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ORIGINAL ARTICLE

α7nAChR Deletion Aggravates Myocardial Infarction and Enhances Systemic Inflammatory Reaction *via* mTOR-Signaling-Related Autophagy

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Abstract— Alpha7 nicotinic acetylcholine receptor (α7nAChR) has been previously reported to play an alleviative role in myocardial infarction (MI). In this study, we investigated its specific mechanism. $\alpha 7nAChR^{-/-}$ mice and its control ($\alpha 7nAChR^{+/+}$) were used for the study of α7nAChR. Left anterior descending coronary artery occlusion was conducted for the creation of mice MI model and lipopolysaccharide (LPS) was used as inflammatory stressor in murine peritoneal macrophages. Triphenyltetrazolium chloride (TTC) staining and echocardiography was used for the detection of infarct size and cardiac function, respectively. Western blot was conducted for the testing of autophagy-related proteins and enzyme-linked immunosorbent assay (ELISA) and real-time polymerase chain reaction (RT-PCR) was used for the testing of proinflammatory cytokines. Rapamycin was used for the induction of autophagy through inhibiting mammalian target of rapamycin (mTOR)-related signaling. We found that knocking out α7nAChR enhanced the cardiac infarct size and damaged cardiac function in MI. α7nAChR deficiency increased the levels of several proinflammatory cytokines in serum and spleen from MI mice as well as murine macrophages under inflammatory stress. α7nAChR deletion decreased the level of autophagy in spleen from MI mice and macrophages under inflammatory stress. Rapamycin alleviated the cardiac function and systemic inflammatory reaction in MI mice as well as inflammatory reaction in macrophages under inflammatory stress, which was attenuated by knocking out α7nAChR. Our current study investigated the mechanism of α7nAChR-mediated cardio-protective and anti-inflammatory effect related to mTOR-related autophagy, which might provide a novel insight in the treatment of MI.

KEY WORDS: alpha7 nicotinic acetylcholine receptor; autophagy; myocardial infarction; mammalian target of rapamycin; lipopolysaccharide.

INTRODUCTION

Alpha7 nicotinic acetylcholine receptor (α7nAChR) belongs to a subtype of nAChRs [1–3]. It has been considered to be a member of superfamily of cys-loop cationic ligand-gated channels [1–3]. α7nAChR is commonly acknowledged to be widely expressed in immune cells such as macrophages as well as neuronal cells, thus playing a vital role in various kinds of diseases [4, 5]. α7nAChR has

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been demonstrated to produce an alleviative effect in the suppression of inflammatory reaction, thus regulating the pathogenesis and progression of several inflammation and immune-related diseases, including atherosclerosis, myocardial infarction (MI), hypertension, inflammatory bowel disease, and multiple sclerosis [6–9]. In MI, although evidence has been provided to prove the therapeutic effect of activating α7nAChR in the treatment of myocardial infarction [10–12], the effect of α7nAChR in MI, also well known as heart attack, is still controversial and the specific mechanism is not yet clarified.

MI is a kind of cardiovascular disease during which coronary artery occlusion, blood flow interruption, and myocardial cell necrosis occur [4, 13]. Inflammation is closely associated with the onset and development of MI. It was reported that an over-triggered systemic inflammatory reaction largely aggravated the severity of MI through the enhancement of cardiac infarct size and damage of cardiac function [14, 15]. As a result, agents targeting on overwhelming inflammatory reaction might provide a potential and effective therapy in the treatment of MI. However, so far, seldom such agents have been successfully applied in the treatment of MI in clinical practice.

Autophagy is a vital metabolic process in organisms through the degradation and recycling of long-lived proteins, damaged organs, and misfolded proteins in cells and organs [16–18]. Autophagy has been reported to be closely involved in the regulation of various kinds of disorders, including atherosclerosis and MI, *via* its function of inflammatory suppression [19–21]. In addition, autophagy process is also associated with the anti-inflammatory effect of α7nAChR in several inflammation and immune-related disorders, indicating the involvement of autophagy in the effect of α7nAChR [22, 23].

Here in our current study, we raised a hypothesis that α7nAChR deletion could aggravate the severity of myocardial infarction and enhanced systemic inflammatory reaction *via* mTOR-signaling-related autophagy. We aimed to explore novel therapies in the attenuation of systemic inflammatory reaction in MI, thus developing new strategy for the treatment of MI.

MATERIALS AND METHODS

Animal Care and Use

C57BL/6 mice were purchased from Shanghai Super-B&K Laboratory Animal Corp., Ltd. (Shanghai, China). $\alpha7nAChR^{+/+}$ and $\alpha7nAChR^{-/-}$ mice on C57BL/6 background (8–10 weeks old, male) were purchased from Jackson Laboratory (Bar Harbor, MA, USA) (B6.129P2-Cnr2$^{tm1Dgen/J}$, Stock Number: 005786). Experimental animals were kept at 22 °C under a 12-h light/dark cycle with unlimited access to water and standard rodent diet. All experiments were approved and conducted in accordance with the Committee of Zhejiang University School of Medicine.

MI Mice Models Creation and Treatment

MI surgery was conducted on 8–9 weeks-old mice through permanent left anterior descending coronary artery occlusion as described previously [24, 25]. In brief, after anesthetized with isoflurane (5%) using a ventilation equipment, mice were tacked and the heart was exposed through a lateral thoracotomy with a 4–5 intercostal incision. A 7-0 prolene suture was used to ligate the left anterior descending coronary artery at 1–2 mm below the ostium. Success of surgery was judged through the observation of paleness around and below the ligation point. The chest cavity was closed with 7-0 nylon sutures and placed in a warming pad until recovery from anesthesia and surgery. For certain group, rapamycin was intraperitoneally administrated to mice at the dose of 2 mg/kg body weight at 5 min before ischemia.

