Genetic Variations in the α_{2A}-Adrenoreceptor Are Associated With Blood Pressure Response to the Agonist Dexmedetomidine

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Background—α_{2A}-Adrenoceptors (α_{2A}-ARs) have important roles in sympathetic cardiovascular regulation. Variants of *ADRA2A* affect gene transcription and expression and are associated with insulin release and risk for type 2 diabetes. We examined whether *ADRA2A* variants are also associated with cardiovascular responses to the selective α_2-AR-agonist dexmedetomidine.

Methods and Results—Seventy-three healthy subjects participated in a placebo-controlled, single-blind study. After 3 infusions of placebo, subjects received 3 incremental infusions of dexmedetomidine (cumulative dose, 0.4 μg/kg). Primary outcomes were changes in systolic blood pressure (SBP) and plasma norepinephrine concentrations, measured as difference of the area-under-the-curve during placebo and dexmedetomidine infusions (ΔAUC). We used multiple linear regression analysis to examine the associations between 9 *ADRA2A* tagging variants and 5 inferred haplotypes and ΔAUC after adjustment for covariates. Homozygous carriers of rs553668 and the corresponding haplotype 4, previously associated with increased α_{2A}-AR expression, had a 2.2-fold greater decrease in AUC_{SBP} after dexmedetomidine (adjusted $P=0.006$); similarly, the maximum decrease in SBP was 24.7$\pm$8.1 mm Hg compared with 13.6$\pm$5.9 mm Hg in carriers of the wild-type allele ($P=0.007$). Carriers of haplotype 3, previously associated with reduced α_{2A}-AR expression, had a 44% smaller decrease in AUC_{SBP} ($P=0.013$). Haplotype information significantly improved the model predicting the decrease in SBP ($P<0.001$). There were similar but nonsignificant trends for diastolic blood pressure and heart rate. Genotypes were not significantly associated with norepinephrine responses.

Conclusions—Common ADRA2A variants are associated with the hypotensive response to dexmedetomidine. Effects of specific variants/haplotypes in vivo are compatible with their known effects on gene expression in vitro. **(*Circ Cardiovasc Genet*. 2011;4:179-187.)**

Key Words: receptors, adrenergic, alpha ■ genetic polymorphism ■ pharmacogenetics ■ receptor ■ variability in drug response

Alpha$_{2A}$-Adrenoceptors (α_{2A}-ARs) are important regulators of sympathetic tone through central and peripheral (presynaptic) sympathetic inhibition. They are also directly involved in several homeostatic functions, including contraction and relaxation of vascular smooth muscle, control of vigilance, anxiety and stress-related behaviors, pain perception, platelet aggregation, lipolysis, and insulin release. Genetically engineered mice that do not express the α_{2A}-AR gene (*ADRA2A*) have a hyperadrenergic phenotype with increased blood pressure, heart rate, and plasma norepinephrine concentrations and develop cardiac hypertrophy and heart failure.[1–4] Moreover, *ADRA2A* knockout mice do not decrease blood pressure in response to an α_2-AR-agonist such as clonidine.[1]

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We and others have systematically defined genetic variation and the haplotype structure of *ADRA2A*.[5,6] Some of the variants identified have distinct functional effects on receptor expression or function in vitro.[6–8] However, studies to define the functional significance of *ADRA2A* genetic variation in cardiovascular regulation in vivo have yielded inconsistent results. Some studies have found associations between *ADRA2A* variants and hypertension,[9–11] whereas others, including 2 genome-wide association studies, have not.[12–15]

Blood pressure is regulated by many factors and mechanisms, and the contributions of single genetic variants to

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blood pressure regulation may be difficult to detect in population-based studies, where many confounding variables remain uncontrolled. A more sensitive approach to defining the functional effects of genetic variants in membrane-bound receptors is the administration of a pharmacological agonist or antagonist in a controlled experimental setting.[16–18] Indeed, Sober et al[11] recently hypothesized that functional *ADRA2A* variants could affect the pharmacological responses to α_2-AR agonists such as clonidine.

Thus, we examined the hypothesis that *ADRA2A* variants affect cardiovascular responses to α_2-AR-agonists by administering dexmedetomidine, a selective α_2-AR-agonist, to healthy volunteers in a highly controlled setting.

Methods

Subjects

The Institutional Review Board of Vanderbilt University Medical Center approved the study protocol, and subjects gave written informed consent. Details of the study methods and subjects have been published previously.[19,20] In brief, we studied unrelated healthy American black and white subjects, aged 18 to 45 years, without clinically significant abnormal findings on medical history, physical examination, or routine laboratory testing. For 5 days before the study, subjects were on an alcohol- and caffeine-free diet (providing 150 mmol of sodium, 70 mmol of potassium, and 600 mmol of calcium daily). All subjects were free of medications and dietary supplements for at least 2 weeks.

Study Procedure

The study was placebo-controlled, single-masked, and was performed at the Vanderbilt University Clinical Research Center. After an overnight fast, intravenous cannulas were placed in antecubital veins bilaterally, one for blood collection and the other for drug infusion. After a 30-minute supine resting period, blood pressure (BP) and heart rate were obtained from the left brachial artery with a semiautomated device (Dinamap MPS; GE Medical Systems, Waukesha, WI), and a blood sample was taken for DNA extraction and measurement of baseline plasma catecholamine concentrations. All heart rate and BP measurements were performed twice and averaged for analysis. After baseline measures, 6 infusions (each infusion lasted 10 minutes and was followed by a 20-minute observation period) were administered (Figure S1, online-only Data Supplement). Placebo (normal saline) was infused during cycles 1 to 3 and dexmedetomidine (Precedex, Abbott Laboratories, Abbott Park, IL) at doses of 0.1, 0.15, and 0.15 μg/kg body weight, respectively, during cycles 4 to 6 (cumulative dose, 0.4 μg/kg). Ten minutes after each infusion, heart rate and BP were measured and a blood sample was drawn for measurement of plasma catecholamine and dexmedetomidine concentrations.

Genotyping

We genotyped 9 *ADRA2A* tag single nucleotide polymorphisms (tagSNPs) representing common genetic variations in black and white Americans[5] (Table S1, online-only Data Supplement). The selection of tagSNPs was based on our previous study of 135 demographically similar black and white residents of Nashville, in whom we sequenced *ADRA2A* (including approximately 2.2 kb of 5′-flanking and 0.1 kb of 3′-flanking regions) and found 41 SNPs, among which we selected 9 tagSNPs by LD-based binning (binning criterion, $r^2 \geq 0.4$) and prevalence criteria (MAF $\geq$5%) that optimally captured genetic variability of *ADRA2A*.[5] We genotyped the 9 tagSNPs by allelic discrimination with TaqMan 5′-nuclease assays[21] on an ABI 7900 HT real-time PCR system (Applied Biosystems, Foster City, CA) using validated TaqMan probes. For genotyping quality control, we included 6 samples with known genotypes previously determined by direct sequencing,[5] and all control samples had concordant genotypes.

Haplotype Assignment

Haplotypes were inferred from the 9 tagSNPs using an expectation-maximization algorithm implemented in the software Powermarker.[5] Because subtypes of haplotypes 4 and 5 were expected to be infrequent, we grouped these subtypes into haplotype families for analysis as previously defined.[5] The variant rs553668 was a tagSNPs for haplotype 4, and rs553668 genotype groups therefore comprised the same patients as corresponding HT4 haplotype groups. Only haplotypes that could be inferred with $\geq$85% probability were assigned; haplotypes with $<$85% probability were handled as missing data.

