Estrogen status affects sensitivity to focal cerebral ischemia in stroke-prone spontaneously hypertensive rats

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Estrogen status affects sensitivity to focal cerebral ischemia in stroke-prone spontaneously hypertensive rats. Am. J. Physiol. Heart Circ. Physiol. 278: H290–H294, 2000.—Estrogen treatment has been shown to reduce ischemic brain damage. Because endogenous estrogen levels fluctuate markedly during the estrus cycle, we investigated the effect of stage of estrus cycle on ischemic brain damage. Halothane anesthetized 3- to 5-mo-old female Wistar-Kyoto rats (WKY) and stroke-prone spontaneously hypertensive rats (SHRSP) in proestrus (high estradiol levels) or metestrus (low estradiol levels) underwent permanent middle cerebral artery occlusion. In SHRSP, infarct volume at 24 h postocclusion was 24% smaller in proestrus compared with metestrus [208.6 ± 9.5 mm³ (n = 7) vs. 272.7 ± 23.8 mm³ (n = 7), respectively, means ± SE; P = 0.0278, unpaired t-test]. In WKY, infarct volumes were similar in proestrus and metestrus [157.0 ± 5.4 mm³ (n = 5) and 131.5 ± 16.5 mm³ (n = 8), respectively; P = not significant (NS)]. Brain swelling (ipsilateral minus contralateral hemispheric volumes) was similar in proestrus and metestrus for SHRSP [138 ± 9 mm³ (n = 6) and 136 ± 10 mm³ (n = 7), respectively] and for WKY [103 ± 15 mm³ (n = 5) and 90 ± 11 mm³ (n = 8), respectively]. Thus the reduction in infarct size in SHRSP is caused by a true attenuation of the infarct volume and not simply by a reduction in brain edema.

METHODS

All experiments were carried out under a project license from the British Home Office and were subject to the Animals (Scientific Procedures) Act, 1986. Breeding and housing of SHRSP and WKY was described previously (5). Two groups were used in each strain: females were either in metestrus (WKY, n = 8; SHRSP, n = 7) or in proestrus (WKY, n = 5; SHRSP, n = 7) at the time of middle cerebral artery (MCA) occlusion (MCAO). Vaginal smears were stained with methylene blue, and the types of cells present were examined under a light microscope to determine the cycle stage (2). Smear results were verified in a separate series of female WKY by radioimmunoassay of plasma estradiol levels and were found to be 15 pg/ml in metestrus and 167 pg/ml in proestrus.

All rats were 3–5 mo old at the time of MCAO. Anesthesia was induced by 5% halothane in oxygen-nitrous oxide (30:70) and maintained by intubation on a ventilator with 1–2% halothane. A 2-mm distal segment of the left MCA was occluded by electrocoagulation using the technique of Tamura et al. (29) with monitoring of physiological variables throughout the MCAO and at 24 h after MCAO as previously described (3). A temperature probe inserted into the temporalis muscle was used to assess brain temperature throughout the experiment.

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the surgical procedure. Twenty-four hours after MCAO, coronal brain sections were stained with hematoxylin-eosin for measurement of infarct size and brain swelling by image analysis (MCID, Imaging Research, St. Catherines, ON, Canada) as described previously (3). Briefly, the volume of infarction and brain swelling for each brain was derived from integration of areas of damage and hemisphere, respectively, over eight coronal levels with end points of 12.5 mm anterior and 0.05 mm posterior to the interaural line (20). Brain swelling was calculated as the difference in volume of the ipsilateral and contralateral hemispheres. Data are presented as means ± SE, and Student’s unpaired t-test (2-tailed) was employed for statistical analysis.

RESULTS

Physiological parameters. Table 1 illustrates the physiological parameters for the experiments in the present study. All physiological variables were maintained within normal limits under anesthesia. Systolic blood pressure (measured pre-MCAO by tail cuff) and both anesthetized and conscious (24 h post-MCAO) mean arterial blood pressures (MAP) were significantly higher in SHRSP than in WKY, as expected.

