A Direct Interaction between Transforming Growth Factor (TGF-β) and Amyloid-β Protein Affects Fibrillogenesis in a TGF-β Receptor-independent Manner*

Received for publication, April 17, 2003, and in revised form, June 30, 2003
Published, JBC Papers in Press, July 16, 2003, DOI 10.1074/jbc.M304080200

Darrell D. Mousseau†§§, Sarah Chapelsky‡§, Gregory De Crescenzo‡, Marina D. Kirkitadze***, Joanne Magoon§, Sadayuki Inoue‡‡, David B. Teplow**, and Maureen D. O’Connor-McCourt§§

From the †Cell Signaling Group, Neuropsychiatry Research Unit, Department of Psychiatry, University of Saskatchewan, Saskatoon SK S7N 5E4, Canada, the §Receptor, Signaling, and Proteomics Group, Health Sector, Biotechnology Research Institute, Montreal H4P 2R2, Canada, the **Department of Neurology, Harvard Medical School, and Center for Neurologic Diseases, Brigham & Women’s Hospital, Boston, Massachusetts 02115, and the ††Department of Anatomy and Cell Biology, McGill University, Montreal H3A 2B2, Canada

Transforming growth factor-β (TGF-β) receptor-mediated signaling has been proposed to mediate both the beneficial and deleterious roles for this cytokine in amyloid-β protein (Aβ) function. In order to assess receptor dependence of these events, we used PC12 cell cultures, which are devoid of TGF-β receptors. Surprisingly, TGF-β potentiated the neurotoxic effects of the 40-residue Aβ peptide, Aβ-(1–40), in this model suggesting that there may be a direct, receptor-independent interaction between TGF-β and Aβ-(1–40). Surface plasmon resonance confirmed that TGF-β binds with high affinity directly to Aβ-(1–40) and electron microscopy revealed that TGF-β enhances Aβ-(1–40) oligomerization. Immunohistochemical examination of mouse brain revealed that hippocampal CA1 and dentate gyrus, two regions classically associated with Aβ-mediated pathology, lack TGF-β Type I receptor immunoreactivity, thus indicating that TGF-β receptor-mediated signaling would not be favored in these regions. Our observations not only provide for a unique, receptor-independent mechanism of action for TGF-β, but also help to reconcile the interpretation of the role of TGF-β in Aβ function. These data support a critical etiological role for this mechanism in neuropathological amyloidoses.

The 39–43-mer amyloid-β (Aβ)1 peptide is derived from the membrane-bound amyloid precursor protein (APP) as an aberrant cleavage product (1). Transgenic APP mouse models exhibit age-related extracellular amyloid deposits (plaques) and neurodegeneration as well as cerebral amyloid angiopathy (CAA) comparable to that found in human Alzheimer’s disease (AD) brain (2, 3). These same models respond to both active and passive immunization against Aβ as evidenced by the reduction in levels of Aβ, the prevention and/or clearance of amyloid plaques, and the improvement in cognitive behavior (4). However, an effective preparation free of significant side effects in humans is still awaited. Indeed, clinical trials involving Aβ vaccination have been discontinued following the development of inflammation in patients brains (5). Cerebral microhemorrhaging has also been observed in similarly immunized mice (6). Although it has been suggested that antibodies capable of recognizing other Aβ epitopes or conformations should be screened (6), perhaps a closer examination of modulators of Aβ fibrillogenesis may reveal a target better suited for immunotherapy.

Among the modulators proposed to date, which include apolipoprotein E, cholesterol, and α2-macroglobulin, we were particularly interested in the cytokine transforming growth factor-β (TGF-β). The TGF-β isoform was recently implicated as a co-factor for amyloid deposition with the observation that cerebrovascular amyloid deposits, which are strikingly similar to those seen in patients with AD and CAA, and the ensuing neuropathological development are accelerated in bigenic mice overexpressing both TGF-β1 and human APP (hAPP), relative to hAPP transgenic mice controls (7). These authors suggested that cerebrovascular amyloid deposition might reflect TGF-β receptor-mediated induction of extracellular matrix deposition. Aβ binding proteins within these extracellular matrix components could enhance the formation and/or stability of Aβ fibrils (8, 9). A link between TGF-β and Aβ deposition was already being considered following the localization of TGF-β immunoreactivity to senile plaques and neurofibrillary tangle-bearing neurons in AD patient brain (7, 10). TGF-β has also been shown to enhance the formation of amyloid deposits in rats when co-injected intracerebroventricularly with Aβ-(1–40) (11) and to increase the number of Aβ plaque-like deposits in hippocampal slice culture in a subfield-selective manner (12). Finally, APP production and Aβ-(1–40)/Aβ-(1–42) processing were promoted by TGF-β1 in transgenic mice (13).

