Journal of Cardiology 73 (2019) 81–88

Contents lists available at ScienceDirect

Journal of Cardiology

journal homepage: www.elsevier.com/locate/jjcc

Original article

Targeted regulation of sympathetic activity in paraventricular nucleus reduces inducible ventricular arrhythmias in rats after myocardial infarction

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ARTICLE INFO

Article history:
Received 24 April 2018
Received in revised form 29 May 2018
Accepted 5 June 2018
Available online 4 July 2018

Keywords:
Myocardial infarction
Sympathoexcitation
Ventricular arrhythmias
Phosphoinositide-3 kinase/AKT pathway

ABSTRACT

Background: The hypothalamic paraventricular nucleus (PVN) is the center of the regulation of autonomic nervous system functions and cardiovascular activity. Phosphoinositide-3 kinase (PI3K)-AKT pathway in PVN contributes to mediate sympathetic nerve activity and is activated in spontaneously hypertensive rats. Overactivation of the sympathetic output was considered as an important mechanism of the arrhythmias. In the present study, we aimed to explore whether targeted regulation of sympathetic activity in PVN could reduce the peripheral sympathoexcitatory and attenuate the ventricular arrhythmias (VAs) in myocardial infarction (MI) rats via PI3K-AKT pathway.
Methods: A stainless steel gauge guide cannula was stereotaxically implanted into the PVN, and 7 days later, rats were randomly divided into the following 4 groups: group A, control + dimethyl sulfoxide (DMSO); group B, control + LY294002; group C, MI surgery + DMSO; and group D, MI surgery + LY294002. Studies were conducted seven days post-MI. Myocardial function, infarct size, inducible VAs by programmed electrical stimulation, renal sympathetic nerve activity (RSNA), and protein level of PI3K and AKT were measured.
Results: MI increased the protein ratios of p-PI3K-to-total-PI3K and p-AKT-to-total-AKT in PVN but can be reduced by LY294002 treatment. Inhibition of sympathetic nerve activity in PVN led to a reversion in plasma norepinephrine, RSNA and inducible VAs in MI rats.
Conclusions: PI3K-AKT pathway in the PVN was a main mechanism in regulating sympathetic activity and arrhythmias in MI rats. Targeted inhibition of sympathetic activity in PVN may be a potential treatment for the VAs via PI3K-AKT pathway.

Introduction

Myocardial infarction (MI) is one of the most serious coronary heart diseases. MI may cause ventricular arrhythmias (VAs) and sudden cardiac death (SCD), which is closely associated with an adverse and sustained increase in cardiac sympathetic nerve activity [1]. The increased sympathetic activity and impaired parasympathetic activity in post-MI patients led to imbalanced autonomic cardiac nerves [2]. Basic research had shown that MI rats present overactivity of the sympathetic nervous system [3]. Overactivation of the sympathetic output was considered as an important mechanism of the arrhythmias [4], and ameliorating the sympathetic nerve tone after MI could potentially reduce the incidence of VAs [5–7].

The hypothalamic paraventricular nucleus (PVN) is the center of the regulation of autonomic nervous system functions and cardiovascular activity. In spontaneously hypertensive rats (SHRs) and heart failure rats, the observed sympathoexcitation was related to the changes in neuronal activity in the PVN [8,9]. Consistently stimulating the PVN could activate the sympathetic output and induce arrhythmia [10]. Previous reports showed that

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https://doi.org/10.1016/j.jjcc.2018.06.003
0914-5087/

in MI rats the frequency of spontaneous action potentials were increased in the PVN presympathetic neurons [11], suggesting that the PVN modulates cardiovascular responses in MI rats via regulating sympathoexcitatory action [12]. Our previous research showed that cardiac nerve sprouting and sympathoexcitatory activity were correlated with VAs [7,13,14]. In the present study, we discussed the relationship between the PVN and VAs after MI and the underlying mechanism.

The phosphoinositide-3 kinase (PI3K)-AKT pathway is a known intracellular signal transduction in the central nervous system (CNS) [15]. Activated PI3K-AKT pathway appears to be a potential mechanism of the pathogenesis of neurogenic hypertension [16]. In contrast, blood pressure and plasma norepinephrine (NE) level were reduced in SHRs with LY294002 (a PI3K inhibitor) treatment, which meant that down-regulation of the PI3K-AKT pathway reduced the baseline sympathetic outflow [17]. Thus, we hypothesize that PVN mediated peripheral sympathetic nerve activity via the PI3K-AKT pathway. We aimed to investigate whether targeted regulation of sympathetic activity in the PVN could decline the peripheral sympathoexcitatory and attenuate the VAs induced by MI.

Materials and methods

Animals

Male Sprague-Dawley rats (8 weeks old, 220–270 g) obtained from Vital River Laboratory (Beijing, China) were used in this study. All experimental procedures were performed in accordance with the guidelines for animal care and study procedures established by the Shandong University (Jinan, China) Institutional Animal Care and Use Committee. Rats were housed on a 12-h light/dark cycle with the lights on from 8 a.m. with free access to normal rat chow and drinking water.

