Published in final edited form as:

Auton Neurosci. 2011 October 28; 164(1-2): 89–95. doi:10.1016/j.autneu.2011.07.004.

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

ED Wittwer¹, Z Liu², ND Warner¹, DR Schroeder³, AM Nadeau³, AR Allen¹, CJ Murillo¹, RL Elvebak¹, BM Aakre¹, and JH Eisenach¹

¹Department of Anesthesiology, Mayo Clinic, Rochester, MN, USA

²Department of Cardiology, Medical School of Zhejiang University, Hangzhou, China

³Department of Biostatistics and Health Sciences Research, Mayo Clinic

Abstract

Variation in the beta-1 and beta-2 adrenergic receptor genes (ADRB1 and ADRB2, resp.) 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; 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 increases 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.

Keywords

beta-1 adrenergic receptor; beta-2 adrenergic receptor; heart rate; head-up tilt; fainting; genetic model

Correspondence: Erica D. Wittwer, M.D., Ph.D. Department of Anesthesiology, Mayo Clinic, 200 First St. SW, Rochester, MN 55905, Phone: 507-993-6765; Fax 507-255-7300, Wittwer.Erica@mayo.edu.

This work was presented at the 21st Intl. Symposium on the Autonomic Nervous System

CONFLICTS OF INTEREST/DISCLOSURES: none

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

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., 2005; Eisenach et al., 2004). Position 27 of the ADRB2 gene has also been shown to influence physiologic characteristics, but less is known 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 towards a lower HR in young people with the Arg16 + Gln27 haplotype (Castellano et al., 2003; Ranade et al., 2002; Snyder et al., 2006a; Snyder et al., 2006b; Wilk et al., 2006).

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 response to HUT.

Methods

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, resp.) 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, resp.). 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 hours 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 (Charkoudian, 2001; Minson et al., 2000).

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 hours. The participants fasted for at least 4 hours. 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.

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 seconds of HUT as previously described (Ramirez-Marrero et al., 2008). If subjects were not feeling well or exhibiting 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.

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.

Statistical Analysis

Values are expressed as means $\pm$ 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 (position 49 and 389) and the two ADRB2 SNPs (position 16 and 27). Three common genetic models were evaluated where 0 = no effect, 1 = effect, 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 position 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 this study was adequately powered.

Results

Demographics

There were 72 females and 48 males, with a mean ($\pm$ SE) age of 25.6 ± 0.6 years and a mean BMI of 24.1 ± 0.2 kg/m². The genotype distribution for ADRB1 and ADRB2 are as follows: For ADRB1 position 49, 96 subjects were Ser homozygotes, 20 subjects were heterozygous, 2 subjects were Gly homozygotes, and for 2 subjects the genotype was undetermined. For position 389, 62 subjects were Arg homozygotes, 41 were heterozygous, 8 were Gly homozygotes, and for 9 subjects the genotype was undetermined. For ADRB2 position 16, 35 subjects were Arg homozygotes, 26 were heterozygotes and 59 were Gly homozygotes. For position 27, 58 subjects were Gln homozygotes, 26 were heterozygotes and 36 were Glu homozygotes. For position 164, 115 subjects were Thr homozygotes, 2 subjects were heterozygotes, and for 3 subjects the genotype was undetermined.

ADRB2 Position 16

As shown in Figure 1A, for position 16 of ADRB2, baseline HR values fit a recessive model with Gly16 homozygotes and heterozygote subjects having lower baseline HR (61 ± 1 and 58 ± 2 bpm, respectively) compared with Arg16 homozygotes, i.e., subjects with two copies of the minor allele, (66 ± 1 bpm). Heart rate during HUT also fit a recessive model, with Gly16 homozygotes and heterozygote subjects having lower HUT HR (78 ± 2 and 75 ± 2 bpm, respectively) compared with Arg16 homozygotes (86 ± 2 bpm).

