INTRANASAL DELIVERY OF MACROMOLECULES TO THE RODENT BRAIN VIA OLFACTORY PATHWAYS.

A THESIS SUBMITTED IN TOTAL FULFILMENT

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THE DEGREE OF DOCTOR OF PHILOSOPHY

BY

Anthony Neil Pollard

Bachelor of Science (Honours)

Department of Human Physiology,

Centre for Neuroscience;

School of Medicine, Flinders University;

Adelaide, South Australia

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Dedicated to my Nanna,

Muriel Estella Bartle

1912-2006

THESIS SUMMARY

One of the major limitations in drug development and gene therapy for brain diseases is the natural defensive structure called the blood brain barrier (BBB), which prevents therapeutic polypeptide drugs and viral vectors from entering the brain. Intranasal delivery of therapeutic gene products into the brain offers a non-invasive alternative towards a feasible gene and protein therapy for neurological diseases. From recent studies involving axonal transport, it is tempting to speculate that therapeutic macromolecules including neurotrophic factors and viral vectors can be delivered into the brain by peripheral neurons, such as olfactory receptor neurons (ORNs), which span the BBB. It is thought that the nasal pathway into the brain involves two general mechanisms; intracellular (intraneuronal) or extracellular routes of transport. However the pathways involved have not yet been fully characterized.

In this study I firstly investigated the temporal and spatial localisation pattern of both biotinylated and I^{125} labelled ciliary neurotrophic factor (CNTF) following nasal delivery into Sprague-Dawley rats. Results showed that intranasal delivered CNTF was transported to several brain regions by both intracellular/axonal pathway through ORNs and the extracellular trigeminal pathway. Excess unlabelled CNTF competed for receptor binding in the olfactory mucosa confirming receptor mediated intracellular transport to the olfactory bulb via ORNs. Denervation of the olfactory mucosa prior to CNTF delivery failed to prevent CNTF transport to trigeminal and hypothalamic brain regions. Intranasal delivered CNTF was biologically active, resulting in activation of the STAT3 signalling pathway in the thalamus and hypothalamus.

To examine the functional activity of intranasal delivered CNTF, I conducted a weight loss trial using an obese Zucker rat (OZR) model to test whether CNTF treatment caused body weight loss. Intranasal administration of CNTF resulted in

reduced body weight in the CNTF treated OZR group compared to the BSA control group during the 12 day trial and for 3 days after. Intranasal delivery of CNTF may be a valuable method for the treatment of obesity.

In the second study, I investigated the temporal and spatial expression of Enhanced Green Fluorescent Protein (EGFP) transferred by a single nasal delivery of either a recombinant adenovirus vector (Ad5CMV-EGFP) or an adeno-associated virus vector (AAV2-EGFP) into Sprague-Dawley rats. Adenovirus mediated EGFP expression was localized in ORNs throughout the olfactory epithelium after 24 hours. EGFP in the ORNs appeared to be anterogradely transported along their axons to the olfactory bulb and transferred in glomeruli to second-order neurons. EGFP was transferred to several brain regions including the cortex, hippocampus, and brainstem after 7 days. EGFP expression co-localized with Olfactory Marker Protein and was confirmed with EGFP immunofluorescence labelling and western blotting. AAV expressed EGFP localized in similar olfactory and brain regions 6 weeks after delivery. mRNA levels suggested that the AAV-EGFP construct was only incorporated into olfactory mucosa cells and the viral vector was not present in olfactory bulb and brain regions.

In conclusion, this simple and non-invasive polypeptide and gene delivery method provides ubiquitous macromolecule distribution throughout the rodent brain and may be useful for the treatment of neurological disorders.

DECLARATION

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

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Anthony N Pollard

March 2009

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PERSONAL PUBLICATIONS AND CONFERENCE PRESENTATIONS

Personal Publications (2005-2009)

Refereed Journal Articles

1) **Pollard AN**, Aguilar-Salegio EA, Wang YJ, Sun Y and Zhou XF. (2009) Delivery of CNTF along olfactory pathways and the implication in the therapeutic treatment of obesity. (Submitted to J Cell Molec. Med.).

2) **Pollard AN**, Wang YJ, Aguilar-Salegio EA, and Zhou XF. (2009) Adenoviral and AAV vector gene delivery of enhanced-GFP to the brain via the olfactory receptor neurons. (in manuscript).

3) Aguilar-Salegio EA, **Pollard AN**, Smith M and Zhou XF. (2009) Depletion of macrophages prevents regeneration of pre-conditioned adult dorsal root ganglion neurons after spinal cord injury. (Submitted to Brain Behav Immun.)

4) Wang YJ, Valadares D, Sun Y, Wang X, Zhong JH, Liu XH, Majd S, Chen L, Gao CY, Chen S, Lim Y, **Pollard AN**, Aguilar E, Gai WP, Yang M, Zhou XF. (2009) Effects of proNGF on Neuronal Viability, Neurite Growth and Amyloid-beta Metabolism. **Neurotox Res.** 2009 Aug 13. [Epub ahead of print]

5) Wang YJ, Thomas P, Zhong JH, Bi FF, Kosaraju S, **Pollard AN**, Fenech M, Zhou XF. (2009) Consumption of grape seed extract prevents amyloid-beta deposition and attenuates inflammation in brain of an Alzheimer's disease mouse. **Neurotox Res.** 15:3-14.

