Amyloid beta oligomers induce impairment of neuronal insulin receptors

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ABSTRACT **Recent studies have indicated an association between Alzheimer's disease (AD) and central nervous system (CNS) insulin resistance. However, the cellular mechanisms underlying the link between these two pathologies have not been elucidated. Here we show that signal transduction by neuronal insulin receptors (IR) is strikingly sensitive to disruption by soluble Aβ oligomers (also known as ADDLs). ADDLs are known to accumulate in AD brain and have recently been implicated as primary candidates for initiating deterioration of synapse function, composition, and structure. Using mature cultures of hippocampal neurons, a preferred model for studies of synaptic cell biology, we found that ADDLs caused a rapid and substantial loss of neuronal surface IRs specifically on dendrites bound by ADDLs. Removal of dendritic IRs was associated with increased receptor immunoreactivity in the cell body, indicating redistribution of the receptors. The neuronal response to insulin, measured by evoked IR tyrosine autophosphorylation, was greatly inhibited by ADDLs. Inhibition also was seen with added glutamate or potassium-induced depolarization. The effects on IR function were completely blocked by NMDA receptor antagonists, tetrodotoxin, and calcium chelator BAPTA-AM. Downstream from the IR, ADDLs induced a phosphorylation of Akt at serine^{473}, a modification associated with neurodegenerative and insulin resistance diseases. These results identify novel factors that affect neuronal IR signaling and suggest that insulin resistance in AD brain is a response to ADDLs, which disrupt insulin signaling and may cause a brain-specific form of diabetes as part of an overall pathogenic impact on CNS synapses.—Zhao, W. Q., De Felice, F. G., Fernandez, S., Chen, H., Lambert, M. P., Quon, M. J., Krafft, G. A., Klein, W. L. Amyloid beta oligomers induce impairment of neuronal insulin receptors. *FASEB J.* 22, 246–260 (2008)**

Key Words: insulin resistance · calcium · NMDA receptor · receptor loss · Akt serine^{473} · tyrosine phosphorylation

The insulin receptor (IR) is a protein tyrosine kinase playing a pivotal role in regulation of peripheral glucose metabolism and energy homeostasis. Impairment of peripheral IR functions is characterized by reduced ability of insulin to stimulate glucose utilization (insulin resistance), a syndrome that is associated with type II diabetes, hypertension, and obesity (1–3). Insulin receptors also occur in the brain, where they are abundantly distributed in synaptic membranes of the cerebral cortex and hippocampus (4–7). CNS IRs diverge from their peripheral counterparts both in structure and function (4, 8). Unlike those from the periphery, the neuronal IRs do not seem to be involved in glucose metabolism but rather in more diverse brain functions, including synaptic activities required for learning and memory (8–13).

Recently, the intriguing suggestion has been put forward that CNS insulin signaling can manifest a novel type of insulin-resistant diabetes that may be linked to Alzheimer's disease (14–18). Although attempts to correlate insulin resistant type 2 diabetes mellitus (T2DM) and AD have yielded paradoxical results, likely due to interactions of factors such as cerebrovascular complications and ApoE genotypes (19, 20), there is a significant body of evidence indicating that insulin resistance (21–26), along with impaired brain energy utilization (15, 27, 28), is present in AD. Major impairments in insulin and insulin-like growth factor (IGF) gene expression and signaling have been observed in the brains of AD patients (29, 30). AD-like changes have been observed in an experimental animal model of insulin resistance (18 17). Supporting evidence also comes from AD transgenic mice, in which hyperinsulinemia develop at the age of 13 months (31), an age exhibiting high amounts of Aβ deposits and cognitive impairment (32). Administration of rosiglitazone, a

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PPARγ agonist used for treating insulin-resistant type II diabetes, prevents hyperinsulineamia and rescues memory deficits in these mice (33).

Decline of IR function can originate with aberrations in expression or phosphorylation state of the receptor. For example, in certain familial forms of insulin resistance, defective insulin receptor function is caused by the severe lesion of IR alleles and substantial decreases in IR levels (34). Alternatively, suppression of IR enzymatic activity has been linked to negative regulatory factors stimulated by IR signaling as well as other pathways. Elevated tyrosine phosphatase activity, *e.g.*, attenuates net IR tyrosine kinase activity, while phosphorylation of certain serine (pSer) and threonine (pThr) residues within the receptor conformationally inhibits the capacity for tyrosine phosphorylation (pTyr). Additionally, IR activity can be inhibited by a downstream negative feedback pathway, in which pSer of insulin receptor substrate 1 (IRS1) and Akt Ser^{473} ($pSer^{473}$) suppress IR pTyr by inducing IR pSer and pThr (35–39). Sustained enhancement of these negative feedback loops are known to be caused by oxidative stress and proinflammatory factors and to contribute to development of insulin resistance in peripheral tissues (39–43). Because AD is a brain disorder putatively characterized in early stages by synaptic failure (44), IR impairment in AD may be coupled locally to synaptic pathology. Recent evidence strongly implicates Aβ oligomers as the proximal pathogenic trigger. Soluble Aβ oligomers (also known as amyloid-beta derived diffusible ligands, or ADDLs) are markedly elevated in the brain and cerebrospinal fluid (CFS) of postmortem AD patients (45, 46) and appear to play a critical role in the synaptic failure and memory deficits of early AD (47–50). ADDLs induce pathological changes that include oxidative stress and tau hyperphosphorylation (51–53). Acting as gain-of-function pathogenic ligands, both synthetic ADDLs and those derived from AD brain bind specifically to synaptic spines of primary hippocampal cultures. Synaptic binding results in structural deterioration and receptor loss (54–56). ADDLs disrupt activity-dependent synaptic plasticity, including long-term potentiation (LTP) and reversal of long-term depression (LTD) (47, 57). A different Aβ oligomer preparation, considered naturally secreted oligomers, has been shown to impair LTP and memory formation in experimental animals (58–60).

The current study addresses whether direct interactions between neurons and ADDLs might give rise to abnormalities in insulin signaling. Experiments have characterized IR expression and protein tyrosine kinase activity in highly differentiated cultures of hippocampal neurons, whose synapses are known to be targeted by ADDLs (54, 56). Following short-term exposure to ADDLs, these neurons exhibited a striking loss of dendritic IRs and capacity for IR tyrosine autophosphorylation. Decreased IR autophosphorylation also occurred in human IR overexpressed in NIH3T3 cells, suggesting possible direct association between ADDLs and IRs. Consistent with decreased receptor function, ADDLs increased the level of neuronal Akt $pSer^{473}$ phosphorylation, a downstream negative feedback regulator of IR and PI3 kinase activities (39, 61). Marked reductions in dendritic IR levels and insulin-evoked tyrosine kinase activity caused by synaptotoxic, disease-associated ADDLs could provide a molecular mechanism to explain the origin of insulin resistance in AD.

MATERIALS AND METHODS

Materials

Synthetic Aβ1–42 peptide was purchased from American Peptides (Sunnyvale, CA, USA). 1,1,1,3,3,3,-hexafluoro-2-propanol, DMSO, Papain, poly-L-lysine, glutamate, APV, TTX, okadaic acid, and protease and phosphatase inhibitor cocktails were purchased from Sigma Aldrich (St. Louis, MO, USA). Phenol red-free Ham's F12 medium was purchased from BioSource (Camarillo, CA, USA). Culture medium and reagents, including DMEM, Neurobasal, Neurobasal A, B-27, bFGF, fetal bovine serum, L-glutamine, and penicillin/streptomycin, were from Invitrogen (Carlsbad, CA, USA). Insulin (Humulin, 100 U) was from Eli lily (Indianapolis, IN, USA). BAPTA-AM, precast electrophoresis gels, Alexa fluorophores-labeled secondary antibodies, and ProLong Gold antifade mounting medium were purchased from Invitrogen. Electrophoresis buffers were purchased from Bio-Rad (Hercules, CA, USA). BAC Micro reagent, Protein A- and G-sepharose beads, SuperSignal chemiluminescent reagents were purchased from Pierce (Deerfield, IL, USA). Anti-IRα, anti-IRβ, anti-IGF-1R, and antiphospho-tyrosine (PY20) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antiphospho-tyrosine (4G10) antibody was purchased from Upstate Biotechnology (Charlottesville, VA, USA). Antiphospho IR1150/1151, anti-Akt, antiphospho Akt473 antibodies were purchased from Cell Signaling (Danvers, MA, USA). Antiphospho IR1162/1163 antibody was purchased from Calbiochem. Anti-ADDL antibody (NU1 and NU2) was prepared according to previous reports (62).

Preparation of ADDLs

ADDLs were prepared with synthetic Aβ1–42 according to the procedure described previously (45). In brief, the peptide was dissolved in 1,1,1,3,3,3,-hexafluoro-2-propanol to 1 mM and stored as a dried film at −80°C after evaporation of the solvent. The dried peptide film was resuspended in DMSO to a final concentration of 5 mM, vortexed thoroughly, and sonicated for 10 min. The solution was then diluted with ice-cooled phenol red-free Ham's F12 medium to 100 μM and placed at 4°C overnight to form Aβ oligomers. The oligomer solution was centrifuged briefly, and the supernatant was collected as soluble Aβ oligomers (ADDLs).

