Cortical and Putamen Age-Related Changes in the Microvessel Density and Astrocyte Deficiency in Spontaneously Hypertensive and Stroke-Prone Spontaneously Hypertensive Rats

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Abstract: Cerebral small vessel disease (SVD) is a major contributor to dementia in the elderly, and hypertension represents a major cause for developing the disease. However, little is known about its development and progression. Modifications of large cerebral arteries due hypertension are thought to participate to the development of small ischemic infarcts, but the status of the small vessels before the establishment of hypertension is not well defined. Using spontaneously hypertensive rats (SHR) and stroke-prone SHR (SP-SHR) as a models for SVD, we analysed the effect of hypertension on the microvasculature in the cortex and putamen, and on its relationship with astrocytes in animals aged 2 to 9 months. Compared with the normotensive Wistar-Kyoto rats (WKY), the densities of the collagen type IV-positive capillaries were significantly higher in both brain areas of young SHR and SP-SHR. In contrast, the expression of the astrocytic marker GFAP was significantly lower in these animals, whereas astrogliosis was observed after 6 months in their cortex only. To investigate if chronic hypoxia occurs due to the lower number of astrocytes in young SHR and SP-SHR, we evaluated the levels of HIF-1α in both brain regions. The accumulation of HIF-1α was not observed at the youngest ages, but was apparent in neurons of 9-month-old SHR and SP-SHR. Our results indicate that the brains of young SHR and SP-SHR rats show evidence of cellular imbalance between microvessels and astrocytes at the neurovascular unit that may lead to their higher vulnerability to hypoxic events at older ages.

Key Words: Hypertension, brain capillaries, SHR, SP-SHR, astrocyte, hypoxia, neurovascular unit.

INTRODUCTION

Cerebral small vessel disease (SVD) is an important cause of stroke, cognitive decline and dementia (Lis 1997, Paglieri *et al.*, 2004, Paglieri *et al.* 2008). This pathology is associated with diffuse white matter abnormalities and small deep cerebral ischemic infarcts. The molecular mechanisms involved in the development and progression of SVD are not yet completely understood. Only few studies have so far investigated the mechanisms involved in the SVD. Arterial hypertension (AH) is one of the strongest risk factor for stroke (Lammie 2002, Roman *et al.*, 2002, Vermeer *et al.*, 2003) as well as for SVD (Liao *et al.*, 1996, Longstreth *et al.*, 1996, Paglieri *et al.*, 2004, van Dijk *et al.*, 2004). Spontaneously hypertensive rats (SHR) and stroke-prone spontaneously hypertensive rats (SP-SHR) represent animal models of chronic hypertension and have been used to evaluate the hypertension-related changes occurring in the brain. SHR have increased immunoreactivity for extracellular matrix proteins, in particular for collagen type IV in cerebral arterioles (Nag and Kilty 1997) that may be associated with their hypertrophy. Studies comparing brain neuroarchitecture of SHR with normotensive controls, the Wistar-Kyoto rats (WKY), provided controversial data (Sabbatini *et al.*, 2001). Some investigators found a reduced number of arterioles and capillaries in the pons, medulla oblongata, and mesencephalon of SHR (Sokolova *et al.*, 1985), while others observed no rarefaction of capillaries in 21 examined brain areas of SHR (Werber *et al.*, 1990) or increased capillary densities in the sensory motor cortex (Lin *et al.*, 1990) and the dorsolateral geniculate nucleus (Gesztelyi *et al.*, 1993). AH induces impaired angiogenic responses by increased plasma levels of vascular endothelial growth factor (VEGF). As VEGF plays a key role as physiological regulator of angiogenesis, these results suggest that SHR exhibit a dysfunction of endothelial cells and an impaired vascular growth. An increased expression of VEGF and its receptor VEGFR1 (Flt-1) was also observed in the frontal cortex of adult SP-SHR (Jesmin *et al.*, 2004). In these animals abnormal angiogenesis may result in structural alterations such as capillary rarefaction, increased arteriolar length and tortuosity. Thus, there is a discrepancy between the expression of angiogenic factors on one hand, and the absence of angiogenesis of cerebral small vessels in these hypertensive animals on the other hand.

Angiogenesis and vascular remodelling in the brain may induce an imbalance in the neurovascular unit (NVU) if the other collaborative cells of the unit are not regulated in parallel. Indeed, microvessel-neuron communication implies cellular and functional interactions of blood vessels with non-neuronal cells including endothelium, pericytes, astrocytes, oligodendrocytes and microglia. In the NVU, astrocytes are the major intermediary cells linking vessels and neurons (Allan 2006, Koehler *et al.*, 2006). They provide neuronal nutrition, maintain ionic, neurotransmitter, and metabolic

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Received: August 17, 2009, Revised: September 4, 2009, Accepted: September 8, 2009

1567-2026/09 $55.00+.00

homeostasis, and contribute to neuroimmune functions (Holash *et al.*, 1993, Ridet *et al.*, 1997). It is not known if a disturbance of the vessel-astrocyte interaction causes hypoxia, although it is well described that general hypoxia induces HIF-1α in rat brain tissue (Bergeron et al, 1999).

