THE UNIVERSITY OF ADELAIDE

Neuroimmunopharmacology of Opioids

Jacob Henry Lloyd Thomas (PhD Candidate)
12 December 2017

Discipline of Pharmacology
School of Medicine

Supervisors:
Professor Mark R Hutchinson
Professor Andrew A Somogyi

Table of Contents

Abstract

Opioids are an extremely important part of medical practice, and for thousands of years, continue to provide relief from severe acute and chronic pain. Intriguingly, however, the use of opioids activates endogenous counter-regulatory mechanisms resulting in the release of proinflammatory mediators from central immune cells that facilitates a wide-range of effects on opioid pharmacodynamics including: opposition of acute and chronic opioid analgesia, opioid analgesic tolerance, opioid- induced hyperalgesia, development of opioid dependence, opioid reward, and opioid respiratory depression. Until recently, the counter regulation of opioid-induced analgesia had been had been attributed to neuronal receptors where the beneficial and detrimental actions of opioids were thought to be inseparable. It is now apparent from molecular and rodent data that opioids have non-neuronal, non-classic, non-stereoselective sites of action. Therefore, the purpose of this thesis was to further identify this non-classical, non-stereoselective site of action and examine the kinetics of opioid binding.

The first study examined, using in vivo, in vitro, and in silico techniques, the potential involvement of toll-like receptor (TLR) 2 and TLR4 in the pharmacodynamic actions of opioids. $TLR4^{-/-}$ animals demonstrated significantly altered analgesia, tolerance, withdrawal responses to opioids compared with wildtype mice. A range of saturation, displacement, and kinetic binding experiments were subsequently conducted to assess the non-stereoselective binding of opioids to TLR4. However, at concentrations required for classical μ receptor binding, no TLR4 binding of classical opioid antagonists was observed.

The second study assessed if many of the key drugs that target opioid induced immune activation have any direct action at opioid receptors or whether they can modify opioid receptor activation. Classical binding and functional studies were conducted in cells overexpressing the human μ receptor and in rat brain membranes. Of all the tested ligands, only amitriptyline and WZ811 (CXCR4 antagonist) were shown to have direct binding at sites defined by the classical antagonists diprenorphine and (-)-naloxone. All of the tested ligands had no detectable efficacy for the m receptor or any allosteric modulatory effects. This study confirms that ligands that alter in vivo opioid pharmacodynamic responses (increase opioid analgesia and reduction of tolerance) do not do so via modifying μ-opioid receptor activity.

The final study employed the use of [^{3}H](+)-naloxone, a stereoisomer of (-)-naloxone that has been shown to have extensive effects on classical opioid pharmacodynamics. Mouse brain membranes and HEK293 cells overexpression systems were used in order to unmask any non-stereoselective (+)-opioid isomer binding sites. However, using traditional [^{3}H] binding experiments, (+)-naloxone had no detectable binding at sites at low nM concentrations. Due to the lipophilicy of (+)-naloxone and the subsequent high level of non-specific binding, no specific binding was detectable at μM concentrations.

In sum, this thesis provides additional evidence that TLR4 plays a significant role in the pharmacodynamic responses to opioids. The kinetic assessment of TLR4 as a non-stereoselective opioid site of action reveals that this site does not bind opioids at nM concentrations. As such, this does not rule out the direct involvement of TLR4 in opioid binding, but rather suggest that TLR4 has disparate binding characteristics compared with

classical μ-opioid receptor binding. Further assessment of this complex system is required, particularly at higher concentrations. However, the present finding can assist with the future discovery of a non-classical opioid binding site that is involved in opioid induced central inflammatory events.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I acknowledge that copyright of published works contained within this thesis (as listed below) resides with the copyright holders of those works.

Thomas, J., Hutchinson, M.R., 2012. Exploring neuroinflammation as a potential avenue to improve the clinical efficacy of opioids. Expert Rev Neurother 12, 1311–1324. doi:10.1586/ern.12.125

Thomas, J., Mustafa, S., Johnson, J., Nicotra, L., Hutchinson, M., 2015. The relationship between opioids and immune signalling in the spinal cord. Handb Exp Pharmacol 227, 207–238. doi:10.1007/978-3-662-46450-2_11

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Jacob Thomas Date

Acknowledgments

I would like to sincerely thank my supervisors Professor Mark Hutchinson and Professor Andrew Somogyi for allowing me to undertake this project. Their enthusiasm and immense knowledge has really driven me to complete this PhD. Their ongoing encouragement, patience and guidance over the years are greatly appreciated and I could not have wished for better supervisors.

The research presented in this thesis would have been impossible without the financial support from the ARC funding body as well as support from the Faculty of Health Sciences Divisional PhD Scholarship. The opportunities to present my work at numerous national and international conferences were generously supported by the Australasian Society for Clinical and Experimental Pharmacologists and Toxicologists Travel Grant, Faculty of Health Sciences Postgraduate Travelling Fellowship, The School of Medical Sciences Postgraduate Travel Award and Mark Hutchinson.

Thanks to all my friends and family, you guys have been the best. Understanding some of the PhD struggles, and knowing to only ask “when are you going to finish” every now and then! Furthermore, I would also like to thank everyone in the neuroimmunopharmacology group for being top-notch people and willing to help with anything at any given time (except for you, Jon). You guys have kept me coming in every day and made this journey more than worthwhile!

Thanks again to everyone

Abbreviations

3BM	3-Biotinylated morphine
5-HT	Serotonin
AP1	Activator protein-1
B_{max}	Total binding sites
CNS	Central nervous system
DAMGO	[D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin
GIRK	G protein-activated inwardly rectifying K^+ channels
GPCR	G_i/G_o protein-coupled receptors
IC_{50}	Concentration inhibition 50% of ligand binding
IFN-β	Interferon-β
IKK	Inhibitor of NF-kB
IL-	Interleukin
IRAK	IL-1 receptor-associated kinase
IRF3	Interferon regulatory factor 3
K_d	Dissociation constant
K_i	Inhibitory constant
K_{off}	Equilibrium dissociation rate constant
K_{on}	Association rate constant
LBP	LPS binding protein
LPS	Lipopolysaccharide
M3G	Morphine-3-glucuronide
MAPK	Mitogen-activated protein kinase
MPE	Maximum possible effect
MyD88	Myeloid differentiation primary response gene 88

NA	Noradrenaline
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
PAG	Periaqueductal gray
PAMP	Pathogen-associated molecular patterns
PI3K	Phosphatidylinositol 3-kinase
PKA	Protein kinase A
PLC	Phospholipase C
ROS	Reactive oxygen species
RVM	Rostral ventromedial medulla
S-DDD	Sold defined daily doses
TIR	Toll/interleukin-1 receptor
TLR	Toll-like receptors
TNF-α	Tumor necrosis factor-α
TRAF6	Tumor necrosis factor receptor 6
TRIF	TIR-domain containing adaptor inducing IFN-b
UGT	Uridine diphosphate glucuronosyltransferase
WT	Wildtype