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Hashimoto, Akinori
Maeda, Hitoshi
Yokoyama, Mitsuhiro

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AUGMENTATION OF PARASYMPATHETIC NERVE FUNCTION IN PATIENTS WITH EXTRINSIC BRONCHIAL ASTHMA
- EVALUATION BY COEFFICIENCY OF VARIANCE OF R-R INTERVAL WITH MODIFIED LONG-TERM ECG MONITORING SYSTEM -

Akinori HASHIMOTO, Hitoshi MAEDA, and Mitsuhiro YOKOYAMA

First Division, Department of Internal Medicine,
Kobe Unibersity School of Medicine

INDEXING WORDS

parasympathetic nerve activity; nocturnal asthma; coefficiency of variance of R-R interval ($cv_{R\text{-}R}$); long-term ECG monitoring system

SYNOPSIS

Patients with bronchial asthma (BA) have evidence of increased vagal cholinergic tone. Although the cardiovascular-respiratory tests used in the past might have been biased by concomitant chest disease, coefficiencies of variance of the R wave to R wave (RR) interval ($CV_{R\text{-}R}$) on electrocardiogram (ECG) have been applied to many diseases as a method of functional evaluation of the parasympathetic nerve system. In 24 hour recordings of ECG (Holter ECG), continuous values of $CV_{R\text{-}R}$ have been determined for each one-minute period with a recently developed long-term ECG monitoring system. To examine functional augmentation of parasympathetic nerve in patients with BA, we applied this system in the present study to determine the alteration of parasympathetic nerve function during an inhaled methacholine provocation test in 14 patients and 7 healthy subjects. $CV_{R\text{-}R}$ values were calculated from the collection of a 24-hour electrocardiogram. Bronchial response, which was measured by methacholine inhalation, was evaluated with the baseline value of respiratory resistance (Rrs-cont), the cumulative dose producing a 35% decrease in respiratory conductance (PD35Grs) and the minimun cumulative dose required to start to decrease respiratory conductance from baseline (Dmin). The serum concentrations of epinephrine and norepinephrine were measured at the basal and double-increased levels of respiratory resistance during the inhalation test.

The increased values of $CV_{R\text{-}R}$ in asthmatic patients were intimately associated with

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Authors' names in Japanese: 橋本彰則, 前田　均, 横山光宏

augmented respiratory resistance. The disparity of CV_{R-R} values (ΔCV) during the resistance levels from basal to double in the asthmatic group was significantly greater than that in the control group. The absolute change of CV_{R-R} values in the asthmatic group was significantly larger than that in the control group. There was a significant correlation between ΔCV and Dmin ($r=0.536$, $p<0.05$), and between ΔCV and PD35Grs ($r=0.552$, $p<0.05$). The serum concentration of epinephrine and norepinephrine were not significantly different during the methacholine inhalation provocation test. We conclude that alteration of the values of CV_{R-R}, which were calculated with a modified long-term ECG recording system, may reflect the change of parasympathetic nerve function and the severity of bronchial hyperreactivity, and this system can be a useful non-invasive method for evaluation of increased vagal tone in patients with bronchial asthma.

INTRODUCTION

Bronchial asthma is a disease characterized by bronchial hyperreactivity (BHR) to various stumuli and by the reversibility of airway narrowing that occurs spontaneously or as a result of therapy.[1] Factors such as epithelial damage,[2,3] allergic inflammation,[4,5] and abnormal function of bronchial smooth muscle[6] can contribute to the physiopathology of BHR in bronchial asthma.

