High Potassium Diets Protect Against Dysfunction of Endothelial Cells in Stroke-Prone Spontaneously Hypertensive Rats

TOKUICHIRO SUGIMOTO, LOUIS TOBIAN, AND MUKUL C. GANGULI

SUMMARY Two lines of evidence strongly support the hypothesis that high potassium diets protect arterial endothelial cells from hypertensive damage. Stroke-prone spontaneously hypertensive rats (SHRSP) fed normal (0.75%) K or high (2.1%) K and normotensive Wistar-Kyoto rats (WKY) were examined in an endothelial function study and a histological study. In the endothelial function study, aortic rings were suspended in tissue baths to monitor isometric tension. Rings contracted with norepinephrine were tested with acetylcholine and sodium nitroprusside. In normal K SHRSP (blood pressure, 156 mm Hg), endothelium-dependent acetylcholine relaxation was severely depressed by 49% (p<0.001), whereas in high K SHRSP (blood pressure, 155 mm Hg), normal values were preserved. Endothelium-independent nitroprusside relaxation was virtually the same in both the SHRSP groups (high K vs normal K diet). Since indomethacin did not improve the impaired acetylcholine relaxation in normal K SHRSP, the cyclooxygenase products do not appear to have affected the endothelium-dependent relaxation in the normal K SHRSP. Thus, the endothelium-dependent relaxation response was much decreased in the normal K SHRSP and was preserved in the high K SHRSP. Thus, a high K diet appears to protect the aortic endothelium from a hypertension-induced dysfunction. In the histological study, aortic and mesenteric intimal lesions were assessed blindly under the microscope and graded from 0 to 60 for aortic and from 0 to 40 for mesenteric lesions. Aortic intimal lesion scores were 28 in normal K SHRSP (blood pressure, 209 mm Hg) and 13 in high K SHRSP (blood pressure, 207 mm Hg; -54%; p<0.001). Mesenteric scores were 18 in rats on the normal K diet and 10 in rats on the high K diet (-45%; p<0.001). Scores of high K SHRSP equaled those of WKY. Thus, a high K diet prevented the hypertensive intimal lesions without lowering the blood pressure. Endothelium protection by a high K diet seems a very likely partial explanation for the markedly reduced lesions in the high K SHRSP. (Hypertension 11: 579-585, 1988)

KEY WORDS: potassium, arterial lesions, stroke-prone spontaneously hypertensive rats, endothelium-derived relaxing factor, endothelial cell injury, platelet-derived growth factor

STROKE-PRONE spontaneously hypertensive rats (SHRSP) are regarded as an experimental model of severe hypertension. The development of strokes and stroke-induced death in SHRSP was remarkably reduced by potassium supplements in the diet, and this effect was obtained without any blood pressure reduction. Therefore, the protective effect of a high K diet against hypertensive arterial damage was somehow independent of blood pressure-lowering activity. Since functional injury of the endothelium has been proposed as a key factor in atherosclerosis development, hypertensive arterial lesions might also involve a functional injury to the endothelium. On the basis of the data presented herein, we propose a hypothesis that arterial endothelium is protected from such functional hypertensive injury by high K diets.

Recently, arterial endothelium was found to have the capacity to produce and release a factor, endothelium-derived relaxing factor (EDRF), that relaxes arterial smooth muscle. Investigators have noted that this endothelium-dependent relaxation is impaired in several different types of experimental hypertension. A decreased production and release of EDRF caused by the hypertension is a likely mechanism for this. Thus, examining the endothelium-dependent relaxation should be a good gauge by which to test endothelial function and should be a good measure of functional...
hypertensive endothelium injury. In the present report, we examined the effect of high K diets on endotheli-
dependent relaxation as well as on the hyperten-
sive intimal lesions in high salt-fed SHRSP.

