The Role of G Protein Activation in the Toxicity of Amyloidogenic Aβ-(1–40), Aβ-(25–35), and Bovine Calcitonin

Dawn L. Rymer and Theresa A. Good‡

From the Department of Chemical Engineering, Texas A & M University, College Station, Texas 77843-3122

More than 16 different proteins have been identified as amyloid in clinical diseases; among these, β-amyloid (Aβ) of Alzheimer's disease is the best characterized. In the present study, we performed experiments with Aβ and calcitonin, another amyloid-forming peptide, to examine the role of G protein activation in amyloid toxicity. We demonstrated that the peptides, when prepared under conditions that promoted β-sheet and amyloid fibril (or protofibril) formation, increased high affinity GTPase activity, but the nonamyloidogenic peptides had no discernible effects on GTP hydrolysis. These increases in GTPase activity were correlated to toxicity. In addition, G protein inhibitors significantly reduced the toxic effects of the amyloidogenic Aβ and calcitonin peptides. Our results further indicated that the amyloidogenic peptides significantly increased GTPase activity of purified Goα, and Goα subunits and that the effect was not receptor-mediated. Collectively, these results imply that the amyloidogenic structure, regardless of the actual peptide or protein sequence, may be sufficient to cause toxicity and that toxicity is mediated, at least partially, through G protein activation. Our abilities to manipulate G protein activity may lead to novel treatments for Alzheimer's disease and the other amyloidoses.

The amyloidoses are complex, multiform disorders characterized by the polymerization and aggregation of normally innocuous and soluble proteins or peptides into extracellular insoluble fibrils. More than 16 biochemically unique proteins, including transthyretin, α-synuclein, calcitonin, βA-macroglobulin, gelsolin, amylin, and β-amyloid, have been isolated as the fibrillar components of disease-associated amyloid deposits (1–5). These proteins share no conserved primary structural motives or other structural homologies, but their fibrils all possess some common structural features (3, 6, 7). All amyloid fibrils contain β-sheet structures in which the polypeptide chains are orthogonally aligned in the fibril directions (8–10). With Congo Red staining, amyloids show a green birefringence under polarized light, and under electron microscopy, their morphology consists of bundles of nonbranching, long filaments about 5–12 nm wide (4, 5, 11).

The most characterized amyloid-forming peptide is β-amyloid (Aβ) of Alzheimer's disease. The toxicity of Aβ has been directly linked to structure and amyloid content. In an aggregated state (containing fibrils, protofibrils, and low molecular weight intermediates), Aβ has been consistently shown to be toxic to neurons in culture (12–18). Although there is some disagreement as to the exact structure of the aggregated species associated with toxicity, whether it be a protofibril (14, 19), a diffusible, nonfibrillar ligand (20), or some other low molecular weight intermediate (19), toxicity is associated with peptide structures that are part of the aggregation pathway associated with amyloid formation. In addition, Aβ neurotoxicity has been shown to be attenuated by Congo Red and rifampicin, which bind to and selectively inhibit the formation of Aβ amyloid fibrils (21–24). Clearly, all of these observations imply a causal link between Aβ fibril formation and neurodegeneration.

Various research groups have hypothesized potential molecular mechanisms of β-amyloid toxicity, but there is no consensus. Cellular responses to Aβ that have been postulated to result in toxicity encompass destabilization of calcium homeostasis, membrane depolarization, increased vulnerability to excitotoxins, increased membrane permeability due to free radical generation, blockage or functional loss of potassium channels, and direct disruption of membrane integrity (17, 18, 25–36). The preceding plethora of observed biochemical responses to Aβ suggests that perhaps a more common, fundamental pathway is initially being activated and that this pathway subsequently diverges to produce many unique intracellular responses.

Analogous to Aβ, calcitonin is another model amyloid peptide associated with medullary carcinoma of the thyroid (37–43). The fibrils of human calcitonin have also been shown to be neurotoxic (44–46), suggesting that the amyloidoses may possess a shared mechanism of toxicity related to their secondary and macromolecular structures.

