

Gender-Linked Brain Injury in Experimental Stroke

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Background and Purpose—Premenopausal women are at lower risk than men for stroke, but the comparative vulnerability to tissue injury once a cerebrovascular incident occurs is unknown. We hypothesized that female rats sustain less brain damage than males during experimental focal ischemia and that the gender difference in ischemic outcome can be eliminated by ovariectomy.

Methods—Age-matched male (M), intact female (F), and ovariectomized female (O; plasma estradiol: 4.1 ± 1.6 pg/mL compared with 7.4 ± 1.5 in F and 4.0 ± 1.1 in M) rats from two different strains, normotensive Wistar and stroke-prone spontaneously hypertensive rats, were subjected to 2 hours of intraluminal middle cerebral artery occlusion, followed by 22 hours of reperfusion. Cerebral blood flow (CBF) was monitored throughout the ischemic period by laser-Doppler flowmetry. Infarction volume in the cerebral cortex (Ctx) and caudoputamen (CP) was determined by 2,3,5-triphenyl-tetrazolium chloride staining. In a separate cohort of M, F, and O Wistar rats, absolute rates of regional CBF were measured at the end of the ischemic period by quantitative autoradiography using [14 C]iodoantipyrine.

Results—F rats of either strain had a smaller infarct size in Ctx and CP and a higher laser-Doppler flow during ischemia compared with respective M and O rats. Mean end-ischemic CBF was higher in F compared with M and O rats in CP, but not in Ctx. Cerebrocortical tissue volume with end-ischemic CBF <10 mL/100 g/min was smaller in F than M rats, but not different from O rats.

Conclusions—We conclude that endogenous estrogen improves stroke outcome during vascular occlusion by exerting both neuroprotective and flow-preserving effects. (*Stroke*. 1998;29:159-166.)

Key Words: cerebral blood flow ■ estrogens ■ stroke, experimental ■ gender ■ neuroprotection

The risk of stroke is lower in premenopausal women relative to men of the same age,^{1,2} but the incidence of cerebrovascular events rapidly increases in women after menopause.³ Historically, these epidemiological findings have been attributed to estrogen, in part due to the beneficial effect of the hormone in preventing coronary heart disease.⁴ However, the comparative vulnerability of females and males to tissue injury once stroke is ongoing remains understudied. There is some evidence for gender-specific responses to experimental ischemia. Female gerbils have lower incidence of and less severe brain lesions after carotid occlusion compared with males.⁵ Furthermore, thromboembolism induced by photochemical irradiation of the carotid artery differentially affects male and female rats, producing greater inflammatory responses, but less severe infarcts, in female than in male rats.⁶ There is also evidence for gender-specific responses to other types of brain injury, such as cerebral contusion,⁷ hypoxia,⁸ and drug-induced toxicity.⁹

A growing body of evidence indicates that estrogen has multiple vascular effects,^{10,11} all of which could contribute to salvage of neural tissue during ischemic episodes. Single reports indicate that estrogen increases cerebral perfusion in women

with¹² or without¹³ known cerebrovascular disease. Furthermore, we have previously shown that residual cerebral blood flow during global cerebral ischemia in animals can be aug-

See Editorial Comment, page 166

mented by chronic estradiol treatment.¹⁴ It has been proposed that estrogen has tissue antioxidant properties, which could also contribute to neuroprotection during ischemic episodes.⁵ We now utilize a stroke model of transient focal ischemia in two genetically distinct strains of rat to compare ischemic outcome between males and females and examine the role of endogenous female sex hormones in any gender-specific responses. We also measured regional cerebral blood flow during vascular occlusion to determine the contribution of flow preservation in gender-specific stroke outcome.

Materials and Methods

This study was conducted in accordance with the National Institutes of Health guidelines for the care and use of animals in research and the protocols were approved by the Animal Care and Use Committee of the Johns Hopkins University. Age-matched, adult (13 to 15 weeks) male (M), intact female (F), and ovariectomized female (O) rats from two strains, normotensive Wistar (255 to 360 g of body weight,

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Selected Abbreviations and Acronyms

CBF	= cerebral blood flow
Ctx	= cerebral cortex
CP	= caudoputamen
ER	= estrogen receptor
F	= age-matched female rats
IAP	= [¹⁴ C]iodoantipyrine
LDF	= laser-Doppler flowmetric
LD-CBF	= laser-Doppler flowmetry of cerebral blood flow
M	= male age-matched rats
MAP	= mean arterial blood pressure
MCA	= middle cerebral artery
O	= age-matched female ovariectomized female rats
rCBF	= regional cerebral blood flow
SHR-SP	= stroke-prone spontaneously hypertensive rats
TTC	= 2,3,5-triphenyltetrazolium chloride

n=45) and stroke-prone spontaneously hypertensive rats (SHR-SP, 182 to 282 g, n=32), were studied. SHR-SP rats were included in the study in view of the strain's genetic predisposition to stroke, presence of hypertension, and overall similarities to human stroke.¹⁵ A colony of SHR-SP is maintained in our institution from a stock obtained from the National Institutes of Health (Laboratory Sciences Section, Veterinary Resources Program, National Center for Research Resources, Bethesda, Md).¹⁶

