Central insulin signaling is critical for the prevention of insulin resistance. Hyperinsulinemia contributes to insulin resistance, but it is not yet clear whether neurons are subject to cellular insulin resistance. We used an immortalized, hypothalamic, clonal cell line, mHypoE-46, which exemplifies neuronal function and expresses the components of the insulin signaling pathway, to determine how hyperinsulinemia modifies neuronal function. Western blot analysis indicated that prolonged insulin treatment of mHypoE-46 cells attenuated insulin signaling through phospho-Akt. To understand the mechanisms involved, time-course analysis was performed. Insulin exposure for 4 and 8 h phosphorylated Akt and p70-S6 kinase (S6K1), whereas 8 and 24 h treatment decreased insulin receptor (IR) and IR substrate 1 (IRS-1) protein levels. Insulin phosphorylation of S6K1 correlated with IRS-1 ser1101 phosphorylation and the mTOR-S6K1 pathway inhibitor rapamycin prevented IRS-1 serine phosphorylation. The proteasomal inhibitor epoxomicin and the lysosomal pathway inhibitor 3-methyladenine prevented the degradation of IRS-1 and IR by insulin, respectively, and pretreatment with rapamycin, epoxomicin, or 3-methyladenine prevented attenuation of insulin signaling by long-term insulin exposure. Thus, a sustained elevation of insulin levels diminishes neuronal insulin signaling through mTOR-S6K1-mediated IRS-1 serine phosphorylation, proteasomal degradation of IRS-1 and lysosomal degradation of the IR. (Endocrinology 151: 75–84, 2010)
known whether neurons are subjected to cellular insulin resistance after prolonged exposure to insulin.

Insulin resistance is a hallmark feature of type 2 diabetes mellitus (T2DM) and obesity. There are numerous etiologies for insulin resistance, including lipotoxicity, inflammation, endoplasmic reticulum (ER) stress, oxidative stress, and hyperinsulinemia (22). Hyperinsulinemia coexists with insulin resistance in obese and metabolic syndrome phenotypes. High fasting plasma insulin predicts T2DM independent of insulin resistance (23). In vivo and in vitro studies indicate that prolonged exposure to high levels of insulin leads to attenuated insulin signaling (16, 17, 24–30).

In this study, we investigated whether a sustained elevation in insulin levels leads to cellular insulin resistance in neurons. We used a neuronal cell model, mHypoE-46, developed in our laboratory, which was immortalized from the hypothalami of embryonic mice and exemplifies neuronal behavior similar to in vivo neurons. Using this hypothalamic, neuronal cell model, we aimed to determine whether prolonged insulin exposure attenuates insulin signal transduction in neurons and, if so, the molecular mechanisms involved in this process.

Materials and Methods

Cell culture and reagents

The immortalized mHypoE-46 cells were cultured at 37°C in 5% CO₂ in DMEM (Invitrogen Life Technologies, Burlington, Ontario, Canada) containing 5% fetal bovine serum (Hyclone Laboratories Inc., Logan, UT) and 1% penicillin/streptomycin (Invitrogen), as previously described (31). We used human biosynthetic Novolin ge Toronto regular insulin, which was gifted by Novo Nordisk Canada Inc. (Mississauga, Ontario, Canada). Epoxomicin and 3-methyladenine (3-MA) were purchased from Peptides International (Louisville, KY) and Sigma-Aldrich Canada Ltd. (Oakville, Ontario, Canada), respectively, and rapamycin, LY294002, and U0126 were from Cell Signaling Technology Inc. (Danvers, MA). The G protein β-antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA), and the anti-Akt, IRS-1, IRβ, phosphospecific-Akt (ser473), p70-S6K (thr389), and IRS-1 (ser1101) antibodies were obtained from Cell Signaling.