We hypothesised that, in SHR and SP-SHR, early cerebral abnormalities at the NVU such as changes at the level of the microvessels (capillaries and venules) and/or astrocytes may induce small vessel disease. Therefore, in a first step, we aimed to study changes in density of capillaries and of astrocytes during the development of hypertension (2 and 4 months) and established hypertension (6 and 9 months) in the cortex and putamen of the SHR in comparison to the normotensive strain of age-matched Wistar Kyoto rats (WKY). After observing a dysbalance between microvessels and astrocytes, we analysed if an ischemic-like condition develops with time by investigating the accumulation of the hypoxia inducible factor 1α(HIF-1α).

MATERIALS AND METHODS

Animals and Physiological Parameters

The procedure followed for the care and euthanasia of the animals was approved by the local Animal Welfare Committee and was in compliance with the Swiss Guidelines for the Care and Use of Animals. Three-week old male spontaneously hypertensive rats (SHR, n=20), male stroke-prone spontaneously hypertensive rats (SP-SHR, n=20), and male Wistar Kyoto rats (WKY, n=20) were purchased from Charles River Laboratories (Sulzfeld, Germany) and housed in regulated environment with free access to food and water. Five animals per strain were analysed at 2, 4, 6 and 9 months of age.

Animals were weighted and then anaesthetised with 3% isoflurane in a gas mixture of 30% oxygen and 70% nitrogen through a nose mask. Body temperature was maintained at 37°C using a rectal probe coupled with a homeothermic blanket system (Harvard Apparatus, Holliston, MA, USA) during anaesthesia. The femoral artery was catheterized and the mean arterial blood pressure (MABP) was recorded during 5 min using a transducer coupled to a printer. To exclude diabetes as a confounding factor in the development of SVD, blood glucose level was measured in a drop of arterial blood taken from the femoral artery at the time of blood pressure measurements showing normal values in all three strains in the 6 and 9 months-old animals.

Immunofluorescence Staining

At 2, 4, 6 and 9 months of age, brains were dissected and immediately frozen. Coronal sections (10 µm thick) were cut using a cryostat and mounted on Superfrost Plus slides (Menzel, Braunschweig, Germany). Tissue sections were fixed for 1 hour in 10% formaldehyde at room temperature and then blocked with normal goat serum in phosphate-buffered saline (PBS) containing 0.02% Tween 20 for 15 min. To visualize microvessels, brain tissue sections were incubated for 2 hours with rabbit anti-collagen type IV (Col IV) antibody (1/500, MD Biosciences, Zürich, Switzerland) at room temperature. Astrocytes were detected using mouse anti-GFAP antibody (1/1000, Sigma, Saint-Louis, MS, USA). Detection of HIF-1α was performed with rabbit anti-HIF-1α antibody (1/500, Novus Biologicals, Littleton, CO, USA). For the double staining of this factor and neurons, sections were co-incubated with mouse anti-NeuN (1/500, Chemicon International). After washing in PBS, all sections were incubated with secondary Cy3-conjugated anti-mouse and Cy2-conjugated anti-rabbit IgG antibodies (1/300, Jackson ImmunoResearch Laboratories, West Grove, PA, USA) for 1 hour at room temperature. Cell nuclei were counterstained with DAPI (4',6'-diamidine-2-phenylindole) added to the secondary antibodies. The sections processed without primary antibodies served as negative controls. Slides were mounted with FluorSave Reagent (Calbiochem, Merck, Darmstadt, Germany) and immediately processed.

Quantification of the Immunofluorescent Signals

Coronal brain tissue sections localized at the stereotaxic level of the Bregma were analysed. Fluorescent images were analysed under a Laser microscope (Leica DMRE microscope, Leica Microsystem, Wetzlar, Germany). Six regions of interest (ROI) of 0.35 mm^2 were randomly chosen over the left and right temporal cortex and putamen, and the pictures were captured using a video camera (F-View, Soft Imaging System, Münster, Germany) and analysed with the AnalySIS Image computer program (AnalySIS, Soft Imaging System). For Col IV staining, the fluorescent signal was binarized (as white signal on black background) and quantified either as the vascular fraction (percentage of the fluorescent-positive signal in the total ROI) or as the number of Col IV-positive particles in the ROI, reflecting the density of microvessels. The content of Col IV per vessel was calculated by dividing the fraction of Col IV signal by the number of capillaries. For GFAP and HIF-1α, the immuno-fluorescent signal was binarized and calculated as the percentage of ROI. As blood vessels and astrocytes are components of the neurovascular unit (NVU), we estimated the relationship between GFAP-positive astrocytes and Col IV-positive microvessels by calculating the ratio of the fraction of GFAP signal to the number of microvessels.

