Effects of inhibition of ATP-sensitive potassium channels on metabolic vasodilation in the human forearm

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ABSTRACT

Experimental data suggest that vascular ATP-sensitive potassium (K<sub>ATP</sub>) channels may be an important determinant of functional hyperaemia, but the contribution of K<sub>ATP</sub> channels to exercise-induced hyperaemia in humans is unknown. Forearm blood flow was assessed in 39 healthy subjects (23 males/16 females; age 22±4 years) using the technique of venous occlusion plethysmography. Resting forearm blood flow and functional hyperaemic blood flow (FHBF) were measured before and after brachial artery infusion of the K<sub>ATP</sub> channel inhibitors glibenclamide (at two different doses: 15 and 100 μg/min) and gliclazide (at 300 μg/min). FHBF was induced by 2 min of non-ischaemic wrist flexion–extension exercise at 45 cycles/min. Compared with vehicle (isotonic saline), glibenclamide at either 15 μg/min or 100 μg/min did not significantly alter resting forearm blood flow or peak FHBF. The blood volume repaid at 1 and 5 min after exercise was not diminished by glibenclamide. Serum glucose was unchanged after glibenclamide, but plasma insulin rose by 36% (from 7.2±0.8 to 9.8±1.3 m-units/l; P=0.02) and 150% (from 9.1±1.3 to 22.9±3.5 m-units/l; P=0.002) after the 15 and 100 μg/min infusions respectively. Gliclazide also did not affect resting forearm blood flow, peak FHBF, or the blood volume repaid at 1 and 5 min after exercise, compared with vehicle (isotonic glucose). Gliclazide induced a 12% fall in serum glucose (P=0.009) and a 38% increase in plasma insulin (P=0.001). Thus inhibition of vascular K<sub>ATP</sub> channels with glibenclamide or gliclazide does not appear to affect resting forearm blood flow or FHBF in healthy humans. These findings suggest that vascular K<sub>ATP</sub> channels may not play an important role in regulating basal vascular tone or skeletal muscle metabolic vasodilation in the forearm of healthy human subjects.

INTRODUCTION

The control of skeletal muscle blood flow, at rest or during functional (exercise-induced) hyperaemia, is dependent on perfusion pressure and the calibre of resistance vessels. In skeletal muscle, as in the myocardium, blood flow is closely linked to metabolic rate. During exercise, the changing metabolic demands are closely matched by concomitant changes in blood flow, allowing adequate delivery of nutrients at a time of increased need. Blood flow to skeletal muscle may increase as much as 10–15 times above basal levels in the immediate post-exercise period [1], a phenomenon known as metabolic vasodilation. Local factors within exercising muscle play a critical role in this response. The increased metabolic activity in exercising muscle results in the formation and release of metabolites, which diffuse through interstitial fluid to the arterioles and cause vasodilation. These

Key words: ion channels, microcirculation, regional blood flow, vasoconstriction/dilation.
Abbreviation: FHBF, functional hyperaemic blood flow.
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include potassium ions, hydrogen ions, adenosine and inorganic phosphate; raised tissue osmolarity and hypoxia also have this effect [1,2]. Endothelium-derived vasoactive substances, such as nitric oxide and prostacyclin, have also been implicated in the genesis of exercise-induced hyperaemia [3,4]. Changes in the membrane potential of vascular smooth muscle cells appear to be a major determinant of resistance vessel calibre. Indeed, some local chemical and mechanical signals may alter membrane potential through their actions on membrane-bound ion channels [5]. Potassium ion channels, such as the metabolically regulated K<sub>ATP</sub> channel, are believed to be important in this regard. ATP-sensitive potassium channels are found in vascular smooth muscle and the endothelium. They are inhibited by intracellular ATP, and by sulphonyleurea derivatives such as glibenclamide and gliclazide, which are widely used in the treatment of Type II diabetes mellitus. These channels are activated by ADP and by potassium channel openers such as nicorandil and diazoxide.