The alteration of autonomic nerve balance plays another important role in the physiopathology of bronchial asthma.[7,8,9] Although an in-vitro functional abnormality in the parasympathetic nerve system has been shown in bronchial asthma,[10] the in-vivo definitive direct variables, with which the alteration of autonomic nerve function is evaluated, have not yet been clarified. The measurement of beat-to-beat variations of heart rate has been used as one of the indirect methods for assessment of parasympathetic nerve activity in allergic disease,[11] as have the methods of eccrine sweat-gland response[12] and pupillary constrictor responses[13] to cholinergic agonists. Recently, spectral analysis of heart rate variability has been applied to evaluate the physiopathology of bronchial asthma.[14]

A long-term ECG monitoring (Holter ECG) system has been developed for determining arrhythmia and ischemic response in patients with cardiac diseases. With this system, abnormal rhythms and ischemic responses are detected with good reproducibility, in the daily life of patients. Holter ECG has recently been modified to enable the calculation of the coefficiency of variance of the ECG R wave to R wave (R-R) interval (CV_{R-R}) from the beat-to-beat variation of heart rate, with an automatic analyzer[15] which calculates CV_{R-R} from the heart rate each minute of a 24-hour period.

To elucidate the contribution of parasympathetic nerve function to the physiopathology of bronchial asthma, we investigated the alteration of parasympathetic nerve activity in patients with extrinsic bronchial asthma using a modified Holter ECG

system during an inhaled methacholine provocation test. This provocation test is a reliable measurement of the severity of bronchial hyperreactivity. We used methacholine as the inhaler provocation material, because it has a low incidence of cough during tests compared with histamine and acetylcholine. Thereafter, we applied this system to the patients with bronchial asthma whether or not they showed augmented vagal cholinergic function in asthma attacks.

MATERIALS AND METHODS

Subjects

The subjects in the present study were selected from our out-patient clinic at Kobe University Hospital. We studied 14 patients with extrinsic bronchial asthma (seven males, seven females; mean [± SD] age, 37.4 ± 12.3 years old) and seven normal subjects (four males and three females; 26.8 ± 1.5 years old). None had had an acute exacerbation of asthma for at least four weeks. Extrinsic-type asthma was defined by increased Ig E or possessing a positive radioallergosolvent test for mites and/or house dust. Informed consent was obtained from each patient for the study protocol, and the protocol was approved by our Institutional Ehical Bord.

Methacholine inhalation test

The methacholine inhalation test was performed with an Astograph system (TCK-6000M, Chest Co., Tokyo, Japan),[16] which can write dose-response curves of respiratory resistance (Rrs) with tidal breathing during continuous inhalation of methacholine in two fold incremental concentrations from 0.049 to 50 mg/ml for each one-minute period. In this study, we adopted a method using continuous tidal breathing because it is difficult to evaluate beat-to-beat variations of heart rate during the provocation test with the conventional standardized methods with tidal volume breathing in a body box and with maximal forced expiratory flow volume curves. Three indices were evaluated for BHR: the baseline value of the resistance of the respiratory system (Rrs-cont), the cumulative dose producing a 35% decrease in respiratory conductance (PD35Grs), and the minimum cumulative dose required to start to decrease respiratory conductance from the baseline (Dmin). Bronchodilators and xanthines which were not long-acting agents were withdrawn from the patients at least 12 hours before they were examined.

Long-term (Holter) ECG monitoring system

The Holter ECG monitoring system was mechanically modified to simultaneously

record both respiratory pattern and conventional electrocardiogram by a recording component, SM 28® (Fukuda Electronic Co., Tokyo, Japan), and an analyzing component, SCM 270® (Fukuda). The average R-R interval (msec), standard deviation of R-R interval (SD. msec) and coefficiencies of variance of R-R intervals ($CV_{R\text{-}R}$, SD/Average R-R interval × 100, expressed as percentage) were automatically calculated from continuously recorded electrocardiograms. $CV_{R\text{-}R}$ values were calculated at each one-minute interval and adopted for the index of variance of heat rate.