In contrast to its role as a risk factor, TGF-β1 facilitates Aβ clearance and plaque burden reduction following activation of parenchymal glial cells in TGF-β1/hAPP bigenic mouse brain (14). TGF-β has also been shown to protect neuronal cell cultures against Aβ-mediated insult (15–17) as a direct consequence of TGF-β Type II receptor activation (17).

These apparently opposing actions of TGF-β in the brain illustrate the multifunctionality of this cytokine and the dependence of its effects on the specific cellular context in which it is expressed. Additional investigation is needed to characterize the cellular and molecular processes that underlie the ef-

1 The abbreviations used are: Aβ, amyloid-β; TGF, transforming growth factor; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium.
fants of TGF-β during Aβ-mediated pathology. To date, the actions of TGF-β have centered on receptor-mediated events. In this report, we demonstrate a direct interaction between TGF-β and Aβ that promotes Aβ fibrillogenesis and neurotoxicity. Our data support a role for a unique, receptor-independent mode of action for TGF-β and define a new molecular point of intervention for inhibiting Aβ fibrillogenesis.

MATERIALS AND METHODS

TGF-β Receptor Competition Assays—Mink lung epithelial Mv1Lu cells (ATCC: CCL-64) were seeded at 2 × 10⁵ cells/ml in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cell surface receptors were cross-link-labeled with 200 μM [¹²⁵I]TGF-β1 (PerkinElmer Life Science Products) in the presence of increasing concentrations (1–50 μM) of Aβ-(1–40), Aβ-(25–35), and Aβ-(12–28) (BIOSOURCE, Camarillo, CA). Bis(sulfosuccinimidyl) suberate (Pierce) was used as the cross-linking reagent, and the receptors were analyzed by 4–11% gradient SDS-PAGE and autoradiography. Signal intensities were quantitated using the ImageQuant system (Molecular Dynamics, Sunnyvale, CA).

Circular Dichroism Spectroscopy (CD)—Aβ-(1–40) was prepared by dissolution into 50 mM phosphate buffer (pH 7.0) to yield a final concentration of 1 mg/ml. Samples were incubated at ambient temperature (22 °C) without stirring and CD measurements (at 22 °C) were performed periodically using a 0.1 cm pathlength quartz cell (Hellma, Forest Hills, NY) and an Aviv Model 62A DS spectropolarimeter (Aviv Associates, Lakewood, NJ). The scan rate was 1 nm/sec at a bandwidth of 1 nm. Three independent sets of experiments, each comprised of triplicate scans performed from 250–198 nm, were done. The buffer spectrum was subtracted from the scans and the resulting functions were smoothed. Data could not be acquired at wavelengths lower than 198 nm due to saturation of the photomultiplier. However, this range is sufficient to accurately evaluate the secondary structure state of the samples (18). Protein concentrations were determined a posteriori by quantitative amino acid analysis, thus enabling accurate calculation of molar ellipticities (θ).

Neurotoxicity Assay—Rat pheochromocytoma PC12 cells (ATCC: CRL-1721) were cultured on rat tail collagen-coated plates. Both PC12 cells and human neuroblastoma SH-SY5Y cells (ATCC: CRL-2266) were maintained in Dulbecco’s modified Eagle’s medium containing 10% horse serum (PC12) and 5% fetal bovine serum (PC12 & SH-SY5Y) at 37 °C (5% CO₂). Cell culture media was crosslink-labeled with 200 μM [¹²⁵I]TGF-β1 (PerkinElmer Life Science Products) to yield a final concentration of 1 mg/ml (Fc). Incubation was performed during several rinses of cell culture media. Cross-link-labeled samples were allowed to incubate for 3 h at 37 °C, after which samples were washed twice with phosphate-buffered saline, the sections were fixed for 1 h at room temperature with 4% formaldehyde, and then air-dried. The specimen was negatively stained with 2% uranyl acetate in water for 5 min. The sections were air-dried and imaged using a JEM-2000FX electron microscope (JEOL, Ltd., Tokyo, Japan) using an accelerating voltage of 80 kV.

Immunolocalization of TGF-β Receptors in Mouse Hippocampus—Mice (C5H/C57Bl; Charles River Laboratories) were terminated by cervical dislocation, and the brains were quickly removed, frozen in liquid nitrogen and kept at −80 °C until use. Animal maintenance and manipulation was performed according to the recommendations of our institutional ethical committees (Biotechnology Research Institute-NRC). Affinity-purified polyclonal antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) were used for localization of TGF-β RI (V-22, 2 μg/ml), RII (C-16, 1 μg/ml), and RIII (C-20, 2 μg/ml) receptors. Antibody specificity was confirmed using biosensor and Western blot analyses. Serial sagittal sections (8-μm thick) were incubated with the primary antibody in phosphate-buffered saline/1% bovine serum albumin in a moist chamber overnight at 4 °C. The specificity of the immunoreactions was tested by exclusion of the primary antibody as well as by competition, i.e. preblocking the primary antibody with the immunizing peptide (25 μM) prior to primary antibody incubation. The specificity of the immunoreactions was confirmed in our laboratory. Using PC12 cells eliminates the possibility of any event being obscured by TGF-β binding to these three receptors. We analyzed this using [¹²⁵I]TGF-β1 binding on Mv1Lu cells which express relatively high levels of these three receptors. Analysis of variance of all treatment groups revealed significant reductions in binding of [¹²⁵I]TGF-β1 to all three types of receptors, i.e. RI (F(1, 10) = 3.853, p = 0.0044), RII (F(1, 10) = 2.653, p = 0.0101) and RIII (F(1, 10) = 2.824, p = 0.0544). Post hoc analysis indicated that this effect occurred, in a dose-dependent manner, with the physiologically relevant peptide Aβ-(1–40), but not with the Aβ-(12–28) and Aβ-(25–35) fragments (Fig. 1).

