Alzheimer's disease (AD) is the most common cause of dementia in elderly people and constitutes the fourth most common cause of death in Western society (Katzman, 1976). Epidemiological studies have shown that approximately 9% of the general population aged 65 and more than 34% of those aged 85 and older have dementia. Of all these cases, 72% have (probable) AD (Ott et al., 1995). Currently there are more than four million AD patients in the United States, at an annual cost of $100 billion. It is estimated there will be 7.5–14.3 million AD patients by year 2050 (Larson et al., 1992), at an annual cost of $300–350 billion (see Coleman, 1994). Because of the increasing number of patients suffering from AD, there is an urgent need for therapeutic strategies to at least postpone the occurrence of this devastating neurodegenerative disorder.

One of the potential therapeutic strategies for AD is the use of neurotrophins. It has been proposed that the degenerative changes in neurodegenerative disorders including AD are the result of lack of trophic support (Appel, 1981). AD is characterized by the presence of a large number of plaques, neurofibrillary tangles and diminished neuronal activity (see Chapter 26 by Swaab et al. for more details). The rationale for the involvement of neurotrophins in the neurodegenerative process of AD is based on the following observations: i) normally abundant expression of nerve growth factor (NGF) mRNA is found in the cerebral cortex (Korsching et al., 1985; Shelton et al., 1986) and hippocampus (Whittemore et al., 1986); ii) the highest levels of low affinity neurotrophin receptors are present in the basal forebrain area (Goedert et al., 1989; Hefti and Mash, 1989; Higgins et al., 1989; Ernfors et al., 1990); iii) there is a considerable degree of colocalization between choline acetyltransferase (ChAT) and neurotrophin receptors in the basal forebrain of the rat (Dawbarn et al., 1988) and human (Mufson et al., 1991); iv) the ability of intraventricular-administrated NGF to increases ChAT activity in the basal forebrain of neonatal rats (Gnahn et al., 1983; Mobley et al., 1986; Johast et al., 1987); v) protective effects of NGF on fiber sprouting and improved transmitter-dependent functions of basal forebrain cholinergic neurons in adult rats following lesions (Hefti, 1986; Williams et al., 1986), vi) the ability of basal forebrain neurons to retrogradely transport NGF (Seiler et al., 1984), and vii) the ability of exogenous NGF to reverse cholinergic atrophy and memory impairment in old rats (Fischer et al., 1987). In support of the relationship between neurotrophins and the physiological function of basal forebrain neurons, it has been shown that postnatal development of the cholinergic projection is reflected in the approximately ten-fold increase in ChAT activity in the hippocampus.

*Corresponding author. Tel.: 031-20-5665503; fax: 031-20-6961006; e-mail: A.Salehi@nih.knaw.nl
Parallel to the increase in hippocampal ChAT activity, there is an earlier increase in hippocampal NGF and its mRNA levels in the basal forebrain which is most likely retrogradely transported from the hippocampus (Large et al., 1986; Whittemore et al., 1986).

Based on all these observations and because of the fact that the degeneration of cholinergic basal forebrain neurons is one of the earliest and most characteristic processes occurring in the brain of AD patients, intracerebral administration of NGF was proposed and carried out as an experimental treatment for AD (Seiger et al., 1993). In this study a total of 6.6 mg of NGF was delivered into the ventricular system during three months at a rate of 15 μg/h. Based on the obtained data, only the verbal episodic memory showed an improvement which lasted no longer than two and a half months after cessation of the NGF-therapy unlike the other cognitive tests, e.g. delayed face recognition, semantic memory and short-term memory which did not improve at all.

If NGF expression was be related to the process of degeneration in AD, one would expect lower levels of NGF at the production sites of this neurotrophin in the brains of AD patients. However, measurement of the levels of NGF expression in AD did not reveal any decrease in the cerebral cortex (Goedert et al., 1989), cerebrospinal fluid or serum (Murase et al., 1993) of AD patients, while some studies even reported increased levels of NGF-like activity in the cortex of these patients (Crutcher et al., 1993). These observations led to the idea that in AD the failure of basal forebrain neurons to respond to neurotrophins; specially NGF, might not be due to a disorder at the level of the synthesis of neurotrophins, but to a disorder in the expression or functional properties of neurotrophin receptors (Hefti et al., 1986).

