected anteriorly and posteriorly to expose the squamosal bone to the point where the zygoma fuses to the squamosal bone. A hand-held drill was used to make a burr hole in the rostro-dorsal part of the squamosal bone, and the squamosal bone was removed to expose the MCA. The bent tip of a 25-gauge hypodermic needle was used to retract the meninges over the MCA. The MCA was permanently occluded using bipolar electrical coagulation (Cameron Miller; Chicago, IL) at three points. The first occlusion was made just dorsal to the rhinal fissure, the second occlusion was made just ventral to the bifurcation of the MCA to the frontal and parietal cortices, and the third occlusion was made just before the bifurcation of the MCA to the parietal cortex. For the sham occlusion group, all surgical procedures described above were performed except the MCA was not occluded.

Baroreflex testing, autonomic tone measurement, and drug injections. To determine the effect of MCA occlusion on the reflexive changes in heart rate in baroreceptor activation, the baroreceptor reflex was evoked using bolus intravenous injections of the α-adrenergic receptor agonist phenylephrine hydrochloride (PE; Sigma-Aldrich, n = 38). The peak amplitude of the resulting pressor and reflex bradycardia responses evoked by increasing doses of PE (0.025, 0.05, and 0.1 mg/kg) were plotted against each other. Regression lines were obtained by the least-squares method, and the slopes were used to provide an index of BRS. The slopes of the BRS curves and both parasympathetic and sympathetic tone were determined 20 min before and 30, 60, 90, 120, 180, and 240 min after either MCA occlusion (n = 6) or sham (n = 4) treatment.

Four groups of animals (n = 4 animals/group) were administered intravenous bolus injections of 17β-estradiol (1 × 10⁻³ mg/kg; injection volume = 0.2 ml; Sigma-Aldrich), 30 min before, immediately before, or 30 min after MCA occlusion or a sham occlusion. This dose of estrogen has been previously demonstrated from a dose-response relationship to produce optimal changes in cardiovascular and autonomic parameters in both female and male rats (37, 38, 39) and to completely block sympathoexcitation in a rat model of sympathetic hyperreflexia (39). In these four groups, the slopes of the BRS curves and changes in autonomic tones were determined as described above, except that in the two groups that received estrogen 30 min before occlusion or sham, additional measurements were made 10 min before estrogen administration.
To determine the specificity of the estrogen-induced effects on BRS and autonomic tone, the selective estrogen receptor antagonist ICI-182,780 (5 mg/kg; 0.2 ml; 0.9% saline and 0.03% ethanol; Tocris; Bowlin, MO; Ref. 16) was injected intravenously 10 min before estrogen (n = 4 animals/group) or 40 min before either MCA occlusion or sham operation with no estrogen administration (n = 4 animals/group). This dose of ICI-182,780 has been previously shown (37, 39) to completely block the cardiovascular and autonomic effects of the optimal dose of estrogen (1 × 10^{-2} mg/kg). BRS and autonomic tone was measured as above except that in the three groups where ICI-182,780 was administered 40 min before either occlusion (with or without estrogen) or sham, additional measurements were made 10 min before and 10 min after ICI-182,780 injections.

**Histological procedures.** Four hours after sham or MCA occlusion, the animals were transcardially perfused with phosphate-buffered saline (0.1 M; 200 ml), and the brains were removed and sliced into 1-mm coronal sections using a rat brain matrix (Harvard Apparatus). Sections were then incubated in a 2% solution of 2,3,5-triphenol tetrazolium chloride (TTC; Sigma-Aldrich) for 10 min and stored in 10% formalin. Infarct size was determined using the protocol established by Mathews and colleagues (19). Briefly, digital photographs of each section were taken to quantify infarct area using a computer-assisted imaging system (Bioquant; R & M Biometrics). After calibration of the image analysis program, the region of interest (prefrontal cortex beginning 0.2 mm rostral to Bregma) was outlined, and the infarct area was calculated as a percentage of the total ipsilateral hemisphere area.

