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Effects of variation in the human α_{2A}- and α_{2C}-adrenoceptor genes on cognitive tasks and pain perception

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Abstract

Background—The mechanisms underlying interindividual variability in pain perception and cognitive responses are undefined but highly heritable. α_{2C}- and α_{2A}-adrenergic receptors regulate noradrenergic activity and are important mediators of pain perception and analgesia. We hypothesized that common genetic variants in these genes, particularly the *ADRA2C* del322–325 deletion variant, affect pain perception or cognitive responses.

Methods—We studied 73 healthy subjects (37 Caucasians and 36 African-Americans) aged 25.4 ±4.6 years. Pain response to a cold pressor test was measured using a 10 cm visual analog scale and again on the next day, after 3 infusions of the selective α_2-agonist dexmedetomidine. Standardized cognitive tests were administered at baseline and after each infusion. The contribution of *ADRA2C* deletion genotype, dexmedetomidine concentration, and other covariates to pain perception and cognitive responses was determined using multiple linear regression models. Secondary analysis examined the effects of *ADRA2A* and other *ADRA2C* variants on pain perception.

Results—*ADRA2C* Del homozygotes had higher pain scores in response to cold at baseline (6.3 ±1.8 cm) and after dexmedetomidine (5.6±2.2 cm) than insertion allele carriers (4.6±2.1 cm [baseline] and 3.8±1.9 cm [after dexmedetomidine]; adjusted P-values=0.019 and 0.004, respectively). Cognitive responses were unrelated to *ADRA2C* Ins/Del genotype. None of the other *ADRA2A* and *ADRA2C* variants was significantly related to cold pain sensitivity before dexmedetomidine; after dexmedetomidine, *ADRA2A* rs1800038 was marginally associated (P=0.03).

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Disclosures

The laboratory of Mika Scheinin has contract research relationships with Orion Corporation (Espoo, Finland) and Hospira (Lake Forest, IL, USA). Hospira has a license agreement with Orion Corporation concerning dexmedetomidine (Precedex®). Mika Scheinin has also received speaker fees and consulting fees from Orion Corporation. None of the other authors has a conflict of interest relevant to the work presented.

Conclusion—The common *ADRA2C* del322–325 variant affected pain perception before and after dexmedetomidine but did not affect other cognitive responses, suggesting that it contributes to interindividual variability in pain perception.

Key words

α_{2C}-adrenoceptor; α_{2A}-adrenoceptor; pain; genetics; cognition

Introduction

Adrenergic signaling plays an important role in cognition. Processes such as memory, learning, and selective attention are influenced by ascending dorsal noradrenergic bundles from the locus ceruleus in the brainstem where α_2-adrenoceptors (α_2-ARs) play an important role. Also, in the frontal lobe, α_2-ARs mediate increased attention (Coull, 1994; Milstein et al., 2007). The noradrenergic system is also intimately involved in pain perception; descending noradrenergic fibers to the spinal cord from the locus ceruleus and brainstem modulate pain responses via spinal α_2-ARs (Kwiat and Basbaum, 1992; Proudfit, 1988). There are three α_2-ARs subtypes: 2A, 2B and 2C. Studies performed in genetically modified animals have elucidated the specific functions of each α_2-AR subtype.

In mice, the α_{2A}-AR subtype is the chief mediator of central effects such as sedation and analgesia (Galeotti et al., 2004); however, α_{2C}-ARs also have central effects and alter cortical arousal (Puolivali et al., 2002), amphetamine-induced responses, prepulse inhibition, startle responses (Scheinin et al., 2001), and spatial and non-spatial search patterns (Bjorklund et al., 1999). Both α_{2A}- and α_{2C}-AR subtypes mediate effects at the spinal level (Pieribone et al., 1994), but importantly, α_{2C}-ARs are mainly found on the presynaptic terminals where they inhibit the release of neurotransmitters and mediate spinal analgesia (Fairbanks et al., 2002).