To explore if a common, fundamental mechanism of toxicity exists in the amyloidoses, we examined the structure-function relationships of several synthetic Aβ sequences, Aβ-(1–40), Aβ-(25–35), Aβ-(1–16), and bovine calcitonin. We were able to manipulate the secondary and macromolecular structures of these peptides to produce stable amyloid and nonamyloid structures. With these model systems, we demonstrated that the peptides in an amyloid state (with high β-sheet content and the ability to bind Congo Red) altered G protein activity associated with both cell membrane extracts and purified Goα subunits. We showed that the abilities of the peptides to induce GTPase activation were correlated with their toxicities, and the neurotoxicities of the peptides were attenuated by specific and non-specific GTPase inhibitors. In addition, we demonstrated that

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‡ To whom correspondence should be addressed: 337 Zachry, Dept. of Chemical Engineering, Texas A & M University, College Station, TX 77843-3122. Tel.: 979-845-3413; Fax: 979-845-6446; E-mail: tgood@tamu.edu.
significant GTPase activities were still induced even when the cell surface receptors were removed with a nonspecific protease. These results suggest that G protein activation, possibly induced via a protein-membrane interaction, plays an important role in the toxicity of Aβ and other amyloid-forming proteins.

**EXPERIMENTAL PROCEDURES**

**Materials**—Aβ-(1–40), Aβ-(25–35), and Aβ-(1–16) were purchased from BIOSOURCE International (Camarillo, CA), and bovine calcitonin was obtained from Sigma. ATP and GTP were purchased from Aldrich, and [γ-32P]GTP was from TNC Biotechnology (Irvine, CA). Supernatant and Pronase were obtained from Calbiochem and Roche Molecular Biochemicals, respectively. Cell culture reagents were purchased from Life Technologies, Inc. Purified 1,2-dimalonyl-sn-glycerol-3-phosphocholine (DPPC) and cholesterol were obtained from Avanti Polar Lipids (Alabaster, AL). The XK16/70 column and Superfine Sephadex G-50 for size exclusion were acquired from Amersham Pharmacia Biotech. Puriﬁed Gαs and Gαi subunits and epinephrine were purchased from Calbiochem. All other chemicals, unless otherwise speciﬁed, were obtained from Sigma.

**Aβ Peptide Preparation**—The Aβ peptides were prepared analogously to established methods in the toxicity and structural literature for forming β-sheet structures and for forming α-helices. The reaction was carried out by the addition of 800 μl of a 20 μM solution of 10 mg/ml were prepared by dissolving the Aβ peptides in 0.1% (v/v) trifluoroacetic acid in water. After incubating for 1 h at 25 °C, the peptide stock solutions were diluted to concentrations of 0.5 mg/ml. The cells were incubated with the SH-SY5Y and PC12 cells for 24 h, after which time MTT reduction was assessed. MTT was added to the culture medium to yield a final concentration of 0.5 mg/ml. The reaction was stopped by the addition of 800 μl of 20 μM KH2PO4 buffer (4 °C, pH 7.0) containing 5% (w/v) activated charcoal. The released 32P was separated from the nucleotide-bound phosphate by centrifugation (15000 × g, 4 °C, 20 min) prior to the addition of the peptides or controls for the GTPase assays. For the epinephrine control, 200 μM epinephrine in serum-free RPMI was incubated with the plated PC12 cells for 1 h at 4 °C. The cells were washed with a buffer consisting of 10 mM triethanolamine (TEA) and 0.5 mM creatine phosphate, 40 mM GTP, and 0.2% (w/v) bovine serum albumin in 50 mM TEA/HCl buffer (pH 7.4). Following a 5-min preincubation period at 25 °C, the reaction was initiated by the addition of 5–8 μg of membrane protein. After 15 min at 25 °C, the reaction was stopped by the addition of 800 μl of a 20 μM KH2PO4 buffer (4 °C, pH 7.0) containing 5% (w/v) activated charcoal. The released 32P was separated from the nucleotide-bound phosphate by centrifugation (15000 × g, 4 °C, 20 min), and 100 μl of the supernatant was counted on a Topcount Microplate Scintillation Counter (Packard Instrument Co.).

**Low affinity or nonspeciﬁc GTPase activity was measured by adding excess unlabeled GTP (50 μM) to the aforementioned reaction mixture and conducting the reaction as described. Speciﬁc high afﬁnity GTPase activity was calculated as the difference between the total GTPase activity in the absence of unlabeled GTP and the low afﬁnity GTPase activity.**

**Pronase Experiments**—Pronase studies were conducted analogously to established procedures (61–63). Pronase at a concentration of 3 mg/ml in serum-free RPMI was incubated with the plated PC12 cells for 1 h at 4 °C. The cells were harvested with a cell scraper and collected by centrifugation (1600 × g, 4 °C, 20 min). Then the cells were thoroughly washed with PBS and centrifuged again (1600 × g, 4 °C, 20 min) prior to the addition of the peptides or controls for the GTPase assays. For the epinephrine control, 200 μM epinephrine in serum-free RPMI was incubated with these Pronase-treated PC12 cells for 30 min prior to the membrane isolation step.