Hippocampal and cortical neuronal cultures

Primary hippocampal and cortical neuronal cultures were prepared according to established procedures (45). Briefly, hippocampi and cortices from E18 embryonic Sprague-Dawley Rats were dissected and digested in papain (2 mg/ml) for 30 min. After trituration and settlement, the cell suspension was plated in 6-well dishes coated with poly-L-lysine at a density of ~1 million cells/well. For immunocytochemistry, cells were plated onto poly-L-lysine coated coverslips in 24-well culture plates at a density of 70,000 cells per plate. Cells

were maintained in Neurobasal supplemented with 2% B27, 0.5 mM L-glutamine, and 1% penicillin/streptomycin. In certain experiments, hippocampal and cortical cultures were also prepared from 1-day-old postnatal Wistar rats, according to a protocol described elsewhere (63), and maintained in Neurobasal-A complemented with 2% B27, 10 ng/ml b-FGF and 0.5 mM L-glutamine.

ADDL treatment of neurons and immunocytochemistry

Cultured hippocampal neurons (25 DIV) were treated with 100 nM ADDLs or vehicle at 37°C for 30 min. Cells were fixed with 3.7% formaldehyde (in PBS buffer) to the media for 5 min followed by the removal of the entire fix:media solution and replacement with 3.7% formaldehyde for 10 min. Cells were rinsed 3 times with PBS, pH 7.5, incubated with PBS: 10% normal goat serum (NGS) for 90 min at room temperature, and incubated simultaneously with the ADDL-selective NU2 antibody (Lambert *et al.*, ref. 62; 1:500 dilution) and insulin receptor-α (Santa Cruz Biotechnology) antibody (1:500) overnight at 4°C. Neurons were then rinsed 3 times with PBS and incubated for 3 h at room temperature with Alexa Fluor555 anti-mouse IgG and Alexa Fluor488 anti-rabbit IgG (1:1000). After wash, cells on coverslips were mounted with Prolong Gold mounting media, and were observed with a Nikon Eclipse TE 2000-U fluorescence microscope.

Culture and treatments of NIH3T3 cells overexpressing human IRs and IGF-1 receptors (IGF-1R)

The human full-length IR cDNA or IGF-1R was stably transfected into NIH3T3 cells (64, 65). Cells with or without expression of these receptors were cultured in low-glucose (1 g/L glucose) DMEM medium supplement with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cells were cultured to ~80% confluence and serum deprived overnight before using for experimental treatments. To stimulate Tyr phosphorylation of IR and IGF-1R, cells were stimulated with 100 nM insulin, for various lengths of time in the absence or presence of ADDLs. Reactions were terminated by removing the medium, rapid washing cells with precooled PBS and freezing cells on dry ice before preparations of cell lysates.

ADDL and pharmacological treatment of cells for measurement IR pTyr

To increase insulin sensitivity of neuronal IRs, which were constantly exposing to insulin in B27, neurons were changed to Neurobasal medium without B27 supplement for 3–4 h before treatments. In certain experiments, neurons were placed and subsequently treated in physiological Kreb-Ringer's buffer (KRB) containing 20 mM HEPES, 125 mM NaCl, 5 mM KCl, 1 mM Na_2HPO_4, 1 mM $MgSO_4$, 1 mM $CaCl_2$, and 5.5 mM Glucose. ADDLs were added to cells to final concentrations of 50–500 nM with or without the presence of 100 nM insulin. To depolarize neurons, the physiological KRB was replaced with a high-K^+ KRB containing 20 mM HEPES, 75 mM NaCl, 55 mM KCl, 1 mM Na_2HPO_4, 1 mM $MgSO_4$, 1 mM $CaCl_2$, and 5.5 mM glucose. The depolarization was performed following treatment with ADDLs and insulin, and was allowed for 10 min before termination of the reaction. Alternatively, cells were stimulated with 100 μM glutamate in nondepolarizing KRB for 10 min after treatment with ADDLs and insulin. For inhibitor treatment, neurons were preincubated for 15–30 min with 50 μM APV, 20 μM memantine, 50 μM BAPTA-AM, or 20 nM TTX before ADDL and insulin treatment. On termination of reactions, the culture media were removed, cells were rapidly rinsed with precooled KRB or 1× PBS, pH 7.4, and cell lysates were prepared with a lysis buffer containing 10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.8 M EDTA, 0.5 M EGTA, 1% Triton X-100, 0.5% Nonidet P-40, and 1% protease cocktails and collected with a cell scrubber. After lysing in microtubes on ice for 30–60 min with vortex in every 10–15 min, the lysates were spin at 1500 *g* for 5 min, and the supernatants were collected and stored at −80°C before use. Protein concentrations were measured using the BAC Micro reagent.

Detection of IR and IGF-1R phosphorylation with immunoprecipitation

IR or IGF-1R was immunoprecipitated with an anti-IRβ or an anti-IGF-1R antibody in a volume of 300 μl mixture containing 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1% protease inhibitor cocktail, and 1 mg/ml cellular proteins. The mixture was incubated at 4°C overnight with constant rotating followed by incubation with a mixture of UltraLink protein A and G sepharose beads for 2 h at 4°C. After two washes with ice-cooled precipitation buffer, and one with ice-cooled 1× PBS pH 7.4, the precipitated IR was released from the sepharose beads with reducing SDS-sample buffer boiled for 4–5 min, and resolved on 4–20% precast PAGE. After transfer onto a nitrocellulose membrane and blocked with 5% dry milk, the membrane was incubated with combination of two antiphosphotyrosine antibodies (4G10 and Py20) in 1× PBS containing 0.05% Tween-20 (PBS-T) at 4°C overnight. The membrane was washed with PBS-T and incubated with a secondary antibody conjugated with HRP at room temperature for 1 h. The IR phosphorylation signals were detected with SuperSignal chemiluminescent reagents. The total amount of precipitated IR from the identical samples was measured with the anti-IRβ and used for normalization of the phosphorylation extents.

Phosphorylation detection with Western blotting and immunocytochemistry

Phosphorylation extents of IR, and Akt were also determined on Western blots using specific antibodies against the phosphorylated form of each molecule at specific sites. For normalization, a set of duplicated samples transferred to a separate membrane (or on the same membrane that was stripped with a stripping buffer after blotting with the first primary antibody) were incubated with an antiregular IR (or Akt) antibody to detect the total amount of the protein. The ratio of phospho-protein over the regular protein was used to assess the extent of phosphorylation. For immunocytochemical detection of phosphorylated IR and Akt, cells cultured on coverslips and treated with ADDLs and/or insulin were incubated with antipTyr IR or anti-Akt $pSer^{473}$ at 4°C overnight, followed by incubations with secondary antibodies conjugated with Alexa fluorophores. Cells were then washed and mounted on a glass slide with ProLong Gold antifade mounting medium; the image was acquired with a laser scanning confocal microscope.

Coimmunoprecipitation

To detect interaction between ADDLs and insulin receptor, neurons were treated with 500 nM biotin-labeled ADDLs (bADDLs) at 37°C for 30 min. After terminating the reaction, neurons were washed with PBS and lysed in cell lysis buffer described in previous sections. Insulin receptors were immunoprecipitated from the cell lysates with an anti-IRβ antibody (Santa Cruz Biotechnology). The coprecipitated bADDLs

were detected on Western blots with streptavidin conjugated with IRDye800 (Rockland Immunochemicals, Inc. Gilbertsville, PA, USA). Alternatively, the cell lysates were precipitated with 6E10 antibody, and the precipitated bADDLs were detected on Western blots with IRDye800 conjugated streptavidin. The fluorescent signals for bADDLs were acquired and analyzed using a LiCor-Odyssey imager (LI-COR Biotechnology, Lincoln, NE, USA). Coimmunoprecipitation was also performed using an ADDL antibody (NU-1). The precipitated complexes were then blotted with an IR or an IGF-1R antibody on Western blots.

Data analyses

For IRα levels and ADDL binding 40 to 50 images were collected for 5 independent batches of cultures. The acquired immunocytochemical images for IR and ADDL binding were quantified by histogram analysis of the fluorescence intensity at each pixel across the images using Image J (66). Appropriate threshold was applied to both control and ADDL-treated cells. Cell bodies were digitally removed from the images so that ADDL binding or IRα immunostainings on dendritic processes was quantified. Alternatively immunostainings only from cell body compartment were analyzed for assessment of ADDL-induced IRα accumulation. Data from all experiments was then subjected to statistical analysis.

For Western blotting assays, data for each experiment were collected from at least three independent repeats. IR and Akt signals were acquired by densitometric scan. The mean density of each immunoreactive band was measured using Image J. Ratios of the phosphorylated form over the nonphosphorylated form, or the total amount of each protein were calculated, and converted to percent the control (Basal level phosphorylation) samples. The insulin induced phosphorylation was obtained after subtracting values of the basal level (normally 100%). Values from different treatment were then analyzed with one-way or two-way ANOVA using GraphPad Prism software (San Diego, CA, USA).

