High Potassium Intake Inhibits Neointima Formation in the Rat Carotid Artery Balloon Injury Model

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Recently, we reported that elevated extracellular potassium concentration in vitro inhibited proliferation and migration of vascular smooth muscle cells, formation of free radical compounds by macrophages, and reduced platelet sensitivity to agonists. In the present study we analyzed the effects of long-term, in vivo elevation of extracellular potassium concentration resulting from changes in dietary potassium intake on the vascular response to injury. The rat carotid artery balloon injury model was employed in 70 adult Sprague Dawley rats assigned to three groups. Beginning 14 days before surgical placement of the carotid lesion and continuing until death, the animals were fed diets containing either low (0.1% potassium, n = 25), normal (1.5% potassium, n = 19), or high potassium (4.0% potassium, n = 26). Fourteen days postsurgery the animals were killed and the arteries were analyzed to determine quantitatively the ratio of neointimal to medial area. Dietary potassium had a significant effect on arterial plasma potassium concentration (one-way analysis of variance, \( P < .01 \)). Group mean and standard errors were 4.26 ± 0.12 mmol/L for the low-potassium group, 5.22 ± 0.19 mmol/L for normal, and 5.80 ± 0.23 mmol/L for the high-intake group. Increases in dietary potassium attenuated neointima formation significantly (\( P < .05 \), one-way analysis of variance), with the mean ratio of neointimal area to medial area being 0.447 ± 0.106 for the low-intake animals, 0.384 ± 0.116 for normal, and 0.240 ± 0.046 for the high-intake group. These results are consistent with a hypothesis that a high level of potassium intake is effective in inhibiting neointima formation in vivo. Am J Hypertens 2000;13:1014–1020 © 2000 American Journal of Hypertension, Ltd.

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High rates of potassium intake are associated with protection from cardiovascular diseases in populations consuming primitive diets and in vegetarians living in industrialized cultures (for review see references 1–3). In studies in both humans and animals, a strong inverse association between potassium intake and hypertension and stroke has been described.4,5 We have presented results that suggest mechanisms by which potassium could provide a vascular protective effect.

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Increases in potassium concentration inhibit the following: free radical formation from vascular endothelial cells and macrophages; proliferation of vascular smooth muscle cells; vascular smooth muscle cell migration; and platelet aggregation and arterial thrombus formation. We propose that in vivo elevation of dietary potassium intake increases plasma potassium concentration, thereby inhibiting free radical formation, smooth muscle proliferation, and thrombus formation. As a result, the rate of vascular lesion formation may be diminished. This hypothesis has received support from our recent observation of an inverse relationship between dietary potassium intake and occurrence of coronary artery arteriosclerotic lesions in cholesterol-fed rabbits. These cellular functions affected by potassium are also believed to be important in formation of the neointimal proliferative lesions that follow balloon angioplasty in the rat carotid artery. This widely used model of the vascular response to injury was employed in the present study to test the hypothesis that chronic elevation of potassium intake and extracellular potassium concentration inhibit activity of the cells involved in lesion formation. If the hypothesis were valid, the magnitude of neointimal proliferation measured in the rats with a high potassium diet would be less than that in the rats consuming diets with normal or low potassium content.

**MATERIALS AND METHODS**

Three groups of Sprague Dawley rats weighing 250–340 g were used. The animals were housed in a room with a 12-h light/dark cycle beginning with light at 0800 h. For 14 days before surgery they were fed specially formulated diets, containing either 0.1% potassium for the low-intake group (Teklad/Harlan TD 94198, Harlan Teklad, Madison, WI), 1.5% for the normal intake group (TD 94305), or 4.0% for the group eating the high-potassium intake (TD 94199). One-half of the potassium was added as KCl and half as KHCO₃. All had a sodium content of 0.24%. Standard rat laboratory diets produced by Teklad/Harlan have potassium contents of approximately 1.0%.

After the 14 days of modified diet, the rats were anesthetized with sodium pentobarbital, 50 mg/kg intraperitoneal, and under sterile conditions the right carotid artery was exposed via a midline incision in the neck. Using a modification of the technique developed by Clowes and associates, the vessel was isolated, including the initial portion of the external carotid. A 2F Fogarty balloon embolectomy catheter (Edwards Life Sciences, Irvine, CA, EMB60) was introduced into the right external carotid and passed caudally to the aorta. The balloon was inflated with 0.06 mL of air and then withdrawn approximately 30 mm cephalically, then deflated and advanced back into the aorta. The procedure was repeated six times, after which the catheter was removed and the external carotid ligated. Care was taken to not compromise flow through the internal carotid artery. The cervical incision was closed and the animal permitted to recover.

