出國報告（出國類別:進修）

心律不整之深度病理機轉探討

服務機關：國立臺灣大學醫學院附設醫院/綜合診療部

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摘要

很幸運可以獲得這個機會以留職的方式順利在美國接受兩年完整的基礎研究訓練，接受訓練的地點是在美國印第安納大學 Methodist hospital 心臟科的實驗室，修習的主題為心律不整機制的探討，其中又以新興的 SK（small conductance calcium activated potassium channel）通道為研究的主軸。

藉由參與研究的過程，學習到很多研究心律不整的實驗方法，包括 optical mapping、patch clamp、細胞株的養殖、轉染、選殖技術、心臟衰竭兔子的建立、心肌細胞分離等等，也藉由整理研究結果、撰寫文章的過程當中學習到學者對於研究結果的解讀該有的嚴謹態度，在參與每周的實驗室報告以及國際會議參訪時，體驗到與同好分享與交流所得到的回饋，相信這些經驗在這輩子當受用無窮。

目次

本文

目的

本人為心臟科臨床醫師，對於心臟的電生理特別有興趣，在心臟電生理的領域服務病患有五年之久，深感對於心律不整的了解實有不足，尤其在心臟衰竭的病患身上看到許多難以控制的心律不整，植入性去顫器的確是病患的一大福音，但卻無法真正的預防或減少病患被電擊的痛苦，心律不整的研究必須著重在觀察心肌細胞膜電位的變化，擴大到心肌細胞間的交互作用，筆者有感於國內這方面研究能力的不足，故申請至國外專門從事心律不整研究的實驗室學習研究心律不整的方法。

過程

一、實驗室介紹: Krannart Institute of Cardiology, Indiana University

此次進修的實驗室位在美國印第安納州州政府所在地印第安納波里斯的 Methodist Hospital 內，緊鄰心導管室及手術室，但是由於門禁管制，我們平常是不會進導管室及手術室，但如果有需要時，如我們要去取從人體採下來的心臟組織時，很方便就可以取得，並可在最短的時間內將取得的組織加以處理保存。心臟科平時定期的學術會議都在實驗室旁邊的會議室舉行，我們都可以自由參加，對於學基礎出身的研究員來說，是增進臨床知識的管道，對於我們這種本來就是臨床背景的研究員，也可以溫故而知新。

實驗室有兩層樓，在進修的這兩年內感覺實驗室一直處於搬動的狀態，有時候是要合併，有時候是要挪出空間來給新的老師或新的機器使用，有時候是要配合新的法規，雖然對於研究員來說，不喜歡東西被移來移去，但是感覺實驗室是活的，沒有必須墨守的成規，沒有必須堅守的傳統。實驗室有幾位老師級的人物，他們的辦公室就在同一樓層，有問題要討論時很

容易就找得到人，也可以和同儕討論，常常在討論中找到研究的方向，開放的空間有助於彼此的交流與溝通。

二、研究主題

心臟衰竭會造成心肌細胞電生理性質的改變，如鉀離子通道電流下降、鈉離子通道關閉不全、以及鈉鈣離子交換增加，這些性質的改變會使得心肌細胞再極化的能力下降，使得膜電位處於去極化狀態的時間拉長，導致心臟處於容易發生心律不整的狀態，造成病患發生心因性死亡。SK (small conductance calcium-activated potassium current)是一種鉀離子通道，它受鈣離子調控，已知在中樞神經系統扮演控制神經細胞活性的重要角色，但對於其在心肌細胞上的角色還不是非常了解。過去藉由一些動物實驗已經知道這個鉀離子通道在心房及房室結的傳導有一定的影響，對於心室的影響則還處於不明確的階段。在正常的心室，這個鉀離子通道似乎影響不大，但一旦心臟處於衰竭的狀態，其他的鉀離子通道下降時，這個受鈣離子調控的鉀離子通道就相對顯得重要。

三、研究內容

甲、Apamin 的專一性(附件一、二)

Apamin 是 SK 通道的抑制劑，許多實驗藉由給予 apamin 來研究 SK 通道的角色，然而過去某些研究對於 apamin 的專一性並沒有共識，有一些早期的研究顯示 apamin 除了抑制 SK 通道外也會抑制心肌細胞的鈣離子通道及鈉離子通道，因而造成研究上的困惑--當實驗中觀察到細胞或組織對 apamin 的反應，是不是能完全代表 SK 通道呢?因此我們利用 Patch clamp 結合細胞轉染的技術，將 HEK293 細胞株分別轉染各種心肌細胞重要的離子通道，包括鈉離子、鈣離子、IKs、IKr、Ito、以及 IK1，利用 patch clamp 的技術觀察這些通道電流對 apamin 的反應，發現 apamin 只對 SK 電流有很強的抑制效果，證實 apamin 是一個對 SK 專一性很高的

