REVIEW

Clinical relevance of the neurotrophins and their receptors

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ABSTRACT

The neurotrophins are growth factors required by discrete neuronal cell types for survival and maintenance, with a broad range of activities in the central and peripheral nervous system in the developing and adult mammal. This review examines their role in diverse disease states, including Alzheimer's disease, depression, pain and asthma. In addition, the role of BDNF (brain-derived neurotrophic factor) in synaptic plasticity and memory formation is discussed. Unlike the other neurotrophins, BDNF is secreted in an activity-dependent manner that allows the highly controlled release required for synaptic regulation. Evidence is discussed which shows that sequestration of NGF (nerve growth factor) is able to reverse symptoms of inflammatory pain and asthma in animal models. Both pain and asthma show an underlying pathophysiology linked to increases in endogenous NGF and subsequent NGF-dependent increase in BDNF. Conversely, in Alzheimer's disease, there is a role for NGF in the treatment of the disease and a recent clinical trial has shown benefit from its exogenous application. In addition, reductions in BDNF, and changes in the processing and usage of NGF, are evident and it is possible that both NGF and BDNF play a part in the aetiology of the disease process. This highly selective choice of functions and disease states related to neurotrophin function, although in no way comprehensive, illustrates the importance of the neurotrophins in the brain, the peripheral nervous system and in non-neuronal tissues. Ways in which the neurotrophins, their receptors or agonists/antagonists may act therapeutically are discussed.

INTRODUCTION: NEUROTROPHINS AND THEIR RECEPTORS

In mammals there are four members of the neurotrophin family: NGF (nerve growth factor), BDNF (brain-derived neurotrophic factor), NT-3 (neurotrophin 3) and NT-4 (neurotrophin 4) (for a review, see [1]). NGF is produced in sympathetic and sensory target organs and target tissues in the brain, providing trophic support to neurons, which retrogradely transport the protein to the cell body. All of the neurotrophins are synthesized as precursors and are processed either inside the cell by furin or pro-hormone convertases, or extracellularly by plasmin or the MMPs (matrix metalloproteinases) MPP3 or MPP7 to produce mature neurotrophins. Pro-NGF, pro-NT-3, and pro-NT-4 are packaged into constitutive vesicles before secretion; pro-BDNF, however, is mainly packaged into regulated secretory pathway vesicles,

Key words: Alzheimer's disease, asthma, depression, neurotrophin, pain, $p75^{NTR}$ (p75 neurotrophin receptor), Trk.
Abbreviations: ALS, amyotrophic lateral sclerosis; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; Aβ, β-amyloid peptide; apoE, apolipoprotein E; APP, amyloid precursor protein; BDNF, brain-derived neurotrophic factor; ChAT, choline acetyltransferase; CYP, cyclophosphamide; DRG, dorsal root ganglion; LTP, long-term potentiation; E-LTP, early-LTP; GABA, γ-aminobutyric acid; ICV, intracerebroventricular; L-LTP, late-LTP; LTD, long-term depression; MAPK, mitogen-activated protein kinase; MMSE, Mini-Mental Status Examination; MRI, magnetic resonance imaging; nbM, basal forebrain nuclei; NGF, nerve growth factor; NMDA, *N*-methyl-D-aspartate; NT-3, neurotrophin-3; NT-4, neurotrophin-4; $p75^{NTR}$, p75 neurotrophin receptor; PI3K, phophoinositide 3-kinase; PLC-γ, phospholipase C-γ; PS1, presenilin 1; PS2, presenilin 2; VR-1, vanilloid receptor-1.
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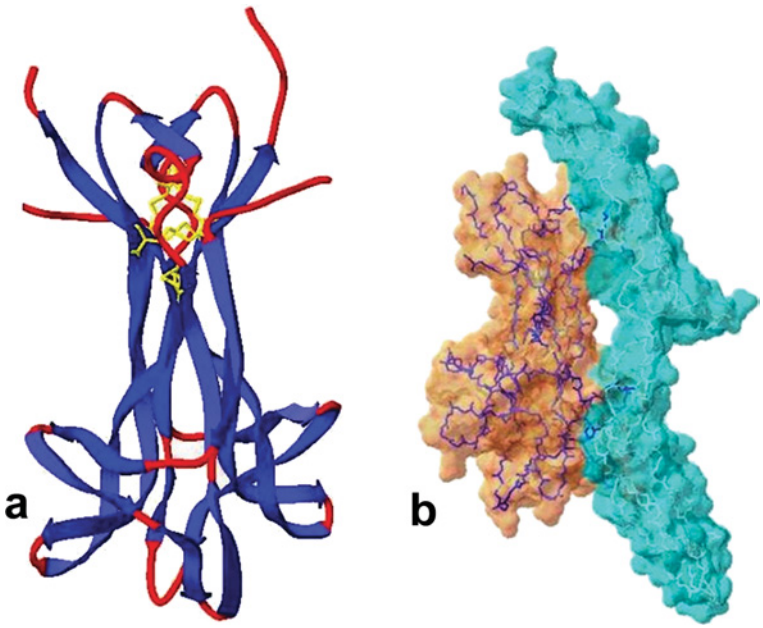

Figure 1 X-ray crystallographic structures of NGF alone and bound to p75^{NTR}

(a) Ribbon cartoon of mature human NGF. This is a homodimer consisting of two strands each 120 amino acids, which non-covalently dimerize to form a 26 kDa protein. Note the N-terminus of the monomers is not apparent (unresolved). (b) Mature human NGF (brown) bound to the extracellular domain of p75^{NTR} (turquoise); both structures have been covered with a theoretical molecular surface. By binding together, the conformational change prevents further binding to a second receptor.

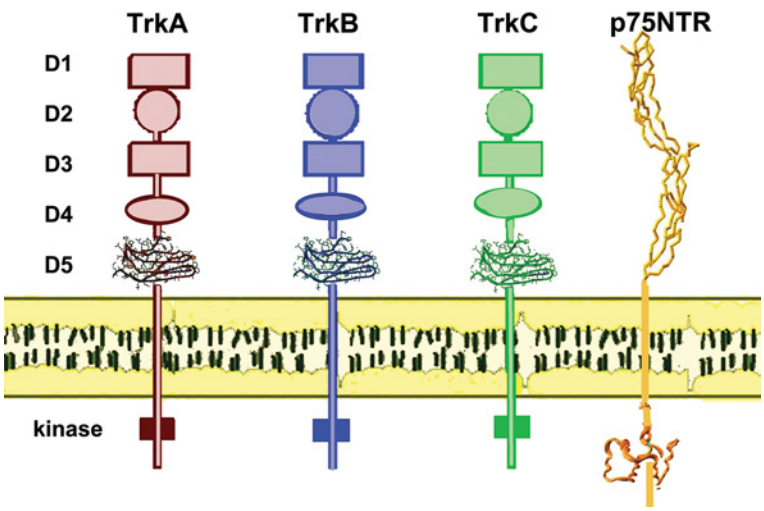

Figure 2 Schematic representation of the structure of the catalytic forms of TrkA, TrkB and TrkC and also the p75^{NTR} protein

Non-catalytic forms of TrkB and TrkC also exist (not shown). The extracellular domain of the Trk receptors can be divided into five distinct domains D1–D5. There is an intracellular kinase domain. p75^{NTR} is a type I transmembrane receptor with an extracellular domain that contains four cysteine-rich domains. The intracellular portion contains two potential TRAF (tumour-necrosis-factor-receptor-associated factor)-binding sites, a type II death domain, a potential G-protein-activating domain and a PDZ-domain-binding motif. The structure of the death domain is shown.

processed and secreted in an activity-dependent manner [2,3]. The structures of the mature proteins have been elucidated by X-ray crystallography and they each appear very similar in conformation. Figure 1(a) shows the structure of mature NGF. Structures of BDNF, NT-3 and NT-4 all appear similar to that of NGF.