Western blot analysis

mHypoE-46 cells were grown to 80–90% confluency, serum starved for 16 h, and then treated with insulin (10 nM) or vehicle for 4–24 h. Sixteen hours before insulin treatment, medium was changed to serum-free DMEM containing insulin pretreatment. At time zero, the cells were washed twice with 1× PBS, placed in fresh insulin-free-serum-free DMEM for 1 h, and then rechallenged with 10 nM insulin. During the reverse time-course experiments, the cells were pretreated with 100 nM insulin for 24 h or vehicle and then washed with 1× PBS and placed in fresh medium. At 2, 4, 6, 8, and 12 h after washing, the cells were rechallenged with 10 nM insulin or water vehicle, and protein was isolated after 15 min. For the inhibitor experiments, the cells were pretreated with 50 μM epoxomicin, 10 nM rapamycin, 10 μM 3-MA, or dimethylsulfoxide (DMSO) vehicle for 1 h, followed by treatment with 100 nM insulin or water vehicle for 8 h. After 8 h pretreatment, the cells were washed with 1× PBS, placed in fresh serum-free DMEM, and rechallenged with 10 nM insulin. Protein was isolated after 15 min. For the phophatase treatments, insulin-treated protein was treated with either 2.5 or 0.25 mM acid phosphatase type 1 from wheat germ (Sigma-Aldrich) for 1–15 min. After treatment, the phosphatases were inhibited with 40 mM ZnSO₄, 3% phosphatase cocktail 1, and 3% phosphatase cocktail 2 (Sigma-Aldrich).

Quantitative RT-PCR

mHypoE-46 neurons were treated with 100 nM insulin over a 48-h time course, and total RNA was isolated from the cells by the guanidinium thiocyanate phenol chloroform extraction method. RNA was treated with Turbo deoxyribonuclease (Ambion, Austin, TX), and first-strand cDNA was synthesized from 4 μg RNA, using the high-capacity cDNA reverse transcription kit (Applied Biosystems Inc., Foster City, CA) following the manufacturer’s protocol. Real-time RT-PCR was performed using a SYBR Green mix containing 0.3× Sybr Green dye, 1× ROX (a passive reference dye), 1× buffer, 3 mM MgCl₂, 0.2 mM dNTP, and 0.5 U Platinum Taq (all from Invitrogen) with 200 ng template and run on the Applied Biosystems Prism 7900HT real-time PCR machine. The primer sequences are as follows: IR sense, 5′-TCC CAT CAA ATA TTG CCA AAA TT-3′, and antisense, 5′-CAG AAA TAG ATA AAT ACT TCC AAT CAC-3′; IRβ sense, 5′-TCC AGA AGC AGC AGG AT-3′, and antisense, 5′-AGG ATT TGC TGA GGT CAT TTA GGT-3′; and ɣ-actin sense, 5′-CCT CCC CAC GCC ATC TTG-3′, and antisense, 5′-CCC GGT CAG TCA GAT CTT CAT-3′. Real-time RT-PCR values were calculated using the standard curve method and normalized to ɣ-actin.

Statistics

The data are presented as the mean ± the SEM and analyzed using GraphPad Prism (GraphPad Software Inc., San Diego, CA). Statistical significance was determined using ANOVA with Bonferroni’s multiple-comparison test.

Results

Characterization and functional analysis of mHypoE-46 neurons

The mHypoE-46 neuronal cells were immortalized and characterized as previously reported (32). The cells ex-
press key genes important for our study, such as the IR, IRS-1 and -2, NPY, and AgRP (33). mHypoE-46 neurons were treated with 10 nM insulin or vehicle over 60 min, and the relative levels of pAkt were analyzed using Western blot analysis. Insulin significantly induced phosphorylation of Akt from 5–60 min (Fig. 1A), indicating that our cells are responsive to insulin and that the classical phosphatidylinositol 3-kinase (PI3K)-Akt signaling pathway is functional in our cell model (Fig. 1B).