Echocardiographic Examination

A 2-D-guided M-mode echocardiography in a Vevo 2100 system (Vevo 2100, Visual Sonics) was used for the analysis of cardiac function as described previously [25]. In brief, mice were anesthetized with isoflurane (5%) using a ventilation equipment. Echocardiographic examination was detected for the analysis of cardiac function. Left ventricular end-systolic diameter (LVESD) and left ventricular end-diastolic diameter (LVEDD) were obtained from at least three separate cardiac cycles. Ejection fraction (EF) and fractional shortening (FS) were calculated according to the equations listed as follows: left ventricular end-systolic diameter (LVESD) = $7.0 \times LVESD^3 / (2.4 + LVESD)$; left ventricular end-diastolic diameter (LVESD) = $7.0 \times LVEDD^3 / (2.4 + LVEDD)$; EF = (LVEDV-LVESV)/LVESV × 100%; EF = (LVEDD-LVESD) / LVESD × 100%.

Triphenyltetrazolium Chloride Staining

TTC staining was conducted for the assessment of infarct size in heart issue as described previously [25]. In brief, at 24-h postinfarction, mice were anesthetized with

isoflurane (5%) and heart issues were quickly isolated from mice. After washed with saline, the heart issues were frozen for 10 min and cut into 5 transverse slices from apex to base of equal thickness (1.5 mm). The slices were then stained in 1% TTC, incubated in 37 °C for 10 min and fixed in 4% (*w*/*v*) paraformaldehyde for 30 min. The infarct size was assessed using ImageJ software.

Primary Peritoneal Macrophages Culture and Treatment

Murine primary peritoneal macrophages were collected as described previously [26, 27]. In brief, $\alpha 7nAChR^{+/+}$ and $\alpha 7nAChR^{-/-}$ mice were injected 10% thioglycollate (i.p.) for 3 days. Murine peritoneal macrophages were collected from the peritoneal cavity by flushing with 5 mL of ice-cold Hanks' balanced salt solution containing 10 U/mL heparin. Cells were plated at a density of 1×10^5/mL in Dulbecco's modified essential medium supplemented with 10% fetal calf serum, and were left to adhere for 4 h in a humidified atmosphere at 37 °C with 5% CO_2. Cells were then washed twice, and the remaining cells were primed with 1 μg/ml lipopolysaccharides (LPS, Sigma, Louis, MO, USA) for 12 h. For certain group, rapamycin was pre-treated (1 μg/L) at 10 min before the challenge of LPS.

Western Blot

Spleen tissues obtained from experimental mice or murine primary peritoneal macrophages were lysed in lysis buffer. Bicinchoninic acid method (Thermo Scientific, Pittsburgh, PA, USA) was conducted for the measurement of total protein concentration. Samples were loaded in 6% or 15% tris/gly gels and transferred on NC membranes through SDS-PAGE (Millipore, Billerica, MA, USA). Blotting was conducted using the rabbit anti-Beclin-1 monoclonal antibody (1:1000; Cell Signaling Technology, Danvers, MA, USA), rabbit anti-LC3 polyclonal antibody (1:1000; Novus Biologicals, Littleton, CO, USA), rabbit anti-p62 antibody (1:1000; Cell Signaling Technology, Danvers, MA, USA), and mouse β-actin (1:5000, Beyotime Biotechnology, Shanghai, China). Membranes were then incubated with a donkey anti-rabbit or donkey anti-mouse secondary antibody (1:10000, LI-COR Biosciences, Lincoln, NE, USA) accordingly. Images were obtained and analyzed using the Odyssey infrared imaging system (LI-COR Bioscience, Lincolin, NE, USA).

Enzyme-Linked Immunosorbent Assay

Blood samples were collected from experimental mice and centrifuged at 3000 rpm (soft) for 30 min at 4 °C. The serum in the upper layer or murine peritoneal macrophages was obtained and the levels of proinflammatory cytokines including IL-1β, IL-6, IL-12, and TNF-α in serum were analysis using commercial available ELISA kits (R&D system, New York, NY, USA) according to the manufacturer's instructions.

Real-time Polymerase Chain Reaction

Total RNA from heart tissues and spleens isolated from experimental mice was isolated by Trizol (Invitrogen, Carlsbad, CA, USA). The first-strand cDNA was synthesized using PrimeScript RT Master Mix (Takara, Otsu, Shiga, Japan) and a $2\Delta\Delta CT$ method was used to analyze the results of PCR with β-actin as the internal reference. 7500 Rea-Time PCR System and the Fast Start Universal SYBR Green Master (Roche, Basel, Switzerland) were used for RT-PCR. Primers used were listed as follows: IL-1β: sense, 5′-GAAATGCCACCTTTTGACAGTG-3′ and anti-sense, 5′-TGGATGCTCTCATCAGGACAG-3′; IL-6: sense, 5′-TAGTCCTTCCTACCCCAATTTCC-3′ and anti-sense, 5′-TTGGTCCTTAGCCACTCCTTC-3′; IL-12: sense, 5′-GGAAGCACGGCAGCAGAATA-3′ and anti-sense, 5′-AACTTGAGGGAGAAGTAGGA ATGG-3′; tumor necrosis factor-α (TNF-α): snese, 5-CAGGCGGTGCCTATGTCTC-3′ and anti-sense, 5′-CGATCACCCCGAAGTTCAGTAG-3′; β-actin: sense, 5′-GTAAAGACCTCTATGCCAACA-3′; and anti-sense, 5′-GGACTCATCGTACTCCTGCT-3′.

Statistical Analysis

All data were presented as mean±SEM. Statistical analysis was conducted by Student's *t* test or one-way analysis of variance (ANOVA) followed by the Bonferroni *post hoc* test for the comparison of two independent groups or multiple comparisons, respectively. $P < 0.05$ was considered as statistical difference.

