Neuroscience Vol. 30, No. 1, pp. 143–152, 1989
Printed in Great Britain

0306-4522/89 $3.00 + 0.00
Pergamon Press plc

NERVE GROWTH FACTOR RECEPTOR IS ASSOCIATED WITH CHOLINERGIC NEURONS OF THE BASAL FOREBRAIN BUT NOT THE PONTOMESENCEPHALON

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Abstract—Sequential immunohistochemical demonstration of nerve growth factor receptor and choline acetyltransferase on the same tissue section in the rat revealed that approximately 92% of all cholinergic neurons in the basal forebrain possessed that receptor. Only 0.9% of the neurons demonstrating nerve growth factor receptor in the basal nuclear complex lacked the cholinergic synthetic enzyme, and a similarly small percentage of cholinergic cells, 7.1%, were choline acetyltransferase-positive but nerve growth factor receptor-negative. Affiliation of nerve growth factor receptor with structural entities morphologically indistinguishable from those demonstrating choline acetyltransferase on separate but corresponding tissue sections was also observed in the telencephalic fiber tracts and terminal fields of basal forebrain cholinergic neurons, including cholinergic puncta in the reticular nucleus of the thalamus. Nerve growth factor receptor was not found in association with choline acetyltransferase-positive somata of the pedunculopontine and laterodorsal tegmental nuclei, however, nor were fibers immunoreactive for nerve growth factor receptor observed originating from those cell bodies.

These results suggest that nerve growth factor receptor, which is probably synthesized in cholinergic basal forebrain somata and transported throughout their dendritic and axonal arbors, has a physiologic role in those cells in the adult nervous system. This does not appear to be the case for phenotypically similar neurons of the pontomesencephalotegmental cholinergic complex.

Currently available experimental evidence suggests that cholinergic neurons in the basal forebrain are preferentially sensitive to nerve growth factor (NGF). When infused into the hippocampus and neocortex, this neurotrophic agent is accumulated by cholinergic nerve terminals in those regions and transported retrogradely to the parent somata in the medial septal nucleus, vertical limb of the diagonal band, and nucleus basalis, but not to other neurons projecting to the cerebral mantle.[29,30] The neuronal uptake and transport of NGF is thought to be receptor mediated,[6,32,41] and experimental evidence supporting such a conjecture has been gathered from cell culture investigations[41] and studies of the peripheral nervous system, particularly the sympathetic and dorsal root ganglia.[32,41] A similar, if not identical, process involving the coupling of NGF to its receptor also has been postulated to operate centrally and to contribute significantly to the overall mechanism by which NGF increases choline acetyltransferase (ChAT) activity in the basal forebrain during development[13,25] and following partial lesions of the septohippocampal pathway.[15]

The presence and differential binding of NGF and messenger ribonucleic acid (mRNA) in the adult brain of normal rats[21,28] suggest that, in addition to its role in the developing organism and in response to injury, this neurotrophic agent may be physiologically important in the mature, non-injured brain as well. If NGF is intraneuronally accumulated and transported by means of a receptor-mediated mechanism in surgically unmanipulated cholinergic neurons in the adult brain, then it would be expected that this receptor would be found normally in relation to central cholinergic somata, fibers and terminal regions. In this regard, NGF receptor has been observed in association with acetylcholinesterase-containing cell bodies in the human basal forebrain[17] and ChAT-positive somata in the basal nuclear complex of developing and adult rats,[2,9] observations compatible with the finding that, following ablation of cortical targets, NGF receptor accumulates in ascending acetylcholinesterase-containing pathways.[31] Similarly, lesions of the fimbria-fornix produce proximal and distal accumulation of NGF receptor in that fiber bundle, suggesting that this growth factor and its receptor are transported both anterogradely and retrogradely.[20]

In none of these previous studies, however, have detailed quantitative estimates been made of the association of NGF receptor with cells demonstrating ChAT-like immunoreactivity in the cholinergic basal forebrain, nor have cholinergic neurons in the pontomesencephalic tegmentum been examined systematically for the presence of NGF receptor. Those two issues are potentially important, however, given that

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Abbreviations: ChAT, choline acetyltransferase; IgG, immunoglobulin G; MAP1, microtubule associated protein1; mRNA, messenger ribonucleic acid; NGF, nerve growth factor; PBS, phosphate-buffered saline.

both the basal forebrain and pontomesencephalotegmental cholinergic systems contain neurons that are similar morphologically and together comprise the majority of centrally projecting cholinergic cells.[3,4,34,35,37-39] The relation of NGF receptor to ChAT-positive fibers and terminal fields in the surgically unmanipulated brain has similarly not been assessed. In this report, we extend earlier observations by addressing these topics.

EXPERIMENTAL PROCEDURES

Experimental animals

Fifteen male and female adult Sprague–Dawley rats (Bantin and Kingman Co., Fremont, CA, U.S.A.) were used. They weighed approximately 250–350 g at the time of being killed and were housed in stainless steel cages under conditions of constant temperature (22°C) and relative humidity (50%). Food and water were provided *ad libitum*.

