Nervous System

Yohimbine Attenuates Baroreflex-Mediated Bradycardia in Humans

Jens Tank, Karsten Heusser, André Diedrich, Robert J. Brychta, Friedrich C. Luft, Jens Jordan

Abstract—α-2 Adrenoceptor stimulation profoundly augments baroreflex-mediated bradycardia presumably through parasympathetic activation. We tested the hypothesis that endogenous α-2 adrenergic tone mediates a similar response. In 10 healthy men (age: 33±3 years; body mass index: 24±1.3 kg/m²), we determined baroreflex control of heart rate and sympathetic traffic after ingestion of the selective α-2 adrenoceptor antagonist yohimbine (20 mg) or placebo. Testing was conducted in a randomized, double-blind, crossover fashion. We measured heart rate, brachial and finger blood pressure, and muscle sympathetic nerve activity. Sympathetic and parasympathetic baroreflex curves were determined using incremental phenylephrine and nitroprusside infusions (0.3, 0.6, 0.9, 1.2, and 1.5 μg/kg per minute). Plasma norepinephrine increased with yohimbine (50±38 ng/L; P<0.05) and was unchanged with placebo (2.2±7.6 ng/L). Blood pressure increased 13±4/8±1 mm Hg with yohimbine and 6±2/3±1 mm Hg with placebo (P<0.01). HR increased 5±1 bpm with yohimbine but did not change with placebo (P<0.01). Ninety minutes after drug ingestion, resting muscle sympathetic nerve activity was similar with yohimbine and with placebo. Baroreflex control of heart rate was decreased with yohimbine (6 ms/mm Hg versus 10 ms/mm Hg; P<0.01) and reset to higher blood pressure and heart rate values. In contrast, yohimbine did not alter the sympathetic baroreflex curve. Yohimbine selectively attenuates baroreflex heart rate control in normotensive young men possibly through parasympathetic mechanisms. (Hypertension. 2007;50:899-903.)

Key Words: receptors ● adrenergic ● baroreflex ● sympathetic nervous system ● adrenergic α antagonists ● yohimbine

Blood pressure and heart rate are regulated by α-2 adrenoceptors through central nervous and peripheral mechanisms.1–5 Pharmacological α-2 adrenoceptor activation in the brain inhibits sympathetic tone and decreases blood pressure.3,6–8 α-2 Adrenoceptors may also regulate parasympathetic nervous system responses. Indeed, α-2 adrenoceptor stimulation with clonidine augments baroreflex-mediated bradycardia in mice and in human subjects.8,9 In mice, atropine abolishes clonidine-induced bradycardia.9 The α-2 adrenoceptor antagonist yohimbine can be applied to test the contribution of endogenous α-2 adrenoceptor tone to cardiovascular autonomic regulation.1,3,10–13 Yohimbine elicits a pressor response through sympathetic activation.1,3,14,15 Thus, α-2 adrenergic receptors tonically suppress sympathetic outflow even under resting conditions.3 Whether endogenous α-2 adrenoceptor tone also affects parasympathetic heart rate regulation in human subjects is unknown. We conducted studies with yohimbine to test the hypothesis that α-2 adrenoceptor tone augments baroreflex heart rate regulation in human subjects.

Methods

Subjects
We studied 10 healthy men (age: 33±3 years; body mass index: 24±1.3 kg/m²). Participants received no medications. Written informed consent was obtained before study entry. All of the studies were approved by the institutional review board.

