Nerve Growth Factor Blocks the Glucose-induced Down-regulation of Caveolin-1 Expression in Schwann Cells via p75 Neurotrophin Receptor Signaling*

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Altered neurotrophism in diabetic peripheral neuropathy (DPN) is associated in part with substantial degenerative changes in Schwann cells (SCs) and an increased expression of the p75 neurotrophin receptor (p75NTR). Caveolin-1 (Cav-1) is highly expressed in adult SCs, and changes in its expression can regulate signaling through Erb B2, a co-receptor that mediates the effects of neuregulins in promoting SC growth and differentiation. We examined the hypothesis that hyperglycemia-induced changes in Cav-1 expression and p75NTR signaling may contribute to altered neurotrophism in DPN by modulating SC responses to neuregulins. In an animal model of type 1 diabetes, hyperglycemia induced a progressive decrease of Cav-1 in SCs of sciatic nerve that was reversed by insulin therapy. Treatment of primary neonatal SCs with 20–30 mM glucose, but not L-glucose, was sufficient to inhibit transcription from the Cav-1 promoter and decrease Cav-1 mRNA and protein expression. Hyperglycemia prolonged the kinetics of Erb B2 phosphorylation and significantly enhanced the mitogenic response of SCs to neuregulin1-β1, and this effect was mimicked by the forced down-regulation of Cav-1. Intriguingly, nerve growth factor antagonized the enhanced mitogenic response of SCs to neuregulin1-β1 and inhibited the glucose-induced down-regulation of Cav-1 transcription, mRNA, and protein expression through p75NTR-dependent activation of JNK. Our data suggest that Cav-1 down-regulation may contribute to altered neurotrophism in DPN by enhancing the response of SCs to neuregulins and that p75NTR-mediated JNK activation may provide a mechanism for the neurotrophic modulation of hyperglycemic stress.

Diabetic peripheral neuropathy (DPN) has a complicated etiology with contributions from metabolic and vascular insults.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF489529.

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The abbreviations used are: DPN, diabetic peripheral neuropathy; Cav-1, caveolin-1; EGFP, enhanced green fluorescent protein; FCS, fetal calf serum; JNK, c-Jun N-terminal kinase; NGF, nerve growth factor; NRG1-β1, human recombinant neuregulin1-β1 (176–246); SCs, Schwann cells; STZ, streptozotocin; DMEM, Dulbecco’s modified Eagle’s medium; CMV, cytomegalovirus; PBS, phosphate-buffered saline; ANOVA, analysis of variance; dn-JNK2, dominant-negative JNK 2; pfu, plaque-forming units.

Cav-1, caveolin-1, EGFP, enhanced green fluorescent protein; FCS, fetal calf serum; JNK, c-Jun N-terminal kinase; NGF, nerve growth factor; NRG1-β1, human recombinant neuregulin1-β1 (176–246); SCs, Schwann cells; STZ, streptozotocin; DMEM, Dulbecco’s modified Eagle’s medium; CMV, cytomegalovirus; PBS, phosphate-buffered saline; ANOVA, analysis of variance; dn-JNK2, dominant-negative JNK 2; pfu, plaque-forming units.
ropathies (21). Thus, decreased Cav-1 levels following nerve injury (22) may remove an inhibitory regulation of Erb B2 and enhance cellular responses to neuregulins. However, whether prolonged diabetes may decrease Cav-1 expression in peripheral nerve and contribute to altered neurotophin in DPN by affecting the response of SCs to neuregulins is unknown.

In this study, we examined the effect of hyperglycemia and neuregulin on the regulation of Cav-1 expression in SCs and the response to neuregulins. We demonstrate that either hyperglycemia or forced down-regulation of Cav-1 is sufficient to alter biologically relevant responses of SCs to neuregulin-1-A1. Additionally, we provide support that p75NTR-dependent activation of c-Jun N-terminal kinase (JNK) can attenuate the effect of hyperglycemia on altering Cav-1 expression and signaling through the neuregulin/Erb B2 ligand-receptor cassette. Collectively, our data suggest that hyperglycemia-induced changes in Cav-1 and p75NTR may contribute to altered neurotophin in DPN by affecting the response of SCs to neuregulins.

**EXPERIMENTAL PROCEDURES**

*Streptozotocin-induced Diabetes and Insulin Therapy*—Male rats (200–250 g) were rendered diabetic with an intraperitoneal injection of 65 mg/kg streptozotocin (STZ) dissolved immediately before injection in 1 ml of sterile 10 mM sodium citrate in phosphate-buffered saline (PBS), pH 4.5. Control animals were treated similarly but received only a 1 ml injection of the sodium citrate vehicle. Fasting blood glucose levels (overnight) were determined after 1 week using a commercial glucometer (Precision QID), and animals exhibiting a blood glucose level of ≥300 mg/dl (16.7 mM) were deemed diabetic and enrolled in the study. If necessary, non-diabetic animals were re-injected, and the procedure was repeated. Two control plus three diabetic animals were randomly assigned to groups maintained for 3, 6, 9, 12, 15, or 17 weeks following their initial designation as diabetic. All animals were maintained in a 12-h light/dark cycle with 50% relative humidity and 22 °C temperature. The slides were washed and coverslips mounted with Permount. Images were captured on a Bio-Rad MRC 1000 confocal microscope.

To obtain a semi-quantitative estimate of the loss of Cav-1 immunoreactivity, its staining intensity was quantitated using the intensity of the green (Cav-1) and red (S100β) signals surrounding the nerve fibers in 60 randomly selected areas of each nerve cross-section was assessed using Adobe Photoshop.

*Schwann Cell Preparation and Culture*—Sciatic nerves were dissected from postnatal day 2 or day 3 Sprague-Dawley rat pups, and SCs were isolated essentially as described (24). The nerves were rinsed with L15 medium (L15) and incubated with 0.1% collagenase in L15 medium for 30 min at 37 °C. The tissue was then cut with L15 medium containing 0.25% trypsin plus 0.1% collagenase for an additional 30 min, and protease activity was inactivated with DMEM containing 5% fetal calf serum (FCS). The cells were triturated through a fire-polished glass pipette and plated onto poly-L-lysine-coated plates (50 μg/ml) in low glucose (5.5 mM) DMEM containing 5% FCS and 2 ng/ml forskolin (complete medium). The cells were grown in the presence of 10 μg/ml cytosine arabinoside for 4 days to rid the cultures of the faster growing fibroblasts and passed upon confluency after approximately 1 week in culture. The Schwann cells were subcultured upon confluency and used for no more than 6 passages.

