The Daily Pattern of Heart Rate, Body Temperature, Locomotor Activity, and Autonomic Nervous Activity in Congenitally Bronchial-Hypersensitive (BHS) and Bronchial-Hyposensitive (BHR) Guinea Pigs

Megumi AKITA¹⁾, Masayoshi KUWAHARA¹⁾, Ryoji NISHIBATA²⁾,
Hiroki MIKAMI²⁾, and Hirokazu TSUBONE¹⁾

¹⁾Department of Comparative Pathophysiology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, and ²⁾Institute of Bio-Active Science, Nippon Zoki Pharmaceutical, Hyogo 673-1461, Japan

Abstract: We studied the characteristics of the rhythmicity of heart rate (HR), body temperature (BT), locomotor activity (LA) and autonomic nervous activity in bronchial-hypersensitive (BHS) and bronchial-hyposensitive (BHR) guinea pigs. For this purpose, HR, BT, LA, and electrocardiogram (ECG) were recorded from conscious and unrestrained guinea pigs using a telemetry system. Autonomic nervous activity was analyzed by power spectral analysis of heart rate variability. Nocturnal patterns, in which the values in the dark phase (20:00–06:00) were higher than those in the light phase (06:00–20:00), were observed in HR, BT and LA in both strains of guinea pigs. The autonomic nervous activity in BHS guinea pigs showed a daily pattern, although BHR guinea pigs did not show such a rhythmicity. The high frequency (HF) power in BHS guinea pigs was higher than that in BHR guinea pigs throughout the day. Moreover, the low frequency/high frequency (LF/HF) ratio in BHS guinea pigs was lower than that in BHR guinea pigs throughout the day. These results suggest that parasympathetic nervous activity may be predominant in BHS guinea pigs.

Key words: body temperature, circadian rhythm, heart rate variability, locomotor activity, radiotelemetry

Introduction

Asthma has been characterized by intermittent reversible airway obstruction, airway inflammation, and airway hyperresponsiveness. Asthma is also thought to

be associated with abnormal autonomic nervous function, because there is markedly increased bronchial sensitivity to cholinergic and non-adrenergic non-cholinergic constrictors, and decreased sensitivity to β_2 -adrenergic and non-adrenergic non-cholinergic dila-

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Address corresponding: M. Kuwahara, Department of Comparative Pathophysiology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

tors [3]. These mediators contribute to the pathogenesis of asthma, not only by regulating the smooth muscle tone in the airways, but also by affecting pulmonary blood flow, endothelial permeability and airway secretions. Although the alteration of autonomic nervous activity has been hypothesized to underlie the incidence of asthma, how this disease affects the cardiovascular autonomic nervous activity is poorly understood.

Bronchial-hypersensitive (BHS) and bronchial-hyposensitive (BHR) strain guinea pigs are spontaneous model animals of airway hyper- and hyposensitivity [11]. They have been developed by genetic selection from Hartley strain guinea pigs on the basis of measurement of the onset time to falling-down due to hypoxemia resulting from bronchoconstriction induced by inhalation of acetylcholine. It is known that BHS guinea pigs have significantly greater airway responses to leukotriene D₄, bradykinin, histamine, and acetylcholine compared with BHR guinea pigs [5, 8, 12, 17, 18]. These findings indicate that BHS and BHR guinea pigs are good congenital model animals for the investigation of airway hypersensitiveness and its related pathophysiology.

We considered that these animal models might provide new insight into the regulatory roles of autonomic nervous function in asthma. Moreover, chronotherapeutic strategies are important to the efficacious treatment of asthma. Therefore, it is useful to investigate the features of diurnal variation of cardiovascular and respiratory autonomic nervous regulation in these animal models. For this purpose, the circadian alteration of heart rate (HR), body temperature (BT), locomotor activity (LA), and autonomic nervous function in BHS and BHR guinea pigs was investigated using a telemetry system and power spectral analysis of heart rate variability (HRV).

Materials and Methods

This study was performed in accordance with the ethical guidelines, which meet the generally accepted international criteria, for animal care, handling, and euthanasia in our institution.

Animals and housing

Seven male BHS and 7 male BHR guinea pigs weighing 500–800 g were used. They were provided by the

Institute of Bio-Active Science, Nippon Zoki Pharmaceutical Co., Ltd. (Hyogo, Japan). They were housed in individual cages (width 40 cm, depth 25 cm and height 20 cm), floored with wood shavings, within a light-proof chamber (Sanyo, MIR-553 or MIR-252, Tokyo, Japan). In the chamber, a light-dark cycle (LD 14:10; lights-on at 6:00) was maintained under constant temperature ($24 \pm 1^\circ\text{C}$). Standard guinea pig pellets (RC4; Oriental Yeast Co., Tokyo, Japan) and water were supplied *ad libitum*.

