Relative contribution of vasodilator prostanoids, NO, and K\(_{\text{ATP}}\) channels to human forearm metabolic vasodilation

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Farouque, H. M. Omar and Ian T. Meredith. Relative contribution of vasodilator prostanoids, NO, and K\(_{\text{ATP}}\) channels to human forearm metabolic vasodilation. *Am J Physiol Heart Circ Physiol* 284: H2405–H2411, 2003. First published February 21, 2003; 10.1152/ajpheart.00879.2002.—Isolated ATP-sensitive K\(^+\) (K\(_{\text{ATP}}\)) channel inhibition with glibenclamide does not alter exercise-induced forearm metabolic vasodilation. Whether forearm metabolic vasodilation would be influenced by K\(_{\text{ATP}}\) channel inhibition in the setting of impaired nitric oxide (NO)- and prostanoid-mediated vasodilation is unknown. Thirty-seven healthy subjects were recruited. Forearm blood flow (FBF) was assessed using venous occlusion plethysmography, and functional hyperemic blood flow (FHBF) was induced by isotonic wrist exercise. Infusion of N\(^6\)-monomethyl-L-arginine (L-NMMA), aspirin, or the combination reduced resting FBF compared with vehicle (\(P < 0.05\)). Addition of glibenclamide to L-NMMA, aspirin, or the combination did not further reduce resting FBF. L-NMMA decreased peak FHBF by 26%, and volume was restored after 5 min (\(P < 0.05\)). Aspirin reduced peak FHBF by 13%, and volume repaid after 5 min (\(P < 0.05\)). Coinfusion of L-NMMA and aspirin reduced peak FHBF by 21% (\(P < 0.01\)), and volume was restored after 5 min (\(P < 0.05\)). Addition of glibenclamide to L-NMMA and aspirin did not further decrease FHBF. Vascular K\(_{\text{ATP}}\) channel blockade with glibenclamide does not affect resting FBF or FHBF in the setting of NO and vasodilator prostanoic inhibition.

DESPITE MANY YEARS OF STUDY, the control mechanisms underlying skeletal muscle blood flow at rest and with exercise are not clearly understood. Early investigators proposed that local muscle metabolites and ions, including lactate, adenine nucleotides, and K\(^+\), were important but that other determinants, such as decreased tissue O\(_2\) tension and pH, increased CO\(_2\) tension, and osmolarity, may also modulate blood flow (34). Methodological advances, including development of pharmacological modulators and analytic techniques, have enabled us to gain further insights. Specifically, there is a growing body of evidence implicating endothelium-derived vasoactive factors and ion channels in the regulation of vascular tone (12, 13, 19, 20, 29, 37). It is apparent that multiple factors are involved, but their relative roles may vary between species, vascular beds, and the nature of the exercise stimulus.

The ATP-sensitive K\(^+\) (K\(_{\text{ATP}}\)) channel is a distinct type of K\(^+\) channel that is metabolically regulated and has a ubiquitous distribution (38). It has been found in the vascular smooth muscle cells of a variety of mammalian species, including humans (23). The activity of K\(_{\text{ATP}}\) channels is determined by many factors, including the intracellular metabolic milieu. In this way, K\(_{\text{ATP}}\) channels provide a potential link between cellular metabolism and vascular tone through their effects on membrane potential. Recently, we demonstrated that isolated vascular K\(_{\text{ATP}}\) channel blockade does not attenuate resting blood flow or exercise-induced hyperemia in the human forearm (17). These findings suggest that vascular K\(_{\text{ATP}}\) channels in the human forearm may not play a critical role in exercise-induced hyperemia under optimal conditions. However, it remains possible that “upregulation” of parallel vasodilator systems may have compensated for K\(_{\text{ATP}}\) channel inhibition, leading to maintenance of blood flow, as has been described in the canine coronary circulation (29). Accordingly, this study was undertaken to examine the interplay between K\(_{\text{ATP}}\) channels and endothelium-derived vasoactive factors in the control of forearm skeletal muscle blood flow by sequential inhibition of vasodilator prostanooids, nitric oxide (NO), and K\(_{\text{ATP}}\) channels.

