Independent Regulation of Alpha₁ and Alpha₂ Adrenergic Receptor–Mediated Vasoconstriction in vivo

Mordechai Muszkat, MDᵃᵇ, Daniel Kurnik, MDᵃ, Gbenga G. Sofowora, MDᵃ, Alastair J.J. Wood, MDᵇ, and C. Michael Stein, MDᵃ

ᵃDepartments of Medicine and Pharmacology, Divisions of Clinical Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee
ᵇDivision of Clinical Pharmacology, Department of Medicine, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Abstract

Background—Vascular α₁- and α₂-adrenergic receptors (ARs) mediate vasoconstriction and are major determinants of peripheral vascular tone. There is wide variability in vasoconstrictor sensitivity to α₁- and α₂AR-agonists among individuals. In previous studies this variability was not explained by identified α₁- and α₂-AR genetic variants. Thus, we hypothesized that adrenergic vasoconstrictor sensitivity is determined by shared constrictor mechanisms downstream of the individual receptors and that α₁- and α₂-AR-mediated vasoconstrictor sensitivity would therefore be correlated.

Methods—Dorsal hand vein responses to increasing doses of the α₁-AR agonist phenylephrine (12 ng/min –12,000 ng/min) and the α₂-AR agonist dexmedetomidine (0.01 ng/min – 100 ng/min) were measured in healthy subjects using a linear variable differential transformer. From individual dose-response curves we calculated the dose of phenylephrine and dexmedetomidine that produced 50% (ED₅₀) of maximum venoconstriction (Eₘₐₓ) for each subject. We examined the correlation between phenylephrine and dexmedetomidine ED₅₀ and Eₘₐₓ before and after adjustment for covariates (age, gender, ethnicity, BMI, blood pressure, heart rate, and baseline plasma norepinephrine concentrations).

Results—In 62 subjects (36 males, 34 African American, 28 Caucasians) the median ED₅₀ for dexmedetomidine was 1.32 ng/min (IQR, 0.45–5.37 ng/min), and for phenylephrine 177.8 ng/min (IQR, 40.7–436.5 ng/min). The Eₘₐₓ for phenylephrine was 90.8% (82.2–99.6%) and for dexmedetomidine 80.0% (64.7–95.2%). There was no correlation between individual sensitivities (ED₅₀) to phenylephrine and dexmedetomidine, before and after adjustment for covariates (p>0.30).

Conclusions—Phenylephrine and dexmedetomidine both produce strong venoconstriction in the dorsal hand vein; however, there is no significant correlation between vascular sensitivity to an α₁-
AR and α2-AR agonist. These findings suggest independent regulation of vascular α1- and α2-AR-mediated responses.

Keywords
α1 adrenoceptors; α2 adrenoceptors; vasoconstriction

INTRODUCTION

Post-synaptic α1- and α2-adrenergic receptors (AR) are widely distributed in the peripheral vasculature [1,2] and both mediate vasoconstriction [1,2]. In humans, direct infusion of the α2-AR agonist, clonidine, into the brachial artery causes vasoconstriction [3,4], and in the forearm vasculature of young healthy men post-synaptic α2-ARs contribute more than α1-ARs to basal vascular tone [5]. In the hand vein, we and others have shown that both α1-AR and α2-AR agonists cause pronounced vasoconstriction, with sensitivity among individuals varying several fold [6–8].

However, the factors contributing to this interindividual variability in vascular sensitivity are poorly understood. Pharmacogenetic studies have not identified α1- and α2-AR variants that affect response to agonists substantially. For example, variants in the gene (ADRA2B) encoding the α2B-AR, an important mediator of vasoconstriction [9], contribute only a small amount to variability in dorsal hand vein responses to the α2-AR agonist, dexmedetomidine [10–12]. Similarly, genetic variants of the α1-AR studied thus far do not explain the large interindividual variability in vascular response to the α1-AR agonist phenylephrine [8].