Ovariectomy was performed at the age of 10 to 12 weeks. Briefly, under halothane anesthesia (1% to 2% via snout mask in O₂-enriched air), the ovary was accessed through a lateral abdominal incision, and the ovarian artery and vein were clamped with a fine surgical hemostat and ligated. The ovary was then resected, surgical wounds closed, and the animal allowed to recover for 2 to 4 weeks. On the day of the experiment, rats were anesthetized as above and instrumented with a femoral artery catheter for monitoring arterial blood pressure and measurement of blood gases. Rectal and temporalis muscle temperatures were controlled at 37±0.5°C using heating lamps. An area 2 to 3 mm in diameter was thinned in the right parietal bone 2-mm posterior and 6-mm lateral to the bregma for placement of a LDF probe (Moor Instruments, Ltd., model MBF3D, England), as previously described.¹⁷ To allow for continuous monitoring of LD-CBF, the head piece of a stereotaxic frame was modified to allow for free rotation around the longitudinal axis of the rat and was equipped with a snout mask for ventilation and with a holder for the LDF probe. The probe was positioned during the control period over an area devoid of visible blood vessels, and its position was not changed throughout the experiment. The signal was allowed to stabilize over a 30-minute period before a baseline reading was taken before vascular occlusion.

MCA occlusion was achieved by modifying an established procedure for proximal occlusion of MCA in the rat with an intraluminal filament.^{18–20} Briefly, the right common carotid artery was exposed through a lateral incision, carefully separated from the vagus and ligated. The external carotid artery was ligated, the occipital branch cauterized, and the pterygopalatine artery exposed and ligated. An occluding filament (4–0 monofilament nylon surgical suture with a heat-rounded tip) was advanced into the internal carotid artery until the LDF signal displayed an abrupt and significant reduction (77%±2% from baseline, n=62); the filament was then secured in place. Measurements were made over 5 minute periods at 5, 15, 30, 60, 90, and 120 minutes after occlusion. Withdrawal of the string at the end of the ischemic period (2 hours) was associated with a rapid restoration of flow. LD-CBF reached 66%±7%, 75%±10%, and 90%±6% of baseline by 5 minutes, and 79%±8%, 93%±7%, and 87%±11% of baseline by 15 minutes after withdrawal of the filament in M (n=20), F (n=22), and O (n=20), respectively. After 22 hours of reperfusion, the animal was reanesthetized with 3% halothane, and a blood sample withdrawn for determination of plasma hormone levels. Plasma 17-β-estradiol and progesterone were measured in triplicate by radioimmunoassay, as previously described.¹⁴ The brain was harvested and sliced into seven 2-mm thick coronal sections for

TTC staining, as previously described.¹⁸ Infarction volumes were measured using digital photography and image analysis software (SigmaScan Pro, Jandel). The infarcted area (unstained) was numerically integrated across each section and over the entire ipsilateral hemisphere. Infarct volume was measured separately in the cerebral cortex and caudoputamen and expressed as a percentage of the volume of the ipsilateral side.

End-ischemic rCBF was measured in additional cohorts of Wistar rats using quantitative autoradiography with [¹⁴C]IAP, as described previously.^{21,22} Animals were instrumented with femoral vascular catheters, and the MCA occluded as in the previous cohorts. At 2 hours of MCA occlusion, arterial blood pressure and blood gases were measured, then 40 μCi of [¹⁴C]IAP (New England Nuclear) in 1 mL of isotonic saline were infused intravenously for 45 seconds. During infusion, fifteen 20-μL samples of free-flowing arterial blood from the femoral artery catheter were collected in heparin-coated sample tubes. With the filament still in place and the laser-Doppler indicating ischemic status, the rat was decapitated 45 seconds after the start of infusion. The brain was quickly removed and frozen at -50°C in 2-methylbutane on dry ice. Each brain was cryostatally sectioned into 20-μm-thick coronal sections at -20° and thaw-mounted onto cover glasses. Sections were apposed for 1 week to film (Kodak, SB-5) with ¹⁴C standards. The concentration of [¹⁴C]IAP in blood samples was determined by liquid scintillation spectroscopy (Beckman, model 3801) after decolorization with 0.2 mL of tissue solubilizer (Soluene-350, Packard Instruments Co., Downers Grove, Ill). Autoradiographic images representing three different coronal levels (+2.7, +0.2, and -1.8 mm from the bregma,²³ six to nine images each) were digitized and regional CBF determined using image analysis software (Inquiry, Loats Associates, Westminster, Md). Rates of rCBF were calculated by the Kety-Schmidt modification of the Fick principle:

