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REGULATION OF THE NORADRENALINE TRANSPORTER  
IN A MODEL NEURONAL CELL LINE:  
EFFECTS OF CELL SIGNALLING PATHWAYS AND  
THE TRICYCLIC ANTIDEPRESSANT DRUG, DESIPRAMINE

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A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR  
THE DEGREE OF DOCTOR OF PHILOSOPHY,  
THE UNIVERSITY OF AUCKLAND, 2007

# The University of Auckland

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FAILURE IS NOT FATAL:  
IT IS THE COURAGE TO CONTINUE THAT COUNTS.

WINSTON CHURCHILL



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## ABSTRACT

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The noradrenaline transporter (NAT) is found in presynaptic noradrenergic nerve terminals. It regulates noradrenergic neurotransmission by mediating reuptake of noradrenaline (NA) from the synapse. The NAT is inhibited by the tricyclic antidepressant agent, desipramine (DMI). While a single dose of DMI rapidly inhibits noradrenaline reuptake, repeated administration is required for therapeutic effects. This thesis aimed to investigate whether DMI has cellular effects in addition to the inhibition of the NAT. To research this hypothesis, the effect of DMI on protein expression and regulation of the NAT in cultured neuronal cells was investigated.

Short-term DMI treatment was shown to increase the expression of phosphorylated CREB in several mammalian neuronal cell lines, as shown by immunocytochemistry. These results were confirmed by western blotting in human neuroblastoma SH-SY5Y cells, but not for the other cell lines. Short-term DMI treatment also appeared to augment the expression of phosphorylated substrates of PKC and MAPK, as investigated by western blotting with kinase specific phospho-protein antibodies. However, there did not appear to be phosphoproteins expressed specifically in response to DMI.

To study the regulation of the NAT, a stable human noradrenergic neuroblastoma cell line, SY5Y cells transfected to express a GFP-NAT fusion protein, was produced for this thesis work. SY5Y-GFP-NAT cells displayed a 3-5 fold increase in [<sup>3</sup>H]noradrenaline uptake with GFP-fluorescence localized to the plasma membrane. The level of GFP-NAT expression was sufficient for biotinylation analysis of surface NAT expression. SY5Y-GFP-NAT cells treated with the PKC activator  $\beta$ -PMA showed a significant reduction in both [<sup>3</sup>H]noradrenaline uptake and surface GFP-NAT expression, confirming that the cell line was suitable for studies of NAT regulation. Treatment with muscarinic receptor agonists was found to reduce NAT activity. This effect was abolished by the depletion of PKC activity, suggesting that the signalling pathway involved PKC. Unlike the effect of  $\beta$ -PMA, muscarinic agonists did not affect cell surface NAT expression.

Regulation of the NAT by the insulin signalling pathway was investigated in SY5Y-GFP-NAT cells. Insulin treatment robustly increased [<sup>3</sup>H]noradrenaline uptake, an effect

blocked by the tyrosine kinase inhibitor, genistein. Genistein treatment alone significantly reduced noradrenaline uptake, suggesting insulin receptor tyrosine kinase regulated basal NAT activity. Further investigations suggested that insulin-dependent regulation of the NAT involved cross-talk between PKC and PI3K signalling pathways.

DMI treatment of 1 to 3 days was shown to reduce cell surface NAT expression in the SY5Y-GFP-NAT cells. This was detected both by GFP fluorescence and surface protein biotinylation. Increased intracellular GFP-NAT was also observed in DMI-treated cells. GFP-NAT associated mostly with recycling endosomes after 1-day of DMI treatment, as shown by co-localization with transferrin conjugated to Alexa-568. GFP-NAT became associated with late endosomes and lysosomes after 2 – 3 days of DMI treatment. A model was developed to explain the effect of DMI on the cellular trafficking of the NAT.

