Depolarization-Stimulated $^{42}$K$^+$ Efflux in Rat Aorta is Calcium- and Cellular Volume-Dependent

Lawrence Magliola and Allan W. Jones

The purpose of this study was to investigate the factors controlling membrane permeability to potassium of smooth muscle cells from rat aorta stimulated by depolarization. The increase in $^{42}$K$^+$ efflux (change in the rate constant) induced by depolarization (application of high concentrations of potassium chloride) was inhibited significantly by the calcium antagonists diltiazem and nisoldipine. Parallel inhibitory effects on contraction were observed. Diltiazem also inhibited potassium-stimulated $^{36}$Cl$^-$ efflux. The addition of 25-150 mM KCl to normal physiologic solution stimulated $^{42}$K$^+$ efflux in a concentration-dependent manner. Diltiazem suppressed potassium-stimulated $^{42}$K$^+$ efflux approximately 90% at 25 mM KCl and approximately 40% at 150 mM KCl. The ability of nisoldipine to inhibit $^{42}$K$^+$ efflux also diminished as the potassium chloride concentration was elevated. The component of efflux that was resistant to calcium antagonists probably resulted from a decrease in the electrochemical gradient for potassium. Cellular water did not change during potassium addition. Substitution of 80 and 150 mM KCl for sodium chloride produced cellular swelling and enhanced potassium-stimulated $^{42}$K$^+$ efflux compared with potassium chloride addition. The addition of sucrose to prevent cellular swelling reduced efflux response to potassium substitution toward that of potassium addition. A hyposmolar physiologic solution produced an increase in the $^{42}$K$^+$ efflux and a contracture that were both prevented by the addition of sucrose. We concluded that the depolarization-mediated $^{42}$K$^+$ efflux has three components: one is calcium dependent; a second is dependent on cellular volume; and a third is resistant to inhibition by calcium antagonists. (Circulation Research 1987;61:1-11)

Stimulation of vascular smooth muscle by norepinephrine (NE) is associated with increases in calcium ($\text{Ca}^{2+}$) and sodium ($\text{Na}^+$) influx and in potassium ($\text{K}^+$) and chloride ($\text{Cl}^-$) efflux across the cell membrane. K$^+$ efflux tends to oppose the inwardly directed, depolarizing currents of Ca$^{2+}$, Na$^+$, and Cl$^-$, thereby stabilizing the membrane potential. The degree of coupling between depolarizing currents and K$^+$ efflux may depend on the kinetics/number of K$^+$ channels for a particular tissue. Coupling would determine the type of vascular smooth muscle response by restricting the excursion of membrane potential from the resting state during stimulation: High K$^+$ conductances ($g_{K^+}$) would produce hyperpolarization and a relaxation; intermediate $g_{K^+}$ would elicit graded depolarizations and a tonic contraction; and low $g_{K^+}$ would likely result in repetitive depolarizations and a phasic contraction. Supportive of this hypothesis is the observation that the K$^+$-channel inhibitor, tetraethylammonium, caused the normally tonic rabbit ear artery to generate action potentials when treated with NE. This effect was accompanied by phasic contractile activity.

Ca$^{2+}$-dependent K$^+$ fluxes have been observed in a wide variety of tissues, including smooth muscle. An elevated flux may represent the opening of K$^+$-selective channels that are activated by an increase in the concentration of Ca$^{2+}$ at the inner surface of the cell membrane. Our laboratory recently reported that NE stimulated a Ca$^{2+}$-dependent $^{42}$K$^+$ efflux from rat and rabbit aorta. NE increases cytoplasmic Ca$^{2+}$ either by stimulating Ca$^{2+}$ influx from the extracellular space or by releasing Ca$^{2+}$ from an intracellular store. In contrast, depolarization by high K$^+$ solutions increases cytoplasmic Ca$^{2+}$ almost exclusively through extracellular Ca$^{2+}$ influx. Moreover, recent evidence suggests that Ca$^{2+}$ influx stimulated by K$^+$ depolarization occurs through a channel that is sensitive to changes in membrane potential, while Ca$^{2+}$ influx induced by NE occurs through a channel activated by receptor occupation. This paper describes experiments in which the $^{42}$K$^+$ efflux from rat aorta stimulated by depolarization was shown to depend on cytoplasmic Ca$^{2+}$, the cellular volume, and a factor(s) resistant to inhibition by Ca$^{2+}$ antagonists.

This study has been communicated in preliminary form.

Materials and Methods

Animals and Tissues

Male Sprague-Dawley rats (200-300 g) were decapitated, and the thoracic aorta was prepared according to a method described previously. Endothelial cells were removed from aortic strips (flux studies) and from ring segments (contractile studies) by gently

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stroking the intimal surface with filter paper while tissues were in dissection solution

