A High Potassium Diet Reduces Infarct Size and Improves Vascular Structure in Hypertensive Rats

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Running head: Dietary potassium and cerebral ischemia
Abstract

High potassium diets can improve vascular function yet the effects of potassium supplementation on ischemic stroke have not been studied. We hypothesized that dietary potassium supplementation would reduce ischemic cerebral infarct size by reversal of cerebral artery hypertrophy. Six-week old male stroke-prone spontaneously hypertensive rats (SHRSP) were fed diets containing 0.79% potassium (LK) or 2.11% potassium (HK), for 6 weeks; Wistar Kyoto (WKY) rats received the LK diet. The HK diet did not reduce blood pressure, as measured by telemetry, in the SHRSP. Cerebral ischemia was induced by middle cerebral artery (MCA) occlusion. The resultant infarct was smaller in the HK-SHRSP than in the LK-SHRSP (55.1±6.3 vs 71.4±2.4 % of the hemisphere infarcted, HK-SHRSP vs. LK-SHRSP p<0.05). WKY rats had smaller infarcts than both SHRSP groups (33.5±4.8 %). The vessel wall of MCAs from LK-SHRSP were hypertrophied compared to WKY rats; this was reversed in the HK-SHRSP. RT-PCR analysis of the cerebral vessels showed that the expression of the platelet derived growth factor (PDGF) receptor-α, PDGF receptor-β, epidermal growth factor receptor (EGFR), collagen I and III was increased in the vessels from the LK-SHRSP compared to WKY and were reduced in the HK-SHRSP. These results suggest that potassium supplementation provides neuroprotection in a model of ischemic stroke independent of blood pressure and possibly through changes in vascular structure.
Key Words

Middle cerebral artery occlusion, cerebral ischemia, smooth muscle cells, spontaneously hypertensive rats, growth factors


Introduction

Stroke is the third leading cause of death in the US. Eighty percent of all strokes are ischemic in nature and result in long-term disability. When cerebral ischemia is induced experimentally by MCA occlusion, the volume of the resulting infarct is greater in stroke prone spontaneously hypertensive rats (SHRSP) compared to that in Wistar Kyoto (WKY) control rats (7). The difference in ischemic infarct size may, in part, be due to differences in cerebral blood vessel structure caused by hypertension. Normally, when an ischemic occlusion occurs, the collateral anastomoses around the occlusion dilate. This allows for adequate perfusion and oxygen delivery to the neuronal tissue to prevent a large infarct. In hypertension, the cerebral microcirculation remolds in such a way that arteries have smaller lumens and thicker walls (3, 5, 15). The anastomoses between the MCA and the anterior cerebral artery (ACA) in SHRSP also have an impaired ability dilate in response to ischemia (4, 6), although the number of anastomoses is constant between SHRSP and WKY rats (4). Thus, when an occlusion occurs there is insufficient tissue perfusion and a large cerebral infarct is produced. Accordingly, any intervention that alters vascular structure in a manner that will promote increased blood flow may reduce the size of the infarct after ischemic stroke.

Epidemiological studies show that potassium intake correlates inversely with the incidence of cerebrovascular events (1, 11, 31, 37). The observations made in humans have been replicated in rats; treatment of SHRSP and spontaneously hypertensive rats (SHR) with potassium-rich diets reduces the incidence of spontaneous hemorrhagic strokes induced by a high sodium diet (14, 34, 35). However, the majority of cerebral
events in the human population are ischemic in nature and the effects of dietary potassium supplementation in a model of cerebral ischemia have not been examined.

Dietary potassium supplementation has also been shown to affect the structure of systemic blood vessels. The aorta, superior mesenteric and carotid arteries from hypertensive rats fed a high potassium diet have thinner walls and larger lumens than untreated controls (14, 18, 32, 34). The structural changes observed in blood vessels from rats fed the high potassium diet might be attributed to direct effects to reduce vascular smooth muscle cell (VSMC) proliferation. Studies using cultured porcine aortic VSMCs suggest that potassium inhibits cell proliferation (22), particularly in response to PDGF (20).

