Smaller Local Brain Volumes and Cerebral Atrophy in Spontaneously Hypertensive Rats

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Spontaneously hypertensive rats (SHR) have enlarged cerebral ventricles from 8 weeks of age onward and smaller brains than age-matched, normotensive Wistar-Kyoto (WKY) rats (controls). At 6–7 months of age local cerebral glucose utilization is apparently lower in many brain areas of SHR relative to WKY rats. These observations led to the hypothesis that there are morphological differences between these two strains of rats in many, if not all, brain areas. This hypothesis was tested in 6–7-month-old SHR and WKY rats by quantitating 1) the volumes of the ventricular system, whole brain, six gray matter structures, and two white matter areas; 2) the thickness of two regions of the cerebral cortex; and 3) the frequency of neuronal nuclei (neuronal frequency) in nine brain areas. Ventricular volume was twofold greater in SHR than in control rats. The volumes of the entire brain and all six gray matter structures plus the thickness of the two cortical regions were 11–25% less in SHR. Neuronal frequency was, however, similar in the two rat strains. The latter finding coupled with the smaller regional tissue volumes indicates appreciably fewer neurons per brain structure in young adult SHR than in controls. These results indicate significant cerebral structural differences between young adult SHR and WKY rats and suggest that structure as well as metabolism are abnormal in the SHR brain. (Hypertension 1993;21:105–111)

KEY WORDS • brain • hydrocephalus • cerebral ventricles • hypertension, essential • hyperkinesis • metabolism • neurons

The spontaneously hypertensive rat (SHR), which was selectively bred from the Wistar rat strain, is not only hypertensive but also hyperactive and hydrocephalic. Relative to age- and sex-matched Wistar-Kyoto (WKY) rats, the normotensive control strain for SHR, total brain weight and volume are about 11% less in 12-week-old male SHR. For 8-month-old animals, Lehr et al. have reported that the midbrain and diencephalon are smaller and the pons is larger in SHR than WKY rats. Of relevance to these observations, magnetic resonance imaging has indicated that brain volume is reduced and ventricular volume is greater in humans with essential hypertension than in age-matched control subjects.

Local cerebral glucose utilization (LCGU) is highly dependent on local neural activity, is commonly used as an indicator of the functional state of cerebral tissue, and varies appreciably among brain areas and conditions. For most brain structures, LCGU is virtually the same in 11–12-week-old SHR and WKY rats, but for the superior cervical, cardiac, and coeliac sympathetic ganglia, LCGU is significantly less in SHR than in WKY rats. At 6–7 months of age, however, LCGU appears to be lower in most gray matter areas of SHR relative to WKY rats. These LCGU findings indicate that function is altered in many parts of the nervous system of young adult SHR, including a number of structures that have little or no role in sympathetic nervous system activity and blood pressure regulation.

The brain of the young adult SHR, thus, seems to differ both morphologically and physiologically from that of the WKY, and these differences appear to involve many parts of the nervous system. The purpose of the present study was to test the hypothesis that the structure of many brain areas differs between 6–7-month-old SHR and WKY rats by using histological sections obtained from previously published work. With freeze-fixed sections, the volume of the ventricular system, the entire brain, six specific gray matter structures, and two white matter areas plus the thickness of two cortical regions were estimated. The frequency of neuronal nuclei in nine brain areas was determined on the perfusion-fixed histological samples. The brain areas range from the temporal cortex and inferior colliculus to the corpus callosum and substantia nigra.

Methods

Animals

The SHR and WKY rats were 1 month old when obtained from Taconic Farms, Germantown, N.Y. They were housed in the same room of the local animal care facility until the time of experimentation. Animal care, surgical preparation, and experimental procedures were done according to institutional and National Institutes of Health guidelines.
of Health guidelines and were approved by the institutional Animal Care and Use Committee of the State University of New York at Stony Brook.

