Increased superoxide anion generation and altered vasoreactivity in rabbits on low-potassium diet

B. C. Yang, D. Y. Li, Y. F. Weng, J. Lynch, C. S. Wingo, and J. L. Mehta. Increased superoxide anion generation and altered vasoreactivity in rabbits on low-potassium diet. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H1955–H1961, 1998.—Potassium reduces blood pressure in populations at high risk of developing hypertension, which suggests that potassium depletion may increase vascular resistance. This study was designed to examine the effect of potassium depletion on the L-arginine-nitric oxide pathway in arterial tissues. New Zealand White rabbits were fed either a control diet, containing a normal amount of potassium, or a low-potassium diet for 1–3 wk. As expected, the low-potassium diet resulted in reduced serum and urinary potassium levels. Carotid arteries were excised, and their contractile and relaxant responses were determined in vitro. Carotid arterial ring contractile responses to norepinephrine was enhanced, and relaxation in response to the endothelium-dependent vasodilator acetylsalicylic acid was attenuated in rabbits fed low-potassium diet (all P < 0.01 compared with responses in rabbits fed control diet). The vasomotor responses were similarly altered in rabbits fed low-potassium diet for 1 or 3 wk. Both the enhanced contraction and attenuated relaxation were abolished by treatment of arterial rings with superoxide dismutase but not by treatment with L-arginine or indomethacin. Carotid artery rings from rabbits fed the low-potassium diet showed ~100% greater superoxide anion formation than those from rabbits fed control diet (P < 0.01), whereas plasma and urinary nitrite levels were similar in both groups of rabbits. These observations indicate that low-potassium diet enhances the sensitivity of the carotid artery to vasoconstrictor stimuli and reduces the sensitivity to endothelium-dependent stimuli. Attenuation of endothelium-dependent relaxation appears to be secondary to increased free radical generation, which may degrade nitric oxide. Altered vasoreactivity may underlie the genesis of hypertension in populations consuming diets low in potassium.

Determination of Vasoreactivity

Carotid arteries were quickly excised, placed in oxygen-saturated (95% O2–5% CO2) Krebs-Ringer buffer (composition in mM: 118 NaCl, 4.7 KCl, 1.3 CaCl2, 1.2 KH2PO4, 1.2 MgCl2, 25 NaHCO3, 1.6 KH2PO4, and 5 glucose) at 37°C, and aerated before use. After 1 wk of stabilization on an ordinary diet, the rabbits were killed by intravenous injection of pentobarbital (100 mg/kg). Venous blood was collected in sodium citrate (9:1, vol/vol) for plasma preparation. Urine was collected and frozen at −70°C. Carotid arteries were isolated and used for determination of vasoreactivity in response to a variety of stimuli and for determination of superoxide anion formation.

Materials and Methods

Animals and Diet

New Zealand White rabbits (weight ~1 kg) were purchased through the Department of Veterans Affairs and were individually housed. After 1 wk of stabilization on an ordinary diet, the rabbits were fed either a control diet containing a normal amount of potassium (382 mmol K/kg diet, 1.49% K; TD 85004, Teklad, Madison, WI) (n = 7) or a low-potassium diet (64 mmol K/kg diet, 0.25% K; TD 87443, Teklad) (n = 7) for 7 days. A second group of rabbits (n = 8) was studied on both diets after 3 weeks of conditioning. Details of control and low-potassium diets have been published previously (30, 35).

At the end of the special-diet period, rabbits were killed by intravenous injection of pentobarbital (100 mg/kg). Venous blood was collected in sodium citrate (9:1, vol/vol) for plasma preparation. Urine was collected and frozen at −70°C. Carotid arteries were isolated and used for determination of vasoreactivity in response to a variety of stimuli and for determination of superoxide anion formation.

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Values are means ± SE for 5 animals in each group. BUN, blood urea nitrogen. *P < 0.05, †P < 0.01 vs. control diet.
response to NE (P < 0.01), with ~50% reduction in the EC50 (1.34 ± 0.22 × 10⁻⁶ M vs. 2.25 ± 0.32 × 10⁻⁶ M in the control diet group, P < 0.05). The contractile response to a single concentration of NE (10⁻⁷ M) is shown in Fig. 1.

Low-potassium diet also resulted in significantly attenuated relaxation of carotid arterial rings in response to both ACh (P < 0.01) and A-23187 (P < 0.05). The concentration-response was shifted to the right (EC50 of ACh: 7.90 ± 0.57 × 10⁻⁸ M vs. 3.61 ± 0.42 × 10⁻⁸ M in the control diet group, P < 0.01; EC50 of A-23187: 1.99 ± 0.67 × 10⁻⁷ M vs. 1.30 ± 0.32 × 10⁻⁷ M in the control diet group, P < 0.05). The most impressive attenuation of relaxation in the low-potassium diet group in response to ACh (10⁻⁷ M) and A-23187 (10⁻⁶ M) is shown in Fig. 1.