Based on these observations, we hypothesized that dietary potassium supplementation would reduce the ischemic cerebral infract size in SHRSP and improve the hypertensive remodeling of the vessel wall. We further proposed that the high potassium diet would have these effects by reducing the expression of the receptors for known VSMC mitogens.
Materials and Methods.

Animals. Male SHRSP were obtained from the breeding colony at the Medical College of Georgia. Male WKY rats were purchased from Harlan (Indianapolis IN). Rats were maintained on a 12-hour light dark cycle, housed two per cage and allowed access to food and water *ad libitum*. SHRSP were fed either a low potassium (LK) or a high potassium (HK) chow containing 0.79% and 2.11% potassium respectively (Zeigler Brothers: Gardners PA) from 6 to 12 weeks of age, WKY rats received the LK chow. Both the HK and LK diets contained 0.16% NaCl. These studies complied with the protocols for animal use outlined by the American Physiological Society and were approved by the Institutional Animal Care and Use Committee. The number of rats used for each experiment was determined based on our previous experience.

MCA occlusion. Rats were anesthetized with sodium pentobarbital (50 mg/kg IP) and body temperature was maintained at 37°C during anesthesia. The MCA was permanently occluded using the intralumenal thread occlusion technique (19). Briefly, the common carotid artery was exposed by a midline incision and the branches of the external carotid artery were cauterized. A 3-0 monofilament thread with a rounded tip was introduced into the carotid artery and advanced cranially into the internal carotid artery over a distance of 19mm, measured from the bifurcation of the common carotid artery. The thread was left in place and the rats were allowed to recover. Blood flow to the region surrounding the MCA was measured using a laser Doppler flow probe to confirm MCA occlusion. Post-occlusion (6 or 24 hours), the rats were anesthetized and decapititated and the brains carefully removed. The area of the infarction was quantified
using 2,3,5-triphenyltetrazolium (TTC) staining as described previously. The area of the brain that stained pink in response to TTC was deemed to be viable tissue and the white area was classified as tissue damaged by ischemia (10).

**RT-PCR analysis.** The MCA, ACA, posterior communicating and ophthalmic arteries from each rat were isolated under a light microscope and snap frozen. RT-PCR analysis was carried out as described previously (23) using primers specific for the epidermal growth factor receptor (EGFR), the PDGF receptor-α (PDGFR-α), the PDGF receptor-β (PDGFR-β), and collagen I and III (Table 1). Optimum annealing temperature, cycle number and template dilution factor were determined for each amplicon prior to experimentation and each amplicon was amplified individually. The results were expressed as arbitrary densitometry units and were normalized to the expression of cyclophillin A, a constitutively expressed gene that we have found to be consistently expressed in the cerebral vasculature.

**Vessel Structure.** The MCA and its branches were isolated and placed in a heated (37°C) chamber containing Ca²⁺ free physiologic salt solution (PSS) (in mM: 119 NaCL, 4.7 KCL, 1.17 MgSO4, 1.18 NaH2PO4, 24 NaHCO3, 5.5 glucose) equilibrated with 21% O2, 5% CO2 and 74% N2. Vessels were cannulated at both ends with micropipetts (50-100 µm tip in diameter) and secured using 10-0 nylon suture. The pipettes were connected to a perfusion system to allow intraluminal pressure to be changed under zero flow conditions. The intraluminal pressure was increased from 0 to 80 mmHg in 10 mmHg increments; the internal and external diameter of the vessel was measured...
after 15 minutes at each pressure using videomicroscopy. This range of pressures allows for the analysis of wall stress and strain and the higher pressures fall within the physiological range for rat cerebral vessels. For each group of rats the MCA and its primary branches were analyzed separately. All mathematical equations used to calculate the passive vessel wall mechanics followed those described previously (2, 29).

