Hypertension Induced by Brain Grafts From Fetal Spontaneously Hypertensive Rats

Christian F. Deschepper, Jin-Sheng Li, Ernesto L. Schiffrin, Sharon A. Welner

Abstract Hypothalami from fetal rats were grafted into the third ventricle of four strains of adult rats. Grafts from spontaneously hypertensive rats (SHR), in contrast to grafts from Wistar-Kyoto (WKY) rats, induced an elevation of systolic blood pressure and a thickening of the media of resistance arteries along with corresponding alterations in the contractile properties of these vessels. However, no cardiac hypertrophy was observed. The resistance arteries of rats grafted with hypothalami from SHR also displayed functional alterations that were similar to what is typically found in the resistance arteries of young prehypertensive SHR, ie, an increase in the sensitivity to cocaine and an impairment in the ability to relax in the presence of acetylcholine. This suggests that the brain may play a causal role in these alterations. Histological examination of sections of brains grafted with previously labeled tissue revealed that (1) there was no brain area that was systematically infiltrated by grafts from SHR and not by grafts from WKY rats; (2) the volume of the transplants appeared larger 2 weeks after the graft than the volume of the tissue originally implanted; and (3) grafts from SHR were slightly larger, displayed more individual foci, and extended farther along the anteroposterior axis than grafts from WKY rats. In addition, gial cultures derived from the hypothalami of SHR had a higher in vitro growth rate than equivalent cultures from WKY rats. It is therefore possible that the ability of brain grafts from SHR to induce hypertension is related to a higher proliferative and/or migratory potential of mononuclear cells within the hypothalamus. (Hypertension. 1994;23[part 1]:765-773.)

Key Words • hypertension, experimental • rats, inbred SHR • brain tissue transplantation • hypothalamus • mesenteric arteries • vasoconstriction

The spontaneously hypertensive rat (SHR) is a genetic animal model generally believed to have many of the same characteristics as essential hypertension in humans. Consequently, many investigators have used this model to try to isolate possible causes of essential hypertension. Whereas there is general agreement that hypertension in SHR results from the interaction of a variety of factors, several lines of evidence indicate that the central nervous system plays an important role in the elevation of blood pressure. For instance, (1) the SHR exhibits a central hyperactivity that tends to elevate sympathetic activity in response to environmentally alerting stimuli; (2) intracerebroventricular administration of catecholamine neurotoxins prevents the development of hypertension in young SHR; (3) intracerebroventricular administration of angiotensin II antagonists, converting enzyme inhibitors, or monoclonal antibodies to atrial natriuretic peptide lowers blood pressure in adult SHR but not in control rats; (4) morphometric differences exist between the hypothalami of SHR and Wistar-Kyoto (WKY) rats; and (5) the hypothalami of SHR have increased Na,K-ATPase-inhibiting activity, which in turn is involved in sympathoexcitatory and pressor responses to sodium loading. Most of these studies suggest that the central defect in SHR is mainly present in the hypothalamic region. Recently, it was shown that transplantation of hypothalamic tissue from fetal SHR into the third ventricle of adult WKY rats induces hypertension in the host rats. This constitutes further evidence that hypothalamic abnormalities contribute at least partially to increases in blood pressure. However, many questions remain about how brain transplants may lead to hypertension.

Our goals were threefold. First, we wanted to investigate whether the model of brain graft-induced hypertension could be replicated independently in our laboratory. Second, we wanted to examine the extent of migration of the transplanted tissue into the grafted brain and use this information to formulate hypotheses about the central mechanisms leading to the development of hypertension in this model. Third, we wanted to test whether some of the functional and morphological changes found in the resistance arteries of SHR could also be found in SHR-transplanted rats.

Methods

Animal Procedures

All animal procedures were approved by the Clinical Research Institute of Montreal (IRCM) Animal Care Committee and conducted according to the recommendations of the Canadian Council on Animal Care. Eight-week-old male WKY or Sprague-Dawley rats and timed-pregnant SHR and WKY female rats were purchased from Taconic Farms. Eight-week-old male Wistar or Long-Evans rats were purchased from Charles River. All rats were housed at the IRCM animal care facility. They were maintained for 8 days before the experiment in a temperature-controlled room (22°C) with 60% humidity and a 12-hour light/dark photoperiod.

