eISSN 1303-1775 • pISSN 1303-1783

Neuroanatomy (2009) 8: 20–25

Original Article

Time to onset of anxiety- and depression-like behaviors in rats after myocardial infarction and association with autonomic control of heart

Published online 14 March, 2009 © http://www.neuroanatomy.org

Caglar MACIT [1]
Guldem MERCANOGLU [2] ✢
Nurhas SAFRAN [3]
Mehmet GUNGOR [4]
Lutfiye EROGLU [4]

Yeditepe University Faculty of Pharmacy [1] Department of Clinical Pharmacy, and [2] Department of Pharmacology, Istanbul; Istanbul University, Istanbul Medical Faculty, [3] Department of Microbiology, and [4] Department Pharmacology and Clinical Pharmacology, Istanbul-TURKEY.

✢ Guldem Mercanoglu, Assist. Prof. Dr.
Yeditepe University
Faculty of Pharmacy
Department of Pharmacology
Kayisdagi-Istanbul, Turkey.
☎ +90 216 578 00 00 /1592
+90 216 578 04 14
✉ guldemiko@yahoo.com

Received 11 September 2008; accepted 5 February 2009

ABSTRACT

Although emotional factors increase the risk of cardiac deaths in patients with coronary artery disease, exact mechanisms underlying the increased risk has not been identified. The aim of the study was to investigate the anxiety-like and depression-like behaviors in rats after myocardial infarction and the association with the autonomic control of heart rate. Anxiety-like and depression-like behaviors were assessed during 28-day post myocardial infarction period. Myocardial infarction was induced surgically by the ligation of left anterior descending artery. Elevated plus-maze and forced swimming tests were chosen for assessment of anxiety and depression, respectively. Autonomic control of heart rate was evaluated by power spectral analysis of heart rate variability. Our findings showed that both anxiety-like and depressive-like behaviors were seen after myocardial infarction. However anxiety-like behaviors were seen in the acute period of myocardial infarction, depression-like behaviors were significant in the late period. Anxiety but not depression was associated with reduced autonomic control of heart rate after myocardial infarction.

These data lead to the conclusion that emotional factors seem to be involved in the prognostic factors in coronary artery disease. Adding of antidepressant/anxiolytic therapy to the reperfusion strategies in patients after myocardial infarction is very important. © **Neuroanatomy. 2009; 8: 20–25.**

Key words [anxiety] [autonomic control of heart] [depression] [myocardial infarction]

Introduction

Coronary artery disease (CAD) is the major cause of cardiovascular mortality worldwide. It is known that acute coronary syndromes are the initial presentation of coronary artery disease in most patients and nearly half of subjects with an acute coronary syndrome have myocardial infarction (MI) [1].

Emotional factors (including major depression, anxiety disorders, hostility and anger) and chronic stressors (including low social support, low socioeconomic status, work stress, marital stress, and caregiver strain) are two main psychosocial factors that contribute significantly to the pathogenesis and prognosis of CAD [2].

Emotional factors become a risk factor for cardiovascular events when occurs in healthy people; on contrary when it occurs as a result of cardiovascular disorder, it becomes a factor worsening the prognosis of the related cardiac disorder [3,4]. Although this association was mainly attributed to impaired autonomic control of heart [5], precise mechanisms underlying this phenomenon have not been completely identified.

The aim of the study was to investigate the anxiety and depressive-like behaviors in rats during 28-day post MI period and examine whether depression and/or anxiety was associated with autonomic control of heart rate.

Material and Methods

Animals

Adult male Sprague-Dawley rats weighing 250-300 g were housed as four rats per cage under standard laboratory conditions with a 12 h light/dark cycle, controlled humidity and temperature and were allowed tap water and commercial chow (standard animal chow) ad libitum. Animals were handled to minimize the effects of non-specific stress and acclimatized to laboratory for 15 day prior to any experimental procedure. The experimental procedure was conducted in accordance with the standards established by the guide for care and use of laboratory animals of EEC Council Directive 86/609 and approved by the local 'Institutional Animal Ethic Committee'.