\[ \text{Wall Thickness} = \text{Outer Diameter} - \text{Inner Diameter} \]

\[ \text{Circumferential Wall Stress} = \frac{\text{Intraluminal Pressure} \times \text{Inner Diameter}}{2 \times \text{Wall Thickness}} \]

\[ \text{Circumferential Wall Strain} = \frac{\text{Inner Diameter} - \text{Inner Diameter at 0 Pressure}}{\text{Inner Diameter at 0 Pressure}} \]

**Blood Pressure and Plasma Potassium.** Blood pressure was continuously monitored by telemetry (25) (Data Sciences, Inc.; 20). Telemetry probes were placed in the abdominal aorta of five-week old rats. Baseline blood pressure was measured for 3 days before the animals were placed on the specialized diets at six week of age. Plasma potassium was measured using the Synchron EL-ISE electrolyte system (Beckman Instruments).

**Chemicals.** All general laboratory chemicals were purchased from Sigma (St. Louis MO).

**Statistics**

Blood pressure was compared using an ANOVA with repeated measures. For the analysis of cerebral infarct size and the mRNA expression a one-way ANOVA was used
with a Newman-Keuls post-test. Lumen diameter, wall thickness, wall area and wall to lumen ratio were compared at the maximum pressure of 80 mm Hg.

Results.

Blood Pressure. There was no difference in the mean arterial pressure between the HK- and LK-SHRSP (Figure 1). The WKY rats had significantly lower blood pressure than both groups of SHRSP.

Plasma Potassium Levels and Body Weights. There was a 17% increase in the plasma potassium levels in the HK-SHRSP compared to the LK-SHRSP (5.65±0.1 vs. 4.82±0.23 mmol/L HK-SHRSP vs. LK-SHRSP p<0.05). The plasma potassium level in the WKY rats was similar to that observed in the LK-SHRSP (4.89±0.1 mmol/L). HK-SHRSP weighed less than the LK-SHRSP (270±4.5 vs 287±3.2 grams HK-SHRSP vs LK-SHRSP p<0.05). The WKY rats weighed 271±11.8 grams.

Cerebral Infract Size. Cerebral ischemia was induced by MCA occlusion. After six hours of ischemia the cerebral infarct was 35% smaller in the HK-SHRSP (n=8) compared to the LK-SHRSP (n=5) (37.0±2.1 vs. 56.7±6.1 % of the hemisphere infarcted HK-SHRSP vs. LK-SHRSP p<0.05). The WKY rats fed the LK diet had smaller infarcts than both the HK and the LK-SHRSP (14.51±1.55 % of the hemisphere infarcted n=9). This difference in cerebral infarct size persisted when the duration of ischemia was extended to 24 hours (Figure 2). The infarct was limited to the cortex and the basal ganglia. MCA occlusion was confirmed by laser Doppler, the percentage drop in blood
flow to the area supplied by the MCA was similar in all groups of rats (HK-SHRSP: 65.4±6.5%, LK-SHRSP: 65.5±4.7%, WKY: 61.4±2.7%).

**Passive Vessel Dynamics.** The structural properties of cerebral microvessels at an intralumenal pressure of 80mmHg are summarized in Table 2. The internal diameter of the MCA was smaller in the LK-SHRSP than the WKY rats. There was a trend toward an increase in the lumen diameter in the HK-SHRSP; however, this did not reach statistical significance. In contrast, the wall thickness and wall to lumen ratio were significantly increased in the LK-SHRSP compared to both the HK-SHRSP and the WKY rats. In primary branches of the middle cerebral artery (i.d. < 160 µm), luminal diameters at a passive distending pressure of 80 mm Hg were similar among all treatment groups. Wall thickness and wall:lumen ratio were increased in the LK-SHRSP by ~30% indicating a hypertrophy of the vascular wall. The wall thickness and wall:lumen ratio was similar in the HK-SHRSP and the WKY rats suggesting that the HK diet reduces vascular hypertrophy in the SHRSP.