Since genetic variants of the respective receptors studied to date do not explain variability in α-AR-mediated vascular response, this variability may be determined by factors that are not directly dependent on the receptor, and are shared by α1- and α2-ARs such as factors that determine the drug concentration at the site of action, or factors that determine the response of pathways downstream of the receptor. If such factors contribute to variability in α1- and α2-ARs responses, then one would expect the sensitivities to vasoconstriction mediated by α1- and α2-ARs to be correlated. However, the possibility that α1-AR and α2-AR vascular sensitivity are co-regulated, has not been studied.

Therefore, to examine the hypothesis that α1- and α2-AR-mediated vasoconstrictor sensitivity are correlated we measured responses to the α1-AR agonist, phenylephrine, and to the highly selective α2-AR agonist, dexmedetomidine, in healthy subjects in vivo using the dorsal hand vein model.

METHODS

Subjects

The Institutional Review Board of Vanderbilt University Medical Center approved the study protocol, and subjects gave written informed consent. Male and female Caucasians and African-Americans were eligible for the study if they were 18 to 45 years of age and had no clinically significant abnormalities according to medical history, physical examination, and laboratory testing.

Sixty-two subjects were studied. Ethnicity, family history of hypertension, and exercise history were determined by self-report, and body mass index (BMI) was calculated. Subjects took no medications for at least 2 weeks, and abstained from alcohol and caffeine for at least 5 days before the study. Each subject received a diet containing 150 mmol/day of sodium, 70 mmol/day of potassium, and 600 mmol/day of calcium for the 5 days prior to each study.
day. Studies were performed in the morning after an overnight fast, in the same temperature-controlled room.

**Measurement of Vascular Responses**

Hand vein responses to the $\alpha_2$-AR agonist dexmedetomidine HCl (Abbott Laboratories, Chicago, IL, USA) [6] and the $\alpha_1$-AR agonist phenylephrine HCL (Elkins-Sinn, Cherry Hill, N.J., USA) [8] were measured on separate days. Venous responses were measured in a dorsal hand vein by use of a linear variable differential transformer (LVDT), (Schaevitz, model 100 MHR) as previously described [13,14]. This instrument, when mounted on the hand, measures and records changes in the diameter of the vein.

Subjects rested on a comfortable bed and remained supine throughout the study. The subject’s arm was placed on a support sloping upward. A 23-gauge needle was inserted into a suitable dorsal hand vein, and an infusion of normal saline was administered for at least 30 minutes. Following three stable baseline measurements of hand vein diameter, drugs were infused into the vein over which the LVDT was mounted.

Phenylephrine (12 ng/min to 12,000 ng/min) or dexmedetomidine (0.01 ng/min to 100 ng/min) were administered in increasing doses with each dose infused for 7 minutes using a Harvard syringe pump and response recorded during the last 2 minutes of each infusion. The total flow rate into the vein was maintained constant by changing the rate of infusion of saline and drug. Heart rate was monitored continuously with a bedside cardiac monitor, and blood pressure was measured in the arm on the side opposite the side receiving the hand vein infusion using a semiautomated device (Dinamap MPS, Johnson and Johnson Medical, Tampa, Fla. USA). A blood sample for measurement of plasma norepinephrine concentration was obtained on the first study day at baseline before drug infusion.

**Analysis of hand-vein response to dexmedetomidine and phenylephrine**

Venoconstriction was expressed as the percentage reduction in vein diameter from maximal dilation, which was defined as the average of three stable baseline measurements of hand vein diameter. Measurements for the phenylephrine and dexmedetomidine hand vein responses were plotted as individual semi-logarithmic dose-response curves and analyzed using a sigmoid dose-response model (Prism 5.01 software). The dose that produced 50% ($ED_{50}$) of maximum venoconstriction ($E_{max}$) was determined for each subject, and these values were converted to molar concentrations to compare sensitivity ($ED_{50}$) to dexmedetomidine and phenylephrine for each subject.

**Statistical analyses**

Continuous parameters were expressed as median and interquartile range (IQR). $ED_{50}$ values are presented in original units (ng/min), and in log transformed molar units [log femtomole/min (log fmol/min)] for the comparison of $ED_{50}$ values for dexmedetomidine and phenylephrine.