Materials and Methods

Experiment 1
Arterial Section Preparation

Male 5-week-old SHRSP rats from our breeding colony were fed a high sodium, Japanese-type chow for 8 weeks. This basic diet, containing 0.75% K and 6% NaCl, was fed to 70 SHRSP rats. Twenty-three of these rats received only this basic diet with no added K, while 47 other SHRSP received this same diet plus 1.36% added K, either in the form of potassium citrate or KCl (final K concentration, 2.11%). Age-matched Wistar-Kyoto rats (WKY) obtained from Taconic Farms (Germantown, NY, USA) were also fed the basic diet with no added K (n = 13).

After 8 weeks, mean arterial pressure was taken with the rats under Inactin anesthesia (100 mg/kg i.p., Byk Gulden Konstanz, West Germany) through a PE-50 catheter inserted into the right carotid artery. Then each rat was perfused intra-aortically for 5 minutes with a phosphate buffer solution (pH 7.4), using a perfusion pressure equal to the mean arterial pressure of that particular rat. The perfusion solution then was switched to 10% neutralized formalin. After 15 minutes of this perfusion fixation, the thoracic aorta and the superior mesenteric artery were cut out and further fixed in 10% neutral formalin for 2 days. These arteries were then dehydrated with 50, 70, 80, 90, and 100% ethyl alcohol for 1 hour and cleared by toluene for 1 hour. Then tissues were infiltrated with paraffin wax and embedded to obtain an arterial cross-section at 5 mm above the diaphragm for the aorta and at 5 mm distal from the abdominal aorta for the superior mesen-
teric artery. Sections (5 μm) were stained for elastic fibers using Verhoeff’s elastica stain. Rats and tissues were processed on a round-robin basis to minimize the effects of artifacts on the results.

Determination of Arterial Intimal Damage Score

Since there were considerable changes in arterial intima, including various degrees of intimal thickening and splitting of the internal elastic lamina, we tried to quantify the severity of these intimal lesions. With the use of 430 × magnification, each microscopic field of artery wall covered an arterial segment about 450 μm in length. One observer (T. Sugimoto) scored nine such segments for each aorta and two such segments for each mesenteric artery. Each slide was given a code number by a third person so that scoring would be done in a completely blind manner. The aortic intima was scored using seven grades of severity of intimal thick-
ening plus internal elastic splitting (0 = no change, 10 = slightest change, 60 = most severe change). The mesenteric artery intima was scored with five grades (0 = no change, 10 = slightest change, 40 = most severe change). The mean value of these scores was designated as the average intimal lesion score for a given artery.

Experiment 2

Tissue Bath Preparation

Male 6-week-old SHRSP were fed either a normal K diet (6% NaCl, 0.75% K; n = 11) or a high K diet (6% NaCl, 2.11% K; n = 9) for 5 weeks. Age-matched WKY (n = 8) were fed standard Purina rat chow containing 0.3% NaCl (St. Louis, MO, USA). The rats were killed by decapitation after direct blood pressure measurement in the right femoral artery under light ether anesthesia. After exsanguination, thoracic aortas were quickly cut out and washed gently in ice-chilled modified Krebs-Ringer bicarbonate solution to remove the remaining blood. The composition of the Krebs-Ringer solution was (mM) NaCl, 118; KCl, 4.7; CaCl2, 2.5; MgSO4, 1.2; KH2PO4, 1.2; NaHCO3, 25.0; edetate calcium disodium, 0.026; glucose, 11.1. After all adhering connective tissue was carefully re-
moved, aortic rings from the middle portion of the thoracic aorta (5 mm wide) were suspended in 10-ml tissue baths filled with warmed (37°C) Krebs-Ringer solution and connected to isometric force transducers (Model FT-02 or FT-03D, Grass, Quincy, MA, USA) coupled to a Grass recorder. The bath solution was aerated with a 95% O2, 5% CO2 mixture throughout the experiment.