The circumferential stress / strain relationship was determined as an indicator of arterial stiffness (Figure 3). In the MCAs, the stress / stain curve was shifted to the right for both the HK-SHRSP and the LK-SHRSP indicating an increase in distensibility compared to vessels from the WKY rats. In contrast, branches of the MCA from the LK-SHRSP were less compliant than vessels from WKY rats. Interestingly, the compliance was increased in the HK-SHRSP with the stress / strain curve mimicking closely the curve obtained from the WKY blood vessels.
Growth Factor Receptor Expression and Smooth Muscle Proliferation. Cerebral vessels were removed from HK-SHRSP and LK-SHRSP and WKY rats for the analysis of growth factor receptor expression by RT-PCR. The mRNA expression of PDGFR-α and PDGFR-β and EGFR was increased in the LK-SHRSP compared to the vessels from the WKY rats (Figure 4 and 5). Feeding SHRSP the HK diet significantly reduced mRNA expression of all three growth factor receptors in the cerebral vasculature.

Collagen Expression. Expression of mRNA for collagen I and III was also increased in cerebral vessels from the LK-SHRSP compared to the WKY rats. The potassium supplemented diet caused a reduction in the mRNA expression of both collagen I and III (Figure 6).

Discussion.

There are three novel findings in this study. First, we have shown that dietary potassium supplementation reduces the size of the ischemic cerebral infarct in SHRSP. We have also shown that that vessel wall thickness is reduced and vascular compliance is increased by dietary potassium supplementation. These structural changes correlate with the observed reduction in growth factor receptor and collagen mRNA expression in the cerebral vasculature and occur independently of changes in blood pressure.

We and others have shown that hypertension exacerbates the damage caused to the brain by cerebral ischemia (7, 10), this is thought to be due to structural differences
in the cerebral blood vessels (3-6, 15). It has been clear for some time that a high potassium diet reduces the number of hemorrhagic strokes in SHRSP (34, 35) and reduces the risk of stroke in humans (1, 11, 31). We have also previously shown that administration of spironolactone, a potassium sparing drug, reduces the ischemic cerebral infarct size in SHRSP (10). The mechanism for the protective effects of high dietary potassium has never been fully elucidated.

Rats were fed the specialized diets for six weeks beginning at six weeks of age. This is the time during which blood pressure is increasing rapidly in the SHRSP. This is also the time-frame over which our previous studies with spironolactone were carried out (10). The current studies show that by increasing the potassium in the diet from 0.79% to 2.11% we can both increase the levels of plasma potassium and significantly reduce the size of the ischemic cerebral infarct. After 24 hours of ischemia the cerebral infarct size was less in the SHRSP that had been fed the high potassium diet, suggesting a protective effect of this diet on ischemic damage. After six hours of ischemia the reduction in ischemic damage was also evident and the level of protection achieved was similar to that obtained with spironolactone treatment (10) and angiotensin converting enzyme inhibition with captopril in SHRSP (9). Similar to the effect of potassium, spironolactone reduced the ischemic infarct size without reducing blood pressure while captopril lowered blood pressure to a level similar to that seen in a normotensive rat. The effects of dietary potassium supplementation on blood pressure have been controversial, with most studies suggesting it only lowers blood pressure by 10 – 20 mmHg (14, 18). These rats were fed a high sodium diet and while potassium
supplementation may have caused a significant reduction in blood pressure it did not render the rats normotensive. The blood pressures reported here were measured by radiotelemetry, this allows for the assessment of even very small changes in blood pressure. Using this sensitive technique we can therefore propose that the beneficial effects of the HK diet in SHRSP given normal amounts of sodium occur independently of changes in blood pressure. Blood pressure lowering after a stroke is currently being investigated as a potential therapy. However when one considers the long-term effects of hypertension on blood vessels the usefulness of this type of intervention is questionable. Classically blood vessels are thought to become stiffer or less compliant with long term elevations in blood pressure, thus it may be necessary to maintain a higher blood pressure to maintain tissue perfusion. Therefore, prevention of the changes in blood vessel structure, as observed here, is perhaps more therapeutically applicable to the at risk patient.

