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

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Running head : Sildenafil and heart rate variability at high altitude
Abstract

Both acute hypoxia and sildenafil may influence autonomic control through transient cardiovascular effects. In a double-blind study, we investigated whether sildenafil could interfere with cardiovascular effects of hypoxia. Twelve healthy men (placebo, PLA n = 6; sildenafil, SIL n = 6) were exposed to 4,350m during six days. Treatment was continuously administered from 6-8 hours after arrival at altitude (3x40mg/day). The autonomic control on the heart was assessed by heart rate variability (HRV) during sleep at sea level (SL), between days 1-2 (D1-2) and days 5-6 (D5-6) in hypoxia. Arterial pressure (AP) and total peripheral resistances (TPR) were obtained during daytime. There were 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/high frequency ratio increased at D1-2 (PLA, \(P = 0.04\); SIL, \(P = 0.02\)) and D5-6 (PLA and SIL, \(P = 0.04\)) versus SL, while normalized high frequency power decreased only in PLA (\(P = 0.04\), D1-2 vs SL). Normalized low frequency power increased at high altitude (PLA and SIL, \(P = 0.04\), D5-6 vs SL). TPR decreased at D2 in PLA (\(P = 0.02\)) and tended to normalize at D6 (\(P = 0.07\), D6 vs D2). Acute hypoxia induced a decrease in parasympathetic and increase in sympathetic tone which tended to be reversed with acclimatization. Sildenafil had no deleterious effects on the cardiovascular response to high altitude exposure and its control by the autonomic nervous system.

Key words: acute mountain sickness, autonomic nervous system, hypoxia
Changes in the autonomic control of heart rate have been extensively studied by electrical nerve activity (14, 30), pharmacological blockade (8), catecholamines concentration (3, 21, 32) or myocardial beta adrenergic and muscarinic receptors (11, 27). Power spectral analysis of heart rate variability (HRV) represents a new tool to assess the sympato-vagal balance (2, 23, 33). Acute exposure to hypoxia causes an increase in resting heart rate (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 heart rate 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 O₂ content (21, 27). During chronic hypoxic exposure, sympathetic and parasympathetic activities have been shown to remain constant (5, 24) or to progressively tend towards normoxic values (15, 32). These autonomic adaptations and the reduced metabolic vasodilatation may participate to 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 (28). 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 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
sympatho-adrenergic tone with sildenafil (1, 25, 26). Since systemic arterial pressure (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 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, 2) sildenafil, by reducing hypoxemia, facilitates the return of autonomic control towards 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 non smoking male (aged 29 ± 6 yrs, height 181 ± 6 cm, body weight 79 ± 11 kg), native from sea level, 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 (\( \dot{V}O_{2\text{max}} = 46.2\pm2.1 \text{ ml/min/kg} \)), unacclimatized to altitude before the experiment. After giving their written informed consent, subjects were randomly assigned to either sildenafil (SIL, n = 6) or placebo (PLA, n = 6) group. Treatment started at 20:00 on day 1 at altitude, then the experimental group was given 40mg sildenafil citrate (Viagra®, Pfizer, New York, NY) three times per day (08:00, 14:00, 20:00) while control group took an identically-appearing placebo, from day 2 to day 6 in hypoxia. The treatment started 6-8 hours after arrival at high altitude. The protocol was approved by the ethics committee of the Necker Hospital, Paris.
Procedures. Recordings were firstly obtained in normoxia (Bobigny, sea level, SL) and then on days 2 and 6 (D2, D6) during a 6-day stay at 4,350m above sea level (Observatoire Vallot, Chamonix). After a night spent in Chamonix (1000m above sea level), the subjects were transported by helicopter within 10 min to the Observatoire Vallot. Heart rate variability was assessed during the night at SL, between D1 to D2 (D1-2), and D5 to D6 (D5-6). Systolic, diastolic and mean arterial pressure, cardiac output, arterial oxygen saturation, breathing frequency and tidal volume were measured at SL, D2 and D6. 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 minutes preceding each measurement. Subjects were asked to go to bed before midnight and to stay in bed and remain as quiet as possible until 06h00.

