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Beta-1 and beta-2 adrenergic receptor polymorphism and association with cardiovascular response to orthostatic screening☆

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ABSTRACT

Variation in the beta-1 and beta-2 adrenergic receptor genes (ADRB1 and ADRB2, respectively) may influence cardiovascular reactivity including orthostatic stress. We tested this hypothesis in a head-up tilt (HUT) screening protocol in healthy young adults without history of syncope. Following brachial arterial catheter insertion, 120 subjects (age 18–40, 72 females, Caucasian) underwent 5 min 60° HUT. Polymorphisms tested were: Ser49/Gly and Arg389/Gly in ADRB1; and Arg16/Gly, Gln27/Glu, and Thr164/Ile in ADRB2. Three statistical models (recessive, dominant, additive) were evaluated using general linear models with analysis for each physiologic variable. A recessive model demonstrated a significant association between Arg16/Gly and: absolute supine and upright HR; HUT-induced change in cardiac index (CI), stroke index (SI) and systemic vascular resistance (SVR); and supine and upright norepinephrine values. Blood pressure was not influenced by genotype. Fewer associations were present for other polymorphisms: Ser49/Gly and the change in SI (dominant model), and Arg389/Gly and supine and HUT norepinephrine (additive model). We conclude that in this population, there is a robust association between Arg16/Gly and HUT responses, such that 2 copies of Arg16 increase supine and upright HR, and greater HUT-induced decreases in CI and SI, with greater increases in SVR and norepinephrine. ADRB1 gene variation appears to impact SI and plasma NE levels but not HR. Whether ADRB2 gene variation is ultimately disease-causing or disease-modifying, this study suggests an association between Arg16/Gly and postural hemodynamics, with sympathetic noradrenergic activity affected in a similar direction. This may have implications in the development of orthostatic disorders.

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1. Introduction

Growing evidence suggests that genetic variation in beta-adrenergic receptors influences intermediate physiologic traits in healthy humans with relevance to distant, more complex phenotypes such as cardiovascular disease. Single nucleotide polymorphisms (SNPs) in the beta-1 adrenergic receptor gene (ADRB1) and the beta-2 adrenergic receptor gene (ADRB2) have been shown to influence cardiovascular function (Brodde, 2008; Eisenach and Wittwer, 2010).

Heart rate control is multifactorial with both adrenergic and vagal influences working in opposition to result in observed heart rate. Due to the predominance of beta-1 receptors in the heart, it is reasonable to postulate that polymorphisms in ADRB1 may influence HR. A previous report showed that individuals homozygous for Ser49 had higher mean resting HR compared with heterozygotes, and Gly49 homozygotes had lower resting HR than either heterozygotes or Ser49 homozygotes (Ranade et al., 2002). For position 389, Gly389 homozygotes had higher resting HR compared with heterozygotes or Arg389 homozygotes (Ranade et al., 2002; Wilton et al., 2008).

Polymorphic variation in ADRB2 has repeatedly been shown to influence sympathoexcitatory responses. We have reported that HR and cardiac output (CO) responses to isometric handgrip exercise are influenced by Arg16/Gly, as individuals who are homozygous for Gly16 exhibit a trend toward slower resting HR, with a greater increase in HR and CO compared with Arg16 homozygotes (Eisenach et al., 2004, 2005). Position 27 of the ADRB2 gene has also been shown to influence physiologic characteristics, but less is known on how these SNPs interact to influence cardiovascular reactivity to orthostatic stress. Furthermore, conflicting data exists regarding the association between resting HR and ADRB2 gene variants, with evidence that the Gly16 allele is associated with a lower resting HR, while one study did not find an association with resting HR and another found a trend toward a lower HR in young people with the Arg16+Gln27 haplotype (Ranade et al., 2002; Castellano et al., 2003; Snyder et al., 2006a, 2006b; Wilk et al., 2006).

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Gene variants that affect the cardiovascular response to head-up tilt (HUT) testing in healthy individuals may provide prognostic or disease-modifying implications for orthostatic disorders. Identifying associations is particularly useful in healthy individuals without history of syncope in order to eliminate potential confounders such as age, medications, and alterations in physical and behavioral lifestyle. For example, a recent analysis of 129 adults aged 18 to 67 years with recurrent syncope found no association between polymorphisms in ADRB1 and ADRB2 and hemodynamic measures during HUT (Sorrentino et al., 2010). However, no information exists regarding these gene polymorphisms and the hemodynamic and catecholamine responses to HUT in healthy non-fainters. The objective of this study was to investigate the impact of ADRB1 and ADRB2 SNPs on cardiovascular and arterial catecholamine responses to orthostatic stress. We hypothesized that ADRB1 and ADRB2 gene variation would influence the hemodynamic and catecholamine responses to HUT.