Figure 1B depicts that the change in cardiac index (CI) from baseline to HUT (or Δ CI) for position 16 fit a recessive model with Gly16 homozygotes and heterozygotes having a smaller decrease in Δ CI (-0.18 ± 0.06 and -0.17 ± 0.06 , respectively) compared with the

Arg16 homozygotes (-0.33 ± 0.06), $p=0.01$. Similarly, as shown in Figure 1C, the change in stroke index (SI) from baseline to HUT (or Δ SI) for position 16 of ADRB2 fit a recessive model with Gly16 homozygotes and heterozygotes having a smaller decrease in Δ SI (-11.9 ± 0.52 and -12.44 ± 0.9 , respectively) compared with the Arg16 homozygotes (-14.0 ± 0.7), $p=0.02$. The change in systemic vascular resistance is shown in Figure 1D. The SVR from baseline to HUT (or Δ SVR) fit a recessive model as well for position 16 with Gly16 homozygotes and heterozygotes having a smaller increase in Δ SVR (1.0 ± 0.2 and 0.95 ± 0.3 , respectively) from baseline to HUT compared with Arg16 homozygotes (1.8 ± 0.3), $p=0.02$. The mean absolute values and ranges for MAP, CI, SI and SVR are reported in Table 2.

As shown in Figure 2, consistent with the genotype effect on HR, plasma NE levels were influenced by genotype at baseline and during HUT. Baseline and HUT plasma NE levels also fit a recessive model with Gly16 homozygotes and heterozygote subjects having lower NE levels. Baseline values were 110 ± 6 and 119 ± 9 pg/mL for Gly 16 homozygotes and heterozygotes, respectively, compared with Arg16 homozygotes (132 ± 9 pg/mL), $p=0.04$. HUT values were 252 ± 11 and 264 ± 19 pg/mL for Gly 16 homozygotes and heterozygotes, respectively, compared with Arg16 homozygotes (298 ± 18 pg/mL), $p=0.03$. Epinephrine levels during HUT were not associated with genotype.

ADRB2 Position 27

For Position 27, as shown in Figure 3, baseline HR values fit a dominant model with subjects with at least one copy of glutamic acid having a lower HR (62 ± 2 and 58 ± 1 bpm, for Glu homozygotes and heterozygotes, respectively) compared with Gln homozygotes (64 ± 1), $p=0.02$. At Position 27 of ADRB2, the HUT HR also fit a dominant model with subjects with at least one copy of glutamic acid having a lower HR (79 ± 2 and 75 ± 2 bpm, for Glu homozygotes and heterozygotes, respectively) compared with Gln homozygotes (82 ± 1 bpm), $p=0.02$. There was no association between position 27 and Δ CI, Δ SI and Δ SVR.

ADRB1

Position 49 of ADRB1 did not have differences between genotypes for baseline hemodynamic or catecholamine levels. During HUT, at position 49 of ADRB1 the change in stroke index (SI) from baseline to HUT, or Δ SI, fit a dominant model with Gly homozygotes having a smaller decrease in SI compared with the Arg heterozygotes or homozygotes (-6 ± 3 , -12 ± 1 and -13 ± 1 , respectively), $p=0.03$. Position 389 had baseline plasma NE levels which fit an additive model with significance such that the Gly homozygotes had a lower mean plasma NE level and with each copy of Arg the mean plasma NE level is increased (92 ± 13 , 107 ± 7 and 127 ± 6 pg/mL for Gly homozygotes, heterozygotes and Arg homozygotes, respectively), $p=0.04$. HUT plasma NE levels also fit an additive model with Gly homozygotes having the lowest mean NE levels (230 ± 36 pg/mL) and with each copy of Arg the mean NE levels increased (256 ± 13 and 281 ± 14 pg/mL for heterozygotes and Arg homozygotes, respectively), $p=0.05$. However, these values were not accompanied by genotype-dependent hemodynamic responses.

