Am J Transl Res 2016;8(2):1295-1301
www.ajtr.org /ISSN:1943-8141/AJTR0021760

Original Article

Ranolazine attenuated heightened plasma norepinephrine and B-Type natriuretic peptide-45 in improving cardiac function in rats with chronic ischemic heart failure

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Received December 13, 2015; Accepted January 29, 2016; Epub February 15, 2016; Published February 29, 2016

Abstract: As a new anti-anginal agent, ranolazinehas been shown to play a cardioprotective role in regulating myocardial ischemic injury. Given that plasma norepinephrine (NE) and brain natriuretic peptide (BNP, also termed B-type natriuretic peptide-45 in rats) are considered neuron-hormones to indicate heart failure progression. This study aims to examine effects of ranolazine on plasma NE and BNP-45 of rats with chronic ischemic heart failure (CHF). CHF was induced by myocardial infarction following ligation of a left anterior descending artery in adult Sprague-Dawley rats. We hypothesized that ranolazine attenuates the elevated levels of NE and BNP-45 observed in CHF rats thereby leading to improvement of the left ventricular function. Results showed that levels of plasma NE and BNP-45 were increased in CHF rats 6-8 weeks after ligation of the coronary artery. Our data demonstrate for the first time that ranolazine significantly attenuated the augmented NE and BNP-45 induced by CHF ($P<0.05$ *vs.* saline control). In addition, a liner relation was observed between NE/BNP-45levels and left ventricular fractional shortening as indication of left ventricular function (r=0.91 and $P<0.01$ for NE; and r=0.93 and $P<0.01$ for BNP-45) after administration of ranolazine. In conclusion, CHF increases the expression of NE and BNP-45 in peripheral circulation and these changes are related to the left ventricular function. Ranolazine improves the left ventricular function likely by decreasing heightened NE and BNP-45 induced by CHF. Therefore, our data indicate the role played by ranolazine in improving cardiac function in rats with CHF.

Keywords: Ranolazine, myocardial infarction, norepinephrine, brain natriuretic peptide

Introduction

Ranolazine is a new kind of anti-anginal agent. By inhibiting the late inward sodium current in heart muscle [1], ranolazine leads to reductions in elevated intracellular calcium levels and thereby decreases tension in the heart wall, which mainly cut oxygen requirements for the muscle [2]. Thus, ranolazine has been observed to decrease ventricular arrhythmias induced by ischemia and ischemia-reperfusion in a rat model [3]. Ranolazine has also used to treat anginal symptoms among patients with symptomatic chronic stable angina pectoris in clinical trials [4].

Additionally, ranolazine has been shown to play a cardio-protective role in regulating myocardial ischemic injury via a variety of mechanisms [5-7]. For example, prior studies using experimental animal models demonstrated that ranolazine reduces myocardial infarct size, improves left ventricular function, decreases ischemia/reperfusion-induced arrhythmias and thereby improve outcomes in myocardial ischemic injury [5-7]. It is noted that those data were largely obtained from an acute rat model of myocardial ischemia and/or heart failure.

A well-known hallmark of chronic congestive heart failure (CHF) is increased sympathetic nerve activity (SNA). Sympathoexcitation is firmly established to play a prominent role in disease progression [8], and is inversely related to disease prognosis [9]. Specifically, the exaggerated SNA lowers fibrillation threshold, which

thus increases the probability of a fatal arrhythmia [10, 11]. As the sympathetic nervous system is activated norepinephrine (NE) is released from the cardiac sympathetic nerve terminals and the neurovascular junction and evokes cardiac contraction and vasoconstriction within a given vascular bed [12]. This leads to an increase in the plasma NE level. In addition to plasma NE, neuron-hormones such as brain naturetic peptide (BNP), renin and arginine vasopressin are elevated in plasma [13-15]. Among these neuron-hormones, NE and BNP are considered markers of heart failure progression [16, 17]. Nevertheless, it is unclear if ranolazine alters heighten NE and BNP regulated by SNA in CHF. Therefore, in this report we used a rat model of CHF induced by ligation of the coronary artery to examine the effects of ranolazine on the levels of plasma NE and BNP [also referred as B-type natriuretic peptide-45 (BNP-45) in rats] over time, namely 6-8 weeks after the ligation surgery. We hypothesized that administration of ranolazine attenuates the augmented concentration of NE and BNP-45 in plasma and this improves left ventricular functions in CHF rats. We further speculated that a close relation in the levels of NE/BNP-45 and cardiac functions would be observed after ranolazine.