**MATERIALS AND METHODS**

**Subjects**

A total of 37 healthy subjects (mean age 24 ± 5 yr, 19 male and 18 female) were recruited for the study. Baseline characteristics are displayed in Table 1. Subjects were asked to refrain from alcohol and caffeine for 24 h before the procedure, and none were taking vasoactive medication. The study was approved by the Southern Health Human Research Ethics Committee, and written informed consent was obtained from all subjects.

**General Methods**

Plethysmographic studies were performed in a climate-controlled vascular research laboratory after an overnight fast. Brachial arterial cannulation was performed under local
Table 1. **Clinical characteristics of study population**

<table>
<thead>
<tr>
<th>Protocol 1 (n = 17)</th>
<th>Protocol 2 (n = 10)</th>
<th>Protocol 3 (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, yr</strong></td>
<td>25 ± 6</td>
<td>23 ± 3</td>
</tr>
<tr>
<td><strong>Gender, M/F</strong></td>
<td>10/7</td>
<td>6/4</td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>22 ± 3</td>
<td>23 ± 3</td>
</tr>
<tr>
<td><strong>Total cholesterol, mmol/l</strong></td>
<td>3.9 ± 0.3</td>
<td>3.8 ± 0.8</td>
</tr>
<tr>
<td><strong>LDL</strong></td>
<td>2.3 ± 0.6</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td><strong>HDL</strong></td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td><strong>TG, mmol/l</strong></td>
<td>1.0 ± 0.8</td>
<td>1.3 ± 1.0</td>
</tr>
<tr>
<td><strong>Glucose, mmol/l</strong></td>
<td>5.1 ± 0.5</td>
<td>5.1 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, number of subjects. Protocol 1, coinfusion of aspirin and glibenclamide; protocol 2, coinfusion of N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA) and glibenclamide; protocol 3, coinfusion of aspirin, L-NMMA, and glibenclamide. M, male; F, female; BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides.

Physiological solutions (0.9% saline or 5% dextrose) were infused at 0.4 ml/min through a 20-gauge, 5-cm polyethylene catheter (Cook, Brisbane, Australia) by computerized syringe pump (Terumo, Tokyo, Japan) for anesthesia. Physiological solutions (0.9% saline or 5% dextrose) were infused at 0.4 ml/min through a 20-gauge, 5-cm polyethylene catheter (Cook, Brisbane, Australia) by computerized syringe pump (Terumo, Tokyo, Japan) for anesthesia.

Drug Infusion Protocols

Glibenclamide lyophilisate (kindly supplied by Aventis Pharma Deutschland) was dissolved in isotonic saline and infused into the brachial artery at 15 μg/min. This agent has been used in similar infusion regimens to inhibit vascular K\textsubscript{ATP} channels in the human forearm (4, 18). Aspirin (Aspisol, Bayer) was diluted in dextrose and infused at 3 mg/min to inhibit cyclooxygenase. It was expected, on the basis of previous work from our laboratory, that this dose would reduce net forearm prostacyclin production in the resting state by ~70% and resting forearm blood flow by ~20% (13). N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA; Clinalfa), an NO synthase inhibitor, was infused at 2 mg/min, as in previous studies (21, 37). All drugs were infused at 0.4 ml/min by computerized syringe pump.

Experimental Protocols

To examine the relative contributions of K\textsubscript{ATP} channels, the vasodilator prostanoids, and NO to resting forearm blood flow and metabolic vasodilation, three experimental protocols were devised (Fig. 1). All drugs were administered for at least 10 min before the first blood flow measurement was recorded. Drug infusions were continued during forearm exercise and the assessment of functional hyperemia. Fasting blood samples were taken for measurement of serum glucose, insulin, and C-peptide levels before and after glibenclamide infusion. In experimental protocols 1, 2, and 3, a sample size of 16, 9, and 7 subjects, respectively, would provide 80% power to detect a 30% change in 5-min hyperemic volume at the 5% significance level.

**Protocol 1** (n = 17). To assess the effect of K\textsubscript{ATP} channel blockade on forearm blood flow after inhibition of local prostanoid synthesis, resting blood flow and functional hyperemia were measured after vehicle infusion, aspirin infusion, and, finally, the combination of aspirin and glibenclamide.