$$C_{\text{brain}}(T) = K_0 \int_0^T C_{\text{blood}} e^{-K(T-t)} dt$$

where C_{brain} is the concentration of the tracer in the tissue at the time of decapitation (T), C_{blood} is the concentration of the tracer in arterial blood, t is the variable time and K (the transfer coefficient) = $(m) \cdot (rCBF)/\lambda$, where rCBF is the rate of blood flow per unit mass of tissue (mL/100 g/min), m is the diffusion equilibrium constant ($m_{\text{IAP}}=1.0$), and λ is the tissue:blood partition coefficient ($\lambda_{\text{IAP}}=0.992$).

Two methods of analysis were used to determine rCBF. First, CBF was measured by sampling 0.08-mm² squares within those regions most vulnerable to MCA occlusion, the frontal and parietal lobes of the cerebral cortex and the medial and lateral aspects of the caudoputamen complex. Flow rates were then averaged from squares assayed from six to nine consecutive brain slices at each of three coronal levels. In the second method, areas with flow rates below 10 mL/100 g/min were isolated by digital image scanning and perimetrically measured for each slice in the entire ischemic hemisphere and in the cerebral cortex alone. Areas were averaged over six to nine images from each brain level and then were numerically integrated across the three coronal levels to obtain an estimate of tissue volume with severely compromised ischemic blood flow.

All values are reported as means±standard errors of the mean unless otherwise indicated. Physiological parameters were subjected to two-way ANOVA. Differences in infarct size, mean residual laser-Doppler flow and autoradiographic cerebral blood flow among groups were determined with one-way ANOVA. Post hoc comparisons were made with Newman-Keuls test. The relationship between residual LD-CBF and infarct size was examined by regression analysis. The criterion for statistical significance is $P \leq .05$.

Results

Physiological data are summarized in Table 1. The MAP was equivalent in all groups within the same strain and was maintained at values similar to baseline throughout the experimental protocol. Baseline MAP was higher in SHR-SP rats compared with all corresponding groups from the Wistar strain. Baseline arterial pH, PaCO₂, and PaO₂, and hemoglobin and glucose concentrations were equivalent among all groups

Summary of Selected Physiological Variables Before (B), During (D), and After (A) Middle Cerebral Artery Occlusion

Group (n)	MAP (mm Hg)		pH	Paco ₂ (mm Hg)	Pao ₂ (mm Hg)	Hb (g/100 mL)	Glucose (g/100 mL)
	Wistar	SHR-SP					
M							
B (25)	95±2	131±3	7.38±0.01	43±1	136±10	13±0.3	114±07
D (25)	94±3	130±3	7.38±0.01	44±1	124±10	13±0.3	107±05
A (20)	92±3	131±5	7.39±0.01	47±2	134±11	12±0.3	121±09
F							
B (27)	99±3	135±3	7.36±0.01	43±2	130±05	13±0.4	112±05
D (27)	93±3	136±3	7.38±0.01	41±1	131±05	13±0.5	117±10
A (22)	88±2	138±3	7.39±0.01	40±1	124±05	13±0.6	133±14
O							
B (25)	94±2	131±5	7.35±0.02	47±1	134±05	14±0.5	104±04
D (25)	90±3	128±5	7.38±0.01	44±1	128±05	13±0.5	104±03
A (20)	90±2	128±5	7.39±0.01	41±1	139±06	13±0.5	106±04

Values are means±SE. SHR-SP indicates stroke-prone spontaneously hypertensive rats; n, number of rats; M, male; F, female; O, ovariectomized.

and were maintained at values similar to baselines throughout the experimental protocols. Plasma estradiol concentrations were 7.36 ± 1.11 , 3.95 ± 1.46 , and 4.11 ± 1.58 pg/mL, and plasma progesterone concentrations were 27 ± 2.8 , 5.9 ± 0.8 , and 16 ± 3.3 ng/mL in F (n=28), M (n=21), and O groups (n=17), respectively.