Left anterior descending (LAD) artery ligation

MI was induced by LAD ligation as described previously [6]. After anesthetizing with ketamine and xylazine combination (8mg/100g ketamine, 5mg/100g xylazine), rats were intubated through a tracheotomy and ventilated with a volume-cycled small-animal ventilator (TOPO ventilator, Kent Scientific, USA). A left thoracotomy in the third intercostal space was performed to expose the heart. The LAD was identified and then ligated about 2 mm distal to its origin with 6-0 prolene sutures, then it was released after 30 minutes. MI was confirmed by regional cyanosis, ST elevation on ECG, and elevation of serum creatine kinase MB (CK-MB) and troponin T

Table 1. Power Spectral Analysis of HRV.

Day	HR		SDNN index (msec)		LF/HF	
	Sham	MI	Sham	MI	Sham	MI
Basal	242±18	245±12	5.7±0.8	6.3±0.2	4.59±0.26	4.37±0.85
3	253±10	224±08*&	5.5±0.3	4.7±0.4*&	4.93±0.55	10.43±0.95*&
5	248±12	218±10*&	6.0±0.9	4.0±0.2*&	4.93±0.61	11.75±0.53*&
7	250±14	207±07*&	6.1±0.4	3.2±0.6*&	4.94±0.72	11.88±0.70*
14	253±13	212±04*&	5.8±0.3	2.5±0.1*&	4.84±0.58	9.29±0.95*&
21	245±11	209±11*	6.2±0.8	2.1±0.5*	4.75±0.32	7.84±0.51*&
28	248±09	205±14*	5.9±0.4	1.9±0.4*	4.84±0.31	7.20±0.73 *&

*(***** p<0.05 compared to sham-control at the same time point; **&** compared to day within a same group)*
***HR:** Heart rate, **SDNN index:** standard deviation of R-R intervals; **LF/HF:** Low frequency to High frequency ratio*

levels. Positive end-expiratory pressure was applied to fully inflate the lungs, and then the chest was closed in layers. The sham-control rats underwent the same procedure except ligation. All surgical procedure was done under aseptic conditions.

ECG records

Before each behavioral test procedure, ECG records were taken using 1-lead electrocardiogram. Animals were lightly anesthetized by ketamine/xylazine combination (10mg/kg ketamine and 3mg/kg xylazine). Subcutaneous electrodes were placed both axillae and over the xiphoid process and connected to the PowerLab data-acquisition system (10T Hardware System, PowerLab, ADI Instruments, UK). ECG was recorded at a rate 200 samples/second during 5 minutes.

Assessment of autonomic control of heart rate

Autonomic control of heart rate was evaluated by power spectral analysis of heart rate variability. An off-line analysis was performed on an ECG processor analyzing system (Nevrokard, Small Animal HR Analysis Software, Japan) using the stored ECG data on a computer [7]. First R waves were identified, and both heart rate (HR) and the R-R interval tachograms as the raw heart rate variability (HRV). From the tachograms HRV was calculated as the standard deviation of R-R intervals (SDNN). The power spectrum of the fluctuation was obtained by Fast Fourier Transform (FFT) analysis. Frequency ranges were set as: 0.04-1.00 Hz for low frequency (LF) and 1.00-3.00 Hz for high frequency (HF). The LF region was considered a measure of both sympathetic and parasympathetic activity. The HF region was associated with parasympathetic activity. LF/HF ratio was used as a measure of sympathovagal balance [8].

Behavioral tests

Anxiety and depression-like behaviors were evaluated before MI (basal) and at the 3, 5, 7, 14, 21 and 28 days after MI. While elevated plus-maze test was used for the evaluation of anxiety-like behaviors, forced swimming test was used for the evaluation of depression-like behaviors.

All tests were performed in the light period (12:00–17:00) in a quiet, dimly lit room.

Elevated plus-maze test

The plus-maze was elevated to height of 50 cm and consisted of two open arms (50 x 10 cm^2) crossed with two similar closed arms with walls of 40 cm height. Each rat was placed at the center of the maze facing one of the closed arms. During 5-min test period, an unaware observer recorded the number of open and closed arms entries and the time spent in the open arms. Subsequently, the percentage of open arm entries and time spent in open arms were calculated [9].

