Circadian rhythms of specific airway conductance and bronchial reactivity to histamine: the effects of parasympathetic blockade

D. Dreher, E.A. Koller

ABSTRACT: In ten healthy, nonsmoking, non-atopic, young volunteers, specific airway conductance and bronchial response to aerosolized histamine were measured plethysmographically at intervals of 4.8 h during two periods of 24 h, i.e. one day without, the other with, a parasympatholytic aerosol (0.20–0.24 mg ipratropium bromide) inhaled 1 h before each measurement, in order to determine the role of the parasympathetic innervation in the circadian rhythms of the airways. Specific airway conductance and bronchial reactivity showed clear circadian variations with corresponding peak times (16.11 and 04.41 h, respectively). Topical vagal blockade markedly increased specific conductance and resulted in a significant reduction of its rhythm amplitude, whereby the strong correlation between specific conductance and heart rate was significantly diminished. On the other hand, bronchial reactivity to histamine was lowered without flattening of its circadian rhythm. It is concluded that central parasympathetic outflow is an essential factor for the circadian rhythm of bronchial tone and, thus, for the increase in bronchial resistance at night.


The importance of chronobiology for diagnosis and treatment of reversible airways obstruction has been increasingly recognized in recent years [1, 2]. Various studies have shown circadian changes in airway calibre in healthy [3-5] and in asthmatic subjects [6-8]. Moreover, day/night variations of bronchial reactivity have been demonstrated for unspecific [9-11] and for antigenic [12] irritants. However, the mechanisms underlying the circadian variations of airway resistance, and the mechanisms mediating the rhythm in bronchial reactivity, are not yet clear. Our aim was to illuminate the role of the autonomous nervous system (in this paper the impact of the parasympathetic lung innervation) with appropriate plethysmographic and statistical methods in healthy subjects.

Bronchomotor tone, i.e. the parasympathetic outflow to the bronchial muscles and glands, results, in the healthy as well as in the asthmatic subject, from central vagal tone and the afferent vagal input arising from the pulmonary receptors [13]. Thus, these vagal receptors initiate, among other effects, the reflex changes in airway calibre due to inspiration and expiration during normal breathing as well as the reflex changes due to increased bronchial reactivity produced by asthmogenic substances [14]. On the other hand, the parasympathetic system is supposed to play an important role in controlling the day/night variations of both airway calibre and bronchial reactivity. Only two studies have investigated the influence of parasympathetic blockade on the circadian rhythm of airway calibre by measuring bronchial resistance [3, 4]. Their findings were opposed; however, neither paper provided direct statistical comparison between the rhythms before and after the parasympatholytic treatment. The role of vagal tone in the nocturnal increase of bronchial reactivity is, to our knowledge, investigated for the first time in this study.

Our experiments were designed to show the role of the parasympathetic lung innervation in both airway calibre and bronchial reactivity in selected healthy subjects, using ipratropium bromide inhaled in high dosage to achieve the topical blockade of the pulmonary parasympathetic innervation. In order to avoid external influences on the endogenous rhythms, the following principles were adhered to: 1) body plethysmography ensured measures independent from the subjects compliance; 2) the chronological design of the study was adjusted to the individual activity pattern; 3) the subjects were kept free from agents (drugs, nicotine, caffeine, etc.) which might affect lung functions or heart rate.
Methods

Subjects

Ten healthy young medical students (7 males and 3 females) gave their informed consent to the experiments. The subjects were 21–29 yrs old, nonsmokers, with no history of allergic diseases and with normal cardiopulmonary functions. Their physical characteristics and sleeping habits are recorded in table 1. Before and during the trials, no medication was allowed; food-intake and physical activity followed the Guidelines of the Assembly of Allergy and Clinical Immunology, A.T.S. [15].