**Solutions**

The physiologic solution had the following composition (in mM). Na⁺ 146.2, K⁺ 5.0, Ca²⁺ 2.5, Mg²⁺ 1.2, Cl⁻ 143.9, HCO₃⁻ 13.5, H₂PO₄⁻ 1.2, and glucose 11.2. Ca²⁺ was lowered to 0.025 mM in the dissection solution. The physiologic solution was adjusted, where appropriate, to contain 25 - 155 mM K⁺ either by adding KCl to normal physiologic solution (K⁺ add) or by replacing NaCl with equimolar KCl in normal physiologic solution (K⁺ sub). The Na⁺ salt of HCO₃⁻ was replaced by the K⁺ salt in a 150 mM K⁺ sub solution. Solutions were made hypoosmolar by reducing the NaCl (total Na⁺ 75 mM) In some instances, an 80 mM K⁺ sub solution was made hyperosmolar by replacing the NaCl (total Na⁺ 35 mM). Solutions were made hypoosmolar by the addition of sucrose. All solutions were bubbled with a 97% O₂-3% CO₂ mixture to obtain a pH of 7.4. Propranolol (3 μM) (Sigma Chemical Co., St. Louis, Mo.), ethylenediaminetetraacetic acid (EDTA) (0.025 mM), and ascorbic acid (1 mM) (Sigma) were added to all solutions for β-receptor blockade and to inhibit oxidation of catecholamines. Aqueous stock solutions were prepared for NE (Sigma) and for diltiazem (DZ) (gift from Marion Laboratories, Kansas City, Mo.). Ethanol stock solutions were prepared for nisoldipine (NIS) (gift from Miles Pharmaceuticals, New Haven, Conn.). Experiments were conducted in subdued lighting when NIS was used because of the drug's sensitivity to light.

**Isotope Fluxes**

Isotopic techniques were similar to those used previously. Open strips of aorta were mounted on stainless steel holders and were incubated for 4 hours at 37°C in physiologic solution containing either 20 μCi/ml ⁴²K⁺ (University of Missouri Research Reactor, Columbia, Mo.) or 20 μCi/ml ⁴²Ca²⁺ and 2 μCi/ml ³⁶Cl⁻ (ICN Biomedicals, Inc., Irvine, Calif.). After equilibration, the strips were passed through a series of vigorously gassed tubes containing nonradioactive solution. Washout curves were calculated (IBM XT) by sequentially adding decay-corrected tissue and tube activities (measured by routine gamma and liquid scintillation methods) in reverse order and by normalizing in terms of initial activity. Results were expressed as the fractional loss per minute or rate constant, k (min⁻¹), for each washout period. Under steady-state conditions, the rate constant for K⁺ efflux gives an estimate of K⁺ permeability.

**Contraction**

Aortic ring segments (3.5 mm wide) were prepared and mounted for the measurement of isometric force according to a standard procedure. Experiments were performed on rings stretched 1.3 × resting diameter. The tension developed 15 minutes after stimulation with chemical agents represents the tonic component of the contraction.

**Ion and Water Contents**

Aortic strips were prepared according to the method employed for flux measurements. Strips were equilibrated for 4 hours at 37°C in normal physiologic solution before initiation of experimental protocols. For measurement of extracellular space, the chelate of ⁶⁰Co²⁺ to EDTA was employed as an extracellular marker. The K⁺ salt of EDTA was used to prepare a 150 mM K⁺ sub solution. Tissues were equilibrated for 15 minutes at 37°C in an experimental solution containing 1 mM Co²⁺ EDTA and ⁶⁰Co²⁺ EDTA (0.5 μCi/ml) and then were transferred to an identical solution for 1 minute at 1°C to reduce evaporative water loss while the tissues were blotted and weighed. The strips were then oven-dried for 20 hours at 105°C to determine water contents and dry weights. Tissues were counted in a gamma well, and the weight of extracellular water was calculated as the ratio of counts in the strip to a weighed amount of the ⁶⁰Co⁺⁺ EDTA solution. The space was calculated on the basis of kg H₂O/kg dry wt. Intracellular water was calculated as the difference between total water and water in the ⁶⁰Co²⁺ EDTA space. Tissues were ashed overnight at 85°C in 30% (wt/vol) H₂O₂. The ashes were dissolved in ionic analysis solution [0.1 N HNO₃, 10 mM La(NO₃)₃]. The Na⁺ and K⁺ were measured by flame photometry (Model 143, Instrumentation Laboratory, Wilmington, Mass.). Cellular ion contents were calculated by subtracting the [(⁶⁰Co²⁺ EDTA space) × (ion concentration in the experimental solution)] from the total ion contents. No corrections were made for Na⁺ adsorbed to the extracellular matrix since the ionic analysis was done to monitor changes rather than absolute levels of Na⁺. Cellular concentrations were calculated by dividing the tissue ion contents by cellular water. Dry weights were corrected for the weight of the sucrose in the extracellular space.

**Statistics**

Data are presented as the mean ± SEM. The Student's t test was used for the comparison of two groups. An analysis of variance was performed when more than two groups were compared simultaneously. If a significant treatment effect was indicated by the analysis of variance, then a modified t statistic was calculated for each comparison. Significant differences among group means were determined using the Bonferroni method. A p < 0.05 was considered significant for all test procedures. A tissue's response to a drug is usually a function of the log of the drug concentration. Equieffective concentrations of a drug are normally distributed on a log scale but not on an arithmetic one. Therefore, the concentrations of Ca²⁺ antagonists required for half-maximal inhibition (IC₅₀) were determined from each concentration response curve by interpolating the log values of drug concentrations that produced inhibitions above and below the 50 percent value. The mean log
Ca\(^{2+}\)- and Cellular Volume-Dependent Ionic Fluxes