Less is known about the specific roles of α_{2A}- and α_{2C}-ARs in mediating central and spinal effects in humans. Effects in the spinal cord are mediated by α_{2A}- and α_{2B}-subtypes, and those in dorsal root ganglia by α_{2B}- and α_{2C}-subtypes (Ongioco et al., 2000; Smith et al., 1995). In humans, non-selective agonists with greater effects at α_{2C}-ARs, for example clonidine, specifically disrupt performance of tasks requiring attention, suggesting that the α_{2C}-AR subtype mediates some specific central effects in humans (Jakala et al., 1999). The lack of subtype-specific agonists and antagonists has made it difficult to further delineate the pharmacological effects mediated by the α_2-AR subtypes in humans.

The identification of common α_2-AR genetic variations that affect receptor function may provide a novel means for defining the functions of the respective subtypes. A common 12 base-pair deletion in the coding region of the α_{2C}-AR gene (*ADRA2C*) results in the deletion of four amino acids (del322–325) and a receptor that has markedly decreased agonist-mediated responses *in vitro* (Small et al., 2002). In a previous study, we showed that this variant affected heart rate responses to a cold pressor stimulus. (Kurnik et al., 2008) The mechanisms underlying this effect are not known.

Cold induced pain is highly heritable, suggesting that it is strongly affected by genetic factors (Nielsen et al., 2008). In view of the central role of α_{2A}- and α_{2C}-ARs in pain perception we addressed the hypotheses that 1) the *ADRA2C* deletion variant affects cold pressor pain perception and cognitive responses, and 2) other genetic variants in *ADRA2A* and *ADRA2C* contribute to interindividual variation in cold pain perception. To augment potential genotypic differences in response, we also repeated the cold pressor test after the administration of dexmedetomidine, a highly selective α_2-adrenergic agonist.

Methods

Subjects

The study was approved by the Institutional Review Board of Vanderbilt University, and all subjects gave written informed consent. The recruitment process and study procedures are described in detail elsewhere (Kurnik et al., 2008). We studied 73 (37 Caucasian and 36 African-American) healthy subjects who were recruited by advertisement and from a volunteer database (Harris et al., 2005). Sixty-seven subjects were enrolled without knowledge of their *ADRA2C* genotype, and in order to enrich the homozygous subgroups in the study population, 6 African-American subjects were enrolled because they were known from a previous study to be homozygous for the *ADRA2C* insertion or deletion allele (3 each with Ins/Ins and Del/Del genotype, respectively).

Study Procedure

Subjects were studied on 2 consecutive study days starting in the morning (between 8:00–10:00 am) in a temperature-controlled room (22°C) at the Vanderbilt University Clinical Research Center, after an overnight fast. On study day 1, following a 30 min supine rest period, a cold pressor test was performed as previously described (Kurnik et al., 2008). Briefly, with the participant supine, the left foot was immersed up to the ankle in a tub filled with a slurry of ice and water (4°C) for 2 min. Pain ratings were obtained immediately after the foot was withdrawn. On study day 2, each subject received a total of 6 infusions, 3 each of saline and dexmedetomidine (0.10, 0.15, and 0.15 μg/kg), in that order. The duration of each infusion was 10 minutes with 20 minutes between any two successive infusions. Dexmedetomidine, the most selective α_2-adrenergic receptor agonist in clinical use, was administered with the objective of enhancing any differences in response between subjects with different α_2-adrenergic receptor genotypes by examining responses under conditions of receptor stimulation. Thirty minutes after completion of the last dexmedetomidine infusion (cumulative dose, 0.4 μg/kg), another cold pressor test was performed. The hemodynamic responses in these subjects have been reported previously (Kurnik et al., 2008).

A standardized short term memory test (immediate serial recall), digit symbol substitution test (DSST) (Lezak, 1982), and reaction time tests (Jakala et al., 1999), were administered at the beginning of study day 2 (10 minutes before the start of the first saline infusion) and then 10 minutes after the completion of each of the three saline and three dexmedetomidine infusions. For immediate serial recall, a classic short term memory test, a list of 15 words was read to the subject, who then recalled as many as possible in any order. The DSST is a timed, written test that requires the subject to translate numbers into symbols using a key. DSST performance reflects psychomotor speed, attention, and perceptual organization (Hindmarch, 1980). The computer-based simple reaction time tests and choice reaction time test assesses motor performance as well as sustained attention and vigilance (Jakala et al.,1999). In addition, each subject reported the subjective intensity of sedation at each of these 7 time points by placing a mark on a 10 cm visual analog sedation scale (VAS), with 0 and 10 cm representing no and full sedation, respectively.

