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Decreases in Cerebral Microvasculature with Age Are Associated with the Decline in Growth Hormone and Insulin-Like Growth Factor 1*

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ABSTRACT

Several reports have demonstrated that cerebral blood flow decreases with age and may contribute to neurodegenerative changes found in aging animals and man. Because GH and insulin-like growth factor 1 (IGF-1) decrease with age and have an important role in vascular maintenance and remodeling, we hypothesized that the decrease in cerebral blood flow is associated with a rarefaction of cerebral blood vessels resulting from a decline in GH and IGF-1. Measurements of vascular density (number of vessels/cortical surface area) in both Brown-Norway and Fisher 344/Brown-Norway rats were made at 5, 13, and 29 months of age using chronic cranial window chambers that allowed viewing of the cortical surface and its corresponding vasculature. Correlations were made with plasma levels of IGF-1. In Brown-Norway rats, arteriolar density decreased from 15.53 ± 1.08 to 9.49 ± 0.62 endpoints/mm^2 in 7- and 29-month-old animals, respectively ($P < 0.05$). A decline was observed also in arteriolar anastomoses [3.05 ± 0.21 to 1.42 ± 0.24 connections/mm^2 in 7- and 29-month-old animals ($P < 0.05$)]. Venular density did not decrease with age. Similar changes were observed in Fisher 344/Brown-Norway rats. The number of cortical surface arterioles was correlated with plasma IGF-1 levels at the time of vascular mapping ($r = 0.772$, $P < 0.05$), and injection of bovine GH (0.25 mg/kg, sc, twice daily for 35 days) to 30-month-old animals increased both plasma IGF-1 and the number of cortical arterioles. These data indicate that: 1) vascular density on the surface of the cortex decreases with age; 2) vascular density is correlated with plasma levels of IGF-1; and 3) injection of GH increases cortical vascular density in older animals. We conclude that GH and IGF-1 have an important role in the decline in vascular density with age and suggest that decreases in vascular density may have important implications for the age-related decline in cerebral blood flow and brain function. (*Endocrinology* **138:** 3515–3520, 1997)

PREVIOUS studies indicate that cerebral blood flow and capillary density decrease with age in rodents, nonhuman primates, and man and have the potential to be important contributing factors in brain aging (1–7). Although the etiology of the decrease in cerebral blood flow has not been determined, the decline does not seem to be related to alterations in mean arterial pressure, because pressure remains constant or increases with age. However, increases in vascular resistance caused by an age-related increase in arteriolar vessel segment length between branches has been reported (8) and, in addition, we have demonstrated a decline in total number of arterioles between the ages of 3 and 30 weeks in skeletal muscle (9), suggesting that these variables contribute to a decrease in blood flow. Because a decline in arteriolar density (or an increase in vessel segment length) with age has the potential to decrease perfusion pressure and tissue blood flow, we hypothesized that the decline in cerebral blood flow observed in aged animals may be related to a decline in arteriolar density.

Although the maintenance of arteriolar density is a complex process involving a number of growth factors, previous studies suggest that both GH and insulin-like growth factor 1 (IGF-1) have important regulatory roles in blood vessel growth and repair (10–13). For example, blood vessels have receptors for GH and IGF-1, and several studies indicate that immunoreactive IGF-1 within vessels increases during periods of growth and repair (14, 15). Furthermore, IGF-1 has been shown to potentiate the actions of several vascular growth factors (16). Although it is well known that both plasma GH and IGF-1 decrease with age and contribute to the decline in protein synthesis and vascular compliance that occurs in aged animals (17–25), the role of these hormones in the age-related decrease in vascular density has not been assessed. Because the decrease in cerebral blood flow seems to be an important factor in brain aging, the regulation of vascular density by these anabolic hormones has the potential to have therapeutic importance for both vascular repair and brain function. Therefore, the goal of this study was to determine: 1) whether a decrease in cerebral cortical vasculature occurs with age; 2) whether the age-related decrease in vasculature is associated with the decline in GH and IGF-1; and 3) whether injection of bovine GH has the potential to reinitiate cerebral vascular growth in aged animals.

