Barium Reduces Resting Blood Flow and Inhibits Potassium-Induced Vasodilation in the Human Forearm

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Background—Increasing extracellular K⁺ concentration within and just above the physiological range hyperpolarizes and relaxes vascular smooth muscle in vitro. These actions involve inwardly rectifying potassium channels (Kir) and Na⁺/K⁺ ATPase, which are inhibited, respectively, by Ba²⁺ and ouabain. The role (if any) of Kir in controlling human resistance vessel tone is unknown, and we investigated this in the forearm.

Methods and Results—Blood flow was measured by plethysmography in healthy men. Drugs and electrolytes were infused in the infused forearm to 50±0.8 μmol/L (mean±SEM) and reduced blood flow by 24±4% (n=8, P<0.001) without causing systemic effects. Ouabain (2.7 nmol/min), alone and with BaCl₂, reduced flow by 10±2% and 28±3%, respectively (n=10). Incremental infusions of KCl (0.05, 0.1, and 0.2 mmol/min) increased flow from baseline by 1.0±0.2, 2.0±0.4, and 4.2±0.5 mL/min per deciliter forearm, respectively. Responses to KCl (0.2 mmol/min) were inhibited by BaCl₂, alone and plus ouabain, by 60±5% and 88±6%, respectively (both P=0.01). In control experiments, norepinephrine (240 pmol/min) reduced blood flow by 24±2% but had no significant effect on K⁺-induced vasodilation. BaCl₂, alone or with ouabain, did not significantly influence responses to verapamil or nitroprusside.

Conclusions—Ba²⁺ increases forearm vascular resistance. K⁺-induced vasodilation is selectively inhibited by Ba²⁺ and almost abolished by Ba²⁺ plus ouabain, suggesting a role for Kir and Na⁺/K⁺ ATPase in controlling basal tone and in K⁺-induced vasorelaxation in human forearm resistance vessels. (Circulation. 2002;105:1323-1328.)

Key Words: potassium ■ vasodilation ■ endothelium-derived factors ■ muscle, smooth ■ vasculature

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We measured effects of Ba$^{2+}$ on K$^+$-induced vasodilation as well as on basal blood flow. Increasing [K$^+$], displaces K$_{IR}$ channel-bound polyamines$^{19}$; in the relevant concentration range, this increases outward K$^+$ current, hyperpolarizing the membrane.$^4$ Increasing [K$^+$], also hyperpolarizes vascular smooth muscle by activating the electrogenic Na$^+$/K$^+$ pump.$^3$ Brachial artery infusion of ouabain was therefore used in some experiments to produce unilateral regional block of Na$^+$/K$^+$ ATPase.$^{20–22}$ Experiments with vasodilators (verapamil and nitroprusside) and vasoconstrictors (norepinephrine) that act by mechanisms distinct from K$_{IR}$ and the Na$^+$/K$^+$ pump were used to control for possible nonspecific effects.

Methods

Human Studies

The St Thomas' Hospital Research Ethics Committee approved all the studies, and subjects gave written informed consent. Studies were performed in healthy normotensive (arterial pressure, 116±4/70±2 mm Hg; mean±SEM), normocholesterolemic (mean serum cholesterol, 3.6±0.2 mmol/L) men (mean age, 33±2 years) who were taking no medication. Studies were performed in the morning in a temperature-controlled room (24±1°C). The left brachial artery was cannulated with a 27-gauge stainless steel needle (Cooper Needleworks) after local anesthesia (≤1 mL 1% lidocaine). Isotonic saline (140 mmol/L sodium chloride) with or without dissolved drugs was infused at 1 mL/min. BaCl$_2$ was administered through a syringe filter (Acrodisc, 0.2 μm, Pall Corporation). Subjects rested supine for 30 minutes, and saline was infused for 12 minutes before measuring baseline flow. Blood flow (milliliter per minute per deciliter of forearm volume) was measured simultaneously in both arms by venous occlusion plethysmography$^{23}$ with the use of electrically calibrated strain gauges.$^{24}$ During measurements, the hands were excluded from the circulation by inflation of wrist cuffs to 180 mm Hg. Upper arm cuffs were intermittently inflated to 220 mm Hg, and the mean of the last 5 readings of each infusion period was used for analysis.