**Data analysis.** All data are presented as means ± SE and were analyzed by a two-way ANOVA for repeated measures followed by a Student-Newman-Keuls post hoc analysis (SigmaStat and SigmaPlot, Jandel Scientific). Statistical comparison of multiple regression line slopes was carried out with analysis of covariance (ANCOVA). Differences in baseline or peak changes in renal or vagal nerve activity were determined using both an ANOVA and a Student t-test. In all cases, differences were considered significant if P < 0.05.

**RESULTS**

For all animals (n = 46), rectal temperature was maintained throughout the experiment at 36.5 ± 0.5°C. Arterial blood gases were measured in three animals from samples taken before occlusion of the MCA and at the end of the experiment (before death). No significant changes in pH (7.4 ± 0.1), arterial PaCO_2 (PaCO_2) (39.2 ± 0.4 mmHg), arterial PaO_2 (PaO_2) (103 ± 6 mmHg), or oxygen saturation (97.8 ± 0.5%) were measured between these two times (P > 0.05). No significant baseline or reflex cardiovascular or autonomic changes were observed in sham-operated rats receiving ICI-182,780 (n = 6; Table 1; P > 0.05). All cardiovascular and autonomic tone changes in sham-operated rats receiving estrogen (n = 6) are depicted in Table 1.

**Effect of stroke and drug treatments on baseline mean arterial pressure and heart rate.** In the period before MCAO, the mean arterial pressures and heart rates of all groups were not significantly different from each other (n = 34; P > 0.05). MCAO had no significant effect on either mean arterial pressure or heart rate at any time point compared with baseline measurements made before the MCAO (n = 4; Figs. 1 and 2) or compared with sham-operated animals (n = 12) at any time point throughout the experiment (Fig. 2 and Table 1; P > 0.05). In addition, mean arterial pressure and heart rate remained unchanged compared with baseline in the MCAO group treated with either estrogen (n = 4; Figs. 1 and 2; P > 0.05) or ICI-182,780 (n = 4; Table 2; P > 0.05).

**Effect of stroke and drug treatments on baseline autonomic tone.** At all time points measured following MCAO, renal sympathetic nerve activity (RSNA) was significantly elevated from a baseline value of 4.1 ± 0.2

| Table 1. Sham operation and baseline cardiovascular and autonomic measurements |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **MAP, mmHg** |
| 30 min | 60 min | 90 min | 120 min | 180 min | 240 min |
| Sham | 94 ± 11 | 97 ± 10 | 94 ± 11 | 100 ± 11 | 100 ± 7 | 96 ± 7 | 94 ± 6 |
| Sham + E | 90 ± 11 | 77 ± 10 | 79 ± 13 | 80 ± 14 | 85 ± 11 | 80 ± 10 | 79 ± 9 |
| Sham + ICI | 101 ± 6 | 112 ± 9 | 118 ± 10 | 113 ± 8 | 114 ± 6 | 96 ± 7 | 94 ± 11 |
| **HR, beats/min** |
| Sham | 335 ± 14 | 361 ± 24 | 336 ± 12 | 345 ± 21 | 347 ± 14 | 368 ± 41 | 346 ± 12 |
| Sham + E | 339 ± 17 | 319 ± 15 | 319 ± 11 | 319 ± 10 | 349 ± 12 | 339 ± 10 | 335 ± 9 |
| Sham + ICI | 360 ± 13 | 370 ± 19 | 377 ± 10 | 370 ± 20 | 390 ± 4 | 404 ± 10 | 428 ± 6 |
| **VPNA, μV/s** |
| Sham | 4.1 ± 0.2 | 4.1 ± 0.3 | 4 ± 0.2 | 4 ± 0.2 | 3.8 ± 0.3 | 3 ± 0.3 | 4 ± 0.4 |
| Sham + E | 5.1 ± 0.4 | 6.2 ± 0.3* | 6.8 ± 0.3* | 6.5 ± 0.3* | 5.5 ± 0.3 | 5.2 ± 0.3 | 5.3 ± 0.3 |
| Sham + ICI | 4.2 ± 0.2 | 3.9 ± 0.2 | 3.9 ± 0.2 | 3.8 ± 0.2 | 3.8 ± 0.2 | 3.8 ± 0.2 | 3.8 ± 0.3 |
| **RSNA, μV/s** |
| Sham | 3.7 ± 0.2 | 4 ± 0.2 | 4 ± 0.2 | 4.1 ± 0.15 | 4 ± 0.3 | 4 ± 0.3 | 4 ± 0.4 |
| Sham + E | 3.7 ± 0.3 | 3.1 ± 0.3 | 3.5 ± 0.3 | 3.7 ± 0.3 | 3.1 ± 0.3 | 3.7 ± 0.3 | 3.7 ± 0.3 |
| Sham + ICI | 4.1 ± 0.1 | 4.1 ± 0.1 | 4 ± 0.2 | 4.1 ± 0.2 | 4 ± 0.05 | 4 ± 0.3 | 4.1 ± 0.2 |
| **BRS, slope** |
| Sham | 0.5 ± 0.04 | 0.5 ± 0.03 | 0.5 ± 0.01 | 0.5 ± 0.04 | 0.5 ± 0.02 | 0.4 ± 0.03 | 0.5 ± 0.05 |
| Sham + E | 0.55 ± 0.1 | 0.8 ± 0.1* | 0.7 ± 0.1* | 0.7 ± 0.1* | 0.6 ± 0.05 | 0.6 ± 0.05 | 0.6 ± 0.05 |
| Sham + ICI | 0.4 ± 0.05 | 0.5 ± 0.05 | 0.5 ± 0.05 | 0.5 ± 0.05 | 0.5 ± 0.05 | 0.5 ± 0.05 | 0.5 ± 0.05 |