RESULTS

Aβ-(1–40) Diminishes [¹²⁵I]TGF-β1 Labeling of Cell Surface Receptors—Three high affinity cell surface receptors for TGF-β have been identified, i.e. the type I, II, and III receptors (RI, RII, and RIII, respectively; Ref. 19). One approach to investigating the mechanism of action of TGF-β on Aβ-mediated effects is to determine the effect of freshly dissolved Aβ on TGF-β binding to these three receptors. We analyzed this using [¹²⁵I]TGF-β1 binding on Mv1Lu cells which express relatively high levels of these three receptors. Analysis of variance of all treatment groups revealed significant reductions in binding of [¹²⁵I]TGF-β1 to all three types of receptors, i.e. RI (F(1, 10) = 3.853, p = 0.0044), RII (F(1, 10) = 2.653, p = 0.0101) and RIII (F(1, 10) = 2.824, p = 0.0544). Post-hoc analysis indicated that this effect occurred, in a dose-dependent manner, with the physiologically relevant peptide Aβ-(1–40), but not with the Aβ-(12–28) and Aβ-(25–35) fragments (Fig. 1).

Aβ-(1–40)-Mediated Neurotoxicity Is Potentiated by TGF-β Isoforms in a Receptor-independent Manner—To examine the receptor-dependence of TGF-β on Aβ-(1–40)-mediated neurotoxicity, we chose to use cells that do not express TGF-β receptors (i.e. PC12 cells) and comparing these to cells that express all three TGF-β receptor types (i.e. SH-SY5Y) (Ref. 20 and confirmed in our laboratory). Using PC12 cells eliminates the possibility of any event being obscured by TGF-β RI receptor-mediated neuroprotection (10, 17). We first confirmed the concentration state of freshly dissolved Aβ-(1–40) using circular dichroism. Aβ underwent a well-defined random coil → β-sheet transition (Fig. 2), as expected of a species undergoing a conformational transition associated with fibril formation (18, 21). We used neutron retraction and mitochondrial conversion of the tetrazolium redox dye, MTT, as early indicators of cytotoxicity. TGF-β1 and TGF-β2 alone had no observable effect on either neutron retraction or MTT dye conversion in PC12 cells (F(2, 29) = 0.3632, p = 0.6985, Fig. 3A), as expected of cells that do not express TGF-β receptors. Also as expected, Aβ-(1–40) treatment caused neutron retraction (Fig. 3A) and inhibition of MTT dye conversion (reduced by 25% relative to untreated PC12 cells, t₀.₀₅,₂₃ = 3.426, p = 0.0023, Fig. 3B). Both TGF-β1 and TGF-β2 potentiated the cytotoxic effect of Aβ-(1–40) as evidenced by further neutron retraction (Fig. 3A) and a further reduction of MTT dye conversion (to 50% of control, F(2, 19) = 4.968, p = 0.0184, Fig. 3B). Aβ-(1–40) also induced a reduction in MTT dye conversion in SH-SY5Y cells (F(5, 22) = 24.78, p = 0.0001, Fig. 3B), however, this effect was not exacerbated by TGF-β which is expected of a cell model expressing all three
TGF-β receptor types. In fact, a marginal reversal of the effect of Aβ-(1–40) was observed during treatment with TGF-βs. These combined data demonstrate that TGF-β receptor-mediated events supersede the receptor-independent events. The reverse peptide, Aβ-(40–1), did not exert any effect on MTT reduction either alone or in combination with TGF-βs (data not shown). The extremely low ratio of TGF-βs to Aβ-(1–40) used during these experiments, i.e. a 1 to 50,000 molar ratio, supports the idea that TGF-β may have a seeding effect and emphasizes the potential physiological relevance of these observations.

