p75 Neurotrophin Receptor Signaling Activates Sterol Regulatory Element-binding Protein-2 in Hepatocyte Cells via p38 Mitogen-activated Protein Kinase and Caspase-3

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Nerve growth factor (NGF) influences the survival and differentiation of a specific population of neurons during development, but its role in non-neuronal cells has been less studied. We observed here that NGF and its pro-form, pro-NGF, are elevated in fatty livers from lepin-deficient mice compared with controls, concomitant with an increase in low density lipoprotein receptors (LDLRs). Stimulation of mouse primary hepatocytes with NGF or pro-NGF increased LDLR expression through the p75 neurotrophin receptor (p75NTR). Studies using HuH7 human hepatocyte cells showed that the neurotrophins activate the sterol regulatory element-binding protein-2 (SREBP2) that regulates genes involved in lipid metabolism. The mechanisms for this were related to stimulation of p38 mitogen-activated protein kinase (p38 MAPK) and activation of caspase-3 and SREBP2 cleavage following NGF and pro-NGF stimulations. Cell fractionation experiments showed that caspase-3 activity was increased particularly in the membrane fraction that harbors SREBP2 and caspase-2. Experiments showed further that caspase-2 interacts with pro-caspase-3 and that p38 MAPK reduced this interaction and caused caspase-3 activation. Because of the increased caspase-3 activity, the cells did not undergo cell death following p75NTR stimulation, possibly due to concomitant activation of nuclear factor-κB (NF-κB) pathway by the neurotrophins. These results identify a novel signaling pathway triggered by ligand-activated p75NTR that via p38 MAPK and caspase-3 mediates the activation of SREBP2. This pathway may regulate LDLRs and lipid uptake particularly after injury or during tissue inflammation accompanied by an increased production of growth factors, including NGF and pro-NGF.

Neurotrophins, such as NGF, are important regulators of the development and maintenance of specific neurons, but their functions in non-neuronal cells and in the regulation of cell metabolism are only emerging (1, 2). NGF acts by stimulating the TrkA (tropomyosin receptor kinase A) in neurons, but NGF can also bind to the p75 neurotrophin receptor (p75NTR) (3, 4). In addition, pro-NGF, which is the larger proform of NGF, activates p75NTR inducing specific responses in neurons, such as modulation of cell viability, reduced neurite outgrowth, and changes in gene expression (5–8). The p75NTR belongs to the large cytokine receptor family that includes receptors for TNF-α, a cytokine that plays a role in tissue injury and inflammation (9). p75NTR is expressed during early brain development and is up-regulated after nerve lesions indicating a role of this receptor in cell injury (10, 11). However, p75NTRs are also present in non-neuronal tissues as shown for white adipose tissue and muscle (12, 13). Previously, it was reported that p75NTR influences glucose metabolism and may play a role in insulin resistance that accompanies type-2 diabetes (14). We have recently shown that stimulation of p75NTR by pro-NGF leads to an increase in LDLR in septal cholinergic neurons and in PC6.3 pheochromocytoma cells (15). The signaling mechanisms and the possible roles of p75NTR in non-neuronal cells in the regulation of lipid metabolism have, however, remained open.

Inflammation accompanies many human lipid disorders, including fatty liver, but the mechanisms and factors involved are not fully understood (9). We observed that livers from lepin-deficient obese ob/ob mice (16) have increased levels of NGF and pro-NGF concomitant with high levels of LDLRs. To study the role of these neurotrophins in lipid metabolism in more detail, we stimulated HuH7 human hepatocyte cells with NGF and pro-NGF that increased LDLR expression in these cells. Using primary hepatocytes from p75NTR gene-deleted mice.

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4 The abbreviations used are: p75NTR, p75 neurotrophin receptor; C, control; DN, dominant-negative; LDLR, LDL receptor; NF-κB, nuclear factor-B; B1SP1, site 1 protease; S2P, site protease; BIFC, bimolecular fluorescence complementation; IP, immunoprecipitation; Boc, t-butoxycarbonyl; Z, benzyloxy carbonyl; fmk, fluoromethyl ketone; SCAP, SREBP cleavage-activating protein; INSIG, insulin-induced gene protein.
mice, we observed that the p75NTR signaling is crucial for the neurotrophin-mediated regulation of LDLRs. To study the mechanisms involved, we focused on sterol regulatory element-binding protein-2 (SREBP2) that is a key factor in the cells determining the expression of LDLR (17, 18). SREBP2 is present latent in intracellular membranes and is proteolytically processed to give rise to a transcriptionally active part that acts as a transcription factor for lipogenic genes (17, 18). Data showed that NGF and pro-NGF affected SREBP2 processing in an unexpected way through the activation of caspase-3 leading to the cleavage of SREBP2 at a caspase-3-sensitive site (19) and with the release of the active fragment of the molecule. Despite increased caspase-3 activity, the hepatocyte cells did not die revealing a novel non-apoptotic function of caspase-3 in lipoprotein metabolism in hepatocyte cells.

**Experimental Procedures**

**Materials**—The source of chemicals and antibodies used are specified below under the appropriate headings.

**Vector Constructs**—Expression plasmid for dominant-negative (DN) caspase-3 has been described (20). DN-p38 MAPKα and DN-MKK6 were from Davis and co-workers (21). Wild-type and mutant LDLR promoter firefly luciferase reporter plasmids were from Addgene (numbers 14940 and 14945), and the expression vector for SREBP2 was from Open Biosystems Inc. (GE Healthcare). Mutant SREBP2 (D468A) was generated using the QuikChange Lightning site-directed mutagenesis kit (Agilent) and was confirmed by sequencing. pBiFC-HA-caspase-3-VC155 containing catalytically inactive (C163A) pro-caspase-3 was cloned into the vector with the amino-terminal part or the carboxyl-terminal part of Venus (VN and VC, respectively) linked to caspase-2 vectors with the amino-terminal part or the carboxyl-terminal part of Venus (VN and VC, respectively) linked to caspase-2 (22) was from Addgene (numbers 49261 and 49262). pBiFC-HA-caspase-3-VC155 containing catalytically inactive (C163A) pro-caspase-3 was cloned into the vector with the corresponding caspase-2-VC construct using standard methods.