**Results**

**Calcium-Antagonist Effects on K\(^{+}\)-Stimulated \(^{42}\text{K}^{+}/\text{^{36}Cl}^{-}\) Efflux and Contraction**

Depolarization with solutions containing high K\(^{+}\) increased the \(^{42}\text{K}^{+}\) efflux from rat aorta, measured as a change in the rate constant (\(\Delta k\) response) (Figure 1). The \(\Delta k\) response diminished significantly (\(p<0.001\)) in the presence of the Ca\(^{2+}\)-antagonist DZ. DZ also inhibited the increase in \(^{36}\text{Cl}^{-}\) efflux that accompanies K\(^{+}\) stimulation (\(p<0.025\)) (Figure 1), and it prevented a contraction stimulated by K\(^{+}\) (Figure 1). We used the amplitude of force development as an estimate of [Ca\(^{2+}\)]. Morgan and Morgan\(^{15}\) found that during K\(^{+}\) depolarization [Ca\(^{2+}\)] and contraction were closely correlated. In general, the concentration-dependent inhibition of K\(^{+}\)-stimulated \(^{42}\text{K}^{+}\) efflux by DZ was similar to changes in contraction during inhibition by DZ (Figure 2A). However, the concentration of DZ required for half-maximal inhibition of K\(^{+}\)-stimulated contraction (0.4 \(\mu M\), antilog of \(-6.41 \pm 0.04\)) was significantly less (\(p<0.05\)) than that required for K\(^{+}\)-stimulated \(^{42}\text{K}^{+}\) efflux (0.6 \(\mu M\), antilog of \(-6.21 \pm 0.08\)). A concentration of DZ as high as 100 \(\mu M\) did not alter basal \(^{42}\text{K}^{+}\) efflux or resting tension. The possibility that micromolar concentrations of DZ could directly inhibit a K\(^{+}\) flux rather than inhibit a Ca\(^{2+}\)-dependent K\(^{+}\) flux by blocking Ca\(^{2+}\) entry prompted us to investigate the relation between K\(^{+}\)-stimulated \(^{42}\text{K}^{+}\) efflux and contraction with the more potent antagonist NIS. Like DZ, NIS inhibited K\(^{+}\)-stimulated \(^{42}\text{K}^{+}\) efflux and contraction but was 100 to 1,000 times more potent than DZ (Figure 2B). Likewise, the concentration of NIS required for half-maximal inhibition of K\(^{+}\)-stimulated contraction (0.6 nM, antilog of...
FIGURE 3. Effect of KCl addition on $^{42}$K$^+$ efflux and contraction in rat aorta. For flux measurements, individual aortic strips that had been equilibrated in $^{42}$K$^+$ were passed through a series of tubes containing graded amounts of KCl added to normal physiologic solution. Effluxes stimulated by addition of KCl were restored to baseline by washing the strips in normal solution before exposure to the next higher concentration. Change in the rate constant for $^{42}$K$^+$ efflux during K$^+$ stimulation was not diminished by repeated challenge with the same concentration of KCl (data not shown). Means ± SEM (representative values) of the rate constants are plotted for control (●) ($n = 8$ rats) and in the presence of 30 μM DZ throughout (□) ($n = 8$ rats). A maximal concentration of NE (3 μM) was added to strips previously exposed to 150 mM KCl. Protocol employed for measuring contractions to graded K$^+$ add was identical to that used for flux measurements. Contractile responses of each ring were normalized by comparing the maximal tension developed by K$^+$ with that induced by 3 μM NE. Repeated stimulation with 80 mM K$^+$ add (up to 5 cycles of challenge and wash) did not cause significant loss of responsiveness to K$^+$ (data not shown). Typical contractile responses are shown. DZ was added 30 minutes prior to KCl application in contractile studies. Dashed lines denote basal tension before KCl application.

Responses to K$^+$ Addition

The addition of 25–150 mM KCl to normal physiologic solution (K$^+$ add) produced concentration-dependent increases in the $^{42}$K$^+$ efflux, as shown in Figure 3. DZ markedly depressed the Δk response to K$^+$, although the refractory component of the Δk response to DZ increased as [K$^+$], was elevated (Table 1). High NIS was also less able to inhibit $^{42}$K$^+$ efflux as [K$^+$] was elevated (Figure 4B). The application of a supermaximal concentration of NE (3 μM) to aortas previously exposed to 150 mM K$^+$ add further increased the Δk response (Figure 3), and unlike K$^+$ stimulation, the Δk response to NE was not inhibited by DZ (Table 1).

A maximal contraction of aortas during K$^+$ add occurred at 50 mM (~80% of NE response) (Figure 3). A progressive loss of response was observed at K$^+$ greater than 50 mM (Table 2). DZ inhibited the contractions developed during K$^+$ add (Figure 3), but the residual contractions increased as [K$^+$], was elevated (Table 2). High NIS was also less able to inhibit contractions as [K$^+$] was elevated (Figure 4A). NE (3 μM) elicited a small, but significant ($p < 0.02$), increase in tension when applied to aortas previously exposed to 150 mM K$^+$ add that was not inhibited by DZ (Table 2).

The addition of 25–150 mM KCl to normal physiologic solution produced a general reduction in total tissue and extracellular water from aortas at 15 minutes with no change in cellular water (Table 3). Cellular K$^+$ content and concentration increased with the amount of KCl added to the physiologic solution (Table 3). Cellular Na$^+$ content and concentration remained near control levels.

