

The Anti-estrogenic Activity of Alzheimer's Amyloid- Associated Peptides: Potential Effects on Hormone Replacement Therapy. A Review and Commentary

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Abstract

Alzheimer's disorder (AD) is an affliction in the advanced age population that affects memory and cognition loss in patients. Due to loss of estrogen hormones during menopause, estrogen replacement therapy (ERT) came into clinical use in the last decade for women diagnosed with AD. However, clinical treatments declined due to unfavorable cardiac side effects. More recent studies have now shown that ERT can be neuroprotective for AD when timed at the onset of menopause. Due to the many physiological activities of estrogen in the brain, ERT has once again gained clinical prominence for AD female patients. In the present report, the process of cell amyloidosis is first addressed followed by description of the structure and function of the Beta amyloid peptide (Abeta) fragments. This was followed by a analysis of the biochemical composition of the Abeta peptide sub fragments including their hydrophobicity/hydrophilicity profiles and amino acid sequences. Finally, the Abeta peptides were compared to a similar positive control peptide in a rodent uterine assay to determine whether Abeta and sub fragments were capable of inhibiting-growth in estrogen-sensitive cells and tissues (i.e. brain). This is the first study to propose that Alzheimer-derived peptide fragments could possibly interfere and/or hinder the effect of estrogen stimulated growth on targeted cells and tissues (hence, ERT) in the brain.

Keywords

Peptides; Beta Amyloid; Alzheimer's; Uterine Inhibition; Amyloidosis; Estrogen-Mediated Growth

Introduction

General

Alzheimer's disorder (AD) is an advanced age affliction of memory loss and/or dementia that can diminish a person's ability to perform normal life functions regarding, work, family, and societal activities. The U.S. Statistic Database has revealed that nearly 5.7 million individuals now have some clinical form of AD [1]. The average illness duration of AD is 8 years following diagnosis; however, it can extend up to 20 years in some patients. Although memory loss is the major characteristic of AD, other changes can include mood changes, sleep effects, speech difficulties, disorientation, struggle to formulate words and/or sentences, unnecessary suspicions and fears, and cognitive decline [2]. Interestingly, two-thirds of AD cases are females compared to only third in male patients. Menopause, which can range from 45 to 55 years of age in women, causes a notable reduction in estrogen and progesterone hormone levels [3]. It has been proposed that estrogen reduction and loss were major contributing factors in the onset and course of AD in perimenopausal patients [4]. One of the causative agents in AD pathophysiology is the beta Amyloid (Abeta) peptide, a toxic peptide.

The role of estrogen (estradiol, E2) in AD induced dementia and cognitive decline in aging women has recently gained renewed clinical research interest during the last decade; this followed a decline in clinical literature reports [5]. The reduction in biomedical clinical studies resulted from published reports that heart disorders were associated with Estrogen Replacement Therapy (ERT) in postmenopausal women diagnosed with AD [6]. In contrast, recent results have now shown that the timing of ERT administration at the onset of menopause rather than postmenopausal, could prevent or delay the decrease in memory loss and cognition in women with AD [7]. Following menopause, estrogen levels appear to be lower in females than in aged-matched male patients. Estrogens synthesized in the postmenopausal period in women are limited to production in the cal cells and adrenal cortex cells [8].

Alpha-fetoprotein and Estrogen Activity

Alpha-fetoprotein is a tumor-associated fetal protein produced during pregnancy.

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Interestingly, AFP in newborn rodents was found to protect the rodent brain against excess estrogen levels that can induce androgenization and masculinization [9]. The presence of high levels of AFP in the postnatal rodent brain is thought to influence developmental neuron circuit patterning necessary for mating behavior; such cells/tissues in the brain are regulated by estrogen [10]. AFP has also been employed as the protein of origin for producing of non-toxic synthetic therapeutic peptides (34 AA length and its fragments) to study their anti-estrogenic activities on growth both *in vitro* and *in vivo* models [11]. In comparison to the toxic Abeta peptides, the non-toxic AFP-derived peptides similarly function to block the action of E2 on estrogen-sensitive brain cell growth responses [10,11].

