Biochimica et Biophysica Acta 1832 (2013) 439–444

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Leptin regulates amyloid β production via the γ-secretase complex

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ARTICLE INFO

Article history:
Received 9 October 2012
Received in revised form 17 December 2012
Accepted 19 December 2012
Available online 26 December 2012

Keywords:
Leptin
Presenilin
γ-secretase
β-amyloid
Alzheimer's disease

ABSTRACT

Alzheimer's disease (AD) is the most common age-related neurodegenerative disease, affecting an estimated 5.3 million people in the United States. While many factors likely contribute to AD progression, it is widely accepted that AD is driven by the accumulation of β-amyloid (Aβ), a small, fibrillogenic peptide generated by the sequential proteolysis of the amyloid precursor protein by the β- and γ-secretases. Though the underlying causes of Aβ accumulation in sporadic AD are myriad, it is clear that lifestyle and overall health play a significant role. The adipocyte-derived hormone leptin has varied systemic affects, including neuropeptide release and neuroprotection. A recent study by Lieb et al. (2009) showed that individuals with low plasma leptin levels are at greater risk of developing AD, through unknown mechanisms. In this report, we show that plasma leptin is a strong negative predictor of Aβ levels in the mouse brain, supporting a protective role for the hormone in AD onset. We also show that the inhibition of Aβ accumulation is due to the downregulation of transcription of the γ-secretase components. On the other hand, β-secretase expression is either unchanged (BACE1) or increased (BACE2). Finally, we show that only presenilin 1 (PS1) is negatively correlated with plasma leptin at the protein level ($p<0.0001$). These data are intriguing and may highlight a role for leptin in regulating the onset of amyloid pathology and AD.

1. Introduction

Alzheimer's disease (AD), the most common age-related neurodegenerative disease, is a continually growing socio-economic burden in industrialized nations, such as the United States, where the median life expectancy now tops 78 years. While a small number of AD cases are familial, due to well-characterized mutations in the amyloid precursor protein (APP) and presenilin (PS1 and PS2) [1], the majority are sporadic, with no clear etiology.

It is now widely accepted that AD pathology is driven, at least initially, by the accumulation of β-amyloid (Aβ), a small fibrillogenic peptide cleaved from the larger, membrane-bound APP [2]. This peptide rapidly oligomerizes, stimulating many downstream events and ultimately leading to neurodegeneration [3]. The production of Aβ is achieved by the sequential proteolysis of APP by the β- and γ-Secretases — both of which have been explored as targets for AD therapeutics. γ-secretase is a multimeric enzyme complex that completes the conversion of APP C-terminal fragments to Aβ peptides through the actions of its catalytic subunit, presenilin. Transcriptional, translational, and post-translational control of β- and γ-secretase expression and assembly is tightly controlled [4–8]. Aβ accumulation in sporadic AD arises from an imbalance between its production and degradation, though the processes underlying this imbalance are unclear and likely intertwined. It is well-documented that lifestyle and overall health impact disease development and progression, however [9–15].

Leptin is a hormone released from adipose tissue that regulates satiety and energy balance via hypothalamic signaling in the brain and subsequent neuropeptide release [16]. Though the effects on other areas of the brain are less clear, leptin has been reported to improve long-term potentiation in neurons and to be neuroprotective [8,17,18], likely via the potentiation of pro-growth and anti-apoptotic signals [19–22]. In addition, leptin has been shown to negatively regulate Aβ production and tau phosphorylation in neuronal cell lines and primary neurons [23,24]. Intriguingly, a recent paper reported that low plasma leptin is associated with increased AD risk in elderly humans [25], through unknown mechanisms.

In this report, we show that plasma leptin is strongly and negatively correlated with brain Aβ levels and presenilin 1 (PS1) expression in mice. In addition, leptin increases APP C-terminal fragment (CTF) and Aβ accumulation and reduces mRNA expression of γ-secretase components in human neuroglioma cells.

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http://dx.doi.org/10.1016/j.bbadis.2012.12.009

2. Materials and methods

2.1. Animals

For leptin measurements, 1–2 month old $APP^{\Delta NLh} \times PS1^{P264L}$ knock-in mice (wild-type, n = 37; heterozygous, n = 49; homozygous, n = 21; total, N = 107) [26] were fed a control (20% fat, n = 46), western (40% fat, n = 14) or ketogenic (80% fat, n = 47) diet (Research Diets) ad libitum for 1 month. Mice were split approximately equally by gender (*M*: n = 52; *F*: n = 55). Animals were euthanized by CO_2 asphyxiation followed by decapitation, and the tissues and blood collected. In an independent set of experiments, 6 (n = 19) and 18 (n = 18) month old male $APP^{\Delta NLh} \times PS1^{P264L}$ knock-in mice (homozygous, n = 18; wild-type, n = 19; total, N = 37) were used for body composition analyses, as well as serum analyses. Mice were euthanized by isoflurane anesthesia, followed by cardiac puncture and decapitation.

