Amyloid Beta Peptide Is an Endogenous Negative Allosteric Modulator of Leptin Receptor

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Keywords

Alzheimer's disease · Leptin receptor · Amyloid beta · Metabolism

Abstract

Introduction: Metabolic dysfunction is now recognized as a pivotal component of Alzheimer's disease (AD), the most common dementia worldwide. However, the precise molecular mechanisms linking metabolic dysfunction to AD remain elusive. **Objective:** Here, we investigated the direct impact of soluble oligomeric amyloid beta (Aβ) peptides, the main molecular hallmark of AD, on the leptin system, a major component of central energy metabolism regulation. **Methods:** We developed a new time-resolved fluorescence resonance energy transfer-based Aβ binding assay for the leptin receptor (LepR) and studied the effect of Aβ on LepR function in several in vitro assays. The in vivo effect of Aβ on LepR function was studied in an Aβ-specific AD mouse model and in pro-opiomelanocortin (POMC) neurons of the hypothalamic arcuate nucleus. **Results:** We revealed specific and high-affinity ($K_i = 0.1$ nM) binding of Aβ to LepR. Pharmacological characterization of this interaction showed that Aβ binds allosterically to the extracellular domain of LepR and

negatively affects receptor function. Negative allosteric modulation of LepR by Aβ was detected at the level of signaling pathways (STAT-3, AKT, and ERK) in vitro and in vivo. Importantly, the leptin-induced response of POMC neurons, key players in the regulation of metabolic function, was completely abolished in the presence of Aβ. **Conclusion:** Our data indicate that Aβ is a negative allosteric modulator of LepR, resulting in impaired leptin action, and qualify LepR as a new and direct target of Aβ oligomers. Preventing the interaction of Aβ with LepR might improve both the metabolic and cognitive dysfunctions in AD condition.

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Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease worldwide, characterized by progressive memory loss and cognitive dysfunction. At the molecular level, AD is defined by the presence of extracellular aggregates of amyloid beta (Aβ) peptide, especially the 42-amino-acid-long form (Aβ₁₋₄₂), and of intracellular neurofibrillary tangles of tau protein, which correlate with cognitive deficits in advanced AD patients [1]. There

is currently no treatment to stop or to revert AD progression, and our limited knowledge on the onset of the disease hampers the development of AD-specific drugs.

Accumulating evidence suggests that AD involves a metabolic component, with pronounced impairment in brain glucose metabolism [2] and altered response to hormones involved in metabolism [3, 4]. The link between metabolic dysfunction and AD is further supported by the fact that type 2 diabetes (T2D) and middle-life obesity significantly increase the risk of developing AD [5, 6]. Altered levels of the hormone leptin and/or dysfunction of leptin signaling is frequently observed in both metabolic disorders like T2D and obesity as well as in AD [7]. These data suggest that metabolic dysfunction might be a noncognitive early symptom of AD. Accordingly, transgenic mice lacking leptin receptor (*db/db* mice) or deprived of leptin production (*ob/ob* mice) show significant cognitive impairment in their adulthood [8–11]. Importantly, mice obtained by crossing the *db* or *ob* mice with an AD ($APP^{\Delta NL/\Delta NL} \times PS1^{P264L/P264L}$) mice model (*db/AD*, *ob/AD*) display more pronounced and earlier impairment in learning and memory performance, as well as earlier metabolic dysfunction [12, 13]. The link between these pathologies seems therefore to be bidirectional, with one facilitating the development of the other. At the molecular level, however, little is known on the mechanisms underlying such interactions.

Leptin is a polypeptide hormone primarily produced by adipocytes that regulates food intake and energy metabolism by acting in the hypothalamus. Leptin effects are mediated by its membrane receptor – LepR, which belongs to the cytokine receptor class I family. Although different isoforms of LepR can be generated by alternative splicing, the long isoform LepRb is thought to be the main isoform mediating leptin signaling (reviewed by Peelman et al. [14]). LepR activation and signaling need to be tightly regulated to maintain glucose and energy homeostasis (reviewed by Roujeau et al. [15]). Excessive leptin levels lead to the development of leptin resistance and metabolic disorders [16, 17].

A growing body of evidence shows that leptin functions go beyond food intake control and also regulate neuronal activity and plasticity. LepR is not only expressed in the hypothalamus, the main center of energy regulation, but also in the cortex and hippocampus, 2 major brain regions affected in AD [18, 19]. Leptin administration is able to improve cognitive performance [20] and confers neuroprotection against glutamate toxicity [21], brain ischemic injury [22], and apoptosis induced by nutrient deprivation [23] or 6-hydroxydopamine [24]. In

humans, leptin replacement therapy of subjects with congenital leptin deficiency rescues structural brain abnormalities, neuronal activity, and cognitive deficits [25–27]. Whereas it is still controversial whether circulating leptin levels are increased or decreased in AD patients [7, 28], a deficit in the leptin response due to decreased expression of LepR and downstream signaling molecules is commonly acknowledged with AD progression [4, 7, 29]. Accordingly, altered food intake and energy expenditure have been reported in AD models [30–32] indicating a deficient leptin response. However, direct information on the functional status of the LepR in AD and on the precise connection between the leptin system and AD-related pathological mechanisms are still missing. Here, we show that LepR is directly targeted by A β oligomers, which dampens leptin signaling through negative allosteric modulation upon binding to LepR, ultimately impacting the responsiveness of hypothalamic neurons to this hormone.

Materials and Methods

Cell Culture

HEK293T and N46 cell lines were maintained in DMEM Glutamax (Invitrogen) culture medium supplemented with 10% FBS at 37 °C (95% O₂, 5% CO₂). Cells seeded in P6 well plates were transfected with plasmids coding to SNAP-LepRa, SNAP-LepRb, or SNAP-EGFR receptors using the jetPEI reagent, according to the supplier's instructions (Polyplus-Transfection, New York, NY, USA).

Preparation of A β Oligomers

Lyophilized A β_{1-42} was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol, aliquoted and reconstituted in anhydrous DMSO and PBS prior to use. The peptide solution was incubated for 24 h at 4 °C to generate oligomers, as previously described [33].

