Limited Effect of Systemic $\beta$-Blockade on Sympathetic Outflow

Jens Tank, André Diedrich, Christoph Schroeder, Mandy Stoffels, Gabi Franke, Arya M. Sharma, Friedrich C. Luft, Jens Jordan

Abstract—Central $\beta$-adrenoreceptors may augment sympathetic outflow. We tested the hypothesis that $\beta$-blockade attenuates central sympathetic outflow by inhibiting central adrenoreceptors. We studied 18 healthy controls (4 female, 14 male; age, 26±6 years, body mass index, 23±3 kg/m$^2$). ECG, brachial, and finger arterial blood pressure, muscle sympathetic nerve activity, and respiration were measured continuously before and during complete $\beta$-blockade. Subjects received a total intravenous dose of 0.21 mg/kg of propranolol in 15 minutes. Spontaneous baroreflex slopes were calculated using the sequence technique (BRSup, BRSDown). The sympathetic baroreflex slope was determined at baseline using phenylephrine and sodium nitroprusside infusions. The subjects underwent cold pressor testing before and during $\beta$-blockade. The R-R interval increased from 861±119 ms at baseline to 952±141 ms during $\beta$-blockade ($P<0.01$). Blood pressure was 117±9/65±8 mm Hg at baseline and 117±10/67±8 mm Hg during $\beta$-Blockade ($P=\text{NS}$). $\beta$-Blockade did not affect baroreflex sensitivity (BRSup: 21±10 versus 28±11 ms/mmHg, $P<0.1$; BRSDown: 17±8 versus 20±8 ms/mmHg, $P=\text{NS}$). Muscle sympathetic nerve activity increased significantly during $\beta$-blockade (number of bursts/100 beats: 32±9 versus 40±14, $P<0.05$), compared with baseline. However, the operating points of the parasympathetic and sympathetic baroreflex during $\beta$-blockade were on the baroreflex curves obtained at baseline. $\beta$-Blockade blunted the heart rate response to cold pressor testing; blood pressure and muscle sympathetic nerve activity responses were similar. Our study demonstrates that propranolol does not cause an acute decrease in sympathetic activity in normotensive young subjects. This, observation is not consistent with an important tonic stimulatory effect of $\beta$-adrenoreceptors in the brain. (Hypertension. 2001;38:1377-1381.)

Key Words: cardiovascular physiology $\bullet$ autonomic nervous system $\bullet$ $\beta$-blockade $\bullet$ muscle sympathetic nerve activity $\bullet$ central adrenoreceptors

Activation of adrenergic pathways within the brain may have a stimulatory effect on the sympathetic nervous system in humans. This notion is supported by human studies on cerebral norepinephrine turnover. In these studies, internal jugular vein norepinephrine spillover was positively correlated with peripheral sympathetic nerve traffic.1 Animal studies suggest that the pathway by which centrally released catecholamines elicit sympathetic activation may involve $\beta$-adrenoreceptors within the brain.2–4 in particular $\beta_2$-adrenoreceptors.5 Functional polymorphisms of the $\beta_2$-adrenoreceptor gene influence blood pressure regulation and venous norepinephrine concentration in humans.6–8 Hence, central $\beta$-adrenoreceptor activity might contribute to the regulation of sympathetic outflow in humans. In a study involving normotensive neurological patients, small amounts of adrenoreceptor agonists and antagonists were applied to a lateral cerebral ventricle.9 Injection of the nonselective $\beta$-adrenoreceptor agonist isoproterenol elicited an increase in heart rate, blood pressure, and core temperature. In contrast, central application of the nonselective $\beta$-adrenoreceptor blocker propranolol was associated with a marked decrease in blood pressure, heart rate, and core temperature. These findings suggest a major effect of $\beta$-adrenoreceptors on sympathetic outflow. However, in another study lipophilic and hydrophilic $\beta$-blockers had similar effects on responses to cardiovascular reflex testing and heart rate variability.10 This finding is not consistent with an important effect of central $\beta$-adrenoreceptors on autonomic regulation because lipophilic agents cross the blood-brain barrier, whereas hydrophilic $\beta$-blockers mainly act in peripheral tissues. We tested the hypothesis that central nervous $\beta$-adrenoreceptors have a tonic stimulatory effect on the sympathetic nervous system and that this effect can be attenuated by systemic $\beta$-blockade. Furthermore, we determined whether or not systemic $\beta$-blockade would influence the response to sympathetic stimulation.
Heart Rate Variability and BRS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline 1</th>
<th>snp 1.6</th>
<th>Baseline 2</th>
<th>phe 1.6</th>
<th>Baseline 3</th>
<th>β-blockade</th>
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<td>Lfnu</td>
<td>69±17</td>
<td>80±12</td>
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<td>73±12</td>
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<td>43±14</td>
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<td>LF/HF</td>
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<td>6.2±4.4</td>
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<td>1.6±0.9</td>
<td>3.6±2.5</td>
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<td>BRSup, ms/mm Hg</td>
<td>14±7</td>
<td>9±5</td>
<td>15±7</td>
<td>21±10</td>
<td>13±8</td>
<td>22±15</td>
</tr>
<tr>
<td>BRSLF, ms/mm Hg</td>
<td>20±9</td>
<td>9±5</td>
<td>20±11</td>
<td>31±20</td>
<td>19±11</td>
<td>29±11</td>
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</tbody>
</table>

