Published in final edited form as:

Biochem Biophys Res Commun. 2009 February 27; 380(1): 98–104. doi:10.1016/j.bbrc.2009.01.041.

Leptin regulates Tau phosphorylation and Amyloid through AMPK in Neuronal Cells

Steven J. Greco, Sraboni Sarkar, Jane M. Johnston, and Nikolaos Tezapsidis *

Neurotez, Inc., Bridgewater, New Jersey, USA

Abstract

Leptin, which serves as a lipid-modulating hormone to control metabolic energy availability, is decreased in Alzheimer's disease (AD) patients, and serum levels are inversely correlated to severity of dementia. We have previously described the effects of leptin in reducing amyloid β protein both *in vitro* and *in vivo*, and tau phosphorylation *in vitro*. Herein, we systematically investigated the signaling pathways activated by leptin, leading to these molecular endpoints, to better understand its mechanism of action. Inhibition of amyloid β production and tau phosphorylation in leptin-treated human and/or rat neuronal cultures were both dependent on activation of AMP-activated protein kinase (AMPK). Direct stimulation of AMPK with the cell-permeable activator, 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), replicated leptin's effects and conversely, Compound C, an inhibitor of AMPK, blocked leptin's action. The data implicate that AMPK is a key regulator of both AD-related pathways.

Keywords

Alzheimer's disease; Leptin; AICAR; β -Amyloid; tau; AMPK; GSK-3 β ; PPAR γ

Introduction

It is widely accepted that A β is associated with neurodegeneration in AD [1;2]. Soluble A β oligomers, prior to plaque buildup, exert toxic effects leading to synaptic loss, neuronal death, and dementia [3]. However, for sporadic AD, representing the overwhelming majority of AD cases, there is an astonishing absence of concrete indication of a particular cause that triggers the A β cascade.

On the other hand, neurofibrillary tangles (NFT), which result from hyperphosphorylation of the microtubule-interacting protein tau, and tau oligomers, termed paired helical filaments (PHF), have also been shown to be associated with microtubule destabilization and neurodegeneration [4].

Compared to A β plaques, the distribution of NFT within the brain correlates better with neurodegeneration and cognitive decline [5]. However, tangles are seen in other tauopathies including supranuclear palsy and frontotemporal dementia and parkinsonism linked to

*Address correspondence and reprints requests to: Nikolaos Tezapsidis, Ph.D., Neurotez, Inc., 991 Highway 22, Suite 200A, Bridgewater, New Jersey 08807, USA., Tel: 908-998-1340, Fax: 908-864-8957, Email: ntezapsidis@neurotez.com.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

chromosome 17 (FTDP-17), which do not present with deposits of A β [6;7]. These findings suggest that tangle formation may be triggered by a number of neurodegenerative insults brought on by earlier events in these CNS disorders; yet again, only speculations exist as to what those may be.

A significant correlation between mid-life obesity and dementia later in life has been reported [8;9]. However, weight loss and a decline of leptin levels are common in AD [10]. Leptin is a peptide hormone synthesized by adipocytes. Within the central nervous system, leptin crosses the blood brain barrier to bind specific receptors in the hypothalamus to mediate food intake, body weight and energy expenditure [11]. However, accumulating evidence suggests that leptin receptors, expressed in abundance in the hippocampus [12], may be mediating other functions as well. The diverse pathways of leptin action suggest that leptin may have diverse signaling pathways.

We have found that leptin can reduce A β levels both *in vitro* and *in vivo* [13]. Additionally, we have shown that leptin can reduce tau phosphorylation in neuronal cultures [14]. Herein, we investigated the signaling pathways activated by leptin to mediate these effects.