Similarly, pain response to the cold pressor tests before (study day 1) and after dexmedetomidine (study day 2) was measured using a 10 cm visual analog scale. On each study day, one subject did not perform the cold pressor test and was excluded from the analysis of cold pressor test data, but included in other analyses. Blood for the determination of plasma dexmedetomidine concentrations was drawn 10 minutes after the completion of the last dexmedetomidine infusion.

Genotyping Procedure

We determined genotypes for 5 common (estimated minor allele frequency >10%) *ADRA2C* variants that were not highly linked (linkage disequilibrium r^2<0.70: *ADRA2C* 322–325 ins/del, rs9790683, rs13118771, rs2416, and rs7434444 (Small et al., 2004)) and 9 relatively common *ADRA2A* variants (rs11195418, rs1800544, rs2484516, rs1800545, rs1800035, rs1800038, +1483T>A, rs553668, and rs3750625) (Kurnik et al., 2006). Genotyping for the *ADRA2C* deletion involved amplification of DNA fragments by polymerase chain reaction (PCR) followed by DNA fragment analysis (Kurnik et al., 2007). The remaining *ADRA2C* variants and all *ADRA2A* variants were genotyped by allelic discrimination with TaqMan 5′-nuclease assays (Livak, 2003) on an ABI 7900 HT real-time polymerase chain reaction system (Applied Biosystems, Foster City, California, USA) using validated TaqMan probes and a 95% quality value threshold. For each variant, genotype results were confirmed by direct sequencing in 17–20 randomly selected samples with complete concordance.

Dexmedetomidine determination

Plasma dexmedetomidine concentrations were measured by reversed-phase high-performance liquid chromatography with tandem mass spectrometric detection (LC-MS/MS; SCIEX API 365 instrument, Foster City, CA, USA). The method was modified from a recently published procedure (Ji et al., 2004). The lower limit of quantitation of the assay was 0.02 ng/mL. The within- and between-run precision of the assay (coefficient of variation) was within 8% in the relevant concentration range. Dexmedetomidine concentrations could not be determined in three subjects due to technical problems.

Data and statistical analysis

Data are expressed as mean and standard deviation. Comparisons of demographic variables and study outcomes among genotypes were performed by Chi-square test, independent t-test, and one-way analysis of variance, or Mann Whitney U and Kruskal-Wallis test if the normality assumption was not met. Multiple linear regression models were used to assess effects of genetic variants on pain perception and, for *ADRA2C* del322–325, on cognitive and sedation scores and on choice reaction time, after saline and dexmedetomidine administration. A response-feature approach was used to model multiple measurements on the same patients (Dupont, 2002). In these analyses, the response feature was the area under the score-time curve (AUC) for memory, digit symbol substitution, VAS sedation, and choice reaction time test, which was calculated for each subject as a summary measure of responses during saline (saline AUC) and dexmedetomidine infusion (dexmedetomidine AUC). Previous study showed that only homozygous deletion carriers were functionally different from carriers of an insertion allele (Kurnik et al., 2008; Small et al., 2002); therefore, we combined subjects who were homozygous (Ins/Ins) or heterozygous (Ins/Del) for the *ADRA2C* 322–325 insertion allele for analysis. An additive effect was assumed for all other genetic variants. Since *ADRA2C* and *ADRA2A* are each partitioned into 10–24 complex haplotypes, our sample size did not allow for formal haplotype analysis, and we restricted our exploratory analysis to single marker analyses. In addition, in view of the exploratory nature of the study, we did not adjust for multiple comparisons. In a sensitivity analysis, we repeated the analysis for pain perception after excluding the 6 subjects pre-selected by known genotype and after excluding white subjects, since the frequency of the deletion allele is about 10-fold higher in African-Americans than in Caucasians. All statistical models were adjusted for age, race, and sex; in addition, adjustment for plasma dexmedetomidine concentrations was included in models that assessed responses after dexmedetomidine administration. Control (saline) outcome measures were included as covariates in models that assessed changes in responses after dexmedetomidine administration.

