Effects of combined inhibition of ATP-sensitive potassium channels, nitric oxide, and prostaglandins on hyperemia during moderate exercise

William G. Schrage,1 Niki M. Dietz,1 and Michael J. Joyner1,2
1Department of Anesthesiology and 2General Clinical Research Center, Mayo Clinic, Rochester, Minnesota

Submitted 30 December 2005; accepted in final form 6 February 2006

Schrage, William G., Niki M. Dietz, and Michael J. Joyner. Effects of combined inhibition of ATP-sensitive potassium channels, nitric oxide and prostaglandins on hyperemia during moderate exercise. J Appl Physiol 100: 1506–1512, 2006. First published February 9, 2006; doi:10.1152/japplphysiol.01639.2005.—ATP-sensitive potassium (KATP) channels have been suggested to contribute to coronary and skeletal muscle vasodilation during exercise, either alone or interacting in a parallel or redundant process with nitric oxide (NO), prostaglandins (PGs), and adenosine. We tested the hypothesis that KATP channels, alone or in combination with NO and PGs, regulate exercise hyperemia in forearm muscle. Eighteen healthy young adults performed 20 min of moderate dynamic forearm exercise, with forearm blood flow (FBF) measured via Doppler ultrasound. After steady-state FBF was achieved for 5 min (saline control), the KATP inhibitor glibenclamide (Glib) was infused into the brachial artery for 5 min (10 μg·dl−1·min−1), followed by saline infusion during the final 10 min of exercise (n = 9). Exercise increased FBF from 71 ± 11 to 239 ± 24 ml/min, and FBF was not altered by 5 min of Glib. Systemic plasma Glib levels were above the therapeutic range (68 ± 2 vs. 90 ± 2 μg/dl). In nine additional subjects, Glib was followed by combined infusion of N3-nitro-l-arginine methyl ester (l-NAME) plus ketorolac (to inhibit NO and PGs, respectively). As above, Glib had no effect on FBF but addition of l-NAME + ketorolac (i.e., triple blockade) reduced FBF by ~15% below steady-state exercise levels in seven of nine subjects. Interestingly, triple blockade in two subjects caused FBF to transiently and dramatically decrease. This was followed by an acute recovery of flow above steady-state exercise values. We conclude 1) opening of KATP channels is not obligatory for forearm exercise hyperemia, and 2) triple blockade of NO, PGs, and KATP channels does not reduce hyperemia more than the inhibition of NO and PGs in most subjects. However, some subjects are sensitive to triple blockade, but they are able to restore FBF acutely during exercise. Future studies are required to determine the nature of these compensatory mechanisms in the affected individuals.

Doppler ultrasound; N3-nitro-l-arginine methyl ester; blood flow; human; potassium; nitric oxide synthase; cyclooxygenase

MUSCLE BLOOD FLOW MUST INCREASE quickly and dramatically, via local vasodilation, to meet the metabolic demands of exercise. The types and relative importance of vasodilator signals contributing to the rise in blood flow in humans are poorly understood. ATP-sensitive potassium (KATP) channels are an intriguing candidate signal for mediating exercise hyperemia, because they are distributed in human limbs (18), and KATP channel agonist administration increases blood flow in the forearm (4, 23). In vascular smooth muscle, these channels cause vasodilation via potassium efflux and smooth muscle hyperpolarization (16). Finally, the fact that potassium levels increase during exercise (18) is consistent with the concept that KATP channels may have an important role in control of muscle blood flow.

In hamsters (14, 20) and rats (23), the KATP channel blocker glibenclamide (Glib) reduced hyperemic response in electrically stimulated muscles, whereas it had no effect on swine skeletal muscle blood flow during treadmill running (7). Studies of porcine coronary blood flow suggest KATP channels contribute to exercise-associated hyperemia (8), especially after inhibition of other vasodilator signals or pathways (7, 9, 10, 15, 17). In contrast, studies in dog hearts do not support an obligatory role for KATP channels in exercise-induced coronary vasodilation (24). These observations raise the possibility that KATP channels actively control skeletal muscle blood flow during exercise, but inhibition of KATP channels may lead to activation of nitric oxide (NO) or prostaglandins (PGs) or adenosine to maintain blood flow as compensation for loss of vasodilation through KATP channels. However, there is little direct evidence to support this attractive “redundancy” hypothesis in animals or humans.