HUT intolerance

Interestingly, thirteen participants did not tolerate HUT for 5 min due to symptoms or sudden hemodynamic signs of impending syncope, none of whom reported previous recurrent syncope or pre-syncope episodes. Demographically, these individuals on average were slightly younger and weighed less than the individuals who tolerated the HUT (23 ± 1 yrs and 65 ± 3 kg compared to 26 ± 1 yrs and 72 ± 1 kg) although there was no difference in height or BMI. The proportion of female:male (10:3) was not different from those who tolerated HUT. Blood pressure values at baseline were not different between individuals

who did not tolerate HUT and those that did. Mean systolic blood pressure, diastolic blood pressure and MAP differed between the intolerant subjects and the tolerant subjects during HUT (114 ± 3 , 68 ± 2 and 82 ± 2 mmHg vs 126 ± 1 , 78 ± 1 and 93 ± 1 mmHg, respectively), $p<0.05$. As shown in Figure 4A the change in pressure from supine to HUT (Δ values) also differed significantly between the HUT intolerant and HUT tolerant subjects, $p<0.0001$. Δ SVR values differed between the tolerant group and intolerant group with the tolerant group having a statistically higher Δ SVR (1.4 ± 0.2 and 0.3 ± 0.5 , respectively). See Figure 4B. The change in HR (Δ HR) and SV (Δ SV) were not different between the groups. There were no differences in Epi and NE values between the groups at baseline, but differences were found in HUT values and Δ values. The HUT tolerant participants had lower increases in plasma Epi levels (HUT value: 68 ± 5 , Δ Epi value: 36 ± 4) compared with the intolerant participants (HUT value: 384 ± 130 , Δ Epi value: 345 ± 124 , $p=0.036$ and $p=0.032$, respectively). Plasma NE levels were higher in the tolerant group (HUT value: 275 ± 10 , Δ NE value: 156 ± 8) compared to the intolerant group (HUT value: 202 ± 19 , Δ NE value: 110 ± 17 , $p=0.016$ and $p=0.049$). See Figure 4C. The groups were assessed for association between tolerance to HUT and genotype and while there was a trend towards more Gly16 homozygotes ($n=10$) in the intolerant group this was not statistically significant ($p=0.15$).

Discussion

To our knowledge, this is the first study to determine an association between ADRB1 and ADRB2 gene variation and the cardiovascular responses to orthostatic screening in healthy individuals with no history of syncope. The major finding was that variation of SNP position 16 in the ADRB2 gene is strongly associated with HR while supine, with Gly 16 homozygotes and heterozygotes demonstrating a lower HR compared with Arg 16 homozygotes in addition to tilt-induced differences in the change in HR, stroke index, and systemic vascular resistance. No relationship with ADRB2 position 16 was found with blood pressure, as it did not differ between the genotype groups. As a possible mechanistic explanation, the NE values while supine and during HUT were also associated with ADRB2 position 16 in a direction consistent with the hemodynamic variables, such as the Arg16 homozygotes having a greater increase in SVR and higher NE during HUT compared with the Gly16 homozygotes.

The next finding was that there was also an association between ADRB2 position 27 and supine and orthostatic HR, but not for any of the other hemodynamic or catecholamine variables. In this context, consideration must be given to the mode of inheritance for the homozygous forms of these SNPs. The association between Gln27 and HR while supine and upright may have been primarily due to linkage disequilibrium with Arg16, as all individuals in our genotyped recruitment pool who are Arg16 homozygotes are also Gln27 homozygotes. Because of this relationship, an additional analysis of position 27 while excluding the Arg16 homozygotes ($n=35$) revealed that the association between HR and position 27 was no longer significant. Although this analysis may have been underpowered due to a reduction in sample sizes, the plots of the data confirmed that the magnitude of the position 27 effect was substantially diminished compared to what it was when the Arg16 + Gln27 homozygotes were included. Taken together, this suggests that the association between position 27 and HR is largely explained by position 16.