It has been established that brachial arterial infusion of diazoxide produces forearm vasodilation, which can be inhibited by glibenclamide [6]. These observations suggest that the forearm skeletal muscle circulation contains a pool of recruitable K<sub>ATP</sub> channels. Previous studies in humans suggest that K<sub>ATP</sub> channels in the skeletal muscle vasculature contribute to the phenomenon of reactive hyperaemia induced by transient limb ischaemia [7–10]. ATP-sensitive potassium channels also appear to be active in the intact human coronary circulation [11,12]. However, the contribution of these channels to non-ischaemic exercise-induced functional hyperaemia in humans has not been investigated. The purpose of the present study was to determine if vascular K<sub>ATP</sub> channels contribute to metabolic vasodilation in the human forearm, by examining the effects of acute inhibition of K<sub>ATP</sub> channels on exercise-induced forearm vasodilation in healthy subjects.

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**METHODS**

**Subjects**

A total of 39 healthy subjects (mean age 22 ± 4 years; 23 male, 16 female) were enrolled in this study after written informed consent was obtained (Table 1). Exclusion criteria included the presence of significant cardiac or non-cardiac disease, the use of vasoactive medication, and the presence of conventional cardiovascular risk factors. The study protocol was approved by the Southern Health Human Research Ethics Committee, and the investigation conformed to the principles outlined in the Declaration of Helsinki.

**General methods**

Subjects were studied in the morning after an overnight fast, and had refrained from alcohol and caffeine for 24 h prior to the procedure. Brachial arterial cannulation was performed under sterile conditions after local anaesthesia was induced, as described previously [3,4]. Physiological solutions (either 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 a computerized syringe pump (Terumo Corp., Tokyo, Japan) for a minimum of 30 min before the first blood flow recording was taken.

**Haemodynamic measurements**

Forearm blood flow was measured using the technique of venous occlusion plethysmography with calibrated mercury-in-silastic strain gauges, as described previously [3,4]. Resting blood flow was measured for a minimum of 2 min, and an average of at least five stable recordings was used for analysis. Forearm metabolic vasodilation (functional hyperaemia) was induced by 2 min of rhythmic non-ischaemic wrist flexion–extension exercise at 45 cycles/min, in time with a metronome. We have shown previously that this form of exercise induces reproducible hyperaemic responses [3]. Upon cessation of exercise, forearm blood flow was measured for 5 min, allowing the construction of a flow versus time curve (Figure 1). Peak functional hyperaemic blood flow (ΔFHBF) was determined, and the total volume repaid to the forearm at 1 and 5 min was estimated from the area under the flow versus time curve at these time points using the trapezium rule [13]. The absolute increase in peak FHBF (Δ peak FHBF) and volume repaid were calculated by sub-

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**Table 1** Baseline characteristics of the study population (n = 39)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gibenclamide (15 µg/ml)</th>
<th>Gibenclamide (100 µg/ml)</th>
<th>Gliclazide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.8 ± 0.4</td>
<td>4.9 ± 0.5</td>
<td>5.1 ± 0.6</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>4.0 ± 0.6</td>
<td>3.8 ± 0.9</td>
<td>4.0 ± 0.7</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>2.4 ± 0.6</td>
<td>2.2 ± 0.7</td>
<td>2.4 ± 0.6</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.2 ± 0.2</td>
<td>1.0 ± 0.4</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>1.0 ± 0.5</td>
<td>0.8 ± 0.4</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22 ± 2</td>
<td>22 ± 3</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>Age (years)</td>
<td>22 ± 4</td>
<td>23 ± 7</td>
<td>22 ± 4</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>13/7</td>
<td>4/3</td>
<td>6/6</td>
</tr>
</tbody>
</table>
extracting resting forearm blood flow immediately before exercise from peak FHBF and total volume repaid at 1 and 5 min. The absolute (Δ) values were calculated to account for variations in baseline forearm blood flow. Forearm blood flow was expressed as ml·min⁻¹·100 ml⁻¹ forearm tissue. Forearm vascular resistance (mmHg·min⁻¹·ml⁻¹·100 ml⁻¹ forearm tissue) was calculated by dividing mean arterial pressure by forearm blood flow.