In humans, limited data suggest little or no role for KATP channels during exercise. For example, Glib modestly reduced the peak blood flow response during reactive hyperemia in most (1–3), but not all (12), studies. One study, using venous occlusion plethysmography to assess blood flow on cessation of exercise, reported no effect of Glib on postexercise blood flow (11). A main limitation to using plethysmography in exercise studies is that blood flow measurements are only possible after exercise is finished, and postexercise flow might not always reflect what was actually happening during exercise. Therefore, no studies in humans have assessed the role of KATP channels during dynamic exercise.

It is unknown whether redundant or parallel signals might be working in human skeletal muscle. Recent work from our laboratory suggests that inhibition of NO during exercise reduces blood flow by ~20% (without compensation), which argues against redundant signaling. However, inhibition of PGs leads to a transient reduction in blood flow (~12%, with compensation) that can be acutely restored during steady-state exercise (within 3 min). The compensating signal for loss of PGs is not known, but KATP channels are one possibility. To our knowledge, no studies in humans have assessed the role of KATP channels, alone or in combination with NO and PGs, during dynamic exercise.

In this context, we tested two main hypotheses in healthy adults. First, inhibition of KATP channels alone would not...
reduce exercise hyperemia significantly. Second, subsequent inhibition of NO and PGs in the exercising forearm would synergistically reduce blood flow more than inhibition of NO and PGs.

METHODS

Subjects

Each subject provided his or her written, informed consent before participation in this study. All protocols and procedures were approved by the Institutional Review Board at Mayo Clinic.

Eighteen healthy volunteers participated in this study. Subjects were normotensive, nonsmoking, nonobese, and not taking any medications other than oral contraceptives. A blood sample was obtained from female subjects \( n = 9 \) <24 h before the study to ensure that none was pregnant. All female subjects were tested during the placebo phase of oral contraception or in the early follicular phase of their menstrual cycle to minimize possible cardiovascular effects of gender-specific hormones. All subjects refrained from caffeine, alcohol, and exercise for 24 h before the study, but they ate a light meal 2–3 h before the study to ensure adequate blood glucose levels.

Instrumentation, Hemodynamic Measurements, and Drug Administration

Heart rate (HR) was measured by three-lead ECG. Blood flow was measured by Doppler ultrasound (see below), and blood pressure was measured directly from a brachial artery catheter in the exercising forearm.

The brachial artery was catheterized under aseptic technique after infiltration of the area with 1–2 ml of 1% lidocaine. A standard 5-cm 20-gauge Teflon catheter was inserted into the nondominant arm and continuously flushed with heparinized saline. A pressure transducer connected to the arterial catheter measured beat-to-beat blood pressure. An 18-gauge venous catheter was inserted into the dominant (nonexercising) arm to sample blood for systemic levels of glucose, insulin, and Glib.

Saline or study drugs were administered via the brachial artery catheter using a three-port connector system that permitted simultaneous measurements of arterial pressure during drug infusions. Saline and study drugs were infused at 1–2 ml/min, and saline infusion at these rates did not alter baseline blood flow. Glib (100 \( \mu \)g/ml, Sigma), \( N^\circ \)-nitro-L-arginine methyl ester (L-NNAME; 2.5 mg/ml, Aerobio/Clinalfa), and ketorolac (trade name: Toradol, 300 \( \mu \)g/ml, Abbott) were diluted in saline immediately before use. The dose of Glib (10 \( \mu \)g·dl\(^{-1}\)·min\(^{-1}\), \( \sim 1 \) mg total) is less than a typical oral dose (2–10 mg) given to treat Type 2 diabetes. Ketorolac was chosen to inhibit cyclooxygenase (600 \( \mu \)g/min for 5 min) instead of indomethacin because our laboratory recently showed it reduced exercise hyperemia in young healthy subjects (6, 21). The dose of ketorolac was chosen as 10–20% of the systemic dose (15–30 mg). L-NNAME was infused at 5 mg/min for 5 min to inhibit NO synthase. The dose of L-NNAME was based on adjustments to whole body intravenous infusions of L-NNAME in previous work (13) and experience in our laboratory with L-NNAME.

