分类号____密级___ UDC___

学校编码: 10384 学号: 24620121154389



硕士学位论文 甲亢性心功能变化中心磷脂对延迟整流 钾离子通道亚基KCNQ1,MIRP1和hERG的影响 Effect of Cardiolipin on Cardiac KCNQ1,MIRP1 and hERG subunits of Delayed Rectifier Potassium Channel in Hyperthyroidism.

Samiullah

指导教师姓名: 叶本兰教授

专业名称: 生理学

论文提交日期: 2015年4月

论文答辩时间: 2015年5月

学位授予日期: 2015年 月

答辩委员会主席:

评 阅 人:

2015年6月

厦门大学学位论文原创性声明

本人呈交的学位论文是本人在导师指导下,独立完成的研究成果。本人在论文写作中参考其他个人或集体已经发表的研究成果,均在文中以适当方式明确标明,并符合法律规范和《厦门大学研究生学术活动规范(试行)》。

另外,该学位论文为((心磷脂在甲亢性心脏功能变化中的作用及其钾离子调控途径的作用机制)课题(组)的研究成果,获得(国家自然科学基金81170727号项目)课题(组)经费或实验室的资助,在(厦门大学医学院生理学科)实验室完成。(请在以上括号内填写课题或课题组负责人或实验室名称,未有此项声明内容的,可以不作特别声明。)

声明人(签名):

年 月 日

DECLARATION

I hereby declare that apart from the sources cited, this dissertation is my own work under the supervision of my supervisor Professor YE Ben Lan. The materials of the dissertation have not been presented and it will not be presented to any other university other than this university for similar or any other degree.

Signature

Candidate:Samiullah

厦门大学学位论文著作权使用声明

本人同意厦门大学根据《中华人民共和国学位条例暂行实施办法》等规定保留和使用此学位论文,并向主管部门或其指定机构送交学位论文(包括纸质版和电子版),允许学位论文进入厦门大学图书馆及其数据库被查阅、借阅。本人同意厦门大学将学位论文加入全国博士、硕士学位论文共建单位数据库进行检索,将学位论文的标题和摘要汇编出版,采用影印、缩印或者其它方式合理复制学位论文。

本学位论文属于:

- ()1.经厦门大学保密委员会审查核定的保密学位论文,于 年 月 日解密,解密后适用上述授权。
- ()2.不保密,适用上述授权。

(请在以上相应括号内打"√"或填上相应内容。保密学位论文应是已经厦门大学保密委员会审定过的学位论文,未经厦门大学保密委员会审定的学位论文均为公开学位论文。此声明栏不填写的,默认为公开学位论文,均适用上述授权。)

声明人(签名):

年 月 日

COPYRIGHTS

All rights reserved. No part of this dissertation may be reproduced, stored in any retrieval system, or transmitted in any form by any means; electronic, mechanical, photographing, recording or otherwise without prior written permission of the author or Xiamen University.

Signature

Candidate Samiullah

Table of Contents

Acknowledgment	I
List of Figures	II
List of Tables	IV
List of Abbreviations	V
List of Recipes	
Abstract in English	1
	4
CHAPTER 1 INTRODUCTION	
1.1Hyperthyroidism	6
1.1.1 Hyperthyroidism and thyroid hormones	
1.1.2 Hyperthyroidism and Mitochondria	7
1.1.3 Hypothyroidism and cardiovascular Risk factors	
1.2 Cardiolipin	11
1.2.1 Role of cardiolipin	11
1.2.2 Cardiolipin synthesis and remodeling	14
1.3 Delayed rectifier K ⁺ Potassium K ⁺ channels	15
1.4 Hyperthyroidism and Delayed rectifier K+ channels	16
1.4.1 The slow component, IKs	16
1.4.2 Atrial Fibrillation	17
1.4.3 The fast component IKr	18
1.4.4 Long QT Syndrome	19
1.4.5 KCNQ1 and HERG are partners	20
CHAPTER 2 MATERIALS AND METHODS	
2.1 Cell culture	21

2.1.1 Cell Line
2.1.2 Cell preparation for experiments
2.1.3 Treatment of H9C2
2.2 Immunoblot PAGE22
2.2.1 Protein Extraction
2.2.2 Determination of protein concentration
2.2.3 Reagents and Buffers Preparation
2.2.4 Gel Preparation
2.2.5 Western Blot
2.2.6 Data analysis
2.3 Genetic experiments
2.3.1 Isolation of RNA
2.3.2 Determination of RNA concentration and purity
2.3.3 cDNA generation from total RNA
2.3.4 PCR thermocycler conditions
2.3.5 Standard PCR
2.3.6 cDNA Agarose Gel Electrophoresis
2.3.7 Primers
2.3.8 Quantitative Real Time PCR
2.3.9 Data analysis
CHAPTER 3 RESULTS
3.1 KCNQ1
3.1.1 KCNQ1 Western Blot Results
3.1.1.1 KCNQ1 in 48 hours T4 treated H9C233
3.1.1.2 Different time period treatment strategy for T4 treatment34
3.1.1.3 KCNQ1in 3 hours Cardiolipin treated H9C235