This research clearly demonstrated a novel finding, that the tricyclic antidepressant drug DMI, affects the cellular distribution of the NAT in the SY5Y-GFP-NAT model cell-line. While specific changes in protein expression in response to DMI were not found, it appears likely that regulation of the NAT by DMI treatment involves cell signalling pathways and post-translational protein modification. The SY5Y-GFP-NAT cell, developed during this research, is a novel cell-line that has proved its value for studies of the regulation and trafficking of the NAT. This cell line should be useful for future studies of potential transporter-protein interactions and additional studies of the cellular actions of DMI.

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## LIST OF ABBREVIATIONS

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[ <sup>3</sup> H]noradrenaline	Tritiated noradrenaline
/	Per
~	Approximately
°C	Degree Celsius
5-HT	Serotonin (5-hydroxytryptamine)
β-PMA	β-phorbol 12-myristate 13-acetate
BDNF	Brain derived neurotrophoic factor
BF	Bright-field
Bp	Base pair
BSA	Bovine serum albumin
C6	Rat glioma cell line
CaMK	Ca <sup>2+</sup> / calmodulin-dependent protein kinases
cAMP	Cyclic adenosine monophosphate
Carb	Carbachol
cDNA	Complementary DNA
CRE	cAMP response element
CREB	cAMP response element binding protein
DA	Dopamine
DAB	3,3'-diaminobenzidine tetrahydrochloride
DAT	Dopamine transporter
DMI	Desipramine
DNA	Deoxyribonucleic acid
ECL	Enhanced chemiluminescence
EDTA	Ethylenediaminetetra-acetic acid (disodium salt)
ERK	Extracellular signal Regulated Kinase
FBS	Fetal bovine serum
FITC	Fluorescein isothiocyanate
g	Grams
G418	Geneticin
GABA	Gamma-aminobutyric acid
GDNF	Glial-derived nerve factor

GEN	Genistein
GFP	Green fluorescent protein
HEK-293	Human embryonic kidney cell line
HRP	Horseradish-peroxidase
IEG	Immediate early genes
Ins	Insulin
kDa	kilodaltons
$K_m$	Substrate affinity constant
KRH	Krebs-Ringer-HEPES
LY	LY294002
mAChR	Muscarinic cholinergic receptor
MACRCKS	Myristoylated alanine-rich C kinase substrate
MAOI	Monoamine oxidase inhibitors
MAPK	Mitogen-activated protein kinase, or ERK-1/2
MCh	Methacholine
MDCK	Madin-Darby Canine Kidney epithelial cell
MEK-1/2	MAPK or ERK kinases
MEM	Minimum Essential Medium
NA	Noradrenaline
NAT	Noradrenaline transporter
NSRI	Noradrenaline specific reuptake inhibitor
PBS	Phosphate buffered saline
PC12	Rat pheochromocytoma cell line
pCREB	phosphorylated CREB
PD	PD98059
PI3K	phosphatidylinositol-3-kinase
PKA	Protein kinase A
PKC	Protein kinase C
PP1/PP2Ac	Protein phosphatase catalytic subunit 1 and 2Ac
PSG	100 unit/ml penicillin, 100 $\mu$ g/ml streptomycin and 4 mM glutamine
RIPA	Radioimmunoprecipitation buffer
rpm	Revolutions per minutes

SD	standard deviation
SDS	Sodium dodecyl sulphate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
SEM	Standard error of means
SERT	Serotonin transporter
SK-N-SH	Human neuroblastoma cell line
SSRI	Serotonin-specific reuptake inhibitor
STS	Staurosporine
SY5Y	Human neuroblastoma cell line
TBS	Tris-buffered saline
TCA	Tricyclic antidepressant
temp	Temperature
TMD	Transmembrane helical domains
Tris	Tris[hydroxymethyl]aminomethane
Tween	Polyoxyethylenesorbitan monlaurate
TX-100	Triton X-100
$V_{max}$	Maximum transport velocity
Wort	Wortmannin
WT	Wild-type/ parental
x	Times