Nerve Growth Factor Induces Rapid Increases in Functional Cell Surface Low Density Lipoprotein Receptor-related Protein*

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The low density lipoprotein receptor-related protein (LRP) is a large endocytic receptor that binds multiple ligands and is highly expressed in neurons. Several LRP ligands, including apolipoprotein E/lipoproteins and amyloid precursor protein, have been shown to participate either in Alzheimer's disease pathogenesis or pathology. However, factors that regulate LRP expression in neurons are unknown. In the current study, we analyzed the effects of nerve growth factor (NGF) treatment on LRP expression, distribution, and function within neurons in two neuronal cell lines. Our results show that NGF induces a rapid increase of cell surface LRP expression in a central nervous system-derived neuronal cell line, GT1-1 Trk, which was seen within 10 min and reached a maximum at about 1 h of NGF treatment. This increase of cell surface LRP expression is concomitant with an increase in the endocytic activity of LRP as measured via ligand uptake and degradation assays. We also found that the cytoplasmic tail of LRP is phosphorylated and that NGF rapidly increases the amount of phosphorylation. Furthermore, we detected a significant increase of LRP expression at the messenger RNA level following 24 h of NGF treatment. Both rapid and long term induction of LRP expression were also detected in peripheral nervous system-derived PC12 cells following NGF treatment. Taken together, our results demonstrate that NGF regulates LRP expression in neuronal cells.

The low density lipoprotein receptor-related protein (LRP) is a large multiligand endocytic receptor expressed abundantly in liver and brain (1). In brain, it is expressed by most if not all neurons and some glial cells (2–4). LRP is the largest endocytic receptor identified to date (~600 kDa; see Ref. 5), synthesized as a single polypeptide chain and cleaved in the trans-Golgi into two subunits (6, 7). A 39-kDa receptor-associated protein (RAP) is a unique LRP ligand antagonist (8, 9), which can also bind and antagonize ligand binding to other low density lipoprotein receptor family members (10–12).

While LRP has been shown to have over 10 ligands (1), four of these (apolipoprotein E (apoE)/lipoproteins, α2-macroglobulin (α2M), tissue factor pathway inhibitor (TFPI), and β-amyloid precursor protein (APP)) have been shown to participate either in Alzheimer's disease pathogenesis or pathology. For example, the e4 allele of apoE is a major risk factor for late onset AD (13–15). While the mechanism for this association is not yet clear, apoE has been found to associate with both Aβ and Tau both in vitro and in vivo (16–19), and recent data suggest that apoE/lipoprotein uptake through neuronal LRP can modify toxic effects of Aβ (20). In addition, studies have shown that isofoms of apoE when conjugated to lipoproteins can differentially influence neurite outgrowth via cell surface LRP (21–24). α2M is one of several constituents of Aβ-containing plaques in AD brain (25, 26). We and others have recently found that α2M complexes with Aβ and mediates the endocytosis of Aβ via cell surface LRP (27, 28). Recent data also show that LRP and TFPI immunoreactivity associates with Aβ-containing plaques in the AD brain (3, 29). Finally, two recent studies suggest a genetic association between certain polymorphisms in LRP and the risk of developing AD (30, 31). Taken together, these findings suggest that LRP could play a role in AD pathogenesis by 1) modifying Aβ uptake and clearance through Aβ interactions with LRP ligands such as α2M and apoE/lipoprotein or 2) direct effects of ligands on cells.

Despite increased understanding of the biology of LRP, its regulation within the nervous system is not well understood. Unlike the low density lipoprotein receptor, the promoter for LRP contains no sterol regulatory element and is not down-regulated by sterols (32). To analyze whether LRP expression is regulated in neuronal cells, we examined the effects of nerve growth factor (NGF) treatment on LRP expression in both a central nervous system-derived neuronal cell line, GT1-1 Trk, and a neuronal crest-derived neuronal cell line, PC12 cells. We found that 1) NGF induces a rapid increase (over minutes) of cell surface LRP; 2) the cytoplasmic tail of LRP is phosphorylated, and the phosphorylation is increased by NGF; and 3) NGF induces a long term increase in LRP mRNA. These studies thus provide new insights into the mechanism of regulation and function of both NGF and LRP in neuronal cells.