In rabbits adapted to a low-potassium diet for 3 wk, the contractile response of arterial rings to NE was similarly enhanced (P < 0.05 vs. contraction in rings from control diet group). The relaxant responses of arterial rings to ACh and A-23187 were also attenuated (P < 0.01) (Fig. 2). The differences in constrictor and relaxant responses in rabbits on 3 wk of low-potassium diet were similar to those in rabbits on 1 wk of low-potassium diet (P = not significant).

Modulation of Altered Vasoreactivity

To determine the contribution of prostaglandins in altered vasoreactivity in rabbits on low-potassium diet, carotid arterial rings were treated with indomethacin (10⁻⁶ M) for 30 min and then exposed to NE (n = 4). As shown in Fig. 3, indomethacin treatment reduced the contractile response to NE in arterial rings in both groups of rabbits; however, the differences in vasoconstriction persisted. Treatment with indomethacin did not significantly affect ACh- or A-23187-mediated relaxation, and the differences in endothelium-dependent relaxation in the two groups persisted (Fig. 4).

To examine the role of the L-arginine-NO pathway, parallel sets of rings from control and low-potassium-diet rabbits were treated with L-arginine (10⁻⁴ M) for

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**Fig. 1.** A: vasoconstriction in response to norepinephrine (NE; 10⁻⁷ M) is greater in rings from rabbits fed low-potassium diet vs. those from rabbits fed control diet for 1 wk. B: endothelium-dependent relaxation in response to 2 different relaxants, ACh and calcium ionophore A-23187, is diminished in rings from rabbits fed low-potassium diet. Data are means ± SE from 7 animals in each group. *P < 0.05 vs. control diet.

**Fig. 2.** Despite adaptation to low-potassium diet for 3 wk, vascular ring contraction in response to NE (A) is still greater in rings from rabbits fed low-potassium diet, and endothelium-dependent relaxation (B) is diminished compared with that in rabbits fed a control diet. Data are means ± SE from 4 animals in each group. *P < 0.05 vs. control diet.
1 h, washed, and then exposed to NE. The contractile response to NE was not altered in L-arginine-treated rings, and the differences between the two groups persisted (Fig. 3). L-Arginine treatment slightly enhanced the relaxation in response to both ACh and A-23187 in rings from the low-potassium-diet group, but the magnitude of relaxation was still significantly less compared with that in the control-diet group (Fig. 4).

To determine whether NO produced in the rings from the low-potassium-diet group was degraded quickly, parallel sets of rings were treated with SOD and then exposed to NE. As shown in Fig. 3, SOD-treatment markedly decreased NE-induced contraction in rings from the low-potassium-diet group (P < 0.05) but did not do so in the control-diet group. This resulted in similar magnitude of contractile response to NE in both groups. SOD treatment also enhanced ACh- and A-23187-mediated relaxation in rings from the low-potassium-diet group (P < 0.05) but not in the control diet group. This resulted in similar magnitudes of both ACh- and A-23187-mediated relaxation in control-diet and low-potassium-diet groups of rabbits (Fig. 4).

Superoxide Anion Formation by Carotid Arterial Segments

The rate of superoxide anion formation by carotid arterial segments was determined by chemiluminescence of lucigenin. The chemiluminescence of lucigenin (counts/min) is positively correlated with the rate of superoxide anion formation (7, 24). The rate of superoxide anion formation in carotid arterial segments from control rabbits was 5,224 ± 1,150 counts·min⁻¹·mg tissue⁻¹. Three weeks of low-potassium diet resulted in a marked increase in superoxide anion formation in carotid arterial segments (9,838 ± 1,013 counts·min⁻¹·mg tissue⁻¹, P < 0.05 vs. that in control-diet group) (Fig. 5).

![Graph showing vascular ring contraction in response to NE](image)

**Fig. 3.** Vascular ring contraction in response to NE is greater in rabbits fed low-potassium diet than in those fed control diet (see Figs. 1 and 2). Treatment of rings with cyclooxygenase inhibitor indomethacin decreases contractile response to NE, but the difference in both groups of rabbits persists. Treatment with L-arginine has no effect on contractile response to NE, and the difference in both groups of rabbits persists. Treatment of rings with superoxide dismutase (SOD) decreases contractile response in rings from low-potassium-fed rabbits without affecting contractile response in control rabbits, which resulted in similar contraction of rings in response to NE in both groups of rabbits. Data are means ± SE from 4 animals in each group. *P < 0.05 vs. control diet; †P < 0.05 vs. buffer.

