Eplerenone inhibites atrial autonomic nerve remodeling via the ERK1/2 MAPK pathway in a rabbit model of atrial fibrillation

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Research Article

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Abstract

Aims:

Aldosterone is closely associated with atrial fibrillation, and mineralocorticoid receptor antagonists (MRAs) have been proved to be effective in preventing atrial structural remodeling. Atrial autonomic nerve system (ANS) plays an important role in atrial fibrillation (AF). However, the effects of MRAs on ANS remodeling in AF and the underlying mechanisms are still unknown.

Main methods:

Twenty-one rabbits were randomized into sham, pacing(P), and pacing + eplerenone(P + E) groups. HL-1 cells were subject to control treatment or rapid pacing with or without eplerenone or U0126 (an inhibitor of ERK1/2). Atrial sympathetic and parasympathetic remodeling was detected by immunohistohistochemical analysis, western blotting and reverse transcription-polymerase chain reaction. Circulating neurohormone levels and atrial electrophysiology were also assessed.

Key findings:

The ERK1/2 MAPK pathway was significantly activated in AF rabbit/HL-1 cell models, resulting in the upregulation of key downstream proteins; this effect was significantly restored by eplerenone($P<0.05$). Eplerenone also prevented the changes in circulating neurohormone levels, reduced the mRNA levels of sympathetic- and parasympathetic-related growth factors, and inhibited the inducibility and duration of AF.

Significance:

Eplerenone inhibited atrial autonomic nerve remodeling and the occurrence of AF by modulating the ERK1/2 MAPK pathway.

Introduction

Atrial fibrillation (AF) represents the most common arrhythmia worldwide, and is related to an increased risk of ischemic stroke or systemic embolism[1, 3]. The initiation and progression of AF result from atrial remodeling, including electrical, structural and contractile remodeling, which has been shown to contribute continuously to the self-perpetuating nature of AF[2]. However, the detailed mechanisms underlying AF that involve the autonomic nervous system(ANS) are not fully understood.

The cardiac ANS is considered to play an important role in the mechanism underlying the initiation and maintenance of AF. Previous studies have suggested that the cardiac ANS plays a key role in the process of “AF begets AF”[4]. Considerable evidence has suggested sympathetic hyper-innervation in the fibrillating atrium, with evidence of increased sympathetic nerve firing in persisted patients, as well as in sustained AF animal models[5–7]. Our previous work also reported extensive sympathetic and vagal

sprouting in both AF and OSA canine models[8–10]. But to date, the exact mechanisms underlying atrial autonomic remodeling are unknown.

Progressive interstitial changes provoked by mitogen-activated protein kinase (MAPK) increase the risk of AF[11]. The three most important members of the MAPK superfamily are p38MAPK, extracellular signal-regulated kinase1/2 (ERK1/2) and c-jun N-terminal kinase (JNK). A previous study showed that the ERK1/2 pathway is associated with the activity of ANS[12]. However, whether ERK1/2 takes part in autonomic nerve remodeling and contributes to atrial fibrillation is unknown.

Recent studies reported that high aldosterone levels might lead to atrial fibrosis and thereby contribute to human AF[13]. Studies in dog models showed that aldosterone blockade can halt fibrosis formation and is presumed to prevent atrial fibrillation[14]. Eplerenone, a selective aldosterone receptor blocker, has therapeutic value in preventing cardiovascular diseases and associated end-organ damage[15]. However, the effects of eplerenone on atrial nerve remodeling associated with AF have not been determined. Therefore, the present study aimed to investigate whether the ERK1/2 MAPK pathway is involved in the effect of eplerenone on atrial nervous remodeling associated with AF.

Materials And Methods

Ethical approval

This study was approved by the Animal Use and Management Ethics Committee of the First Affiliated Hospital of Harbin Medical University. The use of animals and all procedures were in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication 2011; eighth edition). Twenty-one New Zealand white rabbits weighing 2.5-3.0kg were raised in individual cages in the animal center of the First Affiliated Hospital of Harbin Medical University. The temperature in the room was maintained at 23°C under a 12-h light-dark cycle. To determine the effect of eplerenone on a model of atrial fibrillation established by rapid right atrial pacing, the rabbits were randomly categorized into three groups: (1) sham-operated group (sham, n=7) with sutured electrodes and no pacing; (2) rapid atrial pacing group (P , n=7), with sutured electrodes and atrial rapid pacing at 600 beats/min for three weeks; (3) eplerenone treatment group (P+E, n=7) with eplerenone-administered p.o. (Pfizer, Inc., New York, NY, USA) at a dose of 50 mg /day for 28 days and pacing the right atria for three weeks, beginning on the eighth day.

Rabbit AF model

The AF model was established based on our previous studies [17]. The rabbits were anesthetized by the intravenous injection of ketamine (30-35mg/kg; Sigma Aldrich, St Louis, MO, USA) and xylazine (5mg/kg, Sigma Aldrich). After anesthesia, the rabbits were intubated through the nasal cavity and connected to the ventilator. After the pericardium was opened, one end of the unipolar epicardial electrode (Medtronic, US) was sutured to the right atrial appendage and the other side was attached to the pacemaker (Fudan University, Shanghai, China) that was placed in a subcutaneous pocket in the backs of rabbits. After one

week of recovery, the pacemaker began to work to make the right atrial rapid pacing (RAP). Electrocardiogram was randomly checked during the pacemaker operation to ensure the working of a pacemaker. After three weeks of pacemaker operation, atrial electrophysiological tests and sampling were performed. All atrial tissues and serum were stored at -80°C.

Cell culture

To determine whether ERK1/2 MAPK plays a critical role in the autonomic remodeling associated with AF, we used an HL-1 cell model of AF, which was established by using YC-2 stimulator (Chengdu, China). The experimental procedure is based on our previous studies[17].

To investigate the role of eplerenone in the regulation of GAP-43, ChAt and ERK1/2 expression induced by tachypacing in vitro, HL-1 cells were categorized into three groups: (1) control group: cultured for 24 h, no rapid field stimulation or other drug interventions; (2) pacing group (P): rapid field stimulation for 24 h as aforementioned; (3) eplerenone and pacing group (P+E): rapid field stimulation for 24 h with eplerenone (4μm/L, CAS No; 107724-20-9, MedChemExpress, eplerenone concentration screening in Supplementary Materials).

To evaluate the effects of MAPK inhibitors on the expression of GAP-43 and ChAt were also evaluated in cells. The HL-1 cells were divided into four groups: (1) control group: cultured for 24 hours, no rapid field stimulation or other drug interventions; (2) pacing group: rapid field stimulation for 24 hours as aforementioned; (3) pacing and U0126 group: rapid field stimulation for 24 hours with U0126 (10μm/L, an ERK1/2 inhibitor; CAS No;109511-58-2, MedChemExpress, U0126 concentration screening in Supplementary Materials) pretreatment for 1 h; and (4) U0126 group:cultured for 24 h with U0126.

