# 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

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Success is not final,

failure is not fatal:

it is the courage to continue that counts.

Winston Churchill

# ABSTRACT

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 [$^3$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 β-PMA showed a significant reduction in both [$^3$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 β-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 [$^3$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.

# ACKNOWLEDGEMENTS

Above all, I would like to thank my supervisor Associate Professor David Christie (Cell Biology & Biochemistry group, School of Biological, University of Auckland). His logical and scientific way of thinking, wide knowledge and dedication to research have guided me over the course of these years. I am extremely grateful for David's support, encouragement, and for devoting his time on this thesis. I could not have asked for a better mentor.

On research, I would like to thank my co-supervisor Professor Michael Dragunow and Dr. Jeff Greenwood (Department of Pharmacology and Clinical Pharmacology) for their advice on the CREB work and antibodies. Thanks to my advisor Professor Janusz Lipski (Department of Physiology) for his encouragement and advice. Also, many thanks to the people in SBS, especially Dr. Adrian Turner for assistance in microscopy, Dr. Jean-Claude Schellenberg, Dr. Richard Choi, Dr. Christina Buchanan and Lance Xu for advice on 2DGE. Many thanks also to Yves Hsieh and Ivy Li for keeping me sailing. Thanks to Justin Goh and Rawiri for reading my drafts.

To my laboratory team, a big thank you to Jo Dodd for her practical advice and support. Special thanks also to Dr. Julie Lim and Dr. Mark West for their encouragement. Thank you also to Dr. Svetlana Boycheva and all fellow researchers in the Cell biology & Biochemistry group for their kind assistance.

My appreciation also goes to the Health Research Council for funding this project. Financial support including the Graduate Student Travel Award (Australasian Electrophoresis and Proteomics Society Annual Meeting), the Graduate Research Fund and SBS contestable travel fund (the Society for Neuroscience conference), and Dr Marklena Milojkovic Memorial Scholarship, are gratefully acknowledged.

Finally, to Dad and Mum, thank you for your love and faith in me. To Jann and Ken, thanks for running the errands for me from the beginning till the last day of my write-up. This thesis would not be possible without my family.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| [$^3$H]noradrenaline | Tritiated noradrenaline |
| / | Per |
| ~ | Approximately |
| ºC | 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 | $Ca^{2+}$/ 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 μg/ml streptomycine 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 |