For ADRB2, we found that Gly16 homozygotes had a decreased supine and upright HR compared to Arg homozygotes. The heterozygotes at position 16 also demonstrated a lower supine and upright HR. The change in CI and SI from supine to HUT for the Gly16 homozygotes and the heterozygotes at position 16 showed a smaller decrease compared with the Arg16 homozygotes. Additionally, the change in SVR from supine rest to HUT for the Gly16 homozygotes and the heterozygotes was smaller than the change in SVR for the

Arg16 homozygotes. There was no difference in change in MAP, SBP or DBP from supine to HUT among genotypes. Equation 1 demonstrates the relationship the delta variables have with one another, where RAP is right atrial pressure, a value that was not measured and may vary with posture, but probably negligible in this healthy population. Equation 2 demonstrates the delta values from supine rest to HUT for the Gly16 homozygotes and the position 16 heterozygotes when compared to the Arg16 homozygotes:

$$\text{Delta:MAP} - \text{RAP} = \text{HR} \times \text{SI} \times \text{SVR} \quad \text{Equation 1}$$

$$\text{Delta:MAP}(\leftrightarrow) = \text{HR}(\leftrightarrow) \times \text{SI}(\uparrow) \times \text{SVR}(\downarrow) \quad \text{Equation 2}$$

The recessive model fit all cardiovascular indices and implies that with respect to these indices of cardiovascular control, two copies of the minor allele Arg are required to see a difference from the above equation and that those subjects with either no copies of the minor allele or with only one copy follow Equation 2. Plasma NE values mirrored HR in that Gly16 homozygotes and heterozygotes had decreased NE values at baseline and during HUT compared to Arg16 homozygotes.

This study implies that cardiovascular control differs between healthy young subjects based on genotype; however, in these subjects the differences result in equivalent MAP values. Participants with different genotypes appear to be using alternate means to achieve the same end. One genotype may be better able to compensate for a dysfunction that ultimately may result in orthostatic intolerance compared with another genotype based on their intrinsic response to orthostatic stress. For example, while there is no evidence suggesting a causal link between postural orthostatic tachycardia syndrome (POTS) and adrenergic receptor gene variants, ADRB2 variation may affect the hemodynamic manifestation of POTS in an afflicted individual (Nickander et al., 2005). Moreover, previous work has demonstrated lower circulating norepinephrine levels in patients who were homozygous for Gly16 and in patients who were homozygous for Glu27 (Jacob et al., 2006). Our current findings generally support this idea. However, Jacob et al. also found higher blood pressures in patients with POTS who had the aforementioned genotypes.

We found additional evidence that ADRB2 gene variation is associated with resting HR. The baseline HR was significantly lower in Gly16 and Glu27 subjects compared to Arg16 and Gln27 subjects, which is consistent with some but not all prior studies (Castellano et al., 2003; Ranade et al., 2002; Snyder et al., 2006a; Snyder et al., 2006b; Wilk et al., 2006). All Glu27 homozygotes are Gly16 homozygotes as well and this is likely the reason that position 27 was found to be associated with HR. Lower resting HR in healthy individuals has been associated with improved cardiovascular health in outcome studies including the Framingham study and the CORDIS study (Kannel et al., 1987; Kristal-Boneh et al., 2000). The determination of resting HR has several potential areas of chromosomal influence. A genome scan for qualitative trait loci influencing resting HR indicated influences of chromosomes 4 and 10 (Wilk et al., 2002). Prior work has linked position 49 genotype with resting HR control (Ranade et al., 2002), however, our study did not find differences in resting HR based on genotype at position 49 of ADRB1. This emphasizes that the control of resting HR is multifactorial and is likely to have multiple areas of input across the genome.

An interesting and novel finding was revealed from the sub-analysis of the participants who were intolerant of the 5 min HUT. With the variables averaged during the upright position, the intolerant group had an expected decrease in MAP and SVR, while the change in HR and SI was not different than the subjects who tolerated the HUT. Consistent with previous

work on individuals who are prone to syncope, the intolerant subjects had Epi values which rose significantly higher than in the tolerant subjects (Benditt et al., 2003; Takase et al., 2003). However, we found that the NE response in the intolerant subjects was significantly blunted, which has previously not been described in healthy patients.

