CO-LOCALIZATION OF HIGH-AFFINITY NEUROTROPHIN RECEPTORS IN NUCLEUS BASALIS OF MEYNERT NEURONS AND THEIR DIFFERENTIAL REDUCTION IN ALZHEIMER'S DISEASE

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Abstract—It has been suggested that degeneration of neurons in Alzheimer's disease is the result of diminished trophic support. However, so far no evidence has been forwarded that neuronal degeneration in Alzheimer's disease is causally related to insufficient production of neurotrophins. The present study deals with (i) the expression and co-localization of tyrosine kinase receptors (trks) in the human nucleus basalis of Meynert and (ii) alterations of these receptors in Alzheimer's disease in the nucleus basalis of Meynert, an area severely affected in Alzheimer's disease. The expression of trkA, trkB and trkC in the nucleus basalis of Meynert of control and Alzheimer's disease brains was studied using three polyclonal antibodies specifically recognizing the extracellular domain of trkA, trkB and trkC. Brain material of eight controls and seven Alzheimer's disease patients was obtained at autopsy, embedded in paraffin and stained immunocytochemically. Using an image analysis system, we determined the proportion of trk neurons expressing the different trk receptors in controls and Alzheimer's disease patients. In control brains, trkA, trkB and trkC were differentially expressed in numerous nucleus basalis of Meynert neurons. The highest proportion of neurons was found to express trkB (75%), followed by trkC (58%) and trkA (54%). Furthermore, using consecutive sections, a clear co-localization of trk receptors was observed in the same neurons. The highest degree of co-localization was observed between trkA and trkB. In Alzheimer's disease patients, the number of immunoreactive neurons and the staining intensity of individual neurons was reduced dramatically. Reduction in the proportion of neurons expressing trkA was 69%, in trkB 47% and in trkC 49%, which indicated a differential reduction in the amount of trk receptors in Alzheimer's disease.

These observations indicate that nucleus basalis of Meynert neurons can be supported by more than one neurotrophin and that the degeneration of these neurons in Alzheimer's disease is associated with a decreased expression of trk receptors, suggesting a decreased neurotrophin responsiveness of nucleus basalis of Meynert neurons in Alzheimer's disease. Copyright © 1996 IBRO. Published by Elsevier Science Ltd.

Key words: trk receptors, Alzheimer, neurotrophin, nucleus basalis, NGF.

Alzheimer's disease (AD) is the most common cause of dementia in elderly people and is characterized by the presence of senile plaques, neurofibrillary tangles, neuronal atrophy and loss of synapses. One of the brain areas severely affected in AD is the nucleus basalis of Meynert (NBM).^{16,49,70} The cholinergic NBM neurons project extensively to the entire cerebral cortex.^{38,39} It has been proposed that the degenerative changes in AD are the result of lack of trophic support,^{5,22} since normally (i) abundant expression of nerve growth factor (NGF) mRNA is

observed in the cerebral cortex,^{31,60} (ii) a high density of p75 receptors is present in the basal forebrain area,⁵⁴ and (iii) there is a considerable degree of co-localization between choline acetyltransferase and p75 in the basal forebrain of the rat¹³ and human.⁴² This idea was reinforced by studies showing protective effects of NGF on basal forebrain cholinergic neurons in adult rats following lesions²¹ and the ability of basal forebrain neurons to retrogradely transport NGF.⁵⁹ Based on these observations, intracerebral administration of NGF was proposed as an experimental treatment for AD,⁵⁰ which indeed had some positive effects in one patient.⁵⁸ However, measurement of the levels of NGF expression in AD did not reveal a decrease in the cerebral cortex,¹⁹ cerebrospinal fluid or serum,⁴⁵ and some studies even showed increased levels of NGF-like activity in the

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Abbreviations: AD, Alzheimer's disease; BDNF, brain-derived neurotrophic factor; NBM, nucleus basalis of Meynert; NGF, nerve growth factor; p75, neurotrophin receptor; TBS, Tris-buffered saline; trk, tyrosine kinase receptor.