Measurement of R-R intervals and spectral analysis of HRV. R-R intervals were recorded with an accuracy of 1/1000 second with a numeric S810 Polar R-R Recorder (Polar Electro, Kempele, Finland) during sleep from 01h00 AM until wake-up. Then, data were transferred by the telemetric heart rate monitor to a portable computer, via a RS232 Polar 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 less than 10% of artifacts were kept. Heart rate variability was estimated on sets of successive continuous 30-minute periods of R-R intervals free of artifacts, which represent more than the recommended 1024 successive R-R intervals (33). Average data were extracted from each continuous 30-minute periods, and then averaged for each recording. Recordings with one single 30-minute period and the first 15 minutes 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 modelling 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 (a.u., ms²) to estimate their power, and in normalized units (n.u., %) to estimate the relative part of each component in the total power (33). The LF/HF ratio is an index of the sympatho-vagal balance (2, 33).

Breathing pattern could affect spectral power of the HRV (2, 9). However, it was not possible to record breathing frequency during the night. Then, breathing frequency and tidal volume were measured during day time 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-second period during a 5-minute resting period in a sitting position.

*Systemic arterial pressure, cardiac output and peripheral resistances.* Systemic arterial pressure was measured by sphygmomanometry just before medication. Mean arterial pressure (MAP) values were calculated from systolic (SAP) and diastolic (DAP) arterial values with the following equation: MAP = [(SAP - DAP)/3] + DAP. Data correspond to the average of the three daily recordings. Cardiac output (CO) was calculated by a trans-thoracic electrical impedance method using the Physio Flow PF-05 lab1 apparatus (Manatec Biomedical, France), which provides a reasonably accurate and reproducible estimation of
Values were continuously monitored during a 5-minute resting period in a sitting position and averaged over a 15-second period. Total peripheral resistances (TPR) were calculated from MAP and CO using the following equation: TPR = MAP / CO.

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

**Statistical analysis.** Data were analysed using Statview (5.0) statistical package. Results were expressed as mean ± standard error. Differences in the response of each group from SL to D2 and D6 were analysed by the non parametric Wilcoxon’s paired test. The effect of sildenafil treatment was analysed by the non parametric Mann-Whitney $U$-test between the two groups of treatment. Linear regression analyses were used when necessary. A P value <0.05 was taken as evidence of significance.

**RESULTS**

One subject of PLA during the night between days 1-2 and one subject of SIL during the night between days 5-6 were excluded from the statistical analysis because of more than 10% artefacts in R-R interval recordings.

**HRV.** Mean R-R interval decreased in both groups at day 1-2 and day 5-6 when compared to SL, and remained constant during acclimatization (Fig. 1). There was no difference between groups in R-R interval. Total power decreased in acute hypoxia (PLA, -67%, SIL, -68%) and slightly increased towards SL values during acclimatization (Table 1).
LF power tended to decrease from SL to D1-2 in both PLA and SIL groups (-51%, n.s.; -57%, n.s.; respectively), and to increase towards normoxic values at D5-6 (Table 1). The slight increase in LFnu observed on D1-2 became significant at D5-6 in both groups (Fig. 2A). HF power strongly fell at D1-2 (PLA, -88%, SIL, -91%) compared to SL in both groups and then tended to increase during acclimatization without reaching SL values (Table 1). HFnu decreased on D1-2 versus SL and tended to increase towards normoxic values with acclimatization (PLA, +77%, n.s.; SIL, +55%, n.s. D5-6 vs D1-2) (Fig. 2B). The sympathovagal balance (LF/HF ratio) increased in both groups on D1-2, and remained above normoxic values on D5-6 (Fig. 2C). There was no difference between groups in all HRV components.

Breathing frequency (BF) increased significantly at D6 versus SL only in PLA. On D2, BF was higher in PLA than in SIL (Table 2). Tidal volume increased in both groups at D2 versus SL (Table 2).