After surgery the animals consumed the modified diets for an additional 14 days at which time they were anesthetized with sodium pentobarbital, and a blood sample drawn from the abdominal aorta for measurement of sodium and potassium activities (Nova 1 ion selective electrode system, New Bedford, MA), and blood gas data (Instrumentation Laboratories, Lexington, MA; Model 1304 pH/blood gas analyzer). After blood sampling, the carotid arteries were removed, fixed and mounted in paraffin, sectioned (5–7 μm), and stained with hematoxylin and eosin (H&E) for histologic analysis. There were 25, 19, and 26 animals analyzed in the low-, normal-, and high-intake groups, respectively.

Vessel wall morphology was quantified using digital image analysis (Optimas Corporation, Seattle, WA). Investigators blinded to the make-up of the experimental groups conducted the analyses. All sections mounted from a particular artery were inspected microscopically and the section with the largest neointimal area was identified. The selected section was analyzed to determine the area of the entire vessel wall, from the inner (luminal) limit of the wall to the external elastic lamina; the neointima, from the inner limit of the wall to the internal elastic lamina; and the media, from the internal elastic lamina to the external elastic lamina.

Plasma renin activity measurements were made in 0.5 mL of blood placed into iced sodium EDTA tubes and centrifuged for more than 10 min at 4°C. We used 0.2 mL of plasma for the radioimmunoassay procedure. After our initial study we observed that the plasma potassium concentrations were abnormally low. These findings suggested that the stress of anesthesia and surgery had affected the plasma levels of potassium. Accordingly, to obtain a more accurate measurement of the relationship between diet, potassium concentration, and neointima formation, a second, parallel study was conducted using the same strain and age of rats, fed the same three diets for the same time periods as in the first study. However, in the parallel study, under anesthesia using sterile procedures, catheters were implanted into the rats’ abdominal aortae 7 days before the completion of the 4-week feeding period. The presence of the indwelling catheters permitted sampling of blood from the conscious animals on the 28th day of the study in absence of surgical and anesthetic stress. The samples were drawn between 0800 and 0900 h. In addition to sampling blood for
measurement of potassium concentration and plasma renin activity, arterial blood pressure was measured continuously by way of the aortic catheter in the tethered animals for 24 h\textsuperscript{20} before obtaining the blood sample. Ten animals were included in each of the groups eating the low-, normal-, and high-potassium diets, and 11 animals in a group consuming standard laboratory diet.

Statistical comparisons of the groups were made using one-way analysis of variance, and when significant differences were indicated by the initial group analysis, post-hoc comparisons were made between individual group means using Dunnett’s modification of the \( t \) test for independent groups for multiple comparisons to a single control mean.\textsuperscript{21} The nonparametric Kruskal-Wallis one-way analysis of variance was used to test for differences between the neointimal area to medial area ratio data from the three groups, because ratio data are not typically intervalic in nature and are not normally distributed as required for parametric analyses.\textsuperscript{22} When the analysis of variance of the data from the three groups indicated a significant effect of potassium intake on neointimal to medial ratio, the Mann-Whitney \( U \) test was applied post-hoc to test for differences between pairs of groups.\textsuperscript{23} A \( P \) value < .05 was considered indicative of a statistically significant difference among or between groups.

**RESULTS**

The animals gained weight normally on each of the three diets. The animals consuming the low-potassium diet gained 63 ± 6 g during the 4-week period of the experiment, whereas those of the normal- and high-intake groups gained 63 ± 6 and 66 ± 6 g, respectively.