Fig 1 summarizes infarction volumes as a percentage of the cerebral cortex or caudoputamen in Wistar rats. Male rats sustained larger infarcts than females both in the cerebral cortex ($25.7\pm6.8\%$ in M compared with $9.4\pm2.9\%$ in F) and caudoputamen ($41.5\pm4.4\%$ in M versus $19.9\pm4.8\%$ in F). Ovariectomized rats sustained injury volumes that were not different from that of males ($30.2\pm6.4\%$ in the cerebral cortex and $42.4\pm4.0\%$ in CP). Fig 2 represents averaged residual laser-Doppler CBF over the 2 hours of MCA occlusion in the same animals. Female rats maintained a higher percentage of baseline LD-CBF during ischemia compared with male and ovariectomized rats ($37.6\pm3.1\%$ in F rats compared with $29.7\pm0.7\%$ and $27.0\pm1.2\%$ in M and O, respectively). Similar differences in infarct size and ischemic flow between genders that are abolished by ovariectomy were also observed in SHR-SP rats. Although SHR-SP rats had a significantly greater infarct size and a more severe reduction in

LD-CBF during ischemia compared with corresponding Wistar groups, treatment- and gender-specific differences in infarct size were similar between the two strains. Fig 3 compares infarct size in the cerebral cortex and caudoputamen among M, F, and O SHR-SP rats. Male SHR-SP rats sustained larger infarcts compared with females both in the cerebral cortex ($53.1\pm4.2\%$ in M versus $35.6\pm3.9\%$ in F) and caudoputamen ($53.9\pm3.6\%$ in M versus $34.1\pm6.5\%$ in F), a difference that was prevented when female rats were ovariectomized at an early age ($46.5\pm4.0\%$ in the cerebral cortex and $51.4\pm4.8\%$ in caudoputamen in O). Fig 4 summarizes averaged residual LD-CBF over the 2 hours of MCA occlusion in SHR-SP rats. In agreement with an inverse relation between level of perfusion and the severity of injury, F rats maintained a higher relative flow during ischemia compared with M and O rats ($18.9\pm1.4\%$ in F compared with $11.6\pm0.5\%$ and $14.8\pm1.5\%$ in M and O, respectively). Fig 5 depicts the correlation between LD-CBF during ischemia and infarct size in the cerebral cortex, and demonstrates that the relationship holds true for both sexes and strains. Fig 6 demonstrates regional blood flow distribution in representative brain slices using iodoantipyrine autoradiography at the end of 2 hours of MCA occlusion. In this example, blood flow to

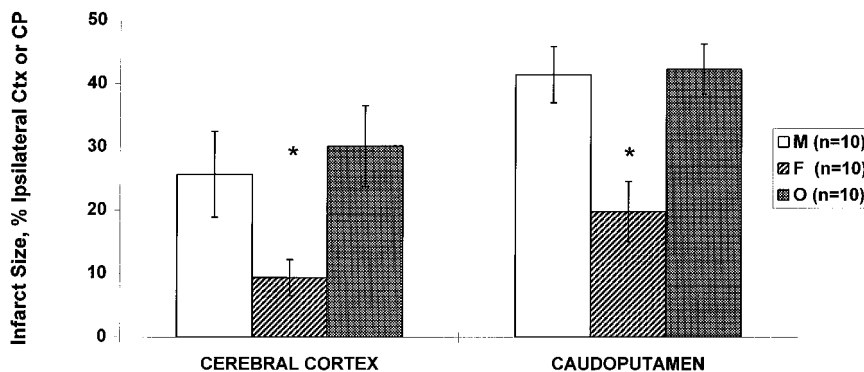


Figure 1. Cerebral infarct after MCA occlusion in Wistar rats. MCA was occluded by an intraluminal filament for 2 hours in age-matched M, F, and O rats. Infarction volumes were measured in brain sections by TTC staining after 22 hours of reperfusion and expressed as a percentage of the ipsilateral Ctx or CP. *Significantly different from M and O groups ($P<.05$)

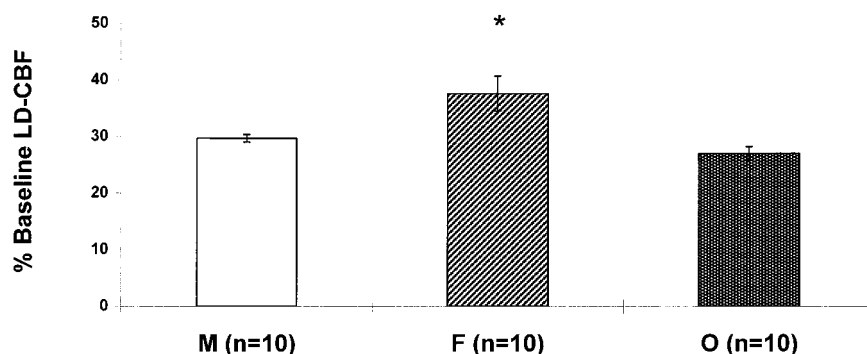


Figure 2. Residual LD-CBF during MCA occlusion in Wistar rats. LD-CBF was measured over the ipsilateral parietal cerebral cortex during MCA occlusion in age-matched M, F, and O rats. Control values were 356 ± 39 , 381 ± 24 , and 322 ± 32 LD perfusion units in M, F, and O, respectively. *Significantly different from M and O groups ($P < .05$).