Table 1. Change in the Rate Constant, Δk, for $^{42}$K$^+$ Efflux Stimulated by KCl

<table>
<thead>
<tr>
<th>Addition</th>
<th>Substitution</th>
<th>S-A*</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 mM K$^+$</td>
<td>0.0133 ± 0.0006†</td>
<td>0.0120 ± 0.0010</td>
<td>-0.0013</td>
</tr>
<tr>
<td>50 mM K$^+$</td>
<td>0.0164 ± 0.0008</td>
<td>0.0221 ± 0.0010</td>
<td>0.0057</td>
</tr>
<tr>
<td>80 mM K$^+$</td>
<td>0.0203 ± 0.0009</td>
<td>0.0406 ± 0.0022</td>
<td>0.0197</td>
</tr>
<tr>
<td>150 mM K$^+$</td>
<td>0.0246 ± 0.0016</td>
<td>0.0505 ± 0.0033</td>
<td>0.0259</td>
</tr>
<tr>
<td>NE§</td>
<td>0.0117 ± 0.0024</td>
<td>0.0236 ± 0.0017</td>
<td>0.0119</td>
</tr>
</tbody>
</table>

Diltiazem||<0.001|<0.001|<0.001|<0.001|<0.001|

<table>
<thead>
<tr>
<th>Addition</th>
<th>Substitution</th>
<th>S-A*</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 mM K$^+$</td>
<td>0.0014 ± 0.0001</td>
<td>0.0023 ± 0.0004</td>
<td>0.0009</td>
</tr>
<tr>
<td>50 mM K$^+$</td>
<td>0.0027 ± 0.0002</td>
<td>0.0068 ± 0.0005</td>
<td>0.0041</td>
</tr>
<tr>
<td>80 mM K$^+$</td>
<td>0.0065 ± 0.0008</td>
<td>0.0146 ± 0.0011</td>
<td>0.0081</td>
</tr>
<tr>
<td>150 mM K$^+$</td>
<td>0.0140 ± 0.0011</td>
<td>0.0136 ± 0.0011</td>
<td>-0.0004</td>
</tr>
<tr>
<td>NE§</td>
<td>0.0117 ± 0.0024</td>
<td>0.0496 ± 0.0023</td>
<td>0.0379</td>
</tr>
</tbody>
</table>

*Values are the difference in the Δk response of strips to K$^+$ substitution and K$^+$ addition.
†Each value is the mean ± SEM of the Δk response at 15 minutes to K$^+$ addition ($n = 8$) or K$^+$ substitution ($n = 6$).
‡Not significant.
§Values denote the Δk response at 15 minutes to NE alone (150 mM K$^+$ plus NE − 150 mM K$^+$) [30 μM].
Responses to K+ Substitution

The substitution of 25–150 mM KCl for NaCl (K+ sub) caused a progressive enhancement of the ΔK response relative to K+ add shown in Table 1 (also compare Figures 3 and 5). DZ inhibited the ΔK response to K+ sub (Figure 5). The residual ΔK response was enhanced during K+ sub compared with K+ add, except at 150 mM (Table 1). High NIS was less able to inhibit the ΔK response to K+ sub compared with K+ add (p < 0.001) (Figure 4B). NE (3 μM) increased the K+ response relative to K+ add shown in Table 1 (also compare Figures 3 and 5). DZ inhibited contractions in the presence of K+ add, then the Ak response to K+ sub (Figure 5) toward that of K+ add (Table 2). The increase in tension was greater when NE was added in the presence of DZ. Like 42K+ efflux, the enhanced responsiveness to NE when DZ was present may be related to differences in basal tension and to a limitation on the tension that can be developed.

The 150 mM K+ sub caused a significant increase in cellular water and a concomitant decrease in extracellular water in two experiments (Tables 3 and 4). Total tissue water did not change. The 80 mM K+ sub produced a significant increase in tension (p < 0.001) when added to aortas already contracted during 150 mM K+ sub (Table 2). The increase in tension was greater when NE was added in the presence of DZ. Like 42K+ efflux, the enhanced responsiveness to NE when DZ was present may be related to differences in basal tension and to a limitation on the tension that can be developed.

The cellular swelling caused by K+ sub was prevented by the addition of sucrose (42 mM) to the high K+ solution (Table 3). Sucrose also reduced the ΔK response to K+ sub (Figure 6) toward that of K+ add (Figure 3). Sucrose reduced the ΔK response after 15 minutes K+ sub in the presence of DZ (p < 0.01). Sucrose decreased the tension elicited by 150 mM K+ sub but not to the level of K+ add (compare Figures 3 and 6). Sucrose appeared to inhibit the NE-stimulated ΔK response of aortas previously exposed to K+ sub, although a small contractile response was observed (Figure 6).

Table 2. K+ Stimulation Contraction in Rat Aorta (Expressed as a Percentage of a Maximal NE Contraction)

<table>
<thead>
<tr>
<th>Control</th>
<th>Addition</th>
<th>Substitution</th>
<th>S-A*</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 mM K+</td>
<td>71 ± 3*</td>
<td>39 ± 4</td>
<td>-32</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>50 mM K+</td>
<td>77 ± 1</td>
<td>86 ± 3</td>
<td>9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>80 mM K+</td>
<td>66 ± 1</td>
<td>93 ± 2</td>
<td>27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>150 mM K+</td>
<td>39 ± 1</td>
<td>92 ± 4</td>
<td>53</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NE</td>
<td>7 ± 2</td>
<td>17 ± 4</td>
<td>10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Diltiazem</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 mM K+</td>
<td>2 ± 1</td>
<td>0 ± 0</td>
<td>-2</td>
<td>NS</td>
</tr>
<tr>
<td>50 mM K+</td>
<td>10 ± 1</td>
<td>2 ± 1</td>
<td>-8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>80 mM K+</td>
<td>14 ± 1</td>
<td>5 ± 1</td>
<td>-9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>150 mM K+</td>
<td>19 ± 1</td>
<td>12 ± 3</td>
<td>-7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NE</td>
<td>6 ± 1</td>
<td>71 ± 4</td>
<td>65</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Each value is the mean ± SEM of the response at 15 minutes to K+ addition (n = 8) or K+ substitution (n = 7)
swelling induced solely by hypotonicity should also increase $^{42}$K$^+$ efflux. A hypoosmolar solution (total Na$^+$ 75 mM) increased the $^{42}$K$^+$ efflux from aortas (Figure 7); however, the response was not sustained despite the continued presence of the hypoosmolar solution. The addition of sucrose to the hypoosmolar solution prevented the increase in $^{42}$K$^+$ efflux (Figure 7). The hypoosmolar solution also produced contractions with a time course parallel to that observed for $^{42}$K$^+$ efflux, and these contractions were prevented by sucrose (Figure 7). NIS reduced only modestly the $\Delta K^+$ response to the hypoosmolar solution, even at concentrations 100 times higher than those that prevented contractions (Figure 8).