In vivo A β Model

Male Swiss CD-1 mice, aged 7–9 weeks and weighing 32 $\pm$ 2 g (Janvier, St Berthevin, France) were housed in the animal facility of the University of Montpellier (CECEMA, registration number D34-172-23), in a temperature and humidity controlled facility on a 12 h/12 h light/dark cycle (lights on at 7:00 h) with access to food and water ad libitum. The amyloid β_{25-35} peptide (A β_{25-35}) and scrambled A β_{25-35} peptide (Eurogentec, Angers, France) were solubilized in sterile distilled water (3 mg/mL) and stored at –20 °C. Before injection, peptides were aggregated by incubation at 37 °C for 4 days. They were administered under isoflurane anesthesia, intracerebroventricularly in a volume of 3 μ L per mouse according to the previously published method [34, 35]. The following day, mice were i.p. injected with either saline (control) or leptin (2 mg/kg body weight) and sacrificed by cervical dislocation after 30 min. Brain tissue was immediately dissected to collect the hippocampus and hypothalamus, and samples were stored at –80 °C until analysis by Western blot.

SDS-PAGE

Detection of signaling molecules was performed in lysates from cells or brain tissues prepared by homogenization in NP40 lysis buffer (50 mM Tris pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 0.25% sodium deoxycholate, containing protease and phosphatase inhibitors). Protein level was quantified by the BCA method (ThermoFisher Scientific), and 50 µg of protein was mixed in 2× Laemmli buffer. Denatured proteins (95 °C, 5 min) were resolved in 10% SDS-PAGE, transferred to nitrocellulose membranes, blocked in 5% nonfat dried milk or 3% BSA in TBS (10 mM Tris-HCl, pH 8, 150 mM NaCl) and immunoblotted with primary antibodies against the phosphorylated and total form of STAT-3 (Cell Signaling, Ref. 9145S and 9139S, Leiden, Netherlands), AKT (Cell Signaling, Ref. 587F11 and 4685), and ERK (P-ERK1/2 sc-7383; ERK2 sc-154; Santa-Cruz Biotechnology, Dallas, TX, USA) proteins (overnight, 4 °C), or against Aβ (6E10, 803001; Biologend, San Diego, CA, USA). Immunoreactivity was revealed using secondary antibodies coupled to 680 or 800 nm fluorophores (1:15,000, diluted in 3% nonfat dried milk in TBS; LI-COR Biosciences, Lincoln, NE, USA), and readings were performed with the Odyssey LI-COR-IR fluorescent scanner (LI-COR Biosciences).

Receptor Subdomains

Recombinant human EGFR extracellular domain (ECD) was purchased from R&D Systems (Ref. 344-ER, Minneapolis, MN, USA). LepR domains were produced using expression vectors coding for the different ECDs of the LepR coupled to a combined FLAG-His tag, as previously described [36]. The recombinant proteins were expressed in Freestyle 293-F cells and purified from the supernatants using the C-terminal 6*His-tag with TALON metal affinity resin (Clontech). Imidazole (Merck) was used for elution and removed using PD-10 gel filtration columns (GE Healthcare). Protein concentrations were determined using the absorbance at 280 nm (Nanodrop; ThermoFisher Scientific, Waltham, MA, USA) and purity via SDS-PAGE. The correct folding of the ECDs into the biologically relevant conformations is attested by previous studies from our team in which the ECDs were used for structural studies by small-angle X-ray scattering [37], for leptin binding studies and to generate single-domain camelid antibodies able to recognize LepR in vitro and in vivo [38–40].

AlphaLISA

Intracellular phospho-ERK1/2 (Thr202/Tyr204), phospho-AKT, and phospho-STAT-3 were measured using the respective AlphaLISA kit (ALSU-PERK-A500, ALSU-PAKT-B500, ALSU-PST3-A500; Perkin Elmer, Waltham, MA, USA). Briefly, 1 day after transfection, HEK293T or N46 cells were trypsinized and plated into 48-well plates and serum-starved for 16 h prior to stimulation. After adding the ligand, plates returned to the incubator for the indicated incubation time. Plates were then placed on ice, medium was aspirated, and cells were lysed by adding 20 µL of lysis buffer (provided by the manufacturer) and using cell scraper. Lysates were added to a 384-well plate to run the assay according to the manufacturer's instructions. The plates were read using the Tecan Infinite M1000 PRO microplate reader (Tecan Group, Ltd., Männedorf, Switzerland).

Time-Resolved Fluorescence Resonance Energy Transfer Binding Assay

Cells expressing SNAP-tagged LepR were fluorescently labelled with the Terbium cryptate fluorophore (Tb; Lumi4-Tb; Cisbio

Bioassays, France) using the SNAP enzyme technology (Keppler et al. [51]). Cells were incubated with 10^{-7} M of Tb-SNAP substrate in Tag-lite labeling medium (1 h, 4 °C), washed several times, detached using a Cell Dissociation buffer (13151014; ThermoFisher Scientific), collected, and distributed into 384-well plate. Cells were then incubated with fluorescently labeled Aβ-FAM oligomers (AS-23525; Anaspec, Fremont, CA, USA), or fluorescently labeled ligands of LepR (leptin-d2, LAN-d2, custom label by Cisbio Bioassays) in the presence or absence of competitors (2–4 h, RT), and the time-resolved fluorescence resonance energy transfer (TR-FRET) signal was read in the plate reader Tecan F500 (Tecan) (ex: 340 nm/em: 520 nm (acceptor Aβ-FAM) or 665 nm (acceptor leptin-d2); and 620 nm (donor)). Data are expressed as acceptor/donor ratio or normalized as percent of maximal binding (maximal TR-FRET ratio = 100%).

