Original Article
The effect of nicotinamide adenine dinucleotide (NADH) on electrical remodeling in atrial fibrillation

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Abstract: Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia in human. The aim of this study is to detect the effect and elucidate the molecular mechanism of nicotinamide adenine dinucleotide (NADH) on ER. Twenty-four rabbits were randomly divided into four groups (n=6), including control group, pacing group, pacing/NADH group and pacing/NADH+rotenone group. Atrial effective refractory period (AERP) was determined in four groups before and after perfusing and pacing. Reverse transcription polymerase chain reaction (RT-PCR) assays were applied to detect the mRNA expression levels of L-type Ca^{2+} channel alpha 1C subunit (CACNA1C), sarcoplasmic reticulum calcium ATPase (ATP2A2) and ryanodine receptor type 2 (RYR2) in right atrium tissues. After perfusing and pacing for 1 hour, the AERP was significantly increased in pacing/NADH group compared with pacing group, while the AERP recover after treatment with rotenone. The mRNA expression levels of CACNA1C and ATP2A2 were significantly up-regulated in pacing/NADH group compared with pacing group, while RYR2 mRNA expression was decreased. In addition, these effects could be reversed after rotenone treatment. Our data suggest that NADH could prolong AERP, and activation of NADH is one potential strategy to prevent AF.

Keywords: AF, NADH, AERP, rotenone, atrium

Introduction

Atrial fibrillation (AF) is a ubiquitous yet diverse cardiac arrhythmia [1-3]. The self-perpetuating nature of AF has been attributed to shortening of atrial refractoriness and a loss of rate adaptation of the atrial refractory period in response to shortening in drive cycle length (DCL), called electrophysiological remodeling (ER) [1, 4, 5]. However, the fundamental mechanisms underlying ER are still unclear. Several study have investigated the mechanisms underlying ER on a cellular level in various animal models of rapid atrial pacing (RAP) and in humans with chronic AF [4, 6]. These data showed that a marked increase in spontaneous Ca^{2+} release and a obvious reduction of L-type Ca^{2+} currents and the transient outward K^+ current Ito [7, 8].

Recently, study found the α1C subunit of the human cardiac L-type Ca^{2+} channel is reversibly inhibited by hypoxia [9, 10]. Chronic AF is asso-ciated with a reduction in atrial myocardial blood flow, and the histopathology of atria in chronic fibrillation is similar to that of chronically ischemic ventricular myocardium [11]. Carnes et al. [12] also found that ascorbate (antioxidant) could attenuate atrial pacing-induced electrical remodeling and decrease the incidence of postoperative atrial fibrillation. All of observations suggest that diminished energy reserves and altered oxidative state may contribute to the occurrence of AF as initiators or triggers.

NADH oxidase is a major source of reactive oxygen species (ROS) in cardiomyocyte [13]. Importantly, NADH can inhibit ventricular ryanodine receptor type 2(RyR2)-mediated Ca^{2+}-induced Ca^{2+} release (CICR) from sarcoplasmic reticulum (SR) membranes [14, 15]. Here, we found that NADH prolong atrial effective refractory period (AERP) in rapid atrial pacing model by decreasing atrium *RyR2* expression and increasing *CACNA1C* and *ATP2A2* expression.

Materials and methods

Ethical approval

New Zealand white rabbits were purchased from the Southern Medical University Animal Center, China. All animals were treated according to the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources for the National Research Council. The study was approved by the ethics committee of West China Hospital of Sichuan University (Sichuan, China).