Materials and methods

All procedures outlined in this study were approved by the Animal Care Committee of this institution and were performed in compliance with the rules and regulations described in the National Institutes of Health Guide for *the Care and Use of Laboratory Animals*.

Coronary artery ligation

Male Sprague-Dawley rats (150 to 200 g) were anesthetized by inhalation of an isoflurane-oxygen mixture (2-5% isoflurane in 100% oxygen), incubated, and artificially ventilated. A left thoracotomy between the fourth and fifth ribs was performed, exposing the left ventricular wall. The left coronary artery was ligated. Age and body weight-matched rats that underwent the same procedure as described except that a suture was placed below the coronary artery but was not tied served as controls. The blood samples were taken for the measurements of NE and BNP-45 6 to 8 weeks after the surgery.

Administration of ranolazine

Ranolazine (Sigma-Aldrich) was givenby intraperitoneal (i.p., 20 mg/kg) injection once a day after the ligation surgery. This dose of ranolazine has been shown to maintain its 24 hrs concentration in plasma that is clinically relevant and to be beneficial to cardiac function in rats with myocardial infarction [6, 18]. In control group, saline was i.p. injected in rats. Accordingly, the rats were divided into three groups: control rats (saline, n=15); CHFrats (saline, n=20); and CHF rats (ranolazine, n=20).

Determination of left ventricular function

The rats were anesthetized by inhalation of an isoflurane-oxygen mixture. Transthoracic echocardiography was then performed 1 week before the experiments. The transducer was positioned on the left anterior chest, and left ventricular dimensions were measured. The left ventricular fractional shortening (LVFS) was determined by echo-cardiographic measurements. If LVFS of animals was <30%, they were considered as CHF and included in this study for data analysis. Also, at the end of each experiment a catheter was inserted into the right carotid artery and was threaded into the left ventricle for measurement of left ventricular end-diastolic pressure (LVEDP) to further determine the rats' cardiac function.

Measurements of NE and BNP-45

A blood sample was withdrawn from aorta and transferred into the tube containing EDTA and aprotinin for anti-coagulation and anti-proteinase. The extraction of NE and BNP-45 from plasma was performed using a standard column. Then, according to the manufacturers' manuals plasma NE were determined by NE ELISA Kit (BioSource Co. USA) and BNP-45 levels were determined by Rat BNP-45 Immuno-Assay Kit (Phoenix Pharm Inc, USA), respectively. Briefly, standard or plasma samples were added to each well. The wells were incubated at room temperature and then aspirated and washed. Respective biotinylated NE/BNP-45 antibodieswere added to each well and incubated, followed by washes. Then, streptavidin-peroxidase conjugate was added and incubated and washed. Afterward, chromogen substrate solution was added to each well and incubated and the reaction was stopped by adding

