A high-potassium diet reduces infarct size and improves vascular structure in hypertensive rats

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Dorrance, Anne M., David M. Pollock, Olga P. Romanko, and David W. Stepp. A high-potassium diet reduces infarct size and improves vascular structure in hypertensive rats. Am J Physiol Regul Integr Comp Physiol 292: R415–R422, 2007. First published August 17, 2006; doi:10.1152/ajpregu.00438.2005.—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 reversing 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 wk; Wistar-Kyoto (WKY) rats were fed 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 infarced (P < 0.05). Infarcts were smaller in WKY rats (33.5 ± 4.8%) than in LK-SHRSP or HK-SHRSP. The vessel wall of MCAs from HK-SHRSP was hypertrophied compared with WKY rats; this was reversed in HK-SHRSP. RT-PCR analysis of the cerebral vessels showed that expression of platelet-derived growth factor receptor-α and -β, epidermal growth factor receptor, and collagen I and III was increased in the vessels from LK-SHRSP compared with WKY rats and reduced in 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.

stroke is the third-leading cause of death in the United States. Eighty percent of all strokes are ischemic in nature and result in long-term disability. When cerebral ischemia is induced experimentally by middle cerebral artery (MCA) occlusion, the volume of the resulting infarct is greater in stroke-prone spontaneously hypertensive rats (SHRSP) than 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 remodels in such a way that arteries have smaller lumens and thicker walls (3, 5, 15). Also, in SHRSP, the ability of the anastomoses between the MCA and the anterior cerebral artery to dilate in response to ischemia is impaired (4, 6), although the number of anastomoses is constant between SHRSP and WKY rats (4). Thus, when an occlusion occurs, tissue perfusion is insufficient, 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.

Epidemiologic studies show that potassium intake correlates inversely with the incidence of cerebrovascular events (1, 11, 31, 37). Observations 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 and 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 a 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 platelet-derived growth factor (PDGF) (20).

On the basis of these observations, we hypothesized that dietary potassium supplementation would reduce the ischemic cerebral infarct size in SHRSP and improve the hypertensive remodeling of the vessel wall. We further proposed that a high-potassium diet would have these effects by reducing 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 12:12-h light-dark cycle, housed two per cage, and allowed access to food and water ad libitum. SHRSP were fed a low-potassium (LK) or a high-potassium (HK) chow containing 0.79% or 2.11% potassium, respectively (Zeigler Brothers, Gardners, PA) from 6 to 12 wk of age; WKY rats were fed the LK chow. 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 on the basis of our previous experience.

MCA occlusion. Rats were anesthetized with pentobarbital sodium (50 mg/kg ip), and body temperature was maintained at 37°C during...
anesthesia. The MCA was permanently occluded using the intraluminal 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 19 mm, 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. After occlusion (6 or 24 h), the rats were anesthetized and decapitated, and the brains were carefully removed. The area of the infarction was quantified using 2,3,5-triphenyltetrazolium staining, as described previously. The area of the brain that stained pink in response to 2,3,5-triphenyl-tetrazolium was deemed to be viable tissue, and the white area was classified as tissue damaged by ischemia (10).

RT-PCR analysis. The MCA, anterior cerebral artery, and 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 epidermal growth factor (EGF) receptor (EGFR), PDGF receptor (PDGFR)-α, PDGFR-β, and collagen I and III (Table 1). Optimum annealing temperature, cycle number, and template dilution factor were determined for each amplicon before experimentation, and each amplicon was amplified individually. The results are expressed as arbitrary densitometry units and were normalized to the expression of cyclophilin 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 physiological salt solution (in mM: 119 NaCl, 4.7 KCl, 1.17 MgSO₄, 1.18 Na₂HPO₄, 24 NaHCO₃, and 5.5 glucose) equilibrated with 21% O₂-5% CO₂-74% N₂. Vessels were cannulated at both ends with micropipettes (50- to 100-μm tip 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 diameters of the vessel were measured after 15 min at each pressure using video microscopy. 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)

wall thickness = OD − ID
Circumferential wall stress
= (intraluminal pressure × ID)/(2 × wall thickness)
Circumferential wall strain
= (ID − ID at 0 pressure)/ID at 0 pressure
where ID is inner diameter and OD is outer diameter.