MCA territory was minimally reduced in the ischemic hemisphere in female brain slices in contrast to the severe reduction in rCBF and formation of a distinctive core of severely compromised blood flow in corresponding areas in male and ovariectomized female brain slices. Fig 7 demonstrates the results of point rCBF measurements for cerebral cortex and caudoputamen in ischemic and contralateral hemispheres. At the end of ischemia, female rats sustained higher rCBF in the caudoputamen compared with M and O rats (62 ± 10 versus 15 ± 7 and 18 ± 6 mL/100 g/min in F, M, and O rats, respectively). However, mean flow rates in the cerebral cortex were not statistically different among groups (94 ± 32 , 40 ± 17 , and 31 ± 7 mL/100 g/min in F, M, and O rats, respectively). Mean flow rates on the contralateral side (both the cerebral cortex and caudoputamen) at the end of ischemia were similar among the three groups (246 ± 70 in M, 237 ± 60 in F, and 213 ± 70 mL/100 g/min in O). In a separate analysis of cerebrocortical tissue volume at high risk for infarction, only 11 ± 7 mm³ of the cerebral cortex in F received less than 10 mL/100 g/min at the end of MCA occlusion compared with 76 ± 21 mm³ in M. However, a similar level of ischemic severity was present in 54 ± 21 mm³ of the cerebral cortex in O. This segment of the cerebral cortex was not statistically different from that of F.

Discussion

The major findings of this study are that (1) female rats sustain smaller cortical and striatal infarcts after MCA occlusion compared with age-matched males of both the normotensive Wistar and the SHR-SP strains, (2) females maintain higher

striatal, but not cortical, CBF than males at the end of vascular occlusion, (3) volume of severely ischemic tissue (CBF < 10 mL/100 g/min) at the end of MCA occlusion is smaller in the cortex of females compared with males, (4) gender differences in infarct size are prevented by ovariectomy, which equalizes plasma estrogen, but not progesterone, in male and female animals, and (5) ovariectomy eliminates gender differences in end-ischemic striatal CBF, but does not affect volume of severely ischemic cortical tissue. These findings provide evidence for gender-specific responses to cerebrovascular occlusion and suggest a dual neuroprotective and flow-preserving effect of endogenous estrogen in the setting of cerebral ischemia and stroke.

The incidence of stroke is lower in premenopausal women relative to men of the same age.¹⁻³ However, little is known about stroke outcome and ischemic mechanisms in males versus females once stroke has taken place and tissue injury is in process. We used an established animal model of reversible occlusion of middle cerebral artery to compare outcome in male and female rats from two genetically distinct strains and to explore the underlying mechanism of any gender-linked ischemic brain injury. Our data indicate that estrogen-primed female rats sustain strikingly smaller brain infarcts, both in the cerebral cortex and striatum, than age-matched males. This observation was consistent across strains and valid even in animals with baseline hypertension, a known risk factor for stroke and vascular pathology. Sex-linked differences in stroke outcome in SHR-SP strain are of particular importance given the similarities between underlying pathology of this strain and human stroke.¹⁵ When corresponding groups from the two strains of rats are compared, SHR-SP rats suffer more severe

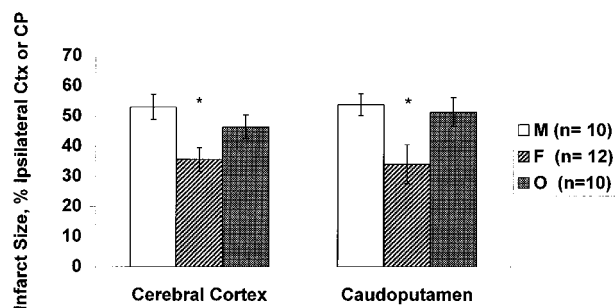


Figure 3. Cerebral infarct after MCA occlusion in SHR-SP. MCA was occluded by an intraluminal filament for 2 hours in age-matched M, F, and O rats. Infarction volumes were measured in brain sections by TTC staining after 22 hours of reperfusion and expressed as a percentage of the ipsilateral Ctx or CP. *Significantly different from M and O groups ($P < .05$).

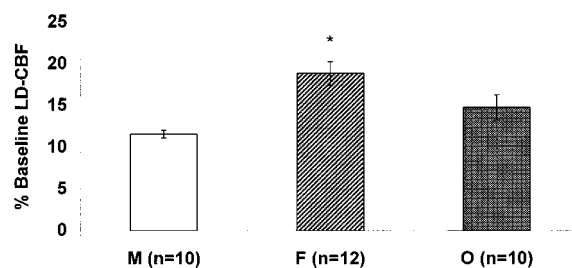


Figure 4. Residual LD-CBF during MCA occlusion in SHR-SP. LD-CBF was measured over the ipsilateral parietal cerebral cortex during MCA occlusion in age-matched M, F, and O rats. Control values were 353 ± 27 , 381 ± 55 , and 416 ± 40 LD perfusion units in M, F, and O, respectively. *Significantly different from M and O groups ($P < .05$).

