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AFFINITY LABELING OF A NERVE GROWTH FACTOR RECEPTOR COMPONENT ON RAT PHEOCHROMOCYTOMA (PC12) CELLS

JOAN MASSAGUE, STEPHEN BUXSER, GARY L. JOHNSON and MICHAEL P. CZECH

Department of Biochemistry, University of Massachusetts Medical Center, Worcester, MA 01605 (U.S.A.)

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Clonal PC12 rat pheochromocytoma cells were sequentially incubated with ^{125}I-labeled nerve growth factor and the photoreactive bifunctional agent hydroxysuccininidyl-*p*-azidobenzoate. This treatment effected the crosslinking of ^{125}I nerve growth factor to a PC12 cell component that exhibits an apparent M_r = 148 000–158 000, and consists of a single polypeptide chain with internal disulfide bonds. The amount of label associated with this M_r = 148 000–158 000 species was proportional to the degree of occupancy of nerve growth factor receptors by ^{125}I-labeled nerve growth factor. Affinity labeling of this species was inhibited by the presence of 0.2 μM unlabeled nerve growth factor during incubation of PC12 cells with ^{125}I nerve growth factor. In membranes prepared from PC12 cells hydroxysuccinimidyl-*p*-azidobenzoate effected the crosslinking of ^{125}I-labeled nerve growth factor to an M_r = 120 000–130 000 species but not to the M_r = 148 000–158 000 component observed in intact cells. The kinetics of ^{125}I nerve growth factor affinity labeling of the M_r = 148 000–158 000 species closely paralleled the time-course of ^{125}I nerve growth factor association to two kinetically distinct forms of nerve growth factor receptors in PC12 cells. The data indicate that the M_r = 148 000–158 000 species affinity-labeled by ^{125}I nerve growth factor is the native form of a component associated with kinetically different nerve growth factor receptors in PC12 cells.

Introduction

The polypeptide hormone nerve growth factor is involved in the survival, development and differentiation of several cell types of neuronal crest derivation [1–3]. These effects presumably result from the interaction of nerve growth factor with specific cell surface receptors. Specific nerve growth factor binding can be demonstrated on membranes from several types of neuronal cells [4–8], and some physical properties of detergent-extracted nerve growth factor receptors have been described [9–12].

We have recently reported the structural characteristics of two nerve growth factor receptor species (M_r = 143 000 and 112 000, respectively) affinity-labeled in membrane preparations from rabbit sympathetic ganglia [13]. An important question arising from these studies relates to the possible precursor-product relationship between the two species affinity-labeled in sympathetic ganglia membranes. Circumstantial evidence for a degradative process affecting the native M_r = 143 000 nerve growth factor receptor present in these membranes was based on the multiple similarities between the respective peptide maps of the two labeled species and the differing relative amounts of the two species in various membrane preparations [13]. However, several protease inhibitors added during membrane isolation did not decrease the proportion of the smaller (M_r = 112 000) form. Therefore, it was also possible that these two species corresponded to different components of the same receptor or to the two kineti-

cally different nerve growth factor receptor forms revealed by kinetic studies [7,14–17].

The present studies were designed to affinity-label native nerve growth factor receptors on the surface of intact cells, in order to investigate (1) the susceptibility of these receptors to fragmentation during cell-disruption and membrane isolation, and (2) whether the two kinetically distinct forms of nerve growth factor receptor identified by radioligand binding studies in various systems [7,13–16] are structurally analogous or correspond to two structurally different receptor entities. We have used the PC12 clonal cell line of rat pheochromocytoma established by Greene and Tischler [18]. In the absence of nerve growth factor, PC12 cells synthesize, store and release catecholamines, proliferate at a normal rate and exhibit other general properties of chromaffin cells [18,19]. Addition of nerve growth factor to PC12 cultures stops cell replication and initiates differentiation of PC12 cells into a sympathetic neuron phenotype that includes neurite outgrowth and increased neurotransmitter synthesizing activity [18,19]. PC12 cells have been extensively used as a model system for the study of the mechanisms of nerve growth factor action and neuronal differentiation. The availability of a nerve growth factor-responsive, intact cell system allows experimentation under conditions in which structural and kinetic integrity of nerve growth factor receptors is best preserved.