Protocol
All of the tests were conducted with the subjects in the supine position after an overnight fast. Subjects underwent testing on 2 separate days in a randomized, double-blind, and crossover fashion. On 1 day, they were studied after placebo ingestion. On another day, they were tested after ingestion of 20 mg of yohimbine. We chose this dose to increase plasma norepinephrine levels by ~30%.16 Subjects ingested test medications with 50 mL of tap water. We continuously recorded respiration, ECG, and transthoracic bioimpedance (Cardioscreen, Medis GmbH) to calculate stroke volume changes. In addition, we assessed beat-by-beat finger (Finapres, Ohmeda) and brachial (Dinamap, Critikon) blood pressure (BP). We inserted 2 catheters in contralateral antecubital veins, 1 for blood sampling and 1 for drug infusions. Muscle sympathetic nerve activity (MSNA) was recorded from the right peroneal nerve. A unipolar tungsten electrode ( uninsulated tip diameter of 1 to 2 mm; shaft diameter of 200 μm) was inserted into the muscle nerve...
fascicles of the peroneal nerve at the fibular head for multunit recordings. Nerve activity was amplified with a total gain of 100,000, bandpass filtered (0.7 to 2 kHz), rectified, and integrated.

On both study days, subjects underwent a controlled respiration test over 6 minutes ~1 hour after drug ingestion. The approach entails computation of the transfer function between MSNA and BP. The magnitude spectra reflects the gain between the 2 signals, and the phase spectra reflects the relationship between the 2 signals. Gain values >1 indicate amplification, and gain values <1 indicate dampening. Negative phase values indicate that the input signal precedes the output signal. Broadband fluctuations in the respiratory rate were initiated when subjects breathed on cue to a random sequence in time displayed on a laptop screen. Thereafter, we started incremental sodium nitroprusside and phenylephrine hydrochloride infusions (0.3, 0.6, 0.9, and 1.2 µg/kg per minute over 6 minutes). The infusion was not increased further when the maximum dose had been given, when diastolic BP had changed >25 mm Hg, or when subjects experienced adverse effects. Blood samples were taken before and 1 hour after drug ingestion. The sensitivity to vasoactive drugs was calculated as the dose increasing or decreasing BP by 12.5 mm Hg.

Data Acquisition and Analysis
Data were analog to digital converted at 5 kHz using the WindaqPro+ software (Dataq Instruments Inc). R-R intervals, diastolic BP and systolic BP values, and respiration were defined offline for the complete records using a program written by 1 of the authors (A.D.) that is based on PV-wave software (Visual Numerics Inc). MSNA bursts were identified after filtering the integrated signal and defining the baseline according to following criteria: (1) signal/noise ratio (set to 2.5); (2) tolerance limits of the skewness of the rising and falling parts of the bursts; (3) latency limit; (4) burst width limit; and (5) no preceding premature beats.

Baroreflex Analysis
We plotted the R-R interval and MSNA over BP during phenylephrine and sodium nitroprusside infusions. Individual baroreflex sensitivities of sympathetic activity and heart rate were determined from the slopes of the steepest part of these curves. Mean baroreflex curves were obtained by relating R-R intervals to systolic arterial pressures, considering the fact that cardiac vagal neurons are excited predominantly by baroreflex afferents during systole. Sympathetic discharges, on the other hand, originate from disinhibition of the sympathetic premotor neurons during the diastolic pressure trough. Therefore, sympathetic activity has been related to diastolic arterial pressure for the determination of sympathetic baroreflex curves.

**BP and MSNA Variability**
Beat-to-beat series were interpolated and resampled at 4 Hz. Power spectra densities of BP variability were estimated by the Welch method with 0 padding, linear trend elimination, and a 50% overlapped Hanning window. Transfer functions between MSNA (defined as “spike rate” using the raw signal) and BP were calculated during the 6 minutes of randomized breathing using a program written in Matlab (The Mathworks) by 1 of the authors (R.J.B.).

**Plasma Catecholamines**
Plasma norepinephrine and epinephrine concentrations were determined with high-performance liquid chromatography with fluorometric detection.

**Statistics**
All of the data are expressed as mean±SEM. Intraindividual differences were compared by the nonparametric Wilcoxon matched-pairs test. Baroreflex curves were fitted using the Boltzmann sigmoidal (Graphpad Prism version 4; y=bottom+(top-bottom)/{1+exp((y0-x)/slope)}). The curve fits were weighted for number and scatter of measurements, and their parameters were compared using the F test. A P<0.05 was considered significant.

