Block of Inward Rectifying K⁺ Channels (Kᵢᵣ) Inhibits Bradykinin-Induced Vasodilatation in Human Forearm Resistance Vasculature

R. Dwivedi, S. Saha, P.J. Chowienczyk, J.M. Ritter

Objective—To investigate the possible involvement of inward rectifying K⁺ channels (Kᵢᵣ) in the response of human resistance vessels to bradykinin in vivo.

Methods and Results—Drugs were administered via the brachial artery in healthy male volunteers and forearm blood flow was measured by venous occlusion plethysmography. Inhibition of Kᵢᵣ by barium chloride (4 μmol min⁻¹) alone or with additional inhibition of Na⁺/K⁺ ATPase (ouabain 2.7 μmol min⁻¹) reduced responses to bradykinin (30 pmol min⁻¹) by 26±8.3% and 36±7.2%, respectively (each P<0.05). Barium with ouabain plus inhibitors of prostaglandin (PG) and nitric oxide synthesis inhibited but did not abolish responses to bradykinin (51±2.8% inhibition; P<0.01); norepinephrine (240 pmol min⁻¹) caused similar reduction of baseline blood flow, as did this combination of inhibitors, but did not significantly inhibit the response to bradykinin. Barium plus ouabain did not significantly reduce responses to acetylcholine or albuterol.

Conclusion—A component of the vasodilator response to bradykinin in human forearm vasculature is mediated by Kᵢᵣ.

Key Words: bradykinin • barium • forearm vasculature • inward-rectifying potassium channels • hyperpolarizing factor

Potassium ion (K⁺) channel activity is an important determinant of the membrane potential of vascular smooth muscle and, thus, of vascular tone. Inward-rectifying K⁺ channels (Kᵢᵣ) in vascular smooth muscle differ from other vascular smooth muscle K⁺ channels (Ca²⁺-activated K⁺ channels, Kᵥ, voltage-dependent K⁺ channels, Kᵥ, and ATP-sensitive K⁺ channels, KᵥATP) in their unique current voltage properties and the consequent hyperpolarizing effect of increased external K⁺. Kᵢᵣ channels play a role in K⁺-mediated dilation of rat coronary and cerebral arteries and contribute to basal vasodilator tone in human forearm resistance vasculature. Kᵢᵣ is more sensitive to Ba²⁺ than other K⁺ channels, half-block being achieved at 2 μmol L⁻¹ at −60 mV in rat cerebral artery vascular smooth muscle. Brachial artery infusion of barium chloride in a dose that increases the local mean plasma concentration of Ba²⁺ to 50 μmol L⁻¹ inhibits the vasodilator response to infused potassium chloride by 60±9%. Electrogenic Na⁺/K⁺ exchange also contributes to hyperpolarizing responses to K⁺, and ouabain, an inhibitor of Na⁺/K⁺ ATPase, inhibits forearm vasodilator responses to KCl by ≈33% in healthy men. The combination of Ba²⁺ plus ouabain in the same doses inhibits responses to K⁺ almost completely. Coinfusion of barium chloride with ouabain thus provides a pharmacological tool to investigate whether increased extracellular K⁺ concentration contributes to vasodilator responses.

Activation of vascular smooth muscle Kᵢᵣ and Na⁺/K⁺ ATPase by K⁺ released from endothelial cells causes endothelium-dependent hyperpolarization in rat hepatic arteries in vitro, but the contribution, if any, of K⁺ to endothelium-dependent vasodilator agonists in vivo is less clearly established. Bradykinin is an endothelium-dependent vasodilator. In addition to releasing prostacyclin and nitric oxide (NO) from endothelial cells, it causes endothelium-dependent hyperpolarization of human coronary artery despite inhibition of prostaglandin (PG) and NO synthesis. When infused via the brachial artery, it is a potent vasodilator in human forearm vasculature by an action on B₂ receptors. Bradykinin-induced vasodilation in this vascular bed is not inhibited by aspirin and is incompletely blocked by L-N⁵-monomethyl-arginine (L-NMMA). Ouabain does not inhibit forearm responses to bradykinin in normotensive subjects but does significantly reduce such responses in patients with essential hypertension. In the present investigation, we determined effects of Ba²⁺ with or without ouabain on bradykinin-induced vasodilation in healthy normotensive men without...
known risk factors for atheromatous vascular disease. Experiments were performed with or without inhibitors of NO and PG synthesis. Norepinephrine was used to control for non-specific physiological antagonism caused by vasoconstriction caused by the inhibitors. Sensitivity of bradykinin to Ba\(^{2+}\) plus ouabain was compared with 2 other endothelium-dependent agonists (acetylcholine and albuterol) that activate NO synthesis by distinct mechanisms in this vascular bed.\(^{20-22}\)