PVN microinjection

After being anesthetized with 3% sodium pentobarbital (30 mg/kg, intraperitoneal injection), the skull was opened by creating two small micro-holes. A stainless steel gauge guide cannula was stereotaxically implanted into the PVN (1.8 mm posterior, 0.4 mm lateral to bregma, and 7.9 mm deep from the top of the skull) [17], and were fixed to the skull. The animals were allowed 7 days to recuperate. The microinjections into the PVN were performed in conscious, freely moving rats with a 1-μL microsyringe connected by a polyethylene tube to an injection needle 0.5-mm longer than the guide cannula. Dimethyl sulfoxide (DMSO) or LY294002 (5 nmol/L) was microinjected into the PVN after the MI surgery according to the experimental design with a 100-nl volume for each PVN and was removed slowly after 5 min every 12 h [18] for 7 days.

Experimental design

According to the design, 87 of the 100 rats were enrolled (13 died after cannula implantation). Rats were completely randomized into the following four groups: group A, control + DMSO (n = 14); group B, control + LY294002 (PI3K inhibitor) (n = 15); group C, MI surgery + DMSO (n = 29); and group D, MI surgery + LY294002 (n = 29). According to the Masson's trichrome staining, rats with an infarct size between 30% and 50% were enrolled [19]. Within 7 days post-MI, 12 rats in group C and 9 rats in group D died, none died in groups A and B. According to the infarct size, 3 rats in group C were excluded (infarct size 50%, n = 2; infarct size 30%, n = 1) and 2 rats in group D were excluded (infarct size 30%, n = 2). The PI3K specific inhibitor LY294002 (Cat No.: A8250; Apexbio, Houston, TX, USA) was dissolved in DMSO at a concentration of 5 nmol/L for PVN microinjection. Twelve hours after the pretreatment, the ligation surgery was performed. On 7 days post-MI, hemodynamic measurements, renal sympathetic nerve activity (RSNA) recording and programmed electrical stimulation were performed to evaluate the sympathetic nerve activity. After the animals were killed, the PVN tissues were used for Western blotting, and blood samples were collected to measure NE.

Animal model

MI surgery was performed seven days after stereotaxic implantation of the PVN. After intubation, a thoracotomy was performed via the left fourth intercostals space, and then the heart was exposed. The left anterior descending coronary artery was ligated 2 mm from its origin through pericardiotomy to induce MI with 6/0 silk sutures [20]. ST elevation on a surface electrocardiogram (ECG) and stiff movement of the left ventricle (LV) were used to confirm coronary occlusion. Rats in the control groups only experienced thoracotomy and pericardiotomy.

Blood pressure measurement

Blood pressure (BP) was evaluated by the tail-cuff method, using a noninvasive automatic BP recorder (BP-98A; Softron, Tokyo, Japan).

RSNA recording

The left RSNA was measured as previously described [17]. A left retroperitoneal incision was made, and we carefully dissected a bundle of the left renal sympathetic nerve from other connective tissues. Then the distal end of the renal nerve was cut, and we put the central end of the nerve on a pair of platinum electrodes connected to power-lab (AD Instruments, Sydney, Australia) to record the RSNA. Integrated RSNA was simultaneously recorded. To insulate the electrodes and the nerve from the surrounding tissue and prevent desiccation of the nerve, we covered the electrodes and the nerve in warm mineral oil. We measured the background noise after sectioning proximal end of the renal nerve, and the experimental data were corrected for this value.

Electrophysiological study

The electrophysiological study was performed as reported previously [14,21]. Electrodes and the epicardial surface of the LV meshed together tightly. The signals were recorded using an animal biological function experiment system (LEAD-7000; JJET, Chengdu, China). Programmed electrical stimulation was then performed to induce VAs as follows: eight paced beats at a cycle length of 120 ms (S1) followed by single (S2), double (S3), and triple (S4) extra stimuli were applied. When the ventricular tachycardia was shown in ECG and the standard programmed stimulation was interrupted. Ventricular tachycardia and ventricular fibrillation were considered nonsustained (lasted <15 beats) and sustained (lasted >15 beats). The VA scores were measured as previously described [19].

Hemodynamic measurements

Right carotid artery cannulation involved a 2F microtip pressure–volume catheter being advanced into the LV to obtain the pressure volume (P–V) data. The LV end-systolic pressure (LVESP), LV end-diastolic pressure (LVEDP), maximal slope of the LV systolic pressure increment (dp/dtmax), diastolic pressure decrement (dp/dtmin), and LV ejection fraction (EF) were calculated with the Lab-Chart Pro software (AD Instruments).

When the study ended, the volume was calibrated using the relative volume unit (RVU) calibration method with fresh heparinized warm blood as previously reported [20].

Immunohistochemistry and Masson's trichrome staining

The brains were collected, embedded in paraffin, and sectioned into several sections at about 4-μm for immunohistochemistry to examine the sympathetic nerve activity as assessed by Fra-like immunoreactivity (Fra-LI). The sections were incubated with a primary antibody for Fra-LI (1:100). The slides were incubated with an ABC Elite kit (Vector Laboratories, Burlingame, CA, USA) and DAB substrate (Vector Laboratories) and then counterstained with hematoxylin. For each animal, the positive neurons within the bilateral borders of the PVN were manually counted similarly for data analysis in three consecutive sections and an average value was reported.