TGF-β Isoforms Bind Directly to Aβ-(1–40) with Low Nanomolar Affinities—We used a Surface Plasmon Resonance-based biosensor to test for a direct physical interaction between TGF-β isoforms and Aβ peptides. The biosensor would detect mass accumulation resulting from binding of the individual TGF-β isoforms to the covalently immobilized Aβ peptides as a change in the refractive index of the surface matrix and would generate a curve recorded in arbitrary resonance units (RUs). Both TGF-β1 and TGF-β2 were observed to bind significantly to freshly dissolved Aβ-(1–40), with the binding of TGF-β2 being greater than that of TGF-β1 (Fig. 4, A and B). The extent of binding of the TGF-β isoforms to Aβ-(12–28) was significantly lower than that observed with Aβ-(1–40), while no detectable binding was observed on surfaces to which Aβ-(25–35) was immobilized. Subsequent biosensor experiments were focused on the physiologically relevant Aβ-(1–40) peptide given the overall lack of interaction of the pharmacological fragments Aβ-(25–35) and Aβ-(12–28) with TGF-β isoforms in the present biosensor study. The specificity of the interaction between injected TGF-β2 and the immobilized Aβ-(1–40) was confirmed by co-injection of the TGF-β ligand specific antibody 3C7 (Celtix Pharmaceuticals, Inc.) (Fig. 5), thus excluding the possibility that the binding to Aβ-(1–40) that we observed might be
due to a protein contaminant in the commercial TGF-β preparation.

The binding of TGF-β isoforms to freshly dissolved Aβ(1-40) was characterized in more detail using the biosensor by varying the concentration of TGF-β1 and TGF-β2. The curves (Fig. 6, A and D) clearly indicated that both TGF-β1 and TGF-β2 bound to Aβ(1-40) in a concentration-dependent manner. Fitting of these binding data using nonlinear least squares analysis and numerical integration of the differential rate equations (22) demonstrated that the binding of TGF-β1 to Aβ(1-40) could not be described well by a simple binding model (A + B → AB), as judged by the variance in the residuals.
between the calculated and experimental data (Fig. 6B). However, the data was well represented by a rearrangement model (A + B → AB → AB\(^+\)) (Fig. 5, A and C) with an apparent \(K_D\) of 60.5 ± 5.2 nM. The fitting of the rearrangement model suggests that the initial TGF-β1-Aβ-(1–40) complex undergoes a kinetically detectable rearrangement, perhaps a structural transition. This observation may point to a direct effect of TGF-β1 on Aβ-(1–40) fibril formation since Aβ-(1–40) is known to undergo a conformational transition from a predominantly random coil to \(β\)-sheet-rich form during fibrillogenesis. In the case of TGF-β2 (Fig. 6B), when the curves derived from all of the TGF-β2 concentrations were taken into account, neither the simple model (Fig. 6E) nor rearrangement model (data not shown) represented the data well. However, the lower concentration curves for TGF-β2 could be fit using a simple model, resulting in an apparent \(K_D\) of 96.1 ± 17.9 nM (Fig. 6, D and F). When the 100 and 150 nM TGF-β2 curves were predicted using the constants derived from the fitting of the lower concentration curves, the experimental curves were found to have significantly higher plateau values than the predicted curves (Fig. 6D), illustrating the complexity of the TGF-β2-Aβ-(1–40) interaction. The greater than predicted RU values at higher TGF-β2 concentrations may result from a change in density of the biosensor matrix due to the formation of aggregates or fibrils on the surface. The interaction between TGF-βs and Aβ-(1–40) is not generalized to growth factors. Indeed, this was confirmed by the absence of binding between Aβ-(1–40) and 150 nM nerve growth factor (NGF; data not shown), which, along with TGF-βs, is a member of the cystine-knot-containing superfamily of growth factors (23). Subsequent binding of TGF-β2 to the same surface confirmed the presence of covalently immobilized Aβ-(1–40) (data not shown).

**TGF-β Isoforms Promote Aβ-(1–40) Fibril Formation In Vitro**—Our biosensor studies confirmed that TGF-βs interact directly with Aβ-(1–40) and suggested a structural transition possibly affecting fibril growth. We used electron microscopy to examine the characteristics of Aβ-(1–40) fibrils formed in the absence and presence of TGF-βs. Lower magnification electron microscopy of Aβ-(1–40) alone showed occasional short strands having a gross morphology characteristic of protofibrils (21) (Fig. 7a). In contrast, protofibrils were more abundant and formed network-like assemblies in the Aβ-(1–40) + TGF-β1 (Fig. 7b) and Aβ-(1–40) + TGF-β2 (Fig. 7c) samples. No detectable structures were discerned with TGF-β1 or TGF-β2 alone (data not shown). The low ratio of TGF-βs to Aβ-(1–40) used during this experiment, i.e., a 1–1000 molar ratio, together with our biosensor data indicating a structural transition in the TGF-β-Aβ-(1–40) complex, suggest that TGF-β may enhance fibrillogenesis by generating a conformationally altered form of Aβ-(1–40) with seeding ability. Higher magnification revealed that the Aβ-(1–40) protofibrils were 2–4 nm in width and were composed of a tight helical structure with a periodicity of 2–3 nm based on the coil of 1 nm wide filaments (Fig. 7, d–f). At this magnification, the tight helical nature of the protofibrils was more evident for Aβ-(1–40) + TGF-β2 than for Aβ-(1–40) + TGF-β1, although further magnification of the Aβ-(1–40) + TGF-β1 sample (Fig. 7c, inset) confirmed the presence of loose helical structures in this sample. This magnification also revealed numerous flexible filaments 1 nm in width in the “interstrand” spaces (data not shown). Interestingly, the morphology of the 3-nm wide helical Aβ-(1–40) protofibrils that predominate in the presence of TGF-β1 and, particularly, TGF-β2 resembles that of in situ amyloid protofibrils obtained using advanced sample preparation methods such as cryofixation and freeze substitution (24).

