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Chronobiology in cardiology

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Summary. - In these years, circadian periodicity in the onset of myocardial ischemia, myocardial infarction, sudden cardiac death and ischemic stroke has been confirmed by several investigators. There has also been reported that there exists a significant circadian rhythm in ventricular arrhythmias in patients with myocardial infarction. These recent advances in chronocardiology depend on a development of a high quality built-in A/D converter in ambulatory ECG monitoring system and a remarkably developed sophisticated software. One of the most current topics in recent years is heart rate (HR) variability. As an index of HR variability, RR50 is frequently estimated by the cosine fitting technique. Next, we analyzed the so-called Lorenz plot for another index of HR variability, that is preceding R-R intervals and coupling intervals were plotted sequentially every 3 hours on the abscissa and ordinate, respectively. Finally, we investigated HR variability by power spectral analysis of R-R intervals both by the maximum entropy method and the fast Fourier transform. Mainly, in this paper, we introduced current topics in HR variability, but recent advances in ambulatory blood pressure (BP) monitoring system are also strikely remarkable. Our newly developed monitoring system of physical activity donated quite a few informations evaluating episodic changes of HR and BP. This device was also useful for finding out the difference between the individual life styles, such as the eveningness versus morningness. In conclusion, these very recent advances in ambulatory monitoring system should be really available for the clinical practice from this time onward.

Key words: blood pressure, chronobiology, chronocardiology, circadian rhythms, coronary ischemic disease, heart rate variability.

Riassunto *(La cronobiologia in cardiologia)*. - In questi anni diversi ricercatori hanno fornito la dimostrazione che esiste un comportamento periodico nell'arco delle 24 ore a riguardo degli episodi di ischemia ed infarto miocardico, morte cardiaca improvvisa e ictus ischemico. È stato anche riportato che esiste una personalità circadiana per le crisi di aritmia ventricolare nei pazienti con infarto miocardico. Queste recenti conoscenze di cronocardiologia dipendono dallo sviluppo di convertitori analogico/digitali di alta qualità, inseriti nei sistemi di monitoraggio ambulatorio elettrocardiografico e di software notevolmente sofisticato. Uno degli argomenti oggetto di studio di questi anni è la variabilità della frequenza cardiaca (FC). Come indice della variabilità FC è frequentemente usato il parametro R-R50 approssimato tramite la funzione coseno. Come ulteriore indice di variabilità FC, di recente, abbiamo applicato i cosiddetti diagrammi di Lorenz in cui venivano graficati ogni tre ore sequenzialmente gli intervalli R-R precedenti agli intervalli R-R copula, rispettivamente nella ascissa e nella ordinata. Infine abbiamo effettuato l'analisi spettrale degli intervalli R-R con il metodo della massima antropia e con la trasformata rapida di Fourier. In generale, in questo lavoro, noi introduciamo gli aspetti correlati della variabilità FC insieme ai più recenti progressi nel monitoraggio ambulatorio della pressione arteriosa (PA). Il sistema di monitoraggio dell'attività motoria consente informazioni sui bruschi cambiamenti di FC e di PA, nonché sullo stile di vita di tipo mattutino o serotino. La conclusione è che i progressi nei sistemi ambulatoriali di monitoraggio dovrebbero essere realmente disponibili da ora in avanti nella pratica clinica cardiologica.

Parole chiave: cardiopatia ischemica, cronobiologia, cronocardiologia, pressione arteriosa, ritmi circadiani, variabilità della frequenza cardiaca.

Introduction

Circadian periodicity has been investigated in recent years in many cardiovascular indices such as heart rate (HR), blood pressure (BP) and some kinds of pathologic phenomena including episodic myocardial ischemia, myocardial infarction and sudden cardiac death. In the last few years, HR variability has also attracted increasing interest as a noninvasive index of vagal activity. Several studies [1, 2] have shown that signs of reduced vagal activity are associated with an enhanced risk of sudden cardiac death. Markedly decreased left ventricular ejection fraction and nonsustained ventricular tachycardia episodes have been known as an independent predictor of sudden cardiac death. In recent years, some investigators have expected such decreased HR variability as the third possible predictor of sudden cardiac death in patients with a variety of diseased state.

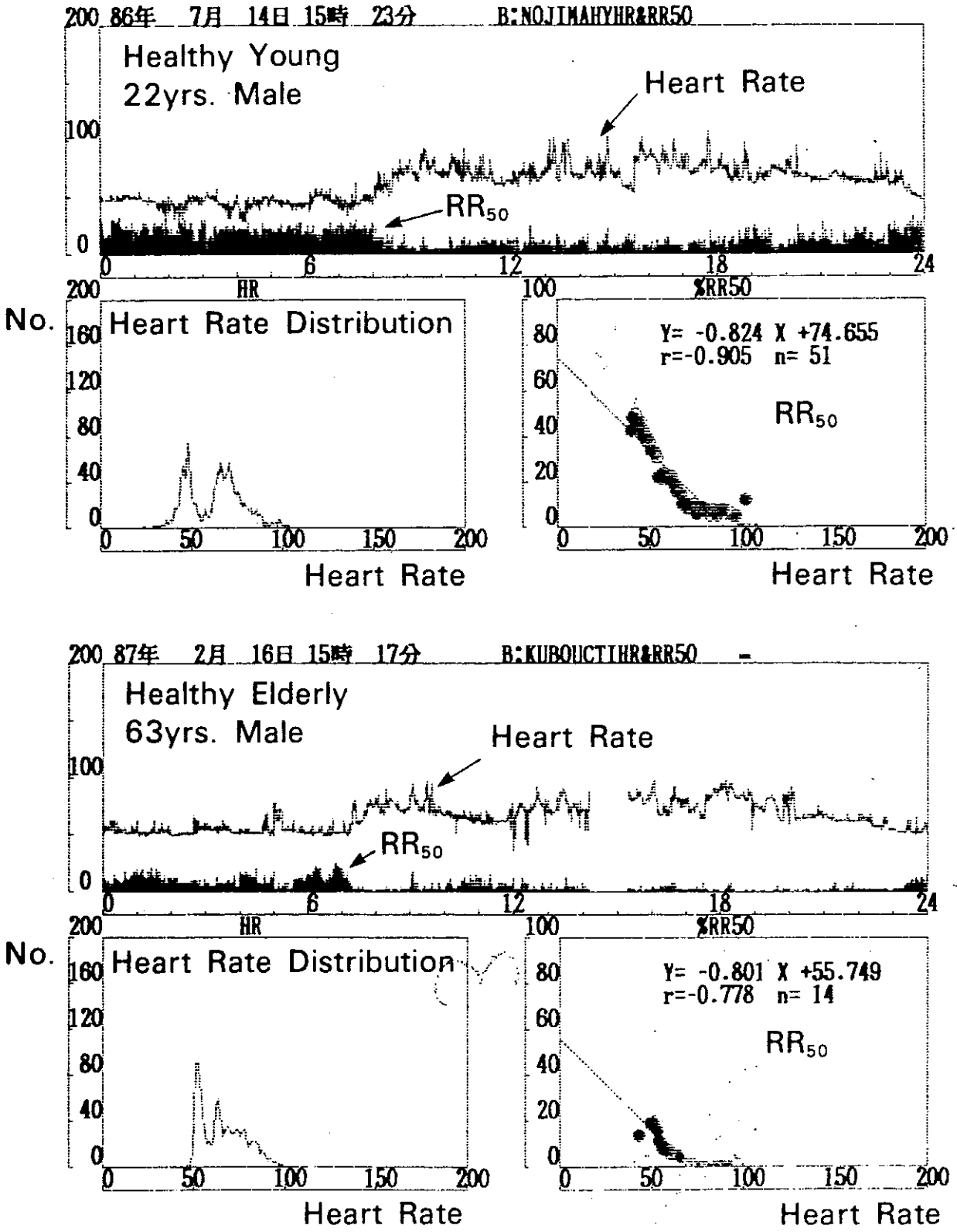

Fig. 1. - Example of RR50 analyses of healthy young (upper) and healthy elderly (lower). In each panel heart rate (HR) trendgram, RR50 trendgram (upper), a nonsequential HR distribution (lowere left) and the slope between HR and RR50 relationship along with RR50 index, which is shown as "n" (lower right), are depicted.

It is also noteworthy to be mentioned that a sufficiently reliable, small and lightweight, and nearly silent, hence less sleep-disturbing, device for ambulatory BP monitoring system has been developed a few years ago [3, 4]. As a result, it became not difficult to observe a circadian profile of BP.

As a background of these remarkable developments, there observed quite a few investigations in cardiology from an aspect of chronobiology.

Methods for clinical chronocardiology

It has been established in experimental studies that there exists an endogenous circadian rhythm in HR. Our previous investigation [5] has also demonstrated that not only HR but also bradyarrhythmia episodes show an endogenous circadian periodicity in rats using records over 7 days of ambulatory electrocardiogram (ECG) and electroencephalogram. However, in clinical practice only 24 h recording is usual. Is it sufficient to analyze the circadian periodicity? It has been known that maximum entropy method (MEM) has the remarkable resolution property and the superiority over the other spectral methods such as the fast Fourier transform (FFT), in particular for short data length. However, even the MEM power spectrum resolves a sharp peak, suggesting a circadian periodicity only when the recording span is longer than 48 h, as shown our previous report [6]. Therefore, the longer the recording span, the sharper becomes the peak. Then we concluded that at least a 48 h record was needed to analyze the circadian periodicity in clinical practice, even if the MEM analysis was applied.