Immunohistochemical procedures

Animals were anesthetized with 50 mg/kg sodium pentobarbital injected intraperitoneally, placed on a bed of ice, and transcardially perfused with 50 ml cold (4°C) phosphate-buffered saline (PBS) followed by 250 ml cold 4% paraformaldehyde with 0.2% saturated picric acid dissolved in 0.1 M phosphate buffer (pH = 7.6). The brains were then postfixed in the latter solution for 2–24 h before being cut on a Vibratome, or they were transferred immediately to 30% sucrose in 0.1 M phosphate buffer for 2–5 days prior to sectioning on a freezing microtome. Brain sections were cut at 50 μm intervals throughout the entire extent of the basal forebrain and pontomesencephalotegmental cholinergic complexes.[3,4,34,35,37-39] On average, every third section was saved, collected into PBS, and stored at 4°C until immunohistochemical processing.

Hybridoma cells secreting the 192-immunoglobulin G (192–IgG) monoclonal antibody against NGF receptor were grown in culture. The antibody was prepared and subsequently purified according to the procedures described in Chandler *et al.*[6] Brain sections to be stained for NGF receptor (n = 10 rats) were first placed overnight into PBS containing 0.3% Triton X100. They were then transferred to a solution of the monoclonal antibody against NGF receptor, diluted 1:100 in PBS containing 0.1% sodium azide as a preservative, and incubated at room temperature for 48 h with gentle agitation. The primary antibody solution was then decanted and the tissue sections reacted according to the protocol outlined in Gould and Butcher[14] with the following modifications. Following three rinses in PBS, sections were incubated in each of the following solutions for 15–30 min: (1) biotinylated anti-mouse IgG diluted 1:50 in PBS and containing 1.5% normal horse serum, (2) 0.3% H_2O_2 in PBS, (3) avidin–biotin-horseradish peroxidase, and (4) 0.05% diaminobenzidine, 0.01% H_2O_2, and 2.5% nickel ammonium sulfate in PBS (chemicals from Vector Laboratories, Burlingame, CA, U.S.A. and Sigma Chemical Co., St Louis, MO, U.S.A.). The nickel ammonium sulfate was used to intensify the reaction product. Biotinylated anti-mouse IgG preadsorbed to rat immunoglobulins (i.e. "species-specific" second antibody: American Qualex Co., La Mirada, CA, U.S.A.) was employed to attenuate spurious staining. Some brain sections were alternatively processed by use of the "double-bridge" peroxidase–antiperoxidase protocol outlined in Houser *et al.*[18] Both the avidin–biotin and peroxidase–antiperoxidase methods yielded immunocytochemical results of essentially equal quality and sensitivity.

In five rats, brains were processed for both NGF receptor and ChAT. Sections were first incubated with the 192-IgG monoclonal antibody against NGF receptor for 48 h as described previously. Following three rinses in PBS, these sections were then reacted in a solution of fluorescein isothiocyanate-conjugated anti-mouse IgG (Sigma Chemical Co., St Louis, MO, U.S.A.), rinsed again in PBS, and then mounted onto uncoated glass slides before being coverslipped under PBS. Following microscopic analyses of NGF receptor immunoreactivity as described in the succeeding discourse, the glass coverslips were removed gently and the brain sections transferred to vials containing PBS. The tissue was then counterprocessed for ChAT. Following 24 h preincubation in 0.3% TritonX PBS, sections were placed for 48 hrs in a solution of the 11/255 monoclonal antibody against ChAT (for characterization see Eckenstein and Thoenen[10]). This tissue was then reacted with biotinylated anti-rat IgG that had been preadsorbed to mouse immunoglobulins (Vector Laboratories, Burlingame, CA, U.S.A.) in order to eliminate cross-reactivity of the secondary antisera to NGF receptor antibody. Other steps in the avidin–biotin procedure were identical to those described previously in this manuscript. Some tissue sections also were processed only for ChAT according to the avidin–biotin or the peroxidase–antiperoxidase methods described above and were used for comparison purposes.

Use of a fluorescent label second antibody to visualize NGF receptor in the double-label procedure resulted in somewhat reduced sensitivity compared to the avidin–biotin and peroxidase–antiperoxidase methods alone, but only with respect to fibers and terminal fields. Efficacy of staining for NGF receptor in cell bodies and proximal processes in the basal nuclear complex was unaffected. Similarly, compared to single-label procedures, the double-label protocol did not alter the number of ChAT-positive cell bodies observed.

Control procedures included elimination of the primary antibody from the reaction sequence and substitution of normal serum immunoglobulins for primary antibodies. In the co-localization studies, some sections that had been reacted with the monoclonal antibody against NGF receptor, which was generated in mouse, were processed with biotinylated anti-rat IgG preadsorbed to mouse immunoglobulins, the secondary antibody used to demonstrate ChAT. In all of these control procedures, specific immunoreactivity was abolished.

Data analysis

Brain sections processed for NGF receptor alone were examined microscopically by use of an Olympus Vanox microscope and evaluated for the presence of cell bodies, fibers and terminals immunolabeled according to the avidin–biotin or peroxidase–antiperoxidase procedures. This material was also photographed and compared to photographs of sections processed immunohistochemically for ChAT at corresponding levels of the basal forebrain and pontomesencephalotegmental cholinergic complexes from the same or different rats.

Material used to evaluate the co-localization of NGF receptor and ChAT was first processed for that neurotrophic receptor by use of a fluorescent-label second antibody as described elsewhere in this manuscript. Major structural landmarks and the precise locations of somata demonstrating NGF receptor were drawn at 100 times magnification onto projection paper by use of a camera lucida apparatus attached to a Zeiss microscope. Comprehensive photomicrographs of the brain regions containing NGF receptor-labeled cells also were made. Following counterprocessing of these same tissue sections for ChAT, the areas of the brain demonstrating NGF receptor were examined again for ChAT-containing somata and the resulting data mapped onto the previously constructed NGF receptor templates, again by use of a Zeiss microscope and drawing tube. Photomicrographs were also taken of the ChAT-positive somata in the identical loci containing cells demonstrating NGF receptor-like immunoreactivity, and

the resulting two photographic series were compared and analysed for the presence of double-labeled neurons.