Statistics

Values are presented as means ± standard error of the mean (SEM). In order to analyse the time and strain effects, the two-way analysis of the variance (ANOVA) followed by the Fisher's protected least-significant difference (PLSD) test was used. A probability $p < 0.05$ was taken as significant.

RESULTS

Evolution of the Body Weight and Development of Hypertension in SHR

Table **1** shows the evolution of the body weight and the mean arterial blood pressure (MABP) in both rat strains from 2 to 9 months of age. SHR and SP-SHR had significantly lower body weights compared with aged-matched normo-tensive WKY rats at all ages. The blood pressure of SHR and

Table 1. Evolution of Body Weight and Arterial Blood Pressure in SHR, SP-SHR and WKY

Age	2 Months	4 Months	6 Months	9 Months
Body weight (g)				
SHR	218 ±15*	328 ± 15*	346 ± 20*	380 ± 11*
SP-SHR	214 ± 8*	307 ± 11*	337 ± 12*	343 ± 15*
WKY	258 ± 12	372 ± 14	440 ± 23	465 ± 21
MABP (mm Hg)				
SHR	143 ± 16	183 ± 14*	186 ± 35*	144 ± 10
SP-SHR	141 ± 11	184 ± 33*	196 ± 20*	205 ± 10*
WKY	114 ± 14	122 ± 6	124 ± 10	122 ± 21

SP-SHR elevated with age, while that of WKY did not change. Compared with age-matched WKY, significant differences in MABP were observed at 4 and 6 months in SHR and at 4, 6, and 9 months in SP-SHR. SP-SHR had higher values than SHR at 6 and 9 months.

Young SHR and SP-SHR Exhibit Higher Collagen IV-Positive Microvessel Densities

The immunostaining of Col IV and GFAP in the cortex and putamen of SHR, SP-SHR, and WKY showed a homogenous distribution of Col IV-positive microvessels and GFAP-stained astrocytic processes (Figs. **1A**, **1B**). A

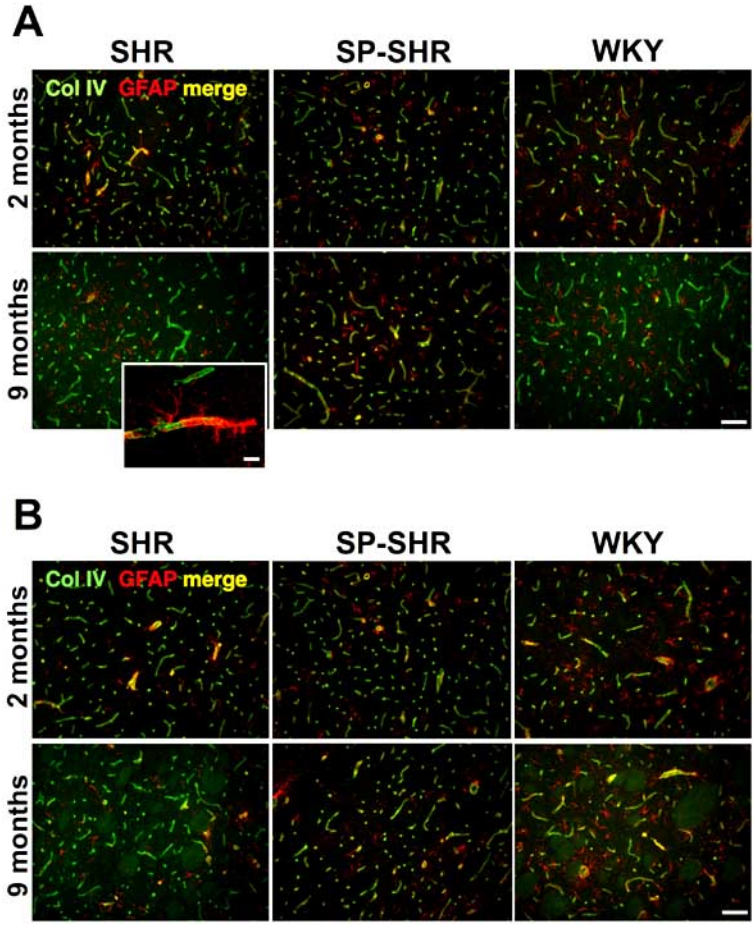

Fig. (1). Representative double immunofluorescent stainings for Col IV and GFAP in 2 and 9-month-old SHR, SP-SHR, and WKY. The Col IV-positive capillaries (in green) and GFAP-positive astrocytes (in red) are shown in the cortex (**A**) and putamen (**B**). Original magnification: 200x, bar=100µm. The high-magnification image shown in (**A**) illustrates GFAP-positive astrocytic endfeet making contact with a capillary (original magnification 400x, bar=100µm).

