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Nerve growth factor receptor molecules in rat brain

(monoclonal antibody/immunoprecipitation/retrograde transport)

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ABSTRACT **We have developed a method to immunoprecipitate rat nerve growth factor (NGF) receptor proteins and have applied the method to detect NGF receptor molecules in the rat brain. Crosslinking ^{125}I-labeled NGF to either PC12 cells or cultured rat sympathetic neurons yielded two radiolabeled molecules (90 kDa and 220 kDa) that were immunoprecipitated by monoclonal antibody 192-IgG. Further, 192-IgG precipitated two radiolabeled proteins, with the expected sizes (80 kDa and 210 kDa) of noncrosslinked NGF receptor components, from among numerous surface-iodinated PC12 cell proteins. These results demonstrate the specific immunoprecipitation of NGF receptor molecules by 192-IgG. We applied the ^{125}I-NGF crosslinking and 192-IgG-mediated immunoprecipitation procedures to plasma membrane preparations of the following areas of rat brain: medial septum, cerebellum, brainstem, hippocampus, cerebral cortex, thalamus, and olfactory bulb. NGF receptor molecules of the same molecular masses as the peripheral receptor components were consistently detected in all of these regions and in preparations from whole brains. Removal of the peripheral sympathetic innervation of the brain did not eliminate these NGF receptor proteins, indicating that the receptor is endogenous to central nervous system tissues. We also observed retrograde transport of ^{125}I-labeled 192-IgG from the parietal cortex to the nucleus basalis and from the hippocampus to the nucleus of the diagonal band of Broca and the medial septal nucleus. These findings demonstrate the presence in brain of NGF receptor molecules indistinguishable from those of the peripheral nervous system.**

Nerve growth factor (NGF) promotes the survival and function of sympathetic neurons and neural crest-derived sensory neurons (1). Recent studies have suggested that NGF is active not only in the peripheral nervous system but also in the brain. Treatment of cultured brain neurons with NGF (2, 3) or intraventricular administration of NGF to neonatal rats (4–6) increases the choline acetyltransferase activity of cholinergic neurons. Radiolabeled NGF injected into the parietal cortex of adult rats is retrogradely transported to large neurons in the nucleus basalis region (7), suggesting the operation of receptor-mediated uptake and transport mechanisms similar to those of peripheral sympathetic and sensory neurons. Endogenous NGF molecules and mRNA encoding NGF have been detected in brain regions containing the magnocellular cholinergic neurons of the basal forebrain (8, 9). However, treatment of these neurons *in vivo* and in tissue culture with anti-NGF antibodies does not reduce their choline acetyltransferase activities (4), and NGF fails to promote fiber outgrowth and survival of cultured brain neurons (3). Such negative results raise the possibility that exogenous NGF administered to the brain behaves as an agonist for a different, brain-endogenous agent by binding to the agent's receptor. The hypothesis that NGF acts physiologically in the brain, therefore, would be strengthened greatly by evidence of specific NGF receptors in brain tissue. Although NGF-binding activity has been reported in embryonic chicken brain (10, 11), there has been no molecular characterization of these binding moieties. Accordingly, we probed for the receptor with a monoclonal antibody, 192-IgG, that binds to rat NGF receptor specifically, without competing with NGF, and which is retrogradely transported within sympathetic neurons (12, 13). We found that 192-IgG immunoprecipitated molecules from rat brain tissues which were indistinguishable from those of the peripheral NGF receptor and that the monoclonal antibody was retrogradely transported within brain neurons. These results demonstrate the presence of NGF receptor molecules in brain tissue and thereby support the hypothesis that endogenous NGF is active within the central nervous system.

MATERIALS AND METHODS

Materials. The 2.5S subunit of NGF was purified from male mouse submaxillary glands (14). Porcine insulin (26.8 units/mg) was provided by Eli Lilly. Guanethidine was a gift from CIBA/Geigy. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) was purchased from Pierce, and formalin-fixed *Staphylococcus aureus* cells (Pansorbin) were obtained from Calbiochem. Electrophoresis reagents were from Bio-Rad. All other chemicals were supplied by Sigma. Sprague–Dawley rats from Chappel Breeders (St. Louis, MO) were used in all experiments.