EXPERIMENTAL PROCEDURES

Materials—α2M was purified from human plasma and activated with methylamine as described (33). Recombinant human NGF was obtained from Promega (Madison, WI). Recombinant RAP was produced as a fusion protein with glutathione S-transferase in bacteria and purified as described before (34). Rabbit anti-LRP-85 polyclonal antisera was a gift from Joachim Herz (University of Texas Southwestern Medical Center at Dallas). All tissue culture media, sera, and plastic ware were from Life Technologies, Inc. Carrier-free Na125I was purchased from NEN Life Science Products. [35S]Cysteine and [32P]orthophosphate...
were obtained from ICN (Costa Mesa, CA). IODO-GEN was from Pierce. All other chemical reagents were from Sigma.

**Cell Culture**—The generation and culturing of GT1–1 Trk cells has been described previously (35). PC12 cells were obtained from the American Tissue Culture Collection. Both of these cell lines were cultured in Dulbecco’s modified Eagle’s medium containing 5% horse serum and 5% fetal calf serum at 37 °C in a humidified incubator with 5% CO2.

**Ligand Binding Assays**—LRP ligands (α2M* or RAP) were iodinated to a specific activity of 5 × 10^7 cpm/ng protein, using the IODO-GEN method as described previously (36). GT1–1 Trk cells were seeded into 12-well culture plates 2–3 days before assay. Lidgand binding buffer for α2M* was Dulbecco’s modified Eagle’s medium supplemented with 6 mg/ml bovine serum albumin and 5 mM CaCl2. Cell monolayers were washed three times with binding buffer and incubated with 125I-α2M* in the absence or presence of excess unlabeled RAP (0.5 μM) for 2.5 h at 4 °C. Cells were then washed three times with PBS (phosphate-buffered saline containing 1 mM CaCl2 and 0.1 mM MgCl2) to remove unbound ligands and were lysed with Laemml sample buffer (36). The radioactivity was quantified by scintillation spectrometry. Binding of 125I-RAP to PC12 cells was carried out in suspension due to the poor attachment of these cells at 4 °C. Briefly, PC12 cells were detached from culture plates and washed with PBS. Binding assays were initiated by incubating 125I-RAP with PC12 cells for 2 h at 4 °C, in the absence or presence of excess unlabeled RAP (0.5 μM) and a final volume of 400 μl. At the end of each assay, PC12 cells were layered over 800 μl of fetal calf serum in an Eppendorf tube and centrifuged for 1 min at top speed. After the removal of supernatant, cell pellets were counted directly for the radioactivity.

**Cell Surface Iodination**—Semi-confluent GT1–1 Trk cells cultured in 6-cm dishes were incubated with or without NGF (100 ng/ml) for 1 h at 37 °C. Cell monolayers were then washed on ice three times with prechilled PBS. The plasma membranes were iodinated using lactoperoxidase as the oxidizing enzyme and glucose/glucose oxidase as an H2O2-generating system (37). The cells were incubated for 30 min at 4 °C in 2 ml of PBS containing 50 μg of glucose oxidase, 100 μg of lactoperoxidase, 1 mg of glucose, and 300 μCi of Na125I. The reaction was terminated by washing cell monolayers two times with MEMH (minimum essential medium supplemented with 20 mM Hepes/NaOH, pH 7.2, lacking bicarbonate). Cells were then lysed and immunoprecipitated as described below.

**Ligand Uptake and Degradation Assay**—GT1–1 Trk cells cultured in 12-well plates were incubated with or without NGF (100 ng/ml) for 1 h at 37 °C. Binding of 125I-α2M* to GT1–1 Trk cells was performed at 4 °C as described above. After removal of unbound ligands, cells were warmed to 37 °C and incubated in Dulbecco’s modified Eagle’s medium for either 1 or 2 h. At the end of each incubation, media overlying cell monolayers were removed and subjected to precipitation with 20% trichloroacetic acid. Trichloroacetic acid-soluble radioactivity was used as the measurement for internalized ligands.