*Immunoblot and Immunoprecipitation Analyses*—After the indicated treatments, the cells were rapidly washed with ice-cold PBS and solubilized in lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 0.5 mM sodium orthovanadate, 40 mM NaF, 10 mM β-glycerophosphate, and Complete protease inhibitors). After removing cell debris, proteins were separated by SDS-PAGE and transferred to nitrocellulose, and the membranes were probed with the indicated primary antibodies. Immunoreactive bands were visualized using a horseradish peroxidase-conjugated secondary antibody and the Amersham Biosciences ECL reagents as described by the manufacturer. Antibodies against phospho-Erb B2 (22352-5-B, Erb B2 (C-18), JNK (FL), and phospho-JNK (G-7) were from Santa Cruz Biotechnology.

**Immunoprecipitations were performed as described previously (25).**

*Cloning of Rat Cavin-1 Promoter and Construction of pLuc-EFEGFP Reporter Plasmids*—A 7-kb rat Cav-1 genomic DNA fragment was isolated from PAC clones (BACPAC Resource, Oakland, CA) and ligated into the SpeI site of pBluescript KS+ (+) vector (Stratagene, La Jolla, CA). A 2680-bp Cav-1 promoter region (upstream of the ATG in exon 1) was identified by DNA sequencing (GenBank accession number AF485929) and showed 79 and 89% identity to the promoter regions of the human and mouse Cav-1 genes, respectively.

To construct the single vector dual-reporter gene system, we used the pGL3-Basic luciferase vector (Promega, Madison, WI) as the backbone. The Cav-1 gene was excised from pEGFP (N1) (Invitrogen) with BamHI and NotI, and the fragment ends were partially filled using the Klenow fragment and dGTP, dATP, and dTTP. The vector pRL-CMV (Promega) was cut with NheI and XbaI to replace the Renilla reniformis luc gene, and the ends were partially filled in using the Klenow fragment in the presence of dCTP, dATP, and dTTP. The efavirenz signal was inserted into the modified pRL-CMV vector, replacing the Renilla luciferase cDNA, and re-named pEGFP-CMV. To obtain the efavirenz gene containing the upstream CMV immediate early promoter, and a downstream poly(A) signal, pEGFP-CMV was digested with BamHI and BgIII, and the resulting fragment was inserted into a Sall site of the pGL3-Basic luciferase vector creating pLuc-EFEGP. To prepare plasmid constructs of the rat Cav-1 promoter, the 2660-bp Cav-1 promoter region was amplified with the flanking primers: 5′-ACGCGTGAGAGGAAATTGCTCTTGCG-3′ and 5′-GGCCGCCGGGGGTCTTTATGTGTTTGGCTTCTCTAGTG-TCATCGATTATCACCAGACAT-3′ (extra MluI and NotI sites shown in boldface) and inserted into the TOPO-PCB 2.1 vector (Invitrogen).

Native SCs were isolated from 6-week-old rat pups (24). To obtain pLuc-EFEGFP plasmid containing a 400-μl mixture containing 4 μl of pLuc-EFEGFP (empty vector control) or the indicated Cav-1 promoter constructs in serum-free low glucose DMEM. After 1 h in
the transfection mixture, the cells were incubated in complete medium for 24 h. Typically, the medium was then changed to low glucose DMEM (5.5 mM) containing 2% FCS, and the cells were treated with 30 mm \( \beta \)-glucose in the absence or presence of 100 ng/ml mouse 2.5S NGF (Harlan Bioproducts, Cincinnati, OH) for an additional 16–24 h. At the end of the treatment, the cells were washed three times with PBS and incubated with 400 \( \mu \)l of luciferase lysis buffer (Promega) at room temperature for 30 min. To measure firefly luciferase activity, 20 \( \mu \)l of the cell lysate was added to 100 \( \mu \)l of the luminol substrate, and light emission was measured in a Packard Luminometer.

Because the reporter construct constitutively expresses EGFP from the CMV immediate early promoter, transfection efficiency was qualitatively assessed by visualizing EGFP expression by fluorescent microscopy and quantified using 250 \( \mu \)l of the cell lysate. The lysate was transferred to a 96-well plate, and green fluorescence was measured in a FL600 Microplate Fluorescence Reader with filter characteristics of 485 nm for excitation and 530 nm for emission.

**Relative Quantification of mRNA by Real Time PCR**—Schwann cells were placed in DMEM, 2% FCS and treated with buffer or the indicated concentration of \( \beta \)-glucose in the absence or presence of 100 ng/ml NGF for 24 h. Three dishes of cells for each treatment were combined to isolate total RNA using the Trizol reagent (Invitrogen) according to the manufacturer’s instructions. Residual genomic DNA was degraded by incubation with RNase-free DNase I for 10 min at 25 °C, and the enzyme was inactivated by incubation at 75 °C for 10 min. To generate cDNA, 45 \( \mu \)l of total RNA was reverse-transcribed in a 100-\( \mu \)l reaction containing 1 \( \times \) MLV buffer (Promega), 0.5 mm dNTPs, 2.5 \( \mu \)g of oligo(dT) 15-mer primer, 120 mm of RNasin, and 300 mm of Moloney murine leukemia virus reverse transcriptase. After 2 h at 42 °C, the polymerase was inactivated by heating at 95 °C for 5 min, and 2 \( \mu \)l of each sample (900 ng) was used directly for real time PCR analysis.

Real time PCR analyses were performed using a Cepheid Smart Cycler System. Table I lists the sequences of the primers and fluorescent hybridization probes and the resulting amplicon size. The 5’-end of the fluorescent hybridization probe for Cav-1 was labeled with 6-carboxyfluorescein and the 3’-end with the BHQ2 quencher dye. The master mix for the multiplex real time PCRs contained 1 \( \mu \)l of cDNA, 159 bp for normalization, and the 5’-end of the fluorogenic hybridization probe, and 1 unit of \( \beta \)-TaqMan buffer, 4 mm MgCl\(_2\), 0.25 mm dNTPs, 120 mm of the \( \beta \)-actin forward and reverse primers, 400 mm of the forward and reverse Cav-1 primers, 250 mm of each fluorogenic hybridization probe, and 1 unit of z-TaqMan polymerase. Template cDNA was added to give a 25-\( \mu \)l total volume, and PCR conditions were one cycle at 95 °C for 2 min followed by 45–50 cycles of 15 s at 95 °C, 30 s at 62 °C, and 30 s at 72 °C.