Materials and Methods

Cells. The clonal line PC12 of rat pheochromocytoma was obtained from Dr. John Wagner, Sidney Farber Cancer Research Institute. The cells were grown in Dulbecco's Modified Eagle medium supplemented with 10% fetal calf serum and 10% heat-inactivated horse serum. Cell cultures were maintained in a 90% air/10% CO_2, water-saturated atmosphere. Cells were used 5–7 days after subpassage.

Preparation of membranes. Cells were removed from 150 mm petri dishes by repetitive washing using a pipet and collected by centrifugation. The cells were washed twice in Puck's saline solution and then transferred to ice-cold lysis buffer (150 mM NaCl, 10 mM Hepes/HCl, 2 mM $MgCl_2$, 1 mM EDTA, pH 7.4). The cells were then incubated in a Parr nitrogen cavitation apparatus for 20 min at 400 lbs/inch^2. The cells were lysed by rapid decompression and the lysate centrifuged for 5 min at $900 \times g$. The supernatant was centrifuged for 20 min at $40\,000 \times g$ and the pellet resuspended using a Dounce homogenizer in 20 mM Hepes, 2 mM $MgCl_2$, 1 mM EDTA, pH 8.0 containing 10% (w/w) sucrose. The suspension was then layered over a discontinuous sucrose density gradient containing 40% sucrose and 20% sucrose. The gradients were centrifuged for 75 min at 27 000 rpm in a Beckman SW-28 rotor and the band at the 20 to 40% sucrose interface collected, diluted in 20 mM Hepes, 2 mM $MgCl_2$, 1 mM EDTA, pH 8.0 amd centrifuged for 30 min at $40\,000 \times g$. The pellets were resuspended in the same buffer at 2–3 mg protein/ml, frozen in a solid CO_2/ethanol bath and stored at −85°C.

Rabbit superior cervical ganglia were obtained from Pel-Freez (Rogers, AR). A plasma membrane-enriched microsomal fraction was prepared from these ganglia according to Banerjee et al. [4]. The characteristics of nerve growth factor receptors in these membrane preparations has been described elsewhere [4,9].

Affinity-labeling protocols. PC12 cells gently detached from polystyrene tissue culture dishes and resuspended with a plastic pipette were washed twice in 10 mM Hepes/Hanks medium containing 5 mM glucose and 1% bovine serum albumin. Cells were resuspended in the latter medium at a concentration of $1.5 \cdot 10^6$ cells/ml and incubated in the presence of ^{125}I nerve growth factor under the conditions specified in each experiment. At the end of this incubation, cells were washed once at 15°C with 10 mM Hepes/Hanks medium unless otherwise indicated, and resuspended in this medium at a final concentration of $1.5 \cdot 10^6$ cells/ml. Hydroxysuccinimidyl-*p*-azidobenzoate, freshly dissolved in dimethyl sulfoxide, was added to these cells at the indicated final concentrations. The cell suspension was immediately transferred to a quartz cuvette and exposed in the cold room to a Hanovia 140 W mercury lamp as previously described [20]. The crosslinking reaction was allowed to proceed for 8 min at the end of which the cell suspension was diluted, pelleted at $1000 \times g$, for 5 min, and washed with 0.25 M sucrose, 10

mM Tris, 1 mM EDTA, pH 7.2, to quench the unreacted hydroxysuccinimidyl-*p*-azidobenzoate. The washed cell pellet was retained for electrophoresis.

Affinity-labeling of membranes from PC12 cells and rabbit superior cervical ganglia was performed at a final membrane concentration of 1 mg/ml, under the same conditions described for affinity-labeling of intact PC12 cells, except that treatment with hydroxysuccinimidyl-*p*-azidobenzoate was conducted at 4°C and terminated by addition of an excess 10 mM Tris, 1 mM EDTA, pH 7.2 and centrifugation of the membranes at 12000 × *g* for 5 min.

