

Effect of the Arg389Gly β_1 -adrenoceptor polymorphism on plasma renin activity and heart rate, and the genotype-dependent response to metoprolol treatment[†]

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SUMMARY

1. A gene–drug interaction has been indicated between β_1 -adrenoceptor-selective beta-blockers and the Arg389Gly polymorphism (rs1801253) in the adrenergic beta-1 receptor gene (*ADRB1*). In the present study, we investigated the effect of the *ADRB1* Arg389Gly polymorphism on plasma renin activity (PRA) and heart rate (HR), as well as genotype-dependent responses to metoprolol and exercise.

2. Twenty-nine healthy male subjects participated in two treatment periods (placebo and 200 mg/day metoprolol). A 15 min submaximal exercise test was performed after each treatment period and PRA and HR were measured before and after exercise.

3. Before exercise, median PRA was lower in Gly/Gly subjects than in Arg/Arg subjects after both placebo ($P = 0.030$) and metoprolol ($P = 0.020$) treatment. After placebo, the exercise-induced increase in PRA was greater in Gly/Gly than Arg/Gly and Arg/Arg subjects ($P = 0.033$). The linear association between $\log(\text{PRA})$ and $\log(\text{metoprolol concentration})$ varied significantly between genotypes ($P = 0.024$). In Gly/Gly subjects, PRA decreased significantly with metoprolol concentration before ($P = 0.025$) and after exercise ($P < 0.001$), whereas in Arg/Gly and Arg/Arg subjects metoprolol concentration had no effect on PRA. The effect of metoprolol

concentration on PRA in Gly/Gly subjects was enhanced by exercise ($P = 0.044$). No significant differences in HR were seen between genotype groups.

4. Resting PRA was lower in Gly/Gly than Arg/Arg subjects and the effect of exercise and metoprolol concentration on PRA was stronger in Gly/Gly subjects than with the other two genotypes. Thus, Gly/Gly heart failure patients may require lower doses of metoprolol than other patients to block neurohumoral hyperactivity.

Key words: adrenergic beta-1 receptor (*ADRB1*) gene, Arg389Gly polymorphism, exercise, healthy subjects, heart rate, metoprolol, plasma renin activity.

INTRODUCTION

Pharmacogenetics is an emerging field in individualized therapy for chronic heart failure (HF). Metoprolol, a beta-blocker with high affinity for the β_1 -adrenoceptors, is used as the first-line treatment of HF. The Arg389-homozygous (Arg/Arg) genotype of the adrenergic beta-1 receptor gene (*ADRB1*) has been associated with increased intracellular adenylyl cyclase activity¹ and increased plasma renin activity (PRA).² This may reflect a more active Arg/Arg receptor phenotype compared with the Gly/Gly phenotype. Furthermore, a genotype-dependent response to beta-blockers has been shown for the *ADRB1* Arg389Gly polymorphism. In HF, the Arg/Arg genotype has been associated with an increased response to beta-blockers with regard to increased left ventricular ejection fraction (LVEF) and decreased heart rate (HR), hospitalizations and mortality.^{3–10} These data are not entirely consistent and the question remains as to whether this polymorphism is a candidate for stratified and individualized beta-blocker therapy in HF.

In the present study, we analysed the physiological differences between the three Arg389Gly genotypes of the *ADRB1* gene. By using a study design wherein healthy male subjects were exposed to both placebo and beta-blocker treatment, we investigated

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whether specific genotypes of the *ADRB1* Arg389Gly polymorphism influenced the response to metoprolol. At the end of each treatment period with either placebo or metoprolol, subjects performed a submaximal exercise test. The exercise test was designed to mimic the pathophysiological conditions associated with HF, characterized by increased neurohormonal activity, even at rest.

β_1 -Adrenoceptors are expressed in both the heart and kidneys, where they act in two distinct ways.^{11–13} In the heart, β_1 -adrenoceptors produce a change in HR upon stimulation, whereas in the kidneys they stimulate renin secretion. Therefore, to investigate the functionality of the *ADRB1* Arg389Gly polymorphism *in vivo*, we measured HR and PRA before and after submaximal exercise.

