Abstract We conducted this study to determine whether physiological changes in potassium concentration affect free radical formation by vascular cells. We assessed the effects of potassium on reactive oxygen species formed by cultured endothelial and monocyte/macrophage cells or freshly isolated human white blood cells by cytochrome c reduction or luminol chemiluminescence, respectively. Reducing potassium concentration of endothelial cell media (normally 5.1 to 6.1 mmol/L) to 3.0 mmol/L exponentially increased the rate of cytochrome c reduction, up to 8.4-fold at 2 hours; raising potassium concentration to 5.3 or 7.0 mmol/L at 1 hour reduced the maximal rate of cytochrome c reduction by 86% or 93%. Subsequent studies were done 30 to 75 minutes after media change. Potassium reduced the rate of cytochrome c reduction by 49% (endothelial cells) to 55% (monocytes/macrophages) between 3.0 and 7.0 mmol/L; the greatest decrement (20% to 26%) occurred between 3.0 and 4.0 mmol/L. Superoxide dismutase reduced the rate of cytochrome c reduction by 62% or 50% in endothelial or monocyte/macrophage cells. Potassium had no effect on the rate of cytochrome c reduction in the presence of superoxide dismutase. Increasing potassium concentration from 1.48 to 4.77 or 7.94 mmol/L also reduced luminol chemiluminescence in human white blood cells challenged by 1 to 10 mg/mL zymosan. We conclude that physiological increases in potassium concentration inhibit the rate of superoxide anion formation by cell lines derived from endothelium and from monocytes/macrophages and reactive oxygen species formation by human white blood cells. (Hypertension. 1994;24:77-82.)

Key Words • potassium • reactive oxygen species • free radicals • superoxide anion • endothelium • macrophages • monocytes • cell line

Potassium intake (60 to 801 mmol/d) and urinary potassium excretion2 are inversely related to the incidence of stroke-associated mortality in adult women. Most studies also find a significant inverse relation between potassium intake (ranges: 23 to 54,3 38 to 71,4 61 to 81,5 61 to 167,7 and 60 to 2008 mmol/d) and blood pressure. Although the correlation between potassium intake and blood pressure is not always statistically significant in the general population after adjusting data,9,10 a much stronger correlation exists when only hypertensive individuals are considered.7 This relation has been reported in clinical intervention studies since the 1920s.8 These studies show that varying potassium intake (ranges: 0 to 300,11 16 to 96,12 30 to 100,13 70 to 190,14 and 80 to 20015 mmol/d) or administering potassium supplements (24,16 48,16 60,17 80,18 and 78 to 10419 mmol/d) reduces blood pressure in hypertension. Potassium may reduce adult blood pressure by transiently increasing sodium excretion through a shift in the pressure-natriuresis curve, leading to a new steady state of reduced blood volume and pressure.15,19,20 Potassium may reduce stroke-associated mortality through reduced blood pressure alone or in combination with other effects, such as an antithrombotic action.21

The antihypertensive effect of potassium changes with age. Whereas increased potassium intake or potassium supplementation lowers blood pressure in young (<36 years) people with elevated22,23 or normal24 blood pressure, dietary sodium, urinary sodium, or changes in sodium intake have little relation to blood pressure.22-26 Moreover, the correlation coefficient of the relation between plasma potassium and diastolic blood pressure is three times greater in young (<36 years) than in old (>49 years) patients.26 It has been proposed that potassium antagonizes the formation of early renovascular lesions25 thought to initiate hypertension in youth.27 Support for this hypothesis has come from animal studies in which increased dietary potassium reduces the incidence of renovascular and aortic fatty-streak lesions in rats.28-30

There is a highly significant inverse correlation between plasma potassium (3.0 to 5.5 mmol/L) and blood pressure in hypertensive patients less than 36 years of age.26 Varying potassium intake from 0 to 300 mmol/d for 5 days causes a corresponding 0.8-mmol/L change in fasting plasma potassium.11 Lowering potassium intake from 96 to 16 mmol/d for 10 days reduces fasting plasma potassium from 4.2 to 3.4 mmol/L.12 Raising potassium intake from 80 to 200 mmol/d for 8 weeks raises fasting plasma potassium from 4.2 to 4.4 mmol/L.15 High potassium meals (100 mmol) further increase plasma potassium over 4 hours, peaking 2 hours after eating at 0.6 mmol/L above fasting levels.30 Moderate potassium meals (25 mmol) do not alter postprandial plasma potassium.30 These studies indicate that changes in dietary potassium can alter fasting plasma potassium by at least 1 mmol/L and peak postprandial plasma potassium by more than 1.5 mmol/L.

