Genetic and Gender Influences on Sensitivity to Focal Cerebral Ischemia in the Stroke-Prone Spontaneously Hypertensive Rat

Hilary V.O. Carswell, Niall H. Anderson, James S. Clark, Delyth Graham, Baxter Jeffs, Anna F. Dominiczak, I. Mhairi Macrae

Abstract—We have investigated genetic transmission of increased sensitivity to focal cerebral ischemia and the influence of gender in the stroke-prone spontaneously hypertensive rat (SHRSP). Halothane-anesthetized, 3- to 5-month-old male and female Wistar-Kyoto rats (WKY), SHRSP, and the first filial generation rats (F1 crosses 1 and 2) underwent distal (2 mm) permanent middle cerebral artery occlusion (MCAO) by electrocoagulation. Infarct volume was measured by using hematoxylin-eosin–stained sections and image analysis 24 hours after ischemia and expressed as a percentage of the volume of the ipsilateral hemisphere. Infarct volume in males and females grouped together were significantly larger in SHRSP, F1 cross 1 (SHRSP father), and F1 cross 2 (WKY father), at 36.6 ± 2.3% (mean ± SEM, P < 0.001, n = 15), 25.4 ± 2.4% (P < 0.01, n = 14), and 33.9 ± 1.6% (P < 0.001, n = 18), respectively, compared with WKY (14 ± 2%, n = 17). Male F1 cross 1 (18.9 ± 2.4%, n = 6) developed significantly smaller infarcts than male F1 cross 2 (32.8 ± 2%, n = 8, P < 0.005). Females, which underwent ischemia during metestrus, developed larger infarcts than respective males. A group of females in which the cycle was not controlled for developed significantly smaller infarcts than females in metestrus. Thus, the increased sensitivity to MCAO in SHRSP is retained in both F1 cross 1 and cross 2 hybrids, suggesting a dominant or codominant trait; response to cerebral ischemia appears to be affected by gender and stage in the estrous cycle. In addition, the male progenitor of the cross (ie, SHRSP versus WKY) influences stroke sensitivity in male F1 cohorts. (Hypertension. 1999;33:681-685.)

Key Words: rats, stroke-prone spontaneously hypertensive ■ cerebral ischemia, focal ■ gender ■ estrus ■ estrogen ■ middle cerebral artery occlusion

The stroke-prone spontaneously hypertensive rat (SHRSP) was established from Wistar-Kyoto rats (WKY) and is an inbred animal model of cerebrovascular disease.1 Spontaneous strokes occur in the SHRSP, and the role of a genetic factor in stroke is highlighted by the resistance of the related SHR to spontaneous strokes despite hypertension and similar diets.

SHRSP exhibit an increased sensitivity to ischemia and an increased volume of infarction after experimentally induced focal cerebral ischemia compared with their reference strain, the WKY. This has been shown by ligation2 or electrocoagulation3-4 of the middle cerebral artery (MCA). One reason for the increased sensitivity of the SHRSP to stroke may be reduced blood flow through collateral vessels between the MCA and anterior and posterior cerebral arteries. The collaterals have been postulated to have an impaired ability to dilate and a smaller internal diameter compared with those in normotensive Wistar rats.5 In addition, more marked ischemic glutamate increases in SHRSP than WKY may be related to stroke sensitivity.6

Recently, we revealed a genetic component to the increased sensitivity after experimental ischemia in the SHRSP. We linked increased volume of infarction 24 hours after MCA occlusion (MCAO) to a locus on rat chromosome 5 with a logarithm of odds (LOD) of 16.6.4 Having previously demonstrated a dominant mode of inheritance for this phenotype in F1 cross 2 hybrid rats (WKY father),7 we now use a different set of reciprocal crosses especially bred for these experiments to examine genetic transmission and any influence of the origin of the Y chromosome (SHRSP male progenitor versus WKY male progenitor) on infarct volume. We also investigate the effects of sex differences and control for estrous status during ischemia induction—the first F1 analysis to do so.

