Effect of exogenous nitric oxide on baroreflex function in humans

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Effect of exogenous nitric oxide on baroreflex function in humans. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H221–H227, 1999.—Nitric oxide (NO) donors inhibit sympathetic neurotransmission and baroreceptor activity and can directly stimulate heart rate (HR) in vitro. To assess whether exogenous NO affects cardiovascular autonomic control in humans, we tested the baroreceptor-cardiac reflex [baroreflex sensitivity (BRS)] and the arterial blood pressure (BP) and HR variability during an infusion of NO donor sodium nitroprusside (SNP, 2 µg·kg⁻¹·min⁻¹) or 5% glucose in 16 healthy subjects. The hypotensive action of SNP was prevented by phenylephrine (PE, 0.9 ± 0.15 µg·kg⁻¹·min⁻¹). The SNP + PE infusion did not affect BRS or HR variability, but it caused a significant reduction in the diastolic and systolic BP low-frequency power. In addition, SNP + PE caused a sustained 12% increase in HR in the absence of changes in brachial and aortic BP. In conclusion, SNP had no effect on the cardiac-vagal limb of the baroreflex in humans but caused a substantial reduction in BP low-frequency power consistent with a decreased baroreflex/sympathetic control of peripheral resistance. The increase in HR in the absence of baroreceptor downloading confirms our previous finding of a direct positive chronotropic effect of NO donors.

NITROVASODILATORS are widely used to test the sensitivity of the arterial baroreflex, since they elicit hypotension by causing vasorelaxation and are thought to have no direct effect on baroreflex transmission/integration, heart rate (HR), or activity of the autonomic nervous system (26). The finding that they exert their action by releasing nitric oxide (NO) has put this belief into question, since it is now well established that NO is a ubiquitous intracellular messenger that, among many other actions, inhibits the vascular (6) and chronotropic response (10, 13) to sympathetic stimulation, enhances cardiac vagal responses (11, 13, 37), and suppresses the gain of the baroreceptor-cardiac reflex (25). In addition, our recent findings show that nano- to micromolar concentrations of NO donors such as sodium nitroprusside (SNP) or 3-morpholinosydnonimine increase the beating rate of isolated guinea pig atria through the activation of an intracellular pathway involving NO, cGMP, and the stimulation of the hyperpolarization-activated current (Ih) (30). Likewise, intravenous administration of the 3-morpholinosydnonimine prodrug mol-
sidomine or SNP causes a significant increase in HR in the anesthetized rabbit after cardiac autonomic denervation and β-adrenergic blockade (20).

Could the extravascular actions of NO donors significantly affect the sensitivity of the arterial baroreflex in humans? To answer this question, we tested the baroreceptor-HR reflex [baroreflex sensitivity (BRS)] and the arterial blood pressure (BP) and HR variability during an infusion of SNP or glucose in a randomized crossover study in young healthy subjects. The hypotensive action of SNP was prevented by simultaneous administration of phenylephrine (PE).

METHODS

Subjects. Sixteen healthy physically trained male students (body mass index = 26 ± 1 kg/m², average age = 23 ± 1 yr) volunteered for the study. All were nonsmokers, had a normal electrocardiogram (ECG) and BP, and were not taking any medication at the time of the study. Subjects abstained from caffeine and alcohol on the day of the study. Each participant gave his informed consent after receiving a detailed account of the purpose and nature of the study. Experiments were carried out with the approval of the Central Oxford Research Ethics Committee.

Protocol. Experiments were performed with the subjects in the supine position in a quiet, darkened laboratory at a controlled temperature of ~23°C. After familiarization with the procedures, subjects were randomized for an intravenous infusion of SNP (starting from 0.125 µg·kg⁻¹·min⁻¹ with incremental increases to a maximal infusion rate of 2 µg·kg⁻¹·min⁻¹) and PE (starting from 0.25 µg·kg⁻¹·min⁻¹ or an equivalent infusion of 5% glucose. The rate of infusion of PE was adjusted to prevent a fall in brachial BP with SNP. Infusions were separated by 45 min. During SNP administration the subjects’ legs were lifted to minimize the decrease in central venous pressure (CVP) induced by venodilatation.