A limitation of our methods is that we performed static measurements of NE in the arterial plasma as an estimate of whole-body sympathetic noradrenergic stimulation and NE spillover into the general circulation, without regard to regional vascular beds (Goldstein et al., 1983). However, our findings are consistent with a study in 15 patients with recurrent syncope of unclear etiology, age 15–66 years, who were placed 70 degrees upright for up to 45 minutes. Of the patients who did not tolerate HUT, in the first 2–3 minutes arterial levels of Epi were significantly higher, and arterial NE levels tended to be lower than those who tolerated HUT (Ermis et al., 2003). This sympathoadrenal imbalance has been demonstrated in patients with a history of neurocardiogenic syncope, with a smaller norepinephrine increase compared with the epinephrine increase (Goldstein et al., 2003). Together with our findings, this supports the concept that a “unitary sympathoadrenal response” to stress has evolved to the idea that adrenomedullary and sympathetic noradrenergic activation are not mutually exclusive (Goldstein and Kopin, 2008) which may affect orthostatic tolerance. As for a genetic influence on this finding, there was a trend toward a greater proportion of the Gly16 allele in the intolerant subjects but this did not reach significance likely due to the low number of intolerant subjects.

Implications

In a broad sense, it appears that from our large recruitment pool of genotyped healthy normotensive individuals, Arg16/Gly affects cardiovascular control across separate physiological studies with minimal crossover among participants. The Gly16 allele is associated with greater beta-agonist mediated vasodilation, both in the isolated forearm (Garovic et al., 2003) and the systemic vasculature during baroreflex inhibition (Hesse et al., 2010). In this context, it is interesting that 10 of 13 individuals who did not tolerate HUT were Gly16 homozygotes, consistent with the previous finding at our institution that Gly16 was associated with decreased orthostatic diastolic blood pressure in individuals with POTS (Nickander et al., 2005). Therefore, Gly16 may contribute to excessive vasodilation.

The Gly16 allele has also been associated with slower resting HR that increases to a greater extent during the handgrip pressor reflex (Eisenach et al., 2005; Eisenach et al., 2004) and cardiac output increases to a greater extent in response to systemic beta-agonist during baroreflex inhibition (Hesse et al., 2010). In the present study, the influence of position 16 can be interpreted in two ways: either the Gly16 allele is associated with lower resting HR, or 2 copies of Arg16 is associated with greater resting HR. These discrepant HR values are preserved during HUT, but the Arg16 homozygotes have a greater fall in stroke volume which reduces cardiac output, accompanied by a greater increase in systemic resistance and sympathetic noradrenergic activity (norepinephrine). Thus, it appears that intact counter-regulatory mechanisms are effective in maintaining blood pressure when upright. Furthermore, the idea of how gene variation influences physiology and disease has emerged into an increasingly complex picture of interactions among DNA, RNA, and regulatory pathways (Feero et al.) which allows for redundant homeostatic adaptations in cardiovascular regulation.

Acknowledgments

We thank study coordination by Pamela A. Engrav, Shelly K. Roberts, and Karen P. Krucker, and Jean N. Knutson. We also thank the study volunteers for their enthusiastic participation.

SOURCE OF FUNDING:

This study was supported by NIH HL-089331, NS-32352, HL-083947, NIH/NCRR and NIH Roadmap for Medical Research 1 KL2 RR024151-01, and Science and Technology Department of Zhejiang Province, China (2006C30049).