Dodecyl sulfate polyacrylamide gel electrophoresis. Affinity-labeled intact PC12 cells were directly solubilized by boiling in the presence of 2% sodium dodecyl sulfate and 50 mM Tris-HCl, pH 6.8, with or without 50 mM dithiothreitol. The resulting sample was centrifuged at 100000 × *g* for 20 min and the supernatant electrophoresed on the discontinous system of Laemmli [21], using 5–10% polyacrylamide gradient gels unless otherwise indicated. Affinity-labeled membrane samples were boiled in 2% sodium dodecyl sulfate, 50 mM Tris-HCl, pH 6.8 and directly applied to the electrophoresis gels described before [13] and subjected to autoradiography on Kodak X-OMAT R film with enhancing screen.

Reagents. Nerve growth factor was prepared from adult male mouse submaxillary glands as described before [22]. Nerve growth factor was labeled with ^{125}I using lactoperoxidase-glucose oxidase system for radio-iodination (Enzymobeads, BioRad Laboratories, Richmond, CA). 50 µl of enzymobeads, reconstituted as per manufacturer's instructions, were mixed with 50 µl of phosphate buffer (0.2 M, pH 7.4), 2 mCi of Na^{125}I and 20 µg of nerve growth factor (final volume of approx. 150 µl). The radio-iodination reaction was started by the addition of 25 µl of 1% β-D-glucose. The reaction mixture was incubated at room temperature for 10 min before the addition of a second 25 µl aliquot of β-D-glucose. After a second 10-min incubation, 300 µl of 6 M guanidine HCl was added to the reaction container and the entire mixture was immediately chromatographed on a 7.5 × 300 mm column of G-25 Sephadex equilibrated with 20 mM Hepes-buffered saline containing 0.1% bovine serum albumin. Fractions collected at the void volume were 95–97% trichloroacetic acid precipitable and migrated as a single band to a position identical with non-iodinated nerve growth factor on SDS-polyacrylamide gels. Specific activity of the ^{125}I nerve growth factor was approx. 20 Ci/g. In the experiments described here, maximal binding of ^{125}I nerve growth factor to PC12 cells or membranes was 10–20% of the total input ^{125}I nerve growth factor. Non-specific binding accounted for 5–12% of the total binding. Hydroxysuccinimidyl-*p*-azidobenzoate was purchased from Pierce Chemical Co. (Rockford, IL)

Results and Discussion

A suspension of intact PC12 cells incubated with 0.15 nM ^{125}I nerve growth factor was exposed to a short treatment with 30 µM hydroxysuccinimidyl-*p*-azidobenzoate and photolyzed to effect covalent crosslinking of ^{125}I nerve growth factor to cell surface components. Electrophoresis of the affinity-labeled material on dodecyl sulfate polyacrylamide gels and autoradiography of these gels revealed one single major labeled band migrating at a position corresponding to M_r = 148000 (Fig. 1, lane A). Inclusion of an excess (0.2 µM) non-radioactive nerve growth factor during incubation of PC12 cells with ^{125}I nerve growth factor inhibited the labeling of this M_r = 148000 species (Fig. 1, lane B). When electrophoresis was performed in the presence of 50 mM dithiothreitol the affinity-labeled species migrated with an apparent M_r = 158000 (Fig. 1, lane D), but no increase in the labeling of this species or appearance of other affinity labeled species was observed. These findings suggest that a component of the cell surface nerve growth factor receptor in PC12 cells has been labeled which consists of a single peptide chain not disulfide-linked to any other membrane component. The increase in apparent M_r upon treatment of this species with dithiothreitol in the presence of dodecyl sulfate suggests a certain degree of molecular compactness provided by internal disulfide bonds. An increase of the apparent M_r in the presence of dithiothreitol has also been noted in nerve growth factor receptors from rabbit superior cervical ganglia membranes

