Taming the “sleeping giant”: the role of endothelin-1 in the regulation of skeletal muscle blood flow and arterial blood pressure during exercise

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Barrett-O’Keefe Z, Ives SJ, Trinity JD, Morgan G, Rossman MJ, Donato AJ, Runnels S, Morgan DE, Gmelch BS, Bledsoe AD, Richardson RS, Wray DW. Taming the “sleeping giant”: the role of endothelin-1 in the regulation of skeletal muscle blood flow and arterial blood pressure during exercise. Am J Physiol Heart Circ Physiol 304: H162–H169, 2013. First published October 26, 2012; doi:10.1152/ajpheart.00603.2012.—The cardiovascular response to exercise is governed by a combination of vasodilating and vasoconstricting influences that optimize exercising muscle perfusion while protecting mean arterial pressure (MAP). The degree to which endogenous endothelin (ET)-1, the body’s most potent vasoconstrictor, participates in this response is unknown. Thus, in eight young (24 ± 2 yr), healthy volunteers, we examined leg blood flow, MAP, tissue oxygenation, heart rate, leg arterial-venous O2 difference, leg O2 consumption, pH, and net ET-1 and lactate release at rest and during knee extensor exercise (0, 5, 10, 15, 20, and 30 W) before and after an intra-arterial infusion of BQ-123 [ET subtype A (ETA) receptor antagonist]. At rest, BQ-123 did not evoke a change in leg blood flow or MAP. During exercise, net ET-1 release across the exercising leg increased approximately threefold. BQ-123 increased leg blood flow by ~20% across all work rates (changes of 113 ± 76, 176 ± 83, 304 ± 108, 364 ± 130, 502 ± 117, and 570 ± 178 ml/min at 0, 5, 10, 15, 20, and 30 W, respectively) and attenuated the exercise-induced increase in MAP by ~6%. The increase in leg blood flow was accompanied by a ~9% increase in leg O2 consumption with an unchanged arterial-venous O2 difference and deoxyhemoglobin, suggesting a decline in intramuscular efficiency after ETA receptor blockade. Together, these findings identify a significant role of the ET-1 pathway in the cardiovascular response to exercise, implicating vasoconstriction via the ETA receptor as an important mechanism for both the restraint of blood flow in the exercising limb and maintenance of MAP in healthy, young adults.

during dynamic exercise of increasing intensity, the O2 demand of the exercising muscle is elevated, requiring a marked increase in skeletal muscle blood flow that is accomplished through a combination of systemic sympathoexcitation and local metabolic vasodilation (24). While vasodilation of active skeletal muscle is an essential component in the cardiovascular response to exercise, it must be balanced with the concomitant need to support mean arterial pressure (MAP). Indeed, leg blood flow values as high as 4 l·kg–1·min–1 have been observed during isolated small muscle mass exercise (37), suggesting that the skeletal muscle represents a “sleeping giant” that must be under tonic restraint to avoid hypotension during intense, whole body exercise (41). Studies using isolated arm and leg exercise modalities have supported this concept, demonstrating a reduction in MAP when additional muscle mass is recruited at near-maximal exercise intensities, even in the face of drastic increases in sympathetic nervous system activity (9, 37, 45, 52). From these observations, the concept has been put forth that exercise-induced metabolic vasodilation must be restrained to some degree to protect against precipitous drops in MAP as the limit of cardiac output is reached.