Relative quantification of the real time PCR was based upon the amplification efficiency of the target and reference genes and the cycle number at which fluorescence crossed a specified background level, cycle threshold (C\(_t\)) (26). Because the experimental goal was to quantify relative changes in Cav-1 mRNA following glucose and NGF treatment, the relative expression ratio (R) was determined by using Equation 1,

\[
R = \frac{(E_{\text{target}})}{(E_{\text{reference}})} \frac{\Delta C_{\text{target}}^{\text{(control–treatment)}}}{\Delta C_{\text{reference}}^{\text{(control–treatment)}}} \quad (\text{Eq. 1})
\]

In this mathematical model, the amplification efficiency (E) in the exponential phase of the real time PCR is given by \( E = 10^{(-1/\text{cycle threshold})} \) and is derived in separate PCR conditions by plotting the \( C_{\text{t}} \) versus the input concentrations of authentic target and reference cDNA templates (26). Over a broad range of DNA concentrations, both Cav-1 and \( \beta \)-actin cDNA showed similar (1.98 and 1.95, respectively) real time PCR amplification efficiencies (data not shown). Because the fluorescent hybridization probes for Cav-1 and \( \beta \)-actin bind in a sequence-specific manner and were labeled with fluorescent dyes with different emission spectra, the two templates were co-amplified together in subsequent reactions. Preliminary optimization experiments indicated no difference existed in the C\(_t\) values at a given amount of Cav-1 and \( \beta \)-actin cDNA regardless of whether the templates were amplified in separate reactions or multiplexed in the same reaction.

**Infection of Schwann Cells with Recombinant Adenoviruses**—Preparation of recombinant adenoviruses co-expressing the green fluorescent protein (27), the p75\(^{\text{TNFR1}}\) mutant chimera, and dominant-negative JNK 2 (28) have been described previously. To prepare the antisense Cav-1 adenovirus, the coding sequence of canine Cav-1 cDNA was amplified by PCR and subcloned in the reverse orientation into pAdTrack-CMV. Blank and recombinant adenoviruses were prepared as described (27), amplified in 283 cells, purified by two rounds of centrifugation over CsCl gradients, and dialyzed against 10 mm Tris-HCl, pH 8.0, 100 mm NaCl, 0.1% bovine serum albumin, 20% glycerol. Schwann cells were infected with adenoviruses for 24 h in complete medium prior to switching the medium to DMEM containing 2% fetal calf serum for glucose treatment.

**Thymidine Uptake Assay**—Native SCs were grown in 6-well plates until they were ~50% confluent. The cells were washed in serum-free DMEM, placed in DMEM containing 2% FCS, and treated with the indicated concentration of glucose in the absence or presence of NGF for 24 h. The cells were then stimulated with 6.3 mm (50 ng/ml) human recombinant neuregulin1-β1 epidermal growth factor domain (amino acids 176–246) (NRG1-β1) (R & D Systems, Minneapolis, MN) for an additional 24 h. \(^{3}H\)Thymidine (1 \( \mu \)C/mi) was added during the final 10 h of the incubation after which the cells were washed twice with ice-cold PBS followed by 2× 15-min washes with 5% ice-cold trichloroacetic acid. The cells were solubilized with 0.5 ml of 0.5 mm NaOH, and 0.4 ml was used to assess the amount of \(^{3}H\)thymidine incorporated into the DNA by scintillation spectrometry. \(^{3}H\)Thymidine uptake was normalized to total protein that was determined from aliquots of the remaining lysate. In some experiments, cells were infected with the indicated titers of Cav-1 antisense adenovirus for 24 h prior to treating the cells with 50 ng/ml NRG1-β1 for an additional 24 h. \(^{3}H\)Thymidine was added for the final 4 h of the incubation, and uptake into DNA was determined as described above.

**RESULTS**

**Cav-1 Is Expressed in SCs and Is Down-regulated in Sciatic Nerve in an Animal Model of DPN**—Streptozotocin-induced diabetes in the rat is a well characterized and widely used animal model for studying DPN in type 1 diabetes (29, 30). Male rats were treated with the drug vehicle or rendered diabetic with STZ, and the sciatic nerves were harvested over a course of 3–17 weeks following the initial designation of the animal as a diabetic. Cav-1 is highly expressed in endothelium (31) and the perineurium of peripheral nerve (22). Thus, biochemical isolation of the total Cav-1 associated with caveolar membrane preparations from sciatic nerve has contributions from multiple cell types that may respond differentially to hyperglycemia. To circumvent this issue, we examined changes in Cav-1 expression specifically in SCs by immunofluorescence analysis of transverse sections of sciatic nerve that were double-labeled with a monoclonal antibody for Cav-1 and a polyclonal antibody against the SC-specific protein, S100β (23). Cav-1 immunoreactivity (Fig. 1A) localized around nerve fibers

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<th>Table I</th>
<th>Probe and primer sequences used for real time PCR</th>
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<tr>
<td>Gene</td>
<td>Forward primer</td>
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<tr>
<td>Cav-1</td>
<td>5′-ATGTCCTGGGGAATAATCGTAGAC-3′</td>
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<td>5′-CAAGATCATGGCTCCTGTG-3′</td>
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cell lysate was added to 100 μl of leukemia virus reverse transcriptase. After 2 h at 42 °C incubation with RNase-free DNase I for 10 min at 25 °C, according to the manufacturer’s instructions. Residual genomic DNA was degraded by isola...
Cav-1 with S100/H9252
euthilium (C). arrows (thick arrow). thin arrows co-staining in capillary endothelium (C) and perineurium then co-stained with a rabbit anti-bovine S100 sections were stained with the Cav-1 2234 monoclonal antibody and arrows) and the perineurium (Fig. 1, C). merged images showing clear co-localization of Cav-1 with S100β (arrows). Scale bar, A–C, 100 μm; D, 10 μm.

throughout the entire nerve section in a pattern very similar to that seen for the S100β immunoreactivity (Fig. 1B). Indeed, merging the images shows co-localized regions of Cav-1 and S100β immunoreactivity surrounding most nerve fibers throughout the section (Fig. 1C), and this co-localization is clearly demonstrated in the higher magnification overlay of the Cav-1 and S100β signals (Fig. 1D). Importantly, S100β immunoreactivity did not co-localize with Cav-1 immunoreactivity associated with endoneurial blood vessels (Fig. 1C, thin white arrows) and the perineurium (Fig. 1C, thick white arrow).