RESULTS

Distribution of nerve growth factor receptor immunoreactivity

Neurons demonstrating NGF receptor were found in association with nuclei containing the basal forebrain cholinergic system (for definition and projection patterns, see Refs 3, 4, 34, 35, 37–39), including the medial septal nucleus, vertical and horizontal limbs of the diagonal band, magnocellular preoptic area, substantia innominata, nucleus basalis, and so-called nucleus of the ansa lenticularis (Fig. 1). At the cellular level, the reaction product was intense and relatively equally distributed throughout the perikaryal cytoplasm and in the proximal processes of those neurons (Fig. 1). Numerous NGF receptor-immunoreactive fibers also were found in the internal capsule (Fig. 2A and C) that probably derived from basal forebrain cholinergic cells.[39] These fibers were morphologically similar, if not identical, to ChAT-positive fibers in the same region (Fig. 2A and C; compare with Fig. 2B and D). Both NGF receptor- and ChAT-containing fibers were of medium caliber, exhibited varicosities, and were present in comparable densities. Some of these fibers were located in the dorsal part of the internal capsule and appeared to be coursing toward cortical regions (Fig. 2A and B).

Other major fiber tracts such as the cingulum and the fimbria-fornix were found to possess NGF receptor-labeled fibers (Fig. 3A and C) that resembled ChAT-containing fibers in the same locations with respect to morphology and density (Fig. 3B and D). Numerous puncta immunoreactive for NGF receptor also were observed in all of the telencephalic targets of the basal forebrain cholinergic system, including the amygdala, cerebral cortex, and hippocampal formation (Fig. 4A). These terminals were again similar to ChAT-positive puncta found in those same regions (Fig. 4B). Some NGF receptor-labeled puncta were seen in the reticular nucleus of the thalamus, but these morphologic entities were less dense than terminals immunoreactive for ChAT in the same nucleus (Fig. 4C, compare with Fig. 4D).

Somata immunoreactive for NGF receptor were observed occasionally within the traditional boundaries of the olfactory tubercle and caudate–putamen nucleus (Fig. 5, see also Fig. 7), usually in caudoventral aspects of the latter cellular configuration (Fig. 5A, see also Fig. 7), although some such cells were present at more intermediate but not rostral levels (Fig. 5B, see also Fig. 7). The number of such neurons, all of which expressed ChAT, did not exceed four on any one brain section analysed and did not surpass 32 in any one brain examined.

Somata immunoreactive for NGF receptor were not detected in the pedunculopontine and laterodorsal tegmental nuclei (see also Fig. 8), which contain ChAT-positive neurons of the pontomesencephalotegmental cholinergic complex (for definition and projection patterns, see Ref. 35). Similarly, no labeling for NGF receptor was seen in the proximal processes of neurons there or in the major projection target of this brainstem cholinergic system,[35]

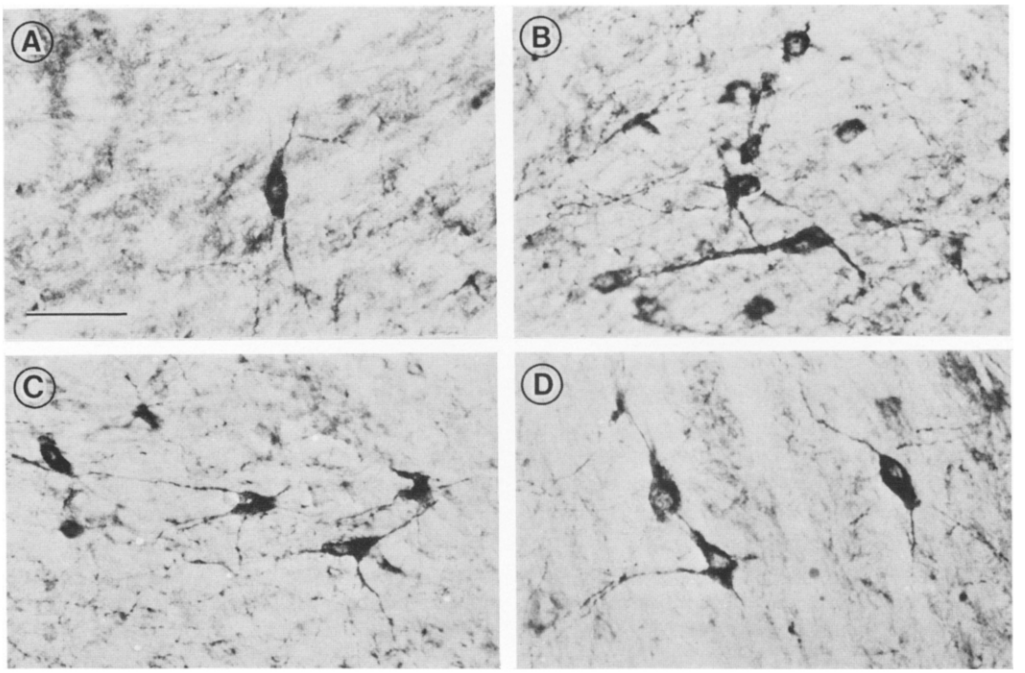

Fig. 1. Neurons demonstrating NGF receptor-like immunoreactivity in the medial septal nucleus (A), vertical limb of the diagonal band (B), substantia innominata (C), and nucleus basalis (D). Avidin–biotin method. Scale bar in A is 50 μm and applies also to frames B–D.