### Methods

The St Thomas’ Hospital Research Ethics Committee approved the studies and all subjects gave written informed consent. Healthy men aged 33±11 years (mean±SD), nonsmokers using no medications, were invited to take part. Subjects were normotensive (blood pressure <130/80 mm Hg) and normcholesterolemic (total cholesterol<5.2 mmol L\(^{-1}\)). Forearm studies were performed as described previously.\(^5\) Blood flow was measured in both arms using venous occlusion plethysmography, and drugs dissolved in physiological saline were infused into the left brachial artery via a 27-gauge steel cannula. Each protocol was performed in a separate group of subjects to limit exposure to barium. Barium infusions (4 \(\mu\)mol min\(^{-1}\)) were given for 6 minutes. In each protocol, saline was first administered for 18 minutes to establish baseline flow. Vasodilators were infused for 3 minutes. Bradykinin (30 \(\mu\)mol min\(^{-1}\)) was administered, followed by saline for 18 minutes, to re-establish baseline flow. Inhibitors were then administered for 3 minutes alone and for a final 3 minutes with bradykinin. Inhibitors were: Ba\(^{2+}\) alone in the first protocol; Ba\(^{2+}\) with ouabain (2.7 \(\mu\)mol min\(^{-1}\)) in the second; Ba\(^{2+}\), indomethacin (0.34 \(\mu\)mol min\(^{-1}\)),\(^{33}\) ouabain, and l-NMMA (64 \(\mu\)mol min\(^{-1}\))\(^{34}\) in the third; and norepinephrine (240 \(\mu\)mol min\(^{-1}\))\(^{35}\) in the fourth. In control experiments to exclude desensitization, bradykinin was infused twice as before but with saline rather than inhibitor. In 2 separate studies, acetylcholine (33 nmol min\(^{-1}\)) or albuterol (1 nmol min\(^{-1}\)) were given instead of bradykinin, and Ba\(^{2+}\) with ouabain tested as a potential inhibitor. Results are expressed as means±SEM. Percent inhibition was calculated for each individual subject as: \([\text{FBF}_{\text{control}}-\text{FBF}_{\text{inhib}}]/\text{FBF}_{\text{control}}] \times 100\%\), where FBF\(_{\text{control}}\) and FBF\(_{\text{inhib}}\) were the blood flow in the absence and presence of inhibitor. Differences were analyzed using Student paired \(t\) test (2-sided). \(P<0.05\) was taken as significant.

### Results

There were no adverse events or electrocardiographic changes. Arterial blood pressure and blood flow in the noninfused arm did not change significantly. Mean blood flows in the infused arm are summarized in Table 1. Bradykinin increased blood flow similarly in each protocol, and each inhibitor reduced blood flow significantly. When co-infused with norepinephrine, the vasodilator effect of bradykinin was not significantly inhibited. Ba\(^{2+}\) alone inhibited the vasodilator response to bradykinin by 26±8.3% \((P<0.05)\), barium plus ouabain inhibited the response by 36±7.2% \((P<0.05)\), and barium plus ouabain, indomethacin, and \(l\)-NMMA inhibited the response by 51±2.8% \((P<0.01)\). When bradykinin was infused twice using the same protocol but in the absence of inhibitors, there was no evidence of desensitization: the response to the first versus second infusion was 8.6±1.7 versus 8.6±1.8 mL min\(^{-1}\) 100 mL forearm\(^{-1}\) \((n=6, P=NS)\). Ba\(^{2+}\) plus ouabain did not significantly reduce vasodilator responses to acetylcholine or to albuterol (Table 2), which also shows the effect of these inhibitors on bradykinin for comparison.

### Discussion

The main finding is that Ba\(^{2+}\) (plasma concentration \(=50 \mu\)mol L\(^{-1}\))\(^{3}\) selectively inhibits forearm blood flow responses to bradykinin. Norepinephrine does not significantly inhibit responses to bradykinin, and Ba\(^{2+}\) plus ouabain does not significantly inhibit acetylcholine or albuterol. This

### Table 1. Effects of Inhibitors of \(K_{\text{IR}}, Na^{+}/K^{+}\) ATPase, NO Synthase, and Cyclooxygenase on Responses to Bradykinin

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Saline</th>
<th>Bk</th>
<th>Saline+Inhibitor</th>
<th>Bk+Inhibitor</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ba (n=7)</td>
<td>2.2±0.4</td>
<td>6.3±1.1*</td>
<td>2.6±0.7</td>
<td>2.0±0.5†</td>
<td>4.9±1.2‡</td>
</tr>
<tr>
<td>Ba/Ouab (n=7)</td>
<td>2.9±0.4</td>
<td>8.6±1.2*</td>
<td>3.0±0.4</td>
<td>2.4±0.4†</td>
<td>5.4±0.9‡</td>
</tr>
<tr>
<td>Ba/Ouab/MMA/Indo (n=8)</td>
<td>2.8±0.4</td>
<td>8.1±1.4*</td>
<td>2.2±0.2</td>
<td>1.4±0.2†</td>
<td>4.2±0.8‡</td>
</tr>
<tr>
<td>NE (n=4)</td>
<td>2.3±0.3</td>
<td>7.8±1.4*</td>
<td>2.4±0.4</td>
<td>1.4±0.2†</td>
<td>6.8±1.3</td>
</tr>
</tbody>
</table>

Ba indicates barium chloride \((K_{\text{IR}}\) inhibitor\); Bk, bradykinin; Indo, indomethacin (cyclooxygenase inhibitor); MMA, \(N^{\text{O}}\)-monomethyl-L-arginine (NO synthase inhibitor); NE, norepinephrine; Ouab, ouabain \((Na^{+}/K^{+}\) ATPase inhibitor). \(*)P<0.001\) compared to baseline; \(†P<0.05\) compared to preceding baseline; \(‡P<0.05\) compared to Bk alone; \(§P<0.05\).