RESULTS

α7nAChR Deletion Enlarges Cardiac Infarct Size and Damages Cardiac Function in MI Mice Models

To determine the effect of α7nAChR on MI, we detected the infarct size ratio in $\alpha 7nAChR^{+/+}$ and $\alpha 7nAChR^{-/-}$ mice with or without left anterior

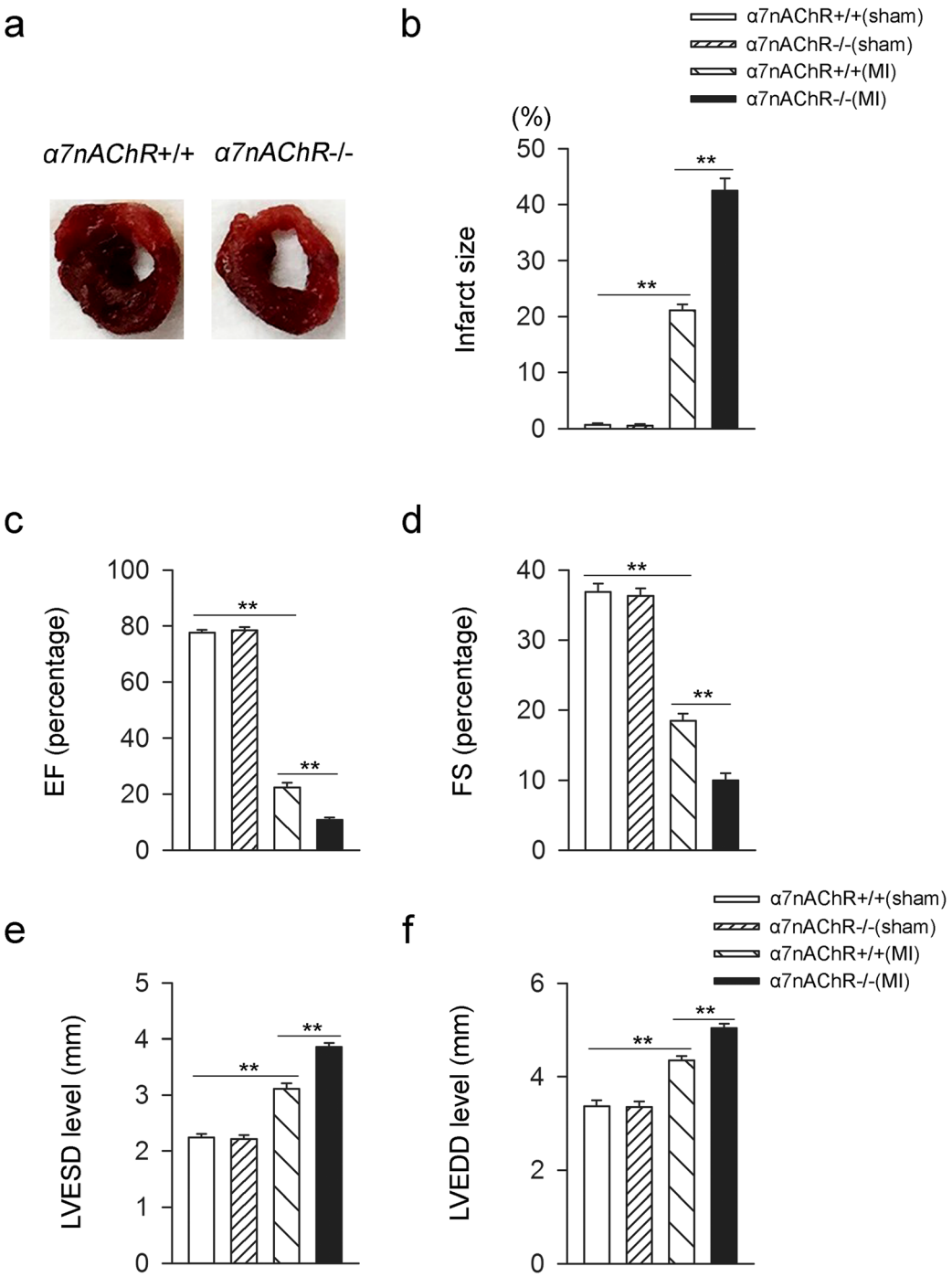

Fig. 1. α7nAChR deficiency enhances the severity of MI *in vivo*. MI mice models were created through permanent left anterior descending coronary artery occlusion. (**a**) Representative images of MI heart from $\alpha 7nAChR^{+/+}$ and $\alpha 7nAChR^{-/-}$ mice isolated at 24-h postinfarction. (**b**) Quantitative analysis of the percentage of infarct size in heart issue ($n = 7$ per group). $^{**}P < 0.01$ *vs.* $\alpha 7nAChR^{+/+}$+MI group; data are presented as mean ± SEM. (**c**–**f**) Quantitative analysis of 2-D guided M-mode echocardiographic detection including the values of EF, FS, LVESD, and LVEDD at 4-w postinfarction ($n = 7$ per group). $^{**}P < 0.01$; data are presented as mean±SEM.