It has been well known that FFT has an advantage in analyzing the power of the periodicity. However, we have already observed that when the MEM spectral peak is sharp, the power of the MEM spectrum is well correlated with the one of the FFT. In clinical practice, cosine fitting technique by least squares is frequently used. Cosine fitting technique has some advantages in contrast to the spectral analysis. A time series only 24 h long is required for the fit of a 24 h cosine. In addition, characteristics of the circadian rhythm, such as acrophase, amplitude and MESOR (midline estimating statistic of rhythm), are able to be quantified. After the fitting of the 24 h cosine function is performed individually (the so-called single cosinor), a population circadian rhythm may be obtained (the so-called population-mean cosinor) as previously reported by Halberg *et al.* [7].

Circadian rhythm of heart rate variability and aging

One of the recent advances in ambulatory ECG monitoring system is a development of a high quality built-in A/D converter. Therefore, there is no need now to insert an A/D converting system to an ambulatory ECG analysing system. In addition, in recent years, sophisticated software permits an analysis of all R-R intervals from a 24 h ambulatory ECG and it can compensate for such confounding factors as ectopic beats and abrupt changes in QRS complexes amplitude along with postural changes. As an index of HR variability, RR50 [8] is frequently used in clinical practice. Subtracting the times between successive normal beats allowed the calculation of normal-normal R-R intervals, and subtraction of each interval from its predecessor allowed the change in interval to be determined. Each change in interval was then compared with 50 ms. If an increase or decrease > 50 ms occurred, a positive or negative count was added to the total being accumulated for the minute. This count per minute was defined as RR50. Circadian periodicity was analyzed by the cosine fitting technique, and RR50-mesor was calculated. Next, correlation between RR50 and HR was analyzed. When there were more than 2 readings of RR50 for the respective HR, average of the readings of RR50 was calculated and it was used as the representative RR50, which was defined as %RR50.

Count of the %RR50 more than 5 during a 24 h day was defined as RR50 index [9]. The slope of the correlation equation between HR and %RR50 was also calculated. Not only RR50 but also standard deviation (SD) and coefficient of variance (CV) of normal QRS R-R intervals were calculated every minute. SD-mesor and CV-mesor were analyzed by the cosine fitting technique.

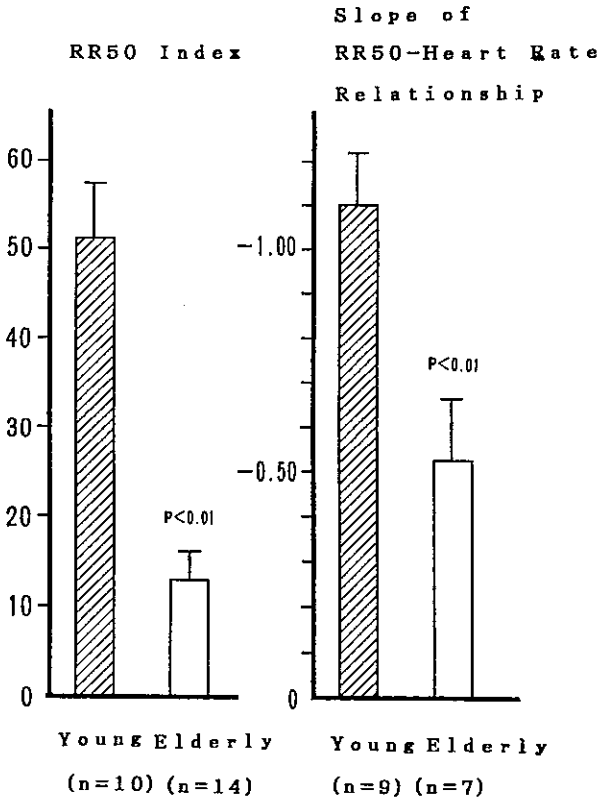

Fig. 2. - Comparison of RR50 index and the slope of RR50-HR relationship between the healthy young and elderly. (Mean and standard deviation (SD).

Fig. 1 shows examples of the RR50 analysis of healthy young (upper) and elderly (lower), which shows age related alterations of RR50, RR50 index and the slope between RR50 and HR relationship. Both RR50 index and the slope were significantly smaller in healthy elderly, as shown in Fig. 2 ($p < 0.01$). SD-mesor, CV-mesor and RR50-mesor were also significantly smaller in healthy elderly ($p < 0.01$, Fig. 3). Please note a remarkable decrease in RR50 in a case of recent myocardial infarction (Fig. 4), which shows that RR50 index is zero. Population cosinor analysis shows the 95% range of acrophase of SD, CV and RR50 (Fig. 5). Any indices of HR variability show that the acrophase locates at night or in the early morning.

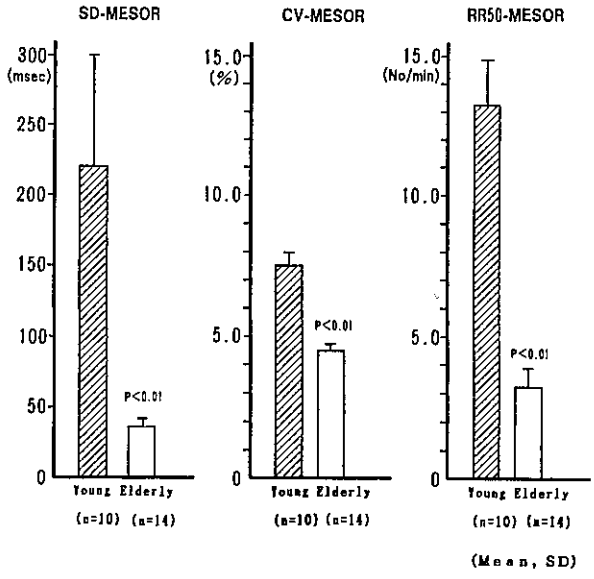

Fig. 3. - Comparison of SD-MESOR, CV-MESOR and RR50-MESOR between the healthy young and elderly.

We tried an application of Lorenz plot for the evaluation of HR variability [10]. Time-series data of R-R intervals was plotted as follows: preceding R-R intervals (RR_n) and coupling R-R intervals (RR_{n+1}) were plotted sequentially on the abscissa and ordinate, respectively. An example of the Lorenz plot is shown in Fig. 6. The plotted points are scattered as an oval form on and around the function $y=x$, depending on the degree of their variance. We analyzed the Lorenz plot every 3 h, and the minor axis (width;W) of the oval was measured. As a result, the width of the oval (W) of the Lorenz plot showed a definite circadian periodicity in the young subjects group (10 men aged from 22-29 years). The fitted cosine function of "W" in the young group was as follows: $y=190(ms)+45(ms)\cos(\omega\ t-0.99\ (radian))$ ($p < 0.001$), whereas the cosine function of "W" in the elderly group was as follows: $y=118(ms)+24(ms)\cos(\omega\ t-0.21\ ((radian))$ ($p=0.13$). Thus, not only the circadian average but also the circadian amplitude of "W" was significantly higher in the young than

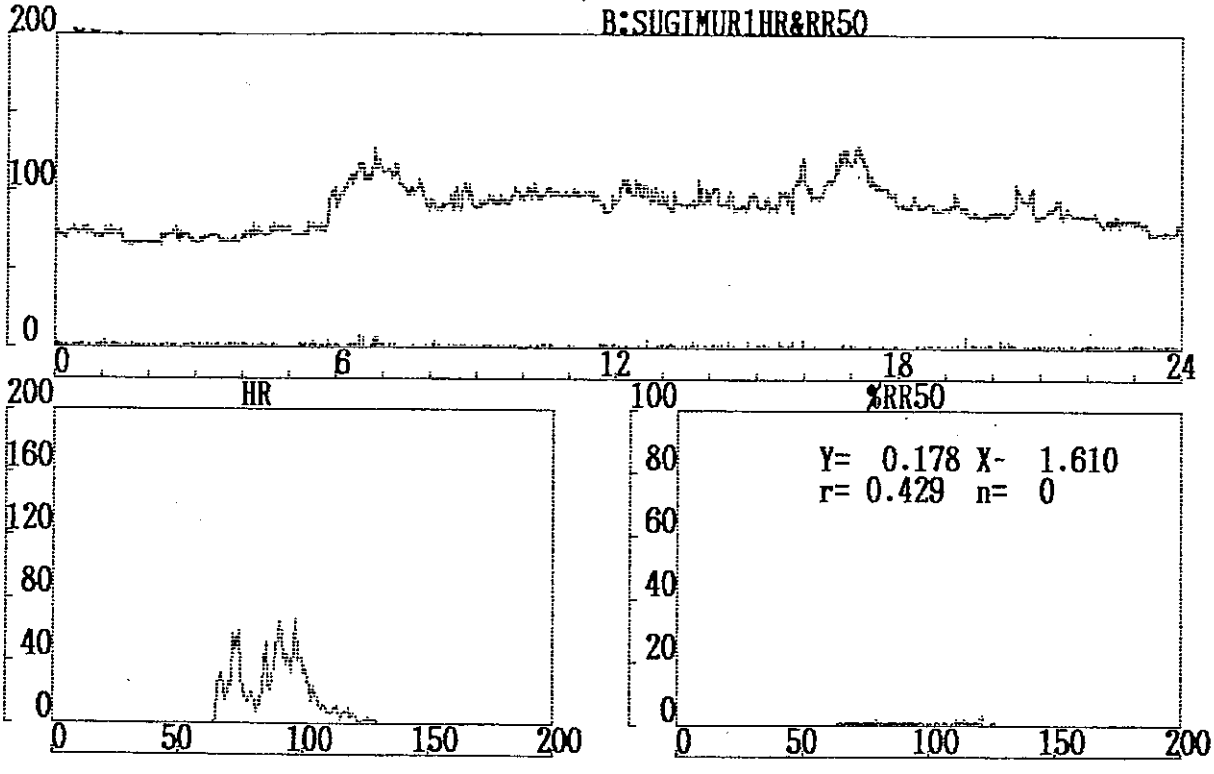

Fig. 4. - Example of an RR50 analysis in a patient with old myocardial infarction. Please note remarkable decrease in RR50.