When cerebral ischemia is induced experimentally in SHRSP and WKY rats, the SHRSP have larger cerebral infarcts than the WKY rats (10). When the cerebral blood vessel structure is compared between the two strains it is clear that vessels from the SHRSP have smaller lumens and thicker walls (3, 4, 6, 15). In this study we also observed a hypertrophy of the cerebral blood vessel wall in the hypertensive rats. In both the small and larger cerebral blood vessels the LK-SHRSP had significantly thicker vessel walls and a greater wall / lumen ratio than the WKY rats (also fed the LK diet). The administration of the HK diet to the SHRSP reduced both of these parameters to similar levels to the WKY rats. Changes in vascular compliance were also evident in
the hypertensive rats. In the MCA’s the stress / strain curve for the SHRSP’s was shifted to the right of the WKY curve, irrespective of diet type. This paradoxical increase in the compliance of the cerebral blood vessels from SHRSP has been reported previously (2). Physiologically, in a normotensive rat, distensibility increases as vessels become smaller. This reduced stiffness reflects the fact that most of the resistance is carried by smaller vessels. In our studies, the branches of the MCA from the SHRSP fed the LK-diet were less compliant than those from the WKY rats as evidenced by a shift the stress / strain curve to the left. The reduction in the distensibility of small cerebral arteries was reversed by the HK diet. The improvement in distensibility of the blood vessels from the HK-SHRSP may result in better perfusion of the brain during ischemia and may be the mechanism by which the cerebral infarct size is reduced. One of the interesting findings of this study is that the MCA and its branches respond to the high potassium diet in different ways in the SHRSP. This difference may be caused by differences in blood flow or the difference in perfusion pressure between the two vessels. The branches of the MCA respond to the HK diet by increasing their compliance, it is possible that this is a protective mechanism to allow these vessels to respond to changes in pressure thereby preventing damage to the smaller down stream vessels.

The increased vessel wall thickness in the hypertensive rats may be due to either an increase in smooth muscle proliferation, increased cell size, or increased extracellular matrix deposition, or a combination of these effects. We used RT-PCR to assess the mRNA expression of the receptors for PDGF and EGF, two known smooth muscle cell
mitogens that have previously been implicated in the pathogenesis of hypertension (27, 28). We have previously shown that EGFR mRNA levels are greater in the cerebral vasculature of SHRSP compared to WKY rats (10). We confirmed that finding here and also showed the expression of PDGFR-α and PDGFR-β is greater in the LK-SHRSP compared to the WKY rats. The expression of all three growth factor receptors was reduced in the SHRSP fed the HK diet. This raises the possibility that VSMC proliferation is reduced in the SHRSP fed the HK diet. Others have shown that addition of potassium to the media of cultured VSMC reduces the proliferation, particularly in response to PDGF (20, 22). Some studies have suggested that potassium itself can modulate gene expression. Recently, the promoter of the Na K-ATPase has been shown to be potassium responsive, such that low potassium concentrations cause an up-regulation in the expression of this gene (39). It is therefore possible that in the rats fed the LK-diet there is an upregulation of gene expression in presence to the low potassium levels and that this is effectively inhibited by the HK-diet. Clearly, several additional studies would need to be carried out to assess if this is the case and the possible involvement of other modulators of gene transcription such as flow, pressure and intracellular calcium cannot be ruled out.

Vascular structure can also be affected by the deposition of collagen in the vessel wall. The expression of both collagen subtypes was increased in the LK-SHRSP compared to the WKY rats and was reduced in the SHRSP fed the HK diet. A reduction in collagen deposition could be the mechanism for the increased compliance seen in the branches of the MCA’s from the HK-SHRSP. There are other possible mechanisms by which a
high potassium diet may affect the outcome of ischemia. Studies in SHRSP suggest dietary potassium supplementation may reduce endothelial permeability to macromolecules (16). Others have reported a reduction in vascular macrophage and monocyte infiltration after potassium supplementation (18); inflammatory cell infiltration is indicative of endothelial damage. A reduction in lipid peroxide formation or cholesterol ester deposition in the in aorta has also been suggested to be responsible for the protective effect of dietary potassium supplementation (17, 32, 33). Clearly, there needs to be more investigation to elucidate the link between the high potassium diet and vascular protection, however, one of the most promising pathways appears to be the reduction of inflammation. We have shown that the levels of the inflammatory cytokine interleukin-1β (IL-1β) are reduced in SHRSP fed the HK-diet (8). Potassium efflux is a necessary component of the IL-1β export process (12, 24) and raising the extracellular potassium concentration inhibits the release of IL-1β from inflammatory cells (12, 24, 36). Interestingly, a change in IL-1β levels fits well with our data for PDGFR-α expression as IL-1β is known to increase the expression of PDGFR-α in VSMC by increasing the expression of C/EBPδ (13).

