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eNOS gene haplotype is indirectly associated with the recovery of cardiovascular autonomic modulation from exercise

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ABSTRACT

Polymorphisms in the endothelial nitric oxide synthase (eNOS) gene decrease expression and activation of eNOS in vitro, which is associated with lower post-exercise increase in vasodilator reactivity in vivo. However, it is unknown whether such polymorphisms are associated with other eNOS-related phenotypes during recovery from exercise. Therefore, we investigated the impact of an eNOS haplotype containing polymorphic alleles at loci −786 and 894 on the recovery of cardiovascular autonomic function from exercise. Sedentary, non-obese, healthy subjects were enrolled [n = 107, age 32 ± 1 years (mean ± SEM)]. Resting autonomic modulation (heart rate variability, systolic blood pressure variability, and spontaneous baroreflex sensitivity) and vascular reactivity (forearm hyperemic response post-ischemia) were assessed at baseline, 10, 60, and 120 min after a maximal cardiopulmonary exercise test. Besides, autonomic function was assessed by heart rate recovery (HRR) immediately after peak exercise. Haplotype analysis showed that vagal modulation (i.e., HF n.u.) was significantly higher, combined sympathetic and vagal modulation (i.e., LF/HF) was significantly lower and total blood pressure variability was significantly lower post-exercise in a haplotype containing polymorphic alleles (H2) compared to a haplotype with wild type alleles (H1). HRR was similar between groups. Corroborating previous evidence, H2 had significantly lower post-exercise increase in vasodilator reactivity than H1. In conclusion, a haplotype containing polymorphic alleles at loci −786 and 894 had enhanced recovery of autonomic modulation from exercise, along with unchanged HRR, and attenuated vasodilator reactivity. Then, these results suggest an autonomic compensatory response of a direct deleterious effect of eNOS polymorphisms on the vascular function.

1. Introduction

Well-conducted in vitro studies showed that some polymorphisms in the endothelial nitric oxide synthase (eNOS) gene lead to lower nitric oxide (NO) synthesis, due to reduction in eNOS expression and/or activation (Nakayama et al., 1999; Joshi et al., 2007; Zhang et al., 2008). However, in vivo, the impact of eNOS polymorphisms on complex multi gene traits depends on several factors and seems to be evident, particularly, when NO production is stimulated (Rossi et al., 2003; Dias et al., 2009), such as by dynamic exercise involving recruitment of large muscle mass (e.g., running) (Neves et al., 2010; Rocha et al., 2012; Silva et al., 2013). In this sense, our group showed that healthy subjects with the 894G>T polymorphism in the eNOS gene have lower vasodilator reactivity to ischemia (Neves et al., 2010) and mental stress (Rocha et al., 2012) than wild type counterparts after a single bout of exercise. Furthermore, haplotype analysis showed that the effect on vasodilator reactivity to ischemia was evident, particularly, when the 894G>T occurred concomitantly with the −786G>T polymorphism in the eNOS gene (haplotype −786C/4b/894T; a haplotype is a combination of alleles in a single chromosome) (Silva et al., 2013). However, it remains unknown how the unfavorable effect of this eNOS haplotype on vascular function interacts with other integrative physiological mechanisms, such as the autonomic regulation of the cardiovascular system.

NO synthesized by eNOS is one of the mediators of cardiovascular autonomic regulation. This occurs because NO acts not only autocrinally in the endothelium, but also paracrinally, since it diffuses from the

Abbreviations: Alpha-LF, index of spontaneous baroreflex sensitivity; ANCOVA, analysis of covariance; ANOVA, analysis of variance; BMI, body mass index; DBP, diastolic blood pressure; DNA, deoxyribonucleic acid; eNOS, endothelial nitric oxide synthase; H1, haplotype 1; H2, haplotype 2; HDL, high density lipoprotein; HF, high frequency; HR, heart rate; HRR, heart rate recovery; HRR_{1min}, heart rate at the first minute of recovery; HRRτ, time to obtain 63% of the total heart rate decay response; LDL, low density lipoprotein; LF, low frequency; LF/HF, ratio between low and high frequency; NO, nitric oxide; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; RMSSD, root mean square of successive beat interval differences; SBP, systolic blood pressure; SEM, standard error of the mean; $V'O_{2peak}$, peak oxygen consumption.

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endothelium to nearby tissues (Paton et al., 2002), modulating, for example, peripheral autonomic receptors (Meyrelles et al., 2003) and central autonomic neurons (Sakai et al., 2000). In addition, eNOS is constitutively expressed in the sinus node (Brahmajothi et al., 2007) and cardiomyocytes (Barouch et al., 2002), where it contributes to the autonomic regulation of the heart (Paton et al., 2002). Consequently, NO acts as a sympatholytic agent, decreasing central sympathetic outflow (Hirooka et al., 2011), and attenuating cardiac response to sympathetic stimulation (Gyurko et al., 2000), as well as increasing the activity of central vagal neurons (Travagli et al., 1994) and enhancing cardiac response to vagal stimulation (Feron et al., 1998; Chowdhary et al., 2004). Hence, taking into account that subjects with eNOS haplotype containing polymorphic alleles have reduced exercise-mediated increase in vasodilator reactivity, it would be expected that they also present altered autonomic regulation during the recovery from exercise. This would demonstrate a direct effect of eNOS polymorphisms on the autonomic regulation, which would corroborate findings about the impact of cardiovascular risk factors on vascular and autonomic function, since in this situation there is parallel deterioration of both phenotypes (Huikuri et al., 1999).