Measurements of Brain Volumes and Thickness

When these animals were 6–7 months old, 12 SHR and 12 WKY rats were prepared for measurement of cerebral blood flow and glucose metabolism by the quantitative autoradiographic method. To begin this procedure the rats were anesthetized with 3% halothane and subsequently maintained on 1.5% halothane in 70% nitrous oxide–30% oxygen. Catheters were placed in the femoral arteries and veins. The wounds were infiltrated with lidocaine hydrochloride and closed with suture. Subsequently, the rats were kept warm by means of a heat lamp set to maintain body temperature at 37°C. The physiological state of these animals was monitored thereafter by regularly measuring arterial blood pressure, blood gases, plasma osmolality, plasma glucose, and body temperature.

The actual experiment was performed 2–3 hours later with the administration of the appropriate radiotracer. At the end of the experimental period, the rats were decapitated. Immediately the brain was rapidly removed and instantly frozen in 2-methylbutane previously cooled to −45°C. The frozen brains were sealed in plastic bags and stored at −70°C until sectioned.

The brains were cut into 20-μm-thick sections in a cryostat at −17°C. Sections were obtained at regular (200 μm) intervals starting at the medulla and ending at the rostral termination of the lateral ventricles. Sections designated for histology were placed on glass slides, dried at 45°C, stained with cresyl violet, and covered. Sets of histological sections from 10 of the 12 SHR and 10 of the 12 WKY rats from the study of Wei et al. were selected for volume and thickness measurements. The sets selected were the ones that provided the most sections of the entire brain and the areas of interest.

The volumes and thicknesses were quantitated by first digitizing the individual histological sections with an image analysis system (model MCID, Imaging Research Inc., St. Catharines, Canada). The areas of the ventricles and the brain were demarcated automatically by the image analysis system on the basis of the marked contrast in optical density at the tissue borders. Tissue areas of interest were outlined by tracing along the gray matter–white matter borders of each structure. These areas were multiplied by the distance between the histological sections to yield a volume. All of these “volumes” for a particular structure were summed to yield a total volume.

The eight structures chosen for volume measurement were long enough to appear on more than five sets of sections (length > 1 mm). In addition, the edges of these areas were clearly demarcated by the cresyl violet stain, and objective assessment of their borders could be readily done. These were the criteria for selecting these areas and for excluding others. The analysis was performed independently by two investigators using the summing procedure indicated above. Tissue volumes were measured for two white matter structures, the anterior commissure and the genu of the corpus callosum, and for six gray matter areas, the substantia nigra, periaqueductal gray matter, the inferior colliculus, the superior colliculus, the caudate-putamen, and the thalamus.

Volume estimates of the cerebral cortex were not made because of the difficulty of defining the boundaries between cortical regions (e.g., the frontal and parietal cortex) from the cresyl violet-stained sections. Instead, the thickness of the frontal and temporal cortex was estimated at specific levels along the rostral-caudal axis and the separate thickness values were averaged for each of these cortical regions. All brain structures were identified according to a rat brain atlas.

Neuronal Frequency

For this part of the study, 6–7-month-old SHR (n = 5) and WKY rats (n = 5) were anesthetized with sodium pentobarbital (40 mg/kg i.p.) and perfusion fixed via the left ventricle using 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer. Fixed brains were cut into a series of 0.2-mm-thick coronal sections with a vibratome. Brain areas of interest were dissected from the vibratomed sections. Five to eight samples of each area of interest were washed in four changes of 0.1 M phosphate buffer, postfixed in buffered 1% osmium tetroxide (wt/vol 0.1 M phosphate buffer), dehydrated in a graded ethanol series, and embedded in EPON resin (EMbed-812, Electron Microscopy Sciences, Fort Washington, Pa.). From each embedded tissue block, 6–8 2-μm-thick sections were cut with a glass knife, placed on a slide, and stained with 1% toluidine blue.

The same image analysis system indicated above, but with software developed for microvessel morphometry (MCID model, Imaging Research) and coupled to a light microscope (Microphor FX, Nikon Corp., Garden City, N.Y.), was used for quantitating the frequency (distribution) of neuronal nuclei. The total area scanned and the number of neuronal nuclei were measured on one of the sections of each brain area of interest from each rat. The number of neuronal nuclei per millimeters squared, hereafter referred to as “neuronal frequency,” was calculated from these numbers. Clearly, neuronal frequency as determined in the present context is a simple indicator of the relative number of neurons, not the total number of neurons, in the field of measurement.