Plasma Catecholamine Determination

Blood was collected into cooled heparinized tubes that were immediately placed on ice until centrifuged at 4°C for 10 minutes at 3000 rpm. Plasma was separated and stored at −20°C in tubes containing 40 μL of reduced glutathione (6%) until assayed. Norepinephrine and epinephrine concentrations were measured by high-performance liquid chromatography using electrochemical detection with dihydroxybenzylamine as internal standard.[22]

Plasma Dexmedetomidine Determination

Plasma dexmedetomidine concentrations were determined by reversed-phase high-performance liquid chromatography with tandem mass spectrometric detection[23] (PE Sciex API4000, PE Sciex, Foster City, CA) as previously described.[23,24]

Data and Statistical Analysis

Data are expressed as means and standard deviations (SD) or 95% confidence intervals (CI). Genotype distribution was tested for deviation from Hardy-Weinberg equilibrium with the use of a χ^2 test with 1 degree of freedom. To generate a summary variable representing the overall response, we plotted outcomes (systolic and diastolic BP, heart rate, and plasma catecholamine concentrations) against time for each subject and determined the area under the curve (AUC) for the 1.5-hour periods of placebo (AUC_{PLAC}) and dexmedetomidine infusions (AUC_{Dex}), respectively, by the trapezoidal rule, assuming linear changes between the measurements. AUC data were normally distributed, and dexmedetomidine responses were assessed as the difference between AUC_{Dex} and AUC_{PLAC} for each outcome variable by paired t test. In a sensitivity analysis, we also assessed the decrease in the outcome measure between the last placebo infusion and the last dexmedetomidine infusion. Because low doses of dexmedetomidine preferentially reduce systolic blood pressure and plasma norepinephrine concentrations,[5,24–26] changes in these variables were the primary outcomes, and changes in heart rate and plasma epinephrine concentrations were secondary outcomes. For single-marker analysis of the nine tagSNPs, we used 1-way ANOVA to compare outcomes by number of variant alleles. When there were 2 or fewer subjects homozygous for the variant allele, we grouped them with heterozygous carriers for statistical analysis. To adjust for potential covariates (age, sex, race, plasma dexmedetomidine concentration, body mass index [BMI], and AUC_{PLAC} for the corresponding outcome), we performed linear regression analyses, assuming an additive mode of inheritance for the SNP. For each of the 5 haplotypes, we created biallelic genotypes by treating the haplotype as a variant allele and grouping the other haplotypes together as the other allele, and repeated the above analyses. In addition, we also performed multiple linear regression analyses including all covariates and all 5 haplotypes with additive haplotypic effects. All tests were 2-tailed. In these exploratory analyses, we did not adjust for multiple comparisons, and probability values of $<$0.05 were considered statistically significant. Analyses were performed

Table 1. Demographic and Baseline Characteristics (n=73)

Parameter	n (%) or Mean±SD
Women	32 (43.8%)
Age, y	25.4±4.6
Race	
White	37 (50.7%)
Black	36 (49.3%)
Body mass index, kg/m^2	25.3±3.7
Systolic blood pressure, mm Hg	113.6±9.2
Diastolic blood pressure, mm Hg	69.0±6.8
Heart rate, bpm	64.5±7.7
Norepinephrine plasma concentration, pg/mL	230.0±95.0
Epinephrine plasma concentration, pg/mL	21.8±17.0

with the statistical software packages R (www.r-project.org) and SPSS (SPSS v.15.0, SPSS Inc, Chicago, IL).

Results

Subjects

We studied 73 healthy subjects; their demographic characteristics and baseline measures are shown in Table 1.

ADRA2A Genotypes and Haplotypes

Genotypes of the 9 *ADRA2A* variants could be determined in 94.5% to 100% of the subjects (mean call rate, 98.2%) and are shown in Table S1 (online-only Data Supplement). Minor allele frequencies were in the expected range in both ethnic groups.[6,27] All genotypes conformed to Hardy-Weinberg equilibrium in each ethnic group (all $P>0.10$). Haplotype families could be assigned to 99.3% of haplotypes with >85% probability, and the haplotype distribution was in the expected range (Table S2, online-only Data Supplement).[5]

Determinants of Outcomes at Baseline and During Placebo

Men (+8.9 mm Hg; 95% CI, 5.3 to 12.4 mm Hg; $P<0.001$), black ethnicity (+4.6 mm Hg; 95% CI, 1.1 to 8.1 mm Hg; $P=0.010$), and a higher BMI (per kg/m^2 unit, +0.6 mm Hg; 95% CI, 0.05 to 1.1 mm Hg; $P=0.033$) were individually associated with higher systolic BP at baseline, and similarly, during placebo infusions. After adjustment for these covariates, none of the *ADRA2A* tagSNPs or haplotypes was associated with systolic BP or any of the other outcomes (diastolic BP, plasma norepinephrine and epinephrine concentrations, and heart rate) at baseline or during the placebo infusions (all $P>0.07$).

ADRA2A Variants and Systolic BP Responses to Dexmedetomidine

During the dexmedetomidine infusions, the AUC for all outcome measures decreased significantly compared with that during placebo infusions (Table 2; all $P\leq0.028$; Figure S2, online-only Data Supplement). For the primary outcome, systolic BP, a higher AUC_{SBP} during placebo was associated with a greater decrease in AUC_{SBP} during dexmedetomidine

Table 2. Cardiovascular Outcome Measures During Placebo and Dexmedetomidine Infusions

	Placebo	Dexmedetomidine	ΔAUC	*P* Value
AUC_{SBP} mm Hg*h	171.3±14.1	160.2±12.4	−11.1±6.3	<0.001
AUC_{DBP} mm Hg*h	103.7±9.1	95.7±8.0	−8.0±5.8	<0.001
AUC_{HR} bpm*hr	97.5±12.1	95.2±12.4	−2.3±5.6	0.001
AUC_{NE} pg*h/mL	346.8±125.1	247.9±108.2	−98.9±76.0	<0.001
AUC_{Epi} pg*h/mL	35.8±21.9	32.9±20.2	−3.0±10.7	0.028

AUC indicates area under the curve; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; NE, norepinephrine; Epi, epinephrine; and ΔAUC, difference in AUC between dexmedetomidine and placebo infusions.

($P=0.007$), but other covariates (age, sex, race, BMI, dexmedetomidine concentration) did not affect the reduction. Table 3 shows ΔAUC_{SBP} in genotype groups by single marker analysis assuming an additive model of inheritance. Three subjects homozygous for rs553668 had a 115% (2.2-fold; 95% CI, 1.5 to 2.8-fold; $P=0.001$) greater decrease in AUC_{SBP} than carriers of 1 of more copies of the major allele, and the difference remained significant after adjustment for covariates (9.6 mm Hg; 95% CI, 2.8 to 16.4 mm Hg*h; $P=0.006$; Figure 1). A similar trend for rs2484516 did not reach statistical significance (adjusted $P=0.07$; Table 3).

Table 4 shows ΔAUC_{SBP} by haplotypes in single haplotype analyses. Homozygous or heterozygous carriers of haplotype 3 (HT3) had a 44% smaller reduction in AUC_{SBP} (ie, a smaller hypotensive response; adjusted $P=0.008$), whereas the 3 subjects with 2 copies of HT4 (already defined above by the rs553668 variant) had 2.2-fold greater reductions in AUC_{SBP} (ie, a larger hypotensive response) than carriers of 1 or no copy (adjusted $P=0.006$; Figure 1). When analyzing races separately in a sensitivity analysis, white and black homozygotes for rs553668/HT4 had a similarly larger ΔAUC_{SBP} compared with the other genotypes (2.3-fold and 2.1-fold for whites and blacks, respectively); however, genotype differences were statistically significant only in whites (unadjusted $P=0.003$), probably reflecting the small sample size for black homozygotes (n=1).

