Mediator Release from Mast Cells by Nerve Growth Factor

(Received for publication, December 21, 1992, and in revised form, April 8, 1993)

Kazuhiko Horigomo‡, Johnny C. Pryor‡, Eric D. Bullock‡, and Eugene M. Johnson, Jr.‡§

From the Departments of ‡Molecular Biology and Pharmacology and §Neurological Surgery, Washington University School of Medicine, St. Louis, Missouri 63110

Nerve growth factor causes mediator release from rat peritoneal mast cells in the presence of lysophosphatidylserine. We have investigated the neurotrophin and receptor specificity involved in this response. Nerve growth factor produced a dose-dependent release of \[^{14}C\] serotonin in the presence of lysophosphatidylserine with an EC\(_{50}\) of \(\sim 1\) nM. Incubation with brain-derived neurotrophic factor and neurotrophin-3 did not produce a response. Northern blot analysis with probes for low affinity nerve growth factor receptor (p75), trkA, trkB, and trkC detected a signal only in trkA mRNA. Western blots of trkA immunoprecipitates from mast cell lysates, probed with anti-phosphotyrosine antibodies, demonstrated expression of functional TrkA protein. To determine whether p75, trkB, or trkC mRNA was present in amounts below the limit of detection for Northern analysis, a sensitive reverse transcriptase polymerase chain reaction protocol was used; again rat peritoneal mast cells demonstrated only trkA. The predominant form of trkA message expressed in rat peritoneal mast cells was smaller than the neuronal form. An 18-nucleotide exon (coding for 6 amino acids in the extracellular domain) in the neuronal message was not found in the predominant mast cell trkA message. PC12 cells, a rat pheochromocytoma cell line, and dissociated rat sympathetic neurons showed both trkA and p75, but not trkB or trkC. Anterior pituitary expressed only trkA, but not trkB and trkC. To confirm the lack of expression of p75 on mast cells, \(^{32}P\) nerve growth factor was chemically cross-linked to mast cells or PC12 cells and then immunoprecipitated with a monoclonal antibody specific for p75, 192-IgG; no p75 was detected.

Thus, mediator release from rat peritoneal mast cells by nerve growth factor was specific and not a general property of neurotrophins, and the response was modulated through the trkA proto-oncogene. To our knowledge, this is the first description of a bone marrow-derived cell type that expresses trkA at both the mRNA and protein levels. These data provide further evidence that p75 is not necessary for nerve growth factor signal transduction.

Nerve growth factor (NGF)\(^1\) is the prototype of neurotrophic factors. These factors have a critical role in development, maintenance, and survival of neurons throughout the life of animals (for reviews, see Snider and Johnson (1989) and Thoenen and Barde (1980)). The biology and pharmacology of NGF have been extensively studied since its discovery and purification from the mouse submaxillary gland. Over the last few years, several highly related factors have been characterized, either by purification and cloning as in the case of brain-derived neurotrophic factor (BDNF) or by molecular biological techniques based on similarities between NGF and BDNF (Leibrock et al., 1989), as in the case of NT-3 (Hohn et al., 1990; Jones and Reichardt, 1990; Maisonnier et al., 1990; Rosenthal et al., 1990), NT-4 (Halbouk et al., 1991), and NT-5 (Berkemeier et al., 1991).

Although these factors are generally described in the context of their actions on neuronal cells, many reports describe effects of NGF on nonneuronal cell types (Cattaneo, 1986; Gee et al., 1983; Morgan et al., 1989; Otten et al., 1989; Thorpe et al., 1987; Thorpe and Perez-Polo, 1987); the physiological relevance of these observations remains poorly understood. Similarly, effects of more recently discovered members of the neurotrophin family on nonneuronal cells have not been explored.

One nonneuronal cell type that responds to NGF is the mast cell (Aloe and Levi-Montalcini, 1977; Aloe, 1988). This response occurs in both differentiated, connective tissue-type mast cells (rat peritoneal mast cells (RPMC)) and mast cell precursors. Indeed, administration of NGF to newborn rats increases the number of mast cells in vivo (Aloe and Levi-Montalcini, 1977). In vitro addition of NGF to peritoneal mast cells results in the release of large quantities of inflammatory mediators, such as histamine and serotonin. Stimulation of histamine release by NGF in vitro does not occur de novo but requires the presence of lysophosphatidylserine (lyso-PS) (Bruni et al., 1982). In vivo subcutaneous injection of NGF produces plasma extravasation (Otten et al., 1985), while intravenous administration induces hypotension (Yan et al., 1989); both responses are consistent with histamine release caused by NGF. Similarly, exposure to antibodies against NGF results in a decreased number of mast cells, consistent with a physiologic role for the factor in vivo (Aloe, 1988).

