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Original Article

Microelectrode array measurement of potassium ion channel remodeling on the field action potential duration in rapid atrial pacing rabbits model

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Abstract: Background: Atrial fibrillation (AF) arises from abnormalities in atrial structure and electrical activity. Microelectrode arrays (MEA) is a real-time, nondestructive measurement of the resting and action potential signal, from myocardial cells, to the peripheral circuit of electrophysiological activity. This study examined the field action potential duration (fAPD) of the right atrial appendage (RAA) by MEA in rapid atrial pacing (RAP) in the right atrium of rabbits. In addition, this study also investigated the effect of potassium ion channel blockers on fAPD. Methods: 40 New Zealand white rabbits of either sex were randomly divided into 3 groups: 1) the control, 2) potassium ion channel blocker (TEA, 4-Ap and $BaCl_2$), and 3) amiodarone groups. The hearts were quickly removed and right atrial appendage sectioned (slice thickness 500 μm). Each slice was perfused with Tyrode's solution and continuously stimulated for 30 minutes. Sections from the control group were superfused with Tyrode's solution for 10 minutes, while the blocker groups and amiodarone were both treated with their respective compounds for 10 minutes each. The fAPD of RAA and action field action potential morphology were measured using MEA. Results: In non-pace (control) groups, fAPD was 188.33 ± 18.29 ms after Tyrode's solution superfusion, and 173.91 ± 6.83 ms after RAP. In pace/potassium ion channel groups, TEA and $BaCl_2$ superfusion prolonged atrial field action potential (fAPD) (control vs blocker: 176.67 ± 8.66 ms vs 196.11 ± 10.76 ms, 182.22 ± 12.87 ms vs 191.11 ± 13.09 ms with TEA and $BaCl_2$ superfusion, respectively, $P < 0.05$). 4-AP superfusion significantly prolonged FAPD. In pace/amiodarone groups, 4-Ap superfusion extended fAPD. Conclusions: MEA was a sensitive and stable reporter for the measurement of the tissue action potential in animal heart slices. After superfusing potassium ion channel blockers, fAPD was prolonged. These results suggest that I_{to}, I_{Kur} and I_{K1} remodel and mediate RAP-induced atrial electrical remodeling. Amiodarone alter potassium ion channel activity (I_{to}, I_{Kur}, I_{K1} and I_{Ks}), shortening fAPD.

Keywords: Atrial fibrillation, microelectrode arrays, rapid atrial pacing, field action potential duration, potassium ion channel blocker

Introduction

The atrial fibrillation (AF) is one of the most common clinical arrhythmias. The mechanism of action in AF remains unclear. Since AF is a result of abnormal atrial structure and electrical activity, research has focused on defining the mechanism responsible AF-induced changes in the electrophysiological properties of atrial tissues and ion channels in the cell membrane. In the electrical remolding of atrial myocytes in AF, a critical event that occurs is "calcium overload". A series of changes of proteins in the channels, mainly involving with the L-type Ca2+ ion channels, the K^+ ion channels and Na+ channels, etc. occurred to the atrial myocytes.

In the past, several studies have applied with the patch clamp technique to measure the action of the ion channels, in the presence and absence of blockers, on myocardial cells. The patch clamp technique has become the conventional method to study the cellular electrophysiology and has gained fruitful achievements. This technique can be used to measure changes in the ion channel currents of cells under stimulation of drugs, which has great sig-

nificance for the study on the channel activation of membrane receptors as well as the further drug screening. The patch clamp technique has a high accuracy and can measure multiple membrane channel currents. The minimum value measured can be ranked to pA (10-12A) level, which proves to be a kind of typical low-noise measurement technique. However, the interface damages resulting from measurement limit the long record of patch clamps. MEA is a type of new technique to use the non-invasive record with 60 sites of the action potential of the tissues. MEA can be used in the electrophysiological testing on of the whole heart anti-arrhythmia and the drugs interfering the myocardial treatment, and also test the cell group and the active tissue slices. The experiments and studies in the recent years proved that [5] RAP has been proved to be a fairly valuable method in the study of atrial fibrillation. In the RAP or AF models, the shortening of atrial effective refractory period (AERP) is the most prominent change of the electrophysiological features. These changes have also been proved in the AF or patients with atrial flutter. Some studies confirmed that the AEFRP shortens also related to the potassium ion channels generic expression and activity changes except for the weakening of currents of $I_{Ca,L}$, I_{to}, I_{Na}, etc at the time of AF [2, 6].