Following 3 weeks of diabetes, little qualitative change was observed in the level of Cav-1 expression between control and STZ-treated animals (data not shown). However, beginning at 6 weeks of diabetes, a progressive decrease in the expression of Cav-1 was evident, and images from a representative animal after 9 weeks of treatment are shown in Fig. 2A. In contrast to the age-matched control, Cav-1 immunoreactivity decreased throughout the nerve but still displayed intense abaxonal localization (22) around some of the nerve fibers. The progressive decline of Cav-1 expression and the loss of its overall co-localization in SCs with prolonged hyperglycemia is clearly evidenced by the decreased intensity of the yellow signal throughout the nerve section upon merging of the images for Cav-1 and S100β.

The decrease in Cav-1 expression was not because of a general decline in SC number because the overall staining intensity for S100β between control and STZ-treated animals was not significantly different. Therefore, changes in the fluorescent intensity of Cav-1 were normalized to S100β to obtain a semi-quantitative estimate of the extent of the decrease in Cav-1. The ratio of Cav-1 to S100β was calculated only where Cav-1 co-localized with specific S100β immunoreactivity and excluded Cav-1 immunoreactivity associated with the endothelium and the perineurium. Fig. 2B shows a scatter plot of the mean ratio of the fluorescent intensity of Cav-1 to S100β from 60 random areas where Cav-1 co-localized with S100β. Data are mean ± S.E. of the ratios for each animal. The data were grouped by time and treatment and analyzed by one-way ANOVA and Tukey’s post hoc test. Asterisks indicate p < 0.001 versus time-matched control.

Fig. 1. Cav-1 is expressed in SCs of sciatic nerve. The nerve sections were stained with the Cav-1 2234 monoclonal antibody and then co-stained with a rabbit anti-bovine S100β antibody. A, Cav-1 immunoreactivity (green) localizes around unstained nerve fibers through the nerve. Thin arrows show examples of staining of capillary endothelium, and the thick arrow shows staining of perineurium. B, S100β immunoreactivity (red). Note lack of staining of capillary endothelium (arrows). C, merged images showing co-localization (yellow) of Cav-1 with S100β surrounding individual nerve fibers but absence of co-staining in capillary endothelium (thin arrows) and perineurium (thick arrow). D, higher magnification images showing clear co-localization of Cav-1 with S100β (arrows). Scale bar, A–C, 100 μm; D, 10 μm.

Fig. 2. Prolonged hyperglycemia decreases Cav-1 immunoreactivity in SCs of sciatic nerve. A, Cav-1 and S100β staining in sciatic nerve from animals receiving drug vehicle (Control) or STZ and maintained for 9 weeks. Arrows indicate several regions of intense Cav-1 staining in STZ-treated animals. B, the fluorescent signal of Cav-1 was normalized to that of S100β from 60 random areas where Cav-1 co-localized with S100β. Data are mean ± S.E. of the ratios for each animal. The data were grouped by time and treatment and analyzed by one-way ANOVA and Tukey’s post hoc test. Asterisks indicate p < 0.001 versus time-matched control.
shown), whereas Cav-1 immunoreactivity was substantially decreased relative to controls (Fig. 2B).

To demonstrate that the down-regulation of Cav-1 was causatively related to hyperglycemia and not a nonspecific effect of STZ toxicity, mice were rendered diabetic with STZ, and after 6 weeks of diabetes, some animals received insulin therapy for 2 weeks. Following 6 weeks of diabetes, average blood glucose was 434 ± 16 mg/dl (−24 mM, n = 7), and blood glucose levels recovered to 245 ± 25 mg/dl (−14 mM, n = 6) after 2 weeks of insulin therapy. As expected, insulin treatment also increased weight gain by 18.6 ± 2%.

The localization of Cav-1 immunoreactivity in sciatic nerve from mice was identical to that present in rats (Fig. 3A) and significantly decreased relative to control animals after 8 weeks of diabetes (Fig. 3B). Importantly, insulin therapy for the final 2 weeks induced a substantial recovery of Cav-1 expression that rebounded to about 80% of control levels. Collectively, these data provide the first evidence that hyperglycemia specifically decreases the expression of Cav-1 in SCs in a physiologically relevant animal model for type 1 diabetes.

Glucose Decreases Cav-1 Expression in Primary SCs—To gain insight into the physiological relevance of hyperglycemia-induced changes in Cav-1 expression, we attempted to recapitulate this effect in vitro using primary neonatal SCs. SCs were exposed to hyperglycemic stress by incubation in DMEM containing 2% FCS supplemented with glucose to a final concentration of 5.5–65 mM. After 16–24 h of glycemic stress, Cav-1 expression was determined by immunoblot analysis following SDS-PAGE. To control for differences in protein loading, Cav-1 levels were initially normalized to the expression of S100 because it did not change with glucose treatment and would not have any contribution from residual contaminating fibroblasts (which are very minimal) in the cultures.

Numerous studies (33–35) have used 30 mM glucose in various cell culture models to investigate acute effects of glucose on signal transduction events and changes in protein and gene expression. Similarly, treatment of SCs with 30–40 mM glucose for 24 h induced a 30–50% decrease in Cav-1 protein expression without changing the expression of S100 (Fig. 4A, upper panels). Although the acute treatment of SCs with 30–40 mM glucose mimicked the effect of chronic hyperglycemia on Cav-1 expression observed in the diabetic animals, these glucose concentrations are above typical in vivo pathophysiological levels. Glucose concentrations from 10 to 20 mM are well within the limits of those observed in diabetic animals and humans, but acute treatment of SCs with 10–20 mM glucose for 24 h did not decrease Cav-1 expression (Fig. 4A, lower panels). This may reflect the short time course of these studies because 30–65 mM glucose dose-dependently down-regulated Cav-1 protein expression but had no effect on the expression of flotillin-1. The down-regulation of Cav-1 by d-glucose is not due to osmotic stress because even high concentrations of l-glucose, a physiologically inactive enantiomer of d-glucose, did not substantially decrease Cav-1 or flotillin-1 expression (Fig. 4A, lower panels).