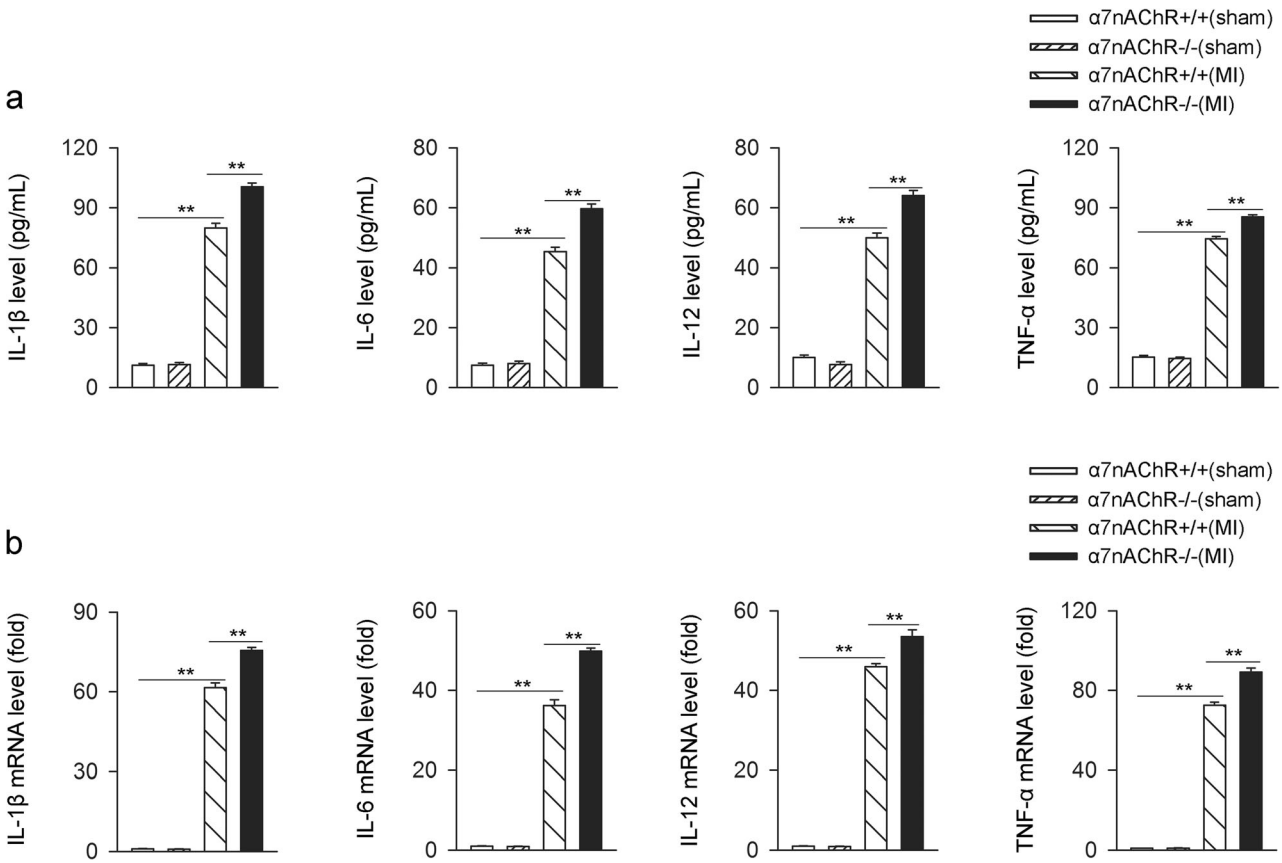

Fig. 2. α7nAChR deficiency enhances inflammatory reaction in MI mice models *in vivo*. MI mice models were created through permanent left anterior descending coronary artery occlusion. (**a**) Quantitative analysis of the levels of several proinflammatory cytokines including IL-1β, IL-6, IL-12, and TNF-α in serum at 24-h postinfarction ($n = 7$ per group). $^{**}P < 0.01$; data are presented as mean±SEM. (**b**) Quantitative analysis of the mRNA levels of several proinflammatory cytokines including IL-1β, IL-6, IL-12, and TNF-α in spleen isolated from experimental mice at 24-h postinfarction ($n = 7$ per group). $^{**}P < 0.01$; data are presented as mean±SEM.

descending coronary artery occluding surgery. We found that knocking out α7nAChR led to the enhancement of infarct size ratio (Fig. 1a, b). To further investigate the effect of α7nAChR in the development of MI, we conducted the echocardiographic examination and assessed the cardiac function of $\alpha 7nAChR^{+/+}$ and $\alpha 7nAChR^{-/-}$ mice with or without left anterior descending coronary artery occluding surgery. We found that α7nAChR deficiency led to the damage of cardiac function including EF, FS, LVESD, and LVEDD (Fig. 1c–f). Taken together, those data demonstrated the protective effect of α7nAChR in the alleviation of infarct size and protection of cardiac function in MI mice models.

α7nAChR Deletion Enhances the Level of Proinflammatory Cytokines in Serum and Spleen from MI Mice Models

Since the overwhelmingly induction of inflammatory responses contributes to the progression of MI, we determine whether α7nAChR could suppress inflammatory reaction on the occurrence of MI. We found that α7nAChR deficiency led to the enhancement of the levels of several proinflammatory cytokines including IL-1β, IL-6, IL-12, and TNF-α in both serum (Fig. 2a) and spleen from MI mice models (Fig. 2b). Those data indicate the anti-inflammatory effect of α7nAChR in MI mice models.