Implanting the transmitter

A telemetric transmitter for electrocardiogram (ECG) and BT (TA10EA-F20, Data Sciences, St. Paul, Mn, USA) or ECG (TA10ETA-F20, Data Sciences, St. Paul, Mn, USA) was implanted under pentobarbital sodium anesthesia (40 mg/kg, i.p.). The paired wire electrodes in a precordial bipolar lead (Apex-Base lead) were placed at the cervical subcutaneous region over the trapezius, and the skin was closed by suture. This transmitter also detects LA with ECG and BT signals. All animals were used one week after the surgery.

HR, BT and LA recordings and analysis

The guinea pig housed cage was placed on a signal receiving board (CTR-86, Data Sciences, St Paul, MN, USA) in the chamber. The signals of HR, BT, and LA were continuously recorded every 5 min by a Data Quest analyzing system (Data Sciences, St-Paul, MN, USA) for 20–30 days. LA was measured as the number of line crossings during a 5-min period. The hourly values for each guinea pig in each period were taken as the average of 12 points of data and then are summarized for groups to get a mean $\pm$ SEM. The averages of these data, both in the light and dark phases, were compared.

ECG recordings and power spectral analysis

The ECG waveform from a signal receiving board (CTR-86, Data Sciences, St Paul, MN, USA) under the guinea pig housed cage was continuously recorded by an ECG processor (Softron, Tokyo, Japan) for 24 h to evaluate the daily variation of autonomic nervous activity. Power spectral analysis was performed on every one hour's ECG. Power spectral analysis was performed on an ECG processor analyzing system (Softron, Tokyo, Japan). The computer program first detected R

Fig. 1. Representative records of heart rate, body temperature, and locomotor activity in BHS (A) and BHR (B) guinea pigs. The circadian rhythms of these parameters show a nocturnal pattern especially in the hypersensitive guinea pig. The shadow indicates the dark period.

waves and calculated the RR interval tachogram as the raw HR variability in sequence. From this tachogram, data sets of 512 points were resampled at 100 msec. This length of the tachogram was selected as a best compromise between the need for a large time series, to achieve greater accuracy in the computation, and easiest for short periods [13]. Any RR intervals before and after artifacts were excluded from analysis. We then applied the Hamming window and the fast Fourier transform to each set of data to obtain the power spectrum of the fluctuation. Squared magnitudes and the products of the computed discrete Fourier transforms were averaged to obtain spectral estimates. We obtained hourly values of the low frequency (LF) power, the high frequency (HF) power, and the LF/HF ratio from recordings. We set LF at 0.07–0.7 Hz and HF at 0.7–3.0 Hz according to a previous study [1]. The data were summarized for groups to get a mean $\pm$ SEM.

The average of these data both in the light and dark phases were compared.

Statistical analysis

All values are expressed as mean $\pm$ SEM. Statistical analyses were made using Student's paired *t*-test or ANOVA to compare the values in the light and dark phases. Student's *t*-test was used to compare between groups.

Results

Typical recordings of HR, BT and LA in BHS (A) and BHR (B) guinea pigs are shown in Fig. 1. Nocturnal rhythms were clearly observed in both guinea pigs. The changes in the 24 h plot of the hourly HR, BT and LA are shown in Fig. 2. There was a clear peak after light-off in HR, BT and LA in BHS guinea pigs. How-

Fig. 2. Changes in hourly averaged values of heart rate, body temperature, and locomotor activity in BHS and BHR guinea pigs during 24 h. The shadow indicates the dark period. Values are mean $\pm$ SEM of 7 guinea pigs.

ever, no clear peak was observed in BT in BHR guinea pigs. HR, BT and LA in the light and dark phases are summarized in Fig. 3. The values of HR, BT and LA in the dark phase (20:00–06:00) were higher than those in the light phase (06:00–20:00) in both guinea pigs. The values of HR in the dark phase in BHS guinea pigs were significantly higher than those in BHR guinea pigs. The values of BT in the light phase in BHS guinea pigs were significantly lower than those in BHR guinea pigs. While the values of LA in BHS guinea pigs were significantly lower than those in BHR guinea pigs throughout the day.

The changes in the 24 h plot of the hourly LF, HF, and LF/HF ratio are shown in Fig. 4. Moreover, LF, HF and LF/HF ratio in the light and dark phases are summarized in Fig. 5. The HF, LF, and the LF/HF ratio in BHR guinea pigs showed no daily pattern. However, the HF and LF in the light phase in BHS guinea pigs were significantly higher than those in the dark phase. Furthermore, the HF in BHS guinea pigs was higher than that in BHR guinea pigs throughout the day. While the LF/HF ratio in BHS guinea pigs was lower than that in BHR guinea pigs throughout the day.