Objectives of the Present Study

As an objective, it was deemed of importance to study the effects of toxic Abeta peptides on estrogen-induced tissues in parallel with non-toxic AFP control peptides (of similar length) using *in vivo* animal models of estrogen-stimulated growth. From an opposite standpoint, such studies would be directed at the effect of Abeta peptides on E2-sensitive cells rather than the effect of E2 on Abeta-affected cells. In light of the proposed neuroprotective effects of estrogen on the Abeta peptide in AD patients, the present study sought to determine the effects of Abeta peptides on non-brain cells. Studies on the effects of A beta peptides on non-brain cells are presently absent in the biomedical literature. Furthermore, there are few if any studies of Abeta peptides directly affecting the function of a reproductive organ, such as the uterus, ovary, mammary gland, or testis, except in ovariectomy studies [12]. In contrast, studies of AFP-derived peptides have been extensively reported concerning their anti-estrogenic activity in rodent reproductive models. Thus, it was determined to assay and compare the effects of toxic Abeta peptides to an established activity of non-toxic AFP-peptide in inhibiting the estrogen growth response in the immature rodent uteri. It is further germane to this report that the gene for the Alzheimer Amyloid Beta-Protein Precursor (APP) has recently been detected in the rat testis and appears to play a role in reproductive cellular differentiation and morphologic changes of the sperm [13]. Finally, it is of further interest that the process of amyloidosis in breast cancer can induce malignant cells to enter a dormant or resting state devoid of cell division, growth, and proliferation [14]. Thus, cancer cells have been reported to undergo growth cessation after entering a dormant state following accumulation of large aggregations of amyloid fibrils within the cancer cell cytoplasm. This would explain the low incidence of breast cancer in women with Alzheimer's disorder [14].

What is Amyloidosis (ALD) and What is its Function?

Amyloid (A) Bodies: A-bodies are insoluble proteinaceous fibrous aggregates formed from cross- β polymerization of β -pleated sheets [15]. The aggregation of A-bodies leads to fibril formations (10 nm fibrils) and to toxic plaque deposits in brain cells. Therefore, amyloid proteins play a central role in the pathogenesis of AD [16] due to the formation and presence of A-bodies resulting from ALD. Such deposits occur in other human neuropathies, such as Parkinson's and Huntington's disease. All these disorders exhibit an unfolded amyloid-protein structure, rather than the natural (tertiary) protein fold. Such strictures are subject to the Unfolded Protein Response (UPR) pathway, a cellular stress response to misfolded or unfolded protein [17]. This latter pathway is associated with heat-shock and glucose-shock proteins produced within the endoplasmic reticulum.

ALD is a by-product of cytoplasmic polypeptide assembly, and A-bodies are a highly organized form of protein aggregation that converts native-fold proteins into β -sheet rich aggregates that are protease K and sodium dodecyl sulfate resistant multimers [14]. Interestingly, A-body peptides are amphipathic, can bind various metals, such as iron, zinc, copper, and cobalt, and can function as cell-membrane disrupters and channel formers [18-20]. These chelated metal proteins not only disrupt cell membranes, but aid in penetrating cell membranes, binding to receptors, and inducing the formation of endocytic vesicles [21]. Metal binding to the amyloid-

peptide sequence is due to the positioning of two histidine residues that aid in producing a tetrahedral symmetrical formation [22]. In the physiological state, the resultant protein fibrils are nontoxic; moreover, the A-body formations are reversible by means of protein chaperone interactions employing HSP70, HSP90, and GRP78 shock-induced disaggregation agents [23,24].

Contrary to popular belief, (ALD) is a common occurrence in eukaryotic cells [14]. A recent report further elucidated ALD as a natural physiological response in mammalian cells responding to multiple stress stimulations [25]. The ALD process enables cells to aggregate protein as fibrils for storage, thereby facilitating adaptation to cellular stresses. As such, ALD enables cells to store large quantities of fibrillary proteins and enter a dormant or resting state, while still remaining viable during extended periods of stimulation from extracellular stressors. Eukaryotic cells frequently encounter environmental stress factors, such as inflammation, hypoxia, high temperatures, H_2O_2 peroxidation, acidosis, pH extremes, oxidative stresses, and other conditions associated with growth dysregulation [24,25]. For example, high extracellular and/or cytoplasmic temperatures activate both the heat/glucose shock response and the UPR. Expression of the chaperone proteins (HSP70/90, GRP78) enables the cell to reduce the total cell volume of misfolded proteins by refolding proteins back to their native folded state [23]. In a similar fashion, transcription factors can activate several genes that respond to hypoxic environments, augmenting oxygen delivery and increasing glucose metabolism during low-oxygen periods [25]. Whatever the environmental stress stimulation may be, both the stress response and the UPR are designed to aid in: 1) restoring cell homeostasis, 2) repairing cell/molecular damage, and 3) sustaining cell viability in situations of environmental stress encounters [25].