We sliced hearts into 3 portions along the top and bottom edges of the infarction for Masson's trichrome staining. Then the mid-left LV was harvested, processed on paraffin-embedded tissues, cut into 8-μm-thick sections, and stained with Masson's trichrome. The infarct size (%) was calculated by division of the components of the infarcted LV by inner and outer LV circumference.

Western blotting

Tissue samples of the PVN were subject to western blotting analysis. Equal amounts of protein samples were fractionated on 8–12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels and transferred into polyvinylidene difluoride membranes. Then, the membranes were incubated at 4 °C overnight with anti-phosphorylated PI3K p85 (Cell Signaling Technology, Boston, MA, USA), anti-PI3K p85 (Cell Signaling Technology), anti-phosphorylated AKT (Cell Signaling Technology), anti-AKT (1:1000; Cell Signaling Technology) and anti-GAPDH (1:5000; CoWin Bioscience, Beijing, China) antibodies. Imaging involved the FluroChem E Imager (Protein-Simple, Santa Clara, CA, USA) with an enhanced chemiluminescence detection kit (Millipore, Billerica, MA, USA).

Enzyme-linked immunosorbent assay

The blood samples and myocardia were processed according to the instructions, and the supernatants were collected and stored at −80 °C. We used a commercial NE enzyme-linked immunosorbent assay kit (USCN Life Science, Wuhan, China) to measure the NE concentration.

Statistical analysis

The statistical analysis was performed with SPSS 16.0 (IBM, Armonk, NY, USA). Quantitative data are presented as the mean ± standard deviation (SD). ANOVA followed by Tukey's test was used to compare differences between groups. Difference between two groups was analyzed by unpaired *t*-tests. A value of $p < 0.05$ was considered significant.

Results

PI3K inhibition attenuated the sympathetic activity in PVN

Fra-LI is an indicator of chronic neuronal activation. Immunohistochemical labeling was performed to measure Fra-LI activity in PVN. Fig. 1 depicts a representative photomicrograph of the PVN. The densities of Fra-LI immunoreactivity in the PVN in MI groups were higher compared with control groups, and could be attenuated significantly in LY294002-treated rats compared with M + D (MI surgery + DMSO) group (M + D vs M + L: 197.4 ± 14.4 vs 128 ± 4.68, $p < 0.05$). The possibility of PVN damage caused by the microinjection has been already excluded (see in supplements).

PI3K inhibition did not affect cardiac function post-MI

In the present study, Masson's trichrome staining was used as a criterion for successful MI model (Fig. 2A). Rats with infarct size between 30% and 50% were enrolled at 7 days post-infarction. As shown in Table 1, HR was higher in MI groups than control groups, and LY294002 treatment MI group reduced the HR more than M + D group (364.8 ± 7.73 beats/min vs 396.8 ± 10.4 beats/min, $p < 0.05$). However, the BP in MI groups was lower than control groups, and we guessed that decreased BP in MI rats may be related to the reduced ejection fraction after LV infarction. There was no significant difference between MI groups in BP. So we demonstrated that inhibition of PI3K-AKT pathway could attenuate the increased heart rate post-MI. There was no significant difference in infarct size between MI groups. The EF, LVESP and dP/dtmax significantly decreased in the MI groups compared with the control groups. Conversely, the difference between M + D and M + L (MI surgery + LY294002) groups in the hemodynamic data had no significant difference (Table 1).

PI3K inhibition attenuated the NE levels and RNSA

To evaluate the peripheral sympathetic activity, we determined the NE levels and the left RSNA. The plasma NE levels (Fig. 2B) and myocardial NE levels (Fig. 2C) were significantly upregulated in the M + D group compared to the control groups (plasma NE, 89.42 ± 11.21 pg/ml vs 26.61 ± 1.03 pg/ml, 25.53 ± 0.93 pg/ml; myocardial NE, 27.36 ± 2.08 pg/mg vs 14.09 ± 1.20 pg/mg and 13.74 ± 1.74 pg/mg, $p < 0.05$). The NE levels were significantly lower in the M + L group than in the M + D group (plasma NE, 37.17 ± 3.73 pg/ml vs 89.42 ± 11.21 pg/ml; myocardial NE, 27.36 ± 2.08 pg/mg vs 20.01 ± 1.45 pg/mg, $p < 0.05$).

As shown in Fig. 3, the RSNA baseline of the M + D group was significantly increased compared with that in the control groups (0.13 ± 0.01 uv·s vs 0.06 ± 0.01 uv·s and 0.06 ± 0.01 uv·s, $p < 0.05$), and the LY294002 treatment MI group had a significantly reduced RSNA baseline compared with the M + D group (M + D vs M + L: 0.13 ± 0.01 uv·s vs 0.09 ± 0.01 uv·s $p < 0.05$). Fig. 3f shows means of the changes in RSNA. The mean value of the change in RSNA relative to the C + D (control + DMSO) level was significantly higher in the MI groups and was attenuated by LY294002 treatment group compared with M + D group (M + D vs M + L: 114.8 ± 12.81% vs 47.04 ± 4.55%, $p < 0.05$).