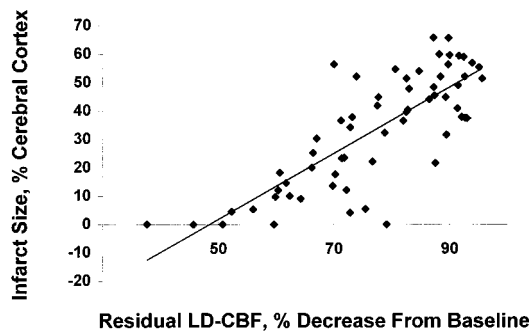


Figure 5. Correlation between residual LD-CBF during MCA occlusion and size of cerebrocortical infarct. LD-CBF was measured in the ipsilateral parietal cerebral cortex over 2 hours of MCA occlusion and expressed as percent change from baseline. Infarct size was measured in TTC-stained brain sections 22 hours after intraluminal filament occlusion of MCA for 2 hours ($r=.79$, $P<.001$).

brain damage compared with Wistar rats. This finding agrees with previous reports that vascular occlusion in genetically hypertensive strains of rat results in larger infarct volume than in normotensive strains.²⁴ This observation suggests that salvage of neural tissue associated with estrogen is likely to be a generalizable phenomenon despite quantitative differences in the magnitude of tissue damage among individual strains.

Our findings with MCA occlusion in the rat are consistent with previous work demonstrating gender-specific responses to experimental ischemic brain injury.^{5,6} Female gerbils are less vulnerable to hippocampal neuronal injury after unilateral carotid occlusion than their male counterparts, consuming smaller amounts of endogenous antioxidants during the ischemic insult.⁵ Li et al have demonstrated differences in severe cortical infarcts, defined as the amount of cytopathology and loss of astrocytic staining, between male and female Fisher rats after thromboembolic injury.⁶ Our findings expand on these earlier observations by demonstrating the deleterious consequences of endogenous estrogen deficiency in stroke and by evaluating the contribution of flow-mediated and flow-independent mechanisms in the more favorable outcome (sparing of neural tissue) enjoyed by female animals after experimental stroke.

The gender difference in infarct size is lost when female animals are ovariectomized at an early age. Ovariectomized female rats sustain a similar infarct size as males, which indicates that salvage of neural tissue in females is due to an action of

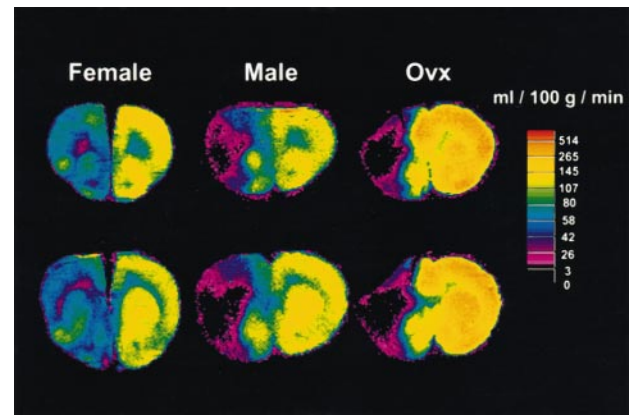


Figure 6. Color-coded distribution of rCBF rates in representative autoradiograms during MCA occlusion. Rates of rCBF were measured at the end of 2 hours of intraluminal filament occlusion of right MCA by [14 C]IAP autoradiography in male, female and ovariectomized (Ovx) female Wistar rats. Coronal sections correspond to +0.2 (upper panel) and -1.8 mm (lower panel) relative to the bregma.

female sex steroids, most likely estrogen because ovariectomized females, which have plasma estrogen levels similar to males lose the advantage enjoyed by intact females and display infarct size similar to that of males. It is unlikely that progesterone was responsible for the improved outcome of stroke in intact versus ovariectomized females because plasma progesterone in ovariectomized rats was lower than that in intact females, but still higher than in males. However, in view of recent reports demonstrating a neuroprotective effect of exogenously administered progesterone in male rats,²⁵ the possibility of an interaction between the two hormones cannot be excluded. In support of such an interaction is the demonstration of induction of progesterone receptors by estrogen in the brain.²⁶ Differences in stroke outcome could not be accounted for by differences in age, halothane concentration, core or head temperatures, or physiological variables such as arterial blood pressure or blood gases, because these parameters were similar among all groups within each strain.