Approximately 10 min after the maximum dose of SNP was reached and 20 min after start of the glucose infusion, ECG, beat-by-beat BP, a breathing signal (chest impedance), and CVP (n = 6) were recorded for 15 min with the subject awake but motionless. After these recordings, aortic flow velocity and pressure were estimated by pulsed aortic Doppler ultrasonography (model SD-50, Vingmed) and radial applanation tonometry (Sphygmocor, PWV Medical), respectively. Venous blood samples for the determination of plasma catecholamines were taken before and at the end of each infusion. In seven subjects BRS was also measured by the Oxford method (4).

Measurements. Three electrodes were placed on the chest to record ECG and breathing-related changes in chest impedance (Minimon 7136, Kontron Instruments). Finger beat-by-beat BP was monitored (Finapres 2300, Ohmeda) using an appropriately sized cuff applied to the middle finger of either hand and positioned at the heart level. Arterial BP was also measured in the arm simultaneously by an automatic device (model UA-251, Copal) and a trained observer with a stethoscope. CVP was measured via an 18-F polyethylene drun-
cartridge catheter inserted percutaneously into a median antecubital vein under local anesthetic and connected to a pressure transducer (Novakıt-Single line MX8003, Medex Medical).

The integral of the intensity-weighted flow velocity profile in the ascending aorta was averaged over at least three respiratory cycles during the last 5 min of each infusion and was used as an index of stroke volume (SV, cm). Left ventricular ejection time (in ms) was taken as mean duration of the intensity-weighted flow velocity profile over this period. Cardiac output (CO, m/min) was calculated as the product of SV and HR, and total peripheral resistance (in arbitrary units) was estimated by dividing mean arterial pressure by CO.

The aortic pulse waveform was derived from the radial pulse waveform (recorded twice for >10 s by application tonometry) by using a validated transfer function (9, 22) implemented in the SphygmoCor software.

Venous blood samples (5 ml), for the estimation of plasma catecholamines, were drawn in chilled syringes before and at the end of each infusion and were immediately centrifuged (Lobofuge 400R, Heraeus Instruments) at 0°C for 10 min. Plasma was kept at -70°C until analysis by HPLC.

All signals, except the radial application tonometry, were recorded on a Power Macintosh computer (8100/550). The analog inputs were sampled at 500 Hz by a real-time data acquisition software program (Acqknowledge 3.2, Biopac Systems) and stored on compact disk. The R wave of the ECG was subsequently triggered, and systolic and diastolic BP were automatically calculated for each R-R interval (RR). The breathing signal was sampled once every cardiac cycle.

BRS. The spontaneous sequence method provides a non invasive measure of BRS by assessing the relationship between spontaneous RR and systolic BP fluctuations, as first described by Bertini et al. (1). Briefly, 15-min time series of systolic BP and RRs were automatically scanned for sequences in which systolic BP and the following RR progressively increased (upslopes) or decreased (downslopes) over at least three consecutive beats. All values for ups- and downslopes were averaged for estimation of total BRS. The minimum change that was accepted for a spontaneous rise or fall in systolic BP was 1 mmHg. Linear regressions relating RR to systolic BP were fitted for each sequence, and their slope was taken as an estimate of the BRS. Only regression lines (lag0) with a correlation coefficient >0.85 were used.

In seven subjects, BRS was also evaluated by the Oxford method (4). Briefly, two to four rapid intravenous injections of phenylephrine hydrochloride (50–70 µg; Knoll) were given at 3-min intervals. The BRS (in ms/mmHg) was obtained from the average slope of at least two regression lines relating beat-to-beat change in RR to the preceding systolic pressure. The spectral power (in ms²) was computed for the high-frequency (HF, 0.15–0.40 Hz) and low-frequency components (LF, 0.04–0.14 Hz). The HF component of the HR variability represents vagally mediated fluctuations in the RR elicited by breathing (i.e., the respiratory sinus arrhythmia), whereas the HF variability of BP reflects breathing-related changes in preload. Vagal and sympathetic efferent activities can contribute to the LF power of the HR variability, whereas the LF component of BP variability is mediated by fluctuations in sympathetic vasomotor tone (reviewed in Refs. 31 and 38a).