**Study drugs**

Glibenclamide lyophilisate (HB 419; Aventis Pharma Deutschland G.m.b.H.) was used as an inhibitor of vascular K<sub>ATP</sub> channels. Its specificity for vascular K<sub>ATP</sub> channels has been demonstrated in humans and animals [6,14]. The formulation used in the present study was suitable for parenteral human use, and did not require the addition of an alkaline vehicle to ensure solubility [12]. Glibenclamide was dissolved in isotonic saline and infused at 15 μg/min in Protocol 1 (see below). Assuming that resting forearm blood flow is 3 ml·min⁻¹·100 ml⁻¹, this infusion rate was expected to result in an estimated local plasma concentration of 500 ng/ml. This level is at the upper end of the concentration range observed in venous blood at a mean of 3 h after administration of a single 20 mg oral dose of glibenclamide to patients with Type II diabetes mellitus [15]. To determine if a dose–response relationship existed, glibenclamide was also infused at 100 μg/min in a separate protocol (see below).

Gliclazide is known to be a potent inhibitor of pancreatic β-cell K<sub>ATP</sub> channels. However, its effect on vascular tone has not been studied previously. Gliclazide (Servier Laboratories) was diluted in 5 % (w/v) dextrose and infused at 300 μg/min. Assuming that resting forearm blood flow is 3 ml·min⁻¹·100 ml⁻¹, this dosing regime would result in a regional plasma gliclazide concentration of 10 mg/l. A single oral dose of 120 mg results in peak venous plasma gliclazide concentrations of approx. 8 mg/l in patients with Type II diabetes mellitus [16]. All drugs were infused at 0.4 ml/min by a computerized syringe pump.

**Experimental protocols**

To examine the contribution of K<sub>ATP</sub> channels to metabolic vasodilation in skeletal muscle, basal and exercise-induced forearm blood flow was measured before and after the brachial artery infusion of sulphonylurea K<sub>ATP</sub> channel inhibitors. Baseline flow was re-established after each exercise period. In all cases, sulphonylurea infusions were administered for 10 min prior to the first blood flow measurement, and continued throughout the period of exercise and during assessment of metabolic vasodilation. Three protocols were utilized, as detailed below.

**Protocol 1 (n = 20)**

Glibenclamide was infused at 15 μg/min. In 10 subjects, venous blood was taken from the infused arm at the end of the study period to determine regional plasma glibenclamide concentrations.

**Protocol 2 (n = 7)**

Glibenclamide was infused at 100 μg/min to assess the effect of an increased dose (and therefore a greater degree of K<sub>ATP</sub> channel inhibition) on forearm blood flow responses.

**Protocol 3 (n = 12)**

Gliclazide was infused at 300 μg/min to examine the effect of an alternative K<sub>ATP</sub> channel blocker on forearm blood flow.

**Biochemical assays**

Fasting blood was taken for measurement of serum glucose, plasma insulin and C-peptide levels before and after sulphonylurea infusion. Blood was also taken from the antecubital vein of the infused arm for plasma glibenclamide determination. These samples were centrifuged immediately at 3000 rev./min for 10 min and the plasma was stored at −80 °C. Analysis for glibenclamide levels was subsequently undertaken by HPLC. The assay method has been validated previously, and involves liquid–liquid extraction of human plasma samples with diethyl ether and chromatographic separation on an ODS column with UV detection [17]. The lower limit of quantification is 5 ng/ml, and the inter-assay coefficient of variation is between 2.1 % and 6.9 %.

**Statistical analysis**

Baseline characteristics are presented as means ± S.D. Other data are expressed as means ± S.E.M. The paired Student’s t-test was used to compare physiological and biochemical data before and after sulphonylurea infusion. The Wilcoxon signed-rank test was used to compare paired data that did not conform to a normal distribution. Statistical significance was accepted if P < 0.05.
RESULTS

Effect of low-dose glibenclamide on forearm blood flow
Glibenclamide infused at 15 µg/min did not alter resting forearm blood flow or forearm vascular resistance in healthy young subjects (Table 2). Compared with vehicle infusion, glibenclamide also did not affect the peak or sustained phase of the hyperaemic response induced by 2 min of isotonic forearm exercise (Table 2). However, there was a small increase in mean arterial pressure at the termination of the glibenclamide infusion (from 81.2 ± 1.6 to 83.1 ± 1.6 mmHg; \( P = 0.02 \)).