**Protocol 2** (n = 10). The effect of K\textsubscript{ATP} channel blockade on forearm blood flow after inhibition of NO synthesis was examined by measuring basal blood flow and FHBF after vehicle, L-NMMA infusion, and the combination of L-NMMA and glibenclamide.
Protocol 3 (n = 10). The effect of KATP channel blockade on forearm blood flow after inhibition of prostacyclin and NO synthesis was examined by measuring basal blood flow and FHR after vehicle, coinfusion of L-NMMA and aspirin, and then infusion of aspirin, L-NMMA, and glibenclamide.

Statistical Analysis

Baseline characteristics are presented as means ± SD. Other data are expressed as means ± SE. Repeated-measures analysis of variance with robust standard errors was used to compare physiological data during vehicle and drug (single and combined) infusions. The paired Student’s t-test was used to compare biochemical data. Statistical significance was accepted if P < 0.05.

RESULTS

Effect of Aspirin and Glibenclamide on Forearm Blood Flow

Intra-arterial infusion of aspirin reduced resting forearm blood flow by 22% (from 2.3 ± 0.2 to 1.8 ± 0.2 ml·100 ml⁻¹·min⁻¹, P < 0.05; Table 2). There was an associated 36% increase in forearm vascular resistance (P < 0.05). Cointfusion of glibenclamide did not result in any additional change in resting forearm blood flow or vascular resistance (Table 2).

Aspirin infusion produced a 13% reduction in peak FHRB, which remained significant after the decrease in basal forearm blood flow was taken into account (P < 0.05; Table 2). There was a corresponding 16% increase in minimum forearm vascular resistance (P < 0.05). A trend to reduction in total and absolute hyperemic volume at 1 min was noted; it attained significance by 5 min.

Addition of glibenclamide to aspirin did not further alter exercise blood flow parameters (Table 2). Glibenclamide did not alter serum glucose levels (5.1 ± 0.1 and 5.1 ± 0.1 mmol/l), but insulin increased by 51% (from 6.1 ± 0.7 to 9.2 ± 1.0 μU/ml, P < 0.0001) and C-peptide by 16% (from 0.6 ± 0.1 to 0.7 ± 0.1 nmol/l, P = 0.001). Contralateral forearm blood flow and mean arterial blood pressure did not change significantly during drug infusions.

Effect of L-NMMA and Glibenclamide on Forearm Blood Flow

Infusion of L-NMMA decreased resting forearm blood flow by 37% (from 2.7 ± 0.4 to 1.7 ± 0.2 ml·100 ml⁻¹·min⁻¹, P < 0.05; Table 3) and increased basal forearm vascular resistance by 60% (from 40.8 ± 6.3 to 65.9 ± 9.4 mmHg·ml⁻¹·100 ml⁻¹·min⁻¹, P < 0.05). When glibenclamide was combined with L-NMMA, no further reduction in resting forearm blood flow was noted compared with L-NMMA alone (Table 3). The mean value after combined infusion was not significantly different from that after L-NMMA alone. L-NMMA reduced peak FHRB by 26% (from 17.0 ± 2.3 to 12.6 ± 1.0 ml·100 ml⁻¹·min⁻¹) and increased minimum forearm vascular resistance by 23% (from 6.4 ± 0.9 to 7.9 ± 0.7 mmHg·ml⁻¹·100 ml⁻¹·min⁻¹, P < 0.05 for both parameters; Table 3). L-NMMA signifi-