Preparation of rapid atrial pacing (RAP) rabbit model

24 adult New Zealand white rabbits were used in this study. The rabbits were anesthetized with pentobarbital (25 mg/kg). Twenty-four rabbits were randomly divided into four groups (n=6), including control group, pacing group, pacing/NADH group and pacing/NADH+rotenone (NADH oxidase blocker) group. The heart of rabbits was removed by the Langendorff technique as described previously [16]. Then, control group was continuously perfused with Tyrode buffer at a constant flow (8 mL/min), pacing group perfused with Tyrode buffer and pacing for 1 hour, pacing NADH group perfused with Tyrode buffer containing NADH (0.2 mmol/l) and pacing for 1 hour, and NADH+rotenone group perfused with Tyrode buffer containing NADH (0.2 mmol/l) and Rotenone (20 nmol/l) and pacing for 1 hour. Tyrode buffer contained the following constituents: NaCl 135 (mmol/l), KCl 4.5 (mmol/l), $CaCl_2$ 1.8 (mmol/l), $MgCl_2$ 1.0 (mmol/l), NaH_2PO_4 0.33 (mmol/l), HEPES 10 (mmol/l), Glucose 10 (mmol/l).

Atrial effective refractory period (AERP)

Two bipolar electrodes were localized in the epicardium of right atrium. The higher electrode was connected to a modified stimulator, and pacing performed at 500 beats/min with a pulse width of 0.5 ms and an amplitude of 4V. The other one was used to monitoring the right atrial epicardial electrogram, which was continuously recorded at a paper speed of 50 mm/s. The stimulator was used to deliver extrastimulus pacing via the external programmer to determine AERP during a basic pacing drive of 300 ms. A standard drive train of 8 extrastimuli (S_1) was followed by a single S_2, all at twice diastolic threshold. The initial S_1-S_2 coupling interval was begun at a shorter interval than the expected AERP and increased incrementally in 10 ms steps until atrial capture was achieved. The AERP was defined as the longest S_1-S_2 that failed to capture the atrium and was taken as the mean of two measurements. Pacing was sustained for 1 hour. AERP was determined before and after perfusing and pacing.

RNA isolation and semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR)

All right atria tissue of rabbits was extracted by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. RNA samples were purify with chloroform and isopropyl alcohol, and finally dissolved in diethyl pyrocarbonate (DEPC)-treated water. cDNA was synthesized using Super Script First Strand cDNA System (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The specific primers are shown in **Table 1**. RT-PCR reaction was performed using the Takara EmeraldAmp PCR Master Mix (DRR-300A, Takara, Dalian, Japan) according to the manufacturer's instructions. The temperature procedure was performed at 98°C for 2 min, 98°C for 10 s, 55°C for 30 s, and 72°C for 30 s, with 30 cycles, finally 72°C for 5 min. Electrophoresis of the amplified products was performed on 2% agarose gels containing ethidium bromide. Gels were photographed with a gel imaging system (Tanon Science and Technology, Shanghai, China), and the density of each band was analyzed with Gel-Pro Analyzer (software version 4.0; United Bio., NJ USA).

Real-time quantitative PCR (qPCR)

qPCR was performed using the SYBR® Premix EX TaqTMIIPCR Kit (Takara, Dalian, Japan) according to the manufacturer's instructions on the Roche LightCycler® 480II (Roche, Germany). The primers are shown in **Table 2**. The temperature procedure was performed at 95°C for 5 min, 95°C for 10 s, 60°C for 30 s, with 45 cycles, finally 72°C for 5 min. Data was collected and analyzed by SDS2.3 Software (Applied Biosystems, Foster City, USA). The mRNA expression levels of *CACNA1C*, *ATP2A2* and *RYR2* was normalized internally by using the CT of the housekeeping gene *GAPDH*. The relative quantitative value was expressed by the $2^{-\Delta\Delta Ct}$ method.