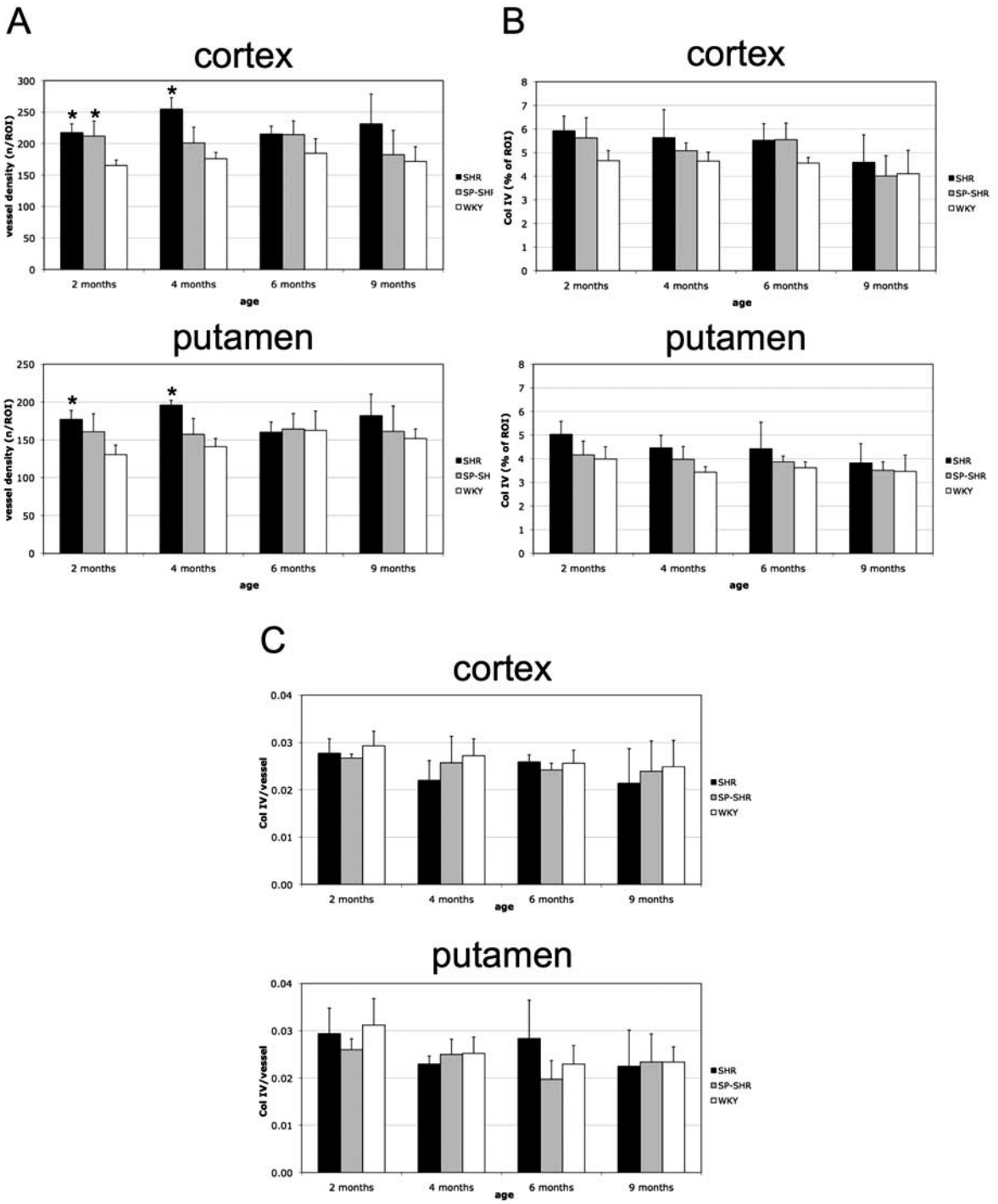

Fig. (2). Quantification of the Col IV signal in the cortex and putamen of SHR, SP-SHR, and WKY at increasing ages. Capillary densities (**A**), Col IV-positive fractions of the ROI (**B**) and the collagen content per vessel (**C**) were determined from the immunofluorescent stainings and compared between the three rat strains at the ages of 2, 4, 6 and 9 months. The densities of the capillaries differed significantly between SHR, SP-SHR and WKY in the cortex as well as in the putamen at 2 months, between SHR and WKY at 4 months of age in both brain regions. *: $p<0.05$ compared to age-matched WKY.

gliovascular interaction between astrocytic perivascular endfeet and one microvessel is illustrated in more detail (Fig. (**1A**), insert). Quantification of the Col IV immunofluorescent signal revealed a higher density of Col IV-positive microvessels in SHR and SP-SHR compared to age-matched WKY in both brain areas (Fig. **2A**). The differences observed were significant in the cortex for the 2 and 4-month-old SHR and for the 2-month-old SP-SHR, and for 2 and 4-month-old SHR in the putamen. The fractions of Col IV signal per ROI were also increased in SHR and SP-SHR,