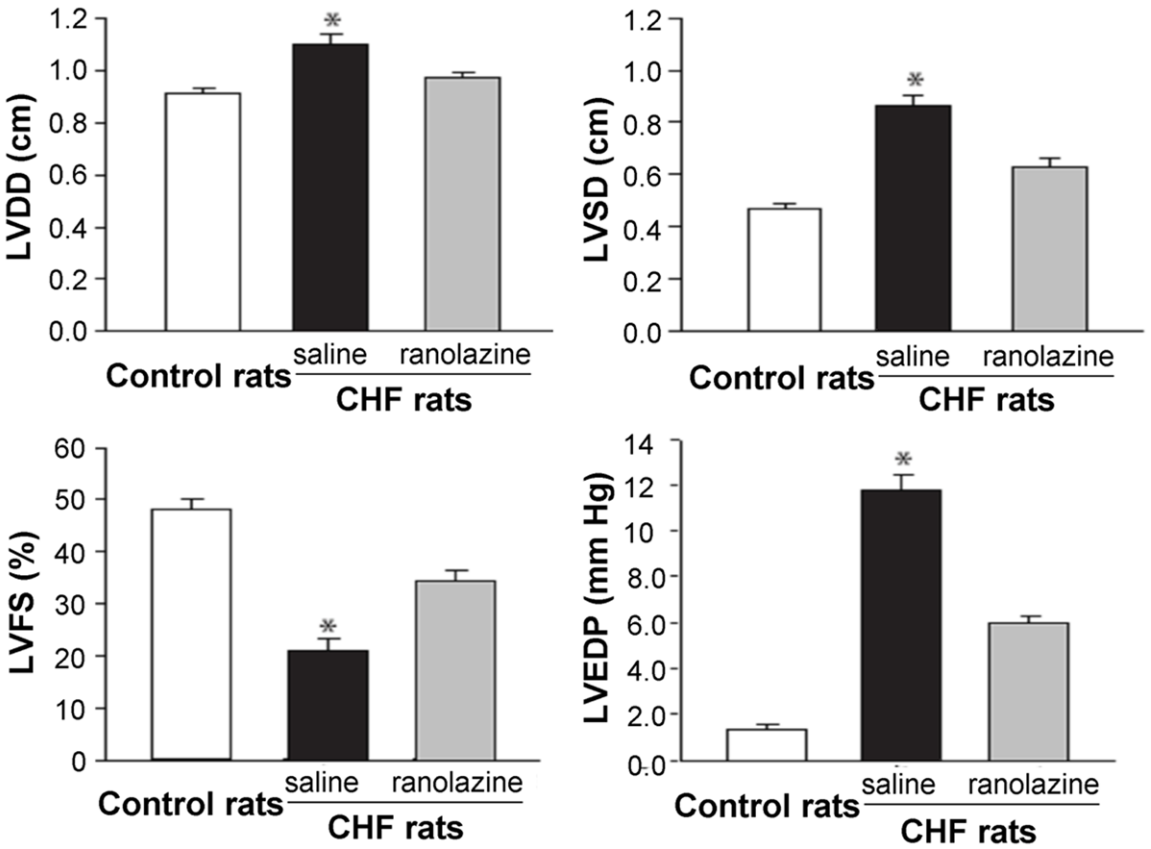

Figure 1. Echocardiographic measurements are demonstrated. LVDD: left ventricular diastolic dimension; LVSD: left ventricular systolic dimension; and LVFS: left ventricular fractional shortening. Left ventricular end-diastolic pressure (LVEDP) is also shown. Values are mean ± SE. *P<0.05 vs. control rats and CHF rats that received i.p. injection of ranolazine. The number of rats=15 in control; 20 in CHF with saline; and 20 in CHF with ranolazine.

stop solution. After this, the optical density was detected immediately by using a microplate reader.

Statistical analysis

The data of NGF measurements were analyzed using a two-way repeated-measure analysis of variance. As appropriate, Tukey post hoc tests were utilized. Values are presented as means ± SE. For all analyses, differences were considered significant at $P<0.05$. All statistical analyses were performed by using SPSS for Windows version 15.0 (SPSS, Chicago, IL).

Results

General and echocardiographic measurements

There were no significant differences in body weight among three groups. i.e., body weight was 525±8 g in control rats (n=15); 515±10 g in CHF rats with saline injection (n=20); and 521±12 g in CHF rats with ranolazine (n=20, $P>0.05$ among three groups). Heart weight was increased in CHF rats with saline (1.82±0.04 g, $P<0.05$ *vs.* other groups) as compared with other two groups (1.45±0.02 g in control rats; and 1.63±0.02 g in CHF with ranolazine).