RESULTS

ADDL binding and insulin receptor levels: For ADDLs to impair insulin receptors, ADDL binding sites, and insulin receptors should occur on the same neurons. We investigated this possibility using cultures of mature hippocampal neurons, which have been shown to develop clusters of ADDL binding sites specifically at synapses (53, 54). ADDLs were added to cultures at 100 nM and incubated for 30 min to allow for complete binding. ADDL binding sites exhibited the same punctate distribution previously seen (**Fig. 1**). Insulin receptors, identified using antibodies against the outward-directed alpha subunit, also distributed in a punctate manner. ADDL binding occurred on neurons that expressed insulin receptors, although not on all of them (~40% of neurons had insulin receptors but no ADDL binding). Most significantly, however, the subcellular distribution of insulin receptors was strikingly different on neurons with and without bound ADDLs (Fig. 1*A–C*). Neurons with ADDL binding showed virtual absence of insulin receptor immunoreactivity on their dendrites. Reciprocally, dendrites with abundant insulin receptors showed no ADDL binding. By image analysis, dendrites with ADDL binding had ~70% less insulin receptor immunoreactivity than the ADDL-free cells (Fig. 1*B*).

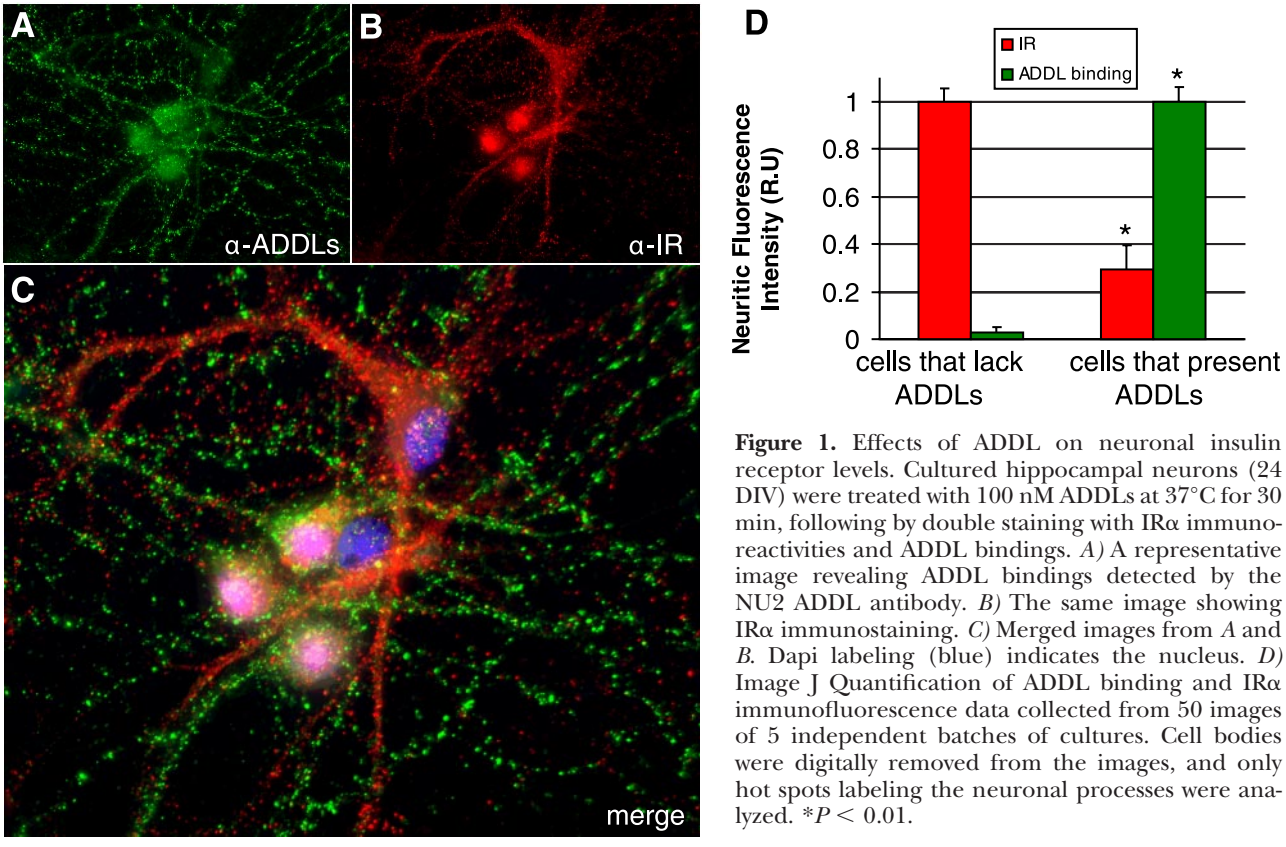

Figure 1. Effects of ADDL on neuronal insulin receptor levels. Cultured hippocampal neurons (24 DIV) were treated with 100 nM ADDLs at 37°C for 30 min, following by double staining with IRα immunoreactivities and ADDL bindings. *A)* A representative image revealing ADDL bindings detected by the NU2 ADDL antibody. *B)* The same image showing IRα immunostaining. *C)* Merged images from *A* and *B*. Dapi labeling (blue) indicates the nucleus. *D)* Image J Quantification of ADDL binding and IRα immunofluorescence data collected from 50 images of 5 independent batches of cultures. Cell bodies were digitally removed from the images, and only hot spots labeling the neuronal processes were analyzed. $*P < 0.01$.

ADDL-induced neuronal IR redistribution

Differential distribution of ADDL binding sites and insulin receptors strongly suggests ADDLs cause dendritic insulin receptors to down-regulate. However, an alternative possibility is that ADDLs attached only to dendrites that did not express insulin receptors. This alternative is not the case, as we observed control cultures (not exposed to ADDLs) expressed insulin receptors in 100% of their dendrites (40 neurons selected by phase and then assessed for insulin receptor signal; a typical branched neuron expressing insulin receptors is shown in **Fig. 2*F***). This clearly indicates that ADDLs did not target dendrites lacking insulin receptors but rather caused dendritic insulin receptor down-regulation. Significantly, ADDL-bound neurons that lacked dendritic insulin receptors exhibited high levels of receptors within their cell bodies (Fig. 2*A*). In fact, in ADDL-positive neurons, insulin receptor immunoreactivity in cell bodies was elevated ~3-fold compared to levels in ADDL-free cells (Fig. 2*B*). The possibility thus exists that ADDLs triggered a major redistribution of insulin receptors without causing reduction in total receptor level, a possibility supported by Western blot data presented below. Consistent with the concept of redistribution, it recently has been reported that ADDLs trigger down-regulation of surface NMDA and EphB2 receptors, which, like insulin receptors, are synaptic proteins implicated in memory mechanisms (56).

Inhibition of neuronal insulin receptor activity by ADDLs

We next investigated whether ADDLs also caused loss of neuronal insulin receptor function. Our experiments focused on the impact of ADDLs on insulin-induced receptor protein tyrosine kinase activity, measured by receptor autophosphorylation. Hippocampal and cortical neuronal cultures were incubated with or without ADDLs (50 nM total Aβ) for 60 min. In the absence of ADDLs, stimulation of cells with insulin for 5 min caused a large increase in IR autophosphorylation (**Fig. 3**). After 60 min, IR autophosphorylation was still robust but not as large. When ADDLs were present, the level of autophosphorylation due to insulin was greatly reduced (>80%) in both hippocampal and cortical cultures (Fig. 3), demonstrating that CNS neurons exposed to ADDLs develop major deficiencies in their response to insulin.