This project applied MEA technique to observe the influence of potassium channel blocker on the field action potential of the atrial appendage of rabbits after 24 h rapid atrial pacing and also examined the feasibility of the application of MEA technique to study the actions of ion channel blockers and amiodarone on the myocardial tissue to assess the therapeutic potential of these agents to treat AF.

Materials and method

Experimental animals and groups

The Institutional Animal Care and Use Committee of Xiniiang Medical University reviewed and approved the experimental design of all animal experiments. 40 white New Zealand rabbits, weighing 2.5-3.0 kg, were used in this study. These rabbits were randomly divided into three groups: 1) pacing group (n = 8), 2) pacing + potassium channel blocker group (TEA group, 4-AP group and $BaCl_2$ group, each group n = 8, and 3) pacing + amiodarone (n = 8).

Reagents and devices

Composition of modified Tyrode solution: 144 mmol/L NaCl; 5.0 mmol/L KCl; 1.8 mmol/L $CaCl_2$; 1 mmol/L $MgCl_2$; 0.33 mmol/L NaH_2PO_4; 10 mmol/L glucose, and 5.0 mmol/L HEPES adjusted to pH 7.4. Tetraethylammonium (TEA), 4-Aminopyridine (4-AP) and amiodarone (AMI) were purchased from Sigma (USA). The microelectrode arrays (MEA60 System, MEA 1060 Amplifier, MC-card A/D and stimulus generator were purchased from MCS (GmbH, Germany).

Rapid atrial pacing (RAP) model

3% sodium pentobarbital 30 mg/kg was intravenously injected along the ear edge to anaesthetize the rabbits. Following this, 1,000 U of heparin was intravenously injected to prevent blood clotting. The rabbits were then laid on the desk at with face upwards and the right neck veins were surgically exposed. The electrophysiology instrument LEAD-2000 (Jinjiang Electronic Technology, Sichuan, China) was applied to record the ECG. Under the monitoring of intracardiac ECG and surface ECG, a 6F bipolar electrode (with a distance of 5 mm between two electrodes) was topically applied to the right atrium to stimulate pacing. The ideal intracardiac ECG profile will appear as a tall A-wave and small V-wave. Once this profile was achieved, the electrode conductivity was fixed. Rapid atrial pacing was made at a fixed frequency of 600 times/minute for 24 hours. AF was defined as irregular atrial rates faster than 500 beats/minute (bpm) associated with irregular atrioventricular conduction lasting > 5 seconds.

Sample preparation

After 24 hours of rapid atrial pacing (RAP), the chest was quickly opened and the heart was surgically removed and placed in the modified Tyrode solution filled with oxygen at 4°C. Blood that had accumulated inside the hard was drained through extrusion at a pace comparable to a regular heartbeat. Following this, the heart was dissected and the atrial appendage was excised and sectioned at a thickness of 500 μm per slice. The sections were placed in the MEA recording system via a sliver wire ring and the temperature was regulated by a temperature controller TCO2 (Multichannel Systems, Germany) at 37°C.