Pregnant mothers (either SHR or WKY) were killed by decapitation at the 16th day of gestation. Their embryos were quickly removed and placed on ice. The anterior hypothalami...
were dissected out under a stereomicroscope, using fine dissecting instruments, and placed in ice-cold Dulbecco’s modified essential medium (DMEM) supplemented with 10 mmol/L HEPES buffer containing 10 mg/mL Hoechst bisbenzimide 33342. Hoechst-phosphate-buffered saline containing 10% sucrose, frozen in isopentane cooled to —30°C with dry ice, and stored at —70°C until ready for further processing. A pneumatic pulse transducer was secured on the tail and connected to a calibrated polygraph.

The results presented in the present article originate from three experimental rat groups. Experiment 1 included 6 unoperated rats, 7 adult WKY rats implanted with WKY fetal hypothalamic tissue (G-WKY), and 7 adult WKY rats implanted with SHR fetal hypothalamic tissue (G-SHR). Blood pressure was measured in all rats 1 week before transplantation and then at repeated intervals. Measurements were made in conscious animals by tail plethysmography as follows. Rats were warmed to 37°C for 30 minutes and then placed in an acrylic restrainer. A pneumatic pulse transducer was secured on the tail and connected to a calibrated polygraph.

The results presented in the present article originate from three experimental rat groups. Experiment 1 included 6 unoperated rats, 7 adult WKY rats implanted with WKY fetal hypothalamic tissue (G-WKY), and 7 adult WKY rats implanted with SHR fetal hypothalamic tissue (G-SHR). Blood pressure was measured in all rats 1 week before the graft, all rats were killed by decapitation. Trunk blood was collected on EDTA for subsequent measurement of plasma atrial natriuretic factor concentration and plasma renin activity. Total body weight and heart weight were measured on a toploading balance. Arteries were dissected out from the mesentery and kept on ice for subsequent analysis on a myograph. Experiment 2 included 6 adult WKY rats implanted with WKY hypothalamic tissue and 24 adult rats from other strains (6 WKY, 6 Wistar, 6 Long-Evans, and 6 Sprague-Dawley) implanted with SHR hypothalamic tissue. Body weights and systolic blood pressure were measured 1 week before and 4 weeks after the graft. One day before death, all WKY host rats were briefly anesthetized with ether, and an arterial catheter was surgically placed into the carotid artery. After 24 hours of recovery, the animals were housed individually and allowed to rest 2 hours in a quiet room. Five hundred microliters of blood were then drawn from the arterial line for subsequent plasma catecholamine and corticosterone (ACTH) determinations.

A third group of WKY rats was used for histology. In this group the tissue to be transplanted was previously labeled by incubation for 1 hour at 37°C in HEPES-buffered DMEM containing 10 μg/mL Hoechst bisbenzimide 33342. Hoechst-labeled hypothalamic tissue (from either SHR or WKY fetal rats) was consequently implanted into 2x5 adult male WKY rats (five rats for each type of graft). After 2 weeks the rats were anesthetized with pentobarbital (50 mg/kg body wt) and perfused transcardially with 4% paraformaldehyde in phosphate-buffered saline. The brains were removed, postfixed in the same fixative for 6 hours at 4°C, rinsed overnight in phosphate-buffered saline containing 10% sucrose, frozen in isopentane cooled to —30°C with dry ice, and stored at —70°C until ready for further processing.

Histology

Frozen brains were mounted onto a cryostat chuck in sagittal orientation. Sectioning was started at the level of the anterior commissure/optic chiasm. Approximately 150 to 200 coronal sections (20 μm thick, three per slide) were collected onto gelatin-coated slides. This represented a 3- to 4-mm length of brain and ranged from the anterior up to the pretectal medial hypothalamus. One slide out of each three was stained with 0.5% cresyl violet and compared with a rat brain atlas. Sections spaced at 0.5-mm intervals were selected, and adjacent sections were examined under UV illumination for visualization of the transplanted tissue. In each section the cross-sectional area occupied by the transplanted tissue was determined by direct examination under the light microscope and comparison of the coordinates to a micron-engraved standard slide. By examining serial sections, we evaluated the general shape taken by the transplanted tissue mass (cylinder, sphere, cube, etc) and used this information to calculate the volume occupied by the transplanted tissue in each 0.5-mm interval. Calculations of the volume occupied by the transplanted tissue were then used to obtain a semiquantitative estimate of the volume occupied by the transplanted tissue in the entire 3 to 4 mm of brain region.