**Results**
Heart rate, BP, MSNA, and plasma norepinephrine levels at baseline were identical on both study days (Table 1). Ninety minutes after drug intake, BP changed 13±4/8±1 mm Hg with yohimbine and 6±2/3±1 mm Hg with placebo (P<0.01). Heart rate changed 5±1 bpm with yohimbine and 1±1 bpm with placebo (P<0.01). Resting MSNA increased slightly with yohimbine when compared with baseline but was not different from the corresponding measurement on placebo (Table 2). Plasma norepinephrine concentration increased with yohimbine (50.2±38.3 ng/L; P<0.05) and did not change with placebo (−2.2±7.6 ng/L). Venous plasma norepinephrine concentrations 60 minutes after drug intake were 174±18 ng/L with placebo and 206±59 ng/L with yohimbine (P<0.01). Plasma epinephrine did not change with either intervention.

Phenylephrine and nitroprusside sensitivities did not change significantly with yohimbine (Figure 1, left). With yohimbine, the set point of baroreflex heart rate regulation

**Table 1. Hemodynamic Data During Baseline at the 2 Study Days**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Day</th>
<th>Yohimbine Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>59±3</td>
<td>58±2</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>115±4</td>
<td>114±3</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>65±3</td>
<td>64±3</td>
</tr>
<tr>
<td>MSNA, bursts·min⁻¹</td>
<td>33±2</td>
<td>28±3</td>
</tr>
<tr>
<td>NE, ng/L</td>
<td>176±20</td>
<td>156±27</td>
</tr>
</tbody>
</table>

NE indicates plasma norepinephrine concentration.

**Table 2. Parameter Changes During Yohimbine and Placebo Compared With Baseline**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>30 Minutes</th>
<th>60 Minutes</th>
<th>90 Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Yohimbine</td>
<td>Placebo</td>
</tr>
<tr>
<td>ΔHR, bpm</td>
<td>0.1±1.0</td>
<td>0.3±1.0</td>
<td>1.5±1.1</td>
</tr>
<tr>
<td>ΔSystolic BP, mm Hg</td>
<td>1.4±1.0</td>
<td>1.2±1.0</td>
<td>2.8±1.0</td>
</tr>
<tr>
<td>ΔDiastolic BP, mm Hg</td>
<td>0.1±1.1</td>
<td>0.9±0.8</td>
<td>−0.9±0.8</td>
</tr>
<tr>
<td>ΔMSNA, bursts·min⁻¹</td>
<td>−1.2±1.2</td>
<td>−0.2±1.2</td>
<td>0.9±1.7</td>
</tr>
</tbody>
</table>

Data are presented as mean values±SEM of absolute parameter changes; 30, 60, and 90 minutes indicate 30, 60, and 90 minutes after drug or placebo intake, respectively.

*P<0.05, Wilcoxon matched pairs test versus baseline.
†P<0.05 yohimbine versus placebo.
was reset to higher BP and heart rate values such that the
baroreflex curve was shifted downward and rightward (Figure 1, middle). Mean individual baroreflex sensitivity was 10
ms/mm Hg with placebo and 6 ms/mm Hg with yohimbine
(Figure 2; \( P < 0.01 \)). The minimal heart rate during baroreflex
loading with phenylephrine was 53±1 bpm with placebo and
60±1 bpm with yohimbine (\( P < 0.01 \)). The sympathetic
baroreflex curve was reset to higher BP values (Figure 1,
right). However, the range of the mean baroreflex curve and
individual sympathetic baroreflex sensitivity was similar with
placebo and with yohimbine. The slope of stroke volume
changes during phenylephrine and nitroprusside infusion
plotted against the corresponding R-R intervals was steeper
during yohimbine compared with placebo (Figure 3).