2.2. Plasma and serum measurements

Plasma was obtained from whole blood by low speed centrifugation (1500 ×*g*, 10 min). Leptin was measured in plasma using a commercially-available ELISA (Millipore, Billerica; MA), according to manufacturer's instructions. Serum was obtained from whole blood by allowing it to clot at 4 °C overnight followed by centrifugation (3000 ×*g*, 30 min). Serum was collected and either analyzed immediately or aliquoted and stored at -80 °C. Levels of total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and non-esterified fatty acids (NEFA) in sera were measured colorimetrically using commercially available kits (Wako Chemicals; Richmond, VA). Serum adiponectin, leptin, and insulin levels were all evaluated by ELISA in accordance with the manufacturer's assay protocol (R&D Systems; Minneapolis, MN for adiponectin; R&D Systems for leptin; and Crystal Chem Inc.; Downers Grove IL for insulin). To measure total pools of adiponectin, serum samples were first denatured (boiled in SDS buffer for 5 min) to break down large complexes.

2.3. Cell culture

H4 neuroglioma cells stably overexpressing $APP^{\Delta NLh}$ were grown in Opti-MEM (Invitrogen; Carlsbad, CA) with 10% fetal bovine serum (HyClone; Logan, UT) and 1% penicillin-streptomycin, supplemented with hygromycin (200 μg/mL) as described [27,28]. H4 wild-type cells were grown in the absence of hygromycin. SH-SY5Y neuroblastoma cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. Upon reaching confluence, serum was removed and the cells were treated with recombinant human leptin (Sigma-Aldrich; St. Louis, MO) for 24 h. H4-conditioned media, containing Aβ, were collected, and the cells were lysed in RIPA buffer (1% Triton X-100/0.5% sodium deoxycholate/ 0.1% SDS/50 mM Tris/150 mM NaCl + Protease Inhibitor Cocktail (Amresco; Solon, OH), pH = 8.0). Two independent experiments were performed.

2.4. Aβ ELISA

Frozen brain tissue was homogenized in 2% SDS with Complete Protease Inhibitor Cocktail using an AHS200 PowerMax homogenizer. Insoluble material was removed by centrifugation (20,800×*g*, 30 min., 14 °C) and the supernatants frozen until use. Total Aβ was measured by sandwich ELISA as previously described [27]. Briefly, SDS extracts were diluted in AC buffer (0.2 M sodium phosphate (pH 7), 0.4 M NaCl, 2 mM EDTA, 0.4% Block Ace (Serotec; Raleigh, NC), 0.4% BSA, 0.05% CHAPS, 0.05% NaN_3) for analysis. A standard curve was prepared from recombinant human $A\beta_{1\text{-}42}$ diluted in AC buffer. Standards and samples were measured at least in duplicate. 384-well plates (Immulon 4HBX; Thermo Scientific; Waltham, MA) were coated with 0.5 μg Ab9/well (against human $A\beta_{1\text{-}16}$) and blocked with Synblock (Serotec) according to the manufacturer's instructions. After incubation with the samples and standards, Aβ was detected with biotinylated-4G8 (against $A\beta_{17\text{-}24}$; Covance; Princeton, NJ), followed by incubation with 0.1 μg/mL NeutrAvidin-HRP (Pierce Technologies; Rockford, IL). The plate was developed with 3′,3′,5′,5′-tetramethylbenzidine (TMB; Kirkeguard and Perry Laboratories; Gaithersburg, MD) and the reaction stopped with 6% *o*-phosphoric acid. The absorbance at 450 nm was measured with a BioTek multiwell plate reader. H4 cell culture media was assayed for $A\beta_{40}$ using a commercially-available ELISA kit (BioSource; Carlsbad, CA). The $A\beta_{42}$ levels in the media were below detection limits. Data were averaged over 4-5 replicates.