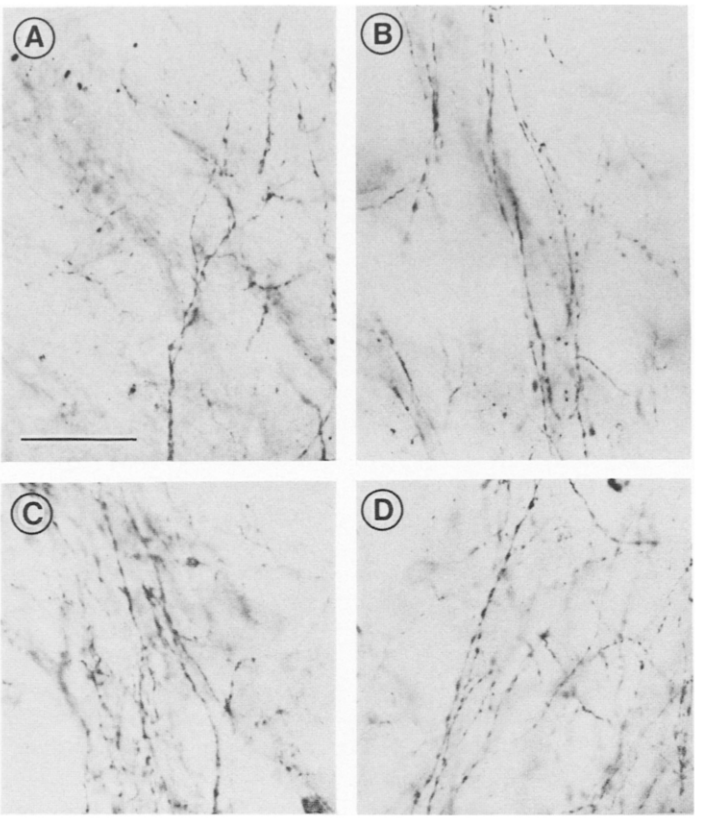

Fig. 2. NGF receptor-labeled (A,C) and ChAT-labeled (B,D) fibers in the internal capsule. Peroxidase–antiperoxidase method. Fibers illustrated in A and B are located in the dorsal part of the internal capsule and appear to be coursing toward the neocortex. Fibers in C and D are found in the internal capsule intermingled with many nucleus basalis cells (not shown) from which they may derive. Scale bar in A is 50 μm and applies also to B–D.

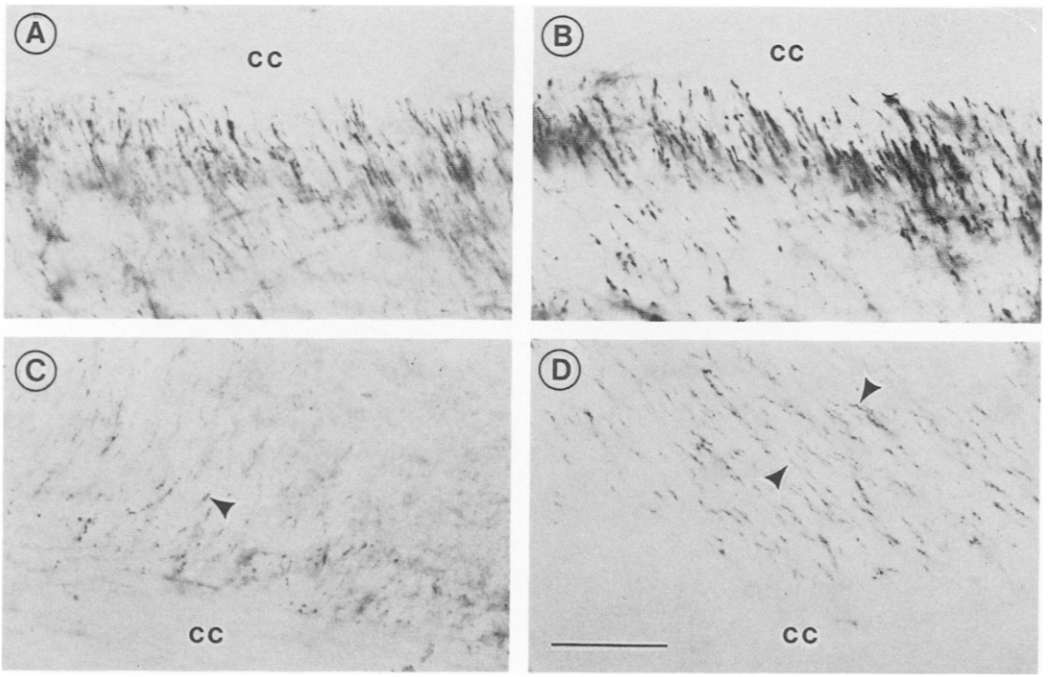

Fig. 3. Fibers immunoreactive for NGF receptor (A,C) and ChAT (B,D) in the dorsal fornix (A,B) and the cingulum (C,D). Peroxidase–antiperoxidase method. Arrowheads in C and D point to individual fibers. Abbreviation: cc, corpus callosum. Scale bar in D is 50 μm and applies also to A–C.

