Effects of Nitroglycerin Treatment on Baroreflex Sensitivity and Short-Term Heart Rate Variability in Humans

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OBJECTIVES
We set out to determine the effect of sustained treatment with nitroglycerin (GTN) on neural modulation of heart rate in humans.

BACKGROUND
Acutely, exogenous and endogenous nitric oxide reduces sympathetic, while increasing vagal outflow. An animal study showed loss of these effects during nitrate tolerance.

METHODS
A total of 29 healthy men (age range, 18 to 32 years) received transdermal GTN (0.6 mg/h/24 h) or no therapy for six days in a parallel controlled trial. The reflex regulation of heart rate was assessed with the spontaneous baroreflex sensitivity (BRS) method. Heart rate variability was calculated both in time (standard deviation of mean RR interval [RRSD]) and frequency domains (Fast Fourier Transformation) over 10-min intervals.

RESULTS
Systolic blood pressure was unchanged after continuous GTN, whereas mean RR interval decreased significantly (from 839 to 781 ms, p < 0.05). Nitroglycerin blunted BRS (p < 0.05). When compared with untreated subjects, RRSD was significantly lower after GTN, whereas the ratio of low to high frequencies was increased (all p < 0.05).

CONCLUSIONS
Chronic GTN reduces tonic and reflex vagal heart rate modulation, resulting in greater relative sympathetic influence. Importantly, such changes in the regulation of chronotropic oscillations might have negative prognostic implications in both heart failure and coronary artery disease. Furthermore, because chronic GTN alters the blood pressure/heart rate relationship, our data suggest caution when using these variables as pharmacodynamic markers for the development of nitrate tolerance. (J Am Coll Cardiol 2002;40:2000–5)

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Nitroglycerin (GTN) and other nitric oxide (NO) donors are commonly used vasodilators. Sustained administration of GTN can cause generalized neurohormonal activation, including an increase in plasma norepinephrine, renin, arginine vasopressin, and aldosterone levels (1,2). Although these responses were initially considered one mechanism of nitrate tolerance (1), this link has yet to be definitively established. Nevertheless, it does appear that chronic nitrate therapy can alter the function of the autonomic nervous system.

Interactions between vascular and autonomic effects of NO donors are complex. When nitrovasodilators, including organic nitrates, are given systemically, the reduction in arterial blood pressure acts as a reflex stimulus to sympathetic outflow (3). However, at the same time, the acute administration of these NO donors also exerts sympathoinhibitory actions at multiple central and peripheral sites, and augments efferent vagal tone (4–10). Interpretation of autonomic responses to NO donors is further complicated by the fact that their direct acute effects on the autonomic nervous system might not be sustained during chronic therapy. For example, evidence from a porcine model suggests that long-term nitrate therapy causes a loss of this central sympathoinhibitory effect of NO and is associated with more pronounced increases in sympathetic outflow in response to stimulation (10). The purpose of the present experiment was to determine the impact of continuous nitrate therapy on parameters of the modulation of heart rate such as heart rate variability (HRV) and baroreflex sensitivity (BRS) in healthy humans.

METHODS

Study population. Twenty-nine healthy men (age range, 18 to 32 years) participated. All subjects abstained from caffeine and physical exercise on study days and from any drug, including supplemental vitamins, for the duration of these investigations. Exclusion criteria included hyper- or hypotension, concurrent illness, use of medications, and smoking. The University of Toronto Human Subjects Review Committee approved the study protocol, and written informed consent was obtained from all volunteers.

Measurement of spontaneous baroreflex and short-term HRV. Studies occurred at the same time of the day. Subjects sat in a quiet, temperature- and humidity-controlled environment for 10 min before measurements were obtained. A finger cuff was placed on the right medium
finger for continuous noninvasive beat-to-beat recording of blood pressure (Ohmeda 2300 Finapres, Reudxle, Ontario, Canada). Subjects were instructed to breathe regularly at a frequency of 0.2 Hz. Blood pressure and heart rate (lead II of the electrocardiogram) were recorded continuously for 10 min, acquired digitally at a frequency of 1,000 Hz, and stored into disk using a Labview-based software for subsequent analysis (11). All subjects were in sinus rhythm, and measurement periods excluded any extrasystoles. The standard deviation of RR intervals (RRSD) was calculated for each subject as a time domain index of tonic overall RRSD was also considered as mean short-term HRV. The RRSD is also considered a prognostic indicator in patients with heart disease, although, for this purpose, longer time intervals (i.e., 24 h) have generally been used. The utility of shorter measurement periods, such as those utilized in the present study as prognostic indicators, remains controversial. Spontaneous BRS was assessed to determine reflex vagal control, using previously reported methods (12). Briefly, sequences of three or more beats, during which systolic blood pressure and the RR interval of the immediately following beat changed in parallel, were isolated, identified, and categorized as either up sequences (rising systolic blood pressure and RR interval) or down sequences (decreasing systolic blood pressure and RR interval). For each study day and subject, the mean value of the slope (gain) of these relationships, that is, the change in millisecond of RR interval per mm Hg change in systolic blood pressure, was calculated separately for both up and down sequences. This method reliably estimates the gain of the baroreflex control of heart rate at physiologic levels of arterial blood pressure (13). Because of the rapidity with which they occur, these heart rate responses to spontaneous rises and falls in blood pressure are felt to be mediated, respectively, by phasic vagal cholinergic activation and vagal withdrawal (14).

