Heritability of Intracerebral Hemorrhagic Lesions and Cerebral Aneurysms in the Rat

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Background and Purpose—Under certain conditions, the Brown Norway (BN) rat is susceptible to intracerebral hemorrhagic vascular (ICV) lesions within the cerebral cortex, whereas the Long-Evans (LE) rat is prone to develop aneurysms in the circle of Willis. The incidence of these 2 pathological phenotypes was studied in progeny of different BNXLE crosses to determine their heritability in these new rat models. In addition, a possible link between ICV lesion occurrence and either the susceptibility to spontaneous rupture of the arterial internal elastic lamina (IEL) or basal plasma angiotensin-converting enzyme (ACE) activity was also studied in back-cross (BC) F1XBN rats, the only second-generation group with a high incidence of ICV lesions.

Methods—To induce cerebrovascular lesions, rats were submitted to experimental hypertension associated with ligation of 1 carotid artery. After death, the brain was examined for cerebral lesions. Numbers of arterial IEL ruptures were determined microscopically with the use of en face preparations. Plasma ACE activity was determined before the induction of hypertension.

Results—In general, groups that developed ICV lesions presented a low incidence of aneurysms. ICV lesion incidence was similar in F1 hybrids and BC(F1XBN) and greatly decreased in F2 and BC(F1XLE) rats compared with BN rats. No cerebral aneurysms developed in F1 rats. Aneurysmal incidence was 24% (20% ruptured) in LE, 42% (59% ruptured) in F2, and 50% (75% ruptured) in BC(F1XLE) rats. In BC(F1XBN) rats, neither the incidence of IEL rupture nor the plasma ACE activity was higher in the rats with ICV lesions. However, the mean blood pressure level was higher in these rats, and peak blood pressure was higher in rats with the most severe grades of ICV lesions.

Conclusions—These data suggest a polygenic and dominant mode of inheritance of ICV pathology. The formation of aneurysms in the circle of Willis tended to be favored, and their rupture was clearly increased by the presence of BN rat alleles within the LE rat genome. These data may provide the basis for future studies to determine, in new rat models, which genes are involved in these pathologies. (Stroke. 2000;31:2678-2684.)

Key Words: cerebral aneurysm ■ intracerebral hemorrhage ■ rats

We have previously shown that, compared with Long-Evans (LE) rats, Brown Norway (BN) rats are prone to develop extensive intracerebral hemorrhagic vascular (ICV) lesions within the cerebral cortex when rendered hypertensive1 and are highly susceptible to the spontaneous rupture of the arterial internal elastic lamina (IEL), mainly in the caudal and abdominal aorta.1 In addition, we have described a higher incidence of these IEL ruptures2 in the caudal artery of the genetically stroke-prone spontaneously hypertensive rat (SHR)3 compared with stroke-resistant SHR. These data suggested that the susceptibility of the arterial IEL to rupture is a genetically determined trait, independent of hypertension, which may be linked to the occurrence of intracerebral hemorrhagic lesions.1 To test this hypothesis, we performed different crosses between BN and LE rats, and in back-cross (BC) F1XBN rats, the only second-generation group to present significant numbers of ICV lesions, we studied the incidence of IEL ruptures in the abdominal aorta and renal arteries and the occurrence of cerebrovascular lesions.

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In humans, the deletion/insertion polymorphism of the ACE gene partly determines the variation in plasma angiotensin-converting enzyme (ACE) levels between individuals.4 An association between ACE polymorphism and various cardiovascular disorders has been investigated,5,6 and conflicting results were obtained for the association between ACE gene polymorphism and stroke in humans.7–11 Different levels of plasma ACE activity have also been demonstrated in various strains of rats and shown to be primarily genetically determined.12 Indeed, plasma ACE activity levels are higher in the BN rat, which is also prone to intracerebral vascular lesions, than in the LE rat. To test a possible link between these 2 parameters, in the present study we determined plasma ACE activity levels before the induction of hypertension, which thus indirectly reflects the polymorphism of the ACE gene, and the occurrence of cerebrovascular lesions in BC(F1XBN).
We recently reported that LE rats are more susceptible than BN rats to the development of experimental cerebral aneurysms in the arteries of the circle of Willis. Aneurysms were induced by ligation of 1 common carotid artery, a procedure that modifies the regional cerebral blood flow, associated with hypertension. Thus, BN and LE rats present opposing susceptibilities to ICV lesions and cerebral aneurysms. We performed different crosses between these 2 rat strains and studied the occurrence of these 2 cerebral pathologies in the progeny to determine their heritability. Recently, data on the inheritance of different stroke phenotypes in the rat have initiated studies for genetic markers for stroke severity and latency to stroke. Thus, data from the present study on the heritability of pathological phenotypes in new rat models may be useful for the future study of determinant genes in these cerebrovascular diseases in the rat.