**MTT Reduction Assay**—SH-SY5Y and PC12 cell viability was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. MTT is reduced by viable cells to form blue formazan crystals, and inhibition of this reaction is indicative of cell viability. After incubation of the SH-SY5Y and PC12 cells for 24 h, after which time MTT reduction was assessed. MTT was added to the culture medium to yield a final concentration of 0.5 mg/ml. The cells were allowed to incubate with the MTT for 4 h in a CO2 incubator after which time 100 μl of a 5:2:3 N,N-dimethylformamide/SDS/water solution (pH 4.7) was added to dissolve the formed formazan crystals. After 18 h of incubation in a humidified CO2 incubator, the results were read using an Emax Microplate reader at 595 nm ( Molecular Devices, Sunnyvale, CA). Viability is reported relative to control cells unexposed to the peptides.

**GTPase and Toxicity Inhibition**—Pertussis toxin (PT) (100 ng/ml), GDPβS (600 μM), and suramin (20 μM) were incubated with the PC12 and SH-SY5Y cells for 24, 3, and 3 h, respectively, at 37 °C prior to the peptide additions for the GTPase or toxicity assays. The peptide solutions for these assays also contained the same inhibitors at the same concentrations. Control cells were treated identically except for the presence of peptide.

**Reconstitution of Goα and Goi Vesicles**—Vesicles consisted of 82% (w/w) DPPC and 18% (w/w) cholesterol, and they were prepared by mixing the DPPC and cholesterol in chloroform and evaporating off the solvent under nitrogen in 50 °C. The PT was added at 1 μg/ml. The lipid film, producing a final lipid concentration of 1 mg/ml. The resulting DPPC/cholesterol suspension was sonicated for 10 min. Subsequently, 1.2 volumes of these DPPC/cholesterol vesicles were combined with 0.8 volume of Goα or Goi in a 10 mM NaHepes buffer (pH 8.0)
containing 1 mM EDTA, 0.1 mM dithiothreitol, and 0.1% (v/v) Genapol. This mixture was gel-filtered using an ÄKTA Explorer (Amersham Pharmacia Biotech) with Sephadex G-50 in a XK16/70 column at a flow rate of 0.5 ml/min according to procedure of Pedersen and Ross (65). The elution buffer consisted of 20 mM NaHepes buffer (pH 8.0), 1 mM EDTA, 0.1 mM dithiothreitol, 0.1 M NaCl, and 2 mM MgCl2.

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**RESULTS**

**Peptide Secondary and Macromolecular Structures**—The structures of the Aβ peptides under the employed solvation conditions have been well characterized and were not re-examined. Under these conditions, Aβ-(1–40) and Aβ-(25–35) have been shown to be amyloidogenic (containing fibrils and protofibrils) and to contain extensive β-sheet structures, whereas Aβ-(1–16) has been demonstrated to be nonamyloidogenic and predominantly random coil (47–50).

Because the solution structures of bovine calcitonin are not as well documented, we identified conditions that promoted the formation of β-sheet structure and amyloid using CD spectroscopy and Congo Red binding assays. As determined by CD (Fig. 1A), both 40 and 80 μM bovine calcitonin in water containing 5 mM CaCl2 and 1 mM MgCl2 adopted structures with −55 ± 10% β-sheet and only 15 ± 10% α-helix. Incubating the peptide in deionized water alone at these same concentrations (Fig. 1A) produced structures devoid of β-sheet character with 95 ± 10% α-helical contents. As depicted by Congo Red difference spectra (Fig. 1B), the 40 and 80 μM water solutions of bovine calcitonin with 5 mM CaCl2 and 1 mM MgCl2 significantly bound and increased the high affinity GTP hydrolysis. *, **, and *** indicate that the increases in the rates of hydrolysis relative to the untreated control cells were significant at p < 0.002, p < 0.0005, and p < 0.0001, respectively.