Beat-to-beat forearm blood flow (FBF) was measured as described previously (6, 19, 21, 22). Briefly, a 4-MHz pulsed Doppler probe (model 500V, Multigon Industries, Mt. Vernon, NY) measured brachial artery mean blood velocity (MBV) proximal to the catheter insertion site. The probe insonation angle was 60°. A linear 7.0-MHz echo Doppler ultrasound probe (model 128XP, Acuson, Mountain View, CA) was placed proximal to the pulsed Doppler probe to measure brachial artery diameter at rest and at several time points corresponding to saline or drug infusions (see Fig. 1). FBF was calculated by multiplying MBV by brachial artery cross-sectional area (6, 21). Forearm vascular conductance (FVC) was calculated as

\[
\frac{\text{FBF}}{\text{MAP}}
\]

Fig. 1. Experimental time line. In protocol 1 (A), subjects \( n = 9 \) performed forearm exercise for 20 min with either saline or glibenclamide (Glib) once steady-state exercise had been attained (minute 5). Glib was infused from minute 5 to minute 10 during exercise, which was replaced with saline the final 10 min. In protocol 2 (B), the effects of triple blockade during exercise were assessed in 9 more subjects. As in protocol 1, Glib was infused during minutes 5–10 of exercise. After 2 min of saline, a 5-min infusion of \( N^\circ \)-nitro-L-arginine methyl ester (L-NNAME) plus ketorolac was followed by the final 3 min of saline with exercise (\( n = 7 \)). In 2 subjects, L-NNAME plus ketorolac was infused first, followed by Glib at minute 12 of exercise (not shown). 

**Dynamic Forearm Exercise**

Rhythmic forearm exercise was performed using a weight corresponding to 10% of maximal voluntary isometric contraction. The weight was lifted 4–5 cm over a pulley by squeezing a handgrip at a duty cycle of 1-s contraction and 2-s relaxation (20 contractions/min) in time to a metronome (6, 21).

**Experimental Protocol**

Drug infusion protocols. The experimental protocol is illustrated in Fig. 1. After 2 min of baseline recording with saline infusion (Rest), subjects began 20 min of dynamic forearm exercise.

**PROTOCOL 1 (GLIB ONLY; \( N = 9, 3 \) FEMALE SUBJECTS).** Saline was infused during the first 5 min (control exercise), at which point saline was replaced with Glib for 5 min (minutes 5–10 of exercise). At 10 min, Glib was changed to saline for the last 10 min of exercise; no other drugs were given.

**PROTOCOL 2 (TRIPLE BLOCKADE; \( N = 9, 6 \) FEMALE SUBJECTS).** In the remaining subjects, seven received Glib as in protocol 1, but 2 min after Glib ended, we infused both ketorolac and L-NNAME over 5 min (minutes 12−17 of exercise), followed by 3 min of saline. Two other subjects received combined L-NNAME + ketorolac first, followed by Glib. Because all three drugs have prolonged half-lives (hours), the protocol was designed such that the second drug infusion resulted in triple-blockade condition during exercise.

**Data and Statistical Analysis**

Data were collected and stored on a computer at 200 Hz and analyzed offline with signal-processing software (WinDaq, DATAQ Instruments, Akron, OH). MAP, HR, and brachial artery MBV signal were averaged in 30-s intervals, or 10 contraction-relaxation cycles.
Table 1. Blood values in response to exercise and Glib infusion

<table>
<thead>
<tr>
<th>Variable</th>
<th>Protocol 1</th>
<th>Protocol 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Glib</td>
</tr>
<tr>
<td>Blood glucose, mg/dl</td>
<td>86±4</td>
<td>89±2</td>
</tr>
<tr>
<td>Insulin, U</td>
<td>5±1</td>
<td>5±1</td>
</tr>
<tr>
<td>Glib, pg/ml</td>
<td>NM</td>
<td>217±32</td>
</tr>
</tbody>
</table>

Values are mean ± SE for 12 subjects. All blood samples were obtained from a venous catheter in the dominant arm. Blood glucose did not change significantly with exercise or glibenclamide (Glib) infusion. Insulin increased 40−50% by the end of exercise. Systemic Glib levels were high by the end of Glib infusion and remained so through the end of exercise. NM, not measured. *Value different from Rest and End Glib, P < 0.05.