The $\Delta K^+$ response of aortas to isoosmolar K$^+$ sub was enhanced by reducing the NaCl (low Na$^+$-high K$^+$) (Figure 9). The addition of sucrose (95 mM) to the low Na$^+$-high K$^+$ solution reduced cellular water to control levels and reduced the $\Delta K^+$ response toward that of K$^+$ add (compare Figures 3 and 9, Tables 3 and 4).

![Figure 5. Effect of KCl substitution on $^{42}$K$^+$ efflux and contraction in rat aorta. See Figure 3 legend for details but n = 6 rats for both groups.](image)

### Table 3. Water and Ion Distribution of Rat Aorta in Normal and High K$^+$ Solutions

<table>
<thead>
<tr>
<th>Water (kg H$2$O/kg dry wt)</th>
<th>Total $^{40}$Co$^{2+}$ EDTA (mmol/kg dry wt)</th>
<th>Total ion (mmol/kg dry wt)</th>
<th>Total $^{42}$K$^+$ (mmol/kg cell H$2$O)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water (kg H$2$O/kg dry wt)</td>
<td>K$^+$</td>
<td>Na$^+$</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-----------------------------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>5 mM K$^+$</td>
<td>2.75 ± 0.05*</td>
<td>1.67 ± 0.06</td>
<td>1.04 ± 0.08</td>
</tr>
<tr>
<td>25 mM K$^+$ add†</td>
<td>2.67 ± 0.12</td>
<td>1.49 ± 0.08</td>
<td>1.19 ± 0.13</td>
</tr>
<tr>
<td>50 mM K$^+$ add</td>
<td>2.33 ± 0.05§</td>
<td>1.31 ± 0.07§</td>
<td>1.00 ± 0.05</td>
</tr>
<tr>
<td>80 mM K$^+$ add</td>
<td>2.50 ± 0.07§</td>
<td>1.50 ± 0.12</td>
<td>1.00 ± 0.08</td>
</tr>
<tr>
<td>150 mM K$^+$ add#</td>
<td>2.38 ± 0.05§</td>
<td>1.37 ± 0.05§</td>
<td>1.01 ± 0.05</td>
</tr>
<tr>
<td>25 mM K$^+$ sub§</td>
<td>2.68 ± 0.08</td>
<td>1.54 ± 0.09</td>
<td>1.14 ± 0.10</td>
</tr>
<tr>
<td>50 mM K$^+$ sub</td>
<td>2.57 ± 0.13</td>
<td>1.45 ± 0.09</td>
<td>1.12 ± 0.09</td>
</tr>
<tr>
<td>80 mM K$^+$ sub</td>
<td>2.51 ± 0.07</td>
<td>1.39 ± 0.11</td>
<td>1.12 ± 0.05</td>
</tr>
<tr>
<td>150 mM K$^+$ sub#</td>
<td>2.68 ± 0.10</td>
<td>1.28 ± 0.06‡</td>
<td>1.40 ± 0.10‖</td>
</tr>
<tr>
<td>150 mM K$^+$ sub + SU**</td>
<td>2.44 ± 0.10</td>
<td>1.42 ± 0.05</td>
<td>1.02 ± 0.08‡†</td>
</tr>
</tbody>
</table>

*Values represent the means ± SEM Each aorta was divided into 4 strips, and strips from several rats were pooled. For each treatment, 6 strips were randomly selected from the pool.
†Addition
‡p < 0.002 vs. 5 mM K$^+$
§p < 0.05 vs. 5 mM K$^+$
‖p < 0.01 vs. 5 mM K$^+$
§§p < 0.001 vs. 150 mM K$^+$ sub
††p < 0.05 vs. 80 mM K$^+$ sub
‡‡p < 0.01 vs. 80 mM K$^+$ sub
§§§p < 0.001 vs. 150 mM K$^+$ sub

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Table 4. Water and Ion Distribution of Rat Aorta in Normal and High K⁺ Solutions

