行政院國家科學委員會專題研究計畫成果報告

在豬隻心房顫動模式中，奇趣電流通道在蛋白、訊息核醣核酸及組織學層次之變化

Changes of the Funny (Pacemaker) Current Channels at Protein, mRNA and Histology Levels in a porcine Model of Atrial Fibrillation

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一、中文摘要

本研究有兩個主要的目的
第一：研究奇趣電流在心臟各不同部位的分佈
第二：研究在心房顫動的豬隻模式中，奇趣電流通道在心房各部位及肺靜脈的變化。

奇異電流通道又稱為節律電流通道，由於它對於動作電位第四期自動去極化的貢獻，使得心肌細胞可以產生自主性的動作電位。在心房顫動方面，我們過去就曾證實，心房組織中的奇異通動的訊息核醣核酸有明顯的增加，然而對於此通道之蛋白或其在組織中的分佈仍未明瞭，因此本研究擬以豬隻之心房顫動模型為材料，藉以研究此通道在心臟中的分佈，並研究此通道在心房顫動時的變化。

首先，我們以植入節律器並對右心房作快速刺激(每分鐘六百次)來造成豬隻之心房顫動。在右心房刺激四週後，將節律器關閉，豬隻可呈現持續性心房顫動，而在心房顫動持續兩週後，我們取出其心臟作為研究材料，我們自下列區域採集心肌組織：右心耳，右心房側壁，左心房側壁，左心耳，右上肺靜脈，左上肺靜脈，房室結竇房結，本研究中我們所採到的組織進行①西方墨點分析②反轉錄-聚合酶連鎖反應③免疫組織切片染色。

西方墨點分析顯示，奇趣電流通道在心房組織，竇房結，房室結及肺靜脈都有分佈而且分佈的情形十分平均，心房顫動組織並未顯示上升。

免疫組織染色，我們證實奇趣電流通道在房室結有分佈，在肺靜脈區也有反應，但在心房組織中，主要的反應是集中在血管之平滑肌。反轉錄-聚合酶連鎖反應在研究中不成功。

總之，本研究首先探討了奇異電流通道在心房組織的分佈，在心房顫動時，未見其顯著上升。

關鍵詞：心房顫動, 奇趣電流

Abstract

There are two specific aims in the present study.

1. To investigate the distribution of funny current channels in different part of atrial tissue.
2. To investigate the changes of funny current channels in a porcine model of atrial fibrillation.

Funny current is a hyperpolarization activated current. It contributes to the spontaneous depolarization in phase 4 of action potential and it plays important roles in the generation of normal sinus rhythm. In our past studies, we have shown that the

mRNA level of funny current channels is increased in human subjects with atrial fibrillation. However, information regarding the changes in protein and histological levels are not available. Therefore, we propose to use the porcine model of atrial fibrillation to investigate the distribution of funny current channels as well as its changes in atrial fibrillation.

First, we established a porcine model of atrial fibrillation. A transvenous AOO pacemaker was implanted in adult pigs. The pacing rate was set at 600 per minute. After pacing for 4 weeks, the pacemaker was turned off and the pig developed persistent atrial fibrillation. The pigs were sacrificed two week after turning off the pacemaker and atrial tissues were collected. We obtained atrial tissue from the following positions: right atrial appendage, right atrial free wall, left atrial appendage, left atrial free wall, AV node, atrial tissue extending into the pulmonary veins. With the above-obtained tissue, we performed Western blotting analysis, reverse transcription-polymerase chain reaction, and immunohistochemical studies.

Our results showed that the funny current channel protein was present in the pulmonary venous tissue. The channel protein was evenly distributed in the atrial tissue. We also found that the amount of funny current channel protein was not significantly different between pigs with atrial fibrillation and control pigs. In immunohistochemitry studies, the AV nodal tissue showed reaction with the antibody as expected. However, the funny current channel protein in the atrial tissue was strongly associated with vascular smooth muscle. The pulmonary venous tissue was alos studied. It showed diffuse mild reaction with the funny current channel antibody. In RT-PCR experiments, we failed to amplify the funny current channel RNA because the funny current cDNA sequence was not available in the GenBank and human primers did not work on the porcine model.

In conclusion, our study demonstrated for the first time the distribution of funny current channel in the atrial and pulmonary venous tissues. We found that funny current channels existed not only in pacemaker tissues but also working atrial myocardial tissue. This channel was also present in the pulmonary venous tissue. We also showed that the funny current channel was present in large amount in the vascular smooth muscle cells. This finding is also novel. It is possible that these channels also play important roles in smooth muscle contraction.

Keywords: Atrial fibrillation, funny current channel

二、緣由與目的

Atrial fibrillation is the most common type of tachyarrhythmias in humans. However, the pathophysiological mechanism of atrial fibrillation remains unclear.

Recent mapping and ablation studies have identified a critical area, which is important for the initiation and maintenance of atrial fibrillation. Haissaguerre et al. reported that spontaneous firing foci in the proximal pulmonary veins are responsible for the initiation of atrial fibrillation in humans. Radiofrequency catheter ablation of these foci resulted in clinical improvement of this arrhythmia.

In normal hearts, spontaneous firing is characteristic of cardiomyocytes in the sinus node, atrioventricular node, Purkinje fibers and ectopic foci. The ionic mechanism for spontaneous heart beats had puzzled the electrophysiologists for years. It has been observed that cardiomyocytes from rabbit sinoatrial node exhibited hyperpolarization induced inward current. This current was called “funny” current. Finally, the ion channel responsible for the funny current was cloned in 1998. This channel is expressed in large amount in pacemaker tissues and contributes to spontaneous firing of cardiomyocytes.