These time points were the last 30 s each primary section of exercise (rest, control exercise, end of drug infusions, end of exercise), and they denoted by  ▽ in Fig. 1.

Data for FBF and FVC were expressed in the following manner: 1) absolute levels; 2) normalized responses, where no blood flow was defined as 0%, and the 5-min steady-state FBF level was defined as 100%. This approach reduces the variability of hyperemic responses between subjects due to the range of absolute workloads and therefore absolute blood flow. We believe that the latter approach most accurately reflects the relative contribution from KATP channels, NO, and PGs. We also expressed the data as the change in FBF above baseline FBF (or FVC) and the percent of change above baseline FBF, where baseline FBF was defined as 0%, and the 5-min steady-state FBF was defined as 100%. Despite the manipulations of data, the pattern of changes in FBF and FVC were similar. Thus select graphs and tables are presented for simplicity.

This study included data from 9 subjects from each protocol (Glib only and triple blockade), with the primary analysis to test whether patients’ measurements changed over time. Data were analyzed using generalized estimating equations (GEE) (25) to take into account the clustering of data within subjects. An unstructured working correlation structure was used for the GEE analysis. The GEE approach is easily implemented, is not as sensitive to model assumptions, and has relatively rapid computing time. Because model assumptions are less important using GEE vs. ANOVA, we elected to use GEE. We also analyzed the data with ANOVA modeling, and those results support the present conclusions equally.

A model was run for each dependent variable, FBF, FVC, and HR, and the independent variable was time. There were two participants who had extreme measurements that could have highly influenced the models. Thus, as a secondary analysis, we ran all the GEE models excluding the two outliers. All data are expressed as means ± SE. Significance for all comparisons was P < 0.05.

RESULTS

Subjects

The 18 subjects (9 women) were all young (27 ± 1 yr), lean (height 171 ± 3 cm, weight 72 ± 3 kg, body mass index 24 ± 1 kg/m²) and normotensive (125 ± 27/3 ± 2 mmHg). Resting blood glucose was 88 ± 2 mg/dl. The average 10% exercise workload was 3.5 ± 0.3 kg.

Cardiovascular Response to Forearm Exercise

Blood glucose was 88 ± 2 mg/dl at rest and did not change with exercise or Glib infusion (P = 0.50, Table 1). HR, MAP, and the absolute and normalized FBF and FVC data are summarized in Table 2. HR and MAP did not change during exercise and/or drug infusion (P = 0.28). Brachial artery diameter was 0.42 ± 0.02 cm and 0.35 ± 0.02 cm at rest in the two protocols, and it did not change from rest to exercise or with drug infusion (P = 0.99).

Protocol 1: Effects of Glib on Exercise Hyperemia

The effects of Glib on FBF are summarized in Fig. 2. Blood flow increased from 71 ± 11 to 239 ± 24 ml/min in response to dynamic exercise (~3-fold) and did not change significantly with Glib infusion or the subsequent 10 min of saline after Glib infusion.