**Neurotrophin receptors**

Two types of receptors for neurotrophins have been identified: the low affinity neurotrophin receptors or p75 (Chao et al., 1986), a 75 KDa protein interacting with all neurotrophins, and the recently characterized tyrosine kinase receptors (trkA, B and C), a family of transmembrane proteins with a specific affinity for particular members of the neurotrophin gene family (Kaplan et al., 1991a, b; Klein et al., 1993). TrkA binds NGF and neurotrophin-3 (NT-3; Kaplan et al., 1991a, b), while trkB and trkC transduce biological responses of brain-derived neurotrophic factor (BDNF) and neurotrophin 4/5 (NT 4/5; Klein et al., 1991; Soppet et al., 1991; Squinto et al., 1991) and NT-3 (Lamballe et al., 1991; Klein et al., 1994) respectively. TrkB and trkC are also found as truncated proteins lacking the intracellular kinase domain, while trkA is not found in a truncated form. Consistent with the regional specificity of NGF, trkA mRNA is found in specific areas of the central and peripheral nervous system, while trkB and C are widely distributed throughout the brain (Merlio et al., 1992; Altar et al., 1994). The distribution of the neurotrophin receptors is consistent with the effects of the neurotrophins. Unlike BDNF and NT3, the spectrum of neurons influenced by NGF is quite restricted. In the peripheral nervous system it affects sympathetic and sensory neurons and in the central nervous system it mostly influences cholinergic neurons in the basal forebrain. In situ hybridization studies have revealed that the full-length forms of trkB and trkC are mainly found in neurons, but not in glial cells. However, the truncated forms of these receptors are widely expressed in non-neuronal cells (Frissen et al., 1993; Altar et al., 1994). The mechanism of action of neurotrophins is yet to be completely revealed. However, it has been shown that NGF binds to trkA through dimerization causing activation of its kinase resulting in autophosphorylation (Kaplan et al., 1991a, b; Jing et al., 1992; for a complete overview on the mechanism of action of NGF on trk receptors see Chapter 4 of this volume by Kaplan). An interesting consequence of NGF action is upregulation of trkA gene expression (Meakin et al., 1992). Li et al. (1995) showed that NGF administration in vivo activates trkA receptors and increases both trkA and ChAT mRNA.
Conversely, infusions of a NGF antibody suppress the expression of both genes.

NGF is the most potent neurotrophin in its effect on the number and size of basal forebrain cholinergic neurons (Kaliatsos et al., 1994). A widely observed neurochemical alteration in AD is the loss of ChAT activity in the cerebral cortex (Rosor et al., 1982), which is due to degeneration of cholinergic neurons in the basal forebrain (Whitehouse et al., 1981).

The nucleus basalis of Meynert in Alzheimer’s disease

Although neuropathological changes in the brains of AD patients are far more extensive and do not only entail the loss of cholinergic inputs (Bowen et al., 1983; Palmer et al., 1986; Joffe et al., 1987), cholinergic neurons are involved in cognition and memory processes (Collerton, 1986), and cholinergic degeneration could thus be responsible for at least some of the manifestations of the disorder. The cholinergic neurons of the basal forebrain are located in band-like structures comprising the septal nucleus, diagonal band (including vertical and horizontal) of Broca and the nucleus basalis of Meynert (NBM). The NBM, or the ch4 division of basal forebrain cholinergic system according to the Mesulam’s nomenclature (Mesulam et al., 1984), is one of the most prominent subcortical structures in the human basal forebrain. Together with the septal nuclei and the diagonal band of Broca, it is the major source of cholinergic innervation for the hippocampus, amygdala and cerebral cortex (Parent et al., 1981; Ribak et al., 1982; Hedreen et al., 1984; Mesulam et al., 1984). Bowen et al. (1976) reported a selective loss of ChAT activity in different parts of the AD brain. This was followed by the finding that neurons in the basal forebrain specially the NBM, are selectively degenerated in AD (Whitehouse et al., 1981; Nakano and Hiran, 1982; Nagai et al., 1983; Mann et al., 1984). In addition to ChAT, the levels of acetylcholine (Richter et al., 1980), high affinity choline uptake (Rylett et al., 1983) and acetylcholine synthesis (Sims et al., 1980) are reduced in AD. However, AD is not the only disorder in which NBM degeneration is found. There are other neurological disorders causing deterioration of memory and cognitive functions, e.g. Creutzfeldt-Jakob’s disease (Arendt et al., 84), Parkinson’s disease (Arendt et al., 1983; Whitehouse et al., 1983), Pick’s disease (Uhl et al., 1983), Korsakoff’s disease (Arendt et al., 1983; Perry, 1986) dementia with argyrophilic grains or atypical progressive supra nuclear palsy (Masliah et al., 1991) and progressive supranuclear palsy (Tagliavini et al., 1983), all of which display significant NBM degeneration.