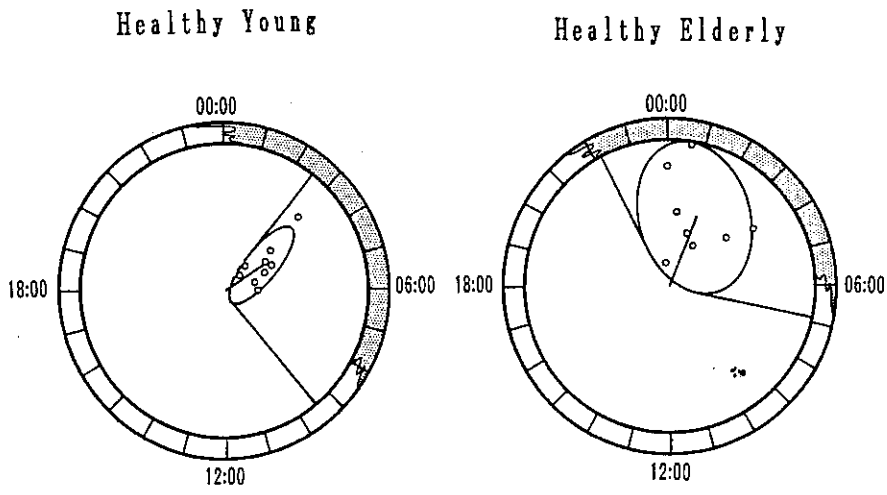

Fig. 5. - Comparison of the population-mean cosinor analysis of RR50 between the healthy young and elderly.

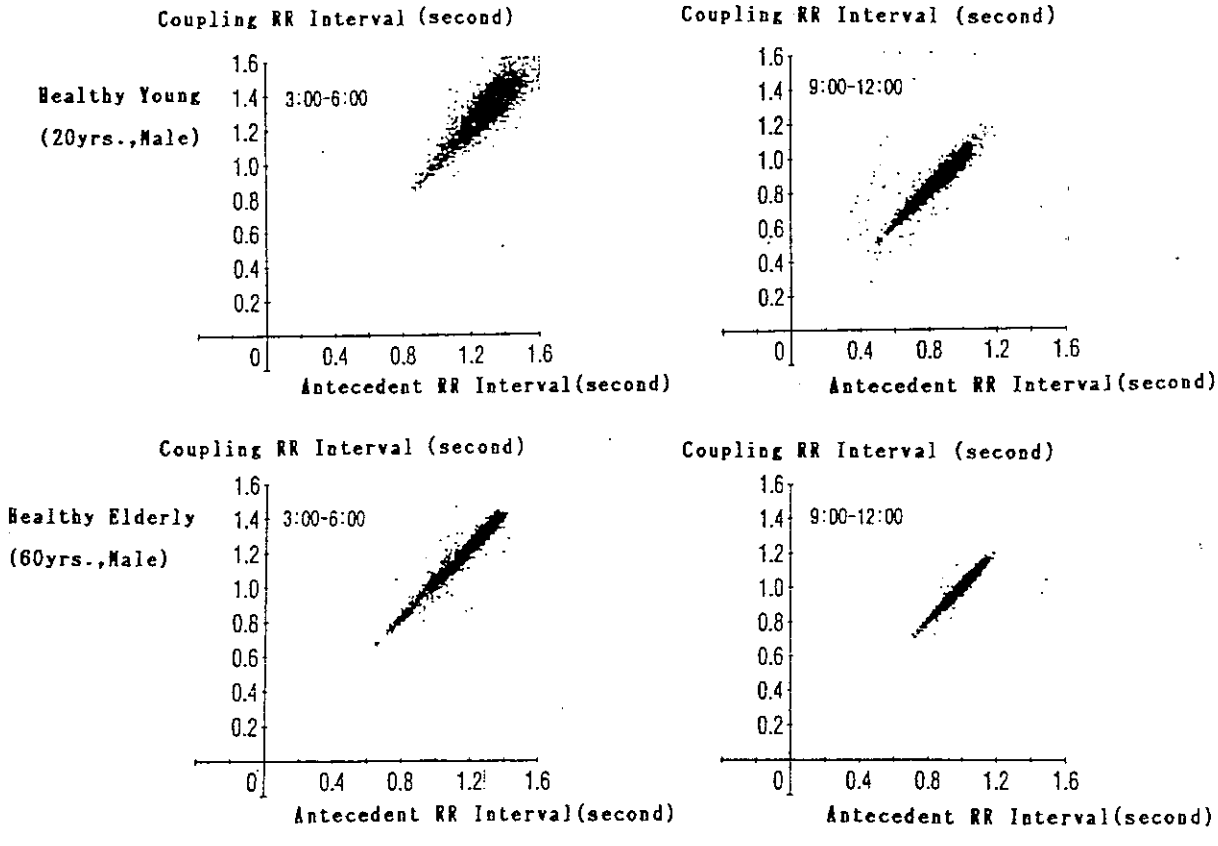

Fig. 6. - Example of the Lorenz plot of R-R intervals. Comparison of the healthy young (upper) and elderly (lower) is shown. Left panels show the plots between 03:00-06:00 and right panels show the ones between 09:00-12:00.

the elderly (Fig. 7). The Lorenz plot has been known as one of the mathematical methods for the analysis of "chaos", that is complex periodicity in random signals. Recently, use of the Lorenz plot was advocated to analyze ECG rhythm [11]. Our previous investigation showed that the "W" closely correlated with RR50. The "W" during sleeping span also correlated CVRR and a phase 2 baroreflex sensitivity index, investigated by Valsalva maneuver. The "W" during an awakening span correlated with plasma renin activity at rest. These results may suggest that "W" reflects the regional autonomic functions. Therefore, we speculated that the Lorenz plot of R-R intervals offered a new insight into the autonomic cardiovascular functions.

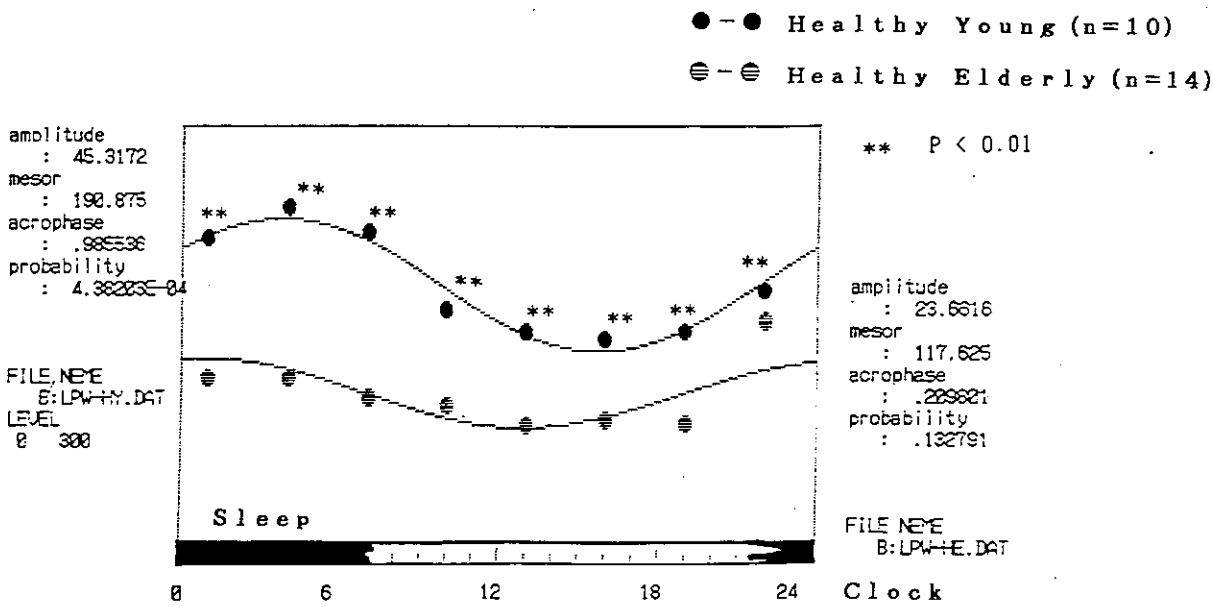

Fig. 7. - Circadian periodicity of "width" of the Lorenz plot. Please note the difference between the healthy young and elderly.