BRET Assay

HEK293T cells were transfected with expression plasmids coding for LepR fused to *Renilla* luciferase (Rluc) enzyme and LepR fused to yellow fluorescent protein (YFP), at 1:1 DNA ratio, or with increasing amounts of the energy acceptor (YFP-fused LepR) for saturation curves. Cells were plated in white 96-well microplates, and assays were performed 48 h after transfection, as previously described [41]. Briefly, culture medium was removed and replaced by phosphate-buffered saline, followed by addition of Rluc substrate (coelenterazine h, 5 µM; Ref. UPR30783; Interchim, Montluçon France) and incubation for 5 min before addition of leptin, when indicated. Plates were immediately read using a lumino/fluorometer (Mithras; Berthold Technologies), which allows sequential integration of Rluc (475–495 nm) and YFP (517–543 nm) emission signals. Bioluminescence resonance energy transfer (BRET) signal is expressed as excited YFP signal/Rluc signal ratio × 1,000 to obtain miliBRET units (mBU). BRET₅₀ value refers to the acceptor/donor ratio concentration required to generate half-maximal BRET signal and were obtained from saturation curves.

Electrophysiology

Pomc-Cre:ROSA-tdTomato mice were generated by breeding Pomc-Cre mice to ROSA-tdTomato mice purchased from The Jackson Laboratories (Pomc-cre line: 005965 – STOCK Tg(Pomc1-cre)16LowI/J; ROSA-tdTomato line: 007914 – B6.Cg-Gt(ROSA)26Sor^{tm14(CAG-tdTomato)Hze/J}). Both lines were maintained on C57BL6 background, and Pomc-Cre driver and tdTomato Cre reporter mice were used in the heterozygous state for experiments.

Electrophysiological recordings were performed on living brain slices containing the tuberal region of the hypothalamus from 8- to 12-week-old transgenic *Pomc-Cre::tdTomato* mice as previously described [42]. Briefly, mice were anesthetized with isoflurane and decapitated. The brain was removed and rapidly placed in ice-cold artificial cerebrospinal fluid (ACSF) containing the following (in mM): 120 NaCl, 3.2 KCl, 1 NaH₂PO₄, 26 NaHCO₃, 1 MgCl₂, 2 CaCl₂, 2.5 glucose (290 mOsm, pH 7.4) and bubbled with 95% O₂–5% CO₂. Three 250-µm coronal hypothalamic slices containing the arcuate nucleus (ARC) were cut using a vibratome (VT1200; Leica). Slices were incubated at 34 °C in oxygenated ACSF for a recovery period of 1 h, and then at room temperature until recording. For the Aβ experiments, scrambled control Aβ or Aβ_{1–42} were added to ACSF during the incubation period.

For patch-clamp recording, individual brain slices were placed in a recording chamber (Warner Instruments), immobilized by a

nylon grid and continuously perfused at 3 mL/min with oxygenated ACSF which is maintained at 32.8 °C by a heater controller (TC-344C; Warner Instruments). Pro-opiomelanocortin (POMC) neurons were visualized and identified using a $\times 40$ magnification in an upright fluorescent microscope with infrared differential interference contrast (Leica DM-LFSA) and a digital camera (Hamamatsu Photonics). Recording pipettes were pulled from borosilicate glass capillaries (1.5 mm outer diameter; 1.12 mm inner diameter; World Precision Instruments) using P1000 Flaming Brown and had resistance from 4 to 6 M Ω when filled with an internal solution containing the following (in mM): 123 K-gluconate, 2 MgCl₂, 8 KCl, 0.2 EGTA, 4 Na₂-ATP, 0.3 Na-GTP, and 10 HEPES, pH 7.3 with KOH. Pipettes were placed in contact with a POMC neuron using a PCS-5400 micromanipulator (Thorlabs). Whole-cell patch-clamp recordings were performed in the current-clamp mode by using a Multiclamp 700B Amplifier, digitized using the Digidata 1322A interface and acquired using pClamp 10.2 software (Molecular Devices). Data were filtered at 1 kHz and sampled at 5 kHz.

To study the effect of A β on neuronal response of POMC neurons to leptin or Glp1, leptin (20 nM) or Glp1 (20 nM) was applied to the perfusing system after a stable baseline recording. Recordings were analyzed with Clampfit 10.2 from pClamp software (Molecular Devices). For each recording, membrane potential and mean firing rate were determined before and during the bath application of leptin. Neurons were considered responsive when a change of >20% in firing rate was observed. The peak response was determined, and the number of spikes was counted 2 min before and after the peak effect.

Data and Statistical Analysis

Data are presented as mean with SEM of the indicated *n* number of independent experiments. Nonlinear regression and statistic tests were performed using GraphPad 6 software (GraphPad Software, version 6.0b). For data sets with equal variances, the parametric Student *t* test was used to compare 2 groups, while analysis of variance (ANOVA) test, followed by Tukey's or Dunnett's multiple comparison test, was used to compare >2 groups. Two-way ANOVA, followed by Tukey's multiple comparison test, was used to evaluate the interaction factor of the independent variables (control vs. A β groups) on the dependent variable (leptin-induced response). Values of *p* < 0.05 were considered statistically significant.

Results

A β Impairs Leptin Signaling *in vitro* and *in vivo*

Leptin is known to activate several signaling pathways including the JAK2/STAT-3, PI3K/AKT, and ERK1/2 pathways [43, 44]. In order to study the impact of soluble A β _{1–42} oligomers (oA β) – the pathologically most relevant species of A β in AD – on LepR function, we evaluated leptin signaling in the N46 mouse hypothalamic cell line [45] and in human embryonic kidney (HEK293) cells ectopically expressing the signaling competent LepRb isoform. Preparations of oA β were characterized by Western blot, revealing the presence of oA β migrating at an apparent MW of 17 kDa and the absence of high MW

A β aggregates (online suppl. Fig. S1a, b; see www.karger.com/doi/10.1159/000508105 for all online suppl. material). Stimulation of cells with leptin time-dependently increased the phosphorylation of STAT-3, AKT, and ERK1/2 with peak levels between 5 and 15 min (Fig. 1a, b). Preincubation of cells with oA β largely diminished or prevented the leptin-induced effects in both cell types (Fig. 1a–d). The inhibitory effect of oA β was not due to cell toxicity, as no significant cell death was detected up to 1 μ M oA β (online suppl. Fig. S1c), or to decreased cell surface expression of LepRb (online suppl. Fig. 1d), known to impact the amplitude of LepR signaling [46]. oA β preincubation had no effect on Il-6-induced STAT-3 phosphorylation (Fig. 1e, f), or on serum-induced AKT and ERK1/2 activation (Fig. 1g, h), excluding a general inhibitory effect of A β _{1–42} on these signaling pathways. The inhibitory effect of oA β on leptin (1 nM)-induced signaling at peak levels (15 min) was concentration-dependent with IC₅₀ values in the low nanomolar range (Table 1), as assessed directly in cell lysates using the quantitative AlphaLISA technique (Fig. 1i–k).