抑制劑，而這個結果也印證在兔子的心肌細胞上，證實在實驗上觀察到給予 apamin 前後的變化確實是可以代表 SK 通道的影響，這個結果將對後人欲使用 apamin 來研究 SK 通道提供重要的依據。

乙、SK 通道在急性心搏過慢之心肌細胞的變化(附件三)

SK 通道在正常心室並不明顯，但在心臟衰竭的細胞會上升，前人為了徹底了解 SK 通道的變化，以將房室結阻斷的方式控制兔子心臟的速率，意外發現 SK 電流的上升與單純心臟衰竭相比，似乎有更明顯的現象，為了觀察單純房室結阻斷導致心搏過慢對 SK 通道的影響，我們將健康兔子的心臟取出，以 Eppendorff 灌流的技術維持心臟的活性，將房室結阻斷，結合 optical mapping 的技術觀察 SK 通道在這些心搏過慢心臟的變化，發現即使是健康的心臟，只要經過兩個小時的緩慢起搏，SK 通道就有上揚的情形，但其臨床意義有待進一步釐清。

丙、SK 通道在人類心臟衰竭細胞的驗證

既然在動物實驗中可以觀察到 SK 電流在心臟衰竭的心肌細胞會增加，相對於其他鉀離子通道的下降，更顯得異常重要，我們想要了解這個現象在人類是否也有和動物實驗一樣的結果、相對於其他重要的鉀離子通道對膜電位有多少比例的影響。我們利用 optical mapping 的技術，將接受心臟移植病患的衰竭心臟染色，在光學顯微鏡下觀察 apamin 對膜電位變化以及鈣離子釋放的反應，配合免疫組織化學技術，證實 SK 通道在人類的衰竭心臟也有像動物實驗中觀察到的上升的情形，另外也意外觀察到這個通道似乎對電流在心肌組織橫向傳導的速度也有影響力，這個發現有待進一步研究來了解其機轉及可能造成的影響。

丁、臨床上常用藥物對 SK 通道的影響(附件四)

既然 SK 通道在心臟衰竭時會上揚，影響其膜電位的穩定及電流的傳導，我們會擔心臨床上常用的藥物，可能會干擾到這個電流而不自知，因此

我們利用 HEK293 細胞株轉染 SK 通道的模式，觀察這個 SK 電流會不會受到臨床上常用藥物的影響。我們選用在臨床上最常使用在心臟衰竭病患的藥物 amiodarone 來測試，結果證實 amiodarone 在一般的治療濃度下確實會抑制 SK 電流，其下游代謝物也有一樣的效果，這個發現喚起臨床醫師對這個離子通道的重視，其他藥物對於 SK 通道的影響也應該要測試，未來新的藥物的開發如果有可能要使用在心臟衰竭的病患也應該測試其對 SK 通道的影響。

戊、SK 通道在 calmodulinopathy 病患的影響(附件五)

這兩年來自世界不同角落的醫師紛紛報導一些早發性心因性猝死的病患，先是一個個案報告，緊接著較大規模篩檢，報導 calmodulin 基因突變造成病患在很年輕的時候就有很嚴重的心律不整，這些病患的鈣離子通道以及 ryanodine receptor 都受到突變的 calmodulin 影響，而使這些病患的心肌細胞處於不穩定的狀態，SK 通道受鈣離子調控正是透過 calmodulin 來決定 SK 通道的開啟及關閉，我們想要了解在這些病患的心肌細胞中 SK 通道的變化。我們利用轉染及選殖技術先培養出帶有穩定 SK 電流的細胞株，再將這些從病患身上找到的突變 calmodulin 轉染至細胞株內，觀察其對 SK 電流的影響，發現 SK 電流會受到抑制，為了進一步了解這些突變的 calmodulin 是如何影響 SK 電流，我們利用免疫螢光染色及共軛焦顯微鏡觀察 SK 蛋白質在這些細胞中的分布，了解到這些突變的 calmodulin 影響 SK 電流是藉由改變 SK 通道的開啟與關閉，排除 SK 通道蛋白質的製造及細胞內傳輸受到影響的可能性。

四、國際會議參訪

甲、Gordon Research Conference (Cardiac Arrhythmia Mechanism) (Mar, 2013; Ventura, CA) (附件三)