**Materials and Methods**

**Animals and Crossbreeding Procedures**

All rats used for this study were males with the exception of female progenitors. Inbred BN rats were supplied by Iffa-Credo, L’Arbresle, France and outbred LE rats were supplied by CEJ, Le Genest Saint-Isle, France.

LE and BN rats were mated to produce F1 hybrids, and the F2 cohort was obtained by mating rats from the F1 generation. Hybrid F1 were mated with either BN or LE rats to obtain BC rats: BC(F1XBN) and BC(F1XLE), respectively. BC(F1XBN) rats were obtained from 3 consecutive series of crosses, spanning a period of several years.

Animal care complied with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research. The studies were performed under authorization No. 006235 of the Ministère de l’Agriculture, France.

**Experimental Protocol**

The surgical procedure was performed under anesthesia by injection of pentobarbital (40 mg/kg IP). Rats were rendered hypertensive by unilateral nephrectomy at the age of approximately 11 to 14 weeks followed, 1 week after surgery, by a weekly subcutaneous injection of deoxycorticosterone acetate (25 mg/kg, Sigma) and 1% saline to drink. To modify blood flow in arteries of the circle of Willis, the left common carotid was ligated in 2 locations approximately 5 mm apart and cut between the ligatures. This procedure was performed at the same time as nephrectomy.

The animals were kept until they died spontaneously and were then autopsied. The brain was removed from the skull, and the presence of ICV lesions within the cerebral cortex and of cerebral aneurysms in the arteries of the circle of Willis was determined with the use of a dissecting microscope. The mean diameter of aneurysms was measured with a calibrated micrometer in the microscope eyepiece.

The criteria for ICV grades were as follows. We attributed grade 1 to the presence of 1 or 2 small round hemorrhagic spots (Figure 1a) or very small lacunar areas, grade 2 to more diffuse hemorrhagic areas (Figure 1b) or lacunae and also to important edema of the right cortex, and grade 3 to large hemorrhages (Figure 1c) that were obviously responsible for the animal’s death.

**Blood Pressure Measurements**

Systolic blood pressure was recorded weekly in conscious rats by the indirect tail cuff and transducer method (BP recorder 8006, Apelex) in BC rats originating from the F1XBN mating.

**Plasma ACE Activity Measurements**

During the surgical procedure, blood was sampled via the jugular vein. Plasma ACE activity was thus measured before rats were rendered hypertensive, in 2 groups of BC rats (F1XBN) (n=23), with the use of a previously described fluorometric assay. Briefly, blood was sampled from the jugular vein at the time of surgery (see above). The method is based on the conversion of the substrate analogue hippuryl-His-Leu to hippurate and His-Leu, which is quantified spectrofluorometrically (at 500 nm, F 2000, Hitachi spectrofluorometer) by formation of a fluorescent adduct with o-phthaldialdehyde (Sigma). Plasma ACE activity was expressed as nanomoles hippuryl-His-Leu cleaved per milliliter of plasma per minute of incubation. Different concentrations of His-Leu (Bachem)
incubated with o-phthaldialdehyde and treated under the same conditions were used as standards.