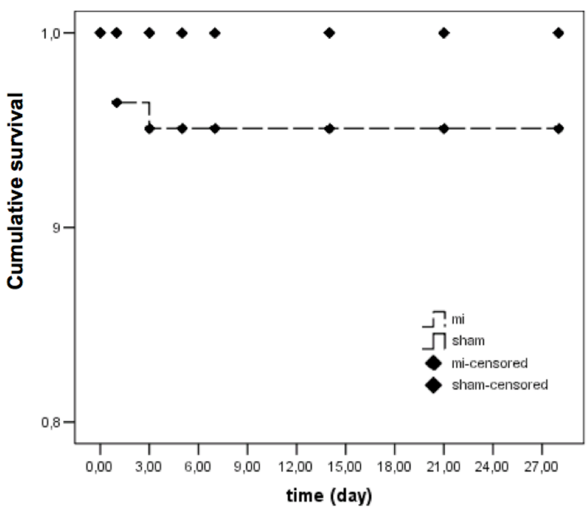

Figure 1. Kaplan-Meier survival curves during study period. No death was seen in the operational control (sham) group. In the LAD ligation grup (MI) Deaths were seen within the sub-acute period of MI (first 72 hour post MI). No more death was seen after this period. In this group survival ratio was greater than 95%.

Forced swimming test

The test procedure described by Porsolt et al. was followed [10]. Each rat was placed individually in a glass cylinder (40 cm height, 18 cm diameter) containing tap water (25 °C) 18 cm from bottom. Animals were forced to swim for 15 min, and the total immobility time was measured. Immobility was assigned as floating in the water without struggling, and making only those movements necessary to keep the head above water. Animals were exposed to pretest for 15 min, 24 h prior to the 5-min swimming test.

Statistical Analysis

All the variables were expressed as mean±SD. Differences among groups were assessed using mixed-design ANOVA (one way for independent groups and one repeated measures) with post-hoc Scheffe's test. Correlation coefficients were calculated by Pearson correlation analysis. Kaplan-Meier survival curves over the follow-up period were constructed and analyzed by the generalized Savage (Mantel-Cox) test. A value of $p<0.05$ in a two-tailed distribution was considered statistically significant.

Results

A total of 24 rats were subjected to the MI or sham operation. Mortality was followed up for 28 days after operation. No death was seen in the operational control group (sham) during the study. Except 4 peri-operative deaths within the first 72 hours after LAD ligation procedure, no more death was seen in the MI group in that period (Figure 1).

Elevated plus-maze test

Analysis of elevated plus-maze data was done according to two criteria: percentage of open arm entries and percentages of time spent in open arms.

Results of elevated plus-maze test are shown in Figure 2. As seen in this figure, baseline percentages of both open arm entries and time spent in open arms were not different between two groups. In the operational control group (sham) the percentage of open arm entries decreased at 3-day post MI period. However, this ratio did not reach to statistical significance ($p=0.08$) and return to the baseline at the 5th day of MI. On contrary, percentages of time spent in open arms did not significantly change in this group during the study.

In the MI group, percentages of both open arm entries and time spent in open arms were decreased significantly in a time dependent manner ($p<0.05$ for both comparisons). This decrease was prominent during the first 7-day post MI period. Moreover, there was a positive correlation between time spent in open arms in elevated plus-maze and the SDNN indexes (Figure 3).

Forced-swimming test

Analysis of forced-swimming test data revealed that the duration of immobility was significantly different between two groups during the study period ($p<0.05$) (Figure 4). The immobility time of animals in the

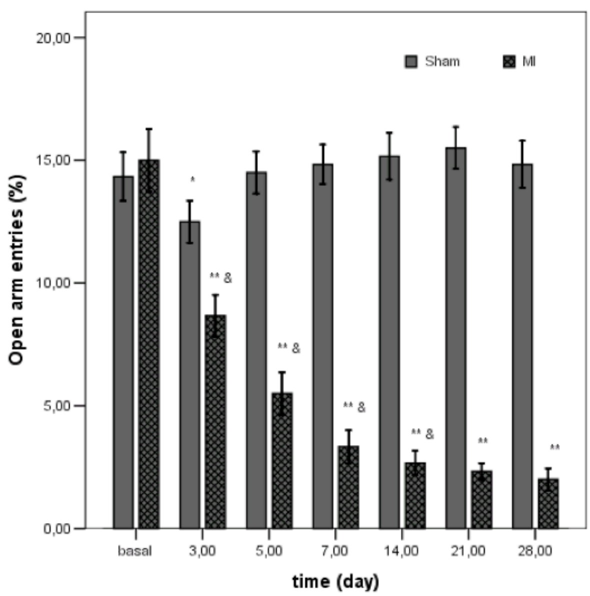

Figure 2a. The percentage of open arm entries of groups in the elevated plus-maze test. In the operational control group (sham) the percentage of open arm entries was decreased significantly at the 3rd day after operation however it turned to the basal level at the 5th day after operation. On contrary percentage of open arm entries in MI group was decreased immediate after MI. This trend contiuned during the study period also. (*p<0.05 compared to basal value; **p<compared to days within a same group; &p<0.05 compared to sham group at the same time point)