Table 2. Effect of aspirin and combination of aspirin and glibenclamide on exercise hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>ASA</th>
<th>ASA + Glib</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting FBF, ml·100 ml⁻¹·min⁻¹</td>
<td>2.3 ± 0.2</td>
<td>1.8 ± 0.2 *</td>
<td>1.7 ± 0.2 *</td>
<td>0.003</td>
</tr>
<tr>
<td>Resting FVR, units</td>
<td>45.5 ± 4.9</td>
<td>61.7 ± 7.2 *</td>
<td>66.6 ± 6.8 *</td>
<td>0.002</td>
</tr>
<tr>
<td>Peak FHFBR, ml·100 ml⁻¹·min⁻¹</td>
<td>14.2 ± 1.4</td>
<td>12.3 ± 1.2 *</td>
<td>11.9 ± 1.0 *</td>
<td>0.005</td>
</tr>
<tr>
<td>ΔPeak FHFBR, ml·100 ml⁻¹·min⁻¹</td>
<td>12.4 ± 1.4</td>
<td>10.0 ± 1.0 *</td>
<td>9.9 ± 1.0 *</td>
<td>0.0004</td>
</tr>
<tr>
<td>Minimum FVR, units</td>
<td>7.3 ± 0.6</td>
<td>8.5 ± 0.9 *</td>
<td>8.8 ± 0.8 *</td>
<td>0.001</td>
</tr>
<tr>
<td>Volume restored at 1 min, ml/100 ml</td>
<td>9.3 ± 0.9</td>
<td>8.7 ± 0.8</td>
<td>8.4 ± 0.8</td>
<td>0.13</td>
</tr>
<tr>
<td>ΔVolume restored at 1 min, ml/100 ml</td>
<td>7.6 ± 0.8</td>
<td>6.6 ± 0.7</td>
<td>6.5 ± 0.8</td>
<td>0.06</td>
</tr>
<tr>
<td>Volume restored at 5 min, ml/100 ml</td>
<td>29.9 ± 3.5</td>
<td>26.0 ± 2.6 *</td>
<td>24.2 ± 2.3 *</td>
<td>0.04</td>
</tr>
<tr>
<td>ΔVolume restored at 5 min, ml/100 ml</td>
<td>21.2 ± 3.6</td>
<td>15.2 ± 2.6 *</td>
<td>14.7 ± 2.3 *</td>
<td>0.01</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>89.8 ± 1.3</td>
<td>90.1 ± 1.4</td>
<td>91.0 ± 1.5</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Values are means ± SE. ASA, aspirin; Glib, glibenclamide; FBF, forearm blood flow; FVR, forearm vascular resistance; FHRB, forearm hyperemic blood flow; MAP, mean arterial pressure. P values were calculated using repeated-measures ANOVA. *P < 0.05 compared with vehicle by post hoc analysis. Addition of glibenclamide did not significantly alter blood flow parameters to a greater extent than aspirin alone.

Table 3. Effect of L-NMMA and combination of L-NMMA and glibenclamide on exercise hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>L-NMMA</th>
<th>L-NMMA + Glib</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting FBF, ml·100 ml⁻¹·min⁻¹</td>
<td>2.7 ± 0.4</td>
<td>1.7 ± 0.2 *</td>
<td>1.6 ± 0.2 *</td>
<td>0.002</td>
</tr>
<tr>
<td>Resting FVR, units</td>
<td>40.8 ± 6.3</td>
<td>65.9 ± 9.4 *</td>
<td>82.3 ± 22.3</td>
<td>0.0003</td>
</tr>
<tr>
<td>Peak FHFBR, ml·100 ml⁻¹·min⁻¹</td>
<td>17.0 ± 2.3</td>
<td>12.6 ± 1.0 *</td>
<td>12.4 ± 1.0 *</td>
<td>0.03</td>
</tr>
<tr>
<td>ΔPeak FHFBR, ml·100 ml⁻¹·min⁻¹</td>
<td>14.7 ± 2.4</td>
<td>10.9 ± 1.0</td>
<td>10.8 ± 1.3</td>
<td>0.09</td>
</tr>
<tr>
<td>Minimum FVR, units</td>
<td>6.4 ± 0.9</td>
<td>7.9 ± 0.7</td>
<td>8.7 ± 1.2</td>
<td>0.08</td>
</tr>
<tr>
<td>Volume restored at 1 min, ml/100 ml</td>
<td>9.1 ± 1.1</td>
<td>7.1 ± 0.8 *</td>
<td>7.0 ± 0.8 *</td>
<td>0.007</td>
</tr>
<tr>
<td>ΔVolume restored at 1 min, ml/100 ml</td>
<td>6.9 ± 1.2</td>
<td>5.5 ± 0.8</td>
<td>5.5 ± 0.8</td>
<td>0.08</td>
</tr>
<tr>
<td>Volume restored at 5 min, ml/100 ml</td>
<td>27.8 ± 3.2</td>
<td>19.5 ± 2.1 *</td>
<td>18.1 ± 1.5 *</td>
<td>0.003</td>
</tr>
<tr>
<td>ΔVolume restored at 5 min, ml/100 ml</td>
<td>16.5 ± 3.5</td>
<td>11.2 ± 2.0 *</td>
<td>10.2 ± 1.4 *</td>
<td>0.05</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>92.7 ± 1.7</td>
<td>93.5 ± 2.4</td>
<td>94.9 ± 1.8</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Values are means ± SE. P values were calculated using repeated-measures ANOVA. *P < 0.05 compared with vehicle by post hoc analysis. Addition of glibenclamide did not alter blood flow parameters to a greater extent than L-NMMA alone.
cantly reduced total hyperemic volume at 1 and 5 min by 22 and 30%, respectively.