![Graph showing calcium ionophore A23187-mediated relaxation](image)

**Fig. 4.** ACh- (A) and A-23187-mediated (B) vascular ring relaxation is not affected by treatment with indomethacin or L-arginine but is enhanced in rings pretreated with SOD. This enhancement is seen only in rings from rabbits fed low-potassium diet, resulting in similar relaxation of rings pretreated with SOD in response to ACh and A-23187 in both groups of rabbits. Data are means ± SE from 4 animals in each group. *P < 0.05 vs. control diet; †P < 0.05 vs. buffer.

![Graph showing superoxide anion formation](image)

**Fig. 5.** Rings from rabbits fed low-potassium diet produce almost twice as many superoxide anions as rings from rabbits fed control diet (P < 0.05). Data are means ± SE from 4 animals in each group.
As shown in Table 2, plasma and urinary nitrite levels were similar in groups of rabbits given control diet or low-potassium diet. There was no difference in rabbits fed low-potassium diet for either 1 or 3 wk.

**DISCUSSION**

Substantial evidence indicates an important role for potassium in the regulation of blood pressure (2, 3, 9, 10, 23, 26, 29, 34). The present study demonstrates for the first time that a low-potassium diet results in greater contraction of arterial tissues in response to the \(\alpha\)-agonist NE and attenuated relaxation in response to two different endothelium-dependent vasodilators, ACh and the calcium ionophore A-23187. This study also shows that although plasma and urinary nitrite levels are not affected by a low-potassium diet, vascular superoxide anion formation is almost twice as high in rabbits fed low-potassium diet as in rabbits fed the control diet. We suggest that the enhanced degradation of NO by large amounts of superoxide anions may be a basis for altered vasoreactivity. The critical role of enhanced superoxide anion formation in enhanced vasoconstriction and attenuated relaxation became obvious as the altered vasoreactivity in rabbits given the low-potassium diet was "normalized" by incubation of the arterial tissues with SOD.

As expected, rabbits on the low-potassium diet exhibited a significantly lower plasma concentration (−65% of that in rabbits on control diet) and a pronounced decrease in urinary potassium (−18% of that in rabbits on control diet) in the present study.

Potassium supplementation generally reduces blood pressure, especially in populations with a high risk of developing hypertension. Mechanisms for the blood pressure-lowering effects of potassium are thought to be attenuation of the sodium-mediated increase in renal vascular resistance (34) and activation of Na\(^+\)K\(^-\) ATPase in the central nervous system (25) and in the vascular tissues (4). Sudhir et al. (27) have suggested preservation of endothelial function and improvement in arterial compliance with potassium supplementation. McCabe et al. (14) have shown inhibition of free radical formation by potassium. A low potassium concentration, on the other hand, results in a high rate of free radical formation (14). Potassium depletion has also been reported to increase the expression of \(\alpha\)-adrenergic receptors (16), which increase Na\(^+\)/H\(^+\) exchange in the proximal tubule, inhibit water transport in the collecting tubule, and alter blood pressure regulation. In the present study, a reduction in dietary potassium intake enhanced the sensitivity of systemic artery rings to the \(\alpha\)-adrenergic agonist NE, resulting in a greater contractile response. Concurrently, the relaxant response to two different endothelium-dependent vasodilators, ACh and A-23187, was diminished, suggesting a decreased synthesis of NO and/or its greater degradation. The greater contractile response to NE may reflect either a primary increase in expression of \(\alpha\)-adrenergic receptors (16) or a decrease in the activity of NO at baseline (11).

Vascular endothelial cells play an important role in regulating smooth muscle tone via release of vasodilators, such as NO and prostacyclin, and vasoconstrictors, such as endothelin, thromboxane A\(_2\), angiotensin II, and superoxide anion (13, 15). Under physiological conditions, a balance is present between the vasodilator and vasoconstrictor species. We hypothesized that the altered vasoreactivity in rabbits fed the low-potassium diet may be due to either excessive production of vasoconstrictor thromboxane A\(_2\) or alterations in NO formation or activity. Indomethacin inhibits the formation of both prostacyclin and thromboxane A\(_2\) and inhibits vasoconstriction in response to NE and thromboxane A\(_2\) analog as well as vasorelaxation in response to ACh in the isolated rat aorta (31). In keeping with this concept, treatment of arterial rings with indomethacin in the present study resulted in a qualitatively similar reduction in vasoconstrictor response and a nonsignificant decrease in vasorelaxant response in control and low-potassium-diet groups, which excludes the involvement of cyclooxygenase products in the altered vasoreactivity by low-potassium diet. Because plasma and urinary nitrite levels were similar between the control and low-potassium-diet groups, and because the abnormal vasoreactivity in rabbits on a potassium diet was not affected by treatment of rings with L-arginine, the precursor of NO, it is unlikely that a substrate deficiency accounts for the abnormal vasoreactivity.