Data are expressed as means ± SE (n = 4 per group). MAP, mean arterial pressure; HR, heart rate; VPNA, vagal parasympathetic nerve activity; RSNA, renal sympathetic nerve activity; BRS, baroreflex sensitivity; E, estrogen; ICI, estrogen receptor antagonist ICI-182,780. *Significant difference compared with prestroke (−30 min) value (P < 0.05, ANOVA).
μV/s to a peak value of 7.5 ± 0.2 μV/s 90 min after MCAO (Fig. 3A; \( P < 0.05 \)). Just before the termination of the experiment 4 h post-MCAO, RSNA remained significantly elevated (5.1 ± 0.2 μV/s; Fig. 3A; \( P < 0.05 \)).

Intravenous estrogen administration 30 min before or immediately before MCAO completely blocked the increase in RSNA observed at all time points following MCAO (Fig. 3A; \( P > 0.05 \)). Estrogen administered 30 min after MCAO, during the time in which RSNA values were significantly increased, resulted in a recovery of RSNA levels to prestroke values within 90 min of estrogen injection (Fig. 3A; \( P > 0.05 \) at 120, 180, and 240 min following MCAO). Coinjection of the estrogen receptor antagonist ICI-182,780 with estrogen before or after MCAO completely blocked the estrogen-induced attenuation of the enhanced sympathetic activity (Table 2). In fact, injection of ICI-182,780 only before MCAO resulted in significantly higher RSNA levels than those measured following MCAO alone (Table 2; \( P < 0.05 \)). In addition, in all groups receiving ICI-182,780, the enhanced RSNA level returned to prestroke (baseline) values (Fig. 4), an effect that was not measured in the MCAO group (Fig. 3A).

Compared with baseline (prestroke) values, MCAO resulted in a significant decrease in vagal parasympathetic nerve activity (VPNA) from a baseline value of 4.3 ± 0.2 to 1.9 ± 0.3 μV/s 60 min after MCAO (\( n = 4; P < 0.05 \)) and remained significantly decreased until the end of the experiment (2.7 ± 0.2 μV/s at 240 min post-MCAO; Fig. 3B; \( P < 0.05 \)). Similar to the estrogen-induced effects on RSNA, intravenous estrogen administration 30 min before or immediately before MCAO completely blocked the decrease in VPNA observed at all time points following MCAO (Fig. 3B; \( P > 0.05 \)). Estrogen administered 30 min after MCAO and during the time in which VPNA had begun to decrease resulted in a recovery of VPNA levels to prestroke values within 90 min following estrogen injection (Fig. 3B; \( P > 0.05 \)).
Coinjection of the estrogen receptor antagonist ICI-182,780 with estrogen before or after MCAO completely blocked the ability of estrogen to prevent the decline in VPNA values (Table 2; *P*= 0.05). In contrast to the effect on RSNA, injection of ICI-182,780 only before MCAO did not result in any greater decrease in VPNA following MCAO (Table 2; *P*= 0.05) compared with MCAO alone.