**Mouse Hippocampal Field CA1 and Dentate Gyrus Lack TGF-β1 RI Receptors**—The hippocampus is a structure particularly vulnerable during amyloid pathology. The increases in amyloid plaque burden that occur following co-treatment of hippocampal slices with Aβ and TGF-βs (12) and in TGF-β1/ hAPP biviscenic mice (7, 14) were found to be subfield-specific, indicating the context-dependent nature of TGF-β action. Our observations indicate that TGF-β enhances Aβ-(1–40)-mediated neurotoxicity in a receptor-independent manner. We therefore examined the expression of all three TGF-β receptors within the hippocampal formation to determine if the vulnerability of particular subfields correlates with the pattern of TGF-β receptor expression. Although no TGF-β RI receptor expression was detected in field CA1 and dentate gyrus (Fig. 8A), expression was detectable, albeit weak, in the stratum pyramidale of fields CA2-CA3, while much stronger staining was found in the stratum lucidum of fields CA2-CA3 (Fig. 8C) throughout the hilus of the dentate gyrus. TGF-β RI receptors were expressed throughout the hippocampal formation particularly in the stratum pyramidale of fields CA1-CA3 and the strata moleculare and granulosum of the dentate gyrus, with sparse staining also observed in the neuropil (Fig. 8, B and D). We were unable to detect TGF-β RII receptor expression in the hippocampus. However, examination of the caudate putamen and inner granular layer of the cerebellum revealed diffuse TGF-β RII receptor staining throughout these regions confirming the ability of this antibody to immunoreact with the TGF-β RII receptor (data not shown). Experiments were performed in parallel to verify the specificity of the antibodies for the various TGF-β receptors. Competition by preadsorbtion of the antibody with the relevant immunizing peptide diminished immunostaining in all cases (data not shown).

**DISCUSSION**

The role of TGF-βs in Aβ function has been the subject of much speculation given that it apparently exerts both beneficial and deleterious effects in neuronal cells through the same high-affinity cell surface receptor system (7, 10, 14, 17). We now show that the Aβ-(1–40) peptide decreases \(^{125}\)I-TGF-β binding to all three types of TGF-β receptors. These results agree with those of Huang et al. (25) who showed that Aβ-(1–40) decreases TGF-β receptor binding but has little effect on TGF-β signaling (confirmed in our laboratory, data not shown), and those of Bodmer et al. (26) who also observed that APP does not diminish TGF-β signaling. In explanation of these results,
Huang et al. (25) hypothesized that Aβ-(1–40) acts as a classical receptor antagonist, i.e. that it interacts directly with a TGF-β receptor. We propose that these binding displacement data are more readily interpreted as resulting from ligand sequestration due to a direct interaction of ligand with Aβ-(1–40) rather than from Aβ-(1–40) interacting with a receptor. Ligand sequestration by Aβ-(1–40) would likely reduce binding to all three receptor types equally, as observed. In contrast, if Aβ-(1–40) was acting as an antagonist at a given receptor type, then it would likely interfere selectively with binding to that receptor type.

In support of our hypothesis, we provide evidence that TGF-β ligands interact directly with Aβ-(1–40) and, at seeding concentrations, enhance the formation of structures having a gross morphology characteristic of protofibrils (Figs. 6 and 7). A tightly coiled or “beaded” substructure reminiscent of the flexible, “beaded” protofibrillar intermediates characterized by Walsh et al. (21), was particularly evident when Aβ-(1–40) was coincubated with the TGF-β2 isoform, although a looser helical structure was also observed when Aβ-(1–40) was coincubated with the TGF-β1 isoform (Fig. 7). The pathological relevance of this direct physical interaction was suggested by the ability of
TGF-β Promotes Aβ Protofibrils and Neurotoxicity

Fig. 8. TGF-β RI receptors are not expressed in mouse hippocampal field CA1 and dentate gyrus. A–D, sagittal sections of brain obtained from a 10-month old female mouse. TGF-β receptors were immunolocalized using antibody V-22 (panels A and C; RI receptors) and antibody C-16 (panels B and D; RII receptors). Immunolocalization was visualized by the secondary antibody-mediated diamino-benzidine coloration. Methyl green was used as the counterstain.