These results were further confirmed *in vivo* in a previously described A β -specific AD model, which is based on i.c.v. administration of the oligomeric A β _{25–35} fragment [34, 35]. Because A β _{25–35} is more prone to form oligomers and aggregates than A β _{1–42} while retaining comparable neurotoxic properties (reviewed by Millucci et al. [47]), it is the preferred and validated model for *in vivo* investigation of A β effects in rodents due to the rapid development of cognitive impairment compared to full-length A β _{1–42}. In this accelerated AD model, cognitive impairment is observed as early as 7 days post-injection, as also confirmed in our study using the Y-maze spontaneous alternation test (online suppl. Fig. 2a). In order to evaluate the acute effect of A β on leptin signaling, A β _{25–35}-treated mice received intraperitoneal administration of leptin (or saline as control) 1 day after the i.c.v. injection of A β _{25–35}. The same protocol was performed in mice injected with the scrambled A β _{25–35} peptide (A β _{scr}) as control. ERK1/2, STAT-3, and AKT activation was determined 30 min later in the hypothalamus and hippocampus, 2 brain areas relevant for metabolic control and cognitive performance, respectively (Fig. 1l–o, online suppl. Fig. 2b, c). At this time point, there was no detectable leptin-induced ERK1/2 activation in those tissues (data not shown). In A β _{25–35}-injected animals, the basal STAT-3 phosphorylation level was increased in the hypothalamus and hippocampus compared to A β _{scr}-injected animals (Fig. 1l–o, online suppl. Fig. 2b, c). Basal AKT phosphorylation was similar in both groups (Fig. 1l–o,

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Table 1. Inhibitory effect of A β on leptin-induced signaling pathways

	pIC ₅₀	I _{max} , %
P-STAT-3	9.7 $\pm$ 0.5	69.6 $\pm$ 7.7
P-AKT	8.8 $\pm$ 0.4	96.2 $\pm$ 15.5
P-ERK	7.7 $\pm$ 0.3	88.3 $\pm$ 9.4

Concentration-response curves were analyzed by non-linear regression, and the potency of A β inhibition on leptin-induced signaling activation is expressed as pIC₅₀ $\pm$ SEM. Maximal inhibition (I_{max}) is expressed as a percentage (mean $\pm$ SEM) of the maximal activation induced by leptin in the absence of A β .

online suppl. Fig. 2b, c). The effect of A β _{25–35} on STAT-3 activation could not be determined in the hippocampus as no leptin-induced STAT-3 activation was observed under our experimental conditions (Fig. 1n). In the hypothalamus, leptin treatment induced STAT-3 and AKT phosphorylation, as expected, in A β _{scr}-injected animals, and this effect was abolished in A β _{25–35}-injected animals (Fig. 1l, m). In the hippocampus, leptin-induced AKT activation was similarly decreased in A β _{25–35}-injected animals (Fig. 1o). Altogether, these data indicate that A β oligomers induce spontaneous STAT-3 activation which is insensitive to leptin stimulation and inhibit leptin-induced AKT activation.

A β Binds Directly to the LepR

A β oligomers have been shown to interact with various cellular components like lipids, sugars, and proteins [48–50]. To explore the possibility of a direct binding of oA β to LepR, we developed a TR-FRET-based oA β binding assay. The assay is based on the energy transfer between the energy donor (terbium-labeled SNAP-LepR) and en-

ergy acceptor (fluorescently labeled A β _{1–42} oligomers, oA β -FAM), which occurs only if both are in close proximity to each other (<10 nm; Fig. 2a). SNAP is an O6-alkylguanine-DNA alkyl transferase, that catalyzes its own covalent binding to fluorescent derivatives of benzylguanine such as Lumi4-Tb [51]. Stoichiometric labeling of LepR is achieved by incubating cells expressing N-terminally SNAP-tagged LepR with Lumi4-Tb. The biological activity of both components, SNAP-LepR and oA β -FAM, has been previously validated [33, 52]. Successful generation of oligomeric A β -FAM was monitored by Western blot showing a similar migration pattern compared to unlabeled oA β preparations (online suppl. Fig. S1b). Using this binding assay, we observed saturable binding of the oA β -FAM tracer to Lumi4-Tb-labelled SNAP-LepRb with nanomolar affinity (K_d = 169 $\pm$ 64 nM; Fig. 2b). Non-specific binding was defined in the presence of an excess of nonlabeled oA β (1 μ M). A β -FAM binding was observed in a time- and A β -FAM concentration-dependent manner (Fig. 2c), with K_{on} and K_{off} values of 5.6 $\times$ 10⁴ M⁻¹ min⁻¹ and 0.007 min⁻¹, respectively. The calculated K_d of 131 nM (K_d = K_{off}/K_{on}) is in good agreement with the K_d value determined at equilibrium.

Unlabeled oA β showed high-affinity binding to LepRb in the subnanomolar range (pK_i = 10.7 $\pm$ 0.4) as determined in competition binding experiments with the oA β -FAM tracer (Fig. 2d). Similar results were obtained with the short LepRa isoform (pK_i = 10.5 $\pm$ 0.3), which contains the same ECD as LepRb (Fig. 2e). Taken together, these results show that oA β binds to the ECD of LepR with high affinity. Unlabeled oA β binds with sub-nanomolar affinity and the fluorescently labeled oA β -FAM with nanomolar affinity.