Table 2. Cardiovascular and autonomic baseline measurements following ICI-182,780 or estrogen + ICI-182,780 administered 30 min before MCAO

<table>
<thead>
<tr>
<th></th>
<th>–30 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>180 min</th>
<th>240 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg, MCAO</td>
<td>99 ± 13</td>
<td>95 ± 10</td>
<td>84 ± 12</td>
<td>91 ± 8</td>
<td>85 ± 7</td>
<td>84 ± 8</td>
<td>83 ± 10</td>
</tr>
<tr>
<td></td>
<td>MCAO + ICI</td>
<td>103 ± 11</td>
<td>96 ± 6</td>
<td>100 ± 6</td>
<td>100 ± 9</td>
<td>103 ± 7</td>
<td>98 ± 9</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>MCAO + ICI</td>
<td>103 ± 11</td>
<td>96 ± 6</td>
<td>100 ± 6</td>
<td>100 ± 9</td>
<td>103 ± 7</td>
<td>98 ± 9</td>
</tr>
<tr>
<td></td>
<td>333 ± 20</td>
<td>348 ± 11</td>
<td>359 ± 20</td>
<td>377 ± 26</td>
<td>377 ± 29</td>
<td>360 ± 15</td>
<td>365 ± 21</td>
</tr>
<tr>
<td></td>
<td>360 ± 20</td>
<td>379 ± 20</td>
<td>370 ± 18</td>
<td>376 ± 9</td>
<td>372 ± 9</td>
<td>370 ± 13</td>
<td>344 ± 18</td>
</tr>
<tr>
<td>VPNA, μV/s, MCAO</td>
<td>3.6 ± 0.5</td>
<td>2.3 ± 0.5</td>
<td>2.4 ± 0.5</td>
<td>2.6 ± 0.2</td>
<td>3 ± 0.2</td>
<td>3.1 ± 0.5</td>
<td>3 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>MCAO + ICI/E</td>
<td>3.5 ± 0.2</td>
<td>3 ± 0.5</td>
<td>2.5 ± 0.4</td>
<td>2.6 ± 0.2</td>
<td>2.6 ± 0.6</td>
<td>3 ± 0.7</td>
</tr>
<tr>
<td>RSNA, μV/s, MCAO</td>
<td>4.6 ± 0.3</td>
<td>6.8 ± 0.8</td>
<td>8.2 ± 0.7</td>
<td>7.9 ± 0.3</td>
<td>6 ± 0.3</td>
<td>4.9 ± 0.3</td>
<td>4.9 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>MCAO + ICI/E</td>
<td>4.9 ± 0.3</td>
<td>5.9 ± 0.5</td>
<td>7.3 ± 0.1</td>
<td>7.1 ± 0.2</td>
<td>5.9 ± 0.2</td>
<td>4.8 ± 0.4</td>
</tr>
<tr>
<td>BRS, slope, MCAO</td>
<td>0.5 ± 0.05</td>
<td>0 ± 0.01</td>
<td>0 ± 0.02</td>
<td>0 ± 0.13</td>
<td>0.15 ± 0.01</td>
<td>0.2 ± 0.05</td>
<td>0.2 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>MCAO + ICI/E</td>
<td>0.5 ± 0.03</td>
<td>0.4 ± 0.05</td>
<td>0.25 ± 0.1</td>
<td>0.25 ± 0.1</td>
<td>0.25 ± 0.05</td>
<td>0.25 ± 0.05</td>
</tr>
</tbody>
</table>

Data are means ± SE; *n* = 4 rats per group. MCAO, middle cerebral artery occlusion. *Significant difference from pre–30 min (–30 min; ANOVA) value.