Study Protocol

The thoracic aortic rings were given 2.0 g of basic tension, which was found to produce optimal isometric force development in a preliminary study using rats of the same age. After a stabilization of the baseline (20–30 minutes), norepinephrine was added to the tissue bath to obtain about 1.0 g of additional tension, which was about 50% of the maximal contraction. Then increasing doses of acetylcholine (10−10 up to 10−7 M) were added to the tissue bath every 4 minutes to ob-
serve the dose responsiveness for endothelium-depen-
dent relaxation. After the highest dose of acetylcholine was given, the aortic ring was rinsed two times by changing the bathing solution, followed by another 20 to 30 minutes of stabilization. The tissue was then con-
tacted again with norepinephrine to obtain 1.0 g of additional tension. Then, in order to observe an endothelium-independent relaxation, sodium nitroprusside was applied in increasing doses (10−10 up to 10−7 M). The amount of the relaxation was expressed as the percentage of relaxation caused by a relaxant, using the contraction from norepinephrine as the baseline. To examine the possibility that some cyclooxygenase product was involved in the endothelium-dependent effect of acetylcholine, some rings were tested after a preincubation for 30 minutes with 10−3 M indomethacin.10

Chemicals

Norepinephrine bitartrate, acetylcholine chloride, sodium nitroprusside, and indomethacin were obtained
from Sigma Chemical (St. Louis, MO, USA). Norepinephrine and acetylcholine were dissolved in deionized water and stored in small vials of a 10 mM solution at −70°C. Sodium nitroprusside was dissolved in deionized water (10 mM) and stored in the dark at 4°C. Up to 2 months of storage was confirmed not to decrease the biological activity of these chemicals. During an experiment, each stock solution was diluted in the modified Krebs-Ringer bicarbonate solution and up to 100 μl was added to the tissue bath. Indomethacin was dissolved in 5 mM Na₂CO₃ solution on the day of the experiment, and 200 μl was added to the tissue bath. This treatment did not change the pH of the bath solution.

**Statistical Methods**

The results were expressed as means ± SE. Differences in a given pair of the three groups were analyzed by Student's unpaired t test. A p value of less than 0.05 was regarded as significant.

**Results**

**Experiment 1**

Table 1 shows the body weight and mean arterial pressure in each group of rats after 8 weeks on the three diets. Body weights of both SHRSP groups were significantly lower than that of the WKY group. Mean arterial pressure was much lower in the WKY compared with both SHRSP groups. Among the two SHRSP groups, there was no difference in the blood pressure (209 vs 207 mm Hg). Figure 1 shows some examples of aortic intimal changes. Panel A depicts an aortic segment with no intimal lesions (score = 0). Panel B shows slight intimal changes in a segment with an apparent increase in the intimal cellularity (score = 10). Panel C shows severe lesions, with considerable thickening of the intima and splitting of the internal elastic lamina (score = 50). Average scores for intimal injury are shown in Figure 2. As can be seen in Figure 2A, the aorta score for normotensive WKY averaged 14. There are two groups of SHRSP with closely matching blood pressures (209 vs 207 mm Hg). The SHRSP on the normal 0.75% K intake had an average lesion score of 28, whereas the SHRSP on the high 2.1% K diet for 8 weeks had an average lesion score of only 13. Thus, even though the blood pressure was equally high in the two groups, the lesion score was reduced 54% in rats on the high K diet (p<0.001).

**TABLE 1. Body Weight and Mean Arterial Pressure of the Rats in Experiment 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Mean arterial pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal K SHRSP</td>
<td>218 ± 8*</td>
<td>209 ± 7*</td>
</tr>
<tr>
<td>High K SHRSP</td>
<td>236 ± 3*†</td>
<td>207 ± 3*</td>
</tr>
<tr>
<td>Normal K WKY</td>
<td>363 ± 8</td>
<td>138 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SE.

* p < 0.001, compared with WKY values.
† p < 0.05, compared with normal K SHRSP.