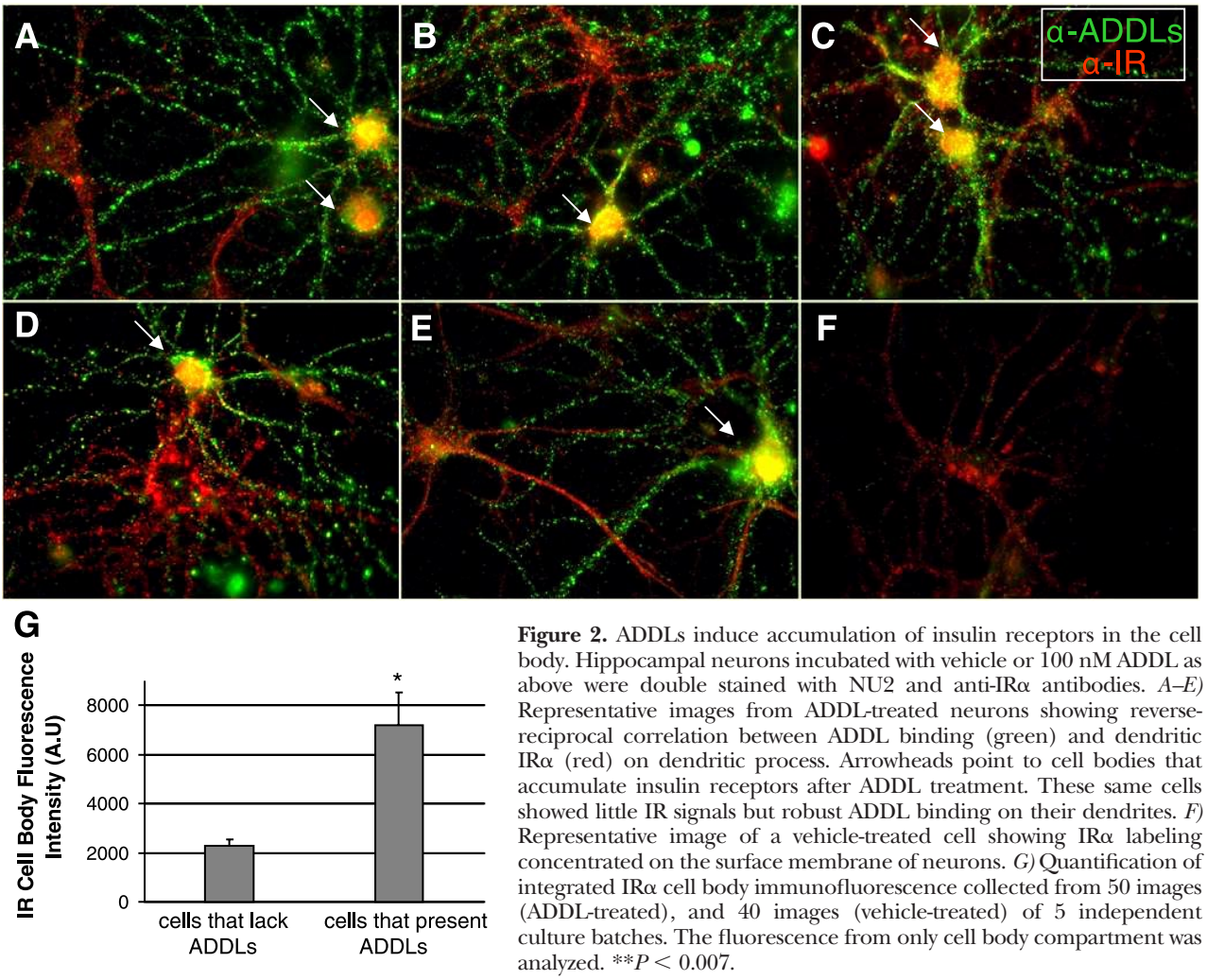

Figure 2. ADDLs induce accumulation of insulin receptors in the cell body. Hippocampal neurons incubated with vehicle or 100 nM ADDL as above were double stained with NU2 and anti-IRα antibodies. *A–E)* Representative images from ADDL-treated neurons showing reverse-reciprocal correlation between ADDL binding (green) and dendritic IRα (red) on dendritic process. Arrowheads point to cell bodies that accumulate insulin receptors after ADDL treatment. These same cells showed little IR signals but robust ADDL binding on their dendrites. *F)* Representative image of a vehicle-treated cell showing IRα labeling concentrated on the surface membrane of neurons. *G)* Quantification of integrated IRα cell body immunofluorescence collected from 50 images (ADDL-treated), and 40 images (vehicle-treated) of 5 independent culture batches. The fluorescence from only cell body compartment was analyzed. $^{**}P < 0.007$.

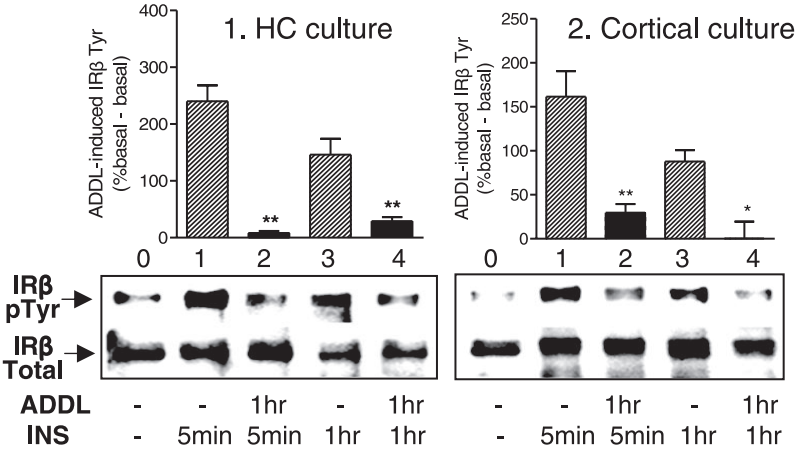

Figure 3. Inhibition of neuronal IR Tyr phosphorylation (pTyr) by ADDLs: Hippocampal (HC, 1) and cortical neuronal cultures (2) (14–20 DIV) prepared from postnatal day 1 rats were "starved" in B27-free Neurobasal basal medium for 3–4 h and treated with 50 nM ADDLs or vehicle in the presence and absence of insulin (100 nM). IR was immunoprecipitated with an IRβ antibody and the pTyr detected on Western blots with antipTyr antibodies (4G10 and Py20). Lane 0: basal condition; Lane 2–4: treated with insulin and/or ADDLs.

ADDL-induced inhibition of IR autophosphorylation is associated with NMDA receptor activity

ADDL-induced synapse pathology and formation of reactive oxygen species have previously been linked with NMDA receptor activity (51, 56, 63, 68–70). We, therefore, examined the effects of glutamate stimulation as well as depolarization on IR function. IR phosphorylation was examined using antibodies against phosphorylated IR Tyr1150/1151 (**Fig. 4**) or Tyr 1162/1163 (see below, **Fig. 5**). These sites play a critical role in stabilizing IR tyrosine kinase activity. As shown in Fig. 4, insulin stimulated a substantial increase in IRβ-pTyr1150/1151. This increase was reduced 37% by glutamate and 24% by potassium-induced depolarization. A one-way ANOVA showed significant treatment effects ($F2,8=11.6$, $P<0.01$, $n=3$).

We next investigated the combined effects of ADDLs and glutamate on IR inhibition. Aβ oligomers are known to target excitatory synapses (45, 56, 57, 67–70) and stimulate Ca2+ influx, either directly (72) or indirectly (51). It previously has been reported that increased Ca^{2+} inhibits IR autophosphorylation in synaptic membranes (7) and adipocytes (73). Since activation of NMDA receptors by glutamate is known to promote Ca^{2+} influx, the possibility arises that ADDLs in tandem with excitation-driven Ca^{2+} influx might be particularly harmful to IR function. As shown in Fig. 5*A*, both glutamate and ADDLs inhibited receptor autophosphorylation at IRβpTyr1162/1163. This inhibition was augmented when they were applied together. Inhibition was confirmed using immunocytochemistry (Fig. 5*D*). Basal and insulin-induced activity was seen in the soma and dendritic processes of the cultured hippocampal neurons. In the presence of ADDLs and glutamate, the insulin-induced IRp-Tyr1162/1163 was similar to control neurons, to which only vehicle was added (Fig. 5*D*, the first panel). Overall, the effects of ADDLs and neuronal activity combine to cause major reduction in IR phosphorylation at the activity-stabilizing domain. Most significantly, preapplication of the specific NMDA receptor antagonists APV or memantine preserved receptor function. This protection was evident whether neurons were exposed to ADDLs, glutamate, or both together. The data thus lead to the major conclusion that ADDL impairment of IR function is mediated by NMDA receptor activity. Protection against ADDLs afforded by memantine may likely be relevant to the therapeutic effect of this drug in AD patients (51, 71, 75).

As shown in Fig. 5*B*, when ADDL-treated neurons were depolarized with high K^+, near-maximal inhibition in IR autophosphorylation was observed. Because high K^+ can trigger Ca^{2+} influx, we tested the effects of

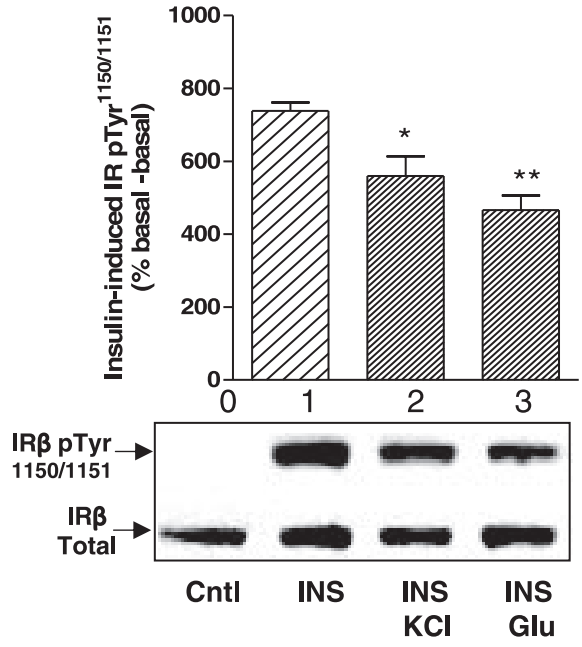

Figure 4. Effects of glutamate and depolarization on neuronal IR activity. Hippocampal cultures (18–20 DIV) were "starved" in Kreb-Ringer's buffer (KRB) for 3–4 h and treated with 100 nM insulin for 1 h. Cells were either changed to high K^+ (55 mM) KRB to depolarize neurons, or were stimulated with 100 μM glutamate, each treatment lasting for 10 min. The amount of phosphorylation of IR at Tyr1150/1151 was determined by anti-IR-pTyr1150/1151 antibody and then normalized to the total amount of IR immunoreactivity. The normalized ratios were converted to percent control. After subtracting the basal value, data were analyzed with one-way ANOVA. Both treatments caused a loss of insulin-induced pTyr at Tyr1150/1151. $*P < 0.05$; $**P < 0.01$, $n = 3$.