The neurotrophins act via two different receptor types: the pan neurotrophin receptor, p75^{NTR} (p75 neurotrophin receptor), and the family of tyrosine kinase receptors (Trk) (Figure 2). All the neurotrophins bind with similar affinity to p75^{NTR}, but each binds specifically with high affinity to different Trk receptors (for a review,

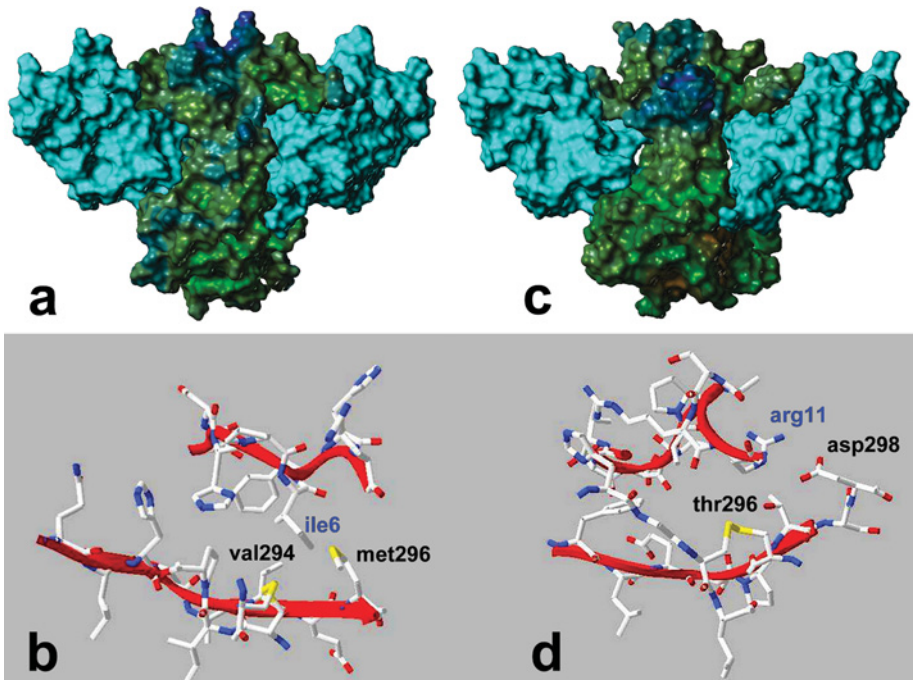

Figure 3 X-ray crystallographic structures of NGF–TrkAD5 (a) and NT-4–TrkBD5 (c), and interactions between the N-terminus of the neurotrophin with the D5 domain of the receptors (b and d)

(a) Mature human NGF bound to two TrkAD5 domains. D5 is the domain of the extracellular portion of TrkA nearest the membrane. It is a 13 kDa monomer. (b) Interactions between the N-terminus of NGF and TrkAD5. Hydrophobic interactions between Ile^{6} of NGF and Met^{296} TrkA are shown. (c) Mature human NT-4 bound to two TrkBD5 domains. (d) Interactions between the N-terminus of NT-4 and TrkBD5. A salt bridge is able to form between Arg^{11} of NT-4 and Asp^{298} of TrkB.

see [4]). $p75^{NTR}$ is a member of the tumour necrosis factor family with a single transmembrane region. The extracellular portion contains four cysteine-rich domains, and the X-ray structure has recently been determined [5] (Figure 1b). It has an intracellular Type II death domain which is implicated in apoptosis (for a review, see [6]).

There are three Trk receptors: TrkA, TrkB and TrkC (Figure 2). TrkA binds NGF, TrkB binds BDNF and NT-4, and TrkC binds NT-3 (for a review, see [1]). The intracellular kinase domains are all very similar, with approx. 87 % homology. The extracellular portion of each of these Trk receptors can be subdivided into five distinct domains D1–D5. D5, the region closest to the membrane, is able to bind its neurotrophin ligand without the other domains being present [7,8] (Figure 3). The X-ray crystal structures of the domains alone [8,9], and bound to their respective ligands, have all been solved [7,10].

Neurotrophins exist as homodimers and are able to pull together two tyrosine kinase receptors. This results in transphosphorylation of tyrosine residues in the intracellular kinase domain of the receptors. Phosphorylated tyrosines act as docking sites for signalling molecules which regulate cell growth and survival through the Ras, PI3K (phophoinositide 3-kinase) and PLC-γ (phospholipase C-γ) pathways (for reviews, see [4]). $p75^{NTR}$ is able to modify the selectivity of binding of the neurotrophins to Trk receptors. It also signals independently through NF-κB (nuclear factor κB), Akt and JNK (c-Jun N-terminal kinase) (for reviews, see [6]). It interacts with several adaptor proteins associated with the induction of apoptosis and cell survival.

It is evident that the neurotrophins play a vital role in development, but also in the maintenance of the neuronal systems throughout life. Recently BDNF and TrkB have been shown to play a prominent role in synaptic plasticity and memory.

SYNAPTIC PLASTICITY

Synaptic plasticity is a mechanism by which adult, as well as developing, animals may learn about their environment and adapt to it. Memory acquisition is dependent on the hippocampus and associated limbic structures. It has been characterized as being short-term, lasting between seconds and minutes, or long-term, which may persist for hours or longer and for which protein synthesis is required.

A role for BDNF has emerged in connection with regulation in synaptic function and synaptic plasticity. It has been known for over a decade that BDNF applied to neurons and muscle cells, in culture, potentiates neurotransmitter release at presynaptic terminals [11] and, furthermore, that BDNF potentiates excitatory

synaptic transmission in hippocampal neurons [11,12]. It has been shown to play a critical role in learning outcome in a large number of studies [13,14] and to have a role in hippocampal-dependent cognitive performance. Thus spatial memory was found to be superior in rats housed in enriched rather than impoverished environments [15] and after training [16–18]. Disruption of BDNF expression by use of antisense oligonucleotides [17,18], in heterozygous BDNF knockout animals [19] or by use of anti-BDNF antibodies [20] caused severe impairment in spatial memory and learning. Similarly impaired learning was evident in the conditional TrkB knockout [21] and in mice overexpressing the truncated form of TrkB [22].

LTP (long-term potentiation) [23] is associated with memory formation and the consolidation of long-term memory. LTP is used as a measure of increase in synaptic efficacy at the synapse. It occurs with concomitant depolarization of the postsynaptic membrane, in conjunction with the binding of the excitatory neurotransmitter glutamate to the NMDA (*N*-methyl-D-aspartate) receptor, a calcium permeable ion channel; however, dependent on the levels of calcium and the timing of activation, such stimulation may also lead to LTD (long-term depression) of synaptic strength. LTP may be divided into two phases, E-LTP (early-LTP) and L-LTP (late-LTP) phases. E-LTP, which can last for 1–2 h, can be induced by a single burst of high-frequency stimulation or tetanus, designed to mimic a physiological burst of neuronal activity in the hippocampus. L-LTP, which lasts many hours, requires repeated bursts of stimulation. E-LTP activity involves protein phosphorylation, whereas L-LTP is protein synthesis dependent.