Values are mean±SD. Measurements are given at the maximal doses of nitroprusside (snp) and phenylephrine (phe) (1.6 μg·kg⁻¹·min⁻¹) and during complete β-blockade compared to baseline. LFnu indicates low-frequency power of R-R interval variability in normalized units (0.04 to 0.15 Hz); Hfnu, high-frequency power of R-R interval variability in normalized units (0.15 to 0.4 Hz); LF/HF, low-frequency to high-frequency power ratio; BRSup, baroreflex sensitivity for upslopes of systolic blood pressure calculated with the sequence technique; and BRSLF, baroreflex sensitivity calculated as mean value of the transfer function in the low-frequency band.

Methods

Study Subjects

We studied 18 normal subjects after obtaining their informed written consent (4 women, 14 men; age, 26±6 years; body mass index, 23±3 kg/m²). The subjects were included in the study after a review of their medical history and a comprehensive physical examination. The experimental protocol was approved by the local institutional review board.

Experimental Protocol

The subjects abstained from caffeine-containing products and smoking for ≥3 days before testing. They did not ingest any medications. Subjects were studied in the supine position during morning hours. Two intravenous lines were placed in large antecubital veins, one for drug administration and the other for drug application. Respiration, transthoracic bioimpedance, and ECG were measured continuously (Cardioscreen, Medis GmbH). Beat-by-beat blood pressure (Finapres, Ohmeda) and brachial arterial blood pressure (Dinamap, Critikon.) were determined. Sympathetic activity was assessed by microneurography. After a stable baseline was reached, subjects underwent cold-pressor testing by placing one hand in ice water (50% ice/50% water) for 1 minute. After this testing, incremental infusions of sodium nitroprusside and phenylephrine hydrochloride infusions (0.2, 0.4, 0.8, and 1.6 μg·kg⁻¹·min⁻¹ over 5 minutes) were given. The infusions were stopped after the maximum dose had been given or after diastolic blood pressure had changed by >15 mm Hg. After returning to baseline values, bolus injections of propranolol were applied every 3 minutes (0.01, 0.02, 0.04, 0.06, and 0.08 mg/kg) in incremental doses. Subjects received a total intravenous 0.21 mg/kg propranolol dose in 15 minutes. Cold pressor testing was repeated during β-blockade.

Microneurography

Muscle sympathetic nerve activity was recorded from the right peroneal nerve. A unipolar tungsten electrode ( uninsulated tip m; shaft diameter, 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), and integrated.

Data Acquisition and Analysis

Data were analog to digital converted at 500 Hz using the Windaq Pro+ software (Dataq Instruments Inc). R-R intervals, diastolic BP and systolic BP values, and respiration were defined off-line (PV-wave software, Visual Numerics Inc). Muscle sympathetic nerve activity bursts were identified after filtering the integrated signal and defining the baseline according to following criteria: (1) signal-to-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 (short duration=artifact; long duration=skin sympathetic nerve activity or afferent activity), and (5) no preceding premature beats.

Spectral Analysis and Baroreflex Sensitivity

Pharmacological baroreflex sensitivity (BRS) was determined from the steepest part of the sigmoidal curve obtained during phenylephrine and sodium nitroprusside infusions. The spontaneous BRS was calculated using the sequence technique and cross spectral analysis as described elsewhere.11,12 BRSLF and BRSHF are the baroreflex sensitivity calculated as the mean value of the transfer function in the low- and high-frequency bands, respectively. BRSup and BRSDown are the baroreflex sensitivity for upslopes and downslopes, respectively, of systolic blood pressure calculated with the sequence technique.

Statistics

Differences between measurements were tested with Wilcoxon’s matched pairs signed-rank test. Nonlinear regression and linear regression analysis were used if indicated. If not otherwise indicated, results are presented as mean±SD. Significant differences were considered if P<0.05.

