point, and angles between two lines are calculated. Changes of the respective distances or angles in a series showing the moving jaws during mastication thus reproduce the movement pattern.

Efficiency of pin implantation

The use of dental pins as markers in cineradiography allows an exact procedure in measurements and analysis in small animals. Their standardized size, and shape, and the stability of their position, make all marks comparable, which is an advantage over the amalgam or lead markers which were formerly used. Moreover, from one pin two marks are established. This is especially important for the fixation of markers in small objects. The best measurable point can thus be chosen. Simultaneous application of several pins per moving element allows determination of more marker points, thus decreasing the error of interpretation. Furthermore, the reproducibility of measurements is greatly enlarged because the form of the pins is stable.

The use of a hand driver with the pin and the linked plastic shank is very effective for surgical installation in small objects. Screws, as have been applied in *Tenrec*[6] or friction grip pins[11,12] are difficult to manipulate.

The dental gold plated pins do not produce adverse tissue reactions. For normal implantation into the compact bone adhesive, which often damages the cells, is not necessary. Therefore, pins can remain in the animal for life. Our observations in living animals show that the pins are encapsulated within a few days so the surrounding chewing muscles and vessels are soon protected against friction.

We expect that the implantation of self-shearing pins will also be useful in other movement studies using cineradiography for example studies of rib movements during respiration, or gill arch movements of fishes.

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Blood pressure and impairment of endothelium-dependent relaxation in spontaneously hypertensive rats

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Summary. Correlation between hypertension and impairment of endothelium-dependent relaxation was demonstrated using aortae from certain strains of rats with various levels of spontaneous hypertension. It was also observed that the impairment of endothelium-dependent relaxation is the secondary change due to hypertension, and the level and duration of hypertension is the determinant factor of the impairment.
Key words. Spontaneously hypertensive rats; strains: ages; aorta; endothelium-dependent relaxation.

Endothelium-dependent relaxation in animal models of genetic hypertension has been reported to be impaired as compared with that of control normotensive animals[1–11]. This may indicate the involvement of a genetically-induced impairment of endothelium in the initiation of hypertension. However, the decreased endothelium-dependent relaxation can also be observed in thoracic aortae that have been made hypertensive by coarctation[11]. In addition, the decreased endothelium-dependent relaxation of blood vessels of hypertensive rats can be normalized by antihypertensive treatment[8,11]. These two reports[8,11] indicate that impairment is secondary to elevated blood pressure. Thus, the contribution of decreased endothelium-dependent relaxing activity in the initiation of hypertension is still controversial. In the present study, the age-related impairment of endothelium-dependent relaxation was studied using different strains of spontaneously hypertensive rats with various levels of blood pressure. All of the strains used in the present study are genetically established from the same origin; Wistar Kyoto rats (WKY)[12]. We report here that the impairment of endothelium-dependent re-

laxation is directly related to both the degree and the duration of hypertension.

Materials and methods

Wistar Kyoto rats (WKY), conventional spontaneously hypertensive rats (SHR), stroke-prone SHR (SHRSP) and a malignant type of SHRSP (M-SHRSP) were originally obtained from Dr Okamoto[12] and successively bred in our animal facility. Great care was taken in breeding each strain to maintain its respective blood pressure. Systolic blood pressure was measured by the tail cuff method. Briefly, animals were preheated at 40 °C for 5 min and then the systolic blood pressures were measured automatically by a rat blood pressure instrument (Natume, Tokyo). The width of the tail cuff was 10 or 15 mm.