TGF-β RI receptor expression was detected in fields CA2-CA3 through to the hilus (H) of the dentate gyrus (DG), but was conspicuously absent in field CA1 and the DG itself (A). In contrast, TGF-β RII receptor expression (B) was observed in all subfields of the hippocampus, i.e. CA1-CA3, hilus (H) of the dentate gyrus (DG), and subiculum (S). Higher magnification (C–D) of the CA1-C2 junction clearly revealed that TGF-β RI immunoreactivity was absent in the stratum pyramidale of CA1 (cell soma are stained with methyl green), but present in the stratum pyramidale and particularly the stratum lucidum (SL) of field CA2 (C). TGF-β RII immunolocalization demonstrated the presence of staining throughout the CA1 and CA2 (D). Similarly, TGF-β RI immunoreactivity was absent in the DG but present in field CA3 (E), whereas TGF-β RII immunoreactivity was evident in both CA3 and DG (F). Scale bars; 500 μm (A and B), 120 μm (C and D). Hip, hippocampal fissure. These results are representative of at least three separate experiments.

TGF-βs to potentiate Aβ-(1–40)-mediated neurotoxicity in TGF-β-receptor-null PC12 cell culture, but not in SH-SY5Y cell cultures which do express TGF-β receptors (Fig. 3B). This receptor-independent effect was marginally greater with TGF-β2 than with TGF-β1 (Fig. 3). Ren and colleagues (17) had previously used another TGF-β-receptor null cell culture, e.g. NT2/D1, to examine the role of TGF-β in Aβ-function. They did show conclusively that NT2/D1 cells express TGF-β type II receptors upon differentiation, which subserves the neuroprotective effect of TGF-β. However, TGF-βs did not exacerbate Aβ-induced cytotoxicity in undifferentiated NT2/D1 cells. This is easily explained by the fact that these authors made use of the Aβ-(25–35) fragment of Aβ, which does not physically interact with TGF-βs (present study) and, thus, does not allow for our proposed TGF-β receptor-independent events. The importance of TGF-β receptor-independent events is underscored by our demonstration of the low “seeding” ratios of TGF-β to Aβ-(1–40) required to promote Aβ-(1–40) protofibril formation and neurotoxicity. The accumulation and stabilization of protofibrillar populations are believed to be obligate factors in Aβ fibrillogenesis (27). In fact, recent observations suggest that the plaque deposit might simply represent the clinical end point in the Aβ cascade, with most of the neurotoxicity being mediated by smaller oligomeric/protofibrillar conformations (28–31). Several proteins have been shown to codeposit in amyloid plaques and a subset of these appear to modulate Aβ fibrillogenesis (reviewed in Ref. 32). Yet, relatively high ratios of modulator to Aβ are often required for enhancement of fibrillogenesis, and contradictory results have been obtained for the effects of several of these proteins on Aβ fibrillogenesis, putting into question the relevance of these results to the roles of these modulators in vivo (see Ref. 33).

Optimal TGF-β receptor-mediated signaling hinges on recruitment of ligand by the TGF-β RIII receptor, subsequent binding to the RII receptor, and transphosphorylation of the RI receptor, the latter event being an absolute requirement for signaling (19, 34). Our demonstration of a lack of detectable RI receptor expression in the hippocampal CA1 pyramidal layer and dentate gyrus (Fig. 8) suggests that signaling from either TGF-β1 or -β2 would not be favored in these two subfields, both of which are vulnerable during amyloid insult (30). We have also previously shown that binding of the TGF-β1 isoform to RII receptors can occur independently of RIII receptors, while binding of the TGF-β2 isoform to RII receptors appears to require RIII receptors (35). Our present demonstration of a lack of detectable TGF-β RIII receptor immunoreactivity in the hippocampus therefore suggests that TGF-β2 binding to receptors would not be favored in this structure, facilitating the interaction of this isoform of TGF-β with Aβ-(1–40). In support of the relevance of the TGF-β2-Aβ interaction to fibrillogenesis and neurotoxicity in vivo, it has been observed that the distribution of fibrillar Aβ deposits in brains from two APP transgenic mouse models (36–38) as well as the regional vulnerability of AD brains (39) parallels the regional distribution of TGF-β2 immunoreactivity in mouse (40) and rat (41) brain, i.e. frontal and entorhinal cortices and hippocampus. The fact that degenerative changes of the CA1 pyramidal layer induced by co-treatment of hippocampal slices with Aβ and TGF-βs were limited to the TGF-β2 isoform (12) further emphasizes the importance of this isoform in neurodegeneration. Taken together, these receptor localization data support the hypothesis that the direct, receptor-independent effect of TGF-β is an important component of its amyloidogenicity. This molecular mechanism does not mitigate, but rather complements, the regionally selective nature of TGF-β’s receptor-dependent effect, for example, on extracellular matrix deposition by cerebrovascular cells or on Aβ clearance by parenchymal glial cells. Our present cell culture data suggest that TGF-β receptor-mediated events would supersede the receptor-independent events. One may also speculate as to the possibility of isof orm-specific pathologies (e.g. TGF-β1 and cerebrovascular amyloid angiopathy & TGF-β2 and AD-like neurodegeneration) based on regional and/or cell-type specific TGF-β isoform and receptor expression (7, 10, 12, 14, 40, 41).

