The Arg16Gly polymorphism of the β_2-adrenergic receptor and the natriuretic response to rapid saline infusion in humans

Eric M. Snyder[1], Stephen T. Turner[1], Michael J. Joyner[2], John H. Eisenach[2] and Bruce D. Johnson[1]

Departments of [1]Internal Medicine and [2]Anaesthesiology, Mayo Clinic College of Medicine, Rochester, MN 55905, USA

The β_2-adrenergic receptors (β_2ARs) play a role in renal Na^+ regulation. Subjects homozygous for glycine at amino acid 16 (Gly16) of the β_2AR have been shown to have enhanced β_2-mediated vascular relaxation when compared to subjects homozygous for arginine (Arg16). However, Gly16 subjects have been shown to have higher blood pressure than Arg16 subjects. Given the dominant role of the kidneys in long-term blood pressure regulation, we sought to determine whether there were differences in renal Na^+ handling between Gly16 ($n = 17$) and Arg16 ($n = 14$) subjects (Gly16: age, 30 ± 2 years; body mass index (BMI), 25 ± 11 kg m^{-2}; Arg16: age, 30 ± 2 years; BMI, 25 ± 1 kg m^{-2}). We measured urinary Na^+ content before and for 3 h following rapid intravenous saline infusion (30 ml kg^{-1} in $\sim$16 min). Prior to the infusion, there were no differences in 24-h Na^+ excretion between Gly16 and Arg16 subjects (Gly16, 183 ± 21 mmol; Arg16, 184 ± 20 mmol); however, systolic blood pressure (SBP) was significantly higher in Gly16 than Arg16 subjects with no differences observed in diastolic blood pressure (DBP) or mean arterial pressure (MAP) (SBP: Gly16, 117 ± 3 mmHg; Arg16, 109 ± 2 mmHg; DBP: Gly16, 78 ± 2 mmHg; Arg16, 77 ± 2 mmHg; MAP: Gly16, 90 ± 2 mmHg; Arg16, 89 ± 2 mmHg). With rapid saline infusion, MAP increased in both genotype groups (Gly16, 6.7%; Arg16, 3.4%; $P > 0.05$). In the 3 h following Na^+ infusion, Na^+ excretion was less in Gly16 when compared to Arg16 subjects, with a trend towards significance when expressed as total Na^+ excreted (Gly16, 66 ± 7 mmol; Arg16, 85 ± 9 mmol; $P = 0.07$), and a significant difference when expressed as a fraction of the administered load (Gly16, 0.18 ± 0.02; Arg16, 0.28 ± 0.03; $P < 0.01$). These results suggest that the Arg16Gly polymorphism of the β_2AR is associated with differences in natriuretic response to rapid saline infusion, which may influence long-term regulation of blood pressure.

Corresponding author E. M. Snyder: Division of Cardiovascular Diseases, Mayo Clinic and Foundation, 200 1st Street, SW Rochester, MN 55905, USA. Email: snyder.eric@mayo.edu

The β_2-adrenergic receptors (β_2ARs) are located throughout the body including the heart, blood vessels and kidneys. In the heart, the β_2ARs have been localized to the ventricular walls and primarily regulate ventricular contractility (Bristow *et al.* 1989; Brodde, 1991; Bittner *et al.* 1997; Grandy *et al.* 2004). The β_2ARs have also been localized to the vascular tissue and are the primary adrenergic receptor causing vasodilatation upon stimulation with endogenous catecholamines (O'Donnell & Wanstall, 1984; Gaballa *et al.* 1998; Chruscinski *et al.* 1999; Iaccarino *et al.* 2002). The β_2ARs are also located in the kidneys and enhance sodium reabsorption through increased renal epithelial sodium channel (ENaC) activity (Koepke & DiBona, 1986; Shimkets *et al.* 1994; DiBona & Kopp, 1997; Pradervand *et al.* 1999; Snyder, 2000; Wallace *et al.* 2001, 2004).