2.5. qRT-PCR

RNA was extracted from leptin-treated wild-type H4 or SH-SY5Y cells using TRIzol® (Invitrogen). RNA from the SH-SY5Y cells was subsequently purified by RNeasy columns (Qiagen; Valencia, CA). Expression of the γ-secretase components was determined by two-step qRT-PCR, using iScript (BioRad; Hercules, CA) reverse transcription, followed by qPCR with PerfeCTa FastMix™ (Quanta; Gaithersburg, MD). The geometric mean of the C_T values for 18S rRNA, β-actin, and GAPDH was used as an internal control to calculate and compare relative expression ($2^{-\Delta\Delta C_T}$). Gene specific primer sets were obtained from IDT (Coralville, IA; Table 1). All measurements were performed in triplicate. Data was averaged from 2 to 3 independent experiments.

2.6. Immunoblots

Protein levels of APP and the γ-secretase components were determined by Western or spot blot, using antibodies directed against APP (CT20) [27], PS1 (Calbiochem; Gibbstown, NJ), PEN2 (Abcam; Cambridge, MA), or APH1A (Pierce; Rockford, IL). The antibody directed at the C-terminus of nicastrin was a kind gift from Dr. Paul Fraser [29]. Immunoreactive bands were visualized with Super Signal West Dura chemiluminescence HRP substrate (Pierce) and exposed to film. Densitometric analyses were performed using Scion Image or Image J. Expression of the γ-secretase components was standardized to β-actin (Sigma-Aldrich) expression in the same lane or spot, respectively. Spot blot measurements were performed in triplicate.

Table 1
qPCR gene-specific primers.

Target Gene	Direction	Sequence
PS1	Forward	5′-AAATGGGTGCTTTGTGGTCTCTGC-3′
	Reverse	5′-CAGGCTGATGGCTGTTTGTGTGTT-3′
PS2	Forward	5′-ATGGACAGCTCATCTACACGCCAT-3′
	Reverse	5′-AGAGCACCACCAAGAAGATGGTCA-3′
PEN2	Forward	5′-TCTCGCGCAAACGTCCATAACTGA-3′
	Reverse	5′-AATGGACACTCGCTCCAGGTTCAT-3′
Nicastrin	Forward	5′-AGTGCCCAAATGATGGGTTTGGTG-3′
	Reverse	5′-TCATAAGCCAAACCATTGCCCAGC-3′
APH1A	Forward	5′-ATCCGCCAGATGGCCTATGTTTCT-3′
	Reverse	5′-AGCAGGATAATGGCTGCTGTCAGA-3′
APH1B	Forward	5′-TTATGTCTTCACCATCGCCACCGA-3′
	Reverser	5′-TTGCCATGAACCAAACAAGGGACG-3′
BACE1	Forward	5′-ATGTTGCCACTCTGCCTCATGGTA-3′
	Reverse	5′-TGAGCAGGGAGATGTCATCAGCAA-3′
BACE2	Forward	5′-TGAGGACCTTGTCACCATCCCAAA-3′
	Reverse	5′-TGGCCAAAGCAGCATAAGCAAGTC-3′
18S rRNA	Forward	5′-TTAGAGTGTTCAAAGCAGGCCCGA-3′
	Reverse	5′-TTGGCAAATGCTTTCGCTCTGGTC-3′
β-Actin	Forward	5′-ACCAACTGGGACGACATGGAGAAA-3′
	Reverse	5′-TAGCACAGCCTGGATAGCAACGTA-3′
GAPDH	Forward	5′-TCGACAGTCAGCCGCATCTTCTTT-3′
	Reverse	5′-ACCAAATCCGTTGACTCCGACCTT-3′

PS1, PEN2, nicastrin and APH1 protein levels were analyzed in a subset of mice (N = 65 for PS1, PEN2, and APH1; N = 16 for nicastrin).

2.7. Body Composition Analysis

Body composition was measured using a Bruker minispec LF90 time domain NMR analyzer (Bruker Optics; Billerica, MA) as described previously [30].

2.7.1. Statistics

Data were analyzed by linear regression using SPSS® for Windows (Hewlett Packard; Houston, TX). Correlations were analyzed as Pearson product moment for parametric (plasma leptin, brain Aβ) or Spearman rank order for nonparametric (immunoblot) variables. RT-PCR data were analyzed using a Mann-Whitney U nonparametric ANOVA. Aβ secretion values were analyzed by parametric ANOVA followed by Dunnett's test for multiple comparisons to a control group. The effect of body weight, fat, and muscle on Aβ was analyzed by parametric ANOVA. The effect of leptin administration on plasma leptin was analyzed by a one-tailed, nonparametric, Mann–Whitney test. When necessary, manual adjustment for multiple comparisons was performed using the Holm–Bonferroni procedure. All mice were analyzed together as a single population to maximize power.