The hypothesis of oA β -FAM binding to the ECD of LepR was further confirmed as coincubation with the purified ECD of LepR prevented oA β -FAM binding to Lep-

Fig. 1. oA β impairs leptin signaling. **a** Representative Western blot analysis of A β _{1–42} (1 μ M, 2 h) effect on leptin-induced phosphorylation of STAT-3, AKT, and ERK signaling molecules after 5, 15, 30, and 45 min of incubation with leptin (5 nM) in HEK293T cells expressing LepRb receptor. **b** Quantification of data shown in **a**; data are expressed as mean $\pm$ SEM of 5–7 independent experiments. * p < 0.05; ** p < 0.01; *** p < 0.001 by two-way ANOVA test. **c** Representative Western blot analysis of A β _{1–42} (1 μ M, 2 h) effect on leptin-induced (5 nM, 15 min) phosphorylation of STAT-3, AKT, and ERK signaling molecules in N46 cell line expressing LepRb receptor. **d** Quantification of data shown in **c**; data are expressed as mean $\pm$ SEM of 3 independent experiments; * p < 0.05; ** p < 0.01; *** p < 0.001 by two-way ANOVA test. **e** Representative Western blot analysis of A β _{1–42} (1 μ M, 2 h) effect on IL-6-induced phosphorylation of STAT-3. **f** Quantification of data shown

in **e**; data are expressed as mean $\pm$ SEM of 3 independent experiments. **g** Representative Western blot analysis of A β _{1–42} (1 μ M, 2 h) effect on fetal bovine serum (FBS, 2%)-induced phosphorylation of AKT and ERK in nontransfected HEK293T cells. **h** Quantification of data shown in **g**; data are expressed as mean $\pm$ SEM of 3 independent experiments. **i–k** Dose-dependent inhibition by A β _{1–42} (2 h) of leptin-induced phosphorylation of STAT-3 (**i**), AKT (**j**), and ERK (**k**) in HEK293T cells expressing LepRb, quantified by Alpha-LISA assay; n = 3–5. **l–o** Phospho- and total levels of STAT-3 and AKT in the hypothalamus (**l**, **m**) and hippocampus (**n**, **o**) of mice injected i.c.v. with A β _{25–35} or with control scrambled A β (A β _{scr}) following i.p. administration of saline (control) or leptin (2 mg/kg body weight, 30 min); n = 5 mice per group; * p < 0.05, ** p < 0.005, *** p < 0.0001 by two-way ANOVA test. Representative blots are shown in online supplementary Figure S2. ns, nonsignificant.

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Rb-expressing cells (Fig. 2f). A similar quantity of the ECD of the EGFR was without any effect (Fig. 2f). By using previously described subdomains of the ECD of LepR as competitors [38] in the oA β -FAM binding assay, we found that subdomains comprising the immunoglobulin (Ig)-like motif and the fibronectin III (FNIII) domains competed oA β binding to LepR (Fig. 2g). When using fluorescently labeled leptin (leptin-d2) as tracer, only addition of subdomains containing the leptin binding-competent cytokine receptor homology (CRH)2 domain [53] expectedly prevented leptin-d2 binding to LepRb, thus validating the assay and the functionality of the CRH2

domain (Fig. 2h). Binding of oA β -FAM to Ig-like and FNIII domains was further confirmed in an in vitro binding assay by probing oA β -FAM binding to wells coated with different LepR ECDs (Fig. 2i). This strongly suggest direct binding of oA β -FAM to the Ig-like and FNIII domains of LepR and excludes the participation of any cellular component like lipids, sugars, or other proteins. Collectively, these data indicate that oA β binds specifically to the Ig-like and FNIII domains of the ECD of LepR. These sites are distinct from the orthosteric high-affinity leptin binding site located in the CRH2 domain, thus indicating a possible allosteric binding mode of oA β .

Fig. 3. oA β allosteric modulation of leptin binding to LepR. **a** Competition of leptin-d2 binding to Tb-SNAP-LepR in HEK293T cells by increasing concentrations of nonlabelled leptin or A β measured by TR-FRET. **b** Competition of leptin-d2 binding to Tb-SNAP-LepR by increasing concentrations of nonlabelled A β . **c** Dis-

sociation kinetics of leptin in the absence or presence of A β (1 μ M). **d** Competition of LAN-d2 binding to Tb-SNAP-LepR by increasing concentrations of nonlabelled LAN or A β measured by TR-FRET. Data are expressed as mean $\pm$ SEM of at least 3 independent experiments, each performed in triplicates.

Fig. 2. oA β binding to leptin receptor. **a** Scheme illustrating the TR-FRET-based A β -FAM binding assay to SNAP-tagged LepR labelled with Lumi-Tb, indicating the 4 subdomains of the extracellular domain of LepR (CRH1, Ig-like, CRH2, and FNIII). **b** Saturation binding curve of A β -FAM to Lumi-Tb-SNAP-LepR ($n = 4$). Nonspecific binding was defined in the presence of an excess of nonlabeled oA β (1 μ M). **c** Association kinetics of A β -FAM binding to Lumi-Tb-SNAP-LepR ($n = 3$). **d, e** Competition of A β -FAM binding to Tb-SNAP-LepRa (**d**) or Tb-SNAP-LepRb (**e**) by increasing concentrations of nonlabelled A β . Data are expressed as mean $\pm$ SEM of at least 3 independent experiments, each performed

in triplicates. **f** Competition of A β -FAM binding to Tb-SNAP-LepR by purified extracellular domain (ECD) of LepR or EGFR receptors; **** $p = 0.0001$ by one-way ANOVA, $n = 4$ independent experiments, each performed in triplicates. **g, h** Competition of A β -FAM (**g**) or leptin-d2 (**h**) binding to Tb-SNAP-LepR by purified subdomains of the LepR extracellular domain; $n = 5$ independent experiments, each performed in triplicates; * $p < 0.05$, **** $p < 0.0001$ by one-way ANOVA. Experiments shown in panels **b–h** were performed in HEK293T cells transfected with SNAP-LepR. **i** ELISA binding of A β -FAM to 96-well black plate wells coated with purified subdomains of the LepR extracellular domain; $n = 5$.