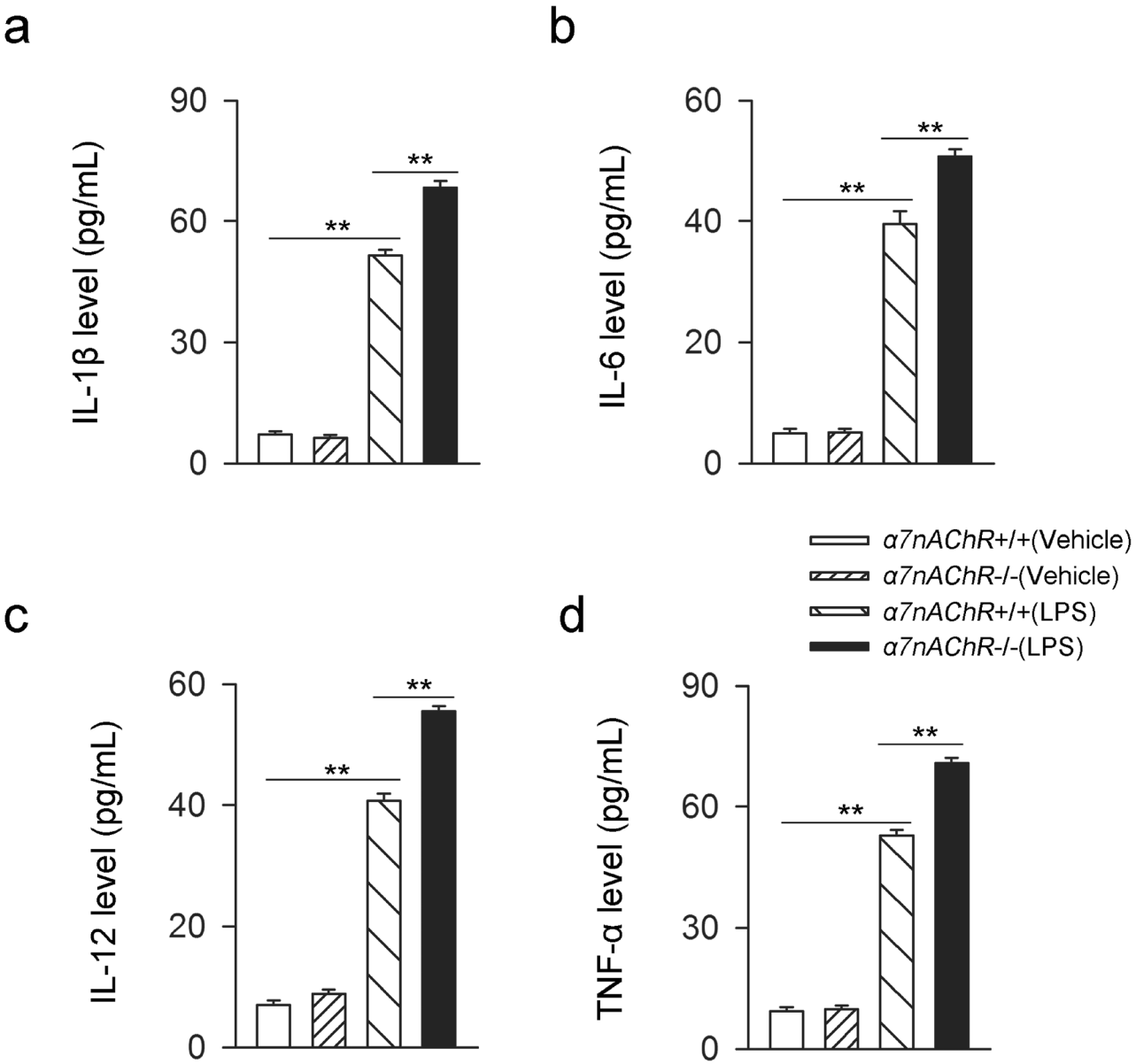

Fig. 3. α7nAChR deficiency increases inflammatory reaction in peritoneal macrophages *in vitro*. Murine peritoneal macrophages were isolated from *α7-nAChR*$^{+/+}$ and *α7nAChR*$^{-/-}$ mice and challenged with LPS (1 μg/mL) as inflammatory stressor. (**a**–**d**) Quantitative analysis of the levels of several proinflammatory cytokines including IL-1β, IL-6, IL-12, and TNF-α in murine peritoneal macrophages after the challenge of LPS for 12 h. $^{**}P < 0.01$; data are presented as mean±SEM.

α7nAChR Deletion Enhances the Level of Proinflammatory Cytokines in Peritoneal Macrophages Under Inflammatory Stress

Since macrophages are one of the major sources of overall inflammatory reaction, we then detected the role of α7nAChR in macrophages. LPS was administrated as inflammatory stress. We found that α7nAChR deficiency led to the significant enhancement of the levels of several proinflammatory cytokines including IL-1β, IL-6, IL-12, and TNF-α in peritoneal macrophages under inflammatory loading (Fig. 3a–d).