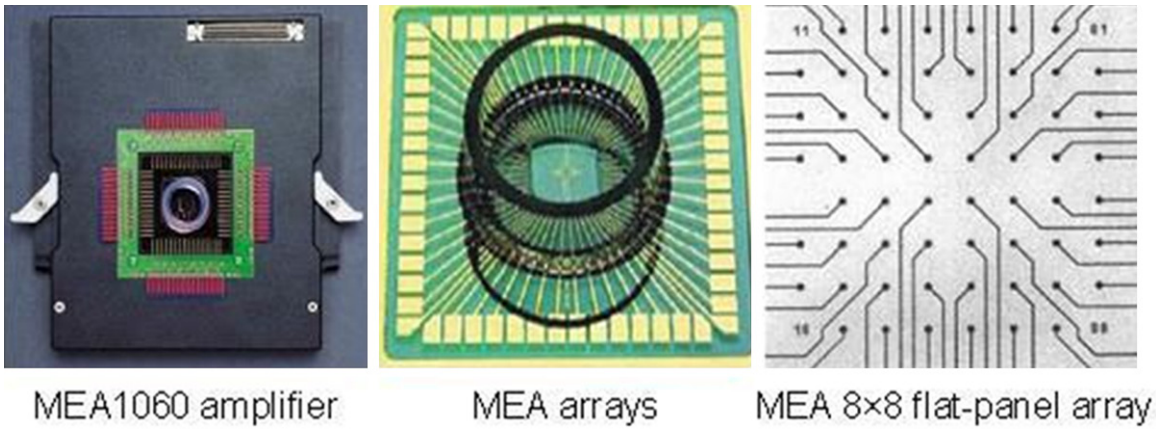

Figure 1. Architecture of the MEA60 system.

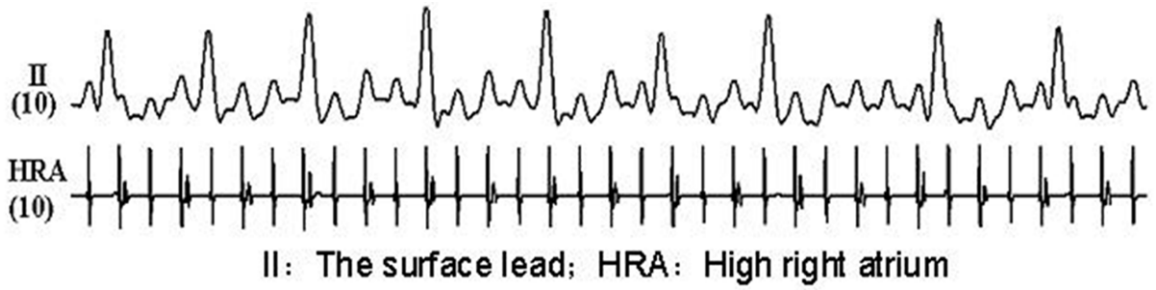

Figure 2. Rapid pacing induced atrial fibrillation model in rabbit (600 beats/min).

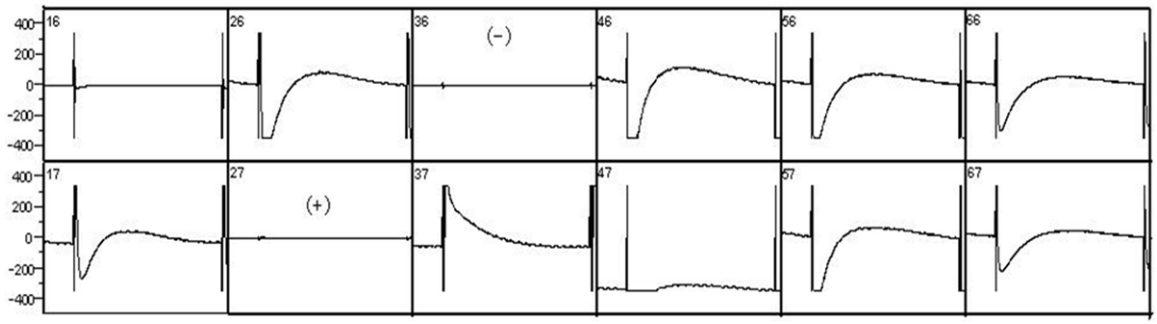

Figure 3. fAPD of right atrial appendage in rabbits after RAP with MEA recording.