Figure 1 further shows left ventricular diastolic dimension (LVDD); left ventricular systolic dimension (LVSD); and left ventricular fractional shortening (LVFS) in three groups of rats. The coronary ligation significantly increased LVDD and LVSD and decreased LVFS ($P<0.05$ *vs.* control rats) and administration of ranolazine significantly attenuated increases in LVDD and LVSD, and a decrease in LVFS ($P<0.05$ *vs.* CHF rats with saline injection). Also, LVEDP in three groups of rats was shown in **Figure 1**. LVEPD was significantly elevated in CHF rats and largely recovered after application of ranolazine.

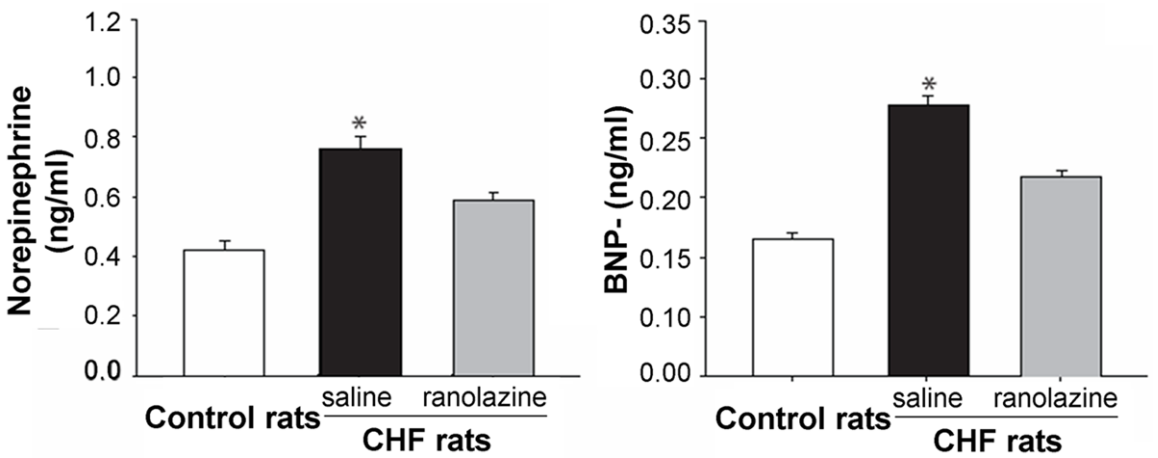

Figure 2. The levels of plasma norepinephrine (NE) and B-type natriuretic peptide-45 (BNP-45) in three groups of rats. Values are mean ± SE. *P<0.05, indicates CHF rats with saline injection (n=20) vs. control rats (n=15) and CHF rats that received i.p. injection of ranolazine (n=20). There were no significant differences in NE and BNP-45 levels in control rats and CHF rats that received i.p. injection of ranolazine.

Levels of NE and BNP-45

Figure 2 demonstrates the levels of plasma NE and BNP-45 in three groups of rats. The coronary ligation significantly elevated both NE and BNP-45 levels in plasma (P<0.05 *vs.* control rats). Moreover, administration of ranolazine significantly attenuated increased NE and BNP-45 evoked by the ligation (P<0.05 *vs.* CHF rats with saline injection for NE and BNP-45) as compared with saline injection in CHF rats.

NE/BNP-45 levels vs. left ventricular function

In addition, in order to determine if the role played by ranolazine in regulating cardiac function was via changes of NE and BNP-45, we performed a liner relationship analysis. **Figure 3** further shows a relationship between plasma NE/BNP-45 levels and LVFS. NE levels (r=0.91 and P<0.01) and BNP-45 levels (r=0.93 and P<0.01) were observed to have a liner relation with LVFS.