6) Wang YJ, **Pollard AN**, Zhong JH, Dong XY, Wu XB, Zhou HD and Zhou XF. (2009) Intramuscular delivery of a single chain antibody gene reduces brain amyloid-beta burden in a mouse model of Alzheimer's disease. **Neurobiol of Aging.** 30:364-376.

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2. **Pollard AN***, Zhou FH, Zhong JH, Oliver J and Zhou XF. (2007) Intranasal delivery of ciliary neurotrophic factor to the rat brain along olfactory pathways. Society for Neuroscience (SfN) 37th annual meeting,. San Diego, Calif, US, Nov 3-7. (Poster presentation)

3. **Pollard AN***, Zhong JH, Wang YJ and Zhou XF. (2006) Adenoviral Vector Gene Delivery to the Brain via the Olfactory Sensory Receptor Neurons. Australian Neuroscience Society (ANS) 26th annual meeting, Sydney, Australia, Jan 31-Feb 3. (Poster presentation)

4. Wang YJ*, **Pollard AN**, Zhou HD, Zhong JH, and Zhou XF. (2006) Characterization of an Alzheimer's Disease Mouse Model Bearing Mutant Genes of Amyloid Precursor Protein and Human Presenilin 1. Australian Neuroscience Society (ANS) 26th annual meeting, Sydney, Australia, Jan 31-Feb 3. (Poster presentation)

5. **Pollard AN***, Li L, Zhou FH, Zhong JH, Wu XB and Zhou XF. (2005) AAV-Mediated Gene Delivery of GFP to the Brain via the Olfactory Pathway. Australian Neuroscience Society (ANS) 25th annual meeting, Perth, Australia, 30 Jan - 2 Feb 2005. (Symposium talk)

* Presenting author.

GENERAL ABBREVIATIONS

Abbreviation	Full name
AAV	adeno associated virus
ABC	avidin-biotin conjugate
AdV	adenovirus
ALS	amyotrophic lateral sclerosis
BBB	blood brain barrier
BCA	bicinchoninic acid
BSA	bovine serum albumin
cDNA	complementary DNA from mRNA
cm, mm, μm	centimetre, millimetre, micrometre
CNS, PNS	central nervous system, peripheral nervous system
rhCNTF	recombinant human ciliary neurotrophic factor
CO_2	carbon dioxide
CPM/mg	counts per minute/ milligram
CSF	cerebral spinal fluid
DAB	3'3'-diamino-benzidine tetrachloride
DAPI	4',6-diamidino-2-phenylindole
DNA	deoxyribonucleic acid
ECL	enhanced chemiluminescence
EDTA	ethylenediaminetetraacetic acid
EGFP	enhanced green fluorescence protein
H&E	haematoxylin & eosin
h, min, s	hour, minute, second
H_2O_2	hydrogen peroxide
HCl	hydrogen chloride
HRP	horse-radish peroxide
IGF	insulin growth factor
ICV, IM, IN, IPa, IT, IV	intracerebroventricular, intramuscular, intranasal, intraparenchymal, intrathecal, intravenous
IL	interleukin
KDa	kilo daltons
Kg, g, mg, μg, ng	kilogram, gram, milligram, microgram, nanogram

L, ml, µl	litre, millilitre, microlitre
M, mM, µM, nM	molar, millimolar, micromolar, nano molar
mRNA	messenger ribonucleic acid
MW	molecular weight
NaCl	sodium chloride
$NaNO_3$	sodium nitrate
NaOH	sodium hydroxide
NHS	normal horse serum
NGF	nerve growth factor
^oC	degrees celsius
OMP	olfactory marker protein
ORN	olfactory receptor neuron
OZR	obese Zucker rat
PAGE	polyacrylamide gel electrophoresis
PB	phosphate buffer
PBS	phosphate buffered saline
PBST	PBS + 0.1% tween-20
RT-PCR	reverse transcription polymerase chain reaction
pH	hydrogen ion concentration
pSTAT3	phospho- signal transduction and activation of transcription 3
RT	room temperature
SD	Sprague Dawley
SDS	sodium dodecylsulphate
pSTAT	phosphorylated signal transduction and activation of transcription
TBS	tris buffered saline
TCA	trichloracetic acid
WGA	wheat germ agglutinin
$ZnSO_4$	zinc suphate

BRAIN REGION NOMENCLATURE

Abbreviation	Full name
AOB	accessory olfactory bulb
AOV	anterior olfactory nucleus, ventral part
ArcM	arcuate nucleus, medial part
CA2	field CA2 of hippocampus
CA3	field CA3 of hippocampus
Cb	cerebellum
CPu	caudate putamen (striatum)
DEn	dorsal endopiriform cortex
DG	dentate gyrus
EPL	external plexiform layer of olfactory bulb
GL	glomerular layer of olfactory bulb
GCL	granular cell layer of olfactory bulb
IC	inferior colliculus
IPL	internal plexiform layer of olfactory bulb
LC	locus coeruleus
LEnt	lateral entorhinal cortex
LH	lateral hypothalamus
MCL	mitral cell layer of olfactory bulb
MOB	main olfactory bulb
ONL	olfactory nerve layer
Pr5VL	principal sensory trigeminal nucleus, ventrolateral part
PVP	paraventriular thalamic nucleus, posterior part
sp5	spinal trigeminal tract
VCA	ventral cochlear nucleus, anterior part
VMH	ventromedial hypothalamic nucleus
VPL	ventral postereolateral thalamic nuclei