**Animal Experiments**—All animal procedures were approved by the ethics committee and carried out in accordance with the European Communities Council Directive (86/609/EEC). All mice were maintained in a temperature- and light-controlled (6 a.m. to 6 p.m. light) environment and received a standard diet.

**Analyses of Liver Samples from ob/ob Mice**—Genetically obese leptin-deficient ob/ob mice (The Jackson Laboratory, Bar Harbor, ME) were used as a model for fatty liver (16). Liver tissue from control C57Bl6 and ob/ob mice was homogenized, and an equal amount of protein was subjected to immunoblotting as described below using anti-NGF (1:1000; Alamone Labs, Jerusalem, Israel) and anti-pro-NGF antibodies (1:300; Alamone Labs). β-Actin was used as a control.

**Preparation of Mouse Primary Hepatocytes**—Wild-type and p75NTR gene-deficient mice (The Jackson Laboratory) weighing 30–40 g were anesthetized by intraperitoneal injection of sodium pentobarbital. Liver perfusions were performed as described previously for rats (23). The mouse abdomen was opened and heparin (500 IU/mg body weight) was injected into the inferior vena cava. After 2 min, a 25-gauge Teflon cannula was inserted into the portal vein, and the liver was perfused with a medium containing 119 mM NaCl2, 25 mM NaHCO3, 4.7 mM KCl, 1.3 mM CaCl2, 0.65 mM MgSO4, 1.5 mM NaH2PO4, 5.6 mM glucose, 10 mM Hepes–NaOH, pH 7.4, equilibrated with O2/CO2 (19:1) at a temperature of 37 °C and a flow rate of 5 ml/(min × g). After a stabilization period of 5 min, the perfusion medium was supplemented with 0.8 mg/ml collagenase (Clostridium histolyticum, Sigma). After 15 min, the liver was removed and placed in a Petri dish containing perfusion medium supplemented with collagenase. Hepatocytes were detached using a stainless steel comb, and the remaining vascular parts were removed. The hepatocyte suspension was centrifuged at 50 × g for 5 min at room temperature. The pellets were collected and resuspended in 50 ml of a Dulbecco’s modified Eagle’s medium supplemented with 5% fetal calf serum. Cells were plated at a density of 5 × 10⁶ cm⁻² on collagen-coated well plates and stimulated with 5 ng/ml pro-NGF.

**Cell Culture and Transfections**—Huh7 human hepatocytes were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal calf serum (Gibco/Life Technologies, Inc., Paisley, UK) at 37 °C and 5% CO2 (24). Cells were transfected with 2–4 μg of expression plasmids using transfection or FuGENE reagents (Bio-Rad, Espoo, Finland). Cells were stimulated with different concentrations of NGF or pro-NGF (cleavage-resistant, mutant protein) (Alamone Labs) for various periods of times or with 1 μM simvastatin (Sigma). In some experiments, the broad range caspase inhibitor Boc-aspartyl (Ome)-fluoromethyl ketone (Boc; Calbiochem), the caspase-3 inhibitor Z-DEVD-fmk (BD Biosciences), and the SREBP1 site 1 protease (S1P) inhibitor (23), PF-429242 (Tocris Bioscience, Bristol, UK), were used.

Cell viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Calbiochem) assay as described before (25, 26). In control experiments, staurosporine was used to activate caspase-3 and induce cell death (25). Hoechst 33342 (Sigma) was employed to stain nuclei. To localize caspase-2 in hepatocytes, we double-stained the cells using anti-caspase-2 antibodies (diluted 1:100; Santa Cruz Biotechnology, H119) in conjunction with anti-GM130 (1:200; BD Biosciences, clone 35) to label the Golgi compartment.

**Nuclear Fractionation**—Nuclear and cytosolic fractions were prepared from cells by low speed centrifugation as described elsewhere (27). Fractions were further analyzed using immunoblotting.

**Membrane Isolation**—Human Huh7 cells in 10-cm plates were stimulated with 50 ng/ml NGF or 10 ng/ml pro-NGF for 16 h or with 1 μM staurosporine for 3 h. Cell fractionation was performed by scraping off the cells in ice-cold buffer containing 10 mM KCl, 1.5 mM MgCl2, 1 mM EDTA, 1 mM EGTA, 250 mM sucrose, 20 mM Hepes, pH 7.4, and supplemented with freshly added 1 mM DTT and protease inhibitors, 10 μg/ml aprotinin, and 0.5 mM PMSF and homogenization at 4 °C using a Dounce homogenizer. The homogenate was centrifuged at 800 × g for 10 min at 4 °C, and the resulting supernatant was centrifuged again at 10,000 × g for 20 min at 4 °C to get the mitochondria pellet, followed by centrifugation at 100,000 × g for 1 h at 4 °C to produce the membrane pellet and the cytosolic fraction. Membranes were resuspended in a small amount of buffer (20 mM Hepes and 1 mM EDTA, pH 7.4, supplemented with 0.1% Nonidet P-40, 1 mM DTT, 10 μg/ml aprotinin, and 0.5 mM PMSF). Protein content was determined.
using the Bradford assay; 0.5 μg was used for caspase-3/7 assay and immunoblotting.

Caspase 3/7 Assay—Caspase-Glo assay kit (Promega) was employed for the determination of caspase 3/7 activity in cells essentially as described (28). Membrane and cytosolic fractions obtained from stimulated Huh7 cells were incubated at room temperature for 90 min, and the luminescence was measured using a luminometer (GloMax 20/20, Promega, Biofellows, Helsinki, Finland).