Discussion

This study has demonstrated the rhythmicity of HR, BT, LA and autonomic nervous activity in BHS and

Fig. 3. Light and dark phase values of heart rate (HR), body temperature (BT), and locomotor activity (LA) in BHS and BHR guinea pigs. Values are mean $\pm$ SEM of 7 guinea pigs. *: significant difference between light and dark phase, #: significant difference between BHS and BHR guinea pigs.

Fig. 4. Changes in 24 h plots of LF, HF, and LF/HF ratio in power spectral analysis of heart rate variability in BHS and BHR guinea pigs. The shadow indicates the dark period. Values are mean $\pm$ SEM of 7 guinea pigs. LF=low frequency, HF=high frequency.

BHR guinea pigs. Nocturnal rhythms were observed in HR, BT and LA in both strains of guinea pigs. The autonomic nervous activity in BHS guinea pigs showed a daily pattern, although BHR guinea pigs did not show such a rhythmicity. Moreover, the HF in BHS guinea pigs was higher than that in BHR guinea pigs throughout the day. These results suggest that the parasympathetic nervous activity may be predominant in BHS guinea pigs.

The power spectral analysis of HRV is a noninvasive tool for quantifying the relative amount of sympathetic and vagal activity distributed to the heart. Recently, we showed that the power spectrum of HRV in guinea pigs resembled the power spectra derived from humans and other animal species [1]. Moreover, there are no clear diurnal variations in LF, HF, and LF/HF ratio in normal Hartley guinea pigs. Therefore, we have suggested that there is no diurnal variation in the autonomic nervous activity in guinea pigs. However, in this study we observed diurnal variations in LF and HF in BHS guinea pigs, although this tendency was very weak in BHR guinea pigs. There is no obvious explanation for these discrepancies. One possible explanation is that BHS and BHR guinea pigs become much more homogenous than normal Hartley guinea pigs because BHS and BHR guinea pigs have been genetically selected from Hartley strain guinea pigs. This possibility is supported by the fact that in spite of normal Hartley

Fig. 5. Light and dark phase values of LF, HF, and LF/HF ratio in power spectral analysis of heart rate variability in BHS and BHR guinea pigs. Values are mean $\pm$ SEM of 7 guinea pigs. *: significant difference between light and dark phase, #: significant difference between BHS and BHR guinea pigs.

guinea pigs having different individual characteristics of rhythmicity in HR, BT and LA, as reported previously [2], nocturnal rhythms in HR, BT and LA were clearly observed in all BHS and BHR guinea pigs. Moreover, BT and LA in BHS guinea pigs were lower than those in BHR guinea pigs throughout the day. This difference might be dependent on autonomic nervous function of these animals, because HRV data suggested the dominance of parasympathetic nervous activity in BHS guinea pigs.

HRV is physiologically influenced by respiratory signals such as respiratory rate, tidal volume, and minute ventilation. Hayano *et al.* [7] reported that increase in the respiratory interval increased the HF amplitude. We observed that respiratory rate of BHS guinea pigs was faster than that of BHR guinea pigs (data not shown). However, the HF in BHS guinea pigs was higher than that in BHR guinea pigs throughout the day. Therefore, we consider that the difference in the HF between BHS and BHR guinea pigs is associated with the autonomic nervous activities.

In human asthmatic patients, dysautonomia has been well documented [9, 15]. Because the rate of discharge of the sinoatrial node is under vagal control, the possibility exists that an alteration in autonomic nervous control in airway caliber may be reflected by a parallel change in control of the HR [10]. Moreover, blood pressure responses mediated by sympathetic nervous system may reflect the altered sympathetic airway tone. Some investigators have suggested that increased parasympathetic activity might be associated with asthma and allergies using HRV analysis data [6, 16]. If so, alteration in the resting HR towards bradycardia should exist in asthmatic patients. However, both increased and decreased resting HR has been found in the asthmatic patients [4, 14]. Our results of the analysis of HRV also suggest that parasympathetic nervous activity may be predominant in BHS guinea pigs. This result is reasonable, because BHS and BHR guinea pigs have been selected by airway responsiveness to acetylcholine [11]. However, HR in BHS guinea pigs was not lower than that in BHR guinea pigs. We have also suggested that HR fluctuation in normal guinea pigs is largely dependent on LA. However, this possibility is not likely to explain the difference in HR between BHS and BHR guinea pigs, because the values of LA in BHS guinea pigs were significantly lower than those in

BHR guinea pigs. Therefore, further studies will be needed to clarify the mechanism in the control of resting HR in asthmatic patients.

In conclusion, we assessed the diurnal variations of HR, BT, LA and autonomic nervous function in BHS and BHR guinea pigs. Nocturnal rhythms were observed in HR, BT and LA in both guinea pigs. The cardiorespiratory system in BHS guinea pigs was regulated predominantly by the parasympathetic nervous system. These results suggest that BHS and BHR guinea pigs may be useful animal models for future studies on the chronobiology of asthma.

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