Statistics. Values (means ± SE) were logarithmically transformed where appropriate and compared by repeated measures of ANOVA. The Wilcoxon signed rank test was used to compare CVP, BRS by the Oxford method, the LF/HF ratio, and catecholamines.

RESULTS

One subject was excluded because of anxiety and hyperventilation during the experiment and one because of irregular heart rhythm.

Hemodynamic data. The average finger and brachial BP measurements and the estimated aortic BP are shown in Table 1. The mean infusion rate of PE that was needed to prevent the fall in brachial BP during SNP infusion was 0.90 ± 0.15 µg·kg⁻¹·min⁻¹.

As expected, systolic BP increased progressively from the center (aorta) to the periphery (finger). During the infusion of SNP + PE, aortic BP did not change (Fig. 1), whereas finger BP increased significantly (Table 1). Conversely, diastolic BP did not differ between measurement sites or treatments (Table 1).

Doppler-derived SV and CO were mildly increased by SNP + PE [9.58 ± 5.47 vs. 10.18 ± 6.17 cm (P < 0.05) and 5.14 ± 0.28 vs. 6.08 ± 0.34 m/min (P < 0.01), respectively], whereas total peripheral resistance was reduced (17.1 ± 1.1 vs. 14.1 ± 1.2 arbitrary units, P < 0.01). Left ventricular ejection time did not differ between treatments (303 ± 6 vs. 293 ± 5 ms for 5% glucose and SNP + PE, respectively, P = 0.16), whereas CVP showed a borderline reduction with the combined infusion (3.3 ± 0.8 vs. 1.9 ± 1.0 mmHg, P = 0.08).

Table 1. Aortic and brachial BP and BRS during intravenous infusion of 5% glucose or SNP + PE

<table>
<thead>
<tr>
<th></th>
<th>%Glucose</th>
<th>SNP + PE</th>
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<tbody>
<tr>
<td>Finger BP, mmHg</td>
<td>137 ± 3</td>
<td>151 ± 4*</td>
</tr>
<tr>
<td>Systolic</td>
<td>68 ± 3</td>
<td>64 ± 3</td>
</tr>
<tr>
<td>Diastolic</td>
<td>125 ± 3</td>
<td>127 ± 3</td>
</tr>
<tr>
<td>Brachial BP, mmHg</td>
<td>67 ± 3</td>
<td>67 ± 2</td>
</tr>
<tr>
<td>Systolic</td>
<td>104 ± 3</td>
<td>104 ± 3</td>
</tr>
<tr>
<td>Diastolic</td>
<td>66 ± 3</td>
<td>69 ± 3</td>
</tr>
<tr>
<td>Estimated aortic BP, mmHg</td>
<td>14.53 ± 1.17</td>
<td>13.97 ± 1.62</td>
</tr>
<tr>
<td>Systolic</td>
<td>13.92 ± 1.95</td>
<td>16.10 ± 2.03</td>
</tr>
<tr>
<td>Diastolic</td>
<td>13.96 ± 1.32</td>
<td>12.68 ± 1.48</td>
</tr>
<tr>
<td>BRS, ms/mmHg</td>
<td>17.37 ± 2.5</td>
<td>16.59 ± 2.7</td>
</tr>
</tbody>
</table>

Values are means ± SE. SNP, sodium nitroprusside; PE, phenylephrine; BP, arterial blood pressure; BRS, baroreflex sensitivity; upslope, slope of correlation lines between R-R interval lengthening in response to spontaneous increases in systolic blood pressure; downslope, slope of correlation lines between R-R interval shortening in response to spontaneous decreases in systolic blood pressure. *P < 0.01 vs. 5% glucose.
There was no significant increase in circulating catecholamines as a result of either infusion: the difference in norepinephrine plasma level before and at the end of the SNP − PE infusion was 9 ± 62 pg/ml (P = 0.74) and 21 ± 62 pg/ml with 5% glucose (P = 0.94), whereas for epinephrine it was 9 ± 67 pg/ml with SNP − PE (P = 0.17) and 1 ± 3 pg/ml with 5% glucose (P = 0.83).