Amyloid-converting motif: In the normal physiological response to stress described above, cells induce an amyloid state in their cytoplasmic proteins by invoking and activating a discrete peptide 30-42 stretch of amino acids (AAs) on proteins termed the "Amyloid-Converting Motif" (ACM) [26]. Studies show that the AA-sequence stretch of the ACM is crucial in converting cell proteins into A-bodies by interacting with a nuclear ribosomal intergenic spacer noncoding RNA (rIgS RNA). For example, rIgS RNA interacting with the toxic β -amyloid peptide (1-42 AA-sequence stretch) is directly involved with inducing plaque formation in cells of AD patients; such patients exhibit an ACM-like sequence in the process of initiating amyloidogenesis *in vivo* [26]. Therefore, the ACM comprises peptide sequences derived from proteins and can be divided into two distinct sub motifs consisting of an arginine/histidine (R/H)-rich sequence and a highly amyloidogenic AA sequence that binds Congo red, thioflavin, and Amylo-Glo dyes. The latter domain displays AA (single-letter code: (X=any AA) di- and tripeptide clusters, such as KXL, LXX, GXG, and GXL/I, as well as $HX_{5-9}H$, the latter of which lies within or adjacent to the R/H-rich areas on the peptide [25,26]. Such ACM sequences are found in one to three distinct regions on many diverse proteins, such as CDK1, residues 100-130; HAT1, residues 228-260; HDAC2, residues 1-33; pVHL, residues 104-140; APP, residues 1-42; α_2M , residues 1,314-1,365; and ApoE; residues 200-299 [27].

ABeta42 is a Cell Membrane Fusion Peptide: Cell membrane fusion peptides are cell membrane disrupting molecules that can insert and form channels in the bilipid layer leaflets of the plasma membrane [28]. Examples in nature of such peptides include: Viral peptides, Calcein, Diphtheria toxin fragments, γ -hemolysins, leucocidins, and the β -amyloid (Abeta-42) peptides. Such Linear Peptides can insert into bilipid cell membrane in a tilted oblique position (70° angle), which perturbs the cell membrane bilayer [29]. The peptides induce lipid destabilization by influencing the cholesterol and acidic phospholipid compartmental organization to form large conformational spaces. Membrane permeability (insertion) of the peptide can fill and occupy these open spaces and induce vesicle fusion with the membrane bilayer which disrupts the lamellar leaf organization of the phospholipids. Lipid destabilization provides the peptides with the ability to adopt multi-stable positions

in the presence of lipids followed by the formation of peptide aggregates at or within the cell membrane bilayer [30]. Studies of peptidic fusogenic agents have documented that the process requires only 1.0 minute accomplishing fusion and permeabilization into the cell membrane. The peptides are then able to translocate through the membrane having access to wide conformational spaces (channel) induced in the deep lipid core of the second bilayer. Lipids in 5 to 50 excess molar ratios to peptides are known to induce striking increases in the diameter of liposome particles exposed to fusogenic peptides [31]. Finally, since biological membranes are heterogeneous, locally high peptide concentrations can transiently occur, and such peptide concentrations can be detected under physiological conditions *in vivo*.

Structure Comparison of the Abeta Peptide to AFP-Derived Peptide:

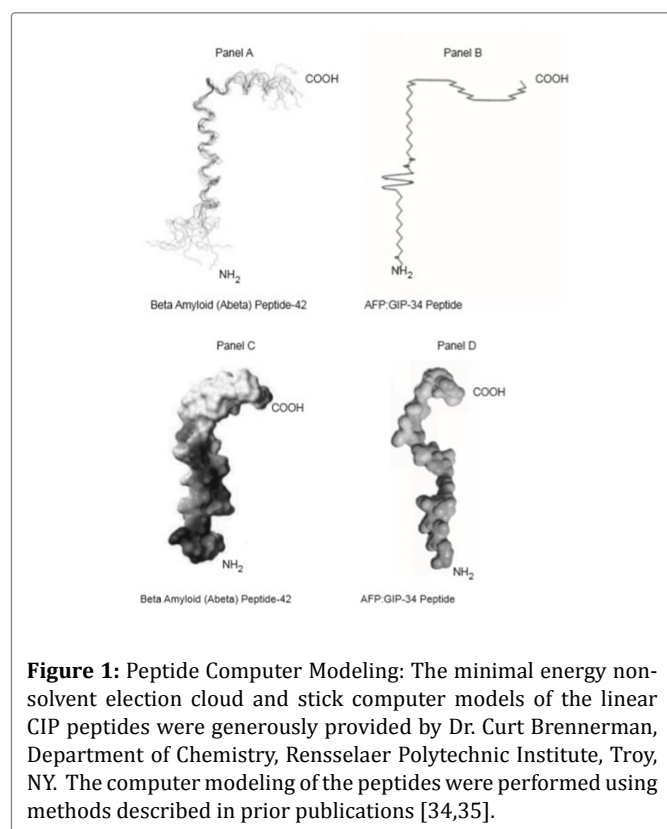
Biochemistry of Abeta Peptide Fragments: Progressive neurodegenerative diseases such as Alzheimer's disorder (AD) and Parkinson's disease involve amyloidosis as described above, in which insoluble toxic protein fibers are deposited in cell/tissues which impair their function [32]. Aggregated amyloid fibers in AD are produced as intracellular, proteinaceous deposits which exhibit a cross-beta sheet and beta turn secondary structure (Figure 1); such fibers are identified by a green birefringence when stained with Congo Red and increased fluorescence when complexed with thioflavin [15]. Beta-amyloid peptides (Abeta) are cleaved by specific secretase enzymes form a larger transmembrane amyloid precursor protein (APP), and are found deposited in the brain of patients suffering from AD; such plaques are linked to neurotoxicity [34]. The transmembrane APPs are cleaved by beta and gamma-secretase enzymes within the plasma membrane bilayer generating various Amino Acid (AA) length amyloid peptides with a 42 AA sequence peptide being the most toxic. Additional Abeta peptides include a 40 AA residue and a 25-35 residue in addition to the more numerous 42 AA fragments. It is the accumulation, aggregation and localization of the cleaved peptide segments within the cell plasma membrane lipid bilayer that contributes to neuronal death. Interestingly, various fragments of the Abeta peptide (residues 1-28, 25-35, 38-42) show biophysical and biochemical properties similar to the full length 42

AA peptide [34]. It has also been reported that metal ion binding (Cu^{2+} , Fe^{2+} , Ni^{++} and Zn^{2+}) is implicated in modulating the Abeta peptide-to-plasma membrane interactions involving membrane phosphatidylcholine and phosphatidylserine in the course of lipid bilayer disruptions [18-20,35]. The amino-acid coordination of the divalent cation metal binding involves the double histidine residues present either in tandem or close juxtaposition at AA positions 13-14 of the 42 AA peptide [22]. Both enhanced deposition of aggregated Abeta and the generation of Reactive Oxygen Species (ROS) are thought to be consequences following coordination of metal ion binding since Abeta 1-42 have a very high affinity for Cu^{++} and Zn^{++} at His13 and His14. Thus, the histidine clusters in the Abeta segment have been found to constitute high affinity sites for binding of Abeta peptides on immobilized metal chelates columns [19]. It is interesting that, the AFP-derived GIP-34 control peptide can also bind copper & zinc [22]. In addition to metal binding, the Abeta peptide can complex to alpha 2-macroglobulin for cellular uptake, and demonstrates high affinity binding to both the alpha-7-nicotinic acetylcholine brain cell surface receptor and to the carboxy-terminal domain of serum apolipoprotein-E.

Abeta and GIP-34 AA Sequences Composition: The Abeta toxic peptide has a 42-amino acid (AA) sequence comprised of the following AA single letter codes: DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA [36]. The 42-mer Abeta peptide contains 19 hydrophobic amino acids, 8 intermediate amino acids, and 15 hydrophilic amino-acids, making it an amphipathic peptide with multiple dipolar ions (Zwitterions) (Table 1). In comparison, the peptide AA sequence for the 36-mer AFP-derived synthesized peptide has been determined to be LSEDKLLACGEGAADIIGHLICIRHEMTPVNPVGVN (GIP-34) composed of residues 447-480 of the α -fetoprotein full length polypeptide [22]. In comparison to Abeta-42, the 36-mer GIP-peptide contains 16 hydrophobic amino acids, 7 intermediate amino acids, and 11 hydrophilic amino acids, also making it an amphipathic peptide. The 42-AA Abeta contains slightly more hydrophobic and hydrophilic AAs than does the GIP-36. However, the Abeta-42 also contains 6 more total AAs than the GIP-36. In nature, the amphipathic peptides have an advantage in that they bind either positive or negative charged surfaces or cell membranes. A structural image comparison of Abeta-42 and GIP-34 revealed that the 2 peptides show similar configurations as displayed in the minimal energy computer models (Figure 1).