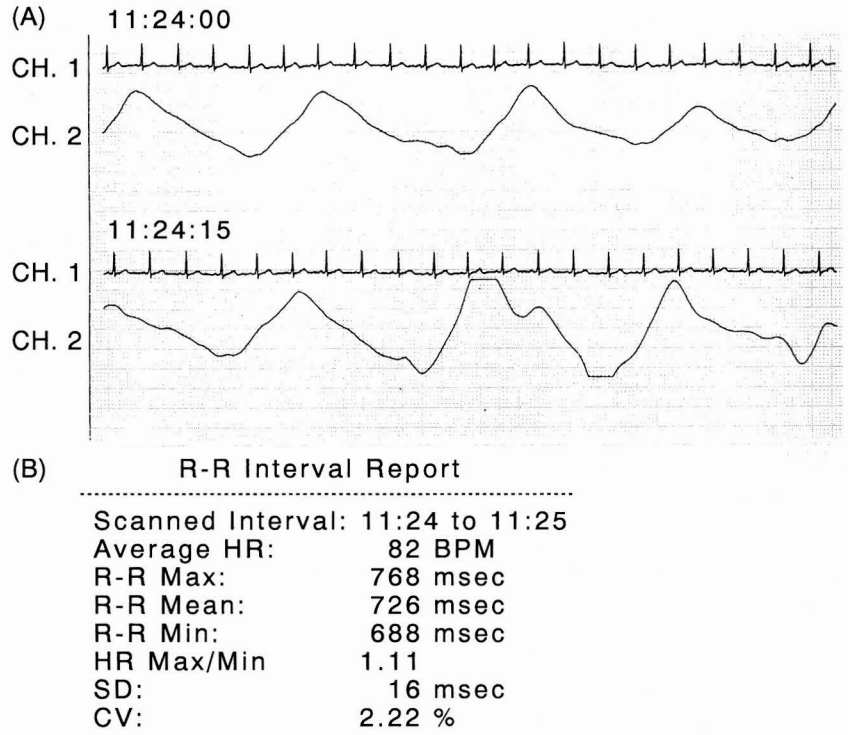

Fig. 1. Sample ECG recording sheet. Example of recording (A). ECG recording and respiratory pattern are shown at channels 1 (CH 1) and 2 (CH 2), respectively. CV values were calculated from the measurements of R-R intervals for one minute from the Holter ECG. All R-R intervals for one minute were measured and analyzed, and given in the summary sheet of R-R intervals (B). Abbreviations; R-R interval=The interval from R wave to R wave on electrocardiogram; BPM=Beats per minute; SD=Standard deviation; CV=Coefficiency of variance of RR interval on electrocardiogram.

Recording of Holter ECG and measurement of $CV_{R\text{-}R}$

The Holter ECG recording was performed first with the patients in various positions, including recumbent resting position, sitting and standing resting condition, and then was applied during the methacholine inhalation test. One week later, Holter ECG recordings during another methacholine inhalation test were performed after two puffs (40μg) inhalation of ipratropium bromide, in order to evaluate the mechanism of changes of $CV_{R\text{-}R}$ values during methacholine provocation tests. One overnight Holter ECG recording was performed on all asthmatic subjects for analyzing the alteration of parasympathetic nerve function in the physiopathology of nocturnal asthma. The variance of heart rate recorded by the Holter ECG monitoring system was analyzed with the SM28® and SCM270® components.

Measurements of serum concentrations of epinephrine and norepinephrine

To evaluate the alteration of autonomic nerve balance, serum concentrations of epinephrine and norepinephrine were determined 10 minutes before the inhaled provocation test and at the time when the patient's Rrs level reached a twice that of his/her basal Rrs level. Seven BA patients were randomly selected for this evaluation. Catecholamines were measured by high-pressure liquid chromatography.

Statistical analyses

Results are presented as mean values $\pm$ SD. Statistical analyses of Dmin and PD35Grs values were performed on logarithmically transformed data, because they showed logarighmically normal distribution. Statistical evaluations were performed by student's t test for paired and unpaired data. A p-value less than 0.05 was considered significant.

RESULTS

A sample recording sheet of electrocardiogram and respiratory pattern is shown in Figure 1(A), from 11:24:00 to 11:24:30. One ECG channel showed electrocardiogram and another showed respiratory pattern. The average heart rates per minute, maximum R-R interval, mean R-R interval and minimum R-R interval were analyzed, and standard deviation (SD) and coefficiency of variance (CV) were calculated (Fig. 1(B)).