Table 1. Clinicopathological data of controls and Alzheimer's disease patients

Patient no.	Sex(m/f)	Age(years)	PMD*(h)	Brain weight(g)	GDS†	Cause of death and clinical diagnosis
Controls						
(1) 93161	m	39	4	1274	—	Cardiac failure
(2) 93026	m	50	nd	1646	—	Bladder carcinoma, shock due to sepsis
(3) 92047	m	53	14	1410	—	Unknown
(4) 92046	f	54	12.45	1080	—	Traffic accident
(5) 93150	m	64	nd	1417	—	Pancreatic carcinoma with hepatic metastasis
(6) 92049	m	71	5.40	1250	—	Sudden death
(7) 92048	f	72	4.35	1210	—	Heart failure
(8) 94115	f	81	7.15	1350	—	Colon carcinoma with brain metastasis
Mean ± S.E.M.	—	60.5 ± 5	7.9 ± 2	1329.6 ± 60		
Alzheimer						
(9) 90262	m	49	4.25	1426	7	AD, epilepsy, diabetes, chronic alcoholism
(10) 91092	f	54	3.15	1055	6	AD, cachexia
(11) 91214	m	58	4.45	1350	7	AD, cachexia, pulmonary insufficiency
(12) 91091	f	60	3.45	1060	7	AD, sudden death
(13) 93341	f	63	2.15	1034	7	AD
(14) 94210	m	64	5.55	1218	3	AD, aspiration pneumonia
(15) 83170	f	70	13.35	780	nd	AD, status epilepticus
Mean ± S.E.M.	—	59.7 ± 3	5.2 ± 1.4	1132 ± 82		

*PMD, *post mortem* delay.†GDS, global deterioration scale.⁵³

cortex of AD patients.¹¹ These observations led to the idea that the failure of NBM neurons in AD to respond to NGF might be due to changes in the expression or functional properties of neurotrophin receptors.^{22,23} Two types of receptors for neurotrophins have been identified: p75,⁸ a 75,000 mol. wt protein interacting with all neurotrophins, and the recently characterized tyrosine kinase receptors (trkA, trkB and trkC), a family of transmembrane proteins with a specific affinity for individual neurotrophins.^{26,27,28} TrkA binds NGF and neurotrophin-3,^{9,26,27,48} while trkB and trkC transduce biological responses of brain-derived neurotrophic factor (BDNF), neurotrophin-4/5^{28,64,65} and neurotrophin-3,^{30,32} 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 trkC are widely distributed throughout the brain.^{4,37} *In situ* hybridization reveals that the full-length forms of trkB and trkC are found mostly in neurons, but not in glial cells. However, the truncated forms of these receptors are expressed widely in non-neuronal cells.¹⁷

The present study was conducted to compare the distribution and assess the co-localization of individ-

ual trks in the NBM of control brains and to study alterations in the expression of these receptors in AD.

EXPERIMENTAL PROCEDURES

Subjects

Brains of 15 human subjects were obtained at autopsy (see Table 1 for details and clinico-pathological information). The material consisted of eight non-demented controls (60 ± 5 years old) without any primary neurological or psychiatric disorder and seven Alzheimer patients (58 ± 3 years old). The patients were clinically assessed and diagnosed as "probable AD" by excluding other possible causes of dementia by history, physical examination and laboratory tests according to the NINCDS-ADRDA criteria.³⁶ The clinical diagnosis of AD and the integrity of the controls was neuropathologically confirmed by F. C. Stam (Netherlands Brain Bank), W. Kamphorst (Free University) or D. Troost (Academic Medical Center; all in Amsterdam). The neuropathological examination of AD patients showed extensive neocortical and hippocampal senile plaques, neurofibrillary tangles and dystrophic neurites. There were no significant differences in age (Student's *t*-test, *P* = 0.89), *post mortem* delay (*P* = 0.25) or brain weight (*P* = 0.07) between controls and AD patients. The hypothalamus containing the NBM was dissected and fixed in 4% formaldehyde at room temperature for about one month. The fixed hypothalamus was dehydrated in graded ethanols, embedded in paraffin and cut serially in 6-µm thin frontal sections. Sections were mounted on chrome-aluminium sulphate-coated slides, deparaffinized, hydrated and stained either by Thionine (0.5%) or immunocytochemically. Every 50th section was stained with Thionine for anatomical orientation.⁶⁸ Since the NBM

is a very extensive cell system, immunocytochemical stainings were performed on a standardized part of the NBM, i.e. the medial and lateral subdivisions of ch4a, according to Mesulam's nomenclature,³⁹ based upon a standardized location of the fornix, the anterior commissure, the optic tract and the supraoptic nucleus.¹⁶