Insulin pretreatment attenuates insulin-mediated phosphorylation of Akt

Insulin can induce insulin resistance both in vivo and at the cellular level, leading to decreased IR signaling and reduced PI3K activity (17, 24, 30, 34). To determine the effects of hyperinsulinemia on neuronal insulin signaling, we pretreated mHypoE-46 neurons with a supraphysiological concentration of insulin (1000 nM) for 48 h. After pretreatment, the cells were washed twice, placed in fresh medium for 1 h, and then rechallenged with 10 nM insulin to determine IR responsiveness. Using Western blot analysis, we determined that insulin pretreatment attenuated insulin-mediated Akt phosphorylation at 5 min by 100 ± 5% (Fig. 1C). The same effect was seen at 15 min (data not shown).

After attaining a state of cellular insulin resistance, we determined the minimal concentration and time of insulin pretreatment required to significantly attenuate insulin signaling. The mHypoE-46 neurons were pretreated with 10–1000 nM insulin for 4–24 h before rechallenge with insulin (10 nM). A minimum of 100 nM insulin for 8 h was required to significantly block insulin signaling, as indicated (Fig. 1D), and 10–1000 nM insulin pretreatment for 8 and 24 h attenuated insulin activation of phospho (p)-Akt (Fig. 1D; 100 and 1000 nM at 8 h, 70 ± 3.8% and 70 ± 9.4%; 100 and 1000 nM at 24 h, 83 ± 9.4% and 89.5 ± 10%).

To determine the time required after removal of insulin for restoration of insulin signaling, we performed a reverse time course in which the cells were pretreated with 100 nM insulin for 24 h and then washed and placed in fresh medium. From 2–12 h after pretreatment removal, the neuronal cells were rechallenged with insulin (10 nM) to determine their responsiveness, and pAkt was analyzed using Western blot analysis. Insulin signaling through Akt was significantly decreased for up to 6 h after removal of insulin pretreatment (Fig. 2A). Interestingly, when we analyzed IRβ and IRS-1 protein levels during the reverse time course, we found that both IRβ and
levels were significantly increased (Fig. 3A; 10, 100, and 1000 nM at 4 h, 2.25 ± 0.085-, 2.79 ± 0.082-, and 3.76 ± 0.1-fold; 10, 100, and 1000 nM at 8 h, 1.82 ± 0.07-, 2.55 ± 0.05-, and 2.99 ± 0.09-fold), and relative p70-S6K levels were also significantly increased (Fig. 3B; 10, 100, and 1000 nM at 4 h, 2.97 ± 0.18-, 2.95 ± 0.18-, and 4.51 ± 0.19-fold; 10, 100, 1000 nM at 8 h, 1.62 ± 0.14-, 2.32 ± 0.12-, and 2.95 ± 0.27-fold). By 24 h, pAkt and pS6K1 levels returned to basal.

IRβ protein expression was significantly decreased at 8 and 24 h by 100 and 1000 nM insulin (Fig. 3C; 100 and 1000 nM at 8 h, 45 ± 2.4 and 63 ± 0.7%; 10 and 1000 nM at 24 h, 71 ± 5.3 and 76 ± 4%). IRS-1 protein expression was significantly decreased at 8 h by 1000 nM insulin and at 24 h by 100 and 1000 nM insulin (Fig. 3D; 1000 nM at 8 h, 41 ± 7%; 100 and 1000 nM at 24 h, 53 ± 0.2 and 63 ± 8.2%). To determine whether the decrease of IRβ and IRS-1 by insulin was occurring only at the level of the protein, quantitative real-time RT-PCR was performed to analyze the levels of IR and IRS-1 mRNA. mHypoE-46 cells were treated with 100 nM insulin over a 48-h time course. IRβ and IRS-1 mRNA expression levels were unchanged with insulin treatment from 4–48 h (Fig. 3, E and F). This indicates that prolonged insulin treatment acts to decrease IR and IRS-1 protein levels as opposed to altering levels of IR and IRS-1 mRNA expression.

