• 計畫中文名稱	性別差異於心房及肺靜脈心肌之電生理特性、鈣離子調控與心房顫動之病理生理機轉		
• 計畫英文名稱	Gender Differences on the Electrophysiological Characteristics and Calcium Homeostasis in Atrium and Pulmonary Vein Cardiomyocytes---Implications in the Pathophysiology of Atrial Fibrillation		
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• 中文摘要	心房顫動是臨床上最常見的一種心律不整原因。研究發現性別差異是造成心律不整的一個重要的風險因子。心房顫動在男性的盛行率遠高於女性。肺靜脈心肌細胞已知在心房顫動的發生中扮演著重要的病理角色，而且肺靜脈具心肌細胞特性並具有高度心律不整活性。研究已知女性有相對較低的肺靜脈誘發的心房顫動發生率。這些結果推測男性與女性的肺靜脈可能具有不同的心律不整活性。異常的鈣離子調控可能對於肺靜脈誘發心房顫動的發生扮演重要角色。鈉鈣離子流交換增加以及 Ryanodine Receptor (RyR) 的功能異常可能誘發心房顫動與肺靜脈之心律不整活性，然而對於性別差異影響肺靜脈與心房心肌細胞之鈣離子調控並未清楚地研究。先前的研究已知鈣離子亮點(spark)在心房顫動的病理生理學機轉扮演重要的角色。因此，性別差異產生的鈣離子亮點可能引起不同的心房及肺靜脈心肌細胞之電生理與心律不整活性。FK-506 可造成 RyR 功能失調，進而引發心律不整。K201 則是一種 RyR 安定物質，被發現可抑制心房顫動。K201 可能經由調控鈣離子恆定而降低肺靜脈之心律不整活性，相反地 FK-506 則可能損害 RyR 功能而促進肺靜脈之心律不整活性，透過這些藥理的研究，我們可以探討性別差異如何影響肺靜脈心肌與心房細胞之鈣離子調控。因此，本研究第一年的研究目的在探討性別差異對於肺靜脈心肌與心房細胞的細胞型態、電生理特性之影響。第二年的實驗中，我們將探討在雄兔與雌兔肺靜脈心肌與心房細胞中，RyR 的功能、鈣離子調控、鈣離子亮點與鈣離子調控蛋白(Calmodulin Kinase、鈉鈣交換離子流、RyR、sarcoplasmic reticulum ATPase、Phopholamban)表現量，並評估這些差異與性別之間是否具關聯性。第三年的研究中我們將分別於雄兔與雌兔探討不同濃度的 RyR stabilizer (K201、Magnesium) 與 dysregulator (FK-506、Ryanodine、 Ouabain) 對電生理特性、離子流、鈣離子調控、鈣		

	離子亮點的影響，並比較公兔與母兔心房細胞與肺靜脈心肌細胞對這些藥物的反應是否有差異。實驗方法：第一年實驗─記錄公母兔 (2-3公斤，6 個月)心房與肺靜脈細胞之組織動作電位。藉著酵素灌流可分離兔子之單一心房與肺靜脈心肌細胞，全細胞箝定實驗記錄細胞之動作電位、L 型鈣離子流、鈉鈣交換離子流、暫時性外向鉀離子流與內向及延遲加強型鉀離子流，並加以比較分析。共軛焦顯微鏡偵測公母兔心肌細胞型態、及大小，並加以比較分析。第二年實驗─藉著酵素灌流可分離兔子之心房與肺靜脈心肌細胞，使用共軛焦顯微鏡偵測暫時性鈣離子流、鈣離子儲存、及鈣離子亮點的方法來測量鈣離子亮點及細胞內鈣離子濃度的變化。利用組織免疫染色及西方墨點法，PCR 去偵測並測量 Kir 2.2, mink, HERG, Kv4.3, RyR、Calmoduline Kinase 及其磷酸化態、鈉鈣交換離子流、dihydropyridine 接受器、sarcoplasmic reticulum ATPase、與 Phopholamban 之蛋白質與 RNA 表現量。第三年實驗─公母兔的心房與肺靜脈單一細胞及組織接受 K201(0.1, 1, 10 μM) magnesium sulfate (1.8 mM, 5.4 mM) or FK506 (0.01, 0.1, 1 μM), ryanodine (0.1, 1, 10 μM) and ouabain (0.1, 1, 10 μM)1,10μm)藥物前後，利用傳統電極記錄及全細胞箝定技術記錄其動作電位、L 型鈣離子流、鈉鈣交換離子流、暫時性外向鉀離子流與內向及延遲加強型鉀離子流。利用共軛焦顯微鏡偵測細胞在接受上述藥物前後之暫時性鈣離子流、鈣離子儲存、與鈣離子亮點。
• 英文摘要	Atrial fibrillation (AF) is the most common cardiac arrhythmia seen in clinical practice and induce cardiac dysfunction and stroke. Gender difference has been found to be an important risk factor of atrial fibrillation. The prevalence of AF was considerably greater in men than in women. Pulmonary veins (PVs) were known to be important pathological role in the genesis of AF. PVs have been shown to contain cardiomyocytes with a high arrhythmogenic activity. It has been shown that female gender has an relatively lower incidence of AF from pulmonary veins (PVs). These findings suggest the possible different arrhythmogenic activity between male and female PVs. However, the understating about the gender differences of PVs and atrium is not clear. Abnormal calcium regulation has been suggested to play an important role in the genesis of atrial fibrillation and PV arrhythmogenesis. Enhanced Na+/Ca2+ exchanger (NCX) and dysfunction of ryanodine receptor (RyR) have been suggested to induce AF and PV arrhythmogenesis. Nevertheless, the information about the gender difference in calcium regulation of PV and atrium was limited. Furthermore, Ca2+ spark has been reported to have a role in the pathophysiology of AF. It is possible gender difference may have different calcium spark to induce dissimilar atrial electrophysiology and PV arrhythmogenesis. FK-506 may induce RyR dysfunction to enhance a sarcoplasmic reticulum (SR) Ca2+ leak to induce AF. K201, the RyR stabilizer has been shown to reverse abnormal RyR and found to inhibit atrial fibrillation with unclear mechanisms. It is possible that K201 may reduce PV arrhythmogenic activity though its regulation on calcium homeostasis and FK-506 may impair PV RyR with an increase of arrhythmogenesis. Through the pharmacological studies, we have the chances to investigate the gender differences in calcium regulation in PV and atrial cardiomyocytes. The purposes of this study in the first year are to investigate the gender difference on the cell morphology and electrophysiological characteristics in the PVs and atrial cardiomyocytes. In the second year experiment, we will investigate the RyR function, calcium regulation, calcium spark and expressions of calcium regulation proteins (calmodulin kinase, NCX, RyR, SERCA, phsopholamban) in PV and atrial cardiomyocytes from male and female rabbits and evaluate whether these differences are gender related. In the third study, we will evaluate the effects of different concentrations of RyR stabilizer (K201, magnesium) and dysregulator (FK-506, ryanodine, ouabain) on the electrophysiology, ionic currents, calcium regulation and calcium spark in PV and atrial tissue preparations and single cardiomyocytes and compare these pharmacological

	responses among atrial and PV cardiomycocytes with and without pacemaker activity in male and female rabbits. Methods: The first year experiment- Transmembrane action potentials (APs) were recorded in atrium and PVs from male and female rabbits (2-3 Kg, 6 months). Single cardiomyocytes are isolated from rabbit PVs and atrial appendage through perfusion of Tyrode solution containing digestive enzymes. Whole-cell clamp techniques are used to study the L-type calcium current (ICa-L), transient inward currents, NCX currents, transient outward currents (Ito) and delayed (IK) and inward rectified outward potassium (IK1) current in female and male PV and atrial cardiomyocytes. Confocal microscopy is used to measure the cell size and number of cell bifurcations from female and male single cardiomyocytes. Second year experiment: Single cardiomyocytes are isolated from rabbit female and male PVs and atrial appendage through perfusion of Tyrode solution containing digestive enzymes. Confocal microscopy is used to measure the intracellular calcium ([Ca2+]i) transient, [Ca2+]i store, and Ca2+ sparks with fluorescence. Immunolabeling with confocal microscopy and western blot are used to detect and measure the Kir 2.2, mink, HERG, Kv4.3, RyR with and without phosphorylation, Calmoduline kinase with and without phosphorylation, NCX, SERCA, and phospholamban. Third year experiment: The APs and ionic currents (L-type calcium current (ICa-L), transient inward currents, NCX currents, Ito, IK and IK1 current are obtained from isolated male and female rabbit PV and atrial tissue preparations and single cardiomyocytes before and after the administration of K201 (0.1, 1, 10 μM), magnesium sulfate (1.8 mM, 5.4 mM) or FK506 (0.01, 0.1, 1 μM), ryanodine (0.1, 1, 10 μM) and ouabain (0.1, 1, 10 μM) using the conventional microelectrode recording and whole-cell clamp techniques. Confocal microscopy is used to measure the [Ca2+]i transient, [Ca2+]i stores and Ca2+ sparks with fluorescence before and after drug administrations.