Figure 1. Baroreflex control of R-R interval (RRI, top) and muscle sympathetic nerve activity expressed in normalized units (MSNA, bottom) obtained during sodium nitroprusside and phenylephrine infusions. The operating point during β-blockade is on the baroreflex curves obtained before blockade. Data are mean±SEM.
Results

Responses to Phenylephrine and Nitroprusside

Blood pressure was $120 \pm 9/67 \pm 8$ mm Hg at baseline, $106 \pm 8/50 \pm 4$ mm Hg during infusion of $1.6 \mu g \cdot kg^{-1} \cdot min^{-1}$ nitroprusside, and $142 \pm 8/84 \pm 7$ mm Hg during infusion of $1.6 \mu g \cdot kg^{-1} \cdot min^{-1}$ phenylephrine ($P<0.01$). The R-R interval decreased from $919 \pm 129$ ms at baseline to $703 \pm 72$ ms during infusion of $1.6 \mu g \cdot kg^{-1} \cdot min^{-1}$ nitroprusside ($P<0.01$). The R-R interval increased to $1073 \pm 161$ ms during infusion of $1.6 \mu g \cdot kg^{-1} \cdot min^{-1}$ phenylephrine ($P<0.01$). Muscle sympathetic nerve activity (normalized area under the burst per 100 beats) increased 2-fold during nitroprusside infusion (range, 143% to 243%) and decreased to <50% of the baseline activity during phenylephrine infusion (range, 7% to 53%). Heart rate variability decreased during nitroprusside infusion and increased during phenylephrine infusion. The low frequency to high frequency ratio in normalized units was lowest during $1.6 \mu g \cdot kg^{-1} \cdot min^{-1}$ phenylephrine and highest during $1.6 \mu g \cdot kg^{-1} \cdot min^{-1}$ nitroprusside (Table). We plotted heart rate and normalized muscle sympathetic nerve activity against systolic blood pressure. The resulting baroreflex curves showed a typical sigmoidal shape characterized by a threshold, a linear part, and a saturation (Figure 1). Systolic blood pressure variability in the low frequency range increased markedly during nitroprusside infusion. It did not change when blood pressure was raised with phenylephrine (Figure 2).

Responses to Propranolol

Heart rate, blood pressure, and muscle sympathetic nerve activity at baseline and during β-adrenoreceptor blockade in a typical subject are illustrated in Figure 3. The R-R interval increased from $861 \pm 119$ ms at baseline to $952 \pm 141$ ms during β-blockade ($P<0.01$) (Figure 4-top). Blood pressure was $117 \pm 9/65 \pm 8$ mm Hg at baseline and $117 \pm 10/67 \pm 8$ mm Hg during β-blockade (Figure 4, top). Muscle

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Figure 2. Changes in systolic blood pressure (SBP) oscillations in the low frequency range (LFsys) plotted against SBP during sodium nitroprusside and phenylephrine infusions. Data are mean±SEM.

Figure 3. Heart rate (upper trace), finger arterial blood pressure (middle trace), and integrated muscle sympathetic nerve activity (lower trace) at baseline and after propranolol bolus injection.

Figure 4. Top, Systolic blood pressure (SBP), diastolic blood pressure (DBP), and R-R interval (RRI) before and during step-wise β-blocker bolus application. Bottom, Changes in normalized muscle sympathetic nerve activity (MSNA) with incremental bolus doses of propranolol. Data are mean±SEM.
sympathetic nerve activity increased significantly during β-blockade (number of bursts/100 beats: 32±9 at baseline, 40±14 during complete blockade, \( P<0.05 \)) (Figure 4-bottom). However, the operating points of the parasympathetic and sympathetic baroreflex during β-blockade were on the baroreflex curves obtained with phenylephrine and nitroprusside (see Figure 1). Propranolol tended to increase spontaneous baroreflex sensitivity (BRSup: 21±10 versus 28±11 ms/mmHg, \( P<0.1 \); BRSDown: 17±8 versus 20±8 ms/mmHg, \( P=\text{NS} \); BRSLF: 15±5 versus 28±11 ms/mmHg, \( P=\text{NS} \); BRSHF: 16±6 versus 20±8 ms/mmHg, \( P=\text{NS} \)). Low frequency and high frequency power of heart rate variability did not change significantly during β-blockade. However, the high frequency power tended to increase, and the low frequency power tended to decrease (see Table). Systolic blood pressure variability in the low frequency range was similar before and during β-blockade. β-Blockade blunted the heart rate response to cold pressor testing. Blood pressure and muscle sympathetic nerve activity responses to cold pressor testing were similar before and during β-blockade (Figure 5).

Discussion

The main finding of the study was that acute β-blockade with propranolol did not lead to a major reduction in muscle sympathetic nerve activity at rest or during cold-pressor testing. Instead, muscle sympathetic nerve activity increased slightly. The relationship between blood pressure and muscle sympathetic nerve activity was not shifted from the parasympathetic baroreflex curve that was determined before blockade. These findings are not consistent with a strong tonic stimulatory effect of central β-adrenoreceptors on the sympathetic nervous system.

