Autonomic control of the cardiovascular system during acclimatization to high altitude: effects of sildenafil

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Cornolo, Jérémy, Pascal Mollard, Julien V. Brugniaux, Paul Robach, and Jean-Paul Richalet. Autonomic control of the cardiovascular system during acclimatization to high altitude: effects of sildenafil. J Appl Physiol 97: 935–940, 2004.—Both acute hypoxia and sildenafil may influence autonomic control through transient cardiovascular effects. In a double-blind study, we investigated whether sildenafil (Sil) could interfere with cardiovascular effects of hypoxia. Twelve healthy men (placebo (Pla) n = 6; Sil, n = 6) were exposed to an altitude of 4,350 m during 6 days. Treatment was continuously administered from 6 to 8 h after arrival at altitude (3 × 40 mg/day). The autonomic control on the heart was assessed by heart rate variability (HRV) during sleep at sea level (SL) and between day 1–2 and day 5–6 in hypoxia. Arterial pressure (AP) and total peripheral resistances (TPR) were obtained during daytime. There was no statistical difference between groups in HRV, AP, and TPR throughout the study. Hypoxia induced a decrease in R-R interval and an increase in AP in both groups. Low frequency-to-high frequency ratio increased at day 1–2 (Pla, P = 0.04; Sil, P = 0.02) and day 5–6 (Pla and Sil, P = 0.04) vs. SL, whereas normalized high-frequency power decreased only in Pla (P = 0.04, day 1–2 vs. SL). Normalized low-frequency power increased at high altitude (Pla and Sil, P = 0.04, day 5–6 vs. SL). TPR decreased at day 2 in Pla (P = 0.02) and tended to normalize at day 6 (P = 0.07, day 6 vs. day 2). Acute hypoxia induced a decrease in parasympathetic and increase in sympathetic tone, which tended to be reversed with acclimatization. Sil had no deleterious effects on the cardiovascular response to high-altitude exposure and its control by the autonomic nervous system.

Changes in the autonomic control of heart rate (HR) have been extensively studied by electrical nerve activity (14, 30), pharmacological blockade (8), catecholamine concentration (3, 21, 32), or myocardial β-adrenergic and muscarinic receptors (11, 28). Power spectral analysis of HR variability (HRV) represents a new tool to assess the sympathovagal balance (2, 23, 33). Acute exposure to hypoxia causes an increase in resting HR, which contributes to offset the reduced arterial oxygen content and to maintain oxygen transport to tissues. From the methods listed above, including HRV analysis, the altitude-induced increase in HR has been attributed to an increased and dominant sympathetic tone (5, 6, 17, 20) associated with a lesser vagal activity (5, 6, 24, 32). Despite this increased sympathetic tone, peripheral resistances decrease because of the local vasodilatation due to the reduced blood oxygen content (19). However, in chronic hypoxia, resting HR tends toward normoxic values due to the restoration in oxygen content (21, 28). During chronic hypoxia exposure, sympathetic and parasympathetic activities have been shown to remain constant (5, 24) or to progressively tend toward normoxic values (15, 32). These autonomic adaptations and the reduced metabolic vasodilatation may participate in an increase in peripheral resistances in chronic hypoxia (19, 21). However, few HRV studies attempted to record autonomic activity during the early phase of acclimatization to hypoxia (5, 15, 24, 32).

The present study is part of a large study on the effects of sildenafil citrate (Viagra) on hypoxia-induced pulmonary hypertension (27). Vasodilator effects of sildenafil on pulmonary circulation are linked to a prolonged availability of cyclic GMP (4). However, sildenafil could induce a modest and transient decrease in systemic resistances and arterial pressure (AP) in healthy men and in patients with chronic heart failure or erectile dysfunction (1, 12, 16, 26). Then a small increase in HR is sometimes observed as a consequence of a baroreflex activation (1, 12, 26). However, there is no consensus about a possible increase in sympathoadrenergic tone with sildenafil (1, 25, 26). Because systemic AP is increased by altitude exposure and the effect of sildenafil is proportional to the baseline level of AP (34), effects of both sildenafil and hypoxia on the cardiovascular system may be additive. Consequently we postulate that 1) exposure to acute altitude hypoxia may exacerbate the effects of sildenafil on the cardiovascular system and 2) sildenafil, by reducing hypoxemia, facilitates the return of autonomic control toward basal normoxic values and may accelerate acclimatization. To our knowledge, no study has reported the effects of sildenafil treatment on the autonomic control of the cardiovascular system at high altitude.