Tissue Culture. PC12 cells, dissociated E21 (embryonic day 21) rat superior cervical ganglion (SCG) neurons (15), Vero (African green monkey kidney) cells, and L-929 mouse fibroblasts were prepared and cultured as described (16).

Dissection of Brain Regions and Preparation of Plasma Membranes. Brains were removed from adult male rats. The medial septal region was obtained by punching out a ventral-to-dorsum core of tissue with a borer (5 mm diameter) centered on the optic chiasm and then discarding the section of cerebral cortex. All other regions were dissected along standard anatomical landmarks; the sample designated as cerebral cortex consisted of occipital and parietal cortices. A plasma membrane-enriched microsomal fraction was prepared for each experiment from 30–100 animals (17), and the protein content of each sample was determined by the method of Lowry *et al.* (18), with bovine serum albumin (BSA) as the standard.

Monoclonal and Polyclonal Antibodies. Monoclonal antibody 192-IgG was purified from ascites by means of the Bio-Rad monoclonal antibody purification system. Monoclonal antibodies MCβ1 (19) and 151 (20) were purified from tissue culture supernatants by affinity chromatography on Pharmacia protein A-Sepharose CL. Polyclonal antibodies

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Abbreviations: NGF, nerve growth factor; ^{125}I-NGF, ^{125}I-labeled NGF; EDAC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; SCG, superior cervical ganglion.

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directed against 192-IgG were developed by immunization of New Zealand White rabbits (Boswell, Pacific, MO) with purified 192-IgG and were affinity-purified on 192-IgG-Sepharose 4B.

Iodination Procedure. NGF and 192-IgG were radioiodinated (21) with $Na^{125}I$ from Amersham. Specific activities of 1.8–2.3 Ci/μmol (1 Ci = 37 GBq) for NGF and 1.1–1.4 Ci/μmol for 192-IgG were attained. PC12 cells were surface-radioiodinated by lactoperoxidase/glucose oxidase catalysis (22); 2×10^7 cells were treated with 1 mCi of $Na^{125}I$.

Crosslinking of ^{125}I-labeled NGF (^{125}I-NGF) to the NGF Receptor and Electrophoresis. The NGF receptor proteins were affinity-labeled by a modification of the protocol of Grob *et al.* (23). PC12 cells, Vero cells, L-929 cells, or plasma membrane preparations were suspended in 0.5 ml of 20 mM KH_2PO_4/160 mM NaCl, pH 7.0, and combined with ^{125}I-NGF (2 nM final concentration). Cultured SCG neurons were washed and maintained in NGF-free medium for 18 hr and then exposed to the ^{125}I-NGF while still attached to the tissue culture dish. After 60 min at 37°C, EDAC was added (20 μl) and the mixture was incubated for 20 min at 22°C. The crosslinking reaction was quenched with 50 mM Tris Cl (pH 7.6). The cells were then solubilized either in $NaDodSO_4$ sample buffer [4% $NaDodSO_4$/2% (vol/vol) 2-mercaptoethanol/20% (vol/vol) glycerol/0.008% bromophenol blue/0.125 M Tris Cl, pH 6.8] for electrophoresis or in *n*-octyl glucoside solution for immunoprecipitation (see below). Electrophoresis in 7% polyacrylamide/0.19% *N,N'*-methylenebis(acrylamide) gels was performed according to Laemmli (24).

Immunoprecipitation. Surface-labeled PC12 cells, ^{125}I-NGF-crosslinked PC12 cells, or ^{125}I-NGF-crosslinked membrane proteins were solubilized at 22°C for 60 min in phosphate-buffered saline (PBS: 20 mM KH_2PO_4/160 mM NaCl, pH 7.4) containing 2% *n*-octyl glucoside, 0.5% BSA, 1 mM iodoacetamide, and 1 mM phenylmethylsulfonyl fluoride (PMSF). The mixtures were clarified by ultracentrifugation at 100,000 × *g* for 60 min; at this point, surface-labeled proteins were further treated by mixing with Pansorbin (10%, wt/vol), followed by centrifugation (8000 × *g*, 3 min) to remove species that bound directly to the Pansorbin. The supernatants were combined with 192-IgG or other antibodies (10 μg/500 μl of solution) for 60 min at 22°C. Molecules bound to 192-IgG were immunoprecipitated with 40 μl of 10% (wt/vol) Pansorbin preadsorbed with anti-192-IgG antibodies (5 μg of antibodies/μl of 10% Pansorbin). After 45 min at 22°C, the Pansorbin was pelleted by centrifugation (8000 × *g*, 60 sec) and washed three times with PBS/1.3% *n*-octyl glucoside/0.5% BSA/0.5 M sucrose/1 mM iodoacetamide/1 mM PMSF, followed by an additional wash with PBS/1.3% *n*-octyl glucoside/1 mM iodoacetamide/1 mM PMSF. Immunoprecipitated materials were eluted by boiling in 100 μl of $NaDodSO_4$ sample buffer, with or without 2% 2-mercaptoethanol, and electrophoresed.