Biochemical measurements

Laboratory measurements were performed under blinded conditions. Three sets of blood samples were collected from the hearts of rabbits after three weeks of rapid atrial pacing. The samples were centrifuged at 3500×g for 15 min. Norepinephrine (NE), acetyl choline (ACH) and aldosterone (ALD) level measurement kits were purchased from Jiancheng Biological Technical Institute (China) and measurements were performed according to the manufacturer's instructions.

Atrial electrophysiological measurements

The electrophysiological measurements were performed before the collection of blood samples according to the method described in our previous study[17]. After anesthetization, intubation and mechanical ventilation, left side thoracotomy was performed. A four-electrode was sutured to the free wall of the right atrial appendage. The tail end of the electrode was connected to the Prucka32 lead electrophysiological recorder to record the body surface and intracardiac electrocardiogram synchronously. AF was induced by burst stimulation applied to the right atrium with 10 Hz, 2-ms stimuli for 1–10 s. If atrial fibrillation lasted for 30 s, synchronous direct current cardioversion was performed.

The duration of atrial fibrillation was recorded for 30 s, and the electrophysiological test was continued after 1 minutes.

Immunohistochemical analysis

Rabbit atrial tissue specimens were fixed using 10% formalin and sliced into sections of 3-5 μm thick and then embedded in paraffin. The specimens were stained with hematoxylin and eosin (HE) and Masson's stains and examined under a light microscope. Cardiomyocytes appear red and collagen appears blue in the Massen's staining. Myocardial collagen volume fraction (CVF) was analyzed and measured using a digital medical image analysis system (HPISA-1000, Olympus, Shinjuku, Japan). CVF was calculated as myocardial collagen area/total area of the field, and values of this parameter were randomly selected from each of five tissue sections. HE and Masson's staining was performed to evaluate fibrotic deposition in rabbit atria.

To observe the remodeling of the atrial autonomic nerve, atria tissue wax block was incubated with anti-growth-associated protein 43 (GAP-43, Abcam), anti-tyrosine hydroxylase (TH, Abcam), anti-nerve growth factor (NGF, Abcam) and anti-choline acetyltransferase (ChAT, Abcam) overnight at 4℃. The atrial tissue was rinsed three times, and then peroxidase-conjugated goat anti-rabbit IgG (1:1000, Abcam) was added and incubated for 10 minutes. Finally, a diaminobenzidine solution was used to visualize the specimen. The immunohistochemical analysis of the atrial autonomic nerve was performed using a digital medical image analysis system (HPISA-1000, Olympus, Shinjuku, Japan).

Real-time quantitative polymerase chain reaction

After atrial tissue was homogenized, total RNA was extracted using TRIzol (CWbio. Co. Ltd, Beijing, China) and a real-time quantitative polymerase chain reaction was performed according to a previous study[18].

Western blotting

Proteins were extracted from rabbit atria tissue specimens or HL-1 cells. The protein content was determined by using the Bradford method according to our previous study[19]. The same amount of protein (40μg) was loaded per lane and separated by 8–12% sodium dodecy1 sulfate-polyacrylamide gel electrophoresis. The proteins were then transferred to polyvinylidene difluoride membranes. The membranes were incubated overnight at 4°C with primary antibodies against ChAT (1:500, Abcam), GAP-43 (1:500, Abcam), Pan-actin (1:1000, sigma) and ERK1/2(1:500, CST). The bands were visualized using Super-Signal West Femto Chemiluminescent Substrate (Thermo Scientific, Waltham, MA, USA) and quantified by scanning densitometry(Chemi-DOC, Bio-Rad, Laboratories, Hercules, CA, USA).

Data and statistical analyses

Quantitative data are presented as mean ± standard error of the mean. One-way analysis of variance was performed for comparing continuous parameters among three or more groups and Tukey’s post-hoc test

was performed to test significant differences. A P value of <0.05 was considered statistically significant. Figures were constructed using the GraphPad Prism 8.0 software. The data and statistical analyses comply with the recommendations on experimental design and analysis in pharmacology.

Results

orphological changes

HE staining was performed to assess the morphological changes of atrial myocytes (Fig.1A). In the sham operation group, HE staining showed properly arranged atrial myocytes, aligned nuclei and less stroma around the nuclei. Conversely, the AF group showed disordered and loose arrangement or breakage of cardiomyocytes, heterotypic nuclei, and connective tissue hyperplasia, with myocardial cell spacing significantly widened. Eplerenone treatment alleviated these changes. As shown in figure1B, RAP exacerbated interstitial fibrosis in atria. CVF was significantly higher in the AF group than the sham group and was reversed by eplerenone treatment(Fig.1C).

Atrial electrophysiological measurements

In the right atrial rapid pacing rabbit model, atrial fibrillation inducibility was significantly increased in the AF group compared with the sham group at baseline. However, compared with the AF group, the incidence of AF was markedly reduced in the eplerenone treatment group (Fig. 2B). Similar to AF inducibility, atrial fibrillation duration was increased in the AF group and was reduced by eplerenone(Fig. 2C).

Measurement of the vagus and sympathetic nerve activity

Serum NE, ACH, and ALD levels are important indicators of parasympathetic and sympathetic activity. Serum NE, ACH and ALD were detected from blood samples collected after electrophysiological test in rabbit models. As shown in figure 3A, serum NE level was distinctly increased in the AF group compared with the sham group. However, the change was partly reversed by eplerenone treatment (Fig. 3A). Serum ALD and ACH levels were significantly increased and reversed in the eplerenone treatment group (Fig.3B, C).

mRNA expression of Cx40, Cx43, M2 , β1, and β2 receptors

The expression of Cx40 and Cx43 was significantly increased in the AF atria group compared with the sham group and was reversed in the eplerenone treatment group (Fig.4A, B). Moreover, the changes were observed in the ratio of Cx40/Cx43 (Fig.4C). These finding indicated that gap junction was impaired after atrial rapid pacing for three weeks, and that the damage could be reversed by eplerenone.

The mRNA levels of M2, β1, and β2 receptors were increased markedly in the AF group compared with the sham group. However, eplerenone treatment could reverse these changes (Fig.4D, E, F).

Protein expression of ChAt, GAP-43 , ERK1/2

The expression of ERK1/2, ChAt, and GAP-43 proteins was significantly upregulated in the AF group compared with the sham group at baseline and was reversed by eplerenone treatment (Fig.5A, B, C, D, E, F).