**Incidence of Ruptures of the IEL**

The incidence of IEL ruptures was studied in 3 different consecutive series of BC(F1XBN). The method used was previously described and based on the microscopic examination of the luminal side of en face preparations of the arteries where tears in the IEL could be visualized with a light microscope and quantified. Briefly, at the time of death or as soon as possible afterward, a catheter was placed in the aorta, the iliac arteries were cut, and the kidney was removed to facilitate the perfusion in situ of the abdominal aorta and the renal artery with formalin followed by orcein and Groat’s hematoxylin to stain the IEL and the endothelial nuclei, respectively. With the use of a dissecting microscope, the arteries were opened and maintained flat by small pins in a sucrose solution (5%). Arteries were then rinsed, dehydrated in graded alcohols, cleared in xylene, and mounted on slides with Eukitt. The total number of IEL ruptures in each artery was recorded.

**Statistical Analysis**

Statistical significance of differences between groups was studied by either a 1-way ANOVA followed by the Fisher test, Student’s t test, regression analysis, or $\chi^2$ analysis according to the type of parameter considered.

**Results**

**Incidence of Intracerebral Hemorrhagic Lesions and Cerebral Aneurysms**

Figure 1 illustrates brains that display either ICV lesions or aneurysms in arteries of the circle of Willis. The hemorrhagic vascular lesions observed in the cerebral cortex occurred on the right side, ie, contralateral to the carotid ligation, and presented different grades of severity, which are shown in Figure 1a through 1c. We did not perform an extensive histological study of these intracerebral lesions, and only some of the brains have been examined. Microscopic features of these ICV lesions were very similar to those described in the brain of renovascular hypertensive rats. Figure 1d shows a coronal slice of a brain where a large hemorrhage had occurred. Microscopically, these areas often contained small subarachnoid arterioles showing extensive fibrinoid necrosis (Figure 2a) with intermingled collagenous material and a complete loss of the normal structure of the media. Several arterioles were occluded and contained hemosiderin deposits (Figure 2b). The dimensions of some of these vessels were greatly enlarged and resembled aneurysms (Figure 2c). Examples of a small intact aneurysm in the anterior part of the circle of Willis (Figure 1e) and a large ruptured aneurysm in its posterior part (Figure 1f) are illustrated. Sections of aneurysmal walls of ruptured aneurysms in the posterior part of the circle of Willis are shown in Figure 2d and 2e. Extensive changes in the media with fibrin and collagenous deposits and a large increase in the lumen diameter had occurred (Figure 2e).

The incidence of ICV lesions and aneurysms is shown in Figure 3. In general, groups that developed ICV lesions presented a low incidence of aneurysms in the circle of Willis. As previously reported, BN hypertensive rats showed a high incidence (approximately 80%) of ICV lesions, approximately 20% of which were large cerebral hemorrhages, whereas in the LE group only 1 rat showed a small lesion. Both ICV lesion incidence and grades of severity were significantly higher in BN than in LE rats ($P\leq0.001$ by $\chi^2$ analysis). In F1 hybrids, a high incidence of ICV lesions, not significantly different from that in the BN group, was observed, with a similar occurrence of the various degrees of severity. In the second-generation groups, the F2 and BC(F1XLE) rats developed much fewer and less severe ICV lesions ($P\leq0.001$) than BN and F1 rats, whereas BC(F1XBN) rats developed similar levels. BC(F1XBN) rats showed higher levels of ICV lesions and higher degrees of severity than F2 and BC(F1XLE) rats ($P\leq0.001$). As for the incidence of aneurysms in the arteries of the circle of Willis (Figure 3), in agreement with previous data,
BN rats did not develop aneurysms, whereas approximately 25% of LE rats displayed cerebral aneurysms. In F1 hybrids, no aneurysms were observed. The aneurysmal incidence was lower in BC(F1XB) rats than in rats from F2 (P<0.01) and BC(F1XLE) (P<0.001) cohorts. The proportion of rats that developed aneurysms was higher in F2 (42%) and in BC(F1XLE) (50%) rats than in LE rats (25%), although differences were not statistically significant by χ² analysis. The percentage of ruptured aneurysms was increased in F2 (59%) and in BC(F1XLE) (75%; P<0.05) groups compared with the LE group (20%).

The size of aneurysms (only measured in F2 and BC(F1XLE) rats; n=28) varied in the different animals. Aneurysmal rupture occurred in aneurysms of the largest size, ie, ruptured aneurysms (mean diameter=2.9±1.4 mm) were significantly larger (P<0.001 by Student’s t test) than unruptured aneurysms (mean diameter=0.9±1.1 mm).