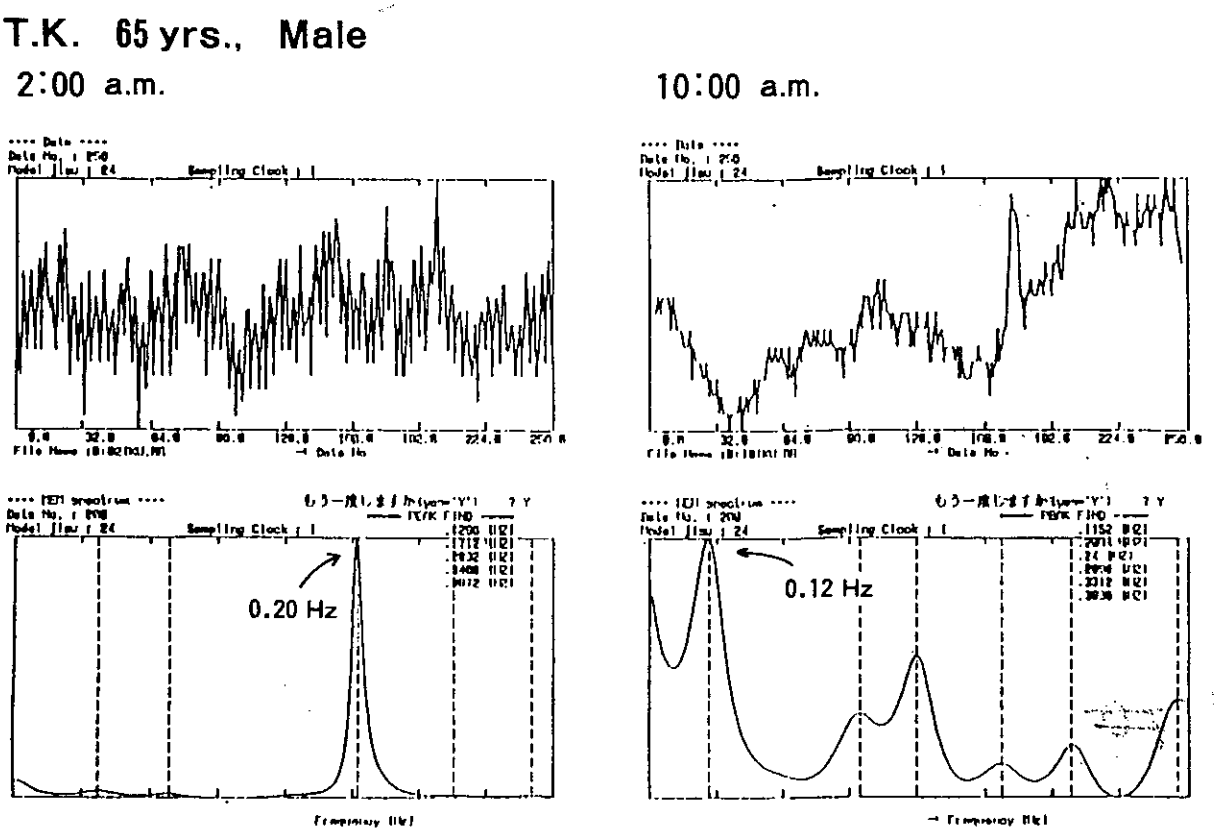

Fig. 8. - Example of power spectral analysis of HR variability by the maximum entropy method (MEM). Left panels show a sequential record of RR intervals around 02:00 (upper) and its MEM spectrum (lower). Right panels show the one around 10:00 (upper) and its MEM spectrum (lower).

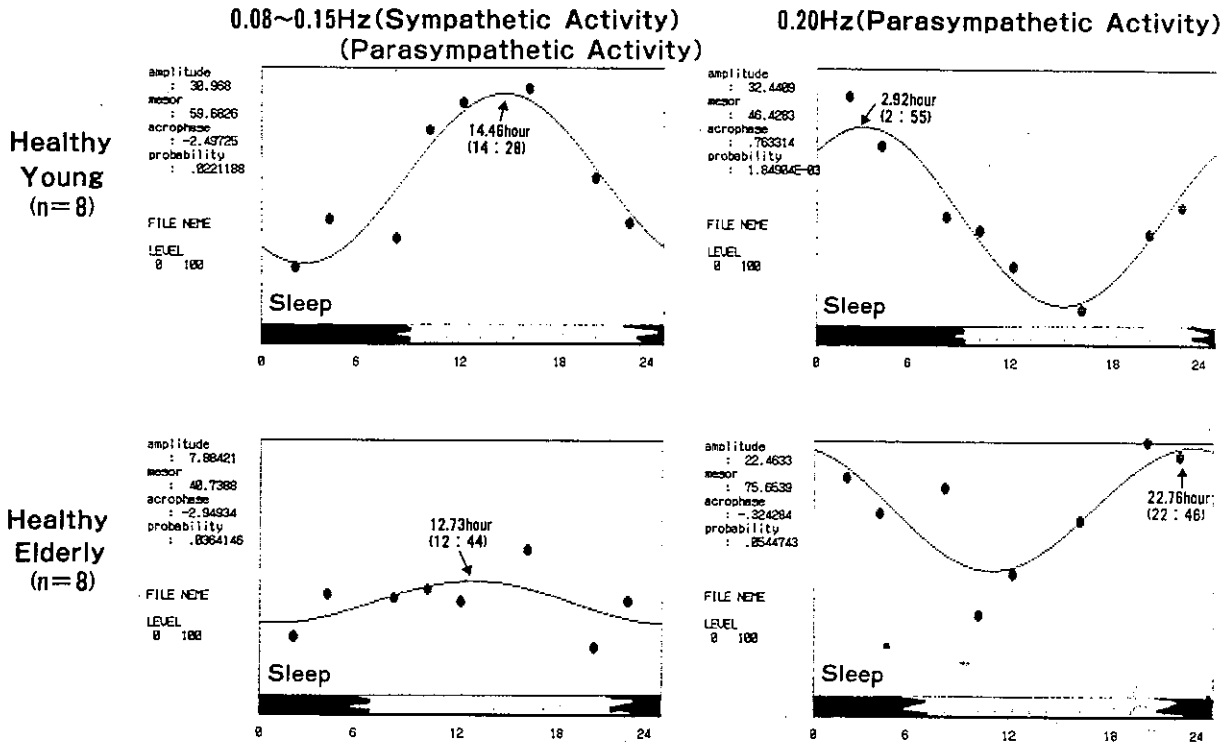

Fig. 9. - Comparison of the circadian rhythm of the lower frequency spectra (left) and the higher frequency spectra (right) between the healthy young (upper) and elderly (lower).

Recently, autonomic cardiovascular functions were frequently evaluated by power spectral analysis of HR variability. It has been known that HR power spectral density in humans contains two major components, reflecting respiratory sinus arrhythmia (around 0.25 Hz) and Mayer-wave-like sinus arrhythmia (around 0.10 Hz).The amplitude of these two components reflects quantitative and specific informations of vagal cardiac function and β-adrenergic function together with vagal modulation, respectively. There are two ways to analyze the power spectral analysis of HR variability. One of them is the MEM analysis. The principle of this method and its advantages over spectral analysis by FFT have been reported in previous studies [12]. An example is shown in Fig. 8 and the circadian variation of the two components is demonstrated in Fig. 9. The lower frequency spectra show a circadian variation with an acrophase in the afternoon (at 14:28 and at 12:44, young and elderly), and the higher frequency spectra show a circadian variation with an acrophase at night (at 02:55 and at 22:46, young and elderly). Another way of power spectral analysis of HR variability is FFT. R-R intervals were arranged equidistantly in series by Newton's first-degree interpolation method. Instantaneous HR was checked every 300 ms. Mean HR was substructured, and a Hanning window was applied to the time-series data of HR. The sequential series of HR had about a 5 min span, which meant a 1024-point spectrum and was processed by the FFT algorithm. This FFT analysis can show the frequency between the lowest 0.00326 Hz, and the criticals, approximately 0.8 Hz. An example is shown in Fig. 10.

In addition, we computed the slope of the regression line, called a $1/f^x$ fluctuation, between the logarithm of frequency and the logarithm of power density of the HR FFT spectrum. This slope was computed as an absolute value of the exponent reading of abscissa on the $1/f^x$ plot. An example

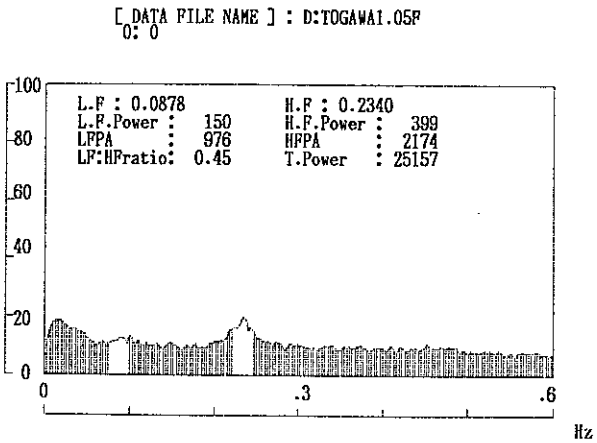

Fig. 10. - Example of the fast Fourier transform (FFT) analysis of HR variability.