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Materials and Methods

Animals

Brown-Norway and Fisher 344/Brown-Norway male rats were obtained from the National Center for Toxicological Research (Jefferson, AR). The animals varied from 5–30 months of age and from 130–600 g

in BW. Rats were housed in an animal satellite facility that is fully accredited by the American Association for Accreditation of Laboratory Animal Care. These facilities comply with all USPHS-NIH policies and standards for laboratory animal care and to those of the Institutional Animal Care and Use Committee at Bowman Gray School of Medicine of Wake Forest University. Rats were maintained on a 12-h light, 12-h dark cycle, and food and water were available *ad libitum*.

Surgery

To observe arterioles and venules on the cortical surface, a chronic cranial window was implanted. Animals were anesthetized with a mixture of ketamine hydrochloride and xylazine (6 mg/100 g and 0.8 mg/100 g BW, respectively, im). The head was secured in a stereotaxic apparatus and a midsagittal incision was made over the frontal and parietal portions of the scalp. The soft tissues were retracted and an air-powered turbine drill was used to cut an 8-mm circle through the skull. The skull and exposed tissues were kept moist and cool by repeated applications of artificial cerebrospinal fluid (NaCl, 124 mM; KCl, 5 mM; NaH_2PO_4, 1.24 mM; $MgSO_4$, 1.3 mM; $CaCl_2$, 2.5 mM; $NaHCO_3$, 26 mM; D-glucose, 10 mM). The skull was removed, and the dura was excised, exposing the pial membrane. A 9-mm window made from 1.5-mm-thick cover glass (Fisher Scientific, Raleigh, NC) was secured to the skull using cyanoacrylic glue applied around the external edges of the window. The animals were allowed to recover for 2 weeks to assure complete recovery from surgical trauma and to allow tissue adaptation to the window.

Microvascular mapping protocol

During the mapping session, rats were lightly sedated with ketamine and xylazine (3 mg/100 g and 0.4 mg/100 g BW, respectively, im). A video photograph was taken of the cortical microvasculature, printed on a Mitsubishi model P71U Video Copy Processor, and used as a road map for the analysis sessions. The mapping protocol consisted of videotaping the cortical surface vasculature at two magnifications. The entire area was recorded at low magnification (111×) for cortical surface area measurements, and then the cerebral arterioles, venules, and anastomoses were recorded individually at high magnification (760×). The length, average diameter, and tortuosity of each arteriole were measured by techniques previously reported (26, 27). These tapes were used to count the number of vessels and anastomoses and measure vessel length. Vessel number (as determined by endpoints where the arterioles enter and the venules exit the cerebral parenchyma) were normalized to surface area of cortex.

Relationship between plasma IGF-1 and vascular density

At the time of vascular mapping, blood samples were drawn from the tail vein of a subset of animals in each age group for analysis of IGF-1. IGF-1 concentrations were measured in plasma after extraction, as previously described (28). Briefly, plasma was acidified, extracted in 10 vol petroleum ether, purified on a C-18 column (Prep-Sep, Fisher Scientific, Atlanta, GA), and analyzed by a specific RIA using antiserum obtained from the National Pituitary Program and NIDDKD. Thr^{59}IGF-1 (Bachem, Inc., Torrance, CA) was radiolabeled with ^{125}I using a lactoperoxidase, glucose oxidase procedure (25). Data were expressed in relation to rhIGF-1 standards (Bachem, Inc.).

GH replacement

For investigation of the effects of GH on cerebral vascular density, 8-month- and 30-month-old Fisher 344/Brown-Norway (F344/BN) rodents were implanted with cranial windows and baseline cortical vascular measurements recorded, as previously described. Young animals were injected with saline alone, whereas old animals were injected with either bovine GH (0.25 μg/kg) or saline twice daily for 35 days. At the end of this period, vascular measures were reassessed and changes in vascular density recorded.

Data analysis

Data on age-related changes in cerebral vascular measures were analyzed by Multivariate ANOVA using SAS (SAS Institute, Cary, NC). Because multivariate analysis indicated statistical significance ($P < 0.05$), univariate tests were performed on individual measures using ANOVA followed by the Student's Newman-Keuls *post-hoc* test, as appropriate. Effects of GH or vehicle on changes in arteriolar density were analyzed by one-way ANOVA.