Plasma Ba$^{2+}$ Analysis

In preliminary dose-ranging studies, 19-gauge plastic cannulas were inserted into the medial antecubital veins draining each forearm. Venous blood (10 mL) was sampled at baseline, during the final 30 seconds of the infusion of BaCl$_2$, and 1 hour after infusion for determination of plasma Ba$^{2+}$ concentration. Samples were immediately centrifuged (1600 g for 5 minutes), and plasma was stored at −18°C in Ba$^{2+}$-free tubes. Samples were analyzed in duplicate in the Trace Element Unit, Southampton Hospital, with the use of inductively coupled plasma (ICP) mass spectrometry (Perkin Elmer-Sciex Elan 5000 ICP mass spectrometer), detection limit of 10 nmol/L, with a 0.5-mL plasma sample. Routine serum chemistries including creatinine, electrolytes, and liver function tests, in addition to Ba$^{2+}$ concentration, were measured at baseline and 1 week after BaCl$_2$ infusion.

In preliminary dose-finding experiments, cumulative infusions of intra-arterial BaCl$_2$ (0.25 to 2.0 μmol/min, each for 4 minutes) were administered. From these pilot studies, a dose of BaCl$_2$ of 4 μmol/min for up to 6 minutes was chosen for the following protocols.

Effects of BaCl$_2$ and Ouabain on Basal Forearm Blood Flow

After measuring basal forearm blood flow as above, BaCl$_2$ (4 μmol/min) was infused into the brachial artery for 6 minutes and blood flow was measured in both arms (n=8). In 6 of these subjects, plasma Ba$^{2+}$ determinations were made as above. In 10 separate experiments, ouabain (2.7 nmol/min) was infused for 15 minutes and blood flows were measured as described elsewhere.$^{21}$ Ouabain was continued for a further 3 minutes, coincubed with BaCl$_2$ (4 μmol/min).

Effects of Ba$^{2+}$ (With or Without Ouabain) on Forearm Responses to K$^+$ or Verapamil

After measuring basal forearm blood flow, cumulative doses (0.05, 0.1, 0.2 mmol/min, n=8) of KCl were infused into the brachial artery, each dose for 3 minutes. Venous blood samples (10 mL) were taken from both arms during the final 30 seconds for determination of plasma K$^+$ concentration.

In separate experiments (n=8), KCl (0.2 mmol/min) was infused for 3 minutes followed by saline for 15 minutes, during which blood flow returned to baseline. BaCl$_2$ (4 μmol/min) was then infused for 6 minutes-alone for 3 minutes and during a second 3-minute infusion of KCl (0.2 mmol/min). In control experiments, after blood flow was measured at baseline, verapamil (80 nmol/min) was infused with saline followed sequentially by saline alone, BaCl$_2$ alone, and verapamil with BaCl$_2$ (n=7).

In further separate experiments (n=6), blood flow was measured as before at baseline and during KCl (0.2 mmol/min), followed by a 15-minute saline recovery period. Ouabain and BaCl$_2$ were infused as described above and continued during a final 3-minute KCl infusion (0.2 mmol/min).

Lack of Effect of Norepinephrine on Forearm Blood Flow Response to KCl

Baseline blood flow was measured, followed by KCl infusion for 3 minutes (0.2 mmol/min, n=5). Saline was then infused alone for a

<table>
<thead>
<tr>
<th>TABLE 1. Plasma Barium Concentrations</th>
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<tbody>
<tr>
<td>Mean (SEM) Ba$^{2+}$ Concentration, μmol/L</td>
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<tr>
<td>Arm</td>
</tr>
<tr>
<td>-----</td>
</tr>
<tr>
<td>Mean (SEM)</td>
</tr>
</tbody>
</table>

Mean (±SEM) plasma barium concentrations (μmol/L; n=6) at baseline, immediately after brachial artery administration of BaCl$_2$ (4 μmol/min for 6 min, both arms), 1 hour after stopping the infusion, and 1 week later. (*P<0.005 concentration in infused arm vs baseline, Student’s paired t test).
Blood Flow Response to Nitroprusside

Infused agents at the doses used. BaCl2 caused no acute ECG changes and had no significant effect on serum electrolytes and liver function tests measured 1 week after the infusion.