NMDA receptors are embedded in the postsynaptic density, in the postsynaptic membrane and associate with a number of scaffold (including PSD-95) and signalling proteins [including Syn-GAP (GTPase-activating protein) and calmodulin]. Thus stimulation of these receptors may initiate a variety of signalling pathways. In addition, glutamate is able to activate a second subclass of receptor, the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor, also present on the postsynaptic membrane. This receptor mediates most of the rapid EPSC (excitatory postsynaptic current). In addition to postsynaptic mechanisms of synaptic plasticity, synaptic modification can also be brought about by presynaptic means such as altered neurotransmitter release.

BDNF mRNA levels are increased with induction of LTP (for a review, see [24]). BDNF knockout mice show impaired LTP in hippocampal slices which can be rescued by application of exogenous BDNF [25,26]. These mice show little discernable L-LTP [27], although E-LTP can be induced [26,27]. Mutational analysis of phosphorylation sites of TrkB shows that inhibition of phosphorylation at the PLC-γ-binding site results in reduction in LTP in the hippocampus, whereas mutation of the Shc site, essential for activation of MAPK (mitogen-activated protein kinase) and PI3K, does not affect this [28]. Bearing in mind that BDNF, like all the neurotrophins, binds to the generic $p75^{NTR}$, inhibition of $p75^{NTR}$ does not stop BDNF-related LTP [29].

BDNF, in addition to enhancing synaptic transmission through NMDA receptors, was shown to cause TrkB-dependent suppression of AMPA-receptor-mediated currents in cultured hippocampal cells [30]. BDNF and TrkB receptors also potentiated GABA (γ-aminobutyric acid)-receptor-mediated responses, via TrkB, and PLC-γ, early in development when GABA is excitatory rather than inhibitory [31,32]. BDNF is also known to have an effect on modulation of voltage-dependent potassium channels, such as Kv1.3 [33], by protein phosphorylation and to alter the activity of G-protein-activated inward rectifying potassium channels (Kir3). In addition, one group has reported that, at nanomolar concentrations, BDNF was able to excite neurons in the hippocampus, cortex and cerebellum [34]. This was reported to be associated with TrkB-mediated gating of the Nav1.9 sodium channels, suggesting a direct activation of NMDA receptors [35].

The morphological correlate of the induction of LTP at the synapse is the increased formation of synapses at dendritic spines or axon collaterals. It is known that synaptic density is increased as a consequence of learning (for a review, see [36]). BDNF knockout mice show reduced numbers of axon collaterals and axonal varicosities in the hippocampus [37].

In order to fulfil a regulatory role in synaptic function, it is essential that BDNF itself should be able to be regulated in an activity-dependent manner. Unlike the other neurotrophins, which are largely processed via the non-regulated secretory pathway, BDNF has been suggested for some time to be sorted to the regulated secretory pathway [38,39], probably in a similar manner to prohormones, that is via a receptor-mediated mechanism requiring a sorting signal motif. Recently this sorting motif receptor-mediated mechanism has been revealed [40]. BDNF has been shown to contain a motif (Ile^{16}, Glu^{18}, Ile^{105} and Asp^{106}) within the mature protein which is able to interact with two basic residues in the sorting receptor carboxypeptidase E. It has been shown that mutation of these residues in BDNF resulted in mis-sorting of pro-BDNF to the constitutive pathway in AtT-20 cells. Wild-type BDNF co-localized with POMC (pro-opiomelanocortin) in a punctate pattern along cell processes, whereas mutated BDNF was mostly perinuclear. Furthermore mutation of NGF, to complete a similar motif for carboxypeptidase E binding, caused partial redirection from the constitutive to the regulated pathway [40]. Cortical neurons from carboxypeptidase E knockout mice showed no activity-dependent secretion of BDNF [40]. The amount of cleavage of pro-BDNF

to the mature form was reduced, although there was still a substantial amount of mature BDNF (50% of total) released via the constitutive pathway. Pro-BDNF is reported to be processed less efficiently by intracellular proteases than the other neurotrophins [3,39]; however, the amounts released, either pro- or mature BDNF, will presumably affect the binding of the neurotrophin to the receptors, with the pro-form binding preferentially to $p75^{NTR}$ rather than TrkB [3,41]. Increased secretion of pro-BDNF may therefore have a negative effect on synaptic plasticity. Since increased neuronal activity would result in an increase in BDNF synthesis and probably an increase in the pro- form due to less time for processing, then the processing of pro-BDNF by extracellular proteases such as plasmin may be important for regulation of L-LTP in the hippocampus [42].

Previous to the discovery of the sorting motif in the mature region of BDNF, it was reported that one of two common BDNF polymorphisms ($Val^{66} \rightarrow Met$; Val66Met) in the pro-region was associated with a marked impairment in regulated secretion of BDNF [43]. Hippocampal neurons in culture transfected with the Met^{66} form of BDNF, unlike the Val^{66} form, did not show localization in dendritic processes and secretogranin II-positive vesicles. Thus BDNF sorting from the Golgi complex was affected. Met^{66} BDNF formed clusters in perinuclear regions, whereas Val^{66} BDNF co-localized with synaptic markers [43,44]. There was a consequent marked reduction in activity-dependent secretion of BDNF from neurons after potassium depolarization. Whether this is a sorting mechanism similar to that described above (carboxypeptidase E mediated) is not known; however, the neurotrophin pro-domain is important for correct folding of the mature protein and misfolding may result in trafficking from the Golgi being affected.

Either way the result is clearly important for synaptic function, since subjects with this polymorphism are less successful in tests of episodic short-term memory and show changes of glucose utilization with functional MRI (magnetic resonance imaging) [43,44]. A further study of hippocampal volume, using MRI in normal subjects, showed bilateral age-independent reductions of hippocampal grey matter in Met-BDNF carriers. Lower volumes in the prefrontal cortex were also evident [45]. Interestingly BDNF knockout mice, in adulthood, have been shown to have reductions in cortical volume [46]. This suggests hard-wired changes at an early stage and that impairment of short-term memory is not simply attributable to alterations in neural transmission during testing. These brain regions normally show high BDNF levels and are known to undergo synaptic plasticity throughout life, and have been reported variously to show reductions in BDNF levels in a number of neurodegenerative diseases or associated models [43,44,47,48].

From these studies we know that BDNF is extremely important in memory function in the hippocampus. Thus, although BDNF and TrkB are widely distributed throughout the brain, it is clear from the role of BDNF as a synaptic modulator that it is able to act in a discrete and highly regulated manner.

The involvement of neurotrophins in many diseases of the central nervous system is well documented. Owing to space constraints, however, we describe only one example of neurodegeneration, Alzheimer's disease, and one of a neuropsychiatric disease, depression.

ALZHEIMER'S DISEASE

Symptoms and neuropathology

Alzheimer's disease is the fourth most common cause of death in the Western world after heart disease, cancer and stroke. It is the most common form of dementia in the elderly; approx. 5–10% of people over 65 years of age and approx. 20% over 80 years of age have Alzheimer's disease. In the U.K., approx. 750000 people have dementia, of which approx. 450000 have Alzheimer's disease. The numbers of people worldwide are difficult to assess, but without doubt these will increase dramatically in the next couple of decades, as we all are living longer.

The symptoms may be grouped into: (i) the neuropsychological element, including amnesia, and also other elements such as aphasia (loss of articulate speech), apraxia (the inability to carry out tasks) or agnosia (the inability to recognize things); (ii) the neuropsychiatric component associated with psychiatric disturbances and behavioural disorders; and (iii) deficits in activities of daily living [49]. The defining neuropathology involves neuronal loss and extracellular deposits of amyloid plaques and intracellular deposition of hyperphosphorylated tau or neurofibrillary tangles (Figure 4); for a review, see [50]).