Discussion

The levels of plasma NE and BNP have recently been considered as indication for screening, diagnostic, prognostic, treatment and monitoring treatment of patients with HF, because cardiac secretion of NE and BNP driven by sympathetic nerves increase with the progression of HF [16]. Indeed, a prior animal study suggests that BNP mRNA levels in ventricular myocardium in HF are accompanied with change of its concentrations in plasma [19]. Thus, in the current report we specifically examined the concentrations of plasma NE and BNP-45 in CHF rats with chronic administration of ranolazine and further determined if alterations of NE and BNP-45 were closely related to the left ventricular function.

The previous studies demonstrate that the SNA is exaggerated in CHF rats after the ligation of the coronary artery as it is in patients with CHF. Of note, the ligation also leads to an increase in left ventricular end diastolic pressure, left ventricular volume, plasma NE, BNP, renin and arginine vasopressin [13-15]. All the changes observed in this model are very similar to those in human CHF. Thus, a rat model of the coronary artery ligation was used to evoke CHF in the present study. Consistent with the prior findings, we have observed that the LVFS was significantly decreased with increases of LVDD and LVSD after induction of CHF (**Figure 1**). Another index of cardiac function LVEDP was also significantly increased in CHF (**Figure 1**). Also, the levels of plasma NE and BNP-45 were significantly elevated in CHF rats compared with control animals (**Figure 2**). Importantly, data of the present study show that ranolazine attenuated amplified NE and BNP-45 induced by CHF and improved the left ventricular function (**Figures 1** and **2**). In addition, a linear relationship analysis shows that there was a close relation between the left ventricular function and NE as well as BNP-45 levels (**Figure 3**).

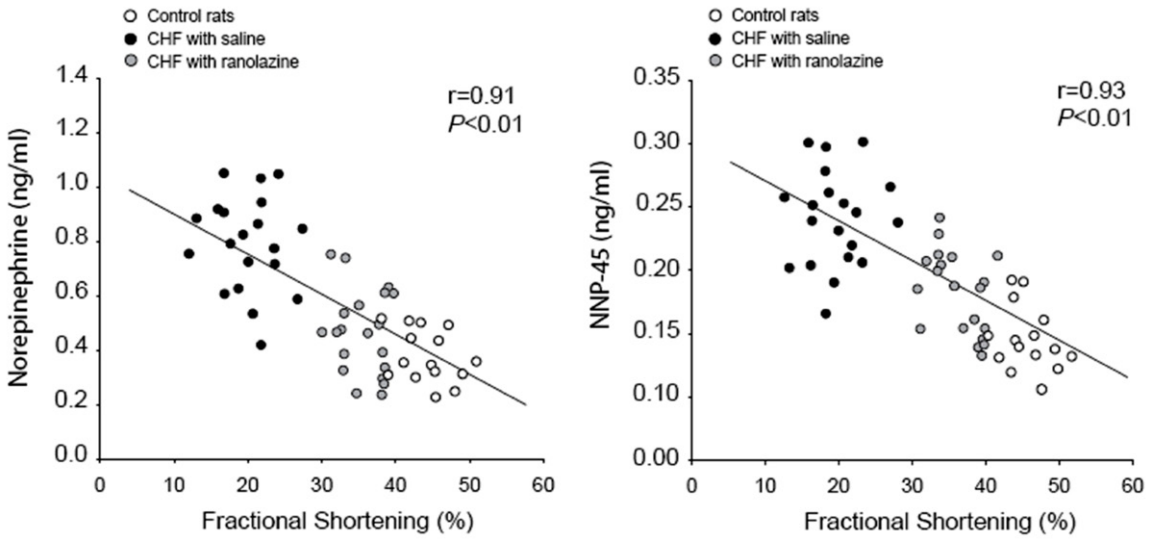

Figure 3. There is a liner relationship between plasma NE levels and left ventricular function indicated by the left ventricular fractional shortening (LVFS); and between plasma BNP-45 levels and LVFS. The number of rats = 15 in control; 20 in CHF with saline; and 20 in CHF with ranolazine.