Allosteric Binding Mode of A β to the LepR

After having established that oA β binds to LepR at a site distinct from the high-affinity leptin binding site in the CRH2 domain, we wanted to know whether leptin binding to LepR is allosterically modulated by oA β . We first measured the effect of oA β on the binding of the leptin-d2 tracer to the Lumi4-Tb-labelled SNAP-LepRb. oA β concentration dependently inhibited leptin-d2 binding ($pIC_{50} = 8.7 \pm 0.4$) with a maximal inhibition of $25.2 \pm 2.4\%$ compared to the reference competitor leptin (Fig 3a). This partial inhibition is compatible with an allosteric binding mode. Competition of the leptin-d2 tracer by leptin in the presence of different oA β concentrations showed that oA β concentration dependently diminishes the E_{max} but not the affinity (pK_i) of unlabeled competitor leptin for LepR (Fig. 3b; Table 2). Finally, we performed kinetic ligand dissociation experiments to determine the off-rate (K_{off}) of leptin in the absence or presence of oA β , a commonly used experimental setting to demonstrate an allosteric binding mode. oA β increased the K_{off} of leptin by 24%, from 0.0006373 to 0.0007885 min^{-1} ($p = 0.02$) (Fig. 3c), proportionally decreasing therefore the half time ($t_{1/2}$) of bound leptin and revealing a negative allosteric binding mode of oA β . The antagonist LAN is a mutated leptin [54] known to bind LepR at the same site and with the same affinity as leptin but without activating LepR [55]. The oA β was similarly able to compete binding of the fluorescently labeled LepR antagonist LAN-d2 with a maximal inhibition of $26 \pm 6.3\%$ and a pIC_{50} of 7.7 ± 0.7 (Fig. 3d). Collectively, these results indicate a negative allosteric effect of oA β on leptin binding to LepR.

A β Is a Negative Allosteric Modulator of LepR Function

We next evaluated the effect of oA β on LepR function. The first events induced by leptin binding are intramolecular conformational changes and clustering of preformed LepR dimers [41, 56]. Both events can be monitored with the previously described BRET assay measuring the proximity between 2 LepR fusion proteins, fused either to the Rluc energy donor or the YFP energy acceptor at their C-termini [41] (Fig. 4a).

To evaluate the effect of oA β on preexisting LepR dimers, we performed BRET donor saturation experiments by coexpressing a constant amount of the LepR-Rluc and increasing amounts of LepR-YFP. Progressive interaction and saturation of the LepR-Rluc donor by the LepR-YFP acceptor is observed, and oA β (1 μM) does not modify this basal BRET signal at any donor/acceptor ratio (Fig. 4b). Accordingly, the calculated BRET₅₀ value, cor-

Table 2. Efficiency and affinity of leptin binding to LepR in the absence or presence of A β

	Leptin	Leptin + A β (0.3 μM)	Leptin + A β (1 μM)	Leptin + A β (3 μM)
pK_i	10 ± 0.1	9.7 ± 0.2	10 ± 0.3	9.4 ± 0.3
B_{max} , %	100	90.7 ± 5.6	$79.6 \pm 6.5^*$	$58.8 \pm 5.7^*$

* $p < 0.05$ compared to leptin group by F test. Concentration-response curves were analyzed by non-linear regression, and the affinity of leptin to LepR is expressed as $pK_i \pm \text{SEM}$. Maximal leptin-d2 binding (B_{max}) is expressed as percentage (mean $\pm$ SEM).

responding to the natural relative propensity of the 2 receptors to interact with each other is unaffected by oA β (BRET₅₀ = 357.9 ± 66.7 and 325.0 ± 60.9 ; without and with oA β , respectively). Taken together, these results suggest that, despite the fact that oA β binds to the inactive form of LepR in the absence of ligand, it does not interfere with LepR dimerization in the basal state nor does it induce any detectable conformational change.

We then determined the effect of oA β on the leptin-activated LepR. Leptin increased the BRET signal in a concentration-dependent manner at a LepR donor/acceptor ratio close to the BRET₅₀ as previously reported [41] (Fig. 4c). In the presence of oA β , the leptin response curve was right-shifted, and the maximal BRET value was diminished (Fig. 4c). At 1 nM leptin (EC_{50}), oA β concentration dependently diminished the leptin-induced BRET with a pIC_{50} in the nanomolar range ($pIC_{50} = 7.7 \pm 0.2$) reaching a plateau corresponding to approximately 50% of the leptin BRET signal (Fig. 4d). This result suggests that oA β significantly modifies the conformation of LepR adopted in the presence of leptin.

We then validated the negative allosteric action of oA β on LepR function by evaluating the effect of oA β on different LepR signaling pathways (Fig. 4e–g). Leptin induced the phosphorylation of STAT-3, AKT, and ERK1/2 with pEC_{50} values of 9.4 ± 0.3 , 10.3 ± 0.2 and 9.0 ± 0.3 , respectively, in transfected neuronal N46 cells. The presence of different oA β concentrations showed a tendency to shift the concentration-response curves to the right and significantly diminished the maximal potency (E_{max}) by 50–70% of the leptin response depending on the pathway (Fig. 4e–g; Table 3). Altogether, these data support the definition of oA β as a negative allosteric modulator (NAM) of LepR on the STAT-3, AKT, and ERK signaling pathways.

Fig. 4. oAβ allosteric modulation of leptin-induced LepR conformational change and signaling. **a** Scheme of BRET assay between LepR-fused *Renilla* luciferase (Rluc, donor) and LepR-fused-YFP (acceptor). **b** Representative BRET donor/acceptor saturation curve in the absence (control) or presence of Aβ. **Insert** BRET₅₀ values. Data are expressed as mean ± SD; similar results were observed in 3 independent experiments, each performed in triplicates. **c** Concentration-response curve of leptin-induced BRET signal in the absence or pres-

ence of Aβ. Data are expressed as mean ± SEM of 4 independent experiments, each performed in triplicates. **d** Concentration-dependent curve of Aβ on leptin-induced BRET signal. Data are expressed as mean ± SEM of 3 independent experiments, each performed in triplicates. **e–g** Allosteric modulation of Aβ on leptin-induced phospho-STAT-3 (**e**), phospho-AKT (**f**), and phospho-ERK (**g**) measured by AlphaLISA. Data are expressed as mean ± SEM of 5 independent experiments, each performed in duplicates.