Table 2. Hemodynamic values for forearm exercise and drug infusions in protocols 1 and 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Protocol</th>
<th>Baseline</th>
<th>Steady-State Exercise</th>
<th>End Glib</th>
<th>Saline</th>
<th>Saline or End Drugs</th>
<th>End of Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>1</td>
<td>61±3</td>
<td>60±4</td>
<td>60±3</td>
<td>61±3</td>
<td>62±3</td>
<td>60±3</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>1</td>
<td>59±2</td>
<td>68±2</td>
<td>58±1</td>
<td>57±2</td>
<td>57±2</td>
<td>56±3</td>
</tr>
<tr>
<td>Brachial artery diameter, cm</td>
<td>1</td>
<td>93±2</td>
<td>94±3</td>
<td>95±2</td>
<td>96±3</td>
<td>96±3</td>
<td>96±3</td>
</tr>
<tr>
<td>FBF, ml/min</td>
<td>1</td>
<td>20±1</td>
<td>23±9</td>
<td>29±3</td>
<td>23±3</td>
<td>24±3</td>
<td>24±6</td>
</tr>
<tr>
<td>Normalized FBF, % control exercise</td>
<td>1</td>
<td>97±6</td>
<td>99±4</td>
<td>95±4</td>
<td>89±3</td>
<td>87±5</td>
<td>87±5</td>
</tr>
<tr>
<td>FVC, ml·min⁻¹·100 mmHg⁻¹</td>
<td>1</td>
<td>76±13</td>
<td>262±35</td>
<td>249±41</td>
<td>262±47</td>
<td>259±41</td>
<td>263±44</td>
</tr>
<tr>
<td>Normalized FVC, % control exercise</td>
<td>1</td>
<td>29±4</td>
<td>94±5</td>
<td>97±7</td>
<td>98±5</td>
<td>96±5</td>
<td>84±4</td>
</tr>
</tbody>
</table>

Values are means ± SE. Data are summarized from same time points as 30-s average forearm blood flow (FBF) data. Heart rate (HR) and brachial artery diameter were similar before and during exercise. Blood pressure did not change at the onset of exercise, but increased significantly by 20 min after infusion of Nω-nitro-L-arginine methyl ester plus ketorolac. Various expressions of blood flow and vascular conductance are also displayed for comparison (explained in METHODS). FVC, forearm vascular conductance. *Different from control exercise value, P < 0.05.

J Appl Physiol • VOL 100 • MAY 2006 • www.jap.org
Similar conclusions are supported by expressing data in relative terms (to steady-state exercise) for FBF ($P = 0.40$) or FVC ($P = 0.72$; Table 2).

**Protocol 2: Effects of Triple Blockade on Exercise Hyperemia**

The effects of Glib, 1-NAME, plus ketorolac on forearm blood flow are summarized in Fig. 3. Blood flow increased from 53 ± 13 to 149 ± 37 ml/min in response to dynamic exercise (~3-fold; $P < 0.01$) and did not change significantly with Glib infusion ($P = 0.29$). Addition of 1-NAME plus ketorolac significantly reduced FBF in both absolute ($P < 0.01$) and relative expression ($P < 0.001$) of FBF. Similar conclusions are supported by expression of FBF ($P = 0.02$) or FVC ($P = 0.03$) in terms relative to steady-state exercise (Table 2).

**Outlier Responses to Triple Blockade**

Not all subjects responded the same to triple blockade of NO, PGs, and $K_{ATP}$ channels (protocol 2). In two of nine subjects in protocol 2, FBF decreased dramatically shortly after the start of the triple-blockade infusion. It is not likely that this was a drug-order effect, because one subject received Glib ($P = 0.85$). Similar conclusions are supported by expressing data in relative terms (to steady-state exercise) for FBF ($P = 0.40$) or FVC ($P = 0.72$; Table 2).
followed by l-NAME plus ketorolac (Fig. 4A), and the other subject received the reverse order (Fig. 4B).

**DISCUSSION**

We tested the hypothesis that K<sub>ATP</sub> channels are important contributors to vasodilation in exercising human skeletal muscle and that triple blockade of K<sub>ATP</sub> channels, NO, and PGs would synergistically reduce muscle blood flow. The main novel findings in this study are that K<sub>ATP</sub> channels appear relatively unimportant for the normal blood flow response to moderate exercise in humans. Second, K<sub>ATP</sub> channels do not appear to interact with NO or PGs in most subjects, because the reduction in FBF with triple blockade was no greater than with double blockade of NO and PGs (21). However, in a subgroup of subjects, triple blockade of NO, PGs, and K<sub>ATP</sub> channels resulted in a dramatic transient fall in blood flow, suggesting that in some humans the importance of K<sub>ATP</sub> channels may increase after inhibition of NO and PGs, but other signals can compensate the blood flow reduction.