Ischemic status, as well as reperfusion, was confirmed in each animal by continuous on-line monitoring of laser-Doppler (LD)-CBF. LD-CBF during ischemia, an indication of residual tissue perfusion after arterial occlusion, correlated well with infarct size and was higher in F compared with M and O rats in both strains. This observation suggests that differences in

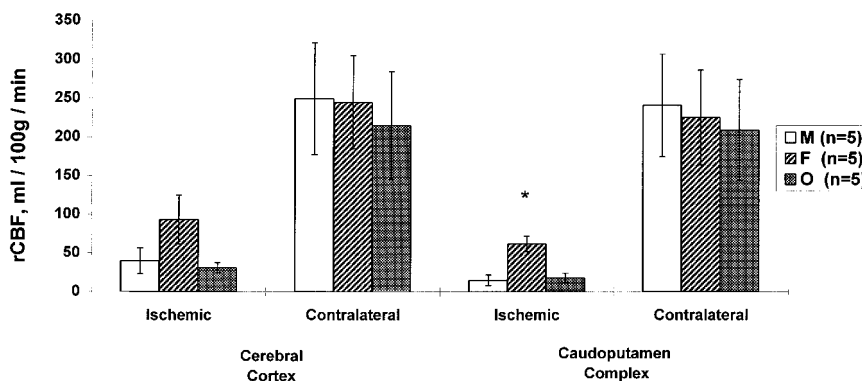


Figure 7. rCBF rates during MCA occlusion in Wistar rats. Flow rates were measured by the iodoantipyrine autoradiographic technique at the end of 2 hours of unilateral intraluminal filament occlusion of MCA. Flow rates in representative areas of the cerebral cortex and caudoputamen were averaged across the ischemic and nonischemic contralateral hemispheres and compared among M, F, and O rats. *Significantly different from M and O groups ($P<.05$).

residual tissue perfusion may account for gender differences in infarct size and that estrogen acts to enhance tissue perfusion during vascular occlusion. The latter view agrees with our previous finding that chronic exogenous administration of 17- β -estradiol, the principal biologically active estrogen in mammals, augments residual CBF during global incomplete ischemia in the rabbit.¹⁴ However, in a gerbil model of global cerebral ischemia, differences in cortical CBF were not apparent between males and females despite differences in neuronal injury.⁵ Because LD-CBF estimates only the relative change in CBF, we used ¹⁴C autoradiography to quantify end-ischemic CBF within MCA-dependent territory and to determine whether residual regional CBF was indeed higher in intact females compared with males and ovariectomized females. In caudoputamen, end-ischemic CBF was clearly preserved in females when endogenous estrogen is present compared with males or ovariectomized females. Blood flow preservation is, therefore, a likely explanation for the smaller infarction volume observed within this region in estrogen primed females. It is unlikely that preservation of CBF in the striatum is a reflection of differences in baseline CBF, because flow in the nonischemic, contralateral region was similar among the three groups. This conclusion is also consistent with our previous observation that baseline blood flow does not increase with exogenous estrogen administration,¹⁴ at least not in anesthetized animals. In conscious animals, acute estrogen administration has been reported to increase CBF in many brain areas, including the cerebral cortex, hippocampus, basal ganglia and cerebellum, both in males and females.²⁷ Using positron-emission tomography scanning, estrogen has recently been shown to augment cognitive activation of regional CBF in young women.²⁸

In contrast to our finding in the caudoputamen, end-ischemic cortical flow was not different among the three groups, suggesting that flow-preservation is an unlikely explanation for the differences observed in infarct size in this region. To further dissect flow-mediated mechanisms in cortical tissue sparing in estrogen-primed animals, we compared tissue volumes with rCBF of less than 10 mL/100 g/min among groups, as a means of equalizing the contribution of flow deficit to the development of cerebrocortical tissue infarction. These regions with a blood flow rate approaching the ischemic threshold for neuronal cell death^{29,30} represent areas of the cortex destined for infarction. Our analysis indicates that despite a clear histological preservation of viable tissue within cortex in intact females versus males and ovariectomized females, the volume of ischemic tissue was smaller in the cortex of intact females compared with males, but the volume of similarly profoundly ischemic tissue in the cortex of ovariectomized females was not different from that of intact females. This finding suggests that flow preservation is a likely explanation for the difference in infarct size between males and females. However, the difference between intact and ovariectomized females cannot be fully explained by flow-mediated mechanisms, and likely represents a flow-independent neuroprotective effect of estrogen. This process is apparent in that, whereas estrogen-competent females have smaller areas with severely ischemic flow than males, ovariectomy does not alter this volume of severely low flow tissue destined for infarction. Thus, for similar areas of severely ischemic flow, intact females sustain

smaller infarction volumes than do estrogen-depleted females. Therefore, we conclude that non-flow-mediated mechanisms of neuroprotection are at work in ischemic cortex in females.