The first reports claiming a severe neuronal loss in the NBM in AD (Whitehouse et al., 1981, 1982; Arendt et al., 1983; Tagliavini et al., 1983) were followed by a number of other publications in which the degree of cell loss in the NBM was reported to vary from a 75% cell loss (Etienne et al., 1986) to no neuronal loss at all (Pearson et al., 1983; Rinne et al., 1987). Estimates of the neuronal loss in the NBM during normal aging vary greatly, ranging from a loss of 23–70% of the neurons (McGeer et al., 1984; Lowes-Homml et al., 1989) to no neuronal loss at all (Chui et al., 1984). However, several studies have indicated that neuronal atrophy rather than cell loss is the main phenomenon in the NBM as well as the cortex of AD patients (Pearson et al., 1983; Rinne et al., 1987; Reguer et al., 1994). The concept of neuronal atrophy rather than cell death being the main hallmark of AD may, of course, have important consequences for therapeutic strategies, especially neurotrophin therapy.

Using the size of the Golgi apparatus (GA) as an indicator of neuronal activity (Salehi et al., 1994; 1995a, b, c), we measured the size of this organelle in NBM neurons of controls and AD patients. In AD we found a very clear reduction in the GA size (Salehi et al., 1994) which seems to be ApoE genotype dependent (Salehi et al., submitted). Based on this and other studies (Pearson et al., 1983; Rinne et al., 1987) it seems that the process of degeneration of NBM neurons in AD is associated with atrophy and decreased neuronal activity rather than cell death.
Neurotrophin receptors in AD

As indicated in one of the previous sections, there are two types of receptors for neurotrophins. P75, which is a receptor protein interacting with all neurotrophins, and trk receptors, which interact specifically with certain neurotrophins (see also Chapter 4 of this volume).

P75

P75, also designated the low affinity neurotrophin receptor, is a member of a superfamily of cell surface proteins, including the tumor necrosis factor receptor (Loetscher et al., 1990), CD40 and APO-I (Fas antigen) a lymphocyte antigen involved in apoptosis (Oehm et al., 1992). Although the physiological function of p75 is not completely clear, the possible roles of this receptor include (i) increasing either the affinity (Hempstead et al., 1991; Klein et al., 1991) or (ii) the specificity of the trk receptors to neurotrophins (Ip et al., 1993). Moreover, it has been shown that trkA-expressing cells become increasingly responsive to NGF when transfected with p75 (Hempstead et al., 1991; Verdi et al., 1994). In addition to trophic effects, p75 may in some circumstances play a role in cell death (Majdan et al., 1997). For instance, Van der Zee et al. (1996) showed that blocking of ligand binding to p75 may reduce normal developmental loss in the basal forebrain cholinergic neurons. In the line with this observation Yen et al. (1997) recently showed that p75 receptor knockout mice show an increase in basal forebrain cholinergic neuronal size and ChAT activity, as can also be observed following NGF infusion. However, it is not clear whether p75 can regulate the process of apoptosis in the adult aging brain.

The gene p75 is localized on chromosome 17 (17q12–q22; Buxser et al., 1983), which is close to the breakpoint found in acute leukemia and to the von Recklinghausen neurofibromatosis gene (Seizinger et al., 1987). There is a large body of evidence showing that p75 is clearly expressed in the human basal forebrain (Hefti et al., 1986; Allen et al., 1989; Goedert et al., 1989; Treanor et al., 1991).