Adding all *ADRA2A* haplotypes into a model predicting ΔAUC_{SBP} that included all other covariates improved the model significantly, increasing the coefficient of determination R^2 (the percent of the variability of ΔAUC_{SBP} explained by the model) from 19.2% to 32.2% ($P<0.001$). In this model, HT3 carrier status was associated with a smaller ($P=0.013$) and HT4 with a larger hypotensive SBP response ($P=0.047$) to dexmedetomidine (Table S3, online-only Data Supplement). When HT4 was modeled as a recessive trait, taking into account the post hoc observation that only homozygous HT4 carriers had greater hypotensive responses, the association between HT4 and ΔAUC_{SBP} was stronger ($P=0.007$).

In a sensitivity analysis, as an additional assessment of responses to dexmedetomidine, we analyzed the reduction in systolic BP between the last placebo infusion and the last dexmedetomidine infusions (ΔSBP). In this analysis,

Table 3. Single-Marker Analysis of Decrease in Systolic Blood Pressure and Norepinephrine Plasma Concentrations After Dexmedetomidine

		ΔAUC_{SBP}, mm Hg*h, Mean±SD					ΔAUC_{NE}, pg/mL*h, Mean±SD				
		No. of Minor Alleles					No. of Minor Alleles				
	SNP	0	1	2	*P* Value	Adjusted *P* Value	0	1	2	*P* Value	Adjusted *P* Value
1	rs11195418	−11.0±6.2 n=65	−10.1±4.1 n=5	−12.5 n=1	0.92	0.90	−104.5±76.5 n=65	−29.1±54.5 n=5	−66.3 n=1	0.15	0.72
2	rs1800544	−9.5±5.0 n=23	−12.3±6.1 n=32	−11.1±6.3 n=18	0.26	0.49	−119.2±80.2 n=23	−86.1±63.6 n=31	−95.1±88.3 n=18	0.28	0.12
3	rs2484516	−10.5±5.8 n=64	−13.6±7.5 n=4	−25.3 n=1	0.06*	0.07*	−101.3±76.4 n=64	−99.9±75.1 n=4	−153.5 n=1	0.79	0.46
4	rs1800545	−11.4±6.5 n=42	−10.8±6.4 n=28	−10.8±4.9 n=3	0.93	0.13	−106.3±81.2 n=42	−89.6±71.5 n=27	−79.3±19.7 n=3	0.61	0.41
5	rs1800035	−10.8±6.3 n=67	−15.3±5.5 n=6	n.a.	0.090	0.18	−97.2±77.8 n=67	−118.2±52.8 n=6	n.a.	0.52	0.53
6	rs1800038	−10.9±6.1 n=67	−12.8±4.1 n=3	n.a.	0.58	0.73	−98.4±78.4 n=67	−127.1±47.7 n=3	n.a.	0.53	0.99
7	rs34303217	−10.8±6.1 n=69	−17.1±8.7 n=4	n.a.	0.051	0.53	−99.4±77.7 n=69	−91.1±42.2 n=4	n.a.	0.83	0.81
8	rs553668	−10.1±5.8 n=51	−12.1±5.8 n=19	−22.8±7.4 n=3	0.002	0.028	−104.6±77.1 n=51	−88.6±69.7 n=19	−69.3±111.4 n=3	0.59	0.17
9	rs3750625	−11.5±6.4 n=53	−9.1±4.6 n=15	−9.9±6.5 n=2	0.38	0.052	−100.9±85.6 n=53	−97.1±47.1 n=15	−85.9±22.8 n=2	0.96	0.82

AUC indicates area under the curve; SBP, systolic blood pressure; NE, norepinephrine; SNP, single-nucleotide polymorphism; and n.a., genotype not represented in cohort.

Decrease of systolic BP and NE was assessed as difference between the area under the curve for the respective variables between placebo infusions and dexmedetomidine infusions (ΔAUC_{SBP} and ΔAUC_{NE}, respectively). Uncorrected *P* values are from multiple linear regression analyses assuming an additive model of inheritance. Adjusted *P* values are after adjustment for the covariates AUC_{SBP} or AUC_{NE} during placebo, respectively, dexmedetomidine plasma concentrations, age, sex, race, and body mass index.

*The single subject homozygous for the variant allele was grouped with the heterozygotes for statistical analysis.

Figure 1. Differences in AUC (ΔAUC) between placebo and dexmedetomidine infusions for different outcomes by haplotype 4. Subjects homozygous for haplotype 4 (defined by rs553668) had a greater reduction in systolic blood pressure (upper panel; adjusted $P=0.006$), diastolic blood pressure (middle panel; adjusted $P=0.007$), and heart rate (lower panel; adjusted $P=0.035$) compared with carriers of 0 or 1 copy. Horizontal lines represent means; whiskers, standard error of the mean.

haplotype 4 was also associated with a greater hypotensive response, and haplotype 3 with a smaller response (Figure 2), with a magnitude similar to that observed with the summary measure ΔAUC_{SBP}. Carriers of HT3, compared with noncarriers, had a 39% (0.61-fold) smaller hypotensive response (ΔSBP=8.8±6.5 mm Hg and 14.5±6.1 mm Hg, respectively; $P=0.015$), and subjects homozygous for HT4 had an 82% (1.8-fold) greater hypotensive response (ΔSBP=24.7±8.1 mm Hg compared with 13.6±5.9 mm Hg; $P=0.007$; Figure 2) compared with carriers of other haplotypes.

For the second main outcome, change in plasma norepinephrine concentrations between placebo and dexmedetomidine infusions, ΔAUC_{NE} was not affected by *ADRA2A*

Table 4. Single-Haplotype Analysis of Decrease in Systolic Blood Pressure and Norepinephrine Plasma Concentrations After Dexmedetomidine

	ΔAUC_{SBP}, mm Hg*h (Mean±SD)					ΔAUC_{NE}, pg/mL*h (Mean±SD)				
	No. of Haplotype Copies					No. of Haplotype Copies				
Haplotype	0	1	2	*P* Value	Adjusted *P* Value	0	1	2	*P* Value	Adjusted *P* Value
HT1	−12.1±7.7 n=23	−11.5±6.2 n=25	−9.5±5.0 n=23	0.17	0.77	−107.8±74.7 n=23	−77.6±65.7 n=24	−119.2±80.2 n=23	0.60	0.46
HT2	−10.6±6.4 n=65	−15.3±5.5 n=6	n.a. n=0	0.09	0.18	−99.6±76.6 n=65	−118.2±52.8 n=6	n.a. n=0	0.56	0.52
HT3	−11.6±6.2 n=63	−6.1±7.2 n=7	−9.5 n=1	0.034*	0.008*	−101.2±71.8 n=63	−73.9±70.6 n=7	−291.3 n=1	0.99*	0.81*
HT4	−10.1±5.8 n=51	−12.1±5.8 n=19	−22.8±7.4 n=3	0.002	0.028	−104.6±77.1 n=50	−88.6±69.7 n=19	−69.3±111.4 n=3	0.44	0.18
HT5	−11.2±6.6 n=40	−10.8±6.4 n=28	−10.8±4.9 n=3	0.79	0.16	−110.6±79.0 n=40	−89.6±71.5 n=27	−79.3±19.7 n=3	0.49	0.35

AUC indicates area under the curve; SBP, systolic blood pressure; NE, norepinephrine; HT, haplotype, and n.a., not represented in cohort.