The arterial plasma potassium concentration of the animals was measured from samples drawn from the abdominal aorta under anesthesia immediately before removal of the carotid artery. Some animals became hypoxic before the sample could be drawn; samples from hypoxic animals (\( pO_2 < 40 \) mm Hg) were eliminated from analysis because of the effect of hypoxia to cause egress of potassium from the intra- to the extracellular compartment. Dietary potassium content had a significant effect on arterial plasma potassium concentration (\( P < .01 \), one-way analysis of variance). Group mean potassium concentrations were 3.01 ± 0.12, 3.50 ± 0.13, and 3.62 ± 0.15 mmol/L for the low-, normal-, and high-intake groups, respectively. In the parallel study, plasma potassium concentration measurements made from blood drawn from the indwelling catheters were higher, due to reduced stress at the time of sampling, and due to the timing of the sampling in relation to the rats’ circadian rhythms. As in the previous study, there was a significant relationship between diet and group mean potassium concentrations (\( P < .01 \), one-way analysis of variance). The data are presented in Fig. 1. Group mean and standard errors were 4.26 ± 0.12 mmol/L for the low-intake group, 5.22 ± 0.19 mmol/L for the normal group, and 5.80 ± 0.23 mmol/L for the high-intake group (high versus low, \( P < .01 \); high versus normal, \( P < .05 \); normal versus low, \( P < .01 \)). The potassium concentration of the group eating standard lab diet averaged 4.98 ± 0.12 mmol/L.

Plasma renin activity (PRA) was measured in some animals in each group. A significant inverse relationship was observed between the rate of potassium intake and the PRA value (\( P < .02 \), one-way analysis of variance). The low-intake group mean value was 5.0 ± 0.8 ng Al/mL/h (\( n = 7 \)), the normal intake group mean was 3.6 ± 0.9 (\( n = 6 \)), and the mean value for the high-intake rats was 2.7 ± 0.4 (\( n = 6 \), \( P < .04 \) v low). Values from the parallel study were similar; group mean values were 5.4 ± 0.9, 3.6 ± 0.5, and 2.1 ± 0.3 ngAl/mL/h for the low-, normal-, and high-intake groups, respectively (\( P < .02 \), one-way analysis of variance).

Mean arterial pressure of the rats used in this study (Table 1) was unaffected by the range of potassium in the diets (\( P > .50 \), one-way analysis of variance).

Sections of normal carotid artery had an intact layer of endothelial cells, a media containing elastin fibers,
and a normal adventia. In balloon-injured artery sections from the low- and normal-intake groups there was extensive neointima development (Fig. 2A and B, respectively). The neointima comprised smooth muscle cells with abundant extracellular matrix. In the normal-intake group the character of the neointima was similar to that of the animals in the low-intake group. The neointimal response in the high-potassium–intake group was significantly attenuated, compared with that of the other groups (Figure 2C).

For each vessel the areas of the neointima, the media, and the total wall were quantified using digital image analysis. The ratio of neointimal area to medial area was calculated for each animal. A consistent, significant inverse relationship was observed between the level of potassium intake and the neointimal to medial area ratio (P < .05; one-way analysis of variance; Figure 3). The group mean values for the ratio of neointimal area to medial area for the low-, normal-, and high-intake groups were 0.447 ± 0.106, 0.384 ± 0.116, and 0.240 ± 0.046, respectively; comparison of individual groups revealed significant differences between the high-intake group and the normal-intake group (P < .04), and between high and low groups (P < .01), but not between the normal and low-potassium groups.

### DISCUSSION

The rat carotid artery balloon injury model has been studied during the last two decades. Medial smooth muscle cell replication and migration to the subintima have been reported by several groups, leading to the proposal that, initially, smooth muscle cell proliferation begins in the media, followed by migration of smooth muscle cells to the intima, after which comes an extended period of smooth muscle cell proliferation in the intima. Previously, we studied the effects of changes in potassium concentration on vascular smooth muscle cell proliferation in vitro. Increasing potassium concentration from 3 to 7 mmol/L reduced the cell numbers on Day 7 of incubation by 61% and raising the concentration from 3 to 4 mmol/L reduced the count by 18%. The antiproliferative effects of elevation of potassium were confirmed by the thymidine incorporation data. Migration of vascular smooth muscle cells from the media to the subintima stimulated by platelet derived growth factor (PDGF) is an important component in the response of the arterial wall to balloon injury. Recently we observed that raising potassium concentration from 3 to 6 mmol/L significantly inhibited PDGF-stimulated migration of coronary artery cells in vitro. These effects of small increases in potassium concentration on vascular smooth muscle cell function indicate that potassium can exert a direct effect on smooth muscle growth and migration in vitro, and show that the effect of as small as a 1-mmol/L increase in potassium concentration from 3 to 4 mmol/L has an effect that may be functionally significant.