Amino Acid Sequence Matches: An amino acid sequence match was performed to search for amyloid-associated protein matches to the AFP-derived peptide (Table 2). The GIP-36 amino acid sequence was subjected to a FASTA search in the Genbank (GCG Wisconsin Program) database. FASTA employs a Z-based statistics algorithm in order to demonstrate identities and similarities (relationship) between protein and/or peptide amino acid sequences. The GCG search found identity/similarity sequence matches of GIP-36 to that of Alzheimer's-associated proteins such as serum Amyloid-beta, acetyl cholinesterase (Ache) receptors and co-transporters. Other matches for Amyloid-associated proteins to GIP-36 included apolipoprotein-E, muscarinic Ache, and tissue plasminogen. These amino-acid matches provide evidence that the GIP-36 peptides contain short recognition cassettes comparable to the AA sequences of Amyloid-like peptides and related AD proteins. The Ache receptor matches further lend credence to the proposal that AFP-derived peptides may be structurally-related to the Abeta peptides involving cholinergic functions [4].

Physiologic Roles of Estrogen in Humans: Estrogens such as E2 are known to play important roles in female reproductive activities as observed in both *in vitro* and *in vivo* studies [12]. Such reports have demonstrated that E2 modulates multiple physiological effects in various areas of the brain. For example, E2 can activate basal cholinergic functions related to memory and learning in brain regions such as the hippocampus and neocortical forebrain cells. In comparison, a consistent decrease in cholinergic activity



Amino Acid *	Hydrophobicity Index	Abeta Peptide		Growth Inhibitory Peptide	
		Number of AA	Totals	Number of AA	Totals
Ile (I)	+4.5	3	+13.5	4	+18.0
Val (V)	+4.2	6	+25.2	2	+8.4
Leu (L)	+3.8	2	+7.6	4	+15.2
Phe (F)	+2.8	3	+8.4	0	0
Cys (C)	+2.5	0	0	2	+5.0
Met (M)	+1.9	1	+1.9	1	+1.9
Ala (A)	+1.8	4	+7.2	3	+5.4
Totals	+21.5	19	+63.8	16	+53.9

I: Hydrophobic Amino Acids

Gly (G)	-0.4	6	-2.4	5	-2.0
Thr (T)	-0.7	0	0	1	-0.7
Ser (S)	-0.8	2	-1.6	1	-0.8
Trp (W)	-0.9	0	0	0	0
Totals	-2.8	8	-4.0	7	-3.5

II: Intermediate Amino Acids

Tyr (Y)	-1.3	1	-1.3	0	0
Pro(P)	-1.6	0	0	2	-3.2
His (H)	-3.2	3	-9.6	2	-6.4
Gln (Q)	-3.5	1	-3.5	1	-3.5
Asn (N)	-3.5	1	-3.5	1	-3.5
Glu (E)	-3.5	3	-10.5	2	-7.0
Asp (D)	-3.5	3	-10.5	1	-3.5
Lys (L)	-3.9	2	-7.8	1	3.9
Arg (R)	-4.5	1	-4.5	1	-4.5
Totals	-28.5	15	-51.2	11	-35.5

III: Hydrophilic Amino Acids

*Single Letter Amino Acid Code

Table 1: A hydrophobicity index list is presented for the amino acids present on the Beta-Amyloid (Abeta) peptide versus the alpha-fetoprotein growth inhibitory peptide (GIP)

is observed in these same brain areas in AD female patients. E2 further affects functions in signaling molecules, transduction agents, neurotransmitters, and in proteins such as apolipoprotein-E, and choline acetyltransferase (Table 2); E2 can also affect activities such as Abeta protein deposition, oxidative stress, and cognitive functioning. Mechanism of such E2 functions involve activities such as: 1) anti-angiogenesis; 2) mitochondrial anti-oxidative effects; 3) neuronal cell dendritic outgrowths; and 4) neurotransmitter-associated cognition. It is noteworthy that the E2 receptors (ER α , ER β) are expressed on glial and neuron cells in the neocortex and hippocampus of the brain [39]. Neurons present in-such brain areas are specifically involved in cognitive activities in forebrain cholinergic neurons of the nucleus basal are; such activities include memory, attention, and cognitive encoding. The alpha and beta E2 receptors themselves are encoded on different chromosomes, and are expressed in affected areas in the brains of AD patients [40]. Furthermore, the E2 receptor beta was found to promote Abeta degradation via autophagy [41]. Finally, E2 can also reduce the neuronal generation of Abeta peptides [42].