References

- Benditt DG, Ermis C, Padanilam B, Samniah N, Sakaguchi S. Catecholamine response during haemodynamically stable upright posture in individuals with and without tilt-table induced vasovagal syncope. *Europace*. 2003; 5:65–70. [PubMed: 12504643]
- Bray MS, Krushkal J, Li L, Ferrell R, Kardia S, Sing CF, Turner ST, Boerwinkle E. Positional genomic analysis identifies the beta(2)-adrenergic receptor gene as a susceptibility locus for human hypertension. *Circulation*. 2000; 101:2877–2882. [PubMed: 10869257]
- Brodde OE. Beta-1 and beta-2 adrenoceptor polymorphisms: functional importance, impact on cardiovascular diseases and drug responses. *Pharmacol Ther*. 2008; 117:1–29. [PubMed: 17916379]
- Castellano M, Rossi F, Giacche M, Perani C, Rivadossi F, Muiesan ML, Salvetti M, Beschi M, Rizzoni D, Agabiti-Rosei E. Beta(2)-adrenergic receptor gene polymorphism, age, and cardiovascular phenotypes. *Hypertension*. 2003; 41:361–367. [PubMed: 12574108]
- Charkoudian N. Influences of female reproductive hormones on sympathetic control of the circulation in humans. *Clin Auton Res*. 2001; 11:295–301. [PubMed: 11758795]
- Eisenach JH, Barnes SA, Pike TL, Sokolnicki LA, Masuki S, Dietz NM, Rehfeldt KH, Turner ST, Joyner MJ. The Arg16/Gly {beta}2-adrenergic receptor polymorphism alters the cardiac output response to isometric exercise. *J Appl Physiol*. 2005; 99:1776–1781. [PubMed: 15994241]
- Eisenach JH, McGuire AM, Schwingler RM, Turner ST, Joyner MJ. The Arg16/Gly beta2-adrenergic receptor polymorphism is associated with altered cardiovascular responses to isometric exercise. *Physiol Genomics*. 2004; 16:323–328. [PubMed: 14665698]
- Eisenach JH, Wittwer ED. Beta-Adrenoceptor gene variation and intermediate physiological traits: prediction of distant phenotype. *Exp Physiol*. 2010; 95:757–764. [PubMed: 20382665]
- Ermis C, Samniah N, Lurie KG, Sakaguchi S, Benditt DG. Adrenal/renal contribution to circulating norepinephrine in posturally induced neurally mediated reflex syncope. *Am J Cardiol*. 2003; 91:746–750. [PubMed: 12633816]
- Feero WG, Guttmacher AE, Collins FS. Genomic medicine--an updated primer. *N Engl J Med*. 2001–2011; 362
- Garovic VD, Joyner MJ, Dietz NM, Boerwinkle E, Turner ST. Beta(2)-adrenergic receptor polymorphism and nitric oxide-dependent forearm blood flow responses to isoproterenol in humans. *J Physiol*. 2003; 546:583–589. [PubMed: 12527744]
- Goldstein DS, Holmes C, Frank SM, Naqibuddin M, Dendi R, Snader S, Calkins H. Sympathoadrenal imbalance before neurocardiogenic syncope. *Am J Cardiol*. 2003; 91:53–58. [PubMed: 12505571]
- Goldstein DS, Kopin IJ. Adrenomedullary, adrenocortical, and sympathoneural responses to stressors: a meta-analysis. *Endocr Regul*. 2008; 42:111–119. [PubMed: 18999898]
- Goldstein DS, McCarty R, Polinsky RJ, Kopin IJ. Relationship between plasma norepinephrine and sympathetic neural activity. *Hypertension*. 1983; 5:552–559. [PubMed: 6345364]
- Hesse C, Schroeder DR, Nicholson WT, Hart EC, Curry TB, Penheiter AR, Turner ST, Joyner MJ, Eisenach JH. beta2-Adrenoceptor gene variation and systemic vasodilatation during ganglionic blockade. *J Physiol*. 2010; 588:2669–2678. [PubMed: 20519311]
- Jacob G, Garland EM, Costa F, Stein CM, Xie HG, Robertson RM, Biaggioni I, Robertson D. Beta2-adrenoceptor genotype and function affect hemodynamic profile heterogeneity in postural tachycardia syndrome. *Hypertension*. 2006; 47:421–427. [PubMed: 16461848]
- Kannel WB, Kannel C, Paffenbarger RS Jr, Cupples LA. Heart rate and cardiovascular mortality: the Framingham Study. *Am Heart J*. 1987; 113:1489–1494. [PubMed: 3591616]
- Kristal-Boneh E, Silber H, Harari G, Froom P. The association of resting heart rate with cardiovascular, cancer and all-cause mortality. Eight year follow-up of 3527 male Israeli employees (the CORDIS Study). *Eur Heart J*. 2000; 21:116–124. [PubMed: 10637085]