### Table 2. Effects of Inhibition of \(K_{\text{IR}}\) and \(Na^{+}/K^{+}\) ATPase on Responses to Bradykinin, Acetylcholine, and Albuterol

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Saline</th>
<th>Bk</th>
<th>Saline+Ba/Ouab</th>
<th>Agonist+Ba/Ouab</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bk (n=7)</td>
<td>2.9±0.4</td>
<td>8.6±1.2*</td>
<td>3.0±0.4</td>
<td>2.4±0.4†</td>
<td>5.4±0.9‡</td>
</tr>
<tr>
<td>Ach (n=6)</td>
<td>2.4±0.6</td>
<td>9.0±3.7*</td>
<td>2.8±1.0</td>
<td>2.5±0.8†</td>
<td>8.7±3.7</td>
</tr>
<tr>
<td>ALB (n=8)</td>
<td>3.3±0.5</td>
<td>6.9±1.3*</td>
<td>3.4±0.6</td>
<td>3.1±0.5†</td>
<td>5.2±0.9</td>
</tr>
</tbody>
</table>

Ach indicates acetylcholine; ALB, albuterol. \(*)P<0.001\) compared to baseline; \(†P<0.05\) compared to preceding baseline; \(‡P<0.05\) compared to Bk alone; \(§P<0.05\).
implies $K_{ir}$ in the vasodilator response to bradykinin in human forearm resistance vasculature. Ba$^{2+}$ with ouabain in the doses used almost completely abolishes vasodilator responses to K$^{-}$, but in the present experiments confusion of ouabain with Ba$^{2+}$ does not completely inhibit the response to bradykinin, and responses to bradykinin are not abolished even when Ba$^{2+}$ and ouabain are given with indomethacin and l-NNMMA in doses that block forearm PG synthesis and nitric oxide-mediated responses to acetylcholine. The simplest explanation is that bradykinin dilates this vascular bed partly but not entirely through activation of $K_{ir}$. The residual vasodilator response to bradykinin in the presence of inhibitors points to the possible involvement of a direct vasodilator action of bradykinin on vascular smooth muscle or of mediator pathways distinct from NO, prostaglandins, or K$^{+}$ ions.

Activation of $K_{ir}$ by bradykinin could be via increased K$^{-}$ concentration in the interstitial extracellular space in resistance arteries. The present experiments do not define the cellular origin of such increased interstitial K$^{-}$ concentration. This could be the endothelium, as in rat hepatic artery in vitro, consistent with an EDHF/K$^{+}$-mediated mechanism of bradykinin in the forearm in vivo. This agrees with the observation that a dose of tetraethylammonium expected to give a plasma concentration of $\approx 2 \times 10^{-4}$ mol L$^{-1}$ inhibits but does not abolish responses to bradykinin in this vascular bed. This concentration of tetraethylammonium could inhibit K$^{+}$ efflux from endothelium via an action on $K_{ir}$ channels, where it produces half the maximum block at $\approx 2 \times 10^{-4}$ mol L$^{-1}$.

**Limitations**

A constraint was our concern to limit the exposure of volunteers to Ba$^{2+}$. The total infused dose of 24 $\mu$mol is less than one-tenth the chronic oral reference dose calculated by the Environmental Protection Agency. Limiting the dose of Ba$^{2+}$ in this way meant that we were not able to explore its effects on a range of doses of bradykinin. A limitation of norepinephrine as a control is that it can inhibit $K_{ir}$ in vascular smooth muscle; other vasoconstrictors may influence $K_{ir}$ by an effect on membrane potential. Another limitation is that the present experiments do not identify the cellular distribution of the $K_{ir}$ channel involved in the vasodilator action of bradykinin in the forearm. This may be important because this channel is expressed not only in vascular smooth muscle but also in endothelial cells.

In conclusion, a dose of Ba$^{2+}$ that selectively inhibits $K_{ir}$ inhibits forearm vasodilator responses to bradykinin in healthy men, evidence that a component of this response is mediated by activation of $K_{ir}$. This is consistent with bradykinin acting through K$^{-}$ and/or another $K_{ir}$-dependent EDHF in human forearm resistance vessels in vivo.

**Acknowledgments**

This work was supported by the British Heart Foundation.

**References**


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Arterioscler Thromb Vasc Biol. published online December 9, 2004;
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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