Much of the understanding of the molecular mechanisms involved has come from two sources: firstly, the determination of the major protein constituents of the plaques and tangles, i.e. amyloid [a 4 kDa peptide called Aβ (β-amyloid peptide)] and tau respectively, and secondly the discovery of families of early-onset Alzheimer's disease sufferers. We know that most of these families (or pedigrees) now discovered are due to mutations in one of three genes, *APP* (amyloid precursor protein), *PS1* (presenilin 1) or *PS2* (presenilin 2) (online mutation database at http://www.alzforum.org). The majority of familial Alzheimer's disease results from mutations in *PS1*; however, approx. 90–95% of cases of Alzheimer's disease are sporadic and have no known aetiology. One known risk factor, however, is the presence of a particular isoform of the protein apoE (apolipoprotein E), apoE4. A common factor linking these four genes is the production or deposition of Aβ. APP

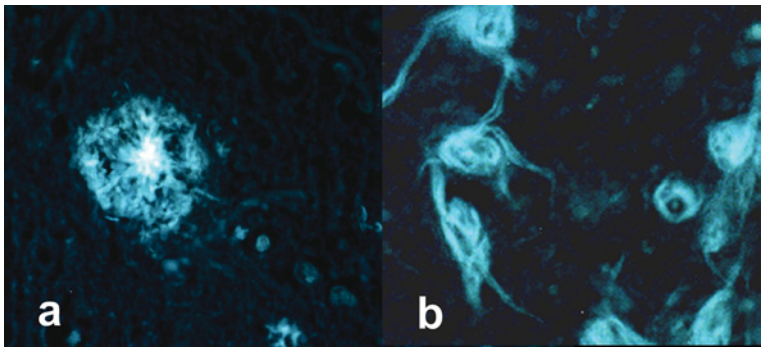

Figure 4 Neuropathological changes seen in Alzheimer's disease

A section of Alzheimer cortex, stained with Thioflavin S, viewed with a fluorescence microscope. (a) An amyloid plaque, and (b) neurofibrillary tangles.

is processed by enzymes [β-secretase or BACE (β-site APP-cleaving enzyme) and γ-secretase (presenilin is the catalytic subunit of γ-secretase)]. ApoE4 is known to increase fibrillization and deposition of Aβ into plaques. Moreover, recently BACE activity has been shown to be increased in sporadic Alzheimer's disease [51,52].

Cholinergic function and the basal forebrain

Early memory loss seen in Alzheimer's disease coincides with the early loss of cholinergic function [53,54]. Cholinergic function, as measured by markers such as ChAT (choline acetyltransferase), choline uptake at terminals or acetylcholine synthesis all showed a marked decline at a very early stage in the disease [55], and numerous studies provide a direct link between cholinergic function and memory. The cortex and hippocampus are innervated by cholinergic neurons originating from the nbM (basal forebrain nuclei) and memory function is impaired following either lesions that interrupt this cholinergic innervation or administration of anticholinergics [56]. By contrast, acetylcholinesterase inhibitors, which inhibit the breakdown of the neurotransmitter acetylcholine, act as a therapeutic solution for some Alzheimer's disease patients. Originally, side-effects, such as nausea and liver failure, limited the use of inhibitors such as Tacrine (Cognex). However, second-generation cholinesterase inhibitors such as donepezil (Aricept) and galantamine (Reminyl) are much better tolerated, providing relief from symptoms in perhaps one-quarter to one-third of patients for months or even years.

More than 90 % of the cholinergic cells of the basal forebrain are p75^{NTR}-immunoreactive [57,58]. Assessment of the numbers of basal forebrain cholinergic cell bodies has revealed a wide range of loss, with largest reductions seen in early onset patients [59–61]. One study reported a substantial reduction of p75^{NTR}-immunoreactivity (cell area and fibre staining) in neurons of the nbM [61]. This differs from an earlier study [59] examining number and size of p75-immunoreactive neurons in different regions of the nbM in older patients (aged 82–94 years). These showed comparatively little loss of p75^{NTR}-immunoreactive cells (28–34 % in the CH4 nucleus and no significant change in the CH2) in averaged scores from seven Alzheimer's disease patients (Figure 5). However, lowest scores and greatest reduction in cell area were in the youngest patients. Dementia score correlated best with accumulated neuronal losses in the intermediate and posterior regions [59], areas often reported as most affected [60]. In general, the loss of cortical cholinergic activity is greater by far than would be expected from the cell loss in the nbM.

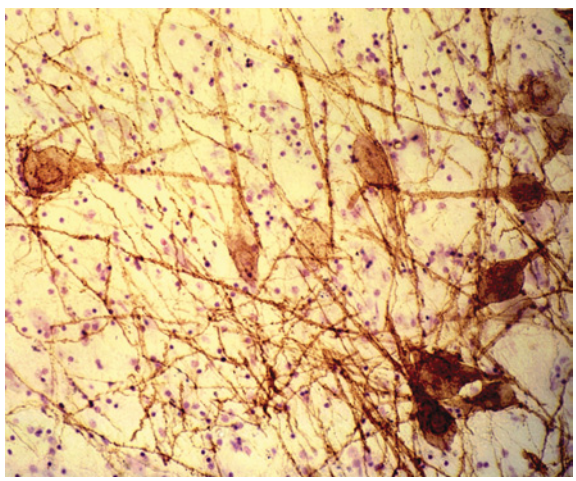

Figure 5 Neurons of the nbM stained with an antibody to p75^{NTR}

Both cell body and fibres are stained. The staining has a punctuate appearance and can be seen at the cell surface and also in the perinuclear region.

p75^{NTR} mRNA [62] and protein (ligand binding and immunocytochemistry) in basal forebrain were found to be unchanged in Alzheimer's disease [59,63]; by comparison, the number of TrkA positively staining cells have been shown to be reduced [64,65]. In the nbM, one study [64] has shown a reduction in the number

of neurons immunoreactive for Trk A, TrkB and TrkC in brains of Alzheimer's disease patients (69, 47 and 49 % respectively). This should be seen in the perspective of the findings that 75 % of nbM neurons show TrkB immunoreactivity, 58 % show TrkC and 54 % show TrkA.