Although sympathetic vasoconstriction is one of the primary regulators of vascular tone at rest, the preponderance of data from studies in both animals and humans has demonstrated substantial blunting of sympathetic vasoconstriction in the exercising skeletal muscle vasculature (8, 12, 39, 43, 48, 53). In the face of this loss of regional sympathetic control, it is likely that nonadrenergic vasoconstrictor pathways may contribute significantly to the balance of skeletal muscle blood flow and MAP during exercise. One such vasoconstrictor that may be relevant in this regard is endothelin (ET)-1, the most potent endogenous vasoconstrictor (58). In the periphery, ET-1 provokes a sustained vasoconstrictor response by binding to ET subtype A (ETA) and B (ETB) receptors located primarily on the vascular smooth muscle (4, 58). This endothelin-derived peptide is released in response to a variety of stimuli, including increases in pulsatile stretch (28), shear stress (32), hypoxia (23), and a reduction in pH. Since these physical and chemical stimuli are among the host of changes that take place within the skeletal muscle during exercise (27), ET-1 has been implicated as an important signaling molecule in the response to exercise. Indeed, circulating concentrations of ET-1 have been documented to increase in an intensity-dependent manner during exercise (29), suggesting a potential role of ET-1 in blood flow distribution and the support of MAP. Previous work in animals has evaluated the tonic ET-1-mediated restraint on vascular tone via ET_A and concomitant ET_A/ET_B receptor blockade during exercise and reported that ET_A receptor inhibition improved exercising muscle blood flow (34). Recently, our group (54) identified an attenuated vasoconstriction in response to an intra-arterial infusion of ET-1 compared with rest during dynamic knee extensor exercise in young, healthy humans. However, the role of endogenous ET-1, and specifically the role of the ET_A receptor, in the regulation of exer-
Excercising limb blood flow and MAP has yet to be investigated during exercise in humans.

Therefore, the present study sought to determine the endogenous contribution of the ETA receptor in the regulation of blood flow and MAP during increasing intensities of dynamic exercise. We hypothesized that 1) intra-arterial, local ETA receptor blockade would enhance exercising skeletal muscle blood flow in an intensity-dependent manner and 2) the reduction in vascular tone induced by ETA receptor blockade would attenuate the increase in MAP evoked by exercise of increasing intensity.

**METHODS**

**Subjects**

Eight young (24 ± 2 yr), healthy subjects were enrolled in the present study. All subjects were nonsmokers and were normally active. Subjects were not taking any prescription medication and were free of overt cardiovascular disease, as indicated by a health history. Protocol approval and written informed consent were obtained according to University of Utah and Salt Lake City Veterans Affairs Medical Center Institutional Review Board requirements. All data collection took place at the Utah Vascular Research Laboratory located at the Veterans Affairs Salt Lake City Geriatric, Research, Education, and Clinical Center.

**Protocols**

Subjects reported to the Utah Vascular Research Laboratory at 0800 hours on the experimental day. After 30 min of supine rest, two catheters [common femoral artery (CFA) and femoral vein] were placed using sterile techniques, as previously reported (1, 5, 57). After catheter placement, subjects rested for ∼30 min and then underwent the protocol as shown in Fig. 1. Due to the duration of the study, subjects were given 1/2 cup of corn flakes and 1/2 cup of skim milk 10 min before the start of the control (saline) and ETA receptor blockade (BQ-123) trials to prevent hypoglycemia (Fig. 1). All data collection took place with subjects in a semirecumbent position (60° reclined), and all experiments were performed in a thermoneutral environment.

Due to the long-lasting effects of BQ-123, the drug administration portion of the protocol always occurred after the saline portion of the protocol. Thus, to determine whether the observed changes in leg blood flow and MAP were the consequence of an exercise ordering effect rather than the effect of the drug per se, subjects returned to the laboratory on a separate day to undergo an exercise time control experiment. For this visit, catheters were not placed, and no drugs were administered. Apart from these differences, the timeline for this study was identical to the drug infusion day, with the addition of leg blood flow measurements immediately before and after a light meal to examine possible postprandial hemodynamic effects.

**Drug Infusions**

Thigh volumes were determined anthropometrically and then used for the calculation of drug doses. A selective ETA receptor antagonist (BQ-123, Clinalfa, Calbiochem-Novabiochem, Läufelfingen, Switzerland) was prepared in normal saline (0.9% NaCl) and administered intra-arterially into the CFA at 10 mmol·min⁻¹·1⁻¹ of thigh volume (infusion rates: 0.8–1.5 ml/min). This dose has been documented to induce an apparent plateau in vasodilation in both the forearm (14, 18) and quadriceps (47) without affecting MAP. BQ-123 has been documented to have a high affinity for the ETA receptor (20) and to effectively counteract the vasoconstrictor effect of ET-1 infusion in the human forearm (17). During the control trial, normal saline (0.9% NaCl) was administered intra-arterially into the CFA at the same infusion rates as BQ-123.