Our finding that female animals maintain higher tissue perfusion during MCA occlusion may be explained by one or more of the following mechanisms: (1) cerebrovascular anatomical differences, eg, greater number and/or diameter of cerebral blood vessels providing extensive collateral flow in females; (2) increased small vessel and capillary density in response to estrogen's angiogenic properties;³¹ (3) functional differences in vascular reactivity; or (4) availability of vasoactive mediators, allowing for increased vasodilator capacity under low flow conditions in estrogen-primed vessels. The latter two mechanisms seem likely in that direct effects of estrogen on both endothelium and vascular smooth muscle have been reported.^{10,11,32}

A direct effect of estrogen on the blood vessel wall is suggested by its ability to relax isolated blood vessels^{10,11} and by evidence for the presence of ER in isolated arteries and vascular cells.³³ Expression of classic ER has been demonstrated in cultured human and bovine endothelial cells,³⁴ and estrogen-binding sites have been described in human endothelial and rat aortic vascular smooth muscle cells.³⁵ Potential mechanisms for estrogen-induced vasodilation include estrogen-mediated release of nitric oxide and prostacyclin from vascular endothelium¹⁰ and VSM membrane hyperpolarization,³⁶ possibly by cGMP-dependent phosphorylation of K⁺ channels.³⁷ Of interest in this regard is the presence of estrogen-responsive elements on genes regulating rate-limiting enzymes in the biosynthesis of both prostacyclin and nitric oxide.¹⁰ Furthermore, vascular reactivity has been shown to be impaired in ovariectomized animals, and it is restored by chronic estrogen treatment.³⁸ Thus, estrogen may protect the brain during ischemic episodes by preserving and augmenting vasodilator mechanisms.

Finally, the cortical neuroprotection observed in intact versus ovariectomized females is likely to represent alternative and significant nonvascular mechanisms. Estrogen exhibits an array of neuronal effects that could potentially alleviate ischemic brain damage, and there is a widespread pattern of ER and associated estradiol targets within neural tissue.^{26,39,40} ER mRNA has been identified in brain areas not generally associated with reproductive function.⁴¹ Furthermore, glial cells contain receptors for estrogen and respond to the hormone both in vivo and in vitro.⁴²

In summary, we have demonstrated that female rats sustain more favorable outcome after ischemic brain injury, possibly due to both neuroprotective and flow-preserving effects of estrogen. This finding provides a possible explanation for the well-documented, but poorly understood, clinical observation that premenopausal women are at lower risk for stroke than men of the same age. Our finding may also explain the steep rise in stroke incidence in women after menopause. It is yet to be determined whether exogenously administered estrogen interacts with mechanisms of ischemic brain damage to protect neurons. Such a finding would provide a rationale for the use of estrogen to improve stroke outcome in postmenopausal women. Furthermore, understanding the mechanisms of estrogen-me-

diated neuroprotection helps elucidate the generalized mechanisms of stroke damage and provides new strategies for stroke injury prevention in both sexes.

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Editorial Comment

This study fills an important gap in our current stroke literature. It raises important questions and provides new information about the influence of gender on brain injury in experimental stroke. Until recently, there has been little attention to gender difference in most areas of illness or disability, with the exception of areas that were specifically gender linked. Consequently, we have limited information about the influence of gender in most health conditions. Before 1985 women were rarely included in clinical trials. That situation has changed in recent years. The National Institutes of Health has instituted its specific "Guidelines for Inclusion of Women and Minorities in Clinical Trials," and women are routinely included in trials.

Although stroke has long been regarded as a problem of concern predominantly in men, there is a steep increase in the incidence of stroke in postmenopausal women. Moreover, since women have a longer life expectancy than men, the incidence of stroke is higher in older women. The mean age of our population is increasing. As we enter the 21st century a dramatic shift is occurring. As the population ages and women comprise a larger percentage of the population, the issue of gender difference in stroke will become even more important. Therefore, it becomes imperative that we are able to address the problem of stroke in both genders of our population.

Moreover, now that there is a newly available treatment for stroke, issues such as the role that gender plays in the size of the

lesion and any changes in response to treatment must be characterized. Certain sex hormones such as estrogen are known to have effects on the brain. Effects such as alteration of regional cerebral blood flow, vasodilator effects, augmentation of cognitive activation and increased neuronal branching in vitro, and cognitive protection in Alzheimer's disease have all been attributed to estrogen. All of this information may play an important role in either augmenting or modifying response to therapies instituted in the acute stroke situation in women. Therefore, it is important to raise the question of and provide the answers to the role, either beneficial or potentially deleterious, of sex hormones that may influence the response to cerebral ischemia.

This study addresses gender and its relation to brain injury in an animal model of focal cerebral ischemia. The authors' conclusions that endogenous estrogen improves stroke outcome by providing both neuroprotective effects and blood flow following focal cerebral ischemia are carefully discussed in detail in the paper. The resulting information provides guidance as the field of clinical stroke research moves forward aggressively in testing therapeutic interventions to prevent and/or limit disabilities resulting from stroke.

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