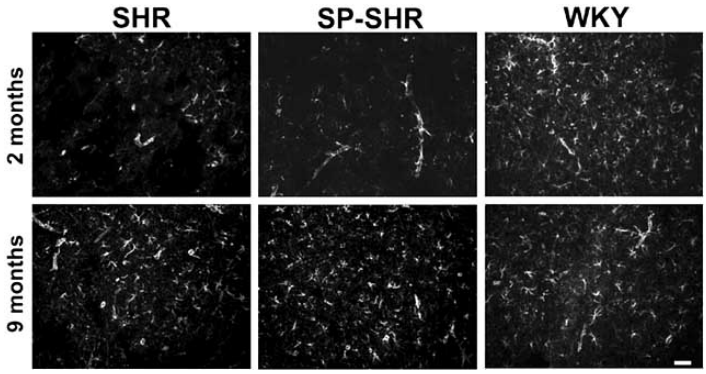

Fig. (3). Representative immunofluorescent staining of GFAP in the cortex of 2 and 9-month-old SHR, SP-SHR, and WKY. Lower GFAP signal is easily observed in 2-months old SHR as well as SP-SHR compared with WKY, but similar staining is seen in 9 months old SHR, SP-SHR, and WKY. Original magnification: 200x, bar=100μm.

but without statistical significant differences when compared with WKY (Fig. **2B**). Therefore, when we calculated the ratio of the immunofluorescent signal of Col IV per microvessel (Fig. **2C**), reflecting the level of Col IV in the vessel matrix, all three rat strains showed similar collagen content at all ages analysed.

Young SHR and SP-SHR Show Smaller GFAP Immunofluorescent Fractions than WKY

The astrocytic part of the NVU was visualized by the immunofluorescent signal of GFAP. As illustrated in the cortex in Fig. (**3**), less GFAP-positive cells were observed in 2-month-old SHR and SP-SHR compared to WKY. However, in 9-months old SHR and SP-SHR, higher GFAP-positive areas were observed in this brain area. The quantification of the GFAP-positive signal (Fig. **4A**) confirmed that the fraction of GFAP was significantly reduced in the cortex of SHR and SP-SHR compared to WKY at the ages of 2 and 4 months. The GFAP fractions significantly increased after 6 and 9 months in SHR and SP-SHR and reached values similar to those determined in age-matched WKY. In the putamen also, significant lower GFAP-positive fractions were measured in 2 and 4-month-old SHR and SP-SHR