**Role of K<sub>ATP</sub> Channels in Exercise Hyperemia**

Despite the fact that these K<sub>ATP</sub> channels can be opened in the forearm with agonists, we hypothesized Glib would not change FBF. This reasoning was based on previous work in humans, where Glib produced minimal effects on reactive hyperemia or postexercise blood flow (2, 3, 11, 12). One could argue that our findings are different than previous studies in that we infused Glib during exercise instead of at rest before exercise. However, in all cases, Glib does not alter baseline FBF, and it has little or no effect on reactive hyperemia or postexercise blood flow. Taken in context with our results, it appears inhibition of K<sub>ATP</sub> channels does not alter multiple measures or manipulations of human muscle blood flow. Our results are consistent with the lack of effect of Glib exercise hyperemia in running pigs (7) but contrast with electrically stimulated skeletal muscle in animals (20, 23). The lack of effect of Glib infusion on FBF are also in agreement with animal studies of the coronary circulation (8, 9, 15, 17). In the heart, inhibition of K<sub>ATP</sub> channels generally did not change the blood flow response to treadmill exercise in pigs unless several other inhibitors were also present (10, 15, 17). It is certainly reasonable to propose that results differ due to variation in K<sub>ATP</sub> channel expression between species and/or muscle beds.

Many in vitro microvascular studies using K<sub>ATP</sub> channel inhibition support an important role for these channels in regulation of vascular tone (reviewed in Ref. 16). Our data suggest that in humans these channels are not obligatory for exercise hyperemia. We are limited in our conclusions about K<sub>ATP</sub> channels across all levels of exercise, because K<sub>ATP</sub> channels could certainly be more important at higher levels of work. We used 10% or the maximum forearm work, which is moderate and sustainable work for 20 min. Future work will need to test this question in a larger muscle group at higher exercise intensities. Presently, though, K<sub>ATP</sub> channels do not appear normally active in mediating the hyperemic response to moderate exercise in humans.

**Role of Triple Blockade in Exercise Hyperemia**

As shown in Fig. 3, triple blockade during exercise reduced FBF to levels similar to “double blockade” of NO and PGs our laboratory reported previously (21). These results confirm our laboratory’s previous work showing a 15–20% reduction in exercise hyperemia with double blockade, and they extend our understanding with regard to the relationships (or lack thereof) between multiple vasodilator mechanisms. Remarkably, exercising skeletal muscle exhibits the ability to maintain >80% of normal blood flow response despite “losing” these three potentially important signals, highlighting the complex nature of physiologic systems that evoke exercise hyperemia.

Contrary to our hypothesis, triple blockade did not synergistically reduce FBF to levels far below l-NAME plus ketorolac. These results are consistent with studies of how the coronary circulation responds to exercise, where it appears many systems work either redundantly or “in parallel” with each other to make certain that cardiac muscle is adequately perfused despite loss of multiple vasodilator pathways. Merkus and colleagues (17) inhibited NO, K<sub>ATP</sub> channels and adenosine receptors (but not PGs) without showing a significant impairment in coronary blood flow during exercise. Conversely, Ishibashi and colleagues (15) abolished the exercise-induced coronary vasodilation in dogs. In light of these species differences, similar approaches need to be tested to fully assess
the interactions of vasodilator signals in exercising human skeletal muscles.

**Outlier Responses to Triple Blockade**

As highlighted in Fig. 4, not all subjects responded the same to triple blockade of NO, PGs, and KATP channels (protocol 2). In two of nine subjects in protocol 2, FBF decreased dramatically shortly after the start of the triple-blockade infusion. Their results are likely due to local responses, because systemic measures of pressure, HR, and insulin and blood glucose levels were similar to the whole group. One of these subjects was female, one was male, and both were 25 yr old. None of the subjects reported any history of cardiovascular disease, nor were they taking any medications. It is not likely that this was a drug-order effect. At least two possible explanations may account for their responses.