In a series of experiments, SHRSP were subjected to an antihypertensive treatment with oral administration of hydralazine hydrochloride (Apresoline®). This treatment was instituted at the weaning period (5 weeks of age) to control the development of hypertension, and was continued until the animals were sacrificed at the age of 16 weeks. The concentration of the drug in the drinking water was 40 mg/l for ages between 5 and 10 weeks and 60 mg/l for ages between 11 and 16 weeks. Thoracic aortae were dissected from the animals and ring preparations (1 mm wide) were made, avoiding the branched portion. In some experiments the endothelium was removed by rubbing the lumen of the aorta with soft rubber. The preparations were mounted in organ baths filled with a modified Tyrode's solution using thin tungsten wire (diameter 30 µm). The other end of the tungsten wire was connected to a force-displacement transducer (Shinkoh, U-gage). The composition of the modified Tyrode's solution was as follows: NaCl, 137 mM; KCl, 5.4 mM; $CaCl_2$, 2.0 mM; $MgCl_2$, 1.0 mM; $NaHCO_3$, 11.9 mM; NaH_2PO_4, 0.4 mM; glucose, 5.6 mM. It was equilibrated with a gas mixture of 95% O_2 and 5% CO_2 at 36 °C. Tension changes were measured isometrically at the basal stretch tension of 800 mg.

Statistics. All results are given as mean ± SE. Mann-Whitney U-test and two-way analysis of variance were used to assess statistical significance, and p values less than 0.01 were considered to be significant in the present studies.

Results and discussion

The systolic blood pressures of each strain of rats at various ages are shown in figure 1. The systolic blood pressure of all animal strains increased with age. The increase was steepest in M-SHRSP, and the blood pressure at the age of 12 weeks of age was 262 ± 7 mmHg (n = 11). However, no further measurement of the

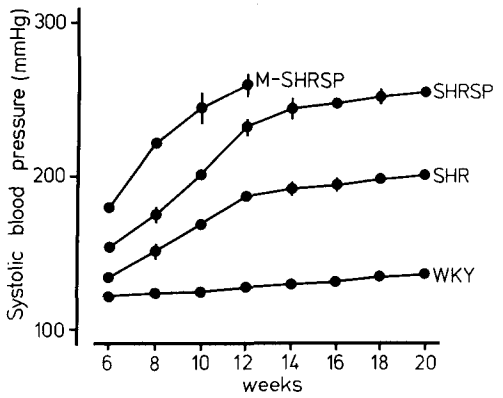

Figure 1. Relationship between age and systolic blood pressure of various strains of spontaneously hypertensive rats and normotensive rats. SHR, SHRSP, M-SHRSP and WKY indicate spontaneously hypertensive rats, stroke-prone SHR, malignant strain of SHRSP and Wistar Kyoto rats, respectively. Blood pressure of M-SHRSP over 16 weeks of age could not be measured because of the high incidence of stroke. Each point represents mean value of 7 to 22 rats with ± SE. Variation in age-dependent elevation of the blood pressure among these four strains were statistically significant ($p < 0.01$).

blood pressure in this strain could be performed, because the incidence of stroke increased and this influenced the blood pressure markedly. In WKY, on the other hand, the blood pressure rose slowly and reached 137 ± 2 mmHg (n = 17) 20 weeks after birth. The age-dependent elevations in the blood pressure of SHRSP and SHR lie between the curves for the M-SHRSP and WKY. Statistical analysis revealed that the difference in the age-dependent elevation of the blood pressure among these strains was significant ($p < 0.01$).

The endothelium-dependent relaxation was obtained by applying 10^{-5} M acetylcholine to aortic preparations contracted in the presence of 5×10^{-7} M noradrenaline. It was ascertained in noncumulative experiments that the concentration of acetylcholine (10^{-5} M) was maximal and supramaximal respectively in WKY and SHRSP preparation. It was also ascertained that relaxation disappeared with the removal of the endothelium.

The endothelium-dependent relaxation induced by acetylcholine decreased with the increase of age in all preparations. The decrease in relaxation was fastest in the M-SHRSP aorta, which showed a marked decrease 12 weeks after birth (fig. 2). The endothelium-dependent relaxation of SHRSP began to decrease at the age of 12 weeks, and that of SHR, between 12 and 16 weeks of age. In the WKY aorta, on the other hand, no significant decrease in the relaxation could be observed before the age of 16 weeks, and only a slight decrease was observed at 20 weeks of age (fig. 2). In age-matched groups, the impairment of endothelium-dependent relaxation occurred in the following order; M-SHRSP > SHRSP > SHR > WKY. The variation in the age-dependent decrease in the relaxation among the preparations from these strains was statistically significant ($p < 0.01$) as ascertained by two-way analysis of