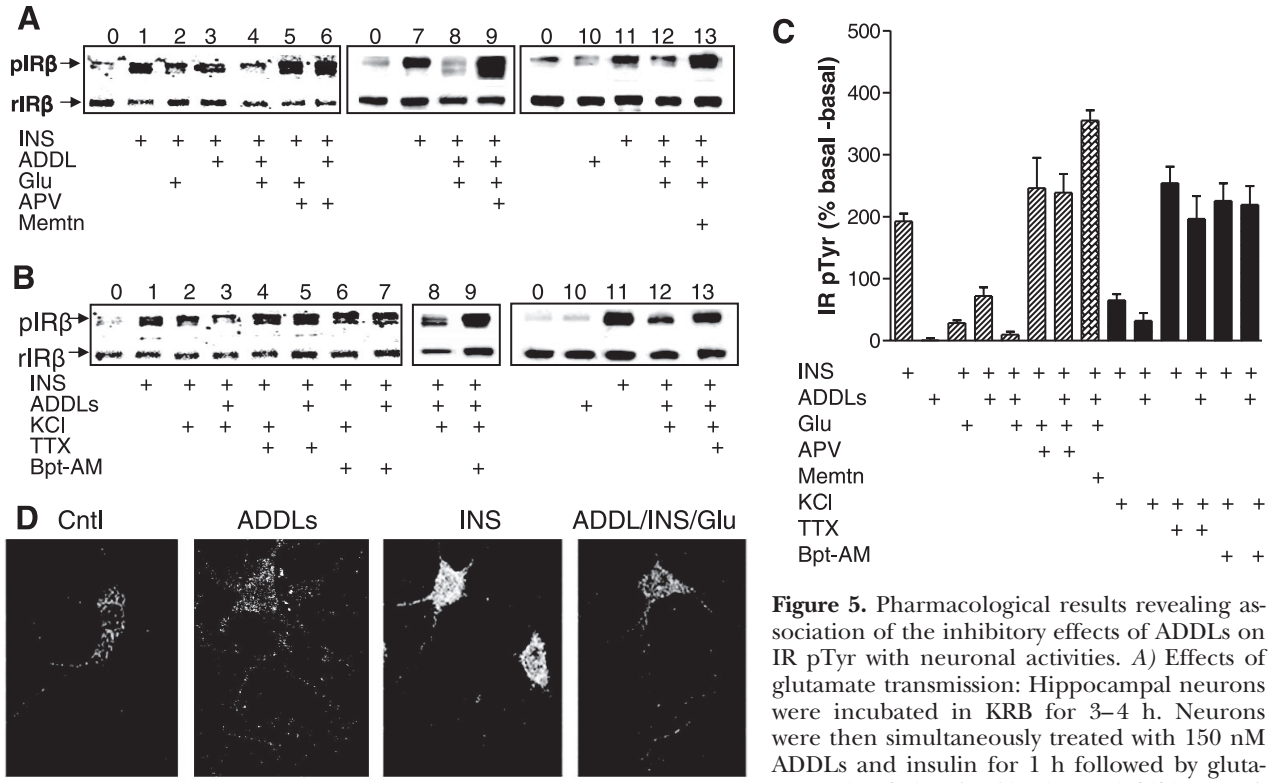

Figure 5. Pharmacological results revealing association of the inhibitory effects of ADDLs on IR pTyr with neuronal activities. *A)* Effects of glutamate transmission: Hippocampal neurons were incubated in KRB for 3–4 h. Neurons were then simultaneously treated with 150 nM ADDLs and insulin for 1 h followed by glutamate stimulation for 10 min. To inhibit NMDA receptor activities, 50 μM APV or 20 μM memantine was incubated with neurons for 30 min prior to ADDL, insulin, and glutamate treatments. IRβ-pTyr was examined using the anti-IRβ-pTyr$^{1162/1163}$ antibody (1:1000), which was normalized to the total amount of IRβ immunoreactivity. *B)* Effects of depolarization: Neurons were treated with ADDLs and insulin as described above and depolarized with high K^+ for 10 min. To block the depolarization and Ca^{2+} signals, neurons were preincubated with 20 nM TTX, or 50 μM BAPTA-AM in Ca^{2+}-free KRB for 30 min. IRβ-pTyr was examined as (*A*). *C)* Summary of pharmacological data: Semiquantification of IR pTyr extents was performed by measuring the densitometry intensities of IR pTyr under different conditions. The intensities of IR pTyr bands were normalized with those of total IR corresponding to each sample. The ratio from different treatments was converted to % control (basal). After subtracted the basal level, data reflecting normalized IR pTyr extents from several independently repeated experiments were pooled and compared with one-way ANOVA. Glu: glutamate; TTX: tetrodotoxin; Bpt-AM: BAPTA-AM. Memtn: Memantine. $^{**}P < 0.01$, $n = 3$–14. *D)* Immunocytochemical staining of IRβ-pTyr$^{1162/1163}$. Hippocampal neurons (DIV 20) were fixed with 4% formaldehyde following experimental treatments. Cells were incubated with the anti- IRβpTyr$^{1162/1163}$ antibody followed by an alexa555-labeled secondary antibody.

the cell permeable, fast Ca^{2+} chelator BAPTA-AM. Chelation of Ca^{2+} completely prevented the IR inhibition caused by ADDLs and depolarization. Blockade of action potentials by TTX also preserved IR function, suggesting that the effect of KCl and ADDLs may be mediated by the release of glutamate. The pharmacological data from several independently repeated experiments are summarized in Fig. 5*C*. Overall, the results show the important involvement of NMDA receptors in ADDL-induced impairment of IR signaling and suggest that interplay may exist between ADDLs in AD brain and regulatory factors that normally control IR activity.

Akt serine^{473} phosphorylation (Akt-pSer^{473}) is greatly enhanced by ADDLs

Inhibition of IR autophosphorylation can occur physiologically through negative feedback regulation by Akt (39). Activation of Akt kinase is triggered by insulin in a biphasic manner, first by phosphorylation at threonine 308 (pThr^{308}) and then at serine 473 (pSer^{473}). While pThr^{308} plays an important role in glucose transport and cell survival, pSer^{473} exerts potent inhibitory effects on IR activity (39, 76). In nerve cells pSer^{473} is known to be increased by activation of NMDA receptors (43, 77). High levels of Akt-pSer^{473} have been detected in diabetes-associated memory deficits in rats (78), AD (79, 80), and other neurodegenerative diseases (81, 82). Here, we tested whether increased Akt-pSer^{473} is associated with ADDL action. First, as expected, insulin progressively stimulated Akt-pSer^{473} in hippocampal cultures, with a minor response at 10 min (**Fig. 6*A***), followed by a marked increase by 60 min. ADDLs in the absence of insulin also stimulated Akt-pSer^{473}, as much as seen by insulin at 60 min. With ADDLs present, the levels of Akt-pSer^{473} were essentially maximal, as further addition of insulin for either 10 or 60 min promoted no significant increase. Confocal immunofluorescence confirmed insulin- and ADDL-induced increases in Akt-pSer^{473} in dendritic processes and soma, with Akt-pSer^{473} localized mainly