Effects of Ba2+ and Ouabain on Basal Flow

In pilot experiments, cumulative, rising doses of BaCl2 (0.25, 0.5, 1.0, and 2.0 μmol/min, n=6), each infused through the brachial artery for 4 minutes, had no significant effect on baseline flow (percent change in baseline flow, −10±10%, −9±7%, −2±14%, and 1±15%, respectively, P=NS). Plasma Ba2+ concentration at baseline (ie, before BaCl2 infusion) was 0.28±0.04 μmol/L and during the final 30 seconds of infusion (at 2.0 μmol/min) was 22±3.4 μmol/L and 1.7±0.9 μmol/L, in the infused and noninfused arms respectively.

BaCl2 (4 μmol/min for 6 minutes) reduced baseline blood flow by 24±4% (n=8, P<0.001, Figure 1). Plasma Ba2+ concentration in venous blood draining the infused arm was 50±8 μmol/L during the final 30 seconds of infusion (Table 1).

Ouabain (2.7 nmol/min) reduced baseline blood flow by 10±2% (n=10, P<0.05) and coinfusion of BaCl2 with ouabain reduced baseline flow by 28±3% (n=10, P<0.0005 compared with the effect of ouabain alone, Figure 1).

Effect of K+ on Forearm Blood Flow

All subjects reported warmth and tingling in the infused arm during KCl infusion, and in preliminary experiments discomfort limited the maximum dose that was consistently tolerated to 0.2 mmol/min. KCl (0.05, 0.1, and 0.2 mmol/min) increased blood flow by 1.03±0.24, 1.99±0.4, and 4.21±0.46 mL/min per deciliter forearm over baseline (n=8, P<0.0001, ANOVA; Figure 2). The concentration of K+ in plasma from venous blood from the infused arm at the end of the infusion of KCl was 6.3±0.2 mmol/L, whereas the concentration in plasma from venous blood draining the noninfused arm was 4.0±0.2 mmol/L (n=6, P<0.0001).

Effects of Ba2+(With or Without Ouabain) on Forearm Response to K+ or Verapamil

KCl (0.2 mmol/min) alone increased forearm blood flow by 3.83±0.56 mL/min per deciliter forearm over baseline (n=8, Table 2) and by 1.43±0.28 mL/min per deciliter forearm when coinfused with BaCl2 (60±9% reduction; P=0.01, Figure 3A). Verapamil (80 nmol/min) increased forearm blood flow by 4.50±0.84 mL/min per deciliter forearm when infused with saline and by 4.46±0.86 mL/min per deciliter forearm when infused with BaCl2 (n=7, Table 3 P=NS). In separate experiments, KCl (0.2 mmol/min) increased forearm blood flow by 4.49±0.68 mL/min per deciliter forearm over
baseline when infused alone and by 0.57±0.23 mL/min per deciliter forearm when coinfused with BaCl₂ and ouabain (88±6% reduction, n=6, P<0.005; Figure 3B and Table 4).

Responses to KCl were measured before and during vasoconstriction with norepinephrine. Norepinephrine (240 pmol/min) reduced baseline blood flow by 24±2% (n=5, P<0.05). KCl (0.2 mmol/min) increased forearm blood flow by 3.01±0.45 mL/min per deciliter over baseline when infused alone and by 3.79±0.64 mL/min per deciliter of forearm when coinfused with norepinephrine (P=NS, Figure 3C).