Habitual changes in potassium intake may elicit greater changes in plasma potassium. The human body contains 2460 mmol of potassium,31 35 times the average daily adult potassium intake, most of which is excreted.32 Animal studies have shown that body potassium stores can change by 23% and that potassium balance is not achieved weeks after potassium intake is altered.33
Physiological studies also demonstrate that changes in plasma potassium are an obligatory component of potassium homeostasis. Aldosterone alone is incapable of controlling potassium excretion, and the changes in aldosterone secretion and plasma aldosterone levels are largely mediated by changes in plasma potassium. Dietary potassium readily alters plasma potassium up to 4.2 mmol/L. The ability of potassium intake to alter plasma potassium above 4.2 mmol/L is directly related to dietary sodium.

Our recent efforts focus on the following question: To what extent can small changes in potassium concentration ([K⁺]) affect biologic systems in ways that can protect against vascular lesions? In this study we tested the hypothesis that small changes in [K⁺] affect free radical formation by cultured endothelial cells, cultured monocytes/macrophages, and freshly isolated human white blood cells.

**Methods**

We used two methods to analyze the effects of changes in [K⁺] on reactive oxygen species formation. For cultured cell lines the rate of reactive species formation was measured by the reduction of cytochrome c. Confirmatory studies on human white blood cells used a reaction with luminol.

**Cell Culture Techniques**

Bovine pulmonary artery endothelial cell line CPA 47 and mouse peritoneal monocyte/macrophage cell line TIB 186 were obtained from American Type Culture Collection. Cells lines were grown to confluence on 75-cm² tissue culture flasks with 10% FBS. The [K⁺] of Ham's F-12K with 10% FBS is 5.1 mmol/L, and that of RPMI 1640 with 10% FBS is 6.1 mmol/L. Monocytes/macrophages were grown in RPMI 1640 (Sigma), which has 400 mg/L KCl with 10% FBS. The [K⁺] of Ham's F-12K with 10% FBS is 5.1 mmol/L, and that of RPMI 1640 with 10% FBS is 6.1 mmol/L. Cells were plated onto six-well plates at a density of 250,000 cells per well and grown for up to 14 days. Experiments were done 3 to 5 days after plating, which was found in preliminary experiments to be the optimal time to observe potassium-sensitive reactive oxygen species formation.

**Measurement of Reactive Oxygen Species Formation in Cultured Vascular Cells**

Measurement of reactive oxygen species formation by endothelial and monocyte/macrophage cell lines was performed in solutions containing [K⁺] in the range of 3.0 to 6.0 mmol/L with 1.0 mg/mL ferricytochrome c (Sigma) at 37°C. Solutions (buffered to pH 7.4 with NaOH) consisted of 25 mmol/L HEPES in a 1:1 (vol/vol) mixture of Eagle's minimum essential medium (without t-glutamine, NaHCO₃, or phenol red; Sigma) and a solution containing (mmol/L) NaCl 112.2, MgCl₂ 1.0, CaCl₂ 1.2, D-glucose 5.6, and KCl 0.6 to 8.6, with added NaCl as a substitute for KG to help maintain osmolarity and ionic strength. Eagle's minimum essential medium contains 5.4 mmol/L KCl and the white blood cells were resuspended in 2.0 mL buffered physiological saline solution containing 4.0 mmol/L potassium. A cell count was performed on the final mixture using a Coulter counter. Samples having less than 6 x 10⁶ polymorphonuclear neutrophils plus monocytes were not used. One tenth milliliter of the cell suspension was mixed with 0.1 mL of a solution containing 0.9 mL of a solution of luminol, opsonized zymosan particulate (zymosan A from Saccharomyces cerevisiae, Fluka), and buffered physiological saline solution containing either 2.0, 5.0, or 8.0 mmol/L K⁺. The final [K⁺] of the mixture was measured with a Nova 1 Na-K⁺ ion-selective electrode instrument (Nova Biomedical Corp). The cells phagocytize the zymosan particles and release reactive oxygen species in the process. Reaction of each free radical ion with the luminol produces a photon that is recorded by the scintillation counter set in the out of coincidence mode. Using this procedure the rate of reactive species formation was described quantitatively, and the effect of changes in [K⁺] on the process was determined.

**Statistical Analysis**

Data were analyzed statistically by one- or two-way ANOVA, with a value of P < 0.05 taken to indicate a significant effect, using the two-tailed assumption. When indicated by the results of the ANOVA test, secondary analyses of subgroups were undertaken using Dunnnett's modification of the t-test for multiple comparison of group means to a single control group mean. For brevity and comparison, some figures present results from both endothelial and monocyte/macrophage cell lines, but separate ANOVAs were performed on each cell line.