**GTPase Activity**—By using Aβ-(1–40), Aβ-(25–35), and bovine calcitonin in water with 5 mM CaCl2 and 1 mM MgCl2 as models of peptides with amyloidogenic structures and Aβ-(1–16) and bovine calcitonin in deionized water as models of peptides without amyloidogenic structures, we examined the relationship between peptide structure and GTPase activity. We found that the rate of high affinity GTP hydrolysis in PC12...
membranes increased by 31 ± 12% on average with exposure to the peptides containing extensive β-sheet and amyloid contents relative to the rate of hydrolysis of control cells unexposed to peptides (Fig. 2A). In all cases, the increases in GTPase activity were significant relative to the control cells (p < 0.001). The rates of GTP hydrolysis were significantly greater for Aβ-(25–35) and 80 μM calcitonin (p < 0.001), the two peptides with the greatest amyloid content, than for Aβ-(1–40) and 40 μM calcitonin, suggesting that the extent of macromolecular structure influences the process. Similarly, Aβ-(1–16) and bovine calcitonin in deionized water, the peptides devoid of amyloid and β-sheet structures, did not significantly alter the GTPase activities of the PC12 cells relative to untreated controls (Fig. 2A) (p > 0.2).

To ensure that the observed phenomena were not isolated to PC12 cells, we examined the GTPase activities of SH-SY5Y membranes exposed to bovine calcitonin. We found similar trends to those observed with the PC12 cells (Fig. 2B). The rate of GTP hydrolysis increased from 16.0 ± 0.5 pmol/mg/min for the control cells to 23.8 ± 0.6 and 18.7 ± 0.6 pmol/mg/min for the cells exposed to 80 and 40 μM bovine calcitonin in water with 5 mM CaCl₂ and 1 mM MgCl₂, respectively (p < 0.002). The nonamyloidogenic 80 μM calcitonin in water did not significantly alter GTPase activity relative to the controls (16.0 ± 0.3 pmol/mg/min, p > 0.4).

Toxicity—In parallel to the GTPase activity experiments, we examined the relationship between peptide structure and toxicity using our model peptides. As seen in Fig. 3, A and B, analogous to our GTPase results, we found that exposure to the peptides containing extensive β-sheet and amyloid contents resulted in significant PC12 (Fig. 3A) and SH-SY5Y (Fig. 3B) cell toxicity (p < 0.001). Conversely, Aβ-(1–16) and bovine calcitonin in deionized water, the peptides devoid of amyloid and β-sheet structures, did not significantly alter cell viability relative to untreated controls (p > 0.2).

GTPase Inhibition—To identify the family or families of G proteins activated by the amyloid-forming peptides, we investigated the effects of GDPβS and suramin, two nonspecific G

**FIG. 3.** The toxicities of the Aβ and calcitonin peptides as a function of their solution structures. The abilities of the PC12 (A) and SH-SY5Y (B) cells to reduce MTT were taken as indications of cell viability. The data are reported as the percentage of the MTT reduced by the cells untreated with these solutions. The means ± S.D. of 8–10 determinations are depicted. The Aβ-(1–40), Aβ-(25–35), and calcitonin/salt solutions, which contained substantial amyloid contents and β-sheet structures, always significantly inhibited the ability of the cells to reduce MTT (*, p < 0.001), but the nonamyloidogenic bovine calcitonin in deionized water and Aβ(1–16) did not significantly alter cell viability (p > 0.2).
protein inhibitors (66–73), and PT, a specific inhibitor of the G_{i} and G_{o} families of G proteins (74–78). As illustrated in Fig. 4A, GDPβS, suramin, and PT were each able to inhibit significantly the increases in GTP hydrolysis observed in PC12 membranes exposed to the amyloidogenic Aβ-(1–40), Aβ-(25–35), and bovine calcitonin (p < 0.005). Analogously, the inhibitors significantly reduced the rate of GTP hydrolysis induced by amyloidogenic calcitonin in membranes from SH-SY5Y cells (Fig. 4B, p < 0.001).