Several polymorphisms of the β_2AR have been studied in humans. The most functional variant of the β_2AR appears to be an isoleucine substitution for threonine at position 164; however, this occurs in less than 3% of Caucasians (Green *et al.* 1995). An arginine (Arg) to glycine (Gly) substitution at amino acid 16 and a glutamine to glutamate substitution at amino acid 27 have also been described and studied in detail (Green *et al.* 1993, 1994, 1995). Human studies suggest that subjects homozygous for Arg at amino acid 16 (Arg16) may have reduced β_2AR function when compared to subjects homozygous for Gly at this position (Gly16) (Dishy *et al.* 2001; Garovic *et al.* 2003; Tang *et al.* 2003; Eisenach *et al.* 2005; Snyder *et al.* 2005a, 2006).

Specifically, Arg16 subjects show attenuated vasodilatation during infusion of a β-agonist in the

brachial artery and venous circulation when compared to Gly16 subjects (Dishy *et al.* 2001; Garovic *et al.* 2003). Although Arg16 subjects tend to have reduced vasodilatation when compared to Gly16 subjects, we have previously found that the Arg16 group had lower arterial blood pressure, which was not dependent on differences in cardiac output between the genotype groups (Bray *et al.* 2000; Snyder *et al.* 2005*a*). Therefore, our observed difference in blood pressure between the genotype groups might be attributed to differences in renal sodium handling (Snyder *et al.* 2005*b*).

In this context, we sought to determine whether there was an association between genetic variation of the β_2AR at amino acid 16 and the natriuretic response to rapid fluid loading. We hypothesized that subjects homozygous for glycine at amino acid 16 of the β_2AR would have greater sodium reabsorption and therefore less sodium excretion when compared to subjects homozygous for arginine at this position following rapid fluid loading.

Methods

Subjects

The protocol was reviewed and approved by the Mayo Clinic Institutional Review Board, all participants provided informed consent prior to participation, and all aspects of the study conformed to the Declaration of Helsinki. Caucasian subjects who were matched for age, sex and level of physical activity were recruited from a pool of subjects previously genotyped for the Arg16Gly polymorphism of the β_2AR (Bray *et al.* 2000).

Protocol

The study involved two separate experimental sessions, one for screening and the second for rapid saline infusion. The screening tests included an incremental cycle ergometry test to exhaustion (to rule out cardio-vascular abnormalities), a complete blood count (CBC, to rule out anaemia) and, in women, a pregnancy test. The maximal cycle ergometry test was performed while oxygen uptake ($\dot{V}_{O_2}$) and the elimination of carbon dioxide were measured continuously (Medical Graphics, St Paul, MN, USA). Two-minute increments were used for the cycle ergometry test until a perceived exertion rating of 18 (out of a possible 20) was achieved (Borg, 1982). Following the screening tests, the subjects were given a 24-h urine collection container for the assessment of urine Na^+ and creatinine (Cr) levels prior to their saline infusion test. At the end of the screening session, the subjects were instructed by a dietician how to maintain their usual sodium intake until the completion of the study.

Saline infusion

The subjects were instructed to report to the laboratory in a fasting state and all saline infusions were performed between 07.00 and 09.00 h. An 18-gauge venous catheter was inserted into a prominent antecubital vein for the rapid saline infusion and for blood sampling. Baseline measurements of cardiac output (CO), heart rate (HR) and systolic (SBP) and diastolic blood pressure (DBP) were obtained prior to the infusion. The subjects were instructed to void their bladders immediately before the infusion, and warmed saline was then infused intra-venously (30 ml kg^{-1} over ~15 min). During the saline infusion, oxygen saturation (S_{aO_2}), blood pressure (BP), HR and physical symptoms were continuously monitored and recorded every 2 min. Catecholamine (adrenaline and noradrenaline) levels were measured before and immediately after saline infusion from samples collected via the venous catheter. Following the infusion, repeat measures of CO and HR were obtained. Urine was collected every hour for 3 h following the infusion for the assessment of Na^+ and Cr levels.

Data collection

Assessment of cardiovascular function. CO was assessed with a previously validated 8- to 10-breath acetylene rebreath technique using a 5-l anaesthesia bag containing 0.7% C_2H_2 and 9% He (Johnson *et al.* 2000; Bell *et al.* 2003). Briefly, a pneumotachograph was connected to a non-rebreathing Y-valve (Hans Rudolph, KC, MO) with the inspiratory port connected to a pneumatic switching valve (Hans Rudolph) which allowed for rapid switching from room air to the test gas mixture. Gases were sampled using a mass spectrometer (Perkin-Elmer) which was integrated with custom-made analysis software for the assessment of CO.