Despite the lack of changes in BP and circulating catecholamines, the infusion of SNP + PE was associated with a 12% increase in HR (61 ± 2 vs. 54 ± 1 beats/min with 5% glucose, P = 0.0001; Fig. 1).

BRS. The sensitivity of the baroreceptor-HR reflex, assessed by the spontaneous sequence method, was not different during the SNP + PE infusion (Fig. 2, Table 1). Likewise, in the subgroup where the BRS was tested by the Oxford method, no differences were observed between treatments (Table 1).

Spectral analysis of the HR and BP variability. Although there was a significant reduction in the mean RR with the SNP + PE infusion, the RR variance and the spectral power in the LF and HF bands were unchanged (Table 2). Likewise, the breathing frequency (0.26 ± 0.01 vs. 0.27 ± 0.01 Hz with SNP + PE) and the center frequency of the LF component (0.09 ± 0.005 vs. 0.10 ± 0.005 Hz) did not differ between infusions.

Conversely, the variance and the LF power of systolic and diastolic BP variability were significantly reduced during the SNP + PE infusion (Fig. 3, Table 2). The LF power of the BP variability did not differ significantly between treatments; however, there was a trend toward a reduction in the HF component of diastolic BP variability (P = 0.075) during the SNP + PE infusion, whereas the HF power of systolic BP tended to increase (P = 0.15; Table 2). The latter is probably a reflection of the increase in finger systolic BP during SNP + PE infusion. Indeed, normalization of the spectral power by the systolic BP (i.e., spectral power/systolic BP × 100) virtually abolished the trend for an increase in HF power (1.33 ± 0.26 vs. 1.49 ± 0.17 for glucose vs. SNP + PE, P = 0.43), whereas it accentuated the reduction in systolic BP LF power with SNP + PE (13.57 ± 3.73 vs. 5.52 ± 0.94, P = 0.0045).

Table 2. R-R interval and blood pressure variability

<table>
<thead>
<tr>
<th></th>
<th>5% Glucose</th>
<th>SNP + PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-R variability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In R-R variance</td>
<td>8.41 ± 0.24</td>
<td>8.40 ± 0.22</td>
</tr>
<tr>
<td>In LF power</td>
<td>7.06 ± 0.28</td>
<td>7.01 ± 0.22</td>
</tr>
<tr>
<td>In HF power</td>
<td>6.89 ± 0.34</td>
<td>6.69 ± 0.27</td>
</tr>
<tr>
<td>LF/HF ratio</td>
<td>1.73 ± 0.46</td>
<td>1.77 ± 0.33</td>
</tr>
<tr>
<td>BP variability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In Variance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP</td>
<td>4.07 ± 0.10</td>
<td>3.38 ± 0.15†</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>2.37 ± 0.08</td>
<td>1.98 ± 0.12†</td>
</tr>
<tr>
<td>In HF power</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP</td>
<td>0.34 ± 0.22</td>
<td>0.72 ± 0.18</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>−0.78 ± 0.19</td>
<td>−1.25 ± 0.20</td>
</tr>
<tr>
<td>In LF power</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP</td>
<td>2.58 ± 0.25</td>
<td>1.98 ± 0.16*</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>1.18 ± 0.19</td>
<td>0.50 ± 0.16†</td>
</tr>
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</table>