Table 1. Primer sequences

<table>
<thead>
<tr>
<th>Product</th>
<th>Forward</th>
<th>Reverse</th>
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<tbody>
<tr>
<td>EGFR</td>
<td>GCT CCC AGT ACC TAC TCA</td>
<td>CAT AAG GCT TGG ACC CAA</td>
</tr>
<tr>
<td>PDGFR-α</td>
<td>GAG AAA GGA TGG TGG TGA</td>
<td>ACA TCG GTG GTG ATC TCA</td>
</tr>
<tr>
<td>PDGFR-β</td>
<td>AGT GGA TGG CCC CAG A</td>
<td>CCT CTA GGA GGC CCT CA</td>
</tr>
<tr>
<td>Collagen I</td>
<td>CAC TGG TGA ATG GAG CAA</td>
<td>TGG TGG GTG AAA CAG CA</td>
</tr>
<tr>
<td>Collagen III</td>
<td>CAC TGG TGA TGG AGC AA</td>
<td>TGG TGG GTG AAA CAG CA</td>
</tr>
<tr>
<td>Cyclophilin</td>
<td>TGG TGG TCT TGC CAT TGC TG</td>
<td>TGG TGG TTG TGG CCG CCT GC</td>
</tr>
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All sequences are 5’ to 3’. EGFR, epidermal growth factor; PDGFR, platelet-derived growth factor receptor.
rats (4.89 ± 0.1 mmol/l) was similar to that in the LK-SHRSP. HK-SHRSP weighed less than LK-SHRSP: 270 ± 4.5 vs. 287 ± 3.2 g (P < 0.05). The WKY rats weighed 271 ± 11.8 g.

Cerebral infarct size. Cerebral ischemia was induced by MCA occlusion. After 6 h of ischemia, the cerebral infarct was 35% smaller in the HK-SHRSP compared with the WKY-SHRSP (n = 8) than in the LK-SHRSP (n = 5): 37.0 ± 2.1 vs. 56.7 ± 6.1% of the hemisphere was infarcted (P < 0.05). The WKY rats fed the LK diet had smaller infarcts (14.51 ± 2.7% for WKY) than in the HK-SHRSP and LK-SHRSP, indicating hypertrophy of the vascular wall. The wall thickness and wall-to-lumen ratio were similar in the HK-SHRSP and the WKY rats, suggesting that the HK diet reduces vascular hypertrophy in the SHRSP.

The circumferential stress-strain relation was determined as an indicator of arterial stiffness (Fig. 3). In the MCAs, the stress-strain curve was shifted to the right for the HK-SHRSP and LK-SHRSP, indicating an increase in distensibility compared with 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 RT-PCR analysis of growth factor receptor expression. The mRNA expression of PDGFR-α, PDGFR-β, and EGFR was increased in vessels of the LK-SHRSP compared with vessels of the WKY rats (Figs. 4 and 5). In HK-SHRSP, mRNA expression of all three growth factor receptors in the cerebral vasculature was significantly reduced.

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

DISCUSSION

There are three novel findings in this study. We have shown that dietary potassium supplementation reduces the size of the ischemic cerebral infarct in SHRSP. We have also shown that vessel wall thickness is reduced and vascular compliance is increased by dietary potassium supplementation. These structural changes correle with the 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 brain damage caused by cerebral ischemia (7, 10), which 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 also previously showed that administration of spironolactone, a potassium-sparing drug, reduces the ischemic cerebral infarct size in SHRSP (10). The mechanism for the protective

![Diagram of stress-strain curves for HK-SHRSP, LK-SHRSP, and WKY rats.](http://ajpregu.physiology.org/)

**Fig. 3.** Stress-strain curves for HK-SHRSP, LK-SHRSP, and WKY rats. SHRSP were fed the specialized diets for 6 wk as described previously; WKY rats were fed the LK diet. MCA and its branches were removed from the rats and mounted in a small vessel arteriograph. **A:** stress-strain curves for MCAs. MCA from the HK-SHRSP and LK-SHRSP were more compliant than those from WKY rats. **B:** stress-strain curves for branches of the MCA. Vessels from LK-SHRSP were less compliant than those from HK-SHRSP and WKY rats ($n = 4$ in each group).

![Bar graph showing expression of platelet-derived growth factor receptor (PDGFR) α and β in HK-SHRSP, LK-SHRSP, and WKY rats.](http://ajpregu.physiology.org/)

**Fig. 4.** Expression of platelet-derived growth factor receptor (PDGFR) α and β is increased in HK-SHRSP compared with WKY and is reduced in LK-SHRSP. mRNA expression was measured using RT-PCR, and results ($n = 6–8$) were normalized for cyclophilin expression. *$P < 0.05$ (by 1-way ANOVA with Newman-Keuls post test).
effects of high dietary potassium has never been fully elucidated.