NGF and amyloid pathology

Contrary to expectation, target tissue (i.e. cortex and hippocampus) levels of NGF were not found to be reduced [66,67], although NGF levels in the basal forebrain in Alzheimer's disease were decreased [68]. However, more recently, there was a reported reduction in NGF [69] in the frontal cortex of undemented patients with senile plaques. In addition, a significant decrease was seen in serum NGF in patients with mild cognitive impairment compared with Alzheimer's disease patients [70]. The authors suggested that the availability of NGF might be reduced at the onset of neurodegenerative processes. Measurements of NGF, by means of ELISA, will in most cases not be able to distinguish between mature NGF protein and the uncleaved or pro- form. It has been shown that, in human brain, the predominant form of NGF is that of pro-NGF, and this has now been reported to be increased in Alzheimer's disease [71] and binds preferentially to the $p75^{NTR}$ [41]. Since there is evidence to show that pro-NGF binding to $p75^{NTR}$ may, under certain circumstances, be associated with apoptosis [72], the apparent increased levels in Alzheimer's disease may have implications in terms of cholinergic vulnerability. In addition, $p75^{NTR}$ has been shown to bind Aβ [73], again implying a negative effect on cell survival. NGF- or TrkA-null mice tend to die *in utero* or soon after birth; however, cholinergic function is markedly reduced in these animals and in those heterozygous (non-lethal) for NGF deletion in the cortex, hippocampus and basal forebrain [74,75], and this loss correlates with deficits in spatial memory function [74]. Transgenic mice, which produce antibodies to NGF, show an age-dependent accumulation of antibodies. These mice (AD11) show an expected cholinergic deficit in the basal forebrain; however, surprisingly they also show cortical cell loss, amyloid plaques and hyperphosphorylated tau in cortical and hippocampal neurons [76]. In addition, they exhibit behavioural deficits. Furthermore, not only does intranasal administration of NGF restore cholinergic function and reduce numbers of amyloid plaques, but so also does intraperitoneal injection of the cholinesterase inhibitor galantamine [77]. Transgenic mice, aged 2, 6 and 6.5 months, were assessed after administration of NGF (1.2–12 μg on alternate days) or galantamine (3.5 mg/kg of body weight, daily). Both therapies restored the number of ChAT-immunopositive neurons to normal and both reduced numbers of amyloid plaques. In addition, NGF prevented deposition of hyperphosphorylated tau in the younger mice, but with no effect on deposited APP in cerebral vessels. Galantamine removed APP deposition, but had no effect on hyperphosphorylated tau. The acetylcholinesterase inhibitors tacrine and physostigmine were reported to have no effect on pathology; exactly why is unclear, although galantamine is also known to have modulatory effects on nicotinic receptors.

If the availability of NGF is somehow related to amyloid production then there must be a mechanism to explain this. The stimulation of PKC (protein kinase C)-coupled M1 and M3 muscarinic acetylcholine receptors in studies of NGF-responsive cortical cells results in an increase in cleavage of APP via α-secretase rather than β-secretase. This results in a decrease in Aβ production. When such stimulation is reduced then β-secretase, rather than α-secretase, cleavage is favoured. Cortical cholinergic hypoactivity may thus result in an increase in Aβ production [78]. It is also possible that the retrograde transport of NGF from the cortex and hippocampus to the cholinergic cell bodies may be compromised. In Down's syndrome, or trisomy 21, there is an extra copy of the APP gene present, and it is likely that the increased amyloid burden is responsible for the early amyloid pathology seen and the fact that most Down's patients have Alzheimer's disease by their fourth decade. Retrograde transport of NGF from the hippocampus to the septum is virtually abolished in aged rats [79] and in a mouse model of Down's syndrome [80]. Neither of these is a true model of Alzheimer's disease; however, it would explain how target levels of pro-NGF could be increased yet basal forebrain levels of NGF decreased in Alzheimer's disease. Of interest perhaps is the finding of a reduction of TrkA immunoreactivity in Down's syndrome basal forebrain [81].

NGF as a therapy

Numerous *in vitro* studies show that NGF increases survival rate of cholinergic neurons and activity of the enzyme ChAT [82,83]. *In vivo*, in rats, lesion of the fimbria-fornix, the fibres connecting the septum and the hippocampus, prevents retrograde transport of NGF and results in cholinergic cell loss and atrophy, whereas ICV (intracerebroventricular) administration of NGF eliminates this degeneration and resultant cognitive deficits [84,85]. Aged rats, with reduced cholinergic function and presenting with behavioural deficits, when treated intracerebroventricularly with NGF have increased cholinergic function and improved spatial memory [86]. However, NGF is a protein and it will not cross the blood–brain barrier. Therefore, in order to be used as a therapeutic, it would need to be administered directly into the brain.

A small clinical trial of three Alzheimer's disease patients took place in Sweden in the early 1990s [87]. These patients were given purified mouse NGF into the cerebral ventricles for up to 3 months continuously. Two patients were given up to 6.6 mg in total. Results were variable, but some improvement was seen in scores on

some of the cognitive tests: in one patient, glucose metabolism was increased; and in a second, cortical blood flow was increased. The two patients administered the higher dose also showed increased [^{11}C]nicotine binding in the cortex. However, there were side-effects of weight loss and pain. These symptoms stopped when the NGF was withdrawn. Further studies in rats [88,89] and primates [88] with ventricular administration of rhNGF (recombinant human NGF) have since revealed a dose-related (non-malignant) Schwann cell hyperplasia. This consisted of atypical hyperplastic tissue attached to the dorsal and lateral surface of the medulla and the spinal cord. This was reversible, was reduced after 8 weeks and was completely abolished after 52 weeks [89]. It is likely, however, that this undesirable side-effect occurs in humans and suggests that ICV administration of NGF is not a good therapeutic solution. This side effect was due to the presence of $p75^{NTR}$, since these hyperplastic cells are immunoreactive for $p75^{NTR}$ but not TrkA [89].

ICV administration of NGF is intrusive and relies on an endless supply of protein for infusion. Ways to circumvent this problem have been explored: for instance, intranasal application of NGF has been considered. Following injection of ^{125}I-labelled NGF into the olfactory bulb of rats, radiolabelled NGF is retrogradely transported to basal forebrain cholinergic cells [90] and also following nasal administration [91]. This may be a route worth exploring further, although efficiency and also compliance need to be considered. Perhaps more immediately applicable is the use of implants. A large body of work has been carried out using fibroblasts engineered to express NGF. These were able to rescue cholinergic function in fimbria-fornix-lesioned rats [92] and in aged rhesus monkeys with atrophy in the cholinergic basal forebrain [93]. These, and other, studies have shown that such fibroblasts were able to sustain NGF production for at least 18 months [92,94]. This work has been pioneered by Mark Tuszynski at the University of California at San Diego and has now been extended to a Phase I clinical trial [94]. Since April 2001, eight patients diagnosed as suffering from the early stages of Alzheimer's dementia have been surgically implanted with their own modified fibroblasts, transfected with Moloney leukaemia virus vectors expressing NGF. Implantation was at five sites along the length of the nbM. Six of the subjects received cells into both the right and left nbM. The first two subjects were only sedated and, due to movement in surgery, suffered subcortical haemorrhage. Both suffered hemiparesis, and one subject died. Subsequent operations were carried out successfully under general anaesthesia. Subjects were monitored for 18–24 months and no weight loss or pain was seen in the six patients. Slowing down of decline in cognition was seen using both the MMSE (Mini-Mental Status Examination) and ADAS-cog (Alzheimer's Disease Assessment-Scale-Cognitive). As would be expected, improvements were greater after the initial 6-month period. Average rate of decline overall in MMSE score, which was previously 6.1 points/year, slowed to 3 points/year. PET (positron emission tomography) scans showed significant increases in glucose utilization in cortical regions receiving cholinergic inputs in four out of the six patients. Histopathology carried out on the brain of the one subject who died 5 weeks after surgery revealed that the patient had Lewy body disease, with numerous plaques, tangles and Lewy bodies in the brain stem, substantia nigra and cortex. The differentiated fibroblast cells were still evident with robust sprouting of cholinergic fibres into the fibroblast grafts.

Despite the intrusive and hazardous nature of this type of operation, it would appear that, at least for up to 2 years, these NGF-producing implants provide therapeutic potential. It will be interesting to see if a longer time frame in a larger trial will provide a robust positive response. These patients were, of necessity, early on in their illness, making definitive diagnosis difficult. As early diagnosis becomes more achievable, perhaps with the assistance of PET scan visualization of amyloid plaques, this sort of operation may be a useful treatment for some patients.