**Exercise Model**

The knee extensor paradigm implemented in this study has been previously described (2, 25, 38, 56). Subjects exercised at 60 rpm for 3 min at each of six exercise intensities (0, 5, 10, 15, 20, and 30 W) with 3 min of recovery after two successive exercise intensities (Fig. 1).

**Measurements**

Ultrasound Doppler assessments. Measurements of CFA blood velocity and vessel diameter were performed in the infused leg using a Logiq 7 ultrasound Doppler system (General Electric Medical Systems, Milwaukie, WI) operating in duplex mode. The Logiq 7 was equipped with a linear array transducer operating at an imaging frequency of 14 MHz. The CFA was insonated 2–3 cm proximal to the bifurcation of the CFA into the superficial and deep branches. The blood velocity profile was obtained using the same transducer with a Doppler frequency of 5 MHz operated in the high-pulsed repetition frequency mode (2–25 kHz). Care was taken to avoid aliasing the blood velocity spectra by using scale adjustments, especially during exercise. All blood velocity measurements were obtained with the probe appropriately positioned to maintain an insonation angle of 60° or less (26). The sample volume was maximized according to vessel size and was centered within the vessel on the basis of real-time ultrasound visualization. At all sample points, arterial diameter (in cm) and angle-corrected, time-averaged, and intensity-weighted mean blood velocity (Vmean) values were calculated using commercially available software (Logic 7). Using measured arterial diameter and Vmean, leg blood flow was calculated according to the following equation:

\[ \text{leg blood flow (in ml/min)} = \frac{\pi \times \left(\text{vessel diameter}^2\right) \times V_{\text{mean}}}{60} \]

Arterial blood pressure, vascular conductance, and heart rate assessment. Arterial blood pressure measurements were collected continuously from the indwelling catheter placed in the CFA with the pressure transducer placed at the level of the catheter (Transpac IV, Abbott Laboratories). MAP (in mmHg) was calculated as follows:

\[ \text{MAP} = \frac{\text{Systolic arterial pressure}}{\text{Diatostic arterial pressure}} \]

On the time control experiment day, MAP was measured noninvasively using finger photoplethysmography (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands). Leg vascular conductance was calculated as leg blood flow/MAP. BQ-123-induced changes in leg blood flow, arterial pressure, and leg vascular conductance were calculated as the difference between values during the BQ-123 and saline trials. Heart rate was monitored from a standard three-lead ECG recorded in duplicate on the data-acquisition device (Biopac, Goleta, CA) and the Logic 7.
Near-infrared spectroscopy. To determine muscle microvascular oxygenation (saturation) and extraction [deoxygenated hemoglobin (HHb)] (11) of one specific exercising muscle, near-infrared spectroscopy (NIRS) was used on the vastus lateralis (15). This technique is based on the absorbance and reflection of near-infrared light in muscle tissue by hemoglobin, myoglobin, and cytochrome c oxidase (7). Frequency-domain, multidistance NIRS (Oxiplex TS, ISS, Champaign, IL) was used to quantify the absolute concentrations of oxyhemoglobin (HbO2), HHb, and total hemoglobin (tHb) (all expressed in μM) (19). From these measured variables, microvascular oxygenation (saturation) was calculated according to the following equation: saturation = [HbO2/([Hb] + [HHb]) × 100.

Before use, in both conditions, the probe was calibrated using a block with known absorption characteristics to calculate the absorption and scattering coefficients. Before placement, the lateral portion of the thigh was cleaned, and double-sided adhesive tape was used to seat the diode, which was covered and further secured with coban (3M, St. Paul, MN). Data were acquired at 0.5 Hz, and 30-s averages were calculated for each stage, during the last minute of exercise (2:30–3:00 min).

Blood chemistry. A lippanel was obtained for all subjects by standard techniques. In the last 30 s of each exercise intensity, femoral arterial and venous blood samples (3–4 ml) were collected. Arterial and venous blood (1 ml) were presented anaerobically to a GEM 4000 blood gas analyzer and cooximeter (Instrumentation Laboratories, Bedford, MA) to obtain arterial and venous Hb and HbO2 saturations, Po2, hematocrit, lactate, and pH. Arterial and venous blood O2 content (in ml/dl) were calculated as: blood O2 content = 1.39 (Hb) × (O2 saturation/100) + 0.003 × Po2. Leg O2 consumption (Vao2; in ml/min) was calculated as follows: Vao2 = (arterial blood O2 content – venous blood O2 content) × leg blood flow. The remaining blood was spun down for plasma samples and stored at −80°C until analysis.