Blood pressure was recorded using the auscultation technique with the same technician performing all measures. Mean arterial pressure (MAP) was calculated using the equation: $MAP = DBP + 1/3(SBP - DBP)$. Systemic vascular resistance (SVR) was calculated from MAP using the equation: $SVR = MAP/CO$. Heart rate was assessed using 12-lead electrocardiography (Marquette Electronics, Milwaukee, WI, USA).

Urine and plasma sodium and creatinine levels. Urinary Na^+ and Cr levels were determined with a 24-h collection prior to the saline infusion and a 3-h collection immediately following the saline infusion. For each urine collection, the time of collection and the volume and amount of Na^+ and Cr were recorded and assessed. In addition, plasma Na^+ and Cr levels were determined prior to the saline infusion. Both urine and plasma Na^+ and Cr levels were assessed using ion-selective electrodes on

a Hitachi 911 analyser (Roche, Indianapolis, IN, USA). The fractional excretion of sodium (FE_{Na}) was determined using the following equation:

$$FE_{Na} = \left(\frac{(U_{Na} \times P_{Cr})}{(P_{Na} \times U_{Cr})} \right) \times 100$$

where U_{Na} and U_{Cr} are the urinary sodium and creatinine concentrations, respectively, and the P_{Na} and P_{Cr} are the plasma sodium and creatinine concentrations, respectively.

Catecholamines. Adrenaline (ADR) and noradrenaline (NA) levels were assessed according to methods developed in the Mayo Clinic General Clinical Research Center immunochemical core laboratory and the methods of Sealey (1991). For ADR, our intra-assay coefficients of variation were 12.2% and 3.6% at 13.8 and 242 pg ml^{-1}. Inter-assay coefficients of variation were 8.5% and 6.3% at 179 and 390 pg ml^{-1}.

Data analysis

All statistical comparisons were performed using the SPSS statistical software package (v. 12.0, Chicago, IL, USA). Group demographics were compared using an independent samples Student's *t* test with an α level of 0.05 to determine significance. A repeated measures ANOVA was performed to determine within group responses to rapid saline infusion, also with an α level of 0.05 to determine significance. An independent samples *t* test was used to compare group differences in HR, BP and the natriuretic response to saline infusion with an α level of 0.05 to determine significance for each. Before each *t* test and ANOVA, a Levene's test was performed to determine the homogeneity of the data. All data are presented as means ± S.E.M., unless otherwise stated.

Results

Subjects

Individuals who were homozygous for Arg16 ($n = 14$) and Gly16 ($n = 17$) agreed to participate in the study and had no exclusion criteria. All subjects were healthy non-smokers, and not on medications. There were no differences between the genotype groups in age, weight, body mass index, body surface area or cardiovascular fitness; however, the Gly16 group was taller than the Arg16 group (Table 1).

Physiological response to rapid saline infusion

At baseline, the Gly16 group had a higher systolic blood pressure than the Arg16 group but there were no significant differences in DBP, MAP, HR or S_{aO_2} (Table 2).

Table 1. Subject characteristics

Characteristic	Arg16 ($n = 14$)	Gly16 ($n = 17$)
Sex (female/male)	4/10	3/14
Age (year)	30 ± 2	30 ± 2
Height (cm)	174 ± 2	180 ± 2*
Weight (kg)	75 ± 4	82 ± 3
Body mass index (kg m^{-2})	25 ± 1	25 ± 1
Body surface area (m^2)	21 ± 1	22 ± 1
$\dot{V}_{O_2 peak}$ (ml kg^{-1} min^{-1})	37 ± 2	40 ± 2

Values are means ± S.E.M. *$P < 0.05$ Arg16 *versus* Gly16.