**Metabolism Labeling and Immunoprecipitation**—Metabolic labeling with [35S]sulfoxytyrosine has been described before (36). Labeling with [35S]Phosphoprotein was performed as described above, except the labeling was for 3 h, and phosphatase inhibitors (1 mM glycercophosphate, 1 mM sodium orthovanadate, and 5 mM sodium fluoride) were included in the lysis buffer. Immunoprecipitation with anti-LRP-85 antibody was performed as described previously (36).

**Northern Blot and Ribonuclease Protection Assay**—GT1–1 Trk cells or PC12 cells were cultured without or with NGF (100 ng/ml) for various periods of time as indicated in the experiments. Total cellular RNA was isolated, and 10 μg of RNA from each condition were subjected to Northern blot analyses. The blot was probed with a mouse cDNA probe, re-probed with a mouse cDNA probe against 18 S rRNA.

## RESULTS

### NGF Induces a Rapid Increase of Cell Surface LRP—GT1–1 Trk cells are immortalized hypothalamic neurons that are stably transfected with the NGF receptor TrkA (35, 39). These cells possess several neuronal specific properties including expression of neuron-specific structural proteins and proteins present in synaptic vesicles (39). They also display electrical properties of neurons, including possession of spontaneous action potential and expression of a functional γ-aminobutyric acid type A receptor-cliochloride channel complex (40). We have previously demonstrated that NGF-mediated TrkA signaling in these cells results in changes in cell morphology and gene expression very similar to that seen in PC12 cells (22, 35). Since we have previously shown that LRP is expressed by these cells and that NGF-mediated neurite outgrowth is augmented by apoE3-containing lipoproteins through LRP (22, 41), we asked whether NGF treatment results in a change in the amount of LRP on the surface of these cells.

GT1–1 Trk cells were treated with NGF (100 ng/ml) for different periods of time, and the amount of 125I-α2M* binding in the presence or absence of RAP (500 nM) was performed at 4 °C to determine the amount of cell surface LRP. Although α2M* may potentially bind to two cell surface receptors (LRP and α2M* signaling receptor), only binding to LRP is inhibited by RAP (42). We found that NGF treatment rapidly increased the amount of RAP-inhibitable binding of 125I-α2M* to the cell surface (Fig. 1). Greater than a 50% increase was seen at 10 min, with an almost 2-fold increase by 20 min. Maximal effects were seen by 1 h. In several such experiments performed, the maximal increase in 125I-α2M* binding ranged between 1.6- and 2.3-fold after 1 h of NGF treatment. Minor variations among experiments were most likely due to the state of cellular growth at the time of the experiments. In addition, similar increases in cellular ligand binding via LRP were also observed in PC12 cells upon NGF treatment (data not shown).

To further characterize the increase in 125I-α2M* binding to the cells in the presence and absence of NGF, we determined the amount of specific 125I-α2M* binding to cells over the concentration range of 0.1–8 nM (Fig. 2A). Saturation of specific binding was seen at concentrations of 2 nM. Scatchard analysis of the binding data (Fig. 2B) revealed that NGF did not significantly alter the apparent affinity of 125I-α2M* binding (Kd, 0.27 nM in the presence and 0.26 nM in the absence of NGF); however, it did increase the total number of binding sites per cell (Vmax was 8000 sites/cell in the presence and 4900 sites/cell in...
mediated ligand uptake, we performed a single cycle endocytosis.