However, the results regarding alterations in the expression of p75 receptors in AD are rather controversial (Goedert et al., 1986; Higgins et al., 1989; Ernfors et al., 1990). Based on Northern blot (Goedert et al., 1989) and receptor binding (Treanor et al., 1991), the expression of p75 in NBM neurons appears to be unaltered in AD. Kojima et al. (1992) showed that NGF is able to upregulate the expression of p75 receptors in cultured cholinergic neurons. It is interesting that in advanced aging, and also in AD, cortical neurons express p75 receptors. This seems to be specific for AD, since it was not observed in other neurodegenerative disorders like Parkinson or Pick’s disease (Mufson et al., 1992). One possible reason for the lack of decreased expression of p75 in the NBM may be that, although the level of NGF is reduced in the NBM of AD patients, p75 expression can be regulated by neurotrophins other than NGF, e.g. BDNF or NT-3. Brukes et al. (1994) showed that removal of neurotrophin-producing hippocampal target neurons during CNS development induces atrophy and neuronal loss in the basal forebrain. In addition to NGF, intraventricular administration of BDNF resulted in attenuation of the reduction in p75 expression.

Trk receptors

1) Nucleus basalis of Meynert

We used three polyclonal antibodies raised against the external domain of the human trkA, trkB and trkC receptors with peptides corresponding to the amino acid 76-96 of human trkA, and mouse trkB (anti-trkBout), and the amino acid 88-108 of rat trkC (anti-trkCout; the antibodies were a generous gift from D. Kaplan, Montreal Neurological Institute). The crossreactivity of the trk antibodies with other peptides was ruled out by using trk-overexpressing NIH-3T3 cells (Hoehner et al., 1995). Furthermore, this point was studied by our group using 911 cells transfected with trkA cDNA. Our experiment showed that antibodies against either trkB, or trkC are unable to stain human embryonic retinoblast cells expressing trkA (Salehi et al., 1996; Fig. 1).
Using immunocytochemical methods to stain trk receptors in post mortem human brain of non-demented controls the proportion of neurons found to be stained was found to be 75% for trkB followed by trkC (59%) and trkA (53%). In Alzheimer patients, we observed a very clear reduction in both the number of positive neurons and the staining intensity of individual cells in the NBM. The reduced immunoreactivity for trk receptors was not restricted to atrophied neurons (Fig. 2B), as a decrement in expression was also observed in a majority of the large remaining neurons in the NBM of AD patients. The extent of the reduction was not the same for all three high affinity neurotrophin receptors. Although the staining level was clearly reduced for trkB, the number of positive cells for this receptor was still quite significant in AD. Furthermore, there were many small neurons in the NBM of AD patients which were intensely trkB positive. There was a 71% \( (p = 0.0003) \) reduction in the number of neurons stained with trkA and a reduction by 47.3% \( (p = 0.002) \) of trkB and 50% \( (p = 0.0004) \) of trkC in AD cases (Fig. 3). The reduction in the expression of trkA has recently been confirmed by several laboratories using both immunocytochemistry and protein determination (Boissiere et al., 1997; Mufson et al., 1997).

Thus, in the NBM of AD patients expression of all three trks is dramatically reduced. TrkA is most clearly affected and becomes virtually undetectable in AD patients. TrkB and trkC are expressed in some neurons, although at a lower level, and many NBM neurons cease to express these receptors as well. In the NBM of AD patients three types of neurons have been observed. The first subset of neurons constitutes a major component of the NBM and is characterized by clear atrophy. The second group, comprising a small percentage of NBM neurons, consists of neurons that remain large and are apparently less atrophied in AD. Interestingly, the expression of trks is reduced in both groups of NBM neurons in AD. This is consistent with the observation that the protein synthetic ability of both small and large neurons is reduced in AD (Salehi et al., 1994). In addition to degeneration, signs of possible cellular activation were observed in a third group of galanin-positive NBM neurons (Chan-Palay, 1988). The intensely trkB positive small NBM neurons in AD, as observed here may correspond to this previously identified population of activated neurons.

In our study, the reduction of trkA was more severe than that of trkB. This might be due to differential degeneration of trkA-expressing neurons in AD. However, since neuronal atrophy rather than cell death seems to be the major phenomenon in Alzheimer NBM neurons (Rinne et al., 1987), the changes seen in trk staining are rather due to changes in the expression of these receptors than to cell death.

2) Hippocampus

Of the two family members of NGF, i.e. BDNF and NT-3, BDNF has a much wider distribution in the brain including the target areas of basal forebrain projections. BDNF is capable of exerting trophic actions on hippocampal neurons in vitro (Phillips et al., 1990). Moreover, it has been shown that BDNF enhances the strength of synaptic connectivity in hippocampal neurons (Levine et al., 1995). This led to the idea that BDNF may also promote the function and survival of hippocampal neuronal populations which are severely affected in AD (Wainer et al., 1989). Using monoclonal antibodies against trk receptors, Muragaki et al., (1995) reported a clear expression of trk receptors in the hippocampus of the human adult brain. Furthermore, using in situ hybridization, Altar et al. (1991) reported the expression of trkB mRNA in all hippocampal areas of the adult rat.