3.1.1.4 KCNQ1in 3 hours Cardiolipin treated and 48 hours T4 treated H9	
	36
3.1.1.5 KCNQ1 Relative Expression of 3 hours Cariolipin with 48 hours	T4
treatment and without T4 treatment in H9C2	37
3.1.2 Quantiative RT-PCR	38
3.1.2.1 PCR primers were experimentally determined specifically	38
3.1.2.2 RT-PCR KCNQ1in 48 hours treated with T4	38
3.1.2.3 KCNQ1 in 3 hours Cardiolipin treated H9C2	39
3.1.2.4 KCNQ1in 3 hours Cardiolipin treated, along with 48 hours T4	
treated H9C2	40
3.1.2.5 Relative Expression of KCNQ1 with 3 hours Cariolipin treatment	t in
both t4 treated cells for 48 hours, and only treated with3 hours Cariolipin	
3.2 hERG	42
3.2.1hERG Western blot Results	42
3.2.1.1 hERG in 48in hours T4 treated H9C2	42
3.2.1.2 hERG in 3 inhours Cardiolipin treated H9C2	43
3.2.1.3 hERGin 3 hours Cardiolipin treated along 48 hours T4 treatment	of
H9C2	44
3.2.1.4 hERG Relative Expression of 3 hours Cariolipin with 48 hours Tatreatment and without T4 treatment in H9C2	
3.2.2 Quantiative RT-PCR	
3.2.2.1 PCR primers were experimentally determined specifically	47
3.2.2.2 hERG in 48 hours treated with T4	47
3.2.2.3 hERG in 3 hours Cardiolipin treated H9C2	49
3.2.2.4 hERG in 3 hours Cardiolipin treated, along with 48 hours T4 trea	ted
H9C2	49
3.2.2.5 Relative Expression of hERG with 3 hours Cariolipin treatment in	n
both T4 treated cells for 48 hours, and only treated with3 hours Cariolipin	51
3 3 MIRD1	52

3.3.1 MIRP1 Western blot Results
3.3.1.1 MIRP1in 48 hours T4 treated H9C2
3.3.1.2 MIRP1in 3 hours Cardiolipin treated H9C253
3.3.1.3 MIRP1in 3 hours Cardiolipin treated along 48 hours T4 treatment of
H9C254
3.3.1.4 MIRP1 Relative Expression of 3 hours Cariolipin with 48 hours T4
treatment and without T4 treatment in H9C255
3.3.2 Quantiative RT-PCR55
3.3.2.1 PCR primers were experimentally determined specifically56
3.3.2.2 MIRP1in 48 hours treated with T456
3.3.2.3 Different time period treatment strategy for T4 treatment57
3.3.2.4 MIRP1 in 3 hours Cardiolipin treated H9C259
3.3.2.5 MIRP1in 3 hours Cardiolipin treated, along with 48 hours T4 treated
H9C260
3.3.2.6 Relative Expression of MIRP1in 3 hours Cariolipin treatment in both
T4 treated cells for 48 hours, and only treated with 3 hours Cariolipin61
DISCUSSIONS 63
SUMMARY 69
REFERENCES71

ACKNOWLEDGEMENT

In the name of Allah, the most Beneficent, the most Merciful. You made our consciousness the shore at which Your divine mysteries lap and so enabled us to sense those mysteries. I sent my gratitude on Muhammad (peace be upon him).

I would like to show gratitude to Professor Dr. Ye Ben Lan for accepting me in her laboratory and allowing me to work in scientifically resourceful environment.

My sincere thanks go to Dr.Qi zhi for all his support and guidance throughout the completion of this project. This whole project is manifested with hard luck and mistakes, however, Dr.. Zou Jun always showed a way out.

The opportunity to pursue my graduate studies would be a dream without the scholarship provided by China Scholarship Council.

I am especially grateful to my laboratory fellows for their help and support. Thanks so much to you all especially to Miss Guo Fan and Zhou Li for their complete assistance. My stay in China provided me immense love and care of my Chinese friends without whom life would have become dull and boring .Liu Jing, Yang Han, He Fei, Zhang Yu Jiao who said good bye earlier though, made my early days pleasant here. Zhong Chun Lian, Hu Guang Lei, Hou Qi, Sun Ting, Yan Ya, Zhao Fa Yun, Chen Ji, Xu Jun Ming, Song Ying, Xie Xin Wen, Zhang Bo, Jiang Hai Long, Ye Shi Wei and special thanks to Dai Dong Li and Zhou Xiao Xia for amusing us in boring time. Wish you all a very best of luck in life.

Last but not least to my Late Grandparents whom I have lost during my masters degree here in China, Abbu g,Ammi g, my sisters, Phuppho, and Uncles. Thank you all for your unconditional love, affiliation and support. I dedicate my effort to you all.

Samiullah.