Because we demonstrated that Cav-1 is down-regulated un-
Neurotrophic Regulation of Hyperglycemic Stress

In primary SCs, the expression of S100Ct threshold for background fluorescence is indicated (for 24 h. Total RNA was isolated, and an aliquot of the reverse-transcribed cDNA was prepared for real time PCR analysis as described under Materials and Methods. Importantly, EGFP expression was unaffected by stimuli such as forskolin that increased transcription from the Cav-1 promoter in SCs demonstrating its independence from treatments directed at the target promoter (data not shown).

FIG. 4. Glucose decreases Cav-1 protein and mRNA expression in primary SCs. A, SCs were incubated in medium supplemented with 5.5–65 mM D-glucose or L-glucose. Total cell lysates were prepared after 24 h, and Cav-1 expression was determined by immunoblot analysis following SDS-PAGE. The blot was stripped and re-probed for expression of S100β (upper panels) or flotillin-1 (lower panels). Data shown are representative of those obtained in three experiments. B, SCs were placed in medium containing the indicated concentration of D-glucose for 24 h. Total RNA was isolated, and an aliquot of the reverse-transcribed cDNA was used for multiplex real time PCR analysis. The threshold for background fluorescence is indicated (Ct). For simplicity, a single representative curve for Cav-1 and β-actin from triplicate amplifications for each treatment is shown. Results shown are from one experiment repeated twice with essentially identical outcomes.

To determine whether the effect of glucose on regulating Cav-1 expression may be at the level of transcription, the rat Cav-1 promoter was subcloned upstream of firefly luciferase in pLuc-EGFP, and a series of deletion constructs was prepared. The pLuc-EGFP plasmid contains EGFP under the control of a separate CMV promoter that enables both a qualitative and quantitative assessment of transfection efficiency for normalization. Importantly, EGFP expression was unaffected by stimuli such as forskolin that increased transcription from the Cav-1 promoter in SCs demonstrating its independence from treatments directed at the target promoter (data not shown).

Passage 4 and passage 6 primary SCs were transiently transfected with the various Cav-1 promoter constructs, and luciferase activity and EGFP fluorescence was measured after 48 h. Constitutive Cav-1 promoter activity was found to be substantially greater in passage 6 versus the passage 4 SCs (Fig. 5B) and is consistent with Cav-1 expression increasing in SCs as they mature (22). Similar to results obtained with murine (36) and human (37) Cav-1 promoters, the bulk of promoter activity was associated with Cav1-Pr (−0.5 kb) that contained the first 455-bp upstream of the ATG start codon (Fig. 5A). These results clearly demonstrate that the constructs are functional and that SCs can differentially use the rat Cav-1 promoter to drive expression of the reporter gene. Therefore, to ascertain if glucose could decrease transcription from the Cav-1 promoter, passage 3–5 SCs were transfected with the Cav1-Pr (−0.5kb) construct. Consistent with the effect of glucose on decreasing Cav-1 protein and mRNA expression, 30 mM D-glucose also decreased Cav-1 promoter activity by 50% (Fig. 5B). However, neither L-glucose nor mannitol significantly decreased luciferase activity. These results support that the regulation of Cav-1 promoter activity by D-glucose is not a consequence of osmotic stress and is likely associated with its metabolism and activation of signal transduction events.

Hyperglycemia and Cav-1 Down-regulation Increase Neuregulin-stimulated Thymidine Uptake—The physiological role of Cav-1 in SCs is unclear, but it is well established that the interaction of Cav-1 with Erb B family members can inhibit their intrinsic tyrosine kinase activity (13, 18, 38). Erb B2 and Erb B3 are expressed in neonatal SCs and contribute to both cell survival (39, 40) and myelination (20, 21) during development by interacting with neuregulins (41). To assess directly whether down-regulation of Cav-1 in SCs may regulate signaling through Erb B receptors, SCs were infected with an antisense Cav-1 recombinant adenovirus that specifically reduced the expression of endogenous Cav-1 but had no effect on the expression of flotillin-1 (Fig. 6A). We assessed the effect of forced down-regulation of Cav-1 on the mitogenic response of SCs to human recombinant neuregulin-1-β1 (176–246) (NRG1-β1) by measuring the uptake of radiolabeled thymidine. SCs were infected with 200–300 pfu/ml of a blank adenovirus or the Cav-1 antisense adenovirus for 24 h and then stimulated with 30 ng/ml NRG1-β1 (6.3 nM) for 24 h. The cells were pulsed with [3H]thymidine, and the incorporation of radiolabeled thymidine into DNA was determined. In cells infected with blank virus, NRG1-β1 increased thymidine uptake about 1.6-fold (Fig. 6B). However, in cells infected with the antisense Cav-1 adenovirus, NRG1-β1 significantly enhanced thymidine uptake about 2.4–3.6-fold compared with cells that received buffer only. Importantly, down-regulation of Cav-1 significantly increased NRG1-β1-induced thymidine uptake relative to cells infected with blank virus and stimulated with the growth factor. These results suggest that changes in the ratio of Cav-1 to Erb B2 can enhance the response of SCs to neuregulins.
Next, we addressed whether hyperglycemia affected neu-
regulin signaling in a similar manner. SCs were incubated with
30 mM glucose for 24 h, stimulated with 50 ng/ml NRG1-
H9252 for 24 h, and pulsed with 
[3H]thymidine. Hyperglycemia signifi-
cantly increased NRG1-H9252-induced thymidine uptake com-
pared with cells that received the growth factor but were main-
tained in medium with 5.5 mM glucose (Fig. 7A,
2nd bar versus 4th bar). Changes in osmolarity do not account for this effect
because 30 mM mannitol did not increase the uptake of thymi-
dine in response to NRG1-H9252; the data in Fig. 7A with NGF are
discussed below. Collectively, these data clearly indicate that
both hyperglycemia and down-regulation of Cav-1 are suffi-
cient to increase NRG1-β1 signaling through Erb B receptors in
SCs.