$ED_{50}$ values for dexmedetomidine and phenylephrine in the same individuals were compared using Wilcoxon signed rank test, as were the $E_{max}$ values. The Kruskal-Wallis test was used to compare dexmedetomidine $ED_{50}$ among quartiles of phenylephrine $ED_{50}$ values.

Correlations between the $ED_{50}$ for dexmedetomidine and phenylephrine, the $E_{max}$ for dexmedetomidine and phenylephrine, and between plasma norepinephrine concentrations and the $ED_{50}$ for dexmedetomidine and phenylephrine were calculated using Spearman’s test.
To evaluate the effects of covariates on the relationship between dexmedetomidine and phenylephrine ED$_{50}$ values, we performed multiple linear regression analyses using log transformed dexmedetomidine ED$_{50}$ as outcome and log transformed phenylephrine ED$_{50}$ as the independent variable, adjusting for the covariates age, gender, ethnicity, BMI, mean arterial pressure, heart rate, and plasma norepinephrine concentrations. Similarly, we used multiple linear regression analysis to evaluate the effect of these covariates on phenylephrine log transformed ED$_{50}$.

All tests were two-sided, and p-values < 0.05 were considered significant. Statistical analyses were performed with the statistical software SPSS v. 17 (SPSS Inc, Chicago, IL, USA).

**RESULTS**

**Subjects**

We studied 62 subjects [36 men (56.3%), 34 African Americans (53.1%), and 28 Caucasians (43.8%)] with a median age (IQR) of 25.0 years (22.0–32.0), and BMI 25.3 kg/m$^2$ (22.7–28.3). Demographic and baseline characteristics are shown in Table 1.

**Hand-vein responses dexmedetomidine and phenylephrine**

The median ED$_{50}$ for dexmedetomidine was 1.32 ng/min (0.45–5.37 ng/min) [3.75 log fmol/min (3.27–4.36 log fmol/min)], and the ED$_{50}$ for phenylephrine was 177.8 ng/min (40.7–436.5 ng/min) [5.94 log fmol/min (5.31–6.34 log fmol/min)]. The ED$_{50}$ in molar units was significantly smaller for dexmedetomidine than that for phenylephrine (p<0.001; Figure 1), suggesting greater vascular sensitivity to dexmedetomidine in the dorsal hand vein. The $E_{\text{max}}$ for phenylephrine [90.8% (82.2–99.6%)] was significantly greater than that for dexmedetomidine [80.0% (64.7–95.2%)], (p<0.001; Table 2; Figure 2).

There was no correlation between individual sensitivities (ED$_{50}$) to phenylephrine and dexmedetomidine in all subjects [r= 0.12 (p=0.34)], or when African Americans [r= 0.06 (p=0.74)] and Caucasians [r= 0.21 (p=0.29)] were analyzed separately (Table 3; Figure 3). Dexmedetomidine ED$_{50}$ did not differ significantly among subjects grouped by quartiles of phenylephrine ED$_{50}$ (p=0.22) (Table 4).

Similarly, there was no correlation between individual $E_{\text{max}}$ values for phenylephrine and dexmedetomidine in all subjects [r= 0.15 (p=0.23)], or when African Americans [r= 0.18 (p=0.32)] and Caucasians [r= 0.17 (p=0.39)] were analyzed separately (Table 3).

**Determinants of hand-vein responses to dexmedetomidine and phenylephrine**

In univariate analysis, plasma norepinephrine concentrations and phenylephrine ED$_{50}$ were significantly correlated [r=0.28 (p=0.037)], suggesting that higher plasma norepinephrine concentrations are associated with reduced phenylephrine sensitivity. In contrast, there was no significant correlation between plasma norepinephrine concentrations and dexmedetomidine ED$_{50}$ [r=−0.01 (p=0.96)].

