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 Adrenergic receptor stimulation profoundly augments baroreflex-mediated bradycardia presumably through parasympathetic activation. We tested the hypothesis that endogenous α-2 adrenergic nerve 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 adrenergic 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 adrenergoreceptors through central nervous and peripheral mechanisms.1-5 Pharmacological α-2 adrenergoreceptor activation in the brain inhibits sympathetic tone and decreases blood pressure.3,6-8 α-2 Adrenergoreceptors may also regulate parasympathetic nervous system responses. Indeed, α-2 adrenergoreceptor 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 adrenergoreceptor antagonist yohimbine can be applied to test the contribution of endogenous α-2 adrenergoreceptor tone to cardiovascular autonomic regulation.1,3,10-15 Yohimbine elicits a pressor response through sympathetic activation.1,3,11,15 Thus, α-2 adrenergoreceptors tonically suppress sympathetic outflow even under resting conditions.3 Whether endogenous α-2 adrenergoreceptor tone also affects parasympathetic heart rate regulation in human subjects is unknown. We conducted studies with yohimbine to test the hypothesis that α-2 adrenergoreceptor tone augments baroreflex heart rate regulation in human subjects.

Received June 7, 2007; first decision June 25, 2007; revision accepted August 24, 2007.
From the Franz Volhard Clinical Research Center (J.T., K.H., F.C.L., J.J.), Medical Faculty of the Charité and HELIOS Klinikum, Berlin, Germany; and the Department of Medicine (A.D., R.J.B.), Division of Clinical Pharmacology, Vanderbilt University School of Medicine, Nashville, Tenn.
Correspondence to Jens Jordan, Franz Volhard Clinical Research Center, Haus 129, Franz-Volhard-Clinic, Medical Faculty of the Charité and HELIOS Klinikum Wiltherbergstr 50, 13125 Berlin, Germany. E-mail jens.jordan@charite.de
© 2007 American Heart Association, Inc.
Hypertension is available at http://hyper.ahajournals.org

DOI: 10.1161/HYPERTENSIONAHA.107.095984
fascicles of the peroneal nerve at the fibular head for multiunit recordings. Nerve activity was amplified with a total gain of 100,000, bandpass filtered (0.7 to 2 kHz), rectified, and integrated.17

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.18 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 at 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.).19

Plasma Catecholamines
Plasma norepinephrine and epinephrine concentrations were determined with high-performance liquid chromatography with fluorometric detection.20

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((y50 − 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>
<thead>
<tr>
<th>Table 1. Hemodynamic Data During Baseline at the 2 Study Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>N</td>
</tr>
<tr>
<td>HR, mean±SEM, bpm</td>
</tr>
<tr>
<td>Systolic BP, mean±SEM, mm Hg</td>
</tr>
<tr>
<td>Diastolic BP, mean±SEM, mm Hg</td>
</tr>
<tr>
<td>MSNA, mean±SEM, bursts·min⁻¹</td>
</tr>
<tr>
<td>NE, mean±SEM, ng/L</td>
</tr>
</tbody>
</table>

NE indicates plasma norepinephrine concentration.

<table>
<thead>
<tr>
<th>Table 2. Parameter Changes During Yohimbine and Placebo Compared With Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>ΔHR, bpm</td>
</tr>
<tr>
<td>ΔSystolic BP, mm Hg</td>
</tr>
<tr>
<td>ΔDiastolic BP, mm Hg</td>
</tr>
<tr>
<td>ΔMSNA, bursts·min⁻¹</td>
</tr>
</tbody>
</table>

Data are presented as mean ± 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 magnitude 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 endogenous \( \alpha-2 \) adrenoceptor blockade to human baroreflex regulation. The main finding was that moderate yohimbine doses differentially affected baroreflex heart rate and MSNA regulation. 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, \( \alpha-2 \) adrenoceptor 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 hemodynamic response could result from central nervous system responses, 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 peripheral norepinephrine release from sympathetic nerve endings.\(^2\),\(^14\),\(^21\),\(^23\) The yohimbine pressor response did not sup-
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, α-2 adrenoceptor stimulation with clonidine slightly increased phenylephrine responsiveness, suggesting that α-2 adrenoceptor mechanisms regulate baroreflex BP buffering. Phenylephrine and nitropusside responsiveness did not change with yohimbine, excluding a major change in baroreflex BP buffering.

Although baroreflex buffering function remained intact with α-2 adrenoceptor 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 α-2 adrenoceptor stimulation with clonidine. Similarly, norepinephrine transporter inhibition elicits a selective decrease in baroreflex vaso-
notor control. Animal studies suggest that this phenomenon may also be related to α-2 adrenoceptor 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 vasomotor 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 α-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 vasomotor tone and plasma norepinephrine with yohimbine.

α-2 Adrenoceptor blockade resulted in pronounced changes in baroreflex heart rate regulation. Previously, we observed that α-2 adrenoceptor 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 α-2 adrenoceptor blockade, we observed resetting of the baroreflex heart rate curve to higher BPs and reduction in its maximal steepness. Furthermore, α-2 adrenoceptor 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 α-2 adrenoceptor 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 α-2 adrenoceptor–mediated parasympathetic tone. Indeed, MSNA was nearly abolished during baroreceptor loading. In contrast, the antibradycardic response to yohimbine was exacerbated during baroreflex loading. α-2 Adrenoceptors may directly affect vagal nuclei. α-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 α-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. α-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 β-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 α-2 adrenoceptors. We cannot fully exclude that some of the yohimbine responses resulted from interaction with imidazoline binding sites.

Perspectives
Endogenous α-2 adrenoceptor 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 α-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.\(^4\) Perhaps exogenous \(\alpha-2\) adrenoceptor agonists could remedy the condition. Finally, \(\alpha-2\) adrenoceptor blockade may alleviate excessive cardiac parasympathetic activation. Parasympathetic activation to the heart mediates bradycardia and asystole during neurally mediated syncope.\(^5\) It is possible that the beneficial effect of yohimbine in these patients\(^6\) is explained in part by attenuated parasympathetic activation.

**Acknowledgments**

We thank Dr Jacques Lenders from the Department of General Internal Medicine and Dr Jacques J. Willemsen from the Department of Chemical Endocrinology (University Medical Center St Radboud, Nijmegen, The Netherlands) for the careful determination of plasma catecholamine concentrations.

**Sources of Funding**

This work was supported in part by Deutsche Forschungsgemeinschaft grants. J.J. was the recipient of a Helmholtz Fellowship of the Max-Delbrueck-Center of Molecular Medicine.

**Disclosures**

None.

**References**


Yohimbine Attenuates Baroreflex-Mediated Bradycardia in Humans
Jens Tank, Karsten Heusser, André Diedrich, Robert J. Brychta, Friedrich C. Luft and Jens Jordan

Hypertension. 2007;50:899-903; originally published online September 17, 2007;
doi: 10.1161/HYPERTENSIONAHA.107.095984
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/50/5/899

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/