In order to test whether the reduction of trks observed in the NBM also occurs in the strongly affected hippocampus of AD patients we studied the expression of trkB receptors in the hippocampus of controls and AD patients. Using Western blot on protein extracts from human hippocampus, the trkB antibody was able to recognize two bands of about 95 and 145 KDA corresponding to the truncated and non-truncated forms of the trkB receptor (Fig. 4). Our study showed a considerable
Fig. 1. Immunocytochemical staining of transfected culture of cells with trkA expression vector as a specificity test. TrkA (A), trkB (B) and trkC (C) antibodies were used. Note the clear staining of these cells with trkA and the absence of staining with the trkB and C antibodies. Arrowheads indicate three heavily stained cells with a trkA antibody (from Salehi et al., 1996, with permission from Elsevier Science).
number of cells, including neurons and glia, stained by the anti-trkB antibody in a variety of hippocampal areas. In several hippocampal areas, especially CA1, there was co-localization between trkB staining and neurofibrillary tangles and plaques. Microscopically, there were no clear differences between the number of neurons stained in controls compared with those of AD patients. In both controls and AD patients, CA2-3 and CA4 areas showed the highest (>70%) and CA1 and subiculum the lowest percentage (<50%) of trkB positive neurons. No significant difference ($p > 0.05$) was found in the percentage of trkB positive neurons in any of the hippocampal areas measured between controls and AD patients (Fig. 5).

We have shown that the expression of trkB is significantly reduced in the NBM neurons in AD. However, a decline in the expression of trkB does not occur in the severely affected hippocampus of AD patients. The reason why trkB is significantly diminished in the NBM and not in the hippocampus might be due to the fact that NBM neurons are dependent on the retrograde transport of neurotrophins to their cell bodies via their projections to the cortex. Anyhow, our data suggest that the reduction in the expression of trk receptors is
brain region specific. In addition, the lack of any change in the expression of trkB in the hippocampus in AD indicates that trkB does not play a major role in the pathogenesis of AD. This is in accordance with our observation that trkB receptors are the least affected ones in the NBM of AD patients (Fig. 3).

Conner et al. (1996) reported an increased immunoreactivity of trkB receptors in the hippocampal areas of AD brains. However, counting the number of trkB receptor containing neurons in the hippocampus of AD patients we did not find any significant change. Conner’s data are thus most probably due to the expression of trkB in glia and the immunoreactivity of a large number of plaques and tangles with an anti-trkB antibody and not to an increase in the number of neurons expressing trkB.

**Diminished expression of trk receptors or failure in axonal transport?**

The question whether failure in neurotrophin functions plays a role in the pathogenesis of AD has yet to be answered. However, based on a large number of animal studies, increased supply of neurotrophins to cholinergic neurons could be beneficial for AD patients. Concerning the issue of
Hippocampal area

Fig. 5. Graph depicting the proportion of neurons stained by the trkB antibody in controls and Alzheimer patients. Note the lack of any significant reduction in the proportion of trkB-expressing neurons in Alzheimer’s disease (A. Salehi, unpublished observations).

The role of neurotrophins in the pathogenesis of AD one can defend the thesis that whatever the underlying cause of the reduced expression of trk receptors in NBM neurons may be (either a reduction of trk expression due to diminished transcription of the trk genes or failure in axonal transport), an increased expression of neurotrophins and/or neurotrophin receptors might be beneficial.

Fig. 6. Immunocytochemical staining of Alz-50 in the NBM of an AD patient. Note the staining of cell bodies and fibers indicating clear cytoskeletal alterations (A. Salehi, unpublished observations).
a) Decreased axonal transport