3. Results

3.1. Plasma leptin and Aβ

We made a striking observation during a study examining the effect of high fat diets on Aβ generation in the $APP^{\Delta NLh} \times PS1^{P264L}$ knock-in mouse model of AD: plasma leptin was strongly and negatively correlated with total brain Aβ (Fig. 1; $R^2 = 0.26$, $p < 0.0001$, $N = 105$). This data includes young (2–3 months old) mice of different genotypes, with one or two copies of mutant APP and/or PS1, as well as wild-type controls. In an effort to remove the contribution of endogenous rodent Aβ, we also performed the correlation with the exclusion of animals containing only wild-type rodent APP. This change modestly improved the strength of the observed correlation ($R^2 = 0.35$, $p < 0.0001$, $N = 69$). These data indicate that leptin has a protective effect on some aspect of Aβ metabolism. While the diets used in this study significantly increased plasma leptin levels ($p = 0.02$), there was no effect on brain Aβ ($p = 0.66$), supporting the conclusion that the correlation observed is driven by plasma leptin and not the variation in diet.

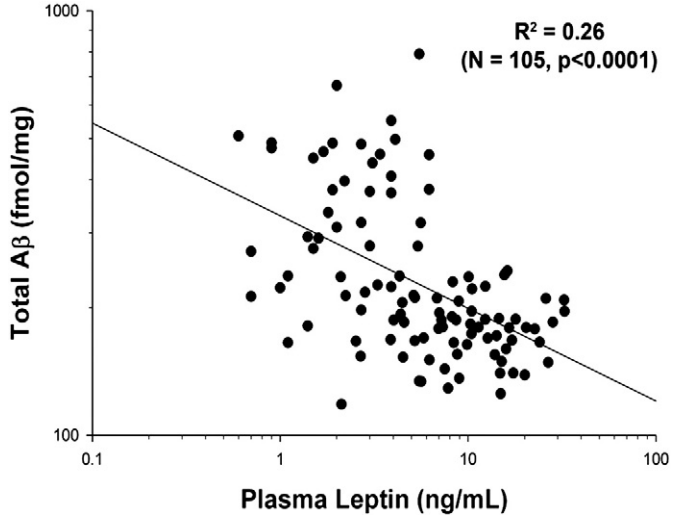

Fig. 1. Plasma leptin negatively correlates with brain Aβ. There was a strong negative correlation between plasma leptin and brain Aβ in young (2–3 month-old) $APP^{\Delta NLh} \times PS1^{P264L}$ knock-in mice ($N = 105$; $p < 0.0001$).

Leptin is an adipose-derived hormone that is important for satiety and energy balance and expenditure, including insulin sensitivity and fatty acid uptake into muscle and liver [31,32]. In addition, circulating levels of leptin are directly influenced by weight and fat mass, which also determine the levels of other adipokines, such as adiponectin. In order to determine whether the observed correlation between plasma leptin and brain Aβ was driven by leptin itself, or by other factors either affecting or affected by leptin, we looked at a separate, small ($N = 37$) cohort of middle aged (6 months) and older (18 months) APP × PS1 knock-in and wild-type mice that were maintained on a control diet (Table 2). The APP × PS1 knock-in mice have significantly elevated Aβ ($p < 0.0005$; consistent with previous reports [26]), triglycerides ($p < 0.03$), non-esterified fatty acids (NEFA; $p = 0.002$), total cholesterol ($p < 0.0005$), high-density lipoprotein (HDL; $p = 0.025$), and low-density lipoprotein (LDL; $p < 0.0005$). Conversely, APP × PS1 mice have significantly reduced serum leptin ($p = 0.04$) and adiponectin ($p = 0.01$), compared with wild-type controls. There was an age-dependent increase in weight ($p = 0.001$), muscle mass ($p < 0.0005$), free fluids ($p < 0.0005$), cholesterol ($p = 0.003$), serum insulin ($p = 0.004$), and Aβ ($p < 0.0005$). There was an age-dependent decrease in triglycerides ($p = 0.009$), NEFA ($p < 0.0005$), and serum adiponectin ($p = 0.015$).

We next examined whether any of these observed effects were associated with changes in brain Aβ. Weight, fat mass, and muscle mass did not significantly correlate with brain Aβ ($p > 0.43$ in all cases), supporting the hypothesis that body composition is not non-specifically related to the level of brain Aβ. Free fluid content was marginally significant ($p = 0.05$), though the reason for this is unclear. Plasma lipids in the form of triglycerides and NEFA were also not significantly correlated ($p > 0.5$) with Aβ. Similarly, plasma insulin was not correlated with brain Aβ ($p = 0.21$). Consistent with previous reports, cholesterol was strongly correlated with Aβ, particularly total cholesterol and LDL ($p < 0.0005$ for both). These data support a specific role for leptin as a regulator of Aβ in the brain.