First, it is possible that, in a subpopulation of humans, NO, PGs, and KATP channels mediate the majority of the exercise hyperemic response, such that loss of all three pathways produces a large decrease in FBF. Alternatively, either NO and PGs, or KATP channels might be critical only with loss of the other pathways. Our results cannot determine whether or not either of these ideas is correct. In either case, it appears other signals can compensate acutely to restore blood flow. Possible candidates for this recovery include adenosine from the muscles, ATP from red blood cells, or perhaps other vascular signals like endothelium-derived hyperpolarizing factor.

**Experimental Considerations**

It is important to consider potential limitations to this study. With regard to experimental design, the moderate exercise intensity and dose of Glib may limit the breadth of our conclusions. First, we chose the moderate workload; therefore, we cannot rule out an important role for KATP channels at higher exercise intensity. Second, our research design did not include sequential inhibition of the three pathways, such that subjects received single, then double, and then triple blockade with each of the drugs. We chose this design (21) given the general lack of effect of Glib alone as seen in previous work (3, 4, 12). Although we cannot exclude an intermediate effect with some Glib and either L-NAME or a combination of ketorolac and double blockade, our data suggest that a small effect would have been likely and extremely hard to detect. Third, the dose of Glib we chose might not be great enough to fully inhibit KATP channels of the forearm, which may underestimate the importance of KATP channels in exercise hyperemia. We think this is unlikely for the following reasons. First, we chose 10 \( \mu g \cdot dl^{-1} \cdot min^{-1} \) based on previous work that showed this dose could shift the response curve to the KATP channel opener, diazoxide (4, 12) and on work that showed a small significant effect on reactive hyperemia (3). Second, because systemic venous levels of Glib were above the therapeutic range (for individuals with Type 2 diabetes), it is likely the local concentration of Glib in the exercising forearm was even greater and that would have reduced FBF had the KATP channels been activated with exercise. Third, systemic insulin levels increased by ~50%, suggesting that we inhibited KATP channels (of note, blood glucose had fallen to 70 mg/dl 20 min postexercise). A final consideration is that exercise hyperemia per se diluted the Glib to an ineffective concentration. We think this is also unlikely because the local forearm dose during exercise was approximately six times greater than a whole body systemic dose. Although testing our effective Glib dose with diazoxide would have been ideal, we believe that the stated rationale above suggests that KATP channels were effectively inhibited. Together, Glib appears to exert little or no effect on basal FBF, reactive hyperemia, or postexercise blood flow. Thus the most likely explanation for our results is that we had effectively inhibited the KATP channels, but they do not play a large role (when inhibited alone) in several measures of FBF (resting or exercise or reactive hyperemia).

Another potential consideration is that insulin has been reported to cause forearm vasodilation (5), which could mask vasoconstriction in response to Glib. However, this increase in FBF occurs with systemic insulin levels that are 30–50 times higher than in the present study (5), so the small increase in insulin from Glib most likely did not mask any vasoconstrictive due to Glib.

In summary, we infused the KATP channel inhibitor Glib into the exercising forearm, and we report no effect on exercise hyperemia. Moreover, addition of inhibitors of NO and PGs did not reduce blood flow further than with inhibition of NO and PGs. These results suggest that these three vasodilator mechanisms do not work in concert with each other to control muscle blood flow in exercising human muscle, nor do they comprise the majority of the dilator pathways. These results are specific to humans, because many animal studies of both skeletal muscle and coronary circulation report different interactions between KATP channels and other vasodilator signals. Despite these findings, a small portion of subjects exhibited a striking sensitivity to triple blockade, suggesting that some humans rely heavily on NO, PGs, and KATP channels to mediate the exercise hyperemic response to dynamic exercise.

**ACKNOWLEDGMENTS**

The authors thank Shelley K. Roberts, Karen P. Krucker, Diane E. Wick, John H. Eisenach, and Christopher P. Johnson for assistance. We also thank the volunteers for their time.

**GRANTS**

This study was supported by National Heart, Lung, and Blood Institute Grants HL-69692 (W. G. Schrage) and HL-46493 (M. J. Joyner) and by General Clinical Research Center Grant RR-00585.

**REFERENCES**