Values are means ± SE. HF, high frequency (0.15–0.40 Hz); LF, low frequency (0.04–0.15 Hz); * P < 0.05; † P < 0.01.
Little information is available on the effect of NO donors on the sensitivity of the baroreflex, and the findings are not entirely consistent with the studies in which endogenous NO synthesis was suppressed. For instance, although infusion of S-nitrosothiols in conscious rats produced a significant reduction in the baroreceptor reflex-mediated tachycardia, this effect was only partially dependent on NO (12). In normotensive subjects a 25-min infusion of SNP (1.5 µg·kg⁻¹·min⁻¹) caused a resetting of the carotid baroreceptor-HR reflex with no changes in its sensitivity (15). Likewise, in conscious rats, SNP (10–20 µg·kg⁻¹·min⁻¹) shifted the BP-RR relationship toward lower BPs without affecting the gain of the baroreflex (28). In the same animals, however, SNP reversed the increase in baroreflex gain after inhibition of endogenous NO synthesis, suggesting that the effect of NO on BRS is already maximum at endogenous NO concentrations or the biological activity of SNP is too low to affect baroreflex function. Indeed, the inhibitory effect of NO donors on baroreceptor activity has only been seen when high doses of these agents (i.e., ≥100 µM) were delivered directly to the carotid sinus (27, 41).

In our study the sensitivity of the baroreceptor-HR reflex was not affected by infusion of SNP + PE (Table 1, Fig. 2). This was demonstrated by using the spontaneous sequence method (1) and the RR response to PE bolus injections (4). The first technique evaluates the RR response to spontaneous changes in systolic BP, and thus it has the advantage of bypassing the use of vasoactive drugs and the possible interference of their extravascular effects on the assessment of BRS. With this method, however, BRS is calculated from the RR response to a narrow range of BP changes. This may underestimate the inhibitory effect of SNP on BRS, since in vitro studies have shown that NO donors are most active in suppressing baroreceptor activity when the carotid sinus pressure is high (27). To circumvent this limitation, we also tested BRS by the Oxford method (4). In these experiments, systolic BP was raised by 15 ± 1 mmHg; nevertheless, the slope of the relationship between RR and systolic BP did not change with SNP, and the maximal increase in RR was the same: 276 ± 26 and 271 ± 27 ms during glucose and SNP + PE infusion, respectively.

It is well established that the infusion of SNP alone causes a baroreflex-mediated increase in muscle sympathetic activity (35) and circulating catecholamines (16). Besides eliciting tachycardia, reflex changes in sympathetic activity have been shown to increase baroreceptor discharge (14). In addition, by dilating arterial and venous capacitance vessels, SNP reduces preload and CVP, and this in turn might affect BRS (24). To prevent these changes we raised the subjects’ legs during SNP infusion and we “damped” BP by simultaneous administration of PE. Experimental work in dogs suggests that α-adrenergic agonists might influence baroreceptor firing by inducing carotid vasoconstriction (33); in humans, however, changes in vascular smooth muscle tone induced by PE or nitroglycerin have no significant effect on baroreceptor activity (2). Likewise, PE infu-
Hajduczok et al. (18) showed that increases in flow (at constant pressure and strain) stimulate carotid sinus nerve activity in anesthetized dogs. These findings suggest that, even in the absence of changes in aortic BP, the increase in CO with the SNP + PE infusion might have caused some degree of baroreflex activation. This is, however, unlikely to have occurred in our study, since a physiologically significant flow-mediated increase in carotid sinus nerve activity has only been demonstrated in the presence of hypotension (18).

Kelly et al. (23) showed that nitrovasodilators can significantly decrease aortic BP (i.e., the BP at the site of the baroreceptors) in the absence of changes in brachial BP. In our subjects, aortic BP was, as expected, significantly lower than brachial BP, but the gradient between these two sites did not differ between treatments (Table 1). Conversely, SNP + PE infusion elicited a significant increase in the gradient between brachial and finger systolic BP (Table 1), as previously observed by Bos et al. (3) with the infusion of SNP alone.