3.2. Effect of leptin on cells in culture

In order to determine if leptin has a direct effect on Aβ production in neural cells, we treated $APP^{\Delta NLh}$-overexpressing H4 neuroglioma cells with leptin and measured the effect on APP processing and Aβ generation. There was a dose-dependent decrease in the amount of Aβ generated in cells treated with leptin in the physiological range observed in mice (10–50 ng/mL): there was a significant reduction in cells treated with 50 ng/mL leptin (Fig. 2A; $p = 0.024$). This was concomitant with accumulation of APP α- and β-C-terminal fragments (Fig. 2B). These two events are hallmarks of reduced γ-secretase

Table 2
Effect of APP/PS1 mutations.

	Wild-type		APP/PS1	
	6 months	18 months	6 months	18 months
Weight (g)[b]	38 ± 1.8	47.4 ± 1.9	40.8 ± 1.9	45.2 ± 1.9
Fat (g)	7.85 ± 1.37	10.1 ± 1.45	6.43 ± 1.45	9.82 ± 1.45
Muscle (g)[b]	25.91 ± 0.74	31.37 ± 0.78	28.67 ± 0.78	30.01 ± 0.78
Free fluids (g)[b]	1.14 ± 0.19	1.99 ± 0.2	1.2 ± 0.2	2.02 ± 0.2
Triglycerides (mg/dL)[a,b]	38.1 ± 4.6	25.5 ± 4.8	49.3 ± 4.6	36 ± 5
NEFA (mEq/dL)[a,b,c]	1.42 ± 0.09	1.04 ± 0.1	1.76 ± 0.09	1.33 ± 0.1
Cholesterol (mg/dL)[a,b]	70.9 ± 7.8	91.6 ± 8.1	98.8 ± 7.8	129.7 ± 8.5
HDL (mg/dL)[a]	69 ± 7	70.7 ± 7.3	86.2 ± 7	87.3 ± 7.7
LDL (mg/dL)[a]	15 ± 3.7	17.4 ± 3.8	25.4 ± 3.7	35.8 ± 4
Insulin (ng/mL)[b]	6.6 ± 2.1	14.6 ± 2.3	5.8 ± 2.1	11.1 ± 2.3
Leptin (ng/mL)[a]	3.67 ± 1.52	7.59 ± 1.52	1.36 ± 1.52	3.17 ± 1.63
Adiponectin (ng/mL)[a,b]	787 ± 44	569 ± 46	566 ± 44	550 ± 49
Total Aβ (pM)[a,b]	1312 ± 76	3834 ± 70	1732 ± 79	81936 ± 9972

[a] Significant effect of genotype ($p \leq 0.05$).
[b] Significant effect of age ($p \leq 0.05$).
[c] NEFA = non-esterified fatty acids.

activity ([33]; i.e. loss of product and accumulation of substrate), and led us to ask the question of how leptin affects this process.

γ-Secretase is a multimeric complex, whose four main components (presenilin (PS), nicastrin, PEN2, and APH1) are individually and coordinately regulated, both transcriptionally and post-translationally [4]. Because leptin binds to cell surface receptors, stimulating intracellular signaling and transcriptional regulation [34], we examined the possibility that leptin regulates transcription of γ-secretase components by measuring mRNA expression by quantitative RT-PCR after leptin treatment of wild-type H4 cells. We found that leptin treatment reduced expression of many of the components of the enzyme complex (PS1, PEN2, nicastrin, and APH1B) by ~30–50% (Fig. 3; $p<03$ in all cases). Expression of PS2, another presenilin isoform, was unchanged ($p=0.4$). We saw the same effect in SH-SY5Y cells, a human neuroblastoma cell line (data not shown). Finally, because recent reports have postulated that leptin regulates Aβ via β-secretase regulation [23], we measured the effect of leptin treatment on BACE1 and BACE2 mRNA expression in H4 cells. Leptin treatment had no effect on the transcription of BACE1 (Fig. 3; $p=0.3$), but led to an increase in the expression of BACE2 ($p=0.004$). While this is a potentially interesting result, an increase in BACE2 expression due to plasma leptin levels would not account for the decrease in Aβ observed in cells treated with leptin. Together, these data indicate that leptin inhibits expression of γ-secretase components, leading to a reduction of Aβ.