Materials and Methods

Reagents and Antibodies

Minimum essential medium (MEM) was purchased from ATCC (Manassas, VA). Trypsin-EDTA and penicillin solution were purchased from MP Biomedicals (Solon, Ohio). Fetal bovine serum (FBS), all-trans retinoic acid (RA) and human recombinant leptin were purchased from Sigma-Aldrich (St. Louis, MO). 5-Aminoimidazole-4-carboxamide ribonucleoside (AICAR) was purchased from Cell Signaling Technology (Danvers, MA). Compound C, 2-Hydroxy-4-(((4-methylphenyl)sulfonyloxy)acetyl)amino)-benzoic acid (S3I-201), 5-(2,2-Difluoro-benzo[1,3]dioxol-5-ylmethylene)-thiazolidine-2,4-dione (522DB13D), 1L6-Hydroxymethyl-chiro-inositol-2-(R)-2-O-methyl-3-O-octadecyl-*sn*-glycerocarbonate (1L6HCl), 2-(4-Chloro-phenyl)-4-(4-fluorophenyl)-5-pyridin-4-yl-1,2-dihydropyrazol-3-one (24C44F5P) and H-Trp-Glu-OH (G3335) were purchased from EMD Chemicals (Gibbstown, NJ). The final Concentrations and incubation times for all inhibitors or activators were based on previous reports [14;15;16;17;18;19;20], with additional information presented as Table 1. All kinase inhibitors were used at or in excess of previously identified inhibitory concentrations; with concentration-dependent inhibition of the target kinases confirmed by immunoblot (data not shown).

Rabbit anti-Akt (pSer⁴⁷³), -Jak2 (pTyr^{1007/1008}), -AMPK α (pThr¹⁷²), -GSK-3 β (pSer⁹), -PPAR γ (81B8), p38 MAPK (pThr¹⁸⁰/Tyr¹⁸²) (28B10) mAb, tau (pSer³⁹⁶) mAb and tau (tau46) mAb were purchased from Cell Signaling. PHF-tau mAb (clone AT8) was purchased from Pierce Biotechnology (Rockford, IL). PHF-1 mAb was a gift from Dr. Peter Davies, Albert Einstein College of Medicine (Bronx, NY). Rabbit anti-tau (pThr¹⁸¹) was purchased from Sigma-Aldrich. Rabbit anti- α -tubulin mAb was purchased from Affinity BioReagents (Golden, CO).

Culture and Neuronal Induction of Cell Lines

The human neuroblastoma cell line, SH-SY5Y, was purchased from ATCC. Cell culture was performed according to manufacturer's specific guidelines. Cells were propagated in MEM containing 10% FBS. Neuronal differentiation was performed as described previously [14].

Culture of Rat Primary Neurons

Primary rat cortical neurons were purchased from BrainBits LLC (Springfield, IL), and cultured as per manufacturer's instruction and as described previously [14].

Protein Extraction and Immunoblotting

Neuronal cells were treated with leptin (100 nM; 1600 ng/ml) for 4 hrs or AICAR (2 mM) or specific inhibitors for 1 hr, unless otherwise specified, and then harvested by scraping. Protein extraction from cell pellets and immunoblots were performed as described previously [14]. All primary antibodies, except tau-pSer³⁹⁶, total tau (1:500), and PHF-tau, AT8 (1:200), and secondary antibodies were used at final dilutions of 1:1,000 and 1:10,000, respectively.

A $\beta_{(1-40)}$ Quantification

A $\beta_{(1-40)}$ levels in cell culture media were determined using the Human β -Amyloid 1-40 Colorimetric Immunoassay kit (Invitrogen; Carlsbad, CA), according to manufacturer's specific instructions. A $\beta_{(1-40)}$ levels were calculated from a standard curve developed with OD at 450 nm using 8 serial dilutions of known concentration.

Statistical Analysis

Statistical data analyses were performed with analysis of variance and Tukey-Kramer multiple comparisons test. Densitometric analyses were performed using the UN-SCAN-IT gel 6.1 software (Silk Scientific; Orem, UT). $p < 0.05$ was considered statistically significant.

