



Exercise reduces angiotensin II responses in rat femoral veins

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ABSTRACT

The control of blood flow during exercise involves different mechanisms, one of which is the activation of the renin-angiotensin system, which contributes to exercise-induced blood flow redistribution. Moreover, although angiotensin II (Ang II) is considered a potent venoconstrictor agonist, little is known about its effects on the venous bed during exercise. Therefore, the present study aimed to assess the Ang II responses in the femoral vein taken from sedentary and trained rats at rest or subjected to a single bout of exercise immediately before organ bath experiments. Isolated preparations of femoral veins taken from resting-sedentary, exercised-sedentary, resting-trained and exercised-trained animals were studied in an organ bath. In parallel, the mRNA expression of prepro-endothelin-1 (ppET-1), as well as the ET_A and ET_B receptors, was quantified by real-time PCR in this tissue. The results show that, in the presence of L-NAME, Ang II responses in resting-sedentary animals were higher compared to the other groups. However, this difference disappeared after co-treatment with indomethacin, BQ-123 or BQ-788. Moreover, exercise reduced ppET-1 mRNA expression. These reductions in mRNA expression were more evident in resting-trained animals. In conclusion, either acute or repeated exercise adapts the rat femoral veins, thereby reducing the Ang II responses. This adaptation is masked by the action of locally produced nitric oxide and involves, at least partially, the ET_B-mediated release of vasodilator prostanoids. Reductions in endothelin-1 production may also be involved in these exercise-induced modifications of Ang II responses in the femoral vein.

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1. Introduction

The control of blood flow during exercise involves different mechanisms, including the activation of the sympathetic nervous system and the release of local vasoactive mediators [40]. Additionally, prior evidence indicates the participation of the renin-angiotensin system in the active modification of vascular tone thereby contributing to the exercise-induced redistribution [17]. In the cardiovascular system, angiotensin II (Ang II), which is considered an important effector of this system, may work independently or in association with the sympathetic nervous system [2]. Moreover, depending on the vascular territory, Ang II responses may be modulated by other local mediators such as prostanoids, nitric oxide (NO) and endothelin-1 (ET-1) [4,15,35].

To achieve a better understanding of circulatory redistribution during exercise, it is necessary to understand the venous bed in detail. The venous bed is considered the primary compartment of capacitance in mammals because it stores approximately 60–80% of blood volume during rest [33]. This blood volume is rapidly shifted through the veins toward the heart during exercise-induced circulatory redistribution [38]. In this regard, some authors have proposed that active venoconstriction evokes a rapid self-contained blood transfusion to the stressed volume, maintaining or increasing the end-diastolic volume during exercise [32]. However, Rowell [34] argues that venoconstriction would cause a proportionally much larger alteration in resistance to flow, thereby impairing the venous return.

Although Ang II is considered a potent venoconstrictor agonist, little is known about its effects on the venous bed during exercise. Trained rats subjected to a single bout of exercise exhibited increased Ang II responses on the portal vein but not on the inferior vena cava, which suggests a territory-specific adaptation [3]. Interestingly, the portal vein receives the blood volume from the splanchnic territory, where previous studies agree that active venoconstriction participates in exercise-enhanced venous return

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[10,32]. Thus, for a better understanding of the effects of exercise on the venous bed, it is necessary to investigate veins that received blood from musculocutaneous circulation where the absence of appreciable venoconstriction may actually be beneficial because it impedes an uncontrolled increase in the resistance to the centripetal flow [34].

Therefore, the present study aimed to assess the Ang II responses in the femoral vein taken from sedentary and trained rats at rest or subjected to a single bout of exercise immediately before organ bath experiments. The involvement of prostanoids, NO and ET-1 in exercise-induced modifications was also investigated in the femoral vein.

2. Material and methods

2.1. Animals

One hundred forty-two male Wistar rats (350–450 g) were housed in plastic cages (50 cm × 40 cm × 20 cm) with five animals per cage. Food and water were available *ad libitum*. During the exercise protocol, rats were maintained in the training room under a 12 h light-dark cycle, with lights on at 07:00 h. Room temperature was maintained at 25 °C. Rats were used in accordance with ethical principles [9], and the study was approved by the Research Ethics Committee of the School of Medicine at Marília (Protocol n° 351/09).

2.2. Exercise protocol

The exercise protocol used was based on a previous study [25]. Briefly, animals were subjected to the maximal exercise test on a treadmill (Movement Technology LX 170) to determine their ability to run on the treadmill. Based on the results of this test, the animals were randomly assigned to sedentary or trained groups with a similar average of maximal exercise capacity in both groups. Then, the animals designated as trained were exercised 5 days per week for 1 h per day for 8–12 weeks. The exercise intensity was progressively increased by a combination of time and velocity, attaining 1 h per day at a velocity correspondent to 60% of maximal exercise by the third week. This protocol has been defined as constituting low-intensity physical training [21,25]. All of the animals used in the present study increased their maximal exercise capacity from the first to the sixth week, when the maximal exercise tests were repeated. This improvement of physical conditioning was considered an indirect indication of the efficacy of the physical training.