Assessment of antibodies specificity

Three rabbit polyclonal antibodies used in this study have been raised against the external domain of trkA, trkB and trkC receptors using peptides corresponding to amino acids 76–96 (QQHLQHLELRNLRGLGELRNL) of human trkA (anti-trkA_{out}), 76–96 (QKRLELLNEDDVEAY-VGLKNL) of mouse trkB (anti-trkB_{out}) and 88–108 (WRGLHTLNAVDMELYTGLQKL) of rat trkC (anti-trkC_{out}). The trk antibodies were a generous gift from Dr D. Kaplan (ABL-Basic Res. Program, Frederick, MD, U.S.A.). The specificity of these antibodies for the receptors had previously been established by Western blot¹⁴ and cross-linking analysis.³ Western blot showed a distinct band at a molecular weight of 140,000, two bands at 110,000 and 145,000, and two bands at 140,000 and 100,000 identified by the trkA, trkB and trkC antibodies, respectively.¹⁴ Moreover, the cross-reactivity of any of the trk antibodies with other peptides has been ruled out by using trk-overexpressing NIH-3T3 cells.²⁵

Two additional control experiments were performed in this study to ascertain the specificity of the immunocytochemical reaction on tissue sections of the human brain.

Adsorption of antisera

Antibody adsorption was performed according to the method of Van der Sluis and colleagues.⁶⁹ The trkA and trkB peptides (gift from Dr D. Kaplan) used to generate the antibodies were applied as 20 spots (100 ng per spot) and fixed using 5% buffered paraformaldehyde as a fixative on a gelatin-coated nitrocellulose membrane (3 mm Whatman, 0.2% gelatine) overnight at room temperature. After five adsorption runs, using fresh strips for each run, the adsorbed antibodies were applied to the human NBM sections using the same dilution (1:600) as non-adsorbed serum.

Cross-reactivity controls

Cross-reaction of the trkB and trkC antisera with trkA peptide was assessed using trkA-transfected cells. In brief, the 2.6-kb trkA cDNA (a gift from Dr D. Kaplan) was cloned into the expression vector pcDNA I/amp (Invitrogen Co.) containing the cytomegalovirus promoter, a strong transcriptional activator that efficiently drives the expression of trkA. Human embryonic retinoblasts (cell line 911; kindly provided by Dr F. Fallaux) were transfected using the calcium phosphate precipitation method.²⁰ After 48 h, the transfected cells were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer and stained with either trkA, trkB or trkC antisera, using the same procedure as for the human brain sections.

Immunocytochemistry

After rehydration in a series of ethanols, the sections were incubated with antibodies against trkA, trkB and trkC (1:600) in Tris-buffered saline (TBS; pH 8.5) for 1 h at room temperature followed by overnight incubation at 4°C. Subsequently, they were washed in TBS (pH 7.6; 3 × 10 min) and incubated with goat anti-rabbit (1:100) in TBS (pH 8.5) for 30 min. Finally, the sections were washed in TBS (pH 7.6; 3 × 10 min) and incubated with peroxidase-antiperoxidase (1:1000) in TBS (pH 7.6) for 1 h. As a chromogen, 3',3'-diaminobenzidine (Sigma) was used. Staining enhancement was obtained by adding ammonium nickel sulphate to the chromogen buffer (2.2 mg/ml). The reaction was terminated by washing the sections in TBS. They were then dehydrated, cleared in xylene and coverslipped.

In order to study the level of co-localization between the trk receptors, adjacent sections from the NBM of three control brains (nos 93161, 93026 and 92046; Table 1) were stained by antibodies against either trkA, trkB or trkC. Using a camera lucida attached to a microscope (Axioskop Zeiss, Germany) it was possible to retrieve NBM neurons in adjacent sections and to reveal whether they were also stained by another trk antibody.