The modified Tyrode solution was perfused into the atrial tissue for 30 min at a constant speed (3 ml/minute) with mixed air of 95% O_2 + 5% CO_2. The stimulus generator then stimulated a pulse with a strength greater than 2 times the threshold, with a wave 2 ms wide and frequency of 1 Hz. The sub-windows No. 27 and No. 36 were set to be the positive and negative controls of the stimulation electrodes respectively. After the specimen became stable for 30 minutes, we began to record the influence of potassium channel blockers on the field action potential duration (fAPD) of the atrium.

Microelectrode arrays (MEA) system

The MEA system contains 60 titanium nitride electrodes arranged in 8 × 8 arrays within a central area (0.78 mm × 0.78 mm) on a piece of transparent 50 mm × 50 mm quartz glass. The tip of electrode had a diameter of 10 μm and the distance between neighboring electrodes was 100 μm. The electrodes are connected by

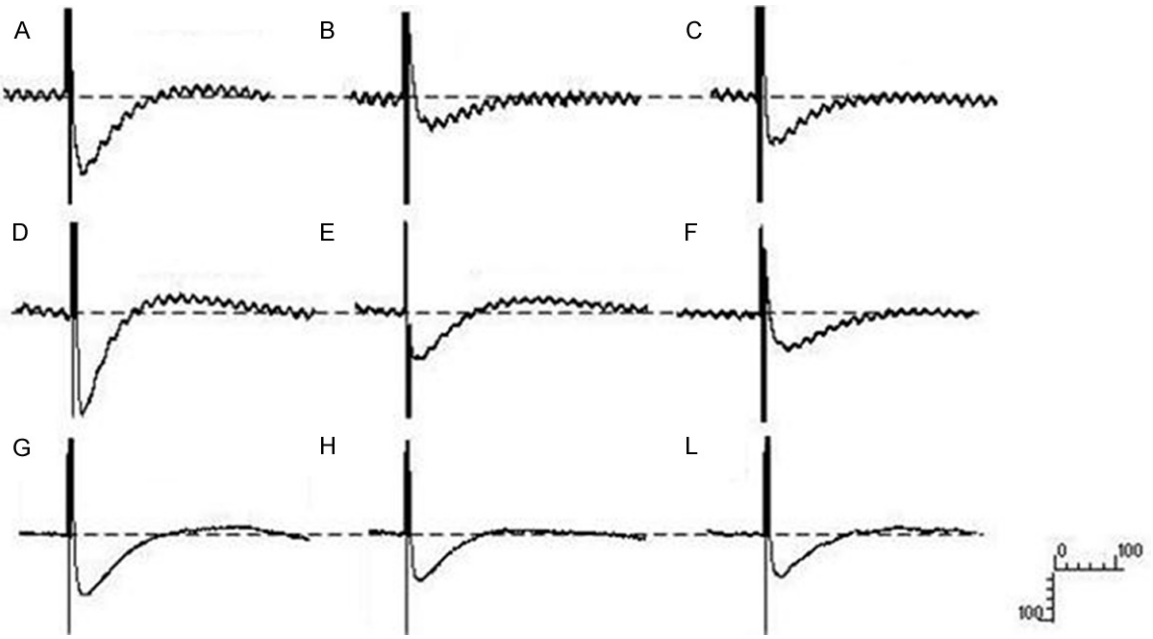

Figure 4. Atrial fAPD before and after administering TEA, 4-AP and $BaCl_2$ in rabbits. A: Before administering TEA; B: 20 mmol/LTEA; C: Washing; D: Before administering 4-AP; E: 5 mmol/L 4-AP; F: Washing; G: Before administering $BaCl_2$; H: 10^{-4} mol/L $BaCl_2$; L: Washing.