Biologic Variables in Plasma

Plasma catecholamines were measured by radioenzymatic assay, as described previously. Plasma renin activity was determined by radioimmunoassay measuring the amount of angiotensin I generated after plasma was incubated for 1 hour at 37°C. The radioimmunoassays for plasma atrial natriuretic factor and plasma ACTH were performed as described previously.

Cell Culture

Pregnant SHR or WKY rats were decapitated at the 21st day of gestation. After fetal brains were removed, the diencephalon and frontal cortex were dissected out and enzymatically dissociated to establish glial-enriched primary cultures exactly as described previously. These cultures (consisting mostly of astrocytes and containing very few contaminating neurons) were maintained in a 50:50 mixture of DMEM and Ham’s F-12 Nutrient Mix containing 10% fetal calf serum (Gibco Canada). Eight days after the initial dissociation, the cells were passaged with trypsin, seeded in 35-mm poly-lysine–coated wells (500 000 cells per well), and maintained in the same medium. Cells were collected 1, 2, 3, and 7 days after plating and sonicating them in 0.5 mL of buffer containing 20 mmol/L NaCl, 5 mmol/L EDTA, and 10 mmol/L Tris, pH 7.8. The DNA content in these samples was subsequently determined by measuring with a fluorometer the amount of fluorescence emitted in the presence of ethidium bromide using various concentrations of salmon sperm DNA as standards.

In Vitro Studies on Arteries

Third-order branch arteries were quickly isolated from the mesentric beds of decapitated rats and investigated as described in detail elsewhere. Briefly, vessels were mounted as ring preparations on isometric myographs (Living Systems Instrumentation) by threading them onto two tungsten wires. They were maintained in perfusing physiological salt solution warmed to 37°C and bubbled with 95% O2 and 5% CO2. The distance between the wires and wall and media widths were measured directly under a microscope with the vessels under no tension and set at 0.9 Lm (where Lm is the internal circumference the vessels would have had in vivo when relaxed and under a transmural pressure of 100 mm Hg). After a rest period the vessels were stimulated according to the following protocol: (1) a cumulative dose-response curve to norepinephrine (from 0.01 to 10 μmol/L, 3 minutes per concentration), (2) a similar curve in the presence of 3 μmol/L cocaine HCl, (3) a cumulative dose-response curve to vasopressin (from 10 pmol/L to 0.1 μmol/L), (4) a cumulative dose-response curve to endothelin-1 (ET-1, from 10 pmol/L to 0.1 μmol/L, 6 minutes per contraction), and (5) two cumulative dose-response curves to acetylcholine (from 1 mmol/L to 0.1 mmol/L) after contraction with 10 μmol/L norepinephrine, without or with prior (15 minutes) and concomitant exposure to 10 μmol/L indomethacin. After each activation the vessels were washed with physiological salt solution for 15 minutes. Media
I before graft after graft

FIG 1. Line graphs show time course of systolic pressure in rats from experiment 1. Weeks are calculated vs day of graft implantation (arrow). Each point corresponds to mean±SEM; n=6-7. WKY indicates Wistar-Kyoto rats; G-SHR, WKY host rats implanted with fetal hypothalamic tissue from spontaneously hypertensive rats (SHR); and G-WKY, WKY host rats implanted with WKY tissue. *P<.05 vs animals in the same group at week -1; **P<.05 vs other G-WKY of WKY at the same time point.

thickness, active media stress, active wall tension, and active effective pressure were calculated with the appropriate equations as described previously. With the use of the INPLOT program for IBM-compatible computers (GraphPad Software), the values from each dose-response investigation were fitted to a nonlinear curve using the logistic function. In addition, the program was used to calculate the pD2 value (defined as −log EC50) and the maximal response for each curve.

Calculations and Statistical Analyses

Statistical analyses were performed using the INSTAT statistical computer program. Differences across time in experiment 1 were tested by one-way ANOVA for repeated measures. In other comparisons involving more than two groups, means were compared by one-way ANOVA followed by the post hoc Newman-Keuls test to assess statistical significance. When two groups were compared, results were analyzed with the Student's t test or nonparametric Mann-Whitney U statistic test (experiment 3).