Our *a priori* hypothesis was that the Gly/Gly genotype would be associated with decreased PRA and HR before and after submaximal exercise compared with the Arg/Arg and Arg/Gly genotypes. We also hypothesized that having one or two gain-of-function Arg alleles would predict an increased response to metoprolol (i.e. the Arg/Arg genotype being associated with the greatest response to metoprolol, especially with increased neurohormonal activity after exercise). We believe that by further elucidating the physiological and pharmacological effect of the *ADRB1* Arg389Gly polymorphism, we will be able to show that the different genotypes of the receptor lead to different phenotypes. This may influence the effect of the selective beta-blocker metoprolol on β_1 -adrenoceptors and, consequently, the treatment of HF patients.

METHODS

Ethics

The present study was approved by the local ethics committee of Rigshospitalet–Copenhagen University Hospital (H-A-2008–064) and by the Danish Medicines Agency (EudraCT no. 2008-001908-23). It was conducted in accordance with good clinical practice (GCP). Monitoring was done by the GCP Unit of the University of Copenhagen. The study has been registered with www.clinicaltrials.gov (NCT00885651) and the Danish Data Protection Agency (2007-58-0015).

Study population

One hundred and ninety-one healthy, male, Caucasian subjects were screened for the *ADRB1* Arg389Gly polymorphism. This was done to ensure that all three genotypes (Gly/Gly, Arg/Gly, Arg/Arg) would be equally represented in the clinical study of 30 subjects. Our study was based on the findings from a study of 18 healthy subjects.² Therefore, we estimated that 30 healthy subjects in the present study would be sufficient to reproduce the findings described earlier.

Of the 191 subjects, 16 (8%) were Gly/Gly, 71 (37%) were Arg/Gly and 104 (54%) were Arg/Arg (no deviation from Hardy–Weinberg equilibrium; $P = 0.18$). Only eight Gly/Gly subjects from the screening were willing to participate in the clinical study and we ended up with eight Gly/Gly, 10 Arg/Gly and 12 Arg/Arg subjects.

Subjects were required to be between 20 and 30 years of age, to have a body mass index of 20–25 kg/m², to be non-smokers,

to be free of chronic diseases and to not be on continuous medication. Before the start of the study, each subject underwent a physical examination by a medical doctor that included electrocardiogram and blood pressure measurements. At this pretrial examination (Fig. 1), subjects, if included, performed an indirect maximal exercise test on a stationary bicycle.¹⁴ This enabled us to measure maximal oxygen uptake indirectly through maximal workload. All subjects were randomized to one of two treatment arms, both including treatment with placebo and metoprolol (randomized cross-over design).

Treatment Arm 1 consisted of 1 week of placebo treatment (Period 1; one tablet per day for 3 days and two tablets per day for 4 days), followed by 1 week of metoprolol treatment (Period 2; one tablet of 100 mg/day for 3 days and two tablets of 100 mg/day for 4 days; Fig. 1), followed by 3 days of withdrawal treatment (100 mg/day metoprolol; not illustrated in Fig. 1). Treatment Arm 2 consisted of 1 week of metoprolol treatment (Period 1; one tablet of 100 mg/day for 3 days and two tablets of 100 mg/day for 4 days), followed by Period 2 (3 days of withdrawal treatment with one tablet of 100 mg/day metoprolol and 4 days of two placebo tablets per day; Fig. 1). Therefore, Period 2 of Treatment Arm 2 was different from Period 1 of Treatment Arm 1.

After each treatment period (Periods 1 and 2), subjects performed a 15 min exercise test on the stationary bicycle (Fig. 1). The workload of these submaximal tests was 60–80% of the maximal workload from the pretrial test. Submaximal tests after the second treatment period were performed identically to those performed after the first treatment period, but only within individual subjects (intra-individually). Therefore, the workload of the submaximal tests could differ between subjects (interindividually). Blood samples were taken from a cubital venous catheter and were drawn just before exercise ($T = 0$) with the subject in a supine