insulation wires and extend to link to the amplifier (**Figure 1**). The electrode Ag/AgCl was placed into the perfusion liquid as a reference. The microelectrodes placed in the bottom of the MEA chips will record the original signals obtained from the electrical activity of the tissue slices of the heart, and these signals will be modulated through MEA 1060 (with a gain 1,200 times) and digitally recorded by the MC-card. The software application MC-Rack was used to obtain parameters of the filed potentials in myocardial tissue. These parameters include: the first positive peak value (FPpre), the first maximum negative peak value (FPmin), the last positive maximum peak value (FPmax) as well as the field potential duration (FPdur, the time from FPmin to FPmax).

Statistical methods

The statistical software SPSS13 was used to analyse the statistical significance of the data. The metering data was represented by mean ± standard deviation ($\bar{x}$ ± s). A *P* value less than 0.05 is considered to be statistically significant.

Results

AF model

After rapid atrial pacing 24 hours, the success rate of inducing AF in atrial sections was 75% and the duration of AF was 5-10 s. See **Figure 2**.

MEA measurements of fAPD in rabbits undergoing RAP

The atrial appendage tissue No. 27 (+) and No. 36 (-) of the normal group, perfused with modified Tyrode solution, was electrically stimulated with 2 V at a pulse width of 2 ms and duration of 100 ms to continuously stimulate the specimen for 30 minutes. The MEA shows the No. 27 and No. 36 display a straight line without any biological electrical signals during the period of the experiment (**Figure 2**). Due to the different sizes and placement positions of the myocardial tissue lumps, the data was measured in subwindow No. 67 with steady field potential signals. The shape of the atrial appendage is similar to the membrane potential curve placed upside down in the atrial action potential. After 24 h of RAP, the fAPD is 173.91 ± 6.83 ms (**Figure 3**).

The influence of TEA, 4-AP and $BaCl_2$ on the fAPD of atrial appendage of RAP in rabbits

The tissue slices of the atrial appendage from rabbits that underwent 24 hours of RAP were placed in the MEA electrode measurement area. The specimen was perfused with modified Tyrode solution at a constant speed. The

Figure 5. Atrial fAPD before and after administering AMI in rabbits. A: Before administering AMI; B: 2 × 10^{-6} mmol/L C: Washing.

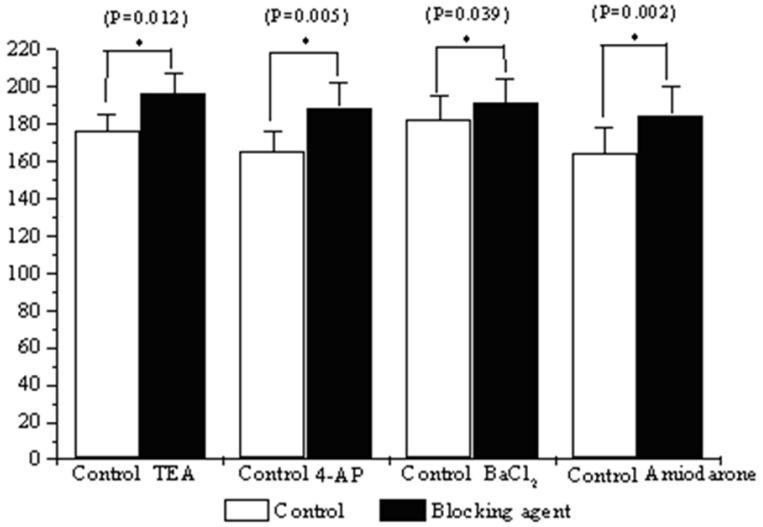

Figure 6. Atrial fAPD before and after administering TEA, 4-AP, $BaCl_2$ and AMI in rabbits.