Decrease of systolic BP and NE was assessed as difference between the area under the curve for the respective variables between placebo infusions and dexmedetomidine infusions (ΔAUCSBP and ΔAUCNE, respectively). Uncorrected *P* values are from multiple linear regression analysis assuming an additive model of inheritance. Adjusted *P* values are after adjustment for the covariates AUCSBP or AUCNE during placebo, respectively, dexmedetomidine plasma concentrations, age, sex, race, and body mass index. Except placebo AUCSBP and placebo AUCNE, respectively ($P<0.021$ in all analyses), no other covariate was significantly associated with the outcomes.

*For statistical analysis, the single subject homozygous for HT3 was grouped with the 7 heterozygous carriers.

variants in single marker (all probability values >0.17; Table 3) or haplotype analyses (all probability values >0.46; Table 4). Accordingly, adding all haplotypes to a model with ΔAUC_{NE} as outcome and all other covariates did not improve model fit ($P=0.66$).

ADRA2A Variants and Secondary Outcomes

For secondary outcomes, in single marker analyses, the same markers associated with a greater hypotensive response for SBP (rs553668 and HT4) were also associated with a greater decrease in diastolic BP (ΔAUC_{DBP}; $P=0.040$) and heart rate (ΔAUC_{HR}; $P=0.033$; Figure 1). However, after adjustment for covariates, these associations were weakened and remained significant only under a recessive mode of inheritance ($P=0.007$ and $P=0.035$ for ΔAUC_{DBP} and ΔAUC_{HR}, respectively). HT3, the haplotype associated with a smaller decrease in SBP after dexmedetomidine, was also weakly associated with a smaller decrease in DBP (ΔAUC_{DBP}; $P=0.15$; adjusted $P=0.042$) but not heart rate response (ΔAUC_{HR}; $P=0.12$; adjusted $P=0.11$) in single haplotype analyses. None of the variants or haplotypes was associated with the decrease in plasma epinephrine concentrations (ΔAUC_{Epi}).

Discussion

In this study, we systematically defined the effects of common *ADRA2A* variants on cardiovascular responses to the selective α_2-AR agonist, dexmedetomidine. Our main findings are that *ADRA2A* variants previously associated with changes in α_{2A}-AR expression contribute to the interindividual variability in BP and heart rate responses to dexmedetomidine, with particular genotypes or haplotypes being associated with approximately 40% smaller and 100% greater hypotensive responses, respectively.

The 3′-UTR SNP rs553668 (G>A), formerly identified as the *Dra*I restriction fragment length polymorphism, defines the haplotype 4 family and has a minor allele frequency of approximately 15% in white and 20% to 30% in black populations.[5,6] Some earlier studies reported a higher prevalence of the rs553668 variant allele in various populations with high blood pressure but several recent studies, including 2 large genome-wide analyses, did not confirm these findings. The molecular mechanisms for potential phenotypic effects of this variant have been partially elucidated.

In cell lines transfected with different *ADRA2A* haplotypes, 3 of 4 haplotypes containing the variant rs553668 allele were associated with increased α_{2A}-AR mRNA transcription.[6] The rs553668 variant also resulted in increased α_{2A}-AR expression on pancreatic islet cell membranes and thus decreased insulin secretion; moreover, it is associated with an increased prevalence of type 2 diabetes mellitus.[8] Thus, the observation that rs553668/HT4 are associated with increased cell surface α_{2A}-AR expression is biologically concordant with our finding of increased responses to the α_2-AR agonist dexmedetomidine. Interestingly, beyond SBP, reductions in the secondary outcomes diastolic BP and heart rate were also more pronounced in subjects carrying this variant.

ADRA2A haplotype 3 is characterized by a minor allele at a single SNP (rs1800544) in the absence of other SNPs. This

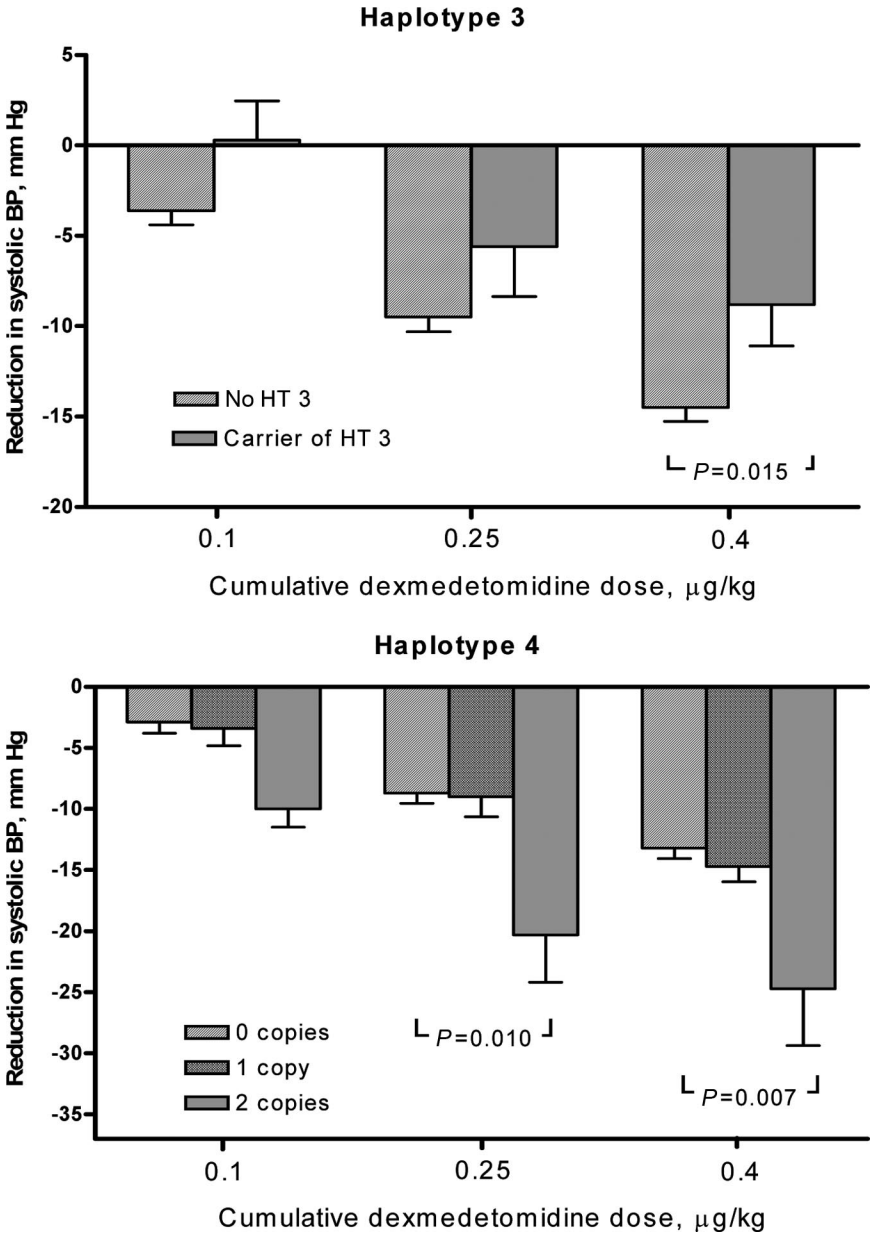

Figure 2. Reduction in systolic blood pressure (BP) during dexmedetomidine. Bar graphs depict the reduction in systolic BP from the last placebo infusion after each of 3 dexmedetomidine infusions, stratified by number of copies of haplotype 3 (upper panel) and haplotype 4 (or rs553668 minor alleles; lower panel). For statistical analysis, the single subject homozygous for HT3 was grouped with the heterozygous carriers. Error bars represent standard errors of the mean.

haplotype occurs in 8% to 11% of black Americans but has not been described in white populations.[5,6] In the present study, carriers of haplotype 3 had significantly smaller hypotensive responses to dexmedetomidine. Interestingly, in a study with haplotype-transfected cell lines, Small et al[6] found this same haplotype (designated as haplotype 1 in their nomenclature) to have the lowest transcription (about 60% of the α_{2A}-mRNA of the wild-type haplotype) and receptor expression (about 20% of the wild-type haplotype) compared with other haplotypes. Thus, the smaller hypotensive response to dexmedetomidine that we observed in carriers of haplotype 3 is concordant with its in vitro effects of lower α_{2A}-AR expression.