Plasma ET-1 concentrations were evaluated via quantitative enzyme immunoassay (R&D Systems, Minneapolis, MN), with a sensitivity of <1 pg/ml. Intra- and interassay coefficient of variation were 4.5% and 5.5%, respectively. Using arterial and venous plasma ET-1 or lactate concentrations with corrections for leg blood flow, "net ET-1 release" and "net lactate release" were calculated (21, 49) according to the following equation: net ET-1 or lactate release = (venous concentration – arterial concentration) × [leg blood flow × (101 – (hematocrit/100))].

Statistical Analyses

Statistics were performed with the use of commercially available software (SigmaStat 3.10, Systat Software, Point Richmond, CA). A 2 × 7 repeated-measure ANOVA was used to identify significant changes in measured variables within and between drug groups and across exercise intensities. When a significant main effect was found (α < 0.05), the Holm-Sidak method was used for α adjustment and post hoc analysis. All group data are expressed as means ± SE.

RESULTS

Subject Characteristics

Subject characteristics are shown in Table 1. All subjects were normotensive, with laboratory values within normal range.

Effect of BQ-123 at Rest and Exercise

At rest, the intra-arterial infusion of the ETA receptor inhibitor BQ-123 did not significantly change heart rate, MAP, leg blood flow, leg vascular conductance, leg VO2, leg arterial-venous O2 difference, NIRS signal, pH, net lactate release, or net ET-1 release (Figs. 2 and 3 and Tables 2 and 3). During exercise, BQ-123 induced a significant intensity-dependent increase in leg blood flow and vascular conductance (Figs. 2 and 3) accompanied by an intensity-dependent reduction in mean MAP (Figs. 2 and 3) and no change in heart rate (Table 2) compared with the saline trial. The vasodilation provoked by BQ-123 during exercise led to an increase in leg VO2 without an alteration in leg arterial-venous O2 difference (Table 2). Muscle microvasculature saturation increased significantly after ETA blockade, with no change in tissue HbO2, tissue HHb, or tissue tHb (Table 3). Additionally, a significant increase in net ET-1 release and no significant changes in net lactate release or pH were observed during BQ-123 infusion during exercise compared with the saline trial (Table 2).

Exercise Time Control Experiment

All eight subjects who participated in the drug infusion protocol returned to the laboratory for an exercise time control protocol. Measurements were highly reproducible, with no significant differences in leg blood flow or MAP between the two exercise bouts (Fig. 4).

DISCUSSION

There are several key findings from the present study. During isolated small muscle mass (knee extensor) exercise, leg blood flow and vascular conductance were significantly elevated in the exercising limb after ETA receptor inhibition, demonstrating a substantial (~20%) ET-1-mediated restraint of skeletal muscle blood flow. This increase in exercising leg blood flow was accompanied by an increase in leg VO2 and microvascular O2 saturation but with unchanged arterial-venous O2 difference or tissue HHb, suggesting a diminished intramuscular efficiency (i.e., greater VO2 for a given work rate) after ETA receptor inhibition (BQ-123). BQ-123 also blunted the intensity-dependent rise in MAP associated with exercise, suggesting that the ET-1 pathway contributes significantly to the exercise pressor response. A significant rise in net ET-1 release across the exercising leg was also observed, further supporting the regulatory role of this pathway during exercise. Taken together, these findings identified a significant contribution of the ET-1 pathway in the cardiovascular response to exercise, implicating vasoconstriction via the vascular ETA receptor as an important mechanism for both restraint
of exercising limb blood flow and the support of MAP in healthy, young adults.

**ETA-Mediated Restraint of Skeletal Muscle Blood Flow**

With increasing exercise intensity, one of the primary ways in which skeletal muscle blood flow is elevated to meet the metabolic demands of the exercising muscle is by reducing vascular tone in the exercising skeletal muscle. Indeed, the attenuated efficacy of infused sympathomimetics during exercise provides evidence that sympathetic vasoconstrictor pathways are at least partially inhibited during exercise (12, 48, 53, 54). However, this increase in skeletal muscle blood flow must be balanced with the concomitant need to support MAP (40).