3.3. Effect of leptin on γ-secretase expression in the brain

We next wanted to determine if leptin also affects the γ-secretase components at the protein level, focusing on those components that were affected at the mRNA level. PS1 protein expression was significantly, inversely correlated with plasma leptin (Fig. 4A; $p<0.0001$), demonstrating that this regulation occurs in vivo. Brain expression of PEN2 ($p<0.73$) and APH1A ($p<0.59$) were not significantly correlated with plasma leptin, suggesting that leptin does not directly affect expression of these molecules at the protein level. In addition, PS1 expression was positively correlated with brain Aβ (Fig. 4B; $p<0.004$), supporting a causative effect on amyloid accumulation. We also examined a subset of mouse brain samples by Western blot. Interestingly, nicastrin displayed a size shift to a lower molecular weight (Fig. 4C) in mice with higher plasma leptin levels (Fig. 4D; $p=0.03$). This change is consistent with the accumulation of the immature, partially deglycosylated form of nicastrin [29], which requires presenilin for its maturation through the endoplasmic reticulum. Similarly, treatment of H4 cells with exogenous leptin induced a shift to the immature form of nicastrin (Fig. 4E), though this change did not reach significance (Fig. 4F; $p>0.09$ for all pairwise comparisons). Together, these findings show that leptin is an important regulator of APP processing and Aβ production through its regulation of PS1 transcription. Finally, we performed a validation analysis in a randomly-selected subset of ~50% of the animals. This yielded identical results for the relationships between plasma leptin and Aβ ($R^2=0.33$, $p<0.0001$, $N=57$), plasma leptin and PS1 ($R^2=0.21$, $p<0.0001$, $N=51$), and PS1 and Aβ ($R^2=0.16$, $p<0.004$, $N=52$).

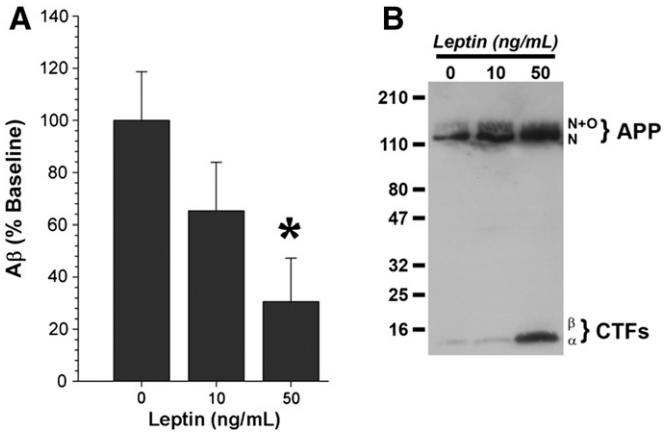

Fig. 2. Leptin treatment inhibits Aβ production in cell culture. Leptin reduced the Aβ concentration in conditioned media from $APP^{\Delta NLh}$-overexpressing H4 neuroglioma cells after 24 h of treatment (A; *$=p<0.05$), concomitant with accumulation of APP C-terminal fragments (CTFα and β: B), indicative of reduced γ-secretase activity. Two independent experiments were performed. A is an average of the two experiments, while B is a representative immunoblot of the results.

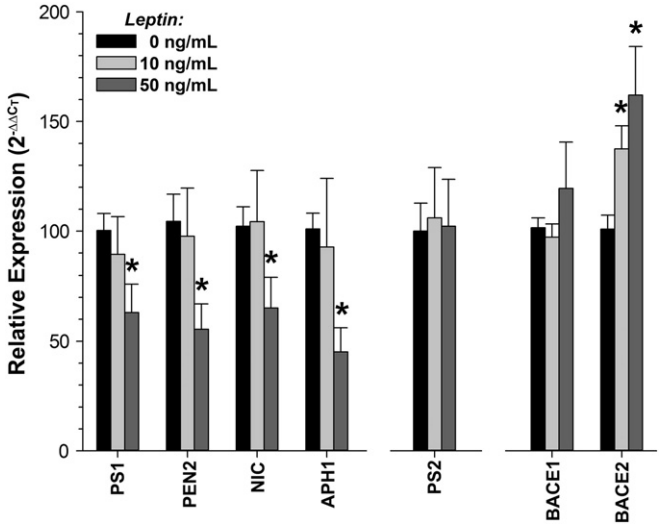

Fig. 3. Leptin treatment reduces mRNA levels of γ-secretase components in cell culture. mRNA expression of the γ-secretase components PS1, PEN2, nicastrin, and APH1 were decreased after leptin treatment in wild-type neuronal tissue culture cells. On the contrary, there was an overall increase in the expression of BACE2 ($p=0.014$), one of the β-secretase enzymes. There was no change in PS2 or BACE1 mRNA expression ($p>0.12$). The data are an average of 2–3 independent experiments.