3. Results

Subject Characteristics

We studied 73 subjects, and their demographic characteristics are shown in Table 1. Among the 37 Caucasian subjects, 3 were heterozygous for the *ADRA2C* deletion variant, whereas among the 36 black subjects, 12 were heterozygous and 12 were homozygous. Genotypes for all other *ADRA2C* and *ADRA2A* variants are shown in Table 3.

Cold Pressor Pain Perception

The mean pain visual analog scale (VAS) score during the cold pressor test was 4.8 ± 2.1 cm (study day 1). Subjects with the *ADRA2C* Del/Del genotype had significantly higher pain scores in response to cold on study day 1 (6.3 ± 1.8 cm) than subjects with the Del/Ins and Ins/Ins genotypes (4.6 ± 2.1 cm; P=0.012). This difference remained significant after adjustment for age, sex and race (P=0.019). Dexmedetomidine lowered mean pain scores significantly (from 4.8 ± 2.1 cm to 4.1 ± 2.1 ; P<0.001); however, pain scores after dexmedetomidine remained significantly higher in Del homozygotes (5.6 ± 2.2 cm) compared to carriers of an Ins allele (3.8 ± 1.9 cm; P=0.004). Subjects with a higher pain score on the control day had a greater decrease in pain after dexmedetomidine (P=0.004). The change in pain score after dexmedetomidine administration was not significantly associated with *ADRA2C* genotype (P=0.13) or dexmedetomidine concentrations (P=0.43; Figures 1 and Table 2).

The association of *ADRA2C* genotype with pain response was almost identical when the 6 subjects pre-selected by genotype were excluded (P= 0.012 and 0.005 for VAS pain score before (day 1) and after dexmedetomidine (day 2), respectively), or when the analysis was restricted to African-Americans (P=0.039 and 0.012 for VAS pain score before (day 1) and after dexmedetomidine (day 2), respectively).

Other *ADRA2C* variants were not associated with cold pressor pain perception before or after dexmedetomidine (Table 3). Similarly, genetic variation in *ADRA2A* was not associated with cold pressor pain sensitivity before dexmedetomidine; after dexmedetomidine (day 2), only *ADRA2A* rs1800038 was associated with cold pressor pain sensitivity, but this finding was based on only 3 variant carriers and was of borderline statistical significance (P=0.03; Table 3).

Visual analog scale (VAS) sedation response

The sedation score was significantly higher after dexmedetomidine than after saline (AUC [area under the score-time curve] of the VAS, 520.8 ± 187.5 cm min compared to 273.1 ± 171.9 cm min; P<0.001). *ADRA2C* genotype was not significantly associated with the sedation score after saline (P=0.09) or dexmedetomidine (P=0.29), nor with the change between the two (P=0.72). A lower sedation score during placebo infusions was associated with a greater increase in sedation score after dexmedetomidine (P=0.002; all P-values adjusted for age, race, sex and dexmedetomidine concentration).

Memory response

The memory score decreased significantly with dexmedetomidine infusion (AUC=697.0 ± 146.0 correct answers minutes during saline and 630.0 ± 173.7 correct answers minutes during dexmedetomidine, P<0.001). *ADRA2C* deletion genotype was not significantly associated with the memory score after saline (P=0.34), after dexmedetomidine (P=0.35), nor with the change between the two (P=0.75; all P-values adjusted for age, race, sex and dexmedetomidine concentration).

Digit symbol substitution test (DSST) response

There was no significant difference between DSST score AUC after saline (1587.7 ± 380.8 correct answers minutes) and dexmedetomidine (1644.7 ± 305.7 correct answers minutes; P=0.12). The DSST scores after saline (P=0.14), dexmedetomidine (P=0.09), and the change between the two (P=0.54; all P-values adjusted for age, race, sex and dexmedetomidine concentration) were not significantly associated with the *ADRA2C* deletion.