As discussed above, ERT is best administered to women approaching menopause in order to replace hormones (estrogen, progesterone) levels that are gradually being reduced. ERTs are used to dampen the physiological effects in menopausal women undergoing hot flashes, night sweats, and bone loss (osteoporosis). The hormone treatments were utilized to protect or delay the onset of AD and other dementias [43,44]. Thus, ERTs are meant to protect against the loss of sex steroid hormones and could benefit women with lowered E2 blood levels. Thus, the timing of HRT at or near the onset of menopause is now deemed critical for treatment efficacy. Beneficial follow-up effects have even been observed in women as long as 10 years following the HRT regimen. E2s can be administered by oral pills, and transdermal skin patches [45]. The interests in the

use of HRT before or near the onset of menopause have now been re-vitalized with increased clinical adaptations. However, the anti-estrogenic effects on Abeta peptides on potential E2-sensitive brain cells could affect E2 dose levels administered during HRT.

Estrogen Functions in AD Animal Models: It has been found that physiological levels of E2 reduce the generation of Abeta peptide in cultured human neuroblastoma cells and in primary cultures of rat, mouse, and human embryonic cerebrocortical neurons [45]. Moreover, brain Abeta peptides (1-40 and 1-42) in ovariectomized rats were increased nearly two-fold compared to controls; concomitantly, uterine atrophy was accompanied by decreased serum E2 levels [12]. Estradiol was also found to block the intracellular calcium changes in neurons following interaction with Abeta peptides [47]. The estrogen neuroprotection against Abeta toxicity was found to require expression of cell membrane forms of alpha-and/or beta Estrogen Receptor (ER) followed by activation of the MAP kinase pathway [48]. Studies in transgenic mice and guinea pigs suggested that the mechanisms involved in the generation and/or the clearance of Abeta [1-42] were the preferential targets for the estrogen protective action [49,50]. Other mechanisms of E2 neuro protection have included: 1) the previously discussed antioxidant effects of E2; 2) activation of neuroprotective transcription genes; and 3) cross talk among various intracellular signaling cascades [51]. Overall, the neuro protection by estrogen against Abeta toxicity signifies that the mammalian brain contains estrogen-sensitive tissues that are still subject to Abeta neurotoxicity following estrogen reduction. It is further known that the brain is highly sensitive to estrogen stimulation via various feedback systems such as the pituitary-hypothalamic axis and surrounding areas of several brain nuclei regions.

AFP-derived GIP-34	L	S	E	D	K	L	L	A	C	G	E	G	A	A	D	I	I	I	G	H	L	C	I	R	H	E	M	T	P	V	N	P	G	V	G	Q	Iden (%) Sim (%)	Total (%) Iden. Plus Sim
Serum Amyloid-P	L	S	E	Q	R	L	L																													71/29 (7)	100	
Human Ache	L	S	E	D	C	L	Y																													57/29 (7)	86	
Xenopus Ache Receptor	L	S	G	D	K	L	L																													85/0 (7)	85	
Actinidin Cysteine Protease	L	S	E	Q	E	L	I	G	C	G	G																									55/27 (11)	82	
Human Apolipoprot-E	L	S	A	G	K	L	Q	C	G	E	N	X	S	E																						47/33 (15)	80	
Human Amyloid-βeta					K	L	V	F	F	A	E	G	X	A	-	-	I	I	G	X	L	M	V													47/26 (19)	73	
Mouse transthyretin					K	L	L	I	C	G	E	P																								75/0 (8)	75	
Rat Muscarinic Ache Receptor									C	G	G	P	E	A	A	V	T	V	G	S	A	C	A	G	H	E	X	W	P	A	-	P	G	A	A	42/23 (26)	65	
Human tissue plasminogen activator																	I	I	I	Y	H	L	C	I	I	Y										70/0 (10)	75	
Mouse acetyl- cholinesterase									C	G	N	G	P	S	R	R	I	R	A	L	D	C	I	R	L	G	R	K	S	G	A	S	G	V	G	37/15 (27)	52	
Baboon APOE																						C	I	R	N	L	L	T	P	S	R					60/10 (10)	70	
Rat Notch-2																								H	E	M	Q	P	L	R	P	G	A			60/20 (10)	80	
Ves. Ache Transporter																									E	M	M	P	D	T	P	G				63/25 (8)	88	
Sheep Prion Protein																																P	G	V	G	N	80/20 (5)	100