Figure 2 demonstrates the traces of alteration in resistance of respiratory system (Rrs) and of CV values during the methacholine inhalation provocation test in a BA patient (upper left panel) and a healthy subject (upper right panel). Typical traces of the response curve of CV values and Rrs before (lower left panel) and after (lower right

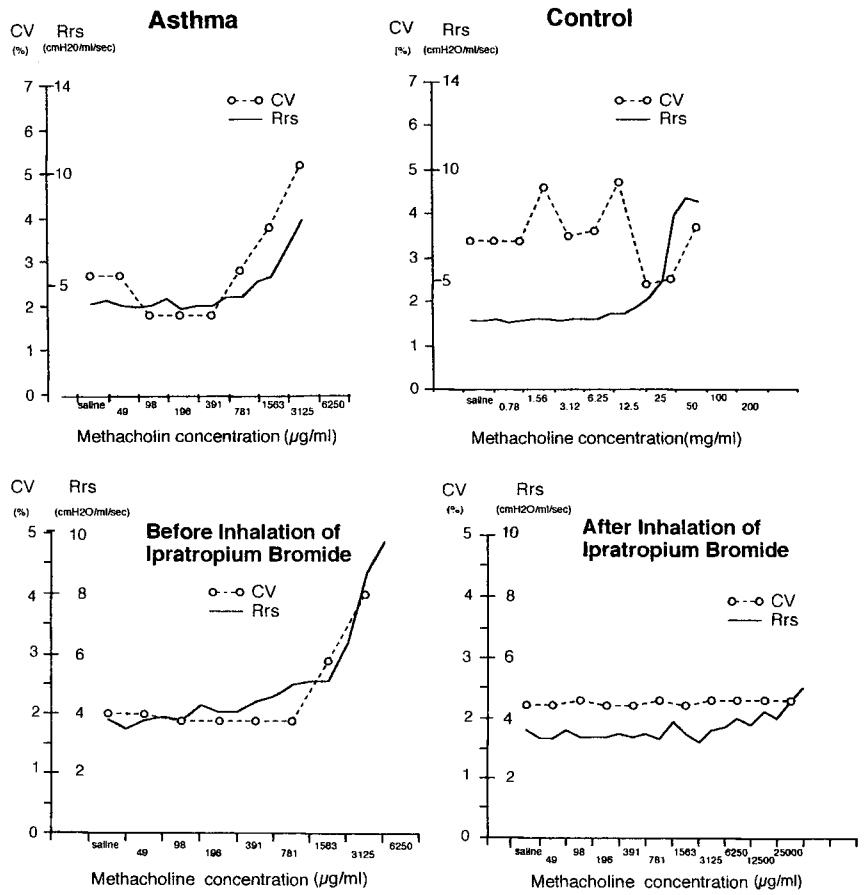

Fig. 2. Typical traces of alteration in CV values and respiratory resistance (Rrs) during the inhaled methacholine provocation test in an extrinsic BA patient (upper left) and a healthy subject (upper right). The methacholine concentration of the upslope point of Rrs was markedly different between a BA patient (781μg/ml) and a healthy subject (25mg/ml). Both the traces of CV values and Rrs elevated according to the increased concentration of inhaled methacholine in the BA patients. The alteration of CV and Rrs values during the inhaled methacholine provocation test before (lower left) and after (lower right) two puffs (40μg) of inhalation of ipratropium bromide in a BA patient. No fluctuation of CV and Rrs values after inhalation of ipratropium bromide in the BA patients was demonstrated. Abbreviations; CV=Coefficiency of variance of RR interval on electrocardiogram; Rrs=resistance of respiratory system.