Methods

All experiments were carried out under license from the Home Office and were subject to the Animals (Scientific Procedures) Act, 1986. Breeding and housing of SHRSP and WKY have been previously described.8 Two parental crosses were performed espe-
processed for hematoxylin-eosin staining as previously described. MCAO procedure. Twenty-four hours after MCAO, tissue was probe inserted into the temporalis muscle was used to reflect the estrous cycle was determined by smear test, which was carried out stated) were in metestrus at the time of MCAO. The stage of the may have on the volume of infarction, all females (unless otherwise

Values are mean±SD. All groups were maintained at normocapnia (36–42 mm Hg), normal physiological pH (7.4), and normal body temperature (37–37.5°C). Similar plasma glucose levels were observed in all groups except for female SHRSP, in which plasma glucose was elevated (significantly higher than female WKY, male SHRSP, and female F1 cross 1 rats; *P<0.05, ANOVA followed by Bonferroni correction for 16 comparisons).

Physiological Parameters

Table 1 shows physiological parameters for all the experiments. Plasma glucose levels in SHRSP females were significantly elevated during anesthesia at the time of MCAO but were normal by 24 hours after ischemia. All other physiological variables were within normal limits during anesthesia at the time of MCAO. Mean arterial blood pressure (MAP) during anesthesia was higher in SHRSP and F1 cross 1 rats than WKY and F1 cross 2 rats for both males and females (as seen in conscious MAP values, Figure 3).

Infarct Volume

Infarct volume in SHRSP (36.6±2.3%, n=15) was significantly greater than in WKY (14±2%, n=17) (mean±SEM, male and female grouped together, Figure 1). The increased sensitivity to ischemia after MCAO was retained in both first-generation crosses: F1 cross 1, 25.4±2.4% (n=14); F1 cross 2, 33.9±1.6% (n=18) (P<0.01, P<0.001, respectively, compared with WKY, ANOVA followed by t test with Bonferroni corrections for 6 pairwise comparisons, Figure 1).

When males and females were split into separate groups, two way ANOVA demonstrated significance for strain and sex but not for an interaction between the two. The t test with a Bonferroni correction for 16 pairwise comparisons was performed. Females exhibited significantly larger infarcts than males in WKY, SHRSP, and F1 cross 1 (Table 2). A comparison between females taken at random (13.5±3.3%, n=5) compared with those taken during metestru (30.2±2.8%, n=8) revealed a significant influence of stage in estrous cycle on infarct size (Figure 2, P=0.003, unpaired two-tailed t test).

Male F1 cross 1 rats exhibited significantly smaller infarcts than male F1 cross 2 rats (Table 2) (P<0.005).

Blood Pressure

Conscious MAP values recorded 24 hours after ischemia are presented in Figure 3. As expected, MAP values in SHRSP (167±6 mm Hg, n=12) were significantly higher than in WKY (134±4 mm Hg, n=12) (P<0.001). In addition, MAP was significantly higher in F1 cross 1 rats (155±7 mm Hg, n=9) compared with F1 cross 2 rats (131±5 mm Hg, n=16) (P<0.05). However, MAP plotted against infarct volume for
the F₁ hybrids shows that animals with the highest MAP values did not have the largest infarct volumes (Figure 4). In fact, an inverse correlation actually reached statistical significance (Pearson correlation, P<0.05, r=−0.4).

**Discussion**

This is the first study in which the two first filial generations, produced by crossing SHRSP and WKY, have been compared in terms of their blood pressures and sensitivities to a focal cerebral ischemic insult. The key finding is that increased sensitivity to ischemia is retained in both crosses of the first filial generation that were bred especially for these experiments. This indicates a dominant or codominant mode of inheritance in accordance with our earlier study demonstrating a spread of infarct volumes in the second filial (F₂) generation ranging from the minimum seen in the WKY to the maximum in the SHRSP.⁴