<table>
<thead>
<tr>
<th>Total (mmol/kg dry wt)</th>
<th>⁴⁰Co⁺ (mmol/kg dry wt)</th>
<th>EDTA (mmol/kg dry wt)</th>
<th>Total (mmol/kg cell H₂O)</th>
<th>Na⁺ (mmol/kg cell H₂O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 mM K⁺</td>
<td>2.53 ± 0.06*</td>
<td>1.68 ± 0.07</td>
<td>0.85 ± 0.05</td>
<td>119 ± 4</td>
</tr>
<tr>
<td>80 mM K⁺ sub</td>
<td>2.35 ± 0.05</td>
<td>1.29 ± 0.04</td>
<td>1.05 ± 0.03</td>
<td>257 ± 5</td>
</tr>
<tr>
<td>80 mM K⁺ + DZ sub</td>
<td>2.61 ± 0.04</td>
<td>1.64 ± 0.07</td>
<td>0.97 ± 0.06</td>
<td>280 ± 6</td>
</tr>
<tr>
<td>150 mM K⁺ sub</td>
<td>2.47 ± 0.02</td>
<td>1.42 ± 0.05</td>
<td>1.05 ± 0.04</td>
<td>399 ± 6</td>
</tr>
<tr>
<td>150 mM K⁺ + DZ</td>
<td>2.51 ± 0.04</td>
<td>1.45 ± 0.03</td>
<td>1.06 ± 0.05</td>
<td>408 ± 3</td>
</tr>
<tr>
<td>80 mM K⁺ sub (low Na⁺)!</td>
<td>2.51 ± 0.03</td>
<td>1.42 ± 0.05</td>
<td>1.06 ± 0.03</td>
<td>253 ± 1C</td>
</tr>
<tr>
<td>80 mM K⁺ + DZ (low Na⁺)</td>
<td>2.72 ± 0.03</td>
<td>1.57 ± 0.07</td>
<td>1.15 ± 0.05</td>
<td>285 ± 5</td>
</tr>
<tr>
<td>80 mM K⁺ + DZ (low Na⁺) + SU#</td>
<td>2.31 ± 0.07</td>
<td>1.51 ± 0.08</td>
<td>0.80 ± 0.02**</td>
<td>268 ± 7</td>
</tr>
</tbody>
</table>

Values represent the means ± SEM. Each aorta was divided into 4 strips, and strips from several rats were pooled. For each treatment, 8 strips were randomly selected from the pool.

*Values represent the means ± SEM. Each aorta was divided into 4 strips, and strips from several rats were pooled. For each treatment, 8 strips were randomly selected from the pool.

$p<0.001$ vs. 5 mM K⁺
$p<0.01$ vs. 5 mM K⁺
$\Delta$30 μM
$p<0.02$ vs. 5 mM K⁺
$\Delta$75 mM Na⁺.
$\Delta$95 mM

substantially inhibited the Δk response to low Na⁺-high K⁺ (Figure 9) but did not reduce cellular swelling (Table 4).

The contractile response of aortas to K⁺ sub was not altered by lowering Na⁺ (Figure 9). The addition of sucrose to the low Na⁺-high K⁺ solution decreased slightly the contractile response of rings. DZ markedly inhibited contractions produced by low Na⁺-high K⁺ whether sucrose was present or not.
Discussion

This study provides evidence suggesting that stimulation of K⁺ permeability by depolarization with KCl is a complex process governed by cytoplasmic [Ca²⁺], cellular volume, and a factor(s) resistant to inhibition by Ca²⁺ antagonists.

**Ca²⁺-Dependent K⁺ Efflux**

We observed correspondence between ⁴²K⁺ efflux and contraction under conditions where cellular [Ca²⁺] was altered either by varying the degree of depolarization or by varying the concentration of Ca²⁺ antagonists. Van Breemen reported that contraction of rabbit aorta stimulated by K⁺ depolarization depended almost exclusively on an influx of extracellular Ca²⁺. Moreover, DZ and NIS were shown to inhibit K⁺-induced contractions by selectively blocking Ca²⁺ influx through potential-operated ion channels. We suggest that an increase in cytoplasmic [Ca²⁺] mediated by depolarization-dependent Ca²⁺ influx stimulates both ⁴²K⁺ efflux and contraction. The inhibition of ⁴²K⁺ efflux by DZ and NIS probably was not due to direct action on a K⁺ channel because NIS produced significant inhibition of ⁴²K⁺ efflux at a concentration of only 0.1 nM. Furthermore, the difference in potency between NIS and DZ for Ca²⁺ entry blockade probably would not mirror a difference between these drugs for K⁺ channel blockade, as our data indicate, unless both processes were controlled by a common mediator such as Ca²⁺.

This study confirms and extends earlier work in which we demonstrated a Ca²⁺ dependency for NE-stimulated ⁴²K⁺ efflux from rat aorta. Others have reported that the activation of ⁸⁶Rb⁺ effluxes (a K⁺ substitute) from rabbit ear artery by either K⁺ or NE required Ca²⁺-Ca²⁺-activated K⁺ effluxes from many cell types, including smooth muscle, and may underlie the K⁺ effluxes observed in these studies (see discussion of Smith and Jones). Taken collectively, these studies support the hypothesis that the activation of K⁺ channels is determined primarily by the cytoplasmic [Ca²⁺]. An increase in cytoplasmic [Ca²⁺] could result from either an influx of extracellular Ca²⁺ stimulated by Ca²⁺ depolarization or by NE or the release of an intracellular Ca²⁺ store by NE.

The K⁺-stimulated ⁴²K⁺ efflux was less sensitive to inhibition by Ca²⁺ antagonists compared with contraction. This difference implies that the K⁺ channels possess a greater sensitivity to Ca²⁺ than the contractile elements (contractile proteins and regulatory system), thus implication is supported by experiments in which flux and contraction were compared during the loss and recovery of responsiveness to Ca²⁺ alterations. Specifically, the contractile response to NE in both rat and rabbit aorta was lost more rapidly than the flux response when Ca²⁺ was removed from the physiologic solution. Furthermore, the flux response to NE in the rat aorta was restored more rapidly than the contractile response when Ca²⁺ was replaced in zero Ca²⁺ solution. Alternatively, an influx of Ca²⁺...
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through a potential-operated channel may elevate \([\text{Ca}^{2+}]\) in the immediate vicinity of the K⁺ channel to a greater extent than in the cytoplasm where the contractile and \(\text{Ca}^{2+}\)-buffering elements are located.