To begin to address the mechanism by which hyperglycemia
increased the mitogenic response of SCs to NRG1-β1, we ex-
amined its effect on Erb B2 activation by monitoring receptor
phosphorylation using an antibody directed against phospho-
Erb B2. Treatment of SCs with 50 ng/ml NRG1-
H9252 induced
maximal phosphorylation of Erb B2 within 2–5 min, and re-
ceptor activation substantially decreased after 60–120 min
(Fig. 7B). Therefore, we hypothesized that the increased mito-
genic potential of NRG1-β1 in the presence of high glucose may
relate to changes in the kinetics of Erb B2 activation. SCs were
incubated in medium containing 5.5 (basal), 20, or 30 mM
sugar for 24 h and then stimulated with 50 ng/ml NRG1-β1
for 5 or 120 min. Cell lysates were prepared, and Erb B2
phosphorylation was determined by immunoblot with the phos-
pho-Erb B2 antibody. Total Erb B2 levels from the same sam-
ple were determined in a parallel blot. Compared with cells
incubated in basal glucose, 20 mM glucose had no effect on the
level of Erb B2 phosphorylation following 5 min of stimulation
(Fig. 7B). In contrast, 20 mM glucose signifi-
cantly prolonged the magnitude of receptor phosphorylation
after 2 h of NRG1-β1 stimulation; identical results were also
seen with 30 mM glucose (data not shown). Differences in re-
ceptor activation were not due to increased/decreased expres-
sion of Erb B2 because total Erb B2 levels remained un-
changed. However, a caveat to this issue is that we cannot rule
out that hyperglycemia may have enhanced surface expression
of the receptor. Nevertheless, these results suggest that phys-
io logically relevant levels of hyperglycemia may increase bio-
logical responses of SCs to neurolgulins by prolonging the ki-
netics of Erb B2 activation.
NGF Inhibits the Glucose-induced Down-regulation of Cav-1

We have proposed previously that neurotrophins may modulate the response of SCs to hyperglycemic stress through p75NTR signaling (42). To examine if NGF may antagonize the effect of hyperglycemia on down-regulating Cav-1, SCs were placed in medium supplemented with 30 mM glucose in the absence or presence of 100 ng/ml NGF for 24 h. Cells were treated with PBS or 50 ng/ml NRG1-β1 for an additional 24 h, and [3H]thymidine incorporated into DNA was normalized to total protein. Results are mean ± S.E. (n = 6) and were analyzed by one-way ANOVA with Tukey’s post hoc test. Asterisk and number sign indicate p < 0.05 compared with the indicated treatment. B, SCs were incubated for 0–120 min with 50 ng/ml NRG1-β1, and the kinetics of Erb B2 phosphorylation was determined by immunoblot analysis using an antiphospho-Erb B2 antibody (p-erb B2). The blot was stripped, and total Erb B2 levels were determined. C, SCs were incubated in DMEM, 2% FCS containing 5.5 or 20 mM glucose for 24 h and then stimulated with 50 ng/ml NRG1-β1 for 5 or 120 min. Cell lysates were prepared, and phospho-Erb B2 and total Erb B2 were determined by immunoblot analysis.

NGF Inhibits the Glucose-induced Down-regulation of Cav-1 through p75NTR-mediated JNK Activation—We have proposed previously that neurotrophins may modulate the response of SCs to hyperglycemic stress through 75NTR signaling (42). To examine if NGF may antagonize the effect of hyperglycemia on down-regulating Cav-1, SCs were placed in medium supplemented with 30 mM glucose in the absence or presence of 100 ng/ml NGF. Cell lysates were prepared after 24 h, and Cav-1 expression was determined by immunoblot analysis. Glucose treatment decreased Cav-1 protein expression by about 45%, and the addition of NGF at the time of glucose treatment partially antagonized the glucose-induced down-regulation of Cav-1. In contrast, the addition of NGF 8 h after the cells were placed in high glucose had little effect on increasing Cav-1 expression (Fig. 8A).

The NGF-induced inhibition of Cav-1 protein down-regulation was not immediately apparent (Fig. 8B). Eight hours after treatment with high glucose, NGF partially antagonized the glucose-induced down-regulation of Cav-1, which suggests that NGF was not rapidly induced in SCs. To further probe the NGF effect on Cav-1 expression, SCs were transfected with Cav-1 expression plasmids and then co-incubated with high glucose and NGF for 24 h. Cells were then stimulated with high glucose and NGF for 5 h, and luciferase activity was measured. Results are mean ± S.E. (n = 6) and were analyzed by one-way ANOVA with Tukey’s post hoc test. Asterisks indicate p < 0.01 (**) or p < 0.05 (*) compared with control. Number signs indicate p < 0.01 (###) or p < 0.05 (##) compared with glucose alone.
tion by glucose was also manifest on Cav-1 mRNA levels. The relative expression of Cav-1 transcripts in glucose-treated cells decreased by 0.48 ± 0.06-fold compared with control cells incubated in 5.5 mM glucose (Fig. 8B). Co-treatment of SCs for 24 h with 30 mM glucose plus 100 ng/ml NGF led to a leftward shift in the C5 for Cav-1 and corresponded to a relative increase of 5.1 ± 1.7-fold in Cav-1 transcripts compared with cells treated with 30 mM glucose alone. Consistent with these results, NGF also attenuated the effect of glucose on decreasing transcription from the Cav-1 promoter. SCs were co-transfected with Cav1-Pr(−0.5 kb) and, after 24 h, treated with 30 mM glucose in the absence or presence of 100 ng/ml NGF. To determine whether NGF would increase transcription from the Cav-1 promoter in a time-dependent manner, the neurotrophin was added coincident with 30 mM glucose or 4–6 h afterward. Hyperglycemia significantly decreased transcription from the Cav-1 promoter, and the co-addition of NGF with glucose (NGF-16 h) or 4 h later (NGF-12 h) significantly blocked the glucose-induced decrease in transcription (Fig. 8C). In contrast, addition of NGF 6 h after glucose (NGF-10b) attenuated but did not significantly reverse its effect on inhibiting transcription from Cav1-Pr(−0.5 kb). Curiously, treating the cells with NGF alone (glucose 0 h) had little effect on luciferase activity but increased Cav-1 mRNA levels 1.44 ± 0.01-fold relative to those present in control cells (Fig. 8B). The reason for this discrepancy is unclear. Interestingly, glucose did not decrease transcription from the Cav-1 promoter when it was added 6 h after NGF treatment (glucose 10 h) suggesting that NGF-induced signaling events can prevent glucose-induced changes in Cav-1 gene expression.