Results

Upstream signaling events mediated by leptin to regulate tau phosphorylation

Tau phosphorylation at AD-related sites in human neuroblastoma SY5Y cells has been reported to increase with RA-induced differentiation (RA-SY5Y) [21]. These changes have been attributed to an increase in the absolute levels of tau during differentiation, rather than hyperphosphorylation of the protein. Due to this increased basal expression, RA-SY5Y cells represent a convenient culture system to monitor changes in human tau phosphorylation.

We previously utilized RA-SY5Y to examine the cause-effect relationship between leptin, and tau phosphorylation [14]. We reported a reduction of tau phosphorylation by leptin treatment. This effect was also achieved by AICAR treatment, a cell-permeable activator of the downstream signaling protein AMPK and conversely antagonized by an AMPK inhibitor. Herein, we expanded these studies to other signaling molecules, known to act upstream or downstream of AMPK and explored their role in regulating tau phosphorylation following leptin treatment of neuronal cells in culture.

RA-SY5Y were treated with leptin in the presence or absence of inhibitors (Table 1) to known kinases. Phosphorylation of tau at several different epitopes served as our experimental endpoint (Figure 1). Comparisons were made relative to cells treated with leptin alone or vehicle (non-treated). Inhibitors of AMPK, Akt (protein kinase B) or p38 MAP kinase significantly ($p < 0.05$) impeded leptin's ability to reduce tau phosphorylation (panels I–III, lanes 4, 6 and 7) but notably, the PI3K inhibitor did not (panels I–III, lane 5). Cells treated with specific inhibitors in the absence of leptin did not show a significant change in tau phosphorylation (data not shown).

We next explored whether any of the above kinases become phosphorylated following leptin treatment (Figure 2). Typically, phosphorylation results in refolding, and this triggers a change in kinase activity. Leptin significantly increased ($p < 0.05$) the phosphorylation of Janus kinase

2 (Jak2), AMPK, p38 and Akt (Figures 2A through 2E) compared to vehicle (non-treated). In parallel, treatment with AICAR, caused similar changes to the above kinases, with the exception of Jak2 (Figure 2B) which was not affected since AMPK is downstream of Jak2.

We lastly examined whether leptin regulates glycogen synthase kinase-3 β (GSK-3 β), a direct substrate for Akt and known inducer of tau hyperphosphorylation [22]. Phosphorylation of GSK-3 β at Ser⁹ by Akt leads to enzymatic inactivation and both leptin or AICAR treatment (Figure 2F) significantly ($p<0.05$) increased the phosphorylation at that site compared to vehicle (Figure 2G).

In addition to the neuroblastoma cells we investigated the effect of leptin and AICAR on AMPK and Akt in primary cortical neurons. In agreement with the results described above, leptin increased phosphorylation of pSer⁴⁷³ Akt by 30 $\pm$ 4 % (n=3) and pThr¹⁷²AMPK by 75 $\pm$ 9% (n=3). In addition, AICAR increased phosphorylation of pSer⁴⁷³ Akt by 32 $\pm$ 3 % (n=3) and pThr¹⁷²AMPK by 71 $\pm$ 6% (n=3).

In summary, the data confirmed leptin's ability to reduce phosphorylation of tau and further implicate the involvement of several principal kinases, among which are AMPK, p38 MAP kinase and Akt.

Leptin regulates A β release via AMPK

From our previous work, it was shown that leptin can reduce A β levels both *in vitro* and *in vivo* [13]. Mechanistically, this is achieved by changes in the lipid composition of membrane lipid rafts, presumably due to leptin's lipolytic activity. This results in a compromised β -secretase (BACE) activity towards APP cleavage, ultimately reducing cellular production of A β [13]. Leptin was also capable of promoting the LRP-directed, apoE-dependent A β uptake, further contributing to the lowering of extracellular levels of A β [13].

Based on our findings, we decided to investigate how the pathways leading to A β production and tau phosphorylation, both of which are modulated by leptin, are interconnected. The peroxisome proliferator-activated receptor- γ (PPAR γ) is a transcription factor known to regulate BACE, a key enzyme in amyloid precursor protein (APP) processing [23]. PPAR γ levels have been shown to increase *in vivo* with leptin administration [24].