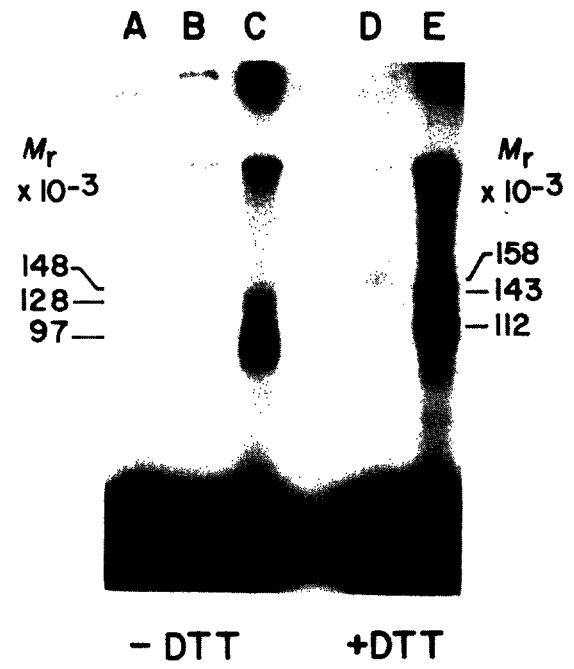

Fig. 1. Dodecyl sulfate polyacrylamide electrophoresis of PC12 cells and rabbit superior cervical ganglia membranes cross-linked to ^{125}I nerve growth factor. PC12 cells ($1.5 \cdot 10^6$ cells) (lanes A and D) and rabbit superior cervical ganglia membranes (200 μg membrane protein) (Lanes C and E) were incubated at 23°C for 60 min in 10 mM Hepes/Hanks medium, pH 7.4, in the presence of 0.15 nM ^{125}I nerve growth factor. At the end of this incubation, the unbound hormone was washed out and cross-linking to cell-or membrane-bound ^{125}I nerve growth factor was effected by 30 μM hydroxysuccinimidyl-*p*-azidobenzoate under the conditions dexcribed under Materials and Methods. Samples of affinity-labeled PC12 cells or ganglia membranes were solubilized in the presence of 2% sodium dodecyl sulfate with (lanes D and E) or without (lanes A and C) 50 mM dithiothrietol. They were then electrophoresed on 5–15% polyacrylamide gradient slab gels. An autoradiogram obtained after 2 week exposure of the resulting fixed, dried gel is shown. Sample on lane B corresponds to PC12 cells treated identically to sample on lane A except that 0.2 μM unlabeled nerve growth factor was present during incubation of cells with ^{125}I nerve growth factor. Protein standards included in a parallel run were: myosin (M_r = 200000), β-galactosidase (M_r = 116000), phosphorylase *b* (M_r = 94000), bovine serum albumin (M_r = 68000), ovalbumin (M_r = 45000) and carbonic anhydrase (M_r = 31000). Radioactive material that did not penetrate the stacking gels is observed at the top of the autoradiogram, specially on lanes corresponding to affinity-labeled superior cervical ganglia membranes (lanes C and E). The affinity-labeling of most of this material is non-inhibitable by 0.2 μM nerve growth factor (not shown), but this material contains some apparently oxidized M_r = 143000 nerve growth factor receptor species that only penetrates the separating gels in the presence of 50 mM dithiothreitol (compare amount of M_r = 128000 species on lane C vs. amount of M_r = 143000 species on lane E).

[13], in receptors for other peptide hormones [23–25] as well as in proteins known to contain internal disulfide bonds [26]. Treatment of samples with *N*-ethylmaleimide after reduction with dithiothreitol did not modify the electrophoretic migration of specifically labeled bands.

In the experiment shown in Fig. 1, membranes from rabbit superior cervical ganglia covalently crosslinked to ^{125}I nerve growth factor by hydroxysuccinimidyl-*p*-azidobenzoate were electrophoresed in parallel with affinity-labeled PC12 cell material (lanes C and E). Hydroxysuccinimidyl-*p*-azidobenzoate typically effects the affinity-labeling of two nerve growth factor receptor species in cervical ganglia membrane preparations [13]. An affinity-labeled M_r = 143000 species (M_r = 128000 in the absence of dithiothreitol) (Fig. 1, lanes C and E) has been identified as a native component of the nerve growth factor receptor in rabbit superior cervical ganglia membranes [13]. The analogy between the electrophoretic properties of this species and the M_r = 148000–158000 species from PC12 cells labeled in the present studies may be due to a substantial degree of structural similarity between these two putative nerve growth factor receptor components. The second ^{125}I-labeled nerve growth factor species in sympathetic ganglia membranes exhibits an M_r = 97000 in the absence of dithiothreitol and an M_r = 112000 in the presence of dithiothreitol (Fig. 1, lanes C and E, and Ref. 13). On the basis of its variable appearance and of comparative peptide mapping analysis, this second labeled species has been proposed to be a product of degradation of the native M_r = 128000–143000 nerve growth factor receptor species from rabbit sympathetic ganglia [13].