Coinfusion of glibenclamide and L-NMMA did not further attenuate functional hyperemia (Table 3). Glibenclamide did not significantly alter serum glucose (5.0 ± 0.1 and 4.8 ± 0.1 mmol/l) but did increase insulin by 49% (from 5.0 ± 0.7 to 9.1 ± 1.2 μU/ml, *P = 0.002) and C-peptide by 23% (from 0.6 ± 0.1 to 0.8 ± 0.1 nmol/l, *P = 0.01). Systemic hemodynamic parameters did not change significantly during the study.

**Effect of Triple Drug Infusion on Forearm Blood Flow**

Compared with vehicle infusion, the combination of L-NMMA and aspirin reduced resting forearm blood flow by 28% (from 1.8 ± 0.1 to 1.3 ± 0.1 ml·100 ml⁻¹·min⁻¹) and increased basal forearm vascular resistance by 28% (from 52.1 ± 3.5 to 72.2 ± 5.6 mmHg·ml⁻¹·100 ml⁻¹·min⁻¹, *P < 0.05 for both parameters; Table 4). Addition of glibenclamide to the combination of aspirin and L-NMMA did not result in any additional change in these resting parameters (Table 4).

Infusion of L-NMMA with aspirin reduced peak FHBF by 21% (from 14.1 ± 1.2 to 11.2 ± 0.6 ml·100 ml⁻¹·min⁻¹, *P < 0.05; Table 4) and increased minimum forearm vascular resistance by 19% (from 6.5 ± 0.7 to 8.0 ± 0.6 mmHg·ml⁻¹·100 ml⁻¹·min⁻¹, *P < 0.05). There was a trend to reduction in total hyperemic volume at 1 min; it was significant by 5 min.

Addition of glibenclamide did not decrease functional hyperemia to a greater extent than the combination of L-NMMA and aspirin (Table 4). There was no change in serum glucose (4.9 ± 0.2 and 4.9 ± 0.1 mmol/l), but insulin increased by 28% (from 7.6 to 9.8 μU/ml, *P = 0.047) and C-peptide by 13% (from 0.6 ± 0.1 to 0.7 ± 0.1 nmol/l, *P = 0.12). Mean arterial blood pressure and contralateral forearm blood flow did not change significantly during the study.

**DISCUSSION**

In this study, we demonstrate that brachial arterial infusion of aspirin and L-NMMA, alone or in combination, produces a decrease in resting forearm blood flow and exercise-induced functional hyperemia. However, addition of glibenclamide to aspirin or L-NMMA, or a combination thereof, did not result in a greater reduction of resting forearm blood flow or functional hyperemia. These findings imply that vascular K<sub>ATP</sub> channels do not contribute to exercise-induced functional hyperemia in the forearm circulation after inhibition of NO and prostanoid synthesis. Furthermore, our observations confirm that NO and the vasodilator prostanoids are important participants in regulation of forearm blood flow.