Vasodilator responses to nitroprusside were measured in the presence and absence of Ba²⁺ and ouabain (Table 4). Nitroprusside (3.3 and 33 nmol/min) increased forearm blood flow, respectively, by 4.13±0.95 and 8.55±1.14 mL/min per deciliter of forearm when infused with saline alone and by 4.19±0.39 and 9.30±2.06 mL/min per deciliter of forearm when coinfused simultaneously with ouabain and BaCl₂ (P=NS; Figure 3D).

**Discussion**

**Ba²⁺ as a Probe of Kᵢᵣ Function in Human Forearm Resistance Vasculature**

Electrophysiological and molecular studies have established the presence of Kᵢᵣ2.1 channels in isolated vascular smooth muscle.⁷,⁸,²³ However, Kᵢᵣ channels are blocked by Ca²⁺ and Mg²⁺, each present in millimolar concentrations in extracellular fluid, and other signal transduction mechanisms could overwhelm any contribution to basal tone from Kᵢᵣ in vivo. Ba²⁺ can be used to probe the functional role of Kᵢᵣ channels.²

We investigated the possible role of Kᵢᵣ channels in human resistance vasculature in vivo by using brachial artery administration of BaCl₂ with bilateral measurement of forearm blood flow. Doses were selected to produce concentrations

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**TABLE 3. Effects of Ba²⁺±Verapamil on Forearm Blood Flow**

<table>
<thead>
<tr>
<th>Arm</th>
<th>Baseline 1 (Mean (SEM) Forearm Blood Flow (mL · min⁻¹ · dL forearm⁻¹))</th>
<th>Verapamil (80 nmol/min)</th>
<th>Baseline 2</th>
<th>Ba²⁺ (4 μmol/min)</th>
<th>Verapamil and Ba²⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infused</td>
<td>2.10 (0.22)</td>
<td>6.60 (1.03)†</td>
<td>2.59 (0.31)</td>
<td>1.85 (0.23)*</td>
<td>6.31 (0.98)†</td>
</tr>
<tr>
<td>Noninfused</td>
<td>2.55 (0.30)</td>
<td>2.28 (0.28)</td>
<td>2.50 (0.54)</td>
<td>2.38 (0.46)</td>
<td>2.47 (0.43)</td>
</tr>
</tbody>
</table>

Mean (±SEM) blood flow (mL · min⁻¹ · dL forearm⁻¹) is shown before and during brachial artery infusions of verapamil (80 mmol/min) and BaCl₂ (4 μmol/min) separately and in combination (n=7).

*P<0.05, †P<0.01 compared with preceding baseline.

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**Figure 3.** Specificity of inhibition of K⁺-induced vasodilation by Ba²⁺±ouabain. Each panel shows the increase above baseline in forearm blood flow (ΔFBF, mL · min⁻¹ · dL forearm⁻¹; mean±SEM) during brachial artery infusion of vasodilators (KCl or nitroprusside) alone (solid bars) and with (open bars) vasoconstrictor (Ba²⁺±ouabain or norepinephrine). Doses used were: KCl (0.2 mmol/min); BaCl₂ (4 μmol/min); ouabain (2.7 nmol/min); norepinephrine (240 pmol/min); nitroprusside (3.6 nmol/min). a, Responses to KCl±BaCl₂ (n=8; *P=0.01); b, Responses to KCl±BaCl₂ with ouabain (n=6; **P<0.005). c, Responses to KCl±norepinephrine (n=5). d, Effects of nitroprusside±BaCl₂ with ouabain (n=4).
that are pharmacologically active locally in the infused forearm but not systemically, and no significant effects on blood pressure or on blood flow in the contralateral (noninfused) forearm were observed. The dose of Ba\(^{2+}\) administered during each study was less than the daily reference dose, and no adverse effects were observed. The mean concentration of Ba\(^{2+}\) measured in plasma from the infused arm was 50±0.8 μmol/L, sufficient to block K\(_{IR}\) channels.2,4,7-9 Ouabain and other drugs were similarly administered through the brachial artery in doses that are not active systemically20-22 and produced no change in blood flow in the noninfused arm.