METHODS

Subjects. Twelve healthy, nonsmoking men (aged 29 ± 6 yr, height 181 ± 6 cm, body weight 79 ± 11 kg), native from sea level (SL), participated in this double-blind placebo-controlled study. Subjects were recruited after being interviewed with a standardized scheme to ascertain their medical history. A complete 12-lead ECG was performed to exclude subjects with significant cardiac abnormality. None of them was affected by cardiovascular or pulmonary diseases. All subjects were moderately trained (maximal oxygen uptake = 46.2 ± 2.1 ml.min⁻¹.kg⁻¹) and unacclimatized to altitude before the experiment. After giving their written, informed consent, subjects were randomly assigned to either a sildenafil group (Sil; n = 6) or a placebo group (Pla; n = 6). Treatment started at 2000 on day 1 at altitude, then the experimental group was given 40 mg of sildenafil citrate (Viagra, content (21, 28). During chronic hypoxia exposure, sympathetic and parasympathetic activities have been shown to remain constant (5, 24) or to progressively tend toward normoxic values (15, 32). These autonomic adaptations and the reduced metabolic vasodilatation may participate in an increase in peripheral resistances in chronic hypoxia (19, 21). However, few HRV studies attempted to record autonomic activity during the early phase of acclimatization to hypoxia (5, 15, 24, 32).

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Pfizer, New York, NY) three times per day (at 0800, 1400, and 2000), whereas the control group was given an identically appearing placebo from day 2 to day 6 in hypoxia. The treatment started 6–8 h after arrival at high altitude. The protocol was approved by the ethics committee of the Necker Hospital, Paris, France.

Procedures. Recordings were first obtained in normoxia [in Bobigny, France (SL)] and then on days 2 and 6 during a 6-day stay at 4,350 m above SL (Observatoire Vallot, Chamonix, France). After a night spent in Chamonix (1,000 m above SL), the subjects were transported by helicopter within 10 min to the Observatoire Vallot. HRV was assessed during the night at SL between days 1 and 2, and days 5 and 6. Systolic (SAP), diastolic (DAP), and mean AP (MAP), cardiac output (CO), arterial oxygen saturation (SaO2), breathing frequency (f), and tidal volume were measured at SL on day 2 and day 6. Throughout the study, temperature was kept constant at 20–23°C. Subjects were not allowed to drink coffee and were asked to rest during the 15 min preceding each measurement. Subjects were asked to go to bed before midnight and to stay in bed and remain as quiet as possible until 0600.

Measurement of R-R intervals and spectral analysis of HRV. R-R intervals were recorded with an accuracy of 1/1,000 s with a numeric S810 Polar R-R Recorder (Polar Electro, Kempele, Finland) during sleep from 0100 until wake-up. Then, data were transferred by the telemetric HR monitor to a portable computer, via a RS232 Polar interface, for further spectral analysis of HRV with the Polar precision telemetric HR monitor to a portable computer, via a RS232 Polar S810 Polar R-R Recorder (Polar Electro, Kempele, Finland) during 30-min periods were recorded with an accuracy of 1/1,000 s with a numeric interface, for further spectral analysis of HRV with the Polar precision performance SW 4. From time series of R-R intervals and visual inspection, original recordings were corrected by either omitting or inserting beats with the use of the Polar software. Only original recordings with <10% of artifacts were kept. HRV was estimated on sets of successive continuous 30-min periods of R-R intervals free of artifacts, which represent more than the recommended 1,024 successive R-R intervals (33). Average data were extracted from each continuous 30-min period and then averaged for each recording. Recordings with one single 30-min period and the first 15 min of each recording were excluded from the analysis.

It has been shown that specific characteristics of the power spectrum of the HRV can be used to quantify sympathetic and parasympathetic control on the heart (2, 23, 33). Power spectrum was obtained by an autoregressive modeling technique. Two frequency bands were considered: the low-frequency (LF) band (0.04–0.15 Hz) and the respiratory high-frequency (HF) band (0.15–0.40 Hz). LF was considered as a marker of both sympathetic and parasympathetic activity (2, 23, 33). Because HF is a result of respiratory sinus arrhythmia mediated by the vagus, its amplitude reflects the respiratory modulation of cardiac vagal outflow. Both LF and HF influences were expressed as absolute units (LFnu, HFnu, ms²) to estimate their power, and in normalized units (LFHnu, HFHnu, %) to estimate the relative part of each component in the total power (TP) (33). The LF-to-HF ratio (LF/HF) is an index of the sympathovagal balance (2, 33).