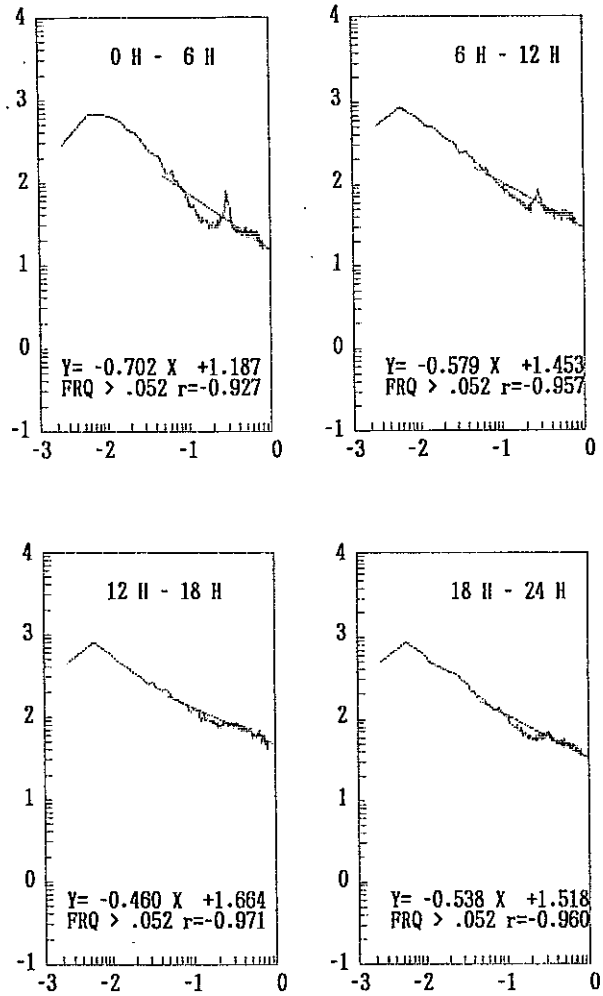

Fig. 11. - Example of the circadian variation of the $1/f^{x}$ fluctuations of HR variability. The slope of the regression line called a $1/f^{x}$ fluctuation, between the logarithm of frequency and the logarithm of power density of HR FFT spectra, is shown every 6 h.

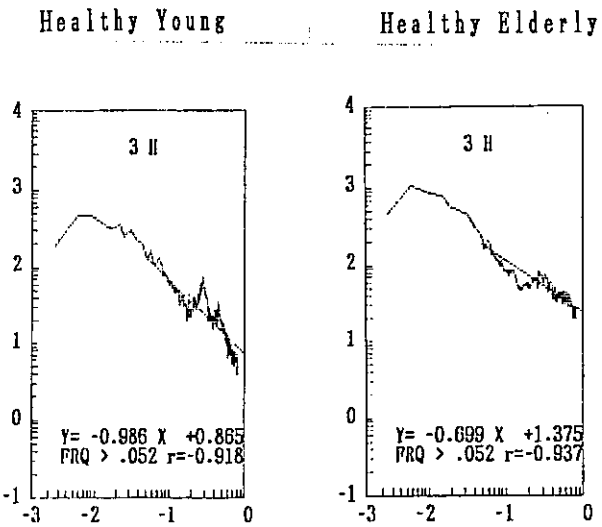

Fig. 12. - Comparison of a $1/f^{x}$ fluctuation of HR variability between the healthy young and elderly.

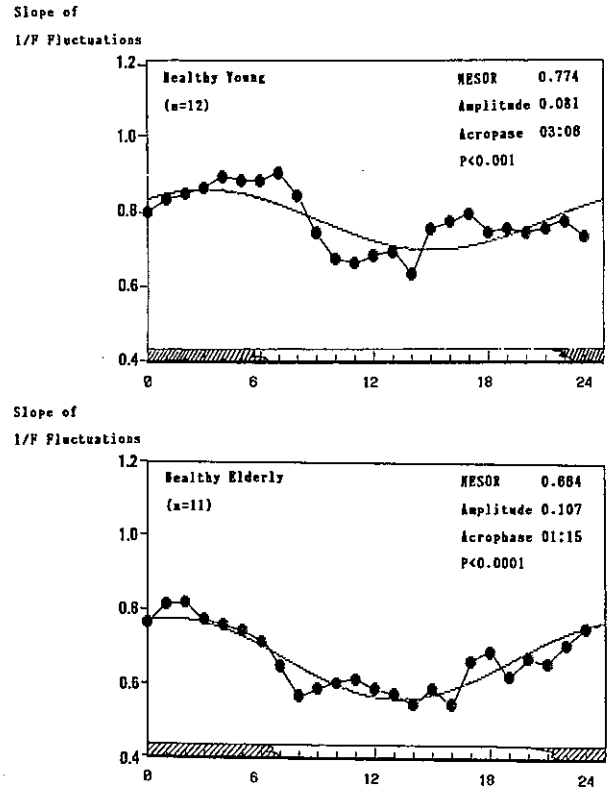

Fig. 13. - Comparison of the circadian rhythm of the $1/f^{x}$ fluctuations of HR variability between the healthy young and elderly.

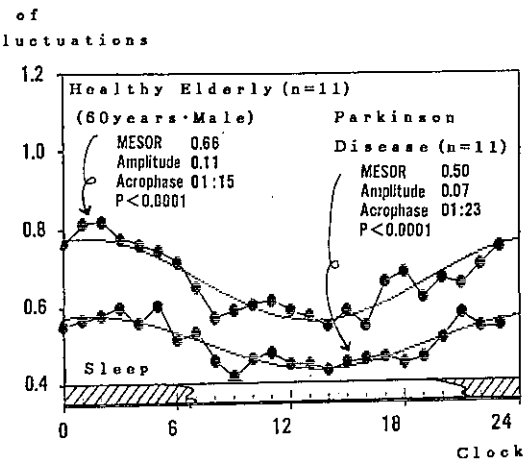

Fig. 14. - Comparison of the circadian rhythm of the $1/f^{x}$ fluctuations of HR variability between the healthy elderly and patients with Parkinson's disease.

of the circadian variation of the $1/f^{x}$ plot every 6 hs is shown in Fig. 11, and an effect of aging of the slope analyzed every hour is shown in Fig. 12. Both healthy young volunteers (12 men aged from 20 to 28 years) and elderly volunteers (11 men aged from 61 to 66 years) showed evident circadian rhythmicity as shown in Fig. 13. The fitted cosine

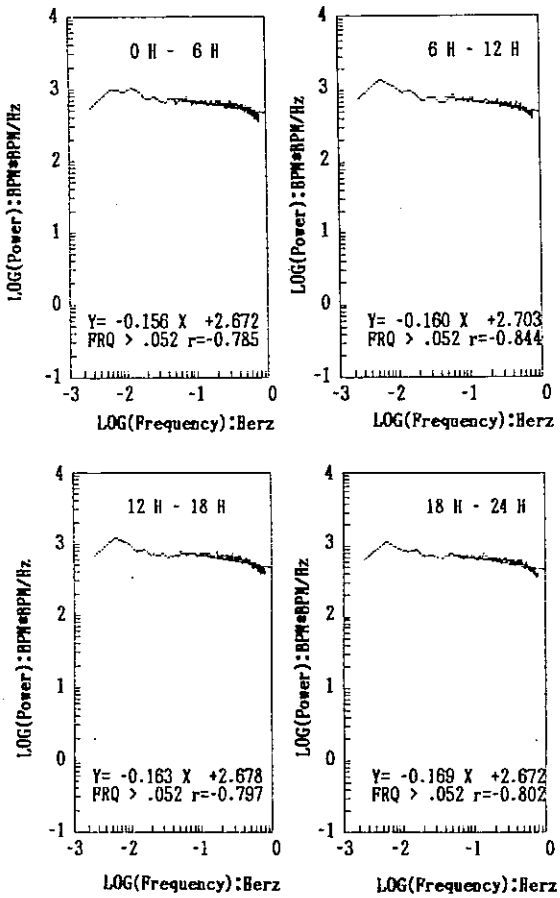

Fig. 15. - Disappearance in the circadian rhythmicity of the $1/f^x$ fluctuations of HR variability in a patient with atrial fibrillation.

function of the slope in the young group shows, MESOR, 0.774; amplitude, 0.081 and acrophase, 03:06. The one in the elderly group shows, MESOR; 0.664, amplitude; 0.107 and acrophase; 01:15. The slope of the $1/f^x$ plot in patients with Parkinson's disease also showed a significant circadian periodicity, but it was significantly smaller than the one in the elderly group, as shown in Fig. 14. Additionally, please not, as regards patients with atrial fibrillation the slope is close to "white", as shown in Fig. 15.

Circadian rhythm of cardiac arrhythmias and antiarrhythmic agents

Quite a few investigations as regards a circadian variation of cardiac arrhythmias have been done these few years. However, it still remains controversial whether the occurrence of ventricular arrhythmias exhibits a circadian periodicity under actual life. Many investigators have emphasized a decrease in both the frequency and the complexity of ventricular arrhythmias during the night, although other authors have demonstrated a nocturnal increase in the frequency of ventricular arrhythmias in patients with specific heart disease. It has also been observed that the frequency and the complexity shows a great deal of day-to-day variation. Recently several studies in patients with coronary heart disease have shown a definite circadian periodicity in ventricular arrhythmias. Lucente *et al.* [13] showed significant circadian rhythm of ventricular arrhythmias in 94 patients with recent myocardial infarction. A significant circadian rhythm of ventricular tachycardia was also found in the total population with acrophase at 14:29. These findings are not incompatible with a preliminarly report by Lown *et al.* [14], showing that incidence and grade of ventricular arrhythmias were reduced during the sleep span.

Irwin *et al.* [15] demonstrated a clear circadian rhythm in the occurrence of symptomatic paroxysmal supraventricular tachycardia in untreated patients. The peak incidence of tachycardia was found at 16:00. As an explanation for the circadian rhythm, they speculated the participation of the fluctuations in autonomic tone. Initiating a supraventricular tachycardia is critically dependent on atrioventricular nodal conduction time, which is modulated by the autonomic nervous system. As to circadian rhythm in bradyarrhythmia episodes, there is no big controversy; it appears usually during sleep [16].