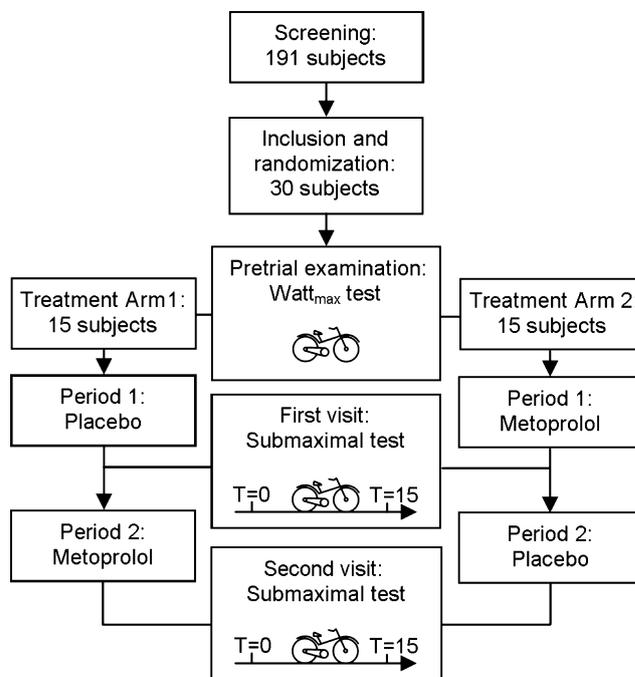


Fig. 1 Schematic illustration of the selection of subjects and assignment to treatment arms.

position (after 30 min rest) and in the final seconds of the 15 min submaximal test (after exercise; $T = 15$). Blood samples were put on ice immediately after they had been drawn, then centrifuged at 1714 *g* for 10 min at 4°C. The buffy coat was isolated and stored at -20°C. Each participant's HR was recorded using a Polar RS400 pulse watch (Polar Electro, Lake Success, NY, USA).

Twenty-nine subjects completed the study (seven Gly/Gly, 10 Arg/Gly, 12 Arg/Arg). One subject (Gly/Gly) was excluded owing to a defective venous catheter.

Measurements

The DNA from venous blood samples was purified using the MaxwellTM 16 automated DNA purification system (Promega, Madison, WI, USA) according to the manufacturer's instructions (2009 manual). The rs1801253 (Arg389Gly) polymorphism was genotyped by reverse transcription-polymerase chain reaction using an ABI 7900 HT (Applied Biosystems; Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. An 11.25 μ L dilution containing 10 ng genomic DNA was mixed with 12.5 μ L Universal PCR Master Mix (TaqMan; Life Technologies; assay number c_8898494-10) and 1.25 μ L SNP Genotyping Assay Mix (TaqMan). Amplification was performed over 40 thermal cycles as follows: 10 min at 95°C, 15 s at 92°C and 1 min at 60°C. Results of the fluorescence profile for each well were analysed with Sequence Detection Software (SDS 2.3; Applied Biosystems).

Plasma renin activity measurements were performed in the presence of 50 pmol sheep angiotensinogen and PRA was thus independent of endogenous substrate variations. Briefly, 25 μ L plasma was incubated at 37°C for 3 h with sheep angiotensinogen in a total reaction volume of 100 μ L. The assay was calibrated against the international reference renin preparation 68/356 and results could therefore be expressed in terms of renin concentration (mIU/L). The enzymatic angiotensin I-generation step and the subsequent angiotensin I radioimmunoassay step were performed in the same tube. Under these assay conditions, renin activity is proportional to renin concentration. The intra-assay and between-day coefficients of variation were < 5% and 8-10%, respectively.

The plasma concentration of metoprolol was measured by HPLC and tandem mass spectrometry (LC-MS/MS) with on-line solid phase extraction. A validation program was executed according to US Food and Drug Administration guidelines.¹⁵ Chromatography was performed on an Acquity UPLC system (Waters, Milford, MA, USA) supplied with an HP1100 pump (Agilent Technologies, Santa Clara, CA, USA). Mass spectrometry detection was performed on an API 365 triple quadrupole (AB Sciex, Concord, Canada) upgraded with an Ionics EP10 + mass analyser (Ionics Mass Spectrometry Group, Bolton, ON, Canada).

Statistical analysis

A non-parametric ordinal test (Kruskal-Wallis test) was used for continuous variables to test differences between genotypes (subject characteristics, covariates, PRA and HR). For PRA and HR, this was done for values obtained before and after exercise ($T = 0$ and 15 min). The distributions of PRA and HR were

found to be skewed, so both outcomes were log-transformed before further analysis.