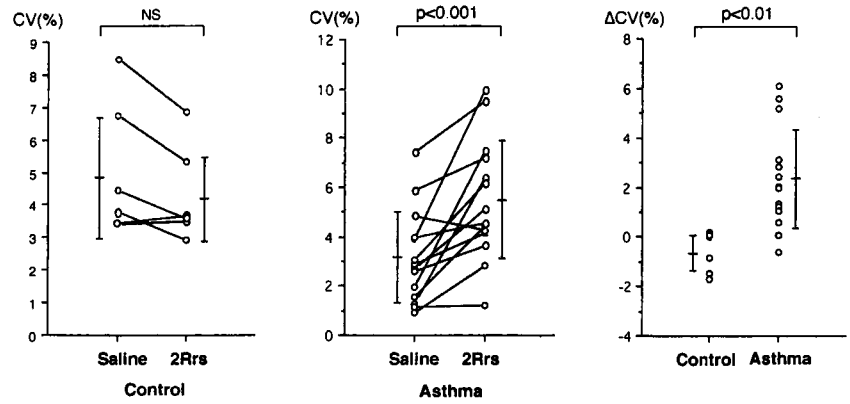

Fig. 3. The change of CV values during the methacholine inhaled provocation test: Absolute change of CV values during the methacholine provocation test in a healthy subject (left) and BA patient (middle). On the right, the percentage changes of $CV_{R\text{-}R}$ from basal level to the point of twice-increased respirarory resistance (2 Rrs) in the healthy subjects and in the BA patients are shown. The patients with BA showed absolute and relative increased values of $CV_{R\text{-}R}$ compared to that of the healthy subjects. Abbreviations; $CV_{R\text{-}R}$=Coefficiency of variance of R-R interval on electrocardiogram; 2 Rrs=Twice-elevated values of resistance of respiratory system from basal level during inhaled methacholine provocation test.

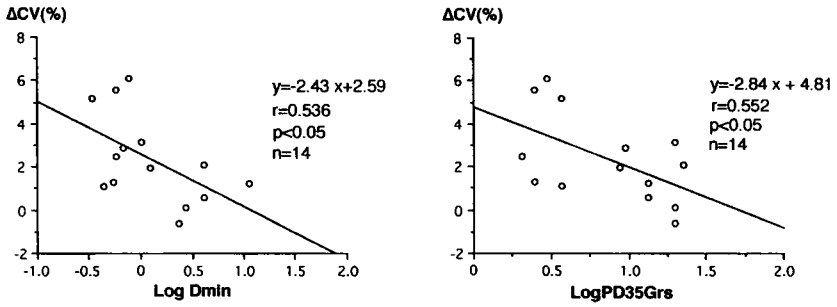

Fig. 4. The relationship between the parameters of bronchial reactivity and responsibility and the changes of CV values from basal level to twice-increase of respiratory resistance. CV values-Log Dmin (left) and CV values-Log PD35Grs (right). The intimate correlations between CV values and the severity of bronchial reactivity and between CV values and the severity of bronchial responsibility are exhibited. Abbreviations; CV=Coefficiency of variance of RR interval on electrocardiogram; Dmin=The minimum cumulative dose required to start to decrease respiratory conductance from baseline; PD35Grs=The cumulative dose producing a 35% decrease in respiratory conductance.

panel) inhalation of ipratropium bromide (40μg) are shown. The CV values and Rrs curve were not changed even at higher concentrations of methacholine after ipratropium bromide inhalation in the patients with BA.

$CV_{R\text{-}R}$ values in the asthmatic group (BA group) and in the normal healthy group (Control group) are shown in Fig. 3. We arbitrarily determined the endpoint of the inhaled methacholine test at the point of twice-values of increased Rrs from basal level during saline inhalation (2 Rrs). The mean CV value in the control group was not significantly different between a saline inhalation and a methacholine inhalation (4.8 $\pm$ 1.9%, 4.2 $\pm$ 1.3%, NS, respectively). The mean CV values in the BA group were, however, significantly changed from 3.2 $\pm$ 1.8% at the point of saline inhalation to 5.5 $\pm$ 2.4% at the point of 2 Rrs during the methacholine inhalation ($p<0.001$). The comparison of percentage changes in $CV_{R\text{-}R}$ from basal level to 2 Rrs revealed a significant difference between the control group and the BA group (-0.7 $\pm$ 0.7%, 2.4 $\pm$ 2.0%, respectively, $p<0.01$). The concentration of catecholamines in both epinephrins and norepinephrins was not significantly different at the point of basal level and 2 Rrs during the inhaled methacholine provocation test in the BA group.