Results

Blood Pressure and Biologic Variables in Grafted Rats

In experiment 1 blood pressure was measured repeatedly before and after the day of transplantation (Fig 1). Within each group statistical differences over time were tested by one-way ANOVA for repeated measures. In either unoperated or G-WKY rats, blood pressure rose slightly over time, but the values were not significantly different. In contrast, blood pressures in the G-SHR group were significantly higher than they were before the graft (P<.05), starting from week 1. Differences between groups were tested by one-way ANOVA for every time point. At weeks 3 and 5, blood pressures of G-SHR were statistically higher (P<.01) than blood pressures of G-WKY or unoperated rats. No differences were found in the latter two groups at any time point. Additional biologic variables were measured 5 weeks after graft placement (Table 1). No statistically significant differences were detected in either body weight, corrected heart weight, plasma atrial natriuretic factor, or plasma renin activity.

In experiment 2 different rat strains were grafted with SHR hypothalamic tissue (Fig 2). Before the graft there was no statistical difference (as tested by one-way ANOVA) in blood pressure in any of the rat strains. Four weeks after the graft blood pressure was statistically higher (P<.01) in all rat groups grafted with SHR tissue than in WKY rats grafted with WKY tissue. The WKY rats grafted with either SHR or WKY tissue were catheterized, and arterial blood was collected for plasma catecholamine and ACTH determinations. Plasma values (mean±SEM) in G-WKY rats versus G-SHR were 0.64±0.02 versus 0.66±0.02 nmol/L for epinephrine, 2.3±0.02 versus 1.95±0.04 nmol/L for norepinephrine, and 16.8±2.25 versus 14±2 fmol/L for ACTH. No statistical differences were detected between the two groups on any of these measures.

Morphology and Contractile Responses of Resistance Arteries

Table 2 shows that media thickness and the mediolumen ratio were significantly higher in G-SHR than

<table>
<thead>
<tr>
<th>Animal</th>
<th>BP, mm Hg</th>
<th>Body weight, g</th>
<th>Heart, mg/100 g</th>
<th>ANF, fmol/mL</th>
<th>PRA, (nmol/L)/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untouched</td>
<td>108±3.8</td>
<td>440±8.1</td>
<td>345±14</td>
<td>77±5.7</td>
<td>2.45±0.37</td>
</tr>
<tr>
<td>G-WKY</td>
<td>115±4.0</td>
<td>468±12.4</td>
<td>312±11.4</td>
<td>58.7±6.2</td>
<td>1.96±0.38</td>
</tr>
<tr>
<td>G-SHR</td>
<td>146±4.7*</td>
<td>488±15.0</td>
<td>322.8±8.5</td>
<td>53.8±5.5</td>
<td>3.15±0.30</td>
</tr>
</tbody>
</table>

BP indicates blood pressure; ANF, atrial natriuretic factor; PRA, plasma renin activity; G-WKY, Wistar-Kyoto (WKY) host rat grafted with WKY tissue; and G-SHR, WKY host rat grafted with tissue from spontaneously hypertensive rat. All values are mean±SEM, and correspond to animals from experiment 1 killed 5 weeks after graft placement.

*P<.01 vs G-WKY.
were statistically higher in G-SHR than G-WKY arteries. The effect of cocaine was greater in G-SHR mesenteric arteries (pD2, 6.15±0.05) than G-WKY arteries (pD2, 6.0±0.05). In addition, G-SHR mesenteric arteries were more sensitive (P<.01) to vasopressin (pD2, 8.98±0.1) and ET-1 (pD2, 8.27±0.09) than G-WKY arteries (pD2, 8.67±0.07 and 7.95±0.06 for vasopressin and ET-1, respectively).

Table 3 summarizes the maximal responses of resistance arteries to norepinephrine, ET-1, and vasopressin. The active wall tension and active effective pressure were statistically higher in G-SHR than G-WKY arteries. However, no differences in active media stress were detected between the two groups.

