Contrasting effects of phentolamine and nitroprusside on neural and cardiovascular variability

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Received 11 December 2000; accepted in final form 28 March 2001

Van de Borne, Philippe, Mohsen Rahnama, Silvia Mezzetti, Nicola Montano, Alberto Porta, Jean Paul Degaute, Virend K. Somers. Contrasting effects of phentolamine and nitroprusside on neural and cardiovascular variability. Am J Physiol Heart Circ Physiol 281: H559–H565, 2001.—The relative contributions of a central neural oscillator and of the delay in α-adrenergic transmission within the baroreflex loop in the predominance of low-frequency (LF) cardiovascular variability during sympathetic activation in humans are unclear. We measured R-R interval (RR), muscle sympathetic nerve activity (MSNA), blood pressure (BP), and their variability in 10 normal subjects during sympathetic activation achieved by BP lowering with sodium nitroprusside (SNP) and α-adrenergic blockade using phentolamine. SNP and phentolamine induced comparable reductions in BP (P > 0.25). Despite tachycardia and sympathetic activation with both SNP and phentolamine, LF variability in RR, MSNA, and BP increased during SNP and decreased during phentolamine (SNP: RR +20 ± 6%, MSNA +3 ± 5%, systolic BP +9 ± 6%, diastolic BP +7 ± 5%; phentolamine: RR –2 ± 7%, MSNA –34 ± 6%, systolic BP –16 ± 8%, diastolic BP –13 ± 4%, P < 0.05 except systolic BP, where P = 0.09). Thus LF variability is reduced when sympathetic activation is induced by α-adrenergic blockade. This suggests that α-adrenergic transmission within the baroreflex loop may contribute importantly to the predominance of LF cardiovascular variability associated with sympathetic excitation in humans.

IN HEALTHY HUMANS, heart rate, blood pressure (BP), and muscle sympathetic nerve activity (MSNA) display low-frequency (LF) oscillations of ~0.10 Hz and faster oscillations at the respiratory frequency [high frequency (HF)] (28). The mechanisms underlying LF oscillations in human cardiovascular variability are not well understood. What is known is that, in closed-loop conditions, arterial baroreflex deactivation and the consequent sympathetic activation are accompanied by a relative enhancement of the LF oscillations in the variability patterns of heart rate, MSNA, and BP compared with the HF oscillations (27, 28).

There is evidence that a resonance phenomenon, due to delay in the α-adrenergic vasoconstrictor response mediated by the baroreceptors, contributes to the LF oscillations (2, 4–6, 9, 11). However, LF components have also been reported in the absence of a functional baroreflex loop. First, LF components were found in the variability of the discharge of single medullary neurons (24) and in sinoaortic denervated cats (25). Second, oscillations in sympathetic nerve discharge synchronous with BP, persist at high cervical spinal transection (12, 14, 21, 30). Third, substantial LF oscillations were found in R-R interval (RR) variability of two subjects with left ventricular assist devices, in whom the RR variability could not be attributed to the minimal BP variability (7). Thus a functional baroreflex loop may not be mandatory for the genesis of LF oscillations.

The relative contributions of a central neural oscillator and of the delay in α-adrenergic transmission within the baroreflex loop in the predominance of LF cardiovascular variability during sympathetic activation are not known in humans.

We anticipated that the analysis of the effect of sympathetic activation, achieved by pharmacological BP lowering with α-adrenergic blockade, on LF oscillations in MSNA might help to distinguish between these mechanisms. β-adrenergic blockade decreases the LF variability of BP in animals (8, 19, 20). However, this reduction could be due to 1) the suppression of the LF variability in MSNA as a result of the interruption of the baroreflex loop at the neurovascular junction or 2) the interruption of transfer to the vascular beds of an intrinsic increase in the LF power in MSNA during sympathetic activation. Thus enhanced LF oscillations in MSNA during α-adrenergic blockade would suggest that the rise in LF oscillations during...
sympathetic activation is mainly generated centrally. In contrast, reduction in LF oscillations in MSNA would suggest that α-adrenergic transmission within the baroreflex loop contributes importantly to enhanced LF oscillatory power associated with sympathetic activation. We therefore determined changes in neural and cardiovascular variability during BP reductions induced by phentolamine (an α-adrenergic receptor blocker) and compared these changes with those induced by sodium nitroprusside (SNP; a direct arterial and venous dilator). We further ensured the effectiveness of α-adrenergic blockade with phentolamine by examining the effects of phenylephrine, an α-agonist.