這是一個之前在臺灣從來沒有聽說過的會議，參加之後覺得非常的值回

票價，這個會議的報名人數非常有限，必須及早報名，參加的人主要以年輕前衛的學者為主，只有一間大教室，為期滿滿兩天(從早到晚)的課程，聽取優秀的學者們談論他們的最新研究，感覺醍醐灌頂，這些研究的初步成果很多都還沒有發表在文獻上，但是學者們對於他們自己研究的主題的專注性令人佩服。這是我到美國後參加的第一個學術會議，我將我在心搏過慢兔子心臟上觀察到 SK 通道電流變化的發現在會議上報告，與與會的學者交流，獲得很多肯定及寶貴的意見，也幸運獲得大會賞識，獲頒獎狀一只(附件六)。

乙、Cardiac electrophysiology Society (Nov, 2013; Denver) (附件一)

這個會議是 AHA(American Heart Association)的會前會，也是以電生理學的領域為主，我將對 apamin 專一性的研究結果發表在這個會議中，吸引一些也很仰賴 apamin 的實驗室朋友的注意，彼此交流研究的進展及困難處，受益良多。

丙、Heart Rhythm Society (May 2014, San Francisco)(附件五)

這個會議基本上是每年電生理學領域的醫師必到的大會，會議的內容比較偏向臨床議題，參加人數非常多，同一時間有許多活動在進行，每次參加這種大會就會有劉姥姥進大觀園的感受，我除了在會中發表 calmodulin 突變對 SK 通道的影響，也利用時間了解目前在這個領域的新進展，在實驗室做基礎研究將近有兩年的時間，感覺對臨床領域的活動有些生疏。在會中遇到好多來自臺灣的同好，藉機了解臺灣的狀況，為回國做準備。

心得

由於這次去的實驗室是一個已經營運有年且經費充足的實驗室，加入成為這個實驗室的一員，開始自己的研究感覺毫無困難。實驗室的主持人開明而支持的

態度，更使得整個兩年的研究過程充實而愉快。在這兩年和老闆的相處當中，雖然沒有辦法像老闆一樣有源源不絕的研究靈感，但是深深為老闆作研究的態度折服。老闆雖然已經是著名期刊的主編，對於新的發現從來沒有先入為主的觀念，對於研究結果的解讀以及主動頻繁地與其他學者交流的態度也令我嘖嘖稱奇，對於其他學者的批評能虛心接受，但對自己研究主題的信念卻又屹立不搖，我想，我沒有辦法把美國優渥的研究環境帶回臺灣，但至少我可以學到老闆做研究的精神，把這個精神帶回臺灣，希望能繼續在臺灣進行心律不整相關的研究，期能為人類心律不整的治療盡一份心力。

建議事項

這次出國進修兩年的時間，學習到許多先進的實驗方法，體會到學者應有的研究態度，擴大了自己的視野，期間也藉機和外國同事交流，推展臺灣的知名度，覺得是一個非常好的國民外交方式，個人認為，此次進修，受益良多。

不過在這過程之中遇到一些小小的困難，在此提出，或許有改善的方法

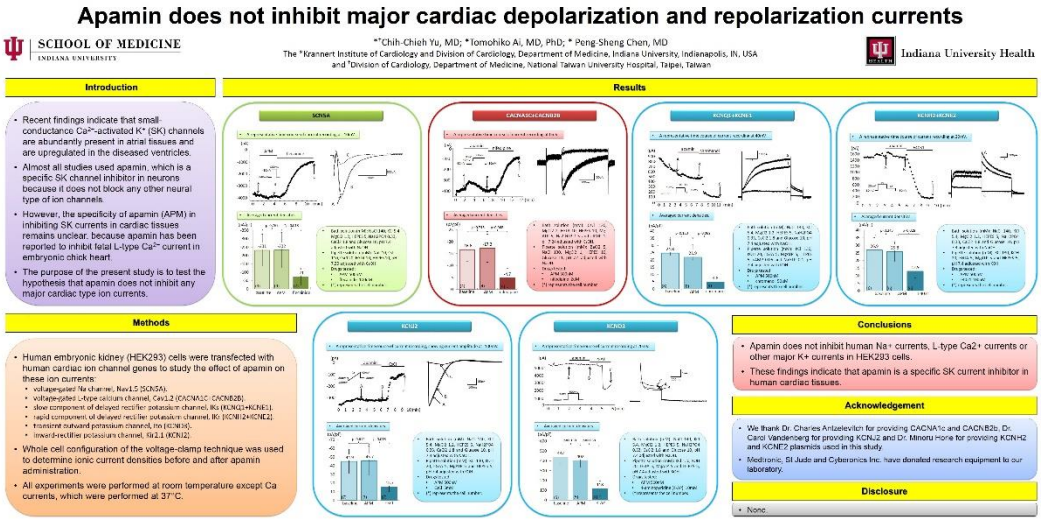
一、對於出國進修職員的補貼。在一個實驗室要開始全新的研究計畫，如果想要有研究成果，一年的時間往往是不夠的，但現行制度對職員的補貼只有一年，是否考慮可將補貼延長至兩年?