Serum glucose was unchanged, but plasma insulin was increased by 36% (from 7.2 ± 0.8 to 9.8 ± 1.3 m-units/l; \( P = 0.02 \)) (Figure 2A) and C-peptide concentrations were

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vehicle</th>
<th>Glibenclamide</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting FBF (ml · min(^{-1} ) · 100 ml(^{-1} ))</td>
<td>2.0 ± 0.2</td>
<td>1.9 ± 0.2</td>
<td>0.43</td>
</tr>
<tr>
<td>Resting FVR (units)</td>
<td>46.1 ± 3.2</td>
<td>50.9 ± 5.2</td>
<td>0.34</td>
</tr>
<tr>
<td>Peak FBF (ml · min(^{-1} ) · 100 ml(^{-1} ))</td>
<td>16.6 ± 1.5</td>
<td>16.9 ± 1.4</td>
<td>0.62</td>
</tr>
<tr>
<td>( \Delta ) Peak FBF (ml · min(^{-1} ) · 100 ml(^{-1} ))</td>
<td>14.6 ± 1.5</td>
<td>15.0 ± 1.4</td>
<td>0.61</td>
</tr>
<tr>
<td>Minimum FVR (units)</td>
<td>6.0 ± 0.6</td>
<td>6.0 ± 0.8</td>
<td>0.99</td>
</tr>
<tr>
<td>Volume repaid at 1 min (ml/100 ml)</td>
<td>12.4 ± 1.2</td>
<td>12.8 ± 1.2</td>
<td>0.51</td>
</tr>
<tr>
<td>( \Delta ) Volume repaid at 1 min (ml/100 ml)</td>
<td>10.6 ± 1.2</td>
<td>11.0 ± 1.2</td>
<td>0.51</td>
</tr>
<tr>
<td>Volume repaid at 5 min (ml/100 ml)</td>
<td>41.4 ± 4.4</td>
<td>41.0 ± 3.8</td>
<td>0.86</td>
</tr>
<tr>
<td>( \Delta ) Volume repaid at 5 min (ml/100 ml)</td>
<td>31.9 ± 4.3</td>
<td>31.5 ± 3.6</td>
<td>0.87</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>81.2 ± 1.6</td>
<td>83.1 ± 1.6</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Table 3 Effects of high-dose glibenclamide (100 µg/min) on resting and exercise haemodynamics (\( n = 7 \))

FBF, forearm blood flow; FVR, forearm vascular resistance; MAP, mean arterial pressure; \( \Delta \) indicates value at hyperaemic blood flow minus that at resting flow.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vehicle</th>
<th>Glibenclamide</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting FBF (ml · min(^{-1} ) · 100 ml(^{-1} ))</td>
<td>2.2 ± 0.1</td>
<td>2.3 ± 0.2</td>
<td>0.43</td>
</tr>
<tr>
<td>Resting FVR (units)</td>
<td>42.2 ± 1.5</td>
<td>41.9 ± 2.2</td>
<td>0.92</td>
</tr>
<tr>
<td>Peak FBF (ml · min(^{-1} ) · 100 ml(^{-1} ))</td>
<td>13.5 ± 1.4</td>
<td>13.3 ± 1.7</td>
<td>0.75</td>
</tr>
<tr>
<td>( \Delta ) Peak FBF (ml · min(^{-1} ) · 100 ml(^{-1} ))</td>
<td>11.3 ± 1.4</td>
<td>10.9 ± 1.7</td>
<td>0.57</td>
</tr>
<tr>
<td>Minimum FVR (units)</td>
<td>7.1 ± 0.6</td>
<td>7.5 ± 0.8</td>
<td>0.25</td>
</tr>
<tr>
<td>Volume repaid at 1 min (ml/100 ml)</td>
<td>9.5 ± 1.3</td>
<td>9.2 ± 1.4</td>
<td>0.75</td>
</tr>
<tr>
<td>( \Delta ) Volume repaid at 1 min (ml/100 ml)</td>
<td>7.3 ± 1.1</td>
<td>7.0 ± 1.3</td>
<td>0.67</td>
</tr>
<tr>
<td>Volume repaid at 5 min (ml/100 ml)</td>
<td>26.2 ± 3.7</td>
<td>26.5 ± 3.9</td>
<td>0.89</td>
</tr>
<tr>
<td>( \Delta ) Volume repaid at 5 min (ml/100 ml)</td>
<td>15.9 ± 3.1</td>
<td>14.9 ± 3.8</td>
<td>0.60</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>91.1 ± 2.6</td>
<td>92.8 ± 1.4</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Figure 2 Effects of glibenclamide infusion at 15 µg/min (A) and 100 µg/min (B) on serum glucose, plasma insulin and C-peptide