The identification of a receptor-independent interaction between TGF-βs and Aβ defines a molecular mechanism that provides for a potent amyloidogenic action of TGF-β. We suggest that a specific targeting of the Aβ-TGF-β interaction would diminish Aβ protofibril formation, which was recently suggested to be more relevant to neurotoxicity than late stage plaque formation.

Acknowledgments—We thank Drs. A. E. G. Lenferink and J. Zwaagstra for critical review of the article, S. Grothe for help with the biosensor experiments, and A. Migneault for help in preparation of the figures. We wish to thank the Electron Microscopy Center, McGill University (Montreal, Canada) for the use of its facilities.

REFERENCES

1. Selkoe, D. J. (1998) Trends Cell Biol. 8, 447–453
2. Sturchler-Pierrat, C., Abramowski, D., Duke, M., Wiederhold, K. H., Mistl, C.,
38722

TGF-β Promotes Aβ Protofibrils and Neurotoxicity

Rothacher, S., Ledermann, B., Burki, K., Frey, P., Paganetti, P. A., Wardel, C., Calhoun, M. E., Jucker, M., Probst, A., Staufenbiel, M., and Sommer, B. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 13287–13292

3. Calhoun, M. E., Burgermeister, P., Phinney, A. L., Stalder, M., Tolnay, M., Wiederhold, K. H., Abramowski, D., Sturchler-Pierrat, C., Sommer, B., Staufenbiel, M., and Jucker, M. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 14088–14093

4. Schenk, D. (2002) Nat. Rev. Neurosci. 3, 824–828

5. Haass, C. (2002) Nat. Med. 8, 1195–1196

6. Pfeifer, M., Boncristiano, S., Bondolfi, L., Stalder, A., Deller, T., Staufenbiel, M., Mathews, P. M., and Jucker, M. (2002) Science 298, 1579

7. Wyss-Coray, T., Masliah, E., Mallory, M., Conen, L., Johnson-Wood, K., Lin, C., and Mucke, L. (1997) Nature 389, 603–606

8. Gupta-Bansal, R., Frederickson, R. C., and Brunden, K. R. (1995) J. Biol. Chem. 270, 24627–24637

9. Castillo, G. M., Ngo, C., Cummings, J, Wight, T. N., and Snow, A. D. (1997) J. Biol. Chem. 272, 35388–35393

10. Flanders, K. C., Ludecke, G., Engels, S., Cissel, D. S., Roberts, A. B., Kondaiah, R. J., Cheifetz, S., Boyd, F. T., and Andres, J. L. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 824–828

11. Frautschy, S. A., Yang, F., Calderon, L., and Cole, G. M. (1996) Neurobiol. Aging 17, 311–317

12. Harris-White, M. E., Chu, T., Balverse, Z., Sigel, J. J., Flanders, K. C., and Frutschi, S. A. (1998) J. Neurosci. 18, 10366–10374

13. Lesne, S., Docagne, F., Gabriel, C., Liot, G., Lahiri, D. K., Buee, L., Flawinska, L., Delacourte, A., Mackenzie, E. T., Buisson, A., and Vivenz, D. (2003) J. Biol. Chem. 278, 18408–18418

14. Wyss-Coray, T., Lin, C., Yan, F., Yu, G. Q., Rohde, M., Conen, L., Masliah, E., and Mucke, L. (2001) Nat. Med. 7, 612–618

15. Prehn, J. H., Bindokas, V. P., Jordan, J., Galindo, M. F., Ghadge, G. D., Roos, R. P., Boise, L. H., Thompson, C. B., Krajewski, S., Reed, J. C., and Miller, R. J. (1996) Mol. Pharmacol. 49, 319–328

16. Ren, R. F., and Flanders, K. C. (1996) Brain Res. 732, 16–24

17. Ren, R. F., Hawver, D. B., Kim, R. S., and Flanders, K. C. (1997) Mol. Brain Res. 48, 315–322

18. Kirkland, M. D., Condon, M. M., and Teplow, D. B. (2001) J. Mol. Biol. 312, 1103–1119

19. Massagut, J. (2000) Nat. Rev. Mol. Cell. Biol. 1, 169–178

20. Massagut, J., Chenuetza, S., Boyd, P. T., and Andres, J. L. (1990) Ann. N. Y. Acad. Sci. 593, 59–72

21. Walsh, D. M., Hartley, D. M., Kusumoto, Y., Fezoui, Y., Condon, M. M., Lomakin, A., Benedek, G. B., Selkoe, D. J., and Teplow, D. B. (1999) J. Biol. Chem. 274, 25945–25952