Coyle et al.¹² previously postulated that the mode of inheritance for susceptibility to infarction was recessive. However, Coyle’s group crossed female SHRSP with an outbred male Wistar rat, which introduced genetic heterogeneity into the cross. By mating two inbred strains, as in the present study, minimal genetic variation should occur. It is known that genetic heterogeneity exists between various WKY originating from different colonies.¹³ However, no genetic heterogeneity exists within our colony of WKY as shown by repeated microsatellite screenings.⁴¹⁴ In addition, Coyle’s group used only one cross (SHRSP mother/grandmother), whereas in the present study we generated reciprocal crosses to show that increased sensitivity to ischemia was retained in the first generation. Finally, Coyle’s group ligated the MCA, whereas in the present study the MCA was electrocoagulated. These experimental differences may account for the different conclusions regarding modes of inheritance.

The significant difference in infarct size between F₁ cross 1 and F₁ cross 2 animals (Figure 1) suggests that the origin of the cross influences the response of the F₁ rats to ischemia. In particular, male F₁ cross 1 rats had significantly smaller infarcts than male F₁ cross 2 rats. Since male F₁ cross 1 rats inherit their X chromosome (Xw) from a WKY mother and their Y chromosome (Ys) from an SHRSP father, one or other of these chromosomes may confer additional protection. To examine this idea further, we categorized male F₂ rats from our previous study⁴ in terms of the origin of their Y chromosome. F₂ males inheriting their X chromosome from the SHRSP had an infarct volume of 23.1±1.6% (n=20), and those inheriting their Y chromosome from the WKY had an infarct volume of 27.5±0.8% (n=11) (P=0.06, unpaired two-tailed t test).

Given that hypertension is a known risk factor for ischemic stroke, a relationship between high blood pressures and large infarct volumes in these animals might be expected. However, Figure 4 illustrates a significant negative, not positive, correlation between blood pressure and infarct volume. The lack of a positive correlation highlights the concept that in these animals, large infarcts are not secondary to hypertension. This is supported by the findings that young SHRSP whose hypertension have not yet fully established still develop significantly larger infarcts after MCAO compared with age-matched WKY²; that SHRSP treated chronically

**TABLE 2. Infarct Volume for Male and Female WKY, SHRSP, F₁ Cross 1, and F₁ Cross 2 Rats**

<table>
<thead>
<tr>
<th>Rat Group</th>
<th>Male n</th>
<th>Female n</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>8±2.4</td>
<td>9±2.0</td>
<td>8.026</td>
</tr>
<tr>
<td>SHRSP</td>
<td>30±1.8</td>
<td>8±2.9</td>
<td>0.014</td>
</tr>
<tr>
<td>F₁ cross 1</td>
<td>18±2.4*</td>
<td>6±2.8</td>
<td>0.043</td>
</tr>
<tr>
<td>F₁ cross 2</td>
<td>32±2.0</td>
<td>8±2.4</td>
<td>0.045</td>
</tr>
</tbody>
</table>

Values are mean±SEM and expressed as percent ipsilateral hemisphere. Two-way ANOVA showed significance for strain and sex but not for an interaction between the two. ANOVA was followed by t test with Bonferroni correction for 16 pairwise comparisons. Male F₁ cross 1 was significantly lower than male F₁ cross 2 (*P<0.005).

Figure 2. Infarct volume for female F₁ cross 1 rats taken at random (n=5) or at metestrus (n=8) (*P<0.005, unpaired t test).

Figure 3. MAP at 24 hours after MCAO in male (open symbols) and female (closed symbols) WKY, SHRSP, F₁ cross 1 rats, and F₁ cross 2 rats. **P<0.05, ***P<0.001 versus WKY, †P<0.05, †††P<0.001, ANOVA followed by t test with Bonferroni correction for 6 pairwise comparisons.