The time after deoxycorticosterone acetate–salt treatment corresponding to 50% mortality was 14 weeks for BN rats, 17 weeks for LE rats, 12 weeks for F1 rats, 18 weeks for F2 rats, 17 weeks for BC(XBN) rats, and 14 weeks for BC(XLE) rats.

IEL Ruptures, Blood Pressure, Plasma ACE Activity and ICV Lesions in BC(F1XBN) Rats

BC(F1XB) rats were chosen for this part of the study because they were the only second-generation group to present considerable numbers of ICV lesions of varying severity as well as a large range of numbers of IEL ruptures.

The Table shows in BC(F1XB), without or with ICV of different severity grades, the mean values per group of the total number of IEL ruptures in the abdominal aorta and the renal artery, quantified at autopsy; peak and mean values of systolic blood pressure recorded weekly throughout the experimental period; and plasma ACE activity measured before induction of hypertension.

Figure 4 illustrates en face preparations of abdominal aortas from BC(F1XB) rats with a low (Figure 4a) and a high (Figure 4b) incidence of ruptures of the IEL. There were no statistically significant differences between the level of IEL ruptures in either of the arteries studied in BC rats without ICV lesions and in those with the highest degree of ICV lesions. Indeed, several rats with large hemorrhagic lesions displayed very few IEL ruptures (1 rat showed only 7 IEL ruptures in its abdominal aorta), while several rats without ICV showed high levels of IEL ruptures (1 rat showed 47 IEL ruptures in the abdominal aorta). A significantly decreased number of IEL ruptures in the renal artery was observed even in the low and intermediate ICV lesion groups (grades 1 and 2) compared with the group without ICV lesions.

As for the level of plasma ACE activity, measured before the start of the experiment, there was no significant difference between groups. Moreover, there was no correlation between plasma ACE activity and levels of IEL ruptures in either of the arteries studied.
However, in BC groups with the 2 highest degrees of ICV lesion severity (grades 2 and 3), a significantly ($P \leq 0.001$) higher peak value of blood pressure was found compared with the group of BC rats lacking ICV lesions. BC rats with the intermediate degree of severity of ICV lesions also showed a statistically higher mean value of all recorded blood pressure measurements than BC rats without lesions ($P \leq 0.001$).

**Discussion**

The involvement of genetically controlled parameters in the variability of the phenotypic expression in different pathophysiological mechanisms has been shown by the comparison of phenotypes in various rat strains. The BN differs from other rat strains with respect to many phenotypic characters. In the present study we used the contrasting susceptibilities of BN and LE rat strains for 2 distinct experimentally induced cerebrovascular pathologies, hemorrhagic vascular lesions in the cerebral cortex and aneurysms in arteries of the circle of Willis, to reveal the capacity of the transmission of these 2 pathological phenotypes by studying the progeny resulting from different crosses between these 2 rat strains and to investigate any possible link between ICV pathology and the susceptibility to IEL rupture or the level of plasma ACE activity as a marker of ACE genotype, 2 characters that also differ markedly between BN or the level of plasma ACE activity as a marker of ACE gene polymorphism, which partly determines ACE plasma levels, and stroke has led to inconclusive data. The previously established difference in levels of plasma ACE activity between BN and LE rats offers the opportunity to study a possible predictive role for the genetically determined basal plasma ACE activity in ICV pathology in the rat. This was investigated in 2 consecutive series of BC(F1XBN) rats. Our results showed that plasma ACE activity measured before surgical intervention did not differ between the group lacking ICV lesions and the 2 groups with the highest degrees of lesion severity. Thus, the basal level of plasma ACE activity does not appear to be a predictor of the occurrence of ICV pathology (mostly of a hemorrhagic type) in the hypertensive rat. A recent large prospective study had reported that the ACE gene polymorphism is not associated with the subsequent risk of stroke, which is, however, primarily of the ischemic type in humans.