stimulating electrode stimulated a continuous pulse for 30 min at 2 V, with a wave width of 2 ms and duration of 100 ms. The surface perfusion of modified Tyrode solution with 20 mmol/L TEA was made to block the delayed rectifing K^+ currents (IK). After perfusion of TEA to the atrial tissue for 10 minutes, the fAPD of the atrial appendage was extended by 10.55 ms (P = 0.012; n = 8) from 176.67 ± 8.66 ms to 196.11 ± 10.76 ms. Under the identical experimental conditions, 5 mmol/L 4-AP perfusion to the tissue specimen to block the transient outward K^+ currents (I_{to}), and the fAPD of the atrial appendage was extended by 19.18 ms (P = 0.005; n = 8) from 169.38 ± 10.56 ms to 188.56 ± 13.82 ms. The perfusion of modified Tyrode solution with 10^{-4} mol/L $BaCl_2$ to the specimen to block the inward rectifier potassium currents (Kir), extended the fAPD in the atrial appendage by 8.89 ms (P = 0.039; n = 8) from 182.22 ± 12.87 ms to 191.11 ± 13.09 ms (**Figures 4**, **6**).

Action of AMI on fAPD in rabbits undergoing RAP

Atrial sections were perfused with modified Tyrode solution containing 2 × 10^{-6} mmol/L AMI for 10 min. The data shows that the fAPD was extended 17.62 ms (P = 0.002; n = 8) from 167.38 ± 13.67 ms to 185 ± 15.14 ms (**Figures 5**, **6**).

Discussion

MEA measurements of fAPD in rabbits from the pacing group

Changes in the action potential duration (APD) are associated with a corresponding change in the AERP in induced AF canine model by rapid pacing [2]. The goad model experiments report that the refractory period became remarkably short and the AF onset duration is related to the operational shortening of the effective refractory period with AF onset in 24 hours [7]. The present study found that under the conditions of AF, APD synchronized with the shortening of AERP, which is one of the electrophysiological parameters of the electrical remodeling of atrial myocytes. The results of this study showed that the field potential of the atrial appendage is similar to the membrane potential and is inversely proportional to the action potential of the atrial myocytes.

Influence of potassium blockers and AMT on the fAPD in rabbits

I_{to} gradually decreased along with the pacing duration while I_{Kur} and I_{K1} had no such changes

with and without rapid pacing with the patch clamp technique [2]. I_{Kur} is important to the atrial repolarization and ADP. The weakening of I_{Kur} can lead to the extension of APD and AERP and the I_{Kur} channel subtype Kv1.5 is mainly expressed in the atrium, which plays a crucial role in the atrial repolarization. The suppression of these channels may prevent the occurrence of AF. Kv1.5 potassium channel blocker drugs extend the APD of the atrial myocytes affected by electrical remolding. This action has become a major focus of new Class III anti-arrythmia drugs to treat AF [8].

Paroxysmal atrial tachycardia for 30 minutes in rats and rapidly increases mRNA expression of Kv1.5 [9]. However, other researchers found the Kv1.5 channel protein expression is reduced and the expression of Kv1.5 mRNA has no effect on the atrial myocytes of patients with paroxysmal AF. The underlying mechanism to explain this contradictory result may be that Kv1.5 mRNA expression become abnormal in during AF [10]. The functional absence of Kv1.5 resulted from the sudden changes of KCNA5 expression, which is a type ne dangerous factor to the damages of repolarization and AF [11]. The present experiment perfused 20 mmol/L TEA to atrial section, leading to an extension of fAPD by 10.55 ms. In addition, the amplitude of the negative phase wave was reduced significantly. According to the results, we concluded that I_{Kur} participates in potassium channel remolding of the electrical remolding of AF. Both of experiments and clinical studies demonstrate that the I_{to} of RAP in atrial tissues is remarkably reduced [2]. The results showed that treatment with 5 mmol/L 4-AP blocked I_{to} and extended the fAPD of atrial appendages in rabbits under RAP. According to these results, we further concluded that the regular electrophysiological indexes APD and AERP extend synchronously and then weaken or reverse the electrical remolding of AF.