**Basic Mechanisms of Vasodilation**

In a previous report from our laboratory, isolated K<sub>ATP</sub> channel inhibition did not decrease forearm blood flow (17). We hypothesized that parallel vasodilator systems may have compensated for K<sub>ATP</sub> channel inhibition, resulting in maintenance of forearm blood flow at rest and during metabolic vasodilation. The other vasodilator pathways examined in this study were those leading to the synthesis of NO and the vasodilator prostanoids. The primary physiological action of NO is through activation of the cytosolic soluble guanylate cyclase, leading to an increase in cGMP and smooth muscle cell relaxation. In contrast, the vasodilator prostanoids (primarily prostacyclin) produce smooth muscle cell relaxation by receptor-mediated activation of adenylate cyclase, leading to an elevation of intracellular cAMP. Thus both of these important vasoactive substances lead to vasorelaxation through different signaling pathways. K<sup>+</sup> channel activation is thought to represent the third fundamental mechanism of vasorelaxation by causing smooth muscle cell membrane hyperpolarization (25). A number of vasodilator substances may lead to hyperpolarization by opening K<sub>ATP</sub> channels, including NO and prostacyclin in some instances (10, 30, 43). For these reasons, we chose to inhibit the activity of these three distinct vasodilator pathways to examine their contribution to vascular tone.

### Table 4. Effect of aspirin and L-NMMA and combination of L-NMMA, aspirin, and glibenclamide on exercise hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>ASA + L-NMMA</th>
<th>ASA + L-NMMA + Glib</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting FBF, ml·100 ml⁻¹·min⁻¹</td>
<td>1.8 ± 0.1</td>
<td>1.3 ± 0.1*</td>
<td>1.2 ± 0.1*</td>
<td>0.007</td>
</tr>
<tr>
<td>Resting FVR, units</td>
<td>52.1 ± 3.5</td>
<td>72.2 ± 5.6</td>
<td>78.6 ± 7.2</td>
<td>0.008</td>
</tr>
<tr>
<td>Peak FHFB, ml·100 ml⁻¹·min⁻¹</td>
<td>14.1 ± 1.2</td>
<td>11.2 ± 0.6*</td>
<td>11.1 ± 0.9*</td>
<td>0.02</td>
</tr>
<tr>
<td>ΔPeak FHFB, ml·100 ml⁻¹·min⁻¹</td>
<td>12.3 ± 1.1</td>
<td>10.0 ± 0.6*</td>
<td>9.9 ± 0.9*</td>
<td>0.04</td>
</tr>
<tr>
<td>Minimum FVR, units</td>
<td>6.5 ± 0.7</td>
<td>8.0 ± 0.6</td>
<td>8.6 ± 1.0</td>
<td>0.009</td>
</tr>
<tr>
<td>Volume restored at 5 min, ml/100 ml</td>
<td>8.3 ± 0.7</td>
<td>7.9 ± 0.4</td>
<td>7.0 ± 0.6</td>
<td>0.06</td>
</tr>
<tr>
<td>ΔVolume restored at 5 min, ml/100 ml</td>
<td>6.6 ± 0.7</td>
<td>5.3 ± 0.4</td>
<td>5.8 ± 0.5</td>
<td>0.29</td>
</tr>
<tr>
<td>Volume restored at 5 min, ml/100 ml</td>
<td>22.8 ± 1.7</td>
<td>17.8 ± 1.5*</td>
<td>17.0 ± 1.2*</td>
<td>0.01</td>
</tr>
<tr>
<td>ΔVolume restored at 5 min, ml/100 ml</td>
<td>14.0 ± 1.6</td>
<td>11.4 ± 1.2</td>
<td>10.9 ± 1.0</td>
<td>0.13</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>84.7 ± 2.4</td>
<td>86.9 ± 2.4</td>
<td>88.1 ± 2.9</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P values were calculated using repeated-measures ANOVA. *P < 0.05 compared with vehicle by post hoc analysis. Addition of glibenclamide did not alter blood flow parameters to any greater extent than L-NMMA and aspirin in combination.