Immunoblotting—Tissue and cells were lysed in a buffer containing 150 mM NaCl, 1 mM EDTA, and 50 mM Tris-HCl, pH 7.5, supplemented with 1% Nonidet P-40, 0.25% sodium deoxycholate, 1% SDS, and protease inhibitors (24, 29). Protein concentrations were determined using the Bradford assay, and an equal amount of protein per sample was subjected to SDS-PAGE and then blotted onto nitrocellulose filters (Amersham Biosciences, Helsinki, Finland). The filters were incubated for 1 h in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, supplemented with 0.1% Tween 20, and 5% skimmed milk or 5% BSA at room temperature and then overnight at 4 °C with primary antibodies as follows: anti-p75NTR intracellular region (1:1000, Abcam, ab32888); anti-p75NTR extracellular region (1:5000, Millipore, 1554); anti-caspase-3 (1:1000, Cell Signaling, 9665); anti-caspase-3 cleaved form (1:1000, Cell Signaling, 9661); anti-caspase-2 (1:500, Enzo Life Sciences, 11B4); anti-SREBP amino-terminal region (1:500, BD Biosciences, 557037); anti-SREBP amino-terminal region (1:1500, Abcam, ab3062); anti-LDLR (1:1200; Cayman Chemical, 10007665); anti-p38 MAPK (1:1000, Cell Signaling, 9212); anti-phospho-p38 MAPK (1:1000, Cell Signaling, 9216); anti-histone H3 (1:1000, Cell Signaling, 9715); rabbit anti-GFP (1:500, Chemicon); mouse anti-GFP (1:500, Roche Applied Science); mouse anti-binding immunoglobulin protein (1:1000; BD Biosciences); and anti-β-actin (1:5000; Sigma). After washing, the filter was incubated with horseradish peroxidase-conjugated secondary antibodies (1:2500; Jackson Immunoresearch, Espoo, Finland), followed by detection using enhanced chemiluminescence (Pierce, Helsinki, Finland). Quantification was performed using ImageJ.

Immunoprecipitation (IP)—IP of endogenous caspase-2 was done using cell lysates in RIPA lysis buffer containing 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 50 mM Tris-HCl, and 0.1% SDS, pH 8.0, supplemented with protease inhibitor mixture (Roche Applied Science) essentially as described (24, 27). Preclearing was done followed by an overnight incubation with anti-caspase-2 antibody. G-agarose (Roche Applied Science, Germany) was added and incubated in a rotor at 4 °C for 2 h, and the agarose beads were collected by centrifugation, washed three times, and dissolved in loading buffer, and the immunoprecipitates were analyzed by immunoblotting using anti-caspase-3 and anti-caspase-2 antibodies. Blots were quantified by densitometry.

Bimolecular Fluorescence Complementation (BiFC) Assay—BiFC-HA-VN173 caspase-2 containing the amino-terminal part of mVenus (VN) was transfected into Huh7 cells in conjunction with pro-caspase-3-VC155 (22). To avoid cell death after transfection, we used the catalytically inactive caspase-3. After 16 h, cells were fixed and analyzed by a fluorescent microscope to detect interaction of caspase-2-VN with caspase-3-VC.

LDL Uptake—Uptake of lipoprotein particles was examined as described (15, 24) by incubation of cells for 6 h at 37 °C in the presence of 5 μg/ml 3,3’-dioctadecyldiodocarbocyanine-LDL (Molecular Probes).

Gene Promoter Assays—Cells were transfected for 24 h with the plasmids encoding wild-type or mutant (lacking the SREBP2-responsive element) LDLR promoters upstream of firefly luciferase. As a control for transfection efficiency, we used the Renilla luciferase pRL-TK vector (Promega). Cells were stimulated with 50 ng/ml NGF, 5 ng/ml pro-NGF, or 1 μM simvastatin for 24 h followed by measurement of firefly and Renilla luciferase activities using a luminometer (Promega, Biofellow, Helsinki, Finland) as described (29, 30). Results are shown as fold increase in firefly luciferase activity normalized to that of Renilla luciferase. The activity of the NF-κB promoter was similarly studied. To block NF-κB, we expressed the mutant (S32A/S36A), dominant-negative inhibitor of κB-α (DN-IκBα), which is not phosphorylated by the IκB kinase and thus not subsequently degraded (28, 30).

RNA Isolation and Quantitative PCR—RNA was extracted from control and pro-NGF-treated Huh7 cells using the RNeasy mini kit (Qiagen) followed by cDNA synthesis. LightCycler 480 SYBR Green I Master (Roche Applied Science) real time quantitative-PCR assays were performed on a LightCycler 480 (Roche Applied Science) with a 96-well block as described (24, 27). The results show the averages of three replicate experiments normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The following primer sequences were used: LDLR, forward, 5’-GACGTGGCGTGTAACATCTG-3’; reverse, 5’-CTGGCACGGCAAATGCTTGTTG-3’; GAPDH, forward, 5’-TTCGTCTAGGTTGTTGAACCA-3’; reverse, 5’-CTGTGGTCATGGGTGTGAAAC-3’. Quantification and Statistics—Statistical comparisons were done using one-way ANOVA followed by a Bonferroni post hoc test for three or more groups. The Student’s t test was used in experiments with two groups with GraphPad Prism version 4.0 (GraphPad Software, La Jolla, CA). Values are expressed as means ± S.E., and p ≤ 0.05 was considered significant.

FIGURE 1. pro-NGF and NGF are increased in livers of leptin-deficient mice. Livers were obtained from wild-type (wt) and ob/ob genetically obese mice, and immunoblots were done using specific antibodies against NGF, pro-NGF, and LDLR as described under “Experimental Procedures.” β-Actin as control. Upper panels, LDLR levels. Lower panels, relative NGF and pro-NGF levels. *p < 0.01 for WT versus ob/ob mice for LDLR. **p < 0.01 for WT versus ob/ob mice for NGF.
NGF Activates SREBP2 and Stimulates LDLR via Caspase-3

**Results**

**NGF and Pro-NGF Are Increased in the Liver of ob/ob Mice**—To investigate the possible involvement of NGF and pro-NGF in lipid metabolism, we analyzed livers obtained from control and leptin-deficient ob/ob mice. These mice are obese and display an increase in lipid accumulation in the liver and other peripheral tissues (16). Immunoblots showed that the levels of NGF and pro-NGF are significantly higher in the livers of ob/ob mice compared with controls concomitant with an increase in LDLR levels (Fig. 1). These findings suggest a role of NGF and pro-NGF in the regulation of LDLR and lipoprotein metabolism in liver tissue.

**NGF and Pro-NGF Increase LDLR Expression in Huh7 Cells**—To study the links between elevations in NGF and pro-NGF and the lipid accumulation in liver tissue, we examined human Huh7 hepatocyte cells that were stimulated with the neurotrophins. Data showed that NGF increased the LDLR levels in Huh7 cells in a time- and concentration-dependent manner (Fig. 2A). SREBP2 is an integral membrane protein retained in the endoplasmic reticulum bound to the (SCAP) insulin-in...
duced gene protein (INSIG) complex (17). Under conditions of low cholesterol, SCAP-SREBP2 is transported to the Golgi compartment where it is sequentially cleaved by site 1 and site 2 proteases (S1P and S2P, respectively) to release the active amino-terminal fragment of SREBP2 that moves from the cytosol to the nucleus (17, 18). We were therefore interested to study whether SREBP2 is involved in the effect observed with NGF.