二、美國是一個高社會福利的國家，國民必須繳納高額的稅金給政府，部分進社會福利系統，部分成為未來自己失業或退休的福利金，這些對於我們這些短期研究員來說根本不應繳交，因為我們既沒享受社會福利，也不會在美國退休，美國和許多國家(包括中國大陸、日本)都有簽約，短期學員不需繳納這些稅款，但臺灣並不在這個名單之內，是否請有關單位進行了解評估，比照其他國家的方式，可能為未來學員減輕負擔。

以上幾點是筆者的小小建議，回國後希望能將在美國所學應用在國內，臺灣的學術研究環境不比美國優渥，相關單位的補助也少許多，但是這牽涉到臺灣的整個經濟狀況以及上位者對學術研發的態度與支持，筆者希望能在現行有限的

資源當中，努力將所學應用在實務上，才不辜負此次出國進修的宗旨。

附件一


Apamin does not inhibit major cardiac depolarization and repolarization currents
SCHOOL OF MEDICINE
INDIANA UNIVERSITY
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Introduction
Recent findings indicate that small conductance Ca2+-activated K+ (SK) channels are abundantly present in atrial tissues and are upregulated in the diseased ventricles.
Almost all studies used apamin, which is a specific SK channel inhibitor in neurons because it does not block any other neural type of ion channels.
However, the specificity of apamin (APM) in inhibiting SK currents in cardiac tissues remains unclear, because apamin has been reported to inhibit fetal L-type Ca2+ current in embryonic chick heart.
The purpose of the present study is to test the hypothesis that apamin does not inhibit any major cardiac type ion currents.
Results
SCN5A
CACNA1C/CACNB2B
KCNQ1/KCNE1
KCNH2/KCNE2
Methods
Human embryonic kidney (HEK293) cells were transfected with human cardiac ion channel genes to study the effect of apamin on these ion currents:
voltage-gated Na channel, Nav1.5 (SCN5A).
voltage-gated L-type calcium channel, Cav1.2 (CACNA1C/ CACNB2B).
slow component of delayed rectifier potassium channel, IKs (KCNQ1+KCNE1).
rapid component of delayed rectifier potassium channel, IKr (KCNH2+KCNE2).
transient outward potassium channel, Ito (KCND3).
Inward-rectifier potassium channel, Kir2.1 (KCNJ2).
Whole cell configuration of the voltage-clamp technique was used to determine ionic current densities before and after apamin administration.
All experiments were performed at room temperature except Ca currents, which were performed at 37°C.
KCNJ2
KCND3
Conclusions
Apamin does not inhibit human Na+ currents, L-type Ca2+ currents or other major K+ currents in HEK293 cells.
These findings indicate that apamin is a specific SK current inhibitor in human cardiac tissues.
Acknowledgement
We thank Dr. Charles Antzelevitch for providing CACNA1c and CACNB2b, Dr. Carol Vandenberg for providing KCNJ2 and Dr. Minoru Horie for providing KCNH2 and KCNE2 plasmids used in this study.
Medtronic, St Jude and Cyberonics Inc. have donated research equipment to our laboratory.
Disclosure
None

附件二

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PLOS ONE

Apamin Does Not Inhibit Human Cardiac Na^+ Current, L-type Ca^{2+} Current or Other Major K^+ Currents

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Abstract

Background: Apamin is commonly used as a small-conductance Ca^{2+}-activated K^+ (SK) current inhibitor. However, the specificity of apamin in cardiac tissues remains unclear.

Objective: To test the hypothesis that apamin does not inhibit any major cardiac ion currents.

Methods: We studied human embryonic kidney (HEK) 293 cells that expressed human voltage-gated Na^+, K^+ and Ca^{2+} currents and isolated rabbit ventricular myocytes. Whole-cell patch clamp techniques were used to determine ionic current densities before and after apamin administration.

Results: Ca^{2+} currents (CACNA1c+CACNB2b) were not affected by apamin (500 nM) (data are presented as median [25th percentile;75th percentile] (from −16 [−20;−10] to −17 [−19;−13] pA/pF, P = NS), but were reduced by nifedipine to −1.6 [−3.2;−1.3] pA/pF (p = 0.008). Na^+ currents (SCN5A) were not affected by apamin (from −261 [−282;−145] to −268 [−379;−132] pA/pF, P = NS), but were reduced by flecainide to −57 [−70;−47] pA/pF (p = 0.018). None of the major K^+ currents (I_{Ks}, I_{Kr}, I_{K1} and I_{to}) were inhibited by 500 nM of apamin (KCNQ1+KCNE1, from 28 [20;37] to 23 [18;32] pA/pF; KCNH2+KCNE2, from 28 [24;30] to 27 [24;29] pA/pF; KCNJ2, from −46 [−48;−40] to −46 [−51;−35] pA/pF; KCND3, from 608 [505;748] to 606 [454;684]). Apamin did not inhibit the I_{Na} or I_{CaL} in isolated rabbit ventricular myocytes ($I_{Na,}$ from −67 [−75;−59] to −68 [−71;−59] pA/pF; $I_{CaL,}$ from −16 [−17;−14] to −14 [−15;−13] pA/pF, P = NS for both).