PI3K inhibition attenuated the arrhythmia susceptibility

To elucidate the physiological effect of attenuated sympathetic activity, ventricular pacing was performed. No rats experienced spontaneous VAs during the placement of the electrodes, and none died during the electrophysiological study. As shown in Fig. 4, the arrhythmia score in control rats was very low (0.12 ± 0.12, Fig. 4, lower panel). The M + D group exhibited significantly increased vulnerability for ventricular tachyarrhythmia compared with the control groups; in contrast, LY294002 administration significantly decreased the vulnerability of ventricular tachyarrhythmia (M + D vs M + L: 75% vs 40%, $p < 0.05$).

PI3K/AKT pathway protein expression in the PVN

To explore the possible pathway involved in the regulation of sympathoexcitation, the effects of the PI3K/AKT pathway were

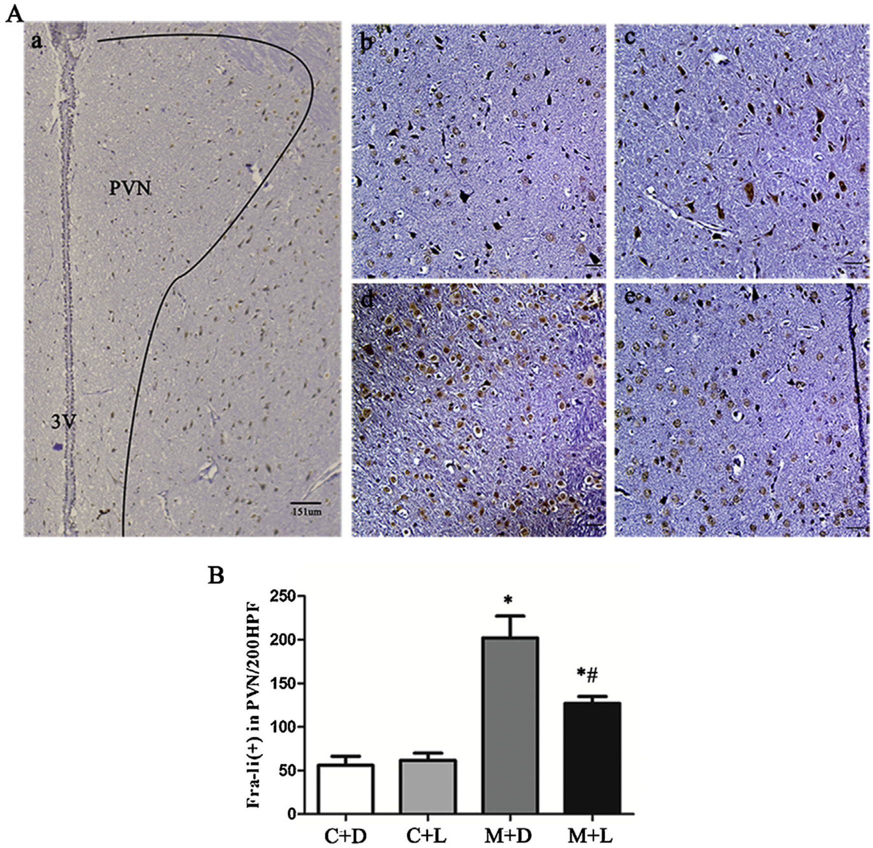

Fig. 1. Effect of PVN microinjection of LY294002 on Fra-like (Fra-LI) positive neurons. (A) A representative image of Fra-LI immunoreactivity in the PVN, an indicator of chronic neuronal excitation (magnification/200). (a) A lower magnification of the PVN. (b) Control + DMSO. (c) Control + LY294002. (d) MI + DMSO. (e) MI + LY294002. (B) Densitometric analysis of Fra-LI positive neurons in PVN. $^*p < 0.05$ compared with the control + DMSO and control + LY294002 groups and $\#p < 0.05$ compared with the MI + DMSO group. PVN, paraventricular nucleus; MI, myocardial infarction; 3V, 3rd ventricle; DMSO, dimethyl sulfoxide; C + D, control + DMSO; C + L, control + LY294002; M + D, MI surgery + DMSO; M + L, MI surgery + LY294002.

assessed in the present study. The protein relative level of PI3K and AKT were significantly higher in the PVN of MI rats, as indicated by a 2-fold increase in the p-PI3K-to-total-PI3K ratio (M + D vs C + D and C + L (control + LY294002): 0.79 ± 0.06 vs 0.40 ± 0.04 and 0.44 ± 0.03, both $p < 0.05$, Fig. 5) and a 1.5-fold increase in the p-AKT-to-total-AKT ratio (M + D vs C + D and C + L: 0.88 ± 0.05 vs 0.59 ± 0.06 and 0.53 ± 0.03, both $p < 0.05$, Fig. 5), manifesting that MI significantly activated the PI3K-AKT pathways in the PVN.