In recent years, several studies have shown that signs of reduced parasympathetic activity were associated with an enhanced risk of sudden cardiac death. Some other studies, as a representative Cast [17], showed that antiarrhythmic agents did not necessarily result in a good goal in patients with acute myocardial infarction. Therefore, we investigated the effect of some kinds of antiarrhythmic agents on the HR variability. Fig. 16 shows an example of RR50 analysis before and after the treatment of aprindine. Before treatment RR50 was very small and RR50 index was zero (Fig. 16, upper panel), but after the treatment not only circadian variation of RR50 but also RR50 index increased (Fig. 16, lower panel). Effect of some kinds of antiarrhythmic agents on HR variability analyzed by RR50 is summarized in Fig. 17. Aprindine alone increased HR variability, propafenone decreased it and mexiletine and SUN-1165 showed no changes. Such notable advantage of aprindine was confirmed again by Lorenz plot. The "W" of the plot depicted in the 1st quadrant, increased after aprindine administration (Fig. 18). Circadian variation of the "W" before and after the treatment also confirmed the increase in HR variability throughout a 24 h day, as shown in Fig. 19. Coupling intervals, which were analyzed by the Lorenz plot and were shown in the 4th quadrant, were prolonged after the aprindine administration (Fig. 20). These results seem to show an implication how we should use some kinds of antiarrhythmic agents in clinical practice.

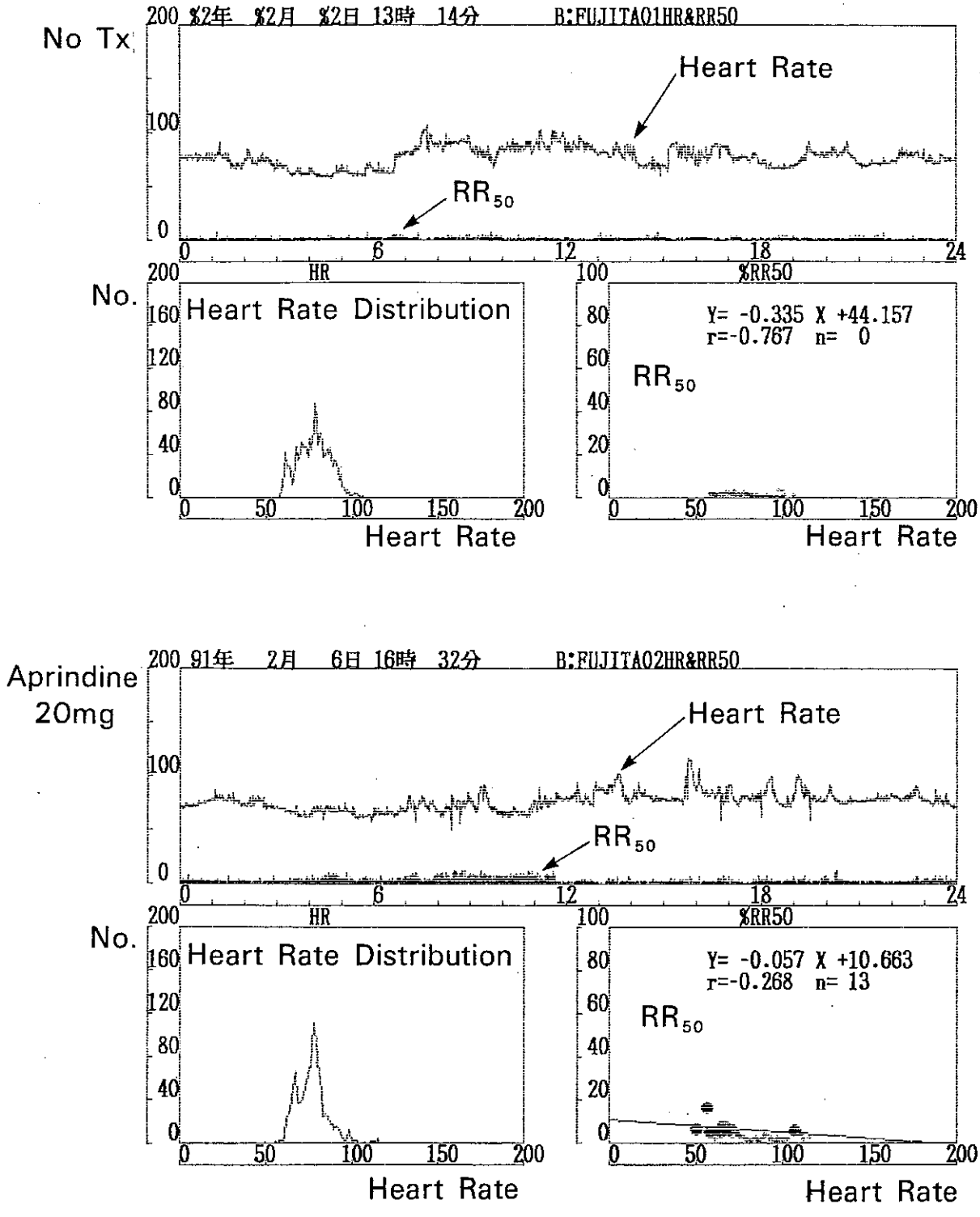

Fig. 16. - Example of an effect of antiarrhythmic agents on RR50. RR50 trendgram, which is shown just below the HR trendgram, and RR50 index, shown as "n" (right lower) in each panel, increase after the treatment with a class Ib antiarrhythmic agent (aprindine).

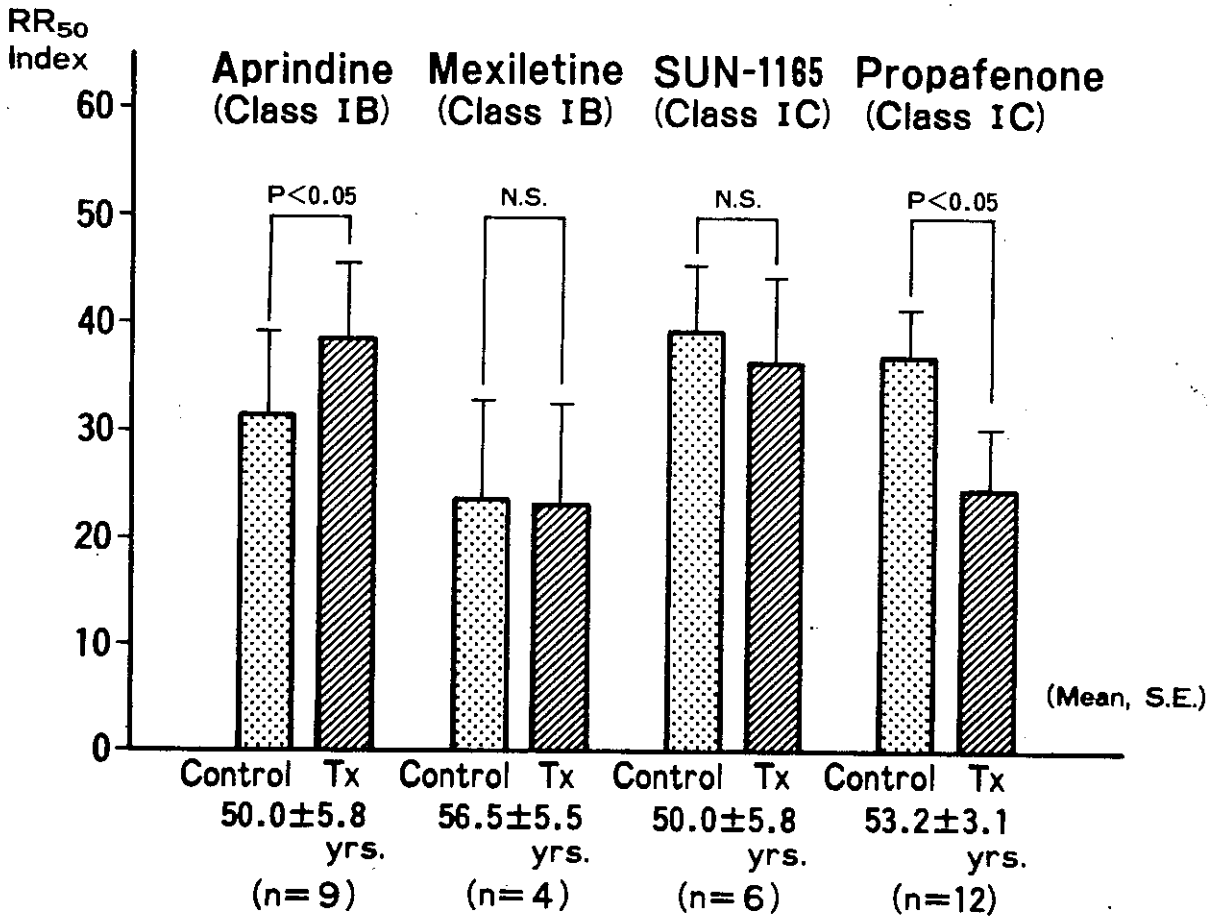

Fig. 17. - Comparison of the effect of some kinds of antiarrhythmic agents on HR variability.