In addition, for each measurement period, the analog output of the electrocardiogram amplifier was stored for spectral analysis by Fast Fourier Transformation. Details of this technique have been reported elsewhere (11). Low-frequency harmonic (LF) (0.05 to 0.15 Hz) and high-frequency harmonic (HF) (0.15 to 0.50 Hz) components of spectral power were calculated. High-frequency components can be considered, after normalization, an index of parasympathetic nervous system activity (15,16). High frequency was expressed in normalized units as obtained by dividing the absolute power of this HF over the power within 0.05 and 0.5 Hz (LF + HF) and multiplying by 100 (HFnu) (15,16). At rest, heart rate is influenced mostly by vagal modulation, but a constant, reciprocal interaction between vagal and sympathetic activity also exists (15). The LF/HF ratio has been proposed as a model to estimate the reciprocal changes of sympathetic and parasympathetic neural contributions to heart rate modulation (sympathovagal balance) (15,16), even if this interpretation has been criticized (17).

**Study protocol.** The study was designed as an open-label, parallel, controlled trial. Two groups of subjects were studied. The GTN group comprised 15 volunteers. On visit 1, after acquisition of baseline values (pretreatment), the first dose of GTN was administered in the form of a 0.6 mg/h transdermal patch (Transderm Nitro, Novalis, Dorval, Quebec, Canada). Subjects were then given a six-day supply of transdermal GTN and were instructed to wear each patch continuously over 24 h and replace it with a new one every morning at 9 AM. On visit 2, after six days of continuous GTN therapy, posttreatment data were acquired. Fourteen additional volunteers were recruited to undergo a measurement of BRS and HRV at baseline and after one week. Subjects in this control group were not treated with transdermal GTN, but underwent the same measurement protocols. The computer files containing the data recorded on visit 1 and visit 2 for each volunteer in both groups were assigned a code and subsequently analyzed at the end of the study. The investigator responsible for all analysis was blinded to the coding system.

**Statistical analysis.** Results for BRS were not normally distributed. A Wilcoxon signed rank test was used to compare BRS slopes between visits within the GTN group. A paired t test was used to compare all other predetermined variables of interest before and after treatment within groups. For comparisons between groups, analysis of covariance (ANCOVA) was used. For this analysis, BRS data were logarithmically transformed. A value of p < 0.05 was set as the threshold for significance. All results are expressed as mean ± SE unless otherwise noted. Statview (SAS Institute Inc., SAS Campus Drive, Cary, North Carolina) was employed for all statistical analyses.

**RESULTS**

**Baseline values.** There were no differences between the GTN and control groups with respect to mean values of age, baseline blood pressure, BRS during both up and down sequences, RRSD, and parameters of sympathetic and parasympathetic nervous activity (Tables 1 and 2). No significant difference was found in the number of baroreflex sequences per minute per heart rate either between the two groups or between visits within each group.
There was a nonsignificant effect of GTN treatment on baroreflex sensitivity. The slopes of the relationship between spontaneous changes in blood pressure and changes in RR interval were significantly lower on visit 2 compared with visit 1 in the GTN group (p < 0.05, Table 2). Continuous treatment with transdermal GTN caused a significant reduction in the sensitivity of the spontaneous baroreceptor reflex. In particular, the slopes of up sequences were significantly lower on visit 2 as compared with visit 1 (p < 0.01) (Table 1, Fig. 1), while those for down sequences were also lower, although not significantly so (p = 0.1). The ANCOVA revealed that the overall differences between groups in RR interval and BRS (including both up and down sequences) were significant (p < 0.05).