**Hemodynamics.** SAP increased in both groups on D2 compared to SL. During acclimatization SAP remained above sea level values, especially in PLA (PLA, $P = 0.02$; SIL, $P = 0.07$; D6 vs D2) (Table 2). DAP and MAP increased in both groups on D2 vs SL, and then remained above SL during acclimatization (PLA, $P = 0.02$; SIL, $P = 0.02$; D6 vs SL) (Table 2 and Fig. 3). There was no difference between groups in SAP, DAP, and MAP. Cardiac output increased in both groups on D2 compared to SL (Table 2). In PLA, TPR significantly decreased on D2 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).

**$\text{SaO}_2$.** As expected, arterial oxygen saturation ($\text{SaO}_2$) decreased in both groups in hypoxia and tended to increase during acclimatization (PLA, +2.8%, n.s.; SIL, +2.2%, n.s.;
D6 vs D2) (Table 2). Although no difference in SaO₂ was found in D2 and D6 between groups, SaO₂ was significantly higher in SIL than in PLA at D3, D4 and D5 (results not shown, 28).

**DISCUSSION**

To our knowledge, no previous study had evaluated the effects of sildenafil on the autonomic control of heart rate 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 sildenafil treatment on the autonomic control of the cardiovascular system was reported in hypoxia. LF/HF ratio 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 day 1-2 tended to be reversed from day 5-6, with no specific effect of sildenafil on acclimatization process. The present study was part of a large study that evidenced the suppressing effects of sildenafil on hypoxia-induced pulmonary hypertension: pulmonary pressure and resistances decreased with sildenafil, with no effect on cardiac output, systemic pressures and left heart function (28).

*Effects of sildenafil treatment in hypoxia on heart rate and heart rate variability.* No difference was observed between groups neither in the cardiovascular system nor in spectral components of the HRV. Total power (TP) decreased in PLA but not significantly in SIL at day 1-2 which could be associated with the lack of increase in breathing frequency 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 (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 systolic, diastolic and mean arterial pressure 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 hours after arrival at 4,350m, 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, LFnu, 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 100-200mg single oral dose used by Jackson et al. (16) that induced a decrease in TPR was higher than our treatment. While TPR decreased at day 2, the increased AP was mainly due to the rise in cardiac output secondary to the increased heart rate and sympathetic tone.

Furthermore sildenafil could have direct cardiac effects, independently of AP changes, as nNOS may have chronotropic and inotropic effects (10). The increase in heart rate and sympathetic activity in hypoxia was sometimes associated with a decrease in nNOS expression (22). Consequently sildenafil, by increasing the bioavailability of cGMP, could limit the hypoxia-induced increase in HR as it was 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 difference appeared between day 2 and day 5 (28). Finally, in both groups, the decrease in R-R interval from sea level to day 1-2 was correlated with the fall in arterial oxygen saturation (PLA, $r = 0.72, P = 0.01$; SIL, $r = 0.83, P = 0.0006$).

Since mechanical effects of respiration on HRV are lower in supine position (31), assessment of the cardiac autonomic control with night recordings seems valid. Voluntarily limiting BF at 15 c/min (0.25Hz) in hypoxia reduces LFnu and increases HFnu (6). In the present study BF increased only in PLA in hypoxia to reach 0.33Hz, becoming higher than in SIL at day 2 (0.27Hz). In fact HFnu was significantly reduced in hypoxia only in PLA probably because of the increased BF in this group (6). However, we did not observe a significant difference between groups in HFnu at day 1-2 which could be explained by the quite similar values for BF (0.33Hz and 0.27Hz). Consequently, breathing pattern may not have significantly influenced HRV.

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

*Effects of hypoxia on heart rate and heart rate variability.* According to some previous studies, TP decreased in hypoxia (6, 15, 32). 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 firstly 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 LFnue reported by some authors in acute hypoxia (5, 6, 20) was not found in the present study at day 1-2. These authors estimated LFnue using a frequency range between 0.01-0.15Hz (20) or 0.03-0.15Hz (5, 6) which is lower than ours (0.04-0.15Hz). Thus, the part of the total power below 0.04Hz could have influenced the lack of increase in LFnue at day 1-2.