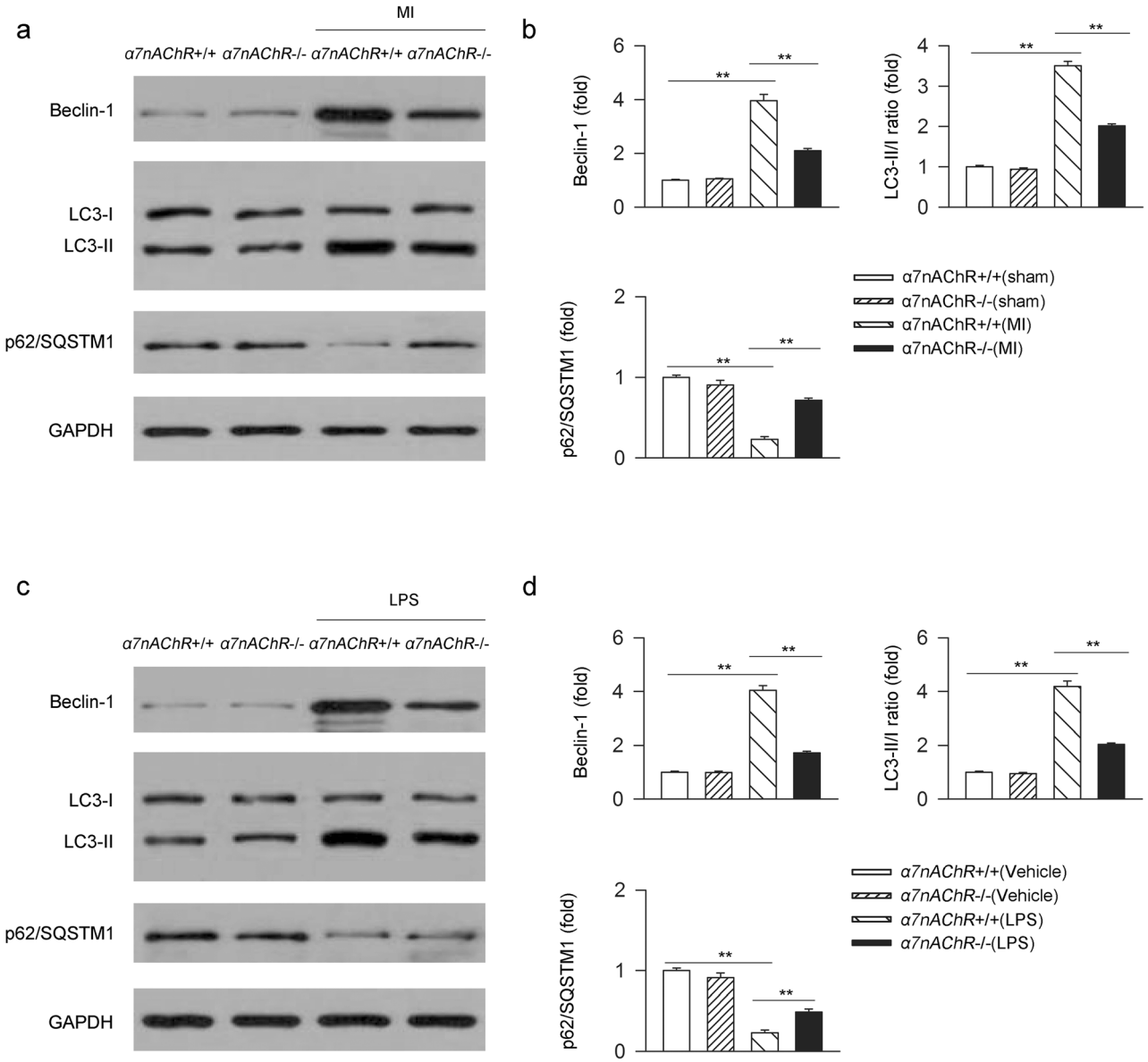

Fig. 4. α7nAChR deficiency results in the dysfunction of autophagy *in vivo* and *in vitro*. (**a**) MI mice models were created through permanent left anterior descending coronary artery occlusion and spleens were isolated at 24-h postinfarction. Levels of autophagy-related proteins including Beclin-1, LC3-II/I, and p62 were detected by Western blot. (**b**) Quantitative analysis of the relative Beclin-1, LC3-II/I ratio, and p62 expression ($n = 6$ per group). $^{**}P < 0.01$; data are presented as mean±SEM. (**c**) Murine peritoneal macrophages were isolated from $\alpha 7nAChR^{+/+}$ and $\alpha 7nAChR^{-/-}$ mice and challenged with LPS (1 μg/mL) as inflammatory stressor. Levels of autophagy-related proteins including Beclin-1, LC3-II/I, and p62 were detected by Western blot. (**d**) Quantitative analysis of the relative Beclin-1, LC3-II/I ratio, and p62 expression ($n = 6$ per group). $^{**}P < 0.01$; data are presented as mean±SEM.

α7nAChR Deletion Is Detrimental in the Induction of Autophagy in Spleen from MI Mice Models and Peritoneal Macrophages Under Inflammatory Stress

Autophagy has been previously reported to produce an alleviative role in the pathogenesis and progression of MI. As a result, we detected the level of autophagy-related proteins including Beclin-1, LC3-II/I, and p62 in spleen isolated from $\alpha 7nAChR^{+/+}$ and $\alpha 7nAChR^{-/-}$ mice with or without left anterior descending coronary artery occluding surgery. We found that α7nAChR deficiency significantly decreased the level of Beclin-1 and LC3-II/I ratio while

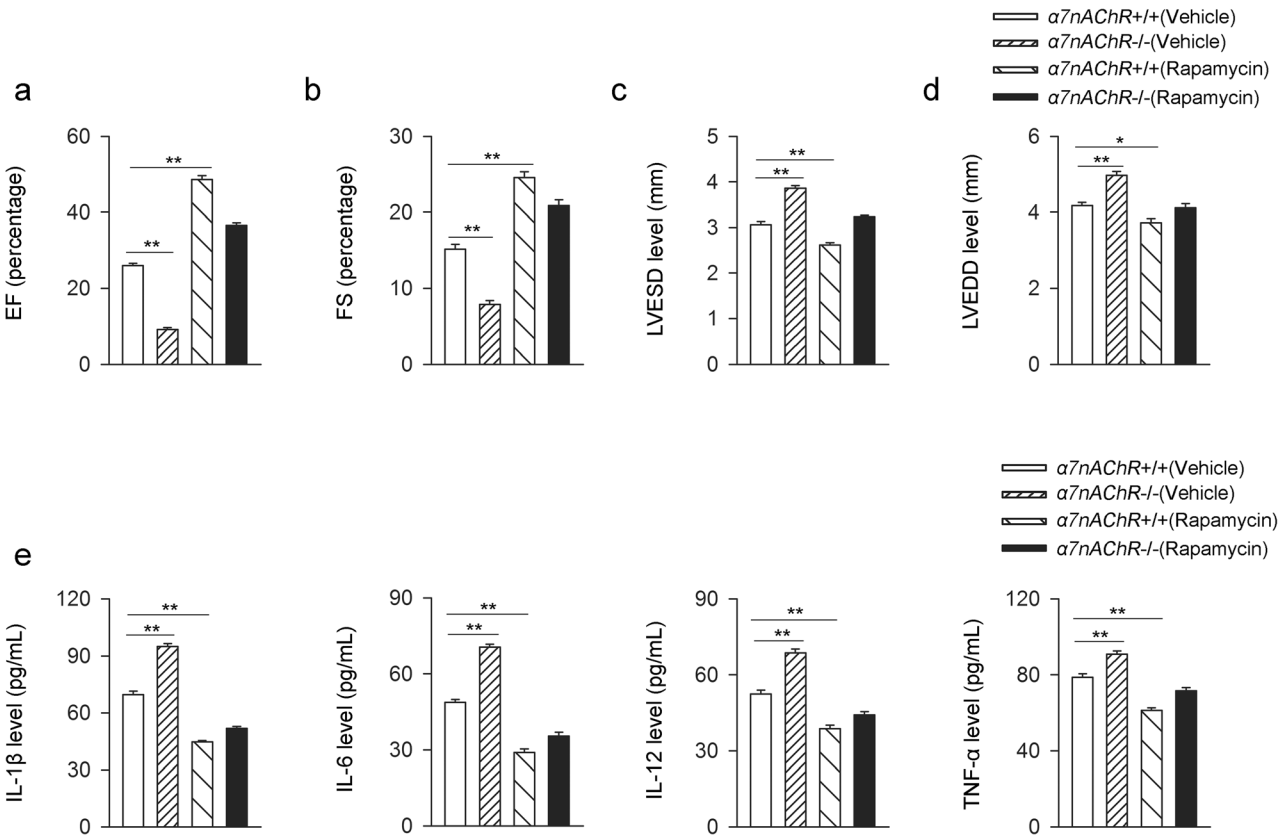

Fig. 5. α7nAChR deficiency attenuates the protective effect of rapamycin in MI mice models *in vivo.* MI mice models were created through permanent left anterior descending coronary artery occlusion and spleens were isolated at 24-h postinfarction. Mice were intraperitoneally injected with rapamycin (2 mg/kg body weight) at 5 min before ischemia. (**a**–**d**) Representative images of MI heart from WT and CB2R KO mice isolated at 24 h postinfarction. Quantitative analysis of 2-D guided M-mode echocardiographic detection including the values of EF, FS, LVESD, and LVEDD at 4-w postinfarction ($n = 7$ per group). $^{*}P < 0.05$, $^{**}P < 0.01$; data are presented as mean±SEM. (**e**) Quantitative analysis of the levels of several proinflammatory cytokines including IL-1β, IL-6, IL-12, and TNF-α in serum at 24-h postinfarction ($n = 7$ per group). $^{**}P < 0.01$; data are presented as mean±SEM.

increased the level of p62 in MI mice models (Fig. 4a, b). Similar trends of changes were detected in peritoneal macrophages under inflammatory stress (Fig. 4c, d). Those data indicates that α7nAChR contributes to the induction of autophagy in macrophages under the challenge of inflammatory stress.