4. Discussion

The data presented herein support a role for the circulating hormone leptin in the regulation of γ-secretase and, hence, Aβ accumulation in the brain. Our data indicate that leptin transcriptionally regulates PS1 mRNA expression in neuronal cells (Fig. 3), thereby reducing PS1 protein levels in the brain (Fig. 4A). This regulation appears to be specific to the PS1 isoform of presenilin as there was no change in PS2 expression upon addition of leptin to neuronal cells (Fig. 3). These changes likely impact catalytic activity of the γ-secretase complex. Indeed, it has been demonstrated previously that alterations in PS1 protein levels impact formation of stable γ-secretase complexes, ultimately affecting expression and modification of the other components, which in turn regulates γ-secretase activity [4]. Consistent with these reports, we also observed a decrease in mRNA expression of other γ-secretase components, including nicastrin, PEN2, and APH1 (Fig. 3), though these changes did not extend to the protein level. In addition, we have observed a leptin-dependent accumulation of immature, partially-glycosylated nicastrin, both in cell culture and mouse brain extracts (Fig. 4C,E), indicating that levels of active protein may be affected, even if total protein is not. Based on this data, it is likely that the changes in PEN2, nicastrin, and APH1 mRNA levels are a result of PS1 downregulation and not a direct result of leptin treatment Another possibility is that leptin downregulates the gene expression of the γ-secretase components, but that these changes only affect the steady-state protein level of PS1.

Our findings highlight a potential role for leptin in the etiology of AD. These results have profound implications on the AD field. Currently, there are no widely accepted peripheral biomarkers of AD [35,36], making pre-diagnosis difficult. The current standard of cognitive testing relies on the detection of existing memory deficits, and may preclude advance preventative strategies. Plasma leptin could