In multiple linear regression analyses, phenylephrine ED$_{50}$ was not associated with dexmedetomidine ED$_{50}$ ($\beta$=0.25, p = 0.31), and neither was any of the other covariates (all p-values > 0.15). With phenylephrine ED$_{50}$ as the dependent variable, the association between phenylephrine ED$_{50}$ and plasma norepinephrine was weakened after adjustment for covariants, and of borderline statistical significance (p=0.063).
DISCUSSION

The major new finding of this study is the lack of correlation between the vascular sensitivities (ED$_{50}$) for dexmedetomidine and phenylephrine in vivo in humans. Therefore, our findings suggest that using the hand vein model with $\alpha_1$- and $\alpha_2$-AR-specific agonists provides independent information about $\alpha_2$-AR and $\alpha_1$-AR-mediated vasoconstrictor responses.

Interactions between the signaling pathways of $\alpha_1$, $\alpha_2$- and $\beta_2$-ARs were suggested by previous in vitro studies [15,16]. Simultaneous activation of $\alpha_{2B}$-ARs and $\beta_2$-ARs decreased the threshold concentration of epinephrine required for $\alpha_{2B}$-AR down-regulation, and this was associated with up-regulation of GRK3 expression [15]. Co-activation of $\alpha_2$-ARs and $\beta_2$-ARs resulted in a facilitatory interaction which led to increases in calcium influx from the extracellular compartment [16]. Thus, we tested the hypothesis that variability in downstream constrictor pathways or other factors that are shared by $\alpha_1$- and $\alpha_2$-ARs contribute to the interindividual differences in $\alpha_1$- and $\alpha_2$-AR-mediated vasoconstrictor responses in the human hand vein. Our findings suggest that the large variability among individuals in both $\alpha_1$- and $\alpha_2$-AR-mediated hand vein vasoconstrictor responses is not explained by interindividual differences in factors that are shared by both receptors.

We found that in the dose-range of agonists used in this study (selected previously to elicit maximal response in the hand vein without systemic hemodynamic effects [6,8]), the human dorsal hand vein is more sensitive to dexmedetomidine than to phenylephrine, since a lower molar concentration was required to produce half the maximal constriction. Also, although both agonists resulted in pronounced venoconstriction ($E_{\text{max}} > 80\%$), phenylephrine resulted in a larger median maximal venoconstrictor effect than dexmedetomidine.

We found a negative correlation between resting plasma norepinephrine concentrations and vascular sensitivity to phenylephrine. This finding is consistent with down-regulation of vascular $\alpha_1$-ARs by norepinephrine. The modulating effect of endogenous non-selective adrenergic agonists such as norepinephrine, which activates both $\alpha_1$-ARs and $\alpha_2$-ARs in vivo, on hand vein responses has not been defined. We have previously shown that hand vein response to $\alpha_1$-AR-mediated venoconstriction is stable over time (and is thus used to produce background vasoconstriction in studies of hand vein vasodilation) [17]. These two observations - the previously reported lack of desensitization with short-term exposure to an exogenous agonist such as phenylephrine, and our current finding of a negative correlation between resting plasma norepinephrine concentrations and vascular $\alpha_1$-AR sensitivity - would be most consistent with near maximal $\alpha_1$-AR desensitization at baseline conditions in vivo.

However, plasma norepinephrine concentrations are affected by several factors, including BMI, gender and ethnicity [18], and after adjustment for these and other covariates, the association between resting plasma norepinephrine concentrations and vascular sensitivity to phenylephrine was weakened and of borderline significance. Thus, it is unclear if there is a direct relationship between phenylephrine sensitivity and norepinephrine concentrations, or if it is due to factors that affect norepinephrine concentrations. To determine the specific relationship between endogenous sympathetic tone and $\alpha_1$-AR-mediated local vascular response would require a study designed specifically for that purpose.