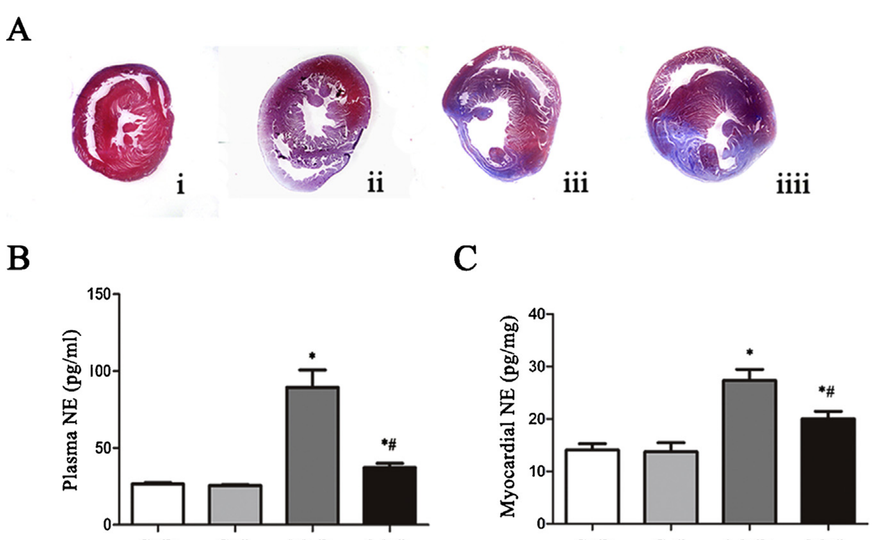

Fig. 2. (A) Original color images of Masson's trichrome staining of the infarcted area in control + DMSO (i), control + LY294002 (ii), MI + DMSO (iii), and MI + LY294002 (iv). Effects of LY294002 microinjection into the PVN on the NE concentration, (B) plasma NE, (C) myocardial NE. Each column with a bar represents the mean + SD. $^*p < 0.05$ compared with the control + DMSO and control + LY294002 groups and $\#p < 0.05$ compared with the MI + DMSO group. NE, norepinephrine, PVN, paraventricular nucleus; MI, myocardial infarction; DMSO, dimethyl sulfoxide; C + D, control + DMSO; C + L, control + LY294002; M + D, MI surgery + DMSO; M + L, MI surgery + LY294002.

Table 1
Hemodynamic data based on pressure–volume 7 days after MI.

	C + D	C + L	M + D	M + L
No. of rats	14	15	14	18
HR (beats/min)	334.5 ± 7.2	326.0 ± 9.6	396.8 ± 10.4*	364.8 ± 7.7*,#
SBP (mmHg)	111.0 ± 3.7	109.3 ± 3.2	89.7 ± 53.2*	92.0 ± 3.5*
DBP (mmHg)	81.0 ± 2.6	80.3 ± 2.4	68.7 ± 2.6*	72.7 ± 2.9
LVESP (mmHg)	112.9 ± 3.1	110.7 ± 2.4	94.8 ± 2.1*	97.6 ± 0.9*
LVEDP (mmHg)	4.8 ± 0.5	4.7 ± 0.4	13.9 ± 2.0*	12.7 ± 1.3*
+dp/dtmax (mmHg/)	5564 ± 177	5636 ± 72	3514 ± 86*	3560 ± 149*
−dp/dtmin (mmHg/s)	3997 ± 112	4086 ± 73	2679 ± 89*	2801 ± 87*
EF (%)	67.3 ± 1.9	64.0 ± 2.6	36.2 ± 1.7*	38.3 ± 2.3*
Infarct size (%)	. . .	. . .	44.7 ± 4.6	43.2 ± 4.1

Values are means ± SEM. HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVESP, left ventricular end-systolic pressure; LVEDP, left ventricular end-diastolic pressure; dP/dtmax and dP/dtmin maximal slope of the systolic pressure increment and the diastolic pressure decrement; EF, left ventricular ejection fraction. MI, myocardial infarction; C + D, control + dimethyl sulfoxide; C + L, control + LY294002; M + D, MI surgery + dimethyl sulfoxide; M + L, MI surgery + LY294002.
* *p* 0.05 compared with control groups.
\# *p* 0.05 compared with MI + DMSO group.

LY294002 treatment group in MI rats resulted in a significant reduction in the relative p-PI3K-to-total-PI3K ratio of 0.58 ± 0.04 compared with 0.79 ± 0.06 for the relative p-PI3K-to-total-PI3K ratio in the M + D group. Treatment with LY294002 decreased the p-AKT-to-total-AKT ratio (M + D vs M + L: 0.88 ± 0.05 vs 0.61 ± 0.01, $p < 0.05$) in the M + L group compared with the M + D group. However, the p-PI3K-to-total-PI3K ratio (0.40 ± 0.04 vs 0.44 ± 0.03, $p > 0.05$) and the p-AKT-to-total-AKT ratio (0.59 ± 0.06 vs 0.53 ± 0.03, $p > 0.05$) were similar between the C + D and C + L groups. These findings supported the hypothesis that in the PVN the PI3K-AKT pathway was activated post-MI and could be reversed by LY294002 treatment into the PVN.