Effect of stroke and drug treatments on BRS and autonomic function. Within 30 min after MCAO, BRS was significantly depressed (from 0.6 ± 0.05 to 0.4 ± 0.07 beats/min·mmHg⁻¹; Fig 4; *P* < 0.05). This decline in BRS continued and reached a peak value of 0.2 ± 0.05 at 2 h post-MCAO and remained significantly depressed for the remainder of the experiment (Fig 4; *P* < 0.05). Injection of estrogen alone 30 min before MCAO resulted in a significant increase in the BRS 10 min after injection and remained significantly elevated for 3 h after MCAO (Fig 4; *P* < 0.05). A similar finding in sham-operated animals was observed (Table 1; *P* < 0.05). Injection of estrogen immediately before MCAO completely prevented the stroke-induced depression in BRS (Fig 4; *P* > 0.05) associated with MCAO alone. Estrogen injected 30 min after MCAO when the BRS was already significantly depressed, resulted in a re-
covery of the BRS to prestroke values within 90 min of estrogen injection (Fig. 4; \( P > 0.05 \)).

Injection of ICI-182,780 before MCAO resulted in a similar decrease in BRS as observed following MCAO alone (Table 2; \( P > 0.05 \)). Coinjection of ICI-182,780 with estrogen 30 min before or immediately before MCAO blocked the estrogen-induced recovery of the MCAO-induced depression of the BRS (Table 2; \( P < 0.05 \)). When estrogen and ICI-182,780 were coinjected 30 min after MCAO, a more severe depression of BRS (0.05 ± 0.02) was observed (Table 2; \( P < 0.05 \)) compared with MCAO alone (Fig. 5A) and remained significantly lower than prestroke values (\( P < 0.05 \)).

In addition to BRS, reflex changes in autonomic tone (autonomic function) was tested in response to a rise in arterial pressure following phenylephrine injection (data not shown). MCAO resulted in a significant attenuation in the reflex increase in VPNA (35 ± 5%) and reflex decrease in RSNA (25 ± 5%) throughout the experiment compared with sham-operated animals (48 ± 5 and 55 ± 6% respectively; \( P < 0.05 \)). Estrogen administration before or after MCAO resulted in a complete blockade of the attenuated reflex autonomic tone changes observed at all time points. Coinjection of estrogen with ICI-182,780 blocked the estrogen-induced effect on reflex autonomic tone changes following MCAO. Finally, injection of ICI-182,780 alone before MCAO resulted in a significant increase in the infarct area by 50 ± 6% (Fig. 5, A and B; \( P < 0.05 \)).

**DISCUSSION**

The results of the present study demonstrate an estrogen-induced recovery of autonomic function in rats when treated before or after MCAO. Estrogen pretreatment completely blocked the stroke-induced changes in BRS, autonomic tone, and reflex function associated with a reduced infarct size. Whereas estrogen administered 30 min after MCAO was not associated with a reduction in infarct size or prevention of sympathoexcitation and depressed BRS, it was associated with a recovery of function within 90 min of administration. Taken together, these results indicate that estrogen has the potential to improve stroke outcome, which may be independent of an ability to prevent stroke-induced cell death in the insular cortex.

**Estrogen-induced reduction in infarct size.** The exact events that lead from ischemia to cell death are not fully understood. However, convincing evidence supports the suggestion that excitotoxicity follows the hypoxic and hypoglycemic conditions encountered in stroke (18). Estrogen has been shown to protect against

---

**Fig. 5.** A: digital photomicrographs illustrating extent of infract size within prefrontal cortex following MCAO, estrogen pretreatment followed by MCAO (E + MCAO), or ICI-182,780 pretreatment followed by MCAO (ICI + MCAO). B: graphic representation of the percent change in infract size with respect to MCAO (100%) in animals administered estrogen at 30 min before, immediately before, or 30 min after MCAO, estrogen, and ICI-182,780 30 min before the MCAO or ICI-182,780 30 min alone before MCAO. *Significant difference compared with prestroke (−30 min) value (\( P < 0.05 \); ANOVA).
ischemia-induced excitotoxic injury. Earlier in vitro evidence suggested that estrogen protected primary cortical neurons from glutamate toxicity (42). The exact mechanism of this protective effect of estrogen is currently under investigation, including a role in the modulation of bcl-2 expression, promoting the outgrowth of neurites, sprouting of neurons, synaptogenesis, and the expression of neurotrophic factors such as nerve growth factor and nerve growth factor receptors (21, 23, 43). These mechanisms of estrogen-mediated neuroprotection may be involved in reducing the size of the MCAO-induced infarct and may even play a role in the functional recovery of cells in the ischemic zone.