There are several methodological considerations regarding the measurement of vascular $\alpha_1$-AR and $\alpha_2$-AR responses to agonist in vivo. Systemic administration of either $\alpha_1$-AR or $\alpha_2$-AR agonists increases and decreases blood pressure, respectively, and thus results in the activation of homeostatic cardiovascular reflexes that would confound the measures of local vascular response. In particular, activation of central $\alpha_2$-ARs results in a decrease in...
sympathetic tone, a factor that would mask the direct vasoconstriction mediated by peripheral $\alpha_2$-ARs [4,19]. Thus, although vascular $\alpha_2$-ARs are functional, it has been difficult to establish their importance in the vasculature relative to $\alpha_1$-ARs.

Accordingly, in order to define $\alpha_1$-AR and $\alpha_2$-AR vascular sensitivity it is necessary to minimize the effects on blood pressure and sympathetic activity that occur after systemic administration of agonist. Thus, we used the dorsal hand vein model [13,14], that allows the direct infusion into the vessel studied of low doses of drugs that act on $\alpha_1$- and $\alpha_2$-ARs locally minimizing systemic effects [6,13] and thus avoiding reflex cardiovascular responses that occur after systemic administration.

We defined $\alpha_1$- and $\alpha_2$-AR sensitivity using highly selective agonists, but cannot rule out the possibility of some non-selective $\alpha$-AR activation. However, this is unlikely. First, phenylephrine and dexmedetomidine are both highly selective for their respective $\alpha$-ARs, as compared to other $\alpha$-AR agonists for in vivo use [20,21]. Second, non-selective $\alpha$-AR activation by either agonist would be expected to increase the correlation between responses to dexmedetomidine and phenylephrine, whereas our study showed lack of correlation between responses.

The clinical implications of the current study are speculative. Since our findings suggest independent regulation of $\alpha_1$- and $\alpha_2$-AR-mediated vasoconstriction, dual blockade of both receptors peripherally could theoretically be used clinically to achieve an antihypertensive or vasodilating effect. However, while $\alpha_1$-AR antagonists are extensively used in the treatment of hypertension, $\alpha_2$-AR antagonists such as yohimbine cause an increase in blood pressure, presumably because of the blockade of central pre-synaptic $\alpha_2$-ARs, resulting in increased sympathetic outflow. Currently, no selective, peripherally acting post-synaptic $\alpha_2$-AR antagonist is available for use in humans, precluding the use of $\alpha_2$-AR antagonists as potential antihypertensive agent [23].

In summary, we found that phenylephrine and dexmedetomidine both produce powerful venoconstriction in the dorsal hand vein in vivo, but that there was no correlation between $\alpha_1$- and $\alpha_2$-AR vascular sensitivity. The large variability among individuals in $\alpha_1$- and $\alpha_2$-AR vascular sensitivity is not explained by differences in shared pre- or post-receptor factors.

Acknowledgments

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Abbreviations and symbols

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>$E_{\text{max}}$</td>
<td>Maximal venoconstriction, expressed as the percentage reduction in vein diameter from maximal dilation</td>
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<tr>
<td>$ED_{50}$</td>
<td>The dose of agonist that produced 50% of maximum venoconstriction ($E_{\text{max}}$) for each subject</td>
</tr>
<tr>
<td>AR</td>
<td>adrenergic receptors</td>
</tr>
<tr>
<td>LVDT</td>
<td>linear variable differential transformer</td>
</tr>
<tr>
<td>IQR</td>
<td>interquartile range</td>
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</table>

*J Hypertens.* Author manuscript; available in PMC 2012 February 1.
Reference List


Figure 1. Hand vein sensitivity to dexmedetomidine and phenylephrine
The horizontal line represents the median, the error bars represent the IQR
Data are presented in log fmole/min.
P<0.001 comparing the sensitivities to phenylephrine and dexmedetomidine
Figure 2. Maximal effect ($E_{\text{max}}$) for dexmedetomidine and phenylephrine

$E_{\text{max}}$ = Maximal venoconstriction, expressed as the percentage reduction in vein diameter from maximal dilation

The horizontal line represents the median and the bars represent the IQR; P<0.001 comparing $E_{\text{max}}$ for dexmedetomidine and phenylephrine
Figure 3. Relationship between log ED$_{50}$ for phenylephrine and dexmedetomidine