Overall, to the best of our knowledge data of the current report for the first time demonstrate that ranolazine improves the cardiac function likely by attenuating heightened NE and BNP-45 induced by CHF.

In CHF, neuron-hormones including NE and BNP are elevated in the peripheral circulation with an increase of SNA [9, 12-15]. This induces abnormal cardiovascular regulation during physiological stresses, including low cardiac outputs for a given level of physical exertion as well as elevated sympathetic tones to maintain adequate blood pressure [20]. Specifically, renal vasoconstriction is enhanced and the rise in active muscle blood flow is attenuated [21]. The reduced blood supply to the kidney leads to excessive stimulation of renin secretion and inappropriate salt and water retention [22]. Interestingly, a recent study has shown [23] that ranolazine added to conventional therapy in symptomatic patients with chronic coronary artery disease extends exercise duration with effectively reducing angina frequency. The reduced skeletal muscle blood flow is an important contributor to exercise intolerance in the patients [24], however, it remains unclear if ranolazine likely contributes to improve exercise activity by a mechanism by which sympathetic nerve-regulated blood flow directed to skeletal muscle tissue is partly reinstated. In the current study, we show that ranolazine significantly attenuated heightened NE and BNP induced by CHF, suggesting that ranolazine is beneficial to the impaired peripheral blood flow observed in CHF.

With respect to the mechanisms responsible of ranolazine to play a role, it is generally accepted that ranolazine inhibits the late sodium currents in cardiac myocytes and this leads to reductions in elevated intracellular calcium levels [1]. This decreases tension in the heart wall and oxygen requirements for the muscle [2]. Thus, ranolazine has significant anti-arrhythmic properties [4].

In addition, ranolazine, considered as a limited fatty acid oxidation inhibitor, activates pyruvate dehydrogenase thereby diverting the cardiac energy source from lipids to glucose which requires less oxygen to maintain cardiac function during ischemia [25]. It has been shown that ranolazine significantly reduces infarct size and cardiac troponin T release in rats subjected to left anterior descending coronary artery occlusion-reperfusion [26]. In contrast, a study using rat isolated perfused heart reported that ischemic protection by ranolazine was not mediated by inhibition of fatty acid oxidation during ischemia and reperfusion [27].

Moreover, ranolazine has been reported to play a role in antagonizing α1-, β1-, and β2-adrenergic receptors without significant effects on heart rate and arterial blood pressure in patients with chronic angina [28, 29]. A recent study has reported that ranolazine significantly attenuat-

ed an increase in blood pressure induced by either phenylephrine or isoproterenol in dogs after autonomic ganglionic blockade with hexamethonium, but not the effects was not observed in normal animals [30]. However, significant anti-adrenergic effects of ranolazine have recently been shown in endothelium of the isolated rat intrarenal arteries whereas it played a small role in antagonizing calcium channels [31]. Nonetheless, additional studies need to clarify the precise mechanisms for chronic administration of ranolazine to attenuate NE and BNP in peripheral circulation that we found in the current study.

In conclusion, our data demonstrate that CHF significantly augments the levels of NE and BNP-45 in plasma as compared with controls. Also, NE and BNP-45 responses to CHF are closely related with the LV function. The worse function of the LV induces greater NE and BNP-45 in plasma. Chronic administration of ranolazine significantly attenuated enhanced concentration of NE and BNP-45 induced by CHF and improved the LV function in CHF rats. Overall, the evidence of our study provides strong support for the proposition that ranolazine plays a role in improving worsened cardiac function in CHF. Thus, ranolazine is a potential therapeutic agent to ameliorate consequences of CHF induced by myocardial infarction.

Disclosure of conflict of interest

None.

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References

[1] Noble D, Noble PJ. Late sodium current in the pathophysiology of cardiovascular disease: consequences of sodium-calcium overload. Heart 2006; 92: iv1-iv5.