Immunoblots of cell extracts from Huh7 cells showed an increase in the levels of processed SREBP2 after NGF treatments detected by an antibody against the carboxyl-terminal region of SREBP2 (Fig. 2B). The cholesterol-lowering drug, simvastatin, also induced cleavage of SREBP2 in the Huh7 cells (Fig. 2B) in line with its known effect on LDLRs (31). To study the levels of active SREBP2, we used an antibody against the amino-terminal region of the molecule. Results showed that the level of nuclear SREBP2 was increased in NGF-treated Huh7 cells (Fig. 2C). In addition, there was an increase in LDLR gene transcription induced by NGF as made evident by an enhanced LDLR gene promoter activity (Fig. 2D). In contrast, the mutant LDLR promoter lacking the SREBP2-responsive element showed no activation by NGF further supporting an involvement of SREBP2 in LDLR expression by NGF treatment (Fig. 2D). An enhanced expression of LDLRs is usually reflected in an increase in LDLRs at the plasma membrane followed by an elevated lipoprotein uptake by the cells (32). We observed that treatment with 50 ng/ml NGF resulted in an enhanced uptake of lipoprotein particles by Huh7 cells in support of such a model (Fig. 2E). In addition, treatment with 5 ng/ml pro-NGF produced a similar increase in lipoprotein uptake suggesting an involvement of p75NTRs.

Involvement of p75NTR in the Regulation of LDLRs in Hepatocytes—Using immunoblots, we observed that the Huh7 cells express p75NTR in appreciable amounts (Fig. 3A), whereas no TrkA expression could be detected (data not shown).
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Stimulation of Huh7 cells with pro-NGF, known to activate p75NTR, also increased the LDLR mRNA and LDLR protein levels in the cells (Fig. 3, B and C). Employing the LDLR promoter reporter, we observed an increase upon pro-NGF stimulation suggesting an increased LDLR gene activity (Fig. 3D). Together, these results show that p75NTR signaling induced by either NGF or pro-NGF is essentially involved in the regulation of LDLR expression in Huh7 hepatocyte cells. To

FIGURE 4. p75NTR activation induces SREBP2 cleavage in a caspase-3-dependent manner. A, schematic structure of SREBP2 in the membrane, its interaction with the SCAP-INSIG complex, and the protease cleavage sites. In addition to the classical S1P and S2P proteases, there is a caspase-3 site at Asp-468 close to the membrane. Mutation of this site to alanine (D468A) was done as described under “Experimental Procedures.” 8, 5 ng/ml pro-NGF increased the levels of cleaved caspase-3 (17 kDa) in a time-dependent manner in Huh7 cells. C, 50 ng/ml NGF increased caspase-3 cleavage that was blocked by using 50 μM Boc, a broad caspase inhibitor (left), and SREBP2 cleavage that was blocked by 1 μM of the specific caspase-3 inhibitor, Z-DEVD-fmk (DEVD). D, cells were transfected with the expression plasmids encoding DN-caspase-3 and further stimulated with 50 ng/ml NGF for 16 h. GFP-expressing plasmid was used as a control. Expression of DN-caspase-3 blocked the increase in cleaved SREBP2 induced by NGF. Left, immunoblot. Right, quantification. Values are mean ± S.E., n = 4. ***, p < 0.001 for NGF versus C, and for NGF + DN-Casp3 versus NGF. E, cells were transfected with mutant SREBP2-D468A (mut-SREBP2) expression plasmid for 24 h followed by stimulation with 5 ng/ml pro-NGF for 16 h. GFP-expressing plasmid was used as a control. Levels of LDLR were determined by immunoblotting. Left, immunoblots. Right, quantification. Values are mean ± S.E., n = 6. *, p < 0.05 for pro-NGF versus C in GFP-expressing cells. There was no increase in LDLRs in mut-DREBP2-expressing cells. The inset shows the levels of full-length (FL) SREBP2 in control and mut-SREBP2-expressing cells.
To corroborate this, we then studied primary hepatocytes isolated from wild-type and p75NTR gene-deleted mice. Data showed that pro-NGF is able to increase LDLR levels in wild-type primary hepatocytes but not in those lacking p75NTRs (Fig. 3E).

**SREBP2 Is Cleaved in a Caspase-3-dependent Manner Downstream of p75NTR**—Apart from the S1P and S2P protease cleavage sites mentioned above, SREBP2 has also a canonical caspase-3 cleavage site at Asp-468 (Fig. 4A). However, the physiological role of this caspase-3 site has remained enigmatic (19). We observed that addition of pro-NGF or NGF induced cleavage (activation) of caspase-3 in Huh7 cells as shown by immunoblotting (Fig. 4, B and C). The broad range caspase inhibitor, Boc-aspartyl (Ome)-fluoromethyl ketone (fmk) as well as the more specific caspase-3/7 inhibitor, Z-DEVD-fmk, significantly reduced this cleavage of caspase-3 induced with NGF (Fig. 4C). Most importantly, Z-DEVD-fmk also blocked the increase in cleaved SREBP2 induced by NGF in Huh7 cells (Fig. 4C).

To study this further, we expressed the DN caspase-3 construct (20) in the cells that inhibited the SREBP2 cleavage caused by NGF treatment (Fig. 4D). These results support the notion that caspase-3 is involved in NGF-induced cleavage of SREBP2 in Huh7 cells.
NGF Activates SREBP2 and Stimulates LDLR via Caspase-3

![Diagram](image)

**FIGURE 6.** p75NTR activation increases caspase-3 activity in the membrane fraction. Huh7 cells were stimulated with 5 ng/ml pro-NGF or 50 ng/ml NGF for 16 h followed by cell isolation of cytotoxic and membrane fractions. Caspase 3/7 activity was determined as described under “Experimental Procedures.” In some experiments 1 μg/ml staurosporine was added for 3 h to increase caspase activity. A and B, NGF and pro-NGF stimulated caspase-3/7 activity in the membrane fraction (B) but was not significant in the cytosolic fraction (A). Staurosporine, in contrast, stimulated caspase-3/7 activity mainly in the cytosol. The results are given as relative luminescence units (RLU), indicative of relative caspase activity. Values given are mean ± S.E., n = 4–5. *, p < 0.05 for pro-NGF versus C, and for NGF versus C in the membrane fraction. **C, D** shows that the caspase-3 site in SREBP2 is essentially involved in the regulation of LDLRs in the Huh7 cells.