ED$_{50}$ - The dose of phenylephrine and dexmedetomidine that produced 50% of maximum venoconstriction ($E_{\text{max}}$) for each subject

ED$_{50}$ is expressed in fmol/min

Spearman rho for the correlation: $r = 0.12$ (p=0.34)
### Table 1

Subject characteristics and baseline measurements [Number (percentage); Median (IQR)]

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>36 (56.3%)</td>
</tr>
<tr>
<td>Race: African Americans: Caucasians</td>
<td>34 (53.1%): 28 (43.8%)</td>
</tr>
<tr>
<td>Family history of hypertension</td>
<td>23 (35.9%)</td>
</tr>
<tr>
<td>Regular exercise, &gt;4 times/week</td>
<td>23 (35.9%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>25.0 (22.0–32.0)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.3 (22.7–28.3)</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>109.5 (102.8–116.3)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>61.0 (56.0–67.0)</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>78.3 (72.6–82.3)</td>
</tr>
<tr>
<td>Heart rate (beats per minute)</td>
<td>60.0 (53.0–66.0)</td>
</tr>
<tr>
<td>Plasma norepinephrine (pg/mL)</td>
<td>156.0 (127.0–202.0)</td>
</tr>
</tbody>
</table>
Table 2

Hand vein responses to phenylephrine and dexmedetomidine

<table>
<thead>
<tr>
<th></th>
<th>Phenylephrine</th>
<th>Dexmedetomidine</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED$_{50}$ (ng/min)</td>
<td>177.8 ng/min (40.7–436.5)</td>
<td>1.3 ng/min (0.45–5.37)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>E$_{max}$ (%)</td>
<td>90.8 (82.2–99.6)</td>
<td>80.0 (64.7–95.2)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as median (IQR)

E$_{max}$ – Maximal venoconstriction, expressed as the percentage reduction in vein diameter from maximal dilation

ED$_{50}$ - The dose of phenylephrine and dexmedetomidine that produced 50% of maximum venoconstriction (E$_{max}$) for each subject

* P-value for the comparison of ED$_{50}$ of phenylephrine and dexmedetomidine in fmole/min (see also Figure 1)
### Table 3

Correlation between measures of phenylephrine and dexmedetomidine vascular response

<table>
<thead>
<tr>
<th></th>
<th>ED₅₀ Rho (p-value)</th>
<th>Eₘₐₓ Rho (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects (n=62)</td>
<td>0.12 (p=0.38)</td>
<td>0.15 (p=0.23)</td>
</tr>
<tr>
<td>African Americans</td>
<td>0.06 (p=0.74)</td>
<td>0.18 (p=0.32)</td>
</tr>
<tr>
<td>Caucasians (n=28)</td>
<td>0.21 (p=0.29)</td>
<td>0.17 (p=0.39)</td>
</tr>
</tbody>
</table>

Eₘₐₓ – Maximal vеноconstriction, expressed as the percentage reduction in vein diameter from maximal dilation

ED₅₀ - The dose of phenylephrine and dexmedetomidine that produced 50% of maximum vеноconstriction (Eₘₐₓ) for each subject
Table 4

Comparison of dexmedetomidine ED$_{50}$ in quartiles of phenylephrine ED$_{50}$

<table>
<thead>
<tr>
<th>Phenylephrine ED$_{50}$ quartile</th>
<th>1$^{st}$ quartile (lowest quartile)</th>
<th>2$^{nd}$ quartile</th>
<th>3$^{rd}$ quartile</th>
<th>4$^{th}$ quartile (highest quartile)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexmedetomidine ED$_{50}$ (ng/min, median, IQR)</td>
<td>0.64 (0.30–1.89)</td>
<td>3.23 (0.26–34.57)</td>
<td>1.20 (0.43–5.08)</td>
<td>1.63 (0.52–9.48)</td>
<td>0.22</td>
</tr>
</tbody>
</table>

ED$_{50}$ - The dose of phenylephrine and dexmedetomidine that produced 50% of maximum venoconstriction (E$_{max}$) for each subject.