Discussion

In this study, we sought to determine whether targeted regulation sympathetic activity in the PVN, which is an

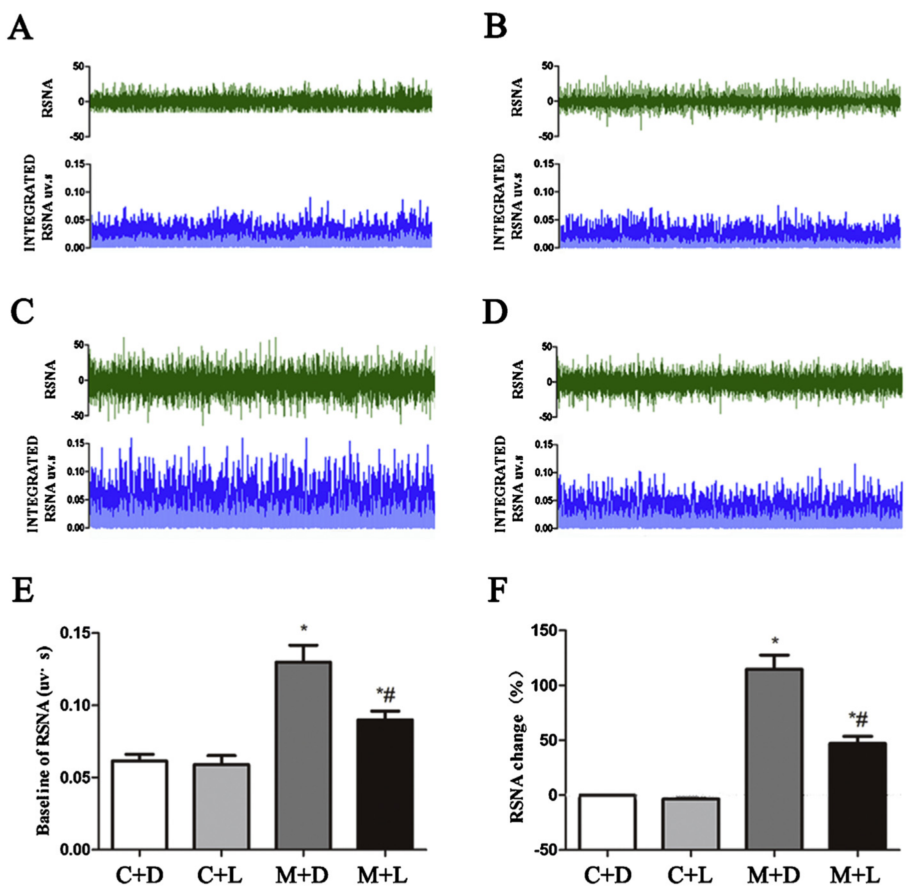

Fig. 3. Typical recordings from an individual rat of the left renal RSNA, integrated RSNA: (A) control + DMSO group; (B) control + LY294002 group; (C) MI + DMSO group; (D) MI + LY294002 group; (E) baseline of RSNA; (F) Changes in RSNA relative to the control + DMSO group level. Values are the mean ± SD. *$p < 0.05$ compared with the control + DMSO and control + LY294002 groups and #$p < 0.05$ compared with the MI + DMSO group. RSNA, renal sympathetic nerve activity; MI, myocardial infarction; DMSO, dimethyl sulfoxide; C + D, control + DMSO; C + L, control + LY294002; M + D, MI surgery + DMSO; M + L, MI surgery + LY294002.

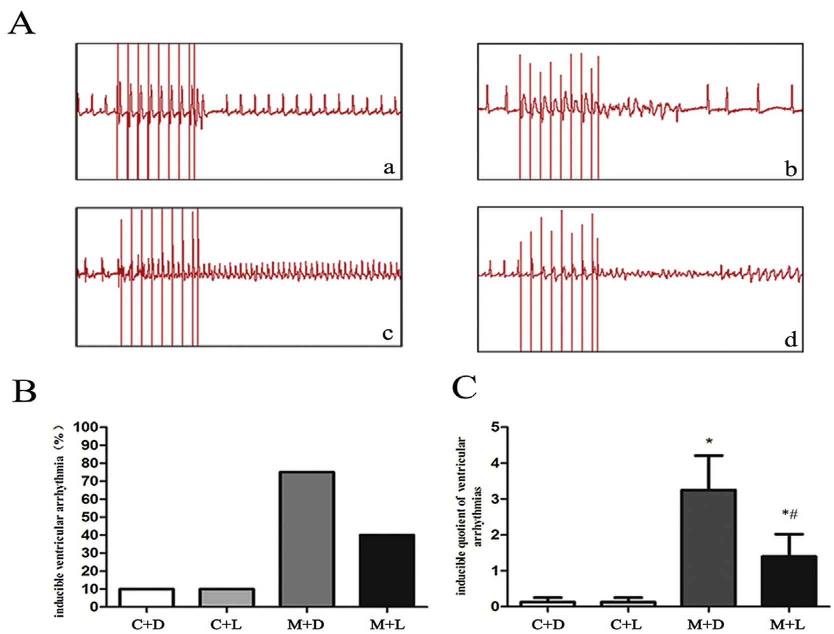

Fig. 4. (A) Recordings of typical inducible ventricular arrhythmias with (a) ventricular premature beats, (b) nonsustained ventricular tachyarrhythmia, (c) sustained ventricular tachycardia and sustained ventricular fibrillation (d). (B) The percentage of inducible total ventricular arrhythmias/stimulation times. (C) Comparisons of the arrhythmia score between the 4 groups at 7 days post-MI. *$p < 0.05$ compared with the control + DMSO and control + LY294002 groups and #$p < 0.05$ compared with the MI + DMSO group. MI, myocardial infarction; DMSO, dimethyl sulfoxide; C + D, control + DMSO; C + L, control + LY294002; M + D, MI surgery + DMSO; M + L, MI surgery + LY294002.