One assumption of our study was that systemic application of propranolol would lead to sufficient β1 and β2 adrenoceptor blockade in the brain. The doses of propranolol used in this study are considered to induce complete blockade of cardiac β-adrenoreceptors. Intravenous application of propranolol in the doses employed in this study diminished isoproterenol-induced lipolysis in skeletal muscle. The isoproterenol dose-response curve was shifted at least 100-fold to the right. The pharmacological properties of propranolol make it highly likely that we reached both, peripheral and central nervous β-adrenoreceptor blockade. Propranolol is a highly lipophilic β-adrenoreceptor blocker. Therefore, the drug easily crosses the blood brain barrier. More direct evidence for central binding of β-adrenoreceptor blockers stems from recent positron emission tomography studies. In these studies, S-[18F]fluorocarazolol was used to visualize central nervous β-adrenoreceptors. Systemic application of pindolol markedly reduced S-[18F]fluorocarazolol uptake in the brain.

We used microneurography to study the effect of nonselective β-adrenoreceptor blockade on sympathetic regulation. This method is widely accepted to quantify sympathetic activity at rest and during physiological or pharmacological interventions. Medications that change blood pressure mainly through peripheral effects cause a compensatory baroreflex-mediated change in muscle sympathetic nerve activity. Phenylephrine-induced vasoconstriction leads to reduction in sympathetic nerve traffic.

Repressor responses to sodium-nitroprusside or prazosin are associated with a marked increase in sympathetic activity. In contrast, medications that exhibit strong central nervous system effects change blood pressure and sympathetic traffic change in the same direction. For example, the sympathetic stimulant yohimbine increases blood pressure and muscle sympathetic nerve activity by >100%. Moderate doses of sympatholytic drug, such as clonidine or moxonidine, decrease blood pressure and muscle sympathetic nerve activity substantially. It is more difficult to characterize any central autonomic effects of drugs that have strong effects in peripheral tissues. Propranolol is such a drug. In the periphery, propranolol attenuates β-adrenergic transmission to the heart. In addition, propranolol blocks presynaptic β2-adrenoreceptors at postganglionic adrenergic neurons. This effect may decrease norepinephrine release. The peripheral effects that would tend to decrease blood pressure may explain the slight increase in muscle sympathetic nerve activity observed in our study.

The fact that sympathetic activity increased, rather than decreased, does not completely exclude a central inhibitory effect of propranolol. We, therefore, compared the effect of propranolol to the effect of phenylephrine and nitroprusside. The change in muscle sympathetic nerve activity with phenylephrine and nitroprusside results mainly from compensatory baroreflex-mediated changes in sympathetic activity. If propranolol had a central inhibitory effect, the relationship between muscle sympathetic nerve activity and blood pressure should be shifted from the sympathetic baroreflex curve obtained with phenylephrine and nitroprusside. This approach was used to demonstrate that NO inhibition causes a central increase in sympathetic tone. In the event, the relationship between sympathetic activity and blood pressure was located almost precisely on the baroreflex curve. Moreover, the increases in blood pressure and in sympathetic traffic were identical before and during β-blockade, even though the heart rate increase was blunted. However, we observed moderate changes in heart rate variability and in spontaneous baroreflex sensitivity. These changes may be in part centrally mediated.

The findings of our study are contrary to the study by Tangri et al. They showed profound reductions in blood...
pressure and heart rate after application of β-adrenoceptor blocker into cerebral ventricles. One possible explanation is that basal sympathetic activity in our subjects was low and that it could not be reduced further. However, we observed that phenylephrine reduced muscle sympathetic nerve activity by >50%. Another, more likely explanation for the discrepancy is that systemic or local application of a β-blocker influence different central nervous system structures. Some of the structures may attenuate, whereas others may augment, sympathetic tone. Our study demonstrates that propranolol does not cause an acute decrease in sympathetic activity in normotensive young subjects. This observation is not consistent with an important tonic stimulatory effect of β-adrenoceptors in the brain. Our findings do not exclude the possibility that more chronic changes in central β-adrenergic neurotransmission occur during long-term pharmacological interventions. However, in a recent study in patients with severe heart failure, chronic treatment with carvedilol did not lead to a reduction in systemic or cardiac norepinephrine spillover. We have not excluded the possibility that disease states or genetic heterogeneity in β-adrenoceptor function might influence the effects of β-blockade on sympathetic tone.

Acknowledgments
This work was supported in part by Deutsche Forschungsgemeinschaft grant Jo 284/4–1. Dr Jordan is recipient of a Helmholtz fellowship of the Max-Delbrueck-Center of Molecular Medicine.

References
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_Hypertension_. 2001;38:1377-1381
doi: 10.1161/hy1201.096120

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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