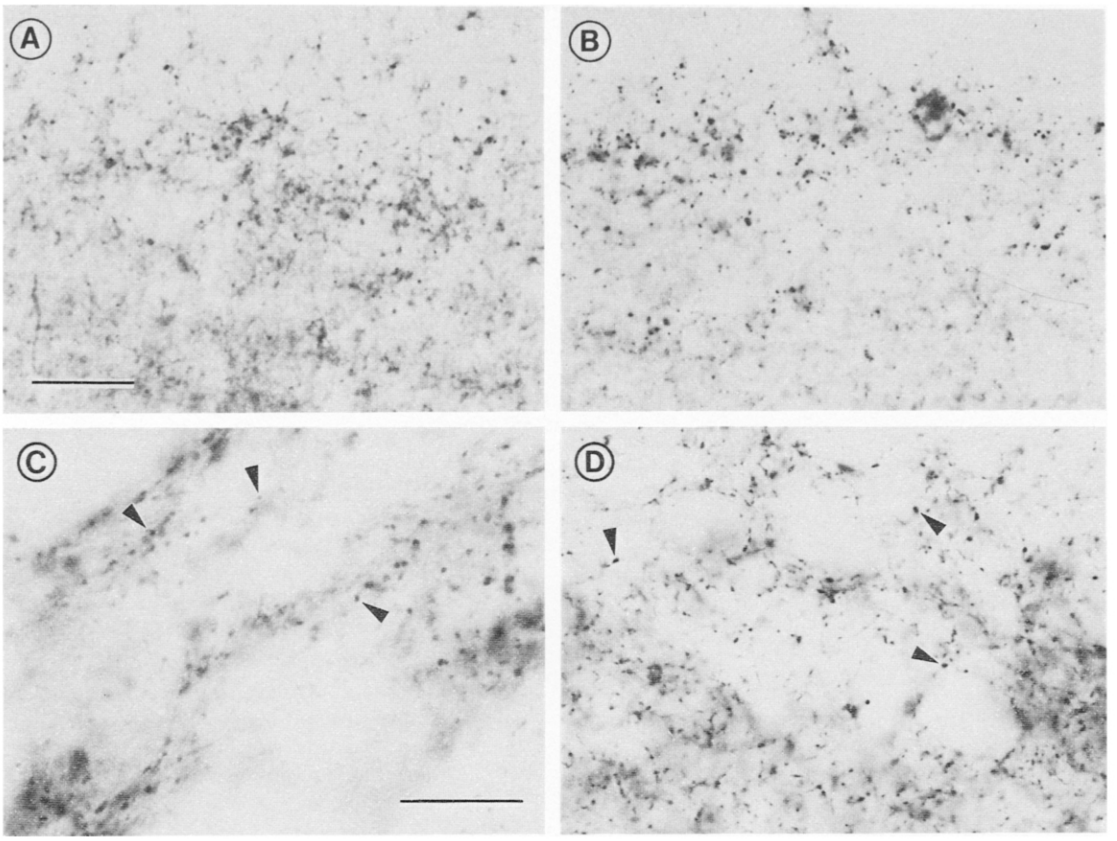

Fig. 4. Puncta immunoreactive for NGF receptor (A,C) and ChAT (B,D) in the dentate gyrus (A,B) and the reticular nucleus of the thalamus (C,D). Peroxidase–antiperoxidase method. Arrowheads in C and D point to presumed terminals of comparable sizes. Scale bar in A is 30 μm and applies also to B. Scale bar in C is 25 μm and applies also to D.

specifically the thalamus, except for the reticular nucleus as described in the foregoing commentary.

Co-localization of nerve growth factor receptor and choline acetyltransferase

The double-label procedure permitted unequivocal identification of ChAT-positive neurons expressing NGF receptor (Fig. 6), and quantitative analyses of all brain sections examined revealed that a mean of 92% of the ChAT-containing cells in the basal nuclear complex demonstrated NGF receptor (see also Fig. 7). Although virtually all, on average 99.1%, of the neurons expressing NGF receptor in the basal nuclear complex were cholinergic (Fig. 7), individual brain sections sometimes contained one–three cells that demonstrated that receptor but were not immunoreactive for ChAT (Fig. 7). In any case, the number of ChAT-positive but NGF receptor-

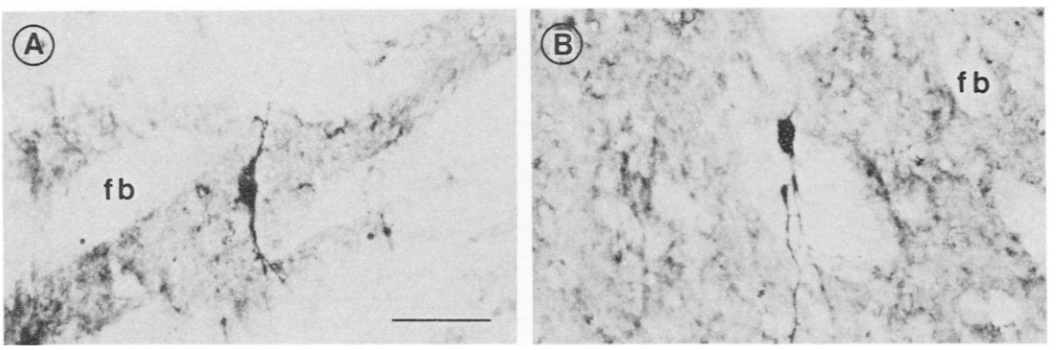

Fig. 5. Somata demonstrating NGF receptor-like immunoreactivity at caudoventral (A) and more intermediate (B) echelons of the caudate–putamen complex. Avidin–biotin method. The cells in A and B are located, respectively, at the approximate levels of the farthest right and middle brain sections illustrated in Fig. 7. Abbreviation: fb, fiber bundle penetrating striatum. Scale in A is 50 μm and applies also to B.