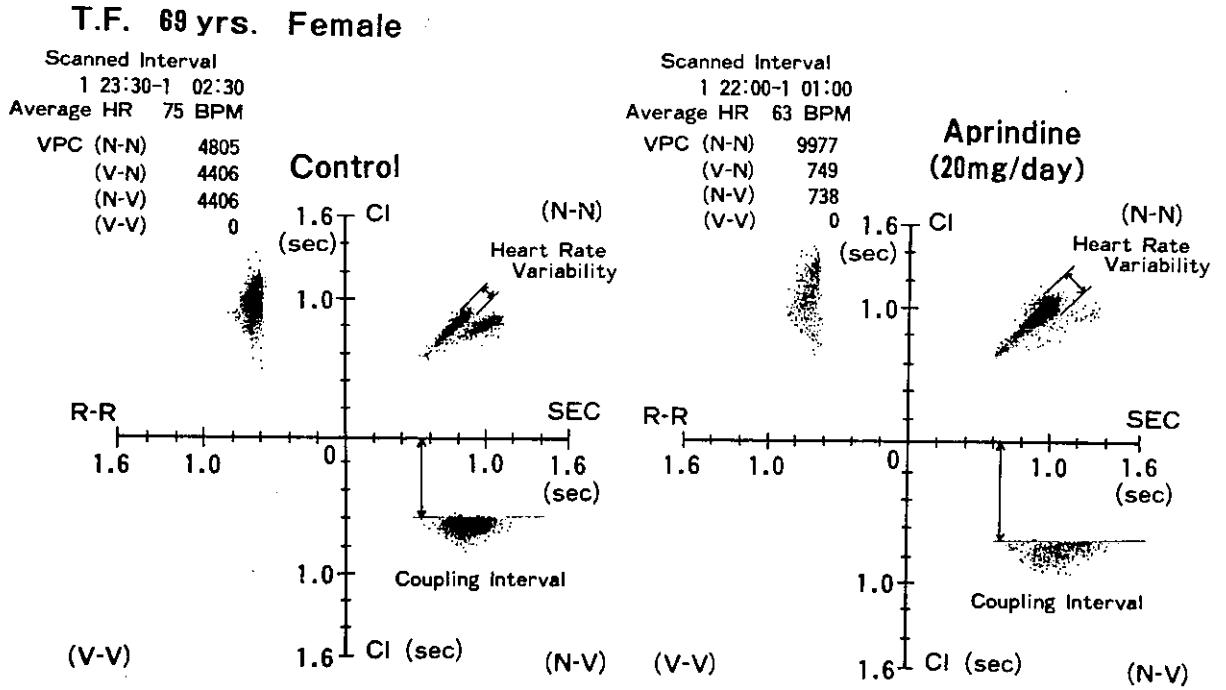

Fig. 18. - Confirmation of the effect of aprindine on HR variability by Lorenz plot. After the administration of aprindine, the width of the plot which is one of the indices of HR variability, increases (1st quadrant). Increase in coupling intervals of ventricular arrhythmias is also observed after aprindine (4th quadrant).

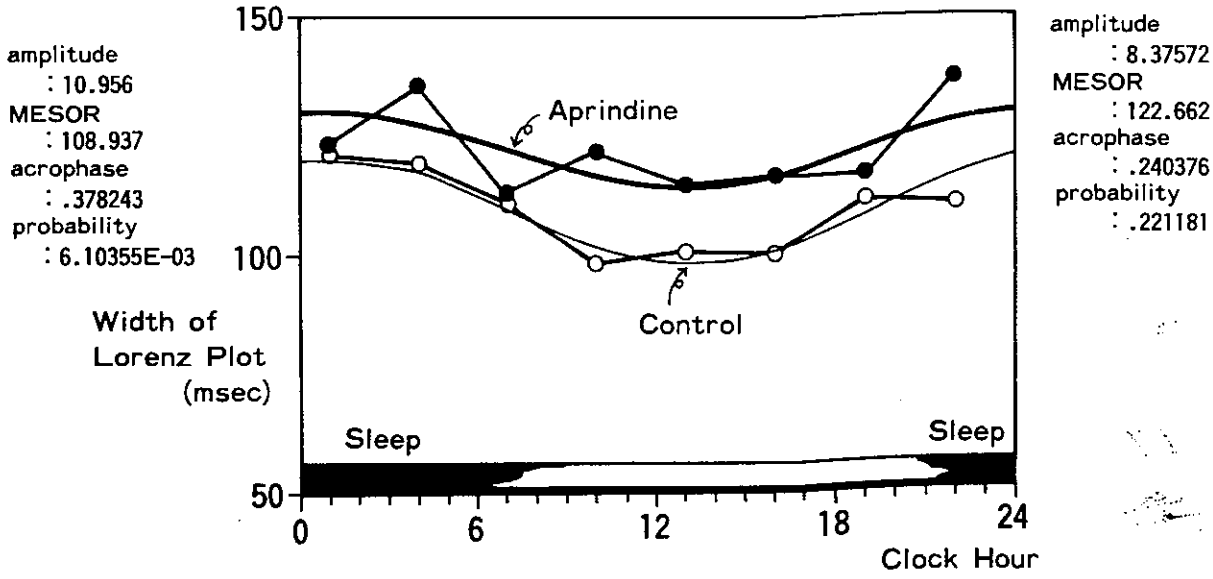

Fig. 19. - Comparison of the circadian rhythm of the width of Lorenz plot between before and after the treatment with aprindine.

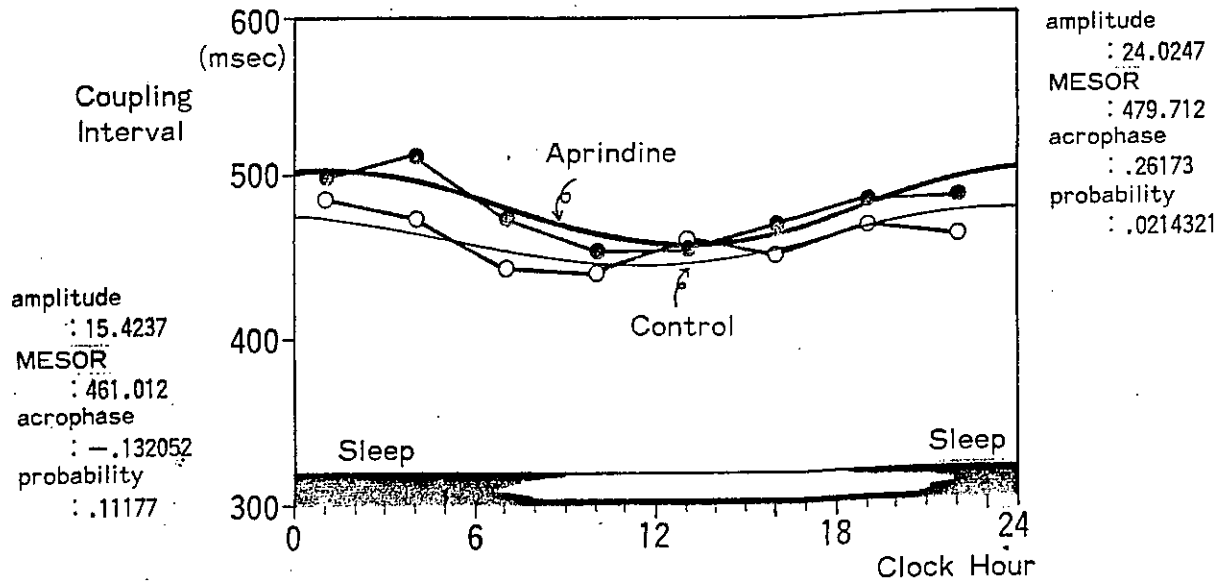

Fig. 20. - Comparison of the circadian rhythm of the coupling intervals of ventricular arrhythmias between before and after the treatment with aprindine.

Circadian rhythm of episodic myocardial ischemia

Prinzmetal's variant form of angina is characterized by transient episodes of ST-segment elevation caused by coronary artery spasm; episodes occur at rest, both with and without angina, in the early morning. Yasue *et al.* [18] demonstrated by coronary angiography that the tone of the large coronary artery was high in the early morning and low in the afternoon in the patients, and α-adrenergic receptors played an important role in the genesis of the circadian variation in the tone of the coronary artery. Many other investigators also observed a definite circadian periodicity of anginal episodes and most episodes occurred in the early morning hours, particularly in the late half of sleep span.

Recent studies show that not only such episodes of ST-elevation but also ischemic ST depression show circadian variation with the peak occurrence in the early morning. The increase in myocardial ischemia both with symptomatic and asymptomatic is particularly enhanced in the first 1 to 3 h after awakening. These circadian rhythms in the frequency of ischemic episodes are of particular interest, because they are

similar to the nonfatal myocardial infarction and sudden cardiac death. The recent study by Muller *et al.* [19] demonstrated a marked circadian rhythm in the frequency of myocardial infarction onset, with a peak from 06:00 to 12:00. In addition, these authors described a prominent circadian variation of sudden cardiac death, with an increase incidence from 07:00 to 11:00. Mazzetti *et al.* [20] analyzed the onset of myocardial infarction in 172 patients, and observed a circadian rhythm with an acrophase at 08:51. He disclosed that circadian rhythm was not observed in 70 patients with anterior myocardial infarction, while in 102 patients with inferior myocardial infarction a circadian rhythm was shown, with an acrophase at 08:55.

Such circadian pattern in the occurrence of cardiac disease may well be influenced by many of the endogenous and exogenous periodic rhythms observed in humans. Morning increases in HR, BP, serum catecholamine levels and cortisol may contribute to increase myocardial oxygen demand. Another possible explanation for the circadian pattern in the onset of cardiac disease could be represented by platelet hyperaggregability and by a decrease in the function of the fibrinolytic system, which contribute to a hypercoagulative state. Sensi *et al.* [21] showed significant circadian rhythms with acrophases at 07:41 (collagen-induced platelet aggregation) and at 10:29 (epinephrine-induced platelet aggregation). A decrease in coronary blood flow in the early morning has also been reported. In addition, in patients with cardiac diseases an increased deterioration in the morning of left ventricular function has been reported.