RESULTS

Crosslinking of ^{125}I-NGF to the NGF Receptor and Immunoprecipitation of the Complex by 192-IgG. Binding of ^{125}I-NGF to PC12 cells followed by crosslinking with EDAC resulted in the specific labeling of 90-kDa and 220-kDa receptor molecules (see immunoprecipitation results below). Labeling of both species was eliminated by addition of excess nonlabeled NGF but not by inclusion of insulin or cytochrome *c*, and no labeling occurred on cell lines lacking the NGF receptor (L-929 fibroblasts and Vero cells, data not shown). Because 192-IgG and NGF do not compete with each other for binding to the NGF receptor (12, 13), we expected that 192-IgG would bind these ^{125}I-NGF-crosslinked receptor molecules. Therefore, ^{125}I-NGF-labeled PC12 cells were solubilized with *n*-octyl glucoside, allowed to bind 192-IgG, and precipitated with anti-192-IgG/Pansorbin. Antibody 192-IgG immunoprecipitated both the 90-kDa and the 220-kDa ^{125}I-NGF–receptor complexes (Fig. 1*A*, lanes 1 and 2). These same radioligand-labeled species were immunoprecipitated by MCβ1 [a monoclonal antibody directed against the NGF molecule itself (19)], thus verifying that they comprise NGF receptor component proteins labeled with ^{125}I-NGF (autoradiogram not shown). No labeled receptor was obtained when the 192-IgG was either omitted (lane 5) or replaced with 151, a monoclonal antibody directed against the epidermal growth factor (EGF) receptor of PC12 cells (20) (lanes 3 and 4). Comparison of ^{125}I-NGF-crosslinked, 192-IgG-immunoprecipitated molecules from PC12 cells (lane 6) and cultured rat SCG neurons (lane 7) suggests that the NGF receptor proteins of PC12 cells are identical to those of sympathetic neurons.

The results of ^{125}I-NGF crosslinking and 192-IgG immunoprecipitation indicated that 192-IgG binds NGF receptor proteins but did not eliminate the possibility that 192-IgG might bind also to other proteins sharing a common epitope with the NGF receptor. We therefore indiscriminately labeled cell surface proteins by iodination of intact PC12 cells and then solubilized the cells and immunoprecipitated with 192-IgG. Although numerous proteins were labeled by the iodination (autoradiogram not shown), 192-IgG mediated the immunoprecipitation of only two molecules (80 kDa and 210 kDa) which differed in molecular mass from those obtained in the earlier experiments by ≈13 kDa, the molecular mass of NGF monomer (Fig. 1*B*). Thus, 192-IgG binds specifically and exclusively to NGF receptor molecules. Consistent with other reports (23, 25), the apparent molecular masses of the NGF receptor proteins were lower (60 kDa and 180 kDa) under nonreducing conditions of electrophoresis (Fig. 1*B*, lane 1, and unpublished observations of ^{125}I-NGF-crosslinked molecules); this may be due to internal disulfide bonds maintaining a more compact structure of the molecule.