I_{Kir} was called as I_{K1} in the cardiomyocytes, the most feature was strong inward rectification. It was reported that Kir2.1, together with Kir2.2 and Kir2.3, constitute the heterologous multimeric to express in the cardiac muscles and had relation with AAF. This indicates that that I_{K1} is an important factor of initiating and maintaining atrial fibrillation [12]. The results of the study demonstrate that the supply of 10^{-4} mol/L $BaCl_2$ blocked I_{Kir}, extending fAPD by 8.89 ms. This suggests that RAP in rabbits may cause the reduction of I_{Kir} and shortens AERP and maintenance of AF. After a specific blocker for I_{Kir} was used, fAPD was extended and the corresponding electrophysiological index AERP of electrical remodeling was also extended, inhibiting the occurrence of AF.

AMI has many unique pharmacological properties including ion channel blocking and electrophysiological action of anti-arrhythmia drugs. At present, AMI is the only drug to extend APD and ERP and increase ERP/APD without inverse frequency independence. It could work both in the open state and closed state of cardiomyocytes, but the main restriction lied to the I_{ks} in the open state. This may be a key reason for it to maintain the extension of APD at a rapid heartbeat and the crucial point to exert its strong medicine properties. Amiodarone can also block the rapid activation of I_{Kur} and I_{K1}. After treating with AMI, our data shows that the atrial fAPD was extended by 17.62 ms. Therefore, the MEA technique may objectively reflect the electrophysiological properties of myocardial tissue slices. Moreover, MEA may be used in the electrophysiological testing for research and development of the drugs for cardial intervention therapy.

The maintenance of normal heart function depends on the mutual coordination of multiple ion channels in the cardiomyocytes. Therefore, the channels related to the arrhythmia are often targets of anti-arrythmia intervention. At the same time of recording the action potential of tissue cellular groups by MEA, the cellular electrical activity in various locations of the tissue can be measured before and after drug intervention. MEA has several advantages over the patch clamp technique as it can directly reflect the electrophysiological activity of the cardiomyoctes. In this study, MEA was combined with intracardiac electrophysiology to measure the extension of fAPD after administration of potassium channel blockers in rabbits undergoing RAP. It stated the remolding of potassium channel participated the electrical remolding mechanism of AF from another angle. Our results were consistent with the patch clamp technique and thus validated the feasibility of using MEA to assess arrhythmia. Compared with patch clamp technique, the advantages of MEA chips are found in the long term, real-time and damage-free measurement

of the signal coupling and conduction between cells. The patch clamp technique has no ability to monitor signals passing between cardiomyocytes coordinating the functional activities of the heart. At present, there have been no reports showing fAPD data from atrial tissue in normal experimental animals or under pathological conditions using MEA. In our animal AF model induced by RAP, the changes in electrophysiology and ion channels are similar to the clinical pathology of human patients with AF.

Study limitations

The changes of the electrophysiological properties of atrial cells are closely related to the levels and functions of multiple ion channels. The high selectivity of the block to the related function of the ion channels may be a development direction for the clinical treatment of atrial fibrillation. Due to the asynchronous changes of the various potassium ion channels in the pathological progression, future studies are required to determine the degree that various antiarrhythmia drugs block the various ion channels to facilitate data from MEA and the patch clamp technique.

Conclusion

MEA was a sensitive and stable measure of the tissue action potential in animal heart slices. The fAPD electrophysical properties were similar to APD and atrial AERP. I_{to}, I_{Kur} and I_{K1} have remodeled and mediate RAP-induced atrial electrical remodeling. Amiodarone can inhibit potassium ion channels (I_{to}, I_{Kur}, I_{K1} and I_{Ks}), and extend fAPD. These findings indicate a potential antiarrhythmic effect of amiodarone during AF as a result of electrical remodeling of the atrium.

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Disclosure of conflict of interest

None.

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