Recent studies in rat sciatic nerve indicate that p75 as well as trkA are retrograde carrier molecules for neurotrophins (Curtis et al., 1995; Ehlers et al., 1995). Retrograde transport of NGF is mediated by trkA (Ehlers et al., 1995) and appears to be independent of p75 (Curtis et al., 1995). The involvement of trkB and trkC in retrograde signaling has not yet been fully resolved, but it is not inconceivable that these molecules also act as retrograde transporters. The abundant expression of trkB in nerve fibers in the NBM (Salehi et al., 1996) suggests that trkB is actively transported along axons. Recent in vitro studies indeed revealed diminished transport of NGF in human basal forebrain neurons in AD (Mufson et al., 1995; Scott et al., 1995). Whether this is due to a decrement of trkA in NBM neurons in AD remains, however, to be determined. Diminished expression of trkA in NBM neurons could result in reduced retrograde transport of NGF from the cortex to the NBM. It is therefore not unreasonable to postulate that diminished trkA expression may directly contribute to the neuronal atrophy and cholinergic dysfunction which are characteristic of AD. In this respect it is important to underscore that in the NBM of AD patients all three trks are reduced simultaneously. This would presumably result in a much more profound effect on neuronal morphology and function than the relatively minor effects observed in the knock-out mice with deletions of single trk gene.

One of most important neuropathological hallmarks of AD are NFTs, which also affect NBM neurons. The density of NFTs correlates with the severity of the disease (Arriagada et al., 1992). NFTs are composed of paired helical filaments (PHFs), whose main component is hyperphosphorylated tau, a microtubule-associated protein (MAP) involved in microtubule formation and stabilization (Lee and Trojanowski, 1992; Lee, 1995). Earlier studies have shown that the NBM is heavily stained by the antibody Alz-50, which reacts with abnormally phosphorylated tau and is therefore regarded as an indicator of early cytoskeletal alterations. In addition to Alz-50, NBM neurons are intensely stained by the 60e (against NFTs), tau-1 (against tau) and 3-39 antibodies (against ubiquitin). However, Alz-50 shows the most intense staining of neurons and neuropil threads in the NBM of AD patients (Swaab et al., 1992; Fig. 6). In physiology tau proteins play a major role in the maintenance of neuronal morphology and in axonal transport. Abnormal tau phosphorylation could result in a dysfunction of axonal transport. Thus, the primary event underlying the neuronal atrophy in the NBM could be a change in axonal transport due to cytoskeletal changes, followed by diminished trafficking of the neurotrophin/trk complex. Diminished trophic support would subsequently lead to decreased protein synthesis and down-regulation of trk gene expression. Based on this concept either cells expressing NGF should be implanted close to the NBM neurons or a method has to be developed to increase or facilitate the retrograde transport of neurotrophins in order to improve the effect of neurotrophin therapy (NGF administration).

b) Primary decrease in the expression of trk receptors in NBM neurons

There are also several studies that suggest that the decrease in trk expression may be a primary event in the process of degeneration of NBM neurons. Nearly 30 years ago (Olson et al., 1969) it was shown that most patients with Down syndrome who die after the age of 30 develop a neuropathology undistinguishable from AD and that most of them will be affected by AD by the age of 45 (Wisniewski et al., 1985). Holtzman et al. (1993) used an animal model of Down syndrome, the trisomy 16 mouse, to test the effect of neurotrophins on basal forebrain cholinergic neurons. Mouse chromosome 16 contains a cluster of genes including the amyloid precursor protein, one of the glutamate receptor genes and superoxide dismutase (SOD1). Cholinergic neurons derived from these animals show a very clear age-related atrophy. NGF is able to reverse trisomy 16-induced atrophy of basal forebrain cholinergic
neurons and stimulates hypertrophy of these neurons. Interestingly, the level of NGF in the hippocampus of those animals which received a transplant (basal forebrain cell suspensions) from trisomy 16-mice was not different from that of controls. Based on these observations the authors concluded that the trisomy 16 atrophy is due to an abnormality intrinsic to these neurons. The same study showed that NGF is able to cause a 1.8-fold increase in trkA mRNA in the transplanted basal forebrain cells. Interestingly, the atrophy occurred in an environment in which there was no deficit of NGF. This study suggests that a factor other than a decreased level of NGF is responsible for neuronal atrophy. Since increased expression of trk is associated with reversal of atrophy, reduced trk might be a good candidate for such a function.

Using autoradiography, Strada et al. (1992) reported a significant reduction in the density of NGF binding sites in AD. The same study showed a decrease in AChE staining in the NBM of AD patients, in parallel with though to a much lesser degree, the decrease in the density of NGF binding sites. This difference was even bigger in the striatum. Based on these observation the decrease in NGF binding sites in the NBM seems to precede the degeneration observed in the NBM.