In the present study, we established a pig model of persistent atrial fibrillation. This model was used for studying the funny current channels in atrial tissue

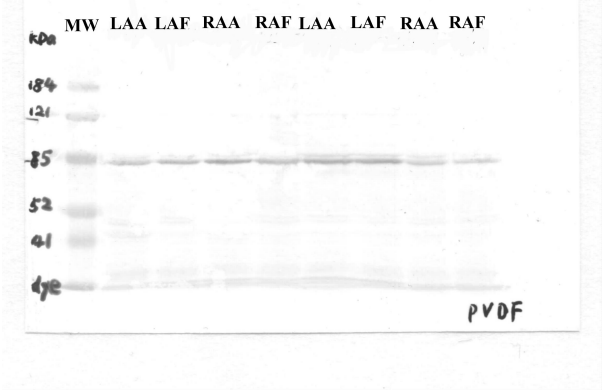
三、結果

Porcine model of atrial fibrillation:

Of the twelve pigs, 6 pigs with active pacing for 4 weeks all showed Af at the end of pacing. The Af persisted after turning off the pacemaker for 2 weeks until sacrifice. For the other 6 pigs with shamed-operation, sinus rhythm was observed 4 weeks after the operation. These pigs had sinus rhythm through out the study period.

Western blotting

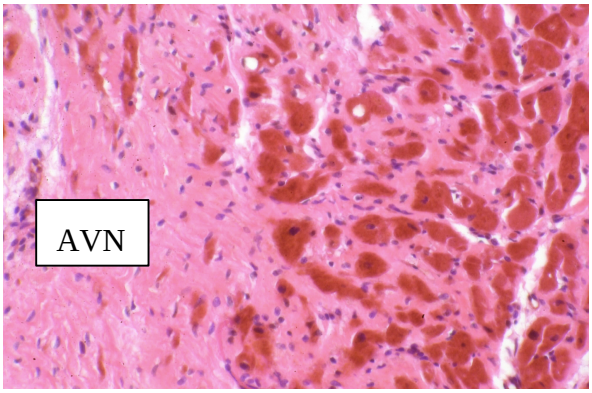
A representative results of Western blotting was shown in the following figure:

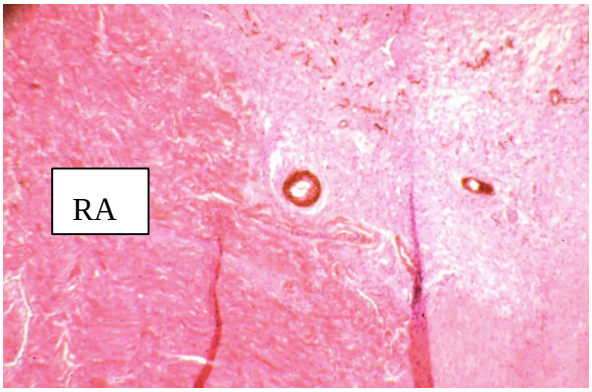


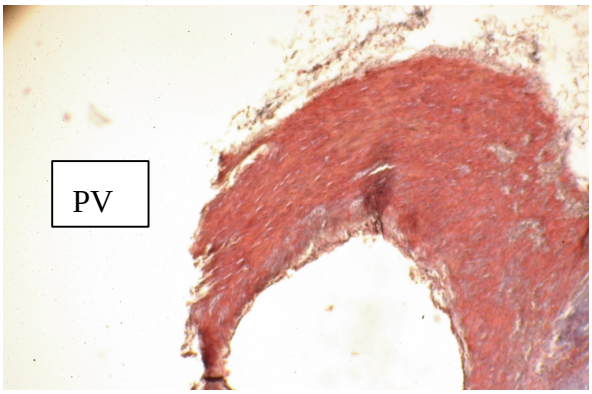
The Western blotting results showed a single band with predicted molecular weight. We found that the funny current channel protein was present in the pulmonary venous tissue. The channel protein was evenly distributed in the atrial tissue. We also found that funny current channel protein amount was not significantly different between atrial fibrillation and control pigs.

Immunohistochemistry

We performed immunohistochemitry studies on procine atrial, pulmonary vein and AV nodal tissue. The AV nodal tissue showed reaction with the antibody as expected. However, the funny current channel protein in the atrial tissue tissue was strongly associated with vascular smooth muscle. The pulmonary venous tissue was alos studied. It showed diffuse mild reaction with the funny current channel antibody. The microslide pictures are shown as the followings.







RT-PCR

We performed RT-PCR for the detection of funny current channel mRNA. Because the funny current cDNA sequence was not available in the GenBank, we designed the primer pair according to the human cDNA sequence. However, the human primers did not work on the porcine model. All the PCR reaction showed no band or bands with wrong size.

四、討論

In the present study, we successfully established a porcine model of atrial fibrillation. The provided a good model for the study of human atrial fibrillation.

Our study demonstrated for the first time the distribution of funny current channel in the atrial and pulmonary venous tissues. We found that funny current channels existed not only in pacemaker tissues but also working atrial myocardial tissue. This channel was also present in the pulmonary venous tissue.

In immunohistochemical studies, we further demonstrated that the funny current channel was present in large amount in the vascular smooth muscle cells. This finding is also novel. It is possible that these channels also play important roles in smooth muscle contraction.

The RT-PCR experiment was not successful. This is attributed to the lack of database in pigs. We may try this part of experiment when more information on pig genome is available.

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附件：封面格式

行政院國家科學委員會補助專題研究計畫成果報告

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