### Acetylcholine-Induced Relaxation of Precontracted Resistance Arteries

Wire myograph-mounted mesenteric arteries were maximally contracted with 10 μmol/L norepinephrine. The vessels were then exposed to increasing concentrations of acetylcholine, and active wall tension was measured (Fig 4). Acetylcholine induced a dose-dependent relaxation; at 10⁻⁴ mol/L acetylcholine, the active wall tension of G-WKY arteries was down to 18% of its control value. However, for every tested dose of acetylcholine, G-SHR arteries relaxed significantly less (P<.001) than G-WKY arteries. Addition of 10 μmol/L indomethacin abolished all differences in the relaxation response of G-SHR and G-WKY arteries.

### Characteristics of Implanted Tissue

The fate of the transplant into the host brain was examined in animals from experiment 3. Transplanted tissue in G-WKY and G-SHR animals was visualized directly under UV illumination in a fluorescence microscope as shown in Fig 5. The distribution of transplanted tissue was determined for each examined brain (Table 4). In all cases, tissue was found in the third ventricle, although the tissue was not limited to the ventral hypothalamic part of the ventricle and extended often into the dorsal part of the third ventricle and less frequently into the lateral ventricles (one example is shown in Fig 5). Moreover, some tissue also infiltrated

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**Table 2. Morphological Characteristics of Resistance Arteries**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>G-WKY</th>
<th>G-SHR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen diameter, μm</td>
<td>231±8</td>
<td>222±9</td>
<td>NS</td>
</tr>
<tr>
<td>Media, μm</td>
<td>11±1</td>
<td>14±2</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Media/diameter, %</td>
<td>5±0.4</td>
<td>7±0.7</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

G-WKY indicates Wistar-Kyoto (WKY) host rat grafted with WKY tissue; G-SHR, WKY host rat grafted with tissue from spontaneously hypertensive rat. All values are from experiment 1 and are mean±SD. Differences were tested with Student's t test.

**Table 3. Maximal Responses of Resistance Arteries to Vasoconstrictive Agents**

<table>
<thead>
<tr>
<th>Parameter and Agent</th>
<th>G-SHR</th>
<th>G-WKY</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active wall tension, mN/mm</td>
<td>2.8±0.4</td>
<td>2.5±0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Active media stress, kPa</td>
<td>216±16.6</td>
<td>230±15.9</td>
<td>NS</td>
</tr>
</tbody>
</table>

NE indicates norepinephrine; CO, cocaine; ET-1, endothelin-1; and AVP, vasopressin. All values are mean±SD. Differences were tested with Student's t test.

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FIG 4. Line graph (left) shows acetylcholine (ACh)-induced relaxation of resistance arteries maximally precontracted with 10 μmol/L norepinephrine. Values represent percentage of active wall tension observed in the absence of ACh. All points are mean±SD (n=6). In the absence of any cyclooxygenase inhibitor (left), resistance arteries from Wistar-Kyoto (WKY) host rats implanted with fetal hypothalamic tissue from spontaneously hypertensive rats (SHR) (G-SHR) relaxed less (*P<.001) than arteries from WKY host rats implanted with WKY tissue (G-WKY) at all ACh concentrations tested. Addition of indomethacin (right) abolished all differences between the two groups.

the parenchyma surrounding the third ventricle, ie, the thalamus and lateral hypothalamus. For comparison of the transplants from SHR or WKY rats, three parameters were calculated semiquantitatively: (1) the volume occupied by the transplanted tissue, (2) the anteroposterior extension of the tissue mass, and (3) the number of tissue foci (defined as well-circumscribed masses of tissue that did not appear connected to other foci of transplanted tissue in contiguous serial sections). The volume occupied by the SHR transplants was generally larger than their WKY counterparts, but the difference was not significant, probably because of the relatively large dispersion of values within each group. However, SHR transplants had a greater number of individual foci and extended farther along the anteroposterior axis than WKY transplants.

Growth Rate of Cultures of Glial Cells

The growth rate of secondary cultures of glial cells was determined by measuring the accumulation of DNA in wells over several days. The increase in DNA concentration was linear over 7 days (Fig 6). At the seventh day, the amount of DNA was significantly higher in diencephalic cultures from SHR than in similar cultures from WKY rats. In contrast, no differences were detected in cultures derived from the cortex of either SHR or WKY rats.