\(^1\)The abbreviations used are: NGF, nerve growth factor; RT, reverse transcriptase; AMV, avian myeloblastosis virus; BDNF, brain-derived neurotrophic factor; bps, base pairs; BSA, bovine serum albumin; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; lyso-PS, lysophosphatidylserine; MCM, mast cell medium; NMPC, non-mast peritoneal cells; NT, neurotrophin; RPMC, rat peritoneal mast cells; PCR, polymerase chain reaction; SGC, dissociated superior cervical ganglion neurons from neonatal rat; Mops, (N-morpholino)propanesulfonic acid; PAGE, polyacrylamide gel electrophoresis.
The nature of the receptor that mediates the NGF response in mast cells has not been structurally characterized. Two cell surface molecules are known to bind NGF in other cell types (for review, see Bothwell (1991), Chao (1992), and Meakin and Shooter (1992)). One molecule is a 75-kDa (p75) protein that binds NGF with low affinity (\(10^{-9} \text{ M}\)) and has historically been referred to as "the NGF receptor," or, more aptly, the low-affinity NGF receptor. However, the binding of NGF to p75 alone appears insufficient to mediate NGF signal transduction and the existence of another component to the functional NGF receptor has been hypothesized (Hosang and Shooter, 1985). Recent work has demonstrated that the component critical to signal transduction is the trkA proto-oncogene (Kaplan et al., 1991b). Other highly related tyrosine kinases (trkB and trkC) are receptors for BDNF and NT-3, respectively (Klein et al., 1989; Lamballe et al., 1991). Thus, the neurotrophins, like many other growth factors (epidermal growth factor, fibroblast growth factor, platelet-derived growth factor, insulin, and stimulating activity factor-1), act through tyrosine kinase receptors.

Considerable uncertainty exists regarding the functional role, if any, of p75 in NGF transduction and the most thoroughly studied NGF-responsive neurons (sensory, sympathetic, and forebrain cholinergic) and other cells (PC12) express p75. Whether p75 is necessary for a biological response is currently a point of debate. Transfection of trkA (Cordon-Cardo et al., 1991) and trkB (Klein et al., 1991; Squinto et al., 1991) into fibroblasts confers sensitivity to NGF and BDNF, respectively. However, such transfections are not physiologic; whether NGF can elicit a response without p75 in normal cells is uncertain.

In this paper, we address the issue of specificity of neurotrophin action on mast cells and the receptor species required to initiate the effects. We show that NGF, but not BDNF or NT-3, was capable of producing mediator release. We examine the receptor species in the cell by sensitive methods at the mRNA and protein level and demonstrate that RPMC expressed trkA, but not p75, trkB, or trkC. Thus, we conclude mediator release by RPMC is not a general property of neurotrophins. Further, we show TrkA alone is sufficient for NGF responsiveness in this normal, endogenous cell type; p75 for mediator release by RPMC is not required for signal transduction, at least in RPMC.

Part of this work has been previously reported in abstract form (Pryor et al., 1992).

**MATERIALS AND METHODS**

**Reagents**—All reagents, unless otherwise noted, were purchased from Sigma.

**Cell Preparation**—The method of collection and purification of mast cells has been described previously (Sullivan et al., 1975). RPMC were harvested from adult female Sprague-Dawley rats (250-300 g, Harlan Sprague-Dawley, Indianapolis, IN) or from male or female Sprague-Dawley rats (retired breeders, 300-530 g, Sasco, Omaha, NE). The peritoneal cells were suspended in mast cell medium (MCM), a phosphate-buffered saline solution, pH 6.8, containing 150 mM NaCl, 3.7 mM KCl, 3.0 mM Na,HPO,, 3.5 mM K,HPO, 0.9 mM CaCl, 0.1% glucose, 0.1% bovine serum albumin (BSA), 10 units/ml heparin. After purification by BSA density gradient centrifugation, mast cell purities of 85-95% were obtained. At least 98% of cells were viable as determined by trypan blue exclusion. Non-mast peritoneal cells (NMPC) were harvested from the BSA gradient supernatant by dilution with MCM and centrifugation. For Western blots, primary cultures of peritoneal mast cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. PC12 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 5% horse serum (HyClone, Logan, UT). Rat superior cervical ganglion (SCG) neurons were prepared by the method of Johnson and Argiro (1983) with modifications described by Martin et al. (1988). Cultures were maintained at 37 °C in humidified atmosphere of 5% CO.2.