Using the size of the GA as an indicator of metabolic activity of neurons, we found a very clear reduction in the size of the GA in the NBM of AD patients (Salehi et al., 1994). However, the reduction in the GA size was much less severe than the decreased expression of trk receptors in the NBM, which suggests that decreased expression of trk receptors may be a primary factor in the change in metabolic activity of NBM neurons in AD. In a recent study measuring the area covered by Alz-50 staining, we found a clear sex difference in the severity of early cytoskeletal alterations in the NBM of AD patients (Salehi et al., submitted). Female AD patients showed much more severe cytoskeletal alterations than males. This is in agreement with new epidemiological data showing the higher prevalence of AD in women compared with men (Brayne et al., 1995; Fratiglioni et al., 1997). Performing a longitudinal study, Fratiglioni et al. (1997) also reported a clearly increased risk of AD for women compared with men. Furthermore, Mcmillan et al. (1996) found that ovariectomy in adult female Sprague-Dawley rats caused a significant reduction in ChAT and trkA receptor expression, which were both reversed by short-term estrogen therapy. This is in accordance with previous data showing the colocalization of low-affinity neurotrophin receptors with estrogen receptors in the basal forebrain of rats (Torrance-Allerand, 1996). There is a large body of data indicating the beneficial effect of estrogen therapy in female Alzheimer patients (Paganini-Hill and Anderson, 1994; Paganini-Hill, 1996; Torran-Alle ran, 1996). Improvements in attention, memory, calculation, orientation and social interaction following administration of estrogens were reported (Honjo et al., 1989). Based on these observation the lack of activation of trk-containing neurons by estrogens occurring in post-menopausal women may lead to a much more severe pretangle formation, which is the first step toward the development of one of the main neuropathological hallmarks of AD, i.e. neurofibrillary tangles.

**Colocalization of trk receptors in the basal forebrain and its possible significance**

Information on the distribution of neurotrophin receptors in the human brain has so far primarily been available for p75. Recently, trkA expression was shown to occur in the adult human brain, primarily in the basal forebrain, and in scattered neurons in the putamen and peripheral nervous system (Shelton et al., 1995). A truncated form of trkB was shown to be expressed in the human cerebellum and hippocampus (Allen et al., 1994), and trkC is expressed in the human basal forebrain, hippocampus and neocortex (Shelton et al., 1995).

TrkA is restricted to the NBM and not expressed in the adjacent hypothalamic nuclei. In contrast, trkB and C are expressed in NBM neurons and hypothalamic nuclei, including the supraoptic nucleus and tuberomammillary nucleus (Salehi et al.,
1996). This is consistent with and extends previous observations on the anatomical distribution of these receptors in the rat (Altar et al., 1994). In particular the expression of trkB and C in the human supraoptic nucleus is in agreement with the expression of trkB and trkC in the rat supraoptic neurons (Altar et al., 1994; Sobreviela et al., 1994).

The clear cytoplasmic expression of trkB and C observed in our study is consistent with the cellular localization in rat (Sobreviela et al., 1994) and human NBM neurons (Boissiere et al., 1994; Muragaki et al., 1995). Trk receptors are clearly colocalized in the cytoplasm of individual NBM neurons. Co-expression of more than one trk receptor in NBM neurons implies that these neurons can be supported by two neurotrophins at the same time. In the rat, NGF and BDNF are both able to prevent the degeneration of basal forebrain neurons following fimbria fornix transection, which suggests that these neurons can indeed respond to both neurotrophins (Hefti, 1986; Morse et al., 1993). In addition, NGF and BDNF exert a similar physiological response in the embryonic septal cholinergic neurons (Alderson et al., 1990). Based on overlap of mRNA expression patterns for different trks, co-expression of trk receptors probably occurs also in rat peripheral sensory neurons, hippocampal neurons and basal forebrain neurons (Merlio et al., 1992; McMahon et al., 1994). At the same time as our publication of immunocytochemical data regarding the colocalization of two trk receptors in the same NBM neuron in the human brain (Salehi et al., 1996), Moshnyakov et al. (1996) reported the presence of two or even three trk receptors in a single rat trigeminal ganglion neuron, using a method based on the reverse transcriptase-polymerase chain reaction.