**METHODS**

**Subjects.** We studied 10 normal male subjects aged 30 ± 5 yr (range 22–38 yr). None were taking any medication. Informed written consent was obtained from all subjects. The Ertme Hospital Human Subjects Review Committee approved the study.

**Measurements.** Systolic (SBP) and diastolic BP (DBP) were measured every minute with a physiocontrol Colin BP-880 sphygmomanometer. Finger BP (Finapres), electrocardiogram (Siemens), and respiration (Respitrace) were recorded on a MacLab 8/s data acquisition system. Sympathetic nerve activity to muscle (MSNA) was recorded continuously by obtaining multunit recordings of postganglionic sympathetic activity, measured from a nerve fascicle in the peroneal nerve posterior to the fibular head as described previously (10).

Electrical activity in the nerve fascicle was measured using tungsten microwire electrodes (shaft diameter 200 μm, tapering to an uninsulated tip of 1–5 μm). A subcutaneous reference electrode was inserted 2–3 cm away from the recording electrode, which was inserted into the nerve fascicle. The neural signals were amplified, filtered, rectified, and integrated to obtain a mean voltage display of sympathetic nerve activity.

**Protocols and interventions.** The primary goal of the interventions was to induce comparable baroreflex deactivation through similar BP reductions with SNP and phentolamine. Measurements were taken during a baseline period of 10 min followed by an infusion of 0.8–1.5 μg·kg⁻¹·min⁻¹ phenylephrine. This infusion was started 10 min after the second bolus of phentolamine and continued for 5 min. In 2 of 10 subjects, the infusion of phentolamine was repeated in the absence of phentolamine. This allowed us to determine the effects of phenylephrine on baseline cardiovascular parameters and to compare these effects with those induced by phenylephrine after the infusion of phentolamine.

The effectiveness of α-adrenergic blockade by phentolamine was further confirmed by studying the effects of 0.9 μg·kg⁻¹·min⁻¹ phenylephrine on cardiovascular measures recorded during baseline conditions in four additional healthy volunteers.

Technically excellent studies examining the effects of phentolamine and SNP on MSNA were obtained in 9 of 10 subjects. A loss of sympathetic nerve recording occurred after the end of the phentolamine session in one subject. Excellent recordings of the effects of phenylephrine on MSNA after phentolamine were therefore obtained in 8 of 10 subjects.

**Data analysis.** Sympathetic bursts were identified by a careful inspection of the mean voltage neurogram. The amplitude of each burst was determined, and sympathetic activity was calculated as bursts per minute multiplied by the mean burst amplitude. Changes in MSNA were calculated as the percent change from baseline. A single observer (M. Rahnama) made measurements.

Analog-to-digital conversion was performed over 10 min at 300 sample/s for the electrocardiogram, BP, MSNA, and respiratory signals. The data were then analyzed off-line with a personal computer (IBM 433DX/7). The principles of the software for data acquisition and autoregressive spectral analysis have been described elsewhere (1, 23, 27, 28). The signal of MSNA was time integrated over each RR. SBP and DBP were determined in correspondence with the R wave while the signal of respiratory activity was sampled once every cardiac cycle. These procedures produced four time series (neurogram, systogram, diastogram, and respirogram), which were synchronized with the tachogram. Stationary segments devoid of arrhythmias and artifacts were analyzed with autoregressive algorithms by a single observer (S. Mezzetti). These algorithms automatically provide the number, center frequency, and power of the oscillatory components. Anderson’s test (1) verified that all information contained in the time series had been extracted in the computation, and Akaike’s test (1) allowed the determination of the optimal model order fitting the data. Previous studies (23, 27, 28, 32, 34) have shown that two major oscillatory components are detectable in short-term RR and MSNA and BP variability. One of these oscillatory components is synchronous with respiration and is called HF oscillation. The other component is described as LF oscillation and has a center frequency of ~0.10 Hz, but can vary considerably (from 0.04 to 0.15 Hz) (23, 27, 28, 34). In this study, the LF and HF components were expressed in normalized units. The normalized LF and HF units were obtained by calculating the absolute variability of each LF and HF component as a percentage of the total power after subtracting the power of the very LF component (frequencies below nominal 0.03 Hz) (23, 27, 28, 34).