**Results**

**Effect of [K⁺] on Reactive Oxygen Species Formation by Endothelial Cells**

The time dependence of the rate of reactive oxygen species formation and the effects of [K⁺] were determined in cultured endothelial cells (cell line CPA 47). Fig 1 illustrates results from three groups (five assays per group) of endothelial cells after exposure to a [K⁺] of 3 mmol/L at time = 0. One hour after exposure, the [K⁺] of replacement solutions used for two of the groups was increased, whereas the [K⁺] of the replacement solutions of the third group was not changed. The rate of reactive oxygen species formation by all groups rose throughout the first hour. The rate of rise was exponential. During the second hour, the rate of reactive oxygen species formation by the group exposed to 3 mmol/L K⁺ continued to rise exponentially, but the rate of reactive oxygen species formation by either of the other groups returned approximately to initial values. By the end of 2 hours the rate of reactive oxygen species formation by the group continually exposed to 3 mmol/L K⁺ was 8.4 times the initial rate, but the group that was containing identical media were used as time controls; separate control blanks were used for each [K⁺]. The absorbance was measured at 550 nm with a Spectronic 501 spectrophotometer (Milton Roy) and an extinction coefficient for 21 mmol/L · cm.

**Measurement of Reactive Oxygen Species in Freshly Isolated Human White Blood Cells**

The protocols used in human studies were approved by the Institutional Review Board on June 22, 1990. Blood was obtained from 10 fasted, healthy volunteers from the medical school community who had not used any medication for more than 10 days. Blood (10 mL) was drawn from the antecubital vein and centrifuged immediately at room temperature; the buffy coat was removed; remaining red blood cells were lysed; and the white blood cells were resuspended in 2.0 mL buffered physiological salt solution containing 4.0 mmol/L potassium. Measurement of reactive oxygen species formation was described quantitatively, and the effect of changes in [K⁺] on the process was determined.
Comparative Effects of [K⁺] on Reactive Oxygen Species Formation

Fig 2 presents comparative effects of changes in [K⁺] on the rate of reactive oxygen species formation by endothelial and monocyte/macrophage cells. Increased [K⁺] from 3 to 7 mmol/L caused highly significant decreases (P<.001, ANOVA) in the rate of reactive oxygen species formation by both endothelial and monocyte/macrophage cell lines. The two cell lines had similar responses: increasing [K⁺] from 3 to 7 mmol/L reduced reactive oxygen species formation by 55% in endothelial cells and 49% in monocytes/macrophages in the 30- to 75-minute interval. It should again be noted that the effects of increased [K⁺] during this interval are much less than they would be had the low [K⁺] stimulus been applied for a longer period of time (compare with Fig 1). Virtually all of the effect of increased [K⁺] on the monocytes/macrophages occurred between 3 and 6 mmol/L (Fig 2), which is consistent with the longer-term observations in the endothelial cells (compare with Fig 1).

Effect of [K⁺] on Reactive Oxygen Species Formation by Human White Blood Cells

The effect of [K⁺] on reactive oxygen species formation by freshly isolated human white blood cells can be assessed from the data presented in Fig 3. The effects of [K⁺] and zymosan were both highly significant (P<.001, two-way ANOVA), although interaction between the two was not. Analysis of the [K⁺] effect within zymosan groups revealed a significant (P<.05, Dunnett’s test) inhibitory effect of the highest [K⁺] versus the lowest at all zymosan concentrations, a significant effect between the middle and lowest [K⁺] in three of the five zymosan concentrations, and a significant difference between the highest and middle [K⁺] at 7.5 mg/mL. At 7.5 mg/mL, zymosan the rate of reactive oxygen species formation in the presence of 4.77 mmol/L K⁺ was 12% less than in the presence of 1.48 mmol/L K⁺ (P<.05), whereas free radical formation at 7.94 mmol/L K⁺ was 26% less than at 1.48 mmol/L (P<.05).
Effects of Superoxide Dismutase on the Rate of Cytochrome c Reduction by Endothelial Cells and Monocytes/Macrophages

<table>
<thead>
<tr>
<th>Endothelial cell line (CPA 47)</th>
<th>Rate of Cytochrome c Reduction, (nmol/pg protein)/min</th>
<th>SOD Absent</th>
<th>SOD Present</th>
<th>Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. ([K^+] = 3) mmol/L</td>
<td>2.26±0.06</td>
<td>0.86±0.01</td>
<td>1.40±0.05*</td>
<td></td>
</tr>
<tr>
<td>B. ([K^+] = 5) mmol/L</td>
<td>1.77±0.02</td>
<td>0.78±0.04</td>
<td>0.99±0.02*</td>
<td></td>
</tr>
<tr>
<td>C. Effect of ([K^+]) (A–B)</td>
<td>0.49±0.04†</td>
<td>0.08±0.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Monocyte/macrophage cell line (TIB 186)</th>
<th>Rate of Cytochrome c Reduction, (nmol/pg protein)/min</th>
<th>SOD Absent</th>
<th>SOD Present</th>
<th>Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. ([K^+] = 3) mmol/L</td>
<td>2.44±0.00</td>
<td>1.12±0.05</td>
<td>1.32±0.00*</td>
<td></td>
</tr>
<tr>
<td>B. ([K^+] = 5) mmol/L</td>
<td>1.57±0.02</td>
<td>1.09±0.03</td>
<td>0.48±0.06*</td>
<td></td>
</tr>
<tr>
<td>C. Effect of ([K^+]) (A–B)</td>
<td>0.87±0.03†</td>
<td>0.03±0.06</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SOD indicates superoxide dismutase. The rate of cytochrome c reduction was measured in endothelial cells and monocytes/macrophages in the absence or presence of SOD and in the presence of 3 (A) or 5 (B) mmol/L \([K^+]\). Comparisons are made of the effects of SOD (Δ) at different \([K^+]\) and the effects of different \([K^+]\) (A–B) in the presence vs absence of SOD.