Autonomic nerve remodeling

Sympathetic and parasympathetic remodeling in the atria of rabbits was further examined, and RAP-induced extensive sympathetic and parasympathetic sprouting and change in the distribution of nerve fibers were observed. TH is an important marker expressed on sympathetic nerves, whereas ChAT is a marker for parasympathetic nerves. NGF plays an important role in nerve growth and injury repair. GAP-43, a marker of newly formed nerve fibers, can induce on nerve sprouting. We detected the mRNA expression of ChAT, GAP-43, TH and NGF, which were sharply increased in the atria of the AF group compared with the sham group. However, eplerenone treatment alleviated these changes (Fig.5H, I, J, K). In addition, TH, GAP-43, NGF and ChAT positive fiber density was higher in both left and right atria of AF rabbits than that in sham rabbits. However, eplerenone treatment reversed these changes (Fig.6).

ERK1/2 MAPK mediated the over-expression of GAP-43 and ChAt in HL-1 cells

Consistent with animal experiment data, the protein expression of p-ERK1/2, GAP-43, and ChAt in HL-1 cells was obviously increased in the pacing group and was attenuated by eplerenone treatment (Fig.7A, B, C, D, E). In addition, ERK1/2 sinhibitors significantly suppressed the upregulation of GAP-43 and ChAt proteins (Fig.7F, G, H).

Discussion

Main findings

We have demonstrated that significant parasympathetic and sympathetic nerve remodeling were present in vitro and in vivo AF model. Eplerenone markedly suppressed atrial autonomic nerve remodeling by inhibiting the ERK1/2 MAPK signal pathway.

Excess aldosterone levels are involved in atrial structural remodeling in atrial fibrillation

The pathological features of atrial remodeling in atrial fibrillation are atrial fibrosis and atrial enlargement[20]. Aldosterone is closely related to atrial fibrosis and contributes to human AF[21]. A previous study suggests that aldosterone may provide a substrate for atrial fibrosis and thereby promotes AF[22]. Aldosterone upregulate profibrotic mediators and collagen synthesis, which can be prevented by mineralocorticoid receptor blockers[22]. Our findings are consistent with the previous clinical and animal studies. The increase in serum aldosterone level was obvious in the AF group. Masson's staining revealed that CVF was significantly increased in the AF group, indicating that RAP in rabbits resultes in obvious atrial fibrosis. However, these changes were attenuated by eplerenone. Furthermore, Cx40 and Cx43 levels

were significantly increased in the AF group, indicating that gap junction was impaired after atrial rapid pacing and lead to abnormal transmission and atrial fibrillation[23].

Atrial ANS remodeling and AF

Atrial ANS remodeling may be an important basis of atrial fibrillation, and the activation of the sympathetic nerve and parasympathetic nerve has different effects on atrial electrophysiology[24]. Although previous studies have assessed the role of atrial fibrillation in the progression of atrial fibrillation (“ AF begets AF ”), studies have explored the effects of atrial fibrillation on autonomic nerve activity, atrial ANS remodeling and reflex function (“AF begets ANS remodeling”)[20]. Autonomic nerve remodeling promotes the occurrence and development of atrial fibrillation. Atrial fibrillation itself promotes the remodeling and activation of autonomic nerves, which may further promote the progress of arrhythmias, thus forming a mutually reinforcing vicious circle. Lu et al.[25] reported that ablation of major atrial parts could prevent both autonomic nerve remodeling and electrical remodeling induced by RAP. Sympathetic fibers and sympathetic activity were significantly increased in canine atrial fibrillation models, leading to increased susceptibility to atrial fibrillation[5]. Ogawa et al.[26]also reported that sympathetic and parasympathetic nerve activities are strengthened before the onset of atrial arrhythmias in dogs with pacing-induced congestive heart failure. In the present study, we also found that autonomic nerve sprout was significantly increased in RAP rabbits and was reversed by eplerenone treatment. In order to elucidate the specific mechanism underlying the autonomic nerve remodeling in atrial fibrillation, we investigated the effects of sympathetic and parasympathetic nerves on atrial fibrillation.

Previous findings hint that the vagal nerve plays a more pronounced role in the initiation and maintenance of AF[27], and its distribution pattern can alter atrial electrophysiology[28]. As one of the specific markers of cholinergic neurons, choline acetylase (ChAT) is synthesized in the soma of cholinergic neurons, transported to the nerve endings, and finally released[29]. In our study, we found that the mRNA and protein expression of ChAt significantly increased both in vivo and in vitro AF models and was inhibited by eplerenone or ERK1/2 inhibitors. Cholinergic M2 receptors are mainly distributed in atrial tissues and are major mediators of parasympathetic nerve control of cardiac function. ACh bonds to the muscarinic cholinergic receptor and activates the ACh-regulated potassium current ($I_{K\text{-}ACh}$), which mediates shorting of ERP[30]. Shortened ERP can significantly increase the incidence of atrial fibrillation. In the present study, we found that vagus nerve sprouting and M2R were upregulated in RAP rabbits, indicating that the upregulation of M2R participated in parasympathetic remodeling in RAP models and lead to an increased susceptibility to atrial fibrillation. In addition, parasympathetic activity was detected. Serum ACH and ALD levels were significantly increased in AF models. However, eplerenone could reverse these changes.

Although the sympathetic effect on atrial electrophysiological parameters appears to be less significant in normal subjects, it is more important in the development of remodeled atrial fibrillation[31]. Sharifov et al.[27] reported that combined intravenous infusion of isoproterenol and acetylcholine increased the

susceptibility and duration of AF compared with acetylcholine alone. Similarly, another study reported that several sympathetic fibers in the atria and pulmonary veins in dogs with atrial fibrillation are associated with the high incidence of atrial fibrillation[32]. Atrial heterogeneous sympathetic hyperinnervation was found in experimental dogs with ventricular myocardial infarction, which might account for the increased AF vulnerability[33]. Gould et al. [34]reported that TH expression and NE content in atrial fibrillation patients were higher than those in the sinus rhythm group, which provides direct evidence for the presence of autonomic nerve, especially sympathetic nerve remodeling in patients with atrial fibrillation. In our study, we found that TH positive fiber density was higher in both left and right atria of AF rabbits than that in sham rabbits. Furthermore, we attempted to elucidate the possible mechanism of sympathetic nerves enhancement in AF models. GAP-43, a marker of nerve sprouting, was increased both in the atria of AF animal models and HL-1cells. However, these changes were reversed by eplerenone. Similar to GAP-43, the mRNA levels of β1 and β2 receptors were up-regulated by RAP in rabbits. However, these changes could be reversed by eplerenone.