Morphometry

An image analysis system (Kontron), which was mounted with a CCD video camera (Sony XC-77CE) connected to an Axioskop microscope (Zeiss), was used for cell counting. The microscope was equipped with an X and Y scanning stage (Märzhauser) and a Z motor (Zeiss), both under the control of the image analyser. The Z motor and the video camera were connected to a Kontron Autofocus unit.

Two to three sections per subject were used to determine the proportion of stained neurons for each trk. In each section the analysis consisted of seven steps: (i) outlining the NBM area in an image loaded with a ×2.5 objective, (ii) superimposing a grid of rectangular areas "seen" through a ×40 objective by the TV camera, (iii) storage of the coordinates of all those fields of the grid which were covered by the NBM outline, (iv) systematic random selection of 45 fields by the computer, (v) automatic loading of the images of randomly selected fields at ×40 objective into the image analyser, (vi) manual labelling (either positive or negative) of each neuronal profile containing a nucleolus by the observer (in case of doubt, the section was scanned in the Z-direction to identify the nucleolus) and (vii) calculation of the total number of stained and non-stained profiles.

Statistical methods

The differences in mean values of cell counts between controls and AD patients were tested using the Mann-Whitney non-parametric test. Differences in age, *post mortem* delay and brain weight between controls and AD patients were tested using Student's *t*-test. One-way analysis of variance (ANOVA) was used to test the differences in the amount of reduction of all three trk receptors in AD. A *P* value < 0.05 was considered to be significant.

RESULTS

Specificity of antisera

Sections incubated with pre-immune serum of the three rabbits used to raise the individual trk antibodies did not result in any detectable immunohistochemical staining. The results of two additional control experiments determined the specificity of the immune reaction on tissue sections of human brain. Firstly, adsorption of trkA and trkB antisera with the homologue peptides used to raise these two antibodies abolished all immunostaining observed with the non-adsorbed sera on tissue sections of the NBM. However, as a second control, cultured cells expressing trkA were immunocytochemically stained with the three antisera. As shown in Fig. 1A, trkA antibody stained numerous trkA-transfected cells, while trkB and trkC antibodies did not exhibit any staining (Fig. 1B, C). This strongly argues that the trkB and trkC antibodies are specific and do not cross-react with the trkA receptor protein as it is expressed in a cellular context.

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 trkB and trkC antibodies. Arrowheads indicate three heavily stained cells with trkA antibody.

Fig. 2. Immunocytochemical staining of trkA in NBM neurons in a control (A) and an AD patient (B). Note the clear reduction in staining of both the large and small neurons in the AD patient. Scale bar = 35 μ m.

Expression of tyrosine kinase receptors in control subjects

All three antibodies stained numerous magnocellular neurons in the NBM area in a heterogeneous way. The lowest proportion of immunoreactive neurons was observed for trkA (54%; Fig. 2A) and the highest for trkB (75%; Fig. 3A), with an intermediate value for trkC (58%; Fig. 4A). TrkA expression was restricted to neurons in the NBM area, whereas trkB- and trkC-positive cells were also observed in other areas of the human hypothalamus, e.g. in the tuberomammillary and supraoptic nucleus. Other hypothalamic nuclei such as the nucleus tuberalis lateralis did not show immunoreactivity for any of the trk

receptors. Interestingly, immunoreactivity for all trk receptors was found in the perinuclear cytoplasm of neurons. TrkB was also expressed in many fibres in the NBM area (Fig. 5). The virtual lack of trkA and trkC immunoreactivity in the fibres might be simply due to the higher level of trkB present in fibres and/or the relatively thin sections used (6 μ m). Trk immunoreactivity in the NBM was observed in a variety of large, medium and small neurons.

In adjacent sections, a clear co-expression was observed between the trk receptor proteins. The highest proportion of co-localization was found for trkA and trkB: 84% of the neurons ($n = 40$) that stained for trkB (Figs 6A, 7B) were also positive for trkA in the adjacent section (Figs 6B, 7A), and the

Fig. 3. TrkB-immunoreactive neurons in the NBM area of a control (A) and an AD patient (B, C). Note the reduction in immunoreactivity in the NBM neurons in AD (B) and the presence of a number of small neurons still showing clear immunoreactivity in AD (C). Scale bar = 35 μ m.