important locus of presympathetic neurons, was involved in the induction of VA by sympathetic activation post-MI. Our data showed (1) increased peripheral sympathetic excitation as indicated by an increased plasma NE level, RSNA, and inducibility of ventricular tachyarrhythmia in MI rats, (2) significant activation of the PI3K-AKT pathway in the PVN in infarcted rats, and (3) inhibition of PI3K-AKT pathway and suppression of sympathoexcitation post-MI by pharmacological blockade of PI3K by LY294002 through bilateral PVN microinjection, which was accompanied by an attenuated HR,

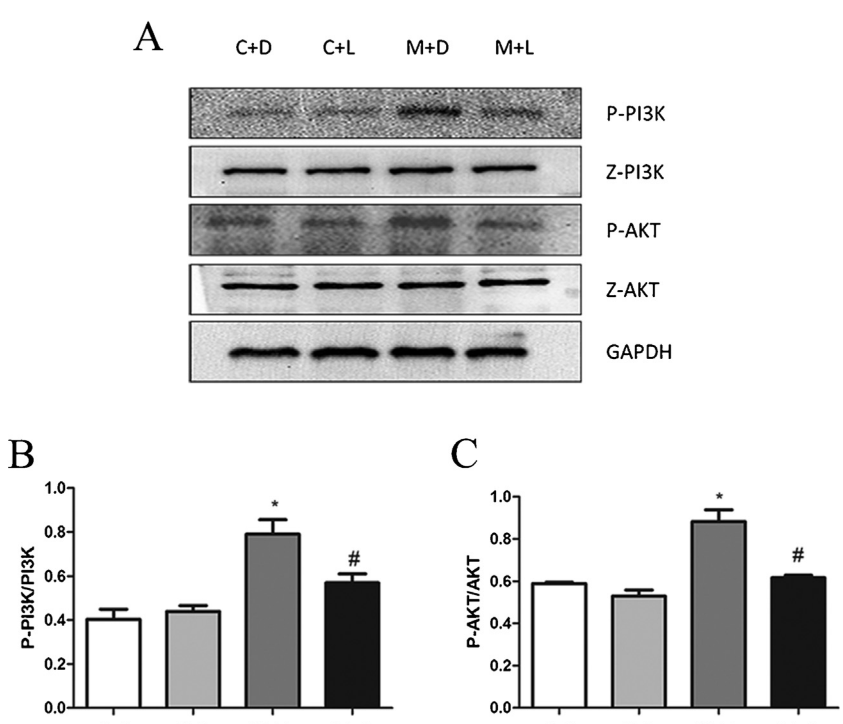

Fig. 5. Western blotting analysis and quantification of protein levels to evaluate PI3K/AKT signaling in the PVN. (A) The western blotting results of PI3K, P-PI3K, AKT, and P-AKT levels. Bar graphs represent the quantitative analysis and differences in the p-PI3K p85α (B) and p-AKT levels, (C) after normalization with the corresponding total proteins in arbitrary units. Each column with a bar represents the mean ± SD. *$p < 0.05$ compared with the control + DMSO and control+LY294002 groups and #$p < 0.05$ compared with the MI + DMSO group. PI3K, phosphatidylinositol 3-kinase; PVN, paraventricular nucleus; MI, myocardial infarction; DMSO, dimethyl sulfoxide; C + D, control + DMSO; C + L, control + LY294002; M + D, MI surgery + DMSO; M + L, MI surgery + LY294002.

plasma NE level, RSNA, and inducibility of ventricular tachyarrhythmia.

Cardiac autonomic nerve disturbances characterized by the imbalanced activation of sympathetic and parasympathetic nerves occurred in patients with cerebrovascular diseases [22]. Generally speaking, overactivation of the sympathetic output was considered as an important mechanism of the arrhythmias [4]. The PVN is one of the most important central sites for autonomic functions and sympathetic tone regulation and played a vital role mediating sympathetic overactivity in heart failure [8]. Local intervention in the PVN showed that reduced RAS (renin–angiotensin system) activity could attenuate sympathetic nerve activity and improve the peripheral symptoms of heart failure [12].

However, the downstream molecular signaling of PVN-induced sympathetic activation remains unclear. The PI3K pathway in the CNS plays a vital role in obesity for the regulation with renal sympathetic and cardiovascular effects of leptin [23]. Consistently, in a subsequent study in normal rats the PI3K-AKT pathway participated in sympathetic excitation induced by angiotensin II [24]. In vitro and in vivo studies have shown that the PI3K subunit p85αprotein and mRNA expression levels are higher in the PVN of SHRs [25] and decreased expression of PI3K p85α results in decreased NE neuromodulation [26]. Targeted administration of LY294002 into the PVN reversed the increase of RSNA and plasma NE level [17]. These findings appeared to indicate that the PI3K-AKT pathway participated in the regulation of the sympathetic outflow in the PVN.