Not only can neuroprotection and infarct size reduction after MCAO be observed after estrogen administration, but regulating the expression of the estrogen-synthesizing enzyme cytochrome P-450 aromatase (CYP19) could also result in similar beneficial effects. Only recently have researchers begun to focus on the anatomic distribution and regulation of aromatase gene expression in the rat brain (31). Recent evidence has been provided to show de novo neurosteroidogenesis in astrocytes, oligodendrocytes, and neurons of the cerebral cortex of the rat brain (48). Cultured astrocytes produce estrogen and express aromatase cytochrome P-450 mRNA (49), and they play a role in estrogen regulation of synaptic plasticity and brain repair (10). In fact, significant increases in aromatase expression by astrocytes has been shown following brain injury (11). This implies that local synthesis within the central nervous system (CNS) nuclei can play a major role in neuroprotection and may even be the source of a “first-line of defense” for neurons in the ischemic and peri-ischemic zones. The present investigation supports this suggestion as the injection of the estrogen receptor antagonist ICI-182,780 at any time before or after MCAO resulted in a significantly larger infarct area. This suggests that endogenous estrogen or estrogen-synthesized de novo in this cortical region must play a significant role in neuroprotection in the male rat brain. In contrast, Sawada and colleagues (40) did not observe a similar finding following administration of ICI-182,780 in male mice. This different result may not only be due to species variability and high dose of ICI-182,780 used (~100-fold greater than that used in the current study), but also due to the use of a chronic stroke model (i.e., infarct sizes were measured 22 h post-MCAO).

It is not a novel finding that estrogen administered before, or even several hours following a stroke, can reduce MCAO-induced infarct size (20, 47) independent of any significant change in cerebral blood flow 24 h postestrogen injection. This is important because estrogen has been shown to have direct vasodilatory effects on both the peripheral and cerebral vasculature (15). It has been speculated therefore that the estrogen-induced reduction in the infarct size is secondary to vasodilation and subsequent enhancement of cerebral blood flow. However, although we did not directly measure cerebral blood flow, many studies have shown that the mechanism by which estrogen provides neuroprotection in forebrain ischemic models is independent of its actions on the vasculature (45, 47). The novel purpose of the present investigation was to correlate this increased cell survival with a functional recovery of the cells in the infarct (or penumbra) zone of the insular cortex. Because the cells in this region are known to regulate autonomic function (33), measuring autonomic tone and autonomic reflexes, such as the BRS, following estrogen-induced neuroprotection provides an indication that the cells protected from ischemic damage are not only viable but can maintain their physiological role. The results of the present investigation support this suggestion but do not indicate where the estrogen is acting in providing this recovery of function, because peripherally administered estrogen has access to all autonomic preganglionic cells in the CNS and, as previously reported (35, 36), can directly enhance sympathovagal balance at these sites.

Estrogen and prevention of stroke-induced autonomic dysfunction. Early evidence suggested that estrogen modulated autonomic tone in the CNS; however, these reports failed to record autonomic tone directly, and their conclusions were based on the result of pharmacological blockade of autonomic postganglionic receptors (2, 12, 22). Our laboratory has previously provided evidence for a role of estrogen in modulating baseline autonomic tone via the CNS (37–39). In ovariectomized female rats, intravenous estrogen injection significantly reduced sympathetic tone within 30 min and significantly increased parasympathetic tone within 5 min of estrogen administration (39). Both effects on autonomic tone were blocked by the prior administration of a potent and selective estrogen receptor antagonist ICI-182,780 (16) into the respective central autonomic preganglionic nuclei (39). Only the estrogen-induced increase in parasympathetic tone was observed in male rats (37, 39). Also, in a vagal stimulation model of autonomic dysfunction, acute estrogen administration before vagal stimulation in both male and saline-replaced female rats blocked the sympathoexcitation and depressed BRS observed following termination of stimulation in these animals (37, 39). This evidence describes a role for estrogen under normal circumstances and in preventing visceral afferent activation-induced autonomic dysfunction independent of pathology. The results presented here demonstrate an additional role of estrogen in protecting against the autonomic dysfunction observed in the presence of an underlying cerebrovascular pathology.