BDNF and Alzheimer's disease

It is worth mentioning finally that BDNF and TrkB are also implicated in the Alzheimer's disease process. Both BDNF mRNA and protein levels were shown to be reduced in Alzheimer's disease hippocampus and temporal cortex [95,96], and protein levels have also been shown to be reduced in the hippocampus and the parietal cortex [97].

Recently, it has been demonstrated that BDNF exists as both pro-BDNF and mature BDNF in human brain and, by Western blotting, a 40% reduction was seen in pro-BDNF levels in Alzheimer's disease parietal cortex compared with controls [98]. BDNF is synthesized in basal forebrain as well as cortex and a reported reduction of 50% was found, by the same group, in BDNF mRNA levels in the nbM of Alzheimer's disease patients [99]. TrkB and TrkC mRNA have been reported to be unchanged in Alzheimer's disease parietal cortex, whereas TrkA was reduced to less than half the normal value [100]; similarly, TrkB immunostaining in the Alzheimer's disease parietal cortex showed a large reduction in TrkA, not TrkB, expression [101]. This may be isoform-specific, since Western blotting has shown a selective reduction in TrkB catalytic form (145 kDa), but not the non-catalytic form, in both frontal and temporal cortex [102]. In addition, as detailed above, one study in the nbM has shown a 47% reduction in the number of neurons immunoreactive for TrkB in the brain of Alzheimer's disease patients [64].

These studies indicate, perhaps, that there is a problem with BDNF and/or the catalytic form of TrkB, but

whether this is the result of generalized transport insufficiency remains to be seen. Whether or not these changes reflect neuronal cell loss or implicate BDNF/TrkB in the disease process is unclear. It is clear that the neurotrophins have a role to play in both the aetiology and the therapeutic aspects of Alzheimer's disease. There are still specifics to be understood in terms of catalytic and non-catalytic forms of the Trk receptors, the pro- and mature forms of the neurotrophins and the role of $p75^{NTR}$ in amyloid and pro-neurotrophin binding. However, the finding that NGF administration to Alzheimer's disease patients improves glucose utilization and cognitive scores is very encouraging and, at the very least, provides a rationale for the use of NGF mimetics in this disease.

BDNF AND DEPRESSION

Depression is a psychiatric disorder of major social consequence. Changes in levels of the neurotrophins have been linked with depression. One of the contributory factors known to be involved with precipitation of onset of depression is stress. There are a number of studies showing a link between NGF levels and anxiety. However, reproducibility of findings has been a problem and thus there is no clarity yet as to the putative role of NGF. Because of the link between BDNF and synaptic plasticity and also the connection with serotonergic function, this review briefly outlines some of the data linking BDNF and depression.

It has been suggested that mood disorders may involve neuronal loss particularly in the cortex and hippocampus [103,104]. Chronic stress has been shown to cause atrophy and cell death in the CA3 hippocampal neurons and reduction of hippocampal volume was seen in brain imaging studies in cases of depression or post-traumatic stress disorder [104,105]. Stress-induced BDNF mRNA reduction has been found in the hippocampus [106]. Lower serum BDNF protein levels were reported in patients with depressive disorders [107], whereas increased serum BDNF concentrations have been observed after treatment with an antidepressant [108], and increased BDNF immunoreactivity was reported in the hippocampus of patients taking antidepressants [109]. There is some evidence to show a correlation between BDNF levels in serum and cerebral cortex [110].

BDNF mRNA levels in the hippocampus are increased significantly after antidepressant medication and electroconvulsive treatment [111–113] and also with physical exercise [113,114]. It has been suggested that antidepressants and electroconvulsive therapy may result in increased neurotrophin expression and thus neuroprotection [113,115]. This agrees with others who show increased expression of BDNF and TrkB mRNA [112] and increased mRNA for NGF [116] in the limbic regions. It has been reported that this treatment causes increased neurogenesis in the hippocampus [103], which would be consistent with the effect of increased BDNF expression. Furthermore, there is some evidence that chronic antidepressant treatment also increases neurogenesis in the hippocampus and that this is accompanied by increased levels of BDNF [103]. In accordance with this, increased BDNF immunoreactivity was found at the time of death in the hippocampus of subjects treated with antidepressants [109].

Finally, we know that changes in brain serotonin levels are associated with depression and drugs which increase serotonin levels are commonly used as treatment. It is interesting, therefore, to consider that BDNF enables survival and is trophic for serotonergic neurons [117] and that serotonin stimulates the expression of BDNF. This interaction between serotonin and BDNF signalling may play a crucial role in depression and anxiety disorders [118].

In summary, BDNF levels are reduced in depression and both antidepressants and electroconvulsive therapy increase BDNF levels and are associated with remission of symptoms. Thus BDNF is clearly closely connected with the symptoms of depression and perhaps therapy.

NGF, BDNF AND INFLAMMATORY PAIN

Nociceptive fibres

Pain involves an unpleasant peripheral experience recognized centrally and, by definition, involves both a peripheral and central component. A description of the involvement of NGF in nociceptive response is given below, along with some of the known contributory effects of BDNF on this pathway.

Primary nociceptive afferent fibres are generally classified as C, Aδ or Aβ, characterized by size, structure and electrophysiological responses. These are a heterogenous groups of fibres, subgroups of which have different thresholds for response to noxious stimuli. C fibres are unmyelinated and slow conducting (comprise approx. 70% of cutaneous afferents) and Aδ are intermediate in diameter, with minimal myelination and of intermediate conduction velocity (approx. 10% of fibres), whereas Aβ fibres are large, myelinated and have fast conduction (the remaining 20% of fibres). Aδ fibres immediately respond to noxious stimuli, such as heat, by inducing a sharp pain; subsequently, C fibres evoke a dull pain. Small-to-medium-sized neurons of the DRG (dorsal root ganglion) give rise to C and Aδ fibres and release of neurotransmitters, such as glutamate, and neuropeptides, including CGRP (calcitonin gene-related peptide) and substance P.

NGF and the hyperalgesic response in man

Numerous studies suggest that the neurotrophins are a central player in a number of inflammatory pain states. Patients with mutations in the *TrkA* gene demonstrate

the relevance of correct NGF signalling pathways in nociception. CIPA (congenital insensitivity to pain with anhidrosis) is a rare autosomal-recessive disorder and is caused by a mutation in the *TrkA* gene [119]. It is a hereditary sensory and autonomic neuropathy (HSAN IV) and is characterized by insensitivity to pain. There is accompanying self-mutilating behaviour, anhidrosis and hyperpyrexia. The clinical phenotype is characterized by involvement of the nervous system, bones with frequent fracture and slow healing and immunological abnormalities. Mutations found are likely to be partial loss-of-function mutations [120], and it is likely that recombinant NGF could restore nociception in some patients. In addition, the *NGF* gene has recently been identified as the gene responsible for loss of deep pain perception in a family in Sweden [121]. A severe reduction in unmyelinated nerve fibres and a moderate loss of thin myelinated nerve fibres were observed in these patients. A mutation in the coding region of the *NGF* gene was identified (a conserved Arg^{100} in the mature protein), which affected peripheral pain pathways, but not mental abilities. This gives rise to a less severe phenotype than that seen with *TrkA* gene mutations and it is likely that it affects the binding to $p75^{NTR}$ rather than TrkA. This is consistent with the observation that $p75^{NTR}$-null mice have a less severe phenotype than TrkA or NGF knockouts, which show loss of sympathetic neurons. By contrast, cell loss in $p75^{NTR}$-null mice is restricted to the sensory nervous system, with no loss of cholinergic basal forebrain cells.