Discussion

Similar to what has been reported previously by Eilam et al,8 our data show that the grafting of fetal hypothalamic tissue in the third ventricle of adult rats reliably induces hypertension in the host rat. The time course of appearance of hypertension and the magnitude of the effect were similar to what had been reported previously. However, there was one major difference between our results and those of Eilam et al. They reported that the hearts of G-SHR were much larger and heavier than those of G-WKY rats or even age-matched SHR. In contrast to their results, we never observed cardiac hypertrophy in any of our G-SHR.
TABLE 4. Characteristics of Transplanted Tissue

<table>
<thead>
<tr>
<th>Animal</th>
<th>Volume, mm³</th>
<th>No. of Foci</th>
<th>AP Extension, mm</th>
<th>Third Ventricle</th>
<th>Lateral Ventricle</th>
<th>Thalamus</th>
<th>Lateral Hypothalamus</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-SHR 8</td>
<td>5.263</td>
<td>3</td>
<td>3</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>G-SHR 9</td>
<td>1.482</td>
<td>7</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>G-SHR 10</td>
<td>1.727</td>
<td>8</td>
<td>2</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>G-SHR 11</td>
<td>3.386</td>
<td>3</td>
<td>2.25</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>G-SHR 12</td>
<td>1.5</td>
<td>5</td>
<td>2.5</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Mean±SEM</td>
<td>2.964±0.78</td>
<td>5.2±1*</td>
<td>2.35±0.2*</td>
<td>100%</td>
<td>40%</td>
<td>80%</td>
<td>100%</td>
</tr>
<tr>
<td>G-WKY 2</td>
<td>3.256</td>
<td>3</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>G-WKY 3</td>
<td>1.35</td>
<td>1</td>
<td>1.5</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>G-WKY 4</td>
<td>0.648</td>
<td>1</td>
<td>1.5</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>G-WKY 5</td>
<td>3.35</td>
<td>2</td>
<td>1.75</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>G-WKY 6</td>
<td>0.345</td>
<td>3</td>
<td>1</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mean±SEM</td>
<td>1.79±0.64</td>
<td>2±0.49</td>
<td>1.5±0.56</td>
<td>100%</td>
<td>20%</td>
<td>80%</td>
<td>60%</td>
</tr>
</tbody>
</table>

*P<.05 vs G-WKY, as assessed by nonparametric Mann-Whitney U statistic.

AP indicates anteroposterior; G-SHR, Wistar-Kyoto (WKY) host rat grafted with tissue from spontaneously hypertensive rat; and G-WKY, WKY host rat grafted with WKY tissue. Signs (+ and −) indicate presence or absence of transplanted tissue in the corresponding region.

One possible explanation may account for this discrepancy. It is unlikely that the cardiac hypertrophy reported by Eilam et al was simply secondary to hypertension, because SHR (that had been exposed to much higher levels of blood pressure and for longer times) did not exhibit the same degree of cardiac hypertrophy. Moreover, other researchers have postulated that central catecholaminergic pathways, in particular within the paraventricular nucleus of the hypothalamus, may play an important role in the activation of sympathetic nerve activity and the development of cardiac hypertrophy. Hypothetically, the grafts in the study of Eilam et al may have been implanted in such a way that they interacted with central areas that induce cardiac hypertrophy independently of hypertension. However, we did observe a significant thickening of the media of small resistance arteries similar to what has been reported in SHR.16,18,19 Such changes are generally considered to be secondary to elevated blood pressure in experimental19-21 and genetic16,18 hypertension, although other factors may also contribute in the latter group. Thus, these findings indicate that the hypertension that develops in G-SHR, albeit moderate, is not labile and is sustained enough as to induce hypertrophy of the smooth muscle layer of the media of resistance arteries.

Because WKY rats and SHR are closely related genetically, we wondered whether the effects of the SHR brain grafts were dependent on the specific genetic background of the host rat. We found that the grafting of SHR hypothalami induced hypertension in four different rat strains and thus was likely to trigger central mechanisms that induced hypertension independently of the specific genetic background of the host rat. Although we did not analyze in detail the precise mechanisms leading to hypertension in the G-SHR, it is known that the brain may control blood pressure via at least two different mechanisms: control of sympathetic tone and neuroendocrine control of the secretion of vasoactive peptides or hormones.3,22 We did not find any changes in the plasma levels of epinephrine and norepinephrine between G-SHR and G-WKY rats. However, these indexes are not the most sensitive, and in particular they have not been reported to be different between SHR and WKY rats.23 More sensitive measurements will be necessary to ascertain whether an increase in sympathetic drive contributes to the hypertension in G-SHR, as is the case in SHR.23,25 In addition, we did not detect changes in plasma atrial natriuretic factor or plasma renin activity. It is therefore unlikely that gross abnormalities in the secretion of these two factors play a significant role in the hypertension of G-SHR.