There were significant relationships between the percentage changes of CV values from basal level to 2 Rrs and log Dmin ($r=0.536$, $p<0.05$), and between the percnetage changes of CV values from basal level to 2 Rrs and log PD35Grs ($r=0.552$, $p<0.05$) (Fig. 4). Therefore, the more increased values in the change of CV from basal level to 2 Rrs, the more augmented state in the bronchial reactivity and the bronchial responsibility.

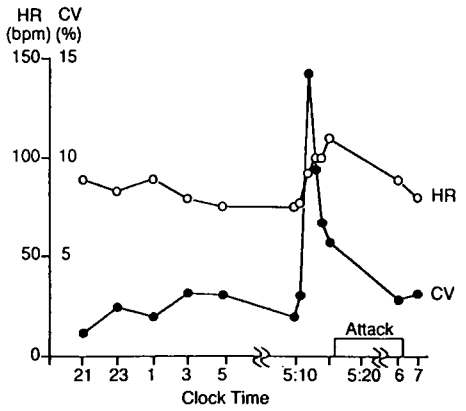

Fig. 5. Time course of CV values and heart rate during a nocturnal attack of asthma in a BA patient (58-year-old, female). CV values were abruptly increased just before the attack, and recovered to the baseline after the cessation of the attack. Abbreviations; HR=Heart rate; CV=Coefficiency of variance of RR interval on electrocardiogram; BPM=Beats per minute.

The chart of CV values and heart rate in a typical asthma attack of a BA patient is shown in Fig. 5. The CV curves in the patient with nocturnal asthma exhibited the abrupt increase just before her asthma attack and gradually decreased in CV values and returned to basal level after the cessation of the asthma attack.

DISCUSSION

There has been much debate over the importance of vagal mechanisms in the pathophysiology of bronchial asthma, and over the several mechanisms that might lead to increased cholinergic activity in asthma:[17] (i) reflex bronchoconstriction due to the stimulation of sensory receptors in the airway (irritant receptors and C-fiber endings) by inflammatory mediators such as histamine, prostaglandins and bradykinins; (ii) increased acetylcholine release due to enhanced neurotransmission in cholinergic ganglia, perhaps because of the release of other neurotransmitters or mediators, or facilitation of acetylcholine release from postganglionic nerve terminals; (iii) an increased effect of cholinergic stimulation of airway smooth muscle, due to an increase in muscarinic receptors or their affinity, or an increased efficacy of coupling of these receptors; (iv) decreased activity of acetylcholinesterase after re-sensitization from allergens.[18]

In the present study, we showed increased vagal tone with the analysis of $CV_{R\text{-}R}$ values. Abnormal control of heart rate in autonomic nerve dysfunction has also been shown in patients with diabetes mellitus[19] and allergic diseases.[20] The measurement of RR interval variance has been widely used as an excellent non-invasive method for evaluation of parasympathetic nerve activity.[15,20] Recently developed electro-mechanic techniques have introduced the revolutionary modification of the measurement of RR interval variations from a conventional ECG recording system to the Holter ECG recording system, which is now usually used for the detection of arrhythmia and ischemic responses. A few problems were encountered in the present study when examining the coefficiency of variance of RR interval ($CV_{R\text{-}R}$) with the Holter ECG system, because the tape of the Holter ECG recording system is elastic and the rotary motion of recording tape is not always completely constant, as in a magnetic tape recording system. Musha et al.[15] achieved good reproducibility in determining the $CV_{R\text{-}R}$ values with both the Holter ECG system and the conventional method. We also confirmed a good relationship in $CV_{R\text{-}R}$ values between the Holter ECG system and the conventional Automonic R-100 (M.E. Commercial Co., Tokyo, Japan) in our preliminary study. We could easily obtain $CV_{R\text{-}R}$ values for each one-minute period for whole day with the Holter ECG system, but not with the Autonomic R-100. The modified Holter ECG system was useful for estimating the parasysmpathetic activity of the BA patients in detail during the methacholine inhaltion test and during the nocturnal

asthma attack.