Conclusions: Apamin does not inhibit human cardiac Na^+ currents, L-type Ca^{2+} currents or other major K^+ currents. These findings indicate that apamin is a specific SK current inhibitor in hearts as well as in other organs.

Citation: Yu C-C, Ai T, Weiss JN, Chen P-S (2014) Apamin Does Not Inhibit Human Cardiac Na^+ Current, L-type Ca^{2+} Current or Other Major K^+ Currents. PLoS ONE 9(5): e96691. doi:10.1371/journal.pone.0096691

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Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Small-conductance calcium activated potassium (SK) channels, which are abundantly present in the central nervous system [1], were first cloned in 1996 by Kohler *et al* [2]. Study of this channel is facilitated by the use of apamin, which has been thought to be a specific inhibitor of SK current in the nervous system [1,3,4]. Subsequent investigations showed that the apamin-sensitive potassium current (I_{KAS}) is present in the atria [5–12]. In addition, while normal ventricles paced at physiological cycle lengths do not express significant I_{KAS} [13], we and others found that I_{KAS} expression is upregulated in failing, ischemic or infarcted human, rabbit and rat ventricles and in normal rabbit ventricles with complete atrioventricular block [14–19]. A common criticism of all these studies is that the specificity of apamin in cardiac type ion channels has not been well established. Some previous studies have shown that apamin inhibits fetal L-type Ca^{2+} currents [20–22] and Na^+ currents [23] in the chick heart, suggesting that apamin may have off target effects on other cardiac ion channels. However, there is no information on the effects of apamin on Na^+, Ca^{2+} and K^+ currents that are responsible for adult human cardiac activation and repolarization. Because I_{KAS} is potentially important in human cardiac arrhythmogenesis, it is important to establish whether apamin is a specific SK current inhibitor as apamin is used to define I_{KAS}. The purpose of the present study was to test the hypothesis that apamin is a specific inhibitor of I_{KAS} in adult human cardiac ion channels. We tested that hypothesis by performing patch clamp studies of major cardiac ion channels expressed in human embryonic kidney (HEK) 293 cells and by testing the effects of apamin on Na^+ and Ca^{2+} currents in rabbit ventricular myocytes.

附件三

Persistent Bradycardia Acutely Upregulates Small-conductance Ca^{2+}-activated K^+ Channels

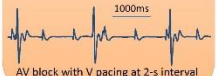
*†Chih-Chieh Yu, MD; *Po-Cheng Chang, MD; *Chia-Hsiang Hsueh, PhD; *Tomohiko Ai, MD, PhD; * Shien-Fong Lin, PhD; * Peng-Sheng Chen, MD
The *Krannert Institute of Cardiology and Division of Cardiology, Department of Medicine, Indiana University, Indianapolis, IN, USA and †Division of Cardiology, Department of Medicine, National Taiwan University, Taipei, Taiwan

Introduction

- Atrioventricular (AV) block and bradycardia is known to cause K current downregulation, leading to prolongation of action potential duration (APD), QT interval and ventricular proarrhythmia.
- Small-conductance Ca^{2+}-activated K+ (SK) channels are present but its currents are not active in normal ventricles during sinus rhythm.
- We hypothesize that (1) acute AV block rapidly prolongs ventricular APD and the duration of intracellular Ca^{2+} (Ca_i) transient (DCa_iT), leading to compensatory SK current activation and (2) apamin, a specific SK current blocker, prolongs the postshock APD in hearts with complete AV block.

Methods

- We studied 8 Langendorff-perfused rabbit hearts.
- AV block was created by radiofrequency ablation and the ventricles were paced at 2-s pacing cycle length (PCL) for one hr in 6 rabbits (bradycardia group, Br group) and at 300 ms PCL in 2 rabbits (control group).
- Dual optical Mapping of action potential and Ca_i was performed before and after 100 nM of apamin.
- The APD_{80} and DCa_iT_{80} (measured to 80% repolarization) were averaged from entire mapped region.