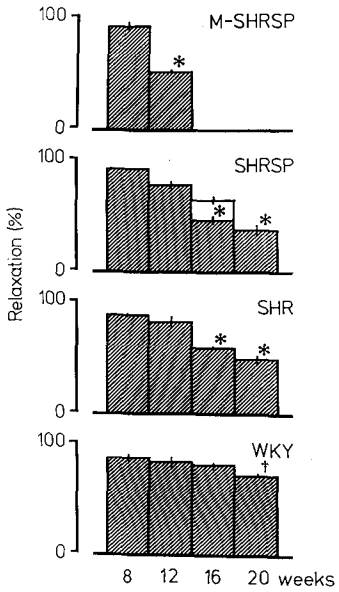

Figure 2. Endothelium-dependent relaxation of aortic rings at various ages. Relaxation was expressed as percentages of the amplitude of the contraction induced by 5×10^{-7} M noradrenaline (mean ± SE, n = 7 to 19). Experiments could not be performed on the aorta of M-SHRSP of 16 to 20 weeks of age, because of the high rate of stroke. The open part of the SHRSP column at 16 weeks indicates increased relaxation brought about by antihypertensive treatment. Aortic rings were contracted by the application of 5×10^{-7} M noradrenaline and 10 min after the contraction achieved its peak, 10^{-5} M acetylcholine was applied to induce endothelium-dependent relaxation. The maximum amplitude of relaxations was measured. The concentrations of the drugs were chosen to produce maximal relaxant responses. * significantly different from the value of WKY of respective age (Mann-Whitney U-test, $p < 0.01$). †significantly different from the value of 8-week-old WKY (Mann-Whitney U-test, $p < 0.01$). The differences in age-dependent decrease in the relaxation among these four strains were statistically significant (Two-way analysis of variance, $p < 0.01$).

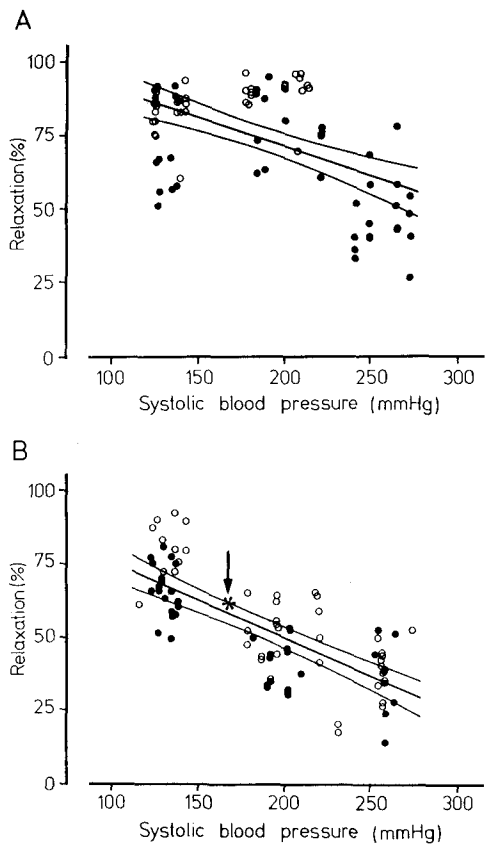

Figure 3. Correlation between blood pressure and endothelium-dependent relaxation. Relaxation was induced by 10^{-5} M acetylcholine and expressed as percentage of the contraction induced by 5×10^{-7} M noradrenaline. All strains of rats are included in this figure. *A* 8-(open circles) and 12-(closed circles) week-old rats. *B* 16- (open circles) and 20- (closed circles) week-old rats. Regression lines are also indicated with area of 99% reliability. Coefficients of correlation are −0.551 ($p < 0.01$) and −0.700 ($p < 0.01$), respectively in *A* and *B*. Asterisk indicated by arrow in *B* shows mean value of the endothelium-dependent relaxation at lowered blood pressure (mean value) of five hydralazine hydrochloride treated SHRSP of 16 weeks of age.

variance. The relationship between the systolic blood pressure and the relaxation induced by acetylcholine is shown in figure 3. The figure shows that the endothelium-dependent relaxation decreased as the blood pressure increased and that the decrease in endothelium-dependent relaxation correlated well with the elevation of blood pressure.