Table 1. - Anthropometric and lung function data, mid-sleep (n=10)

<table>
<thead>
<tr>
<th>Mean</th>
<th>Minimum value</th>
<th>Maximum value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age yrs</td>
<td>23</td>
<td>21</td>
</tr>
<tr>
<td>Height m</td>
<td>1.76</td>
<td>1.65</td>
</tr>
<tr>
<td>VC l</td>
<td>5.11</td>
<td>3.70</td>
</tr>
<tr>
<td>FEV₁, %VC</td>
<td>78</td>
<td>67</td>
</tr>
<tr>
<td>TGV l</td>
<td>3.5</td>
<td>2.5</td>
</tr>
<tr>
<td>sGaw mb-sec⁻¹</td>
<td>0.027</td>
<td>0.19</td>
</tr>
<tr>
<td>PD₁₅ mg</td>
<td>0.22</td>
<td>0.03</td>
</tr>
<tr>
<td>Mid-sleep h-min⁻¹</td>
<td>3.43</td>
<td>2.00</td>
</tr>
</tbody>
</table>

VC: vital capacity; FEV₁: forced expiratory volume in one second; TGV: total gas volume; sGaw: specific airway conductance; PD₁₅: provocative dose producing 15% fall in sGaw.

Study design

In this transversely designed study, interindividual differences in the timing of the circadian system were adjusted by setting the middle of the habitual sleep span ("mid-sleep", MS) as reference for the chronological design of the study. 2 × 5 equidistant blocks of measurements during two periods of 24 h were grouped around mid-sleep, i.e. at: 1) MS -9.6 h; 2) MS -4.8 h; 3) MS; 4) MS +4.8 h; 5) MS +9.6 h. The subjects were asked to stay in bed between the last measurement block (MB) before mid-sleep (MS) and the first MB after MS, except for one interruption for the MB at MS (comparable disruption of sleep in asthmatic subjects was without apparent effect on lung functions [16]), thus resulting in at least 7 h of rest. However, the findings are presented in relation to midnight (midnight = MS -3.72 h). Every MB included consecutive determination of resting heart rate, specific airway conductance (sGaw) and bronchial reactivity to histamine (bHist).

Each of the ten volunteers underwent two experimental periods of 24 h: five subjects were measured without drug on the first day (control period) and with ipratropium on the following day (drug period), whilst the five other subjects began with the drug period and continued with the control period on the third day. During the drug period, ipratropium bromide from a metered-dose aerosolizer (Atrovent) was inhaled during slow maximal inspirations, starting from functional residual capacity. In order to achieve an effective dose of 0.24 mg [17] with a maximum drug effect during the MB, twelve puffs of ipratropium (0.02 mg per puff) were given 1 h before the first MB [18], and ten puffs 1 h before each of the following MBs. A placebo series or double-blind study was excluded, since the volunteers, all medical students, easily distinguished the characteristic taste of ipratropium from that of available placebos. Systematic placebo effects, however, would not affect the cosinor analysis which compares repeated measurements within the same treatment period (see below).

Measurements

Heart rate was determined from the subjects electrocardiogram (ECG) during 1 min after 20 min at rest in the supine position. In this time the subject was allowed to close his eyes but was asked to stay awake.

Measurements of airway resistance (Raw) and thoracic gas volume (TGV) were performed in a constant volume body plethysmograph with on-line data processing (Bodystar FG 90, Dr. Fenyves & Güt). Our modifications of the box included complete lining of the air-conduit system with thermoregulated aluminum tubes in order to avoid deviations from body temperature and standard atmospheric pressure saturated with water vapour (saturated conditions, thus essentially improving the reliability and reproducibility of the measurements. Before each measurement block (MB), the equipment was calibrated by a specifically designed electric piston pump [19]. Determination of sGaw and bronchial reactivity was based on triplets of plethysmographic measurements. Each triplet included three cycles of tidal breathing, each evaluated at 4 points at ±1 l s⁻¹, and was followed by estimation of TGV with closed mouth-shutter. sGaw of one triplet was calculated as the reciprocal of mean Raw of the nearest two measurements multiplied by TGV, thus compensating for intra-individual changes in lung volume [20]. In the present study, the mean standard deviation of sGaw, determined from one triplet, was <7%. sGaw of an MB was obtained from five triplets successively measured within 10 min. 1,500 plethysmographic measurements were thus performed in this study for determinations of sGaw, whilst about 3,000 breathing cycles were evaluated for calculations of bronchial reactivity (see below).