The inability of several protease inhibitors to prevent the appearance of the lower M_r species affinity-labeled by ^{125}I nerve growth factor in sympathetic ganglia membranes [13] made interpretations about the precursor-product relationship between this species and the M_r = 143000 affinity-labeled species difficult. A similar situation has been encountered with receptors for insulin [27] and epidermal growth factor [28] which undergo

limited cleavage by cellular proteases during isolation of membrane fractions. Structural analysis in intact cell systems where receptor integrity is preserved has been useful in elucidating the degradative processes that generate apparent heterogeneity of receptors for insulin and epidermal growth factor [27,29]. The experiment shown in Fig. 2 provides additional evidence for the apparent susceptibility of nerve growth factor receptors to the action of an endogenous protease(s) or glycosydase(s). A membrane fraction was prepared from PC12 cells and was affinity-labeled by sequential incubation in the presence of ^{125}I nerve growth factor and hydroxysuccinimidyl-*p*-azidobenzoate, and electrophoresed on dodecyl sulfate polyacrylamide gels in parallel with a sample derived from affinity-labeled intact PC12 cells. The major species labeled in the PC12 membrane preparation exhibited an $M_r = 130\,000$, lower than the major ($M_r = 158\,000$) species labeled in intact PC12 cells (Fig. 2). This pattern of affinity-labeled species was independent of the concentration of crosslinking agent used. The absence of noticeable $M_r = 130\,000$ labeled species in intact PC12 cells supports the hypothesis that the nerve growth factor receptor undergoes a limited degradative transformation during cell disruption and isolation of membrane fractions.

We sought to determine whether the radioactivity associated with the $M_r = 158\,000$ species labeled by ^{125}I nerve growth factor in PC12 cells was a constant fraction of the total ^{125}I nerve growth factor specifically bound to these cells. PC12 cells were incubated for various periods of time at 37°C with 0.15 nM ^{125}I nerve growth factor. At the end of this incubation cells were washed briefly at 0°C to remove most of the unbound ligand and exposed to hydroxysuccinimidyl-*p*-azidobenzoate and photolysis. At the end of this treatment, the remaining free ^{125}I nerve growth factor was washed out at 0°C and the radioactivity specifically associated with the cells determined in a gamma counter. The affinity-labeled cells were then subjected to electrophoresis in dodecyl sulfate-polyacrylamide gels and autoradiography. Fig. 3 shows an autoradiogram corresponding to this experiment. The radioactivity bound to PC12 cells before electrophoresis and the radioactivity associated with the $M_r = 158\,000$ band in the corresponding gels as