AV block with V pacing at 2-s interval

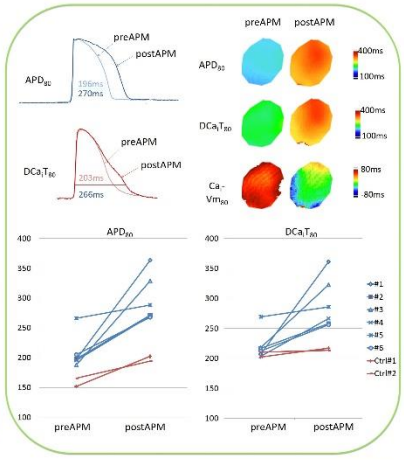
Results

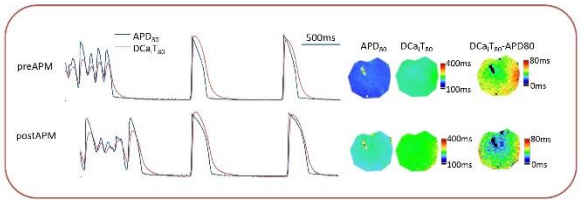
- **2-s PCL**

1. The APD_{80} at 2-s PCL 1hr after AV block in Br and Ctrl gps were 208±29ms and 159±10ms (p=0.011). After apamin infusion, the APD_{80} increased to 298±39ms and 198±6ms (p<0.001), respectively. The delta APD_{80} was significantly larger in Br gp than Ctrl gp (90±53ms vs. 40±15ms, p=0.042).
2. The DCa_iT_{80} at 2-s PCL 1hr after AV block in Br and Ctrl gps were 221±24ms and 206±6ms (p=0.208), respectively, and increased to 292±42 ms and 215±2 ms (p=0.007) after apamin infusion. The delta DCa_iT_{80} was significantly larger in Br gp than Ctrl gp (70±50ms vs. 15±4ms, p=0.014).

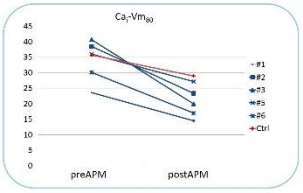
- **Postshock 1-s PCL**

1. We mapped induced ventricular fibrillation (VF) and defibrillation episodes. Only 5 rabbits in Br gp and 1 rabbit in Ctrl gp had VF episodes inducible.
2. Apamin increased the postshock APD_{80} from 152±11ms to 182±21ms (p=0.003) in Br gp and from 159ms to 171s in Ctrl gp, respectively.
3. Apamin increased the postshock DCa_iT_{80} from 189±9ms to 205±18ms (p=0.008) in Br gp and from 199ms to 201ms in Ctrl gp, respectively.





4. The discrepancy between APD_{80} and DCa_iT_{80} (Ca_i-Vm_{80}) was reduced by apamin from 34±10ms to 20±5ms (p=0.001) in Br gp and from 36ms to 29ms in Ctrl gp.



- Disclosure: None

Conclusions

- Persistent bradycardia upregulates SK current in normal rabbit ventricles.
- SK current blockade prolongs the postshock APD in hearts with persistent bradycardia.
- Hearts with AV block may be a useful model in studying the effects of drugs on SK currents.

Limitations

- Apamin is a specific SK current blocker in neurons, but its specificity in cardiomyocyte remains unclear.

附件四

OPEN ACCESS Freely available online

PLOS ONE

Amiodarone Inhibits Apamin-Sensitive Potassium Currents

Isik Turker[1], Chih-Chieh Yu[1,2], Po-Cheng Chang[1], Zhenhui Chen[1], Yoshiro Sohma[3], Shien-Fong Lin[1], Peng-Sheng Chen[1], Tomohiko Ai[1]*

1 Krannert Institute of Cardiology and Division of Cardiology, Department of Medicine, Indiana University School of Medicine, Indianapolis, Indiana, **2** Division of Cardiology, Department of Medicine, National Taiwan University, Taipei, Taiwan, **3** Department of Pharmacology, Keio University School of Medicine, Shinjuku, Tokyo, Japan

Abstract

Background: Apamin sensitive potassium current (I_{KAS}), carried by the type 2 small conductance Ca^{2+}-activated potassium (SK2) channels, plays an important role in post-shock action potential duration (APD) shortening and recurrent spontaneous ventricular fibrillation (VF) in failing ventricles.

Objective: To test the hypothesis that amiodarone inhibits I_{KAS} in human embryonic kidney 293 (HEK-293) cells.

Methods: We used the patch-clamp technique to study I_{KAS} in HEK-293 cells transiently expressing human SK2 before and after amiodarone administration.