Table 1. The specific primers for RT-PCR

Gene	Forward sequences (5'-3')	Reverse sequences (5'-3')	Product (bp)
CACNA1C	GCATAATTGACGTCATTCTCAG	AACACCTGCATCCCGATCACACG	265
ATP2A2	TGAATAAACCGCCTCGG	CAGCACCATCAGCCACT	464
RYR2	GGAAATCCATTCTGAATTCTC	GCAGTCACAAACGGCTCGGTG	483
GAPDH	TCACCATCTTCCAGGAGCG	CTGCTTCACCACCTTCTTGA	572

Table 2. The primers for qPCR

Gene	Forward sequences (5'-3')	Reverse sequences (5'-3')	Product (bp)
CACNA1C	CCAAGCACGGCATCACCA	CACCGAGAACCAGATTTAGA	192
ATP2A2	TTCACAGGCCGGGAGTT	ACACCGTCGCCAGTCATAG	159
RYR2	TAAGAGTTGCGTGGAGTG	GAAGACCGGAAGGTAAAT	163
GAPDH	AGAGCACCAGAGGAGGACG	GGGATGGAAACTGTGAAGAG	103

Table 3. The AERP in rabbit of four group

Group	Before perfusing and pacing (mean ± SEM)	Perfusing and pacing for 1 hour (mean ± SEM)
Control	141 ± 11.2 ms	136 ± 8.9 ms
Pacing	148.0 ± 9.2 ms	116.3 ± 8.7 ms*
Pacing/NADH	137.0 ± 11.1 ms	142.0 ± 8.2 ms**
Pacing/NADH+rotenone	143.5 ± 12.6 ms	126.2 ± 8.6 ms***

*Compared with control group, $P<0.05$; **compared with pacing group, $P<0.05$; ***comparedwith pacing/NADH group, $P<0.05$.

Statistical analysis

Statistical analysis was calculated using SPSS 17.0 (SPSS, Chicago, IL, USA). Values Data were expressed as the mean ± SEM. Each experiment was performed in triplicates and repeated three times. Student's *t* test was used to compare the difference between two groups, and other was analyzed used ANOVA test. Values of $P<0.05$ were considered to be statistically significant.

Results

The effect of *NADH on* AERP in *our group*

AERP was not significantly different in four group before pacing (**Table 3**). After perfusing and pacing for 1 hour, the AERP of pacing group was markedly decreased as comparing with control group (**Table 3**, $P<0.05$). The AERP was also significantly increased in pacing/NADH group compared with pacing group (**Table 3**, $P<0.05$), while the AERP recover after treatment with rotenone (**Table 3**, $P<0.05$).

The mRNA expression levels of *CACNA1C, ATP2A2 and RYR2 in rabbit right atria tissues of four group*

As shown in **Figure 1**, the mRNA expression levels of *CACNA1C* and *ATP2A2* was significantly up-regulated in pacing/NADH group compared with pacing group, while *RYR2* mRNA ex-pression was decreased pacing/NADH group. *CACNA1C* and *ATP2A2* mRNA expression was decreased in pacing/NADH group after treatment with rotenone, and the expression of RYR2 mRNA was increased after treated with rotenone. Moreover, qPCR was also performed to confirm these results. Interesting, the data of qPCR was consistent with the above results (**Figure 2**). These results demonstrated that NADH could decrease CACNA1C and ATP2A2 expression, meanwhile increase RYR2 expression. This effect of NADH could be reversed after treatment with rotenone.