α7nAChR Deletion Attenuates the Alleviative and Anti-inflammatory Effect of Rapamycin in MI Mice Models

We then detected whether autophagy process was involved in the alleviative effect of α7nAChR on the severity of MI and investigated its specific mechanism. We treated mice with rapamycin, an inhibitor of mTOR signaling, for the induction of autophagy. We conducted the echocardiographic examination and assessed the cardiac function of $\alpha 7nAChR^{+/+}$ and $\alpha 7nAChR^{-/-}$ mice undertaking left anterior descending coronary artery occluding surgery with or without the treatment of rapamycin. We found that the administration of rapamycin led to the alleviation of the severity of MI in cardiac function, while knocking out α7nAChR largely attenuated those effects (Fig. 5a–d). We then detected the levels of several proinflammatory cytokines including IL-1β, IL-6, IL-12, and TNF-α in serum and found that rapamycin led to the suppression of the level of proinflammatory cytokines analyzed, while knocking out α7nAChR largely attenuated those effects (Fig. 5e). Those data indicates that autophagy is associated with the alleviated and anti-inflammatory effect of α7nAChR, which is involved in the mTOR signaling pathway.

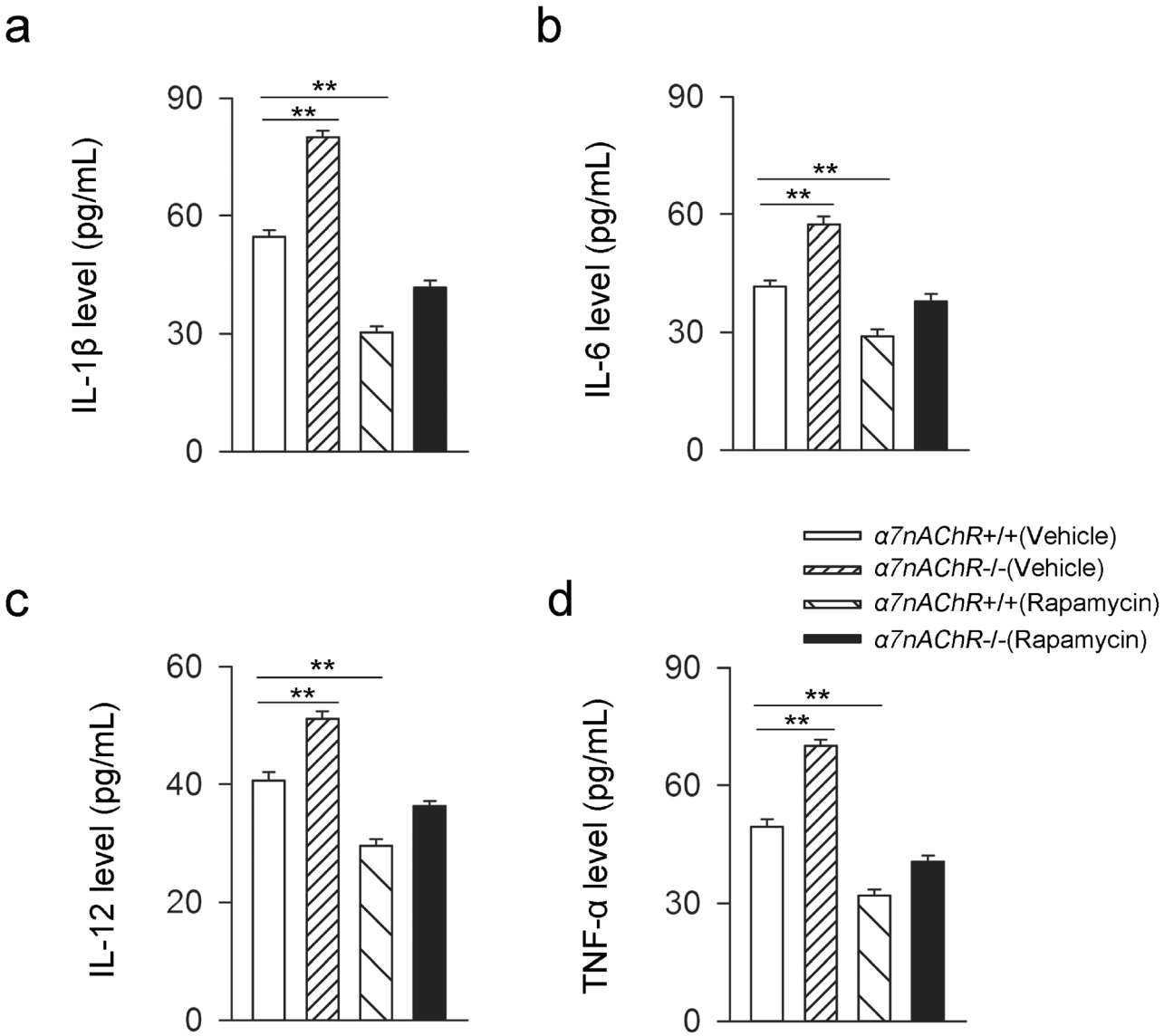

Fig. 6. α7nAChR deficiency attenuates the anti-inflammatory effect of rapamycin in peritoneal macrophages *in vitro*. Murine peritoneal macrophages were isolated from $\alpha 7nAChR^{+/+}$ and $\alpha 7nAChR^{-/-}$ mice and challenged with LPS (1 μg/mL) as inflammatory stressor. Rapamycin was pre-treated (1 μg/L) at 10 min before the challenge of LPS. (**a**–**d**) Quantitative analysis of the levels of several proinflammatory cytokines including IL-1β, IL-6, IL-12, and TNF-α in murine peritoneal macrophages after the challenge of LPS for 12 h. $^{**}P < 0.01$; data are presented as mean±SEM.