Bronchial provocation tests were performed with different doses of histamine solutions prepared according to the standard of the Asthma and Allergic Disease Centers, USA [21]. Within the week preceding the measurement period, the individual threshold dose of histamine causing a 35% fall in sGaw (PD₃₅) was estimated by an interpolation method [22]: firstly, this determination enabled preliminary exclusion of hyperreactive subjects; secondly, PD₃₅ served as provocation dose for the bronchial inhalation test. Instead of repeating the interpolation procedure in the MB, bronchial reactivity was determined as the airway response to the predetermined PD₃₅ (see below). This method, fully described by Gerwais et al. [12], yields higher reproducibility with direct results.
(i.e. without interpolation) and avoids inconstant, cumulative effects of the agent. The bronchial provocation test was carried out outside the body plethysmograph under screen control of respiratory volume and frequency. A French-Rosenthal Dosimeter (John Hopkins School of Medicine, Baltimore) was triggered on inspiration to deliver air during 0.6 s at 0.4 bar to the DeVilbiss No. 646-Nebulizer. After inhalation, triplets of plethysmographic measurements were recorded every 1 or 2 min, until sGaw recovered by increasing values, but at least during 6 min. Bronchial reactivity was defined as the percentage decrease between the lowest sGaw in the MB after inhalation of ten puffs of an isotonic NaCl solution and the lowest sGaw after the ensuing PD35 inhalation, also distributed on ten successive puffs. With an interval of 4.8 h between the histamine inhalation tests neither cumulative nor tachyphylactic effects should be expected [23].

Statistical analyses

Linear regression analysis was used for determination of correlation coefficients. Day/night variations of sGaw and bronchial reactivity to histamine were quantified by the cosinor analysis introduced by Halberg et al. [24], who used multiple regression analysis to fit sinusoidal curves to individual time series data, assuming a 24 h periodicity for circadian rhythms. A rhythm was found to be significant if the amplitude of the curve differed from zero with \( p<0.05 \). Differences between the single rhythm amplitudes and between the single correlation coefficients of the control and the drug period were evaluated by Fisher’s paired t-test. Parameters of group rhythms with and without treatment were estimated by group mean cosinor analysis according to Nelson et al. [25]. Within group cosinor analysis, significance of rhythm detection and differences between group rhythms were assessed by the zero-amplitude and the amplitude-acrophase test, respectively. For all time series modelling, SAS/ETS™ software, together with base SAS® software, was used (SAS Institute Inc.).

Results

Specific airway conductance

The chronogram of sGaw is presented in figure 1. The diagram shows maximum sGaw values at 13.19 h in the second measurement block after mid-sleep (MS +9.6 h); this peak time is not changed by the parasympathetic blockade. The drug, however, shifts mean sGaw from 0.22 to 0.39 (mb·s)\(^{-1}\), i.e. by 80%, as in all subjects the parasympatholytic treatment resulted in a marked bronchodilatation (fig. 2). For each subject, coefficients of correlation were calculated between the deviations of sGaw and heart rate from the period mean. In the control group, the mean coefficient of correlation amounts to 0.78 and is significantly different from zero (\( p<0.001 \)). Pairwise comparison to the individual coefficients after inhalation of the parasympatholytic agent (mean \( r = 0.32, p<0.05 \)) shows that the association between both parameters is significantly reduced in the treatment group (\( p<0.05 \)). In figure 3, the variations from all subjects as compared to the individual period means are depicted, illustrating the loss of association between sGaw and heart rate as the parasympathetic innervation of the airways is blocked.