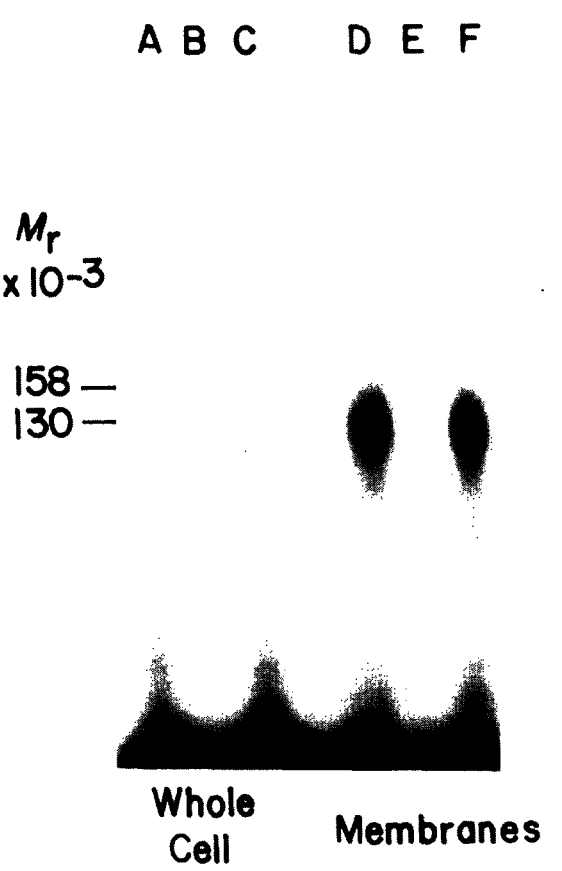

Fig. 2. Affinity labeling of ^{125}I nerve growth factor binding species on membranes from PC12 cells. Intact PC12 cells ($0.7 \cdot 10^6$ cells) or membranes from these cells (100 μg membrane protein) were incubated at 37°C for 60 min in the presence of 0.15 nM ^{125}I nerve growth factor with (lanes B and E) or without (lanes A, C, D and F) 0.2 μM unlabeled nerve growth factor. After washing out the unbound hormones, crosslinking of bound ^{125}I nerve growth factor was performed in the presence of 10 μM hydroxysuccinimidyl-*p*-azidobenzoate (lanes A, B, D and E) or 50 μM hydroxysuccinimidyl-*p*-azidobenzoate (lanes C and F). The affinity-labeled samples were solubilized in the presence of 2% sodium dodecyl sulfate and subjected to electrophoresis on a 6% polyacrylamide gel in the presence of 50 mM dithiothreitol. An autoradiogram obtained after a 10 day exposure of the fixed, dried gel is shown.

plotted in Fig. 3 show that the intensity of labeling of the $M_r = 158\,000$ species was roughly proportional at all time points to the degree of occupancy of nerve growth factor receptors. This observation further supports the concept that the $M_r = 158\,000$ labeled species corresponds to a component of the nerve growth factor receptor(s) in PC12 cells.

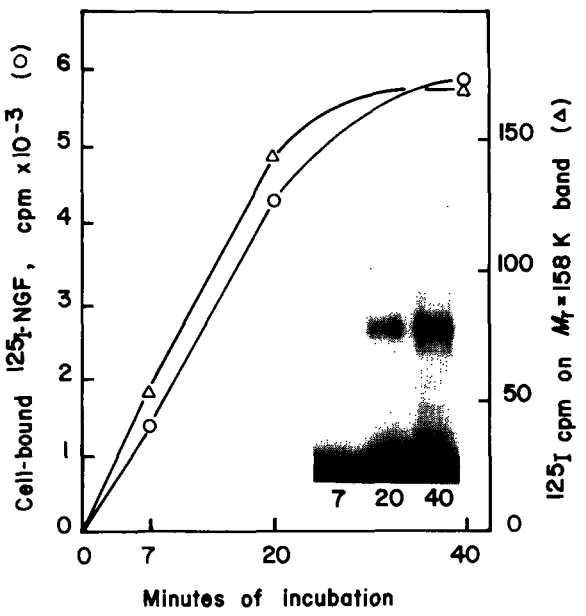

Fig. 3. Correlation between the time-dependent association of ^{125}I nerve growth factor with PC12 cells and labeling of the $M_r = 158000$ species. PC12 cells ($2.5 \cdot 10^6$ cells) in 10 mM Hepes/Hanks medium were incubated with 0.15 nM ^{125}I nerve growth factor for the indicated times at 37°C. They were then added with an excess of 10 mM Hepes/Hanks medium at 15°C, pelleted at $1000 \times g$ for 5 min and the cell pellet resuspended in 10 mM Hepes/Hanks medium at 15°C. The cross-linking reaction was performed in the presence of 30 μM hydroxysuccinimidyl-*p*-azidobenzoate for 8 min and arrested by three consecutive washings at 0°C with 0.2 M sucrose, 10 mM Tris, 1 mM EDTA, pH 7.2. The radioactivity associated with each sample was determined with a gamma counter (circles). Samples were then subjected to dodecyl sulfate-polyacrylamide gel electrophoresis in the presence of dithiothreitol as indicated under Materials and Methods. After autoradiography of the resulting gels (inset) the $M_r = 158000$ region of each lane was excised out and the radioactivity in this region determined with a gamma counter (triangles).