Results

Cerebral arterioles and arteriolar anastomoses decrease with age

Representative views of typical cortical surface vasculature, from 13- and 29-month-old male Brown-Norway rats, are depicted in Fig. 1. These photos clearly indicate a profound rarefaction of the cerebral vascular circulation in the older rat. Pseudocolor-enhanced photographs of arterioles, arteriolar anastomoses, and venules are indicated in Fig. 2

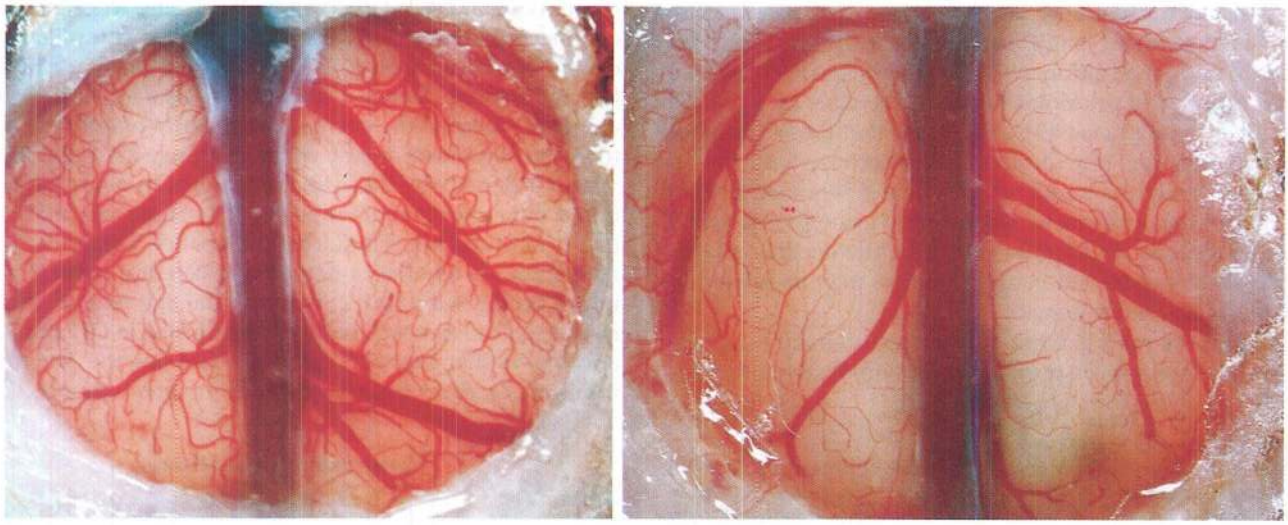

FIG. 1. Representative photographs of the cortical surface microvasculature in 13- and 29-month-old Brown-Norway rats, as seen through the cranial window. The entire parietal cranium has been removed. The cortex visible in this window extends from the frontal to the occipital cortex. Vascular measurements were made over the sensorimotor cortex. C, caudal.

and summarized in Fig. 3. Compared with animals at 5 months of age, cortical surface arterioles per cortical surface area decrease approximately 15% by 13 months of age and 40% by 29 months of age ($P < 0.02$). Arteriole-to-arteriole anastomoses per area of cortical surface also decreased significantly with age ($P < 0.01$). In the young rat, the ratio of arteriolar anastomoses to arterioles was 1:5, whereas in the old animal, this ratio had decreased to 1:6.6. The increased ratio in old animals was the result of a greater loss of arteriolar anastomoses, as compared with the number of arterioles. A decrease in the number of venules per area of cortical surface is also apparent in Fig. 3. However, the rarefaction of cerebral venules was not as consistent as that of arterioles and did not reach statistical significance. The number of venule-to-venule anastomoses was not different among the three age groups. In addition, no differences were observed in the average diameter, tortuosity, or actual length of arterioles or venules among the three age groups.

To test the possibility that the reduction in cortical surface arterioles was specific only to Brown-Norway rats, this study was repeated in F344/BN rats. A 32% decrease in arteriolar density of F344/BN rats was observed between 8 and 32 months of age, which was comparable with the decrease in arterioles observed in Brown-Norway rats (Table 1).