**Figure 1. Examples of aortic intimal lesions in three different aortic segments. Segment A has no lesions (score = 0), whereas Segment B has slight changes (score = 10) and Segment C has severe lesions, with thickening of the intima and splitting of the internal elastic lamina (score = 50). Segment A is from an SHRSP on the high K diet. Segments B and C are from SHRSP on the normal K diet. (Verhoeff's elastica stain; ×256.)**

**Figure 2. Average intimal lesion scores in aorta (A) and superior mesenteric artery (B). BP = blood pressure (in mm Hg).**
In the superior mesenteric artery (see Figure 2B), the lesion score averaged 18 in the 23 SHRSP on the normal K diet compared with a lesion score of 10 in the 47 SHRSP on the 2.1% high K diet. Thus, the lesion score was reduced 45% in those SHRSP on the high K diet, even though the blood pressure was virtually equal in the two groups. In both arteries the score in rats fed the high K diet was about the same as that found in normotensive WKY. In both the aortic and the mesenteric artery, the scores of SHRSP fed the K supplement (high K SHRSP) were significantly lower than those of SHRSP fed no added K (normal K SHRSP; p < 0.001 for all values). These comparisons demonstrate a highly protective effect of the K supplements against intimal injury. The scores of K-supplemented rats were not significantly different from those of normotensive WKY controls. Thus, the K supplements prevented hypertensive arterial intimal lesions in SHRSP and kept them at the same level as those of normotensive WKY.

**Experiment 2**

As in Experiment 1, the high K diet did not lower the blood pressure in the SHRSP in Experiment 2 (normal K SHRSP: 156 mm Hg; high K SHRSP: 155 mm Hg). The blood pressure in the SHRSP in Experiment 1 was about 50 mm Hg higher than that in SHRSP in Experiment 2. Longer salt feeding, a difference in anesthesia, and carotid surgery have all resulted in this difference in blood pressure.

Acetylcholine additions to the tissue bath caused dose-dependent arterial wall relaxation in the three groups (Figure 3). However, the relaxation response was markedly impaired in the normal K SHRSP (blood pressure, 156 mm Hg) at doses higher than 10⁻⁷ M. On the other hand, relaxation was very well preserved in the high K SHRSP, even though the blood pressure was equally high (155 vs 156 mm Hg; Figure 4). Relaxation in the high K SHRSP was very close to that of normotensive WKY at all doses from 10⁻¹⁰ through 10⁻⁷ M (see Figure 3). When doses of acetylcholine required for 50% relaxation were compared (Figure 5) in eight normotensive WKY, a concentration of 32 nmol of acetylcholine per liter of bath solution was required for 50% relaxation. In the nine hypertensive SHRSP fed the 2.1% high K diet, 50% relaxation required an acetylcholine concentration of 36 nmol/L, showing that the normal sensitivity to acetylcholine had been preserved during the high K feeding. On the other hand, the 11 SHRSP fed the 0.75% normal K diet required 100 times more acetylcholine, 3600 nmol/L, to achieve a 50% relaxation, indicating a markedly reduced sensitivity for endothelium-dependent relaxation induced by acetylcholine (see Figure 5). Thus, hypertension appeared to cause a functional injury to
the endothelial cells in rats fed the normal K diet, but this functional injury was prevented by the high K diet. Indomethacin preincubation did not alter the impaired endothelium-dependent relaxation in the normal K SHRSP (data not shown).

Nitroprusside is a vasodilator that relaxes arterial smooth muscle with a mechanism that does not use EDRF. After a 30-minute double washout, these same arterial rings were exposed to nitroprusside. Sodium nitroprusside additions to the bath relaxed norepinephrine-contracted aortic rings dose-dependently in both SHRSP groups. The SHRSP fed either a normal or a high K diet showed an equal $10^{-7}$ M nitroprusside relaxation of about 90% (Figure 6). As shown in the lower panel of Figure 6, the concentration of nitroprusside required for 50% relaxation was not significantly different for SHRSP fed either the normal or the high K diet. Thus, the nitroprusside relaxation, which does not involve endothelial cells, was similar in the normal K and in the high K rats. Conversely, the acetylcholine relaxation, which does involve endothelium, was markedly reduced in the SHRSP fed the normal K diet, but it was preserved intact in the SHRSP fed the high K diet. This finding indicates that the high K diet in the SHRSP preserved the capacity for endothelium-dependent relaxation, whereas the SHRSP fed the normal K intake appeared to have lost a good portion of their capacity for endothelium-dependent relaxation. Thus, a high K diet appears to protect endothelial cells from hypertensive injury, while a normal K intake allows the endothelial cells to undergo hypertensive injury.