With the knowledge that ET-1 provokes a sustained vasoconstriction (16, 34) and circulating levels increase during exercise (29), we sought to evaluate the putative role of the ET\(_A\) receptor in the regulation of skeletal muscle blood flow and the maintenance of MAP in the exercising limb vasculature during increasing intensities of dynamic exercise.

In support of our first hypothesis, BQ-123 administration potentiated the increase in exercising skeletal muscle blood flow in an intensity-dependent manner, demonstrating a clear role for ET\(_A\) receptors in restraining leg blood flow during exercise (Fig. 3). This finding is in contrast to that of McEnery et al. (33), who examined the effect of ET\(_A\) receptor blockade by 10.220.33.6 on October 23, 2017 http://ajpheart.physiology.org/ Downloaded from
on limb blood flow during 15 min of static intermittent handgrip exercise in hypertensive and normotensive individuals. This study reported no differences in forearm blood flow during exercise between blocked and control trials in the normotensive group, suggesting no apparent role of the ET\textsubscript{A} receptor in the regulation of vascular tone during handgrip exercise. The discrepancy between the present study and that of McEniery et al. may be partially attributed to the differences in the limbs that were exercised. Indeed, our group (55) and others (36) have identified substantial differences in the regulation of exercising limb blood flow between the arms and legs, and this limb specificity may partially explain the differing responses to ET\textsubscript{A} receptor blockade between the former study and the present study. Additionally, in the previous study by McEniery et al., the vascular beds perfusing the forearm may not have vasodilated to a degree that challenges the maintenance of MAP. In contrast, the present study used the knee extensor exercise paradigm to exercise the quadriceps, a muscle group with far greater vasodilatory capacity than the arm. With this approach, we identified, for the first time, a functional role of the ET-1 pathway in the regulation of exercise-induced muscle blood flow in the large, ambulatory muscle groups of the leg.

**ET-1 and Intramuscular Efficiency**

The efficiency of skeletal muscle during exercise is defined as the ratio of mechanical output to metabolic cost, as can be calculated from VO\textsubscript{2} (10, 46, 51). After ET\textsubscript{A} receptor blockade, we observed an increase in exercising leg VO\textsubscript{2} for the same amount of work performed in the saline trial, whereas leg arterial-venous O\textsubscript{2} difference and muscle microvascular HHb remained unchanged (Tables 2 and 3). This unique combination of elevated leg VO\textsubscript{2} under the condition of increased leg blood flow, and thus greater O\textsubscript{2} delivery, suggests a decrease in intramuscular efficiency after ET\textsubscript{A} receptor inhibition. This is in agreement with a previous study (22) in humans that used pharmacological manipulation to provoke vasodilation in the exercising muscle and further advances the concept that vasoconstrictor pathways contribute significantly to the optimization of O\textsubscript{2} delivery and VO\textsubscript{2} through restraint of skeletal muscle blood flow during exercise. Whether the documented increase

### Table 2. Impact of BQ-123 infusion on select physiological variables at rest and during exercise

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rest</th>
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<td>66 ± 4</td>
<td>80 ± 5†</td>
<td>87 ± 6†</td>
<td>91 ± 6†</td>
<td>94 ± 7†</td>
<td>97 ± 7†</td>
<td>101 ± 8†</td>
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<tr>
<td>BQ-123</td>
<td>70 ± 4</td>
<td>84 ± 5†</td>
<td>88 ± 5†</td>
<td>95 ± 6†</td>
<td>94 ± 7†</td>
<td>102 ± 8†</td>
<td>110 ± 12†</td>
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<td>146 ± 17†</td>
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<td>248 ± 33†</td>
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<td>122 ± 13†</td>
<td>198 ± 18‡</td>
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<td>289 ± 38‡</td>
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Data are expressed as means ± SE. *Significant difference from saline (P < 0.05); †significant difference from rest (P < 0.05).

