

Role of Opioid Receptors in the Exercise Pressor Reflex in Sensory Neurons

Thesis

Submitted for partial fulfillment of M.D. Degree in
Anesthesiology, I.C.U. & Pain Management

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CERTIFICATION

I, Victor Ruiz-Velasco, Associate Professor of Anesthesiology, Penn State College of Medicine, Hershey, PA, hereby certify to the following:

The experimental work of the doctoral thesis presented by Dr. Bassil Hassan of Cairo, Egypt, entitled, "The Role of Opioid Receptors in the Exercise Pressor Reflex in Sensory Neurons" was performed in my research laboratory, under my supervision at Penn State College of Medicine.

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Acknowledgment

First of all I thank **ALLAH**; the source of all knowledge, by whose abundant grace, this work has come to life.

I would like to express my sincere thanks, deep gratitude, and extreme appreciation to **Prof. Dr. Fawzia Aboul Fetouh**, Professor of Anesthesiology, Faculty of Medicine Cairo University, for giving me the chance to start my research in USA and who generously helped me in carrying out this work with her outstanding support and her indispensable advices and kind encouragement all the time.

I am deeply indebted to **Dr. Victor Ruiz-Velasco**, Associate Professor of Anesthesiology and Pharmacology, Hershey Medical Center, Pennsylvania State University, for giving me valuable chance to be a member in his research team, in HMC anesthesia department, for more than two years. All my work have been done under his direct supervision and guided by his great knowledge. His real support, kind care and advises were the main guide to complete this thesis. I learned a lot of ethics from him inside and outside the medical field.

I wish to express my deep everlasting gratitude to **Prof. Dr. Maged Salah**, Professor of Anesthesiology, Faculty of Medicine Cairo University, whose selfless time, kind care and continuous encouragements were sometimes all that kept me going.

I wish to express my gratitude **Prof. Dr. Ahmed Mukhtar**, Professor of Anesthesiology, Faculty of Medicine Cairo University, for his generous help and support.

I would like express my great appreciation towards **Dr. Khaled Sedeek**; Associate Professor of Anesthesiology, Pennsylvania State University for creating and supporting this link between Cairo University and Pennsylvania State University, giving our anesthesiology residents, a great valuable chance to do their research in USA, gaining unique experience.

I would like to express my deep appreciation towards ***my great parents, sister, and lovely wife*** and to all my family and colleagues, for their continuous encouragement and support to complete this work.

Also I would like to thank all ***my professors and staff members*** in anesthesia department, Cairo University, for providing me a stimulating atmosphere and for giving me the knowledge and the chance to be a member of this great medical staff.

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List of Abbreviations

Abb.	Full term
δ :	Delta
κ :	Kappa
μ :	Mu
AC	Adenylyl cyclase
AgaIVa:	ω -agatoxinIVa
ASIC3:	Acid-sensing ion channel
cAMP:	Cyclic 3' 5' adenylyl cyclase
Cap:	Capsaicin
Ca _v :	Calcium channels
CNS:	Central nerves system
CO:	Cardiac output
C _T :	Comparative C _T Method
DAMGO:	[d-Ala ² -N-Me-Phe ⁴ -Glycol ⁵]-enkephalin
DiI:	1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate
DMSO:	Dimethyl sulfoxide
DRG:	Dorsal root ganglia
EGFP:	Enhanced green fluorescent protein
EPR:	Exercise pressor reflex
GAPDH:	Glyceraldehyde phosphate dehydrogenase
GDP:	Guanosine diphosphate
GIRK:	G protein-coupled inwardly rectifying K

GPCRs:	G protein coupled receptors
GTP:	Guanosine triphosphate
GVIA:	ω -conotoxin GVIA
HR:	Heart rate
Hr:	Hour
HVA:	High-voltage activated
I_h :	Inward currents
IACUC:	Institutional animal care and use committee
IC ₅₀ :	Half-inhibition concentration
I _{MAX} :	Maximum current inhibition
IUPHAR:	International Union of Pharmacology
IVA:	Intermediate voltage activated
LVA:	Low-voltage activated
M6G:	Morphine-6-beta-glucuronide
MAPK:	Mitogen-activated protein kinase
MEM:	Minimum essential medium
Min:	Minute
MOR:	Mu-opioid receptor
Msec:	Millisecond
nH:	Hill coefficient
Nif:	Nifedipine
NOP:	Nociceptin/orphanin FQ peptide receptor
pA:	Picoampere
PCR:	Polymerase chain reaction

PDE:	Phosphodiesterase
pF:	Picofarad
Pi:	Inorganic Phosphate
PLC:	Phospholipase C
PTX:	Pertussis toxin
PNS:	Peripheral nervous system
QRT-PCR:	Quantitative real time-polymerase chain reaction
RGS:	Regulator of G-protein signaling
SD:	Standard deviation
SE:	Standard error
SEM:	Standard error of the mean
Sec:	Second
siRNA:	Small interference ribonucleic acid
SV:	Stroke volume
TM:	Transmembrane
TRPV1:	Transient potential receptor vanilloid 1
TTX:	Tetrodotoxin
TTX-r:	Tetrodotoxin resistant
TTX-s:	Tetrodotoxin sensitive
V:	Volt
VD:	Voltage dependent
VGCCs:	Voltage-gated calcium channels
VGSCs:	Voltage-gated sodium channels
VI:	Voltage independent

Abstract:

Previous studies noticed the ability of opioid to desensitize groups III and IV muscle afferents and can be used to attenuate the exercise pressor reflex (EPR) in pathophysiological conditions as femoral artery occlusion for 72 hr in rats (Tsuchimochi et al. 2010a, b) which is a model similar to the blood flow condition in patient muscles' with peripheral arterial disease (Yang et al. 2000).