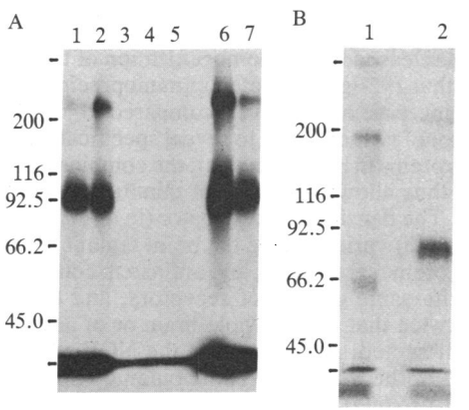

FIG. 1. Immunoprecipitation of ^{125}I-NGF-crosslinked receptor molecules and surface-labeled receptor molecules. Autoradiograms of dried polyacrylamide gels are shown. (*A*) Lanes 1–5: PC12 cells (2×10^7) were combined with 2 nM ^{125}I-NGF, crosslinked in 10 mM EDAC, solubilized, divided into 10 aliquots, and then processed in duplicate for immunoprecipitation with 192-IgG (lanes 1 and 2), anti-EGF receptor monoclonal antibody 151 (lanes 3 and 4), or no primary antibody (lane 5). Lanes 6 and 7: PC12 cells (2×10^6, lane 6) or cultured SCG neurons (3×10^5, lane 7) were crosslinked with ^{125}I-NGF, solubilized, and immunoprecipitated with 192-IgG. All samples were eluted and electrophoresed under reducing conditions. (*B*) PC12 cells (2×10^7) were surface-radioiodinated, solubilized, and immunoprecipitated with 192-IgG. The precipitated proteins were eluted and electrophoresed under nonreducing (lane 1) or reducing (lane 2) conditions. The size markers (values at left in kDa) were reduced. The positions corresponding to the tops of the separation gels and to the tracking dye fronts are also indicated.

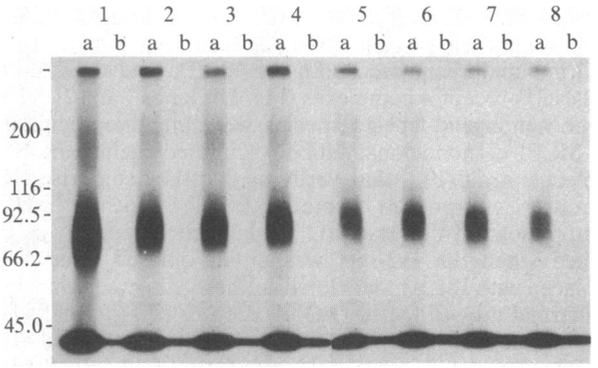

FIG. 2. Immunoprecipitation of ^{125}I-NGF-crosslinked receptor molecules from rat brain. Plasma membrane preparations from SCG (1 mg of protein) and the various brain regions (5 mg of protein in each sample) were combined with 2 nM ^{125}I-NGF [in the absence (lanes a) or presence (lanes b) of 2 μM nonlabeled NGF], crosslinked in 20 mM EDAC, solubilized, and immunoprecipitated with 192-IgG. Only 1/10th of the total sample obtained from SCG neurons was loaded. The samples (and their radioactivities) were SCG (2600 cpm, lanes 1), brainstem (1400 cpm, lanes 2), medial septum (1500 cpm, lanes 3), cerebellum (1400 cpm, lanes 4), thalamus (700 cpm, lanes 5), hippocampus (700 cpm, lanes 6), cerebral cortex (800 cpm, lanes 7), and olfactory bulb (300 cpm, lanes 8).

Immunoprecipitation of ^{125}I-NGF-Crosslinked Receptor from Rat Brain. We then applied the ^{125}I-NGF crosslinking and 192-IgG immunoprecipitation protocols to plasma membrane preparations from rat brain regions (Fig. 2). These procedures revealed radioligand-labeled NGF receptor molecules that, by the criteria of specific labeling by ^{125}I-NGF, apparent size, and 192-IgG binding activity, are indistinguishable from the receptor molecules of sympathetic neurons. The ^{125}I-NGF-crosslinked receptors immunoprecipitated from membrane preparations generally exhibited more size heterogeneity, lower apparent molecular mass of both component molecules, and diminished labeling of the high molecular mass species relative to the low (compare lane 7 of Fig. 1 to lane 1 of Fig. 2). These results are likely caused by proteases released during homogenization of the tissues. We estimate that 192-IgG-mediated immunoprecipitation affords a 50-fold increase in sensitivity compared to ^{125}I-NGF crosslinking alone† (which failed to reveal specifically radioligand-labeled proteins in brain samples); the combination of the two methods thus allows detection of minute quantities of NGF receptor. The density of the receptor (in terms of number per mg of protein) varies among the brain regions (Table 1). The medial septum, cerebellum, and brainstem contained approximately the same density of receptors, and this value was roughly twice that of the whole brain or of any of the other regions dissected. Assuming that the NGF receptors of the brain and of the SCG have equal binding affinities (for both NGF and 192-IgG) and that they are crosslinked to ^{125}I-NGF with the same efficiency, we estimate the density of receptor in membrane preparations from whole brains to be 2% of that in preparations from the SCG.