To assess whether the effect of metoprolol treatment (treatment vs placebo) and exercise differed between genotypes we developed a mixed-effects ANOVA model including treatment (treatment vs placebo), exercise ($T = 0$ vs $T = 15$ min), genotype (Arg/Arg vs Arg/Gly vs Gly/Gly) and interactions between these as covariates (fixed effects). Identification number and period were included as random effects to account for the repeated measurements made on each subject within and between periods.

To assess the effect of metoprolol concentration on PRA and HR we developed a mixed-effects analysis of covariance (ANCOVA) model including log(metoprolol concentration) as the continuous covariate, genotype and exercise as categorical covariates and identification number as a random effect. The ANCOVA model simultaneously fitted six regression lines (each of the three genotypes before and after exercise) and tested whether the slopes were identical across groups. All placebo measurements were excluded from the analysis of metoprolol concentration in the ANCOVA model. Post hoc, a linear regression analysis was used for each of the six regression lines to test the association between log(metoprolol concentration) and log(PRA).

Parameter estimates with 95% confidence intervals (CI) of the effect of exercise and metoprolol (yes vs no) on PRA and HR were obtained from the ANOVA model, whereas parameter estimates (with 95% CI) of the effect of metoprolol concentration on PRA and HR were obtained from the ANCOVA model.

Owing to the repeated-measures design, the analysis could not be further adjusted for experimental error in the metoprolol concentrations. However, the experimental error in the present study was small compared with the biological variation between subjects (expected bias 0.6%). Experimental error was estimated from the repeated measurements of metoprolol concentrations and the average of the measurements for each subject was used in further analyses reported in this paper. Expected bias was estimated as the ratio between the experimental error variance and the total variance (i.e. the variance between measurements on different subjects).

All statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC, USA). A two-sided $P < 0.05$ was considered significant.

RESULTS

Subject characteristics for the three genotypes are presented in Table 1. There were no significant differences in any of the variables between the three genotypes (Gly/Gly, Arg/Gly, Arg/Arg).

Median values of PRA and HR for the three genotypes before and after exercise ($T = 0$ and 15 min) are presented in Table 2. The median level of PRA was significantly lower in Gly/Gly subjects than in Arg/Arg subjects before exercise with either metoprolol treatment ($P = 0.020$) or placebo ($P = 0.030$), whereas no significant differences were found between the genotypes after exercise. No significant differences in the levels of HR were found between the genotypes.

The overall effect of metoprolol (treatment vs placebo) on PRA and HR ($P < 0.0001$ for both variables) did not differ significantly between genotypes either before exercise ($P_{\text{interaction}} = 0.52$

Table 1 Subject characteristics according to the three adrenergic beta-1 receptor gene (*ADRB1*) Arg389Gly polymorphism (rs1801253) genotypes

	Gly/Gly	Arg/Gly	Arg/Arg	<i>P</i> value
Age (years)	23 (22–25)	23 (22–25)	22 (22–24)	0.61
BMI (kg/m ²)	21 (21–26)	24 (21–25)	24 (23–26)	0.31
Maximal workload (W; pretrial visit)	345 (310–345)	310 (275–345)	345 (310–345)	0.72
Maximal oxygen uptake (mL/kg per min)	48 (44–52)	46 (42–49)	47 (40–52)	0.88
Workload (W; Visits 1 and 2)	248 (206–248)	212 (193–242)	242 (217–242)	0.16
Relative workload (W/W _{max} × 100%; Visits 1 and 2)	80 (70–80)	70 (60–70)	70 (70–70)	0.07
Metoprolol concentration (μg/L; Visits 1 or 2)	56 (43–144)	63 (32–80)	58 (42–97)	0.60

Data show median values with the interquartile range in parentheses. *P* values were determined using the Kruskal–Wallis test. BMI, body mass index.