**Statistical analysis.** Changes in cardiovascular variability induced by sympathetic activation with phentolamine were compared with those induced by SNP. Results are expressed as means ± SE. Statistical analysis consisted of Wilcoxon and Mann-Whitney signed rank tests corrected for ties. Significance was assumed at $P < 0.05$.

**RESULTS**

**Contrasting effects of phenolamine and SNP on cardiovascular variability.** Both SNP and phentolamine induced reductions in SBP ($-3 ± 5$ from $117 ± 5$ mmHg vs. $-8 ± 3$ from $116 ± 1$ mmHg, respectively) that did not differ significantly ($P = 0.26$). Changes in DBP also did not differ between SNP ($-9 ± 5$ from $66 ± 4$ mmHg) and phenolamine ($-7 ± 3$ from $71 ± 3$ mmHg, $P = 0.53$).

During SNP, RR decreased ($-130 ± 22$ from $972 ± 41$ ms) and MSNA increased (by $+184 ± 26$%; Table 1 and Fig. 1). As expected, tachycardia and sympathetic
activation were accompanied by increases in normalized LF variability of RR (+20 ± 6 from 52 ± 5%), MSNA (+3 ± 5 from 36 ± 3%), SBP (+9 ± 6 from 61 ± 6%), and DBP (+7 ± 5 from 56 ± 4%) (Table 2 and Fig. 2).

Phentolamine induced a more marked reduction in RR (−203 ± 31 from 995 ± 42 ms) and greater increases in MSNA (+299 ± 31%) \( (P < 0.05 \text{ for RR and MSNA compared with effects of SNP; Table 1 and Fig. 1}) \). Despite greater sympathetic activation with phentolamine, normalized LF cardiovascular variability actually fell (by −2 ± 7 from 53 ± 6% for RR, by −34 ± 6 from 46 ± 2% for MSNA, by −16 ± 8 from 63 ± 8% for SBP, and by −13 ± 4 from 54 ± 5% for DBP, \( P < 0.05 \text{ compared with effects of SNP; Table 2 and Fig. 2}) \).

Thus both phentolamine and SNP decreased BP. This reduction in BP resulted in a greater increase in heart rate and MSNA with phentolamine treatment than with SNP treatment \( (P < 0.05; \text{Table 1}) \). However, these hypotensive agents induced opposite changes in the LF-to-HF ratios of cardiovascular variabilities: the LF-to-HF ratios of RR, MSNA, and BP decreased with phentolamine (RR −0.7 ± 0.6; MSNA −0.8 ± 0.2; SBP −3.2 ± 1.4; and DBP −1.4 ± 0.7) and increased with SNP (RR +3.3 ± 1.2, \( P < 0.01 \); MSNA +0.2 ± 0.2, \( P = 0.01 \); SBP +0.3 ± 1.6, \( P = 0.09 \); and DBP +1.5 ± 0.5, \( P < 0.01 \text{ vs. phentolamine, respectively}) \).