GT1–1 Trk cells also induces a corresponding increase in LRP-

obvious increase in the amount of detectable cell surface LRP

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increase in cell surface LRP molecules, we performed cell

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To more directly assess whether the increase in specific a2M* binding to the cell surface following NGF treatment was due to an increase in cell surface LRP molecules, we performed cell surface iodination experiments. Following incubation at 37 °C for 1 h in the presence or absence of NGF, cell surface proteins were iodinated at 4 °C using lactoperoxidase as the oxidizing enzyme and glucose/glucose oxidase as an H2O2-generating system (37). Cell lysates were then immunoprecipitated with excess anti-LRP antibody and analyzed via SDS-polyacrylamide gel electrophoresis (5% acrylamide; Fig. 3). The amount of 125I-LRP-515 was quantitated under each condition using a PhosphorImager. As seen in Fig. 3, there was an obvious increase in the amount of detectable cell surface LRP induced by NGF (mean increase ± S.E., 2.10 ± 0.24, n = 3; p < 0.05). This increase was similar to the increase in LRP determined by cell surface binding of ligand (see Fig. 2). Taken together, these results clearly demonstrate that NGF treatment acutely induces an increase in the amount of cell surface LRP in GT1–1 Trk cells.

NGF Treatment Results in a Concomitant Increase of LRP-mediated Endocytosis—To examine whether NGF treatment of GT1–1 Trk cells also induces a corresponding increase in LRP-mediated ligand uptake, we performed a single cycle endocytosis and degradation assay. GT1–1 Trk cells, with or without NGF treatment for 1 h, were subjected to ligand binding with 125I-a2M* in the absence or presence of excess unlabeled RAP (see above). After removal of unbound ligands, cells were warmed to 37 °C for either 1 or 2 h. At the end of each incubation, media overlying cell monolayers were removed and subjected to trichloroacetic acid precipitation. Cell-mediated ligand degradation is represented as trichloroacetic acid-soluble radioactivity. As seen in Fig. 4, a2M* degradation was increased in GT1–1 Trk cells treated with NGF: 2-fold at 1 h and 40% at 2 h. Since NGF was not present during the uptake and degradation assay, the smaller increase in 125I-a2M* degradation at 2 h compared to 1 h was likely due to subsequent cycles of ligand uptake for ligand molecules that had initially dissociated. An increase in cell-associated 125I-a2M* was also observed upon NGF treatment (Fig. 4B). These results suggest that NGF treatment of GT1–1 Trk cells results in a concomitant increase of LRP-mediated ligand uptake and degradation, i.e. endocytosis.

NGF Augments Phosphorylation of LRP-85—Phosphorylation of cellular proteins is an important event by which intracellular protein-protein interactions and protein trafficking are regulated. This modification is best known during the initiation of various signal transduction pathways following ligand interaction with cell surface receptors. Since it was not known previously whether or not the cytoplasmic tail of LRP is phosphorylated, we examined this possibility in several cell lines following labeling with [32P]orthophosphate. U87 cells (glioblastoma cell line) and GT1–1 Trk cells were labeled with either [35S]cysteine or [32P]orthophosphate for 3 h at 37 °C. Cell lysates were then immunoprecipitated with an anti-LRP-85 antibody. LRP-85 in both cell lines is specifically labeled with both [35S]cysteine and [32P]orthophosphate (data not shown).

Next, to analyze whether NGF treatment of GT1–1 Trk cells alters the phosphorylation state of LRP, we quantitatively compared the extent of LRP phosphorylation in the absence or presence of NGF. Shown in Fig. 5 is an experiment in which GT1–1 Trk cells are labeled with either [32P]orthophosphate (lanes 1 and 2) or [35S]cysteine (lanes 3 and 4) for 3 h at 37 °C, in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of NGF (100 ng/ml). Cell lysates were then immunoprecipitated with anti-LRP-85 antibody and analyzed via 6% SDS-polyacrylamide gel electrophoresis under reducing conditions. As seen in Fig. 5, the large subunit of LRP (LRP-515) is co-immunoprecipitated with LRP-85 and is labeled with [35S]cysteine but not with [32P]orthophosphate. In contrast, LRP-85 was labeled with both [35S]cysteine and [32P]orthophosphate. Interestingly, the amount of phosphorylation of LRP-85 was increased by 2-fold (quantitated using a PhosphorImager) in the presence of NGF, whereas the amount of [35S]-labeled LRP-85 remained the same in the presence or absence of NGF. The
incubated with or without NGF (100 ng/ml) for 3 h at 37 °C. Binding of radiolabeled ligands, cells were warmed to 37 °C for either 1 or 2 h. At the end of each incubation, media overlying cell monolayers were removed and subjected to precipitation with 20% trichloroacetic acid. Trichloroacetic acid-soluble radioactivity was used as the measurement for cell-mediated ligand degradation (4). The cell monolayers were digested with trypsin, and trypsin-resistant radioactivity was used as the measurement for internalized ligands (4). The increase of LRP-85 phosphorylation was similar in extent to the increase of cell surface LRP upon NGF treatment (2-fold; see above), suggesting that phosphorylation of LRP-85 may serve as a signal for the increase in cell surface LRP.

