Rupture of the Internal Elastic Lamina and Vascular Fragility in Stroke-Prone Spontaneously Hypertensive Rats

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We studied a possible relation between stroke and an enhanced susceptibility to rupture of the arterial internal elastic lamina by comparing stroke-prone spontaneously hypertensive rats with spontaneously hypertensive rats, which have a very low incidence of stroke. We quantified interruptions in the internal elastic lamina in certain arteries and studied the effect of β-aminopropionitrile, an inhibitor of cross-link formation in collagen and elastic fibers, on rupture of the internal elastic lamina and on mortality in these two substrains. To eliminate any influence of higher blood pressure in the stroke-prone rats on the parameters studied, we used antihypertensive treatment to obtain equivalent blood pressures in the two substrains. Results showed that stroke sensitivity was associated with an enhanced early spontaneous rupture of the internal elastic lamina in the caudal artery, an increased susceptibility to β-aminopropionitrile–induced rupture of the internal elastic lamina, and earlier mortality, mainly from aortic rupture, under β-aminopropionitrile treatment. These findings suggest that stroke-prone rats have an enhanced minor connective tissue defect that is expressed by rupture of the internal elastic lamina and may be related, at least in part, to their greater vascular fragility and increased susceptibility to stroke. (Stroke 1991;22:510–515)

Spontaneously hypertensive rats of the stroke-prone substrain (SHRSP) have been shown to be susceptible to the early development of cerebral vascular lesions and are largely considered as a good model for stroke in humans. This substrain was established from the strain of spontaneously hypertensive rats (SHR). Although the severe hypertension and its rapid onset have been shown to be determinant in the occurrence of stroke in SHRSP, other factors such as those related to renal vascular changes, a genetically determined increased cerebrovascular permeability, or an increased vascular fragility may also be implicated.

Spontaneous rupture of the internal elastic lamina occurs in some arteries of rats. In the caudal artery many interruptions of the arterial internal elastic lamina form, especially during the period of rapid growth, whereas in the renal artery they form to a lesser extent and later in life. The propensity for rupture of the arterial internal elastic lamina appears to be in part genetically determined but is also influenced by hemodynamics and environmental factors. In addition, previous studies performed under conditions of hypercholesterolemia and hypertension have suggested that interruptions in the internal elastic lamina may represent areas of structural weakness in the arterial wall and may be relevant to pathologic events later in life. It has recently been shown in rats that thinning and fragmentation of the internal elastic lamina in some cerebral arteries occur at sites that later develop cerebral aneurysms under experimental conditions. It is not clear whether the size of defects in the internal elastic lamina is related to the degree of intimal thickening. Thus, alterations of the internal elastic lamina may be relevant to some aspects of vascular pathology.

β-Aminopropionitrile (BAPN) is a specific inhibitor of the enzyme lysyl oxidase, which is implicated in the formation of cross-links during the synthesis of elastic and collagen fibers. Gaps in the arterial internal elastic lamina morphologically similar to those occurring spontaneously are prematurely induced in weanling rats by BAPN. If this agent is given later in life (5 weeks of age or later), it significantly increases the frequency of rupture of the internal elastic lamina. In addition, recent studies have shown that a genetic susceptibility to rupture of the internal elastic lamina is associated with a decreased aortic lysyl oxidase activi-
studied using five SHRSP and five SHR fed the converting enzyme inhibitor captopril (Squibb France, BAPN free base/kg body wt. All rats were given tap water ad libitum. Arterial blood pressure was measured the week before sacrifice using a tail cuff and pulse transducer. Caudal and renal arteries were fixed by in vivo perfusion of glutaraldehyde under pentobarbital anesthesia and processed for quantification of interruptions in the internal elastic lamina in caudal and renal arteries.

Materials and Methods

For all experiments, SHRSP and SHR were supplied by either the Centre de Sélection et d'Élevage d'Animaux de Laboratoire—Centre National de la Recherche Scientifique (Orléans, France) or IFFA-CREDO (St. Germain sur l'Arbresle, France). We studied the incidence of spontaneous rupture of the internal elastic lamina in caudal and renal arteries using six SHRSP and six SHR 14 weeks of age and five SHRSP and five SHR 25 weeks of age. The rats were fed normal laboratory chow (105, UAR, Villemoisson/Orge, France) and given tap water ad libitum. Arterial blood pressure was measured the week before sacrifice using a tail cuff and pulse transducer. Caudal and renal arteries were fixed by in vivo perfusion of glutaraldehyde under pentobarbital anesthesia and processed for quantification of interruptions in the internal elastic lamina as previously described.81