The purpose of this study is to examine the effect of arterial occlusion on the signaling pathway of the Opioid-mediated modulation of Ca^{2+} channel in rat dorsal root ganglion (DRG) neurons innervating the triceps surae muscles. Our study was on DRG neurons transfected with cDNA coding for EGFP whose expression is driven by the $\text{Na}_v1.8$ promoter region, a Na channel primarily expressed in nociceptive neurons. Determining the involved channels, receptor subtypes and intracellular signaling pathway, may help in the identification of drugs to selectively suppress the enhanced exercise pressor reflex that exacerbates certain human diseases (e.g. heart failure and peripheral arterial disease).

Our results showed significant leftward shift of DAMGO, MOR agonist, concentration-response relationship in rats DRG neurons with femoral artery occlusion for 72 hr compared to those with freely perfused hindlimbs, suggesting that DAMGO has higher potency on the identified rat DRG sensory neurons after

femoral artery occlusion for 72 hr. Thus patients with peripheral arterial disease might need small opiate doses to attenuate the exercise-induced sympathetic response protecting the patients from its hazardous cardiovascular effects.

The Western blot of DRG tissue showed that $G\alpha_{i3}$ significantly expressed followed by $G\alpha_{i2}$, while $G\alpha_{i1}$ and $G\alpha_o$ was not detected in DRG tissue. Our experiments using siRNA approach followed by whole-cell patch clamping suggest that $G\alpha_{i3}$ is an essential signaling element in the pathways that couple Ca^{2+} channels with MOR in EGFP-expressing DRG sensory neurons. Western blotting analysis showed that this enhanced sensitivity to DAMGO did not result from either increased $G\alpha_{i3}$ or MOR expression. Femoral artery occlusion in rats did not affect either Ca^{2+} channel density or fraction of main Ca^{2+} channel subtypes.

In group III and IV muscle afferents, which are an important component of the exercise pressor reflex, N-Type channels ($Ca_v2.2$) is dominated by generating ~50% of the total Ca^{2+} current and could be an important target for treating the excessive undesired cardiovascular response of exercise pressor reflex in patients with peripheral arterial disease.

Key Words: Exercise Pressor Reflex, Mu Opioid Receptors, Dorsal Root Ganglia, Ca^{2+} Channel Current, whole-cell patch clamp.

Aim of the work

The aim of the work is to determine the role of peripheral μ -opioid receptor to inhibit the excitability of rat DRG sensory neurons expressing EGFP (whose expression was driven by the $\text{Na}_v1.8$ promoter region), following rat femoral artery occlusion for 72 hr which is a model likely similar to the blood flow condition in patient with chronic peripheral vascular disease. Then we aimed to determine the specific PTX-sensitive $\text{G}\alpha$ subunit that mediates the functional coupling of MOR and Ca^{2+} channels and also Ca^{2+} channels expressed in this identified DRG neurons which may help in better understanding of their role in mediating the EPR. This study may provide initial step for the development of novel drugs that selectively suppress the exaggerated exercise induced sympathetic reflex that exacerbates certain human diseases (e.g. peripheral arterial disease and chronic muscle ischemia) and to relieve the exercise induced claudicating pain in peripheral vascular disease patients.

REVIEW ARTICLE

Chapter 1: Exercise Pressor Reflex

Exercise leads to reflex increase in cardiovascular and respiratory functions. The cardiovascular response to dynamic exercise is characterized by large increases in heart rate (HR), stroke volume (SV) and cardiac output (CO) (Gallagher et al. 1999). This reflex cardiovascular response after skeletal muscles contraction could be explained by exercise pressor reflex.

The group I ($A\alpha$) and II ($A\beta$) muscle afferents are involved in sensory pathway required to guide motor activity (Houk, 1974), while Group III ($A\delta$) and IV (C) afferents are involved in transmitting the signals of muscle pain and are also involved in mediating exercise pressor reflex response (Coote et al. 1971; Kaufman et al. 1983; Kaufman and Hayes, 2002).

Activation of the exercise pressor reflex leads to transmission of the sensory impulse from skeletal muscle to the spinal cord by group III and IV afferents and subsequently to the cardiovascular centers in the brain stem, with consequent cardiovascular adjustments to exercise predominately via increase in the sympathetic nerve activity to the cardiovascular system and a withdrawal of parasympathetic nerve activity. These actions result in the precise alterations in cardiovascular hemodynamic requirements to meet the metabolic needs of the contracting skeletal muscle. Coordinated activity by this pressor reflex is altered in