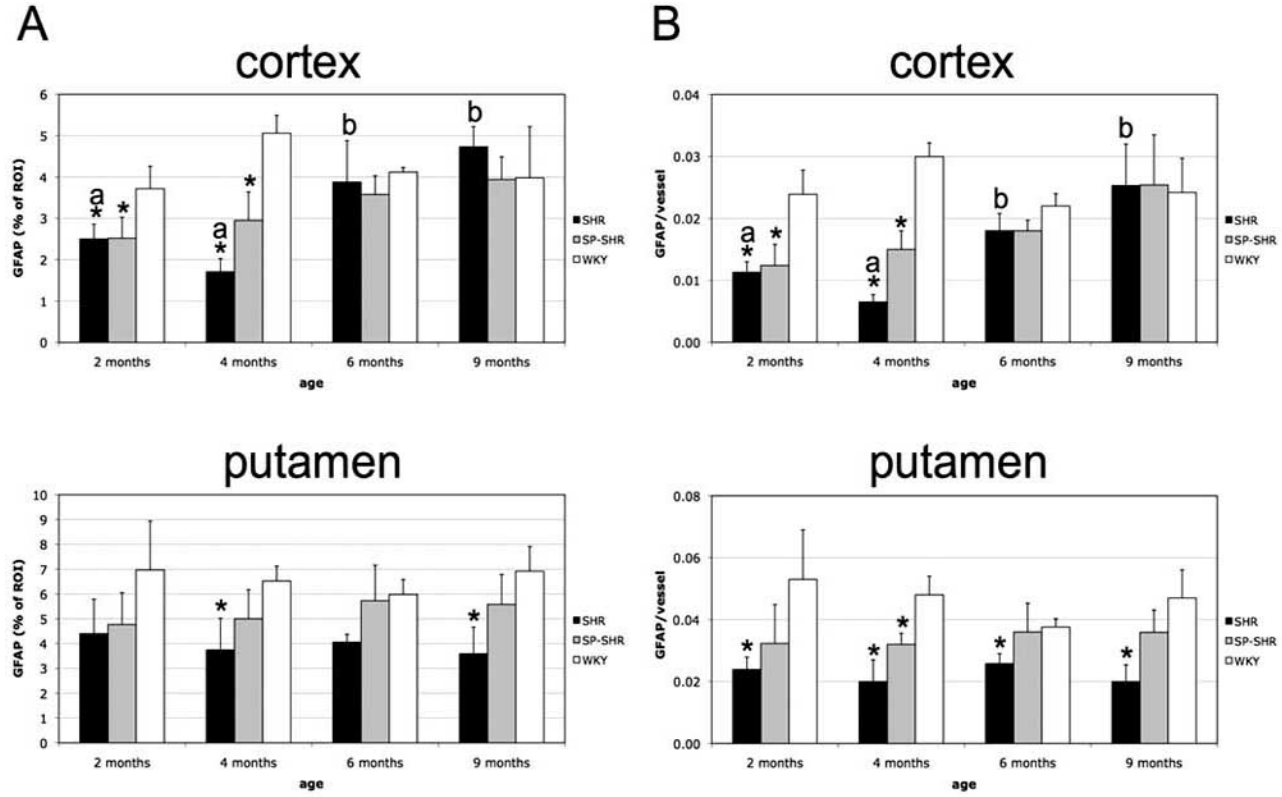

Fig. (4). Quantification of the GFAP immunofluroescent signal. (**A**) Relative GFAP signal (in % of ROI), and (**B**) amount of GFAP per vessel, in the cortex and putamen of SHR, SP-SHR, and WKY at increasing ages. Significant differences between the three strains and age-dependent changes in the cortex of SHR and SP-SHR were observed. *: $p<0.05$ compared to age-matched WKY at age of 2 and 4 months. Different letters indicate age-dependent differences reaching statistical significance within SHR ($p<0.05$).

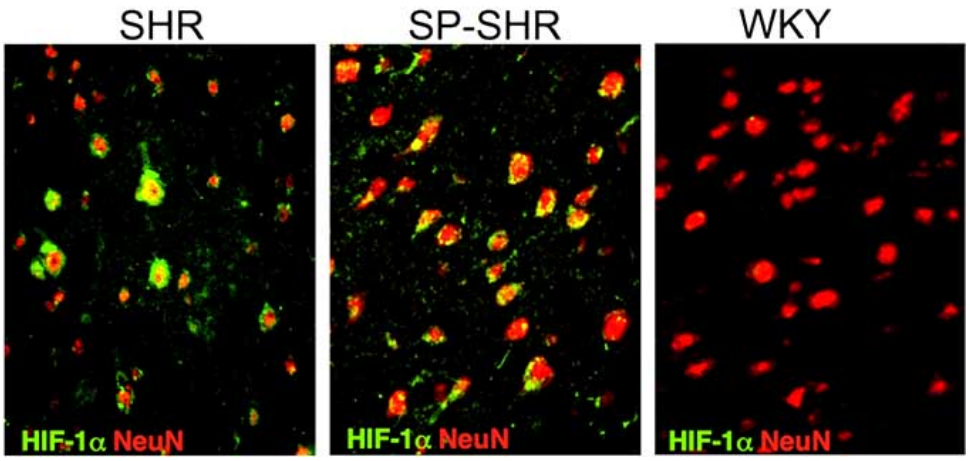

Fig. (5). HIF-1α immunofluorescent signal in the cortex of 9-month-old SHR, SP-SHR, and WKY. Accumulation of HIF-1α (green) was detected mostly in neurons stained with NeuN (red) in SHR and SP-SHR. Original magnification: 200x.

compared to WKY, but no significant increase of the signal was observed over time.

The relation between astroglial processes and blood mirovessels as part of the functional neurovascular units was calculated as an artificial ratio by dividing the fraction of GFAP signal by the number of Col IV-positive vessels (Fig. **4B**). In the cortex, the GFAP/vessel ratio was significantly lower in SHR and SP-SHR than in WKY at 2 and 4 months but increased thereafter to finally reach values similar to those obtained in age-matched WKY. Lower ratio of GFAP/ vessel were also observed in the putamen of SHR and SP-SHR compared to WKY at all ages analysed, with statistically significant differences obtained in SHR.

HIF-1α Accumulates in Neurons of 9-Month-Old SHR and SP-SHR

To investigate if chronic hypoxia occurs due to the lower number of astrocytes in young SHR and SP-SHR, we evaluated the levels of HIF-1α in both brain regions. HIF-1α is constitutively expressed and degraded in normal conditions, but hypoxic conditions stabilize the protein, allowing its detection by immunohistological methods. Cells showing HIF-1α accumulation were found in the brains of all three strains and were identified as neurons using antibody against NeuN, a specific marker for neurons. These HIF-1α positive cells were abundant in the cortex of 9-month-old SHR and SP-SHR as shown in Fig. (**5**). No other cell types, such as astrocytes, showed such an accumulation of HIF-1α. Only SP-SHR exhibited some HIF-1α positive blood vessels (not shown). The quantification of the HIF-1α immunoreactive fraction (Fig. **6**) indicated that very low accumulation of HIF-1α was present in the cortex and putamen of WKY at all ages analysed. In both brain areas, the values measured in 2 to 6-month-old SHR and SP-SHR were not significantly different from the results obtained in WKY. However, the HIF-1α signal increased remarkably in 9-month-old hypertensive rats and became significantly higher compared with with age-matched WKY in both brain regions. At this time point, differences between both brain regions were seen in SHR, with the cortex expressing twice as much HIF-1α as the putamen.