Potassium is also a potent stimulator of aldosterone synthesis; an elevation in plasma aldosterone could potentially have deleterious effects on the vasculature. Plasma aldosterone was measured in the SHRSP and was found not to differ in rats fed the HK and LK diets (data not shown). This is in keeping with other studies from Manger et al showing that in Dahl salt sensitive rats the dietary potassium had to be increased to 4% before there was a significant increase in plasma aldosterone (21).
One of the limitations of this study is that the RT-PCR was used to assess the effects of the HK diets on the levels of the growth factor receptors and collagen I and III. Clearly, the use of Western blotting to assess protein expression would provide a more physiologically relevant measure, however, that has not been possible in this study because of the limited sample size available for analysis. Several studies of protein levels in the cerebral vasculature have utilized a sieving technique to obtain adequate amounts of protein for analysis. We do not feel that this type of analysis is appropriate here as the samples obtained in this way are a mixture of small arteries, veins and capillaries. Therefore, the vessels studied using this technique would not reflect the vessels studied in our structure experiments. It should also be noted that the results we have obtained most probably reflect the changes seen in the MCA and are probably less indicative of any changes in the branches of the MCA.

A second limitation of this study is that only passive vascular structure was assessed. High potassium diets have been shown to improve endothelial function in Dahl rats (30, 38). In these studies endothelium dependent vasodilatation was assessed in conduit vessels from Dahl salt sensitive rats. A high potassium diet was administered in conjunction with the high sodium diet, in both cases the high potassium diet attenuated the increase in blood pressure normally seen with salt loading in these rats, making it difficult to assess if the beneficial effects on the endothelium are dependent on the elevation in potassium or on the reduction in blood pressure. However, the possibility that the HK diet protects against cerebral ischemia in the SHRSP by improving cerebral vessel dilation cannot be ruled out.
The absence of a group of WKY rats treated with the HK diet is also a limitation. For technical reasons we were not able to include this group in the current study. While an effect of the HK diet in the WKY rats would lend considerable weight to the argument that the effect of potassium is blood pressure independent the authors believe that the use of telemetry to measure blood pressure in the rats fed the two diets is sufficient to prove that, at least in the SHRSP, the effects of potassium are not due to changes in blood pressure. In a separate and later study published in abstract form using different instrumentation we have been able to show that HK diets have a similar effect in normotensive rats as they have in the SHRSP (26).

In conclusion, our studies are the first to show that a high potassium diet reduces the amount of damage caused to the brain as a result of cerebral ischemia. Our studies of vascular structure suggest that this reduction of cerebral infarct size may be due to a reduction in vessel hypertrophy in both the MCA and its branches. Interestingly we also observed in an increase in vascular compliance in the branches of the MCA that did not occur in the MCA itself, this change may be protective to prevent the rupture of the smaller intracerebral vessels with changes in pressure. We propose that the mechanism responsible for these changes is a reduction in VSMC proliferation in response to EGF and PDGF and collagen expression. While there are many questions that remain unanswered, it is clear that dietary potassium supplementation affects the vasculature and that these effects are largely protective in nature, suggesting
that the mechanisms of the effects of this simple treatment warrants further investigation.
Acknowledgements

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References.