We therefore explored the roles of PPAR γ and the leptin-AMPK pathway in regulating A β release. RA-SY5Y were treated with leptin, AICAR or vehicle for 6 hrs in the presence or absence of an antagonist or inhibitor to PPAR γ or AMPK, respectively, and soluble A β ₍₁₋₄₀₎ levels within the culture media were determined by ELISA (Figure 3A). A significant ($p<0.05$) decrease in soluble A β was observed in cells treated with leptin (lane 2) or AICAR (lane 5). These effects were negated by co-treatment with AMPK inhibitor (lanes 3 and 6) or PPAR γ (lanes 4 and 7) antagonist. No change was observed in cells treated with inhibitor or antagonist alone (lanes 8 and 9).

These results present a novel finding in which A β production is modulated by AMPK, an energy regulator that we have also linked to phosphorylation of tau.

Tau and A β pathways do not overlap downstream of AMPK

Finally, it was investigated whether leptin-mediated tau and A β pathways differentiate beyond AMPK. Since AICAR increased phosphorylation of Akt (Figure 2E) and was unable to reduce A β levels in the presence of a PPAR γ antagonist (Figure 3A), we hypothesized that these signaling proteins were downstream of AMPK along their respective pathways.

To this end, RA-SY5Y were treated with leptin in the presence or absence of Akt inhibitor (Figure 3B) or PPAR γ antagonist (Figure 3C). Inhibition of Akt was unable to significantly ($p>0.05$) reverse the leptin-induced reduction of soluble A β release (Figure 3B, dark gray bar). Likewise, inhibition of PPAR γ did not significantly ($p>0.05$) reverse the leptin-induced reduction of tau phosphorylation at all examined sites (Figure 3C, right set of bars). This strongly implicates that following activation of AMPK by leptin, a separate pathway involving PPAR γ and BACE exist to regulate A β while another pathway involving Akt and GSK-3 β regulates tau phosphorylation.

Discussion

We have shown that Leptin reduces A β levels both *in vitro* and *in vivo* [13], and inhibits tau phosphorylation in neuronal cultures [14]. Leptin is known to activate AMPK [25;26;27] and our present findings suggest that AMPK mediates Leptin's effect on tau phosphorylation (Figure 1) and A β release (Figure 3). Leptin signaling pathways may reflect differential AMPK responses that are tissue and neuron specific [28]. For example, it has been shown that Leptin activates AMPK in skeletal muscle [27] but inhibits AMPK in the hypothalamus [26]. Nonetheless, Leptin may also act independently of AMPK, through PI3K as shown in hippocampal neurons [12].

The underlying link between AMPK, tau phosphorylation and AD pathobiology has not been previously explored in detail (see Fig 3D for a working model) and it is unknown as to how leptin modulates AMPK activity in the hippocampus. Leptin has been reported to activate AMPK via a Jak2-dependent, signal transducer and activator of transcription 3 (STAT3)-independent pathway in hepatic cells [29]. Inhibition of tau phosphorylation through inactivation of GSK-3 β by Akt has been well-described [30]. AICAR has been shown to transiently activate Akt in hippocampal neurons [31], in agreement with our findings in RA-SY5Y (Figure 2E). However, higher doses of AICAR and longer incubation periods may trigger negative feedback loops or be deleterious to AMPK and Akt. In addition, other signaling pathways may be involved, independent of AMPK.

Surprisingly, inhibition of p38 MAPK, a known inducer of tau phosphorylation, abrogated leptin's reducing effect on tau phosphorylation (Figure 1) but inhibiting PI3K did not. Several reports have demonstrated that p38 can activate Akt [32] and inactivate GSK-3 β [33]. Additionally, AMPK activates p38 in the ischemic heart [34], and this finding was supported in RA-SY5Y through stimulation with AICAR (Figure 2D). Therefore, leptin may also modulate tau phosphorylation through AMPK via an alternative pathway involving p38.