*P<.05, †P<.01, ‡P test, n=3. See text for results of ANOVA.

Discussion

The present study demonstrates that increases in \([K^+]\) within the physiological range can inhibit the rate of free radical formation by vascular cells. In the experiment presented in Fig 1 we followed the time course of reactive oxygen species formed by cultured endothelial cells after removal from cell culture media, which has a high \([K^+]\), and exposure to 3 mmol/L \([K^+]\). This induced an exponential increase in reactive oxygen species formation, which continued for at least 2 hours. Apparent cell mortality prevented us from consistently following this exponential change for longer periods. The most interesting aspect of this experiment is that the formation of reactive oxygen species was nearly eliminated by increasing \([K^+]\) to 5.5 mmol/L, so that a further increase to 7.0 mmol/L had little added effect. Thus, the range over which plasma potassium is correlated to blood pressure in young hypertensive individuals\(^{26}\) is the range over which potassium is most active against free radical formation. Each 1.0-mmol/L increase in \([K^+]\) between 3 and 5.5 mmol/L reduces formation of reactive oxygen species by one third at the 2-hour time point. Greater effects might occur after longer exposures to low \([K^+]\).

Another important aspect of the first experiment is the fact that the effects of reduced \([K^+]\) are more rapidly reversed than induced. The increase in the rate of reactive oxygen species formation induced by 3.0 mmol/L \([K^+]\), which took 1 hour to develop, was immediately reversed by restoring \([K^+]\) to 5.5 mmol/L (Fig 1). This observation raises the question as to whether fasting or postprandial peak values of plasma potassium have special relevance to the protective effects of potassium.

Studies on monocytes/macrophages were limited to shorter time periods, owing to reduced cell adhesion. However, the rate of reactive oxygen species formation by monocytes/macrophages was similar to that of endothelial cells when compared over equal time intervals (Fig 2). Increased \([K^+]\) also reduced reactive oxygen species formation by monocytes/macrophages, and virtually all of the effect occurred below a \([K^+]\) of 6 mmol/L. The effects of potassium were specific for superoxide anion since increasing \([K^+]\) elicited no effect on either endothelial cells or monocytes/macrophages in the presence of superoxide dismutase (Table), and increasing \([K^+]\) from 3 to 5 mmol/L reduced superoxide anion formation by 30% to 64% (Table). Confirmation of these effects on humans comes from studies of theuffy coat of blood drawn from human volunteers (Fig 3). Potassium also inhibited free radical formation by human white blood cells. Although theuffy coat contains several white blood cell types, the effect of \([K^+]\) on human white blood cells was not as strong as in the cultured cells in the preparation used. This is at least partly due to the fact that the measurements were done immediately after exposure to both stimulus and varying \([K^+]\) (compare with Fig 1) but may also partly result from the multiple cell types present in theuffy coat.

![Human PMN's and Monocytes](http://hyper.ahajournals.org/)
and/or the multiple species of free radicals produced. We are presently investigating the cellular basis of these effects, starting with the interaction of potassium with the cell membrane transport mechanisms.

It is generally believed that receptor-mediated uptake of low-density lipoproteins oxidized by free radicals, especially superoxide anion, initiates early vascular lesions, 43–46 which dietary potassium inhibits. 47,48 It has been previously proposed that these early lesion-preventing effects of potassium are specifically mediated by increased plasma potassium. 25 We hypothesized that increased [K⁺] would directly inhibit free radical formation. The present studies support both hypotheses. However, our ability to interpret the significance of this finding is limited because most epidemiologic studies compare between existing vascular disease and existing potassium consumption, and the few available prospective studies are confined to populations older than 40 years. 1–5 By contrast, it takes years or decades for the disease process remains to be established, these results are consistent with the hypothesis that changes in the formation of reactive oxygen species resulting from changes in plasma [K⁺] may contribute to the protective effects of dietary potassium. 26

Acknowledgments

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