A major concern with this approach is the possibility that Ba\(^{2+}\) and ouabain, in the doses studied, could have effects in addition to those on K\(_{IR}\) and Na\(^+/K^+\) ATPase. Bilateral measurement of forearm blood flow coupled with brachial artery administration of subsystemically active doses circumvents confounding from drug actions on the central nervous system or heart, which cause bilateral changes in forearm blood flow.26 Thus, central effects of ouabain resulting in reduction of baseline blood flow in patients with essential hypertension.30,31 Consequently, unequivocal evidence of the presence and function of K\(_{IR}\)-2.1 channels in arterial smooth muscle in vitro4,7,8 does not, of itself, prove the functional importance of these channels under in vivo conditions. The modest vasosconstriction caused in the forearm by ouabain (10±2% reduction in basal blood flow, consistent with previous work20-22) and greater effect of Ba\(^{2+}\), demonstrated here for the first time, are consistent with tonic activity of K\(_{IR}\) on Na\(^+/K^+\) ATPase and, more effectively, on K\(_{IR}\)-channels in vascular smooth muscle in forearm resistance vessels in vivo. Tonic K\(^+-\)related vasodilation could have been underestimated by the inhibition observed (24±4% by Ba\(^{2+}\) alone, 28±3% by Ba\(^{2+}\) plus ouabain) because, due to concerns related to toxicity, we did not perform prolonged infusions. Vasoconstriction caused by Ba\(^{2+}\) may therefore not have reached a plateau. Even so, Ba\(^{2+}\)-induced vasoconstriction was substantial and compares with a maximum vasoconstrictor effect of l-N\(^5\)-monomethyl-l-arginine (which blocks basal endothelium-derived nitric oxide synthesis in this vascular bed) of ≈50% reduction of basal blood flow.29 Another limitation of the study is that it was not practicable to investigate the effect of Ba\(^{2+}\) on K\(^+-\)induced responses throughout a dose range of KCl infusions because responses to lower doses of KCl were small and variable and larger doses could not be used as these caused forearm discomfort.

Tonic vasodilator activity of K\(^+\) within the physiological range could influence arterial blood pressure. Dietary supplementation with potassium salts has been reported to lower blood pressure in patients with essential hypertension.30,31 Conversely, elevated blood pressure has been described in association with low dietary K\(^+\).32 The ability of a small increase in [K\(^+\)], to hyperpolarize vascular smooth muscle could contribute to the hypotensive effect of potassium salts. Local plasma K\(^+\) concentrations (mean, 6.3±0.2 mmol/L)
achieved in venous blood draining the infused arm are in the range observed in voluntary muscle during exercise, supporting a role for KIR and for Na+/K+ ATPase activation in regional blood flow responses during exercise. It is likely that mechanisms similar to those we have observed in forearm vasculature also operate in vivo in cerebral and coronary vessels (which are perfused by plasma of the same composition as are forearm vessels) and in which KIR channels have been detected in vitro. If so, K+-induced vasodilation could be important in pathological conditions such as myocardial or cerebral infarction, in which local elevations of [K+], could reduce resistance vessel tone in and around the infarct. Furthermore, hyperpolarization-induced vasorelaxation by activation of Na+/K+ ATPase is reported to be upregulated when nitric oxide bioavailability is impaired, so K+-induced vasodilation could provide a compensatory mechanism in diseases in which the l-arginine/nitric oxide mechanism is impaired, especially if the KIR mechanism also proves to be upregulated in such disorders.

In conclusion, Ba2+ constricts forearm resistance vessels, and K+-induced vasodilation is selectively inhibited by Ba2+ and almost abolished by Ba2+ plus ouabain. These findings support a role for KIR and Na+/K+ ATPase in controlling basal tone and in K+-induced vasorelaxation in human forearm resistance vessels in vivo. Such a role has important implications for the (patho-) physiological regulation of resistance vessel tone in humans.

Acknowledgments

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References

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