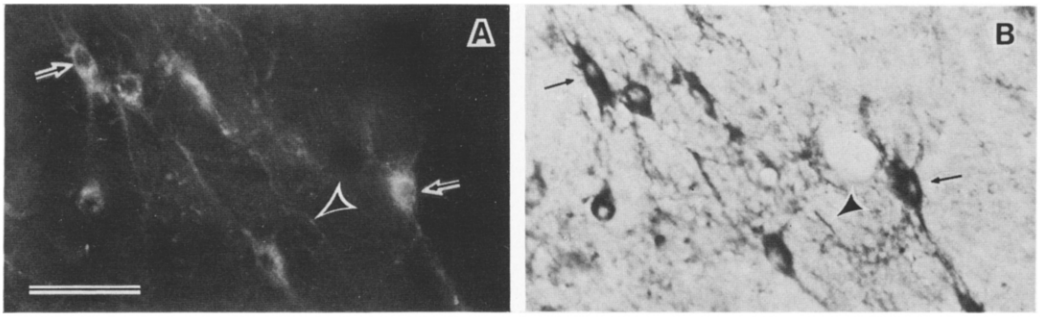

Fig. 6. Somata of the nucleus basalis demonstrating both NGF receptor-like (A) and ChAT-like (B) immunoreactivity. NGF receptor was visualized with a second antibody labeled with fluorescein isothiocyanate and ChAT was demonstrated according to the avidin–biotin procedure. Frames A and B depict the same tissue section. Arrows point to labeled somata. Arrowheads point to the same fiber. Scale bar in A is 50 μm and applies also to B.

negative somata was always greater (mean: 7.1%) in our material than the converse situation (Fig. 7). As intimated previously and unlike the basal forebrain, ChAT-positive neurons in the pedunculopontine and laterodorsal tegmental nuclei were not immunoreactive for NGF receptor (Fig. 8).

DISCUSSION

The present results indicate that NGF receptor in the adult is almost exclusively affiliated with cholinergic neurons in the basal forebrain (see also Refs 2, 9) and with no other cholinergic constellation contained within the brain, including the phenotypically similar cells of the pontomesencephalic tegmentum. This differential receptor distribution suggests that NGF itself may have an important, perhaps specific, role in the physiology of basal forebrain cholinergic neurons, a conjecture having substantial implications