Table 2 Plasma renin activity and heart rate after placebo and metoprolol treatment in the three genotype groups

Genotype		<i>T</i> = 0	<i>T</i> = 15
Plasma renin activity (mL/units per L)			
Placebo	Arg/Arg	22 (10–32)*	52 (31–137)
	Arg/Gly	13 (12–31)	69 (56–87)
	Gly/Gly	10 (6–12)*	58 (54–74)
Metoprolol	Arg/Arg	13 (6–17) [†]	17 (15–37)
	Arg/Gly	8 (4–9)	21 (9–30)
	Gly/Gly	4 (1–11) [†]	17 (1–29)
Heart rate (b.p.m.)			
Placebo	Arg/Arg	67 (61–74)	173 (162–183)
	Arg/Gly	72 (57–80)	183 (174–190)
	Gly/Gly	67 (64–72)	190 (183–195)
Metoprolol	Arg/Arg	53 (48–61)	140 (130–151)
	Arg/Gly	58 (46–66)	142 (136–150)
	Gly/Gly	55 (47–59)	160 (150–165)

Data show median values with the interquartile range in parentheses.

*Placebo plasma renin activity at *T* = 0, difference between Gly/Gly and Arg/Arg: *P* = 0.030 (Kruskal–Wallis test).

[†]Metoprolol plasma renin activity at *T* = 0, difference between Gly/Gly and Arg/Arg: *P* = 0.020 (Kruskal–Wallis test).

and *P*_{interaction} = 0.92, respectively; ANOVA) or after exercise (*P*_{interaction} = 0.27 and *P*_{interaction} = 0.51, respectively). The estimated reductions in PRA and HR with metoprolol treatment for the three genotypes are given in Table 3.

When considering the effect of exercise, the increase in PRA (*T* = 0–15) differed significantly between metoprolol and placebo (*P*_{interaction} < 0.001) and a borderline significant difference was observed between genotypes (*P*_{interaction} = 0.047). The estimated effects of exercise are given in Table 4. After placebo treatment, the exercise-induced increase in PRA was higher in the Gly/Gly group than in the Arg/Gly and Arg/Arg groups (*P*_{interaction} = 0.030), but this was not the case after metoprolol treatment (*P*_{interaction} = 0.57). There were no significant differences in HR responses between genotypes (*P*_{interaction} = 0.36) (placebo) and 0.50 (metoprolol).

When analysing only the values obtained after metoprolol treatment, an overall linear association (*P* = 0.0017) was found between log(PRA) and log(metoprolol concentration), but not (*P* = 0.27) between log(HR) and log(metoprolol concentration). This association between PRA and metoprolol concentration was significantly influenced by genotype (*P*_{interaction} = 0.024,

Table 3 Percentage changes in plasma renin activity and heart rate induced by metoprolol treatment before (*T* = 0) and after (*T* = 15) exercise

	PRA	HR
<i>T</i> = 0		
Arg/Arg (<i>n</i> = 12)	–43 (–72, 18)	–21 (–27, –14)
Arg/Gly (<i>n</i> = 10)	–57 (–81, –6)	–19 (–26, –11)
Gly/Gly (<i>n</i> = 7)	–58 (–84, 7)	–18 (–27, –9)
<i>T</i> = 15		
Arg/Arg (<i>n</i> = 12)	–69 (–85, –36)	–19 (–26, –12)
Arg/Gly (<i>n</i> = 10)	–78 (–90, –52)	–21 (–28, –12)
Gly/Gly (<i>n</i> = 7)	–84 (–94, –59)	–17 (–26, –7)

Data show estimates with 95% confidence intervals in parentheses. Estimates were calculated from ANOVA analysis.

Differences between genotypes (Gly/Gly vs Arg/Gly vs Arg/Arg) are as follows: for plasma renin activity (PRA), *P*_{interaction} = 0.52 (*T* = 0) and 0.27 (*T* = 15); for heart rate (HR), *P*_{interaction} = 0.92 (*T* = 0) and 0.51 (*T* = 15).

Table 4 Percentage increases in plasma renin activity and heart rate induced by 15 min exercise after placebo or metoprolol treatment

	PRA	HR
Placebo		
Arg/Arg (<i>n</i> = 12)	258 (146, 420)	159 (139, 182)
Arg/Gly (<i>n</i> = 10)	364 (208, 599)	168 (145, 194)
Gly/Gly (<i>n</i> = 7)	641 (354, 1108)	183 (153, 217)
Metoprolol		
Arg/Arg (<i>n</i> = 12)	94 (34, 182)	164 (143, 187)
Arg/Gly (<i>n</i> = 10)	135 (56, 254)	163 (140, 188)
Gly/Gly (<i>n</i> = 7)	180 (71, 356)	186 (157, 219)

Data show estimates with 95% confidence intervals in parentheses. Estimates were calculated from ANOVA analysis.