**Probes and Neurotrophic Factors**—The rat trkA and trkB probes were the kind gifts of Dr. Luis Parada, and the p75 and trkC probes were kindly provided by Dr. Steven G. Shooter, University of California, San Francisco. The probes for p75, trkA, and trkC were described by Carroll et al. (1992), and the trkB probe was described by Klein et al. (1989). The actin probe consisted of a HindIII fragment of β-actin. The human glyceraldehyde-3-phosphate dehydrogenase cDNA probe was purchased from Clonetech Laboratories, Inc. (Palo Alto, CA).

NGF was purified (Bocchini and Angeletti, 1969) from male mouse submaxillary glands (Harlan Sprague-Dawley). BDNF and NT-3 were kind gifts from Dr. Qiao Yan of Amgen Inc.

NGF was iodinated by a modification of the lactoperoxidase method (Marchalomi, 1986). NGF, 10 μg, was reacted with 1 μCi of Na[14C] (Amersham Corp.) with approximately 90% incorporation of radiolabeled into protein. This yielded a specific activity of approximately 2000 cpm/mmol NGF.

**[3H]Serotonin Release**—Serotonin release was assayed by using [3H]serotonin-preloaded mast cells (Morrison et al., 1974). Preloading was achieved by incubation of RPMC (1 x 10^6 cells/ml) in MCM with 0.5 μCi/ml [3H]serotonin for 60 min at 37 °C. The cells were washed with ice-cold MCM 3 times, and then 2.5 x 10^6 cells were incubated in 250 μl of MCM containing various concentrations of neurotrophins at 37 °C for 15 min in the presence of 2 μM lypo-PS. Ice-cold MCM (750 μl) was added to stop the reaction. Immediately thereafter, the supernatant was removed. The radioactivity of the supernatant and cell pellet was measured separately by scintillation counting; serotonin release was expressed as a percentage of total radioactivity incorporated.

**Northern Blot Analysis**—Total cellular RNA was isolated from RPMC, NMPC, PC12 cells, and anterior and posterior lobes of the pituitary by guanidinium thiocyanate-phenol-chloroform extraction (Chomczynski and Sacchi, 1987). Samples were separated on a 1.2% agarose gel formed in 2.2 M formaldehyde, 20 mM Mops buffer, pH 7.2, 5 mM sodium acetate and transferred directly to a GeneScreen plus membrane (Du Pont-New England Nuclear) in 0.15 M NaCl, 0.015 mM sodium citrate (1 x SSC). The filters were treated with UV irradiation and prehydrized in 50% formamide, 5 x SSC, 0.04% BSA, 0.04% FeCl, 0.04% polyvinylpyrrolidone, 1% SDS, 125 μg/ml salmon testis DNA at 42 °C for 24 h. Filters were washed twice in 2 x SSC, 0.1% SDS at room temperature for 5 min and twice at 65 °C for 20 min. In the case of trkC, filters were washed under more stringent conditions (0.2 x SSC, 0.1% SDS at 68 °C). The filters were exposed to Kodak XAR-5 film with a Du Pont Cronex intensifying screen at -70 °C. DNA probes were labeled with [α-32P]dCTP to a specific activity of 1-2 x 10^6 dpm/ng by random priming (Boehringer Mannheim).
ACCCACCGCTTT-3', corresponding to residue 15, (5' primer) 5'-CGTGTGAAGTCACCACCCT-3', corresponding to residue 219. Total RNA from the mast cells was contaminated by an unknown substance that inhibited reverse transcription PCR (RT-PCR); therefore, poly(A) RNA was isolated from all cells and tissues by using oligo-dT-30 (Amersham Corp.). First-strand cDNA synthesis was performed with pd(T)19-24 (Pharmacia LKB, Uppsala, Sweden) as a primer and avian myeloblastosis virus reverse transcriptase (AMV-RT) (Promega, Madison, WI). The 20-ml reaction contained 50 mM Tris-HCl, pH 8.3, 50 mM KCl, 10 mM MgCl2, 1 mM dithiotheriol, 10 mg/ml BSA, 1 mM dNTPs, 1.5 mM pd(T)19-24, 10 units of RNase inhibitor (Promega), and 10 units of AMV-RT, and poly(A) RNA corresponding to 5 mg of total RNA. After heating the RNA and primer to 68°C for 5 min and cooling on ice, the remaining reagents were added, and the reaction was incubated at 42°C for 2 h. The reverse transcription was terminated by boiling for 5 min. The PCR cDNA reaction mixture was brought to a final volume of 55 ml with Taq polymerase buffer (Promega), 1.5 mM MgCl2, 0.3 mM dNTPs, 0.025 units/ml TaqI polymerase (Promega), 0.5 mCi of [32P]dCTP (3000 Ci/mmol, Amersham Corp.), and 0.4 mM primers. The original 55-ml reaction mix was divided into 5 aliquots, which were overlaid with a drop of mineral oil. Amplification was performed on a programmable heating block (Perkin-Elmer Cetus Instruments). The reaction cycle consisted of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and elongation at 72°C for 2 min. To examine the progress of the amplification reaction, replicate aliquots were placed in the heat block for various cycle numbers. After amplification, the samples were subjected to electrophoresis on an 8% polyacrylamide gel. Autoradiograms were made by using Kodak XAR film after the gels were dried. As a control for contamination of the RNA samples by genomic DNA, PCR amplification was performed omitting AMV-RT.