In many common inflammatory disease states elevation of the neurotrophins is observed and an ensuing hyperalgesic response. Recent findings suggest that they may be involved in neuroplasticity of the lower urinary tract pathways after cystitis [122–124]. Elevated levels of neurotrophins have been detected in the urine [123] and bladder [124] of women with interstitial cystitis. Recent studies with a chemically [CYP (cyclophosphamide)]-induced bladder inflammation model [122] have demonstrated alterations in neurochemical and electrophysiological properties of micturition reflexes, suggesting considerable reorganization of reflex connections with CYP-treatment. It has been suggested that these changes may be mediated by neurotrophins produced in the bladder with cystitis [122] and that inflammation-induced changes in neurotrophins in the bladder may sensitize afferent and postganglionic nerves, resulting in bladder overactivity.

Mechanisms of inflammatory pain

In experimental conditions involving inflammatory pain, for instance following injections of carrageenan or complete Freund's adjuvant, there is a subsequent rise in endogenous levels of NGF. Adult animals subsequently develop both mechanical and heat hyperalgesia, an increased sensitivity to noxious stimuli or allodynia (painful response to non-noxious stimuli). In addition, mice overexpressing NGF in skin display hyperalgesia to noxious mechanical stimulation, whereas those expressing NGF antisense display hypoalgesia to the same stimuli [125].

Mechanical hyperalgesia may be largely due to central changes, whereas heat hyperalgesia is due to increased sensitization of sensory afferents, i.e. reduced thermal threshold. The mechanisms for this are becoming clearer. In inflammation there is an NGF-dependent enhanced production of a variety of nociception-related molecules, including substance P, CGRP, certain sodium channels, vanilloid receptors [including VR-1 (vanilloid receptor-1; the capsaicin receptor) or TRPV1 (transient receptor potential vanilloid 1)] [126] and also the purinergic receptor P2X3 in DRG neurons [127]. With increased NGF levels, VR-1 activity is increased in peripheral C fibre terminals; this is mediated via TrkA receptors, which results in the recruitment of PLC-γ. As a consequence, this leads to hydrolysis of PIP_2 (phosphatidylinositol-4,5-bisphosphate), a molecule which constitutively inhibits recruitment of the VR-1 receptor. In addition, exogenously applied NGF also results in the rapid sensitization of Nav1.8, a tetrodotoxin-resistant voltage-gated sodium channel expressed only on nociceptive sensory neurons. This contributes to the sensitization of primary sensory neurons after injury [128] and is associated with inflammatory, rather than neuropathic, pain [128]. Studies in Nav 1.8 knockout mice show that it is an essential mediator of NGF-induced thermal hyperalgesia [129]. In addition, p38 MAPK is activated in the soma of C fibre neurons; this probably contributes to the increase in VR-1 activity and maintenance of hypersensitity [130].

NGF sequestration

Deletion of NGF [131], reduction of synthesis [125] or TrkA knockout [75] all result in hypoalgesia to noxious and chemical stimuli. Increases in endogenous levels of NGF may be neutralized by the use of antibodies able to sequester NGF or by the use of a TrkA–IgG fusion molecule comprising two complete extracellular domains of TrkA linked by a heavy chain and hinge region of an antibody [132,133]. Use of this fusion molecule at the site of innervation of the cutaneous saphenous nerve in adult rats resulted in a 25% reduction in nociceptors responding to heat [132,133]. During inflammation there was reported a marked increase (up to 50%) in the proportion of active nociceptors and nociceptors responding to bradykinin [133]. After NGF sequestration, the number of nociceptors responding to application of bradykinin was reduced considerably. There was also a 44% reduction in the innervation density of the skin assessed by quantitative immunocytochemical analysis. Thus endogenous NGF in the adult modulates the terminal arborization of unmyelinated fibres and the

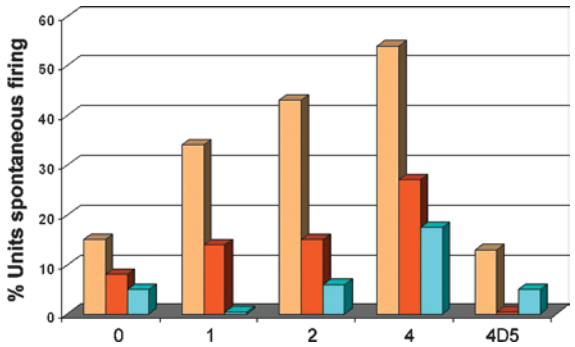

Figure 6 Spontaneous firing of C (beige), Aδ (orange) and Aα/β (turquoise) fibres in the DRG of the guinea pig at 0, 1, 2 and 4 days after administration of complete Freund's adjuvant

Spontaneous firing increased in C and Aδ fibres, but not Aα/β fibres due, in part, to increased levels of endogenous NGF. Administration of 450 ng of TrkAD5 once a day into paw and hindlimb abolished the increase in C and Aδ fibres after 4 days (4D5).

sensitivity of primary afferent nociceptors to thermal and chemical stimuli *in vivo* [132].

An alternative to the use of the TrkA–IgG fusion molecule has been to use recombinant TrkAD5, the TrkA-binding domain of NGF [8]. This domain has been shown to be able to sequester NGF and thereby block its effects [8]. In a study in which guinea pigs were injected into the hind paw with complete Freund's adjuvant, inflammatory response was accompanied by increased spontaneous firing of Aδ and C nociceptive fibres (Figure 6). This is caused by increased levels of endogenous NGF resulting in recruitment of VR-1 and Nav1.8 ion channels that facilitate increased firing of nociceptive neurons. This increase was completely abolished by the administration of TrkAD5 per day [134]. This sequesters endogenous NGF and prevents the hyperalgesic response. The D5 domain has now been shown to suggest the clinical potential of blockade of the action of NGF in a series of models of inflammation, including pancreatitis [135], acute interstitial cystitis [136] and chronic interstitial cystitis, where administration of TrkAD5 recombinant protein has shown a reversal of bladder overactivity and accompanying behavioural changes [137].

A role for BDNF as a modulator of pain

It has been shown that BDNF may be an important neuromodulator in the dorsal horn of the spinal cord [138]. Nociceptive information is relayed there from primary sensory afferents to local and projection neurons. A portion (20 %) of these sensory neurons express BDNF localized to axons and terminals; these are mainly small-to-medium sized neurons and also contain CGRP [139]. In models of inflammatory pain, such as intraplantar injection of Freund's adjuvant, expression of BDNF mRNA and protein is increased markedly [140]. This response is largely attributable to the primary increase in NGF [140,141]. Exogenous application of NGF results in nearly half of all sensory neurons producing BDNF [141], that is those with TrkA receptors. After nerve damage, BDNF is reduced in those injured small/medium-sized DRG neurons and increased in uninjured small neurons [142,143]. If the injury becomes chronic, all these neurons show an increase in BDNF immunoreactivity [144].

BDNF release during pain modulation is mediated by NMDA-receptor activation and is dependent on the pattern of stimulation [145]. Infusion of capsaicin, or short bursts of high-frequency stimulation, induces a dose-dependent release of BDNF in the isolated rat dorsal horn [145], along with substance P and glutamate. This release may be inhibited by an NMDA antagonist. The increased central excitability which results is decreased by a TrkB immuno-adhesion fusion molecule able to sequester BDNF; thus BDNF is sufficient and necessary for the expression of central sensitization in the spinal cord [128]. In addition, TrkB has been shown to be up-regulated in some models of pain [128,146].