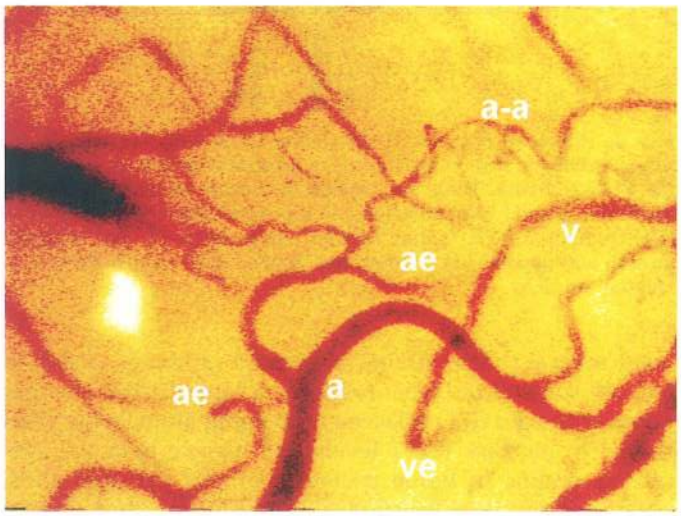

FIG. 2. Pseudocolor-enhanced photograph of cerebral cortical vasculature observed through the cranial window of 25-month-old Brown-Norway/Fisher 344 male rat. Arterioles (a), venules (v), and arteriole-arteriole anastomoses (a-a) are indicated. Arteriolar and venular endpoints (ae and ve) are vessels that descend into the cortical surface.

Arteriolar density is correlated with plasma IGF-1, and GH administration increases arteriolar density in old animals

Analysis of plasma levels of IGF-1 in Brown-Norway rats of various ages indicated a high correlation with cortical arteriolar density at the time of vascular mapping ($r = 0.775$, $P < 0.05$). In response to daily injections of bovine GH, a substantial increase in arteriolar density was observed in 30-month-old F344/Brown-Norway rats. The number of arteriolar endpoints increased by 6.05/mm^2 during the 35-day period in response to GH (12.64 ± 0.70 to 18.73 ± 2.01 endpoints/mm^2, $P < 0.01$, compared with saline-injected animals), whereas only minimal growth was observed in old animals treated with saline (12.47 ± 0.54 to 12.93 ± 0.77 endpoints/mm^2, Fig. 4). No vascular growth was observed in 8-month-old animals treated with saline (data not shown).

Discussion

With advancing age, there is a decline in both glucose and oxygen use by the brain, a reduction in synaptic density, cell loss, and functional changes in both neurons and glia (29). Although the etiology of these changes is poorly understood, numerous investigators have reported a decline in cerebral blood flow, with age, in both animals and man using nitrous oxide, xenon, calcitonin, and PET scanning techniques (1, 3,