In addition, the Gly16 group had a higher CO and a lower SVR compared to the Arg16 group at baseline (Fig. 1). Rapid saline infusion resulted in an increase in ADR and NA levels but there were no differences between the genotype groups in these responses (baseline ADR: Arg16, 20 ± 4 3 pg ml^{-1}; Gly16, 16 ± 3 pg ml^{-1}; post-saline ADR: Arg16, 56 ± 13 3 pg ml^{-1}; Gly16, 33 ± 7 pg ml^{-1}; baseline NA: Arg16, 150 ± 15 3 pg ml^{-1}; Gly16, 192 ± 27 pg ml^{-1}, post-saline NA: Arg16, 329 ± 64 3 pg ml^{-1}; Gly16, 325 ± 49 pg ml^{-1}). Both groups exhibited increases in HR, SBP and MAP with saline infusion (as a percentage increase relative to the baseline level), but there were no differences between the genotype groups in these responses (HR: Arg16, 24 ± 7%; Gly16, 18 ± 4%; SBP: Arg16, 10 ± 3%; Gly16, 8 ± 3%, MAP: Arg16, 4 ± 2%; Gly16, 6 ± 3%). Both groups showed an increase in CO and decrease in SVR with rapid saline infusion, and at the end of the infusion, CO remained higher and SVR lower in the Gly16 group compared to the Arg16 group.

Natriuretic response to rapid saline infusion

There were no differences in 24-h Na$^+$ excretion between the Gly16 and Arg16 groups in the collection that was completed just prior to the infusion (Gly16, 183 ± 21 mmol; Arg16, 184 ± 20 mmol). On average, 2083 ml saline was infused in the Arg16 subjects over 16 min, while 2430 ml saline was infused in the Gly16 subjects over 17 min. The amount of Na$^+$ infused averaged 321 mmol for the Arg16 subjects and 374 mmol for the Gly16 group, because the Gly16 group was slightly heavier (Arg16, 75 ± 4 kg; Gly16, 82 ± 3 kg; $P = 0.16$). Within 3 h after the infusion, the Arg16 group excreted 29% more Na$^+$ than the Gly16 group, but the difference did not reach statistical significance (Fig. 2). The amount of Na$^+$ excreted relative to the amount of Na$^+$ infused was greatest in the Arg16 group (Arg16, 0.28 ± 0.03; Gly16, 0.18 ± 0.02, $P < 0.01$, Fig. 3). There were no differences in FE_{Na} during the 24-h urine collection or following rapid saline infusion; however, there was a trend towards increased FE_{Na} in the Arg16 group (Fig. 4).

Table 2. Physiological response to rapid saline infusion

	Baseline		Fluid challenge	
	Arg16	Gly16	Arg16	Gly16
n	14	17	14	17
Infusion amount (ml)	—	—	2083 ± 233 (15.5 min)	2430 ± 171 (17.1 min)
Sodium infused (mmol)	—	—	321 ± 24	374 ± 13*
HR (beats min^{-1})	62 ± 3	58 ± 2	76 ± 4†	66 ± 3†
SBP (mmHg)	109 ± 2	117 ± 3*	121 ± 4†	126 ± 3†
DBP (mmHg)	78 ± 2	76 ± 2	78 ± 3	79 ± 2
MAP (mmHg)	89 ± 2	90 ± 2	92 ± 3	96 ± 3†
O$_2$ saturation (%)	97 ± 1	98 ± 1	98 ± 1	98 ± 1
Chest tightness (out of 4)	0	0	0.8 ± 0.3†	1.2 ± 0.3†
Shortness of breath (out of 4)	0	0	0.6 ± 0.4†	0.9 ± 0.3†

Values are means ± S.E.M. *$P < 0.05$ Arg16 *versus* Gly16; †$P < 0.05$ compared to baseline. HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.

Discussion

We found that the Arg16 group had a greater natriuretic response to rapid saline infusion when compared to the Gly16 group. Of interest, we also found that the Gly16 group had higher CO and SBP, and lower SVR at baseline and following rapid saline infusion. This is similar to previous results by our group and others demonstrating that CO, and vasodilatation are greater in the Gly16 group when compared to the Arg16 group (Garovic *et al.* 2003; Tang *et al.* 2003; Eisenach *et al.* 2004, 2005; Snyder *et al.* 2005*a*, 2006). It is interesting that there were differences in urinary Na$^+$ content following rapid saline infusion but not at baseline, possibly suggesting an increase in catecholamine levels (as were seen in the present study) are necessary to induce these differences between the genotype groups. This difference following catecholamine stimulation may reflect differences in receptor desensitization and/or density between these genotype groups (Snyder *et al.* 2006).