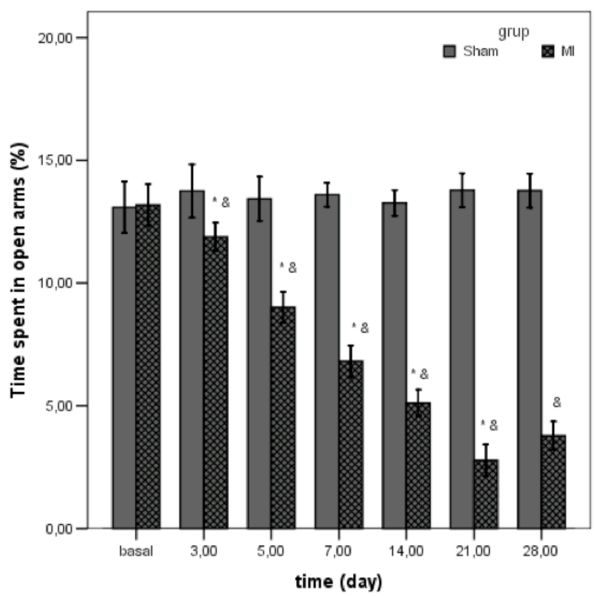

Figure 2b. The percentage of time spent in open arms of two groups in the elevated plus-maze test. In the operational control group (sham) percentage of time spent in open arms did not change during the study. However in the MI group this was decreased immediately after MI. This trend also contiuned during the 21-day period. At the 28th day after MI the percentage of time spent in open arms tended to be increased. (*p<0.05 compared to days within a same group; &p<0.05 compared to sham group at the same time point)

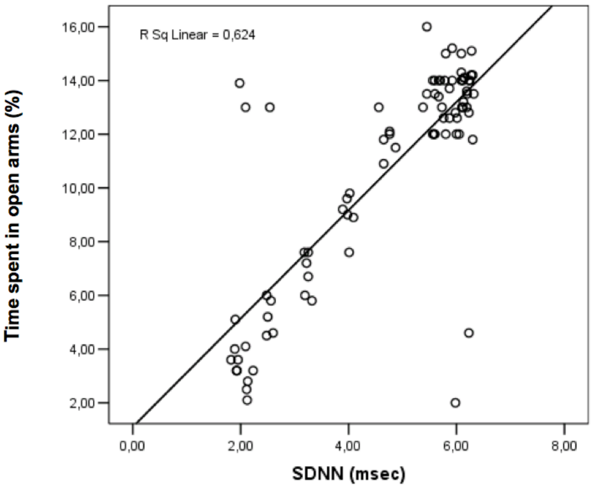

Figure 3. Correlation curve of SDNN with the percentage of time spent in open arms in elevated plus-maze test. Positive correlation was found between the SDNN indexes and percentage of time spent in open arms (R=0.624, p<0.01)

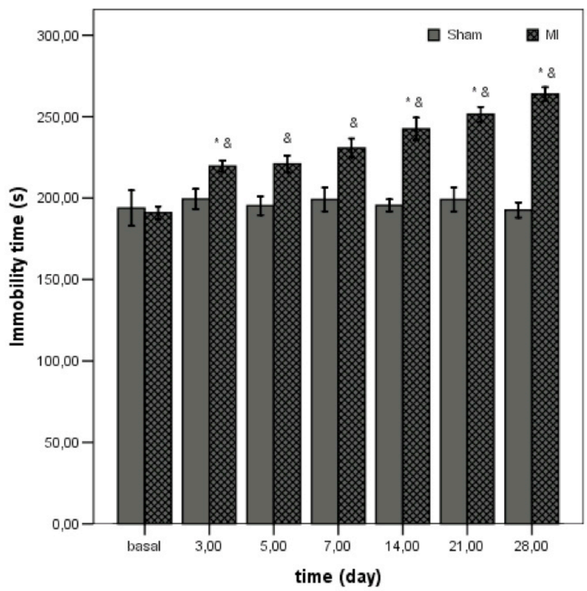

Figure 4. The immobility time of groups in the forced swimming test. In the MI group immobility time increased immediately after MI. This trend continued during the study period too. (*p<0.05 compared to day within a sama group; &p<0.05 compared to sham group at the same time point)

operational control group (sham) was not changed during the study (p>0.05).