The PCR products were directly sequenced to confirm their identities by using a double-stranded DNA cycle sequencing system (Life Technologies, Inc.). Ethidium bromide-stained bands were cut out of the gel, crushed, and soaked for 12 h at 37°C in 0.5 ml of 50 mM Tris, pH 8.0, 0.2% SDS, 0.3 M sodium acetate, 4 mM EDTA. Acrylamide gel electrophoresis was performed omitting AMV-RT.

RESULTS

Neurotrophin Specificity of Mediator Release from Mast Cells—To determine whether mediator release from mast cells was a specific property of NGF or a general property of the neurotrophins, mast cells were exposed to varying concentrations of NGF, BDNF, or NT-3. Increasing concentrations of NGF (in the presence of 2 mM lyso-PS) produced a dose-dependent release of [3H]-labeled serotonin with an EC50 of approximately 0.5 nM (Fig. 1). The dose-response curve of NGF-induced mediator release was repeated several times and consistently yielded EC50s of 0.5-2 nM (data not shown). In contrast, doses of BDNF or NT-3, up to 500 ng/ml (approximately 20 nM), produced no release of labeled serotonin (Fig. 1). Therefore, induction of mediator release appears to be a property of NGF but not of other available neurotrophins.

Examination of Neurotrophin Receptor mRNA Species Expression by Northern Blot—We performed Northern blots on total RNA from mast cells, non-mast peritoneal cells, PC12 cells, or anterior and posterior pituitary, sequentially probing the blots for various neurotrophin receptors and standard mRNA species. Mast cells (Fig. 2, lane 1) demonstrated a large amount of message for trkA but no detectable signal for p75, trkB, or trkC. Non-mast peritoneal cells (lane 2) exhibited no detectable message for p75 but appeared to express very low levels of trkC. PC12 cells (lane 3) showed a signal for p75 and trkA but no demonstrable message for trkB.
14884

Neurotrophin Action on Mast Cells

or trkC. The posterior pituitary (lane 4) exhibited a high level of trkB signal and the anterior lobe (lane 5) demonstrated a high signal for trkB and a very low signal for trkC; however, no signals were observed for p75 or trkA mRNA. The signal for trkA message in mast cells appeared larger than that in PC12 cells.