**Discussion**

In Experiment 1, hypertensive intimal lesion formation in SHRSP was almost completely prevented by the high K diet, with virtually no reduction in blood pressure. The exact mechanism of hypertensive arterial disease is still elusive. However, increased permeability of the endothelium to carbon particles (India ink) and to horseradish peroxidase has been demonstrated in both acute and chronic experimental hypertension,1-11 and an increased turnover of endothelium was observed after 2 to 3 weeks of renal hypertension in rats.12 Therefore, mechanical or functional aberration of endothelium is likely a common early event in arterial hypertension. On the other hand, several chemotactic or mitogenic factors (or both) acting on vascular smooth muscle cells have recently been identified. These are platelet-derived growth factors (PDGFs) coming from platelets and macrophages as well as endothelium.16-20 These growth factors appear to be a major cause of the migration of smooth muscle cells from media to intima and the proliferation of these cells in the intima in atherosclerosis.4 These intimal smooth muscle cells then begin to lay down extracellular matrix including collagen, elastin, and acid mucus polysaccharide.

The release of PDGF from endothelial cells is accelerated in tissue culture when the endothelial cells are in an injured state.4, 7 On the other hand, endothelium also normally produces heparinlike substances that have inhibitory effects on vascular smooth muscle cell growth, but replicating endothelium does not have this property.22 It is not known whether the dysfunction of endothelium caused by hypertension has an effect on the production of these heparinlike substances. Moreover, the functional lesion of endothelial cells caused by hypertension attracts various leukocytes, including monocytes. These monocytes often migrate into the intima and add to the intimal thickening.23, 24 These macrophages also release the platelet-derived growth and attractant factor, which tends to bring vascular smooth muscle cells from media to intima and stimulates their growth in the intima. This is another pathway by which hypertensive endothelial dysfunction can influence intimal arterial lesions. Therefore, aberrations of the production of these growth factors and growth inhibitory factors from endothelial cells, as well as other functional changes of endothelium including increased permeability and increased monocyte attraction, are probably very relevant to the initiation and development of arterial lesions in hypertension. When hypertensive arterial intimal lesions are largely prevented by high K diets without blood pressure reduction, as we observed in Experiment 1, one has to consider that endothelium damage was somehow prevented. When endothelial function is preserved intact in the presence of hypertension, increased permeability and release of PDGF will be prevented and macrophages will not be attracted. The high K diet strongly reduced the degree of intimal injury, including both the thickening of the intimal layer and the splitting of the internal elastic lamina. The degree of injury was reduced to such an extent that the injury score was about the same as that found in the normotensive WKY. Hence, when the number of lesions is greatly reduced with a high K diet, the functional endothelial cell injury probably is also greatly reduced. Once extra cells have migrated into the intima and have grown, it is uncertain whether endothelium...
endothelium-dependent relaxation. Therefore, aortic endothelium likely had sustained a severe functional injury in the normal K SHRSP. In the equally hypertensive high K SHRSP, endothelium-dependent relaxation was severely depressed. Therefore, aortic endothelium likely had sustained a severe functional injury in the normal K SHRSP. In the equally hypertensive high K SHRSP, endothelium-dependent relaxation was severely depressed. Therefore, aortic endothelium likely had sustained a severe functional injury in the normal K SHRSP.