The duration of immobility was increased after induction of MI and further increase was seen during the study period. This increase was prominent especially in the second half of the study period.

Power Spectral Analysis of HRV

Mean heart rate values and results of power spectral analysis of HRV are shown in Table 1. There were no significant differences between the groups in the HRs, SDNN indexes and LF/HF ratios at baseline. HRs of sham-control group did not significantly change during the study. HR values were significantly reduced soon after MI (p<0.05). This trend was also continued during 28-day post MI period.

Power spectral analysis of HRV revealed that; in comparison to sham group, SDNN index was significantly lower in MI induced rats. This trend was also constant during the study period. However, slight increase was seen in the LF values; dramatic decrease was seen in the HF values immediately after MI (Figure 5). These changes were more significant in the first half of the study period. As a result, LF/HF ratio was increased immediately after MI and this trend was also continued during the study period.

Discussion

The present study provides evidence that anxiety like behaviors are seen in the subacute period of MI and anxiety is related to the reduced autonomic control of the heart rate in rats with MI. Autonomic control was significantly reduced (characterized by decreased SDNN index and increased LF/HF ratio) immediately after MI (7 day-post MI period). Most dramatic decrease in the percentages of both open arm entries and time spent in open arms were also seen in the same period. Moreover, correlation between SDNN index and percentage of time spent in open arms was found. Similarly, previous studies showed reduced HRV in patients with generalized anxiety [11], and other stress disorders [12,13]. More recently, Watkins et al. demonstrated the relationship between anxiety and reduced baroreflex control of heart in patients after MI [14].

It is well known that most of early deaths after MI are caused by arrhythmias [15]. It is also known that there is an association between anxiety and risk of sudden cardiac death [16,17]. Moreover, relationship with anxiety and increased prevalence of arrhythmias after MI was shown [18]. In our study, deaths were seen in the subacute period of MI (within first 72 hour of MI) when the anxiety-like behaviors were seen. These deaths may be caused by anxiety-induced arrhythmias but it is impossible to distinguish them from the reperfusion arrhythmias in this LAD ligation model.

Although anxiety tended to be associated with higher HR, in our study HR values were decreased immediately after MI. In some animal MI models, independent from drug effect, a decrease in HR was reported. But these decreases, like in our study, are either slight or low in significance [19,20]. It was also known that increased vagal discharge to reduce work and oxygen consumption of heart for limiting the damage occurs within the first hours of developing MI [21,22].

In this study, decreased autonomic control of HR was mainly secondary to reduced parasympathetic control rather than increased sympathetic control. Because first heart rate recordings were collected before the behavioral tests and second recordings were done under light anesthesia; under these conditions there is no contribution of sympathetic system. Dramatic decreases

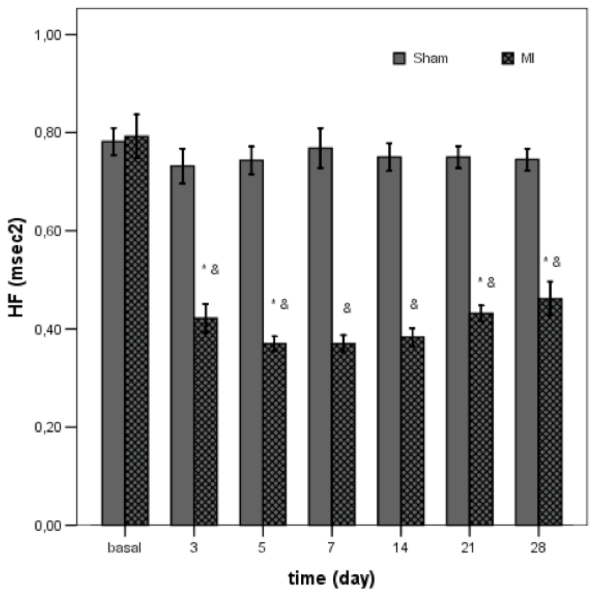

Figure 5a. HF values of two groups in the power spectral analysis of heart rate variability. HF values in the sham group did not change during the study. On contrary significant decrease was seen in the MI group immedialety after LAD ligation. This trend also contiuned during the study (*p<0.05 compared to days within a same group; &p<0.05 compared to sham group at the same time point)