On the other hand, pharmacological studies showed that acute systemic inhibition of NO synthesis leads to vasoconstriction (Charkoudian et al., 2006; Gamboa et al., 2007), which activates baroreflex buffering (Gamboa et al., 2007), increases cardiac vagal modulation (Chowdhary et al., 2000), and decreases muscle sympathetic nerve activity (Charkoudian et al., 2006). Altogether, these compensatory responses tend to maintain blood pressure at normal levels (Charkoudian et al., 2006). Moreover, low level NO blockade during some days in dogs was compensated by the renin–angiotensin–aldosterone system (Seeliger et al., 2001), where renin plasma concentration decreased and sodium excretion increased, contributing to avoid blood pressure elevation. Therefore, it is also conceivable that as vasodilator reactivity is blunted after exercise in the eNOS haplotype containing polymorphism alleles (Silva et al., 2013), the autonomic system could compensate for the vascular deterioration. This would characterize the predominance of an indirect effect of eNOS polymorphisms on the autonomic nervous system.

Given this background, the aim of this study was to investigate the impact of eNOS polymorphisms on the cardiovascular autonomic regulation after a bout of maximal dynamic exercise. In order to get a clearer picture about the relationship among eNOS polymorphisms and autonomic regulation, we decided to investigate specifically a haplotype that is known to be associated with reduced exercise-mediated increase in vasodilator reactivity (haplotype −786C/4b/894T) (Silva et al., 2013).

2. Materials and methods

2.1. Subjects

Subjects were recruited through advertisements in the University and in local newspapers. Approximately 1000 people volunteered to participate, but 107 subjects (84 women and 23 men) met the inclusion criteria and had autonomic data collected satisfactorily. The reasons to exclude subjects were problems in autonomic recordings encompassing cardiac arrhythmias at rest and/or exercise and non-physiological hypotension immediately after peak exercise, which were associated with clinical symptoms (e.g., orthostatic intolerance). Data about vasodilator reactivity from 104 of these subjects were presented previously (Silva et al., 2013). The eligibility requirements were verified through clinical history assessment, physical examination, blood pressure measurement on two different days, biochemical blood analyses, resting electrocardiogram, and maximal cardiopulmonary exercise testing. Subjects had to meet the following criteria to be included in the study: age between 18 and 49 years; women with regular menstrual cycle; absence of any diagnosed disease and no recent infections; body mass index (BMI) between 18.5 and 29.9 kg/m^2; total cholesterol <240 mg/dL; low density lipoprotein (LDL) <160 mg/dL; triglycerides <200 mg/dL; glucose <126 mg/dL; systolic blood pressure (SBP) <140 mm Hg and/or diastolic blood pressure (DBP) <90 mm Hg; do not smoke; do not use medications with exception of oral contraceptives; normal resting and exercise electrocardiograms; and being sedentary (not being engaged in exercise activities lasting 30 min or more, 3 times per week during the last 3 months). After genotyping and estimating haplotypes, only data from haplotype 1 (H1) and haplotype 2 (H2) remained in the study. The study was conducted in accordance with the Declaration of Helsinki, it was approved by the Institutional Ethics Committee and written informed consent was obtained from all volunteers prior to participation in the study.

2.2. Haplotypes

Genomic DNA was extracted from circulating leucocytes using the salting out method (Salazar et al., 1998). The 4b4a genotype was identified directly after a polymerase chain reaction (PCR), while the −786T>C and 894G>T genotypes were identified by PCR followed by restriction fragment length polymorphism (RFLP). Quality control for these assays was assessed by random selection of samples to be re-genotyped by two independent researchers. No misgenotyping was observed. Further details about the genotyping procedures were published previously (Neves et al., 2010; Rocha et al., 2012; Silva et al., 2013). Based on previous studies (Nakamura et al., 1998; Nakayama et al., 1999; Rossi et al., 2003; Metzger et al., 2005; Joshi et al., 2007; Nejatizadeh et al., 2008; Dias et al., 2009; Neves et al., 2010; Silva et al., 2011; Rocha et al., 2012; Silva et al., 2013), the alleles were classified as follows: locus −786, wild = T, polymorphic = C; intron 4, wild = 4b, polymorphic = 4a or 4c; locus 894, wild = G, polymorphic = T. Two haplotypes for each subject were inferred using the software PHASE version 2.1 (University of Oxford, USA) (Stephens et al., 2001). The analysis identified seven types of haplotypes (H1 to H7), as described previously (Silva et al., 2013), but only H1 and H2 were used in the present study. H1 had only wild type alleles, whereas H2 had polymorphic alleles at loci −786 and 894. The volunteers and the researchers were blinded to the genotypes and haplotypes during the study.

2.3. Biochemical blood analyses

Blood was drawn from a vein in the antecubital fossa in the morning, after 12 h of fasting. Cholesterol and its subfractions [high density lipoprotein (HDL) and LDL], as well as triglycerides, were determined by dry chemistry method. Plasma glucose was measured by an enzymatic in vitro test.

2.4. Experimental protocol

The experimental protocol was conducted in the morning, 1 h after a standardized light breakfast. The evaluation was conducted between the 1st and the 12th days after the onset of menstruation. Subjects did not drink alcohol or caffeinated beverages and did not perform intense physical activities within the previous 24 h to the experimental day. All the autonomic and vascular data were recorded in the same session and room. During the experiment, subjects were placed in the supine position in a quiet air-conditioned room (≈24 °C), and rested quietly for 10 min prior to any measurement. Then, resting autonomic modulation was assessed by heart rate (HR) variability, SBP variability, and spontaneous baroreflex sensitivity. Besides, resting vascular reactivity was assessed by venous occlusion plethysmography. After that, subjects were submitted to a cardiopulmonary exercise test, when the cardiac autonomic function was assessed by the HR decay during the transition from peak exercise to an active recovery (i.e., walking), which is known as HR recovery (HRR). Immediately after, subjects were placed again in