Regulation of Tyrosine Hydroxylase in Stress and Parkinson's Disease

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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, or carried out in other institutions. I have included as part of the thesis a statement clearly outlining the extent of collaboration, with whom and under what auspices.

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Acknowledgment of Authorship

I hereby certify that components of the work embodied in this thesis are from published papers of which I am a joint author. My contribution to these papers covers knowledge and experience management, knowledge representation, multi agent system, virtual organisations, and knowledge measurements and warrants inclusion of their parts in the body of my thesis.

Signed (PhD Candidate):_____

Endorsed (Supervisor):_____

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Contents

Abstract

Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the biosynthesis of the catecholamines (CAs). In the periphery, CAs function as major hormones and neurotransmitters in the sympatho-adrenomedullary system to facilitate the fight and flight response to stress. In the central nervous system, the CA-containing neurons are located in the locus coeruleus (LC), medial prefrontal cortex (mPFC) and ventral tegmental area (VTA) and are highly activated during the stress response. CA containing neurons are also located in the substantia nigra (SN) and are involved in the control of motor functions, and loss of these neurons are a major feature of Parkinson's Disease (PD). The rate of CA release is directly coupled to the rate of CA synthesis and this requires the modulation of TH activity. TH activity is primarily regulated by a feedback inhibition by CA and activation by phosphorylation. There are three phosphorylation sites located at the N-terminus of TH, which are known to regulate TH activity, serine 19 (Ser19), serine 31 (Ser31) and serine 40 (Ser40). Whilst there is no direct correlation between Ser19 phosphorylation and TH activity *in vivo,* Ser31 phosphorylation significantly modulates TH activity *in vivo,* and Ser40 phosphorylation abolishes the feedback inhibition and can activate TH up to 4 fold *in vivo*. Alpha-synuclein (αSyn) can also negatively regulate TH activity.

The first part of the study presented in this thesis provides a systematic investigation of TH phosphorylation, TH protein levels and TH activity in the short term response to acute footshock stress in the LC, mPFC and VTA and in the adrenal medulla. The *in vivo* basal stoichiometry of phosphorylation of Ser19, Ser31 and Ser40 in the LC, mPFC and adrenal medulla were determined for the first time. The LC, VTA and adrenal

medulla all had higher basal levels of Ser19 phosphorylation and lower basal levels of Ser31 phosphorylation than the mPFC, while the adrenal medulla had the highest basal levels of Ser40 phosphorylation. Analysis of TH activation *in vivo* over the first 40 minutes after footshock stress showed that there was an increase in Ser31 phosphorylation and a corresponding increase in TH activity in the LC and mPFC. There were no changes detected in the VTA. In the adrenal medulla, there was an early increase in Ser31 phosphorylation and a later increase in Ser40 phosphorylation and a corresponding increase in TH activity. In the adrenal medulla the increased Ser31 and Ser40 phosphorylation was accompanied by increased activation of ERK and PKA. This study has confirmed the important roles of both Ser31 and Ser40 phosphorylation in regulation of TH activity *in vivo.* It was found that the greatest change of TH activity occurs when both Ser31 and Ser40 are phosphorylated. This study has shown that acute footshock stress leads to activation of TH in the LC, pre-synaptic terminals in the mPFC and adrenal medullary chromaffin cells, as well as changes in activity of the hypothalamic-pituitary-adrenal axis.

The second part of the study presented in the thesis examined the interaction between αSyn and TH *in vitro*. A unique TH/ αSyn interaction was detected in which TH and αSyn formed an SDS-resistant complex and this complex was only found associated with aggregated αSyn. This indicates the capacity of TH and αSyn to form a novel oligomeric species. Analysis of deletion mutants of αSyn and TH indicated the involvement of the NAC region of αSyn and the catalytic domain of TH in the formation of the complex. αSyn mutants associated with familial parkinsonism showed a decreased capacity to generate the TH/αSyn complex. The TH in a midbrain dopaminergic neuron is

predominantly in the DA-bound form. This form of TH shows a dramatically reduced capacity to form the TH/ αSyn complex. Activation of TH by phosphorylation at Ser40 could substantially increase the capacity of the TH to form the complex. This indicates that under normal conditions the formation of the TH/ αSyn complex may be low but when TH is activated the level of the complex can increase. This has the potential functional significance in PD as loss of αSyn may promote the activation of TH which in turn will promote the generation of this unique TH/ αSyn complex and the potential capacity to alter the aggregation of αSyn.

Abbreviations list

A	adrenaline
AADC	aromatic amino acid decarboxylase
ACTH	adrenocorticotropic hormone
HPA	hypothalamo-pituitary-adrenal axis
BH_2	dihydrobiopterin
BH_4	tetrahydrobiopeterin
CA	catecholamine
CaMPKII	calcium/calmodulin-dependent protein kinase II
CDK	cyclin-dependent kinase 5
DA	dopamine
DBH	dopamine β-hydroxylase
DOPA	dihydroxyphenylalanie
ERK1/2	extracellular signal-regulated protein kinase 1/2
H_2O	water
LC	locus coeruleus
LPS	lipopolysaccharide
mPFC	medial prefrontal cortex
MPTP	methyl-phenyl-tetrahydropyridine
NA	noradrenaline
Nac	nucleus accumbens
NAC	non-Amyloid-β component
PD	Parkinson's Disease
PKA	cAMP-dependent protein kinase
PKC	protein kinase C
PKG	cGMP-protein kinase
PNMT	phenylethanolamine-N-methyl transferase
PP2A	phosphoprotein phosphatases 2A
PP2C	phosphoprotein phosphatases 2C
SN	substantia nigra
TH	tyrosine hydroxylase
VMAT	vesicular monoamine transporter
VTA	ventral tegmental area
αSyn	alpha-synulcien