Certain abnormalities in the functions of resistance arteries have been reported to occur in SHR. Thus,

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**Fig 6.** Left, Line graph shows growth rate of diencephalon-cultured glial cells from either spontaneously hypertensive rats (SHR) or Wistar-Kyoto (WKY) rats in vitro. Amount of DNA in each well is plotted against number of days in culture; n=3 for each point. Right, Bar graph represents amount of DNA per well for four different types of cultured cells 7 days after initial day of plating. *P<.01 vs all other groups. DIEN indicates diencephalic cultures; CTX, cultures derived from cortex.
cocaine (an inhibitor of monoamine reuptake) increases the sensitivity of resistance arteries to norepinephrine to a greater extent in SHR than WKY rats, suggesting that norepinephrine uptake is greater in the vessels of SHR than of WKY rats. In addition, a cyclooxygenase-dependent substance interferes with endothelium-dependent relaxations of the resistance arteries of SHR.26,27 Although it has been difficult to assess with certainty whether such abnormalities are primary or secondary to hypertension, these two particular alterations have been found in the vessels of young prehypertensive SHR, suggesting that they may be causally related to hypertension. It was therefore of considerable interest to examine whether they could be found in the vessels of G-SHR as well. We observed that cocaine increased the sensitivity of vessels of G-SHR to norepinephrine to a greater extent than that of vessels from G-WKY rats. The magnitude of the difference in sensitivity to cocaine in grafted rats was similar to what has been reported in SHR and WKY rats.18 Likewise, relaxation to acetylcholine was impaired in G-SHR, and differences between arteries from G-SHR or G-WKY rats were abolished by indomethacin, which inhibits the cyclooxygenase-dependent generation of eicosanoids. This was similar to but not as dramatic as what had been observed in SHR, because the resistance arteries of this strain not only relax less but also contract in the presence of high doses (10−7 to 10−6 mol/L) of acetylcholine.26,27 Nonetheless, these results indicate that the secretion of an eicosanoid (probably endothelium-derived) interferes with the acetylcholine-induced relaxation in G-SHR. Thus, two of the functional abnormalities typically found in the resistance arteries of young prehypertensive SHR also exist in the resistance arteries of G-SHR. These findings suggest that the brain may play a causal role in these abnormalities.

We analyzed the effects of norepinephrine (with or without cocaine) on three different calculated variables (ie, active wall tension, active media stress, and active effective pressure) and tested the effects of two additional known vasoconstrictors (ET-1 and vasopressin). Similar to what has been reported in SHR and WKY rats,16,18 the maximal responses to norepinephrine alone and their corresponding pD2 values were not different in G-SHR and G-WKY rats. With the three other treatments, maximal active effective pressures were greater in G-SHR than G-WKY rats (resulting probably from the trends toward higher tension and reduced lumen diameter), but no differences were observed in the maximal active media stress (probably because of the thicker media width). This pattern of response is generally believed to result from structural changes in the vessels rather than from enhanced excitation-contraction coupling28 and is similar to previous observations in SHR.16 In addition, the sensitivity of resistance arteries of G-SHR was increased by all three treatments. Although the literature is not in agreement, such changes have been reported previously in SHR.16,18,25 Arguably, the fact that the sensitivity to three different vasoconstrictive agents is enhanced may result from the impaired endothelium-dependent relaxation response (as described above) rather than from alterations in the efficiency of coupling of receptors to vasoconstrictive agents.