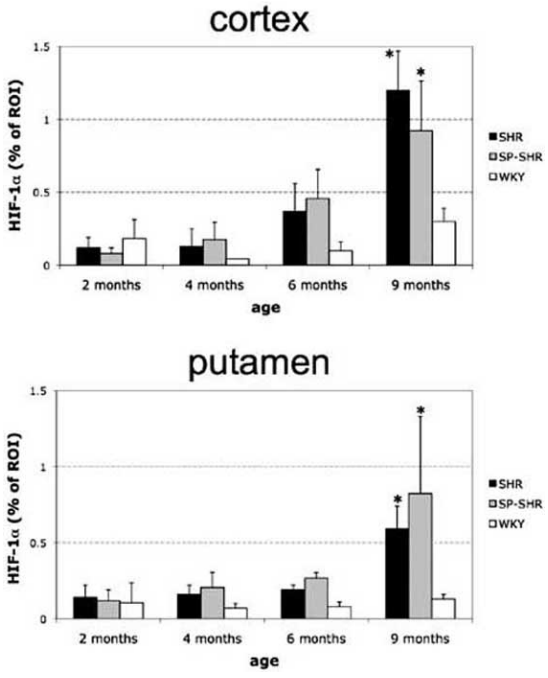

Fig. (6). Time-dependent quantification of the HIF-1α signal in the cortex and putamen in SHR, SP-SHR, and WKY showing the accumulation of this factor in the brain of 9-month-old SHR and SP-SHR. *: $p<0.05$, compared to age-matched WKY.

DISCUSSION

In this study comparing the brains of SHR and SP-SHR with normotensive WKY rats at increasing ages, we demonstrated that 1) pre-hypertensive SHR and SP-SHR are characterized by higher densities of cerebral small vessels in the cortex and putamen, 2) SHR and SP-SHR possess a deficit in astrocytes compared to the number of blood vessels in both regions with astrogliosis developing in the cortex after the age of 6 months, and 3) SHR and SP-SHR show an

accumulation of HIF-1α in neurons in both brain regions at 9 months.

Our findings concerning the microvessel densities in the cortex of SHR are consistent with a previous study reporting a greater frequency of small vessels in the sensorymotor cortex of SHR (Lin *et al.*, 1990). Whereas we found an increased density of capillaries also in the putamen of young adult SHR compared to age-matched WKY rats, other investigators observed no capillary proliferation in this brain area but an increased density in the dorsolateral geniculate nucleus of SHR (Gesztelyi *et al.*, 1993, Lin *et al.*, 1990). Thus, differences in microvessel density in SHR or SP-SHR may not be a general feature of the brain but may affect only some specific brain areas. The mechanisms underlying this induction involve multiple signalling pathways that are rather complex and not clearly understood yet. HIF-1α is known to activate genes needed for energy metabolism and tissue perfusion such as VEGF. Although HIF-1α is rapidly degraded by the proteasome in normoxic conditions, this transcription factor is stabilized under hypoxic conditions (Dery *et al.*, 2005), and transactivated into the nucleus, where it stimulates several target genes (Bergeron *et al.*, 1999, Sharp *et al.*, 2001). HIF-1α did not accumulate in the brains of 2 and 4-month-old SHR or SP-SHR in our study, but only older animals showed accumulation of HIF-1α in neurons (starting at 6 months in the cortex, and frequent at 9 months of age). This shows that the observed microvessel changes cause delayed neuronal ischemia. Another potential inductor of angiogenesis in young SHR and SP-SHR may be angiotensin type II (Ang II) since: 1) Brain Ang II has been shown to be synthesized in the brain of SHR rats (Ganten *et al.*, 1983, Hermann *et al.*, 1984, Hermann *et al.*, 1988) and to up-regulate VEGF (Otani *et al.*, 1998), 2) higher levels of Ang II has been found in the hypothalamus, putamen, cortex and cerebellum of young SHR compared to normotensive WKY or Donryu rats (Duncan *et al.*, 2001, Phillips and Kimura 1986), and 3) the level of brain Ang II changes during development, with high levels during early development and a subsequent decrease with age (Meyer *et al.*, 1990). Thus, the disappearance of Ang II in older SHR may lead to to the lack of significant differences in the microvessel densities in older SHR compared with age-matched WKY. Capillary rarefaction has been described in humans suffering from hypertensive SVD (Brown *et al.*, 2007, Moody *et al.*, 2004), an observation that is in contrast with our data obtained in pre-hypertensive 2-month-old SHR and SP-SHR. However, the density of microvessels decreases over time in SHR and SP-SHR in our study, and the densities obtained after 6 and 9 months were similar to the ones obtained in WKY. Therefore, it is conceivable to hypothesize that the vessel densities in older SHR and SP-SHR may reach values below the ones observed in WKY later in time, reflecting the human situation. Interestingly, a recent study (Heinzer *et al.*, 2008) reported that overexpression of human VEGF in the brain of mice induces angiogenesis with the formation of additional micro-networks. However, this network was connected to higher order vessels whereas an insertion of individual capillaries into the existing vessel structure was absent. As a consequence, the VEGF-induced vascular network did not increase blood flow capacity and did not increase cerebral perfusion in the transgenic mice (Vogel *et al.*, 2004).