![Fig. 1. Variations of sGaw and bRH with and without parasympathetic blockade: mean values±sBM. Time related to midnight (00.00) (n=10). sGaw: specific airway conductance; bRH: bronchial reactivity to histamine.](image)

![Fig. 2. sGaw: effect of parasympathetic blockade on individual period means and individual rhythm amplitudes (n=10). sGaw: specific airway conductance.](image)

![Fig. 3. Relationships between sGaw and heart rate with and without blockade of the parasympathetic lung innervation: deviations from period mean and linear regression analysis with 95% confidence limits (n=50). sGaw: specific airway conductance.](image)
Cosinor analysis was performed on individual data as well as on group data. The individual rhythm amplitudes (half the difference between maximum and minimum) during the control and the drug period are displayed in figure 2. Paired t-test comparison of the individual rhythm amplitudes of sGaw with and without drug reveals a significant reduction of single amplitudes by the parasympatholytic blockade \((p<0.05)\). This reduction, however, is not correlated to the individual increase in mean sGaw \((r = -0.12, p=0.1)\).

The results of group mean cosinor analysis are listed in table 2, and the group rhythms with and without treatment are depicted in figure 4. During the control period, sGaw describes a distinct rhythm with the acrophase (time of maximum) occurring at 16.11 h (MS +12.3 h). After parasympatholytic blockade, circadian variations of sGaw are still detectable \((p<0.05)\), the drug, however, results in a reduction of the group rhythm amplitude from 16 to 4% of the mesor (mean level). Group cosinor analysis verifies a significant difference between the group rhythms with and without parasympatholytic blockade (table 2).

**Table 2. - Results of group cosinor analysis (n=10)**

<table>
<thead>
<tr>
<th>Period</th>
<th>F(p)</th>
<th>Mesor</th>
<th>Amplitude</th>
<th>Acrophase h·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>sGaw mb·s⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>26.37***</td>
<td>0.22</td>
<td>0.03±0.01</td>
<td>16.1±0.13</td>
</tr>
<tr>
<td>drug</td>
<td>5.04*</td>
<td>0.39</td>
<td>0.01±0.01</td>
<td>14.2±0.35</td>
</tr>
<tr>
<td>bRH % sGaw</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>22.0***</td>
<td>37.3</td>
<td>6.7±2.2</td>
<td>04.4±0.13</td>
</tr>
<tr>
<td>drug</td>
<td>13.10***</td>
<td>9.9</td>
<td>5.1±2.6</td>
<td>04.4±0.20</td>
</tr>
</tbody>
</table>

F values obtained from cosinor analysis. One-tailed p values given for the hypothesis of rhythm-amplitude = 0; *: p<0.05; **: p<0.01. Acrophases related to midnight (= mid-sleep-related acrophases +3.4 h), sGaw: specific airway conductance; bRH: bronchial reactivity to histamine.

**Bronchial reactivity to histamine**

During both the control and the drug period, highest bronchial reactivity to histamine is found in the MB at 03.4 h, corresponding to MS (fig. 1). The parasympatholytic drug essentially reduces bronchial reactivity: the minimum individual decrease amounts to 42%, the maximum individual reduction to 85%. The group mean reactivity to the PD₄₃ is reduced by 73%, i.e., from 37.2% during the control period to 9.9% during parasympatholytic blockade.

Group cosinor analysis of the reactivity measurements without treatment reveals a significant circadian periodicity: the acrophase of the group rhythm is found at 04.41 h (MS +0.58 h), the rhythm amplitude is 8% of the mesor (mean level). Correspondingly, during the drug period a clear circadian group rhythm is found with a very similar peak time at 04.42 (MS +0.59 h), whilst the rhythm amplitude of the reactivity is slightly lowered to 6% of the mesor (table 2 and fig. 4). Comparison of the rhythms with and without drug by the amplitude-acrophase test shows no significant difference between the control and drug periods (table 2).