The importance of autonomic imbalance in AF has been shown by studies. The combined firing of sympathetic and parasympathetic nerves has been demonstrated immediately prior to the onset of atrial arrhythmias in animal models of AF[26,35] and in clinical studies[36]. The findings of our study are consistent with those in previous studies. The parasympathetic and sympathetic activity was detected, and serum ALD and ACH levels were significantly higher in AF models, whereas NE increased only a little. The autonomic imbalance was reversed by eplerenone. Therefore, adrenergic and cholinergic nerves are activated in varying degrees during AF, which eventually leads to an imbalance in the automatic nervous system.

NGF has been shown to be a key stimulus underlying the sprouting of both sympathetic and parasympathetic nerves in the heart. An increase in atrial tissue was reported in an animal model of AF and also in the human AF atrium[37-39]. In the present study, the mRNA expression of NGF and nerve staining with NGF were higher in the atria of the AF group, indicating that atrial autonomic nerve remodeling and overactivation may be related to NGF oversecretion.

ERK1/2 MAPK pathway participates in atrial ANS remodeling in AF

MAPK, a large family of serine-threonine kinases including p38 MAPK, ERK1/2, and JNK, participates in atrial structural remodeling in AF[40]. However, the correlation between MAPKs and ANS remodeling is unknown. The expressions of ERK1/2, GAP-43, and ChAt were upregulated in pacing HL-1 cells, whereas the up-regulation of ERK1/2 and MAPK was inhibited by eplerenone. These changes combined with in vivo results suggest that eplerenone can inhibit atrial autonomic nerve remodeling, possibly via the ERK1/2 MAPK pathway.

MRA plays an important role in preventing ANS remodeling

Some studies have found that mineralocorticoid receptor antagonists (MRA) reduces atrial fibrillation[14,41]. Some meta-analysis studies have proved that MRA significantly reduces new-onset AF and recurrent AF[42,43]. In our study, we found that eplerenone played an important role in preventing autonomic nerve remodeling of atrial fibrillation. Our results proved the effectiveness of eplerenone in atrial fibrillation both in vivo and in vitro. We also elucidated the possible mechanism behind the phenomenon.

To our knowledge, this is the first study to show that eplerenone can inhibit atrial autonomic nerve remodeling, and it can ameliorate atrial electrical and structural remodeling. Therefore, eplerenone could be considered a promising novel upstream therapy for AF treatment.

Conclusion

We demonstrated that the ERK1/2 MAPK pathway participates in atrial autonomic nerve remodeling associated with AF, and eplerenone inhibites atrial autonomic nerve remodeling by regulating the ERK1/2 MAPK pathway. Our study provides novel insights into the pharmacological role of eplerenone against AF.

Declarations

Author Contributions Wei Xu was responsible for the data acquisition, analysis, interpretation and writing - original draft of the paper. Wei Xu and Qiang Gao were responsible for the establishment of animal model, atrial electrophysiological detection and tissue sampling. Yue Yuan was in charge of cell culture. Yun Zhang and Jing Shi contributed to the data acquisition, analysis, and interpretation. Qiang Gao and Ding-yu Wang did the required quantitative RT-PCR, western blloting and Immunohistochemical analysis, subsequent data analysis. Yue Li and Guang-zhong Liu were the corresponding author and took overall responsibility for the conducted study and final revision of the manuscript; they contributed to the development of the study design.

Data Availability Not applicable.

Compliance with Ethical Standards

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Conflict of interest The authors declare no conflicts of interest

Ethics Approval The use of animals and all procedures were in agreement with the Guide for the Care and Use of Laboratory Animals (NIH Publication 2011, eighth edition) and were approved by the Animal Care and Use Committee of the Harbin Medical University (Harbin, China)

