β2-Adrenoceptor Genotype Affects Vasopressor Requirements during Spinal Anesthesia for Cesarean Delivery

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Background: Maternal hypotension is common after spinal anesthesia for cesarean delivery. There is wide variability in the incidence and severity of hypotension and in the response to treatment. The β2 adrenoceptor (β2AR) possesses several polymorphic sites. Codons 16 (Arg16Gly) and 27 (Glu27Gln) have been shown to affect desensitization of the receptor. The goal of this study was to determine whether genetic variants of the β2AR alter incidence of hypotension or the amount of vasopressor treatment required during spinal anesthesia for cesarean delivery.

Methods: One hundred seventy healthy women undergoing elective cesarean delivery were studied. Spinal anesthesia was performed with 12 mg hyperbaric bupivacaine, 25 μg fentanyl, 200 μg morphine. Hypotension was treated with ephedrine and/or phenylephrine intravenously, and β2AR genotype at codons 16 and 27 was determined. Analysis of variance was used to compare variables between genotypes, with data expressed as mean ± SD.

Results: Ephedrine or phenylephrine was used in more than 90% of patients, with no difference in the incidence of hypotension between β2AR genotypes. However, there was a significant effect of genotype on the amount of vasopressor required. Gly16 homozygotes received significantly less ephedrine (18 ± 14 mg) than Arg16 homozygotes (28 ± 13 mg) and Arg16Gly heterozygotes (30 ± 20 mg; P = 0.0005). Glu27 homozygotes required significantly less ephedrine than Gln27 homozygotes (14 ± 13 vs. 30 ± 19 mg; P = 0.002). Gln27Glu heterozygotes received less ephedrine than Gln27 homozygotes (23 ± 16 vs. 30 ± 19 mg; P = 0.03).

Conclusions: Glycine at position 16 and/or glutamate at position 27 of the β2AR leads to lower vasopressor use for treatment of hypotension during spinal anesthesia.

MATERNAL hypotension is a common event during spinal anesthesia for cesarean delivery. Despite numerous strategies to avoid maternal hypotension and its potential deleterious effects on uteroplacental perfusion and fetal well-being, including left uterine displacement, administration of varying amounts of crystalloids or colloids,1,2 and the use of “prophylactic” vasopressors such as ephedrine and phenylephrine,3-8 the occurrence of significant hypotension is still a real concern in obstetric anesthesia. Activity and stimulation of the β2 adrenoceptor (β2AR) is an important factor in the regulation of blood pressure and cardiac output, and the β2AR is among the therapeutic targets for the prevention or treatment of spinal hypotension.9,10

The human ADRB2 is encoded on chromosome 5, and its genetic variability has been widely characterized, with 10 different single nucleotide polymorphisms described.11 Several of the polymorphisms affect the function of the receptor. In vitro, substitution of glycine for arginine at residue 16 (Arg16Gly) has been associated with enhanced agonist-induced desensitization, whereas substitution of glutamic acid for glutamine at position 27 (Gln27Glu) has been associated with resistance to desensitization.12,13 These genetic variations are common, with previous reports suggesting that at codon 16, both glycine and arginine are found at 40-60% allele frequencies in most ethnic groups, whereas at position 27, allele frequencies for Gln seem to be in the 60-80% range.14

Recent clinical trials have demonstrated that there are significant differences in the response of individuals to β2AR therapeutic manipulation related to the particular genotype of the β2AR. Asthmatic patients who are homozygous for Arg16 have been shown to have a stronger and more rapid albuterol-evoked response than the carriers of the Gly16 allele.15 The outcome of preterm labor treated with hexoprenaline, a β agonist, seems to be improved in neonates born to women homozygous for Arg16.16 This is consistent with the prediction that the Arg16 allele results in less desensitization in response to β2 agonists.11 In addition, there has been one study assessing the pressor response to laryngoscopy and tracheal intubation according to β2AR gene polymorphism.
phism\(^{17}\); the authors found a greater hemodynamic response after intubation among Glu27 homozygotes compared with those with the two other genotypes at codon 27.

The goal of this study was to examine whether \(\beta_2\)AR genotype influences the incidence and magnitude of maternal hypotension or the response to vasopressors after spinal anesthesia for cesarean delivery in a prospective treatment trial.

