

# Index

<b>INDEX.....</b>	<b>1</b>
<b>SUMMARY.....</b>	<b>3</b>
<b>ABBREVIATIONS .....</b>	<b>5</b>
<b>1 INTRODUCTION .....</b>	<b>7</b>
1.1 MECHANOTRANSDUCTION .....	7
1.2 MECHANOTRANSDUCTION IN INVERTEBRATES.....	8
1.2.1 <i>Mechanotransduction in Caenorhabditis elegans</i> .....	8
1.2.2 <i>Mechanotransduction in Drosophila melanogaster</i> .....	9
1.3 MECHANOTRANSDUCTION IN VERTEBRATES .....	9
1.3.1 <i>Auditory and vestibular system</i> .....	10
1.3.2 <i>Cutaneous mechanotransduction</i> .....	10
1.3.3 <i>A molecular model of mechanotransduction</i> .....	13
1.4 NEUROTROPHINS.....	17
1.4.1 <i>General</i> .....	17
1.4.2 <i>Role of neurotrophins in establishing DRG neuron phenotype</i> .....	20
1.5 AIMS AND HYPOTHESIS .....	22
1.5.1 <i>Part I: Search for a GPI-anchored protease that is involved in sensory mechanotransduction</i> .....	22
1.5.2 <i>Part II: Search for genes that are regulated by the neurotrophin BDNF</i> .....	23
1.5.3 <i>Part III: Search for genes that are regulated by the neurotrophin NT-4</i> .....	24
<b>2 MATERIAL AND METHODS .....</b>	<b>25</b>
2.1 MATERIAL AND ANIMALS.....	25
2.1.1 <i>Technical equipment</i> .....	25
2.1.2 <i>Chemicals and reagents</i> .....	26
2.1.3 <i>Buffers and solutions</i> .....	27
2.1.4 <i>Culture media</i> .....	31
2.1.5 <i>Enzymes and molecular weight markers</i> .....	31
2.1.6 <i>Kits</i> .....	32
2.1.7 <i>Plasmids</i> .....	32
2.1.8 <i>Primers</i> .....	33
2.1.9 <i>Antibodies</i> .....	33
2.1.10 <i>Consumables</i> .....	34
2.1.11 <i>Animals</i> .....	34
2.2 METHODS.....	35
2.2.1 <i>Molecular biology</i> .....	35
2.2.2 <i>Cell culture</i> .....	46

---

2.2.3	<i>Protein chemistry</i> .....	47
2.2.4	<i>In vitro skin nerve preparation (Koltzenburg et al., 1997)</i> .....	51
<b>3</b>	<b>RESULTS</b> .....	<b>54</b>
3.1	SEARCH FOR A GPI ANCHORED PROTEASE THAT IS INVOLVED IN MECHANOTRANSDUCTION.....	54
3.1.1	<i>Binding studies using SPR</i> .....	55
3.2	SEARCH FOR BDNF-REGULATED MECHANOTRANSDUCTION GENES.....	61
3.2.1	<i>Oligonucleotide array analysis</i> .....	61
3.2.2	<i>Suppression subtractive hybridization</i> .....	62
3.2.3	<i>Combined analysis</i> .....	63
3.2.4	<i>In situ hybridization</i> .....	63
3.3	SEARCH FOR D-HAIR SPECIFIC GENES.....	65
3.3.1	<i>Apoptosis in adult NT4 null mutants</i> .....	65
3.3.2	<i>Oligonucleotide array analysis</i> .....	67
3.3.3	<i>Suppression subtractive hybridization</i> .....	68
3.3.4	<i>Combined analysis</i> .....	68
3.3.5	<i>In situ hybridization</i> .....	71
3.3.6	<i>Quantitative PCR</i> .....	74
3.3.7	<i>Functional studies using skin-nerve preparation</i> .....	76
<b>4</b>	<b>DISCUSSION</b> .....	<b>81</b>
4.1	SEARCH FOR A GPI-ANCHORED PROTEASE.....	81
4.2	SEARCH FOR BDNF-REGULATED MECHANOTRANSDUCTION GENES.....	82
4.3	SEARCH FOR D-HAIR MECHANORECEPTOR SPECIFIC GENES.....	85
4.3.1	<i>The T-type calcium channel CaV3.2 is specifically expressed in D-hair mechanoreceptors</i> .....	85
4.3.2	<i>Mechanical sensitivity of D-hair mechanoreceptors is decreased after treatment with mibefradil</i> ..	88
4.3.3	<i>The electrical excitability of D-hairs is decreased after mibefradil treatment</i> .....	89
4.3.4	<i>Model for a function of CaV3.2 in mechanotransduction</i> .....	90
4.3.5	<i>Open questions</i> .....	93
4.3.6	<i>Further experiments</i> .....	93
<b>5</b>	<b>REFERENCES</b> .....	<b>95</b>