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Table 1. Primer sequences, all sequences are 5’ to 3’.
Middle Cerebral Arteries | MCA Branches
---|---
Parameter | WKY | LK-SHRSP | HK-SHRSP | WKY | LK-SHRSP | HK-SHRSP
Inner diameter (µm) | 218±10 | 188±13 | 197±12 | 156±9 | 154±5 | 159±9
Wall Thickness (µm) | 15±1 | 20±2* | 12±1 | 10±1 | 13±1* | 11±1
Wall to Lumen Ratio | 0.139±0.01 | 0.227±0.07* | 0.124±0.02 | 0.124±0.02 | 0.168±0.015* | 0.134±0.009
CSWA (µm²) | 10487±2580 | 13470±2258 | 8106±1468 | 5518±614 | 6903±2105 | 5712±831

Table 2. Passive vessel structure, n=4 in each group (CSWA: cross sectional wall area).
**Figure Legends**

**Figure 1.** Dietary potassium supplementation has no effect on arterial pressure in SHRSP. Blood pressure was measured by radiotelemetry for three days prior to beginning the high and low potassium diets and for the six-week duration of the diet. The average day and night blood pressures were collected for each day and analyzed by a repeated measures ANOVA. The blood pressures for the WKY rats were significantly lower than those for both groups of SHRSP. * significantly different from both groups of SHRSP. (n=6 for the SHRSP fed the High K and Low K diets and n=3 for the WKY rats).

**Figure 2.** A high potassium diet reduces cerebral infarct size in SHRSP. SHRSP were fed the high and low potassium diets for six weeks from six weeks of age, WKY rats received the low potassium diet. Cerebral ischemia was induced by MCA occlusion and the size of the resultant infarct was assessed. The upper panel shows representative brain slices from rats 24 hours after the induction of ischemia, the gray area is viable tissue and the white area is tissue damaged by the ischemia. The lower panel depicts the percentage of the hemisphere infarcted (n=6 in each group results were analyzed using a one way ANOVA with a Newman Keuls post-test *=p<0.05).

**Figure 3.** Stress / strain curves for SHRSP fed the HK and LK diets and WKY rats. SHRSP were fed the specialized diets for six weeks as described previously, WKY rats were fed the LK diet. The MCA and its branches were removed from the rats and mounted in a small vessel arteriograph. **Panel A.** Stress / strain curves for the MCA’s,
the MCA from the SHRSP fed the HK and LK diets were more compliant than those from WKY rats. **Panel B.** Stress / strain curves for the branches of the MCA, the vessels from the LK-SHRSP were less compliant than the vessels from the HK-SHRSP and the WKY rats (n=4 in each group)

**Figure 4.** Expression of PDGFR-α (panel A) and PDGFR-β (panel B) is increased in LK-SHRSP compared to WKY and is reduced in the HK-SHRSP. mRNA expression was measured using RT-PCR and the results were normalized for cyclophillin expression (results were analyzed using a one way ANOVA with a Newman Keuls post-test *=p<0.05, n=6-8).

**Figure 5.** Expression of EGFR is increased in LK-SHRSP compared to WKY and is reduced in the HK-SHRSP. mRNA expression was measured using RT-PCR and the results were normalized for cyclophillin expression (results were analyzed using a one way ANOVA with a Newman Keuls post-test *=p<0.05, n=5-8).

**Figure 6.** Expression of collagen I (panel A) and collagen III (panel B) mRNA is increased in LK-SHRSP compared to WKY rats. The expression of collagen III is reduced in the HK-SHRSP compared to the LK-SHRSP. mRNA expression was measured by RT-PCR and the results were normalized for cyclophillin expression (results were analyzed using a one way ANOVA with a Newman Keuls post-test *=p<0.05, n=5-8).
Figure 1
Figure 2
Figure 5

EGFR-α/cyclophilin mRNA

WKY  HK-SHRSP  LK-SHRSP

P<0.01

P<0.01
Figure 6A

Collagen I/cyclophilin mRNA

WKY | HK-SHRSP | LK-SHRSP

Figure 6B

Collagen III/cyclophilin mRNA

WKY | HK-SHRSP | LK-SHRSP

P<0.01

P<0.05

P<0.01