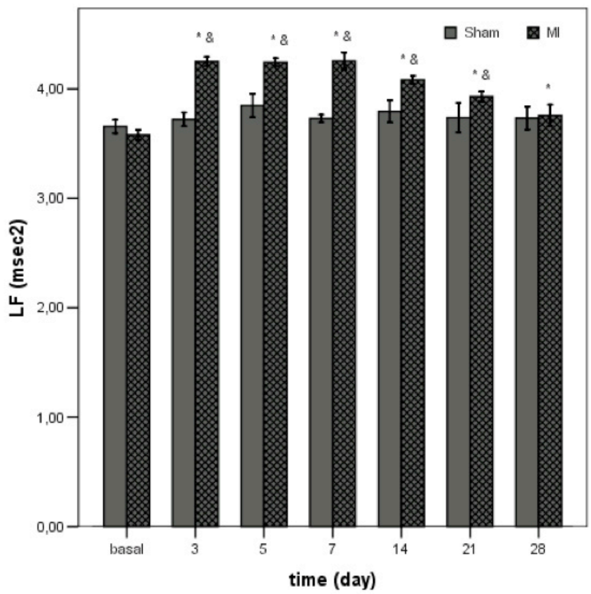

Figure 5b. LF values of two groups in the power spectral analysis of heart rate variability. LF values in the sham group did not change during the study. Slight increase was seen in the MI group immedialety after LAD ligation. LF values were also decreased in a time dependent manner too (*p<0.05 compared to days within a same group; &p<0.05 compared to sham group at the same time point)

in HF region compared to slight increase in LF region in the power spectral analysis of HRV also supports this finding.

We observed depressive-like behaviors in the second half of the study period (after the 14^{th} day of MI). We found no evidence for relationship between depression and impaired autonomic control in rats with MI. No correlation was found between immobility time and SDNN index. However, SDNN indexes were still low in that period. Similar findings were reported in some studies [13,23,24]. However, in other studies, association of depression with reduced HRV in CAD was shown [25,26]. One possible explanation is that the degree of depression could not be determined in this study. Another explanation can be the comorbidity of anxiety-like behaviors.

In summary, our findings show that both anxiety-like and depressive-like behaviors are seen after MI. However, anxiety-like behaviors are seen in the acute period of MI, depression-like behaviors are significant in the late period of MI. Anxiety but not depression is associated with reduced autonomic control of heart rate. With the confirmation of these results in further clinical studies; contribution of emotional factors toward prognostic factors in CAD and addition of antidepressant and/or anxiolytic therapy to the reperfusion strategies after MI would be more important.

Acknowledgements

Authors wish to thank Fehmi Mercanoglu (Professor Dr., Istanbul University; Istanbul Medical Faculty; Department of Cardiology) for his invaluable help and assistance in HRV studies.

Part of this study was represented in the 5^{th} National Neurological Sciences Congress (April 10–14, 2006; Zonguldak, Turkey).

References

[1] Antman EM, Braunwald E. Acute myocardial infarction. In: Braunwald, E; Zipes, D; Libby, P, eds. Braunwald's Heart Disease. 6th Ed., Philadelphia, Saunders Company. 2004; 1184–1288.

[2] Rozanski A, Blumenthal JA, Davidson KW, Saab PG, Kubzansky L. The epidemiology, pathophysiology, and management of psychosocial risk factors in cardiac practice. J. Am. Coll. Cardiol. 2005; 45: 637–651.

[3] Bush DE, Ziegelstein RC, Tayback M, Richter D, Stevens S, Zahalsky H, Fauerbach JA. Even minimal symptoms of depression increase mortality risk after acute myocardial infarction. Am. J. Cardiol. 2001; 88: 337–341.

[4] Frasure-Smith N, Lesperance F, Talajic M. Depression and 18-month prognosis after myocardial infarction. Circulation. 1995; 91: 999–1005.

[5] Schins A, Honig A, Crijns H, Baur L, Hamulyak K. Increased coronary events in depressed cardiovascular patients: 5-HT2A receptor as missing link? Psychosom. Med. 2003; 65: 729–737.

[6] Pfeffer MA. Left ventricular remodeling after acute myocardial infarction. Annu. Rev. Med. 1991; 46: 455–466.