As previously mentioned, S6K1 can serine phosphorylate IRS-1, leading to inhibition of IRS-1. We analyzed whether insulin treatment leads to IRS-1 serine phosphorylation through S6K1, specifically looking at IRS-1 serine 307 and 1101 because these serine residues have been shown to be involved with IRS-1 inhibition (14, 15). Serine 307 showed no phosphorylation with insulin treatment (data not shown), whereas serine 1101 was phosphorylated by 100 nM insulin from 15 min to 4 h (Fig. 4C), indicating a higher molecular weight band on the Western blot, due to hyperphosphorylation of IRS-1; IRS-1 contains over 180 serine residues and thus, depending on the level of hyperphosphorylation, has different electrophoretic mobility (35). Importantly, the phosphorylation of Akt and S6K1 followed a similar temporal pattern to pIRS-1 ser1101 (Fig. 4, A and B), indicating a correlation between the phosphorylation of the three proteins. To determine whether the mammalian target of rapamycin (mTOR)-S6K1 pathway was involved in the phosphorylation of IRS-1 serine phosphorylation, we pretreated the cells with 10 nM rapamycin (an mTOR-specific inhibitor) for 1 h before treatment with insulin (100 nM). Rapamycin did not affect pAkt (Fig. 5A) but did inhibit the phosphorylation of S6K1 by insulin by 100 ± 0.1% and decreased basal pS6K1 significantly below vehicle control levels (Fig. 5B). Rapamycin also inhibited the serine phosphorylation

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**FIG. 2.** Prolonged insulin (Ins) treatment induces a long-term state of cellular insulin resistance. mHypoE-46 cells were pretreated with 100 nM insulin or vehicle for 24 h and then washed and placed in fresh medium. At 2–12 h after insulin removal, the cells were rechallenged with 10 nM insulin (black bars) or vehicle (white bars), and relative pAkt (A), IR protein (B), or IRS-1 protein (C) was analyzed using Western blot analysis. All blots were normalized to Gα (A), IR protein (B), or IRS-1 protein (C) was analyzed using Western blot analysis. At 4 and 8 h after treatment with 10–1000 nM insulin, relative pAkt levels were significantly increased (Fig. 3A; 10, 100, and 1000 nM at 4 h, 2.25 ± 0.085-, 2.79 ± 0.082-, and 3.76 ± 0.1-fold; 10, 100, and 1000 nM at 8 h, 1.82 ± 0.07-, 2.55 ± 0.05-, and 2.99 ± 0.09-fold), and relative p70-S6K levels were also significantly increased (Fig. 3B; 10, 100, and 1000 nM at 4 h, 2.97 ± 0.18-, 2.95 ± 0.18-, and 4.51 ± 0.19-fold; 10, 100, 1000 nM at 8 h, 1.62 ± 0.14-, 2.32 ± 0.12-, and 2.95 ± 0.27-fold). By 24 h, pAkt and pS6K1 levels returned to basal.

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of IRS-1 by insulin by 79 ± 15.7% (Fig. 5C). This indicates that the mTOR-S6K1 pathway is required for IRS-1 serine phosphorylation by insulin, possibly through serine 1101. To confirm that the second band noted in Fig. 4C was due to hyperphosphorylation of IRS-1 by insulin, protein was treated with acid phosphatase (2.5 and 0.25 mU) for 1–15 min at 37°C. Phosphatase treatment at 2.5 and 0.25 mU produced a dose- and time-dependent decrease in IRS-1 serine phosphorylation and diminished the hyperphosphorylated band (Fig. 5D). Two other signaling proteins known to be involved with IRS-1 serine phosphorylation, c-Jun N-terminal kinase and IKK, were analyzed, but neither was found to be phosphorylated by prolonged insulin exposure in our cell model (data not shown).

