Neurobiological Consequences of Stress: Tyrosine Hydroxylase Phosphorylation in Response to Stress

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Statement of Originality

This thesis 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. 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.

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Acknowledgment of Collaboration

I hereby certify that the work embodied in this thesis has been done in collaboration with other researchers. I have included as part of the thesis a statement clearly outlining the extent of collaboration, with whom and under what auspices at the beginning of each research chapters (presented in Chapter 2 ***pg. 57****, Chapter 3* ***pg. 67****, Chapter 4* ***pg. 102****, Chapter 5* ***pg.110*** *and Chapter 6* ***pg. 119****).*

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Acknowledgment of Authorship

I hereby certify that the work embodied in this thesis contains a published paper/s/scholarly work of which I am a joint author. I have included as part of the thesis a written statement, endorsed by my supervisor(s), attesting to my contribution to the joint publication/s/scholarly work.

Lin Kooi Ong

Endorsement of Authorship by Supervisors

*We attest that Research Higher Degree candidate **Lin Kooi Ong** contributed to 1) the conception and design of the research, 2) collection, analysis and interpretation of research data and 3) drafting and revision of significant parts of the work to contribute to the interpretation of the publications entitled:*

The sustained phase of tyrosine hydroxylase activation in vivo

Ong L.K., Sominsky L., Dickson P.W., Hodgson D.M. and Dunkley P.R. Neurochem Res. (2012) DOI: 10.1007/s11064-012-0812-3

The effects of footshock and immobilization stress on tyrosine hydroxylase phosphorylation in the rat locus coeruleus and adrenal gland

Ong L.K., Guan L., Stutz B., Dickson P.W., Dunkley P.R. and Bobrovskaya L. Neuroscience, 192, 20-27 (2011)

The effect of social defeat on tyrosine hydroxylase phosphorylation in the rat brain and adrenal gland

Ong L.K., Bobrovskaya L., Walker F.R., Day T.A., Dickson P.W. and Dunkley P.R. Neurochemical Research, 36(1), 27-33 (2011)

Signal transduction pathways and tyrosine hydroxylase regulation in the adrenal medulla following glucoprivation: an in vivo analysis

Bobrovskaya L., Damanhuri H.A., Ong L.K., Schneider J.J., Dickson P.W., Dunkley P.R. and Goodchild A.K. Neurochemistry International, 57(2), 162-167. (2010)

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E/Prof. Peter Dunkley	A/Prof. Phillip Dickson	Dr Larisa Bobrovskaya

Thesis by Publication

I hereby certify that this thesis is in the form of a series of published papers of which I am a joint author. I have included as part of the thesis written statement from each co-author, endorsed by the Faculty Assistant Dean (Research Training), attesting to my contribution to the joint publications.

Lin Kooi Ong

Prof. John Rostas

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Contents

Thesis Abstract

Stress is part of our daily life. One of the major cell types involved in the stress response are the catecholaminergic cells in the brain, the peripheral nervous system and the adrenal medulla. These cells, which produce adrenaline, noradrenaline and dopamine, are subject to a range of controls each of which is involved in the stress response. The major subject of this thesis is the effect of stress on one of these controls namely biosynthesis of the catecholamines. Tyrosine hydroxylase (TH) is the rate-limiting enzyme in catecholamine biosynthesis. TH is itself subject to a range of regulatory mechanisms, including feedback inhibition by the catecholamines, phosphorylation of serine residues (Ser19, Ser31 and Ser40) which can contribute directly or indirectly to enzyme activation, as well as mRNA expression and protein synthesis which determine the availability of TH. In response to stress catecholaminergic cells are depolarized and extracellular calcium enters leading to the release of catecholamines from these cells and also to the activation of signal transduction pathways that lead to an increase in TH phosphorylation and TH activity. When catecholamines are released from cells during the stress response it has been shown that the concomitant increase in TH activity and catecholamine synthesis maintains catecholamine levels in the cells at a constant level. The phosphorylation of each serine residue does not affect TH activity equally. Ser19 phosphorylation does not increase TH activity directly, Ser31 phosphorylation increases TH activity modestly and Ser40 phosphorylation, which relives the feedback inhibition by catecholamines, increases TH activity substantially. Three phases of TH activation (acute, sustained and chronic) have been identified and the regulatory mechanisms for each phase have been