Difference between genotypes (Gly/Gly vs Arg/Gly vs Arg/Arg) are as follows: for Plasma renin activity (PRA), *P*_{interaction} = 0.030 (placebo) and 0.57 (metoprolol); for heart rate (HR), *P*_{interaction} = 0.36 (placebo) and 0.50 (metoprolol). Differences between the Gly/Gly and Arg/Arg groups were as follows: for PRA, *P* = 0.010 (placebo).

ANCOVA model), indicating a genotype-dependent response to metoprolol treatment as a continuous variable (i.e. metoprolol concentration). When stratifying the analysis for the different genotypes, we found a significant association between log(PRA)

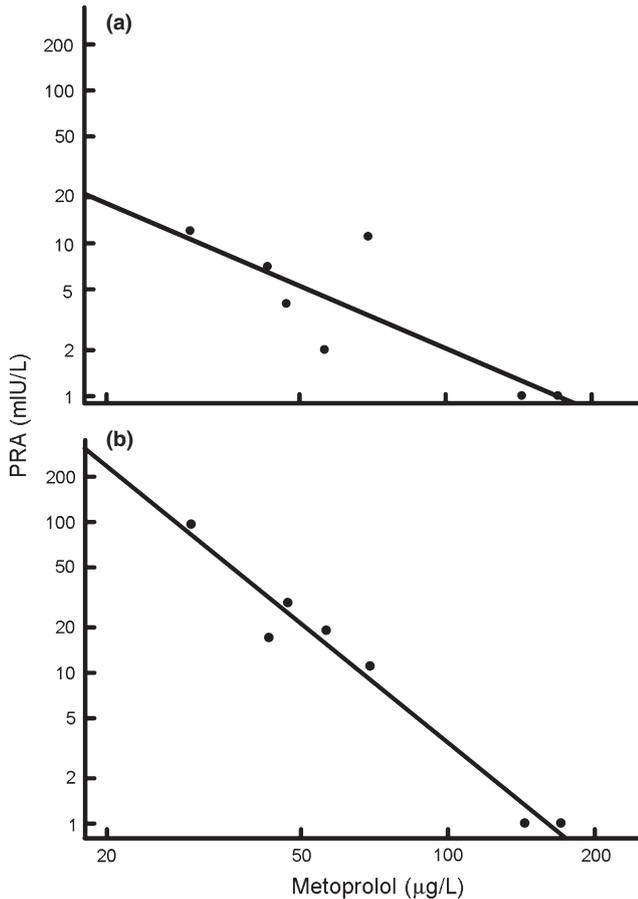
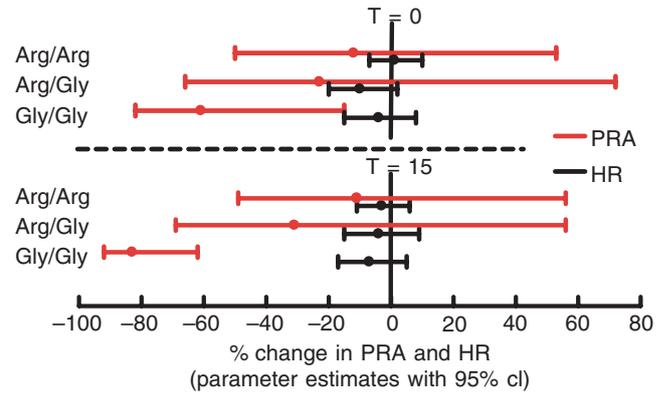


Fig. 2 Associations between log(plasma renin activity) and log(metoprolol concentration) for Gly/Gly subjects (a) at rest ($T = 0$; $n = 7$; $P = 0.025$, $R^2 = 0.67$) and (b) after exercise ($T = 15$; $P < 0.0001$, $R^2 = 0.97$). PRA, plasma renin activity.