Reaction time response

Choice reaction time latency was significantly prolonged by dexmedetomidine (AUC 100.0 ± 22.8 sec min) compared to saline (AUC 93.2 ± 17.1; P<0.001). Choice reaction time latency after saline (P=0.20), after dexmedetomidine (P=0.29), and the difference between the two (P=0.74; all P-values adjusted for age, race, sex and dexmedetomidine concentration) were not significantly associated with the *ADRA2C* deletion.

4. Discussion

Our study is the first to examine the effect of genetic variation in two adrenergic receptors (α_{2C}- and α_{2A}-ARs) on pain perception. Our major finding is that the *ADRA2C* del322–325 genotype is associated with increased pain perception both at baseline and after the administration of the selective α_2-AR agonist, dexmedetomidine. However, *ADRA2C* del322–325 genotype was not associated with short term memory, sedation, and a choice reaction time test during placebo, nor did it influence the effects of dexmedetomidine on these outcomes. None of the other *ADRA2C* or *ADRA2A* variants were consistently associated with cold pain sensitivity.

Little is known about the role of α_{2C}-ARs in man, but studies in animals suggest that α_{2C}-ARs have both cortical and spinal effects (Bjorklund et al.1999; Pieribone et al. 1994; Puolivali et al., 2002; Scheinin et al., 2001). Modulation of pain occurs at both spinal and supraspinal levels, and α_{2C}-ARs in the spinal cord and the brain are thought to play a role. In our study, the *ADRA2C* genotype affected pain perception in response to the cold stimulus but did not affect cortical responses to α_2-AR activation, suggesting an important role of spinal α_{2C}-ARs in the modulation of pain perception during the cold pressor test. The effect of the α_{2C}-AR deletion genotype on pain perception was also present after dexmedetomidine, but the change in pain score was not significantly influenced by the genotypes, suggesting that the *ADRA2C* genotype did not modulate the analgesic effect of dexmedetomidine.

The exact mechanisms through which pain perception could be modulated by the *ADRA2C* deletion variant are not known. The *ADRA2C* deletion variant encodes a receptor with a loss-of-function phenotype *in vitro*, and thus would be expected to be associated with increased neurotransmitter release from the presynaptic nerve terminals and to decreased postsynaptic α_{2C}-AR mediated effects. There is evidence that the postsynaptic α_{2C}-AR mediated effects predominate at the spinal level (Stone et al., 1998).

Both α_{2A}-ARs and α_{2C}-ARs are thought to be important mediators of α_2-AR mediated spinal analgesia (Holmberg et al., 2003). In animal studies, α_{2C}-AR activation in *ADRA2A* knockout mice resulted in significant anti-nociceptive responses (Fairbanks et al., 2002). Thus, the *ADRA2C* deletion variant, given its loss-of-function effect, would be expected to be associated with decreased spinal analgesia. Our findings that subjects with *ADRA2C* Del/Del genotype had significantly higher pain scores in response to the cold pressor test are concordant with that notion.

The α_{2C}-AR mediates cold-induced vasoconstriction, and it is conceivable that the *ADRA2C* deletion variant results in enhanced peripheral vasoconstriction and, through this mechanism,

increased pain in response to cold. We addressed this hypothesis in a different study using laser Doppler to measure skin blood flow in response to a graded decrease in temperature and found no effect of the *ADRA2C* Ins/Del variant on local vascular responses to cold (Friedman et al., 2009).