Neuronal frequency was estimated in nine brain areas, including four where tissue volumes were also assessed. These areas are the genu of the corpus callosum, the caudate-putamen, the medial geniculate body of the thalamus, and the inferior colliculus. One cortical area, the sensorimotor cortex, and one hypothalamic area, the paraventricular nucleus, were chosen. In addition, neuronal frequency was determined in the hippocampus (CA1 area), cerebellar gray matter, and the globus pallidus.

Statistics

For ventricular and total brain volume as well as for cortical thickness, Bonferroni-corrected t tests were used to assess statistical significance. For the regional brain volumes and neuronal frequency, analysis of variance (ANOVA) with repeated measures was used to determine the significance of the differences among areas and between groups (i.e., structure–group interaction). If ANOVA indicated significant variations,
then Bonferroni-corrected t tests were done to find the brain areas of greatest difference. The data are reported as mean±SEM. The level of statistical significance is indicated in the text, table, and figures by the particular probability values obtained.

Results

Volumes of Ventricles, Brain, and Brain Areas

For the animals used for brain and ventricular volume measurements, the mean age of both strains of rats were identical, but the WKY rats were considerably larger than SHR (Table 1). As expected, blood pressure was higher in SHR than WKY rats.

The histologic sections show clear differences in ventricular size between SHR and WKY rats and suggest that there is less brain tissue in SHR than in WKY rats (Figure 1). Quantitatively, the ventricles were approximately twofold larger in SHR than WKY rats, and total brain volume is about 10% less in SHR (Table 1). The ventricular enlargement in SHR, thus, appears to be the result of loss of brain tissue and not of expansion of the ventricles and distension of the brain.

As seen on the histologic sections (Figure 1), the thickness of the frontal and temporal cortex also appears to be less in SHR vis-a-vis WKY rats. Quantitatively, both the frontal and temporal cortices are 20% thinner in SHR (Table 1). The lesser amount of cerebral cortex, therefore, accounts for much of the difference in brain volume between SHR and WKY rats.

For the two white matter areas, the anterior commissure and the genu of the corpus callosum, the tissue volumes are slightly but not significantly smaller in SHR (Figure 2; volume ratio SHR/WKY >0.9). This suggests that the hydrocephalus of SHR is not accompanied by white matter swelling (edema).

The volumes of the six gray matter areas studied (Figure 2) are significantly less in SHR than in WKY rats. The ratios of regional volumes (SHR/WKY) for gray matter are lowest for the inferior colliculus (0.74), periaqueductal gray (0.76), and caudate-putamen (0.77), intermediate for the superior colliculus (0.79) and substantia nigra (0.81), and highest for the thalamus (0.85). These ratios indicate that the size of the SHR-WKY volume differences 1) vary somewhat among these six gray matter areas, 2) differ between gray (sizable difference) and white (small difference) matter of SHR, and 3) are not uniform throughout the brain of SHR.

Neuronal Frequency

Neuronal frequency ranged from around 200 profiles per mm$^2$ in genu of the corpus callosum (white matter) and globus pallidus (gray matter) to 600–800 profiles per mm$^2$ in the other seven areas (Figure 3). In the five SHR and five WKY rats studied, neuronal frequency varied significantly among the nine brain areas ($p<0.001$).

The differences in neuronal frequency between SHR and WKY rats were nonsignificant. The caudate-putamen was the only area in which there was even a hint of an SHR-WKY dissimilarity (Figure 3). In view of the lesser tissue volume in the whole brain and the various areas and the thinner cerebral cortex of SHR, the number of neurons must be less in the brain of SHR than in that of WKY rats.