### Table 3. Impact of BQ-123 infusion on select near-infrared spectroscopy variables at rest and during exercise

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<td>Saline</td>
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<td>BQ-123</td>
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<td>Deoxyhemoglobin, µM</td>
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<tr>
<td>Saline</td>
<td>18.6 ± 3.0</td>
<td>21.3 ± 5.0</td>
<td>21.5 ± 12.0</td>
<td>51.0 ± 12.0</td>
<td>50.2 ± 11.0</td>
<td>49.1 ± 10.0</td>
<td>49.1 ± 12.0</td>
</tr>
<tr>
<td>BQ-123</td>
<td>20.0 ± 5.0</td>
<td>20.2 ± 4.0</td>
<td>21.6 ± 5.0</td>
<td>23.9 ± 6.0</td>
<td>26.3 ± 8.0</td>
<td>28.2 ± 9.0</td>
<td>32.7 ± 11.0</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. *Significant main effect for condition (P < 0.05); †significant main effect for work rate (P < 0.05).
after ETA receptor blockade (Fig. 2). Interestingly, this served an overall reduction in the exercise pressor response "exercise pressor reflex" (35). In the present study, we observed a proportional increase in the leg blood flow to the exercising muscle through elevations in perfusion pressure, an event accomplished through the well-described mechanisms such as the ET-1 pathway, although additional studies are required to address this possibility.

**Perspectives**

In many pathophysiologies, an increase in exercising limb blood flow could be viewed as beneficial, inasmuch as O2 delivery to the exercising tissue is improved. However, the cardiovascular response to exercise appears to be governed by a very complex combination of vasodilatory and vasoconstrictor influences that optimize exercising muscle perfusion while protecting MAP. Data from the present study indicate that the ET-1 pathway significantly contributes to the maintenance of this balance during exercise, as evident by the fact that ETA receptor inhibition produced an increase in limb blood flow that was met with a decrease in MAP and increased leg VO2 (i.e., reduced efficiency) (Figs. 2 and 3 and Table 2). When viewed in conjunction with the marked rise in net ET-1 release during exercise (Table 2), these observed responses appear to indicate that ET-1 provides an important restraining influence to muscle blood flow during exercise, tempering the potential of this “sleeping giant” to vasodilate at the expense of MAP and intramuscular efficiency.

The importance of the ET-1 vasoconstrictor pathway in the cardiovascular response to exercise may be of particular relevance in populations whose phenotype is associated with high circulating levels of ET-1, such as hypertension (44), heart failure (6), chronic obstructive pulmonary disease (13), and the elderly (31). All of these populations are associated with some degree of exercise intolerance, which might be linked to the excessive increase in vascular tone induced by the ET-1 vasoconstrictor pathway. This raises the interesting possibility of ET-1 as a potential therapeutic target to improve skeletal muscle blood flow and ultimately exercise tolerance in these populations, although further studies are necessary to evaluate whether the present observations can be extended to these patient groups.
Experimental Considerations

Due to the slow-acting kinetics of BQ-123 binding and clearance, a dose-response protocol was not possible in the present study. Additionally, these specific properties of BQ-123 required that the study to be ordered so the control (saline) trial always preceded the BQ-123 trial. However, an exercise time control trial on a separate day demonstrated highly reproducible measurements of leg blood flow and MAP during repeated bouts of knee extensor exercise (Fig. 4), providing confidence in the comparison of the control and BQ-123 trials. Due to the noninvasive nature of this exercise control trial, blood samples were not collected, and we thus cannot exclude the possibility of an ordering effect on the outcome measures associated with blood chemistry determinations. Additionally, we acknowledge the possibility that some of the observed changes in peripheral hemodynamics are due to ET<sub>A</sub> receptor activation, as BQ-123 is highly selective for ET<sub>A</sub> receptors. However, a recent study (50) using combined ET<sub>A</sub> and ET<sub>B</sub> receptor inhibition failed to identify a significant role for ET<sub>B</sub> receptors in the regulation of vascular tone in humans. Finally, it is noteworthy that whether the increase in leg VO<sub>2</sub> after ETA receptor inhibition is due to alterations in muscle metabolism or hyperperfusion of the exercising muscle cannot be definitively determined from the present study.

Summary

This study has revealed a significant role of the ET-1 pathway in the cardiovascular response to exercise, implicating vasoconstriction via the ET<sub>A</sub> receptor as an important mechanism for both restraint of blood flow in the exercising limb and maintenance of MAP in healthy, young adults.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