We did not find an association between *ADRA2A* variants and the reduction in plasma norepinephrine concentrations after dexmedetomidine. Possible explanations include the lack of statistical power in view of our sample size and the fact that in non–steady-state conditions, such as occur after dexmedetomidine administration, plasma norepinephrine concentrations are not an ideal measure of sympathetic activity. Norepinephrine spillover studies would be a more precise way of assessing sympathetic activity, but because they involve administration of radiolabeled norepinephrine to measure clearance, they are technically challenging and not feasible for larger study cohorts.

Our findings have several implications. First, variants and haplotypes predicted to affect α_{2A}-AR expression have functional effects on cardiovascular regulation in vivo, concordant with previous findings in vitro. Because the effect size was considerable (with mean differences in hypotensive responses ranging from 0.6-fold to 2.2-fold for particular *ADRA2A* haplotypes), *ADRA2A* genetic variation may contribute to interindividual differences in BP regulation, especially after physiological (stress-induced) or pharmacological activation

(by α_2-AR agonists such as clonidine and dexmedetomidine). Clinically, α_2-agonists are used to date primarily as antihypertensive agents (clonidine) or for sedation and anesthesia during surgery or in the intensive care setting (dexmedetomidine), and predicting interindividual variability in hypotensive BP response (either as therapeutic goal or adverse drug effect) could be of significant clinical importance. Additionally, because *ADRA2A* variants have in vivo functional effects on cardiovascular outcomes, other α_{2A}-AR–mediated outcomes such as platelet function, metabolic regulation, or various central nervous system functions may be similarly affected. In fact, with respect to the α_{2A}-AR–mediated inhibition of insulin secretion from islet cells, a recent study confirmed the association of *ADRA2A* rs553668 with impaired insulin secretion and type 2 diabetes mellitus, and a meta-analysis of genome-wide association studies (GWAS) identified a strong association of rs10885122, a variant approximately 200 kb downstream of *ADRA2A*, with higher fasting glucose.[8,28] In addition, a recent GWAS identified 1 SNP each in whites and blacks downstream *ADRA2A* (about 63 to 70 kb) to be associated with decreased epinephrine-induced platelet aggregation.[29] In contrast, despite the role of α_{2A}-ARs in spinal and central nervous system pain processing, in a previous study we did not find any association between *ADRA2A* variants and pain perception in an experimental pain model.[20]

Our study had several strengths and limitations. We used the highly selective α_2-AR agonist dexmedetomidine to avoid cardiovascular effects mediated by α_1-ARs, as occurs with mixed agonists, for example, clonidine. To account for interindividual differences in dexmedetomidine pharmacokinetics, we measured plasma dexmedetomidine concentrations and adjusted for these in our analyses. We studied healthy subjects in a highly controlled study setting to avoid the confounding effects of environmental factors, disease, and concomitant drugs. This design increased our ability to isolate the functional effects of genetic *ADRA2A* variants; however, our findings cannot necessarily be extrapolated to patients with hypertension or other diseases. Other limitations include the small sample size, resulting in some small genotype groups and thus wide confidence intervals around the estimates of effect sizes. Thus, our findings are preliminary and require validation in larger, clinical cohorts. *ADRA2A* variants and haplotype structure vary between whites and blacks,[5,6,29] but our cohort was underpowered for meaningful separate analyses by race. Nevertheless, the trend toward increased BP reduction in subjects homozygous for rs553668 was similar in magnitude in both races. Indeed, consistency of an association between a variant and a given outcome across multiple ethnic groups with differences in genetic diversity and haplotype structure can be helpful in differentiating truly causal variants from those merely associated with such causative variants by linkage disequilibrium.[30] Additionally, the tagSNPs we used represent well the genetic variability of *ADRA2A* and its 3′-flanking region (approximately 2.2 kb) in our study population, but we did not cover more distant intergenic variants. This may be significant in view of the recently reported association of reduced epinephrine-induced platelet aggregation with 2 more distant variants (rs4311994 in whites and rs869244 in blacks) approximately 63 to 70 kb downstream *ADRA2A*,[29] and another report of a distant downstream variant (202 kb; rs10885122) associated with increased fasting glucose.[28] It is unclear whether these functional associations with distant downstream variants represent linkage disequilibrium with causal variants in closer proximity to or within *ADRA2A* or long-range regulatory elements.[29] Using data available on Hapmap, we found that none of these variants is in significant linkage disequilibrium with rs553668, the variant associated with increased dexmedetomidine responsiveness in our study (all $r^2 \leq 0.125$ for both Caucasians with European ancestry and blacks from Yoruba, Nigeria). Thus, multiple variants within and around *ADRA2A* may be associated with diverse α_{2A}-AR–mediated responses, possibly reflecting tissue-specific effects of specific genetic variants or study-related methodological differences.

In conclusion, this translational study provides evidence that genetic variants in *ADRA2A* are associated with different blood pressure responses to the selective α_2-selective agonist dexmedetomidine. Variants or haplotypes previously linked with greater or lesser gene transcription/expression were associated with approximately 100% greater and 40% lesser responses to the drug, respectively. Future studies of these variants in other cardiovascular settings, for example, stress responses, and other physiological functions mediated by the α_{2A}-AR will be of interest.

Sources of Funding

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Disclosures

The laboratory of Dr Scheinin has contract research relationships with Orion Corporation (Espoo, Finland) and Hospira (Lake Forest, IL). Hospira has a license agreement with Orion Corporation concerning dexmedetomidine (Precedex). Dr Scheinin has received speaker fees and consulting fees from Orion Corporation.

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CLINICAL PERSPECTIVE

The α_{2A}-adrenoreceptor (α_{2A}-AR) is a key regulator of central and peripheral sympathetic tone and thus cardiovascular responses. α_{2A}-AR agonists are in clinical use as anesthetic agents (dexmedetomidine) or antihypertensive agents (clonidine). Functional variants in the α_{2A}-AR gene (*ADRA2A*) have previously been shown to affect receptor expression and function. Furthermore, in recent studies, some variants in or near *ADRA2A* were associated with platelet aggregation response to epinephrine, increased receptor expression, reduced insulin secretion, and increased risk for diabetes mellitus. However, the effects of *ADRA2A* variants on cardiovascular regulation and response to agonists in humans are unclear. In this single-masked, placebo-controlled, translational study in 73 healthy subjects, we infused increasing doses of the selective α_{2A}-AR agonist dexmedetomidine under controlled conditions and measured the decrease in blood pressure, heart rate, and plasma catecholamine concentrations. We then genotyped 9 *ADRA2A* tagging variants, derived haplotypes, and analyzed the response to dexmedetomidine according to *ADRA2A* genotypes and haplotypes. There was substantial interindividual variability in blood pressure responses to dexmedetomidine. Common genetic *ADRA2A* variants and haplotypes previously linked to greater or lesser gene transcription/expression were associated with approximately 100% greater and 40% lesser responses to the drug, respectively. Thus, *ADRA2A* genotype explained some of the interindividual variability in drug response. Our findings may have implications for the prediction of blood pressure reduction (both as therapeutic and adverse effects) in response to α_{2A}-AR agonists such as clonidine and dexmedetomidine. Further studies will be necessary to define the contribution of *ADRA2A* variants to drug response in the clinical setting.