[7] Inagaki H, Kuwahara M, Tsubone H. Effects of physchological stress on autonomic control of heart in rats. Exp. Anim. 2004; 53: 373–378.

[8] [No authors listed]. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation. 1996; 93: 1043–1065.

[9] Sevgi S, Ozek M, Eroglu L. L-NAME prevents anxiety-like and depression-like behavior in rats exposed to restraint stress. Methods Find. Exp. Clin. Pharmacol. 2006; 28: 95–99.

[10] Porsolt RD, Bertin A, Blavet N, Deniel M, Jalfre M. Immobility induced by forced swimming in rats: effects of agents which modify central catecholamine and serotonin activity. Eur. J. Pharmacol. 1979; 57: 201–210.

[11] Thayer JF, Friedman BH, Borkovec TD. Autonomic characteristics of generalized anxiety disorder and worry. Biol. Psychiatry. 1996; 39: 255–266.

[12] Eller NH, Malmberg B, Bruhn P. Heart rate variability and intima media thickness. Int. J. Behav. Med. 2006; 13: 201–213.

[13] Nolan RP, Kamath MV, Floras JS, Stanley J, Pang C, Picton P, Young QR. Heart rate variability biofeedback as a behavioral neurocardiac intervention to enhance vagal heart rate control. Am. Heart J. 2005; 149: 1137.

[14] Watkins LL, Blumenthal JA, Carney RM. Association of anxiety with reduced baroreflex cardiac control in patients after acute myocardial infarction. Am. Heart J. 2002; 143: 460–466.

[15] Pratt CM, Waldo AL, Camm AJ. Can antiarrhythmic drugs survive survival trials? Am. J. Cardiol. 1998; 81: 24D–34D.

[16] Kawachi I, Sparrow D, Vokonas PS, Weiss ST. Symptoms of anxiety and risk of coronary heart disease. The normative aging study. Circulation. 1994; 90: 2225–2229.

[17] Kawachi I, Colditz GA, Ascherio A, Rimm EB, Giovannucci E, Stampfer MJ, Willett WC. Prospective study of phobic anxiety and risk of coronary heart disease in men. Circulation. 1994; 89: 1992–1997.

[18] Sgoifo A, Koolhaas JM, Musso E, De Boer SF. Different sympathovagal modulation of heart rate during social and nonsocial stress episodes in wild-type rats. Physiol. Behav. 1999; 67: 733–738.

[19] Fraccarollo D, Galuppo P, Bauersachs J, Ertl G. Collagen accumulation after myocardial infarction: effects of ETA receptor blockade and implications for early remodeling. Cardiovasc. Res. 2002; 54: 559–567.

[20] Fishbein MC, Maclean D, Maroko PR. Experimental myocardial infarction in the rat: qualitative and quantitative changes during pathologic evolution. Am. J. Pathol. 1978; 90: 57–70.

[21] Adgey AA, Geddes JS, Mulholland HC, Keegan DA, Pantridge JF. Incidence, significance, and management of early bradyarrhythmia complicating acute myocardial infarction. Lancet. 1968; 2: 1097–1101.

[22] Rundqvist B, Elam M, Bergmann-Sverrisdottir Y, Eisenhofer G, Friberg P. Increased cardiac adrenergic drive precedes generalized sympathetic activation in human heart failure. Circulation. 1997; 95: 169–175.

[23] Yeragani VK, Pohl R, Balon R, Ramesh C, Glitz D, Jung I, Sherwood P. Heart rate variability in patients with major depression. Physchiatry Res. 1991; 37: 35–46.

[24] Lehofer M, Moser M, Hoehn-Saric R, McLeod D, Liebmann P, Drnovsek B, Egner S, Hildebrandt G, Zapotoczky HG. Major depression and cardiac autonomic control. Biol. Psychiatry. 1997; 42: 914–919.

[25] Carney RM, Saunders RD, Freedland KE, Stein P, Rich MW, Jaffe AS. Association of depression with reduced heart rate variability in coronary artery disease. Am. J. Cardiol. 1995; 76: 562–564.

[26] Krittayaphong R, Cascio WE, Light KC, Sheffield D, Golden RN, Finkel JB, Glekas G, Koch GG, Sheps DS. Heart rate variability in patients with coronary artery disease: differences in patients with higher and lower depression scores. Psychosom. Med. 1997; 59: 231–235.