**Discussion**

This study was designed to determine the role of vagal motor innervation of the airways in mediating the circadian variations of both airway calibre and bronchial reactivity to histamine in healthy subjects. The choice of body plethysmography for evaluation of changes in airway dimensions based on the experience that other test methods, such as forced expiratory volume in one second (FEV₁) and peak expiratory flow rate (PEFR) depend on the subjects compliance [20]. The latter, however, would probably also show circadian variations. In addition, these tests require initial deep inspirations to total lung capacity and thus alter baseline airway tone and bronchial reactivity to bronchoconstrictor agents [26]. Therefore, in contrast to other authors who used forced expiratory manoeuvres to monitor the airway responses [9-12], we avoided maximum respiratory manoeuvres during the bronchial provocation test also. Circadian rhythms of sGaw and bronchial reactivity were verified by the cosinor analysis, a standard method of chronobiology, which has proved to be appropriate for the evaluation of circadian periodicity in lung functions.
subjects and notes showing a significant difference of Raw running unchanged but on a lower level; the dosage of 418 D. DREHER, E.A. KOLLER circa di an rhythm. 
circa libr e, on the comparison of the rhythms with and without drug, needed bronchial muscle tone and the rhythm in airway day/night pattern of airway resistance in healthy however, was not based on the cosinor n o longer 
particular bronchoconstrictor agent. 
lected acrophases of specific airway resistance (sRaw) at 05.39 h and 03.31 h, respectively; results which correspond well to our findings in healthy students with 04.11 h for the minimum of sGaw. The relative rhythm amplitudes in their groups amounted to 11 and 15%, respectively, whilst the group rhythm amplitude in our study was 16% of the mesor.

Circadian variations of bronchial reactivity have also been demonstrated in several non-plethysmographic studies (see above). De Vuze et al. [9] found that sensitivity to inhaled histamine in eleven patients with bronchial asthma was markedly increased at night, whilst a more recent investigation, on seven asthmatic subjects, demonstrated individual circadian rhythms of airway responsiveness to histamine varying in acrophases, i.e. times of highest reactivity, from 21.8 h to 11.1 h [10]. Reinberg et al. [11], in a group of eight normal subjects, showed that bronchial sensitivity to acetylcholine had a rhythm amplitude amounting to 30% of the mesor with the acrophase occurring at 02.54 h. We also found a distinct circadian rhythm of bronchial reactivity to histamine in normal subjects, with an amplitude of 22% of the mesor and the peak time at 04.41 h. These findings indicate that bronchial responsiveness shows day/night variations which are not restricted to asthmatic subjects or to a specific bronchoconstrictor agent.

Blockade of the parasympathetic lung innervation produced different effects on the circadian rhythms of sGaw and bronchial reactivity to histamine: high doses of inhaled ipratropium bromide (0.24 mg) markedly lessened bronchial muscle tone and the rhythm in airway calibre, on the other hand bronchial reactivity to histamine was lowered without significant flattening of its circadian rhythm. De Mijlau and Ullmer [3] were the first to study the influence of the vagolytic agent ipratropium bromide in a rather low dosage (0.025 mg) on the day/night pattern of airway resistance in healthy subjects; they found with ipratropium circadian variations of Raw running unchanged but on a lower level; the comparison of the rhythms with and without drug, however, was not based on the cosinor method. Gaultier et al. [4] described, in healthy children, a circadian rhythm in sRaw after 0.08 mg of ipratropium bromide, but with a dosage of 0.20 mg the rhythm in airway resistance was no longer detectable. Although based on only three subjects and not showing a significant difference between pre- and post-treatment rhythms by the amplitude-acrophase test, the authors assumed that the anticholinergic drug had suppressed the circadian rhythm, while lower dosages did not reduce the nocturnal increase in sRaw owing to insufficient blockade of parasympathetic activity at night, when vagal tone is highest. Our present study in healthy subjects supports the conclusion that ipratropium bromide inhaled in high dosage effectively reduces the circadian rhythm of airway resistance.