List of Figures

3.1 KCNQ1 protein expression level of control and T4 in H9C2 cells
3.2 KCNQ1 protein expression level of of control and T4 of different hours and analysis of gene expression in H9C2 cells
3.3 KCNQ1 protein expression level of control and CL in H9C2 cells
3.4 KCNQ1 protein expression level of T4 and T4+CL in H9C2 cells
3.5 KCNQ1 protein expression level of CL and T4+CL in H9C2 cells
3.6 KCNQ1 gene expression by northern blot
3.7 KCNQ1 gene expression level of control and T4 in H9C2 cells
3.8 Analysis of KCNQ1 gene expression level of control and CL in H9C2 cells 40
3.9 Analysis of KCNQ1 gene expression level of T4 and T4+CL in H9C2 cells 41
3.10 Analysis of KCNQ1 gene expression level of each CL and T4+CL in H9C2 cells 42
3.11hERG protein expression level of control and T4 in H9C2 cells
3.12hERG protein expression level of control and CL in H9C2 cells
3.13Herg protein expression level of T4 and T4+CL in H9C2 cells
3.14 hERG expression level of CL and T4+CL in H9C2 cells
3.15 hERG gene expression by northern blot
3.16 Analysis of hERG gene expression level of control and T4 in H9C2 cells
3.17 Analysis of hERG gene expression level of control and CL in H9C2 cells 49
3.18 Analysis of hERG gene expression level of T4 and T4+CL in H9C2 cells 50
3.19Analysis of hERG gene expression level of each CL and T4+CL in H9C2 cells 51
3.20MIRP1 protein expression level of control and T4 in H9C2 cells
3.21MIRP1 protein expression level of control and CL in H9C2 cells
3.22MIRP1 protein expression level of T4 and CL in H9C2 cell

3.23MIRP1 protein expression level of CL and T4+CL in H9C2 cells	55
3.24 MIRP1 gene expression by northern blot	56
3.25 Analysis of MIRP1 gene expression level of control and T4 in H9C2 cells	57
3.26Analysis of MIRP1 gene expression level of control and T4 of different hours	58
3.27Analysis of MIRP1 gene expression level of control and CL in H9C2 cells	59
3.28 Analysis of MIRP1 gene expression level of T4 and T4+CL in H9C2 cells	60
3.29 Analysis of MIRP1 gene expression level of each CL and T4+CL in H9C2 cells	61

List of Tables

1.1Cardiovascular risks associated with hypothyroidism	. 10
2.1Primers for hERG.KCNO1 and MIRP1	. 31

List of Abbreviations

C degree Celsius

μg micro gram

μL micro Litre

AF Atrial fibrillation

AP Action potential

BLAST Hepes buffered saline

bp Base pair

BSA Bovine serum albumin

cDNA Complementary deoxyribonucleic acid

CL Cardiolipin

CL 18:2 Di unsaturated acyl chains Cardiolipin

CL14:0 Saturated tetramyristoyl Cardiolipin

CL18:1 Mono unsaturated Acyl chain cardiolipin,

CTP Cell glycosides three phosphate

ddH2O (DDwater) Double distilled H2O

DEPC Dolbecco modified Eagle medium

DMEM Diethyl pyro carbonate/Diethyl dicarobonate

DMSO Dimethyl sulfoxide

DNA Deoxyribonucleic acid

EGTA Ethylene glycol tetra acetic acid

FBS Fetal bovine serum

g gram

h hr

H2O2 Hydrogen peroxide

HBS Basic local alignment search tool

HCl Hydrochloric acid

Ikr Human ethera-go-go-related gene (hERG)

Iks KCNQ1 and KCNE1 together

KCl Potassium chloride

KCNH2 Herg

Kir inwardly rectifying K channel

KvLQT1 KCNQ1 or Kv7.1

L Litre

LQTS Long QT Syndrome

minK or IsK KCNE1

mRNA messenger RNA

Na2SO3 Sodium sulphite

NaCl Sodium chloride

NADPH Nicotinamide adenine dinucleotide phosphate

NaOH Sodium hydro oxide

ng nano gram

OD Optical density

PAGE Polyacrylamide gel electrophoresi

PG Phospholipid n glycerol

PGP Phospholipid n glycerol phosphate

PGRP Peptidoglycan recognition protein

PLA2 Phospholipase A2

PMSF Phenyl methane sulfonyl fluoride

PVDF Polyvinylidene diflouride

qRT-PCR Quantitative Real Time polymerase chain reaction

rapidly (IKr) and slowly (IKs) Delayed rectifier potassium channels.

RNA Ribonucleic acid

RT Reverse transcriptase

RT-PCR Real Time polymerase chain reaction

SDS Sodium dodecyl sulphate

SVR Systemic vascular resistance

T3 Triiodothyronine

T4 3,5,3',5 tetraodothyronine

Degree papers are in the "Xiamen University Electronic Theses and Dissertations Database".

Fulltexts are available in the following ways:

- If your library is a CALIS member libraries, please log on http://etd.calis.edu.cn/ and submit requests online, or consult the interlibrary loan department in your library.
- 2. For users of non-CALIS member libraries, please mail to etd@xmu.edu.cn for delivery details.