Phentolamine slowed the center frequency of the LF components of MSNA and SBP compared with SNP \( (P < 0.05) \). These modifications were not due to changes in the center frequency and variance of respiration \( (P > 0.87) \), and phentolamine did not change the center frequency of the HF components of RR, BP, and MSNA compared with SNP \( (P > 0.32) \).

| Table 1. Effects of phentolamine and sodium nitroprusside on RR, MSNA, SBP, DBP and their variance compared with baseline |
|---------------------------------|-----------|-----------------|-----------|
| Mean Variance                  | RR        | MSNA            | SBP        | DBP        |
| Sodium nitroprusside           | −130 ± 22 ms | +184 ± 26%     | −3 ± 5 mmHg  | −9 ± 5 mmHg  |
| Phentolamine                    | −203 ± 31 ms | +299 ± 31%     | −8 ± 3 mmHg  | −7 ± 3 mmHg  |
| Sodium nitroprusside           | +173 ± 620 ms  | +100 ± 54 au²  | +10 ± 3 mmHg² | +1 ± 2 mmHg² |
| Phentolamine                    | −837 ± 401 ms  | +314 ± 116 au² | −4 ± 3 mmHg² | +1 ± 4 mmHg² |

Values are means ± SE. RR, R-R interval; MSNA, muscle sympathetic nerve activity; SBP and DBP, systolic and diastolic blood pressure, respectively; au, arbitrary units. *\( P < 0.05 \) phentolamine vs. sodium nitroprusside. Wilcoxon signed rank test corrected for ties.
In all subjects, LF oscillations in RR and BP were evident during baseline, SNP, and phentolamine sessions (Fig. 2). These LF oscillations were also present in MSNA during all baseline and SNP recordings. LF oscillations in MSNA were diminished but still evident during phentolamine infusion in six subjects (Fig. 2) and disappeared completely in the other subjects.

Changes in absolute LF and HF spectral powers are shown in Table 2.

**Effects of phentolamine and sodium nitroprusside on the variability of RR, MSNA, SBP, and DBP compared with baseline**

<table>
<thead>
<tr>
<th>Component</th>
<th>Frequency, Hz</th>
<th>Absolute NU</th>
<th>NU</th>
<th>Absolute NU</th>
<th>NU</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR</td>
<td>Sodium nitroprusside</td>
<td>$-0.01 \pm 0.01$</td>
<td>$+457 \pm 407 \text{ms}^2$</td>
<td>$+20 \pm 6$</td>
<td>$-292 \pm 99 \text{ms}^2$</td>
</tr>
<tr>
<td></td>
<td>Phentolamine</td>
<td>$-0.02 \pm 0.01$</td>
<td>$-282 \pm 207 \text{ms}^2$</td>
<td>$-2 \pm 7^*$</td>
<td>$-175 \pm 106 \text{ms}^2$</td>
</tr>
<tr>
<td>MSNA</td>
<td>Sodium nitroprusside</td>
<td>$+0.005 \pm 0.004$</td>
<td>$+46 \pm 15 \text{au}^2$</td>
<td>$+3 \pm 5^*$</td>
<td>$+37 \pm 25 \text{au}^2$</td>
</tr>
<tr>
<td></td>
<td>Phentolamine</td>
<td>$-0.02 \pm 0.01$</td>
<td>$-17 \pm 14 \text{au}^2$</td>
<td>$-54 \pm 6^*$</td>
<td>$+257 \pm 111 \text{au}^2$</td>
</tr>
<tr>
<td>SBP</td>
<td>Sodium nitroprusside</td>
<td>$-0.005 \pm 0.01$</td>
<td>$+5 \pm 1 \text{mmHg}^2$</td>
<td>$+9 \pm 6^*$</td>
<td>$+2 \pm 0.5 \text{mmHg}^2$</td>
</tr>
<tr>
<td></td>
<td>Phentolamine</td>
<td>$-0.04 \pm 0.01$</td>
<td>$-5 \pm 0.5 \text{mmHg}^2$</td>
<td>$-16 \pm 8^*$</td>
<td>$+1 \pm 0.5 \text{mmHg}^2$</td>
</tr>
<tr>
<td>DBP</td>
<td>Sodium nitroprusside</td>
<td>$+0.004 \pm 0.008$</td>
<td>$+1 \pm 1 \text{mmHg}^2$</td>
<td>$+7 \pm 5^*$</td>
<td>$-0.4 \pm 0.3 \text{mmHg}^2$</td>
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<tr>
<td></td>
<td>Phentolamine</td>
<td>$-0.02 \pm 0.01$</td>
<td>$-0.002 \pm 0.7 \text{mmHg}^2$</td>
<td>$-13 \pm 4^*$</td>
<td>$+1.4 \pm 1.4 \text{mmHg}^2$</td>
</tr>
</tbody>
</table>