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Supplemental Materials

Supplemental Materials were not provided with this version

Figures

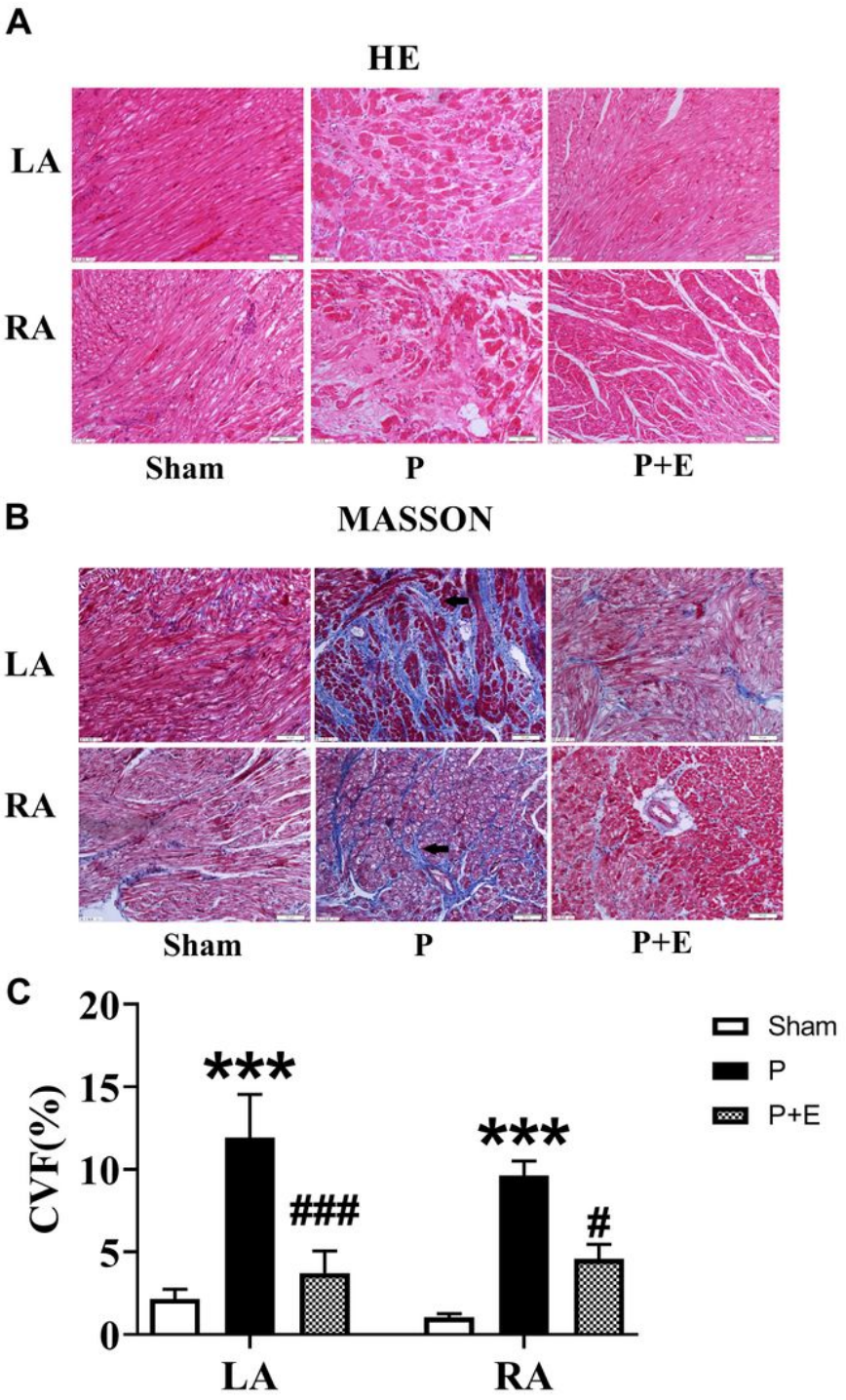

Figure 1

Atrial Morphological changes. P=AF group. P+E=AF+Eplerenone (A) HE stains in the left and right atrial myocardium of the groups (20×magnification). (B) Masson's stain in the left and right atrial myocardium of the three groups (20×magnification). (C) Collagen volume fraction (CVF %) of left and right atria in each group. ***P <0.001 vs. sham group; #P <0.05, ###P <0.001 vs. P group; n = 6 per group.

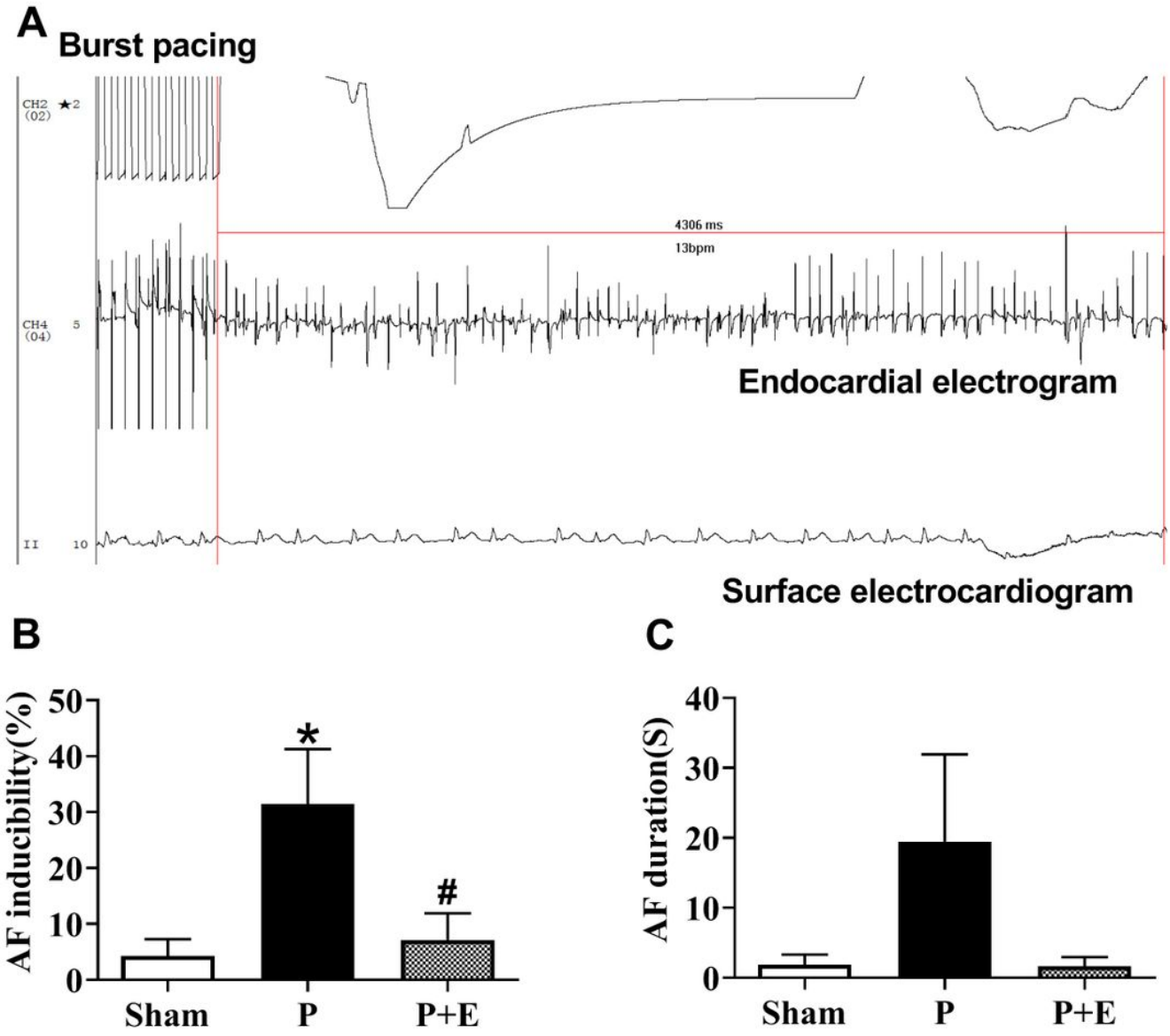

Figure 2

Electrophysiological measurements. (A) AF was induced after rapid-pacing. (B) AF inducibility. (C)AF duration. *P <0.05 versus sham group, #P <0.05 versus AF group, n = 7 per group.