We have previously shown that leptin reduces A β production [13] (Figure 3A). Inhibition of AMPK or PPAR γ reversed this effect, suggesting their role as mediators of the leptin/A β pathway. PPAR γ levels have been shown to increase *in vivo* with leptin administration [24]. The link between PPAR γ and A β has been well described [23]; however the role of AMPK has not. It is unclear whether AMPK directly activates PPAR γ through phosphorylation, or through an indirect mechanism. However, ample evidence suggests that both AMPK and PPAR γ pathways affect lipid metabolism [35;36]. Thus our finding that leptin-induced A β reduction depends on PPAR γ (Figure 3A, lane 4) is consistent with a role for Leptin and AMPK in modulating membrane lipids in neurons as we previously reported [12].

A large study found mid-life central obesity, as the greatest metabolic risk factor for developing dementia later in life [8;9], yet, interestingly, high serum leptin levels in the elderly had significantly less likelihood of cognitive decline [37]. This is in agreement with cross-sectional studies showing that people with dementia have a lower body mass index (BMI) than those without dementia, potentially due to a greater rate of BMI decline during the years immediately

preceding dementia [10]. It is also in agreement with the finding that the levels of leptin are significantly decreased in AD patients, inversely proportional to the severity of dementia [37]. These findings suggest that a potential leptin therapy for AD could in fact be a replacement therapy. Presently, we have demonstrated that leptin regulates two major AD pathways via distinct AMPK-dependent mechanisms in neuronal cells. Leptin, and potentially AMPK activators, may provide a novel therapeutic approach to AD treatment.

Acknowledgements

This work was supported by the National Institute on Aging (SBIR-1R43AG029670) and the New Jersey Commission on Science and Technology.

References

1. Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G. Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science* 1996;274:99–102. [PubMed: 8810256]
2. McLean CA, Cherny RA, Fraser FW, Fuller SJ, Smith MJ, Beyreuther K, Bush AI, Masters CL. Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. *Ann Neurol* 1999;46:860–6. [PubMed: 10589538]
3. Selkoe DJ. Translating cell biology into therapeutic advances in Alzheimer's disease. *Nature* 1999;399:A23–31. [PubMed: 10392577]
4. Alonso A, Zaidi T, Novak M, Grundke-Iqbal I, Iqbal K. Hyperphosphorylation induces self-assembly of tau into tangles of paired helical filaments/straight filaments. *Proc Natl Acad Sci U S A* 2001;98:6923–8. [PubMed: 11381127]
5. Guillozet AL, Weintraub S, Mash DC, Mesulam MM. Neurofibrillary tangles, amyloid, and memory in aging and mild cognitive impairment. *Arch Neurol* 2003;60:729–36. [PubMed: 12756137]
6. Hanihara T, Amano N, Takahashi T, Nagatomo H, Yagashita S. Distribution of tangles and threads in the cerebral cortex in progressive supranuclear palsy. *Neuropathol Appl Neurobiol* 1995;21:319–26. [PubMed: 7494600]
7. Vogelsberg-Ragaglia V, Bruce J, Richter-Landsberg C, Zhang B, Hong M, Trojanowski JQ, Lee VM. Distinct FTDP-17 missense mutations in tau produce tau aggregates and other pathological phenotypes in transfected CHO cells. *Mol Biol Cell* 2000;11:4093–104. [PubMed: 11102510]
8. Whitmer RA, Gunderson EP, Barrett-Connor E, Quesenberry CP Jr, Yaffe K. Obesity in middle age and future risk of dementia: a 27 year longitudinal population based study. *Bmj* 2005;330:1360. [PubMed: 15863436]
9. Whitmer RA, Gustafson DR, Barrett-Connor E, Haan MN, Gunderson EP, Yaffe K. Central obesity and increased risk of dementia more than three decades later. *Neurology* 2008;71:1057–64. [PubMed: 18367704]
10. Gustafson D. Adiposity indices and dementia. *Lancet Neurol* 2006;5:713–20. [PubMed: 16857578]
11. Jequier E. Leptin signaling, adiposity, and energy balance. *Ann N Y Acad Sci* 2002;967:379–88. [PubMed: 12079865]
12. Harvey J. Leptin: a diverse regulator of neuronal function. *J Neurochem* 2007;100:307–13. [PubMed: 17076761]
13. Fewlass DC, Noboa K, Pi-Sunyer FX, Johnston JM, Yan SD, Tezapsidis N. Obesity-related leptin regulates Alzheimer's Abeta. *Faseb J* 2004;18:1870–8. [PubMed: 15576490]
14. Greco SJ, Sarkar S, Johnston JM, Zhu X, Su B, Casadesus G, Ashford JW, Smith MA, Tezapsidis N. Leptin reduces Alzheimer's disease-related tau phosphorylation in neuronal cells. *Biochem Biophys Res Commun* 2008;376:536–41. [PubMed: 18801339]
15. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, Moller DE. Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest* 2001;108:1167–74. [PubMed: 11602624]
16. Siddiquee K, Zhang S, Guida WC, Blaskovich MA, Greedy B, Lawrence HR, Yip ML, Jove R, McLaughlin MM, Lawrence NJ, Sehti SM, Turkson J. Selective chemical probe inhibitor of Stat3,