### Discussion

#### Effect of GTN on BRS

In the control group, no significant change was observed for any measured value on visit 2 compared with visit 1 (Table 1). In the GTN group, systolic blood pressure was unchanged after sustained treatment. As well, there was no significant difference between visits in mean blood pressure (visit 1: 88 ± 2; visit 2: 90 ± 3 mm Hg). However, in this group, the RR interval was significantly reduced on visit 2 (p < 0.05, Table 2). Continuous treatment with transdermal GTN caused a significant reduction in the sensitivity of the spontaneous baroreceptor reflex. In particular, the slopes of up sequences were significantly lower on visit 2 compared with visit 1 (p < 0.01) (Table 1, Fig. 1), while those for down sequences were also lower, although not significantly so (p = 0.1). The ANCOVA revealed that the overall differences between groups in RR interval and BRS (including both up and down sequences) were significant (p < 0.05).

#### Effect of GTN on short-term HRV

Heart rate variability results are presented in Table 2 and Figure 2. The sum of HF and LF power was not changed after GTN treatment. There was a nonsignificant trend for lower HFnu on visit 2 as compared with visit 1 in the GTN group (p = 0.1). Analogous results were obtained with time domain markers of vagally mediated HRV such as pNN50 (number of differences of successive RR intervals greater than 50 ms divided by the total number of intervals, data not presented). Importantly, ANCOVA revealed a significant decrease in RRSD and increase in LF/HF at the end of the treatment with GTN when compared with the control group (p < 0.05).

### Table 1. Blood Pressure and Heart Rate at Baseline and After Transdermal GTN or No Treatment

<table>
<thead>
<tr>
<th></th>
<th>Visit 1</th>
<th>Visit 2</th>
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<tbody>
<tr>
<td><strong>Systolic blood pressure, mm Hg</strong></td>
<td></td>
<td></td>
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<tr>
<td>Control group</td>
<td>112 ± 2</td>
<td>113 ± 1</td>
</tr>
<tr>
<td>GTN group</td>
<td>112 ± 3</td>
<td>115 ± 2</td>
</tr>
<tr>
<td><strong>LF and HF, ms²</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>10.0 ± 1.2 (8.0)</td>
<td>10.2 ± 1.1 (8.8)</td>
</tr>
<tr>
<td>GTN group</td>
<td>12.2 ± 1.4 (11.4)</td>
<td>11.6 ± 1.4 (12.0)</td>
</tr>
</tbody>
</table>

### Table 2. RR Interval and Heart Rate Variability at Baseline and After Treatment With Transdermal GTN or No Therapy

<table>
<thead>
<tr>
<th></th>
<th>Visit 1</th>
<th>Visit 2</th>
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</thead>
<tbody>
<tr>
<td><strong>RR interval, ms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>822 ± 22</td>
<td>839 ± 32</td>
</tr>
<tr>
<td>GTN group</td>
<td>839 ± 13</td>
<td>781 ± 13*</td>
</tr>
<tr>
<td><strong>LF and HF, ms²</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>1,828 ± 475</td>
<td>1,758 ± 406</td>
</tr>
<tr>
<td>GTN group</td>
<td>807 ± 187</td>
<td>839 ± 218</td>
</tr>
<tr>
<td><strong>HFnu, normalized units</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>1,589 ± 403</td>
<td>1,844 ± 435</td>
</tr>
<tr>
<td>GTN group</td>
<td>924 ± 336</td>
<td>478 ± 116</td>
</tr>
</tbody>
</table>

*p < 0.05 compared with control group, analysis of covariance.

GTN = nitroglycerin; HF = high-frequency components of spectral analysis; LF = low-frequency components of spectral analysis.

**Figure 1.** The effect of nitroglycerin (GTN) treatment on baroreflex sensitivity. The slopes of the relationship between spontaneous changes in systolic blood pressure (SBP) and reflex changes in the immediately following RR interval are presented. Up sequences are presented in the upper panel, while down sequences are presented in the lower panel. Pretreatment values (visit 1, solid lines) are compared with post-GTN treatment (visit 2, dashed lines). Thick lines represent the median value for the group on each visit; thinner lines represent the interquartile range.
significant effect on blood pressure (1,18), and the loss of this initial hypotensive response has been repeatedly reported as evidence of the occurrence of GTN tolerance (1,18). While this hypotensive effect of GTN disappeared after continuous treatment with GTN, heart rate tended to remain higher (18,19). This observation was confirmed in our study, in that a significant difference between baseline RR interval and that recorded during treatment in visit 2 was observed.

The neural mechanisms for the dissociation of heart rate and blood pressure effects during chronic GTN therapy have not been established. In the present experiment, GTN therapy was associated with a significant decrease in the sensitivity of the baroreflex vagal control of heart rate. Because blood pressure was not different after prolonged GTN treatment, the described decrease in BRS cannot be attributed to any change in the operating set point of the reflex. In addition, the ratio LF/HF was increased. This parameter is considered, by some investigators (15), a frequency domain representative of the relative balance between sympathetic and parasympathetic modulatory influence on the variability of heart rate, although this effect on baroreceptor sensitivity (BRS) before and after treatment with transdermal nitroglycerin (GTN) or no therapy in the two groups. Right: Corresponding changes in SD of RR intervals (RRSD) in the two groups. Dashed lines = control group; solid lines = GTN group; empty circles = visit 1; solid circles = visit 2. *p < 0.05; analysis of covariance (GTN vs. control group).