GLIB, glibenclamide; CTRL, control. \( * P < 0.05 \) compared with control.
KATP channels and metabolic vasodilation

Figure 3 Effects of gliclazide infusion on serum glucose, plasma insulin and plasma C-peptide
GLIC, gliclazide; CTRL, control. *P < 0.01 compared with control.

**Effect of high-dose glibenclamide on forearm blood flow**
In order to look for a dose–response effect, seven healthy subjects were given a glibenclamide dose of 100 μg/min. Despite the 7-fold increase in glibenclamide dose, no difference in forearm blood flow was noted at rest or after exercise, compared with vehicle controls (Table 3). Plasma insulin rose by 150% from 9.1 ± 1.3 to 22.9 ± 3.5 m-units/l (P = 0.002), and C-peptide increased from 0.7 ± 0.1 to 1.0 ± 0.1 nmol/l (P < 0.001) following glibenclamide infusion (Figure 2B); however, serum glucose was unchanged.

**Effect of gliclazide on forearm blood flow**
Resting forearm blood flow and peak hyperaemia were not altered significantly by intra-arterial gliclazide (Table 4). There was a trend towards an increase in the volume repaid at 1 min after gliclazide, but this was no longer apparent by 5 min. Gliclazide induced a 38% increase in insulin (P = 0.001) and a 12% reduction in glucose (P = 0.009) (Figure 3), although levels were still within the euglycaemic range.

**DISCUSSION**
In the present study we demonstrate that infusion of K_{ATP} channel inhibitors into the forearm circulation does not alter resting blood flow or the vasodilator response to non-ischaemic forearm exercise. In healthy subjects, infusion of either glibenclamide or gliclazide did not attenuate resting or functional hyperaemic forearm blood flow. These observations suggest that vascular K_{ATP} channels do not appear to play an important role in the regulation of basal vasomotor tone or exercise-induced functional hyperaemia in the forearm of healthy human subjects.

K_{ATP} channels and resting skeletal muscle blood flow
The results of this study support the observations of others that inhibition of vascular K_{ATP} channels does not alter resting forearm blood flow in healthy subjects [7–9]. Our findings are also consistent with experimental data in animals using a variety of techniques [18–23], which also suggest that vascular K_{ATP} channels may not be critical to the regulation of basal vascular tone in skeletal muscle vasculature. Since the measurement of blood flow by forearm strain gauge plethysmography primarily reflects skeletal muscle perfusion, our findings have relevance to the skeletal muscle vascular bed. However, the forearm skeletal muscle vasculature may not be representative of all skeletal muscle vascular beds. Blood flow to muscle may vary depending on the type of muscle fibres that constitute its bulk (slow- compared with fast-twitch), and in accordance with its metabolic requirements and function [24,25]. Indeed, K_{ATP} channel inhibition may reduce basal blood flow in the calf in healthy humans [10]. More recently, we have demonstrated that inhibition of K_{ATP} channels in the coronary circulation of patients with atherosclerosis may reduce resting coronary blood flow [12]. Thus it appears that regional differences exist with regard to the importance of mechanisms that control resting vascular tone in humans, not only within the skeletal muscle vasculature, but also between vascular beds of different organs.