[2] Kloner RA, Hines ME, Geunes-Boyer S. Efficacy and safety of ranolazine in patients with chronic stable angina. Postgrad Med 2014; 125: 43-52.

[3] Dhalla AK, Wang WQ, Dow J, Shryock JC, Belardinelli L, Bhandari A. Ranolazine, an antianginal agent, markedly reduces ventricular arrhythmias induced by ischemia and ischemia-reperfusion. Am J Physiol Heart Circ Physiol 2009; 297: H1923-1929.

[4] Banon D, Filion KB, Budlovsky T, Franck C, Eisenberg MJ. The usefulness of ranolazine for the treatment of refractory chronic stable angina pectoris as determined from a systematic review of randomized controlled trials. Am J Cardiol 2014; 113: 1075-1082.

[5] Hale SL, Kloner RA. Ranolazine treatment for myocardial infarction? Effects on the development of necrosis, left ventricular function and arrhythmias in experimental models. Cardiovasc Drugs Ther 2014; 28: 469-475.

[6] Mourouzis I, Mantzouratou P, Galanopoulos G, Kostakou E, Dhalla AK, Belardinelli L, Pantos C. The beneficial effects of ranolazine on cardiac function after myocardial infarction are greater in diabetic than in nondiabetic rats. J Cardiovasc Pharmacol Ther 2014; 19: 457-469.

[7] Reddy NM, Mahajan UB, Patil CR, Agrawal YO, Ojha S, Goyal SN. Eplerenone attenuates cardiac dysfunction and oxidative stress in beta-receptor stimulated myocardial infarcted rats. Am J Transl Res 2015; 7: 1602-1611.

[8] Lincevicius GS, Shimoura CG, Nishi EE, Perry JC, Casarini DE, Gomes GN, Bergamaschi CT, Campos RR. Aldosterone contributes to sympathoexcitation in renovascular hypertension. Am J Hypertens 2015; 28: 1083-1090.

[9] Cohn JN, Levine TB, Olivari MT, Garberg V, Lura D, Francis GS, Simon AB, Rector T. Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. N Engl J Med 1984; 311: 819-823.

[10] Collins MN, Billman GE. Autonomic response to coronary occlusion in animals susceptible to ventricular fibrillation. Am J Physiol 1989; 257: H1886-1894.

[11] Vanoli E, Schwartz PJ. Sympathetic--parasympathetic interaction and sudden death. Basic Res Cardiol 1990; 85: 305-321.

[12] Esler M, Kaye D. Sympathetic nervous system neuroplasticity. Hypertension 2006; 47: 143-144.

[13] Francis J, Weiss RM, Wei SG, Johnson AK, Felder RB. Progression of heart failure after myocardial infarction in the rat. Am J Physiol Regul Integr Comp Physiol 2001; 281: R1734-1745.

[14] Maczewski M, Mackiewicz U. Plasma brain natriuretic peptide correlates with infarct size but not with subsequent remodeling in the rat heart. Cardiovasc Pathol 2007; 16: 79-84.

[15] Palazzuoli A, Deckers J, Calabro A, Campagna MS, Nuti R, Pastorelli M, Pasgui AL, Bruni F, Auteri A, Puccetti L. Brain natriuretic peptide and other risk markers for outcome assess-