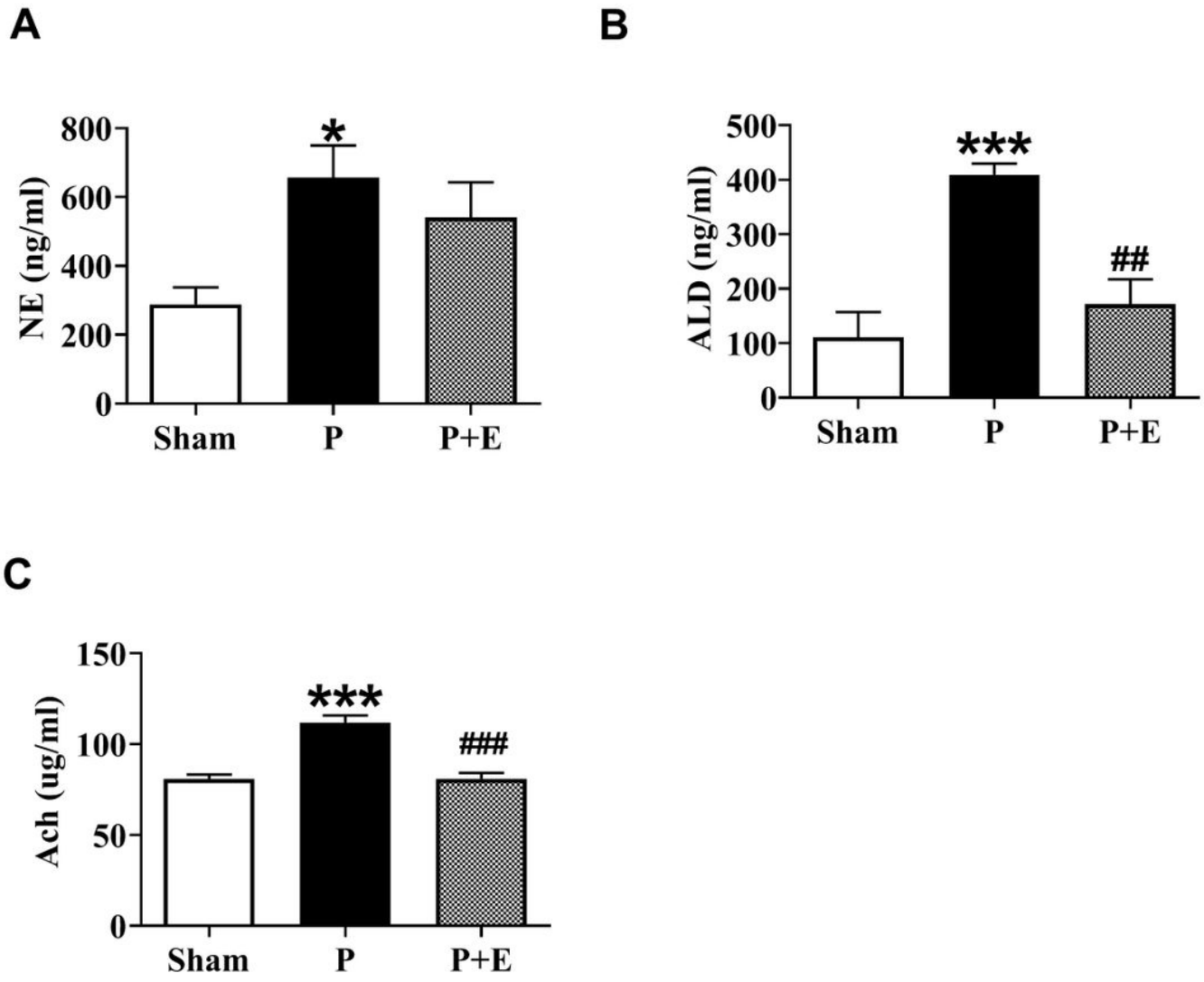

Figure 3

Measurement of vagus and sympathetic nerve activity. (A) Serum NE concentration in each group. (B) Serum ALD concentration in each group. (C) Serum ACH concentration in each group. *P <0.05, ***P < 0.001 vs. sham group, ##P <0.01 , ###P <0.001 vs. P group; n = 6 per group.

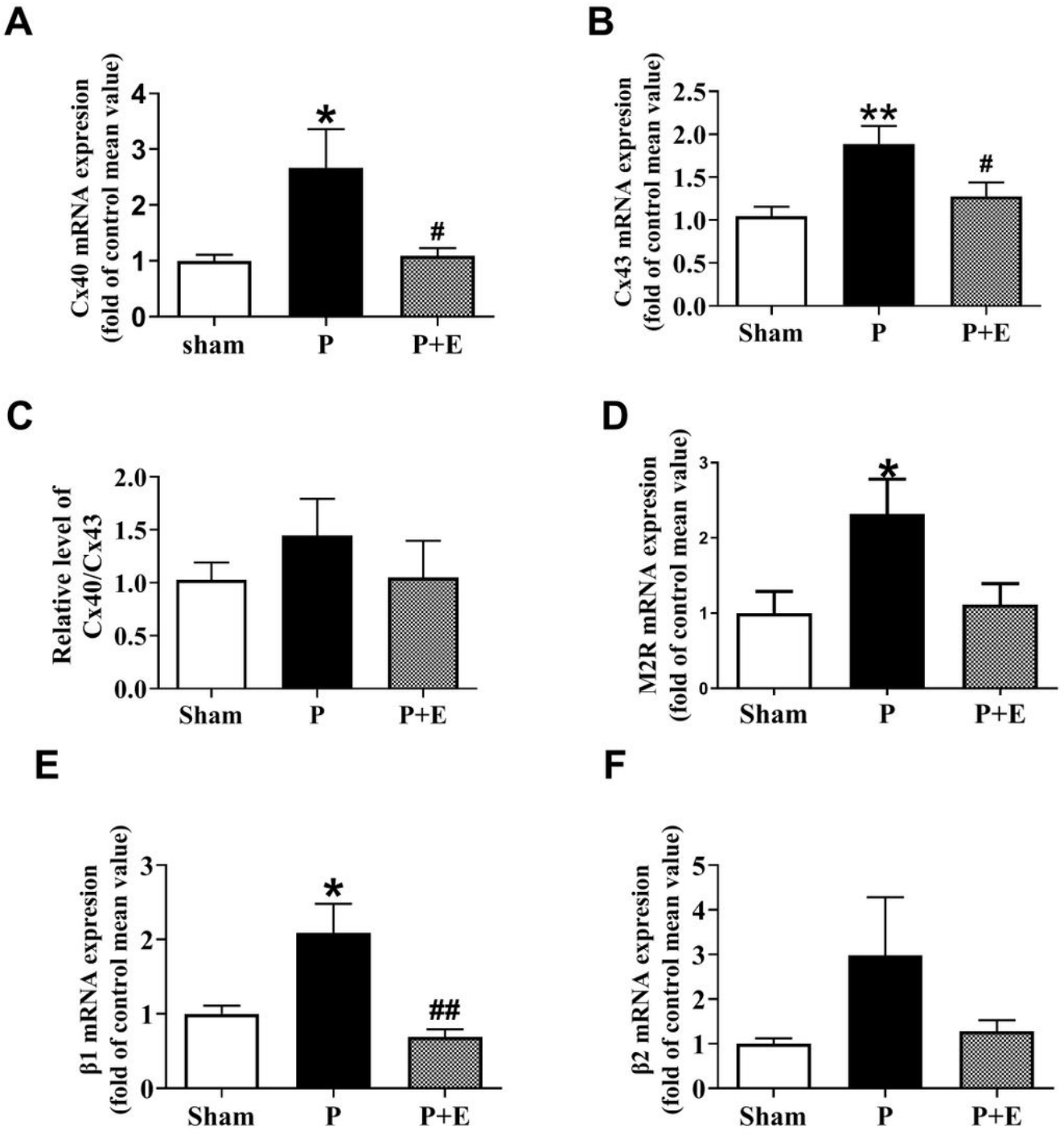

Figure 4

mRNA expression of connexin (Cx)40, Cx43, M2, β1, and β2 receptors in the atria of each group. (A) The mRNA expression of Cx40. (B) The mRNA expression of Cx43 in atrial tissue. (C)The ratio of Cx40/Cx43. (D) The mRNA expression of M2 receptor (E) The mRNA expression of β1 receptor in atrial tissue. (F) The mRNA expression of β2 receptor in atrial tissue. *P<0.05, **P<0.01 vs. sham group, #P<0.05, ##P <0.01 vs. P group; n = 6 per group.