**Pronase Experiments**—To ascertain if the peptide-induced increases in GTPase activity were mediated through something other than a peptide-receptor interaction, GTPase studies were performed with PC12 cells treated with Pronase. Pronase is a nonspecific protease that has been documented to remove cell surface receptors (61–63). As shown in Fig. 5, GTP hydrolysis still increased significantly in the presence of the amyloidogenic bovine calcitonin, Aβ-(1–40), and Aβ-(25–35) relative to control cells treated with Pronase but unexposed to the peptides (p < 0.003). The rates of GTP hydrolysis for the Pronase-treated cells incubated with Aβ-(1–40), Aβ-(25–35), and 80 μM bovine calcitonin in water with divalent cations were increased by 9, 17, and 25%, respectively, compared with the Pronase-treated control cells. We also examined the GTP hydrolysis of Pronase-treated cells with the nonamyloidogenic Aβ-(1–16) and bovine calcitonin in deionized water (Fig. 5). As expected, these peptides did not significantly alter GTPase activity relative to the control cells (p > 0.15).

To demonstrate the effectiveness of Pronase treatment at receptor removal, we incubated cells with 200 μM epinephrine and then measured the rate of GTP hydrolysis. Without Pronase treatment, 200 μM epinephrine increased the rate of GTP hydrolysis in PC12 cells by 100% (data not shown). However, as seen in Fig. 5, after Pronase removal of cell receptors, incubation with epinephrine did not significantly alter the rate of GTP hydrolysis in the Pronase-treated cells relative to Pronase-treated control cells (p > 0.3). These results indicate that the cell receptors had been effectively removed by the Pronase.

**G_{o}i and G_{o}o GTPase Assays**—To demonstrate more specifically that the peptides were interacting with heterotrimeric G proteins, we performed GTPase assays with lipid vesicles containing purified Gα_{o} and Gα_{i} subunits (Fig. 6, A and B). Analogous to the cell membrane GTP results, the amyloidogenic Aβ-(1–40), Aβ-(25–35), and 80 μM bovine calcitonin significantly increased GTPase activity in both the Gα_{i} and Gα_{o} vesicles, but the nonamyloidogenic 20 μM Aβ-(1–16) (Δ) and 80 μM bovine calcitonin in deionized water (■) did not significantly affect GTPase activity in either vesicle system at any of the experimental times. Hydrolysis in the absence of vesicles is also shown (▼). The data presented are averages of triplicate determinations that varied by less than 10%, and the standard deviations of the determinations are indicated by error bars where significant. The zero time, zero protein values represent [32P]GTP contaminating the [γ-32P]GTP.
exposure to the amyloid-forming peptides led to a significant reduction in cell survival relative to the control cells (p<0.0005) in PC12 (A) and SH-SY5Y cells (B). Untreated PC12 and SH-SY5Y cells were exposed to the peptides for 24 h at 37 °C in a humidified 5% CO2 environment in the absence of all pharmacological agents. Treated PC12 or SH-SY5Y cells were preincubated with 100 ng/ml PT, 20 μM suramin, or 600 μM GDPβS for 24, 3, and 3 h, respectively, at 37 °C in a humidified 5% CO2 environment. Following this preincubation, treated cells were then exposed to amyloidogenic peptide solutions containing the same inhibitors for 24 h at 37 °C in a humidified 5% CO2 environment. The data are reported as the percentage of the MTT reduced by the cells incubated with the peptides alone or with the peptides and inhibitors relative to the MTT reduced by control cells unexposed to the peptides. The means ± S.D. of 8 determinations are presented. In the absence of pharmacological reagents, exposure to the amyloid-forming peptides led to a significant reduction in cell survival relative to the control cells (p<0.0001). Incubation of the PC12 and SH-SY5Y cells with PT (open bars), suramin (diagonally striped bars), and GDPβS (cross-hatched bars) protected them from amyloid-induced cell death relative to untreated cells exposed to the amyloids (solid bars). Incubation of the cells with inhibitors in the absence of the amyloid peptides had no significant effect on cell viability (Control Cells). * and ** indicate that the increases in cell viability relative to the untreated cells exposed to the amyloids were significant at p<0.0005 and p<0.0001, respectively.