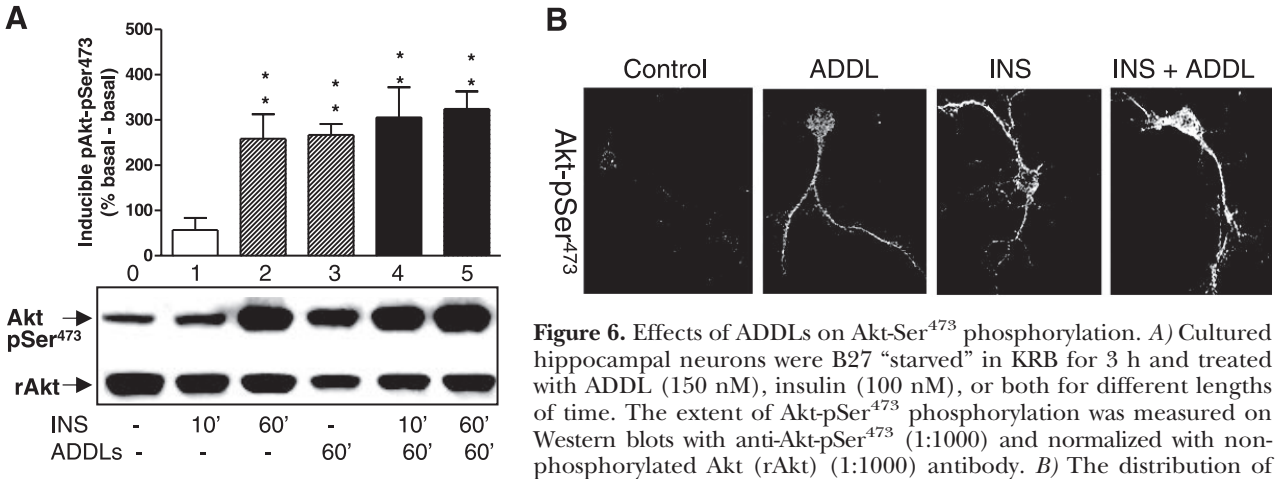

Figure 6. Effects of ADDLs on Akt-Ser^{473} phosphorylation. *A)* Cultured hippocampal neurons were B27 "starved" in KRB for 3 h and treated with ADDL (150 nM), insulin (100 nM), or both for different lengths of time. The extent of Akt-pSer^{473} phosphorylation was measured on Western blots with anti-Akt-pSer^{473} (1:1000) and normalized with non-phosphorylated Akt (rAkt) (1:1000) antibody. *B)* The distribution of Akt-pSer^{473}/rAkt under basal and insulin-stimulated conditions in the absence and presence of ADDLs was measured with fluorescent immunocytochemistry acquired with a confocal microscope.

at surface membranes (Fig. 6*B*). Membrane-localized Akt has been previously observed in response to receptor tyrosine kinase activation (83, 84). Overall, results show that ADDLs induced a large increase in Akt phosphorylation at Ser^{473}, a modification known to be associated with decreased insulin signaling and insulin resistance diseases (39, 76).

Aβ oligomers interact with a receptor complex that includes IRs

Because of the inhibition of IR autophosphorylation by ADDLs, we tested whether ADDLs might interact with a membrane receptor complex that included insulin receptors. This question was addressed first by coimmunoprecipitation experiments using mature hippocampal cultures. We treated hippocampal neurons with biotin-labeled ADDLs (bADDLs) for 30 min, lysed the cells with detergent, and then immunoprecipitated IRs with an IRβ antibody. We tested for coprecipitated bADDLs on Western blots using streptavidin conjugated with an infrared fluorescent dye (IRDye800). As shown in **Fig. 7*A***, streptavidin detected a substantial amount of bADDLs from bADDL-treated cells, as compared to control (vehicle-treated) cells. bADDL signals were observed as a set of distinct bands (Fig. 7*A*) that were identical to those obtained when lysates were immunoprecipitated using the anti-Aβ antibody 6E10 (Fig. 7*B*). Streptavidin also detected bands around 55 kDa and 28–30 kDa from control samples, which we interpret as a non-specific with the primary antibody, but no other bands were evident. These results suggest that the synaptic protein complex to which ADDLs bind (51, 56) includes insulin receptors.

Aβ oligomers inhibit insulin receptor activity in NIH3T3 cells expressing high levels of human insulin receptors

We next investigated receptor impairment by ADDLs using NIH3T3 cells stably transfected with full-length human IR or IGF-1R. The IR response was less sensitive than that found in neuronal cultures, as 100 nM ADDLs for 30 min did not affect insulin-stimulated autophosphorylation (**Fig. 8*A***). However, with longer treatments, this same dose caused significantly reduced IR activity ($P<0.01$). When the ADDL concentration was increased (from 100 to 500 nM), inhibition was evident

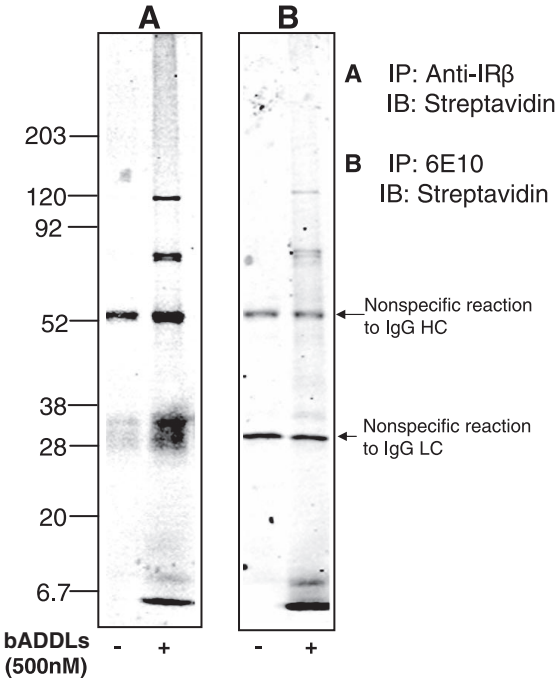

Figure 7. Coimmunoprecipitation of biotin-labeled ADDLs by IR antibody and 6E10: Hippocampal neurons were incubated with 500 nM biotin-ADDLs (bADDLs) at 37°C for 30 min. After removing the reaction medium, cells were rinsed with PBS twice and lysed in ice with cell lysis buffer. Cell lysates were subjected to immunoprecipitation by the IRβ and 6E10 antibodies, respectively. The precipitated complex was resolved on SDS-PAGE and blotted on Western blots with streptavidin conjugated with IRDye800. The fluorescent signals for bADDLs were acquired with a LI-COR Odyssey imager.