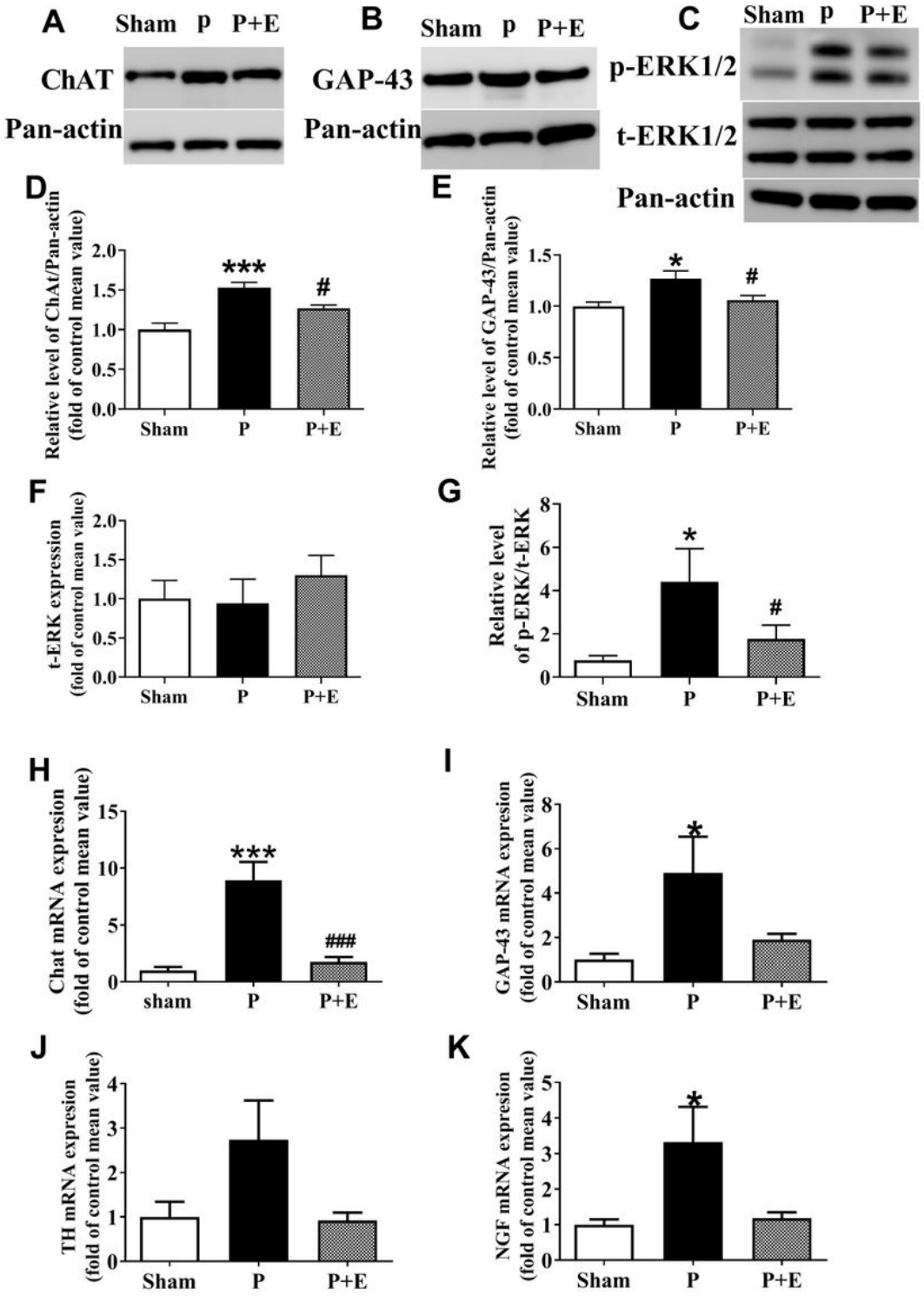

Figure 5

Expression of TH, GAP-43, NGF, ChAT and ERK1/2. (A, B, C, D, E, F, G) Representative immunoblots of ChAt, GAP-43 and ERK1/2 for atrial tissue samples in each group. (H) ChAt mRNA expression in left and right atria. (I)GAP-43 mRNA expression in left and right atria. (J) TH mRNA expression in left and right atria. (K) NGF mRNA expression in left and right atria. *P□0.05, ***P□0.001 vs. sham group, #P<0.05, ###P <0.001 vs. P group; n = 6 each group.