Contraction induced by K⁺ and by low concentrations of NE were inhibited more effectively by DZ than those stimulated by high concentrations of NE. We observed that high concentrations of NE overcame the inhibitory effect of DZ for both K⁺-induced \(\text{K}^+\) efflux and contraction. This differential sensitivity to DZ also was reported for rat and rabbit aorta and is consistent with the concept that high concentrations of NE stimulate the release of an intracellular \(\text{Ca}^{2+}\) pool that is largely resistant to inhibition by DZ. The elevation of \([\text{Ca}^{2+}]\) by NE in the presence of DZ is sufficient to support both an elevated rate of \(\text{K}^+\) efflux and a contraction.

**Volume-Dependent K⁺ Efflux**

Replacement of Na⁺ by K⁺ in the physiologic solution produced cellular swelling in rat aorta. This swelling apparently resulted from a cellular accumulation of KCl due to the greater permeability of the cell membrane to K⁺ than to Na⁺. A similar redistribution of water and ions associated with K⁺ sub was reported by Jones et al for guinea pig and rabbit taenia coli and for rabbit vascular smooth muscles.

Cells swollen by K⁺ sub demonstrated enhanced \(\text{K}^+\) efflux compared with K⁺ add (unswollen cells). The addition of sucrose to prevent swelling reduced the \(\Delta k\) response to K⁺ sub toward that of K⁺ add. These results suggest that a component of the K⁺-stimulated \(\text{K}^+\) efflux is activated by cellular swelling. This conclusion is supported by two additional sets of observations: 1) \(\text{K}^+\) efflux increased transiently in hypoosmolar solutions, and this effect was blocked by the addition of sucrose; and 2) the \(\text{K}^+\) efflux induced by hypoosmolar K⁺ sub was enhanced compared with K⁺ add alone, and this level of response was reduced by the addition of sucrose during K⁺ sub. Many cell types that swell in response to hypotonicity can rapidly restore their volumes. This regulatory volume decrease was attributed to a water coupled KCl loss that was associated with specific increases in K⁺ and Cl⁻ permeability. Such a phenomenon may underlie the increase in \(\text{K}^+\) efflux from rat aortas swollen by hypoosmolar solutions and the enhanced \(\text{K}^+\) efflux during K⁺ sub compared with K⁺ add. The stimulation of \(\text{K}^+\) efflux by hypoosmolar solutions was transient, while the increase in \(\text{K}^+\) efflux during K⁺ sub was sustained. This difference in temporal response may be related to a more rapid volume readjustment in hypoosmolar solutions than during K⁺ sub when K⁺ influx would be increased. Alternatively, the transient release may be due to the rapid inactivation of a stretch-sensitive K⁺ channel (see below).

We have presented data suggesting that K⁺ loss during cellular swelling either is independent of extraacellular \(\text{Ca}^{2+}\), or is activated at a lower \([\text{Ca}^{2+}]\): 1) The stimulation of \(\text{K}^+\) efflux by the application of a hypoosmolar solution was resistant to inhibition by \(\text{Ca}^{2+}\)-antagonists; 2) the residual \(\Delta k\) responses (25–80 mM KCl) were significantly greater during K⁺ sub compared with K⁺ add, and 3) cellular swelling during K⁺ sub was not prevented in the presence of DZ. In contrast, similar residual \(\Delta k\) responses to 150 mM K⁺ sub and K⁺ add suggest that volume-dependent K⁺ loss may be dependent on Ca²⁺ uptake. Nevertheless, the contractions observed in hypoosmolar solutions probably were activated by Ca²⁺ influx since Ca²⁺ antagonists prevented them. Recently, Wang and Chase reported that the swelling of epithelial cells from toad bladder caused an elevation of \([\text{Ca}^{2+}]\) mediated by Ca²⁺ influx.

We considered the possibility that the enhanced \(\Delta k\) response to K⁺ sub might result from low cellular \([\text{Na}^+]\) rather than cellular swelling directly Na⁺ reduced a Ca²⁺-activated \(\text{K}^+\) current when applied to the internal face of membrane patches from smooth muscle, presumably by competing with Ca²⁺ for a common binding site. Relief of Na⁺ blockade in our study is unlikely, however, because the addition of sucrose reduced the \(\Delta k\) response to K⁺ sub even though the cellular \([\text{Na}^+]\) in both groups was similar (Figure 6 and Table 3), and also because the addition of sucrose reduced the \(\Delta k\) response to low Na⁺, 80 mM K⁺ sub compared with isoosmolar 80 mM K⁺ sub despite lower cellular \([\text{Na}^+]\) in the former group (Figure 9 and Table 4).