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Genetic Variations in the α_{2A}-Adrenoreceptor Are Associated With Blood Pressure Response to the Agonist Dexmedetomidine

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Supplemental Material

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SNP position in haplotype	Chromosome position	rs number	Nucleotide change	MAF (%)	
				White subjects	Black subjects
1	112835159	rs11195418	A>G	10.0	0.0
2	112836503	rs1800544	C>G	31.1	62.5
3	112837073	rs2484516	C>G	0.0	8.3
4	112837538	rs1800545	G>A	14.9	31.9
5	112838552	rs1800035	C>G	0.0	8.3
6	112838892	rs1800038	C>A	0.0	4.2
7	112839282	rs34303217	T>A	0.0	5.6
8	112839579	rs553668	A>G	14.9	19.4
9	112839601	rs3750625	C>A	7.4	19.4

Table S1: Minor allele frequencies of 9 *ADRA2A* tagSNPs in white and black subjects. Chromosome positions refer to Genome build GRCh 37.1., August 2009. SNP=single nucleotide polymorphism; MAF=minor allele frequency.

Haplotype family	SNP positions in haplotype	Haplotype frequency (%)	
	1-2-3-4-5-6-7-8-9	White subjects	Black subjects
1	A-C-C-G-C-C-T-G-C	71.4	29.2
2	A-C-C-G-G-C-T-G-C	0.0	8.3
3	A-G-C-G-C-C-T-G-C	1.4	11.1
4	A-G-C-G-C-C-T-A-C A-G-G-G-C-C-T-A-C A-G-C-G-C-C-A-A-C A-G-C-G-C-A-T-A-C A-G-G-G-C-C-A-A-C A-G-G-G-C-C-A-A-C A-G-C-G-G-A-T-A-C	14.9	19.4
5	A-G-C-A-C-C-T-G-C A-G-C-A-C-C-T-G-A G-G-C-A-C-C-T-G-C A-G-C-A-G-C-T-G-C G-G-C-A-C-C-T-G-A	15.7	31.9

Table S2. Definition and distribution of haplotype families. Minor alleles are shadowed in grey. Haplotypes were grouped in haplotypes families as previously defined.[1]

	ΔAUC_{SBP} mmHg*hr	ΔAUC_{DBP} mmHg*hr	ΔAUC_{HR} bpm*hr
HT 2	3.7 (-1.6 to 8.9) P=0.16	1.0 (-3.4 to 5.4) P=0.65	-0.9 (-5.9 to 4.2) P=0.73
HT 3 carrier	-6.1 (-10.8 to -1.3) P=0.013	-3.8 (-7.7 to 0.0) P=0.052	-3.7 (-8.2 to 0.9) P=0.11
HT4	2.9 (0.0-5.8) P=0.047 P_{rec}=0.007	0.9 (-1.5 to 3.2) P=0.47 P_{rec}=0.10	-0.5 (-3.3 to 2.4) P=0.75 , P_{rec} =0.069
HT 5	-1.1 (-3.7 to 1.6) P=0.43	-0.3 (-2.5 to 1.9) P=0.80	-0.1 (-2.7 to 2.5) P=0.93

Table S3. Effect of all haplotypes combined on decrease in blood pressure and heart rate. The table shows means and 95% CI of the β-coefficients. P-values are adjusted for all other haplotypes, age, sex, race, BMI, AUC_{Plac} of the respective outcome, and dexmedetomidine plasma concentrations. P_{rec} denotes P-values derived from analyses in which HT4 was treated as a recessive (rather than additive) trait.

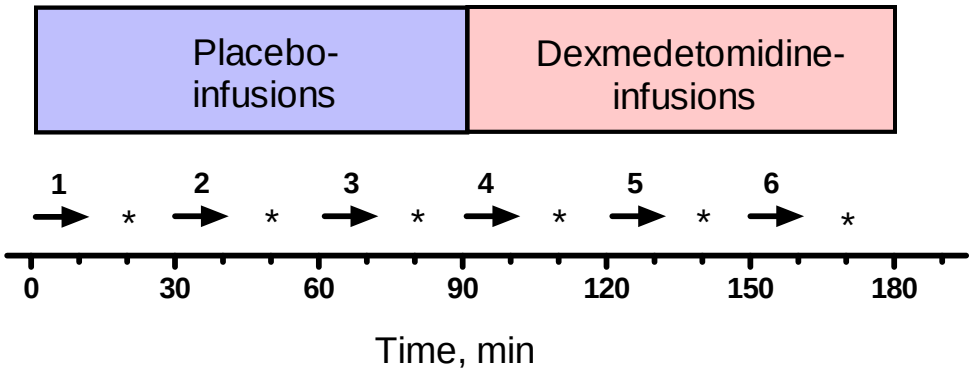

Figure S1. Diagram of study protocol. After baseline measurements, subjects received 6 infusions (10 minutes each, indicated by horizontal arrows). Ten minutes after completion of the infusions, blood pressure was determined and a blood sample taken (indicated by asterix). The first three infusions were placebo, the last three were dexmedetomidine infusions at doses of 0.1, 0.15, and 0.15 mcg/kg body weight, respectively.