Stimulation of p75NTR by Pro-NGF Activates p38 MAPK—We next investigated the signaling cascades that regulate SREBP2 cleavage in cells following p75NTR activation. We observed that the phosphorylation/activation of p38 MAPK was increased by pro-NGF (Fig. 5A). This occurred upstream of caspase-3 as expression of dominant-negative p38 MAPK (DN-p38-α) or the use of the p38 MAPK inhibitor, SB203580, decreased caspase-3 cleavage (Fig. 5, B and C). Blocking the activity of MKK6, a potential activator in the p38 MAPK signaling pathway, using respective dominant-negative constructs (21) also reduced the amount of cleaved caspase-3 in pro-NGF-treated cells (Fig. 5D). Furthermore, SB203580 blocked the increase in LDLR promoter activity brought about by pro-NGF (Fig. 5E). These results identify p38 MAPK cascade as a crucial signaling pathway in the regulation of caspase-3 and LDLR expression induced by pro-NGF.

Caspase-3 Activity Localizes to Intracellular Membranes and Pro-caspase-3 Binds Caspase-2—Results so far indicated that stimulation of p75NTR leads to caspase-3 activation in a p38 MAPK-dependent manner with the subsequent cleavage of SREBP2, releasing the active form of the molecule. As SREBP2 is localized to intracellular membranes (Fig. 4A), we were interested to know whether caspase-3 could also localize to the membranes in Huh7 cells. To study this, we undertook cell fractionation of control and neurotrophin-stimulated Huh7 cells to produce membrane and the cytosolic fractions. A significant increase in caspase-3 activity was observed in the membrane fraction after NGF and pro-NGF treatments, with less obvious changes in the cytosolic fraction (Fig. 6, A and B). In contrast, addition of staurosporine at 1 μM, a concentration that is able to induce cell death, produced a large increase in caspase-3 activity in the cytosol (Fig. 6A).

To confirm that caspase-3 is present in the membrane fraction, we performed immunoblotting of proteins in this fraction. Data showed that pro-caspase-3 (32 kDa) is present in the membrane fraction in control and pro-NGF-stimulated cells (Fig. 6C). The relative level of caspase-3 in the membrane fraction increased following pro-NGF treatment (Fig. 6C), an observation that will require more studies.

As the membrane localization of caspase-3 has not been described before, we searched for possibilities that it may interact with some other caspases in the cell. Immunostaining experiments then showed that caspase-2 localizes largely to the Golgi compartment in human hepatocytes (Fig. 6D), which is in line with previous observations made in other cell types (33). Immunoblotting confirmed the presence of caspase-2 in the membrane fraction isolated from Huh7 cells (Fig. 6C). Using the BiFC assay (22), we observed that caspase-2 and pro-caspase-3 do bind each other in the hepatocytes. This was as shown by the fluorescence produced by interacting split-Venus fragments that were cloned in-frame with the corresponding caspases (Fig. 7A). Immunoprecipitation experiments using...
overexpressing plasmids confirmed the binding of pro-caspase-3 to caspase-2 in these cells (Fig. 7B). Most significantly, stimulation of the cells with pro-NGF significantly reduced the interaction between caspase-2 and pro-caspase-3 (Fig. 7B) suggesting that it can be modulated by some signaling component in the p57NTR pathway. To study whether the activity of p38 MAPK influenced this interaction, we expressed DN-p38\(^{\alpha}/H9251\) (see above) in the cells that were then stimulated with pro-NGF. The results showed that DN-p38\(^{\alpha}/H9251\) is able to restore the binding of pro-caspase-3 to caspase-2 (Fig. 7C) showing that p38 MAPK activity reduces the pro-caspase-3/caspase-2 binding in these cells. This, together with the fact that DN-p38\(^{\alpha}/H9251\) blocks pro-NGF-induced caspase-3 activity in the cells, suggests that phosphorylation of the caspase-2-caspase-3 complex leads to the release of pro-caspase-3 causing activation of caspase-3 at the membrane and the subsequent cleavage of SREBP2. The precise targets for p38 MAPK-mediated phosphorylation in the caspase-2-caspase-3 complex is currently under investigation.

\[ p75NTR \text{ Activation Stimulates NF-\(\kappa/B \) Signaling in Hepatocytes to Promote Cell Survival—} \]

Our finding that caspase-3 is cleaved subsequent to p75NTR activation raised the question whether the hepatocytes undergo cell death upon receptor stimulation. To ascertain this, we stimulated Huh7 cells with increasing concentrations of NGF or pro-NGF. Notably, these treatments did not result in increased cell death as scored by the number of degenerating cells (Fig. 8A). Neurotrophins were previously shown to activate the NF-\(\kappa/B \) signaling in cultured Schwann cells and in primary neurons (30, 34). We observed that both NGF and pro-NGF increased the activity of NF-\(\kappa/B \) in the Huh7 cells, transfected with the NF-\(\kappa/B \) driven-promoter construct (Fig. 8B). Moreover, blocking NF-\(\kappa/B \) signaling using the DN inhibitor of NF-\(\kappa/B \) (I\(\kappa/B \)) rendered the hepatocyte cells more vulnerable to cell death upon NGF or pro-NGF treatments (Fig. 8C). These results lend credence to the view that p75NTR stimulation in Huh7 cells activates NF-\(\kappa/B \) signaling that can promote cell survival preventing apoptosis of these cells. A summary of the pathways activated by p75NTR in the hepatocyte cells is depicted in Fig. 9 to show the dual signaling of p75NTR regulating survival via NF-\(\kappa/B \) and caspase-3-mediated LDLRs via p38 MAPK signaling.