The $CV_{R\text{-}R}$ values in our asthmatics gradually increased in proportion to the rise of respiratory resistance during the methacholine inhalation test, without the alteration of respiratory pattern or of the level in serum cathecholamine. Therefore, the changes of $CV_{R\text{-}R}$ values observed during the methacholine inhalation test might reflect accentuated states of parasympathetic nerve activity in asthmatics, and the augmentation of vagal tone in bronchial asthma was thus also confirmed with the present modified Holter ECG system.

The mechanisms of increased $CV_{R\text{-}R}$ values during the methacholine inhalation test might be as follows: (i) the altered reactivity of airway neural reflex in BA patients; (ii) the direct action of methacholine that delivered to the heart through penetration of the inhaled substances. Neither an increase of $CV_{R\text{-}R}$ values nor an increase of respiratory resistance in proportion to the accumulation of methacholine was shown in the re-examination by a second methacholine inhalation test after the pretreatment of ipratropium bromide. Therefore, the alteration of $CV_{R\text{-}R}$ values during the methacholine inhalation test may not result from the direct effects of methacholine action to heart rate variation through the influence of penetrated methacholine to parasympathetic nerves, because the concentration of ipratropium bromide administered in the present study, 40μg, could completely block the parasympathetic afferent nerve function in the airways.[21] We speculate that the neural reflex is augmented in the patients with bronchial asthma, not the direct contractile action to airway smooth muscle with penetrated methacholine.

The different responses of bronchial smooth muscle to methacholine in the in-vitro and in-vivo systems indicate that autonomic nerves play an important role in the mechanism of bronchial hyperreactivity which characterizes BA.[10] In the presnt study, we observed a strong relationship between Dmin and the disparity of $CV_{R\text{-}R}$ values from basal level to 2 Rrs during the methacholine provocation test; therefore, cholinergic nerves in-vivo participated in the control of bronchial hyperreactivity. However, the detailed mechanisms of parasympathetic nerve function in bronchial hyperreactivity were not elucidated by the present findings.

Augmentation of vagal tone in asthma attacks was shown in the present study. The mechanism of nocturnal asthma is not completerly understood,[18] but recent studies have shown that the alterations of cholinergic and non-adrenergic and non-cholinergic (NANC) nerve functions at night, in combination with airway inflammation, are major factors in the pathogenesis of nocturnal airway narrowing.[22,23] A correlation between increased nocturnal vagal activity and decreased peak expiratory flow rate in asthma has been demonstrated.[24,25] Catterral et al.[26] reported that ipratropium bromide inhalation at 2 AM improved the fall in peak expiratory flow rate overnight, and they concluded that nocturnal bronchoconstriction was partially due to an increase in airway cholinergic

activity at night. In our present study, increased $CV_{R\text{-}R}$ values were observed just ahead of the attack in nocturnal asthma, and therefore our result was compatible with the results on cholinergic hyperreactivity in nocturnal asthma. We suggest that cholinergic hyperreactivity also plays an important role in nocturnal asthma, especially in the triggering of an asthma attack. Several subtypes of muscarinic receptor have been identified in the airway, and both atropine and ipratropium have been known to be non-selective anticholinergic drugs blocking prejunctional (M2) and postjunctional (M3) receptors with the same affinity.[27] More selective anticholinergic agents would be useful drugs for preventing asthma attacks.

In summary, we found that increased parasympathetic nearve activity may play an important role in the pathophysiology of bronchial hyperreactivity in adult bronchial asthmatics with our use of a modified long-term ECG monitoring system. This monitoring system was a noninvasive method for evaluation of parasympathetic nerve activity and made it possible to examine the contribution of parasympathetic nerve function to bronchial hyperreactivity and to the trigger of an attack in nocturnal asthma. We conclude that this modified Holter ECG monitoring system will continue to be a very important and valuable method for the evaluation of parasympathetic nerve activity in the determination of the physiopathology of bronchial asthma. Therefore, we propose that patients with morning dipping could be easily detected by the non-invasive modified Holter ECG system, and the system would be quite helpful for the improvement of their quality of life by revealing their morning dipping for proper treatment and management.

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