Two groups of sedentary and two groups of trained animals were subjected to the organ bath experiments in parallel. One group of sedentary and one group of trained animals were studied at rest, designated resting-sedentary and resting-trained animals, respectively. The other two groups of sedentary and trained animals underwent a single bout of exercise immediately before the organ bath experiments. These animals were designated as exercised-sedentary and exercised-trained, respectively.

2.3. Organ bath studies

Animals were killed in a CO₂ chamber and exsanguinated. The femoral vein (3–4 mm; two rings per animal) was prepared and set up in 2 mL organ baths. Rings were fixed to a stainless-steel hook attached to a stationary support as well as to a hook connected to an isometric force transducer. Rings were bathed in Krebs–Henseleit solution (composition in mmol/L): NaCl 130; KCl 4.7; CaCl₂ 1.6; KH₂PO₄ 1.2; MgSO₄ 1.2; NaHCO₃ 15; glucose 11.1). The solution was kept at pH 7.4 and 37 °C and bubbled continuously with a mixture of 95% O₂ and 5% CO₂. Tension was monitored continuously and recorded using a Powerlab 8/30 data-acquisition system

(ADInstruments, Castle Hill, NSW, Australia). Prior to administering drugs, rings were equilibrated for 60 min at a resting tension of 0.5 g. The time frame from the end of the exercise sessions to the beginning of the Ang II cumulative concentration–response curves was approximately 90 min. The responses (g) evoked by cumulatively adding Ang II (10^{−11} mol/L – 10^{−7} mol/L; Sigma) or ET-1 (10^{−11} mol/L – 10^{−6} mol/L; Sigma) directly into the organ bath were plotted to obtain concentration–response curves. The actions of Ang II were also evaluated by pretreating the rings for 20 min with 10^{−4} mol/L L-NAME and 10^{−5} mol/L indomethacin, non-selective nitric oxide synthase and cyclooxygenase inhibitors (Sigma), respectively, 10^{−6} mol/L BQ-123 (antagonist of endothelin receptor type A – ET_A; Sigma) or 10^{−6} mol/L BQ-788 (antagonist of endothelin receptor type B – ET_B; Sigma). All drugs were administered directly to the organ bath. Non-linear regressions (variable slope) for these curves revealed the *R*_{max} (maximal response; highest point of each concentration–response curve) and the pEC₅₀ (negative logarithm of the concentration that evoked 50% of the maximal response). The pEC₅₀ is indicative of the sensitivity of the system to the drug studied.

2.4. RNA extraction and cDNA synthesis

Total RNA was extracted from frozen femoral vein samples using TRIzol (Life Technologies, Gaithersburg, MD, USA), following the manufacturer's instructions. Total RNA was quantified using a NanoDrop Spectrophotometer – 2000 (NANODROP, USA). The concentrations were adjusted, and the samples were stored at −80 °C until use. cDNA synthesis was carried out using High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems™, USA), following the protocol provided by the manufacturer. All cDNA was quantified using a NanoDrop Spectrophotometer – 2000 (NANODROP, USA). The concentrations were adjusted, and samples were stored at −80 °C.

2.5. Gene expression analysis

All gene expression was measured by qRT-PCR on the Applied Biosystems 7500 Fast Real-Time PCR system (Applied Biosystems™, USA), using the cycling conditions recommended by Applied Biosystems. We used the following assays: preproET-1 (ppET-1) – Assay ID: Rn00561129.m1*, ET_A – Assay ID: Rn00561137.m1*, ET_B – Assay ID: Rn00569139.m1* and GAPDH – Assay ID: Rn99999916.s1.

The threshold values were uniformly set for all assays. All reactions were performed in duplicate. Replicates with standard deviations (SD) higher than 0.5 for the cycle threshold (CT) value were repeated or excluded from the analysis.

The amplification curve of each group was determined, and the CT values were obtained for all genes (ppET-1, ET_A, ET_B and GAPDH). We used the comparative CT method (ΔΔCT method), where we first calculated ΔCT = CT target – CT endogenous controls to normalize the target gene to the endogenous controls. Notably, the Relative Quantification (RQ) of ppET-1, ET_A, ET_B genes was calculated using the control group as a reference and using the 2^{−ΔΔCT} formula, which provides the percentage change, or how much more one gene is expressed in one group relative to another. All CT values were obtained using 7500 software 2.0, and these data were exported to Microsoft Excel (Microsoft, USA) to calculate 2^{−ΔCT} and RQ.

2.6. Statistical analysis

The data are presented as the mean ± SEM. The *R*_{max} and pEC₅₀ values were compared by two-way ANOVA followed by Bonferroni's post-test because one variable was the physical training and

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