The presence of multiple trks in a significant proportion of NBM neurons suggests that the full range of cortical and local neurotrophins could be involved in the maintenance of human NBM neurons. NGF and BDNF are abundantly expressed in the cortex, a major projection area of the axons of NBM neurons (Large et al., 1986; Shelton et al., 1986; Phillips et al., 1990). Studies in trk null-mutant mice provided circumstantial functional evidence for the colocalization of trk receptors. Disruption of trkA or trkC genes has a profound effect on survival of specific subsets of peripheral sensory neurons. In contrast, anatomical studies in these animals have shown that the formation and the survival of CNS neurons in a number of brain areas, including the basal forebrain, were for less affected (Klein et al., 1993; 1994; Smeye et al., 1994). The overall hypothesis emerging from such studies in trk null-mutants is that the survival of many CNS neurons is regulated by multiple neurotrophins and their receptors, while peripheral neurons are often dependent on a single neurotrophin receptor. Multiple trk receptors in human cholinergic forebrain neurons provide compelling anatomical evidence for the notion that certain neuronal populations in the CNS can be supported by more than one neurotrophin. On the other hand, there are some areas of the brain, e.g. the locus coeruleus (Hoogendijk et al., 1995) and the CA1 (West et al., 1994) in which cell death occurs in AD. Unlike the locus coeruleus which shows a clear cell death, the NBM displays little cell death, but significant neuronal atrophy (Rinne et al., 1987). The reason behind this difference might be the fact that NBM neurons have a multiple protection system (trk receptors for a variety of neurotrophins). The locus coeruleus neurons in adults do not express trk receptors other than trkC mRNA (Lamballe et al., 1991). Unlike NT-3, neither NGF nor BDNF are able to promote the survival of noradrenergic neurons of the locus coeruleus (Arenas and Persson, 1994).

**Therapeutic considerations and future strategies**

In contrast to the potential advantages of NGF as a therapeutic factor in neurodegenerative disorders, there have been reports suggesting that NGF induces AD changes. For instance, Garver et al. (1995) showed that NGF is able to activate a protein kinase, i.e. the 42-KDA mitogen-activated protein kinase, which is able to change the mobility of normal tau in SDS gels. Furthermore,
it has been shown that NGF causes an elevation in the brain levels of amyloid precursor protein (APP) mRNA in neonatal animals (Mobley et al., 1988). However, these data are controversial. For instance Cheng and Mattson (1992) showed that NGF is able to prevent the appearance of tangle-like antigenic changes induced by glucose deprivation in hippocampal neurons. Furthermore, it has been shown that the species of APP mRNA induced by NGF are in fact reduced in AD (Ohyagi and Tabira, 1993). In addition, the fact that we found a severe reduction in the number of NBM neurons expressing trkA in AD and the observation of a significantly lower level of NGF in the NBM of AD brains by others (Scott et al., 1995) suggests that overstimulation by NGF is not likely to occur in the NBM.

The delivery of neurotrophic factors is currently being considered as a treatment strategy for several neurodegenerative diseases, including amyotrophic lateral sclerosis, Parkinson’s disease and AD (Hefti, 1994; see Chapter 32 of this volume). The rationale of neurotrophic factor therapy is that this would prevent and/or counteract degeneration of the affected neuronal populations, thereby alleviating some of the aggravating symptoms that are characteristic of neurodegenerative diseases. The first relatively small clinical trial using NGF in AD is on the way (Olson et al., 1992; Seiger et al., 1993). However, the presence of abundant amounts of endogenous NGF in the cortex of AD patients (Scott et al., 1995) supports the notion that a lack of NGF is not the limiting factor in AD. Our finding of a decrement in all three trk receptors in the NBM of AD patients suggests that the NBM neurons in the basal forebrain display a severely reduced responsiveness to neurotrophins. Because it is not known whether decreased trk expression or failure in axonal transport or a combination of both is the key process taking place in the NBM of AD patients, strategies aimed at the development of a neurotrophic factor therapy for AD should include studies on both the regulation of trk gene expression and the axonal transport of trks. The elucidation of regulatory sequences in trk genes may reveal possibilities to manipulate trk gene expression pharmacologically. Finally, in the future it may be possible to deliver copies of the trk gene directly to the affected neurons using gene delivery systems such as viral vectors (Le Gal La Salle et al., 1993; Ragot et al., 1993; reviewed in Hermens and Verhaagen, 1998) or human artificial chromosomes (Sun et al., 1994). Studying neurotrophin transport may also enable us more localized rather than general intraventricular administration of neurotrophins, especially NGF.

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