Discussion

Ventricular Enlargement in Spontaneously Hypertensive Rats

Based on cross sections of the brain at three different levels, Ritter and Dinh$^5$ reported that the ventricles of SHR were enlarged from 8 through 56 weeks of age and suggested that this was mainly the result of cerebral atrophy and not obstruction of the ventricular system. In later work from this group,$^6$ cerebrospinal fluid pressure was found to be normal in SHR, and cerebral ventricular volume of SHR was not diminished appreciably by maintaining lower arterial blood pressure by captopril administration (begun in utero and continued to the time of study). In addition, Ritter et al$^6$ reported that cerebral ventricular cross-sectional area was normal in 22-week-old Sprague-Dawley rats made hypertensive by removing one kidney and partially clipping the renal artery of the other kidney at 4 weeks of age. For these rat models of hypertension, ventricular enlargement or hydrocephalus seems to be independent of blood pressure.

Ventricular volumes have been assessed in vivo by magnetic resonance imaging of SHR and WKY rats ranging in age from 2.5 to 10 months by Bendel and Eilam.$^7$ At 2.5 months of age, the ventricular volumes were identical (15 mm$^3$) in the two strains, but by 3–5 months, mean ventricular volume was significantly greater in SHR (37.5 mm$^3$) than WKY rats (28 mm$^3$). At an age comparable to the animals used in the present study (6–8 months vis-a-vis 6–7 months), mean ventricular volume was 58 mm$^3$ in SHR and 33 mm$^3$ in WKY.
FIGURE 1. Histological sections of the brain of one Wistar-Kyoto (WKY) rat and one spontaneously hypertensive rat (SHR). Panels A (WKY) and B (SHR) were obtained 4.7 mm anterior (rostral) to the interaural line. Panels C (WKY) and D (SHR) were obtained 10.2 mm rostral to the interaural line. The ventricles in panels A and C (WKY) are marked by long, thin arrows. Large ventricles of SHR are apparent (panels B and D) and are not marked by arrows. Differences in size of the various brain areas between SHR and WKY rats are less obvious.

The agreement between these data and that in Table 1 is good, especially since the ventricles collapse slightly in the process of brain removal used in the present study. Although the apparent times for the onset differ somewhat, the in vivo findings of Bendel and Eilam support the time course of SHR hydrocephalus described by Ritter and Dinh.

Recently, Salerno et al have used magnetic resonance imaging to measure ventricular and brain volumes in adult hypertensive men, who were taking antihypertensive medication and were normotensive at the time of study, and a group of healthy, age-matched, normotensive men. They found that the lateral ventricles were larger and the volume of the left hemisphere smaller in the hypertensive men and suggested that hypertension increases the rate of cerebral atrophy. As discussed by Ritter and Dinh, see above and below, hydrocephalus and cerebral atrophy in hypertension may be genetically linked to the elevation of blood pressure but not be driven by it.

The volumes of the two white matter areas were slightly but not significantly less in SHR than WKY rats (Figure 2); neuronal frequency in the genu of the corpus callosum was virtually identical in the two strains of rats (Figure 3). Together these observations indicate that the white matter of SHR was not edematous and that the process of ventricular enlargement does not "force" cerebrospinal fluid into the white matter of SHR.

Smaller Brain Size in Spontaneously Hypertensive Rats

To the best of our knowledge, Lehr et al were the first to observe and quantitate differences in brain size between SHR and WKY rats. They determined for 8-month-old animals that the thickness of the pons and overlying cerebellum was greater in SHR than WKY rats but that the length of the pons, midbrain, and diencephalon was less in SHR than WKY rats. At 12 weeks of age, the volume and weight of the SHR brain is 11-12% less than that of the WKY rat brain. In agreement with the latter results, the data in Table 1 show that total brain volume of 6-7-month-old SHR is 11% less than that of age-matched WKY rats.