**FIG. 7.** The influence of pharmacological agents on cell viability as measured by MTT reduction. The inhibition of G protein activation with PT, GDPβS, and suramin attenuated the cell death caused by the amyloidogenic bovine calcitonin, Aβ(25–35), and Aβ(1–40) in PC12 (A) and SH-SY5Y cells (B). Untreated PC12 and SH-SY5Y cells were exposed to the peptides for 24 h at 37 °C in a humidified 5% CO2 environment. Following this preincubation, treated cells were then exposed to amyloidogenic peptide solutions containing the same inhibitors for 24 h at 37 °C in a humidified 5% CO2 environment. The data are reported as the percentage of the MTT reduced by the cells incubated with the peptides alone or with the peptides and inhibitors relative to the MTT reduced by control cells unexposed to the peptides. The means ± S.D. of 8 determinations are presented. In the absence of pharmacological reagents, exposure to the amyloid-forming peptides led to a significant reduction in cell survival relative to the control cells (p<0.0001). Incubation of the PC12 and SH-SY5Y cells with PT (open bars), suramin (diagonally striped bars), and GDPβS (cross-hatched bars) protected them from amyloid-induced cell death relative to untreated cells exposed to the amyloids (solid bars). Incubation of the cells with inhibitors in the absence of the amyloid peptides had no significant effect on cell viability (Control Cells). * and ** indicate that the increases in cell viability relative to the untreated cells exposed to the amyloids were significant at p<0.0005 and p<0.0001, respectively.

**DISCUSSION**

In previous cell culture studies, the most important predictor of Aβ toxicity was the macromolecular state of the Aβ peptides with only aged or aggregated Aβ peptides (including fibrils, protofibrils, and/or low molecular weight intermediates) consistently eliciting toxic responses (12–18). Additionally, both the L- and D-enantiomers of Aβ exhibited nearly identical structural characteristics and induced similar levels of toxicity, implying that Aβ neurotoxicity was mediated by Aβ fibril features instead of any stereoisomer-specific interactions (79). Compounds such as Congo Red, rifampicin, and recognition peptides that bind to and/or inhibit the formation of amyloid fibrils have also been shown to attenuate the toxicity of Aβ, further establishing the causal link between Aβ structure and function (21–24, 64, 80, 81).

Since the neurotoxic effects of Aβ peptides appear to be linked to peptide structures associated with aggregation and amyloid fibril formation, any plausible molecular mechanism of Aβ toxicity should also demonstrate structural dependence. Our cell membrane and purified subunit data, which suggest that the Aβ toxicity is mediated through a pathway involving peptide-induced G protein activation, possess the required structural specificity. We found that the GTPase activities associated with PC12 membranes and Goα and Goi vesicles only increased with exposure to the peptides containing extensive β-sheet and amyloid contents, Aβ(1–40) and Aβ(25–35). The nontoxic and nonaggregated Aβ(1–16) produced no discernible effects on the GTPase activities of the PC12 membranes or the Goα and Goi vesicles.

Ample evidence exists that suggests that G protein activation and other signal transduction events such as phospholipase D and adenylyl cyclase activation may be associated with the biological activity of Aβ (82–84). Also, the reported changes in K+ and Ca2+ ion channel activity and changes in calcium homeostasis are all consistent with GTPase activation being an early event in the mechanism of action of Aβ (26, 30, 85, 86). However, in very few of these studies has anyone shown the relationship between the biological activity and the structure of the peptide, which is essential in establishing the connection between activity and a toxicity mechanism.

Unlike our results, reports of Aβ-induced harin conductances in N1E-115 neuroblastoma cells (87) and calcium fluxes (85, 88) did not correlate aggregation state of the peptide with activity. When Aβ blockage of the fast-inactivating K+ current was investigated, structure-function relationships were examined, but no structure dependence upon ion channel activity was observed (30). In addition, the data associated with the free radical model of Aβ toxicity such as the ability of Aβ...
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GTPase activity in Pronase-treated cells and G

mediation may also influence aggregation and channel modulations (89–93). Our results do not preclude the result of multiple GTPases, which could result in tremendously diverse intracellular phenomena since G proteins and their effectors have been associated with selective protein phosphorylation, gene transcription, cytoskeletal reorganization, secretion, and membrane depolarization (71, 74, 92, 94–96).

In both our Pronase experiments and in our G

study, few receptors remained, as evidenced by the absence of an epinephrine-induced increase in GTP hydrolysis. Similarly, no receptors were included in the G

subunit preparations. However, in both systems, significant peptide-induced increases in GTPase activity were observed. Non-receptor mediated GTPase activation has been documented with mastoparan, ethanol, and shear stress (59, 97–99). The discovery that our 

Aβ GTPase activities may be membrane-mediated is consistent with a number of other previous findings, which imply the importance of Aβ-membrane interactions to neurotoxicity. Aβ has been shown to interact with the lipid bilayer of the plasma membrane, forming cation-selective channels and disrupting ion homeostasis (25, 26). Interaction with the plasma membrane may also influence aggregation and amyloid formation of the peptide (33, 34, 100, 101).