Fig. 4. Immunocytochemical visualization of trkC in the NBM area in a control (A) and an AD patient (B). Scale bar = 35 μ m.

lowest percentage of co-localization was found for trkB and trkC (66%; $n = 40$; Figs 6C, 6D, 7C, 7D). About 77% of the neurons that stained for trkA ($n = 40$) were also positive for trkC (Figs 7E, F).

Expression of tyrosine kinase receptors in Alzheimer's disease patients

The immunoreactivity of all three trk receptors was dramatically reduced in the NBM of AD brains compared with non-demented controls. This reduction was evident from the decrease in both the number of positive cells and the staining intensity of individual cells. The reduced immunoreactivity for trk receptors was not restricted to atrophied neurons

(Figs 2, 3, 4B), but a decrement in expression was also observed in the majority of the large neurons in the NBM of AD patients (Figs 2, 3, 4B). The extent of the reduction was not the same for all three trk receptors. Although the staining level was clearly reduced for trkB, the number of positive cells for this receptor in AD was quite significant. Furthermore, there were many small neurons in the NBM of AD patients which were intensely trkB positive (Fig. 3C).

Quantification of tyrosine kinase receptor loss

A total of 6796 neurons, i.e. neuronal profiles containing a nucleolus, was studied in controls and

Fig. 5. The presence of immunoreactivity in fibres in the NBM stained with the antibody against trkB in a control subject. Arrowheads indicate a stained fibre. Scale bar = 35 μ m.

AD patients for their trk content. The highest proportion of neurons was found to express trkB, followed by trkC and trkA (Table 2). The proportion of neurons expressing trkA was most strongly reduced (69% reduction) in AD patients, followed by trkC (49%) and trkB (47%) (Fig. 8). The reduction of the amount of stained neurons in AD was not the same for all three trk receptors. One-way ANOVA test showed a significant difference in the amount of reduction between trkA and trkB ($P = 0.004$, $F = 13.6$), and trkA and trkC ($P = 0.017$, $F = 5.34$). However, there was no difference in the magnitude of reduction between trkB and trkC ($P = 0.747$, $F = 0.110$) in AD.

DISCUSSION

Specificity of immunocytochemical staining

The specificity of the trk antibodies used in this study had been established previously by western blot¹⁴ and was further confirmed in the present study by adsorption of the antibodies to the peptides used for immunization and by determining the cross-reactivity in a transfection assay.

Adsorption of antibodies to their homologous peptide completely abolished the immunocytochemical staining on human brain sections. Furthermore, the transfection experiments did not reveal any cross-reaction between trkA expressed in cells in culture

Fig. 6. The presence of co-localization between trkB (A) and trkA (B) and also between trkC (C) and trkB (D) in a control subject at high magnification. Arrowheads indicate the same group of neurons stained with both antibodies. Arrows indicate the same neuron which is stained by trkC (C) and not by trkB (D). Scale bar = 35 μ m.

Fig. 7. The presence of co-localization between trkA (A) and trkB (B), trkB (C) and trkB (D) and also between trkA (E) and trkC (F) in a control subject at low magnification. Arrowheads indicate the same group of neurons stained with different antibodies. Scale bar = 35 μ m.

and trkB or trkC antibodies. Together with the fact that the antibodies identified only partially overlapping populations of NBM neurons, these observations indicate that the three antisera specifically detect genuine trkA, trkB and trkC in human brain sections.

Distribution and co-localization of tyrosine kinase receptors in the basal forebrain and hypothalamus

This is the first study comparing the expression of the three trk receptors (trkA, trkB and trkC) in the human NBM demonstrating a clear co-expression of

Table 2. Proportion of tyrosine kinase receptor-expressing neurons in controls and Alzheimer's disease patients[†]

Subjects	A	Trk B	C
Controls	54.4 ± 4% (1218)	74.7 ± 6% (1613)	58.2 ± 2% (1484)
AD	16.7 ± 3% (977)	39.7 ± 2% (754)	29.6 ± 4% (750)
P [‡]	0.00001*	0.009**	0.004***

[†]Data are expressed as means ± S.E.M. The total number of neurons counted is given in parentheses.