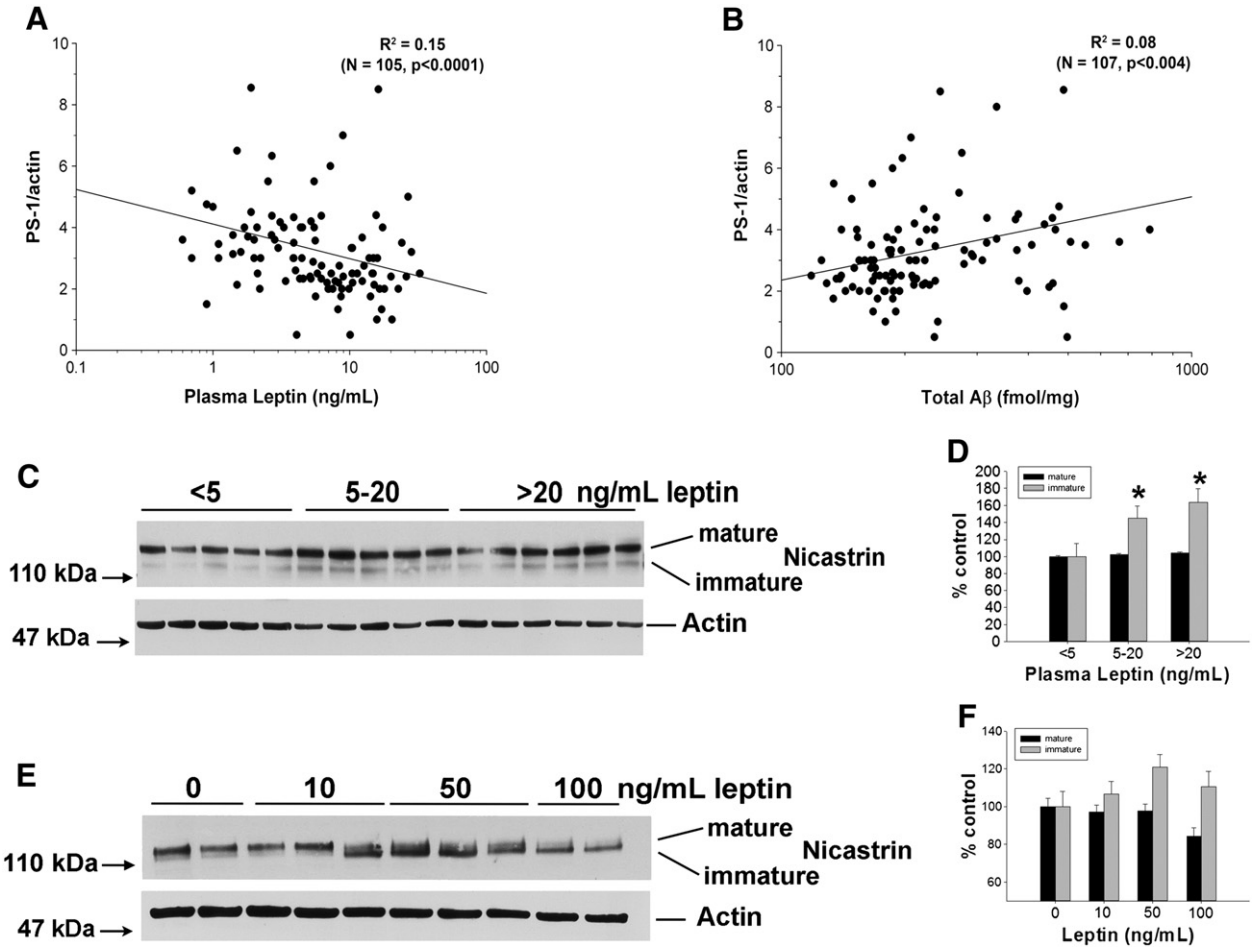

Fig. 4. Plasma leptin negatively correlates with PS1 expression in brain. Plasma leptin was strongly and negatively correlated with brain PS1 protein levels (A: N = 65; $p<0.0001$), but not other γ-secretase components (not shown: N = 65; PEN2, $p<0.727$; APH1A, $p<0.591$). The amount of PS1 protein was positively correlated with brain Aβ (B: N = 65; $p<0.004$). Brain nicastrin (C) displayed a shift to a smaller molecular weight with increasing plasma leptin concentrations (D: N = 16; $p=0.03$), indicating accumulation of an immature form of the protein. Similarly, leptin treatment of H4 cells led to accumulation of the immature form of nicastrin (E), though the change was not significant (F: N = 1 −; $p=0.31$).

predict AD risk several years ahead of the onset of cognitive impairment [25,37], demonstrating that assessment of plasma leptin in middle-aged and elderly individuals holds some promise in this regard. In addition, leptin provides a novel target for prevention, through replacement therapy, as well as therapeutic treatment of AD.

Leptin has been studied extensively in the context of obesity-linked diabetes, a disease that confers a significantly elevated risk for AD through unknown mechanisms [9,38,39]. Because leptin circulates at levels proportional to the amount of adipose tissue, obese individuals have significantly elevated plasma leptin [40]. Under normal circumstances, high levels of leptin should be neuroprotective. However, persistent hyperleptinemia renders the hypothalamus, and likely other areas of the brain, incapable of responding to plasma leptin — a consequence known as leptin resistance [16]. Therefore, diabetic patients are part of a subclass of AD patients that may require unique preventatives and/or therapeutics. Discerning the signaling pathways involved in leptin-mediated PS1 and Aβ regulation may provide a target for these strategies. To this end, an animal model with features of both diseases may help to shed light on these mechanisms.

Finally, another interesting question to address in the future will be the additional ramification(s) of leptin-mediated PS1 regulation. In other words, why is PS1 a target of leptin signaling in non-pathological conditions? While the answer to this question is still unclear, one intriguing possibility is the recently elucidated role for PS1 in autophagy [41,42]. A hormone, such as leptin, that controls cellular energy homeostasis would likely play a role in starvation-induced protein turnover, and control of presenilin expression is one way to achieve that.

5. Conclusions

In conclusion, plasma leptin directly controls PS1 expression via transcriptional regulation. Downregulation of PS1 subsequently decreases expression of the other γ-secretase components, thereby reducing γ-secretase activity and Aβ generation. Low plasma leptin may therefore contribute to the onset of AD and may provide a novel avenue of research into the mechanisms underlying the disease, as well as biomarker and prevention research.

Acknowledgements

We would like to thank Dr. Pete Nelson and Wangxia Wang for the H4 RNA, Chris Holler and Robin Webb for tissue collection, Drs. Fred Schmitt and Elizabeth Head for helpful discussion, Dr. Todd Golde for providing Aβ antibodies, and Dr. Paul Fraser for providing the nicastrin antibody. This study was supported by NSF (DBI004931), NIH (AG005119, NS058382, NS077482-01 RR020171), The Coins for Alzheimer's Research Trust, the Alzheimer's Association (IIRG-10-172905), and the Hibernia Nation Bank/Edward G. Schleider Chair.

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