The molecular basis of volume-induced ionic fluxes has not been determined. Possibly changes in the lateral tension of the plasma membrane (stretch) produce changes in ionic permeability. Such a mechanism may underlie the development of myogenic vascular tone observed in most vascular beds. Stretch could stimulate K⁺ efflux directly or Ca²⁺ influx that would secondarily alter K⁺ efflux through a Ca²⁺-dependent K⁺ channel. The necessity for Ca²⁺ in the development of myogenic tone appears established. A voltage-insensitive, K⁺-selective channel in which the gating depends on membrane stretch was reported recently in membrane patches from embryonic-chick skeletal muscle, but Ca²⁺ was not required for stretch activation.
channels. This component of the efflux need not be associated with an increased [Ca$^{2+}$] and a contraction. We tested the first possibility by calculating the contribution of changes in the electric field to the rate constant, $k$, for K$^+$ efflux. The following relation was used:

$$
efflux = k(V/A)c_0 = P_K\left(c_0\left(\frac{\Delta E_{\text{mem}}}{2FRT}\right)/(1 - e^{-\frac{\Delta E_{\text{mem}}}{RT}})\right)$$

where $P_K$ = permeability to K$^+$, $c_0$ = the free intracellular concentration of ion (mol/cm$^3$), $V/A$ = cell volume to area ratio (0.6 x 10$^{-4}$ cm$^2$), $\Delta E_{\text{mem}}$ = the change in potential across the membrane (V), $z$ = the valence of the ion, $F$ = the Faraday constant (23,064 cal/V/mol), $R$ = the universal gas constant (1.99 cal/K/mol), and $T$ = the absolute temperature ($^\circ$K).

We estimated the degree of depolarization for various K$^+$ solutions. The predicted changes in $k$ for 42 K$^+$ efflux caused by depolarization alone exceeded the observed changes in $k$, produced by K$^+$ add of 25, 50, 80, and 150 mM in the presence of DZ, by 47, 95, 71, and 55% in predicting changes in $k$. We assumed that $P_K$ and $V/A$ did not change during K$^+$ add and that DZ did not alter the membrane potential K$^+$ add did not affect cell water but did increase [K$^+$], which appears on both sides of the above equation and diverts out as a factor. Because the measured changes in $k$ were less than the predicted changes based on an altered electrical gradient, we have no evidence from these studies for a voltage-dependent increase in $P_K$ (or its equivalent K$^+$ conductance) in rat aorta. The residual flux could result from any of the following: 1) alteration of electrochemical gradients across leak channels, 2) the opening of a small percentage of Ca$^{2+}$ channels with a higher resistance to Ca$^{2+}$ channel blockade; 3) the release of intracellular Ca$^{2+}$, which typically shows a high resistance to Ca$^{2+}$ antagonists; and 4) the effect of an increased ionic strength, due to K$^+$ add, to alter Ca$^{2+}$ channel behavior and/or Ca$^{2+}$-antagonist binding.

**Ionic Strength Effects**

The contractile responses to K$^+$ add declined progressively above 50 mM in contrast to isotonic K$^+$ sub. A well-known effect of hyperosmolarity is inhibition of vascular reactivity. Hyperosmolar solutions of both permeant and impermeant solutes inhibited tension elicited by electrical stimulation, by agonists, and by K$^+$ solutions for several smooth muscle preparations. K$^+$ add, even up to 150 mM, did not significantly alter cellular water when measured 15 minutes after application but did increase cellular K$^+$ content (and presumably Cl$^-$), hence the intracellular ionic strength. Ca$^{2+}$-activated force diminished with increasing ionic strength (KCl concentration) in skinned frog muscle fibers. High ionic strength solutions that reduced ATPase activity and reduced supersaturation and solubility of actomyosin from hog carotid arteries are indicative of a reduced interaction between actin and myosin. Also, activation of myo-inositol light-chain kinase by calmodulin was reduced by high ionic strengths.

The contractile responses to K$^+$ add, however, were more resistant to inhibition by DZ and NIS than those caused by K$^+$ sub. The residual contractions may result from a hyperosmolar-induced Ca$^{2+}$ release from intracellular stores or perhaps from a mechanism that is independent of Ca$^{2+}$.

**Ca$^{2+}$-Dependent Cl$^-$ Efflux**

K$^+$-stimulated 36 Cl$^-$ efflux from rat aorta was inhibited by DZ. Smith and Jones reported that NE-stimulated 36 Cl$^-$ efflux from rat aorta was inhibited by Ca$^{2+}$ removal. These studies suggest that Cl$^-$ efflux, like K$^+$, has a Ca$^{2+}$-dependent component. A Cl$^-$ channel, which may be activated by Ca$^{2+}$, was identified in Xenopus laevis oocytes. Such a channel type may underlie the Cl$^-$ flux seen in rat aorta. We estimated that the increase in Cl$^-$ permeability due to K$^+$ stimulation was eightfold greater than for K$^+$. This value compares reasonably well to that estimated for NE stimulation. These large permeability ratios suggest that K$^+$ and Cl$^-$ fluxes are not electrically coupled.

In conclusion, we propose that three components compose the K$^+$-stimulated 42 K$^+$ efflux: one dependent on Ca$^{2+}$ (blocked by Ca$^{2+}$ antagonists), a second dependent on cellular volume, and a third resistant to inhibition by Ca$^{2+}$ antagonists. Future studies will be needed to ascertain whether various modes of activation (Ca$^{2+}$, cellular volume, voltage, etc.) operate through different K$^+$ channels or represent different modes of activation for the same channel in vascular smooth muscle.

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**References**

Magliola L, Jones AW: Depolarization-stimulated $^{42}$K efflux in rat aorta as Ca$^2+$ and cell volume-dependent (abstract). Fed Proc 1985,44:453


Depolarization-stimulated 42K+ efflux in rat aorta is calcium- and cellular volume-dependent.

L Magliola and A W Jones

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