Materials and Methods

After approval from the Columbia University Medical Center Institutional Review Board, New York, New York, and informed written consent from each patient, we recruited 200 women belonging to one of the three major ethnic groups (white, Hispanic, or black, according to self-report) scheduled to undergo elective cesarean delivery during spinal anesthesia at Columbia University Medical Center, with a singleton pregnancy at 37 or more completed weeks of gestation. (Additional information regarding the institutional review board protocol is available on the ANESTHESIOLOGY Web site, [http://www.anesthesiology.org](http://www.anesthesiology.org).) Exclusion criteria included hypertension, gestational hypertension or preeclampsia, other cardiovascular disease, American Society of Anesthesiologists physical status III or IV, or weight above 130 kg. No patient had received steroids, magnesium sulfate, or adrenergic agonists or antagonists during her pregnancy. Patients ate and drank nothing after midnight the night before surgery. On the morning of surgery, all patients received intravenous hydration with 1,000 ml lactated Ringer’s solution preoperatively. If the surgery started after 09:00, patients received an additional 125 ml/h starting at 09:00. Spinal anesthesia was performed in the sitting position at the L3–L4 or L4–L5 interspace. Hyperbaric bupivacaine (0.75%, with 8.25% dextrose), 12 mg, along with 25 \(\mu\)g fentanyl and 200 \(\mu\)g preservative-free morphine were injected intrathecally in a total volume of 2.5 ml via a 25-gauge Whitacre needle. Women were immediately placed in a flat supine position with left uterine displacement. No use of the Trendelenburg position, reverse Trendelenburg, or changes in lateral tilt position were allowed during the study period. Lactated Ringer’s solution was administered at 1,000 ml/h for the first 15 min (total 250 ml) after the spinal anesthetic. An automated blood pressure cuff was programmed to cycle each minute. At each minute interval, hypotension (systolic blood pressure decrease greater than 20% or to less than 90 mmHg) was treated with 5–15 mg ephedrine if the maternal heart rate was less than 120 beats/min or 40–80 \(\mu\)g intravenous phenylephrine if the maternal heart rate was greater than 120 beats/min. A research coordinator who was not involved in the clinical care of the patient recorded the blood pressure, heart rate, and any medications given each minute until delivery. Any degree of hypotension associated with maternal symptoms (dizziness, nausea, decreased consciousness) could be treated at the anesthesiologist’s discretion.

**DNA Collection and Purification, and Genotyping**

Blood samples (10 ml, EDTA tubes) were obtained from all subjects peroperatively. DNA was purified by a Puregene extraction Kit (Gentra, Minneapolis, MN) and tested for quantity, purity, and quality by optical densitometry measure (ratio, 260/280 nm) and gel electrophoresis.

For the identification of the polymorphisms of the **ADRB2** gene, 60 ng DNA was amplified by polymerase chain reaction (96-well microtiter plate block; Biometra, Göttingen, Germany) using specific primers. Primers were chosen in single-copy DNA regions surrounding polymorphisms Arg16Gly and Gln27Glu located in the single exon of **ADRB2** using Oligo6-primer designing software (Molecular Biology Insight, Cascade, CO) with specificity checking by sequence comparison. Sequences of the polymerase chain reaction amplification primers were as follows: forward: **ADRB2-5F**, 5’-GGC-CCAAAATCCGGTACGTCA-3’; reverse: **ADRB2-5R**, 5’-ATCTGGGCTCCGGCAGTAGATATC-3’. Each assay was tested for specificity and reliability by sequencing before extension of its use to the entire cohort.

Polymorphism genotypes were determined by Sanger sequencing reaction and electrophoresis on a fluorescent DNA fragment analyzer apparatus (ABI3100; Applied Biosystems, Foster City, CA)\(^ {18}\) as performed in our previous work. DNA isolation was performed at Columbia University, whereas genotyping was performed (J.-L. B.) at the University of Geneva, Switzerland.

**Analysis**

The dose of vasopressor used was analyzed for ephedrine and phenylephrine separately, and by defining a combined variable (“vasopressor units”) assuming that 5 mg ephedrine is equivalent to 62 \(\mu\)g phenylephrine. One vasopressor unit equals 5 mg ephedrine, 62 \(\mu\)g phenylephrine, or any combination of the two. Only the first 15 min after spinal injection was analyzed, because delivery occurred in many patients between 15 and 20 min. Quantitative data are expressed as mean ± SD. Analysis of variance was used to compare variables between genotypes. Sample size was determined assuming a frequency of 25% Arg16 homozygosity and 10% Glu27 homozygosity among the population of women delivering at Columbia University Medical Center.\(^ {19}\) Enrolling...
No significant differences between genotype groups. No difference in the distribution of genotypes between ethnic groups. Genotypes of each group and the entire cohort were in Hardy-Weinberg equilibrium.

200 women, we expected 80% power to detect 30% variability in response to vasopressors in women carrying the Gly16 or the Glu27 variants as compared with women with the other genotypes.