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

In conclusion HRV analysis has evidenced that 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, sildenafil may have no deleterious effect on the control of heart function at high altitude. By reducing pulmonary pressure and increasing arterial oxygenation, sildenafil may facilitate cardiovascular adaptation to hypoxia and protect against the unwanted effects of high altitude.
AKNOWLEDGEMENTS

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REFERENCES


**Figure legends**

Fig. 1. Mean R-R interval in placebo (PLA) and sildenafil (SIL) group at sea level (SL), during the first (D1-2) and the fifth night (D5-6) at 4350m altitude. Bars and lines show mean ± SE. * p < 0.05, D2 and D6 vs. SL.

Fig. 2. Spectral analysis of heart rate variability in placebo (PLA) and sildenafil (SIL) groups at sea level (SL), during the first (D1-2) and the fifth night (D5-6) at 4350m altitude. 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: LF/HF ratio as a marker of the sympatho-vagal balance. Bars and lines show mean ± SE. * p < 0.05, D2 and D6 vs. SL.

Fig. 3. Mean arterial pressure (MAP) in placebo (PLA) and sildenafil (SIL) group at sea level (SL), during the second (D2) and the sixth day (D6) at 4350m altitude. Bars and lines show mean ± SE. * p < 0.05, D2 and D6 vs. SL.

Fig. 4. Total peripheral resistances (TPR) in placebo (PLA) and sildenafil (SIL) group at sea level (SL), during the second (D2) and the sixth day (D6) at 4350m altitude. Bars and lines show mean ± SE. * p < 0.05, D2 vs. SL.
Figures

Fig. 1

![Bar chart showing Mean RR intervals (msec) for PLA and SIL conditions with D1-2 and D5-6 groups. Significant differences indicated by asterisks.](image-url)
Fig. 2

A

B

C
Fig. 3

MAP (mmHg)

PLA SIL

D5-6

D1-2

SL

MAP (mmHg)

75 80 85 90 95 100 105 110 115

PLA  SIL

SL

D5-6

D1-2

*
Fig. 4

![Bar graph showing TPR (mmHg/min) for PLAs and SILs. The graph compares TPR levels between SL, D1-2, and D5-6 groups. There is a statistically significant difference (p = 0.07) marked with an asterisk (*) between the SL and D1-2 groups in the PLA condition.]
Table 1. Spectral analysis of heart rate.

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<th>PLA</th>
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<th>SIL</th>
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<td></td>
<td>SL</td>
<td>D1-2</td>
<td>D5-6</td>
<td></td>
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<tr>
<td>Total Power (ms²)</td>
<td>17021± 3510</td>
<td>5612± 2210 *</td>
<td>8212± 3036</td>
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<tr>
<td>LFa (ms²)</td>
<td>3625± 818</td>
<td>1785.6± 686</td>
<td>2339± 944</td>
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<tr>
<td>HFau (ms²)</td>
<td>2322± 724</td>
<td>274.7± 144 *</td>
<td>719± 365</td>
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</tbody>
</table>

Values are mean ± SE. Placebo (PLA) and sildenafil (SIL) groups at sea level (SL) and in hypoxia (D1-2, D5-6). Total Power; LFa, Low Frequency power; HFau, High Frequency power. * p < 0.05, D1-2 (night between days 1 and 2) and D5-6 (night between days 5 and 6) vs. SL. No significant difference SIL vs. PLA.
Table 2. Hemodynamics and ventilation.

<table>
<thead>
<tr>
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<tr>
<td></td>
<td>SL</td>
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<tr>
<td>Systolic arterial pressure (mmHg)</td>
<td>129.3± 2.4</td>
<td>138± 4.4 *</td>
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<tr>
<td>Diastolic arterial pressure (mmHg)</td>
<td>75.2± 2.4</td>
<td>83.8± 1.7 *</td>
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<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>
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<tr>
<td>Breathing frequency (c./min)</td>
<td>16.9± 2.2</td>
<td>20.2± 1.4</td>
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<tr>
<td>Tidal volume (ml)</td>
<td>738.8± 100</td>
<td>900.7± 111.4</td>
</tr>
</tbody>
</table>

Values are mean ± SE. Placebo (PLA) and sildenafil (SIL) groups at sea level (SL) and in hypoxia (D2, D6). * p < 0.05, D2 (day 2) and D6 (day 6) vs. SL; # p <0.05, D2 vs. D6. § p < 0.05, SIL vs. PLA.