- Minson CT, Halliwill JR, Young TM, Joyner MJ. Influence of the menstrual cycle on sympathetic activity, baroreflex sensitivity, and vascular transduction in young women. *Circulation*. 2000; 101:862–868. [PubMed: 10694525]
- Nickander KK, Carlson PJ, Urrutia RA, Camilleri M, Low PA. A screen of candidate genes and influence of beta2-adrenergic receptor genotypes in postural tachycardia syndrome. *Auton Neurosci*. 2005; 120:97–103. [PubMed: 15946904]
- Ramirez-Marrero FA, Charkoudian N, Hart EC, Schroeder D, Zhong L, Eisenach JH, Joyner MJ. Cardiovascular dynamics in healthy subjects with differing heart rate responses to tilt. *J Appl Physiol*. 2008; 105:1448–1453. [PubMed: 18756006]
- Ranade K, Jorgenson E, Sheu WH, Pei D, Hsiung CA, Chiang FT, Chen YD, Pratt R, Olshen RA, Curb D, Cox DR, Botstein D, Risch N. A polymorphism in the beta1 adrenergic receptor is associated with resting heart rate. *Am J Hum Genet*. 2002; 70:935–942. [PubMed: 11854867]
- Snyder EM, Beck KC, Dietz NM, Eisenach JH, Joyner MJ, Turner ST, Johnson BD. Arg16Gly polymorphism of the beta2-adrenergic receptor is associated with differences in cardiovascular function at rest and during exercise in humans. *J Physiol*. 2006a; 571:121–130. [PubMed: 16339181]
- Snyder EM, Hulsebus ML, Turner ST, Joyner MJ, Johnson BD. Genotype related differences in beta2 adrenergic receptor density and cardiac function. *Med Sci Sports Exerc*. 2006b; 38:882–886. [PubMed: 16672841]
- Sorrentino S, Forleo C, Iacoviello M, Guida P, D'Andria V, Favale S. Lack of association between genetic polymorphisms affecting sympathetic activity and tilt-induced vasovagal syncope. *Auton Neurosci*. 2010; 155:98–103. [PubMed: 20129829]
- Takase B, Matsushima Y, Umeda E, Satomura K, Katsushika S, Ohsuzu F, Sato T, Kurita A. Endogenous opioids and epinephrine in nitroglycerin provocation tilt test in patients with neurally mediated syncope. *Jpn Heart J*. 2003; 44:493–503. [PubMed: 12906031]
- Wilk JB, Myers RH, Pankow JS, Hunt SC, Leppert MF, Freedman BI, Province MA, Ellison RC. Adrenergic receptor polymorphisms associated with resting heart rate: the HyperGEN Study. *Ann Hum Genet*. 2006; 70:566–573. [PubMed: 16907703]
- Wilk JB, Myers RH, Zhang Y, Lewis CE, Atwood L, Hopkins PN, Ellison RC. Evidence for a gene influencing heart rate on chromosome 4 among hypertensives. *Hum Genet*. 2002; 111:207–213. [PubMed: 12189495]
- Wilton SB, Anderson TJ, Parboosingh J, Bridge PJ, Exner DV, Forrest D, Duff HJ. Polymorphisms in multiple genes are associated with resting heart rate in a stepwise allele-dependent manner. *Heart Rhythm*. 2008; 5:694–700. [PubMed: 18452871]

Figure 1.