Hypertension induces several cerebral vascular changes consisting primarily in increased thickness of the tunica media, accompanied by a decrease of the lumen size and an increase of the media-to-lumen ratios (Amenta *et al.*, 1994, Johansson 1994). These changes may cause a latent status of cellular ischemia. Hypertension has been shown to decrease the activity of matrix metalloproteinases (MMPs), and therefore, to suppress collagen degradation leading to an enhanced collagen deposition in cerebral arteries (Ergul *et al.*, 2004). Since we did not observe any changes in the content of Col IV in the microvessels of 9-month-old SHR and SP-SHR, other cells that compose the neurovascular unit (NVU) such as vascular endothelial cells, pericytes, closely juxtaposed astrocytes or neurons may play a major role in the development of a hypoxia-like condition at this age. In this respect, we observed less GFAP expression in the putamen and cortex of young SHR and SP-SHR until 6 months of age compared to WKY. This result is not consistent with an earlier study (Tomassoni *et al.*, 2004) reporting normal expression of GFAP in the brains of young SHR. This discrepancy may result from the different measurements methods (*in situ* immunofluorescent staining versus Western blot, respectively), and/or by the fact that astrocytes behave in a regionally specific fashion, a characteristic detectable only with *in situ* observations such as immunohistological stainings. The observed decrease of the GFAP fraction may have several causes: less astrocytes may be present in these brain areas, or a similar number of cells are present but the astrocytic morphology may be changed, such as a reduction in the extensions of astrocytic processes. Our observations suggest that brains of SHR and SP-SHR may suffer from an abnormal astrocytic contingent (in number or in structure) that may lead to an impaired function of the NVU, such as reduced exchanges of nutrients and oxygen between blood and brain tissue. This may induce chronic hypoxic conditions. As measured by the accumulation of HIF-1α in the neurons of SHR and SP-SHR at 9 months, such impairment develops in the brain of hypertensive rats, long time before structural changes become apparent. In response to hypoxia, astrocytes undergo hypertrophy and proliferation, with an increased synthesis of the GFAP-containing intermediate filaments (Bernert *et al.*, 2003). The increase of the GFAP fraction observed in the cortex of 9-month-old hypertensive rats may therefore represent reactive astrocytes that attempt to fight against hypoxic or oxidative stress conditions in order to increase the exchanges at the blood brain barrier. Astroglial reaction has been observed in previous studies in the cortex and putamen of hypertensive rats at 6 months, but not at 2 and 4 months of age (Mignini *et al.*, 2004, Tomassoni *et al.*, 2004). The reason why astrogliosis in the putamen is not observed in our study could be due to a different hypoxic status of this brain region, since lower levels of HIF-1α were measured in this brain area (only half the signal measured in the cortex). In humans suffering from small vessel disease, ischemic lesions are mainly localized in subcortical regions, suggesting that the cortex may be protected. Our results suggest that reactive astrocytes may play a role in the protection against hypoxic insults in the cortex. As no sign of demyelination or neuronal death were observed in the 9-month-old hypertensive animals, older animals have to be examined in order to confirm this hypothetical protective role of reactive astrocytes. The role

of reactive astrocytes is still unclear: they have been shown to be neuroprotective, but they can also have deleterious effects, or are even "double edged" in the brain as proposed in several studies (Sen and Levison 2006, Trendelenburg and Dirnagl 2005).

Interestingly, the differences observed in the densities of capillary and of GFAP signal fraction were already significant in young pre-hypertensive SHR and SP-SHR. This raises the question whether factors other than hypertension, such as genetic predispositions, induce cellular dysfunction that may contribute to the increased susceptibility to brain hypoxia in these rat strains. The comparison of the gene expression in the brains of SHR, SP-SHR and WKY may give us some indications about the factors that may be targeted for future preventive therapeutic strategies in patients at risk for developing SVD.

In conclusion, hypertension alone may not be sufficient to initiate the development of subcortical infarcts seen on neuroimaging in patients with SVD. Our observations in young SHR and SP-SHR suggest that abnormalities at the NVU, before hypertension is established may be one trigger for the susceptibility to develop cerebral hypoxia. Given the role of astrocytes in maintaining ionic, neurotransmitter, and metabolic homeostasis in the brain, a better understanding of the mechanisms inducing the imbalance between microvessels and astrocytes observed in the brains of young hypertensive rats may enable us to find strategies for preventing the development of ischemia in hypertensive patients.

ACKNOWLEDGEMENTS

This project was supported by grants from the Stroke Unit, University Hospital Basel and Stiftung für Gesundheit und kardio/neurovaskuläre Forschung (to P.A.L).

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