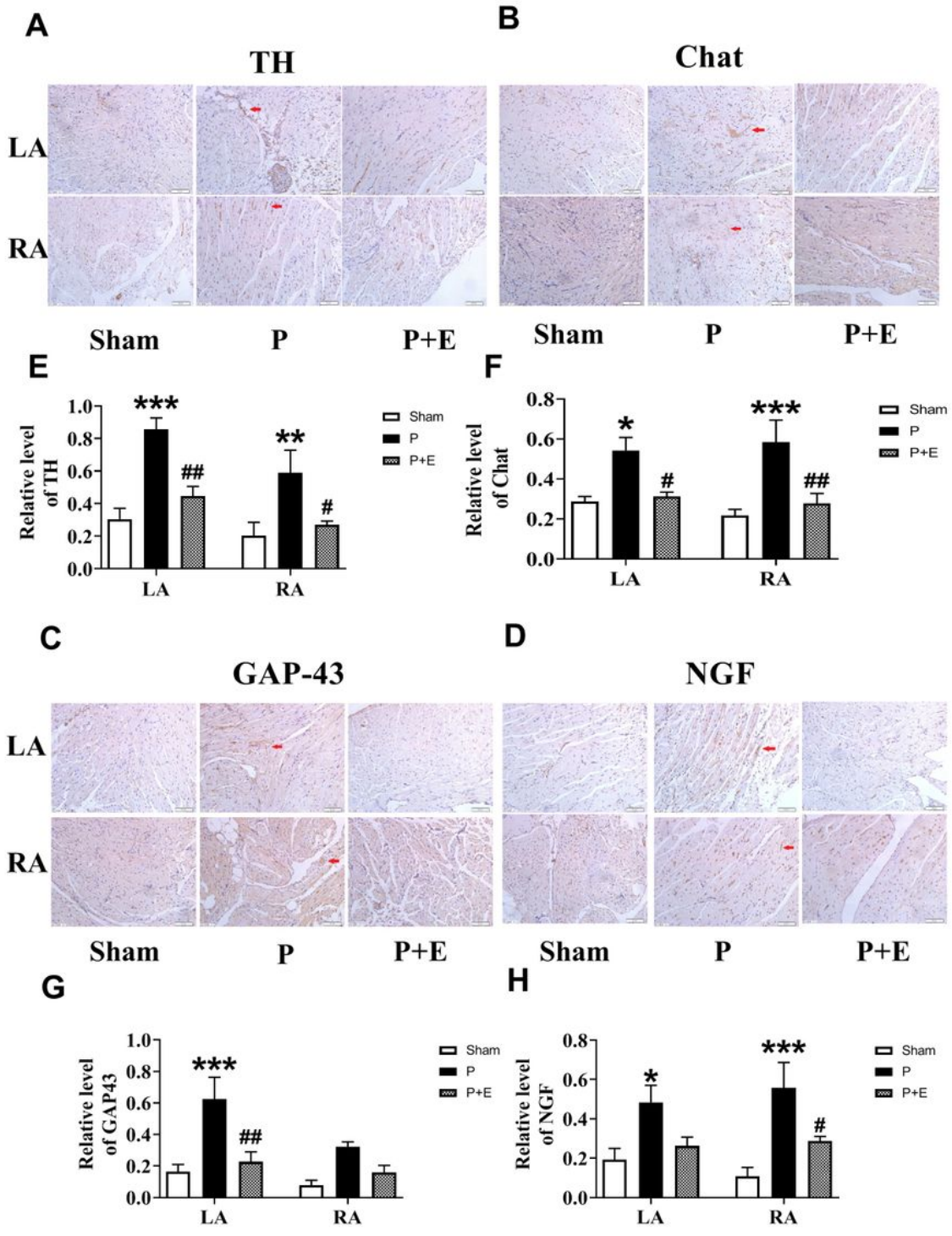

Figure 6

Sympathetic and parasympathetic sprouting and distribution of nerve fibers in the atria of each group. (A) Representative images of tyrosine hydroxylase (TH) expression in left and right atria. (B) Representative images of choline acetyltransferase (ChAT) expression in left and right atria. (C) Representative images of growth-associated protein 43 (GAP-43) expression in left and right atria. (D) Representative images of Nerve Growth Factor (NGF) expression in left and right atria. (E-H) Statistical

results for expression of TH, ChAT, GAP-43, and NGF in left and right atria after atrial rapid pacing for three weeks. The magnification is ×20. *P 0.05, **P 0.01, ***P 0.001 vs. sham group, #P <0.05, ##P <0.01, ###P <0.001 vs. P group; n = 5 each group

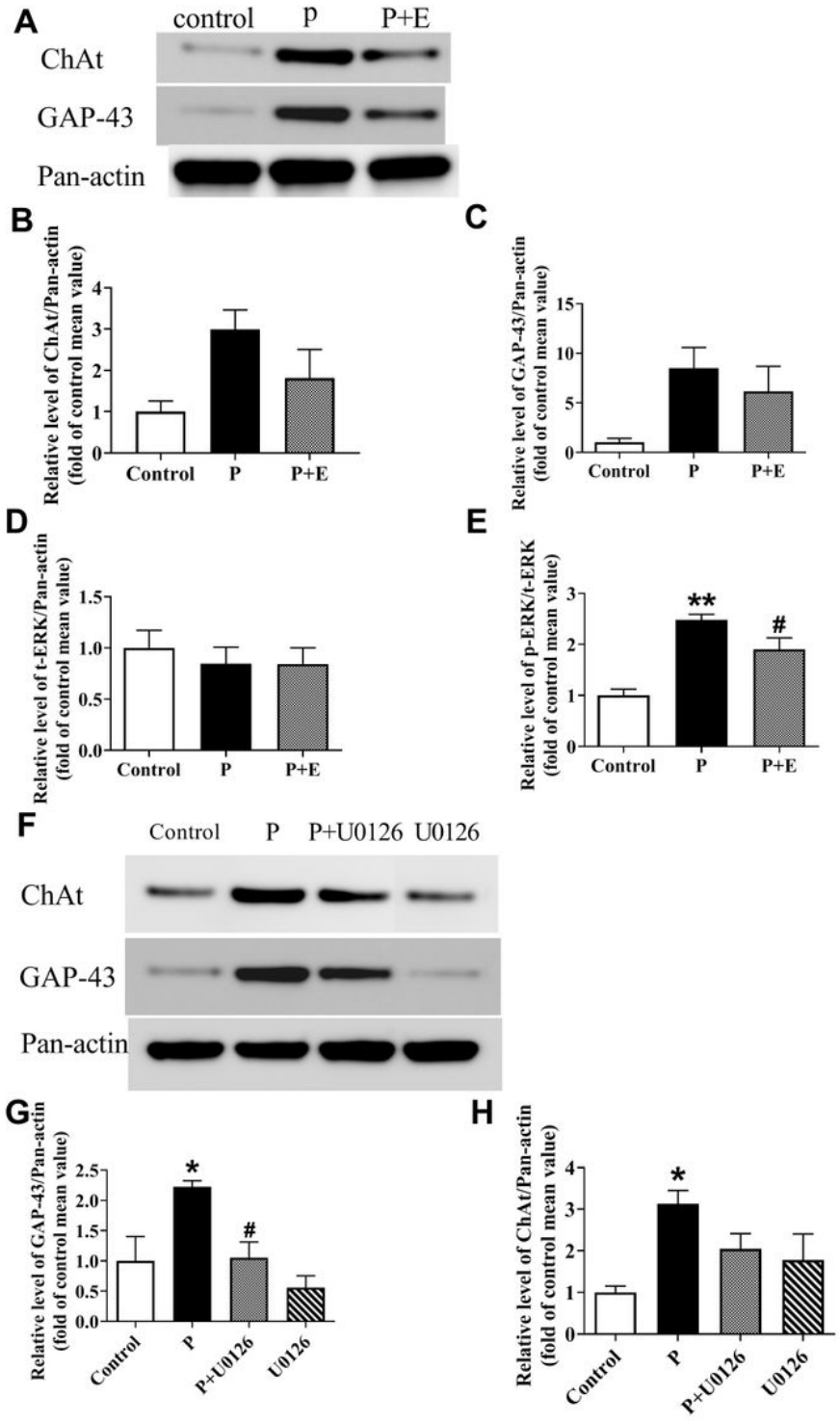

Figure 7

Effects of eplerenone and ERK1/2 inhibitors on the expression of ChAt, GAP-43, t-ERK1/2 and p-ERK/t-ERK in HL-1cell. Data are expressed as mean ±SEM. *P 0.05, **P 0.01 vs. Control group, #P < 0.05 vs. P

group; n = 3 each group

Supplementary Files

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- Highlights.docx