Multiple variants in *ADRA2C* have been described, forming complex haplotypes that affect receptor expression (Small et al., 2004). However, except for the deletion variant, the functional consequences of these individual variants have not been studied. In addition, only a few variants are common and in low linkage disequilibrium (Small et al., 2004). We restricted our analysis to these 5 variants and found only the deletion variant to be associated with cold pain perception. In addition, none of the *ADRA2A* SNPs was consistently associated with pain perception. Importantly, *in vitro* and *in vivo* functions of *ADRA2A* variants have been poorly characterized. Thus, our findings suggest either a lack of a functional effect of these *ADRA2A* variants or a limited role of α_{2A}-ARs in cold pressor pain perception

The α_{2C}-AR has been shown to affect various measures of cognition in mice (Bjorklund et al., 1999; Puolivali et al., 2002; Scheinin et al., 2001), but the role of the α_{2C}-ARs in mediating alertness and other cortical responses in humans is unknown. In our study, dexmedetomidine had significant central effects as evidenced by its effects on sedation, memory, and reaction time. However, central responses to dexmedetomidine were unrelated to the *ADRA2C* del322–325 genotype, suggesting that this genetic variation in the α_{2C}-AR does not contribute substantially to these responses in humans.

Several studies examining experimental pain perception found greater sensitivity to cold pain in blacks than in whites (Rahim-Williams et al., 2007; Kim et al., 2004a), and also in women compared to men (Dixon et al., 2004; Kim et al., 2004b;Compton et al., 2003). We studied healthy volunteers who were free of any medication and had no history of psychiatric illness, under tightly controlled conditions (including standardized diet and salt intake), and found no ethnic or gender differences. Possible reasons for differing findings among studies include methodological differences (for example, many studies did not standardize diet or adjust for covariates).

Our study had some limitations. We studied healthy subjects rather than subjects with chronic pain, thus our findings would need to be tested in that population. However, studying healthy subjects allows for a more homogeneous study population with less potential confounding by diseases and their treatment. Although there is a strong genetic component in pain response to the cold pressor test (Nielsen et al., 2008), other pain modalities may be affected by different genetic components (Nielsen et al., 2008), and therefore our findings cannot be automatically extrapolated to other pain models. Also, we addressed the effect of the *ADRA2C* and *ADRA2A* variants in a hypothesis-driven fashion and thus did not address the potential role of other genetic factors that could alter pain perception and interact with the *ADRA2C* del322–325 variant. In addition, considering the complexity of *ADRA2C* and *ADRA2A* haplotypes, our sample size did not allow a formal haplotype analysis.

In summary, we report that the common *ADRA2C* del322–325 variant is associated with pain perception both at baseline and after administration of the α_2-AR agonist dexmedetomidine but not with other centrally mediated α_2-AR responses such as sedation, memory, and reaction time. Further studies in other experimental pain models and larger populations will be of interest.

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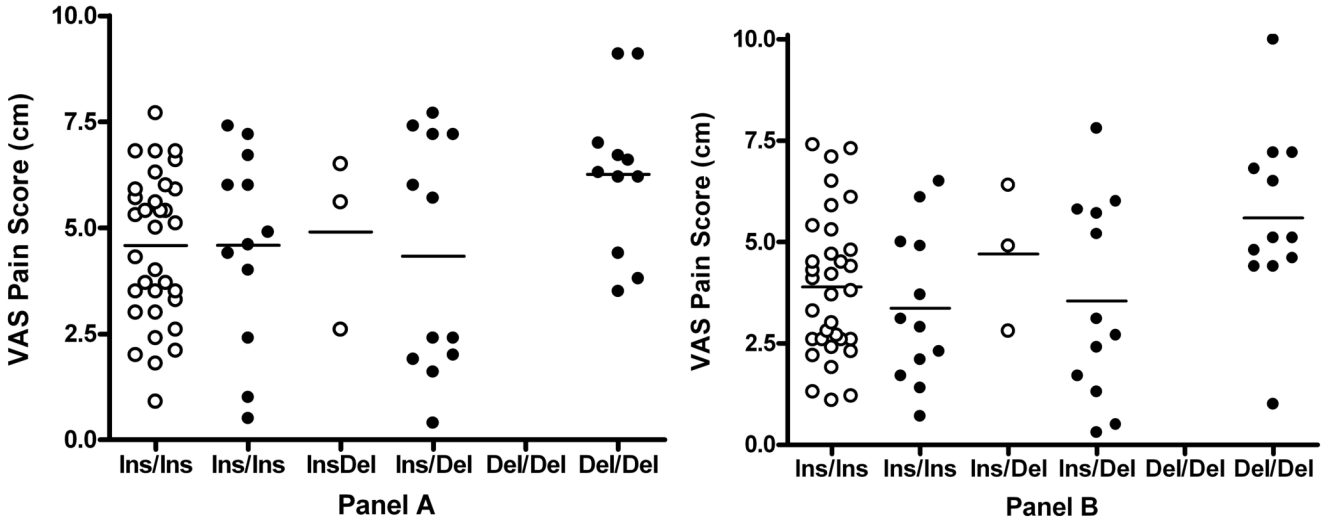