Table 2: GIP-34 Peptide VS Amyloid and Associated Proteins: An Amino Acid (AA) Matching Computer Search

Iden = identity; Sim = similarity; () = number of amino acid in sequence;

Ache = acetyl cholinesterase; apolipoprot. = apolipoprotein-E (APO-E)

Ves = vesicular; AFP = alpha-fetoprotein

The Estrogen-stimulated Uterine Assay

Estradiol-induced growth assay in the immature rodent uterus

A published immature mouse uterine bioassay was presently used to measure the growth inhibitory properties of HAFP peptides in E2 stimulated cell growth [52]. A rat uterine E2-stimulated growth assay, employing E2 administered to 16-18 day rat pups was adapted from the method of Butterstein et al. [53] PeptideGIP-36had been previously reported to suppress estradiol-induced growth in the immature mouse uterus [54]. The GIP peptides previously inhibited (45-51%) E2-stimulated growth in the mouse uterus using 100 ng to 1000 ng peptide per mouse; specifically, GIP-36 and its fragments GIP-12 andGIP-8 significantly suppressed E2-induced uterine growth. These data serve to document the sensitivity and specificity of GIP-36 peptide as an inhibitor of E2-simulated growth in the immature mouse uterus.

Abeta and AFP Peptide Effects on E2-Induced Growth

The non-toxic AFP peptide GIP-36 and the toxic Abeta peptides 42-mer were studied in parallel estrogen induced immature rodent uterine *in vivo* assays. The inhibition of E2-induced growth by AFP-derived peptides was comparable in both mouse and rat assays producing growth inhibitions of21%.to 43%. Clearly, the best growth inhibitions were attained using linear GIP-36 (36 -39 %) and the sub fragment 8-mer GIP (43%). The GIP-14 hydrophobic midpiece, which can bind heavy metal, displayed only about 21 % inhibitory potency in both rodent assays. Interestingly, the addition of zinc to the assay solution slightly enhanced the GIP-36and sub fragments inhibitory potency of E2 induced uterine growth (not shown).

The Estrogen Induced Rat Uterine Growth Assays for Abeta and GIP

A rat version of the mouse uterine growth assay was used to study a comparison of the anti-growth estrogen activity of the AFP peptide versus the Abeta peptide. The immature rat uterine growth assay was useful for assessing agents that interfere with estrogen-stimulated uterine growth over a 24 hr period. The toxic Abeta peptides, which included segments 25-35, 1-40, and 1-42, (Sigma Chemicals), were then subjected to the estrogen-induced immature rat uterine *in vivo* assay. As shown in (Table 3), all Abeta peptide preparations showed E2-growth inhibitory activities from31 to 41 %. That is, the two-fold growth induced by E2 on uterine growth was notably blunted by all toxic amyloid and non-toxic GIP peptides. The 25-35 mer Abeta segment peptide demonstrated the lesser inhibitory activity at 31% comparable to the non-toxic AFP-derived peptides (29 -39 % inhibition). In comparison, the most toxic amyloid peptides of 1-40 and 1-42 segments showed similar or slightly higher inhibitory potency compared to the AFP-derived positive control peptides.

Discussion

The effect of E2 depletion at menopause appears to clinically hasten the onset ofAD regarding memory loss and dementia in female patients [44]. This event can be offset by HRT, especially employing estrogen (E2). AS shown above, estrogen-stimulated cell activation in critical areas of the brain has been localized to the hippocampus and neocortical forebrain neurons regarding cholinergic activities. Such areas are known to be directly involved in memory, attention, and cognitive functions. Understanding the physiologic process of amyloidosis and amyloid body formation has revealed that these actions are normal physiologic processes gone away. In this report it was discussed that peptides/proteins can be converted into

I. Treatment Group	Mouse		
	N#	Ut/BW Ratio	% Inhibition
Saline CONTROL	16	1.00	ZERO
E2 STIMULATION	18	1.76	NA
Cyclic GIP-34	15	1.59	22
Linear GIP-34	16	1.49	36
Linear GIP-12	14	1.54	29
Linear GIP-14	12	1.60	21
Linear GIP-8	11	1.43	43