A previous study indicated that the PI3K-AKT pathway was activated in the hippocampus in MI rats [27]. Consistently, our present study showed that the protein expression of PI3K and AKT in the PVN were significantly upregulated in MI rats. However, Lee et al. demonstrated that PI3K-AKT pathway in the myocardium was down-regulated 7 days post-MI, which further deteriorated the infarction-induced sympathetic innervations [28]. Because of the different roles of the PI3K-AKT pathway in the heart and brain, we adopted targeted drug delivery to the PVN to inhibit PI3K expression and avoid interference with other organs by systematic drug delivery. In the present study, we implanted bilateral stainless steel guide cannulas into the PVN to microinject LY294002. The NE concentration [21] and RNSA [2] were higher in the MI groups than in the control groups, which was consistent with previous studies and suggested enhancement of sympathetic excitability in rats post-MI. Targeted regulation of sympathetic activity by microinjection of LY294002 into the PVN in MI rats ameliorated the peripheral sympathetic excitability. Thus, the PI3K-AKT pathway in the PVN post-MI may play a part in the regulation of sympathetic excitability.

VAs are a major fatal complication of MI. The imbalanced density of sympathetic and parasympathetic innervations is related to abnormal transmitter levels (i.e. acetylcholine and NE) and exacerbates electrophysiological heterogeneity, which increases the susceptibility to VAs and SCD [29,30]. As demonstrated in previous studies [13,14,21,31], asymmetrical cardiac sympathetic innervations may contribute to the increased incidence of VAs, which has confirmed the role of sympathetic nerves in the pathogenesis of VAs. In the present study, we used electrophysiological study to evaluate whether the PI3K-AKT pathway in the PVN was involved in sympathetic activation in the PVN. The programmed electrical stimulation revealed increased inducibility of ventricular tachyarrhythmia in MI groups, which could be decreased by targeted inhibition sympathetic activity in PVN. According to the above findings, targeted inhibition sympathetic activity may be a mechanism responsible for the VAs induced by MI via PI3K-AKT pathway. The possible mechanisms are as follows. One possible mechanism is that microinjection of an exogenous PI3K inhibitor into the PVN ameliorated the central activated sympathetic output induced by the MI, thereby contributing to the rebalance between the peripheral sympathetic and parasympathetic nerves. In the present study, the densities of Fra-LI positive neurons in PVN were reduced after PI3K inhibitor microinjection, which indicated that LY294002 microinjection into PVN could reduce the central sympathetic nerves' activity, and then reduced the plasma NE and RSNA. The exacerbated plasma NE concentrations are an independent prognostic for morbidity and mortality in heart failure [32]. High circulating NE and epinephrine trigger arrhythmias in denervated myocardium via activating supersensitive β-adrenergic receptor (β-AR) signaling pathways [33]. The second mechanism is that MI results in an abnormal cardiac autonomic neural distribution and function. Injection of the PI3K inhibitor into the PVN could reduce the cardiac NE concentration in the MI. NE typically shortens the action potential duration, and excess NE increases the activation of β-ARs and induces subsequent changes in the I_K (K^+ current) and calcium overload [33], which all lead to electrophysiological instability. The increased automaticity and triggers activity induced by excess sympathetic innervation exacerbated electrophysiological heterogeneity and resulted in a higher occurrence of VAs and SCD after MI [34]. Rebalance of the autonomic nervous system by LY294002 administration into the PVN may ameliorate cardiac sympathetic innervation to decrease the inducibility of VAs, although this hypothesis remains to be tested in our future study. The last mechanism is that the hyperactivity of RSNA, a regulation center of the sympathetic tone, may exaggerate sympathetic activation, which acts as a potential trigger for VAs and SCD [35]. RSN has become an interesting intervention target for the treatment of many diseases associated with chronic sympathetic activation. The basic [36] and clinical [37] findings demonstrated that RSN ablation is an effective antiarrhythmic approach to improve VA episodes associated with acute MI. In our results, injection of LY294002 into the PVN reduced the RSNA after MI, which might be beneficial for VAs.

In summary, we discovered that the PI3K-AKT pathway in the PVN was involved in the regulation of sympathetic nerve activity post-MI. Targeted inhibition of sympathetic activity in the PVN can ameliorate peripheral sympathetic activity and reduce the incidence of VAs. These findings have provided new insights into the mechanisms underlying the cardiac electrical irritability that occurs following MI and may represent a novel therapeutic strategy for MI.

Conclusion

Peripheral sympathetic excitation after MI was associated with overactivated sympathetic nerves in the PVN, targeted inhibition of the sympathetic output in PVN via PI3K-AKT pathway may be a main mechanism in regulation of peripheral sympathetic activity and VAs induced by MI.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgments

The work was supported by Science and Technology Development Planning of Shandong Province (2016GSF201034, ZR2015HM051, and 2013GGB14056), the National Natural Science Foundation of China (NSFC, 81570305), National Natural Science Foundation of China Youth Fund (8160020369), Independent Innovation Foundation for Jinan Science and Technology Development Planning (201311020), Natural Science Foundation of

Shandong Province (2017MH067), and Shandong Provincial Health Planning Commission Science and Research Fund (2016WS0457), Chinese Postdoctoral Science Foundation (2016M602154), and the Shandong Taishan Scholarship (Suhua Yan).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jjcc.2018.06.003.

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