Epithelial Na$^+$ channels

The ENaCs are found throughout the body including the lungs and the kidneys (Ciampolillo *et al.* 1996; Wallace *et al.* 2001, 2004; Planes *et al.* 2002). In the lungs the ENaCs are located on type-II alveolar cells and act to move Na$^+$ and water from the alveolar airspace into the interstitium (Planes *et al.* 2002). In the kidneys, the ENaCs have been localized to the distal nephron and are thought to play a role in Na$^+$ and blood pressure regulation (Koepke & DiBona, 1986; Snyder, 2000; Wallace *et al.* 2001, 2004). The importance of the ENaCs in renal sodium regulation and long-term blood pressure control is seen in psuedohypoaldosteronism type-1 and Liddle's syndrome. Psuedohypoaldosteronism type-1 is a loss-of-function mutation of the renal ENaCs which results in salt-wasting, whereas Liddle's syndrome is a gain-of-function mutation of the renal ENaC which results in increased salt-sensitivity

and hypertension in humans (Shimkets *et al.* 1994; Kerem *et al.* 1999; Pradervand *et al.* 1999; Snyder, 2000). Although the renal ENaCs are primarily regulated by aldosterone, recent evidence has suggested that the β_2ARs also play an important role in renal Na$^+$ regulation (Wallace *et al.* 2001, 2004).

β_2-Adrenergic control of renal ENaCs

In the kidneys, the β_2ARs have been localized to the inner-medullary collecting duct (IMCD) and influence sodium reabsorption both locally in response to circulating ADR as well as through central nervous system-mediated mechanisms (Koepke & DiBona, 1986; DiBona & Kopp, 1997; Wallace *et al.* 2004). Stimulation of the β_2ARs results in an increase in cAMP which increases the number of ENaCs on cell surfaces, or the probability that an ENaC will be open (Chen *et al.* 2001; Xi-Juan *et al.* 2002). Wallace *et al.* (2004) have recently elucidated three important facts that relate to β_2AR control of sodium reabsorption: (1) the β_2ARs are located on IMCD cells; (2) stimulation of the β_2ARs on these cells leads to an increase in cAMP; and (3) stimulation of the β_2ARs on IMCD cells augments anion secretion which is nearly abolished with β-blockade.

In the present study we attempted to minimize the catecholamine response by warming the saline to body temperature just prior to the infusion; however, there was an increase in ADR and NA levels with the rapid fluid loading. This increase in catecholamine levels is most probably a result of mental stress caused by the infusion (and the discomfort associated with the infusion, i.e. chest tightness and shortness of breath).

Genetic variation of the β_2AR and blood pressure regulation

Previous research has suggested an association between the Gly16 allele of the β_2AR and elevated blood pressure in humans (Gratze *et al.* 1999; Bray *et al.* 2000; Snyder

et al. 2005*a*). However, other studies have suggested a lack of relationship between genetic variation of the $\beta_2 AR$ and hypertension (Herrmann *et al.* 2002) or have associated the Arg16 allele with elevated blood pressure (Gratze *et al.* 1999). The findings of the present study confirm previous work from our laboratory showing that the Gly16 group has elevated blood pressure when compared to the
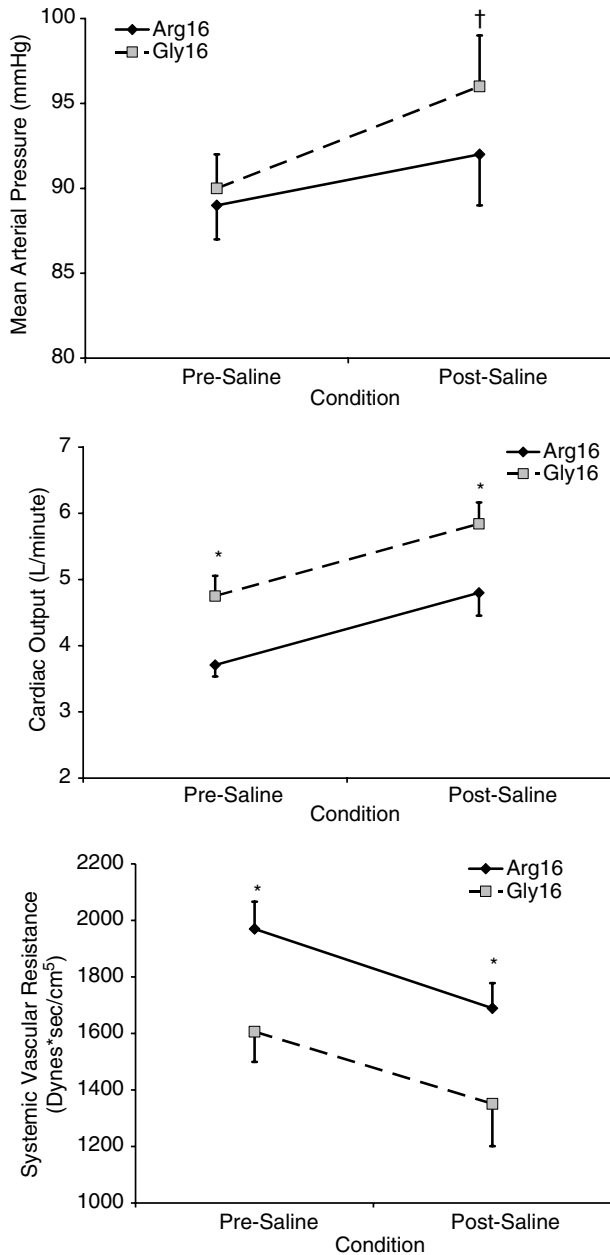