II. Treatment Group	Rat		
	N#	Ut/BW Ratio	% Growth Inhibition
Saline CONTROL	8	0.49	ZERO
E2 STIMULATION	14	1.00	NA
Cyclic GIP-34		0.84	31
Linear GIP-34	12	0.80	39
Linear GIP-12	0	ND	ND
Linear GIP-14	8.	0.85	29
Linear GIP-8	0	ND	ND

III. Treatment Group	Rat		
	N#	Ut/BW Ratio	% Growth Inhibition
Saline CONTROL	8	0.49	ZERO
E2 STIMULATION	10	1.11	NA
Abeta 1-42	6	0.86	40
Abeta 1-40	5	0.85	41
Abeta 25-35	7	0.92	31

Table 3: An assessment of anti-estrogenic activity utilizing Abeta and AFP peptides performed in Rodent Immature Uterine Assays*, **

N# = number of animals utilized

NA = non-applicable;

Ut = uterine; BW = body weight

*previously unpublished data in collaboration with Prof. George Butterstein, Union College, Biology Dept., Schenectady, NY (personal consent communication).

** Percent Inhibition = $100 \times (E - EP) / (E - C)$, (See Ref # 54), where E and EP are the mean uterine

Weight (mg/.G) to body weight X 1000) in the groups given E2 and E2 + Peptide, respectively, and

C is the mean for saline injected controls.

amyloid forms by an RNA translated code into protein AA segments by an amyloid-converting motif AA sequence. Non-AD afflicted cells normally employ the process of amyloidosis for protein storage within cells. However, if over-stressed and non-regulated, amyloid-like proteins can give rise to peptide fragments amounts toxic to their host cells [16,17]. In lieu of recent clinical studies promoting ERT at the onset as opposed to the termination of menopause, present findings have now focused on the potential effects of E2 on brain cell activities involving cholinergic memory loss. However, data from the present report indicates that the Abeta peptide-42, and its sub fragments can themselves block the action of E2 - induced growth on estrogen sensitive cells. In a reverse paradigm, attention in the present report has been directed on whether Abeta peptides themselves can suppress steroid hormone action on E2-sensitive cell responses in cells of animal models. Such studies could have implications which might extend to AD human brain cells in patients receiving HRT.

The observations in the present report indicate that the AD induced Abeta peptides can suppress estrogen-induced growth in a

non-brain animal models. The Abeta peptide is cleaved by secretase enzymes from a Transmembrane (TM) segment from an APP precursor molecule. The TM segments of proteins contain sequences of strongly hydrophobic AAs segments, which are capable of plasma membrane disruption leading to cell toxicity and subsequent apoptosis. Indeed, the structure and function of the toxic Abeta peptides are similar and resemble those of the non-toxic AFP-derived peptides (GIP-34) described herein; such observations justify the use of GIP as an assay positive control peptide. Due to their amphipathic AA structure, these peptides are capable of perturbing the outer bilayer of cell membranes and penetrating into the cell interior by pore/channel formation at the plasma membrane. It is tempting to speculate that the toxicity of Abeta versus the non-toxicity of GIP-34 might be explained by the ratio of hydrophobic versus hydrophilic amino acids in their peptide sequences. Nonetheless, both peptide fragments are capable of inhibiting estrogen-stimulated cell growth in animal models, an action similar to anti-microbial pore-forming peptides [30,55]. It is interesting that Abeta peptides have been reported to be cytotoxic causing subsequent apoptosis while the GIP-36 peptide is cytostatic and produces no observable side effects [56]. The antiestrogenic effect displayed by Abeta in the present report may partially explain why early premenopausal ERTs (low levels of Abeta peptide), are more effective than in late postmenopausal ERT. Finally, the antiestrogenic cell growth effect on target cells/tissues (i.e. cancer cells) described herein may further explain why women with AD experience less breast cancer occurrence than in non-breast cancer patients with AD [14]. This phenomenon might be linked to multiple research reports that AFP-derived GIP inhibits growth of estrogen-sensitive breast cancer cells in pre-clinical studies [14]. It would be of interest that AD-associated Abeta peptides could possess such anticancer growth properties as well. Two such reports in the literature demonstrated that certain Abeta peptides could inhibit cancer growth via an anti-angiogenic mechanism [57,58]. However, Abeta peptides cell toxicity other than in cancer cells might impede their potential use as anti-cancer therapeutic agents. Nonetheless, the "take home" message in the present study is that Abeta peptides can exhibit anti-estrogenic activities.

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Conflicts of Interest

The author declares that there are no known conflicts of interest in the preparation of this manuscript.

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