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Sex Differences in Allodynia:

A Complex Interaction Between 17beta-Oestradiol and the Innate Immune System

Lauren Louise Nicotra

Discipline of Pharmacology
School of Medical Sciences
University of Adelaide

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ABSTRACT

Chronic pain is a debilitating and costly disease that is well known to preferentially affect women, particularly in their child bearing years. In spite of the obvious differences in pathophysiology between the sexes, the underlying mechanisms causing this sex difference in chronic pain have not yet been determined. Contributing to this gap in knowledge is the unrelenting use of preclinical animal pain models along with pain behavioural assessment techniques, which fail to best replicate the clinical pain experience. This translatability issue, along with the continued preferential use of male subjects in preclinical chronic pain investigations has meant that researchers have not yet uncovered what contributes to this sex difference in pain sensitivity, resulting in half the population (females) being inadequately treated for their pain.

Among the numerous mechanisms that have been proposed to underlie this sex difference in chronic pain are both the gonadal hormones, particularly 17β-oestradiol, along with the innate immune system. Although seperately known to play a role in chronic pain, prior to this PhD project, the relationship between these two systems had not be investigated in this debilitating condition. Recent evidence however has revealed not only the presence of oestrogen receptors on the key neuroimmune cells in pain responsive regions of the CNS, but also the exaggerated production of pain producing pro-inflammatory mediators in response to their activation. Given therefore that an association between these two pain-producing systems does exist, this PhD project investigated the relationship between 17β-oestradiol and the innate immue system in exaggerated female chronic pain. Furthermore, based on the significant and reported translatability issues relating to preclinical pain studies in light of the currently

available chronic pain experimental models and assessment techniques, this study further examined this relatinship for the first time using novel translatable preclinical methodologies.

Through a series of key *in vitro* and *in vivo* studies our findings not only present and validate a novel preclinical chronic pain model and behavioural assessment technique that better produces and examines a preclincal neuropathy, but our findings also substantiate previous work demonstrating a significant role of 17β-oestradiol in chronic pain. Importantly this PhD project reveals for the first time not only the capability of the oestrogens to directly bind to key innate immune signalling receptors involved in chronic pain, but that 17β-oestradiol priming of CNS innate immune cells via such direct binding is in part responsible for the exaggerated female pain phenotype.

Overall, the findings of this body of work highlight a fundamental difference in chronic pain pathophysiology between the sexes and further emphasize the importance of treating chronic pain in women during their reproductive years differently from prepubesent female, postmenopausal and male chronic pain sufferers.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma 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. In addition, I certify that no part of this work will, in the future, be used in a submission 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.

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Lauren Nicotra

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ABBREVIATIONS

ABBREVIATION	DEFINITION
5-HT	5-Hydroxytrptamine, serotonin
AIC	Akaike's Information Criterion
AMPA	Alpha-Amino-3-Hydroxy-5-Methyl-4-Isoxazole-Propionic Acid
AP1	Activating Protein 1
ATP	Adenosine Triphosphate
BDNF	Brain-Derived Neurotrophic Factor
CCI	Chronic Constriction Injury
CCL2	Chemokine Ligand 2
CD11b	Cluster of Differentiation Molecule 11B
CFA	Complete Freund's adjuvant
CNS	Central nervous system
COX	Cyclooxygenase
CX_3CL1	Fractalkine
CX_3CR1	CX_3CL1 Receptor
DAMPs	Damage-Associated Molecular Patterns
Di	Dioestrus
DRG	Dorsal Root Ganglion
E	Oestrogen
E1	Estrone
E2	17β-Oestradiol
E3	Estriol
ER	Oestrogen Receptor
ERK	Extracellular Signal-Regulated Kinase
ERα	Oestrogen Receptor Alpha
ERβ	Oestrogen Receptor Beta
FSH	Follicle-Stimulating Hormone
g	Grams Force
GABA	Gamma Aminobutyric Acid
GFAP	Glial Fibrillary Acidic Protein
GLAST	Glutamate–Aspartate Transporter
GLT1	Glutamate Transporter 1
GnRH	Gonadotropin Releasing Hormone
GPR	G Protein-Coupled Receptor
HEK-293	Human Embryonic Kidney-293
HMGB1	High Mobility Group Box-1
HRT	Hormone replacement therapy
HSPs	Heat Shock Proteins

i.t.	Intrathecal
IBS	Irritable Bowel Syndrome
IFN-β	Interferon-Beta
IL	Interleukin
IL-1ra	IL-1 Receptor Antagonist
i.p.	Intraperitoneal
JNK	c-jun N-Terminal Kinase
LAL	Limulus Amebocyte Lysate
LH	Luteinizing Hormone
LPS	Lipopolysaccharide
LTA	Lipoteichoic Acid
MAL	MyD88 Adaptor Like
MAPK	Mitogen-Activated Protein Kinase
MCP-1	Monocyte Chemotactic Protein-1
MD-2	Myeloid Differentiation Factor 2
Met	Metoestrus
MHC	Major Histocompatibility Complex
MIP-2	Macrophage Inflammatory Protein 2
MMPs	Matrix Metalloproteinases
MPQ	McGill Pain Questionnaire
MyD88	Myeloid Differentiation Primary Response Gene 88
N	Sciatic Nerve
NF-κB	Nuclear Factor-κB
NLRs	NOD-Like Receptors
NMDA	N-methyl-D-aspartate
NOD	Nucleotide Binding Oligomerization Domain 1
NR	Nuclear Receptor
OC	Oral Contraceptive Pill
Oest	Oestrus
OV	Ovulation
OVX	Ovariectomy
P	Progesterone
PAG	Periaqueductal Gray
PAMPs	Pathogen-Associated Molecular Patterns
PBS	Phosphate-Buffered Saline
PGE_2	Prostaglandin E_2
PGN	Peptidoglycan
PNS	Peripheral Nervous System
PO	Postoperative
POPNO	Pain of Predominantly Neuropathic Origin

Pro	Pro-oestrus
PSNL	Partial Sciatic Nerve Ligation
ROS	Reactive Oxygen Species
S	Subcutaneous Suture
SARM	Sterile ∝ and Armadillo Motifs
siRNA	Small Interfering RNA
T	Testosterone
TIR	Toll Interleukin-1 Receptor
TIR	Toll/IL1 Receptor
TIRAP	TIR Domain Containing Adaptor Protein
TLR	Toll-Like Receptor
TMD	Temporomandibular
TNF-α	Tumor Necrosis Factor-α
TRAM	TRIF Related Adaptor Molecule
TRIF	Toll-Receptor-Associated Activator of Interferon
TRPV1	Transient Receptor Potential Cation Channel Subfamily V Member 1