- identified through structure-based virtual screening, induces antitumor activity. *Proc Natl Acad Sci U S A* 2007;104:7391–6. [PubMed: 17463090]
17. Bilancio A, Okkenhaug K, Camps M, Emery JL, Ruckle T, Rommel C, Vanhaesebroeck B. Key role of the p110delta isoform of PI3K in B-cell antigen and IL-4 receptor signaling: comparative analysis of genetic and pharmacologic interference with p110delta function in B cells. *Blood* 2006;107:642–50. [PubMed: 16179367]
  18. Hu Y, Qiao L, Wang S, Rong SB, Meuillet EJ, Berggren M, Gallegos A, Powis G, Kozikowski AP. 3-(Hydroxymethyl)-bearing phosphatidylinositol ether lipid analogues and carbonate surrogates block PI3-K, Akt, and cancer cell growth. *J Med Chem* 2000;43:3045–51. [PubMed: 10956212]
  19. de Laszlo SE, Visco D, Agarwal L, Chang L, Chin J, Croft G, Forsyth A, Fletcher D, Frantz B, Hacker C, Hanlon W, Harper C, Kostura M, Li B, Luell S, MacCoss M, Mantlo N, O'Neill EA, Orevillo C, Pang M, Parsons J, Rolando A, Sahly Y, Sidler K, O'Keefe SJ, et al. Pyrroles and other heterocycles as inhibitors of p38 kinase. *Bioorg Med Chem Lett* 1998;8:2689–94. [PubMed: 9873604]
  20. Ye F, Zhang ZS, Luo HB, Shen JH, Chen KX, Shen X, Jiang HL. The dipeptide H-Trp-Glu-OH shows highly antagonistic activity against PPARgamma: bioassay with molecular modeling simulation. *Chembiochem* 2006;7:74–82. [PubMed: 16317783]
  21. Jamsa A, Hasslund K, Cowburn RF, Backstrom A, Vasange M. The retinoic acid and brain-derived neurotrophic factor differentiated SH-SY5Y cell line as a model for Alzheimer's disease-like tau phosphorylation. *Biochem Biophys Res Commun* 2004;319:993–1000. [PubMed: 15184080]
  22. Wang JZ, Grundke-Iqbal I, Iqbal K. Kinases and phosphatases and tau sites involved in Alzheimer neurofibrillary degeneration. *Eur J Neurosci* 2007;25:59–68. [PubMed: 17241267]
  23. Rossner S, Sastre M, Bourne K, Lichtenthaler SF. Transcriptional and translational regulation of BACE1 expression--implications for Alzheimer's disease. *Prog Neurobiol* 2006;79:95–111. [PubMed: 16904810]
  24. Qian H, Hausman GJ, Compton MM, Azain MJ, Hartzell DL, Baile CA. Leptin regulation of peroxisome proliferator-activated receptor-gamma, tumor necrosis factor, and uncoupling protein-2 expression in adipose tissues. *Biochem Biophys Res Commun* 1998;246:660–7. [PubMed: 9618269]