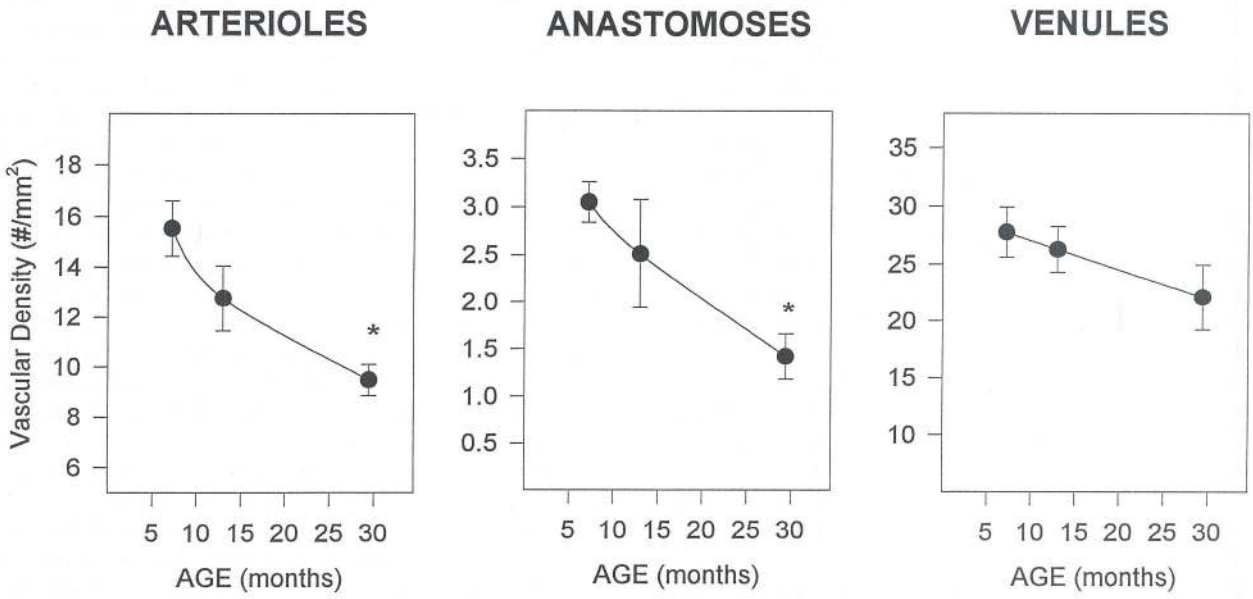

FIG. 3. Summary of arteriolar (*left*), arteriole-to-arteriole anastomotic (*center*), and venular endpoint (*right*) density in male Brown-Norway rats. Data represent mean ± SEM for 18 young, 14 middle-age, and 13 old animals.

TABLE 1. Number of arteriolar endpoints per square millimeter of cortical surface in the F1 hybrid of the Fisher 344 × Brown-Norway rodent

Age	N	Arteriolar Endpoints
Young	15	17.67 ± 2.29
Middle-aged	14	15.54 ± 1.13
Old	15	12.06 ± 0.68[a]

Data represent the mean ± SEM.
[a] $P < 0.05$.

5–7). The implication of these studies is that the loss of metabolic and nutritional support of neurons by blood vessels may be an important factor in both the structural and functional changes known to occur in the central nervous system (CNS) of aged animals. The age-dependent decline in cerebral blood flow potentially results from either a decrease in perfusion pressure and/or a decline in vasculature within the brain. Previous studies indicate that perfusion pressure either is constant or increases with age (30), suggesting that vascular density may be a primary factor that contributes to the decrease in blood flow. To date, analysis of cerebral capillary density, total capillary length, and capillary surface area per unit volume of tissue have been controversial (4, 31–34). The assessment of capillary density in aged animals and the association of this measure with blood flow is a complex process potentially confounded by two factors: 1) there seems to be a disproportionate decrease in the volume of cortical or subcortical structures compared with vasculature in some strains of aged animals (31); and 2) blood flow in the CNS is regulated primarily by arteriolar and arteriole-to-arteriole anastomotic density, rather than capillary density. In the present study, we used a chronic cranial window technique and two strains of animals (Brown-Norway and Fisher 344/Brown-Norway rats) that maintain a relatively consistent cortical volume throughout life (J. Brunso-Bechtold, personal communication) and report that the densities of arterioles and arteriole-to-arteriole anastomoses on the cortical surface decrease with age in both strains. Similar decreases in venular density were noted, but the latter effects were not statistically significant. The finding of reduced arteriolar anastomotic connections in aged animals is in agreement with other studies, indicating that an age-related decrease occurs in the collateral anastomotic potential in the cat after middle cerebral artery occlusion (35). Thus, not only is there an increased resistance to blood flow with age, but a reduction in the ability to maintain homogeneous flow during periods of localized ischemia, which may result in increased risk of neuronal loss in brain regions where vessel rarefaction is prominent. Because the cortical surface vasculature has been shown to mimic the vascular supply to the entire brain (36), the loss of cerebral surface vasculature reported in this study suggests a general rarefaction of microvasculature within the CNS that contributes, at least in part, to the age-related decrease in blood flow, neuronal function, and increased risk of neuronal loss during periods of ischemia.

Previous studies from our laboratory and others demonstrate that both GH and IGF-1 decrease with advancing age (18, 19, 21, 22, 25, 28). These changes have been observed in

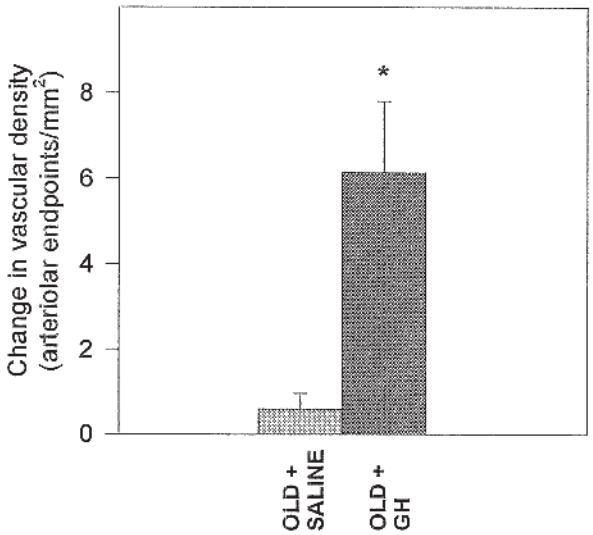

FIG. 4. Effects of bovine GH (25 μg/kg, twice daily) or vehicle administration for 35 days on changes in arteriolar density in 30-month-old F344/BN rats. Data are expressed as the percent increase in arteriolar density from baseline and represent the mean ± SEM of 10 (GH-treated) and 7 (saline-treated) animals/group. No vascular growth was observed in 8- (data not shown) or 30-month-old saline-treated animals.