The kinetics of nerve growth factor binding to PC12 cells and other systems cannot be accommodated by a model of one single receptor class [7,14–17]. For instance, PC12 membranes have been found to contain nerve growth factor binding sites with low apparent dissociation constant ($K_d \sim 8 \cdot 10^{-11}$ M) and high dissociation constant ($K_d \sim 9 \cdot 10^{-9}$ M), respectively [16,17]. These two nerve growth factor binding entities have been further characterized by their susceptibility to dissociate from ^{125}I nerve growth factor at low temperature [16,17]. At 0°C ^{125}I nerve growth factor dissociates from the fast-dissociating sites but not from the slow-dissociating sites if excess unlabeled nerve growth factor is added to the medium. It has been shown that upon exposure of PC12 cells to ^{125}I-nerve growth factor, the relative proportion of slow-dissociating nerve growth factor -binding sites increases with time apparently at the expense of the fast-dissociating sites [16]. These unique kinetic properties have been attributed to a ligand-induced conversion from one state of the nerve growth factor receptor to the other [16]. Slow-dissociating sites, as well as fast dissociating sites, have been found in PC12 cells previously to any exposure of cells to nerve growth factor [17]. It has been unclear whether these two types of binding sites represent two different proteins or two states of chemical or conformational modification of a single receptor protein.

We addressed the question of whether the labeling of the $M_r = 148000–158000$ species can be correlated with the kinetics of ^{125}I nerve growth factor association to nerve growth factor receptors present in two kinetically distinct forms in PC12 cells. Aliquots ($2 \cdot 10^6$ cells) of PC12 cells in suspension were incubated over a 40 min period at 37°C in the presence of 0.75 nM ^{125}I nerve growth factor. Cells were then transferred to an ice bath. Total ^{125}I nerve growth factor bound to these cells was measured by rapid centrifugation (1 min, $12000 \times g$) of one set of aliquots in an Eppendorf microfuge, and counting the radioactivity associated with the resulting cell pellet. Non-specific binding of ^{125}I nerve growth factor was determined in parallel samples exposed to an excess unlabeled nerve growth factor during incubation with ^{125}I nerve growth factor. ^{125}I nerve growth factor specifically bound to PC12 cells under these conditions (Fig. 4, open bars) apparently reflects the occupancy of all classes of binding sites, i.e., 'slow' plus 'fast' dissociating sites [16]. Another set of aliquots was directly exposed to hydroxysuccinimidyl-*p*-azidobenzoate and brief photolysis as described in Methods to affinity-label the nerve growth factor binding sites occupied under these conditions.

In the same experiment, measurement of ^{125}I nerve growth factor specifically bound, and cross-