extensively characterized *in vitro* and *in situ*. The acute phase involves TH phosphorylation which occurs and is mostly reversed over the first hour after exposure to stress. The sustained phase also involves TH phosphorylation via different mechanisms but it occurs from 1 to 24 h after exposure to stress. The chronic phase involves mRNA synthesis and TH protein synthesis and this occurs from 4 to 72 h after exposure to stress. To date, there have been only limited studies that have investigated the acute phase of TH activation in response to stress and no studies that have investigated the sustained phase *in vivo*. Only the chronic phase of TH activation in response to stress has been extensively investigated *in vivo*.

The work presented in this thesis aimed to systematically investigate the different phases of TH activation, especially the acute and sustained phases, by measuring TH phosphorylation and TH protein at different time points in response to a range of stressors *in vivo*. The adrenal medulla and the locus coeruleus (LC) where chosen as representative catecholaminergic cells for these studies. We have compared the profile of TH phosphorylation and TH protein elicited by two stressors tentatively classified as physical (footshock or glucoprivation stress) and two stressors tentatively classified as psychological (immobilization or social defeat stress) in the adrenal medulla and the LC over a 1 h period. We found that the different stressors all induce the acute phase of TH activation, but provide different temporal profiles of TH phosphorylation at Ser19, Ser31 and Ser40, without TH protein synthesis in the adrenal medulla and the LC over the first hour *in vivo*. The physical stressors both activated the catecholaminergic cells to a greater extent when compared to the psychological stressors. We have also compared the profile of TH phosphorylation and TH protein elicited by three different stressors social defeat, glucoprivation or LPS stress in the

adrenal medulla at 24 h. It should be noted that the LPS studies were undertaken with neonatal rats. We found that social defeat or glucoprivation stress do not induce sustained phosphorylation. However, LPS stress induces the sustained phase of TH activation by inducing sustained TH phosphorylation at Ser31 and Ser40 without TH protein synthesis being increased in neonatal rats' adrenal medulla at 24 h. The reason for the difference is unknown, but it is possible that sustained phosphorylation only occurs in neonatal animals or perhaps LPS stress activates the adrenal via a different set of intracellular messengers to the other stressors. Whatever the mechanism this is the first study to demonstrate that the sustained phase of TH activation occurs *in vivo*.

Overall we provided evidence that different catecholaminergic cells respond differently in term of the temporal profiles of TH phosphorylation at Ser19, Ser31 and Ser40, presumably due to differences in the frequency of cell firing and/or the nature of the neurotransmitters released onto these cells, which in turn led to differential activation of signal transduction pathways. In addition, we demonstrated that the activation of TH is associated with the enzymes phosphorylation at Ser31 and Ser40 *in vivo*, an effect that had previously been demonstrated mainly in cultured cells. This thesis has substantially improved our understanding of the mechanism of action of the catecholaminergic cells in mediating stress responses *in vivo*.

Abbreviations list

2DG	2-deoxy-D-glucose
AAAD	aromatic L-amino acid decarboxylase
ACTH	adrenocorticotropic hormone
AM	adrenal medulla
ANOVA	analysis of variance
APG	anterior pituitary gland
CaMKII	Ca^{2+}/ calmodulin-dependent protein kinase II
CDK	cyclin-dependent kinase
CRH	corticotrophin releasing hormone
COMT	catechol-O-methyltransferase
DBH	dopamine-β-hydroxylase
ERK1/2	extracellular signal-regulated kinases 1/2
FS	footshock
HCC	home cage control
HPA	hypothalamic-pituitary-adrenocortical
IMO	immobilization
LC	locus coeruleus
LPS	lipopolysaccharide
MAO	monoamine oxidase
PAGE	polyacrylamine gel electrophoresis
PFC	prefrontal cortex
PKA	cAMP-dependant protein kinase

PND	postnatal day
PNMT	phenylethanolamine-N-methyl transferase
PVN	paraventricular nucleus
SDS	sodium dodecyl sulfate
Ser	serine
SN	substantia nigra
TBST	tris-buffered saline with Tween
TH	tyrosine hydroxylase
VMAT	vesicular monoamine transpoters
VTA	ventral tegmental area