**Chronic Effects of NGF on LRP**—In addition to the rapid changes in the cell surface expression of LRP, we asked whether NGF increases LRP expression at the mRNA level. To examine LRP mRNA expression in GT1–1 Trk cells, we cloned a cDNA fragment of mouse LRP using reverse transcriptase-polymerase chain reaction (400 bp, corresponding to human LRP cDNA 1725–2124). Using this mouse LRP cDNA as a probe for Northern blot analysis, we examined LRP mRNA expression upon NGF treatment. As seen in Fig. 6A, NGF treatment of GT1–1 Trk cells induces an increase in LRP mRNA expression. The initial increase began to be observed at 18 h, and the maximal increase was seen after 24 h of NGF treatment. To test whether NGF treatment also induces LRP expression in PC12 cells, we performed an RNase protection assay using a rat LRP probe that we have described and characterized in previous studies (4). As seen in Fig. 6B, there was a clear increase in LRP mRNA after 52 h of NGF treatment, which persisted. The time required for NGF induction of LRP mRNA expression in PC12 cells is similar to that seen for NGF induction of mRNAs for APP, acetylcholine esterase, and several other mRNA species (43). Coincident with the increase in LRP mRNA in PC12 cells, there was also an increase in LRP protein expression after 52 h of NGF treatment (measured by metabolic labeling with [35S]cysteine; data not shown). Thus, NGF induces not only rapid changes in LRP at the cell surface but also a longer term increase in LRP expression at the mRNA level. The more rapid increase in LRP mRNA in GT1–1 Trk cells compared with PC12 cells may well be due to the fact that GT1–1 Trk cells express more TrkA than PC12 cells (35).

To analyze the overall increase in cell surface LRP expression in PC12 cells, we performed [125I]-RAP (3 nM) binding. Shown in Fig. 7 are results from a representative experiment in which PC12 cells were cultured in the absence or presence of NGF (100 ng/ml) from 1 to 4 days, followed by analysis of the binding of [125I]-RAP (3 nM) in the absence or presence of increasing concentrations of competing RAP (0–500 nM). As seen in Fig. 7, specific binding of [125I]-RAP was increased 3-fold following 2–4 days of NGF treatment. These results are consistent with the findings that NGF induces both rapid changes in cell surface LRP expression as well as longer term increases in LRP mRNA and protein expression concomitant with neuronal differentiation.

**DISCUSSION**

In this report, we demonstrate that upon NGF treatment cellular LRP is up-regulated both acutely on the cell surface and more slowly at the mRNA level in two neuronal cell lines. To our knowledge, this is the first example in which a neurotrophic factor has been shown to rapidly alter the cellular distribution of an endocytic receptor. Neurotrophins have been shown to play important roles in the development, maintenance, and function of neurons in the central and peripheral nervous system (44). Although cellular responses to neurotrophins include long term changes in gene expression, several
NGF Induces LRP Expression

Fig. 6. NGF induces LRP mRNA expression. A, Northern blot analysis of total RNA from GT1–1 Trk cells. GT1–1 Trk cells were treated with NGF for various periods of time as indicated. Total RNA (10 μg/lane) was then separated via denaturing gel, transferred to a nylon membrane, and hybridized with a mouse LRP probe. The same membrane was then stripped and rehybridized with a mouse 18 S rRNA probe. The positions for LRP and rRNAs are labeled. B, RNase protection assay of total RNA from PC12 cells. PC12 cells were cultured in the absence or presence of NGF for the indicated times. Following each culture, total cellular RNA was isolated and used for RNase protection assay (10 μg/lane). Ethidium bromide staining was simultaneously performed as the loading control. The positions for 32P-labeled LRP probe, LRP protected bands, and 28 and 18 S ribosomal RNA are indicated.