In these years, circadian variation in the onset of ischemic stroke has also been confirmed by several investigators. Tsementzis *et al.* [22] reported that ischemic stroke exhibited a peak incidence between 10:00 and 12:00 in 245 consecutive stroke patients. Marler *et al.* [23] also confirmed a morning increase (10:00, 12:00) in onset of ischemic stroke in 1167 patients.

Ultradian as well as infradian periodicities of some cardiovascular emergencies have been also demonstrated [24].

These observations suggest the importance of therapy from the viewpoint of "chronomedicine" for patients not only with coronary artery disease but also with cerebrovascular disorders. The marked increase in ischemic activity soon after awakening should be concerned in particular, because medical regimens frequently adopted may not provide patients with adequate protection at the time span when they are at greatest risk of developing ischemia.

Conclusions

Mainly, in this paper, we introduced our recent investigations in HR variability from a viewpoint of chronobiology. However, as mentioned at the beginning, recent advances in the ambulatory BP monitoring system are strikely remarkable. Not only HR but also BP exhibit a prominent circadian periodicity. To realize an individual circadian profile of BP constitutes an important source of information to be exploited for obtaining objective quantitative measures for prediction, prevention, diagnosis (of the existence and the severity) and treatment of hypertensive states. For example, we experienced that 26 hypertensives out of 100 patients, diagnosed as hypertension in the office, were false positive, when we reconfirmed their BP profiles compared with the 90% prediction limits of normal circadian profiles of systolic and diastolic BPs. We also knew that BP profiles varied along with gender and age [25] and they showed a definite seasonal variation.

We have just observed that our newly developed monitoring system of physical activity [26] (active-tracer, that is a kind of gravitation sensor) donated quite a few informations evaluating episodic changes of HR and BP. This device is also useful for finding out the difference between individual life styles, such as the eveningness versus morningness. It is also easily able to make a difference of circadian profiles BP and HR between the working day and the holiday. The knowledge of these very recent advances in ambulatory BP monitoring system should be truely available for general practitioners, but it is too regretful for us that we have not any spaces here to mention about them.

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REFERENCES

1. MYERS, G.A., MARTIN, G.J., MAGID, N.M., BARNETT, P.S., SCHAAD, J.W., WEISS, J.S., LESCH, M. & SINGER, D.H. 1986. Power spectral analysis of heart rate variability in sudden cardiac death: Comparison to other methods. *IEEE Transactions on Biomedical Engineering BME* **33**: 1149-1156.

2. SINGER, D.H., MARTIN, G.J., MAGID, N., WEISS, J.S., SCHAAD, J.W., KEHOE, R., ZHEUTLIN, T., FINTEL, D.J., HSIEH, A.-M. & LESCH, M. 1988. Low heart rate variability and sudden cardiac death. *J. Electrocardiol.* (Suppl.) S46-S55.

3. OTSUKA, K., CORNELISSEN, G., WATANABE, H., HUNT, S.C. & HALBERG, F. 1991. Time-varying limits for single blood pressures and heart rates of group-synchronized healthy women. *Heart Vessels* **6**: 107-111.

4. KUWAJIMA, I., SUZUKI, Y., SHIMOSAWA, T., OTSUKA, K., KAWAMURA, H. & KURAMOTO, K. 1991. Effect of nifedipine tablets on ambulatory blood pressure in patients aged < 60 and > 65 years with systemic hypertension. *Am. J. Cardiol.* **68**: 1351-1356.

5. OTSUKA, K., SATO, T., SAITO, H., KABA, H., SETO, K., YANAGA, T., OGURA, H. & OZAWA, T. 1986. Role of suprachiasmatic nuclei of the hypothalamus on diurnal rhythm in cardiac arrhythmias. *Heart Vessels* **2**: 15-22.

6. OTSUKA, K., WATANABE, H. & OGURA, H. 1989. Application of the MEM, FFT and cosinor analyses to chronobiology in medicine. *Autonomic Nervous System* **26**: 515-522.

7. HALBERG, F. 1969. Chronobiology. *Ann. Rev. Physiol.* **31**: 675-725.

8. EWING, D.J., NEILSON, J.M.M. & TRAVIS, P. 1984. New method for assessing cardiac parasympathetic activity using 24 hour electrocardiogram. *Br. Heart J.* **52**: 396-402.

9. SATO, Y., ADACHI, M. & OTSUKA, K. 1991. Heart rate variability and chronocardiology. *Ther. Res.* **12**: 3170-3176.

10. OTSUKA, K., TSUKAMOTO, T., OGURA, H. & WATANABE, H. 1991. Circadian rhythm of the fluctuation of RR intervals analyzed by Lorenz plot. In: *New trends in autonomic nervous system research.* M. Yoshikawa, M. Uono, H. Tanabe & S. Ishikawa (Eds). Excerpta Medica, Tokyo.

11. TOYOSHIMA, T., OTSUKA, K., WATANABE, Y. & WATANABE, H. 1991. Circadian variation of A-V nodal conduction in atrial fibrillation: an analysis using RR interval scatter diagram. *Ther. Res.* **12**: 3155-3160.

12. ULRYCH. T.J. & BISHOP, T.N. 1975. Maximum entropy spectral analysis and autoregressive decomposition. *Rev. Geophys Space Physics* **13**: 183-200.

13. LUCENTE, M., REBUZZI, A.G., LANZA, G.A., TAMBURI, S., CORTELLESSA, M.C., COPPOLA, E. IANNARELLI, M. & MANZOLI, U. 1988. Circadian variation of ventricular tachycardia in acute myocardial infarction. **62**: 670-674.

14. LOWN, B., TYKOCINSKY, M., GARFEIN, A. & BROOKS, P. 1973. Sleep and ventricular premature beats. *Circulation* **48**: 691-701.

15. IRWIN, J.M., McCARTHY, E.A., WILKINSON, W.E. & PRITCHETT, E.L. 1988. Circadian occurrence of symptomatic paroxysmal supraventricular tachycardia in untreated patients. *Circulation* **77**: 298-300.

16. OTSUKA, K., IKARI, M., ICHIMARU, Y., SAITO, H., KAWAKAMI, T., OTSUKA, M.K., KABA, H. & SETO K. 1986. Experimental study on the relationship between cardiac arrhythmias and sleep states by ambulatory ECG-EEG monitoring. *Clin. Cardiol.* **9**: 305-313.

17. THE CARDIAC ARRHYTHMIA SUPPRESSION TRIAL (CAST) INVESTIGATORS. 1989. Preliminary report: effect of encainide and flecainide on mortality in a randomized trial of arrhythmia suppression after myocardial infarction. *N. Engl. J. Med.* **321**: 406-412.

18. YASUE, H., OMOTE, S., TAKIZAWA, A., NAGAO, M., MIWA, K. & TANAKA, S. 1979. Circadian variation of exercise capacity in patients with Prinzmetal's variant angina: role of exercise-induced coronary arterial spasm. *Circulation* **59**: 938-948.

19. MULLER, J.E., STONE, P.H., TURI, Z.G., RUTHERFORD, J.D., CZEISLER, C.A., PARKER, C., POOLE, W.K., PASSAMANI, E., ROBERTS, R., ROBERTSON, T., SOBEL, B.E., WILLERSON, J.T., BRAUNWARD, E. & MILIS study group. 1985. Circadian variation in the frequency of onset of acute myocardial infarction. *N. Engl. J. Med.* **313**: 1315-1322.

20. MAZZETTI DI PIETRALATA, M., RIBOLDI, L., SERAFINI, M.G., SCARDABOZZI, E., REBUZZI, C., LANZA, G.A. & REBUZZI, A.D. 1988. *Circadian variation in the frequency of onset of acute myocardial infarction.* Proceedings of the 1 World Conference on Clinical Chronobiology, Monte-Carlo (Abstract 16).

21. SENSI, S., CAPANI, F. & GUAGNANO, M-T. 1988. *Ischemic heart disease. A chronobiological approach.* Proceedings of the 1 World Conference on Clinical Chronobiology, Monte-Carlo (Abstract 30).

22. TSEMENTZIS, S.A., GILL, J.S., HITCHCOCK, E.R., GILL, S.K. & BEEVERS, D.G. 1985. Diurnal variation of and activity during the onset of stroke. *Neurosurgery* **17**: 901-904.

23. MARLER, J.R., PRICE, T.R., CLARK, G.L., MULLER, J.E., ROBERTSON, T., MOHR, J.P., HIER, D.B., WOLF, P.A., CAPLAN, L.R., FOULKES, M.A. 1989. Morning increase in onset of ischemic stroke. *Stroke* **20**: 473-476.

24. CUGINI, P., DI PALMA, L., BATTISTI, P., LEONE, G., MATERIA, E., PARANZI, A., ROMANO M., FERRERA, U. & MORETTI, M. 1990. Ultradian, circadian and infradian periodicity of some cardiovascular emergencies. *Am. J. Cardiol.* **66**: 240-243.

25. OTSUKA, K., WATANABE, H., CORNELISSEN, G., SHINODA, M., UEZONO, K., KAWASAKI, T. & HALBERG. F. 1990. Gender, age and circadian blood pressure variation of apparently healthy rural vs metropolitan Japanese. *Chronobiologia* **17**: 253-265.

26. SATO, Y., OTSUKA, K., YAMANAKA, T., KUBO, Y., NAKAJIMA, S., WATANABE, Y., ADACHI, M., YANAGA, T. & WATANABE, H. 1992. Application of an activity monitor to chronocardiology: Morningness versus eveningness and circadian periodicity. *Ther. Res.* **13**: 764-770.