Most interestingly, we found a marked increase in superoxide anion generation in the carotid arterial segments of rabbits fed a low-potassium diet. This observation suggested that increased degradation of NO by superoxide anions could be the basis for altered vasoreactivity. To test this hypothesis, we treated arterial rings with the superoxide radical scavenger SOD. Indeed, the abnormalities in vascular contraction and relaxation in the low-potassium-diet group were abolished by incubation of arterial rings with SOD. It is unlikely that the large SOD molecule penetrated the endothelial cells and scavenged the superoxide anions. It is, therefore, reasonable to conclude that SOD scavenged superoxide anions extracellularly and prevented the degradation of NO. Previous studies by Lawson et al. (11) showed that direct exposure of rat aortic rings to superoxide anions, formed as a reaction of xanthine plus xanthine oxidase, results in a modest contraction, enhancement of the effect of NE, marked attenuation of NO-mediated vasorelaxation, and endothelial disruption. Collectively, these observations suggest a critical role of enhanced vascular superoxide anion formation.

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<tr>
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<th>Plasma n</th>
<th>Urine n</th>
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<tbody>
<tr>
<td>Control diet</td>
<td>3.99 ± 0.34</td>
<td>14</td>
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<tr>
<td>Low-potassium diet, 1 wk</td>
<td>3.76 ± 0.30</td>
<td>8</td>
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<tr>
<td>Low-potassium diet, 3 wk</td>
<td>3.84 ± 0.71</td>
<td>4</td>
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Values are means ± SE; n, no. of rabbits. Urinary nitrite levels were not measured in rabbits fed low-potassium diet for 1 wk.
in abnormal vasoreactivity in animals fed a low-potassium diet.

Patients with essential hypertension have abnormal endothelium-dependent vascular relaxation (17–20). Studies done thus far (8, 18–20) indicate a defect in the L-arginine–NO pathway that may at least partly account for both the increased vascular resistance under basal conditions and the impaired response to endothelium-dependent vasodilators. Although some studies (21) suggest that the defect in endothelium-dependent vascular relaxation in essential hypertension can be corrected with L-arginine supplementation, other studies (18–20) suggest that the defect in altered vasorelaxation is not related to decreased availability of substrate for NO (19) or to a specific intracellular signalling transduction pathway (20). Higashi et al. (8) found that the renovascular relaxation and the increase in plasma cGMP in response to L-arginine were significantly less in patients with essential hypertension than in normotensive control subjects. Studies in renovascular hypertensive rats by Vega et al. (28) suggest that diminished NO availability and excessive formation of superoxide anions in blood vessels account for increased sensitivity to vasoconstrictors. Our study provides evidence for the concept that enhanced superoxide anion formation in low-potassium states is the basis for rapid breakdown of NO, whereas the synthesis of NO remains intact. This mechanism may underlie the presence of hypertension in populations that consume low-potassium diets.

Our observations also support the hypothesis that inhibition of free radical formation by high concentrations of potassium may have an important role in lowering blood pressure (14). A critical role of increased superoxide anions in abnormal vasoreactivity was shown in experiments in which scavenge of superoxide anions with SOD "normalized" vascular contraction as well as endothelium-dependent relaxation in arterial tissues from rabbits on the low-potassium diet. In previous studies from our laboratory (11), we observed that pretreatment of rat aortic rings with SOD completely blocked the adverse effect of superoxide anions on vascular reactivity.

Two limitations of this study are noteworthy. First, the alterations in carotid artery reactivity may or may not reflect changes in resistance vessels. However, experimental studies have shown qualitative similarity in vasoreactivity in conductance and resistance vessels (24). Second, we did not examine the role of prostacyclin in altered vasoreactivity with low-potassium diet, because there is an important interaction between superoxide anion formation and prostacyclin (5). Nonetheless, treatment of vascular rings with the cyclooxygenase inhibitor indomethacin did not influence vascular reactivity.

In summary, the present study shows that a diet low in potassium significantly enhances arterial contractile response to a-adrenergic stimuli and attenuates endothelium-dependent relaxation responses. These observations can be explained in part by enhanced formation of superoxide anions that degrade NO. These observations also suggest a potentially important therapeutic role of free radical scavengers as well as dietary potassium supplementation in hypertensive subjects, especially those who consume a low-potassium diet.

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