The inhibitors epoxomicin, rapamycin, and 3-MA restored insulin signaling

To determine the mechanisms used by insulin to attenuate insulin signal transduction, three specific inhibitors were used: epoxomicin, rapamycin, and 3-MA. Epoxomicin inhibits the catalytic subunit of the 20S proteasome, preventing proteasomal protein degradation; rapamycin inhibits mTOR, preventing activation of p70-S6K; and 3-MA inhibits autophagy and lysosomal sequestration, the initial steps required for lysosomal protein degradation. mHypoE-46 neurons were pretreated with the inhibitors 50 μM epoxomicin, 10 mM rapamycin, or 10 mM 3-MA or DMSO vehicle for 1 h, followed by insulin (100 nM) or water vehicle treatment for 8 h, after which the phosphorylation of Akt by insulin was analyzed. Consistent with previous results, 100 nM insulin pretreatment in DMSO vehicle control attenuated insulin-mediated Akt phosphorylation. Interestingly, pretreatment with epoxomicin, rapamycin, or 3-MA significantly increased the induction of pAkt by insulin rechallenge after prolonged insulin exposure by 1.98 ± 0.36-, 1.91 ± 0.36-, and 2.27 ± 0.52-fold, respectively, partially restoring insulin signaling (Fig. 6A). This indicates that the three pathways, proteasomal protein degradation, mTOR-S6K1, and lysosomal protein degradation, may be involved with the development of cellular insulin resistance and that prevention of only one of these mechanisms is not sufficient to fully restore insulin signaling. When p70-S6K was analyzed with inhibitor treatments, rapamycin blocked phosphorylation of p70-S6K in control and insulin-pretreated samples, whereas 3-MA restored phosphorylation of p70-S6K in insulin-pretreated samples (Fig. 6B). Epoxomicin restored IRS-1 levels in the presence of 100 mM insulin or water vehicle treatment for 8 h, after which the phosphorylation of Akt by insulin was analyzed. Consistent with previous results, 100 nM insulin pretreatment in DMSO vehicle control attenuated insulin-mediated Akt phosphorylation. Interestingly, pretreatment with epoxomicin, rapamycin, or 3-MA significantly increased the induction of pAkt by insulin rechallenge after prolonged insulin exposure by 1.98 ± 0.36-, 1.91 ± 0.36-, and 2.27 ± 0.52-fold, respectively, partially restoring insulin signaling (Fig. 6A). This indicates that the three pathways, proteasomal protein degradation, mTOR-S6K1, and lysosomal protein degradation, may be involved with the development of cellular insulin resistance and that prevention of only one of these mechanisms is not sufficient to fully restore insulin signaling. When p70-S6K was analyzed with inhibitor treatments, rapamycin blocked phosphorylation of p70-S6K in control and insulin-pretreated samples, whereas 3-MA restored phosphorylation of p70-S6K in insulin-pretreated samples (Fig. 6B).
Discussion

Until recently, the brain was not considered a major contributor to overall glucose homeostasis. Through elegant transgenic experiments, which produced neuron-specific knockouts or overexpression of insulin signaling components, the hypothalamus is now recognized as a critical regulator of insulin action (9). However, exactly how hypothalamic neurons sense insulin and the mechanisms through which neurons develop insulin resistance are not fully understood. We have been able to approach this issue through which neurons develop insulin resistance are not fully understood. We have been able to approach this issue through clonal, immortalized, hypothalamic cell model. It is well documented that prolonged exposure to insulin activates phosphatidylinositol 3-kinase (PI3K) and Akt, in agreement with previous studies performed in myocytes and adipocytes, indicating that in neurons, insulin may also act through the mTOR-S6K1 pathway to inhibit insulin signaling and that this likely involves IRS-1 ser1101 phosphorylation. Of note, IRS-1 ser307 did not appear to be phosphorylated by insulin in our cells (data not shown). Normally, IRS-1 serine phosphorylation may be part of a negative feedback pathway involved in maintaining timely insulin signaling, but in disease states, when insulin levels are continuously elevated, IRS-1 serine phosphorylation may act to provoke cellular insulin resistance.