To preclude the possibility that the NGF receptor molecules detected in brain tissue were actually contained in the sympathetic nerve termini of brain vasculature, we eliminated the sympathetic innervation by two methods. In the first procedure, rats were given a guanethidine treatment that causes virtually complete peripheral sympathectomy (25). The brains of the animals were then dissected into the various regions and assayed for NGF receptor as before (Table 1). Guanethidine sympathectomy caused no net change in the quantity of receptor in the whole brain and failed to eradicate receptor in any of the particular regions. Among the regions, the differences measured between treated and control tissues shift in both directions and are likely due to experimental uncertainty. In the second procedure, the left SCG was surgically removed from adult rats. Five days later, the brains were removed, the brainstem discarded, and the remaining structures bisected into right and left hemispheres. Since each SCG provides unilateral innervation of brain vasculature (26), this procedure removed sympathetic innervation to the left hemisphere of the brain rostral to the brainstem. There was no difference in the amount or apparent size of NGF receptor proteins detected in plasma membrane preparations from the two hemispheres (data not shown). These two sets of data eliminate the possibility that sympathetic innervation can account for the receptors detected in the brain. Control experiments confirmed the specificity of NGF and 192-IgG binding to these brain-endogenous NGF receptors: ^{125}I-NGF crosslinking was blocked by nonlabeled NGF, but not by insulin or cytochrome *c*; the radioligand-labeled molecules were immunoprecipitated by 192-IgG, but not by the anti-epidermal growth factor receptor monoclonal antibody (data not shown).

Retrograde Transport of ^{125}I-labeled 192-IgG. The retrograde transport of NGF and 192-IgG in peripheral sympathetic neurons involves specific receptor-mediated uptake (1, 13). We tested the ability of ^{125}I-labeled 192-IgG to be transported in the brain by injecting trace quantities into the parietal cortex or the hippocampus, areas receiving the projections of cholinergic neurons from the nucleus basalis or from the nucleus of the diagonal band of Broca and medial septal nucleus, respectively. Injection into the parietal cortex

Table 1. Distribution of NGF receptor among brain regions and changes caused by sympathectomy

Region	Receptor density,* cpm/mg of protein	% change after sympathectomy†
Whole brain	0.62 ± 0.11	2
Medial septum	0.96 ± 0.06	−18
Cerebellum	0.95 ± 0.08	−5
Brainstem	0.89 ± 0.13	9
Hippocampus	0.59 ± 0.13	25
Cortex	0.53 ± 0.04	−6
Thalamus	0.45 ± 0.03	−23
Olfactory bulb	0.40 ± 0.04	−24

*In three separate experiments, brains were removed from 100, 32, and 32 rats. The different regions were dissected and pooled separately. Plasma membrane fractions prepared from the various regions (each containing 5 mg of protein) were combined with 2 nM ^{125}I-NGF, crosslinked by reaction in 20 mM EDAC, and subjected to NaDodSO$_4$/PAGE. The portions of the polyacrylamide gel containing the 90-kDa species were cut out to measure the radioactivity. The absolute numbers (corresponding to cpm per mg of protein) from each experiment were normalized to the value of the brain region with the greatest amount of crosslinked ^{125}I-NGF, and the mean ± SD of these normalized values from the three experiments are presented.

†Thirty-two 1-week-old rats were injected subcutaneously with guanethidine (50 mg/kg of body weight) each day for 3 weeks. At 7 weeks of age, treated rats and age-matched control animals were decapitated and their brains were dissected into the various regions. Separate membrane preparations from treated and control tissues were assayed for NGF receptor as described above. The difference between the radioactivity in the 90-kDa species immunoprecipitated from control and treated tissues was calculated and tabulated as the percentage of the control value. A negative number indicates a decrease in the amount of 90-kDa ^{125}I-NGF-crosslinked species in treated tissue compared to control.