[‡]Mann-Whitney U-test.

Fig. 8. Graph depicting the proportion of neurons stained by trk antibodies in controls and AD patients. Note the strong reduction in the proportion of trkA-expressing neurons in AD, which is followed by trkB and trkC. (P values are indicated on Table 2).

these receptors in NBM neurons in controls and a dramatic reduction in AD.

Information on the distribution of neurotrophin receptors in the human brain has so far primarily been available from 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.⁶¹ A truncated form of trkB was shown to be expressed in the human cerebellum and the hippocampus.² TrkC is expressed in the human basal forebrain, hippocampus and neocortex.⁶¹

The present study shows differential staining patterns for each of the trk receptors in the human basal forebrain. TrkA was restricted to the NBM and was not expressed in the adjacent hypothalamic nuclei. In contrast, trkB and trkC are expressed in NBM neurons and in hypothalamic nuclei, including the supraoptic nucleus and tuberomammillary nucleus. This is consistent with and extends previous observations on the anatomical distribution of these receptors in the rat.⁴ In particular, the expression of trkB and trkC in the human supraoptic nucleus is in agreement with the expression of trkB and trkC in rat supraoptic neurons.^{4,63} The clear cytoplasmic expression of trkB and trkC observed in the present study is consistent with the cellular localization in rat⁶³ and human NBM neurons.^{6,44}

Trk receptors were clearly co-localized in 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. So far, no immunohistochemical or *in situ* hybridization studies have revealed the expression of two trk receptors in the same neuron. 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 respond to both neurotrophins.^{21,41} In addition, NGF and BDNF exert a similar physiological response in embryonic septal cholinergic neurons.¹ Based on overlap of mRNA expression patterns for different trk receptors, co-expression of trk receptors probably occurs in rat peripheral sensory neurons, hippocampal neurons and basal forebrain neurons.^{35,37}

The presence of multiple trk receptors in a significant proportion of neurons of the NBM strongly suggests that the full range of cortical and local neurotrophins could be involved in the maintenance of the human NBM. NGF and BDNF are abundantly expressed in the cortex, a major projection area of the axons of NBM neurons.^{33,51,60} Studies in trk null-mutant mice have provided circumstantial functional evidence for co-localization of trk receptors. Disruption of the trkA and trkC genes has a profound effect on the 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 much less affected.^{29,30,62} The overall hypothesis that emerged from studies in trk null-mutants is that 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 the first anatomical evidence for the notion that certain neuronal populations in the CNS can be supported by more than one neurotrophin.

Reduction of tyrosine kinase receptor expression in the Alzheimer's disease brain

In the NBM of AD patients, expression of all three trk receptors was dramatically reduced. TrkA was most clearly affected and had become virtually undetectable in AD patients (Fig. 2B, Table 2). TrkB and trkC were expressed in some neurons, although

at a lower level, and many NBM neurons had ceased to express these receptors as well (Figs 3B, 4B, 8).

Cholinergic dysfunction can certainly not explain all neuropathological features of AD, but numerous studies have indicated that degeneration of cholinergic NBM neurons is an early hallmark of AD^{16,49,70} and involves neuronal atrophy rather than cell death.^{55,56} It should be noted though that a small population of galanin-containing NBM neurons show signs of hypertrophy rather than atrophy.⁷ 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 large neurons which are apparently less atrophied in AD. Interestingly, the expression of trk receptors was reduced in both groups of NBM neurons in AD. This is consistent with the observations that the protein synthetic ability of both small and large neurons is reduced in AD.⁵⁶ In addition to degeneration, signs of possible cellular activation were observed in a third group of galanin-positive NBM neurons.⁷ The intensely trkB-positive small NBM neurons in AD, as observed here, may correspond to this previously identified population of activated neurons (Fig. 3C). Recent studies in rat sciatic nerve indicate that both p75 and trkA are retrograde carrier molecules for neurotrophins.^{12,15} Retrograde transport of NGF is mediated by trkA¹⁵ and appears to be independent of p75.¹² The involvement of trkB and trkC in retrograde signalling has not yet been fully resolved, but it is not inconceivable that these molecules also act as retrograde messengers. The abundant expression of trkB in nerve fibres in the NBM suggests that trkB is actively transported along axons. Recent *in vitro* studies revealed diminished transport of NGF in human basal forebrain neurons in AD.^{43,57} Whether this is due to the decrement in trkA in NBM neurons in AD remains to be determined. Diminished expression of trkA in NBM neurons could result in reduced retrograde transport of NGF from the cortex to the NBM. Therefore, it is not unreasonable to postulate that diminished trkA expression may directly contribute to the neuronal atrophy and cholinergic dysfunction characteristic of AD. In this respect, it is important to underscore that in the NBM of AD patients all three trk receptors are reduced simultaneously. This would presumably result in a much more profound effect on neuronal morphology than the relatively minor effects observed in the single trk receptor knockout mice that have been studied so far.