Breathing pattern could affect spectral power of the HRV (2, 9). However, it was not possible to record f during the night. Then, f and tidal volume were measured during daytime when subjects breathed spontaneously at rest in a sitting position. Ventilation was recorded breath by breath by using an integrated computer system (CPX/D cardiopulmonary exercise system, Medical Graphics, Minneapolis, MN). The breath-by-breath measurements were averaged over a 15-s period during a 5-min resting period in a sitting position.

SAP, CO, and peripheral resistances. SAP was measured by sphygmomanometry just before medication. MAP values were calculated from SAP and DAP values with the following equation: MAP = [(SAP – DAP)/3] + DAP. Data correspond to the average of the three daily recordings. CO was calculated by a transthoracic electrical impedance method using the Physio Flow PF-05 lab1 apparatus (Manatec Biomedical), which provides a reasonably accurate and reproducible estimation of CO (29). Values were continuously monitored during a 5-min resting period in a sitting position and averaged over a 15-s period. Total peripheral resistances (TPR) were calculated from MAP and CO using the equation TPR = MAP/CO.

SaO2, SaO2, was assessed just before medication with pulse oximetry at the earlobe previously vigorously rubbed (Ohmeda Biox 3740). Data correspond to the average of the three daily measurements.

Statistical analysis. Data were analyzed with Statview (version 5.0) statistical package. Results were expressed as means ± SE. Differences in the response of each group from SL to day 2 and day 6 were analyzed by the nonparametric Wilcoxon’s paired test. The effect of sildenafil treatment was analyzed by the nonparametric Mann-Whitney U-test between the two groups of treatment. Linear regression analyses were used when necessary. A P value of <0.05 was taken as evidence of significance.

RESULTS

One subject of Pla during the night between days 1 and 2 and one subject of Sil during the night between days 5 and 6 were excluded from the statistical analysis because of >10% artifacts in the R-R interval recordings.

HRV. Mean R-R interval decreased in both groups at days 1–2 and days 5–6 compared with SL and remained constant during acclimatization (Fig. 1). There was no difference between groups in R-R interval. TP decreased in acute hypoxia (Pla, −67%; Sil, −68%) and slightly increased toward SL values during acclimatization (Table 1). LF power tended to decrease from SL to days 1–2 in both Pla and Sil (−51%, P = not significant; −57%, P = not significant; respectively) and to increase toward normoxic values at days 5–6 (Table 1). The slight increase in LFnu observed on days 1–2 became significant at days 5–6 in both groups (Fig. 2A). HF power strongly fell at days 1–2 (Pla, −88%; Sil, −91%) compared with SL in both groups and then tended to increase during acclimatization without reaching SL values (Table 1). HFnu decreased on days 1–2 vs. SL and tended to increase toward normoxic values with acclimatization (Pla, +77%; P = not significant; Sil, +55%, P = not significant; days 5–6 vs. days 1–2) (Fig. 2B). The sympathovagal balance (LF/HF) increased in both groups on days 1–2 and remained above normoxic values on days 5–6.
Significant variation was observed for TPR in Sil (Fig. 4). There was no difference between groups in all HRV components.

The f increased significantly at day 6 vs. SL only in Pla. On day 2, f was higher in Pla than in Sil (Table 2). Tidal volume increased in both groups at day 6 vs. SL (Table 2).

Hemodynamics. SAP increased in both groups on day 2 compared with SL. During acclimatization SAP remained above SL values, especially in Pla (Pla, P = 0.02; Sil, P = 0.07; day 6 vs. SL) (Table 2). DAP and MAP increased in both groups on day 2 vs. SL and then remained above SL during acclimatization (Pla, P = 0.02; Sil, P = 0.02; day 6 vs. SL) (Table 2 and Fig. 3). There was no difference between groups in SAP, DAP, and MAP. CO increased in both groups on day 2 compared with SL (Table 2). In Pla, TPR significantly decreased on day 2 vs. SL and tended to increase toward normoxic values with acclimatization (+23%, P = 0.07). No significant variation was observed for TPR in Sil (Fig. 4).

\[ \Delta \text{Sao}_2 \]. As expected, \( \Delta \text{Sao}_2 \) decreased in both groups in hypoxia and tended to increase during acclimatization (Pla, +2.8%, P = not significant; Sil, +2.2%, P = not significant; day 6 vs. day 2) (Table 2). Although no difference in \( \Delta \text{Sao}_2 \) was found in day 2 and day 6 between groups, \( \Delta \text{Sao}_2 \) was significantly higher in Sil than in Pla at days 3, 4, and 5 (results not shown; Ref. 27).