NGF treatment also blocked the effect of 30 mM glucose on increasing the mitogenic response of SCs to NRG1-β1 (Fig. 7B). Of note, addition of NGF alone modestly increased NRG1-β1-induced mitogenesis suggesting that NGF may have differential effects on the response to neuregulins depending upon the extent of cell stress. Collectively, these results clearly demonstrate that NGF can attenuate the down-regulation of Cav-1 at the transcriptional, mRNA, and protein levels and functionally antagonize signaling through Erb B2 in cells subjected to hyperglycemic stress.

Neonatal SCs can only respond to NGF through p75NTR because they do not express Trk A (23). Therefore, we sought to identify the signaling mechanisms used to mediate our observed responses to NGF. Although ceramide production and NF-xB activation are two pathways stimulated by p75NTR (42), our preliminary data suggested that ceramide was not involved, and published reports indicate that the activation of NF-xB through p75NTR is absent in primary SCs cultured greater than 1 week (43). p75NTR is also known to activate c-Jun N-terminal kinase (JNK) (44), and NGF treatment of SCs rapidly stimulated JNK phosphorylation (Fig. 9A). JNK was immunoprecipitated with an antibody recognizing the JNK 1–3 isoforms, and JNK activation was determined using an antibody against phospho-JNK 1–3. These results suggest that NGF rapidly activates JNK 2 and/or JNK 3 isoforms since we observed little phosphorylation of JNK 1 despite a robust expression. This rapid activation of JNK by NGF was also observed by measuring phosphorylation of a GST-Jun substrate with an in vitro kinase assay (data not shown).

To address the role of p75NTR-mediated JNK activation in blocking the down-regulation of Cav-1 by glucose, we expressed a mutant p75NTR receptor-chimera (p75NTR-extracellular domain linked to a kinase-negative epidermal growth factor receptor cytoplasmic domain) (28). Expression of the p75NTR receptor-chimera has been demonstrated to inhibit NGF-induced JNK activation and block apoptosis in oligodendrocytes indicating that it functions as a dominant-negative receptor (28). Similarly, adenovirus-mediated expression of the p75NTR receptor-chimera blocked NGF-induced JNK activation in SCs but had no effect on the stimulation of JNK by TNF (data not shown). Moreover, expression of the dominant-negative p75NTR negated the ability of NGF to reverse the down-regulation of Cav-1 by glucose (Fig. 9B). Please note that an antibody against the p75NTR extracellular domain detected both endogenous and mutant receptors shown in the lower panel. These results suggest that the effect of NGF on antagonizing the down-regulation of Cav-1 by glucose requires a functional p75NTR and indirectly suggests that NGF-induced JNK activation is critical for mediating this p75NTR-dependent response in SCs.

To determine more directly whether JNK activity was necessary for NGF to inhibit the down-regulation of Cav-1 by glucose, SCs were infected with a hemagglutinin-tagged dominant-negative JNK 2 (dn-JNK2) adenovirus that is sufficient to block total JNK activity (28). Twenty four hours after infection with a blank or dn-JNK2 adenovirus, the cells were treated with glucose in the presence or absence of NGF for an additional 24 h, and Cav-1 protein expression was determined. In SCs infected with the blank adenovirus, glucose robustly down-regulated Cav-1 expression, and NGF strongly antagonized this effect. These results indicate that viral infection did not alter the response of the cells to glucose and NGF. Furthermore, the expression of flotillin-1 was minimally affected demonstrating that glucose was not non-specifically decreasing expression of other caveolar proteins.

Infection of the SCs with dn-JNK2 adenovirus did not block the glucose-induced decrease in Cav-1 expression indicating that JNK activation is not involved in the effect of glucose on down-regulating Cav-1 expression. In contrast, expression of dn-JNK2 strongly antagonized the ability of NGF to inhibit the glucose-induced down-regulation of Cav-1 that was apparent in cells infected with a blank adenovirus (Fig. 9C). Similar to cells infected with blank virus, the expression of flotillin-1 was not significantly changed. These results strongly suggest that p75NTR-mediated JNK activation is critical in transducing the effect of NGF in modulating the response of SCs to hyperglycemic stress.

**Discussion**

SCs have long been implicated in the pathogenesis of degenerative responses of small and large diameter sensory fibers as well as motor neurons in DPN. The overall axonopathy in DPN has contributions from metabolic changes in neurons, SCs, and vascular endothelium of endoneurial capillaries (30, 45). Our data provide the first evidence that prolonged hyperglycemic stress correlates with a progressive loss of Cav-1 in SCs in a physiologically relevant animal model of type 1 diabetes. These results are consistent with a specific loss of Cav-1 from SCs following axotomy (22) and provide further support that Cav-1 expression is tightly linked to SC differentiation.

The down-regulation of Cav-1 is causatively related to prolonged increases in blood glucose because insulin therapy reversed this effect. Moreover, Cav-1 down-regulation is unlikely to be a nonspecific consequence of diabetes because flotillin-1, which directly interacts with and co-localizes with Cav-1 in caveolae (32), was not decreased by diabetes. Furthermore, Cav-1 expression was not substantially decreased in endothelial cells and the perineurium in diabetic animals. These results suggest that Cav-1 expression in peripheral nerves may be differentially sensitive to hyperglycemia depending upon the cell type.