Nocturnal airway narrowing has been most consistently proposed to be due to increased vagal tone, decreased plasma adrenaline, and/or a fall in plasma corticosteroids [1]. In contrast to the effects of the vagolytic agent in our study, overnight treatment with B-agonists [27] or cortisol infusion [28] led to bronchodilation without reducing the circadian change of airway calibre. Systemic B-adrenergic blockade with high doses of propranolol (160 mg initial dosage + 80 mg before each measurement block) had no effect on the circadian rhythm of airway resistance or on bronchial sensitivity to histamine in our recent, similar designed follow-up study [10]. These findings suggest that vagal bronchomotor tone is the essential part of the autonomous nervous system in mediating the circadian rhythm of airway calibre.

The circadian variations of sGaw are strongly correlated with those of resting heart rate; at night both parameters reflect increased parasympathetic activity [30]. In our experiments the parasympatholytic aerosol inhaled in high doses led to blockade of the vagal bronchomotor fibres and thus to bronchodilation, but did not affect the parasympathetic innervation of the heart. Therefore, we may conclude that the ipratropium aerosol topically blocks the parasympathetic lung innervation. The clinical and therapeutic impact of this conclusion is obvious in nocturnal bronchial asthma: in a recent clinical study, nocturnal airway obstruction was significantly reduced by high doses of an inhaled anticholinergic agent [7].

Parasympathetic blockade significantly lowered bronchial reactivity to histamine by reducing bronchial muscle tone, but the circadian rhythm of bronchial reactivity was not reduced. The persistence of the reactivity rhythm under parasympathetic blockade suggests that the higher vagal activity at night does not cause the nocturnal increase in airway responsiveness to histamine, which therefore depends on other nervous and/or humoral factors. Since in normal subjects our method of bronchoprovocative testing predominantly reflects reactivity of the larger airways [20], these findings might be different in asthmatic patients.

As bronchomotor tone, apart from the influence of the central oscillator, underlies reflex mechanisms in both the normal and the asthmatic subject, it should be pointed out that bronchial reactivity may affect, by vago-vagally mediated reflexes, the bronchial resistance [13, 14]. Corresponding acrophases of bronchial reactivity and airway resistance would support this assumption. We therefore conclude that the circadian rhythm in bronchial reactivity superimposes on the rhythm of airway resistance, thus probably contributing to the aggravation of symptoms of nocturnal asthma in hyperreactive patients. Further, the
Circadian Rhythms of Bronchomotor Tone

Nocturnal increase of bronchial reactivity is of lessened clinical impact if bronchomotor activity and vagally mediated reflex bronchoconstriction are blocked by high doses of a topical parasympatholytic agent.

Conclusions

In healthy, non-atopic, nonsmoking subjects high doses of inhaled ipratropium bromide lead to marked bronchodilatation but do not affect the parasympathetic innervation of the heart. The topically acting parasympatholytic aerosol in high dosage causes a significant flattening of the circadian rhythm in airway calibre and, per analogiam, probably of the excessive rhythm amplitude in nocturnal asthma. In healthy subjects bronchial reactivity to histamine is essentially reduced by the blockade of the parasympathetic lung innervation; its persistent circadian rhythm, however, underlies other nervous and/or humoral factors.

References

déterminer le rôle de l’innervation parasympathique dans les rythmes circadiens des voies aériennes. La conductance spécifique et la réactivité bronchique ont montré clairement des variations circadiennes avec les acrophases analogues (16.11 h et 04.41 h, respectivement). Le blocage vagal des voies aériennes a augmenté sensiblement la conductance spécifique et a provoqué une réduction significative de l’amplitude de son rythme; en outre la corrélation significative entre la conductance spécifique et la fréquence cardiaque a été éliminée par l’aérosol parasympatholytique. D’autre part, la réactivité bronchique à l’histamine a été abaissée sans atténuation significative du rythme circadien. De ces résultats, il est conclu que l’efflux central parasympathique est un facteur essentiel dans le rythme circadien du tonus bronchique et par conséquent dans l’augmentation nocturne de la résistance bronchique.