In summary, inflammatory pain is initiated by increased expression of NGF at the site of injury. This leads to recruitment of ion channels, neuropeptides and other pain-associated receptors, which allow increased firing. This is followed by increased levels of BDNF in the dorsal horn of the spinal cord leading to an increased perception of pain. Since sequestration of NGF and BDNF lead to a reduction in sensitivity to pain, it seems entirely likely that future clinical management of pain could involve mechanisms related to NGF and BDNF.

NGF AND ASTHMA

Asthma is a chronic inflammatory disorder of the airways characterized by recurrent airway obstruction, chronic inflammation of the lungs and hyper-reactivity. This hyper-reactivity is the abnormal response to 'harmless' antigens abundant in the atmosphere. More than one in ten children suffer from allergic asthma and reports indicate that the numbers of people affected are ever increasing.

The mechanism by which sensitization to allergens occurs in vulnerable individuals after repeated exposure to an inhaled allergen has been characterized. Some of the elements involved are described briefly. Initial presentation of allergens results in the differentiation of Th0 cells into Th2 cells. These are able to secrete pro-inflammatory cytokines, IL (interleukin)-4, -5, -10 and -13, which assist in the induction of B-cells to produce IgE. Subsequent re-exposure to the antigen at a later time then results in a biphasic airway obstruction. The early phase occurs in minutes involving oedema and

increased smooth muscle tone. These are related to mast cell activation and degranulation by IgE. Histamine, cytokines, growth factors, including NGF, and toxins are then released. The late phase response occurs hours after exposure. Airways are narrowed and a large number of inflammatory-associated cells infiltrate the lung tissue from the blood. These include lymphocytes, eosinophils and neutrophils. Many of these cells interact to produce a number of pro-inflammatory proteins, resulting in tissue destruction and prolonged inflammation.

Patients suffering from allergic diseases, in particular asthma, show increased levels of NGF in their serum [147]. Possible sources of NGF include a variety of cells in the lung: macrophages, T-cells, mast cells, fibroblasts and epithelial cells, which have all been identified as producing NGF. In biopsies of asthma patients, NGF immunoreactivity was seen in epithelium, fibroblasts, blood vessels and infiltrating cells. Challenge with allergen resulted in an up-regulation of mRNA for NGF [148]. NGF detected in bronchoalveolar lavage from asthmatics [149] was also enhanced further by allergen challenge [150]. In ovalbumin-sensitized and challenged mice, increases were seen in NGF levels in bronchoalveolar lavage fluid and bronchial biopsies [150]. This comprised both an allergen-dependent and -independent release of NGF.

TrkA has been reported to be expressed in several immune cell types, including basophils, mast cells, monocytes and B- and T-cells. In the human lung, TrkA immunoreactivity has been detected in alveolar cells and mucous glands [151] and mast cells from bronchial biopsies [148]. In addition, TrkA mRNA has been detected in human lung mast cells [152].

Data suggest that NGF is an important modulator of sensory neurons of the airways. Transgenic mice overexpressing NGF show marked hyperinnervation of the airways by sensory and sympathetic fibres expressing tachykinins [153], and an increase in substance P immunoreactivity has been seen in the lung tissue. This is consistent with the known NGF-mediated increase in substance P and CGRP in sensory neurons. The mice also exhibited enhanced airway resistance to inhaled capsaicin. Capsaicin is an exogenous agonist of the VR-1 ion channel. Endogenous ligands of this receptor include anandamide (*N*-arachidonoyl-ethanolamine) [154], which is also a cannabinoid receptor ligand, and lipoxygenase products of arachidonic acid, which are mediators of allergic asthma as well as noxious heat [154]. There has also been reported an NGF-mediated increase in the proportion of substance P-positive neurons in the guinea pig trachea [155].

Furthermore, NGF acts as a modulator of many immune cells. It is known to promote survival, proliferation and differentiation of lymphocytes and mast cells, stimulate mast cell degranulation, enhance survival of eosinophils, monocytes and neutrophils and induce release of mediators from T-cells, macrophages, basophils and eosinophils [156–158].

In summary, the neurotrophins have a role in both the early and late phases of this inflammatory response. They are able to influence allergic inflammation by promoting survival, activation and influx of certain inflammatory cells into the lung [159] or by enhancing synthesis of cytokines [150] and pro-inflammatory neuropeptides, such as substance P and CGRP, in sensory neurons [160]. NGF or BDNF application induces airway hyperreactivity by means of neurogenic control of airway smooth muscles [161,162]. In the long-term, such mediators of allergic inflammation can induce neuronal dysfunction which is independent of inflammation, and it is likely that the neurotrophins play a major role in this neuronal plasticity in allergic asthma [159,163]. Finally, from a therapeutic interest it has been shown recently that use of TrkAD5 in a model of asthma is able to normalize the neurogenic response to capsaicin [164].

SUMMARY AND FUTURE WORK

The neurotrophins and their receptors are implicated in many diverse physiological processes, particularly during development. This review presents a diverse, but by no means comprehensive, selection of roles of NGF and BDNF in adult function and dysfunction.

The importance of neurotrophins in physiological functions also suggests that they may have a role in the pathophysiology of certain disease states, perhaps due to a change in their level or in the function of the neurotrophin receptors. In some of these disease states there may be a case for therapeutic intervention either by inhibiting or mimicking the actions of the neurotrophins.

In the near future it is likely that small-molecule neurotrophin antagonists may be useful for the control of inflammatory pain and perhaps also asthma. Increases in endogenous levels of NGF have been shown to result in increased firing of sensory neurons. A TrkA antagonist (unable to cross the blood–brain barrier) will be of immense value as a therapeutic for inflammatory pain states, such as interstitial cystitis, pancreatitis, arthritis and so on. In these conditions the increased NGF levels result in increased BDNF release within the spinal cord, thus leading to an enhanced central perception of pain. Similarly BDNF seems to play a modulatory role supplementing the inflammatory and neurogenic actions of NGF in asthma.

In the CNS, neurotrophin proteins have already been considered as potential therapeutics in a range of disease states. This includes Alzheimer's disease, ALS (amyotrophic lateral sclerosis), Huntington's disease and peripheral neuropathy and, in the future, maybe even depression. BDNF intrathecal infusion and subcutaneous administration has shown some promise in early trials of

ALS, which were not replicated in a larger trial. Similarly early trials with subcutaneous administration of NGF in HIV-associated sensory neuropathy showed promise, which unfortunately did not translate into success in Phase III trials. Thus, to date, neurotrophins have not lived up to their therapeutic potential, probably due to their pharmacokinetic profiles, although these trials have shown us that even large quantities of neurotrophins administered over a period of time do not have significant side-effects. Neurotrophins used as a therapy for neurodegeneration has the added problem that in order for these proteins to reach the brain they must be administered directly, as they will not be able to cross the blood–brain barrier. However, the work of Tuszynski and coworkers [94] has shown that implantation of autologous fibroblasts modified to produce NGF is able to restore function in cases of early Alzheimer's disease. Although this may not be feasible as a treatment for large numbers of patients, it has provided a proof of principal and suggests that small-molecule agonists of the TrkA receptor may be a useful strategy in the future to prevent degeneration of cholinergic basal forebrain neurons and thereby slow the observed decline in cognitive function.

Finally, the design of neurotrophin agonists and antagonists will be dependent on understanding the interactions between these trophic proteins and their receptors. Thus the X-ray crystal structures of the neurotrophins bound to their receptors will be useful starting points for the design and development of small-molecule pharmaceuticals.

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