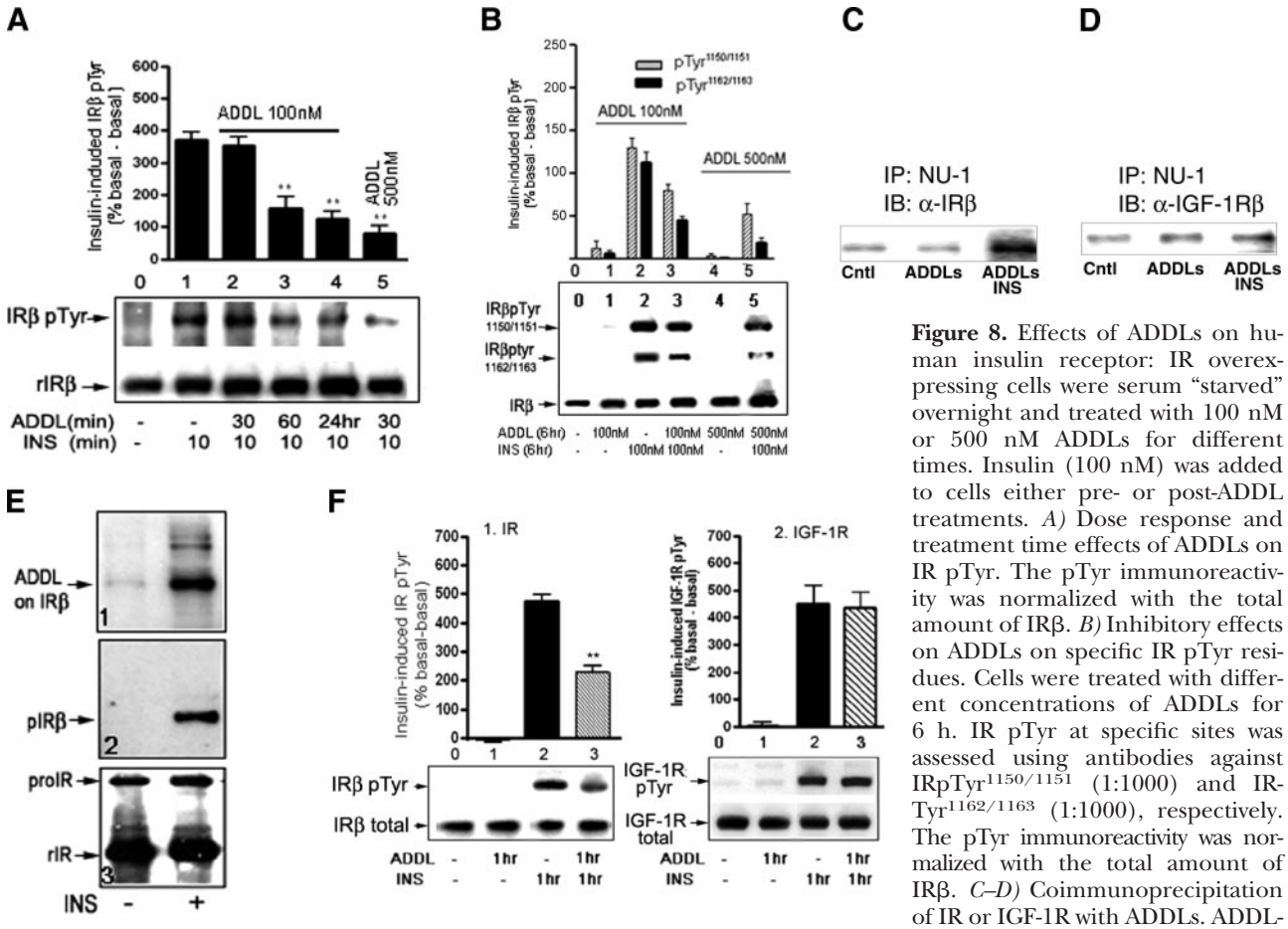

Figure 8. Effects of ADDLs on human insulin receptor: IR overexpressing cells were serum "starved" overnight and treated with 100 nM or 500 nM ADDLs for different times. Insulin (100 nM) was added to cells either pre- or post-ADDL treatments. *A)* Dose response and treatment time effects of ADDLs on IR pTyr. The pTyr immunoreactivity was normalized with the total amount of IRβ. *B)* Inhibitory effects on ADDLs on specific IR pTyr residues. Cells were treated with different concentrations of ADDLs for 6 h. IR pTyr at specific sites was assessed using antibodies against IRpTyr$^{1150/1151}$ (1:1000) and IR-Tyr$^{1162/1163}$ (1:1000), respectively. The pTyr immunoreactivity was normalized with the total amount of IRβ. *C–D)* Coimmunoprecipitation of IR or IGF-1R with ADDLs. ADDL-treated cells were subjected to precipitation by NU-1 antibody. The precipitated complex was resolved on SDS-PAGE and detected with an IR antibody (*C*) or an IGF-1R antibody (*D*). *E)* Ligand blot assay showing ADDL-IR biding. IR overexpressing cells with and without insulin stimulation were lysed and immunoprecipitated by an IRβ antibody. The precipitated IRs were resolved by SDS-PAGE and transferred to a nitrocellulose membrane. *E1)* The blot was incubated with ADDLs (100 nM), followed by blotting with NU-1 antibody. *E2)* INS-induced IR pTyr. The bound ADDLs and antibody were then stripped from the membrane, and the membrane blotted with a 4G10 antibody to visualize IRpTyr. *E3)* Total amount of precipitated IRβ. The membrane was stripped again and the total amount of IR was detected by an anti-IRβ antibody. IP: immunoprecipitation; IB: immunoblotting; proIR: proinsulin receptor; LG: low glucose. *F)* ADDLs inhibited IR pTyr, but not IGF-1R pTyr measured by immunoprecipitation. $^{*}P < 0.05$; $^{**}P < 0.01$; $n = 3$. IRβTyr: tyrosine phosphorylated IRβ; rIR: regular IR; INS: insulin.

by 10 min ($P<0.01$). In addition to total IR pTyr, we also examined the effects of ADDLs on pTyr at residues Tyr$^{1150/1151}$ or Tyr$^{1162/1163}$ within the IR active loop, again finding that, as before, ADDLs caused phosphorylation at these sites to be reduced (Fig. 8*B*).

To test the possibility that ADDLs might interact directly with human IRs expressed in the non-neuronal 3T3 cells, cultures were treated with vehicle or ADDLs in the absence or presence of insulin. In the first experiment, cell lysates were subjected to immunoprecipitation by an ADDL antibody (NU-1). The precipitated complex was blotted with IRβ antibody on Western blots to detect coprecipitated IR. As shown in Fig. 8*C*, NU-1 pulled down a substantial amount of IR from ADDL-treated cells that were stimulated by insulin. In noninsulin treated cells, the amount of IR pulled down by NU-1 was not different from vehicle-treated cell. These results suggest that interaction of ADDLs with insulin receptors in 3T3 cells may be triggered by activation of the receptor. In contrast, this insulin-induced binding phenomenon is not seen in cells overexpressing IGF-1R, in which no differences in the amount of IGF-1R pulled down by NU-1 were observed (Fig. 8*D*). ADDL binding thus is selective to a complex that contains IRs. We note that IR and IGF-1R bands were detected in the vehicle-treated cells subjected to coimmunoprecipitation, most likely due to non-specific attachment of highly abundant IR or IGF-1R from the cells to either NU-1 or to protein A/G beads during immunoprecipitation. In a second approach, we tested the possibility of direct ADDL binding to human IR using ligand blots. ADDLs showed binding to isolated IRs, but, consistent with the immunoprecipitation results, they bound only to IRs from cells that had been insulin-stimulated (Fig. 8*E1*). Controls showed these insulin-stimulated receptors were autophosphorylated (Fig. 8*E2*) and that equal levels of IRs were present in the control and insulin-stimulated preparations (Fig. 8*E3*). Finally, we tested the specificity of ADDLs for IRs compared to IGF-1 Rs in assays of receptor function. In

contrast to the marked inhibition of IRs, the autophosphorylation of IGF-1 Rs was unaffected by ADDLs (Fig. 8*F*), showing a specificity consistent with the pull-down experiments.

DISCUSSION

Our results show that Aβ oligomers (ADDLs) cause rapid and significant disruption of signaling by brain cell insulin receptors. After 30 min exposure of cultured hippocampal neurons to ADDLs, somatic IRs showed marked increases while dendritic IRs were nearly eliminated. Functional responses to insulin stimulation, measured by IR autophosphorylation, were significantly impaired. An increase in Akt-Ser^{473} phosphorylation, which has been linked to insulin resistance diseases, was also triggered by ADDL treatment. Decreased responses to insulin also were elicited by glutamate, and all decreases in response were blocked by memantine and by BAPTA, implicating excessive activity of NMDA-type glutamate receptors in the observed insulin resistance. Results suggest that the buildup of ADDLs known to occur in AD brain (45) leads to impairment of CNS insulin signaling, providing a pathophysiological mechanism to support the emerging concept that AD dementia involves CNS insulin resistance (15, 16, 18, 30, 31, 33).

Loss of dendritic hippocampal insulin receptors caused by ADDLs

Insulin receptors were found to be robustly expressed in mature cultures of hippocampal neurons, providing a CNS model well-suited for investigating cellular mechanisms of insulin resistance potentially germane to AD. Cultured hippocampal neurons have been widely used to investigate synapse cell biology (85), including pathological responses to ADDLs. ADDLs bind with specificity to particular excitatory synapses, inducing deterioration of synaptic structure, composition, and function (54, 56) while also stimulating oxidative stress and AD-type tau hyperphosphorylation (51, 53). Insulin receptors were found to occur in the dendritic arbors of all neurons. Their punctate dendritic distribution was consistent with synaptic occurrence of IRs reported for brain tissue (7) and is in harmony with ability of insulin to rapidly affect mechanisms of synaptic plasticity (10–12).

In the current experiments, hippocampal neurons responded to low-dose ADDLs with a striking loss of IRs from dendrites. Removal of receptors was essentially complete after only 30 min. By image analysis, insulin receptors were decreased 70% on dendrites with bound ADDLs compared to ADDL-free dendrites. In control cultures never exposed to ADDLs, robust IR immunoreactivity occurred on dendrites of all neurons (40 out of 40 inspected); thus, it is impossible for ADDLs to target only neurons whose dendrites lacked insulin receptors. Significantly, neurons affected by ADDLs showed elevated IR levels in their cell bodies, suggesting a rapid redistribution of IR receptors rather than net receptor loss in a short-term ADDL treatment. This conclusion is supported by Western blots, which showed no ADDL-induced changes in total IR levels (see examples in Fig. 5*A–C*). The redistribution of IRs is consistent with reports in which other proteins important for synaptic plasticity, including NMDA receptor subunits and EphB2 receptor tyrosine kinase, also show surface loss caused by soluble forms of Aβ (56, 69). Although the removal of NMDA receptors triggered by Aβ reportedly is mediated by α7 nicotinic receptors (69), the ADDL-induced loss of dendritic IRs was not prevented by the nicotinic antagonist α-bungarotoxin (data not shown). Different mechanisms thus may underlie the two responses. Dendritic IRs may be especially vulnerable to ADDL attack, as the effects are more rapid and occur at lower ADDL doses than reported for NMDA and EphB2 receptors (56). The relationship of surface protein removal to loss of key internal postsynaptic proteins such as PSD95 reported in APP transgenic mice (67) is not yet known. It has been noted that ADDLs cause the actin-binding protein drebrin to be removed from spines and accumulate in the cell bodies (56), suggesting loss of synaptic surface membrane proteins might derive from trafficking deficits associated with cytoskeleton abnormalities.