K_{ATP} channels and functional hyperaemia
A body of experimental evidence suggests that K_{ATP} channels are involved in regulating skeletal muscle blood flow during the increase in muscle metabolism associated with exercise [22,23,26–28]. However, recent experiments in conscious pigs do not provide support for these findings [18]. Glibenclamide at doses sufficient to induce K_{ATP} channel blockade did not reduce the increment in skeletal muscle blood flow to a variety of muscle groups induced by treadmill exercise. The discrepancy between this and previous studies may be related to species differences, the experimental preparation (anaesthetized...
compared with conscious animals) and method by which functional hyperaemia was induced (regional muscle stimulation with electrical stimuli compared with treadmill exercise in intact animals).

There are theoretical reasons to suggest a role for vascular K\textsubscript{ATP} channels in functional hyperaemia. Changes in muscle metabolites associated with muscle contraction may activate these channels, leading to membrane hyperpolarization and vasodilation. Nichols and Lederer [29] have proposed two complementary mechanisms by which these channels may contribute to functional hyperaemia in skeletal or cardiac muscle. The ‘ATP hypothesis’ states that, during increased muscle metabolism, oxygen is consumed at a higher rate by skeletal (or cardiac) muscle, leading to a lower oxygen concentration in vascular smooth muscle within the resistance vessels. The result is a reduced capacity of vascular smooth muscle to generate ATP, and hence activation of K\textsubscript{ATP} channels. The ‘adenosine hypothesis’ states that adenosine released from contracting muscle causes activation of K\textsubscript{ATP} channels in adjacent vascular smooth muscle. Along these lines, other chemical substances released from contracting muscle or the endothelium, such as lactic acid, hydrogen ions, vasodilator prostanoids and nitric oxide, may also activate vascular K\textsubscript{ATP} channels [30–33]. Increased muscle metabolism during exercise may also lead to the formation of osmotically active substances that may mediate vasodilation [1]. Recent data suggest that vasodilation caused by hyperosmolarity in coronary and skeletal muscle microvessels of animals is due to activation of endothelial K\textsubscript{ATP} channels [34,35]. The K\textsubscript{ATP} channel may also mediate the phenomenon known as functional sympatholysis, which may serve to optimize muscle perfusion during exercise [22].

Previous studies in humans have assessed the importance of endothelial mediators and other chemical substances in functional hyperaemia induced by non-ischaemic exercise, but the contribution of K\textsubscript{ATP} channels to this response was not previously known. Our observations in skeletal muscle are not in keeping with previous data from some animal studies [22,23,26–28], but they are consistent with the findings from the only published study to date undertaken in conscious mammals [18]. Apart from species-related and other methodological differences, additional factors may account for the aforementioned differences. It is possible that the lack of effect of isolated vascular K\textsubscript{ATP} channel blockade may relate to an interplay between these channels and parallel vasodilator systems. In theory, the blockade of K\textsubscript{ATP} channels may result in augmentation of other vasodilator mechanisms, thereby masking the role of the blocked pathway. A similar phenomenon has been described in the canine coronary circulation [36].

The type of exercise chosen to stimulate functional hyperaemia in our study may not have induced the required conditions to activate K\textsubscript{ATP} channels, i.e. hypoxia or alterations to nucleotide levels. Indeed, a previous study in humans demonstrated that the oxygen content of deep venous blood increases (rather than decreases) after exercise of forearm muscles [37]. Moreover, intracellular ATP concentrations may be well maintained during muscle contraction, even under ischaemic conditions [38]. Concentrations of other metabolites in the interstitium of the human quadriceps muscle have also been measured using microdialysis techniques during exercise and recovery in conjunction with blood flow [39]. Of the metabolites that are known to activate K\textsubscript{ATP} channels, increases in adenosine and lactate were documented, but only adenosine levels were correlated with increased muscle blood flow. Although adenosine may contribute to exercise hyperaemia, its vasodilator effects may not be mediated by K\textsubscript{ATP} channels in the human forearm [7].