Rats were fed the specialized diets for 6 wk beginning at 6 wk of age. During this period, 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 present studies show that an increase in the potassium in the diet from 0.79% to 2.11% can increase the levels of plasma potassium and significantly reduce the size of the ischemic cerebral infarct. After 24 h of ischemia, the size of the cerebral infarct was less in the HK-SHRSP, suggesting a protective effect of this diet on ischemic damage. After 6 h 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 in a normotensive rat. The effects of dietary potassium supplementation on blood pressure have been controversial, with most studies suggesting that it lowers blood pressure by only 10–20 mmHg (14, 18). These rats were fed a high-sodium diet, and although 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, which 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 high-potassium 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, cerebral infarcts are larger in the SHRSP than in 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 the small and larger cerebral blood vessels, the LK-SHRSP had significantly thicker vessel walls and a greater wall-to-lumen ratio than the WKY rats, which were also fed the low-potassium diet. The administration of the

![Graph](image.png)

Fig. 5. Expression of epidermal growth factor receptor (EGFR) is increased in LK-SHRSP compared with WKY and reduced in HK-SHRSP. mRNA expression was measured using RT-PCR, and results (n = 5–8) were normalized for cyclophilin expression.

![Graph](image.png)

Fig. 6. Expression of collagen I and collagen III mRNA is increased in LK-SHRSP compared with WKY rats. Expression of collagen III is reduced in HK-SHRSP compared with LK-SHRSP. mRNA expression was measured by RT-PCR, and results (n = 5–8) were normalized for cyclophilin expression.
high-potassium diet to the SHRSP reduced both of these parameters to levels similar to those in the WKY rats. Changes in vascular compliance were also evident in the hypertensive rats. In the MCAs the stress-strain curve for the SHRSPs was shifted to the right of the curve for the WKY rats, 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 LK-SHRSP were less compliant than those from the WKY rats, as evidenced by a leftward shift of the stress-strain curve. The reduction in the distensibility of small cerebral arteries was reversed by the high-potassium 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 size of the cerebral infarct 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 high-potassium 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 downstream vessels.

The increased vessel wall thickness in the hypertensive rats may be due to an increase in smooth muscle proliferation, increase in cell size, or increase in 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 previously showed that EGFR mRNA levels are greater in the cerebral vasculature of SHRSP than WKY rats (10). We confirmed that finding here and also showed greater expression of PDGFR-α and PDGFR-β in the LK-SHRSP than in the WKY rats. The expression of all three growth factor receptors was reduced in the HK-SHRSP. This raises the possibility that VSMC proliferation is reduced in the HK-SHRSP. Others have shown that addition of potassium to the medium 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 upregulation in the expression of this gene (39). It is therefore possible that in the rats fed the low-potassium diet there is an upregulation of gene expression in response to the low potassium levels and that this upregulation is effectively inhibited by the high-potassium diet. Clearly, several additional studies are needed to assess whether 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 with the WKY rats and was reduced in the HK-SHRSP. A reduction in collagen deposition could be the mechanism for the increased compliance in the branches of the MCAs 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 that 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 aorta has also been suggested to be responsible for the protective effect of dietary potassium supplementation (17, 32, 33). Clearly, more investigation is needed 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 high-potassium 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, inasmuch as IL-1β is known to increase the expression of PDGFR-α in VSMC by increasing the expression of CCAT/enhancer-binding protein-δ (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 high- and low-potassium diets (data not shown). This is consistent with other studies from Manger et al. (21) 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.

One of the limitations of this study is that the RT-PCR was used to assess the effects of the high-potassium 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 believe that this type of analysis is appropriate here, inasmuch 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 those studied in our structure experiments. Also, our results most probably reflect the changes 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, and in both cases the high-potassium diet attenuated the increase in blood pressure normally seen with salt loading in these rats; therefore, it was difficult to assess whether 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 high-potassium 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 high-potassium diet is also a limitation. For technical reasons, we were not able to include this group in the present study. Although an effect of the high-potassium diet in the WKY rats was not found, 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 using different instrumentation, we showed that high-potassium diets have a similar effect in normotensive rats and in the SHRSP (26).

In conclusion, our studies are the first to show that a high-potassium diet reduces the amount of brain damage caused by 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 the MCA and its branches. Interestingly, we also observed 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, PDGF, and collagen expression. Although many questions 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 warrant further investigation.

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GRANTS

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