We applied transfer function analysis during randomized
breathing to further dissect sympathetic baroreflex regulation.
We calculated the transfer function between BP and MSNA
(H1, neural arc) and the transfer function between MSNA and
bp (H2, peripheral arc). Figure 4 illustrates the magnitude of
the gains for the transfer functions. The filter characteristics
of the neural arc of the sympathetic baroreflex resemble
high-pass filters. In other words, the effect of fast BP
oscillations (\( > 0.1 \) Hz) on MSNA is amplified. In contrast, the
peripheral arc resembles a low-pass filter. The effect of fast
MSNA oscillations (\( > 0.1 \) Hz) on BP is damped. The mag-
nitude of the gain, phase, and corner frequencies of the
transfer function did not change with yohimbine.

Discussion
We used yohimbine to estimate the contribution of endoge-
nous \( \alpha-2 \) adrenoreceptor blockade to human baroreflex reg-
ulation. The main finding was that moderate yohimbine doses
differentially affected baroreflex heart rate and MSNA regu-
lation. Baroreflex curves for heart rate and MSNA were reset
to higher BP values. Heart rate responses were more severely
affected, particularly at lower heart rates that are primarily
regulated through parasympathetic mechanisms. Indeed,
\( \alpha-2 \) adrenoreceptor blockade attenuated baroreflex-mediated bradycardia, reduced parasympathetic baroreflex sensitivity, and
had no influence on sympathetic baroreflex sensitivity.

Similar to previous studies, yohimbine increased BP and
heart rate.1,3,12,16 Furthermore, yohimbine increased cardiac
stroke volume during baroreflex loading. The phenomenon
may be related to increased venous return to the heart or,
more likely, improved cardiac contractility. The hemody-
namic response could result from central nervous system re-
sponses, actions in peripheral tissues, or both mechanisms
combined. In our study, sympathetic vasomotor tone was
unchanged, whereas plasma norepinephrine increased. The
observation suggests that the pressor response to yohimbine
was mediated through a combination of peripheral and central
nervous mechanisms. Yohimbine is known to increase pe-
ripheral norepinephrine release from sympathetic nerve end-
ings.2,14,21–23 The yohimbine pressor response did not sup-

![](image)
press MSNA through baroreflex mechanisms, thus, suggesting central activation of the sympathetic nervous system.

Baroreflex mechanisms attenuate BP responses to vasoactive medication in healthy subjects, a so-called baroreflex BP buffering. In a previous study, $\alpha_2$-adrenergic receptor stimulation with clonidine slightly increased phenylephrine responsiveness, suggesting that $\alpha_2$-adrenergic receptor mechanisms regulate baroreflex BP buffering. Phenylephrine and nitroprusside responsiveness did not change with yohimbine, excluding a major change in baroreflex BP buffering.

Although baroreflex buffering function remained intact with $\alpha_2$-adrenergic receptor blockade, baroreflex heart rate regulation changed substantially. Sympathetic baroreflex responses changed to a lesser degree. In previous studies, the operating point of the sympathetic baroreflex was at a shallow part of the curve during $\alpha_2$-adrenergic receptor stimulation with clonidine. Similarly, norepinephrine transporter inhibition elicits a selective decrease in baroreflex vasoconstrictor control. Animal studies suggest that this phenomenon may also be related to $\alpha_2$-adrenergic receptor stimulation. In the present study, the operating point was on the steep part of the sympathetic baroreflex curve, both with placebo and with yohimbine. Small changes in BP resulted in substantial changes in sympathetic vasoconstrictor tone. Furthermore, the maximal steepness of the curve was unchanged with yohimbine.

In animals, influences of BP on sympathetic activity (neural arc) and sympathetic activity on BP (peripheral arc) can be characterized independently from one another. Transfer function analysis may be a useful approach to estimate open-loop characteristics of the sympathetic baroreflex. The neural arc of the baroreflex had the properties of a high-pass filter. Faster BP changes (>0.1 Hz) are transmitted with higher gains. The peripheral arc acted like a low-pass filter such that the transfer of fast changes in nerve activity (>0.1 Hz) to the vasculature is damped. We found almost identical transfer functions with placebo and with yohimbine. In healthy young subjects, endogenous $\alpha_2$-adrenergic tone may not be sufficient to alter the shape of the sympathetic baroreflex curve or the filter characteristics of the sympathetic baroreflex. It is surprising that the transfer function (peripheral arc) did not change, although we observed dissociation between sympathetic vasoconstrictor tone and plasma norepinephrine with yohimbine.

