細胞核數目相異之肺靜脈與左心房心肌細胞在電生理與離子通道的表現

Electrical Activity and Channel Expression in Pulmonary Vein and Left Atrium Cardiomyocytes of Various Nucleus Number

中文摘要

前言

心房顫動乃是臨床上最常見的心律不整之一，而且會造成嚴重的心臟功能不良以及增加致死率與發病率。過去的研究已知肺靜脈心肌組織是異位節律點的來源，它會引發陣發性心房顫動及異位性左心房心博過速。但何種心肌細胞容易引發心房顫動仍未有定論。

目的

心肌細胞在胚胎發育過程中，會有不同的細胞核數目產生，其機轉尚未完全明瞭。是否不同的細胞核數目有不同的細胞電生理特性，尚未有相關的研究被提出。因此本實驗的目的在探討單雙核心房與肺靜脈心肌細胞的電生理特性與離子通道的差異，與研究不同細胞核數目的心肌細胞在引發心房顫動機轉中所扮演的角色。

材料與方法

年齡約三個月的雄性兔子 (1.5 到 2 公斤，n = 18)，將左心房與肺靜脈的心肌細胞分離出後，以 DAPI 染細胞核，以區分單核或雙核心肌細胞。以全細胞膜電位箝定法與免疫螢光染色去研究單雙核心肌細胞間的電生理特性，以及離子流與離子通道間的差異。

結果

本實驗主要的發現是 (1) 無論是左心房或不具節律性肺靜脈的單核細胞都較雙核細胞的靜止膜電位爲正; (2) 具節律性的肺靜脈心肌細胞中，單核細胞較雙核細胞有較高頻率的節律性; (3) 左心房單核與雙核心肌細胞的 IK1 離子電流密度沒有顯著差異，但肺靜脈單核心肌細胞的 Ik1 離子電流密度較雙核細胞小，相同的，肺靜脈單核心肌細胞的 Kir 2.3 螢光密度也較小; (4) 無論左心房或肺靜脈，單核心肌細胞的 ICa,L 最大離子電流密度都較雙核細胞爲大，而左心房與肺靜脈單核心肌細胞的鈣離子濃度變化也較雙核細胞大; (5) 無論是左心房或肺靜脈，單核心肌細胞都較雙核細胞的 RyR2 螢光密度爲大。

結論

本實驗首次證明左心房與肺靜脈單雙核心肌細胞有不同的電生理特性，且其電生

理特性由不同的離子流與離子通道密度所決定，但仍有許多離子流與離子通道特性尚待驗證。此外，單雙核心肌細胞對於引發心房顫動的藥物反應是否不同也值得探討，以進一步釐清核數目相異心肌細胞的生理反應與心房顫動的關聯性。

英文摘要

Introduction

Atrial fibrillation (AF) is the most important clinical arrhythmia which induces cardiac dysfunction and increases mortality and morbidity. Pulmonary veins (PVs) were known to be important sources of ectopic beats with the initiation of paroxysmal atrial fibrillation and the foci of ectopic atrial tachycardia. However, the characteristics of arrhythmogenic cardiomyocytes in left atrium (LA) and pulmonary vein have not been identified.

Aim

The purposes of this study were to evaluate the electrophysiological difference and ion channel properties between mononucleated and binucleated cardiomyocytes in LA and PV.

Material and Methods

Male rabbits of 3months old (n=18; 1.5-2 kg) were sacrificed. Isolated LA-PV cardiomyocytes were obtaied by enzyme. Whole-cell patch clamp and immunostaining were used to study the electroactivity and ion channel of DAPI-identified mononucleated and binucleated cardiomyocytes in LA and PV.

Results

Compared to binucleated cardiomyocytes, mononucleated cardiomyocytes (n=10) have more positive resting membrane potential than binucleated myocytes (n=17) in LA (-57.9±1.0 mV versus -62.2±1.2 mV, $P<0.05$). Similarly, mononucleated cardiomyocytes (n=10) have more positive resting membrane potential than binucleated cardiomyocytes (n=10) in PV (-56.5±1.1 mV versus -64.0±1.6 mV, $P<0.05$). In pacemaker PV cardiomyocytes, mononucleated myocytes (n=34) have higher frequency of beating rates than binucleated myocytes (n=34) (2.1±0.2 Hz versus 1.3±0.2 Hz, $P<0.05$).
Mononucleated cardiomyocytes (n=19) have smaller IK1 current density than

binucleated cardiomyocytes (n=12) in PV (-2.6±0.2 pA/pF versus -3.5±0.4 pA/pF, P<0.05). Besides, the ICa,L is larger in mononucleated myocytes (n=16) than in binucleated myocytes (n=15) of LA (-14.2±1.3 pA/pF versus -10.9±0.9 pA/pF, P< 0.05). The ICa,L is also larger in mononucleated cardiomyocytes (n=18) than in binucleated cardiomyocytes (n=18) of LA (-9.3±0.7 pA/pF versus -7.0±0.8 pA/pF, P<0.05). In PV,. the RyR2 density of mononucleated myocytes (n=27) is higher than that of binucleated myocytes (n=17) of LA (100.3±4.2 IU/μm2 versus 85.1±3.3 IU/μm2, P<0.05). The RyR2 density of mononucleated cardiomyocytes (n=16) is also higher than that of binucleated cardiomyocytes (n=18) of PV (139.8±5.0 IU/μm2 versus 124.1±5.4 IU/μm2, P<0.05).

Moreover, the mononucleated myocytes (n=20) had a larger [Ca2+]i transient than the binucleated myocytes (n=10) in LA (F-F0/F0, 0.52±0.06 IU versus 0.19±0.05 IU, P<0.05). Similarly, the amplitude of the [Ca2+]i transient of mononucleated cardioimyocytes (n=15) was also larger than that of binucleated cardiomyocytes (n=10) in PV (F-F0/F0, 0.64±0.09 IU versus 0.20±0.03 IU, P<0.05). In addition, the duration of [Ca2+]i transients of mononucleated myocytes (n=20) was longer than that of binucleated myocytes (n=10) in LA (67.9±6.9 ms versus 40.2±3.7 ms, P<0.05). In PV, as compared with binucleated cardiomyocytes (n=10), the mononucleated cardiomyocytes (n=15) also had a longer duration of [Ca2+]i transients (69.2±5.3 ms versus 45.3±5.9 ms, P<0.05)

Conclusions

The study first demonstrate the different electrophysiology characteristics between mononucleated and binucleated cardiomyocytes in LA and PV. Moreover, this study demonstrated the feasibility to examine the response of mononucleated and binucleated cardiomyocytes to drugs that were shown to induce AF.