and log(metoprolol concentration) in Gly/Gly subjects before ($P = 0.025$, $R^2 = 0.67$; Fig. 2a) and after ($P < 0.0001$, $R^2 = 0.97$; Fig. 2b) exercise, but not in Arg/Gly subjects (before exercise: $P = 0.44$, $R^2 = 0.08$; after exercise: $P = 0.55$, $R^2 = 0.05$) or Arg/Arg subjects (before exercise: $P = 0.76$, $R^2 = 0.01$; after exercise: $P = 0.13$, $R^2 = 0.21$). After exercise, the regression line in the Gly/Gly group was different from the regression lines in the Arg/Gly and Arg/Arg groups ($P = 0.001$) due to a strong association between log(PRA) and log(metoprolol concentration) in Gly/Gly subjects (-83% change in PRA for a doubling of concentration; 95% CI: -92% to -62% ; $P < 0.001$) that was not found in Arg/Gly or Arg/Arg subjects (estimated rates of change shown in Fig. 3). Before exercise, the association between PRA and metoprolol concentration also appeared to be stronger in Gly/Gly subjects (-61% change in PRA for a doubling of concentration; 95% CI -15% to -82% ; $P = 0.025$) than in Arg/Gly and Arg/Arg subjects (estimated rates of change shown in Fig. 3), but the regression lines did not differ significantly between genotypes ($P = 0.06$). Furthermore, the association between PRA and metoprolol concentration in Gly/Gly subjects was significantly stronger after exercise than before ($P = 0.044$), indicating that the effect of metoprolol concentration on PRA in Gly/Gly subjects was enhanced by exercise (i.e. physiological stress).



	PRA	HR	
Arg/Arg	-12% (-50%, 53%)	1% (-7%, 10%)	$T = 0$
Arg/Gly	-23% (-66%, 72%)	-10% (-20%, 2%)	
Gly/Gly	-61% (-82%, 15%)	-4% (-15%, 8%)	
Arg/Arg	-11% (-49%, 56%)	-3% (-11%, 6%)	$T = 15$
Arg/Gly	-31% (-69%, 56%)	-4% (-15%, 9%)	
Gly/Gly	-83% (-92%, -62%)	-7% (-17%, 5%)	

Fig. 3 Percentage change in plasma renin activity (PRA) and heart rate (HR) for every doubling of metoprolol concentration. Estimates are calculated from the ANOVA analysis. Differences between genotypes (Gly/Gly vs Arg/Gly vs Arg/Arg) are as follows: for PRA, $P_{\text{interaction}} = 0.060$ ($T = 0$) and 0.010 ($T = 15$); for HR, $P_{\text{interaction}} = 0.41$ ($T = 0$) and 0.73 ($T = 15$). Differences for Gly/Gly versus Arg/Arg are as follows: for PRA, $P = 0.060$ ($T = 0$) and 0.001 ($T = 15$). There were seven, 10 and 12 subjects in the Gly/Gly, Arg/Gly and Arg/Arg groups.

DISCUSSION

In the present study, we found that PRA was significantly lower in Gly/Gly compared with Arg/Arg subjects before exercise (at rest) but not after 15 min of exercise (physiological stress). These differences in resting PRA were significant both with and without metoprolol treatment. We found that the effect of exercise on PRA without metoprolol (i.e. placebo) was significantly higher in Gly/Gly subjects than in Arg/Gly or Arg/Arg subjects. Furthermore, we found that the association between PRA and metoprolol treatment depended on genotype. This interaction was not present when metoprolol treatment was considered as a binary variable (yes vs no) but only as a continuous variable (metoprolol concentration). The interaction was due to a significant inverse association between PRA and metoprolol concentration (the PRA level decreased with increasing metoprolol concentration) in Gly/Gly subjects that was different from the non-significant associations in Arg/Gly and Arg/Arg subjects. This association was stronger after exercise than before exercise and the diminishing effect of metoprolol concentration on PRA was stronger after exercise. No significant differences in HR were found between genotypes.

Our findings provide further information about the physiological consequences of the *ADRB1* Arg389Gly polymorphism. *In vitro*, the Arg/Arg β_1 -adrenoceptor is associated with increased intracellular cAMP production compared with the Gly/Gly

receptor, both before and after agonist stimulation.¹ We found that PRA was lower at rest (i.e. without stimulation) in Gly/Gly subjects than in Arg/Arg subjects with and without metoprolol treatment. This difference was not found after 15 min exercise, suggesting that, when physiologically stimulated, the Gly/Gly receptor can induce the same level of PRA as the other receptor phenotypes.