**Potential limitations**

Insulin has vasodilator properties, which are mediated by endothelial nitric oxide release [40], although other mechanisms (including the activation of K\textsubscript{ATP} channels) may be involved [41]. In theory, the increase in insulin levels could oppose any vasoconstrictor effect of inhibition of vascular K\textsubscript{ATP} channels. However, the vasoactive effects of insulin are delayed in onset and occur at higher concentrations than the low physiological levels observed during the present study [40]. Forearm blood flow in the contralateral arm was not measured routinely in our study. However, in preliminary experiments conducted in our laboratory in which the technique of bilateral venous occlusion plethysmography was employed, a similar glibenclamide infusion protocol did not result in significant alterations to forearm blood flow in the non-infused arm (H. M. O. Farouque and I. T. Meredith, unpublished work). These findings add support to the contention that the small increment in systemic insulin levels observed in the present study was unlikely to have induced significant vasoactive effects.

Mean plasma glibenclamide levels of just above 500 ng/ml during glibenclamide infusion at 15 µg/min were remarkably close to initial estimates. Glibenclamide at doses within the therapeutic range is known to inhibit vascular K\textsubscript{ATP} channels in the human forearm [6]. The highest infused dose of glibenclamide (100 µg/min) would have resulted in estimated total glibenclamide concentrations of 3300–5000 ng/ml (100 µg/min divided by blood flow and multiplied by 1000), assuming that resting forearm blood flow was between 2 and 3 ml \cdot min\textsuperscript{−1} \cdot 100 ml\textsuperscript{−1} forearm tissue. These glibenclamide concentrations are equivalent to 6.7–10 µM (concentration in µg/1 divided by the M, of glibenclamide, which is 494). Glibenclamide concentrations of this magnitude are well above therapeutic levels, and would be expected to interfere with the activity of K\textsubscript{ATP} channels. Moreover,
we examined the response to gliclazide, another potent sulphonylurea K<sub>ATP</sub> channel inhibitor. However, this agent also did not alter forearm blood flow responses. Documentation of insulin release with all protocols provided confirmation of K<sub>ATP</sub> channel blockade. For these reasons, inadequate K<sub>ATP</sub> channel inhibition is an insufficient explanation for the observed results.

There is evidence to suggest that the skeletal muscle microvasculature consists of ‘nutritive’ and ‘non-nutritive’ components [42]. The nutritive circulation is exposed to myocytes, whereas the non-nutritive circulation consists of functional vascular shunts, which may supply connective tissue. In theory, the proportion of nutritive to non-nutritive flow could vary with K<sub>ATP</sub> channel inhibition without altering total blood flow, a situation that would not be detected with venous occlusion plethysmography.

In the present study, forearm blood flow was assessed immediately after exercise, rather than during exercise. It is possible to measure limb blood flow during muscle contraction with venous occlusion plethysmography, but significant technical limitations exist [43]. This is particularly so with strain gauge plethysmography, where artefacts produced by movement of the gauge during exercise result in uninterpretable plethysmographic traces. Therefore, when studying exercise hyperaemia with this technique, it is necessary to examine blood flow changes immediately upon termination of exercise. This is a well accepted method of studying exercise-induced metabolic vasodilation [3,44,45]. Although it is possible that the negative result in the present study was due to type 2 statistical error, we believe this is unlikely. The first protocol (n = 20 subjects) was designed to provide 80% power to detect a 40% change in the mean volume of blood repaid after 5 min with glibenclamide. Pooling and re-analysis of the data to enhance the power of the study (n = 39) did not alter our findings.

**Conclusion**

We demonstrate that vascular K<sub>ATP</sub> channels in forearm resistance vessels do not appear to contribute to the regulation of resting blood flow or functional hyperaemia induced by non-ischaemic isotonic forearm exercise in healthy humans. Whether the failure to attenuate metabolic vasodilation by isolated K<sub>ATP</sub> channel blockade is related to the augmentation of other vasodilator pathways remains to be investigated.

**ACKNOWLEDGMENTS**

We are grateful to Servier Laboratories (France) and Aventis Pharma (Germany) for donating gliclazide and glibenclamide lyophilisate (HB 419) respectively. We thank Michael Zhang and Mauro Baldi for technical assistance. H. M. O. F. was supported by a National Heart Foundation of Australia Medical Postgraduate Research Scholarship (PM 98M 0006).

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