Examination of Neurotrophin Receptor mRNA Expression by RT-PCR—Semi-quantitative RT-PCR was used to assess neurotrophin receptor expression with greater sensitivity. Oligonucleotides were prepared to yield PCR products of 509 base pairs (bp) for p75, 240 bp for trkA, 207 bp for trkB, and 362 bp for trkC. Initially, poly(A') RNA from RPMC, NMPC, PC12 cells, or SCG was reverse-transcribed and subjected to PCR for various cycle numbers by using oligonucleotides to yield PCR products of 509 bp for p75, 240 bp for trkA, 207 bp for trkB, and 362 bp for trkC (Fig. 3A). RPMC demonstrated a large signal for trkB but no p75 signal, even with an increasing cycle number. NMPC showed no detectable signal for p75 or trkA. The small signal seen at 25 cycles in Fig. 3A is almost certainly because of a small amount of contamination by mast cells in the non-mast cell fraction. PC12 and SCG demonstrated signals for p75 and for trkA. In a second set of experiments, poly(A') RNA isolated from RPMC, NMPC, PC12, SCG, or the anterior lobe of the pituitary was subjected to PCR by using oligonucleotides to p75, trkA, trkB, and trkC (Fig. 3B) to confirm data in Fig. 3A and to determine whether other species of the trkA family were present. RPMC demonstrated only trkA message. NMPC showed no detectable signal for any neurotrophin receptor. PC12 and SCG exhibited message for p75 and trkA, but not for trkB or trkC. The anterior pituitary expressed high levels of message for both trkB and trkC. A very faint signal was observed for both trkA and p75 in whole pituitary. Further analysis (data not shown) confirmed a very low level of trkA and p75 mRNA in whole pituitary, estimated to be well below 1% of that in PC12 cells.

In a third set of experiments, the trkA message was analyzed by RT-PCR to determine which of two differentially spliced forms (Barker et al., 1992) was present in RPMC, PC12 cells, and SCG neurons (Fig. 4). The species differ by the presence or absence of 18 nucleotides found to code for a 6-amino-acid portion of the extracellular domain (Meakin et al., 1992; Barker et al., 1992). Semi-quantitative PCR (Fig. 4A) was

FIG. 2. Northern blot transfer analysis of neurotrophic receptor transcripts in rat peritoneal mast cells. Total RNA was isolated and separated on a 1.2% agarose gel containing 2.2 M formaldehyde. Expression of p75, trkA, trkB, trkC, and β-actin, and glyceraldehyde-3-phosphate dehydrogenase (G3PDH) mRNA was assayed by hybridization to specific probes as described under "Materials and Methods." Total RNA isolated from rat peritoneal mast cells (lane 1, 6 μg), non-mast peritoneal cells (lane 2, 12 μg), PC12 cells (lane 3, 12 μg), posterior lobe of pituitary (lane 4, 12 μg), and anterior lobe of pituitary (lane 5, 12 μg). Exposure times were 4-7 days for trkA, p75, and trkB and 10 days for trkC.

FIG. 3. RT-PCR analysis of neurotrophic factor receptor transcripts in rat peritoneal mast cells. A, poly(A') RNA was purified from each of the indicated cells and reverse transcribed by using an oligo(dT) primer and AMV-RT. The resulting cDNA were amplified for the stated cycles in the reaction mixture containing [α-32P]dCTP, and the products were separated by polyacrylamide electrophoresis. The autoradiograph represents a 3-h exposure to the dried gel. PCR amplification was performed that included all reagents except for the AMV-RT. No genomic DNA contamination was detected in any of the poly(A') RNA preparations (data not shown). B, poly(A') RNA was isolated from three independent preparations of rat peritoneal mast cells (lanes 1-3), non-mast peritoneal cells (lanes 4 and 5), PC12 cells (lane 6), cultured SCG cells (lane 7), or whole pituitary (lane 8). The mRNAs corresponding to the neurotrophic factor receptors were amplified by using the primers described under "Materials and Methods." The amplification cycle numbers for p75, trkA, trkB, trkC, and cyclophilin were 26, 26, 28, 26, and 20, respectively. nd indicates that experiment was not done.

~ 12345
performed on cDNA reverse-transcribed from poly(A\(^+\)) RNA isolated from SCG, PC12 cells, and RPMC with oligomer primers designed to amplify the differentially spliced region of trkA. The exon-containing form is represented by a 132-bp base pair band and the exon-lacking form by a 114-bp base pair band. The band amplified from SCG cDNA was exclusively the exon-containing form. PC12 cells expressed predominantly the exon-containing form. Conversely, RPMC expressed predominantly the smaller exon-lacking form. Phosphoimage analysis (Molecular Dynamics, Sunnyvale, CA) consistently shows from 7-10-fold greater levels of the smaller nonneuronal form of the trkA message present in RPMC. The two bands amplified from RPMC were sequenced (Fig. 4B) to verify the PCR-amplified products were from the proposed differentially spliced region.