Our findings indicate that the *ADRB1* Arg389Gly polymorphism may contribute to the different responses to metoprolol treatment observed between individuals, but they also strongly indicate that the concentration of metoprolol plays a key role in this stratified response between genotypes. In a study by Bruck *et al.* that is very similar to ours, 18 healthy subjects were infused with dobutamine.² That study showed that bisoprolol (a β_1 -adrenoceptor-selective antagonist) reduced the PRA and HR response to pharmacological stress (dobutamine) in Arg/Arg subjects, but not in Gly/Gly subjects. Our findings are different from those of Bruck *et al.*,² but are not necessarily contradictory. We found no interaction between genotype and metoprolol treatment (treatment vs no treatment) for either of the dependent variables (PRA or HR). Conversely, we found an interaction between genotype and metoprolol concentration (treatment as a continuous variable) with regard to PRA. These somewhat different findings may be explained by the different study designs (i.e. probably producing differing levels of antagonists and agonists) and we believe that together they illustrate the different dose–response curves for the Arg389Gly receptor phenotypes. With regard to HR, we were not able to reproduce the interaction between the β_1 -adrenoceptor-selective antagonist and the Arg389Gly genotype found by others.^{2,16} This indicates that the mechanisms by which the β_1 -adrenoceptor mediates HR and PRA responses differ and that there may be a site-specific intracellular second messenger system.^{17,18}

Although no interaction was found with regard to HR, we suggest that the Arg389Gly polymorphism may be useful for optimizing the dosing regimens of metoprolol in HF patients (i.e. Gly/Gly patients may require lower doses than those with Arg/Gly and Arg/Arg receptor phenotypes). We also suggest that PRA may be valuable as a biomarker in HF instead of, or in combination with, N-terminal-pro-B-type natriuretic peptide. As the link between the adrenergic and renin–angiotensin–aldosterone systems, PRA reflects the hyperactivity of these systems. This is a crucial factor in the deterioration of the HF syndrome and some studies support the concept of PRA as a causal risk factor. It is known that beta-blockers inhibit PRA and PRA is a risk marker for hypertension, cardiovascular events in patients with stable vascular disease and cardiac death in HF patients independent of angiotensin-converting enzyme inhibitor treatment.^{19–22} That Arg389Gly may be used as a genetic classifier for different metoprolol dosing regimens is nevertheless speculative, as is the indication of PRA as a causal risk factor in HF. These hypotheses need confirmation in larger cohorts of HF patients.

The present study is limited by its small number of subjects. The present study was conceived as a physiological model of the pathophysiological conditions in HF patients, characterized by neurohormonal activity. Obviously, using HF patients instead of healthy subjects would be more representative in this respect. Concerns could be raised about the study's short washout period

(only 4 days), but measurements during the placebo visits indicated that metoprolol was not present at detectable concentrations. The results of the study would have been more robust if all the subjects had been stressed with exactly the same relative workload. The ANCOVA is likely to be biased by experimental error in the metoprolol concentrations. However, although experimental error in the independent variable can severely bias the slope of a regression line towards the null,²³ the expected bias in our data amounts to merely 0.6% and is not likely to have an impact on the results.

In conclusion, we have shown that the *ADRB1* Arg389Gly polymorphism interacts with metoprolol concentration in healthy, Caucasian, male subjects. This interaction occurred only with PRA and not with HR. We found a dose–response relationship between PRA and metoprolol concentration in Gly/Gly subjects that was not found in Arg/Gly or Arg/Arg subjects. This suggests a different receptor–metoprolol relationship between the Arg389Gly genotypes. In addition, PRA values at rest in the Gly/Gly group were lower than in the Arg/Gly and Arg/Arg groups and the PRA response to exercise without metoprolol was higher in Gly/Gly subjects. The Arg389Gly polymorphism may be useful for the development of stratified dosing regimens of metoprolol. It seems that PRA becomes blocked at lower metoprolol concentrations in Gly/Gly subjects rather than in the other two genotypes. This implies that Gly/Gly HF patients require lower doses than Arg/Gly or Arg/Arg patients to optimally block neurohormonal hyperactivity. However, the present study is merely hypothesis generating and all hypotheses made should be properly tested in larger cohorts of HF patients.

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