$\alpha_2$-Adrenergic receptor blockade resulted in pronounced changes in baroreflex heart rate regulation. Previously, we observed that $\alpha_2$-adrenergic receptor stimulation results in resetting of the baroreflex heart rate curve to much lower BP and heart rate values. The normal sigmoidal shape of the baroreflex heart rate curve that prevents an excessive baroreflex-mediated decrease in heart rate was no longer present. The maximal steepness of the baroreflex heart rate curve was unchanged or increased with clonidine. With $\alpha_2$-adrenergic receptor blockade, we observed resetting of the baroreflex heart rate curve to higher BPs and reduction in its maximal steepness. Furthermore, $\alpha_2$-adrenergic receptor blockade attenuated maximal baroreflex-mediated bradycardia.

Changes in cardiac regulation, including reduction in baroreflex-mediated bradycardia, cannot be fully explained by raised sympathetic tone. Previous studies suggested that $\alpha_2$-adrenergic receptor stimulation in human subjects and in mice increases cardiac parasympathetic tone. We propose that changes in heart rate and stroke volume regulation with yohimbine resulted in part from inhibition of endogenous $\alpha_2$-adrenergic receptor–mediated parasympathetic tone. Indeed, MSNA was nearly abolished during baroreceptor loading. In contrast, the antibradycardic response to yohimbine was exacerbated during baroreflex loading. $\alpha_2$-Adrenoceptors may directly affect vagal nuclei. $\alpha_2$-Adrenoceptors are highly expressed in vagus motor nuclei in animals and in human subjects. An interaction between parasympathetic and sympathetic cardiac activation may also occur at the level of the heart. The rather small effects of yohimbine on BP, heart rate, and MSNA are further evidence that $\alpha_2$-adrenergic receptor tone is probably low in younger healthy subjects.

Our results support the concept that different regions in the brain regulate heart rate and sympathetic vasomotor tone, as well as the thresholds and ceiling of the baroreflex. $\alpha_2$-Adrenoceptors seem to be involved as shown in animal studies. Different baseline receptor activities in different brain regions may have contributed to the differential response in the present study.

One limitation of our study is that we did not use $\beta$-adrenergic receptor blockade to confirm that changes in heart rate regulation were parasympathetically mediated. However, resting heart rate is primarily under parasympathetic control, particularly during baroreflex loading. Furthermore, a purely adrenergic mechanism cannot explain the diminished bradycardic response during baroreflex loading. Another limitation of our study is the lack of selectivity of yohimbine for $\alpha_2$ adrenoceptors. We cannot fully exclude that some of the yohimbine responses resulted from interaction with imidazoline binding sites.

**Perspectives**

Endogenous $\alpha_2$-adrenergic receptor tone affects baroreflex heart rate and sympathetic vasomotor regulation in healthy young subjects. The finding may be explained by a combination of sympathetic and parasympathetic mechanisms. Therefore, altered $\alpha_2$-adrenergic tone could contribute to the variability in cardiac parasympathetic regulation in the general population and in patients with cardiovascular disease. The finding may be clinically relevant, because reduced parasympathetic
heart rate regulation is associated with increased cardiovascular morbidity and mortality.34 Perhaps exogenous α-2 adrenoceptor agonists could remedy the condition. Finally, α-2 adrenoceptor blockade may alleviate excessive cardiac parasympathetic activation. Parasympathetic activation to the heart mediates bradycardia and asystole during neurally mediated syncope.35 It is possible that the beneficial effect of yohimbine in these patients16 is explained in part by attenuated parasympathetic activation.

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Disclosures
None.

References