**DISCUSSION**

To our knowledge, no previous study had evaluated the effects of sildenafl on the autonomic control of HR in healthy men during a prolonged stay at high altitude. There were two main findings in our double-blind placebo-controlled study in the present study, no direct effect of sildenafl treatment on the autonomic control of the cardiovascular system was reported in hypoxia. LF/HF increased in hypoxia probably as a result of an increase in the sympathetic tone associated with a fall in the parasympathetic control. These autonomic responses occurring at days 1–2 tended to be reversed from days 5–6, with no specific effect of sildenafl on acclimatization process. The present study was part of a large study that evidenced the suppressing effects of sildenafl on hypoxia-induced pulmonary hypertension: pulmonary pressure and resistances decreased with sildenafl, with no effect on CO, systemic pressures, and left heart function (27).

**Table 1. Spectral analysis of heart rate**

<table>
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<tr>
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<th>Pla</th>
<th>Sil</th>
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<tr>
<td></td>
<td>SL Day 1–2</td>
<td>Day 5–6</td>
</tr>
<tr>
<td>LFau, ms(^2)</td>
<td>3.625±818</td>
<td>1.785±686</td>
</tr>
<tr>
<td>HFau, ms(^2)</td>
<td>2.322±724</td>
<td>274.7±144*</td>
</tr>
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</table>

Values are means ± SE. Placebo (Pla) and sildenafl groups (Sil) at sea level (SL) and in hypoxia (day 1–2, day 5–6). LFau, low-frequency power (in absolute units), HFau, high-frequency power (in absolute units). *P < 0.05, day 1–2 (night between days 1 and 2) and day 5–6 (night between days 5 and 6) vs. SL. No significant difference between Sil and Pla.

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**Fig. 2.** Spectral analysis of heart rate variability in Pla and Sil at SL and during D1–2 and D5–6 at an altitude of 4,350 m. A: low-frequency power (LFnu) as a marker of the cardiac sympathetic activity. B: high-frequency power (HFnu) as a marker of the vagal activity. C: low frequency-to-high frequency ratio (LF/HF) as a marker of the sympathovagal balance. Bars and lines show means ± SE. *P < 0.05, D2 and D6 vs. SL.
Effects of sildenafil treatment in hypoxia on HR and HRV. No difference was observed between groups either in the cardiovascular system or in spectral components of the HRV. TP decreased in Pla but not significantly in Sil at day 1–2, which could be associated with the lack of increase in f in Sil (6). Because there is no difference between groups in TP, LF, and HF power, the influence of breathing patterns on TP may be weak. This could be mainly explained by a large interindividual variability. A decrease in the autonomic control of the heart assessed by a decrease in TP occurs in various diseases such as myocardial infarction (18). Sildenafil did not decrease the autonomic control on the heart conversely to what was shown in normoxia by Fogari et al. (12). In the present study, sildenafil did not exacerbate the decrease in TP due to hypoxia, suggesting that this treatment had no deleterious effect on the control of heart function at high altitude.

Systemic SAP, DAP, and MAP increased in both groups on day 2 without significant differences between groups. In normoxia, however, sildenafil could induce a modest and transient decrease in AP (1, 12, 16, 26), which could be counterbalanced by a reflex increase in HR (1, 12, 26). Because the treatment started 6–8 h after arrival at 4,350 m, a prior increase in AP due to hypoxia could have potentiated the effect of sildenafil on the cardiovascular system (34). Hypoxia increased AP similarly in both groups at day 2. As a consequence, sildenafil did not influence the hypoxia-induced increase in HR, LFn, and LF/HF. TPR decreased at high altitude, which is in accordance with prior studies in acute hypoxia (19). There was no difference between groups, which could be linked to the lack of difference in SaO₂ between groups at day 2 and day 6. Although SaO₂ was higher in Sil than in Pla from day 3 to day 5, we could not relate this difference to HRV and TPR, which were not recorded during acclimatization. Furthermore, the 100- to 200-mg single oral dose used by Jackson et al. (16) that induced a decrease in TPR was higher than our treatment. Although TPR decreased at day 2, the increased AP was mainly due to the rise in CO secondary to the increased HR and sympathetic tone.