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Concept of “Redundant” Vasodilator Pathways

It is apparent that several independent vasodilator pathways contribute to the maintenance of vascular tone and blood flow to cardiac and skeletal muscle. The existence of parallel systems is likely to be advantageous in the regulation of organ blood flow. Specifically, activation of a parallel or backup system to ensure continuity of vasomotor control may compensate for the malfunction of one system due to disease (9). Similar considerations may apply in the experimental setting when the contribution of these pathways to blood flow regulation is assessed. Inhibition of a single regulatory mechanism may lead to erroneous conclusions, inasmuch as increased activation or upregulation of alternative vasodilator systems may obscure the role of the inhibited pathway (34).

There are many examples of such interactions. In a canine model, the isolated inhibition of NO synthesis did not reduce basal coronary blood flow (29). However, in the setting of KATP channel inhibition and adenosine receptor blockade, inhibition of NO synthesis reduced resting coronary blood flow. These results suggest that NO may contribute to basal coronary blood flow regulation in dogs when the activities of other vasodilator systems are impaired. In further experiments by this group of investigators, the blockade of KATP channels alone did not limit exercise-induced coronary vasodilation in dogs. However, the subsequent addition of adenosine receptor blockade, alone or in combination with an NO synthase inhibitor, resulted in attenuation of exercise-induced metabolic vasodilation (14, 29). Along similar lines, a greater dependency on prostanoid-induced vasodilation has been noted in NO synthase knockout mice (8, 22). Other studies have shown that NO may inhibit the production of an endothelium-derived hyperpolarizing factor (2, 39). Thus, in the presence of impaired NO synthesis, vasodilator function may be maintained by endothelium-derived hyperpolarizing factor.

Relative Contribution of KATP Channels to Forearm Blood Flow

Our data suggest that vascular KATP channels do not contribute to resting blood flow or functional hyperemia in the forearm under conditions of impairment in NO- and prostanoid-dependent vasodilation. There are several potential explanations for these findings. Inadequate inhibition of alternative vasodilator pathways could explain the lack of impact of glibenclamide infusion on forearm blood flow. However, the dose of L-NMMA used in this study (2 mg/min) is known to attenuate acetylcholine-induced hyperemia (12), a commonly used measure of NO-related vasodilation. Furthermore, the observed fall in resting forearm blood flow during L-NMMA infusion attests to the reduction in bioavailability of NO. Previous work from our laboratory indicates that aspirin as administered in this study (3 mg/min) reduces production of 6-ketoprostaglandin F1α (a stable metabolite of prostacyclin) in association with a fall in forearm blood flow (13). These data, in addition to the reduction noted in basal forearm blood flow with aspirin infusion, indicate adequate inhibition of vasodilator prostanoid synthesis.

The effects of adenosine and endothelium-derived hyperpolarizing factor were not inhibited in this study. Intra-arterial aminophylline and potassium chloride infusions have been used previously to examine the role of these mediators in the forearm (24, 35). In theory, the added inhibition of one or the other of these vasoactive substances may have altered our results. However, aminophylline and potassium chloride are not ideal pharmacological tools, inasmuch as they have nonspecific effects that may complicate interpretation of the results (24, 35). Glibenclamide caused small increases in insulin levels in all study protocols. The vasodilator action of insulin may have offset any vaso-constriction induced by KATP channel inhibition. This is, however, unlikely, inasmuch as its vasodilator effects are mediated by NO (41), which was also inhibited by infusion of L-NMMA in protocols 2 and 3. The levels of insulin in the present study were in the low physiological range and unlikely to have produced vasoactive effects. Moreover, in previous forearm studies, we found no correlation between baseline insulin levels and resting or exercise-induced forearm blood flow responses (unpublished observations).

Resting Blood Flow

Our observations indicate that vasodilator prostanoids and NO contribute to resting skeletal muscle blood flow and exercise-induced functional hyperemia in healthy humans. The early investigations assessing the role of prostanoids in forearm blood flow regulation used orally administered cyclooxygenase inhibitors (5, 6, 32). These studies did not support a role for prostanoids in the control of basal forearm blood flow. Recent studies have employed brachial arterial infusion of cyclooxygenase inhibitors and have demonstrated significant reductions in basal blood flow of 12–30%, most likely related to more effective blockade of local prostanoid synthesis than in earlier studies (13, 45). These findings are also consistent with data from animal studies examining resting skeletal muscle blood flow (3, 46).