Ischemic damage. Figure 1 illustrates significantly smaller infarct areas in SHRSP in proestrus compared

<p>| Table 1. Physiological parameters for female WKY and SHRSP in metestrus and proestrus |
|---------------------------------|-----------------|-----------|----------------|----------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Body Wt, g</th>
<th>Age, mo</th>
<th>SBP, mmHg</th>
<th>MAP(a), mmHg</th>
<th>MAP(b), mmHg</th>
<th>PaCO₂, mmHg</th>
<th>Brain Temp, °C</th>
<th>Body Temp, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY met</td>
<td>8</td>
<td>222 ± 8</td>
<td>3.98 ± 0.34</td>
<td>133 ± 5</td>
<td>134 ± 8</td>
<td>92.6 ± 3.3</td>
<td>37 ± 1.6</td>
<td>37 ± 0.3</td>
<td>37 ± 0.1</td>
</tr>
<tr>
<td>WKY pro</td>
<td>5</td>
<td>215 ± 5</td>
<td>3.45 ± 0.1</td>
<td>123 ± 4</td>
<td>124 ± 1</td>
<td>87.6 ± 6.9</td>
<td>38 ± 4.3</td>
<td>36 ± 0.4</td>
<td>37 ± 0.2</td>
</tr>
<tr>
<td>SHRSP met</td>
<td>7</td>
<td>192 ± 6</td>
<td>4.27 ± 0.35</td>
<td>155 ± 7*</td>
<td>164 ± 10*</td>
<td>124.4 ± 6.4</td>
<td>39 ± 2.4</td>
<td>38 ± 0.6</td>
<td>37 ± 2.0</td>
</tr>
<tr>
<td>SHRSP pro</td>
<td>7</td>
<td>194 ± 3</td>
<td>3.91 ± 0.16</td>
<td>146 ± 4†</td>
<td>172 ± 10†</td>
<td>102.1 ± 6.2</td>
<td>44 ± 3.6</td>
<td>37 ± 0.3</td>
<td>37 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no of rats. WKY, Wistar-Kyoto rats; SHRSP, stroke-prone spontaneously hypertensive rats; met, metestrus; pro, proestrus; SBP, conscious systolic blood pressure measured by tail cuff before middle cerebral artery occlusion (MCAO); MAP(a), mean arterial blood pressure 24 h after MCAO (conscious); PaCO₂, arterial PCO₂, MAP(b), PaCO₂, and brain and body temperature were measured at time of MCAO (under anesthesia). *P < 0.05, †P < 0.005 vs. WKY in same estrous state (unpaired t-test).

Fig. 1. Areas of infarct (mm²) over 8 coronal planes during metestrus and proestrus in stroke-prone spontaneously hypertensive rats (SHRSP; A) and Wistar-Kyoto rats (WKY; B). Areas of infarct were significantly different between metestrus and proestrous in 5 of the 8 coronal planes examined in SHRSP (*P < 0.05 vs. metestrus at that coronal plane).
with metestrus over five of the eight coronal levels measured. Integration of these areas of damage gave rise to an infarct volume of $208.6 \pm 9.5\, \text{mm}^3$ in proestrus ($n=7$) and $272.7 \pm 23.8\, \text{mm}^3$ in metestrus ($n=7$) in SHRSP ($P=0.028$). There was no significant difference in areas of ischemic damage between proestrus and metestrus in WKY. The volumes of infarction in proestrus ($157.0 \pm 5.4\, \text{mm}^3$, $n=5$) and in metestrus ($131.5 \pm 16.5\, \text{mm}^3$, $n=8$) for WKY were not significantly different ($P=0.26$).

Brain swelling. Brain swelling, measured at the eight coronal levels, is displayed in Fig. 2 for SHRSP and WKY. In SHRSP a significant difference between metestrus and proestrus was apparent at only one coronal level, and the total volume of brain swelling in proestrus ($138 \pm 9\, \text{mm}^3$, $n=6$) was not significantly different from that in metestrus ($136 \pm 10\, \text{mm}^3$, $n=7$) (Fig. 2A). Similarly, in WKY, although the area of brain swelling at one coronal level was significantly higher in proestrus than in metestrus (Fig. 2B), the volume of brain swelling was not significantly different between the two cycle stages [$103 \pm 15\, \text{mm}^3$ ($n=5$) in proestrus and $90 \pm 11\, \text{mm}^3$ ($n=8$) in metestrus]. A significant correlation was found between brain swelling and infarct volume in WKY ($r^2=0.39$, $P=0.02$), but correlation failed to attain significance in SHRSP ($r^2=0.2$, $P=0.13$).