The effect of antihypertensive therapy on spontaneous rupture of the internal elastic lamina in the caudal artery was evaluated in 24 SHRSP and 24 SHR. Five-week-old rats of each strain were separated into four groups of six rats each: group 1 received 100 mg/kg/day i.p. of the angiotensin I-converting enzyme inhibitor captopril (Squibb France, Neuilly/Seine), group 2 received 5 mg/kg/day i.p. of the smooth muscle cell relaxant hydralazine (Sigma Chemical Co., St. Louis), group 3 received 200 mg/kg/day by gavage of the β-blocker atenolol (ICI Pharma, Cergy, France), and group 4 served as controls and received a daily intraperitoneal injection of 0.9% NaCl. Throughout the experimental period (from 5 to 12–13 weeks of age) all rats were given normal laboratory chow with tap water to drink. Blood pressure was measured every 2 weeks. During the experiment one group 3 SHR, one group 1 SHR, and one group 1 SHRSP died. At 12 or 13 weeks of age, the caudal arteries were fixed and processed to quantify interruptions in the internal elastic lamina. The effects of BAPN on this phenomenon were studied by feeding the rats normal laboratory chow supplemented with 1.5 g BAPN monofumarate/kg chow. Rats were ingested daily approximately 100 mg BAPN free base/kg body wt. All rats were given tap water to drink.

The induction of BAPN of premature rupture of the internal elastic lamina in the caudal artery was studied using five SHRSP and five SHR fed the BAPN-containing diet from weaning (3 weeks of age) until the age of 5 weeks. In addition, three SHRSP and three SHR 5 weeks of age served as controls for the quantification of interruptions in the caudal artery internal elastic lamina.

The effects of BAPN on rupture of the internal elastic lamina and mortality in SHRSP and SHR were studied in three consecutive experiments. In experiment 1, 14 SHR (5–7 weeks old) and seven SHRSP (5–6 weeks old) served as controls, and the same number received the BAPN-supplemented diet. When all BAPN-treated SHRSP had died, surviving rats in all groups were killed for a comparative study of the incidence of interruptions in the internal elastic lamina in the caudal and renal arteries.

In experiment 2, BAPN was given later in life (i.e., from about 10 weeks). For the study of mortality, nine SHRSP and seven SHR received the BAPN-supplemented diet and seven SHRSP and six SHR received normal laboratory chow. Blood pressure and body weight were recorded monthly. Two BAPN-treated SHR died accidentally during the experiment. In addition, four SHRSP and four SHR were given the BAPN-supplemented diet from about 10 to 25 weeks of age, and the incidence of interruptions in the internal elastic lamina was compared with that of 25-week-old rats fed normal laboratory chow from the above-described experiment.

To eliminate the factor of higher blood pressure in SHRSP, in experiment 3 we compared the mortality of six SHRSP, the blood pressure of which had been lowered by the administration of 20–30 mg hydralazine/1 drinking water from 6 weeks of age, with the mortality of six SHR. Both groups of rats were fed the BAPN-supplemented diet from 7 weeks of age. The dose of hydralazine used was equivalent to that in the above-mentioned experiment using this compound and did not influence the formation of interruptions in the caudal artery internal elastic lamina. At 23 weeks of age, treatment with BAPN was stopped and the rats were given normal laboratory chow; at 25 weeks of age 1% NaCl was added to the drinking water to precipitate vascular events. Blood pressure and body weight were recorded weekly. Two SHR died accidentally during the experiment.

In all these mortality experiments, whenever possible (80% of cases), rats that died spontaneously were autopsied; several arteries were removed and fixed by immersion in glutaraldehyde solution for future microscopic examination.

The results of quantification are expressed as mean±SD number of interruptions in the internal elastic lamina per centimeter of renal artery or mean±SD percentage length of caudal artery lacking the internal elastic lamina. To compare mean values between groups, the Mann-Whitney U test was used when the parameters are expressed as percentages and Student's t test was used in other cases.

Results

At 14 weeks of age the incidence of spontaneous rupture of the caudal artery internal elastic lamina...
FIGURE 1. Longitudinal semithin sections of caudal artery from rats aged 14 weeks stained with toluidine blue (×115). Arrows indicate interruptions in internal elastic lamina (IEL). a: Stroke-prone spontaneously hypertensive rat; b: spontaneously hypertensive rat.

was significantly higher in SHRSP (27±3%) than in age-matched SHR (17±4%) (Figure 1), whereas at 25 weeks no difference was observed (Figure 2). In the renal artery, no difference in the number of spontaneous interruptions in the internal elastic lamina was observed at either age (14 weeks: SHRSP, 0.5±0.5/cm; SHR, 1.0±1.0/cm; 25 weeks, see Figure 2). Blood pressure was higher and body weight lower in SHRSP than in SHR. Treatment with the three different antihypertensive agents decreased the rise in blood pressure in both substrains, but at the time of sacrifice group 3 SHRSP and group 2 SHR showed blood pressures not significantly different from those of their respective group 4 controls. Results showed that the decrease in the rise and level of blood pressure achieved in all treated groups except those mentioned above did not modify rupture of the caudal artery internal elastic lamina within each substrate (Table 1).