In conjunction with our Aβ GTPase results, our calcitonin experiments suggest that the amyloidosis may share some common steps in the mechanism of toxicity. Analogous to our Aβ findings, we found that amyloidogenic bovine calcitonin in water containing 5 mM CaCl

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and 1 mM MgCl

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increased cell toxicity and increased GTPase activities associated with PC12 membranes and G

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vesicles, whereas the nonamyloidogenic calcitonin in denized water did not significantly alter cell viability or G protein activities of PC12 membranes or G

and G

vesicles. Amyloidogenic bovine calcitonin increased the GTPase activity in Pronase-treated cells and G

and G

vesicles devoid of receptors, and inhibition of GTPase activation attenuated cell toxicity. These results again suggest the potential importance of protein-membrane interactions to amyloid-mediated toxicity and indicate that GTPase activation is an early step in the toxicity of amyloid-forming peptides.

In conclusion, we demonstrated that Aβ and calcitonin peptides altered G protein activity in a structure-specific manner; only the peptides with extensive β-sheet and amyloid contents significantly increased GTPase activity. Both G

and G

activation was observed. At least some of the observed amyloid-induced increases in GTPase activity were not receptor-mediated, pointing to the potential importance of peptide-membrane interactions in the biological activity of the peptides. We demonstrated that the observed increases in G protein activity were linked to neurotoxicity by showing that the amyloidogenic Aβ and calcitonin peptides. These results may help to elucidate the mechanism of toxicity and may lead to novel treatments for the 16 or more diseases associated with amyloid proteins.

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peptides to generate EPR signals were not structure-specific (31). We suggest that amyloidogenic Aβ-induced G protein activation could be an early step in the molecular level mechanism of Aβ toxicity and that activation of alternative G protein pathways could produce many of the observed diverse cellular responses. For example, in our studies, PT had significant inhibitory effects on GTPase activity and toxicity, indicating that the Go

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families of G proteins were being activated (74–78). The results of our purified Go

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subunit GTPase assays confirm this hypothesis. The amyloidogenic Aβ (1–40) and Aβ (25–35) significantly increased the GTPase activity of both the Go

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subunits. Activation of these particular families of G proteins could account for some of the previous ion channel results because they have been linked to certain K

+ and Ca

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+ channel modulations (89–93). Our results do not preclude the result of multiple GTPases, which could result in tremendously diverse intracellular phenomena since G proteins and their effectors have been associated with selective protein phosphorylation, gene transcription, cytoskeletal reorganization, secretion, and membrane depolarization (71, 74, 92, 94–96).

In both our Pronase experiments and in our Go

and Go

subunit experiments, we demonstrated that the presence of receptors was not necessary for the observed amyloidogenic peptide-induced increases in GTPase activity. In the Pronase-treated cells, few receptors remained, as evidenced by the absence of an epinephrine-induced increase in GTP hydrolysis. Similarly, no receptors were included in the Go

and Go

subunit preparations. However, in both systems, significant peptide-induced increases in GTPase activity were observed. Non-receptor mediated GTPase activation has been documented with mastoparan, ethanol, and shear stress (59, 97–99). The discovery that our Aβ GTPase activities may be membrane-mediated is consistent with a number of other previous findings, which imply the importance of Aβ-membrane interactions to neurotoxicity. Aβ has been shown to interact with the lipid bilayer of the plasma membrane, forming cation-selective channels and disrupting ion homeostasis (25, 26). Interaction with the plasma membrane may also influence aggregation and amyloid formation of the peptide (33, 34, 100, 101).

In conclusion, we demonstrated that Aβ and calcitonin peptides altered G protein activity in a structure-specific manner; only the peptides with extensive β-sheet and amyloid contents significantly increased GTPase activity. Both G and G activation was observed. At least some of the observed amyloid-induced increases in GTPase activity were not receptor-mediated, pointing to the potential importance of peptide-membrane interactions in the biological activity of the peptides. We demonstrated that the observed increases in G protein activity were linked to neurotoxicity by showing that the amyloidogenic Aβ and calcitonin peptides. These results may help to elucidate the mechanism of toxicity and may lead to novel treatments for the 16 or more diseases associated with amyloid proteins.

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Dawn L. Rymer and Theresa A. Good

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