Possible mechanisms involved. Nitroglycerin may exert acute NO infusion into isolated carotid sinuses caused a significant inhibition of baroreceptor nerve discharge in response to pressure ramps, suggesting an afferent inhibitory mechanism independent of any hemodynamic and/or direct central effect of NO (23). In our model, diminished baroreceptor impact on central integration might have caused a decrease in efferent vagal discharge, while also decreasing the restraining influence of the baroreceptors on outflow from the central sympathetic oscillations. Such interpretation appears unlikely given that this effect on baroreceptor discharge only occurs with local concentrations of NO donors that are higher than those reached during transdermal therapy (24). At the efferent limb, the demonstrated direct positive chronotropic effect of nitrovasodilators might also have contributed to our observed decrease in RR interval after GTN treatment (25). Finally, the possibility of the persistence of a hemodynamic effect of GTN resulting in reflex sympathetic activation, despite the absence of an effect on systolic and mean blood pressure measured from the brachial artery, has to be considered. For example, the effect of organic nitrates on aortic blood pressure might be more pronounced than at the level of the upper limb (26), leading to a reflex increase in sympathetic outflow.

Central actions of GTN should also be considered. Both animal and human studies have demonstrated an important, physiologic neuromodulator effect mediated by NO synthesis in the central nervous system, resulting in a net increase in parasympathetic and decrease of sympathetic activity (9). Importantly, these effects of NO appear to be lost in the setting of sustained nitrate treatment and tolerance. In animal studies, after induction of tolerance, a marked enhancement in sympathetic outflow in response to glutamate infusion and electric stimulation of somatic nerve efferents was demonstrated (10,19). Zanzerger et al. (10) hypothesized that this abnormality is caused by reduction of endogenous NO synthesis as a result of continuous treatment with an organic nitrate. In support of this hypothesis,
the authors pointed out that sustained nitrate treatment impaired NO production in the lower brain stem, thus causing subsequent loss of the NO-mediated sympathoinhibitory mechanism (10). Interestingly, this process would be analogous to the abnormalities in vascular endothelial NO synthase function observed in our laboratory in the same experimental model of nitrate tolerance (18).

In the present study, sympathetic activity was not measured directly (by measurements of muscle sympathetic activity or cardiac norepinephrine spillover), thus, we cannot conclude definitively that sympathetic modulation of heart rate was increased in absolute terms. Furthermore, the analysis of HRV, while allowing reliable investigation of vagal control, provides less information on sympathetic outflow. The reduction in BRS and RRSD and the increase in LF/HF after treatment with GTN might be an expression of the described loss of inhibitory modulation of the sympathetic nervous system by endogenous NO. However, whether the observed changes were due to unmasking of underlying sympathetic modulation by a decrease in vagal tone, a direct inhibitory effect of increased cardiac sympathetic nerve traffic on parasympathetic modulation of heart rate (27), or both, cannot be established definitively from the available data.

Implications for the assessment of nitrate tolerance. An implication of our results is that blood pressure and heart rate measurements should be used with caution as markers of nitrate tolerance. The loss of blood-pressure-lowering effects of GTN during sustained therapy might be mediated, at least in part, by sympathovagal imbalance (“sympathetic” tolerance) (10,19). Nitrate tolerance is a complex, multifaceted process that involves nervous, neurohormonal, and local vascular abnormalities. Changes in autonomic regulation during chronic therapy might offset the responses to GTN, participating in the loss of its effects on blood pressure and heart rate. Our observations might explain why interventions such as hydralazine failed to prevent this “hemodynamic” (28), while being effective on “vascular,” tolerance (29). Accordingly, blood pressure and heart rate should not necessarily be considered accurate indexes of the development of local processes that are believed to play a relevant role in “vascular” tolerance (e.g., oxidative stress, increased sensitivity to angiotensin II). The cause and effect relationship between autonomic versus local vascular effects of GTN in the development of tolerance has not yet been investigated. However, it has been recently proposed that increased angiotensin II and oxidative stress, and their reciprocal enhancing effects, might be responsible for the observed increase in sympathetic activity after sustained nitrate exposure (30).

The clinical phenomenon of nitrate tolerance remains a problem in a variety of settings. We believe that the present observations extend our understanding of nitrate tolerance, and might have direct clinical and methodological implications in this field.

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