### Results

Although 200 women were studied, genotype and clinical data were available for only 170 because of technical problems that interfered with DNA isolation (20 samples, because of problems with one specific isolation kit), genotyping (4 samples), and technical problems leading to errors in the spinal doses administered (6 cases). There was no difference in maternal demographics (ethnicity, baseline systolic or diastolic blood pressure, height and weight) or neonatal weight according to the genotypes at position 16 or 27 of the β2AR (tables 1 and 2). The indication for cesarean deliveries, which were almost all either elective repeat cesarean deliveries or for breech or transverse presentation, did not differ between genotypes (data not shown). Overall genotype distribution at codon 16 was 29% Gly homozygous, 56% Arg16Gly heterozygous, and 15% Arg16Arg. At codon 27, the distribution was 51% Gln homozygous, 41% Gln27Glu, and 8% Glu homozygous. Genotype distribution within each ethnic group is shown in tables 1 and 2. There were no significant ethnic differences in β2AR genotype at codon 16 or 27. All groups and the entire cohort were in Hardy-Weinberg equilibrium at both polymorphic sites.

Most women (161 of 170, 95%) received some treatment for hypotension. Among Gly16 homozygotes, 6 of 49 (12%) did not require any vasopressor, whereas only 3 of 95 (3%) Arg16Gly heterozygotes and 0 of 26 Arg16Arg homozygotes did not become hypotensive at any time, i.e., did not receive any ephedrine or phenylephrine (P = 0.08, Fisher exact test). At codon 27, 3 of 87 Gln27Glu homozygotes (3%) did not require vasopressor, compared with 4 of 70 (6%) Gln27Glu heterozygotes and 2 of 13 Glu27 homozygotes (not significant).

There was a significant effect of β2AR genotype on ephedrine and vasopressor unit use. Gly16 homozygotes received significantly less ephedrine during the 15 min after the spinal anesthetic (18 ± 14 mg) than Arg16 homozygotes (28 ± 13 mg) and Arg16Gly heterozygotes (30 ± 20 mg; P = 0.0005). Gln27Glu homozygotes required significantly less ephedrine than Gln27 homozygotes (14 ± 13 vs. 30 ± 19 mg; P = 0.002). Gln27Glu heterozygotes received less ephedrine than Gln27 homozygotes (23 ± 16 vs. 30 ± 19 mg; P = 0.03). Vasopressor unit use followed a pattern similar to that of ephedrine (fig. 1). Data for ephedrine, phenylephrine, and vasopressor unit use at 5, 10, and 15 min in all genotypic groups is given in figure 1.

### Discussion

Our findings demonstrate that glycine at position 16 and/or glutamate at position 27 is associated with a
lower requirement for drug treatment of hypotension after spinal anesthesia. The Glu27 “hypertensive” effect is consistent with the recent finding that Glu27 homozygotes have a greater increase in mean arterial pressure and rate pressure product after intubation than glutamine homozygotes and is consistent with what is known about the function of this genotype. This is, however, the first clinical trial demonstrating that genetic variants of an adrenoceptor can affect the response to regional anesthesia and related side effects.

Interindividual variability in response to β2-agonist therapy has been long noted, whether for bronchodilation in the treatment of asthma, tocolysis in the context of preterm labor, or β blockade for treatment of hypertension. An altered response to ephedrine according to β2AR genotype would explain the wide variability in response to vasopressor therapy in the numerous studies attempting to define the optimal strategy to prevent or treat spinal hypotension in the obstetric population. Arg16 homozygotes required more than 50% more ephedrine than Gly16 homozygotes or Arg16Gly heterozygotes. It may be that no study will ever be able to define a one-solution-fits-all strategy to prevent or treat hypotension during spinal anesthesia.

There are several obvious limitations to our current study. We did not standardize to one specific vasopressor treatment (ephedrine or phenylephrine) or use random assignment to one medication or the other. The current protocol was chosen to correspond as closely as possible to clinical practice at the time it was designed (early 2001), when phenylephrine and ephedrine were both commonly used. Because of the study design and the clinical protocol, most of the drug used in this study was ephedrine, but a number of patients received moderate or substantial treatment with phenylephrine, because of either tachycardia or failure of ephedrine to restore blood pressure. Phenylephrine was prepared at a concentration of 40 μg/ml, which we believed was roughly equivalent to 5 mg ephedrine in its effect on systolic blood pressure, the end point in this and most studies of postspinal hypotension. Very recent work suggests that phenylephrine is approximately 80 times...
as potent as ephedrine in this context and that the dose equivalence is 62 μg phenylephrine to 5 mg ephedrine, so we used this conversion factor in our analysis. Similar statistical and numerical results would be obtained using any reasonable dose equivalence (40–80 μg phenylephrine to 5 mg ephedrine) for the two drugs. These limitations related to the use of two drugs to treat hypotension, however, do not alter the finding that β2AR genotype affects clinical response to anesthesia or hypotension treatment, although this factor limits our ability to draw conclusions about mechanism.