†The ratio of specific to nonspecific association of ^{125}I-NGF with SCG membranes (i.e., the radioactivities of samples bound and crosslinked to ^{125}I-NGF in the absence or presence of nonlabeled NGF) is 50-fold greater after 192-IgG immunoprecipitation than before.

led to accumulation of radiolabel in large neurons of the ipsilateral nucleus basalis magnocellularis (Fig. 3*A*). Injection into the hippocampus resulted in heavy labeling of numerous neurons in all sections through the nucleus of the diagonal band of Broca (Fig. 3*B*) and labeling of scattered neurons in the medial septal nucleus (Fig. 3*C*); a few neurons in the nucleus of the contralateral diagonal band of Broca were also labeled (not shown). No accumulation of ^{125}I-labeled 192-IgG outside of these regions was observed; in particular, the thalamus and locus ceruleus were free of label.

DISCUSSION

The monoclonal antibody 192-IgG, developed by Chandler *et al.* (12), binds specifically to the rat NGF receptor without blocking NGF binding and is retrogradely transported by sympathetic neurons (13). It appears to act as an antagonist in two responses of PC12 cells to NGF; namely, neurite regeneration (12) and induction of c-*fos* mRNA (J. Milbrandt, personal communication). Using ^{125}I-NGF and this monoclonal antibody, we have devised procedures to specifically label and immunoprecipitate rat NGF receptor molecules. The apparent sizes of the ^{125}I-NGF-crosslinked species, 90 kDa and 220 kDa, obtained from both PC12 cells and cultured SCG neurons are in agreement with the results of some (23, 28), but not all, other investigators. The major discrepancy is that we do not label the additional species of ≈150 kDa reported by groups using *N*-hydroxysuccinimidyl-4-azidobenzoate (HSAB) as the crosslinking agent (29, 30). This difference may be caused by dissimilar selectivities of EDAC and HSAB for available amino and carboxyl groups. It is significant that the two groups that have affinity-purified the NGF receptor detect only two molecules, of 70–85 kDa and 180–200 kDa (31, 32). It is possible that the 150-kDa species is generated by only the HSAB-mediated crosslinking reaction and is a complex of ^{125}I-NGF (13 kDa), the 80-kDa NGF receptor protein, and a third molecule of ≈60 kDa that binds either NGF or, more likely, the 80-kDa NGF receptor component.

Monoclonal antibody 192-IgG mediated the immunoprecipitation of both 90-kDa and 220-kDa receptor molecules crosslinked by EDAC to ^{125}I-NGF (Fig. 1). Because 192-IgG immunoprecipitated, from numerous surface-iodinated PC12 cell proteins, only two radioactive species with the appropriate sizes for unoccupied NGF receptor molecules (80 kDa and 210 kDa), we conclude that 192-IgG binds these rat NGF receptor proteins specifically and exclusively. That 192-IgG immunoprecipitated a 210-kDa species from surface-labeled PC12 cells that had never been exposed to exogenous NGF argues that this larger form of the receptor is not produced, as has been suggested (33), by the association of two 80-kDa moieties with an NGF dimer (26 kDa).

The ^{125}I-NGF crosslinking and 192-IgG immunoprecipitation procedures have provided clear evidence that NGF receptor molecules are present in the rat brain. The persistence of receptor after chemical or surgical removal of sympathetic innervation of the brain indicates that the NGF receptor molecules detected are endogenous to central nervous system tissue. It appears that NGF receptor is distributed unevenly, but generally, throughout the brain, with the medial septum, the cerebellum, and the brainstem containing twice the density of all other regions. The presumption that NGF receptors exist on forebrain cholinergic neurons and on the central processes of neural crest-derived sensory neurons (2–8) would account for the receptors found in all the regions studied except for the thalamus and cerebellum, two structures for which there are no reported effects of NGF.‡ This wide and relatively uniform distribution of NGF receptors raises the possibility that neurons other than the cholinergic and sensory neurons, or even nonneuronal cells, contain NGF receptor molecules and, therefore, can respond to NGF. The crosslinking/immunoprecipitation procedures performed on grossly dissected samples, however, cannot resolve whether the NGF receptors detected in any region are located on neuronal perikarya, axons, or termini or on nonneuronal cells. Microanatomical analysis of the receptor can be achieved by transport/autoradiography studies such as the one presented in Fig. 3. The retrograde transport of trace amounts of ligands specific for the NGF receptor, namely NGF itself or 192-IgG, involves receptor-mediated uptake at the site of administration and active vectorial translocation to the distant somata (1, 13). The results shown in Fig. 3 indicate, therefore, that NGF receptor-bearing

‡The gross dissection of the thalamus may have included portions of the globus pallidus and some tracts of the septal–hippocampal pathway, which could account for at least some of the NGF receptor molecules detected. The samples of cerebellum, however, should have been free of extraneous tissues.