ment in patients with non-ST-elevation coronary syndromes and preserved systolic function. Am J Cardiol 2006; 98: 1322-1328.
[16] Anand IS, Fisher LD, Chiang YT, Latini R, Masson S, Maggioni AP, Glazer RD, Tognoni G, Cohn JN; Val-HeFT Investigators. Changes in Brain Natriuretic Peptide and Norepinephrine Over Time and Mortality and Morbidity in the Valsartan Heart Failure Trial (Val-HeFT). Circulation 2003; 107: 1278-1283.
[17] Neuhold S, Huelsmann M, Strunk G, Struck J, Adlbrecht C, Gouya G. Prognostic Value of Emerging Neurohormones in Chronic Heart Failure during Optimization of Heart Failure-Specific Therapy. Clin Chem 2010; 56: 121-126.
[18] Jerling M. Clinical Pharmacokinetics of Ranolazine. Clin Pharmacokinet 2006; 45: 469-491.
[19] Suo M, Hautala N, Földes G, Szokodi I, Tóth M, Leskinen H. Posttranscriptional Control of BNP Gene Expression in Angiotensin II-Induced Hypertension. Hypertension 2002; 39: 803-808.
[20] Leimbach WN, Wallin BG, Victor RG, Aylward PE, Sundlöf G, Mark AL. Direct evidence from intraneural recordings for increased central sympathetic outflow in patients with heart failure. Circulation 1986; 73: 913-919.
[21] Middlekauff HR, Nitzsche EU, Hoh CK, Hamilton MA, Fonarow GC, Hage A, Moriguchi JD. Exaggerated Renal Vasoconstriction During Exercise in Heart Failure Patients. Circulation 2000; 101: 784-789.
[22] Dargie H. Sympathetic activity and regional blood flow in heart failure. Eur Heart J 1990; 11: 39-43.
[23] Savarese G, Rosano G, D'Amore C, Musella F, Della Ratta GL, Pellegrino AM, Formisano T, Vitagliano A, Cirillo A, Cice G, Fimiani L, del Guercio L, Trimarco B, Perrone-Filardi P. Effects of ranolazine in symptomatic patients with stable coronary artery disease. Int J Cardiol 2013; 169: 262-270.
[24] Wilson JR, Mancini DM. Factors contributing to the exercise limitation of heart failure. J Am Coll Cardiol 1993; 22: 93A-8A.
[25] Conti CR. Partial fatty acid oxidation (pFOX) inhibition: a new therapy for chronic stable angina. Clin Cardiol 2003; 26: 161-162.
[26] Zacharowski K, Blackburn B, Thiemermann C. Ranolazine, a partial fatty acid oxidation inhibitor, reduces myocardial infarct size and cardiac troponin T release in the rat. Eur J Pharmacol 2001; 418: 105-110.
[27] Wang P, Fraser H, Lloyd SG, McVeigh JJ, Belardinelli L, Chatham JC. A comparison between ranolazine and CVT-4325, a novel inhibitor of fatty acid oxidation, on cardiac metabolism and left ventricular function in rat isolated perfused heart during ischemia and reperfusion. J Pharmacol Exp Ther 2007; 321: 213-220.
[28] Chaitman BR, Pepine CJ, Parker JO, Skopal J, Chumakova G, Kuch J, Wang W, Skettino SL, Wolff AA. Combination Assessment of Ranolazine in Stable Angina (CARISA) Investigator. Effects of ranolazine with atenolol, amlodipine, or diltiazem on exercise tolerance and angina frequency in patients with severe chronic angina: a randomized controlled trial. JAMA 2007; 291: 309-316.
[29] Letienne R, Vie B, Puech A, Vieu S, Le Grand B, John GW. Evidence that ranolazine behaves as a weak beta1- and beta2-adrenoceptor antagonist in the rat [correction of cat] cardiovascular system. Naunyn Schmiedebergs Arch Pharmacol 2001; 363: 464-471.
[30] Zhao G, Walsh E, Shryock JC, Messina E, Wu Y, Zeng D, Xu X, Ochoa M, Baker SP, Hintze TH, Belardinelli L. Antiadrenergic and Hemodynamic Effects of Ranolazine in Conscious Dogs. J Cardiovasc Pharmacol 2011; 57: 639-647.
[31] Deng CY, Kuang SJ, Rao F, Yang H, Fang XH, Shan ZX, Li XH, Zhou ZL, Lin QX, Yang M, Wu SL, Yu XY, Lin SG. Effect of ranolazine on rat intrarenal arteries in vitro. Eur J Pharmacol 2012; 683: 211-216.