**p75 Protein Expression on Mast Cells and PC12 Cells**—To confirm at the protein level that p75 was not expressed by or bound to RPMC, we attempted to demonstrate p75 on RPMC by a very sensitive cross-link-immunoprecipitation protocol. RPMC (120,000 cells) or PC12 cells (2,000 cells) were incubated with \(^{125}\text{I}-\text{NGF}\) and the NGF cross-linked by the carbodiimide, EDC. The cells were solubilized and p75 immunoprecipitated by the monoclonal antibody, 192-1lgG. The autoradiograph (Fig. 5) shows the immunoprecipitates after SDS-polyacrylamide electrophoresis. PC12 cells have the expected broad band at \(~90-100\) kDa. The specificity of the band is shown by the blocking of \(^{125}\text{I}-\text{NGF}\) binding by an excess of unlabeled NGF. No signal was detected in mast cells. The inability to observe the TrkA protein in these experiments is not unexpected because EDC has consistently failed to demonstrate TrkA-NGF complex reliably in cross-linking experiments performed over many years (Hosang and Shooter, 1985; Meakin and Shooter, 1991; Taniuchi et al., 1986).

**TrkA Protein Is Expressed and Functional in Mast Cells**—Experiments were performed to detect TrkA protein phosphorylated on tyrosine residues in response to NGF (Fig. 6). After a 5-min pulse with NGF, cells were washed, lysed, and the cleared lysates were immunoprecipitated with anti-TrkA antibody. Proteins were separated by 7.5% SDS-PAGE with a Bio-Rad mini-gel apparatus and then transferred to Immobilon membranes. Membranes were probed with a monoclonal antibody directed against phosphorylated tyrosine. The Western blot (Fig. 6) indicates that a protein immunoprecipitated by antibody directed against TrkA of approximately 140 kDa is phosphorylated on tyrosine in response to NGF in both PC12 cells (**lanes 1 and 2**) and cultured RPMC (**lanes 4-6**). A response to NGF was not observed in trkA-message-negative Vero cells (**lane 3**). BDNF and NT-3 were not able to induce tyrosine phosphorylation on TrkA in mast cells (data not shown).

**DISCUSSION**

The principal findings reported here are that NGF, but not BDNF or NT-3, caused secretion of serotonin from rat peritoneal mast cells in the presence of lyso-PS and that NGF induced this reaction through interaction with the receptor TrkA, with no involvement of p75, the low affinity neurotrophin receptor. We show that mast cells expressed the TrkA protein that was phosphorylated on tyrosine when the cells
were pulsed with NGF. The first finding demonstrates that mediator release in mast cells is specific for NGF and not a general property of neurotrophins. NGF acts on mast cells to make them more receptive to degranulation by other stimuli, such as IgE, concanavalin A, substance P, or phospho- or lysophospho-compounds such as lyso-PS (Bruni et al., 1982; Tomioka et al., 1988). The second finding suggests TrkA is capable of functioning independent of p75 in these normal cells, provoking a recognized and characterized response.

Although this is the first report of a hemopoietic cell that expresses trkA, many groups have previously reported NGF effects on hemopoietic cells (Cattaneo, 1986; Gee et al., 1983; Morgan et al., 1989; Otten et al., 1989; Thorpe et al., 1987; Thorpe and Perez-Polo, 1987). Indeed, the trkA proto-oncogene was initially cloned from K562, a cell line derived from a patient with chronic myelogenous leukemia in terminal blast crisis (Martin-Zanca et al., 1989). The nature of the receptor mediating other reported actions of NGF on non-mast cells has not been determined.

An additional observation made in these studies was that putative gland expressed a low level of p75 and trkC mRNA, but a high level of trkB mRNA. In primarly the posterior pituitary, p75 is selectively localized to perivascular microglia (Yan et al., 1990). The intensity of p75 immunohistochemical staining in posterior pituitary increases significantly in older rats (Yan et al., 1990), a result consistent with our observations here in that young rats expressed low levels of p75 mRNA. Whether the high levels of trkB are found in the same or a different cell type as is p75 (perivascular microglia) will need to be resolved by anatomical studies.

The discovery of the neurotrophins and the TrkA family of tyrosine kinases suggested a mechanism for signal transduction in response to the neurotrophins. However, the nature of the receptor(s) mediating the physiologic responses to NGF is not completely clear nor are the structural requirements for high affinity binding resolved. The trkA proto-oncogene codes for a 140-kDa glycoprotein that demonstrates tyrosine kinase activity upon binding NGF (Kaplan et al., 1991a, 1991b). The low affinity NGF receptor is a 75-kDa protein with no demonstrable function after NGF binding (Johnson et al., 1986; Radeke et al., 1987).