Table 3. Negative allosteric modulation of A β on leptin-induced signaling pathways

	Leptin	Leptin + A β (0.3 μ M)	Leptin + A β (1 μ M)	Leptin + A β (3 μ M)
P-STAT-3				
pEC ₅₀	9.4 $\pm$ 0.3	9.2 $\pm$ 0.3	9.6 $\pm$ 0.3	9.6 $\pm$ 0.3
E _{max} , %	100	98 $\pm$ 12	65.5 $\pm$ 7.2*	61 $\pm$ 7.5*
P-AKT				
pEC ₅₀	10.3 $\pm$ 0.2	9.8 $\pm$ 0.3	9.8 $\pm$ 0.3	9.9 $\pm$ 0.5
E _{max} , %	100	72.3 $\pm$ 5.8	58.7 $\pm$ 5.8*	51.5 $\pm$ 6.9*
P-ERK				
pEC ₅₀	9.0 $\pm$ 0.3	8.5 $\pm$ 0.2	8.7 $\pm$ 0.2	8.6 $\pm$ 0.4
E _{max} , %	100	95.6 $\pm$ 10	79.1 $\pm$ 7.5*	70.7 $\pm$ 12*

* $p < 0.05$ compared to leptin group by F test. Concentration-response curves were analyzed by non-linear regression, and the affinity of leptin to LepR is expressed as pK_i $\pm$ SEM. Maximal leptin-d2 binding (B_{max}) is expressed as percentage (mean $\pm$ SEM).

A β Impairs Neuronal Response to Leptin

To investigate whether the observed negative allosteric effect of oA β on LepR function has an impact in a native context, we performed patch-clamp experiments on anorexigenic POMC neurons in slices of the hypothalamic ARC, one of the main leptin target cells [57]. To reveal a NAM effect, we studied the effect of oA β on a saturating concentration of leptin (20 nM) in terms of LepR activation and signaling (Fig. 4). We evaluated the effect of oA β in terms of membrane potential and neuronal firing. Under these conditions, leptin depolarized 6 out of 10 POMC cells by 2–6 mV, similar to what was previously reported [58] (Fig. 5a, d). Whereas preincubation of brain slices with scrambled control oA β (A β -scrambled) did not modify the leptin effect (5 out of 8 POMC neurons were depolarized by leptin; Fig. 5b, e), preincubation with oA β abolished the leptin-induced depolarization in 6 out of 6 neurons (Fig. 5c, f). Consistent with the aforementioned data, we found that bath application of 20 nM leptin increased the firing rate of POMC neurons in the presence or absence of scrambled oA β , but this response was markedly inhibited in the presence of oA β (Fig. 5g–i). In order

to verify the specificity of the oA β effect, we performed identical experiments with glucagon-like peptide 1 (Glp1), a hormone that, similarly to leptin, elicits an excitatory response in POMC neurons [59]. As expected, Glp1 depolarized POMC neurons and increased their firing rate (Fig. 6a, c, e). Contrary to what was observed with leptin, the presence of oA β in the bath did not alter the neuronal response to Glp1 (Fig. 6b, d, f). In summary, these data indicate that the inhibitory effect of A β on the membrane depolarization and firing activity of POMC neurons is specific for the leptin-induced response and likely due to the NAM activity of oA β on LepR.

Discussion

Accumulating evidence supports the notion that metabolic dysfunction is an integral part of AD, for which the term type 3 diabetes has been proposed (reviewed by de la Monte [60]). Here, we investigated whether A β oligomers (oA β) could contribute to the metabolic dysfunction observed in AD by targeting a key player of energy and glucose homeostasis, the LepR. We show that oA β directly binds to the Ig-like and the FNIII modules of the ECD of LepR. We show that allosteric binding of oA β interferes with leptin binding and inhibits leptin-induced intracellular signaling in vitro and in vivo, as well as leptin-promoted depolarization of hypothalamic POMC neurons. Thus, oA β behaves as a NAM of LepR function providing a plausible mechanistic explanation for the metabolic dysfunction, in particular for the contribution of the leptin system, observed in AD patients.

Metabolic Dysfunction in AD

Evidence for a link between AD and metabolic diseases in humans comes from different sources. Epidemiological studies show that mid-life obesity and T2D are associated with an increased risk to develop AD, suggesting that metabolic dysfunctions could precede cognitive symptoms [61–65]. Among the genetic risk factors, the ϵ 4 allele of the apolipoprotein E (APOE4) gene, coding for a protein involved in lipid transport and metabolism,

Fig. 5. Electrophysiological analysis of oA β effect on leptin-induced activation of POMC neurons. **a–c** Whole-cell current-clamp recording showing that leptin increased the spontaneous firing activity of the POMC neurons in the absence (**a**) or presence (**b**) of scrambled A β but was ineffective in brain slices preincubated with A β _{1–42} peptide (**c**). **d, e** Membrane potential of ARC POMC neurons before and after bath application of leptin (20 nM)

in the presence of buffer (control, **d**, $n = 6$ from 4 mice), control scrambled A β (**e**, $n = 5$ from 4 mice), and A β _{1–42} peptide (**f**, $n = 6$ from 4 mice). **g, i** Leptin-induced response on spontaneous firing rate in the presence of buffer (**g**, $n = 6$), control scrambled A β (**h**, $n = 5$), and A β _{1–42} peptide (**i**, $n = 6$). p values from paired two-tailed Student t test are indicated.

(For figure see next page.)