Association between ADRB2 position 16 genotype and cardiovascular indices during supine rest and HUT. A. Heart rate and association with position 16 genotype at baseline and HUT. Baseline and HUT HR fit recessive models with Arg16 homozygotes having greater HR compared with Gly16 homozygotes and heterozygotes. B. Change in cardiac index from supine to HUT and the association with position 16. Delta cardiac index values fit a recessive model with Arg16 homozygotes having a greater decrease in CI compared with the Gly16 homozygotes and the heterozygotes. C. Change in stroke index from supine and HUT and the association with position 16. Delta SI values fit a recessive model with Arg16 homozygotes associated with a larger change in SI compared with Gly16 homozygotes and heterozygotes. D. Change in SVR from supine and HUT and the association with position 16. Delta SVR values also fit a recessive model with Arg16 homozygotes associated with a larger increase in SVR compared with the Gly16 homozygotes and heterozygotes. Values are means $\pm$ SE

Figure 2.

Association between ADRB1 position 16 genotype and plasma norepinephrine (NE) levels at baseline and HUT. Baseline NE levels were measured during supine rest. Both baseline and HUT NE values fit a recessive model with Gly16 homozygotes and heterozygotes having lower NE levels compared with the Arg16 homozygotes. Values are means $\pm$ SE

Figure 3.

Association between ADRB2 position 27 genotype and HR during supine rest and HUT. Baseline and HUT HR fit dominant models with Glu27 homozygotes and heterozygotes having lower HR compared with Gln27 homozygotes. Even one copy of the minor allele Glu is associated with a lower HR. Values are means $\pm$ SE.

Figure 4. Hemodynamic variables and catecholamines in participants who were tolerant versus intolerant to 5 min HUT. A. Change from supine rest to HUT in systolic, diastolic and MAP in tolerant versus intolerant subjects. B. Change from supine rest to HUT in SVR and SVRI values in tolerant versus intolerant subjects. C. NE and Epi plasma levels in response to HUT in tolerant subjects versus intolerant subjects. Values are means $\pm$ SE

Statistical genotype models used to fit cardiovascular and catecholamine data for the two ADRB1 SNPs and the two ADRB2 SNPs

Table 1

Receptor	ADRB1			ADRB2			Model
	Position	49	389	16	27		
Major Allele		Ser	Arg	Gly	Gln		
Homozygous Major		SerSer	ArgArg	GlyGly	GlnGln	0	0
Heterozygous		SerGly	ArgGly	GlyArg	GlnGlu	1	0
Homozygous Minor		GlyGly	GlyGly	ArgArg	GluGlu	2	1

Abbreviations: ADRB1 = Beta 1 adrenergic receptor gene, ADRB2 = Beta 2 adrenergic receptor gene, SNP = single nucleotide polymorphisms, Ser = serine, Arg = arginine, Gly = glycine, Gln = glutamine and Glu = glutamic acid. See text for model explanation.

Table 2

Mean values with ranges for MAP, SI, CI and SVR at baseline and during HUT for ADRB2

ADRB2	Parameters	Baseline		HUT	
		Mean	Range	Mean	Range
Arg16 homozygotes	MAP(mmHg)	89	68-100	94	74-109
Heterozygotes		90	77-101	93	77-111
Gly16 homozygotes		89	74-108	91	74-119
Arg16 homozygotes	CI(l/min/m ²)	3.0	2.0-4.3	2.7	2.1-3.7
Heterozygotes		2.7	2.2-4.1	2.5	1.9-3.5
Gly16 homozygotes		2.8	1.8-3.8	2.6	1.7-3.8
Arg16 homozygotes	SI(mL/beat/m ²)	45	36-56	31	24-43
Heterozygotes		47	37-59	34	25-47
Gly16 homozygotes		46	36-58	34	25-47
Arg16 homozygotes	SVR(mmHg*min/L)	17	12-25	20	13-29
Heterozygotes		18	12-22	20	14-27
Gly16 homozygotes		18	9-27	20	9-36

Abbreviations: ADRB1 = Beta 1 adrenergic receptor gene, Arg = arginine, Gly = glycine, MAP = mean arterial pressure, CI = cardiac index, SI = stroke index and SVR = systemic vascular resistance.