Cell types such as sympathetic neurons and PC12 cells synthesize both TrkA and p75 and demonstrate both high and low affinity binding (Koop = 10^{-11} and 10^{-7} M, respectively). Specific binding of NGF on RPMC yields monophasic Scatchard plots with a relatively low affinity (Kd = 4 \times 10^{-8} M) (Cattaneo, 1986). Such binding properties are consistent with the sensitivity of mast cells to NGF and with mediation by a single low affinity receptor. The dose-response curve of NGF on mast cells (Fig. 1) is very similar to that of the mitogenic response of fibroblasts transfected with trkA (Cordon-Cardo et al., 1991). The predominant form of trkA mRNA is missing an 18-nucleotide exon that codes for a conserved 6-amino-acid region present in the extracellular domain of all neuronal TrkA protein (Meakin et al., 1992; Barker et al., 1992). The two different forms of the TrkA protein both display the same slow dissociation kinetics (Barker et al., 1992). Currently, the functional significance of the two different forms of TrkA is not clear.

Confirmation of a role for p75 in high affinity NGF binding is inconclusive at present, but evidence suggests that p75 may not be necessary for NGF signal transduction even if p75 is required for high affinity binding. Polyclonal antibodies raised against the extracellular domain of p75 that block binding of 125I-NGF do not prevent NGF response in NGF-sensitive neurons or PC12 cells (Weskamp and Reichardt, 1991). Additionally, NGF molecules genetically engineered to bind to TrkA, but not p75, maintain biological activity in PC12 cells and bind to fibroblasts transfected with trkA with an affinity comparable with NGF (ibaiez et al., 1992). Recently, transgenic mice completely lacking p75 have been engineered; these animals are viable but demonstrate loss of thermal sensitivity and ulceration of their extremities (Lee et al., 1992). Sympathetic innervation and organ development are at least relatively normal in all tissues in these animals, though the dorsal root ganglia are reported to be somewhat smaller than wild-type animals. However, our current data showing an acute response to NGF in the nanomolar range in a cell possessing only TrkA is consistent with the idea that p75 may have some role in mediating responses seen in other cells at much lower concentrations. Taken on whole, the data on NGF binding and induction of mediator release from mast cells, coupled with the finding of tyrosine phosphorylation of a protein of 140 kDa (immunoprecipitated anti-TrkA antibody) in response to NGF treatment of mast cells, show signal transduction is forced through the receptor TrkA from low affinity interactions. High affinity binding may be functionally distinct from signal transduction and may represent a separate physiological signal, i.e. to regulate receptor number or to regulate access of neurotrophins to the signal transducing receptor, TrkA.

Acknowledgments—BDNF and NT-3 were generously provided by Dr. Qiao Yan and stem cell factor by Dr. Neil Birkett of Amgen Inc., Thousand Oaks, CA. The probes for trkA and trkB were the kind gifts of Dr. Luis Paradja, National Cancer Institute, Frederick, MD. The probes for trkC and p75 were kindly provided by Drs. Steven Carroll and Jeffrey Milbrandt, Washington University School of Medicine, St. Louis, MO. Dr. William Mobley and Mark Grimes, University of California School of Medicine, generously provided anti-trkA antibody 1086. We thank Drs. Steven Estus and Robert Freeman, and Thomas Deckwerth for assistance and insightful commentary, and Patricia A. Osborne for preparation of NGF and editorial advice.

REFERENCES
Aloe, L. (1988) J. Neuroimmunol. 18, 1–12
Aloe, L., and Levi-Montalcini, R. (1977) Brain Res. 133, 358–366
Barker, P. (1986) in Gokcular et al., 341, 787–794
Barker, P. (1986) in Gokcular et al., 341, 787–794
Barker, P. (1986) in Gokcular et al., 341, 787–794
Barker, P. (1986) in Gokcular et al., 341, 787–794
Barker, P. (1986) in Gokcular et al., 341, 787–794
Barker, P. (1986) in Gokcular et al., 341, 787–794
Barker, P. (1986) in Gokcular et al., 341, 787–794
Barker, P. (1986) in Gokcular et al., 341, 787–794
Barker, P. (1986) in Gokcular et al., 341, 787–794
Barker, P. (1986) in Gokcular et al., 341, 787–794
Barker, P. (1986) in Gokcular et al., 341, 787–794
Neurotrophin Action on Mast Cells