Results: Amiodarone inhibited I_{KAS} in a dose-dependent manner (IC_{50}, 2.67±0.25 μM with 1 μM intrapipette Ca^{2+}). Maximal inhibition was observed with 50 μM amiodarone which inhibited 85.6±3.1% of I_{KAS} induced with 1 μM intrapipette Ca^{2+} (n = 3). I_{KAS} inhibition by amiodarone was not voltage-dependent, but was Ca^{2+}-dependent: 30 μM amiodarone inhibited 81.5±1.9% of I_{KAS} induced with 1 μM Ca^{2+} (n = 4), and 16.4±4.9% with 250 nM Ca^{2+} (n = 5). Desethylamiodarone, a major metabolite of amiodarone, also exerts voltage-independent but Ca^{2+} dependent inhibition of I_{KAS}.

Conclusion: Both amiodarone and desethylamiodarone inhibit I_{KAS} at therapeutic concentrations. The inhibition is independent of time and voltage, but is dependent on the intracellular Ca^{2+} concentration. SK2 current inhibition may in part underlie amiodarone's effects in preventing electrical storm in failing ventricles.

Citation: Turker I, Yu C-C, Chang P-C, Chen Z, Sohma Y, et al. (2013) Amiodarone Inhibits Apamin-Sensitive Potassium Currents. PLoS ONE 8(7): e70450. doi:10.1371/journal.pone.0070450

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Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Heart failure is a major public health problem with 300,000 directly attributable deaths annually, in the United States alone. It has a prevalence of 5.8 million in the U.S. and over 23 million worldwide [1]. Ventricular arrhythmias are a major cause of morbidity and mortality in heart failure [2]. Today, many patients with heart failure receive an implantable cardioverter defibrillator (ICD) for primary or secondary prevention of arrhythmic death. However, ICD itself does not reduce the incidence of arrhythmias. Electrical storm (ES) defined as recurrent ventricular arrhythmias in a short period of time, remains a frequent complication and a strong independent predictor of poor outcome even in patients with ICDs [3,4]. Amiodarone is effective in the treatment of recurrent ventricular tachycardia or fibrillation [5] and is commonly used as the first line therapy for ES [6,7]. However, the mechanism behind amiodarone's effectiveness in treating ES remains poorly understood.

Ca^{2+} activated K^+ channels integrate intracellular calcium handling with membrane repolarization in various tissues including brain, peripheral nerve, endothelium, leukocytes, erythrocytes, heart, skeletal and smooth muscle [8]. They are classified into three types based on their conductance pattern: large (BK), intermediate (IK) and small (SK) conductance Ca^{2+} activated K^+ channels. SK channels show weak voltage dependence, susceptibility to the bee venom toxin apamin, and they are highly Ca^{2+} sensitive [9]. However, the role of these channels in the heart is poorly understood.

Xu *et al.* identified three isoforms of SK channels (SK1, SK2 and SK3) in the mouse and human heart, and found that they play important roles in the maintenance of action potential duration (APD) in atrial myocytes and pacemaking tissues [10]. Subsequently, the same group demonstrated that mice engineered to lack SK2 have prolonged atrial APD and higher susceptibility to atrial fibrillation [11]. Interestingly, SK2 expression is strikingly higher in normal mouse, cat and human atria than their respective ventricles [10]. This preferential expression led researchers to

附件五

Calmodulin mutations impede activation of small-conductance Ca^{2+}-activated K^+ current

Chih-Chieh Yu, MD*†, Tomohiko Ai, MD, PhD*†, Zhenhui Chen, PhD*, Matteo Vatta, PhD*, Alfred L. George, Jr., MD ¥, Peng-Sheng Chen, MD*

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Indiana University Health

INTRODUCTION

- Small-conductance Ca^{2+}-activated K^+ (SK) channels are upregulated in heart failure and contribute to the maintenance of repolarization reserve.
- Excessive or inadequate activation of SK current may shorten or prolong the action potential duration and promote ventricular fibrillation.
- The SK channels are gated by intracellular Ca^{2+} through the Ca^{2+}-binding protein calmodulin (CaM).
- We hypothesized that human CaM mutations may affect activation of SK channels.

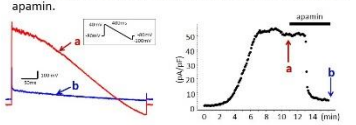
METHODS

- Five previously published CaM mutations were studied:
  - **N54I**, **N98S** : catecholaminergic polymorphic ventricular tachycardia.
  - **D96V** and **D130G**: congenital long QT syndrome with recurrent cardiac arrest.
  - **F90L**: childhood and adolescent onset idiopathic ventricular fibrillation.
- HEK 293 cells that stably express SK2 channels (**SK2 Cells**) were transiently transfected with either wild type (WT) or mutant CaM plasmids.
- Whole-cell voltage clamp recording was used to determine ionic current densities.
- SK2 current was defined by the difference of current before and after 500 nM of apamin.
- Solutions:
  - Bath (mM): NaCl 140, KCl 5.4, $MgCl_2$ 1.2, HEPES 5, NaH_2PO_4 0.33, $CaCl_2$ 1.8 and Glucose 10, pH 7.4 adjusted with NaOH.
  - Pipette (mM): K-Gluconate 144, $MgCl_2$ 1.15, EGTA 1, HEPES 10, free $CaCl_2$ 1.0 uM, pH 7.2 adjusted with KOH.
- The Kruskal-Wallis one-way analysis of variance was used to analyze the differences between groups and Mann-Whitney U test was used for post-hoc analysis.