Unlike the administration of nitrovasodilators or PE alone, the combined SNP + PE infusion did not affect HR variability but produced a significant reduction in the LF power of the diastolic and systolic BP variability. LF BP oscillations have been shown to be a marker of sympathetic vasomotor tone (31). Evidence suggests that they originate in the central nervous system (21), but their frequency and amplitude are modulated by the arterial baroreflex (32). Because microinjection of NO donors in the rostral ventrolateral medulla is associated with an inhibition of basal and reflex sympathetic activity in the anesthetized cat (40), it is conceivable that the reduction in the LF power of the BP variability with the SNP + PE infusion might reflect a central “sympatholytic” effect of SNP. This would not necessarily affect HR variability or the baroreceptor-HR reflex, since 1) cardiac sympathetic tone in young fit subjects at rest in the supine position is expected to be very low and 2) the techniques that we employed to test the arterial baroreflex essentially evaluate vagally mediated changes in HR. Alternatively, the discrepancy between the effect of SNP on HR and BP variability could indicate that the effect of NO on the baroreflex control of HR differs from that of BP. Indeed, in the anesthetized and vagotomized rabbit, SNP (3–6 µg·kg\(^{-1}\)·min\(^{-1}\)) has been shown to inhibit the sympathetic modulation of aortic pressure in response to random changes in carotid sinus pressure without affecting the HR response significantly (29).

NO and HR. The SNP + PE infusion was accompanied by a small increase in HR in all subjects (Fig. 1). Although we cannot categorically rule out that subtle changes in autonomic activity might have contributed to this finding, the absence of changes in brachial and aortic BP, plasma catecholamines, and HR variability argue against this interpretation. Likewise, although high doses of PE can exert a positive chronotropic effect in vitro (39), this is unlikely to have contributed significantly to our findings, since bolus injections of 1–2 µg/kg of this agent only cause a small and inconsistent increase in HR (1–2 beats) in healthy subjects after ganglion blockade (7). Similarly, potential inhibition of cardiopulmonary receptor activity secondary to the borderline reduction in CVP observed during SNP + PE infusion would have no effect on HR (42).

Our recent data show that low concentrations (nanomolar) of NO donors can directly increase the beating rate of isolated guinea pig atria by stimulating the hyperpolarization-activated current (30). Consistent with these in vitro findings, we have shown that molsidomine and SNP elicit a slow linear increase in HR in the anesthetized rabbit after cardiac autonomic denervation and β-adrenergic blockade (20). Whether the administration of NO donors would result in an increase in HR in the presence of an intact nervous system and in the absence of significant changes in BP is difficult to predict, since, besides its direct effect on sinoatrial node activity (20, 30), SNP can suppress the HR response to sympathetic stimulation (1–5 Hz) and enhance the chronotropic effect of vagal nerve stimulation (5 Hz) in isolated guinea pig atria (10, 37). Nevertheless, in our subjects, SNP exerted a small positive chronotropic effect in the presence of an intact autonomic nervous system and in the absence of significant baroreceptor unloading. This suggests that at least part of the increase in HR that accompanies the infusion of NO donors may be independent of the baroreflex-mediated changes in the activity of the autonomic nervous system.

In summary, studies in animals indicate that exogenous NO can decrease baroreceptor activity (27, 41), enhance cardiac vagal responses (11, 37, 38), and inhibit the chronotropic and vasoconstrictor response to sympathetic stimulation (10, 13, 17). Our results in humans show that an infusion of SNP, within the dose range employed to test the arterial baroreflex, does not affect the sensitivity of the cardiac/vagal limb of the reflex or the amplitude of respiratory sinus arrhythmia. Conversely, SNP is associated with a significant reduction in the LF power of the BP variability, consistent with a decreased sympathetic control of peripheral resistance. A sympathoinhibitory effect of NO would profoundly affect the assessment of BRS by SNP, since the HR response to baroreceptor unloading is mainly mediated by an increase in sympathetic activity. Indeed, this action could at least partly explain why the RR shortening after the administration of NO donors has been consistently found to be less prominent than the RR lengthening in response to PE (reviewed in Ref. 8). These findings together with the evidence of a “direct” positive chronotropic effect of NO donors question the suitability of these agents for testing the arterial baroreflex.

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