Figure 1. Change in mean arterial pressure, cardiac output and calculated systemic vascular resistance in response to rapid saline infusion
Mean arterial pressure (top panel), cardiac output (middle panel) and systemic vascular resistance (bottom panel) for Arg16 subjects (*n* = 14; ■—■) and Gly16 subjects (*n* = 17; ◆—◆). *$P < 0.05$ Arg16 *versus* Gly16; †$P < 0.05$ *versus* baseline.
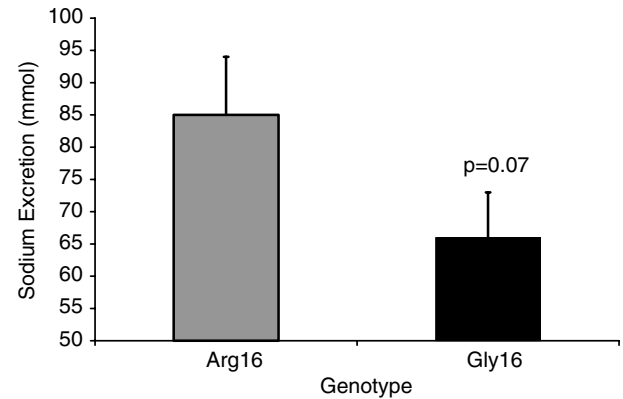

Figure 2. Urinary sodium excretion following rapid saline infusion according to genotype
Na^+ excretion (mmol) for the 3 h following rapid saline infusion in the Arg16 group (■) and the Gly16 group (■).
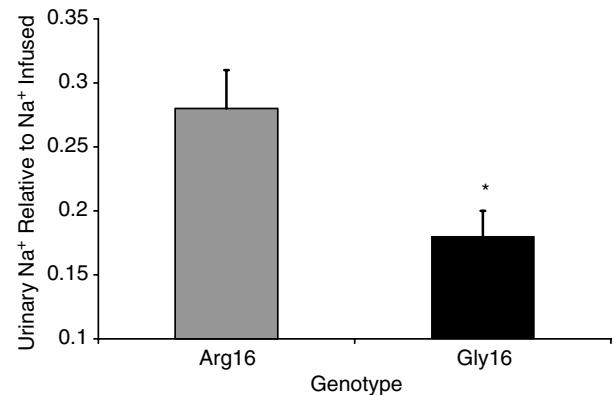