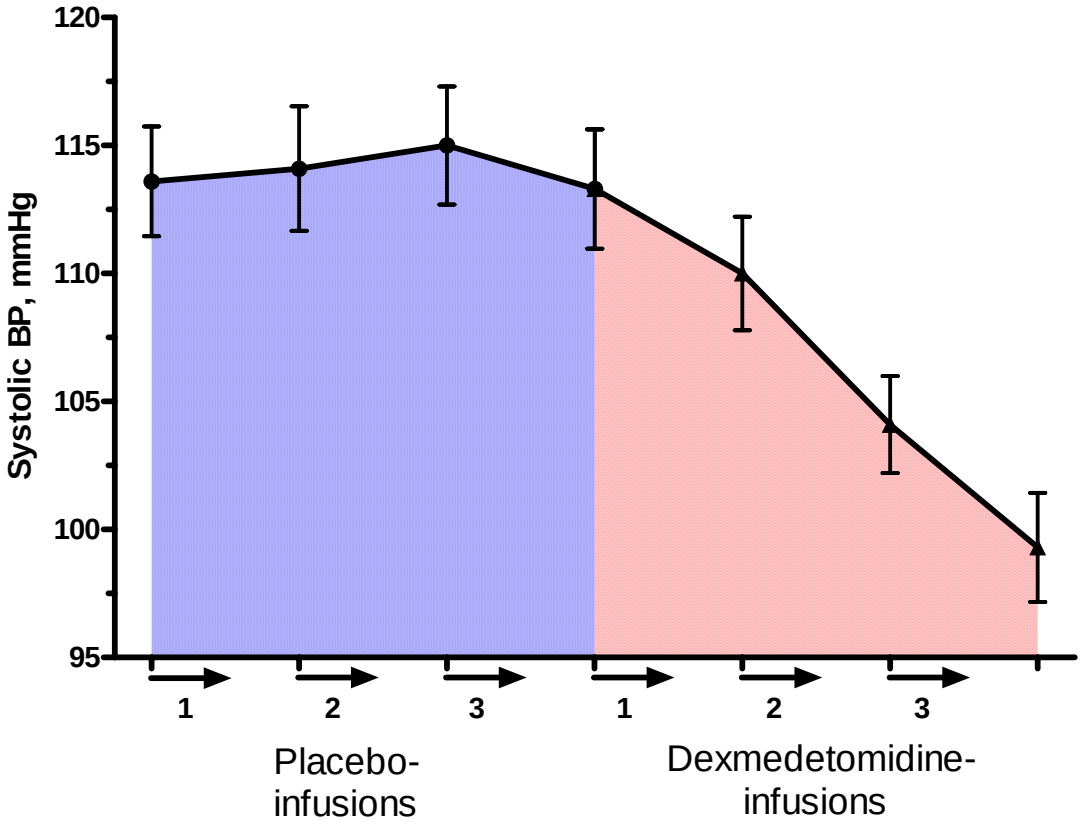

Figure S2. Systolic blood pressure during placebo and dexmedetomidine infusion in 73 subjects. Data points represent the mean, error bars the 95% confidence intervals of systolic blood pressure measured 10 minutes after completion of each infusion (represented by horizontal arrows). The blue and red areas represent the AUC_{SBP} during placebo and dexmedetomidine, respectively.

Supplement reference

1. Kurnik D, Muszkat M, Li C, Sofowora GG, Solus J, Xie HG, Harris PA, Jiang L, McMunn C, Ihrie P, Dawson EP, Williams SM, Wood AJ, Stein CM. Variations in the alpha2A-adrenergic receptor gene and their functional effects. *Clin Pharmacol Ther* 2006; 79:173-85.

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3	A-G-C-G-C-C-T-G-C	1.4	11.1
4	A-G-C-G-C-C-T-A-C A-G-G-G-C-C-T-A-C A-G-C-G-C-C-A-A-C A-G-C-G-C-A-T-A-C A-G-G-G-C-C-A-A-C A-G-G-G-C-C-A-A-C A-G-C-G-G-A-T-A-C	14.9	19.4
5	A-G-C-A-C-C-T-G-C A-G-C-A-C-C-T-G-A G-G-C-A-C-C-T-G-C A-G-C-A-G-C-T-G-C G-G-C-A-C-C-T-G-A	15.7	31.9

Table S2. Definition and distribution of haplotype families. Minor alleles are shadowed in grey. Haplotypes were grouped in haplotypes families as previously defined.[1]

	ΔAUC_{SBP} mmHg*hr	ΔAUC_{DBP} mmHg*hr	ΔAUC_{HR} bpm*hr
HT 2	3.7 (-1.6 to 8.9) P=0.16	1.0 (-3.4 to 5.4) P=0.65	-0.9 (-5.9 to 4.2) P=0.73
HT 3 carrier	-6.1 (-10.8 to -1.3) P=0.013	-3.8 (-7.7 to 0.0) P=0.052	-3.7 (-8.2 to 0.9) P=0.11
HT4	2.9 (0.0-5.8) P=0.047 P_{rec}=0.007	0.9 (-1.5 to 3.2) P=0.47 P_{rec}=0.10	-0.5 (-3.3 to 2.4) P=0.75 , P_{rec} =0.069
HT 5	-1.1 (-3.7 to 1.6) P=0.43	-0.3 (-2.5 to 1.9) P=0.80	-0.1 (-2.7 to 2.5) P=0.93

Table S3. Effect of all haplotypes combined on decrease in blood pressure and heart rate. The table shows means and 95% CI of the β-coefficients. P-values are adjusted for all other haplotypes, age, sex, race, BMI, AUC_{Plac} of the respective outcome, and dexmedetomidine plasma concentrations. P_{rec} denotes P-values derived from analyses in which HT4 was treated as a recessive (rather than additive) trait.

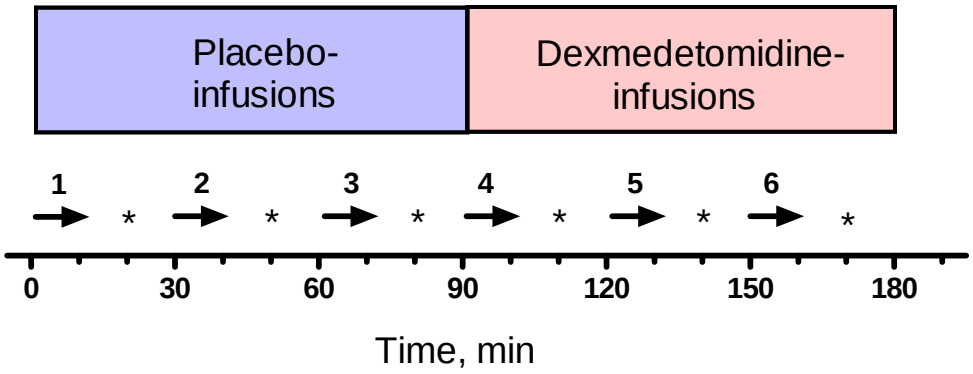

Figure S1. Diagram of study protocol. After baseline measurements, subjects received 6 infusions (10 minutes each, indicated by horizontal arrows). Ten minutes after completion of the infusions, blood pressure was determined and a blood sample taken (indicated by asterix). The first three infusions were placebo, the last three were dexmedetomidine infusions at doses of 0.1, 0.15, and 0.15 mcg/kg body weight, respectively.