RESULTS

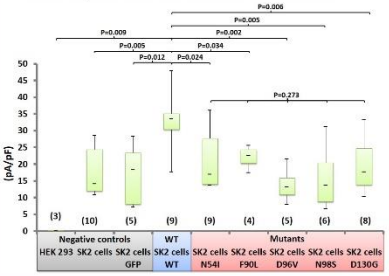
Representative tracings

- Representative tracings (left panel) before (a) and after apamin (b).
- Representative time course (right panel) of SK2 current recorded at 0 mV.
- SK2 current was determined by the difference before and after apamin.



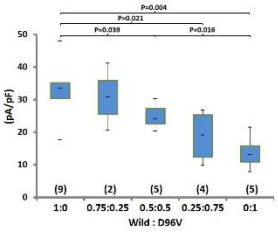
Comparisons between WT and mutant CaMs

- SK2 cells transfected with mutant CaMs had significantly lower SK2 current densities than that transfected with wild type (WT) CaM.
- No significant differences were found among different mutants.
- No significant differences were found among mutants and control cells (HEK 293 cells, SK2 cells with and without GFP transfection).
- Number in parenthesis shows the number of cells studied.



D96V is a loss-of-function mutation

- There is a linear relationship between SK2 current density and the relative amount of WT and D96V CaM in cells with co-transfection.
- Only p values < 0.05 are shown.



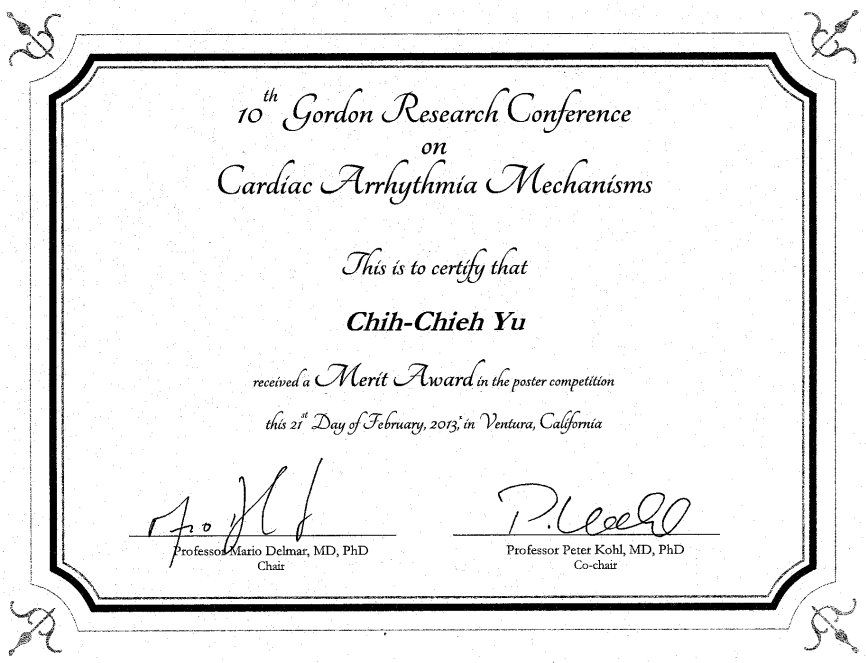
CONCLUSIONS

- Human arrhythmogenic CaM mutations impede activation of heterologously expressed SK2 channels.
- Impaired activation of SK current may contribute to arrhythmogenicity in the setting of CaM mutation.

DISCLOSURE

- Medtronic, St Jude and Cyberonics Inc. have donated research equipment to our laboratory.

附件六



10th Gordon Research Conference
on
Cardiac Arrhythmia Mechanisms

This is to certify that

Chih-Chieh Yu

received a Merit Award in the poster competition

this 21st Day of February, 2013, in Ventura, California

Professor Mario Delmar, MD, PhD
Chair

Professor Peter Kohl, MD, PhD
Co-chair

附件七

INDIANA UNIVERSITY
SCHOOL OF MEDICINE

Chih-Chieh Yu, MD

Has successfully served as
Research Fellow in Cardiac Electrophysiology

From September 1, 2012 August 31, 2014

David Cuss
Chairman, Department of Medicine

Pay-Sly Chen
Director, Division of Cardiology