Platelets may participate in neointima formation by the release of cytokines during contact with the denouethelialized surface of the injured vessel wall. Our previously completed in vitro studies suggested that elevated potassium concentration might inhibit platelet activation and subsequent thrombus formation. We found that platelet sensitivity to thrombin in the midrange of the concentration response relationship was 26% greater in buffer containing a potassium concentration of 1.9 mmol/L than in buffer with a concentration of 5.5 mmol/L. Although we have not directly assessed potassium’s effect on platelet sensitivity in vivo, we have studied in anesthetized dogs thrombus formation in the Folts model of coronary artery thrombosis. We saw a 54% reduction in the rate of thrombus formation associated with an acute elevation of potassium concentration from 3.5 ± 0.1 to 6.1 ± 0.1 mmol/L, and in individual animals elevation of concentration by as little as 1.3 mmol/L totally inhibited thrombus formation. These findings suggest that potassium may attenuate platelet activity in vivo as well as in vitro. In light of our previous in vitro and short-term in vivo work, the range of potassium concentration observed in this study, from 4.26 ± 0.12 in the low-potassium group, to 5.80 ± .23 mmol/L in the high-intake group, may have been large enough to significantly affect the responses to injury of both vascular smooth muscle cells and platelets.

Angiotensin II has been proposed to be a growth-promoting factor for vascular smooth muscle (for review, see reference 27) and angiotensin-converting enzyme (ACE) inhibitors have been used in animal models to prevent increased growth and pathologic changes in arteries. In the rat carotid artery balloon angioplasty injury model, ACE inhibitors reduce neointimal development, although not by inhibiting vascular smooth muscle proliferation, but rather by inhibiting migration to the intima. In our study and in others from several laboratories, a significant inverse relationship has been observed between the rate of K intake and the level of plasma renin activity. The suppression of renin activity and angiotensin II levels by approximately 50% by the high level of K...
intake may have been an additional contributing factor to the effect of potassium observed in the present study.

The three levels of dietary intake chosen were intended to span a range comparable to the extremes in K intake that might be encountered by humans. The level in the normal-intake group diet, 1.5%, is somewhat higher than the 1.0% found in standard laboratory rat diets, and therefore may be considered comparable to the upper range of normal for a human diet. In the United States, the normal daily potassium intake is 60 to 100 mmol/day, and the potassium content is approximately 0.6%.\textsuperscript{34,35} Therefore, the normal-intake group’s diet may be considered comparable to a human intake of approximately one-and-a-half to two times the normal intake level. The low-intake diet of 0.1% is comparable to a diet containing one-sixth the content of the normal Western human diet, whereas the high-intake group’s potassium content might be taken to be analogous to a human diet containing six to seven times the normal potassium content. Humans who consume a diet containing 10 mmol/day may be difficult to document; however, large studies have reported sample group means as

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**FIG. 2.** A) Section of a right common carotid artery 14 days after balloon injury in an animal maintained on the low-potassium diet (0.1%). The lumen (L) is to the right in the figure. There is an extensive neointima (N) luminal to the medial layer of smooth muscle (M). The neointima contains modified smooth muscle cells and abundant extracellular matrix material. The bar (lower left) is 200 μm. B) Section of a common carotid artery 14 days after balloon injury in an animal maintained on the normal diet that contained 1.5% potassium. Neointimal development (N) is similar to that seen in the animals fed the low-potassium diet. The bar (lower left) is 200 μm. C) Section of a common carotid artery 14 days after balloon injury from an animal fed the high-potassium diet. The development of the neointima is significantly less than that seen in the groups fed diets with lower potassium content. The bar (lower left) is 200 μm.
The results of the our present and previous studies are consistent with a hypothesis that elevation of potassium intake is effective in inhibiting neointimal proliferation and/or migration of modified vascular smooth muscle cells in response to balloon angioplasty. The mechanism of the inhibition remains to be determined, although it may be related to the effects of elevated potassium concentration acting directly on the smooth muscle cells, or indirectly by inhibiting platelet function. Alternatively, it may be the result of a reduction in angiotensin II levels caused by suppression of renin release associated with elevation of potassium intake.

REFERENCES