**Discussion**

Lipid metabolism and cholesterol levels are tightly regulated by both transcriptional and post-transcriptional mechanisms to ensure their proper levels in the cell (17, 18, 35). Disturbances in lipid metabolism can lead to increased levels of cell cholesterol and other lipids that are toxic to cells as observed in human disorders, such as cardiovascular and liver diseases (9, 36). We describe here a novel pathway by which the neurotro-
NGF Activates SREBP2 and Stimulates LDLR via Caspase-3

FIGURE 8. NGF and pro-NGF do not induce cell death but increase NF-κB signaling in Huh7 cells. A, Huh7 cells were incubated for 24 h with different concentrations of NGF, pro-NGF, and staurosporine. Cell viability was analyzed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay as described under “Experimental Procedures.” NGF and pro-NGF affect cell viability, whereas staurosporine (STS) reduced it. Values are means ± S.D., n = 4. *, p < 0.05, and **, p < 0.01, for NGF versus C, and ***, p < 0.001, for pro-NGF versus C. C, role of NF-κB activation by NGF and pro-NGF. Cells were transfected with DN-IκBα construct (lacking the phosphorylation sites for IκB kinase) or control GFP plasmid and then stimulated with 5 ng/ml pro-NGF for 24 h. The pro-NGF reduced cell viability only in the cells expressing DN-IκBα. Values are means ± S.E., n = 4. **, p < 0.01, for pro-NGF + DN-IκBα versus pro-NGF and for pro-NGF + DN-IκBα versus DN-IκBα.

FIGURE 9. Schematic summary of the results. Stimulation of p75NTR with pro-NGF or NGF activates p38 MAPK signaling in the hepatocytes resulting in caspase-3 activation in the membrane fraction. Caspase-3 in turn cleaves SREBP2 with the subsequent release of the amino-terminal part of SREBP2 activating LDLR gene expression. The increased of LDLR levels results in an enhanced cellular uptake of LDL particles. Caspase-2 plays a role in the regulation LDLR expression by binding pro-caspase-3 in the cell and probably by localizing it to the membrane. In addition NF-κB signaling is activated by pro-NGF in these cells that promotes cell survival. The precise molecular events controlling the dual signaling of p75NTR in these cells warrant further studies. The neurotrophin-induced pathway for SREBP2 and LDLR regulation described here may be particularly activated during inflammation and tissue trauma and contribute to metabolic disorders.

Aphins, such as NGF and pro-NGF, signaling via p75NTR, can activate SREBP2 that leads to an increased LDLR expression and a stimulation of lipoprotein uptake by Huh7 cells. In this cascade, the SREBP2 is activated by the cleavage at a amino-terminal site subsequent to the activation of caspase-3 and p38 MAPK by p75NTR signaling. The caspase-3 site in SREBP2 was found essential for this activity of the neurotrophins as shown by mutation of this caspase site and by using specific inhibitors blocking the caspase-3 activity. The p75NTR-induced caspase-3 pathway represents a novel physiological mechanism for LDLR regulation that may act in parallel with and partly by-pass the sterol-dependent SREBP2 regulation in cells.

In this context we have recently shown that pro-NGF and NGF elevate LDLRs in neuronal cells leading to an increased LDL uptake by the neurons (15). The underlying mechanism for this increase was not fully delineated, and the interpretation was difficult because septal neurons and PC6.3 cells express both p75NTR and TrkA receptors. We then observed that Huh7 hepatocyte cells express p75NTRs but no significant amounts of TrkA receptors making these cells more ideal to study the role of p75NTR. Using primary hepatocytes from p75NTR gene-deleted mice, we then obtained evidence that this receptor is crucial for the action of NGF and pro-NGF to increase LDLRs in hepatocytes. In addition, activation of p38 MAPK by p75NTR was found to be essential for the subsequent caspase-3 and SREBP2 activation in the hepatocytes.

Surprisingly in these experiments, we observed no overt cell death of Huh7 cells despite an increase in active caspase-3 by pro-NGF. Instead, we found the concomitant activation of NF-κB signaling pathways by p75NTR activation that increased the viability of the hepatocytes.

We hypothesize that some anti-apoptotic genes and factors are induced in the Huh7 cells by p75NTR activation and NF-κB signaling that contribute to cell survival, but the nature of these remains to be established.
Another explanation why activated caspase-3 did not kill Huh7 cells in our experiment is that their activation could occur rather locally in the cell, e.g. adjacent to intracellular membranes. We obtained evidence that caspase-3 activity increased in the membrane fraction following pro-NGF and NGF stimulations. Localization of caspase-3 to intracellular membranes has not been shown before and thus represents an additional mechanism by which the activity of this caspase can be regulated. The presence of various caspases in different cellular compartments, however, is not without precedent, and both caspase-2 and caspase-12 have previously been associated with intracellular membranes (37–39). We observed here that caspase-2 is present also in membranes of hepatocytes and is able to bind pro-caspase-3 in Huh7 cells as shown by immunoprecipitation and employing the BiFC assay for detecting protein-protein interaction in living cells. Most importantly, by using immunoprecipitation the binding of pro-caspase-3 to caspase-2 was reduced by p38 MAPK activated by p75NTR in these cells. It is likely that the caspase-2 binding of pro-caspase-3 could restrict the interaction of caspase-3 to specific substrates in the cell as shown here for SREBP2. Moreover, caspase-2 is present within the Golgi membrane compartment where SREBP2 is also localized following its transport from the ER. However, it is not formally excluded that the cleavage and activation of SREBP2 by caspase-3 may occur at other intracellular membrane compartments as well and particularly under conditions of severe cell stress. The precise targets for activated p38 MAPK to influence the stability of the caspase-3-caspase-2 complex and thereby caspase-3 activation in the cell is currently under investigation.