  25. Ramamurthy S, Ronnett GV. Developing a head for energy sensing: AMP-activated protein kinase as a multifunctional metabolic sensor in the brain. *J Physiol* 2006;574:85–93. [PubMed: 16690704]
  26. Minokoshi Y, Alquier T, Furukawa N, Kim YB, Lee A, Xue B, Mu J, Foulfelle F, Ferre P, Birnbaum MJ, Stuck BJ, Kahn BB. AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. *Nature* 2004;428:569–74. [PubMed: 15058305]
  27. Minokoshi Y, Kim YB, Peroni OD, Fryer LG, Muller C, Carling D, Kahn BB. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature* 2002;415:339–43. [PubMed: 11797013]
  28. Park S, Jang JS, Jun DW, Hong SM. Exercise enhances insulin and leptin signaling in the cerebral cortex and hypothalamus during dexamethasone-induced stress in diabetic rats. *Neuroendocrinology* 2005;82:282–93. [PubMed: 16721034]
  29. Uotani S, Abe T, Yamaguchi Y. Leptin activates AMP-activated protein kinase in hepatic cells via a JAK2-dependent pathway. *Biochem Biophys Res Commun* 2006;351:171–5. [PubMed: 17054914]
  30. Hooper C, Killick R, Lovestone S. The GSK3 hypothesis of Alzheimer's disease. *J Neurochem* 2008;104:1433–9. [PubMed: 18088381]
  31. King TD, Song L, Jope RS. AMP-activated protein kinase (AMPK) activating agents cause dephosphorylation of Akt and glycogen synthase kinase-3. *Biochem Pharmacol* 2006;71:1637–47. [PubMed: 16620785]
  32. Taniyama Y, Ushio-Fukai M, Hitomi H, Rocic P, Kingsley MJ, Pfahnl C, Weber DS, Alexander RW, Griendling KK. Role of p38 MAPK and MAPKAPK-2 in angiotensin II-induced Akt activation in vascular smooth muscle cells. *Am J Physiol Cell Physiol* 2004;287:C494–9. [PubMed: 15084475]
  33. Thornton TM, Pedraza-Alva G, Deng B, Wood CD, Aronshtam A, Clements JL, Sabio G, Davis RJ, Matthews DE, Doble B, Rincon M. Phosphorylation by p38 MAPK as an alternative pathway for GSK3beta inactivation. *Science* 2008;320:667–70. [PubMed: 18451303]
  34. Li J, Miller EJ, Ninomiya-Tsuji J, Russell RR 3rd, Young LH. AMP-activated protein kinase activates p38 mitogen-activated protein kinase by increasing recruitment of p38 MAPK to TAB1 in the ischemic heart. *Circ Res* 2005;97:872–9. [PubMed: 16179588]