Structure of pathogenic oligomers

AD-affected brain tissue shows greatly elevated levels of Abeta oligomers compared to non-AD control brains (45). While the relevance of oligomers to AD has become widely appreciated (49, 50), important aspects of oligomer structure and pathogenic activity remain unresolved. In the current study, dendritic IR loss correlated with the presence of tightly bound ADDLs, known be gain-of-function ligands that attack particular synapses (54, 56). Ligand activity previously was determined by centrifugal ultrafiltration experiments with specific molecular weight cut-off filters to include oligomers smaller than 100 kDa but no smaller than 50 kDa. This size is consistent with the oligomeric 12 mers (54 kDa) found in AD brain (45), which, like the synthetic species, act as pathogenic synaptic ligands (51, 54). However, smaller oligomers, produced metabolically rather than chemically, exhibit extremely potent neurological impact (58–60). At low-nanomolar doses, these small oligomers, particularly trimers, inhibit LTP and memory tasks in animal models (58–60). It may be the case that the small, metabolically produced oligomers assume conformations distinct from those formed by synthetic Aβ. Whether the metabolic oligomers are recognized by the conformation-sensitive antibodies used to test synaptic binding by brain-derived ADDLs has not been reported. In general, the physical nature of Aβ oligomers is difficult to determine. Most biophysical analyses require high concentrations of peptides that lead to aggregation and structural reorganization, possibly accounting for very large

species observed in some experiments (86). On the other hand, oligomers determined to be 50–100 kDa by ultrafiltration or HPLC yield mainly tetramers, trimers, and monomers by SDS-PAGE unless manipulated by crosslinking agents (87) or exposed to biological factors (88). The uncertainties with respect to physical structure make determination of potency dependent on assumptions regarding the active species. In the current experiments, disruption of insulin receptor signaling was significant at ADDLs doses of 50 and 100 nM, normalized to the total amount of Aβ monomer. If normalized to the amount of 12 mer, the pathogenic doses would be, at most, 4 and 8 nM.

Decreased receptor responsiveness to insulin

IR responses to insulin were significantly lowered by ADDL treatment, consistent with removal of IRs from dendritic membranes. The possibility that ADDLs might directly affect IR function is consistent with previous results that kinase activity of semipurified IRs is inhibited by Aβ1–40 (89). However, the inhibitory dose of Aβ1–40 in these experiments was 50 μM, at least 1000-fold greater than used with ADDLs. Whether ADDLs directly interact with IRs is not yet clear. Because, neurons lacking ADDL binding also express insulin receptor (see Fig. 1), the insulin receptor *per se* does not constitute a high-affinity ADDL binding site. However, coimmunoprecipitation of ADDLs with IRs suggests the presence of ADDLs and IR in a complex. Preliminary experiments have shown ADDL binding can be blocked by preincubation of neurons with high doses (1μM) of insulin (data not shown), consistent with possible involvement of IRs as coreceptors. A speculation is that ADDL binding involves a heterologous receptor complex that contains IR and other coreceptors. The specific combination of the complex is not present in all neurons. Some support for direct IR-ADDL interaction comes from transfected 3T3 cells, used because they provided a cell model with very high levels of human IR. These receptors, which responded to ADDLs with decreased autophosphorylation, were found to coimmunoprecipitate with ADDLs and bind ADDLs in ligand blot assays. The interactions appeared to depend on IR tyrosine phosphorylation, as they were markedly increased by prior stimulation of cells with low doses of insulin. ADDL interactions with the IRs of 3T3 cells were relatively specific, as ADDL treatments did not result in binding to IGF-1 receptors nor affect IGF-1 receptor activity.

While the nature of ADDL binding and how it triggers loss of insulin receptors and other synaptic proteins (51, 54, 56, 69, 90, 110) remain to be elucidated, current evidence indicates a mechanism with a high degree of specificity (45, 47, 62). In hippocampal cultures, ADDLs bind only to a neuronal subpopulation, while in cerebellar cultures, there is virtually no binding (45). In synaptosomes, ADDLs bind to cortical but not cerebellar preparations (56). Within neurons, the pattern of ADDL binding is strikingly punctate. These puncta have been identified as spines of excitatory synapses (54, 56). Because binding sites are trypsin-sensitive (47), specificity of binding presumably depends on locally differentiated domains that combine particular proteins and perhaps lipids. Immunoprecipitation data (Fig. 7) indicate these domains could include insulin receptors, and insulin has been found to block ADDL binding (De Felice, unpublished). However, other components must be required for high-affinity binding, as ADDLs do not attach to all cells with insulin receptors (Fig. 1). Some of these components appear to be near or include NMDA-receptors. ADDL binding sites fractionate with postsynaptic densities (56), and an antibody against the NR1 extracellular domain completely blocks ADDL-induced ROS formation and significantly reduces ADDL binding (51). Whatever the molecular combination required for specific binding proves to be, the consequences for synaptic pathology are complex but likely integrated, as insulin, AMPA, NMDA and other synaptic receptors show important regulatory interaction (111–116).

We also found that glutamate and depolarization reduced the responsiveness of IRs to insulin, likely involving Ca^{2+} influx and activation of Ca^{2+}-dependent kinases. These effects are in accordance with existing IR regulatory mechanism, and suggest a possible physiological regulatory feedback between neuronal activity and IR signaling. The inhibition was more severe, when ADDL-treated neurons were stimulated with glutamate and depolarization, and was completely prevented by NMDA blockers memantine and APV. This finding clearly suggests a role of NMDA receptor in mediating the observed IR inhibition. NMDA receptor (NR1) dependence has also been reported in ADDL-induced ROS production (51). Association of ADDLs with NMDA receptor activity strongly suggests a mechanism dependent on elevated intracellular Ca^{2+}, perturbation of which has been implicated in the molecular pathology of Alzheimer's disease (51, 72, 91, 92). We found that chelation of Ca^{2+} with BAPTA-AM completely prevented IR inhibition caused by ADDL treatments. The basis for Ca^{2+}- dependent insulin resistance may involve pSer/pThr of the receptor by protein kinase C or cAMP-dependent kinase, a known intramolecular negative regulatory factor for IR activity (2, 74, 93–98). Protein kinase C in brain cells shows activation and membrane translocation triggered by Aβ (99, 100) and ADDLs (101).

Activation of a possible negative feedback loop: ADDL-induced phosphorylation of Akt at Ser^{473}

ADDLs also were found to affect Akt, a critical signaling molecule of the IR pathway downstream of insulin receptor substrate (IRS) and PI3 kinase. During normal insulin signaling, Akt is first activated by phosphorylation at $threonine^{308}$, which stimulates glucose transport and cell survival events (102–106). With prolonged activation, Akt becomes phosphorylated at $serine^{473}$,

which is a key event in a negative feedback loop that inhibits IR signaling. Akt responds to ADDLs with increased phosphorylation of Ser^{473}. Stimulation of Akt-pSer^{473} occurs at low levels of ADDLs and happens whether or not insulin is present, suggesting possible involvement of a pathway independent of IRs. Consistent with the action of ADDLs, stimulation of Akt-pSer^{473} in neurons is associated with NMDA receptor activation (43, 77). Persistent elevation of Akt-pSer^{473} in brain appears undesirable as abnormally enhanced Akt Ser^{473} phosphorylation is associated with memory deficits (78) and is evident in AD brain (79, 80), Huntington's disease striatal cells (81), and brains of Niemann-Pick type C (NPC) disease animal model (80). Two targets negatively regulated by Akt-pSer^{473} are IRs and PI3K, which become threonine phosphorylated (39, 107, 108). Significantly, high levels of Akt-pSer^{473} are associated with inflammation and peripheral insulin resistance diseases (2, 107, 108), suggesting the possibility that elevated Akt-pSer^{473} induced by Aβ oligomers could contribute to insulin resistance in AD-affected brain.

Pathological outcome of ADDL-induced brain insulin resistance

Our results show that ADDLs compromise insulin signaling in cultured brain cells. It is likely that ADDLs accumulating in AD brain would exert similar effects. The specific consequences of ADDL-induced brain insulin resistance with respect to cognitive function are difficult to predict given that knowledge of precise roles of CNS IRs is at present limited. Various reports have indicated memory-enhancing effects of insulin, learning-associated changes in IR pathways, and impairments of memory and LTP in diabetic animals (10–14). Although specific deletion of the brain insulin receptor reportedly causes no obvious learning and memory impairment (109), the absence of phenotype can be difficult to interpret. The receptor-deficient mice do show tau hyperphosphorylation, a major aspect of AD neuropathology (109), possibly involving dysfunction of GSK3β and downstream molecules such as mTOR and p70 S6 kinase.

Early-stage AD is notably a disease of memory dysfunction for which damage to synaptic plasticity may be of greatest consequence. The specificity of early AD for memory loss plausibly derives from the attack on particular synapses by ADDLs, acting as gain-of-function pathogenic ligands that destroy synaptic plasticity (55). Molecular-level findings presented here show that synaptic pathology induced by ADDLs includes impairment of insulin receptor signaling and suggest that insulin resistance in AD brain is a response to ADDLs, which disrupt insulin signaling and may cause a brain-specific form of diabetes. Damage to this pathway along with other plasticity-associated receptors seems likely to play an important role in the synaptic failure considered to be the mechanistic basis for the memory dysfunction of AD. FJ

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