Eilam et al8 had reported that viable tissue was present in the ventricles of grafted rats, but they did not determine how far the graft actually extended within the host brain. We stained some of our transplants with Hoechst bisbenzimide 33342. This vital fluorochrome stains cell nuclei for long periods of time and does not interfere with cell migration or multiplication; therefore, it has been used previously as a convenient way to monitor the fate of transplanted tissue into rodent brains.20 This technique allowed us to determine that cells originating from the transplant were not confined to the ventral part of the third ventricle but also infiltrated surrounding parenchyma or other ventricular cavities. There was no indication that the SHR transplant reached any particular structure that the WKY transplant did not reach. Of note, we estimated that at the time of the graft, the volume of the transplant was approximately 1 mm3 (indeed, the volume of injection was ±2 to 3 μl and typically consisted of one third tissue and two thirds fluid). The volume occupied by the transplant in the host brain (as estimated by histological examination of sections from brains collected 2 weeks after the graft) was often higher, thus suggesting that the graft could grow and expand in the host brain. Because neurons are postmitotic, it is likely that many of the cells that develop in the transplant are in fact nonneuronal cells. Unfortunately, we could not formally identify these cells with cell markers, because the procedure used for visualization of the Hoechst stain did not allow for optimal preservation of antigens. Eilam et al8 reported that some neurons survived in their grafts. However, their number was small, and the grafts implanted differently in each brain. It is therefore difficult to imagine that hypertension could have resulted from a preferential interaction of neurons within the graft with specialized structures within the hypothalamus. The possibility that nonneuronal cells (and in particular glial cells) play a role in the graft-induced hypertension is intriguing. Moreover, it would be compatible with recent findings, because glial cells have been shown to produce factors that have potent effects on neighboring neurons, including precursors to vasoactive peptide31–33 and various neurotrophic or neurotoxic factors.34,35

Histological analysis revealed that the SHR grafts extended farther along the anteroposterior axis and displayed a significantly greater number of tissue foci. In addition, the average size of the SHR transplants in the host brains was generally larger (although not significantly) than in their WKY counterparts. This suggested that SHR hypothalamic cells had a higher migratory and/or proliferative potential than their WKY counterparts. In view of the possible roles of glial cells, these findings prompted us to compare the growth rate of glial cultures from SHR and WKY rats in vitro. Cultures of hypothalamic SHR cells proliferated more rapidly than equivalent cultures of WKY cells. No differences were found in the growth rate of cortical cells from either strain. Other researchers have reported that the in vitro growth rate of vascular smooth muscle cells36–38 and fibroblasts39 from SHR rats is increased. Our findings indicate that this may also occur with brain cells, although the difference between hypothalamus and cortex suggests that not all cells from SHR will display enhanced growth in vitro. It is far from certain that
enhanced cell growth capacity will necessarily correlate with hypertension.\textsuperscript{50} However, it is striking to observe that enhanced cell proliferation is found in cells derived from the same region (the hypothalamus) where most of the brain genetic alterations can be found.\textsuperscript{3,4,6}

Given the suggestion of a higher growth rate of SHR grafts, one should also wonder whether the grafts might induce hypertension simply by increasing intracranial pressure or by pressing on certain portions of the brain of the host rats. However, Eilam et al\textsuperscript{40,41} have shown that grafting equal volumes of SHR tissue originating from other brain regions (i.e., hippocampus or caudal hypothalamus) did not induce hypertension in the host rats. These observations exclude a nonspecific effect of exogenous brain tissue. In addition, we observed that some of the WKY grafts were as large as some of the SHR grafts, yet only G-SHR became hypertensive. For these reasons, we think it unlikely that hypertension could be explained solely on the basis of a mechanical effect.

Of note, the hypertension in G-SHR was never as marked as in age-matched SHR. For instance, the systolic blood pressure values obtained in our laboratory for 13-week-old SHR are typically around 175 mm Hg. Several explanations may account for this difference. First, one should note that grafted rats received the implants as adults, whereas SHR have been exposed to the influences of the brain throughout development, starting from fetal life. Another consideration concerns the relative importance of the brain in the development of hypertension in SHR. Although this organ plays an important role, it is unlikely to be the only factor involved. For example, other researchers have shown that hypertension can be induced by transplanting SHR kidneys into otherwise normal host adult rats.\textsuperscript{42,43} Finally, we do not know whether the central abnormalities occurring in the brain of grafted animals are identical to what occurs in SHR. However, it is striking to observe that the brain grafts induce two of the central functional abnormalities typically found in young prehypertensive SHR. Therefore, this model may possibly allow one to single out some of the brain abnormalities occurring in SHR and study them independently of other factors. If hypertension may be induced by transplantation of a selected population of cells, this model could be particularly useful in the identification of cellular brain factors that contribute to hypertension.

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References


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