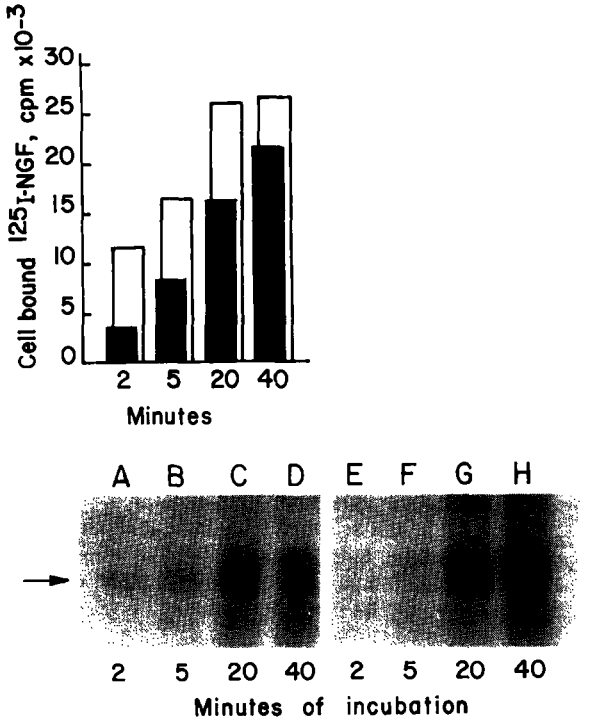

Fig. 4. Correlation between affinity-labeling of the $M_r = 158000$ species and kinetics of ^{125}I nerve growth factor association to slow-dissociating and fast-dissociating nerve growth factor binding sites. PC12 cells ($2.0 \cdot 10^6$ cells) were incubated at 37°C for various times in the presence of 0.75 nM ^{125}I nerve growth factor. The cell samples were then placed on ice and either immediately added with 30 μM hydroxysuccinimidyl-*p*-azidobenzoate or subjected to this treatment after 30 min of exposure to 1.7 μM nerve growth factor. After 8 min of photolysis in the presence of hydroxysuccinimidyl-*p*-azidobenzoate, cells were washed with 0.25 M sucrose, 10 mM Tris, 1 mM EDTA, pH 7.2, solubilized in 2% dodecyl sulfate and subjected to electrophoresis and autoradiography (10 day exposure). The portion of the resulting autoradiogram, comprising the $M_r = 158000$ region (arrow) is shown. Lanes E–F correspond to samples treated with nerve growth factor at 0°C, and lanes A–D correspond to samples that did not receive this treatment. The figure also depicts the radioactivity associated with each sample immediately before the addition of hydroxysuccinimidyl-*p*-azidobenzoate, determined in separate aliquots as described in the text. Open bars represent the radioactivity in samples not treated with nerve growth factor. Solid bars represent the radioactivity in samples treated with nerve growth factor at 0°C.

linking to bound ^{125}I nerve growth factor were also performed in cell samples which after incubation at 37°C in the presence of ^{125}I nerve growth factor were exposed for 30 min at 0°C to excess unlabeled nerve growth factor. Under these conditions, prebound ^{125}I nerve growth factor dissociates from sites defined as fast dissociating sites but remains bound to the slow dissociating sites [16]. Consistently with previous observations [16,17] we noted a time-dependent increase in the fraction of total cell-bound ^{125}I nerve growth factor that can be interpreted as binding to slow-dissociating sites (Fig. 4, solid bars). The autoradiogram in Fig. 4 shows that regardless of whether PC12 cells had been treated at 0°C with excess unlabeled nerve growth factor or not, hydroxysuccinimidyl-*p*-azidobenzoate effected the affinity-labeling of apparently the same molecular species. Furthermore, the time-dependent increase of radioactivity associated with the $M_r = 148000–158000$ species paralleled in each instance the binding of ^{125}I nerve growth factor to PC12 cells (Fig. 4). Thus, at the early time points, substantial labeling of the $M_r = 148000–158000$ species was observed only in cells not treated with native nerve growth factor, but at later time-points the labeling of this species in nerve growth factor treated cells reached the same intensity as in cells not treated with unlabeled nerve growth factor. These observations suggest that regardless of which is the exact molecular basis for the apparent differences between two forms or states of the nerve growth factor receptor in PC12 cells, the $M_r = 148000–158000$ labeled component is common to both of these forms.

It is possible that the $M_r = 148000–158000$ species affinity-labeled in the present studies represents only a minor subpopulation of nerve growth factor receptor components highly susceptible to affinity-labeling with hydroxysuccinimidyl-*p*-azidobenzoate, and that a more abundant form of nerve growth factor receptor escapes crosslinking to ^{125}I nerve growth factor and remains undetectable during electrophoresis and autoradiography analysis. The observed parallelism between the affinity-labeling of the $M_r = 148000–158000$ species and the kinetics of ^{125}I nerve growth factor association to PC12 cells (Fig. 4) argue against this possibility. Rather, the structural similarity between the species affinity-labeled by ^{125}I nerve growth factor in PC12 cells and in sympathetic ganglia membranes, the apparent high affinity of these two species for nerve growth factor, and

their absence from all non-neuronal tissues examined [13], suggest that they are components of physiologically relevant nerve growth factor receptors in these two systems.

Acknowledgments

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