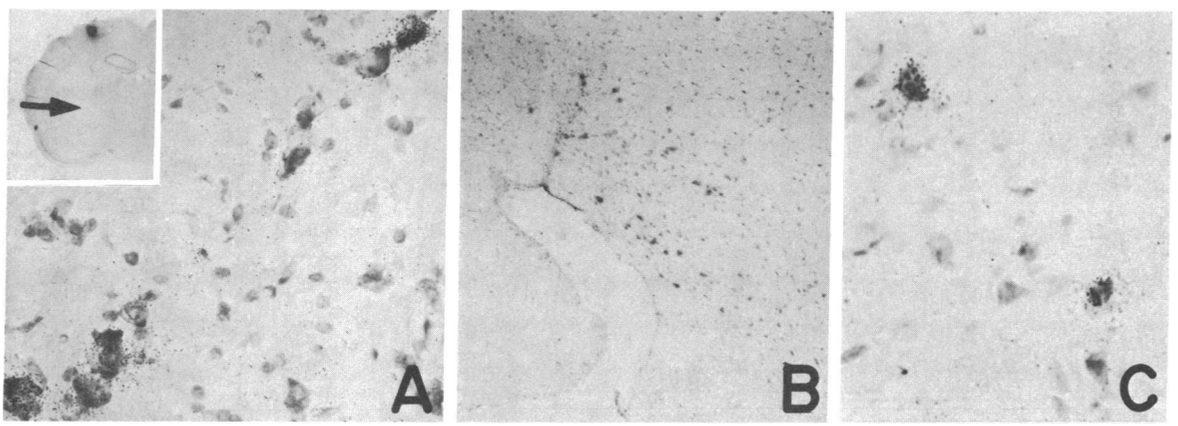

FIG. 3. Retrograde transport of ^{125}I-labeled 192-IgG. Radiolabeled 192-IgG (1 μl; 2 × 10^6 cpm) was injected stereotactically into the parietal cortex or hippocampus of anesthetized adult rats. Injection coordinates were as follows. Parietal cortex: A = 4.5 mm, L = 3.0 mm, V = 1.0 mm. Hippocampus: A = 3.0 mm, L = 2.5 mm, V = 3.3 mm (27). Fourteen hours after the injections, animals were anesthetized and perfused with saline and then with buffered 10% formalin. The brains were embedded in paraffin and serially sectioned (10 μm) in the coronal plane. Every 20th section was mounted, dried, deparaffinized, and coated with Kodak nuclear tracking emulsion NTB-2. Slides were exposed and then developed and stained with toluidine blue. (*A*) Nucleus basalis of rat that received parietal cortex injection. (×1000.) *Inset*: injection site in parietal cortex. (×1.7.) Arrow points to the site depicted at high power. (*B*) Diagonal band of Broca of rat that received hippocampal injection. (×75.) (*C*) Medial septal nucleus of rat that received hippocampal injection. (×1000.)

neurons with perikarya in the nucleus basalis, along the ventromedial border of the globus pallidus, project to the parietal cortex, and those with perikarya in the nucleus of the diagonal band of Broca or the medial septal nucleus project to the hippocampus. The anatomical location of the perikarya and termini of these two sets of cells and the morphological structure of their somatae strongly suggest they are the previously described cholinergic neurons of the nucleus basalis projecting to area 2 of the parietal cortex (34) and the magnocellular cholinergic neurons of the septal–hippocampal pathway (35). These findings complement the demonstration of NGF molecules and NGF-encoding mRNA in brain tissue (8, 9) and strongly support the hypothesis that in the brain, as in the periphery, NGF is elaborated by target cells and binds to specific receptors on the innervating neurons. The interaction of NGF with its receptor may then induce physiological effects on these central nervous system neurons similar to the trophic activity observed in the periphery.

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