The administration of BAPN from 3 to 5 weeks of age led to an increase in interruptions of the internal elastic lamina in the caudal artery that was much greater in SHRSP (37 times that in age-matched untreated SHRSP) than in SHR (11 times that in untreated SHR).

Mortality and rupture of the internal elastic lamina in BAPN-treated SHRSP and SHR in experiment 1 are summarized in Table 2. When rats had received BAPN from 5 weeks of age, the percentage length of caudal artery lacking the internal elastic lamina was increased in treated SHRSP compared with treated SHR, and at 26 weeks of age 100% of the treated SHRSP had died versus only 43% of the treated SHR. Aortic rupture was responsible for death in the rats in which autopsy was possible.

In experiment 2, the treatment of rats with BAPN from 10 weeks of age (when the bulk of growth has been achieved) to 25 weeks still increased rupture of the internal elastic lamina in the caudal artery (Figure 2). In the renal artery, BAPN significantly increased the number of interruptions in the internal elastic lamina in SHRSP but not in SHR. However, although the number of interruptions was higher in BAPN-treated SHRSP than in treated SHR, the difference was not significant.

When BAPN treatment was started at 10 weeks of age, SHRSP died earlier than either BAPN-treated SHR or control SHRSP. All BAPN-treated SHRSP had died by 59 weeks of age compared with only 40% of the BAPN-treated SHR. The mean±SD age at death was 43±9 weeks for BAPN-treated SHRSP, 75±16 weeks for BAPN-treated SHR (p<0.01 compared with BAPN-treated SHRSP), 65±3 weeks for untreated SHRSP (p<0.001 compared with BAPN-treated SHRSP), and 93±8 weeks for untreated SHR (p<0.001 compared with BAPN-treated SHR). In experiment 2, although the time of death was delayed compared with experiment 1, aortic rupture was still
the major cause of death. As shown in Figure 3, blood pressure of BAPN-treated SHRSP throughout most of the experimental period was significantly higher than that of BAPN-treated SHR and thus might play a role in the occurrence of aortic rupture. Nevertheless, BAPN-treated SHRSP had similar or even lower blood pressures than their untreated controls but died earlier.

Results of experiment 3 showed that when the blood pressure of BAPN-treated SHRSP was reduced with hydralazine (Figure 4), the rats survived for >1 year but still died earlier than BAPN-treated SHR. However, the cause of death was no longer aortic rupture; rats died of a variety of pathologies. Some SHRSP showed hind limb paralysis, which may be a consequence of stroke.

Discussion

Our results show that during the period of rapid growth (i.e., up to 14 weeks of age) more interruptions of the internal elastic lamina form spontaneously in the caudal artery of SHRSP than of SHR. Our data confirm the role of some genetically determined factor in the spontaneous rupture of the arterial internal elastic lamina, which has been suggested by the existence of a familial aggregation and various susceptibilities among rat strains. At 25 weeks of age, the difference in the incidence of interruptions in the internal elastic lamina was no longer apparent. This suggests that rupture of the internal elastic lamina in the caudal artery of SHRSP occurs at a higher rate than in SHR only during the period of rapid growth. In the renal artery, no difference was observed between SHRSP and SHR. We have previously shown that size of the kidney, which determines renal blood flow, is important to rupture of the internal elastic lamina in the renal artery. Since SHRSP had a lower body weight and thus smaller kidneys than SHR, this may explain the lack of difference in rupture of the renal artery internal elastic lamina between the two substrains. The formation of interruptions in the internal elastic lamina in the caudal artery did not increase greatly between 14 and 25 weeks of age, whereas it did in the renal artery, in accord with previous observations.

In SHRSP, the higher blood pressure appears not to be responsible for the enhanced spontaneous rupture of the caudal artery internal elastic lamina, which thus probably represents an intrinsic property of this rat substrain. These results are in agreement with previous data; we have shown in other rat models that hypertension does not increase rupture of the internal elastic lamina in the caudal artery.

The great increase in the number of interruptions in the internal elastic lamina observed in both substrains when BAPN was administered from weaning to 5 weeks of age confirmed our previous observation that

### Table 2. Incidence of Rupture of IEL in Caudal and Renal Arteries and Mortality at 26 Weeks of Age of β-Aminopropionitrile-Treated (From 5 Weeks of Age) and Control SHRSP and SHR

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<th>Treated</th>
<th>Control</th>
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<td><strong>Age (weeks)</strong></td>
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<td>SHRSP 16–26</td>
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<td>SHRSP 26–30</td>
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<tr>
<td>Mean±SD</td>
<td>57.4±3.9*</td>
<td>37.4±5.6</td>
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<td>Mortality at 26 weeks of age (%)</td>
<td>SHRSP 100</td>
<td>SHR 43</td>
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SHRSP, stroke-prone spontaneously hypertensive rats; SHR, spontaneously hypertensive rats; IEL, internal elastic lamina.