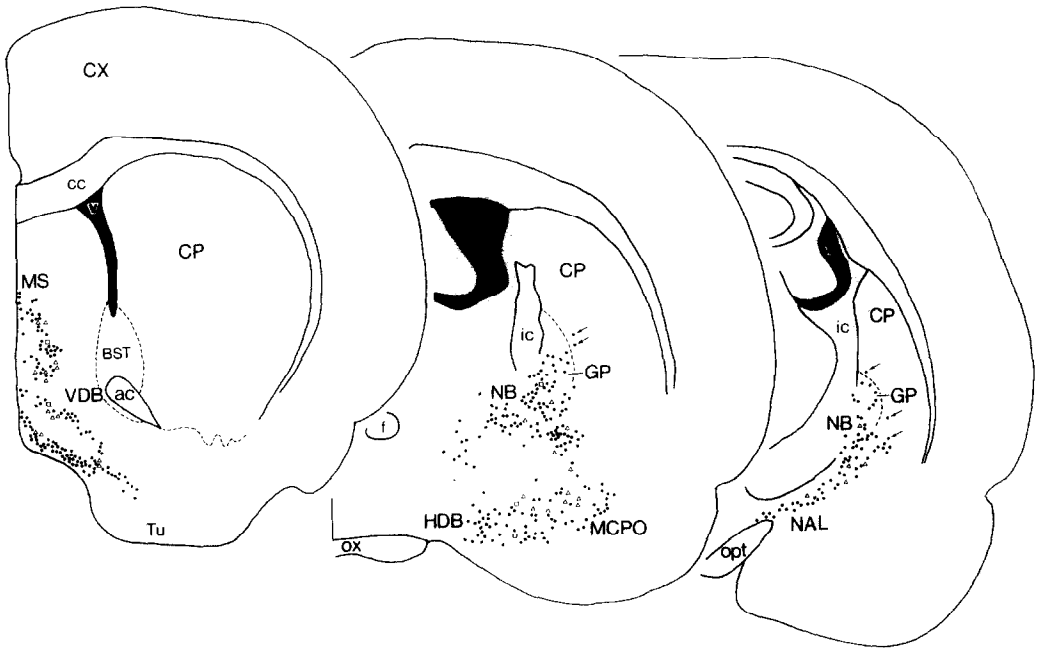

Fig. 7. Representative example of the distribution of NGF receptor- and ChAT-immunoreactive somata in the telencephalon reconstructed from brain sections processed sequentially for both neurochemical indices. Individual sections were chosen to illustrate maximally the major components of the basal forebrain cholinergic system. Drawings are arranged rostro-caudally from left to right and correspond roughly to bregma levels +0.2, −0.92 and −2.3 mm in the stereotaxic atlas of Paxinos and Watson.[27] Each symbol represents one cell. Solid circles represent somata that were labeled for both NGF receptor and ChAT. Open triangles depict cells that were ChAT-positive but NGF receptor-negative. Open squares illustrate somata that were NGF receptor-positive but ChAT-negative. Arrows point to cells lying within the traditional boundaries of the caudate–putamen nucleus. Abbreviations: ac, anterior commissure; BST, bed nucleus of the stria terminalis; cc, corpus callosum; CP, caudate–putamen complex; CX, cortex; f, fornix; GP, globus pallidus; HDB; horizontal limb of the diagonal band; ic, internal capsule; MCPO, magnocellular preoptic field; MS, medial septal nucleus; NAL, nucleus of the ansa lenticularis; NB, nucleus basalis; opt, optic tract; ox, optic chiasm; Tu, olfactory tubercle; v, ventricle; VDB, vertical limb of the diagonal band.

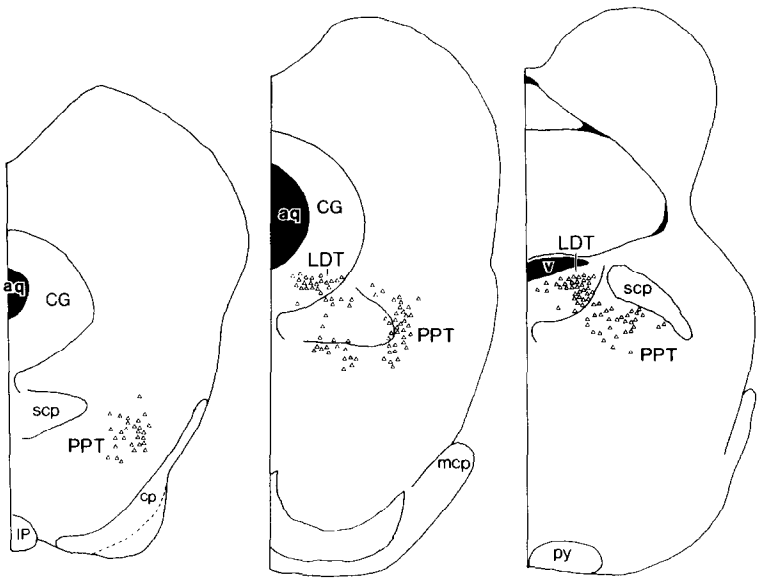

Fig. 8. Representative example of the distribution of cells comprising the pontomescencephalotegmental cholinergic complex at three different levels. Individual sections were chosen to illustrate maximally the different components of that system. Tissue represented was processed sequentially for both NGF receptor and ChAT. Illustrations are arranged rostrocaudally from left to right and correspond roughly to bregma levels −6.8, −8.3 and −8.8 mm in the stereotaxic atlas of Paxinos and Watson.[27] For explanation of symbols, see legend of Fig. 7. Each triangle depicts one cell except for the nuclear group indicated LDT in the farthest right frame, where, because of the high density of cells, each symbol represents one–three neurons. Abbreviations: aq, cerebral aqueduct; CG, central gray; cp, cerebral peduncle; IP, interpeduncular nucleus; LDT, laterodorsal tegmental nucleus; mcp, middle cerebellar peduncle; py, pyramidal tract; PPT, pedunculopontine tegmental nucleus; scp, superior cerebellar peduncle; v, ventricle.

for human neurologic disorders such as Alzheimer's disease (for extensive discussion and critical analysis, see Ref. 36) in which basal forebrain cholinergic neurons are affected prominently[1,7,19] and in which the cells of the pedunculopontine and laterodorsal tegmental nuclei do not undergo degeneration.[40] The selectivity of NGF with respect to basal nuclear cholinergic neurons would be even more extraordinary if it could be shown that the few ChAT-positive cells demonstrating NGF receptor in the olfactory tubercle and neostriatum were actually ectopically located basal forebrain cells.

In partial contradistinction to the previous observations of Hefti *et al.*[17] showing localization of NGF receptor preferentially on the outer membrane of the soma and proximal processes and immediately surrounding the nucleus of basal forebrain neurons, the present results suggest a more uniform distribution of the reaction product throughout the perikaryon, dendrites, and axons of those cells. Species differences (human[17] vs rat in the current experiments) perhaps account for some of these differences, as well as the fact that different monoclonal antibodies were employed in the two studies. Similarly, we observed puncta positive for NGF receptor in the amygdala whereas Hefti *et al.*[17] did not, and, again, the reasons for this discrepancy may be related to species and antibody variances. Nonetheless, to the extent that NGF receptor is co-existent with ChAT-positive projection neurons in the telencephalon, our observations are highly compatible with what is known about the hodology of the basal forebrain cholinergic system.[3,4,34,35,37,39] Indeed, the high percentage of ChAT-positive cells in the basal nuclear complex demonstrating NGF receptor is consonant with our observation that fibers and terminals labeled for NGF receptor in telencephalic sites mirror the topographies of ChAT-positive fibers and terminals found in those same brain regions. The cholinergic basal forebrain has been shown to project to telencephalic loci such as the cingulate and adjacent frontoparietal cortex by means of fibers running through the cingulum.[23,39] Cholinergic projections to the hippocampal formation also have been traced through the fimbria-fornix, and laterally coursing fibers have been folllowed through the internal capsule to innervate lateral cortical targets.[23,39] It would appear likely, therefore, that the NGF receptor-immunoreactivity affiliated with fiber tracts in the internal capsule, cingulum, and fimbria-fornix and with terminals in the amygdala, cortex, hippocampus, and dentate gyrus in the current study derived from NGF receptor-labeled somata in the basal forebrain. This contention is supported by the finding that morphologic similarities exist between NGF receptor- and ChAT-labeled fibers and terminals.