The reduction of trkA in AD is significantly more severe than that of trkB and trkC (see Results section). There was no difference in the degree of reduction in AD between trkB and trkC. This might either be due to differential degeneration of trk-expressing neurons in AD or to a differential alteration of expression of trk receptors in AD. It is not known whether these processes depend on co-

localization of trk receptors. Since neuronal atrophy rather than cell death seems to be the major phenomenon in Alzheimer NBM,⁵⁵ the changes seen in trk staining are, however, due to changes in expression of these receptors rather than to cell death.

The diminished levels of trk expression in AD could be either primary or secondary to structural changes in NBM neurons in AD. NBM neurons are stained heavily by antibodies that detect cytoskeletal alterations, e.g. Alz-50.⁶⁷ The Alz-50 antibody is an indicator of abnormally phosphorylated tau. Tau plays a major role in the maintenance of the morphology of neurons and in axonal transport. Abnormal tau phosphorylation could result in dysfunctional 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. On the other hand, down-regulation of trk expression could be the primary trigger of the neuropathological changes in the NBM in AD.

In contrast to the potential advantages of using NGF as a therapeutic factor in neurodegenerative disorders, there are some reports suggesting possible detrimental NGF-dependent processes in AD. For instance, Garver *et al.*¹⁸ have shown that NGF is able to activate a protein kinase, i.e. a 42,000 mol. wt mitogen-activated protein kinase, which is able to change the mobility of normal tau. Furthermore, it has been shown that NGF causes an elevation in brain levels of amyloid precursor protein mRNA in neonatal animals.⁴⁰ However, these data are controversial. For instance, Cheng and Mattson¹⁰ have shown 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 amyloid precursor protein mRNA which are experimentally elevated by NGF are in fact reduced in AD.⁴⁶ In addition, the fact that we found a severe reduction in the number of NBM neurons expressing trkA in AD and a significantly lower level of NGF in the NBM of AD brains was observed by others,⁵⁷ supporting the idea that overstimulation effects of NGF are not likely to occur in the NBM area of AD patients.

Therapeutical implications

The present findings may have important implications for the application of neurotrophic factors in the treatment of neurodegenerative diseases. The delivery of neurotrophic factors is currently considered as a treatment strategy for several neurodegenerative diseases, including amyotrophic lateral sclerosis, Parkinson's disease and AD.²⁴ 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 underway.^{47,58} However, the presence of abundant amounts of endogenous NGF in the cortex of AD patients¹¹ supports the notion that NGF is not the limiting factor in the neurotrophic signal transduction cascade in AD. The current finding on the decrement in all three trk receptors in the NBM of AD patients suggests that the NBM neurons in the basal forebrain have a severely reduced responsiveness to the neurotrophin family. Future research aimed at the development of a neurotrophic factor therapy for AD should include studies on the regulation of trk gene expression. 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^{34,52} or human artificial chromosomes.⁶⁶

CONCLUSIONS

This study documents a strong and differential reduction of trk receptor expression in the neurons of the NBM of AD patients. The most affected trk receptor was trkA, followed by trkC and trkB. In addition, we observed expression of two trk receptors in the same neurons in the NBM of controls, suggesting that certain neurons in the NBM are able to respond to more than one neurotrophin.²³

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