However, one may have to consider some other possibilities to explain this observation. First, since we tested the EDRF release from endothelium by measuring smooth muscle relaxation, a difference in the smooth muscle response to EDRF between the high K SHRSP and the normal K SHRSP could have resulted in the different extent of relaxation. Since sodium nitroprusside is considered to share a common intracellular mechanism with EDRF for relaxing vascular smooth muscle cells, the finding that relaxation with nitroprusside was the same among the high K and the normal K SHRSP appears to militate against this possibility. Judging from the nitroprusside results, aortic rings from normal K SHRSP would likely have equal responsiveness to EDRF as those from the high K SHRSP. Second, as Lüscher and Vanhoutte showed, impaired endothelium relaxation in spontaneously hypertensive rats could be ascribable to the concomitant release of an endothelium-dependent contracting factor, which was inhibited by indomethacin. In our study the same pretreatment of aortic rings with indomethacin did not restore the acetylcholine relaxation defect in aortic rings from SHRSP fed the normal K diet. Therefore, a cyclooxygenase product did not appear to be involved in the depressed endothelium-dependent relaxation in the normal K SHRSP. The reason for the discrepancy between our experiment and theirs may lie in the differences in the strain of rats, in the NaCl status of the rats, or in the course and magnitude of high blood pressure development. Obviously, we examined a more accelerated hypertension than they did.

The possibility that the aortic structural changes impaired the diffusion of EDRF from endothelium to smooth muscle cells also does not seem very likely. For instance, in cholesterol-fed monkeys, there is a severe impairment of endothelium-dependent relaxation in muscle rings from the iliac artery. Subsequent resumption of a low cholesterol normal diet in these monkeys completely restored endothelium-dependent relaxation, even though gross thickening of the intima of these iliac arteries was still present. Thus, a thickened intima per se does not seem to impair endothelium-dependent relaxation. Therefore, the main mechanism to account for the depressed endothelium-dependent relaxation in the normal K SHRSP probably is the decreased production or release of EDRF, which is likely a result of the functional endothelial abnormality caused by sustained high blood pressure. It is also highly likely that the high K diet prevented most of this functional hypertensive endothelial injury. However, to prove with even greater certainty that high K diets protect endothelium, it would be desirable to have a direct chemical measurement of EDRF release, whenever this becomes available.

If high K diets truly protect endothelium from hypertensive injury, what could be the mechanism? To answer the question, more profound knowledge about the precise mechanism of initiation and development of hypertensive arterial disease is necessary. In any case, one would have to assume some mechanism that does not involve a lowering of the high blood pressure. Moreover, alterations in total body potassium or sodium, in plasma potassium, and in skeletal muscle and aortic potassium or sodium do not appear to be involved in the protective effects of the high K diets. Humoral or neural mechanisms could also be involved in hypertensive arterial changes. Potassium supplements may influence some of these mechanisms. It is known that potassium supplements increase aortic Na\(^+\),K\(^+\)-adenosine triphosphatase (ATPase) activity. There is also evidence that ouabain treatment increases aortic DNA synthesis without raising the blood pressure. Thus, Na\(^+\),K\(^+\)-ATPase activation by potassium may play some role in modulating hypertensive arterial changes.

It has been recently reported that EDRF released from endothelial cells inhibits platelet adhesion to endothelial cells and also inhibits platelet aggregation. Thus, a deficiency of EDRF release, such as would be found in hypertensive arteries, would encourage platelet adhesion to endothelial cells as well as platelet aggregation in the vicinity of the adhering platelets. This effect would strongly increase the likelihood of thrombosis on artery walls. Many infarct-type strokes in hypertensive people or rats are the result of thrombus formation in cerebral arteries. An endothelial dysfunction resulting from hypertension could, by decreasing EDRF release, encourage local thrombus formation through the increase in platelet adhesion and aggregation. High K diets greatly reduce brain infarcts in SHRSP and at the same time protect the endothelium from hypertensive dysfunction. This preservation of EDRF release could be an important factor in reducing these thrombotic infarcts.

In conclusion, high K diets prevent arterial intimal lesions and impairment of endothelium-dependent relaxation in SHRSP without lowering the blood pressure. Endothelium protection by the high K diets is a likely explanation for these effects.

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References

High potassium diets protect against dysfunction of endothelial cells in stroke-prone spontaneously hypertensive rats.
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