It is difficult to discern if hyperglycemia may directly or indirectly affect Cav-1 expression in SCs in the animal model.
However, we demonstrate that acute hyperglycemia recapitulated this response in primary cultures of SCs suggesting that glucose is at least sufficient for decreasing Cav-1 expression. Importantly, the lack of change in flotillin-1 in SCs in vitro would also argue that glucose does not non-specifically down-regulate any protein associated with lipid raft domains. Although the underlying mechanism by which glucose decreases Cav-1 expression is unclear, osmotic damage is not sufficient for this effect because neither L-glucose nor mannitol decreased transactivation from the Cav-1 promoter or increased the mitogenic response of SCs to neuregulins. Indeed, because L-glucose is not efficiently metabolized, our results support that the down-regulation of Cav-1 is a direct consequence of the metabolic conversion of glucose. For example, the glucose-induced production of superoxide anion has been proposed as an initiating event in endothelial cells that stimulates protein kinase C activity, enhances glucose flux through the polyol and hexosamine pathways, and increases the production of advanced glycation end products (33, 46). However, we observed little decrease in Cav-1 immunoreactivity in endothelial cells in vivo suggesting that the glucose-induced generation of superoxide may be insufficient to decrease Cav-1 expression in these cells. This would be consistent with the lack of effect of superoxide generation (47) and hydrogen peroxide (48) in decreasing Cav-1 expression in cultured endothelial cells. Alternatively, increased protein modification with GlcNAc by O-N-acetylglucosamine transferase has been shown to repress transcription in a very gene-specific manner (49). Given the effect of Cav-1 on modulating the response of SCs to neuregulins, it will be important to determine the potential role of oxidative stress and/or the hexosamine pathway in regulating Cav-1 expression in SCs.

The role(s) of Cav-1 in SC biology are not clearly understood, but our results support that changes in its expression can affect the response of SCs to growth factors such as neuregulins. Neuregulins bind Erb B receptors (41) and form a ligand-receptor network that provides potent mitogenic/survival signals to immature SCs (40) and may be essential for myelination during development (20). Previous results have shown that Erb B2 signaling in fibroblasts was inhibited following Cav-1 overexpression (18), and our results are consistent with these studies because down-regulation of Cav-1 increased the mitogenic response of SCs to NRG1-β1. Thus, decreased Cav-1 expression is at least sufficient to increase signaling through Erb B2 in
increase in Cav-1 transcription, mRNA levels, and protein expression in response to the pathophysiology of DPN. The context of these results may contribute to the development of peripheral neuropathies (21). Erb B2 and Erb B3 are highly expressed in neonatal SCs, and the expression of Erb B2 decreases, but is not absent, in mature myelinated SCs (10). The progressive down-regulation of Cav-1 during chronic hyperglycemia may relieve an inhibitory regulation of Erb B2 activity in mature SCs to axon-derived neurotrophins. This altered neurotrophism may increase or prolong Erb B2 activation of downstream effectors such as phosphatidylinositol 3-kinase and contribute to the pathological signaling underlying the rate of demyelination in DPN (21).

Altered neurotrophism in DPN is also associated with an increased expression of p75NTRe in adult SCs within 3–5 weeks of diabetes in the STZ rat model (5). In our in vitro model of hyperglycemic stress, NGF prevented the glucose-induced decrease in Cav-1 transcription, mRNA levels, and protein expression through p75NTRe. Additionally, NGF abolished the effect of glucose in enhancing the mitogenic response of SCs to NGF. Unfortunately, we cannot conclude that inhibiting the glucose-induced down-regulation of Cav-1 is required for NGF to antagonize the effect of hyperglycemia on increasing the cellular response to NGF. However, these data suggest a novel role for the NGF/p75NTRe ligand-receptor cassette in modulating SC response to neurotrophins that may potentially affect regenerative/degenerative responses of these cells to hyperglycemic stress.

It is intriguing that NGF was most effective when added within 4 h following the initiation of glycosic stress, suggesting that early signaling events may be required to antagonize specific aspects of glucotoxicity. DPN is a highly dynamic and progressive dying-back neuropathy that does not occur in a temporally simultaneous fashion (50). Although it is unclear whether p75NTRe signaling may provide a physiologically relevant level of protection during diabetes, our results raise the possibility that glia may differentially respond to p75NTRe signaling depending upon their state of degeneration/regeneration and the duration of hyperglycemic exposure.

The molecular events that couple p75NTRe to downstream events such as apoptosis are complex and not fully understood. However, an important role for JNK activation has been demonstrated through pathways involving the small GTPase, Rac1 (28), or the p75NTRe-interacting protein, NRAGE (51). We demonstrate that NGF treatment transiently activates JNK in SCs and that inhibiting JNK activation negates the effect of p75NTRe in blocking the glucose-induced down-regulation of Cav-1. In contrast, prolonged JNK activation by NGF was associated with apoptosis of oligodendrocytes (28). Thus, these data suggest that transient JNK activation via p75NTRe may provide a mechanism for antagonizing some aspects of hyperglycemic stress, independent of the ability of this pathway to induce apoptosis. Although it is unclear how NGF-induced JNK activation prevents the decrease in Cav-1 by glucose, NGF signaling through p75NTRe leads to increased expression of p53 and its activation by JNK in sympathetic neurons (52, 53). p53 has been shown to up-regulate transcription of the human CAV-1 gene (54), and the rat Cav-1 promoter contains two potential p53 half-sites, at least one of which is well conserved with the human p53 half-site. On the other hand, c-Jun is a well recognized substrate for JNK that is not necessary for p75NTRe-mediated apoptosis of sympathetic neurons (55). It will be interesting to determine whether c-Jun is a potential component in mediating a p75NTRe-dependent response that does not promote apoptosis. Alternatively, given the reciprocal and competitive relationship between phosphorylation versus GlcNAcylation of serine and threonine residues (56), an intriguing possibility is that JNK activation may antagonize a repressive effect of GlcNAcylation on transcription. Indeed, Sp1 would be an attractive candidate for this regulation because it contributes to formation of a transcriptional complex on the human (57) and rat Cav-1 promoter, and enhanced GlcNAcylation of Sp1 represses its transactivating potential (49, 58).

In summary, our results support that hyperglycemia-induced changes in Cav-1 expression and p75NTRe signaling may contribute to altered neurotrophism in DPN by modulating the response of SCs to neurotrophins. Further identification of the biochemical mechanisms that influence growth factor signaling in diabetically stressed SCs may provide insight to help increase the therapeutic efficacy of neurotrophic factors in the treatment of DPN.

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REFERENCES

Neurotrophic Regulation of Hyperglycemic Stress

R. S., eds) pp. 1009–1076, Appleton & Lange, East Norwalk, CT
Nerve Growth Factor Blocks the Glucose-induced Down-regulation of Caveolin-1 Expression in Schwann Cells via p75 Neurotrophin Receptor Signaling

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