*p<0.001 different from SHR by Mann-Whitney U test.
this treatment induces premature rupture in the internal elastic lamina in the caudal artery. In addition, the greater BAPN-induced premature rupture of the internal elastic lamina in the caudal artery of SHRSP than of SHR suggests that the inhibition of lysyl oxidase during growth had a greater adverse effect on the internal elastic lamina of the caudal artery in SHRSP. Previous studies have also reported different sensitivities to BAPN treatment, concerning both aortic aneurysm formation and rupture and rupture of the internal elastic lamina, in different substrains.

In experiments in which BAPN was given from 5 or 10 weeks of age, most rats died of aortic rupture and not of stroke. Feeding BAPN to young animals is well known to induce aortic rupture and aneurysm formation in normotensive rats and in hypertensive turkeys. Recently, MacManus et al induced similar effects in hypertensive rats with low doses of BAPN, which are without effect in normotensive rats.

Although a previous study has reported that BAPN given to adult rats has no effect on aortic structure, our results show that BAPN treatment from 10 to 25 weeks of age still increased rupture of the internal elastic lamina in the caudal and renal arteries (suggesting that some synthesis of elastic fibers still occurs during this period of life). However, no significant difference was observed between SHRSP and SHR, and results were similar to those obtained in control rats of the same age. This suggests that in SHRSP the proposed enhanced connective tissue anomaly, which is expressed by increased rupture of the internal elastic lamina in the caudal artery, is revealed only during the period of rapid growth.

The earlier death of BAPN-treated rats compared with untreated rats while they displayed similar or lower blood pressures shows that an increased rupture of the internal elastic lamina is associated with enhanced vascular fragility. The decrease in blood pressure observed in BAPN-treated rats is in accord with previously published results and is probably due to an increase in arterial distensibility directly related to BAPN’s effect of decreasing cross-linking in newly synthesized collagen and elastin.

Our data do not allow us to relate clearly the increased susceptibility to rupture of the internal elastic lamina and the incidence of stroke. However, since SHRSP showed an enhanced propensity for both spontaneous and BAPN-induced rupture of the arterial internal elastic lamina (which is revealed during the period of rapid growth), we may assume that an increased susceptibility to rupture of the internal elastic lamina in SHRSP is associated with

**FIGURE 3.** Systolic blood pressure as function of age in spontaneously hypertensive rats (circles) and stroke-prone spontaneously hypertensive rats (triangles) either untreated (open symbols) or treated with β-aminohippurinic acid (filled symbols) from 10 weeks of age. **p ≤ 0.05 - 0.001 different from β-aminohippurinic acid-treated stroke-prone spontaneously hypertensive rats of same age by Student’s t test.**

**FIGURE 4.** Systolic blood pressure as function of age in β-aminohippurinate (BAPN)-treated stroke-prone spontaneously hypertensive rats receiving hydralazine (---, ▲) and BAPN-treated spontaneously hypertensive rats (---, O). H, start of hydralazine treatment (20–30 mg/l drinking water) for ▲ only. For both groups: B2, addition of 0.75 g BAPN/kg food; B3, addition of 1.5 g BAPN/kg food; B3, BAPN treatment stopped; S, addition of 1% NaCl in drinking water. **p ≤ 0.05 - 0.001 different from ▲ by Student’s t test.**
an enhanced arterial fragility compared with stroke-resistant SHR. This is in agreement with a unique previous study comparing the physical characteristics of the aorta of SHRSP and SHR from the age of 4 months; the study suggested that the vasculature of SHRSP is more brittle. In addition, compared with SHR, SHRSP showed some biochemical alterations of collagenous protein from the aorta and mesenteric arteries. These molecular differences may account for the increased vascular fragility of the stroke-sensitive substrain.

In a previous study using experimentally induced hypertensive Brown Norway and Long-Evans rats (which show respectively a high and a low susceptibility to spontaneous rupture of the arterial internal elastic lamina), the survival rate was lower in the Brown Norway rats, and some Brown Norway rats showed signs of cerebral hemorrhage. Our present data on spontaneously hypertensive rats are in agreement with these previous results, suggesting that a high propensity for the spontaneous rupture of the arterial internal elastic lamina is related to increased vascular fragility, which under adverse conditions such as hypertension may lead to pathologic events.

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References

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