Discussion

AF is self-perpetuating, suggesting that the tachyarrhythmia causes electrophysiological changes that contribute to the progressive of this disease [17]. The earliest observed change in AF is the abbreviation of the atrial effective refractory period (AERP) [3]. Evidences from animal models of AF support an important role for myocyte calcium overload in initiating the process of atrial electrophysiological remodeling (ER) [18]. Recently study found that oxidative stress is a crucial factor in AF, antioxidants could improve cardiac function in animals with pacing-induced failure [19]. Nevertheless, the mechanisms by which high-rate activity results
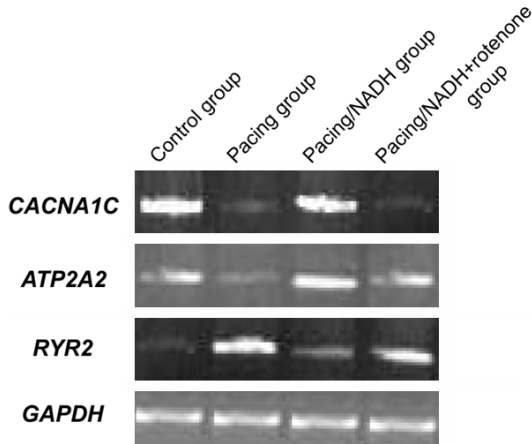
Figure 1. The mRNA expression levels of *CACNA1C*, *ATP2A2* and *RYR2* in rabbit right atria tissues of four group. Expression levels of *CACNA1C*, *ATP2A2* and *RYR2* in rabbit right atria tissues was measured by semi-quantitative reverse transcription-polymerase chain reaction. *GAPDH* was used as a internal control.
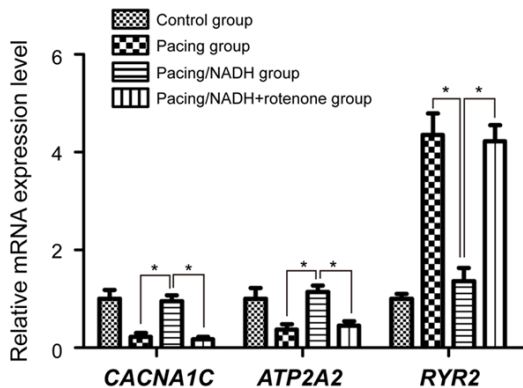

Figure 2. qPCR assay was performed to confirm *CACNA1C*, *ATP2A2* and *RYR2* mRNA expression in rabbit right atria tissues of four group. qPCR: real-time quantitative PCR. *GAPDH* was used as an internal reference. Data were expressed as the mean ± SEM (n=3). *$P<0.05$.

in electrophysiological remodeling are poorly understood.

In cardiac muscle tissue, cytosolic Ca^{2+} is an important dynamic control factor for stimulating the reduction of mitochondrial NAD^+ to NADH. An increase in cytoplasmic Ca^{2+} can activate myofilament, thereby increasing ATP consumption rate, and lead to enhance electron transport and increase ATP production [14, 20, 21]. In cardiac ventricular myocyte, beat-to-beat change in cytoplasmic Ca^{2+} is driven by excitation-contraction coupling [22]. Electrical depolarization of the transversetubule mem-

brane can activate L-type voltage-gated Ca^{2+} channels that enhance inward flux of Ca^{2+} through alpha 1C subunit-DHPR and thereby trigger release of Ca^{2+} from SR [23, 24]. However, the underlying relationship between NADH and Ca^{2+} are not known.

In the present study, we investigated the role of NADH on electrical remodeling in rapid atrial pacing (RAP) rabbit model. We found that the AERP in pacing/NADH group was markedly increased as comparing with pacing group after perfusing and pacing for 1 hour. Interesting, the AERP could return to normal in pacing/NADH group after treatment with rotenone. These results suggest that NADH could prolong AERP, thereby prevent AF occurs.

To better understand the molecular mechanism of NADH on electrical remodeling, we detected the mRNA expression levels of *CACNA1C*, *ATP2A2* and *RYR2* in rabbit right atria tissues of four group by RT-PCR and qPCR. We found the mRNA expression levels of *CACNA1C* and *ATP2A2* was significantly up-regulated in pacing/NADH group compared with pacing group, while *RYR2* mRNA expression was decreased pacing/NADH group. These effects could be reverse after treatment with rotenone. These findings confirmed that NADH could prolong AERP, and activation of NADH is one strategy to prevent AF.

In conclusion, NADH plays an important role in electrophysiological remodeling associated with AF, which provides important insights into one of the mechanisms responsible for initiation of the arrhythmia. Further investigation will be needed to determine if this result is associated with beneficial effects on morbidity and mortality.

Disclosure of conflict of interest

None.

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