Kaplan, D. R., Martin-Zanca, D., and Parada, L. F. (1991b) Nature 350, 158-160

Klein, R., Parada, L. F., Coulier, F., and Barabac, M. (1988) EMBO J. 7, 3701-3709

Klein, R., Nanduri, V., Jing, S., Lamballe, F., Tagley, P., Bryant, S., Cordon-Cardo, C., Jones, K. R., Reichardt, L. F., and Barabac, M. (1991) Cell 66, 395-403

Lamballe, F., Klein, R., and Barabac, M. (1991) Cell 66, 967-979

Lee, K.-F., Li, E., Huber, L. J., Landis, S. C., Sharpe, A. H., Chao, M. V., and Leibrock, J., Lottspeich, F., Hohn, A., Hofer, M., Hengerer, B., Masiakowski, M., Maisonpierre, P. C., Belluscio, L., Squinto, S., Ip, N. Y., Furth, M. E., Lindsay, Marchalonis, J. J. (1989) J. Biol. Chem. 113, 229-305

Martin, D. P., Schmidt, R. E., DiStefano, P. S., Lowry, O. H., Carter, J. G., Martin-Zanca, D., Oskam, D., Mitra, G., Copeland, T., and Barabac, M. (1989) Mol. Cell. Biol. 9, 24-33

Meakin, S. O., and Shooter, E. M. (1992) Trends Neurosci. 15, 322-331

Meakin, S. O., and Shooter, E. M. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 2374-2378

Middemars, D. S., Lindberg, R. A., and Hunter, T. (1991) Mol. Cell. Biol. 11, 143-153

Morgan, B., Thorpe, L. W., Marchetti, D., and Perez-Polo, J. R. (1989) J. Neurosci. Res. 23, 41-45

Morrison, D. C., Rower, J. F., Henson, P. M., and Cochrane, C. G. (1974) J. Immunol. 112, 573-582

Otten, U., Baumann, J. B., and Girard, J. (1985) Eur. J. Pharmacol. 106, 199-201

Otten, U., Ehrhard, P., and Peck, R. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 10059-10063

Pryor, J. C., Horigome, K., and Johnson, E. M., Jr. (1992) Vol. 18, p. 950, Abstr. 401.17, Society for Neuroscience, Wash. D. C.

Radeke, M. J., Misko, T. P., Hau, C., Herzenberg, L. A., and Shooter, E. M. (1987) Nature 325, 983-987

Rosenthal, A., Goeddel, D. V., Nguyen, T., Lewis, M., Shih, A., Laramee, G. P., Nikolins, K., and Winson, J. W. (1980) Neuron 4, 767-773

Snyder, W. D., and Johnson, E. M., Jr. (1989) Ann. Neurol. 26, 489-506

Squinto, S. P., Stitt, T. N., Aldrich, T. H., Davis, S., Bianco, S. M., Radziejewski, C., Glass, D. J., Masiakowski, P., Furth, M. E., Valenzuela, D. M., DiStefano, P. S., and Yancopoulos, G. D. (1991) Cell 65, 885-904

Sullivan, T. J., Parker, K. L., Stenson, W., and Parker, C. W. (1975) J. Immunol. 114, 1473-1479

Taniguchi, M., Schweitzer, J. B., and Johnson, E. M., Jr. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 1950-1954

Thoenen, H., and Barde, Y.-A. (1988) Physiol. Rev. 60, 1284-1335

Thorpe, L. W., and Perez-Polo, J. R. (1987) J. Neurosci. Res. 18, 134-139

Thorpe, L. W., Stetch, R. W., Hashim, D., Marchetti, D., and Perez-Polo, J. R. (1987) J. Neurosci. Res. 17, 125-134

Tomiska, M., Sted, R. H., Niebel, L., Coughlin, M. D., and Bienenson, J. (1988) J. Allergy Clin. Immunol. 82, 599-607

Weiskamp, G., and Reichardt, L. F. (1991) Neuron 6, 649-653

Yan, Q., and Johnson, E. M., Jr. (1987) Dev. Biol. 121, 139-148

Yan, Q., Clark, H. B., and Johnson, E. M., Jr. (1990) J. Neurocytol. 19, 302-312

Yan, Q., Settle, S. L., and Wilkins, M. R. (1991) Clin. Sci. 80, 565-569