The level of systolic blood pressure was clearly determinate in the occurrence of ICV lesions, which was related to the mean value of systolic blood pressure recorded during the experimental period, and the degree of severity of ICV lesions was related to the peak blood pressure. In humans, hypertension is considered a main risk factor of spontaneous...
intracerebral hemorrhage. High levels of blood pressure are also determinant for ICV lesions in stroke-prone SHR, although they are not the only parameter involved. In renovascular hypertensive rats, the level and duration of high blood pressure also affect stroke onset. In addition, the chronic denervation of 1 cerebral hemisphere in stroke-prone SHR increases the incidence of stroke, and although cerebral blood flow is similar in both innervated and denervated hemispheres under control conditions, it is increased in the denervated hemisphere when the arterial pressure is acutely raised.

As for cerebral aneurysms in the circle of Willis, first we confirmed the susceptibility of the LE rat to the induction of cerebral aneurysms with another model of experimental hypertension, uninephrectomy and deoxyxycorticosterone acetate–salt, distinct from the previously used 2-kidney, 1 clip Goldblatt model. In the groups of BC(F1XBN) rats in which blood pressure was measured, no difference in blood pressure was found between rats with and without aneurysms. However, in this group the number of rats displaying aneurysms was small (n = 8). Nevertheless, this suggests that the formation of aneurysms in the circle of Willis of hypertensive rats is more dependent on genetic factors than on the level of blood pressure. This agrees with our previous data showing that normotensive LE rats developed more aneurysms than normotensive BN rats.

In F1 hybrids (BNXLE), no cerebral aneurysms developed, suggesting a recessive mode of inheritance for this phenotype. However, our data may be explained by the fact that numerous F1 rats showed ICV lesions and did not survive long enough to develop aneurysms. In F2 and BC(F1XLE) groups, more rats developed aneurysms and showed a greater susceptibility to their rupture than LE rats, although differences were only statistically significant for rupture. This suggests that the combination of BN genes with those of LE rats tends to enhance the susceptibility to develop cerebral aneurysms and clearly confers a greater susceptibility to their rupture, thus having an additive effect on the phenotypic expression of cerebral aneurysmal disease in the rat. It may be assumed that the genes involved in the vascular fragility in the BN rat may play a role in aneurysmal rupture when associated with the genome of a strain susceptible to the formation of cerebral aneurysms.

Our results concerning the heritability of 2 distinct cerebrovascular diseases in progeny resulting from crosses between rat strains of very different phenotypes may provide a basis for linkage analysis studies in new rat models, which may lead to the identification of genes involved in the pathogenesis of these 2 distinct cerebrovascular diseases.

Acknowledgments
This work was supported by Institut National de la Santé et de la Recherche Médicale (INSERM). The authors would like to thank Catherine Chollet for technical assistance, Liliane Louedec for help in animal husbandry, and Sylvain Roger for photography.

References
The development of animal models of genetically determined cerebrovascular disorders may be helpful in understanding the mechanisms of human inherited disorders. In the study published above, Coutard and colleagues found that Brown Norway rats are more susceptible to the development of intracerebral hemorrhagic lesions, when they are made hypertensive, than Long-Evans rats. In contrast, the latter strain is prone to the development of aneurysms in the circle of Willis under conditions of disturbed flow induced by ligation of 1 carotid artery. To understand the possible mechanism of inheritance of the intracerebral lesions and of the aneurysms, these investigators mated rats from the 2 strains to produce first-generation hybrids. Additionally, they created second-generation hybrids by mating the first-generation hybrids and also created backcross strains by mating first-generation hybrids with the original Brown Norway or Long-Evans rats. The incidence of the intracerebral hemorrhagic lesions enabled them to tentatively conclude that the intracerebral lesions are inherited as a dominant trait, but more than 1 gene is responsible for these lesions.

The results, with respect to the aneurysms of the circle of Willis, were less conclusive, probably because the hybrids died at an earlier stage owing to the presence of intracerebral hemorrhage. The data, nevertheless, suggested that the inheritance mode of the aneurysms was via recessive genes. The authors suggested that subsequent studies may enable the identification of the specific genes involved in determining these disorders. Overall, the data show the great value of genetic techniques in the study of cerebral vascular disorders.

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Stroke. 2000;31:2678-2684
doi: 10.1161/01.STR.31.11.2678

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/31/11/2678

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