2. Methods

2.1. Subjects

This study was performed as part of an ongoing Mayo IRB-approved protocol in our laboratory to evaluate the effect of adrenergic receptor gene variation on cardiovascular traits (*ClinicalTrials.gov*: "High Resolution Phenotyping in Healthy Humans"). Following written informed consent, 138 healthy individuals between 18 and 40 years of age were recruited from a pool of subjects (n>800) that had previously been genotyped for ADRB2 rs1042713 and rs1042714 (Arg16/Gly and Gln27/Glu, respectively) by amplification of the relevant fragment from genomic DNA by polymerase chain reaction as previously described (Bray et al., 2000; Garovic et al., 2003). Additionally, using Taqman™ (Applied Biosystems Inc., Foster City, CA) we added the ADRB2 rs1800888 (threonine/isoleucine, Thr164/Ile), and ADRB1 rs1801252 and rs1801253 (Ser49/Gly and Arg389/Gly, respectively). Importantly, emphasis was placed on recruiting individuals who were double homozygotes for the three common homozygous combinations in ADRB2: Arg16 + Gln27, Gly16 + Gln27, and Gly16 + Glu27. This was done to achieve adequate power to determine potential interactions between ADRB2 positions 16 and 27. The Thr164/Ile polymorphism has a minor allele frequency of 0.02 and only 2 individuals bore 1 copy of the isoleucine minor allele, and provided neither quantitative nor qualitative influence of this SNP on the variables. Exclusion criteria included age over 40 for men, age over 50 or post-menopausal for women, use of tobacco products, use of any medication affecting the cardiovascular system, and any acute or chronic disorder which could affect cardiovascular function. Highly trained athletes were also excluded. The female participants underwent pregnancy test screening within 48 h of the study. Additionally, all women were studied in the early follicular phase of the menstrual cycle (within 7 days from the onset of menses) or in the low-hormone phase of oral contraceptives (7 days of placebo) to minimize variability in autonomic control of cardiovascular function due to reproductive hormones (Minson et al., 2000; Charkoudian, 2001).

2.2. Protocol

All studies were begun between 7 AM and 1 PM. Prior to the study, the participants abstained from caffeine, exercise, and alcohol for 24 h. The participants fasted for at least 4 h. The study room temperature was maintained between 21 and 23 °C. A peripheral intravenous catheter was placed in the dominant arm and a 5-cm, 20 gauge catheter was placed in the brachial artery of the non-dominant arm after local anesthetic (lidocaine 2%). Arterial blood pressure (AP) was measured after the catheter was connected to a pressure transducer. This catheter was used to obtain blood samples for the determination of plasma epinephrine and norepinephrine concentrations via high-performance liquid chromatography (Ramirez-Marrero et al., 2008). A three-lead electrocardiogram was applied to allow continuous HR measurement.

2.3. Head-up tilt

After instrumentation, subjects were secured to a tilt table with belts across the thighs and upper trunk. Feet were positioned flat on a footboard and subjects were instructed to refrain from leg contractions or weight-shifting during the tilt. Subjects rested quietly for 20 min, concluding with an arterial blood sample (5 mL) for catecholamine levels during supine rest. Recording began for 5 min while supine. Then subjects were tilted to 60° for 5 min. An arterial blood sample was repeated during the final 30 s of HUT as previously described (Ramirez-Marrero et al., 2008). If subjects were not feeling well or exhibited hemodynamic signs of impending syncope, the tilt table was returned to supine with simultaneous arterial blood sampling. Hemodynamic data were averaged during supine and HUT.

2.4. Data analysis

Hemodynamic data were digitized at 200 Hz, stored on computer, and analyzed off-line with signal processing software [Windaq; Dataq Instruments, Akron, Ohio, USA for head-up tilt (HUT)]. Modelflow technology (Beatscope) was used for determination of stroke volume and cardiac output. Furthermore, these values were indexed to body surface area because of the variability in gender and height among genotypes. Systemic vascular resistance was calculated from mean arterial pressure ÷ cardiac index.

3. Statistical analysis

Values are expressed as means ± standard error. The association of genotype with the various physiologic response variables was assessed using general linear models with separate analyses performed for each physiologic response including cardiovascular and catecholamine data for the two ADRB1 SNPs (positions 49 and 389) and the two ADRB2 SNPs (positions 16 and 27). Three common genetic models were evaluated where 0 = no effect, 1 = effect, and 2 = twice the effect. The models included a recessive, dominant and additive form. The recessive model assumes that an effect (=1) will only be shown if there are two copies of the minor allele and no effect (=0) if the subject is heterozygous, with one copy of the minor allele and one copy of the major allele, or if the subject is homozygous for the major allele. The dominant model assumes that if one copy of the minor allele is present the effect (=1) will be manifest. Thus, the effect would be seen in heterozygous subjects and subjects who are homozygous for the minor allele. The additive model assumes that with one copy of the minor allele the effect (=1) is present and with two copies of the minor allele (in homozygous individuals) twice the effect (=2) is present. For ADRB1 at positions 49 and 389 glycine is designated as the minor allele. For ADRB2 at position 16, arginine is designated as the minor allele and at position 27, glutamic acid is designated as the minor allele. See Table 1. All subjects were included in these analyses. For subjects who could not tolerate HUT, physiologic responses measured immediately prior to abandoning the HUT maneuver were used for the analysis. Supplemental analyses comparing demographic and physiologic responses between those who were able to tolerate HUT versus not were performed using the chi-square test (or Fisher's exact test) for categorical variables and the two-sample *t*-test for continuous variables. A power analysis based on our laboratory's previous work revealed a minimum sample size well below the 120 subjects that were enrolled. Post-hoc power analysis revealed a power of 1.00, indicating that this study was adequately powered.