Ritter and Dinh indicated, without reporting actual measurements, that the cortex, septum, and hipppocam-
FIGURE 2. Scatterplot shows mean tissue volumes (±SEM) of eight brain areas of spontaneously hypertensive rats (SHR) (n=10) versus those of Wistar-Kyoto (WKY) rats (n=10). In many cases, the standard error bars are smaller than the symbol and cannot be seen. The eight areas chosen for volume measurement were clearly demarcated by the cresyl violet stain. ACM, anterior commissure (white matter); GCC, genu of the corpus callosum (white matter); SNR, substantia nigra; PAG, periaqueductal gray matter; IC, inferior colliculus; SC, superior colliculus; CP, caudate-putamen; TH, thalamus. The line of identity is where the points would lie if SHR volume equaled WKY volume; the line with slope of 0.8 is where the points would lie if the brain volumes of SHR are 80% of those of WKY rats. The points for the six gray matter areas (SNR, PAG, IC, SC, CP, and TH) all lie around the 0.8 line: For these areas the SHR–WKY differences are significant (p<0.005).

FIGURE 3. Bar graph shows frequency of neuronal nuclei or "neuronal frequency," (mean ± SEM) in nine brain areas of spontaneously hypertensive rats (SHR) (n=5) and Wistar-Kyoto (WKY) rats (n=5). The number of neuronal nuclei per millimeter squared were counted on perfusion-fixed tissue sections. GCC, genu of the corpus callosum; CA1, hippocampus; SMC, sensorimotor cortex; MG, medial geniculate body of thalamus; PVN, paraventricular nucleus of hypothalamus; GP, globus pallidus; CP, caudate-putamen; IC, inferior colliculus; and CG, cerebellar gray matter. Differences between SHR and WKY rats are not significant for any brain area.

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confirm, quantify, and extend this suggestion of Ritter and Dinh; the thickness of the cerebral cortex and the tissue volumes of various parts of the pons (periaqueductal gray matter), the midbrain (superior and inferior colliculi plus the substantia nigra), the caudate-putamen, and the thalamus were 15–25% less in SHR than WKY rats. There also may be somewhat less white matter in SHR. The "cerebral atrophy" of SHR, thus, seems to involve many parts of the brain.

The hydrocephalus and lower regional and total brain volumes in SHR could be the result of less nervous tissue growth or greater cerebral tissue loss. The histological data of Ritter and Dinh,5 coronal sections of the forebrain at three different levels from which ventricular and brain cross-sectional areas were measured, suggest that both processes occur. For WKY rats, brain tissue area increased modestly from 4 to 21 weeks and decreased slowly from 21 to 56 weeks. For SHR, there is a lesser rate of increase in tissue area from 4 to 8 weeks, no change in brain area from 8 to 12 weeks, a small decrease from 12 to 21 weeks, and a more pronounced diminution in tissue area from 21 to 56 weeks of age. The difference in brain size between SHR and WKY rats, thus, seems to arise by the earlier cessation of brain growth (possibly occurring around 8 weeks of age) and the earlier onset of tissue loss (apparently beginning about 12 weeks of age) in SHR.

Regarding brain growth and atrophy, genetic and chromosomal mapping of the stroke-prone SHR, which is closely related to SHR, and of the WKY rat has indicated gene loci on two chromosomes that appear to be involved in regulating blood pressure10,19 and a linkage between blood pressure and two autosomal marker loci, the rat growth hormone promoter region and the rat fast nerve growth factor receptor.18 It is possible that genes controlling blood pressure as well as the growth, maintenance, and loss of brain cells are also present on these same chromosomes in SHR and drive the loss of brain tissue reported in several studies, including the present one.

Neuropathology in Spontaneously Hypertensive Rats

Most previous morphological studies of SHR have focused on brain areas involved in blood pressure regulation such as various hypothalamic nuclei, the circumventricular organs, and the nucleus of the solitary tract. Of particular relevance to the present study, Nelson and Boulant18 measured the frequency of neurons in 10 hypothalamic nuclei and two circumventricular organs of 12-week-old animals and found that neuronal frequency was less in SHR than WKY rats for five of the hypothalamic areas (including the paraventricular nucleus) and for both circumventricular organs (the organum vasculosum of the lamina terminalis and the subfornical organ) but was similar for the other five hypothalamic areas. As indicated by these authors, their findings of lower neuronal frequency and smaller brain volume (see above) imply that there is a significant loss of neurons in the hypothalamus of SHR by 12 weeks of age.