Furthermore, sildenafil could have direct cardiac effects, independent of AP changes, as neuronal nitric oxide synthase may have chronotropic and inotropic effects (10). The increase in HR and sympathetic activity in hypoxia was sometimes associated with a decrease in neuronal nitric oxide synthase expression (22). Consequently sildenafil, by increasing the bioavailability of cGMP, could limit the hypoxia-induced increase in HR as previously shown (35). However, we did not find any significant difference between groups in HR increase at altitude at day 1–2 and day 5–6, although a significant...

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**Table 2. Hemodynamics and ventilation**

<table>
<thead>
<tr>
<th></th>
<th>PLA</th>
<th>Sil</th>
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<tbody>
<tr>
<td></td>
<td>SL</td>
<td>Day 2</td>
</tr>
<tr>
<td>Systolic arterial pressure, mmHg</td>
<td>129.3±2.4</td>
<td>138±4.4*</td>
</tr>
<tr>
<td>Diastolic arterial pressure, mmHg</td>
<td>75.2±2.4</td>
<td>83.8±1.7*</td>
</tr>
<tr>
<td>Cardiac output, l/min</td>
<td>4.6±3.4</td>
<td>7.7±0.8*</td>
</tr>
<tr>
<td>SaO₂, %</td>
<td>97.7±0.2</td>
<td>83.9±1.8*</td>
</tr>
<tr>
<td>Breathing frequency, breaths/min</td>
<td>16.9±2.2</td>
<td>20.2±1.4</td>
</tr>
<tr>
<td>Tidal volume, ml</td>
<td>738.8±100</td>
<td>900.7±111.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. Pla and Sil at SL and in hypoxia (day 2, day 6). SaO₂, arterial oxygen saturation. *P < 0.05, day 2 and day 6 vs. SL. †P < 0.05, day 2 vs. day 6.
difference appeared between day 2 and day 5 (27). Finally, in both groups, the decrease in R-R interval from SL to day 1–2 was correlated with the fall in SaO₂ (Pla, r = 0.72, P = 0.01; Sil, r = 0.83, P = 0.0006).

Because mechanical effects of respiration on HRV are lower in the supine position (31), assessment of the cardiac autonomic control with night recordings seems valid. Voluntarily in the supine position (31), assessment of the cardiac autonomic control on the heart, caused a reduction in the circulation.

As already shown in normoxia (1), there is no direct specific effect of sildenafl on the autonomic cardiovascular control in hypoxia. As a consequence, the role of sildenafl, via the nitric oxide pathway, appears highly selective to the pulmonary circulation.

**Effects of hypoxia on HR and HRV.** According to some previous studies (6, 15, 32), TP decreased in hypoxia. This decrease in TP, as a possible marker of the decrease in the autonomic control on the heart, caused a reduction in the absolute value of both spectral components.

We postulate that the decrease in mean R-R interval at day 1–2 may be due to a vagal fall (Pla, r = 0.7, P = 0.005; Sil, r = 0.7, P = 0.008). The increase in LF/ HF at day 1–2, which evidences a dominant adrenergic control, was mainly due to the greater decrease in HF than LF (32). Because LF power is usually considered as a marker of both sympathetic and parasympathetic activities (2, 23, 33), the lack of increase in LF at day 1–2 may have been caused by the net vagal withdrawal. Moreover, the significant increase in LF/ HF reported by some authors in acute hypoxia (5, 6, 20) was not found in the present study at day 1–2. These authors estimated LF/ HF using a frequency range between 0.01 and 0.15 Hz (20) or 0.03 and 0.15 Hz (5, 6), which is lower than our range (0.04–0.15 Hz). Thus the part of the TP below 0.04 Hz could have influenced the lack of increase in LF/ HF at day 1–2.

The decrease in HR with acclimatization was not found to be significant. In fact, the sympathovagal balance remained constant, although a slight increase in HF/ Nu was observed. This adaptation in HF/ Nu could be linked to the increase in parasympathetic tone with acclimatization found by others (8, 15, 32). Sildenafl did not modify this acclimatization process.

In conclusion, HRV analysis has evidenced that if acute exposure to hypoxia is associated with decreased parasympathetic and increased sympathetic tone, then acclimatization seems to be characterized by a progressive shift toward a higher parasympathetic tone. Because autonomic adaptation to hypoxia was not altered with medication, sildenafl may have no deleterious effect on the control of heart function at high altitude. By reducing pulmonary pressure and increasing arterial oxygenation, sildenafl may facilitate cardiovascular adaptation to hypoxia and protect against the unwanted effects of high altitude.