35. Viollet B, Mounier R, Leclerc J, Yazigi A, Foretz M, Andreelli F. Targeting AMP-activated protein kinase as a novel therapeutic approach for the treatment of metabolic disorders. *Diabetes Metab* 2007;33:395–402. [PubMed: 17997341]
36. Semple RK, Chatterjee VK, O’Rahilly S. PPAR gamma and human metabolic disease. *J Clin Invest* 2006;116:581–9. [PubMed: 16511590]
37. Holden KF, Lindquist K, Tylavsky FA, Rosano C, Harris TB, Yaffe K. Serum leptin level and cognition in the elderly: Findings from the Health ABC Study. *Neurobiol Aging*. 2008

Fig. 1.

Leptin-mediated signaling pathways regulating tau phosphorylation. RA-SY5Y were incubated with inhibitors to known downstream effectors of leptin signaling (STAT3, AMPK, PI3K, Akt, p38) in the presence of leptin (100 nM; 1600 ng/ml – lanes 3–7), or non-treated (vehicle – lane 1). Cells treated with leptin alone served as positive control (lane 2). Whole-cell lysates were prepared and analyzed by immunoblot (Panel I) with phosphorylated tau-specific antibodies (pSer³⁹⁶, PHF-1, AT8 or pThr¹⁸¹). Membranes were stripped and re-probed with total tau antibody for normalization. Representative blots are shown, n=3. Normalized tau bands were analyzed by densitometry and results (panels II and III) are presented as the

mean $\pm$ SD percent change, relative to non-treated samples, which were arbitrarily assigned a value of 0.

* vs. non-treated (vehicle – lane 1)

** vs. leptin alone (lane 2)

Fig. 2.

Phosphorylation of downstream signaling proteins activated by leptin and AICAR. **A.** RA-SY5Y were treated with leptin (100 nM; 1600 ng/ml), AICAR (2 mM) or non-treated (vehicle), and signaling proteins (Jak2, AMPK, p38, Akt) implicated in regulating tau phosphorylation were examined by immunoblot using phosphorylation-specific antibodies. Membranes were stripped and re-probed with α-tubulin antibody for normalization. Representative blots are shown, n=3. Normalized bands were analyzed by densitometry and results (**B–E**) are presented as the mean ± SD. Phosphorylation of (**F**) GSK-3β (pSer⁹) in the above cells was measured by immunoblot. Membranes were stripped and re-probed with total GSK-3β for normalization. Bands were analyzed by densitometry and results (**G**) are presented as the mean ± SD.

* vs. non-treated

Fig. 3.

Leptin and AICAR regulate A β production via overlapping signaling pathways. **A.** RA-SY5Y were treated for 6 hrs with leptin (100 nM; 1600 ng/ml – lane 2), AICAR (2 mM – lane 5) and/or inhibitors to each of the following signaling proteins – AMPK (lanes 3 and 6) or PPAR γ (lanes 4 and 7). Non-treated (vehicle – lane 1) cells or cells treated with inhibitor alone (lanes 8–9) served as control. Culture media was collected and assayed for A β _(1–40) by ELISA. Results were normalized to total protein in cell lysates and are presented as the mean A β _(1–40) concentration (pg/ml) $\pm$ SD. **B.** Cells from A were treated with leptin, alone or in the presence of Akt inhibitor (1L6HCl). Culture media was collected and assayed for A β _(1–40) by ELISA. Results are presented as in A. **C.** Cells from A were treated with leptin alone or in the presence of PPAR γ antagonist (G3335). Whole-cell lysates were prepared and analyzed by immunoblot with phosphorylated tau-specific antibodies as described in the legend of Figure 1. **D.** Cartoon depicting the hypothesized signaling pathways activated by leptin and AICAR in RA-SY5Y.

* vs. non-treated (vehicle – lane 1)

** vs. leptin alone (lane 2)

vs. AICAR alone (lane 5)

Table 1

Kinase inhibitors and their inhibitory constants

Kinase	Inhibitor Designation	Inhibition Constant	Concentration Used	Reference
<i>AMPK</i>	Compound C	$K_i=109\text{nM}$	$1\mu\text{M}$	[14]
<i>STAT3</i>	S3I-201	Inhibits STAT3-dependent tumor growth at $100\mu\text{M}$	$100\mu\text{M}$	[15]
<i>PI3K</i>	522DB13D	$\text{IC}_{50}=4.5\mu\text{M}$	$20\mu\text{M}$	[16]
<i>Akt</i>	IL6HCl	$\text{IC}_{50}=5\mu\text{M}$	$10\mu\text{M}$	[17]
<i>p38</i>	24C44F5P	$\text{IC}_{50}=35\text{nM}$	100nM	[18]

Kinase inhibitors were used within the present cell culture model at or in excess of previously identified inhibitory concentrations. In addition, the PPAR γ antagonist, G3335 ($K_D=8\mu\text{M}$), was applied at $30\mu\text{M}$ [19]. Concentration-dependent inhibition of the target kinases were confirmed by immunoblot (data not shown).