Another limitation, possibly more significant, is that the vasopressors may not have been closely titrated to the hemodynamic response. A continuous infusion of phenylephrine or ephedrine would probably provide better pressure control than intermittent boluses, as suggested by recent reports, and this more precise titration of vasopressor should allow more accurate examination of the magnitude of these genotypically determined differences. However, this type of limitation is more likely to result in missing a difference when one does exist (a type 2 error). Very few women did not require any treatment for hypotension, consistent with contemporary practice in which avoidance of any hypotension or very early treatment of initial changes in blood pressure is preferred. Therefore, we cannot truly differentiate between an effect of β2AR genotype on overall cardiovascular and physiologic response to spinal anesthesia versus an altered response to treatment with ephedrine and phenylephrine. Because hypotension was treated in all patients to restore near-baseline blood pressure, it is not possible to determine whether genotype affected the hemodynamic response to spinal anesthesia, resulting in the need for differing amounts of vasopressor in the different genetic groups, or whether the response to a given dose differed by genotype, or to directly assess the degree of hypotension in the absence of treatment.

The initial in vitro descriptions of the phenotypic effect of these β2AR single-nucleotide polymorphisms reported that the Arg16 allele conferred resistance to receptor desensitization relative to the Gly16 allele and that Glu27 led to less desensitization than Gln 27. However, it should be noted that conflicting results have been reported when comparing in vitro and in vivo trials. Desensitization of the Gly16 β2AR has been implicated as a potential mechanism of tachyphylaxis or lack of efficacy of β2-agonist stimulation. However, some recent work in native cells and patients suggests that in vivo, the Gly16 allele may result in less desensitization during β2-agonist stimulation. Some of these discrepancies may be due to haplotype patterns involving multiple polymorphic sites and the known linkage disequilibrium of the naturally occurring receptor, which may explain differences in receptor regulation observed in initial in vitro studies that have subsequently not been observed in all in vivo studies. For example, some of the initial studies on the function of the β2AR polymorphisms examined receptors with arginine at position 16 and glutamate at position 27, a haplotype that is almost never seen in vivo. It should also be noted that pregnancy may have effects on expression and signal transduction of adrenoceptors, so it is possible that any findings on genotype effects in pregnancy may not be directly applicable or replicable in nonpregnant patients.

The effect of β2AR genotype on vascular tone and cardiovascular diseases has been widely studied. An increased risk of cardiac hypertrophy and vascular remodeling in response to hypertension has been reported in subjects carrying the Glu2 allele, and most certainly the rare Ile164 polymorphism, impact on cardiac function and outcomes in patients with congestive heart failure, confirming the hypothesis that there might be an increased catecholaminergic activity in subjects carrying the variants that do not desensitize the β2AR. Using the dorsal hand vein technique with α-agonist preconstriction or venous occlusion plethysmography to assess peripheral blood flow, several studies have demonstrated a large difference in response to β2-agonist-mediated venu- dilation according to β2AR genotype or haplotype. These in vivo studies have shown that the Gly16 β2AR polymorphism attenuates vasodilatory responses to catecholamines in normal human beings, and that desen- sitization seems to be greatest with the Arg16Gln27Thr164 haplotype, followed by the Gly16Gln27Thr164 haplotype, and least with the Gly16Glu27Thr164 haplotype. The mechanistic interpretation of these results is unclear. Epidemiologic studies among whites and African-American patients have not found an association between a specific β2AR genotype and an increased incidence of hypertension. There have been two association studies in a Chinese population, both suggesting an impact of β2AR genotype on the incidence of essential hypertension. The Gly16 allele was found to be a dominant susceptibility allele for essential hypertension in a family-based case-control population of Chinese descent in one study, whereas rare haplotypes seemed to be associated with hypertension in the other study. Unequivocal associations between specific genotypes and cardiovascular risks or response to antihypertensive treatments are nonetheless still lacking, although a recent study suggests a much higher cardiovascular mortality among Arg allele carriers treated with β blockade after surviving an acute coronary syndrome.

In our trial, we decided to study the three most prevalent ethnic groups of women delivering at our institution, namely white, Hispanic, and African-American women. Previous reports have suggested that β2AR genotype distribution differs among these ethnic groups. In the current study, we did not see signif-
icant differences, with all groups having similar distributions of the homozygous and heterozygous genotypes at codons 16 and 27.

In summary, this is the first study examining an effect of β2AR genotype on the incidence and treatment of hypotension after neuraxial anesthesia and sympathetic blockade. Our findings provide evidence that genetic variation of the β2AR affects the hemodynamic response to spinal anesthesia, or the response to vasopressors administered to treat spinal hypotension. These results warrant further studies in a larger sample across all ethnic groups, with more accurately titrated doses of vasopressors. This work illustrates the potential benefits of investigating the genetic causes for variation in response to anesthesia and perioperative drug treatment in the wide spectrum of patients and conditions for which anesthesia is administered.

References

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