Stroke and cardiovascular changes. In the present investigation, MCAO had no measurable effect on mean arterial pressure and heart rate even though sympathetic tone was significantly increased and parasympathetic tone was significantly decreased. This is not a unique finding because several other investigations (1, 6, 13, 46) utilizing a similar protocol were also unable to demonstrate that permanent occlusion of the right MCA had any significant effect on these cardiovascular parameters. For example, in the study by Hachinski and colleagues (13), RSNA and plasma nor-
epinephrine levels were significantly increased independent of a change in mean arterial pressure or heart rate. The studies mentioned above were all performed in anesthetized rats, and whereas this may be a limitation of the model, no correlative evidence exists suggesting that although sympathetic activity is significantly increased following stroke in humans (24), it is not necessarily linked with changes in baseline cardiovascular parameters. Oppenheimer and Martin (27) studied a patient population with a stroke on the left side involving the insular cortex and found no significant difference in their mean arterial pressure compared with controls. Also, Robinson and colleagues (30) found similar findings in his study; however, right-sided stroke in this patient population was associated with a significantly higher heart rate.

Although stroke in humans or rats has not been associated with changes in baseline mean arterial pressure or heart rate, it has been shown to be associated with altered cardiovascular reflex control as measured following activation of the cardiac baroreflex (7, 30). Experimental and clinical evidence suggest that stroke results in a depressed BRS (7, 30). Our results support this finding and indicate that the depressed BRS is due to an increase in sympathetic tone and decreased vagal tone. Estrogen administered before the stroke prevented the attenuated BRS, whereas poststroke estrogen treatment reversed this attenuation. In the current study, the depressed BRS was due to either an attenuated reflex activation (parasympathetic) or inhibition (sympathetic) of autonomic tone in response to an increase in arterial pressure.

In conclusion, the above results strongly suggest a protective effect of estrogen when given before MCAO as well as an ability to reverse autonomic and cardiovascular reflex dysfunction observed following an acute stroke. Although the results presented here are in an anesthetized animal model of acute stroke, we demonstrate that a window of opportunity exists for estrogen therapy in the prevention of autonomic dysfunction. Another interesting finding of this study was the fact that endogenous estrogen may provide a level of protection against the extent of cell death following MCAO in male rats. It remains to be determined, however, whether de novo synthesis of estrogen locally within the CNS or peripheral circulating estrogen is responsible for this protection. Additional investigations are also required to determine the extracortical site of estrogen action within the CNS in providing protection against stroke-induced autonomic dysfunction.

**Perspectives**

The goal of this study was to provide a better understanding of the functional consequences of estrogen-induced neuroprotection in the brain following MCAO in an animal model. This information will greatly help us understand if the key to neuroprotection is in reducing the lesion size or if the protection against functional deficits can be found in other CNS sites. Current studies in our lab are aimed at increasing CNS levels of estrogen in specific autonomic regulatory nuclei before MCAO in an attempt to provide insight into this question. We believe that endogenous estrogen production in central cardiovascular regulatory nuclei may play an important role in neuroprotection and may even lead to the development of new drugs (such as aromatase inducers) that help regulate the production of estrogen in the brain.

This work was supported by Grant 615122 to T. M. Saleh from the Heart & Stroke Foundation of Prince Edward Island. A. E. Cribb is a Scholar of the Canadian Institute for Health Research.

**REFERENCES**


43. Toran-Allerand CD. The estrogen/neurotrophin connection during neural development: is co-localization of estrogen receptors with the neurotrophins and their receptors biologically relevant? Dev Neurosci 18: 36–48, 1996.