Values are means ± SE. LF, low frequency; HF, high frequency; NU, normalized units. *P < 0.05 and †P < 0.01 phentolamine vs. sodium nitroprusside, Wilcoxon signed rank test corrected for ties.

Fig. 2. Power spectral density (PSD) analysis of R-R interval (RR), MSNA, systolic BP (SBP), and diastolic BP (DBP) variability of the recordings depicted in Fig. 1. Nitroprusside (middle), but not phentolamine (right), markedly increased the LF variability of RR, MSNA, and BP compared with baseline (left). Phentolamine prevented, but did not abolish, the increase in LF cardiovascular variability during baroreceptor unloading. HF, high frequency.

In all subjects, LF oscillations in RR and BP were evident during baseline, SNP, and phentolamine sessions (Fig. 2). These LF oscillations were also present in MSNA during all baseline and SNP recordings. LF oscillations in MSNA were diminished but still evident during phentolamine infusion in six subjects (Fig. 2) and disappeared completely in the other subjects.

Changes in absolute LF and HF spectral powers are shown in Table 2.

**Effectiveness of α-adrenergic blockade after phentolamine.** The changes in BP, RR, and MSNA induced by phenylephrine alone were markedly attenuated when phenylephrine was given after administration of phentolamine ($P < 0.05$; Figs. 3 and 4).

**DISCUSSION**

The novel finding of our study is that α-adrenergic blockade eliminates the increased LF variability of sympathetic nerve traffic that accompanies sympathetic activation in healthy humans. This result suggests that 1) α-adrenergic transmission within the baroreflex loop is likely to contribute importantly to enhanced LF oscillatory power associated with sympa-
thetic activation and 2) the rise in LF oscillations during sympathetic activation may not be exclusively dependent on central mechanisms.

Cardiovascular oscillations slower than those of respiration have been reported in all mammalian species, but their origin remains unclear. Respiratory effects on cardiac output produce fluctuations in BP and subsequently in RR via the baroreflex (2–6, 9, 11, 28). The genesis of the LF oscillations in RR is thought to be 1) a consequence of baroreflex buffering of the HF oscillations in BP, resulting in a LF 10-s oscillation because of a resonance phenomenon due to the delay in the sympathetic control loop of the baroreflex and 2) a baroreflex response to the LF oscillations in BP (2–6, 9, 11). Thus it is assumed that respiratory BP waves are the afferent signal responsible for the genesis of LF and HF cardiovascular oscillations. This interpretation is, however, not unequivocal because there is also clear evidence that LF cardiovascular oscillations can be generated in the absence of a functional baroreceptor loop (12, 14, 21, 24, 25, 30), and a central oscillator in the spinal cord has been hypothesized to be responsible for LF oscillations in BP in animals, also called Mayer waves (12, 21, 30). Humans also manifest coherent LF oscillations between MSNA and BP (28). This finding, together with the observations that 1) LF oscillations in BP disappear in the absence of LF oscillations in MSNA (15, 33) and 2) restoration of LF oscillations in MSNA is also seen in BP (33, 35), suggest that LF oscillations in MSNA and BP share a common origin. Cyclic release of norepinephrine by the sympathetic nerve terminals at the neurovascular junction may contribute to LF oscillations in BP. This hypothesis is further supported by the demonstration that LP oscillations in BP are strongly attenuated after acute and
chronic α-adrenergic blockade (8, 19, 20). These studies were performed in animals and did not record LF variability of sympathetic nerve traffic (8, 19, 20). One of the important findings of the present study is the demonstration that the reduction in LF variability in BP after α-adrenergic blockade is not due to suppression of vascular responses to LF oscillations in MSNA because this inhibition of LF oscillatory power is already present in presynaptic intraneural recordings.