α7nAChR Deletion Attenuates the Anti-Inflammatory Effect of Rapamycin in Peritoneal Macrophages Under Inflammatory Stress

We then determined the effect of autophagy on α7nAChR-mediated inflammatory suppression in peritoneal macrophages under inflammatory stress. We treated murine peritoneal macrophages with rapamycin for the induction of autophagy and detected the levels of several proinflammatory cytokines including IL-1β, IL-6, IL-12, and TNF-α. We found that rapamycin led to the suppression of the level of proinflammatory cytokines analyzed, while knocking out α7nAChR largely attenuated those effects (Fig. 6a–d). Those data indicates the association of autophagy with α7nAChR-mediated anti-inflammatory effect, which is involved in the mTOR signaling pathway.

DISCUSSION

α7nAChR has been widely considered to be involved in the systemic anti-inflammatory mechanism, which was called "cholinergic anti-inflammatory pathway" [28]. Previous studies have demonstrated the alleviative effect of α7nAChR in the pathogenesis and progression of MI [10, 29–31]. For example, it was previously demonstrated that post-conditioning with selective α7nAChR agonists, such as PNU-282987 and PNU-120569, markedly reduced infarct size and decreased the levels of inflammatory cytokines, including TNF-α, IL-6, and HMGB1 in a rat model of MI injury, indicating the therapeutic value of post-conditioning α7nAChR agonist in the treatment of MI through the suppression of inflammatory reaction [29, 30]. In addition, Wu et al. reported that eliciting cholinergic anti-inflammatory pathway *via* the activation of α7nAChR exerted an anti-arrhythmogenic effect against ischemic cardiomyopathy-induced ventricular arrhythmia accompanied by downregulation of cytokines, downgeneration of collagens and decrease in sympathetic/parasympathetic ratio, indicating the anti-inflammatory effect of α7nAChR on cardiac protection [10]. To further demonstrate the effect of α7nAChR on MI, we used α7nAChR global knockout mice ($\alpha 7nAChR^{-/-}$) as well as its wild type mice ($\alpha 7nAChR^{+/+}$) for the creation of MI model. We showed that knocking out α7nAChR aggravated the severity of MI through the enhancement of cardiac infarct size and damage of cardiac function. We further demonstrated the level of systemic inflammatory reaction through the detection of several inflammatory cytokines including IL-1β, IL-6, IL-12, and TNF-α in both serum and spleen, the most important inflammatory and immune organ. We found that α7nAChR deficiency enhanced the level of systemic inflammatory response on the occurrence of MI. We then isolated murine primary macrophages and found that α7nAChR deficiency largely increased the level of inflammatory reaction under the challenge of inflammatory stress. Taken together, those data indicated that α7nAChR contributed to the alleviation of MI through the suppression of systemic inflammatory reaction.

Autophagy is regarded as a self-eating cellular catabolic pathway, through which long-lived proteins, damaged organelles, and misfolded proteins are degraded and recycled for the maintenance of cellular homeostasis and normal cellular functions, thus involving the regulation of various kinds of diseases [16]. Autophagy has been demonstrated to suppress inflammation in several disorders [22, 32, 33]. For autophagy in MI, it was previously reported that induction of autophagy produced a protective effect of cardiomyocytes against ischemic injury [34]. In addition, autophagy was also reported to contribute to the alleviation of MI through the suppression of inflammatory response [35]. Based on those previous studies, we found that autophagy was largely enhanced in spleens isolated from MI mice as well as murine macrophages under the challenge of inflammatory stress. In addition, the administration of rapamycin for the induction of autophagy significantly attenuated the severity of MI in restoring cardiac function on the occurrence of MI *via* the suppression of systemic inflammatory reaction as well as inflammatory response in macrophages. Those results demonstrated the alleviative effect of autophagy in MI *via* inflammatory suppression.

α7nAChR has been demonstrated to play an suppressive role of the severity of MI [10, 29–31]. However, the specific mechanisms remain unclarified. Here in our current study, we reported that autophagy was involved in the α7nAChR-mediated cardio-protective and anti-inflammatory effect in MI. We demonstrated that knocking out α7nAChR led to the significant decrease of the level of autophagy in spleen from MI mice models. Similar trends of changes were shown in murine primary peritoneal macrophages obtained from $\alpha 7nAChR^{+/+}$ and $\alpha 7nAChR^{-/-}$ mice under the challenge of inflammatory stress. Rapamycin is widely acknowledged as an effective autophagy inducer targeting on mTOR in various kinds of cells, including macrophages, neurons, and cardiomyocytes, thus functioning in the alleviation of several kinds of disorders *via* inflammatory suppression [36–38]. Here in this study, we further demonstrated that knocking out α7nAChR largely disturbed the alleviative effect of cardiac function damage on the occurrence of MI as well as inflammation-suppressive effect mediated by the administration of rapamycin for the induction of autophagy. Those results indicated that α7nAChR-mediated cardio-protective and anti-inflammatory effect in MI was associated with autophagy.

It has been widely acknowledged that mTOR is an important upstream regulator of autophagy process, receiving activating or inhibiting inputs from stress sensors [39–42]. The mTOR signaling was previous reported to be involved in the regulation of the pathogenesis and progression of MI [43, 44]. Here in this study, we used rapamycin targeting on the inhibition of mTOR signaling. We found that the inhibition of mTOR signaling by rapamycin significantly led to the alleviation of MI severity and

suppression of inflammatory reaction. However, those effects of rapamycin were largely attenuated by knocking out α7nAChR. These data indicate the association of autophagy with α7nAChR-mediated anti-MI and inflammation-suppressive effects, which is involved in the mTOR signaling pathway. However, the alleviative effects of rapamycin on MI severity and inflammatory reaction were also observed in α7nACh-deficient mice despite a significant attenuation compared to those in $\alpha 7nAChR^{+/+}$ mice. In our opinion, the reason might lie in the fact that rapamycin could function in diseases *via* several other signaling pathways despite mTOR signaling [45–47]. As a result, the effects of rapamycin on MI could not be totally abolished by the modulation of α7nAChR. To further figure out this issue, more studies are demanded.

Taken together, here in this study, we demonstrated that α7nAChR deletion could aggravate myocardial infarction and enhance systemic inflammatory reaction, which was proved to be involved in the mTOR-related autophagy process. We believe that this study might provide strong insight in the development of novel drugs in the treatment of MI.

FUNDING INFORMATION

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that they have no conflict of interest.

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