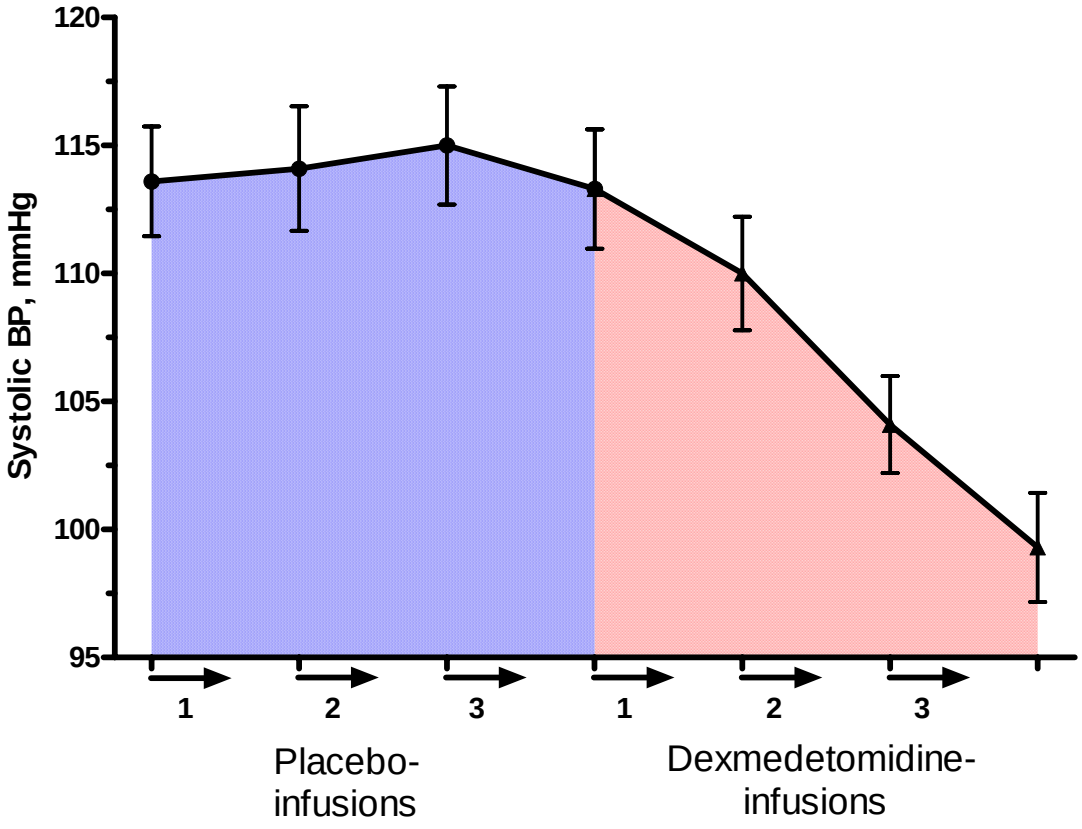

Figure S2. Systolic blood pressure during placebo and dexmedetomidine infusion in 73 subjects. Data points represent the mean, error bars the 95% confidence intervals of systolic blood pressure measured 10 minutes after completion of each infusion (represented by horizontal arrows). The blue and red areas represent the AUC_{SBP} during placebo and dexmedetomidine, respectively.

Supplement reference

1. Kurnik D, Muszkat M, Li C, Sofowora GG, Solus J, Xie HG, Harris PA, Jiang L, McMunn C, Ihrie P, Dawson EP, Williams SM, Wood AJ, Stein CM. Variations in the alpha2A-adrenergic receptor gene and their functional effects. *Clin Pharmacol Ther* 2006; 79:173-85.

Supplemental Material

Kurnik D et al. Genetic variations in the α_{2A}-adrenoreceptor are associated with blood pressure response to the agonist dexmedetomidine.

SNP position in haplotype	Chromosome position	rs number	Nucleotide change	MAF (%)	
				White subjects	Black subjects
1	112835159	rs11195418	A>G	10.0	0.0
2	112836503	rs1800544	C>G	31.1	62.5
3	112837073	rs2484516	C>G	0.0	8.3
4	112837538	rs1800545	G>A	14.9	31.9
5	112838552	rs1800035	C>G	0.0	8.3
6	112838892	rs1800038	C>A	0.0	4.2
7	112839282	rs34303217	T>A	0.0	5.6
8	112839579	rs553668	A>G	14.9	19.4
9	112839601	rs3750625	C>A	7.4	19.4

Table S1: Minor allele frequencies of 9 *ADRA2A* tagSNPs in white and black subjects. Chromosome positions refer to Genome build GRCh 37.1., August 2009. SNP=single nucleotide polymorphism; MAF=minor allele frequency.

Haplotype family	SNP positions in haplotype	Haplotype frequency (%)	
	1-2-3-4-5-6-7-8-9	White subjects	Black subjects
1	A-C-C-G-C-C-T-G-C	71.4	29.2
2	A-C-C-G-G-C-T-G-C	0.0	8.3
3	A-G-C-G-C-C-T-G-C	1.4	11.1
4	A-G-C-G-C-C-T-A-C A-G-G-G-C-C-T-A-C A-G-C-G-C-C-A-A-C A-G-C-G-C-A-T-A-C A-G-G-G-C-C-A-A-C A-G-G-G-C-C-A-A-C A-G-C-G-G-A-T-A-C	14.9	19.4
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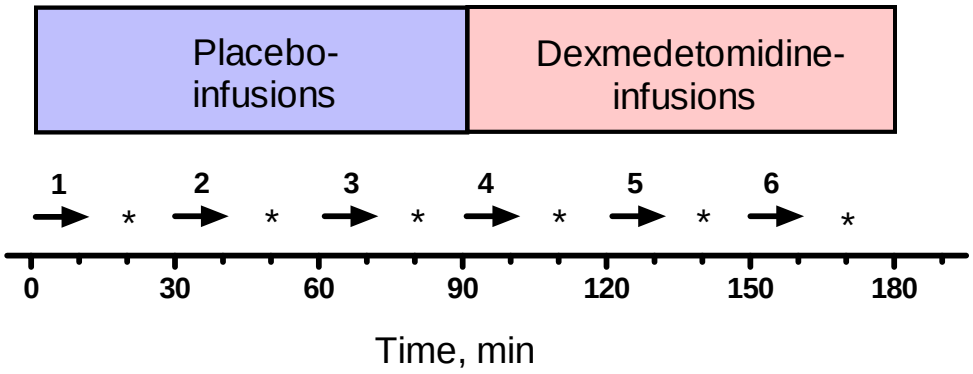

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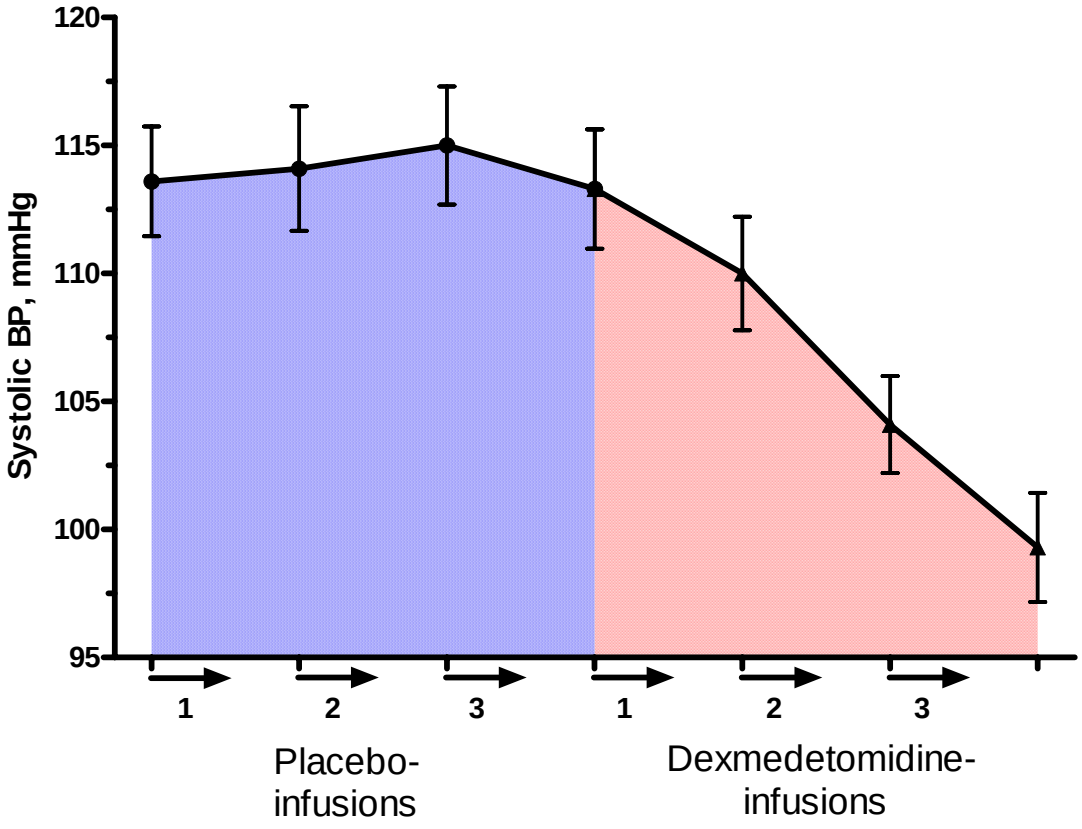

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