Phentolamine did not completely suppress LF oscillations in BP, RR, or MSNA in our study. These oscillations tended to become slower than when sympathetic activation was induced by SNP. This finding is consistent with studies in animals where α-adrenergic blockade did not abolish LF oscillations in BP (6, 11, 20) and also consistent with studies in humans where interruption of the baroreflex loop slowed the LF oscillations during apnea (29). These findings reveal that LF cardiovascular oscillations may persist even in the absence of intact neurovascular transmission within the baroreflex in humans.

Neurally independent LF vasomotor oscillations may conceivably be an autoregulatory property (22, 31). Our observation of persistent LF oscillations in BP in the absence of LF oscillations in MSNA in one-third of the subjects also suggests that LF oscillations in BP may be in part explained by spontaneous fluctuations present in the vasculature during α-adrenergic blockade (3, 22, 31). In this regard, important information could be drawn by the analysis of the phase relationships between oscillations in BP and MSNA. However, this analysis is precluded by the limited number of subjects in whom LF oscillations in BP were both present and coherent with LF oscillations in MSNA after phentolamine administration in our study.

Several studies (3–6, 9, 11) have suggested that a resonance phenomenon in the arterial baroreflex loop may contribute to LF cardiovascular oscillations. A time delay in the response of negative feedback systems generates oscillations when the response delay is such that the output becomes in phase with the input, thereby creating a positive feedback (2, 4–6, 9, 11). The frequency of this oscillation depends on the time delay of the response of the system, i.e., the time of signal transmission between the moment when changes in BP sensed by the baroreceptors are translated into changes in vasomotor tone (3–6, 9, 11). This delay is mainly generated by the time needed for norepinephrine secreted at the neuroeffector junction to induce changes in vasomotor tone. With the use of phentolamine, we attenuated the vascular responsiveness to norepinephrine and thereby extended indefinitely the time delay of the baroreceptor feedback. This intervention suppressed the increase in LF cardiovascular variability in response to baroreceptor unloading and sympathetic activation.

Limitations of the study. Our study has several important limitations. First, the sequence of administration of the vasoactive drugs was not randomized due to the long half-life of phentolamine (17). In mitigation, however, performing all recordings during a single session allowed us to compare the sympathetic responses to baroreceptor unloading while maintaining the same microneurographic recording site. Moreover, vasoactive drugs were administered only after the cardiovascular parameters had returned to baseline levels.

Second, phentolamine inhibits both α1- and α2-adrenoceptors (17), and our study cannot dissociate the contribution of α1- and α2-adrenergic blockade on our results. However, we verified in each subject that the cardiovascular response to a high dose of intravenous phenylephrine was markedly blunted after phentolamine. There is therefore no reason to believe that enhanced neural release of norepinephrine, due to presynaptic α2-adrenergic blockade, may have overridden α1-adrenergic blockade in our study. We cannot exclude, however, that other effects of phentolamine, such as reduced responsiveness to serotonin (17), may have affected our results.

Third, a central effect of phentolamine cannot be ruled out despite observations that intracisternal phentolamine decreases heart rate (18) and that the central effects of phentolamine, evident after an intravenous dose eight times larger than in our study (16), were not observed at lower doses (13, 26). Our data therefore suggest that α-adrenergic transmission contributes importantly to the increased LF neural and cardiovascular variability during sympathetic excitation but do not exclude that central mechanisms participate in generating this LF oscillatory power.

These studies were supported by a Pfizer Research Grant, by the Foundation for Cardiac Surgery, by the National Fund for Research (to P. van de Borne and J. P. Degaute), and by the Marc Hurard Fondation (to S. Mezzetti). V. K. Somers is an Established Investigator of the American Heart Association and was supported by National Institutes of Health Grants MO1-RR-00585, HL-61560, and HL-65176.

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