Interactions of Neurotrophins and their Receptors

by

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ABSTRACT

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In order to investigate the interactions of neurotrophins with their receptors, a number of different domains of the extra-cellular regions of the TrkA, TrkB and TrkC receptors were expressed and the interactions of neurotrophins with these domains were investigated by biosensor. The entire extracellular domains of all three receptors were expressed in the yeast *Pichia pastoris,* while the leucine-rich regions and the immunoglobulin-like domains were expressed as MBP-fusion proteins in *E. coli.* Peptides representing the second leucine-rich regions and purported neurotrophin-binding domain of TrkA, TrkB and TrkC were synthesized. Proteins expressed in *Pichia pastoris* were purified by anion, cation and metal chelating columns; proteins expressed in *E. coli.*, were purified on amylose columns. All recombinant Trk proteins were covalently attached, using EDC/NHS chemistry, to the methyldextran surface of a biosensor cuvette. Extensive kinetics measurements of the interactions of the neurotrophins with immobilized recombinant proteins established a difference in the binding interactions of NGF with TrkA compared with the interactions of BDNF with TrkB and NT-3 with TrkC. All NGF interactions with TrkA proteins showed biphasic kinetics. Interactions of BDNF and NT-3 with TrkB and TrkC showed monophasic kinetics. No interaction of NGF with the immunoglobulin-like domain of TrkA was observed for those proteins expressed in *E. coli*, however the interaction of BDNF was observed with the immunoglobulin-like domain of TrkB when expressed in *E. coli.* Interaction of NGF and BDNF was observed with the leucine-rich domain of TrkA and TrkB respectively. These results differ from previously reported studies, both *in vivo* and *in vitro,* of the interactions of the extra-cellular domains of the Trk receptors with neurotrophins. Previous studies have claimed to establish exclusive interaction of the neurotrophins with either the leucine-rich or immunoglobulin-like domains of the Trk receptors. The interaction studies reported here show a clear interaction of neurotrophins with both leucine-rich and immunoglobulin-like domains of the Trk receptors.

These interactions have similar affinity. This result suggests that the interactions of neurotrophin and receptor may be more complex than previously suggested. It is conceivable that neurotrophins bind initially to the leucine-rich domain of the receptor, followed by movement to the Immunoglobulin-like domain and the initiation of phosphorylation of the intra-cellular domains or internalization of receptor and bound neurotrophin. Kinetics studies of the synthetic peptides failed to show that these represent the exclusive neurotrophin-receptor interaction domain as previously reported. These results suggest that the development of small molecule mimetics of the neurotrophins as a therapy for Alzheimer's and other neurological diseases may be more complicated than previously envisioned.

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Rita Levi-Montalcini, the co-discoverer of the neurotrophin NGF and eventual Nobel Prize winner, began her studies of the protein shortly before the outbreak of the second-world war. She continued her studies in the confines of her home throughout the allied bombing campaign of Italy. After the war, the studies were continued in Europe, the United States and South America. It would be difficult to find a more inspiring story than the trials and difficulties of one scientist in the pursuit of knowledge, than that of Levi-Montalcini. My own project presented in this thesis, has taken me from the United States to Japan, New Zealand and Canada and often seemed so close to failure that it seemed prudent to abandon the entire study. I owe a great deal to those that encouraged me to continue.

An ancient Chinese myth relates the story of an emperor who commissions a noted sculptor to fashion the emperor's likeness out of a piece of jade from a nearby mountain. After many years, the time to present the sculpture to the emperor finally arrived. Upon revelation of the sculpture, the emperor was dismayed to see, not an image of himself, but a figurine of a flock of birds. Naturally the artist was called to account for the apparent lack of deference to the emperor and nature of the commission. He explained that he had not found the emperor's likeness within the jade but instead had found the image of birds. Pursuit of a scientific investigation is quite analogous to the myth.

The study for my thesis began with the idea that the receptor for NGF would be crystallized and its structure determined. It was quickly realized that in order to maximize the possibilities of success, other neurotrophin receptors and even neurotrophins should be crystallized. After the passage of more than 3 years the proteins had been expressed but no diffracting crystals for any receptor or neurotrophin had been obtained (this study is not contained within the following thesis). This situation caused many difficulties for me and came close to terminating the entire thesis. Fortunately a change of concept resulted in a new study, which has led me to a different interpretation of the interaction of neurotrophins and their receptors than that previously espoused. Hence, like the sculptor, I did not find the structure that was originally sought, but from the trials and tribulations of the study a different and in many ways an equally interesting (if not beautiful) result was obtained.

There have been many individuals with whom I have interacted and who have influenced me over the duration of this thesis study. Firstly I would like to thank my wife Siu Lan who has endured stoically these difficult years and who has been my greatest source of encouragement. Without her, this thesis study would have terminated, without success, years ago. I should also like to thank my mother, Eileen, for her support and whose suffering with Parkinson's disease provided me with the initial incentive to pursue development of molecular solutions for human diseases. Without attempting to apportion any degree of direct influence or contribution to the study, the following individuals are now acknowledged for their help and encouragement:
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ABBREVIATIONS

Relevant Abbrevaitions to material in the text are given within the body of the material and in footnotes.

K_D	Equilibrium Dissociation Constant
µM	Micromolar
mM	Millimolar
nM	Nanomolar
K_D	Equilibrium Dissociation Constant
K_{off}	Dissociation Rate
K_{on}	Association Rate
BDNF	Brain Derived Growth Factor
CNS	Central Nervous System
MBP	Maltose Binding Protein
NGF	Nerve Growth Factor
NTF	Neurotrophic Factor
NT-3	Neurotrophin-3
PNS	Peripheral Nervous System
Trk	Receptor for NTF's