Figure 4. MAP at 24 hours after MCAO for individual F₁ hybrids plotted against infarct volume, showing significant negative correlation (r²=0.16, P=0.045, Pearson correlation). Values for male F₁ cross 1 rats are highlighted ■.
with antihypertensive agents still display larger infarcts after MCAO than WKY; and that normotensive rats made acutely hypertensive with deoxycorticosterone acetate–salt do not develop infarcts after MCAO as large as those of SHRSP. In addition, vascular hypertrophy seen after chronic hypertension has been shown not to cause but actually to protect against the incidence of cerebral hemorrhage and ischemic infarct in SHRSP.17

Interestingly, there is a significant negative correlation between high blood pressure and small infarct volume in these animals (P<0.05, Figure 4), and F1, cross 1 rats had significantly higher MAPs (Figure 3) and significantly smaller infarct volumes (Figure 1) than F1, cross 2 rats. In our laboratory, a telemetry study on F2 hybrids originating from the same inbred colonies of SHRSP and WKY revealed that male F1 animals that inherited the Y chromosome from the SHRSP grandfather (Ys) had about a 20 mm Hg higher blood pressure than those that obtained the Y chromosome from the WKY grandfather (Yw). Similarly, in our previous study, four male F2 rats characterized as Ys with small infarcts had significantly elevated MAP values compared with F2 rats characterized as Yw (156±4 mm Hg, n = 12, compared with 141±6 mm Hg, n = 9; P = 0.04, unpaired two-tailed t-test). It is conceivable that the higher blood pressures in the subgroups of animals inhering an SHRSP Y chromosome (Ys) (male F1 cross 1 and a subgroup of male F2 rats) may be exerting a protective effect in these animals, resulting in a consequent smaller infarct volume (Table 2). Indeed, elevating blood pressure during vessel occlusion has been shown to reduce infarct size, and vascular hypertrophy that develops alongside hypertension has been shown to protect against ischemic infarcts.17

The present study is the first F1 genetic analysis to take account of the estrous cycle during ischemia. In many studies on female rats, the stage of the estrous cycle is not controlled for, and the present study demonstrates a significant difference in outcome for the uncontrolled situation (animals taken at random) compared with animals in metestrus. Females in metestrus during ischemia display larger infarcts than those taken at random. In addition, females during metestrus display larger infarcts than males. This is in contrast to the uncontrolled situation, in which females display smaller infarcts than males. In addition, the incidence of spontaneous strokes in SHRSP is lower in females than males. It is therefore apparent that both gender and stage in the estrous cycle influence the outcome of ischemia in these rats. Female sex hormones are therefore clearly influencing stroke sensitivity. Levels of estrogen and progesterone fluctuate between 7 pg/mL and 40 to 50 pg/mL and between 5 to 10 ng/mL and 45 to 50 ng/mL, respectively, during the estrous cycle. The high physiological levels of both estrogen and progesterone at different stages in the 4-day cycle may protect against ischemic damage. It is already known that ovariectomized rats treated chronically with estrogen before a focal cerebral insult suffer smaller infarcts than untreated ovariectomized rats. In addition, progesterone has been shown to exert neuroprotection. Estrogen has been reported to have anti-inflammatory effects in the periphery, including decreasing levels of cytokines such as tumor necrosis factor-α and acting as a free radical scavenger. Estrogen also decreases low-density lipids and increases high-density lipids and promotes nitric oxide-mediated and prostacyclin-mediated vasorelaxation by increasing nitric oxide synthase and prostacyclin production, respectively. Estrogen has been shown to inhibit endothelin-1 production, thereby reducing the extent of endothelin-induced vasoconstriction. Progesterone may mediate its protection by a potent antiedema effect on the brain.11

In conclusion, the increased sensitivity to cerebral ischemia seen in the SHRSP is retained in the first filial generation of rats. Sensitivity to ischemia in F1 hybrids appears to be affected by the origin of the cross as well as by gender and the stage of the estrous cycle. F1 and F2 males with a Y chromosome inherited from the SHRSP male progenitor appear to be less sensitive than those with a Y chromosome inherited from WKY. This decreased sensitivity to ischemia may be at least partly due to the increased blood pressure shown to be present in animals with the Y chromosome inherited from the SHRSP.

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