Fig. 7. Total cell surface increase in LRP following long term NGF treatment. PC12 cells (5 × 10^5 cells/well in 12-well dishes) were cultured in the absence or presence of NGF (100 ng/ml) for 1, 2, or 4 days. Following dissociation of cells from plates, cells were washed, and binding of 125I-RAP (3 nM) in suspension was performed in the absence or presence of unlabeled RAP (1 μM). Each point represents an average of triplicate determinations ± S.E.

recent studies have shown that neurotrophins also have acute effects on neuronal function and physiology. Neurotrophins bind to specific Trk receptors, which results in Trk dimerization, autophosphorylation, and rapid activation within minutes of several downstream intracellular pathways (54). These include Ras-mitogen-activated protein kinase, phosphatidylinositol-3 kinase, and the phosphoprotein SNT. It is the activation of these pathways and resulting changes in the phosphorylation state of many proteins that probably accounts for several of the recently described rapid cellular changes induced by neurotrophins. For example, neurotrophins have been shown to rapidly enhance synaptic transmission in both peripheral and central nervous system through activation of presynaptic neuronal Trk receptors with a resulting increase in neurotransmitter release and synaptic strength (45, 46). In addition, neurotrophins, such as brain-derived neurotrophic factor, have been reported to cause rapid changes in intracellular Ca2+ in primary cultures of hippocampal neurons (44). Furthermore, a recent study by Dugan et al. (47) demonstrated rapid NGF-mediated suppression of free radical formation in both GT1–1 Trk cells and in sympathetic neurons. Taken together, these studies suggest that neurotrophins induce both rapid and long term responses in a variety of cellular processes that lead to changes in cell physiology.

NGF stimulation of cell surface LRP expression was relatively rapid, with peak stimulation occurring ~20–60 min following the NGF addition. Since de novo synthesis of LRP and its trafficking to the cell surface takes ~2 h (48) and the half-life of LRP is relatively long (>10 h), the rapid increase in cell surface LRP is unlikely to be a result of increased LRP synthesis. In addition, the increase in cell surface LRP expression results from an actual increase in cell surface LRP molecules, not simply ligand binding capacity (see Figs. 2B and 3). Furthermore, we have also detected a corresponding increase in the uptake of LRP ligands, the internalization rate of LRP is not likely to be altered upon NGF treatment (49). This increase in cell surface LRP can be best explained 1) by NGF increasing the endocytic recycling rate (exocytosis rate) and/or 2) by NGF triggering transfer of LRP from a pool that is relatively less active or “silent” to a more active endocytic pool. These two possibilities are illustrated in Fig. 8. In the first case (Fig. 8A), NGF treatment of cells results in an increase in the rate constant for exocytosis without changing the rate constant for endocytosis (49). Thus, a net increase in cell surface LRP is observed. If this model is correct, any increase in cell surface LRP must correlate with a concomitant decrease in the amount of LRP within the endosomal pool. In the second case (Fig. 8B), NGF treatment does not alter the exocytosis rate of LRP but instead triggers the movement of a pool of LRP that either does not participate or is less active in endocytosis into a more active endocytic pool. If such a model is correct, an increase in LRP should be observed both at the cell surface and within recycling endocytic vesicles. There is a well studied example in which a growth factor (insulin) rapidly stimulates the translocation of an endocytic receptor (the glucose transporter GLUT-4) to the cell surface. Evidence using techniques such as photoaffinity labeling suggests that this occurs predominantly via increasing the exocytosis rate to a much greater extent than the endocytosis rate (50, 51). Another example of receptor regulation was described for asialoglycoprotein receptor. In this case, a “silent” intracellular pool of asialoglycoprotein receptor has been defined, which normally is not involved in endocytosis but can be recruited by phorbol ester stimulation (52). In future studies, we should be able to distinguish which of these possibilities (or both) exists for NGF stimulation of cell surface LRP expression.