mice, several rodent strains, nonhuman primates, and man and are some of the most robust and reproducible changes noted with age. GH replacement to aging animals has been shown to increase IGF-1 levels and reverse both the age-related decline in tissue protein synthesis (24) and some aspects of immune function (37), suggesting that impairments in GH secretion have physiological relevance for biological aging. In the present study, plasma levels of IGF-1 were positively correlated with cerebral vascular density. These data are consistent with the hypothesis that cerebral vascular density is dependent on adequate levels of GH and/or plasma IGF-1. Our hypothesis is further supported by the fact that vascular growth in older animals was reinitiated in response to daily injections of GH. Previous studies indicate that both endothelial and smooth-muscle cells have receptors for GH and IGF-1 (11, 14, 15), and Hansson *et al.* (15) have shown that immunoreactive IGF-1 is greatly increased in areas of angiogenic activity. In addition, studies indicate that vascular remodeling, evident in response to hypertension, is inhibited by hypophysectomy and restored by GH administration (12). Although the brief duration of GH replacement (35 days) preclude us from reaching the conclusion that qualitative and quantitative aspects of vascular deficiency with age can be completely reversed by GH administration, our results clearly suggest that age-related decreases in GH have an important role in the maintenance of vascular plasticity and that deficiencies in GH and/or plasma IGF-1 concentrations are contributing factors in the decline in vascular density in aged animals.

Both GH and IGF-1 have been shown to stimulate endothelial cell proliferation, tube formation, and angiogenesis in a number of tissues. GH, for example, has been shown to stimulate angiogenesis in chorioallantoic membranes of the chick embryo (13), whereas IGF-1 has been reported to stim-

ulate the growth of endothelial cells in the retina (38) and the proliferation of omental microvessel endothelial cells (16). Similarly, in both rat aortic rings and bovine carotid artery cells, IGF-1 increases angiogenesis, and migration and tube formation of carotid artery cells have been reported in response to IGF-1 (39, 40). Although IGF-1 seems to have the ability to stimulate vascular growth independently, several investigators report that the actions of other growth factors, including tissue plasminogen activator and hepatocyte growth factor, are facilitated by IGF-1 (16). These studies support the hypothesis that GH and IGF-1 regulate vascular growth *in vitro*, and this is the first *in vivo* evidence that the decline in the secretion of these hormones contributes to the vascular deficiency associated with age.

It is well known that metabolic support of neuronal tissue requires adequate blood flow. Angiogenesis, for example, has been hypothesized to be necessary for, and to precede, neurite outgrowth in some models of neuronal damage (41). Other reports suggest that the decreased capacity for neural plasticity in the aged rat results, at least in part, from an inability to generate new cerebral microvessels (42). The dependence of neuronal tissue on an adequate vascular supply indicates that an age-related decrease in blood vessel density may result in alterations in metabolic support for neurons. In addition, arterioles are a source of IGF-1, as well as other growth factors, including NGF, and these factors have the potential to exert important trophic influences on surrounding tissues (10). At the present time, the specific contributions of vascular growth factors to the neurotrophic support of surrounding neurons are unknown. However, we have recently noted a close association between the decline in vessel density, type 1 IGF receptors, and synaptic density in cortex of aged animals, and recent studies suggest that icv administration of IGF-1 increases working memory in old animals. These results indicate a close relationship between vascular density, IGF-1, and neuronal function that contributes to brain aging.

In summary, we have shown that there is a decrease in the number of arterioles and arteriolar anastomotic connections per cortical surface area in two strains of aged rats. There is a correlation between the number of arterioles and plasma level of IGF-1 and, furthermore, administration of GH increases cortical vascular density in aged animals. Our results suggest that the decline in vascular density in aged animals results, at least in part, from the decline in plasma GH and IGF-1.

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