The effect of antihypertensive treatment was also studied by giving hydralazine hydrochloride to SHRSP, as described in materials and methods. The blood pressure of the 16-week-old treated SHRSP was 167 ± 3 mmHg (n = 5), which was significantly lower than the blood pressure of the untreated SHRSP (250 ± 3 mmHg, n = 14). The blood pressure of the treated SHRSP lies between those of the same age of SHR (196 ± 4 mmHg, n = 21) and WKY (133 ± 2 mmHg, n = 22). The endothelium-dependent relaxation of the aorta of treated SHRSP was significantly greater than that of untreated preparations ($p < 0.001$), and was found also to lie between the relaxations of age-matched SHR and WKY (fig. 2). This value is close to the regression line of the relationship between blood pressure and the degree of relaxation shown in figure 3. Thus, it was shown that a good correlation exists between blood pressure and the impairment of the endothelium-dependent relaxation especially in 16- and 20-week-old rats.

Sodium nitroprusside is known to act directly on vascular smooth muscle and induce relaxation. This relaxation is mediated by the production of cyclic GMP[13]. Endothelium-dependent relaxation is also known to be mediated by the production of cyclic GMP in the smooth muscle[14–16].

The relaxation of the endothelium-removed preparation of the SHRSP aorta induced by sodium nitroprusside was either identical with or greater than that of the WKY aorta (data are not shown). Similar results have been reported in the SHR aorta[6] and in SHRSP mesenteric artery[7]. Impairment of endothelium-dependent relaxation may, therefore, not be due to the change in the production of cyclic GMP or to the change in the response of smooth muscle to cyclic GMP. Several expla-

nations for the impairment of endothelium-dependent relaxation can be made: 1) structural damage of endothelium, which causes nonspecific impairment in the ability to produce EDRF, 2) reduced sensitivity to acetylcholine, 3) impairment in the coupling between endothelium and smooth muscle, 4) reduced sensitivity of smooth muscle to EDRF. Since maximal or supramaximal concentrations of acetylcholine, both for WKY and SHRSP preparations, were applied as described above, the difference in the sensitivity of endothelium to this drug would not be involved. Although no further experiment to investigate the mechanism of the impairment of endothelium-dependent relaxation was performed, the structural damage of the endothelium due to the exposure to hypertension[17,18] seems to be the most provable one among the possible causes of the impairment. Recovery from the morphological changes after antihypertensive treatment[18] is also coincident with the recovery of the relaxation observed in the present experiment.

The relationship between the blood pressure of various Kyoto strain rats and the impairment of endothelium-dependent relaxation indicates a causal relationship between blood pressure and the impairment of relaxation. When the blood pressure is high, impairment of the release of EDRF progresses faster than when blood pressure is low, as shown in the age-dependency experiments. It was also observed that the degree of the impairment of endothelium-dependent relaxation increased when the duration of exposure to high blood pressure increased (fig. 2). The effect of antihypertensive treatment also supports the possibility that the decreases in the endothelium-dependent relaxation in spontaneously hypertensive rats are not genetically controlled but are the secondary results of protracted high blood pressure. Thus it can be concluded that impairment of the endothelium cannot be a cause of the initiation of hypertension in spontaneously hypertensive rats of the Kyoto strain, although it may be able to accelerate the elevation of blood pressure.

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Superoxide anion scavenging effect and superoxide dismutase activity of *Ginkgo biloba* extract

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Summary. Ginkgo biloba extract is known to be efficient in diseases associated with free radical generation. The purpose of this work was to study, under in vitro conditions, the action of *Ginkgo biloba* extract (Gbe) against superoxide anion ($O_2^{\cdot-}$), which is directly or indirectly implicated in cell damage.

Gbe appears to have both an $O_2^{\cdot-}$ scavenging effect and also a superoxide dismutase activity. Its antiradical effect was demonstrated by low temperature electron spin resonance and in a non-enzymatic system (phenazine methosulfate-NADH), and its enzymatic activity was shown by polarographic determination.
Key words. Ginkgo biloba; superoxide anion; superoxide dismutase.