DISCUSSION

In a previous study from our group (3), female rats that underwent MCAO in metestrus exhibited greater ischemic damage than rats taken at random in the estrous cycle. The female rats used in that study were the first filial generation produced by crossing SHRSP and WKY and were shown to inherit increased stroke sensitivity from SHRSP (3). The present study illustrates that SHRSP in proestrus exhibit a 24% smaller infarct size compared with SHRSP in metestrus. There was no significant difference in the volume of brain swelling between metestrus and proestrus and no significant correlation between brain swelling and infarct volume in SHRSP. Thus the difference in infarct size between proestrus and metestrus in SHRSP was not simply due to an effect of estrogen on brain swelling. The present results reinforce our previous findings that estrous state can affect the neuropathological outcome of experimental stroke in stroke-sensitive rats, identifying proestrus as a beneficial stage in the estrous cycle.

The magnitude of the difference in infarct size between proestrus and metestrus in SHRSP is comparable with that seen in estrogen-treated and vehicle-treated ovariectomized rats in other studies (8, 25, 31). Examining endogenous hormonal effects by cycle stage...
avoids nonphysiological effects associated with ovariectomy, such as depletion of circulating progesterone. Estrogen and/or progesterone could be responsible for the neuroprotection in proestrus. However, the bulk of current evidence in the literature would favor estrogen. Progesterone has been reported to reduce neuronal damage caused by global ischemia in ovariectomized cats (11) but not following MCAO in ovariectomized rats (21). To date, protective properties of progesterone after MCAO in rats have only been demonstrated in males (18). Given that estradiol treatment wholly reverses the effects of ovariectomy on ischemic damage (35), we propose that estrogen rather than progesterone is the major hormone responsible for protection during proestrus.

Estrogen participates in many cerebral events that may contribute to neuroprotection. Examples include dilatation of cerebral vessels (24), reduction in free radical-induced lipid peroxidation and glutamate toxicity in cell cultures (12), and alteration of the balance between proapoptotic and antiapoptotic genes (15). Other properties of estrogen include modulation of proteins involved in synaptic plasticity (26) and microtubule stability (9), increased expression of neurotrophins (27), and promotion of neurite outgrowth (30). Moreover, estrogen has been shown to directly inhibit N-methyl-D-aspartate receptors (32) and to have anti-inflammatory properties (28). The order of importance of each of these mechanisms in reducing ischemic damage is currently unclear.

On considering the vasodilatory effects of estrogen during cerebral ischemia, several studies demonstrated estrogen-induced cerebral blood flow (CBF) changes (16, 24), whereas a number of others found no evidence for a direct influence on CBF (8, 25). In addition, no change in basal CBF is apparent after ovariectomy (14). We recently demonstrated (4) that the severity and topographical extent of ischemia after MCAO was not different between SHRSP in proestrus and SHRSP in metestrus. This would suggest that flow-independent mechanisms are responsible for the protection against ischemic damage seen in proestrus in the SHRSP.

SHRSP exhibit larger infarcts after an experimental stroke insult than their normotensive counterparts, the WKY (3, 7). Several reasons for this increased stroke sensitivity have been proposed. The ischemia is more severe (6) and glutamate release is higher (10) in SHRSP compared with normotensive rats. In addition, SHRSP have lower superoxide dismutase levels and activity (19), higher levels of lipid peroxidation (17), and an enhanced lipopolysaccharide-induced inflammatory response (13) compared with normotensive rats. It is therefore conceivable that SHRSP may derive greater benefit than WKY from the antioxidant and/or anti-inflammatory effects of estrogen. This may explain why there is a difference in infarct size between metestrus and proestrus in SHRSP but not in WKY. An alternative explanation for the lack of difference in WKY is that SHRSP may require high circulating levels of estrogen (proestrus) to induce protection that is already maximal in metestrus in WKY.

In summary, our results clearly demonstrate that estrous status affects ischemic damage in stroke-sensitive rats. We propose that plasma estrogen levels are responsible for this effect and that flow-independent mechanisms are involved in the neuroprotection.


This work was supported by the Wellcome Trust (Grant 057306/99), the British Heart Foundation (Grant RG97009), the Cunningham Trust (Grant 97/2), and the Robertson Trust.

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Received 7 September 1999; accepted in final form 18 October 1999.

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