The involvement of NO is also in keeping with the results of experimental studies in resting skeletal muscle (16) and previous investigations conducted in the human forearm (21). These studies demonstrate consistent reductions in blood flow of 25–50% after brachial arterial L-NMMA infusion. However, adenosine does not appear to play an important role in regulating resting skeletal muscle blood flow in animals (33) or in the forearm of healthy humans (5).

Functional Hyperemia

The findings of this study indicate that endothelium-mediated vascular control mechanisms are important during functional hyperemia in human skeletal muscle vasculature. The endothelium is able to release a number of paracrine vasodilator factors, such as NO and...
prostacyclin. The release of these mediators may be stimulated by forces exerted by flowing blood acting on the endothelium (shear stress) or in response to neurohumoral influences such as acetylcholine, adenosine nucleotides, and bradykinin (7).

In this study, infusion of aspirin reduced the initial and sustained phases of functional hyperemia. Peak forearm blood flow was reduced by 13% and blood volume repaid after 5 min by 13%. These changes remained significant when the fall in basal forearm blood flow was taken into account. The magnitude of reduction was slightly less than with L-NMMA infusion, although this may in part be a dose-related phenomenon. These changes are in line with results of previous human studies (12, 32, 42, 45). Prostaglandin-like substances have been found in the venous effluent of exercising muscle in dogs and appear to contribute to hyperemia during and after exercise (26). However, contrary findings have been reported from other canine studies (3, 46). Several (12, 15, 21, 31, 42), but not all (44), previous reports indicate that NO is an important mediator of exercise-induced hyperemia in the forearm of healthy humans. There is also supportive experimental evidence that NO-mediated vasodilation in skeletal muscle contributes to exercise hyperemia (27, 28, 36). Our observations are consistent with these reports. There was a significant 25% reduction in peak FHBF and a 30% decrease in blood volume restored at 5 min after NO synthase inhibition. The magnitude of reduction is consistent with that reported in previous studies using the same methodology (11, 12, 42). When the decrease in resting blood flow was taken into account, the changes in functional hyperemia were no longer significant but did show a trend to reduction at 5 min. This was most likely due to the relatively small number of subjects recruited in this protocol. The persistent hyperemia after aspirin and L-NMMA infusion indicates that factors in addition to NO and vasodilator prostanoids are involved in the maintenance of FHBF under normal conditions.

K\textsubscript{ATP} Channels and Vasoregulation

The present findings suggest that K\textsubscript{ATP} channels may not be active in the vasculature of the human forearm at rest or during functional hyperemia. However, this may not be the case in other circulatory beds. We recently demonstrated that K\textsubscript{ATP} channels may be involved in the regulation of coronary blood flow in humans at rest and during metabolic vasodilation (19, 20). These observations indicate that there are differences in the importance of vascular regulatory mechanisms between peripheral and coronary circulations. Although similarities may exist in cellular mechanisms of vascular regulation within skeletal and cardiac muscle, each tissue and associated vascular bed clearly has unique characteristics. Recent data suggest that K\textsubscript{ATP} channels exhibit structural heterogeneity in terms of their molecular composition (40). The implications of these observations for channel function are unknown, but it is conceivable that unique subtypes of K\textsubscript{ATP} channels with different functional characteristics are expressed in the coronary compared with the skeletal muscle circulation. There is also evidence to suggest that the density of sulfonylurea receptors, a component of the K\textsubscript{ATP} channel, is greater in some tissues than in others (1).

Conclusion

The data suggest that vascular K\textsubscript{ATP} channels in forearm skeletal muscle vasculature of healthy subjects do not modulate basal blood flow or exercise-induced hyperemia in the setting of impaired prostanoid- and NO-mediated vasodilation. These findings imply that K\textsubscript{ATP} channels do not appear to play an important role in regulation of forearm blood flow during functional hyperemia. The results of this study also indicate that NO and vasodilator prostanoids are significant contributors to the regulation of forearm blood flow; however, additional mechanisms are clearly involved.

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