It is also not known at present whether a sequence recognition element within the LRP cytoplasmic tail responds to the signal for rapid redistribution or whether a specific endosomal compartment, which may include other endocytic receptors, is responding to NGF. In this respect, it is interesting to note that

G. Bu, Y. Sun, A. L. Schwartz, and D. M. Holtzman, unpublished observation.
the tail of LRP can be phosphorylated and that the phosphorylation increases upon NGF treatment. It is certainly possible that the phosphorylation of LRP may serve as a signal for its redistribution to the cell surface. Recent studies by Breuer et al. (53) have shown that the serine phosphorylation site of the cytoplasmic tail of the 46-kDa mannose 6-phosphate receptor is required for receptor transport to the plasma membrane. Alternatively, phosphorylation of LRP may serve as a signal for triggering the redistribution of the receptor from a “silent” pool to the active endocytic pool. Furthermore, the redistribution of LRP upon neurotrophin treatment could be either specific for LRP or specific for an endocytic vesicle subpopulation in which LRP resides. Thus, it will be important to examine in future studies whether other endocytic receptors (e.g., LRP or specific for an endocytic vesicle subpopulation in which LRP resides) are similarly affected by neurotrophin treatment. Should they be, it would suggest that either specific endosomal vesicle(s) or a common cis-element within the tails of these receptors responds to neurotrophins. On the other hand, if most other endocytic receptors are not regulated by neurotrophin treatment, it would suggest that either an endosomal compartment specific for a subpopulation of endocytic receptors or a cis-element unique to their cytoplasmic tails is responsible for neurotrophin regulation. Understanding these mechanism(s) responsible for the acute regulation and redistribution of cell surface LRP may be facilitated by studies of the effects of NGF on specific intracellular signaling pathways (54).

LRP is a multifunctional endocytic receptor with the ability to bind and endocytose several structurally and functionally distinct ligands including apoE/lipoproteins and APP (55–57). Thus, neurotrophin regulation of LRP may directly influence the catabolism of LRP ligands and their cellular consequences. For example, a principal function of apoE is to mediate lipoprotein binding to cell surface receptors. Thereafter, lipoprotein particles are taken up by cells, and the lipid components are stored and/or reutilized in various cellular functions. Based on results following peripheral nerve injury in which there is a 250–350-fold induction of apoE, a model has been proposed for apoE in transporting lipid and cholesterol to regenerating neuronal growth cones as well as to remyelinating Schwann cells (58). In addition, apoE has also been shown to be up-regulated in the central nervous system within the injured optic nerve (59) and in the hippocampus following perforant pathway interruption (60). Further, since some of the effects of apoE/lipoproteins on neurite outgrowth in vitro also occur in vivo as suggested in some studies (61), this would suggest that apoE/lipoproteins may play a role in structural plasticity after injury or in aging. Since LRP is a principal apoE/lipoprotein receptor expressed on neurons, an increase in cell surface LRP should facilitate the cellular uptake of apoE/lipoprotein. In addition to apoE/lipoprotein, two of the three major isoforms of APP (APP751 and APP770), either in soluble form or transmembrane form, have been shown to be ligands for LRP (56, 57). The receptor-binding domain in APP appears to reside in the 56-amino acid Kunitz domain, which is present in APP751 and APP770 but not in APP695. Since the processing of APP to generate Aβ appears to occur primarily within endocytic compartments (62), it is possible that, upon interacting with APP along the endocytic pathway, LRP may influence the trafficking and processing of APP to generate Aβ. Thus, it will be of interest in future studies to determine the effects of neurotrophins on LRP and cellular Aβ production.

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discussion.

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