Figure 1. Visual analog scale (VAS) pain scores after the cold pressor test for subjects with *ADRA2C* del322–325 Ins/Ins Ins/Del and Del/Del genotypes at baseline (Panel 1) and after dexmedetomidine (Panel 2)

The horizontal line indicates the mean. Closed circles represent African-Americans and open circles Caucasians.

Table 1

Demographic characteristics of the study subjects

Parameter		**Mean ± SD (n=73)**
Age (yrs)		25.4 ± 4.6
Weight (kg)		76.3 ± 15.3
Height (m)		1.73 ± 0.10
Sex (Males/Females)		41/32
Race (Caucasians/African-Americans)		37/36
Plasma dexmedetomidine concentration (ng/mL)		0.171 ± 0.054
ADRA2C genotype	Del/Del (n, %)	12 (16.4%)
	Ins/Del (n, %)	15 (20.5%)
	Ins/Ins (n, %)	46 (63.1%)

Del=deletion allele; Ins=insertion allele

Table 2

Effect of *ADRA2C* del322–325 genotype on pain scores in response to the cold pressor test at baseline and after the α_2-agonist dexmedetomidine

	VAS pain score					
Parameter	**Day 1 (baseline) β Coefficient**	**P**	**Day 2 (after dexmedetomidine) β Coefficient**	**P**	**Decrease in VAS (Day 1 – Day 2) β Coefficient**	**P**
Age	−0.001	0.98	−0.03	0.54	0.033	0.35
Race	−0.15	0.78	−0.65	0.31	0.66	0.11
Sex	0.14	0.78	−0.30	0.59	0.44	0.21
ADRA2C Del/Del vs. Ins/Del and Ins/Ins	1.82	0.019	2.16	0.004	−0.77	0.13
Dexmedetomidine plasma concentration	-	-	1.44	0.80	−2.99	0.43
Day 1 VAS pain score					0.24	0.004

Table 3

Effect of common *ADRA2A* and *ADRA2A* variants on cold pressor pain perception (Additive model)

SNP (WW/Ww/ww)#	Adjusted effect* Baseline [day1]		Adjusted effect* Dexmedetomidine [day2]	
	β coeff	P value	β coeff	P value
ADRA2A				
rs1800035 (67/6/0)	−1.5	0.13	−1.58	0.10
rs1800038 (67/3/0)	−0.77	0.56	−2.84	0.03*
rs11195418 (65/5/1)	1.4	0.08	1.47	0.07
rs1800544 (23/32/18)	0.25	0.51	0.18	0.63
rs2484516 (64/4/1)	−0.50	0.55	−0.11	0.90
rs1800545 (42/28/3)	0.51	0.30	0.39	0.40
plus1483 (69/4/0)	−0.26	0.82	0.15	0.90
rs3750625 (53/15/2)	0.02	0.97	−0.007	0.99
rs553668 (51/19/3)	−0.59	0.21	−.70	0.13
ADRA2C				
rs9790683 (58/13/1)	−0.021	0.97	−0.54	0.36
rs13118771 (58/14/0)	0.15	0.82	−0.40	0.54
promoter-2416 (67/3/1)	−1.51	0.08	−0.87	0.30
rs7434444 (23/18/19)	0.44	0.35	0.38	0.40

* Adjusted for age sex and race; # WW=Wild type homozygote; Ww=Heterozygote; ww=Variant homozygote