Although Eckenstein[9] reported transient expression of NGF receptor in the thalamus of the developing rat, such immunoreactivity was not detected in the adult thalamus in that study, in contrast to the

current observations that puncta demonstrating NGF receptor are found in the reticular nucleus of the thalamus, even though those terminals are less dense than ChAT-positive puncta in the same region. Again, however, our findings in this regard are compatible with currently available neuroanatomic information. Although the thalamic reticular nucleus receives a relatively large cholinergic projection from cells in the pontomesencephalic tegmentum,[35] which lack NGF receptor as demonstrated in the present study, it may receive additional cholinergic input from the basal nuclear complex,[22,23] and this latter neuroanatomic actuality could account for the presence of NGF receptor we observed in that diencephalic nucleus.

The accumulation of NGF receptor-immunoreactivity in cholinergic fiber tracts in response to cortical ablations[31] and fimbria-fornix lesions[20] is also consonant with our findings indicating the normal presence of NGF receptor in those pathways. Although not extensively documented in previous publications, the existence of NGF receptor in fiber bundles and telencephalic terminals of normal adult rats that we observed might reflect the increased sensitivities of the peroxidase–antiperoxidase and avidin–biotin methods used in the current investigation compared to other reports or, in part, to the species employed. The absence of NGF receptor-immunoreactivity in the cholinergic pontomesencephalic tegmentum is compatible with earlier reports of an absence of NGF binding to cells found there.[28]

The relative lack of NGF receptor labeling in the striatum has been reported previously,[17] but the presence of a few cholinergic cells demonstrating NGF receptor that we observed within the traditional borders of the caudate–putamen nucleus, to the extent that such cells are in fact striatal (see above), suggests that some striatal neurons also may be sensitive to NGF (compare with data in Ref. 28). Such a conjecture is compatible with the finding that NGF increases ChAT activity in the striatum during development.[24]

Although receptors for NGF appear to be associated with the vast majority of cholinergic basal forebrain somata, fibers, and terminal fields, such receptors are not found in relation to the somata, fiber tracts, and terminal fields of the cholinergic pontomesencephalic complex, suggesting fundamental, but as yet incompletely understood, neurobiologic differences between the two constellations of cholinergic neurons. Similarly, the nature of the postulated physiologic role of NGF in mature cholinergic neurons of the basal nuclear complex has yet to be established.

One such function could involve the regulation of ChAT in the basal forebrain cholinergic system. Indeed, NGF has been reported to facilitate ChAT synthesis in basal nuclear neurons during development,[13,25] in response to injury,[15] and in cell culture.[16] It has a less pronounced effect on ChAT synthesis in the non-lesion mature brain (see Ref. 15), however. Furthermore, NGF antibodies do not decrease ChAT synthesis during development,[13] suggesting that this growth factor in the normal basal forebrain may not alter ChAT production.

Another potentially important role for NGF in the physiology of cholinergic basal forebrain neurons might encompass the regulation of neurite formation during normal phases of structural plasticity in the mature brain.[5,36] This neurotrophic agent has been shown to enhance neurite outgrowth following cholinergic denervation of the neocortex[5] and in hippocampal slices.[12] Because microtubules are essential for neurite elongation, it is conceivable that NGF stimulates such outgrowth in basal forebrain cholinergic neurons by increasing sytheses of tubulin and microtubule associated proteins according to a ribonucleic acid-dependent mechanism similar to that postulated for NGF stimulation of the production of these same cytoskeletal proteins in other cell systems.[8,11] Preliminary observations of this laboratory showing that the microtubule associated protein 1 (MAP1) has a distribution in the rat forebrain similar to that of cholinergic terminals is a finding not incompatible with such a conjecture.

Deductions based on the present and previous observations[31] suggest that NGF receptor is normally transported throughout the fibrous arbors of cholinergic neurons of the basal nuclear complex. It seems possible, therefore, that presumed NGF receptor-mediated processes such as the regulation of ChAT synthesis and the stimulation of neurite outgrowth in telencephalic cholinergic neurons may occur, perhaps within narrow margins, in the mature, non-injured rat brain. Since the cholinergic basal forebrain system has been implicated repeatedly in learning and memory processes (for reviews, see Refs 1 and 7), it is conceivable that NGF receptor-mediated mechanisms subserve, in part, functions related to cognition. The apparent facilitatory effect of NGF on behavior in certain maze-learning tasks is consistent with such a notion,[33] but caveats exist.[26]

Acknowledgements—We thank Drs Eric Shooter and Felix Eckenstein for their generous gifts, respectively, of hybridoma cells secreting the 192 IgG antibody against NGF receptor and a monoclonal antibody against ChAT. The assistance of Dr Jean de Vellis with cell culture preparations is greatly appreciated, and Mr Justin Oh is thanked for his excellent technical assistance. This research was supported by USPHS grant NS 10928 to L.L.B.

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(*Accepted* 22 *November* 1988)