Figure 3. Urinary sodium excretion relative to sodium load with rapid saline infusion according to genotype
relative Na^+ excretion for the 3 h following rapid saline infusion in the Arg16 group (■) and the Gly16 group (■). *$P < 0.01$ Arg16 *versus* Gly16.
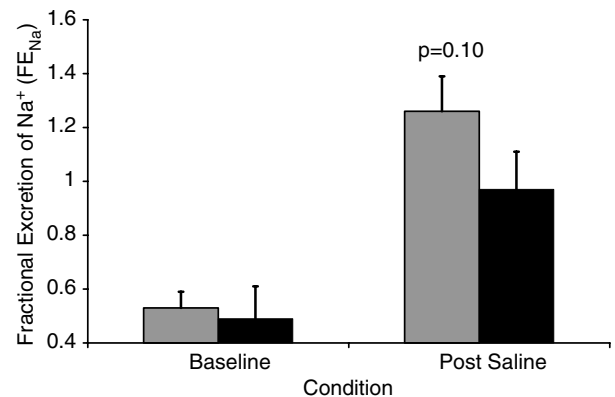

Figure 4. Fractional excretion of sodium according to genotype
Fractional excretion of Na^+ in the Arg16 group (■) and the Gly16 group (■).

Arg16 group (Snyder *et al.* 2006). Because of the contribution of Na$^+$ reabsorption, CO and SVR to blood pressure regulation, the observed difference in blood pressure could be the result of differences in CO, although we have previously found that this does not fully explain the genotype related-differences (Snyder *et al.* 2005*a*). Genotype-related differences in SVR would also not explain why the Gly16 group has previously been shown to have elevated blood pressure. Previous work by us (Snyder *et al.* 2006) and others (Tang *et al.* 2003) suggests that the Gly16 group has enhanced receptor function, which would lower SVR as a result of peripheral vasodilatation, and lead to lower blood pressure.

Interestingly, we observed genotype-related differences in the natriuretic response to rapid saline infusion. Urinary Na$^+$ excretion and FE$_{Na}$ were borderline significant ($P = 0.07$ and 0.10, respectively); however, the amount of Na$^+$ excreted relative to the amount of Na$^+$ infused did reach statistical significance ($P < 0.01$). These findings of differences in the natriuretic response to saline infusion suggest that the genotype-related difference in blood pressure may be related to not only augmented CO but also increased Na$^+$ reabsorption in the Gly16 group.

Limitations

A possible limitation to this study is the use of variation at one site of the β_2AR for comparison, rather than several sites. Although the use of single nucleotide polymorphisms (SNPs) is falling out of favour, we and others have been able to repeatedly reject the null hypothesis when examining phenotypic variation according to position 16. Of interest, recently Lanfear *et al.* (2005) found that Arg16 subjects had higher mortality rates when compared to Gly16 subjects following acute coronary syndrome when discharged with β-blockade; however a specific reason for this difference was unclear. This larger clinical study underscores the importance of smaller mechanistic studies using SNPs to explain phenotypic differences which become particularly important in the clinical setting.

Implications

In a companion paper in this issue by Eisenach *et al.* (2006) salt restriction modified the genotype effect on vascular function, in agreement with previous observations (Garovic *et al.* 2003; Eisenach *et al.* 2004). Specifically, Eisenach *et al.* found that salt restriction increased SVR in Gly16 subjects and found that this intervention eliminated the previously observed augmentation in forearm blood flow in the Gly16 subjects relative to the Arg16 subjects (Garovic *et al.* 2003). This is an important finding and is probably related to the results of the present study. One could hypothesize that, under conditions of normal Na$^+$ intake, Gly16 subjects have enhanced vascular relaxation

which acts as a protective mechanism due to the higher Na$^+$ reabsorption compared to Arg16 subjects. In contrast, with Na$^+$ restriction, the Gly16 subjects have a reduction in vascular relaxation because of an attenuation in Na$^+$ reabsorption. This hypothesis is supported by the work of Naslund *et al.* (1990), who found that Na$^+$ restriction decreased forearm blood flow in response to addition of isoprenaline (isoproterenol), which was related to β_2AR function *in vitro*. The study by Eisenach and colleagues and the present study add to the growing body of evidence that common polymorphisms of the β_2AR influence BP regulation.

Conclusion

We found that subjects homozygous for glycine at amino acid 16 of the β_2AR had an attenuated natriuretic response to rapid saline infusion when compared to subjects homozygous for arginine at this position. In addition, we found that the Gly16 group had higher baseline SBP when compared to the Arg16 group. These findings suggest that the elevated blood pressure observed in the Gly16 group in this and previous studies may be a result of increased renal Na$^+$ reabsorption due to enhanced β_2AR function and increased renal ENaC activity.

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