In addition to the attenuation of insulin signaling, IRS-1 serine phosphorylation has been shown to target the protein for proteasomal degradation. It was first observed by Haruta et al. (20) that insulin causes phosphorylation of IRS-1 through the mTOR-S6K1 pathway and targets it for degradation. Recently, Xu et al. (36) found that this occurs via phosphorylation of multiple serine residues through truncated IRS-1 constructs that the key serines reside between 1 and 574 amino acids, with a key residue being serine 527. We have found that the mTOR-S6K1 pathway is involved with attenuated insulin signaling, al-
though not due to degraded IRS-1. This does not rule out the involvement of IRS-1 serine phosphorylation targeting for proteolysis in our model, because there are other pathways through which insulin can cause IRS-1 serine phosphorylation, such as PKC. IRS-1 was degraded through the ubiquitin-proteasomal pathway, because treatment with the 26S proteasome-specific inhibitor epoxomicin prevented degradation, in agreement with previous studies. Sun et al. (21) first showed that chronic insulin induces IRS-1 degradation through the proteasomal pathway, because the 26S proteasome inhibitor lactacystin prevented insulin-induced degradation. The mechanism through which IRS-1 degradation occurs was later analyzed by Xu et al. (36) who found that a specific E3 ligase complex is involved with IRS-1 degradation. The CUL7 E3 ligase complex specifically bound serine-phosphorylated IRS-1, and the overexpression of CUL7 and another member of the complex, Fbw8, increased the decay of IRS-1. Importantly, they found that the CUL7 knockout mouse has 5-fold increased IRS-1 levels and increased activation of the Akt and MAPK pathways by insulin. By preventing the degradation of IRS-1 by prolonged insulin exposure, using epoxomicin, we restored insulin signaling through the Akt pathway. Additionally, we found that decreased IRS-1 protein levels correlated with decreased insulin signal transduction. Taken together with the previous studies, this suggests that IRS-1 degradation plays a crucial role in the development of cellular insulin resistance.

As early as 1974, it was shown that prolonged insulin exposure decreases the concentration of the IR, both in vivo and in vitro (16). Numerous studies then demonstrated that hyperinsulinemia down-regulates the IR, lead-

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**FIG. 5.** Rapamycin inhibits insulin-induced S6K1 and IRS-1 ser1101 phosphorylation. mHypoE-46 cells were pretreated with DMSO vehicle or 10 nM rapamycin (Rap.) for 1 h, followed by treatment with 100 nM insulin (Ins) or vehicle. Protein was isolated at 15 min and analyzed using Western blot analysis with p-specific antibodies against Akt (A), S6K1 (B), and IRS-1 ser1101 (C). All blots were probed with Gβ as a loading control, and data are shown with SEM (n = 4 independent experiments). D, mHypoE-46 cells were treated with 100 nM insulin (black bars) or vehicle (white bar) for 30 min. The isolated protein was then treated with 2.5 or 0.25 mU acid phosphatase (Phosph.) for 1, 5, and 15 min at 37 C or left untreated. Relative pIRS-1 ser1101 was normalized to Gβ and shown with SEM (n = 3 independent experiments). ***, P < 0.001; **, P < 0.01; *, P < 0.05.
vehicle, 50 µM epoxomicin (Epox), 10 nM rapamycin (Rap), or 10 µM 3-MA for 8 h. The cells were washed and allowed to recover for 1 h before rechallenge with 10 nM insulin (black bars) or vehicle (white bars). Western blot analysis was used to measure pAkt (A), pS6K1 (B), IRβ protein (C), or IRS-1 protein (D); all blots were normalized to G3B as a loading control (Ctrl). Data are shown with SEM (n = 4–7 independent experiments). ***, P < 0.001; **, P < 0.01; *, P < 0.05.