22. De Crescenzo, G., Grothe, S., Lortie, R., Dehanne, M. T., and O’Connor-McCourt, M. D. (2000) Biochemistry 39, 9466–9476

23. Wiesmann, C., and de Vos, A. M. (2000) Nat. Struct. Biol. 7, 440–442

24. Inoue, S. (2001) Int. Rev. Cytol. 210, 121–161

25. Huang, S. S., Huang, P. W., Xu, J., Chen, S., Hsu, C. Y., and Huang, J. S. (1998) J. Biol. Chem. 273, 37640–37644

26. Bodner, S., Podlisny, M. B., Selkoe, D. J., Heil, I., and Fontana, A. (1990) Biochem. Biophys. Res. Commun. 171, 880–887

27. Ramirez-Alvarado, M., Merkel, J. S., and Regan, L. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 8979–8984

28. Chiu, D. H., Tanahashi, H., Ozawa, K., Ikeda, S., Checler, F., Ueda, O., Suzuki, H., Araki, W., Inoue, H., Shirotani, K., Takahashi, K., Gallyas, F., and Tabira, T. (2000) Nat. Med. 6, 560–564

29. Hartley, D. M., Walsh, D. M., Ye, C. P., Dohl, T., Vasquez, S., Vassilev, P. M., Teplow, D. B., and Selkoe, D. J. (1999) J. Neurosci. 19, 8876–8884

30. Klein W. L., Krafft G. A., and Finch C. E. (2001) Trends Neurosci. 24, 219–224

31. Kirkitadze, M. D., Bitan, G., and Teplow, D. B. (2002) J. Neurosci. Res. 69, 567–577

32. McLaurin, J., Yang, D., Yip, C. M., Fraser, P. E. (2000) J. Struct. Biol. 130, 259–270

33. Teplow, D. B. (1998) Amyloid: Int. J. Exp. Clin. Invest. 5, 121–142

34. Blobe, G. C., Schemmann, W. P., Pepin, M. C., Beauchemin, M., Moustakas, A., Lodish, H. F., and O’Connor-McCourt, M. D. (2001) J. Biol. Chem. 276, 24627–24637

35. De Crescenzo, G., Grothe, S., Zwaagstra, J., Tsai, M., and O’Connor-McCourt, M. D. (2001) J. Biol. Chem. 276, 29632–29643

36. Johnson-Wood, K., Lee, M., Motter, R., Hu, K., Gordon, G., Barbour, R., Khan, K., Gordon, M., Tan, H., Ganes, D., Lieberburg, I., Schenk, D., Seubert, P., and McConlogue, L. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1550–1555

37. Irizarry, M. C., Cheung, B. S., Rebeck, G. W., Paul, S. M., Bales, K. R., and Hyman, B. T. (2000) Acta Neuropath. (Berl.) 100, 451–458

38. Holtzman, D. M., Fagan, A. M., Mackey, B., Tenkova, T., Larue, L., Paul, S. M., Bales, K., Ashe, K. H., Irizarry, M. C., and Hyman, B. T. (2000) Ann. Neurol. 47, 739–747

39. Arnold, S. E., Hynan, L. T., Flory, J., Dasch, A. R., and Van Hoesen, G. W. (1991) Cereb. Cortex 1, 103–116

40. Flanders, K. C., Ludecke, G., Engels, S., Cissel, D. S., Roberts, A. B., Kondaiah, P., Lafyatis, R., Sporn, M. B., and Unsicker, K. (1991) Development 113, 183–191

41. Poulsen, K. T., Armanini, M. P., Klein, R. D., Hynes, M. A., Phillips, H. S., and Rosenthal, A. (1994) Neuron 13, 1245–1252
Molecular Basis of Cell and Developmental Biology: A Direct Interaction between Transforming Growth Factor (TGF)-βs and Amyloid-β Protein Affects Fibrillogenesis in a TGF-β Receptor-independent Manner

Darrell D. Mousseau, Sarah Chapelsky, Gregory De Crescenzo, Marina D. Kirkitadze, Joanne Magoon, Sadayuki Inoue, David B. Teplow and Maureen D. O'Connor-McCourt

J. Biol. Chem. 2003, 278:38715-38722.
doi: 10.1074/jbc.M304080200 originally published online July 16, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M304080200

Find articles, minireviews, Reflections and Classics on similar topics on the JBC Affinity Sites.

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 39 references, 16 of which can be accessed free at http://www.jbc.org/content/278/40/38715.full.html#ref-list-1