Many human metabolic diseases are characterized by elevated lipid content and increased cell toxicity. Inflammation is part of the metabolic disorders that are accompanied by an enhanced production of various cytokines and growth factors (9). Previous studies have shown that NGF levels are increased in various inflammatory diseases (40–43) and after nerve injury (44). Data obtained here using a genetically obese ob/ob mouse model showed that NGF and pro-NGF levels are increased in fatty liver together with increased LDLRs. These results indicate a role of p75NTRs in lipid metabolism in hepatocytes and possibly in metabolic disorders. During the preparation of this manuscript, Baeza-Raja et al. (45) reported that p75NTR signaling is involved in obesity and energy regulation in adipocytes. In particular, it was shown that p75NTR gene-deleted mice are protected against the effects of high fat diet, including insulin resistance and fatty liver (45). Our results on the role of p75NTR in inducing LDLRs and lipoprotein uptake into hepatocytes are in accordance with this notion. In addition we show a novel pathway for p75NTR in lipid regulation that involves activation of p38 MAPK and the caspase-3-dependent SREBP2 activation. In the future, it would be important to study whether this signaling pathway is active in human lipid disorders and whether it may contribute to cytokine-mediated metabolic diseases.

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References
1. Sofroniew, M. V., Howe, C. L., and Mobley, W. C. (2001) Nerve growth factor signaling, neuroprotection, and neural repair. Annu. Rev. Neurosci. 24, 1217–1281
2. Allen, S. J., and Dawbarn, D. (2006) Clinical relevance of the neurotrophins and their receptors. Clin. Sci. 110, 175–191
3. Chao, M. (2003) Neurotrophins and their receptors: a convergence point for many signalling pathways. Nat. Rev. Neurosci. 4, 299–309
4. Huang, E. J., and Reichardt, L. F. (2003) Trk receptors: roles in neuronal signal transduction. Annu. Rev. Biochem. 72, 609–642
5. Dechent, G., and Barde, Y. A. (2002) The neurotrophin receptor p75NTR: novel functions and implications for diseases of the nervous system. Nat. Neurosci. 5, 1131–1136
6. Barker, P. A. (2004) p75NTR is positively promiscuous: novel partners and new insights. Neuron 42, 529–533
7. Ibáñez, C. F., and Simi, A. (2012) p75 neurotrophin receptor signaling in nervous system injury and degeneration: paradox and opportunity. Trends Neurosci. 35, 431–440
8. Hempstead, B. L. (2014) Deciphering pronerveotrophin actions. Handb. Exp. Pharmacol. 220, 17–32
9. Brenner, C., Galluzzi, L., Kepp, O., and Kroemer, G. (2013) Decoding cell death signals in liver inflammation. J. Hepatol. 59, 583–594
10. Heumann, R., Lindholm, D., Bandtlow, C., Meyer, M., Radeke, M. J., Misko, T. P., Shooter, E., and Thoenen, H. (1987) Differential regulation of mRNA encoding nerve growth factor and its receptor in rat sciatic nerve during development, degeneration, and regeneration: role of macrophages. Proc. Natl. Acad. Sci. U.S.A. 84, 8735–8739
11. Shulga, A., Magalhães, A. C., Autio, H., Plantman, S., di Lieto A., Nykjær, A., Carlstedt, T., Rissing, M., Arumüüe, U., Castrén, E., and Rivera, C. (2012) The loop diuretic bumetanide blocks posttraumatic p75NTR upregulation and rescues injured neurons. J. Neurosci. 32, 1755–1770
12. Peeraully, M. R., Jenkins, J. R., and Trayhurn, P. (2004) NGF gene expression and secretion in white adipose tissue: regulation in 3T3-L1 adipocytes by hormones and inflammatory cytokines. Am. J. Physiol. Endocrinol. Metab. 287, E331–E339
13. Deponti, D., Buono, R., Catanarzo, G., De Palma, C., Longhi, R., Menervi, R., Bresolin, N., Bassi, M. T., Cossu, G., Clementi, E., and Brunelli, S. (2009) The low-affinity receptor for neurotrophins p75NTR plays a key role for satellite cell function in muscle repair acting via Rhoa. Mol. Biol. Cell 20, 3620–3627
14. Baeza-Raja, B., Li, P., Le Moan, N., Sachs, B. D., Schächtrup, C., Davalos, D., Vagena, E., Bridges, D., Kim, C., Saltiel, A. R., Olefsky, J. M., and Arumae, U. (2003) GDNF-deprived sympathetic neurons die via a novel mechanism. J. Neurophysiol. 90, 2876–2886
15. Do, H. T., Bruelle, C., Pham, D. M., Jauhiainen, M., Eriksson, O., Korhonen, L. T., and Lindholm, D. (2015) Nerve growth factor (NGF) and pro-NGF increase low-density lipoprotein (LDL) receptors in neuronal cells partly by different mechanisms: role of LDL in neurite outgrowth. J. Neurochem. 136, 306–315
16. Lindström, P. (2007) The physiology of obese-hyperglycemic mice (Ob/Ob mice). Scientific World J. 7, 666–685
17. Goldstein, J. L., DeBoese-Boyd, R. A., and Brown, M. S. (2006) Protein sensors for membrane sterols. Cell 124, 35–46
18. Zelcer, N., Hong, C., Boyadjian, R., and Tontonoz, P. (2009) LXR regulates cholesterol uptake through Idol-dependent ubiquitination of the LDL receptor. Science 325, 100–104
19. Wang, X., Zelenski, N. G., Yang, J., Sakai, J., Brown, M. S., and Goldstein, J. L. (1996) Cleavage of sterol regulatory element binding proteins (SREBPs) by CPP32 during apoptosis. EMBO J. 15, 1012–1020
20. Yu, L. Y., Jokitalo, E., Sun, Y. F., Mehlen, P., Lindholm, D., Saarma, M., and Arumaa, U. (2003) GDNF-deprived sympathetic neurons die via a novel
NGF Activates SREBP2 and Stimulates LDLR via Caspase-3

nonmitochondrial pathway. *J. Cell Biol.* **163**, 987–997

21. Enslen, H., Raingeaud, J., and Davis, R. J. (1998) Selective activation of p38 mitogen-activated protein (MAP) kinase isoforms by the MAP kinase kinases MKK3 and MKK6. *J. Biol. Chem.* **273**, 1741–1748

22. Bouchier-Hayes, L., and Green, D. R. (2010) Real time with caspase-2. *Cell Cycle* **9**, 12–13

23. Eriksson, O., Pollesello, P., and Geimonen, E. (1999) Regulation of total mitochondrial Ca2+ in perfused liver is independent of the permeability transition pore. *Am. J. Physiol.* **276**, C1297–C1302