FIG. 6. The inhibitors epoxomicin, rapamycin, and 3-MA partially restore insulin (Ins) activation of the Akt pathway. mHypoE-46 cells were pretreated with vehicle or 100 nM insulin and DMSO vehicle, 50 µM epoxomicin (Epox), 10 nM rapamycin (Rap), or 10 µM 3-MA for 8 h. The cells were washed and allowed to recover for 1 h before rechallenge with 10 nM insulin (black bars) or vehicle (white bars). Western blot analysis was used to measure pAkt (A), pS6K1 (B), IRβ protein (C), or IRS-1 protein (D); all blots were normalized to G3B as a loading control (Ctrl). Data are shown with SEM (n = 4–7 independent experiments). ***, P < 0.001; **, P < 0.01; *, P < 0.05.

Insulin. This indicates that IR degradation also plays a key role in the development of cellular insulin resistance.

Although there are no current studies that have studied the effects of sustained insulin exposure upon neurons, there have been some recent studies that have demonstrated the induction of hypothalamic insulin resistance by nutrients. Ono et al. (40) showed that mice fed a HFD for 1 d had decreased tyrosine-phosphorylated IRS-1 and attenuated suppression of hepatic glucose production (HGP) by insulin; interestingly, IRS-2 was not affected by a HFD. They went on to show that overexpression of p70-S6K induced IRS-1 serine 1101 phosphorylation, which corresponds well with the data presented in our study; attenuated the induction of pAkt by insulin; and blunted the suppression of hepatic glucose production (HGP) by insulin. p70-S6K overexpression also led to insulin resistance and increased IRS-1 serine phosphorylation under hyperinsulinemic-euglycemic clamp conditions. Additionally, expression of dominant-negative S6K1 restored the ability of insulin to suppress HGP on a HFD and restored induction of pAkt and IRS-1 tyrosine phosphorylation by insulin. Zhang et al. (41) found that HFD induces neuronal insulin resistance and that this was mediated by the IKKβ-nuclear factor-κ-light-chain-enhancer of B cells (NFκB) pathway. Interestingly, they found that AgRP neuron-specific IKKβ knockout in mice prevented the negative effects of a HFD. Posey et al. (42) demonstrated that icv infusion of palmitic acid, a prevalent free fatty acid, decreased hypothalamic insulin signaling, which was mediated through the IKKβ-NFκB pathway. Ozcan et al. (43, 44) have found that the obese models, either diet-induced or genetic (ob/ob and db/db), have increased levels of ER stress markers and that icv administration of chemical chaperones that can prevent ER stress, 4-phenylbutyric acid or tauroursodeoxycholic acid (TUDCA), normalize the obese, diabetic phenotype in mice as well as normalizing insulin sensitivity. These studies further indicate that central insulin resistance plays an important role in the development of obesity and T2DM, again stating the need for further investigation of the mechanisms involved in the pathogenesis of neuronal insulin resistance.

Taken together, the experiments in this study have demonstrated that prolonged insulin exposure precipi-
tates in attenuated insulin signaling in neuronal cells. This occurs through three specific mechanisms: the inactivation of IRS-1 by serine phosphorylation, the proteasomal degradation of IRS-1, and the lysosomal degradation of the IR. The inhibition of any one of these mechanisms is sufficient to improve insulin signaling in neuronal cells. Thus, hyperinsulinemia strongly affects neuronal insulin signaling and, because central insulin actions are critical to the regulation of energy and glucose homeostasis, the central effects of insulin resistance requires more attention. The mechanisms illustrated in this study have been similarly demonstrated in studies using peripheral cells; hence, we have determined that there are possible parallel effects of insulin upon neuronal and peripheral cells. The proposition that there are similar mechanisms involved in the development of peripheral and neuronal insulin resistance is important when considering treatments for the pathophysiological states involving insulin resistance, such as obesity and T2DM; treatments that target peripheral insulin resistance may also improve central insulin resistance.

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