For 6–7-month-old animals, the data in Table 1 plus those in Figures 2 and 3 indicate that tissue volume of many, perhaps most, brain gray matter areas is less in SHR than WKY rats but that neuronal density in all brain areas examined is similar for both rat strains. Since gray matter volume was less in SHR than in WKY
rats but neuronal frequency was equal in SHR and WKY rats, the number of neurons per brain structure was almost certainly less in SHR than WKY rats at 6–7 months of age for such diverse parts of the brain as the cerebral cortex, thalamus, hypothalamus, pons, and inferior colliculus.

The frequency of capillary profiles (capillarality) has been reported to be identical in 6–7-month-old SHR and WKY rats for six of eight brain areas, including the genu of the corpus callosum, the inferior colliculus, and the paraventricular nucleus, but to be dissimilar in the sensorimotor cortex (SHR greater than WKY) and subfornical organ (SHR less than WKY).13 Coupled with the observed smaller volumes of gray matter, these findings suggest that at 6–7 months of age, fewer capillaries are present in a number of brain areas of SHR than in those of WKY rats and that either fewer capillaries develop or fewer are retained during the maturation of these brain areas in SHR.

In an attempt to assess the role of the sympathetic nervous system in chronic hypertension, Kadekaro et al13 measured LCGU in the superior cervical, cardiac, and coeliac ganglia and 44 brain areas in SHR and WKY rats. LCGU was significantly less in the cardiac and coeliac ganglia of SHR at 7 weeks of age and in all three ganglia of SHR at 12 weeks of age. For these sympathetic ganglia, the differences were due to a combination of decreased LCGU in SHR over this period of time and either raised or unchanged LCGU in WKY rats. With respect to the central nervous system, LCGU was similar in 12-week-old SHR and WKY rats for 41 of 44 brain areas and higher in SHR for the cuneate (medullary), vestibular (pon-ти, and fastigial (cerebellar) nuclei, three structures that are not strongly involved with the sympathetic nervous system.

In 5–7-month-old rats, however, local cerebral glucose utilization, which is an indicator of local neural activity and metabolism, is apparently 25–30% lower in SHR than WKY rats for nearly all gray matter areas.14,20 The latter findings, along with those of Kadekaro et al,13 suggest that neural metabolism in SHR is lowered at 7–12 weeks in the sympathetic nervous system and is depressed by 5 months of age in cerebral gray matter.

The lower rates of LCGU (which is calculated and expressed in micromoles per 100 grams of tissue per minute) in 6–7-month-old SHR does not appear to correlate with the general similarity in neuronal and capillary frequency in SHR and WKY rats. Within the brain, metabolism appears to be higher at axon terminals and dendritic processes, where it is involved in the restoration of transmembrane ion gradients and the uptake, release, and metabolism of neurotransmitters.11,12 Lowering of these neural activities may be part of the cause of the apparently depressed LCGU in SHR. Glucose metabolism also supports the development and maintenance of neurons and glia; the lowered LCGU rates in SHR may be partly the result of a diminution in these processes in brain cells.

Spontaneously hypertensive rats are hyperactive from 4 to 6 weeks of age onward.2,4 Observations on humans indicate a linkage between hyperactivity and both lowered cerebral glucose utilization21 and cerebral atrophy.22 The similarity between hyperactive humans and SHR with respect to cerebral atrophy and lowered glucose metabolism are apparent.

In summary, our morphometric observations show that there is significantly less tissue throughout the brain of young adult SHR than in age-matched WKY rats; this difference, when considered along with other published data,5–9 appears to be the result of reduced cerebral tissue growth and widespread cerebral atrophy beginning around 8–12 weeks of age in SHR. The similarity in neuronal (present study) and capillary profile frequencies15 within the brain suggests that neurons, capillaries, and (probably) glia are lost or do not develop fully in the various areas of the SHR brain. The hypometabolism, hypertension, and hyperactivity of SHR may be linked to this apparent cerebral atrophy.

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