24. Do, H. T., Tselykh, T. V., Mäkelä, J., Ho, T. H., Olkkonen, V. M., Bornhauser, B. C., Korhonen, L., Zelcer, N., and Lindholm, D. (2012) Fibroblast growth factor-21 (FGF21) regulates low-density lipoprotein receptor (LDLR) levels in cells via the E3-ubiquitin ligase Mylip/Idol and the Canopy2 (Cnpy2)/Mylip-interacting saposin-like protein (Msap). *J. Biol. Chem.* **287**, 12602–12611

25. Korhonen, L., Nääpänkangas, U., Steen, H., Chen, Y., Martinez, R., and Lindholm, D. (2004) Differential regulation of X-chromosome-linked inhibitor of apoptosis protein (XIAP) and caspase-3 by NMDA in developing hippocampal neurons; involvement of the mitochondrial pathway in NMDA-mediated neuronal survival. *Exp. Cell Res.* **295**, 290–299

26. Ammoun, S., Lindholm, D., Wootz, H., Akerman, K. E., and Kukkonen, J. P. (2006) G-protein-coupled OX1 orexin/hcrtr-1 hypocretin receptors induce caspase-dependent and -independent cell death through p38 mitogen-stress-activated protein kinase. *J. Biol. Chem.* **281**, 834–842

27. Hyrskyliuoto, A., Bruelle, C., Lundh, S. H., Do, H. T., Kivinen, J., Rappou, E., Reijonen, S., Waltimo, T., Petersén, Å., Lindholm, D., and Korhonen, L. (2014) Ubiquitin-specific protease 14 reduces cellular aggregates and protects against mutant huntingtin-induced cell degeneration: involvement of the proteasome and ER stress-activated kinase Ire1α. *Hum. Mol. Genet.* **23**, 5928–5939

28. Hyrskyliuoto, A., Pulli, I., Törnqvist, K., Ho, T. H., Korhonen, L., and Lindholm, D. (2013) Sigma-1 receptor agonist PRE084 is protective against mutant huntingtin-induced cell degeneration: involvement of calpastatin and the NF-κB pathway. *Cell Death Dis.* **4**, e646

29. Do, H. T., Bruelle, C., Tselykh, T., Jalonen, P., Korhonen, L., and Lindholm, D. (2013) Reciprocal regulation of very low-density lipoprotein receptors (VLDLRs) in neurons by brain-derived neurotrophic factor (BDNF) and Reelin: Involvement of the E3 ligase Mylip/Idol. *J. Biol. Chem.* **288**, 29613–29620

30. Kairisalo, M., Korhonen, L., Sepp, M., Prruusniel, P., Kukkonen, J. P., Kivinen, J., Timmusk, T., Blomgren, K., and Lindholm, D. (2009) NF-κB-dependent regulation of brain-derived neurotrophic factor in hippocampal neurons by X-linked inhibitor of apoptosis protein. *Eur. J. Neurosci.* **30**, 958–966

31. Knopp, R. H. (1999) Drug treatment of lipid disorders. *N. Engl. J. Med.* **341**, 498–511

32. Brown, M. S., and Goldstein, J. L. (1986) A receptor-mediated pathway for cholesterol homeostasis. *Science* **232**, 44–47

33. Mancini, M., Machamer, C. E., Roy, N., Nicholson, D. W., Thornberry, N. A., Casciola-Rosen, L. A., and Rosen, A. (2000) Caspase-2 is localized at the Golgi complex and cleaves golgin-160 during apoptosis. *J. Cell Biol.* **149**, 603–612

34. Carter, B. D., Kalschmidt, C., Kalschmidt, B., Offenhäuser, N., Böhm-Matthaei, R., Baeuerle, P. A., and Barde, Y. A. (1996) Selective activation of NF-κB by nerve growth factor through the neurotrophin receptor p75. *Science* **272**, 542–545

35. Hong, C., Duit, S., Jalonen, P., Out, R., Scheer, L., Sorrentino, V., Boyadjian, R., Rodenburg, K. W., Foley, E., Korhonen, L., Lindholm, D., Nimpf, J., van Berkel, T. J., Tontonoz, P., and Zelcer, N. (2010) The E3 ubiquitin ligase IDOL induces the degradation of the low density lipoprotein receptor family members VLDLR and ApoER2. *J. Biol. Chem.* **285**, 19720–19726

36. Nabel, E. G. (2003) Cardiovascular disease. *N. Engl. J. Med.* **349**, 60–72

37. Degterev, A., Boyce, M., and Yuan, J. (2003) A decade of caspases. *Onco- genes* **22**, 8543–8567

38. Wootz, H., Hanson, L., Korhonen, L., Nääpänkangas, U., and Lindholm, D. (2004) Caspase-12 cleavage and increased oxidative stress during motoneuron degeneration in transgenic mouse model of ALS. *Biochem. Biophys. Res. Commun.* **322**, 281–286

39. Vakifahmetoglu-Norberg, H., and Zhivotovsky, B. (2010) The unpredictable caspase-2: what can it do? *Trends Cell Biol.* **20**, 150–159

40. Aloe, L., Probert, S. D., Leon, A., and Levi-Montalcini, R. (1994) Nerve growth factor and autoimmune diseases. *Autoimmunity* **19**, 141–150

41. Rüttimann, R. S., Söderström, S., Korhonen, L. T., and Lindholm, D. B. (1998) Overstimulation of nerve growth factors in postinfectious and autoimmune diseases. *Pediatr. Neurol.* **18**, 231–235

42. Aalto, K., Korhonen, L., Lahdenne, P., Pelkonen, P., and Lindholm, D. (2002) Nerve growth factor in serum of children with systemic lupus erythematosus is correlated with disease activity. *Cytokine* **20**, 136–139

43. Lindholm, D., Heumann, R., Meyer, M., and Thoenen, H. (1987) Interleukin-1 regulates synthesis of nerve growth factor. *Cell Death Dis.* **149**, 141–150

44. Baeza-Raja, B., Sachs, B. D., Li, P., Christian, F., Vagena, E., Davalos, D., Le Moan, N., Ryu, J. K., Sikorski, S. L., Chan, J. P., Scadeng, M., Taylor, S. S., Houslay, M. D., Baillie, G. S., Saltiel, A. R., et al. (2016) p75 neurotrophin receptor regulates energy balance in obesity. *Cell Rep.* **14**, 255–268