Fig. 6. Electrophysiological analysis of oA β effect on Glp1-induced activation of POMC neurons. **a, b** Whole-cell current-clamp recording showing that Glp1 increased the spontaneous firing activity of the POMC neurons in both the absence (**a**) or presence (**b**) of $A\beta_{1-42}$ peptide. **c, d** Membrane potential of ARC POMC neurons before and after bath application of Glp1 (20 nM) in the presence of buffer (control, **c**, $n = 7$ from 4 mice), or $A\beta_{1-42}$ peptide (**d**, $n = 6$ from 4 mice). **e, f** GLP-1-induced response on spontaneous firing rate in the presence of buffer (**e**, $n = 7$), or $A\beta_{1-42}$ peptide (**f**, $n = 6$). p values from paired two-tailed Student t test are indicated.

shows the strongest association with AD, increasing up to 15-fold the risk to develop AD (reviewed by Uddin et al. [66]). $A\beta$ -induced neuroinflammatory response has been hypothesized as one of the connecting factors between AD and metabolic dysfunction, as it leads to impaired brain insulin signaling [2, 67], while a more direct molecular mechanism has not been described up to now. Here, we define LepR as a direct target of $A\beta$, revealing as well the first endogenous allosteric regulator of LepR.

A β Interaction with LepR and Other Receptors

In addition to LepR, other receptors have been proposed to be targeted by $A\beta$, although this issue remains controversial due to technical limitations and to the tendency of $A\beta$ oligomers to interact with the cell membrane at high (micromolar to millimolar) concentrations [68]. Among the membrane proteins with evidence for direct binding to $A\beta$ are the cellular prion protein (Prpc), the leukocyte immunoglobulin-like receptor LILRB2, the

Nogo-66 NgR1 receptor, and the ephrin type B receptor (EphB2) [69]. We recently developed a TR-FRET-based oA β binding assay with largely improved specificity and robustness compared to previous assays allowing the detection of A β binding proteins with higher confidence [70]. The assay restricts the interaction detection window to <10 nm (the maximal distance for energy transfer), thus minimizing the background signal of nonspecific interactions. This test allowed us to characterize the binding of oA β to the nicotinic cholinergic α 7nACh receptor, another previously suggested A β target [33], and to identify LepR as a direct target of oA β (present study). Competition experiments showed that unlabeled oA β displaced the oA β -FAM tracer with a K_i value of $\sim$ 0.1 nM for the short and long LepR isoforms. Binding of the oA β -FAM tracer itself was of lower, but still measurable, affinity ($K_d \sim$ 150 nM). Because the oligomeric states of labeled and unlabeled A β were very similar, we suspect that steric hindrance due to the introduction of the fluorescent group is at the origin of the reduced affinity of oA β -FAM. These nanomolar oA β concentrations are compatible with those reported in AD patients [71]. Competition and direct binding experiments with the different modules of the ECD of LepR excluded the leptin binding-competent CRH2 subdomain, and unambiguously pointed towards the Ig-like and FNIII subdomains as oA β binding domains. In our oA β -FAM binding experiments, however, we detected only one apparent binding site, which suggests that oA β probably displays very similar affinities for both domains. Previous studies showed that targeting specifically these 2 latter domains with neutralizing nanobodies leads to reduced LepR signaling [38], indicating their critical role in LepR function. Immunoglobulin and FNIII repeat domains were also previously suggested as A β binding sites in the LirB2 [72] and the EphB2 receptor [73], respectively, suggesting a preferential affinity of A β to this type of domains. The presence of hydrophobic surfaces in those domains, as shown for the LirB2 receptor [62], might be a common feature between A β binding receptors. Whether this is the case for LepR awaits to be investigated, as no high-resolution structure of the LepR is available yet, apart from the isolated CRH2 subdomain.

Similarly to other cytokine receptors, like the growth hormone receptor [74], functional LepR comprises at least 2 receptor units constitutively interacting with each other in the basal state and has 2 asymmetrically placed ligand binding sites. Binding of leptin to the high-affinity binding site in the CRH2 subdomain primes low-affinity interaction of leptin with the Ig-like subdomain [37]. It is thus tempting to speculate that oA β interferes

with leptin binding to this secondary site, hampering LepR activation. Nevertheless, A β binding to FNIII subdomains can also have important consequences for LepR activation, as they are involved in LepR dimerization through the formation of an intrareceptor disulfide bond involving Cys 604 [37]. Therefore, oA β binding to this domain could interfere with the leptin-induced stabilization of the active LepR conformation, which is in line with our BRET results. Whether one or the other domain is more important for the inhibitory effect of A β on LepR function remains to be elucidated. The negative allosteric nature of oA β binding to LepR was first revealed in a series of competition and kinetic binding assays that showed a partial inhibitory effect of A β on leptin binding and an increased K_{off} of leptin in the presence of oA β , 2 established experimental settings to reveal an allosteric binding mode [75, 76]. The NAM nature of the A β effect was further confirmed on the functional level by the observed right shift of the leptin concentration curve in the BRET assay monitoring the leptin-induced conformational change and clustering of LepR, as well as in leptin concentration-response curves of STAT3, AKT, and ERK activation, where oA β significantly decreased the maximal efficiency of leptin to activate the 3 pathways. Variation of pIC_{50} values for the different pathways indicate a differential sensitivity towards the inhibitory effect of oA β , most likely reflecting different capacities of the oA β -stabilized LepR conformation to activate the 3 signaling pathways.

Consequences of the Negative Allosteric Modulation of A β on LepR

The relevance of such effect was evidenced in a physiologically relevant context by evaluating the response of hypothalamic neurons to leptin in the presence of oA β . Membrane depolarization and neuronal activity of POMC neurons in the ARC nucleus, which have been shown to mediate leptin effect on feeding and glucose homeostasis [57, 77], were significantly impaired by oA β , but not by the scrambled control oA β peptide. Depolarization of arcuate POMC neurons by leptin has been shown to occur through activation of TRPC channels by the PI3K/AKT pathway [78]. A β impairment of the leptin-induced AKT pathway activation probably underlies the absence of neuronal depolarization in response to leptin in our system. Interestingly, A β did not alter the depolarization and firing response of these neurons to Glp1, indicating that A β specifically targets the LepR. The effect of A β is not restricted to the depolarizing action of leptin, as A β has also been shown to impair leptin-in-

duced hyperpolarization observed in other neuronal types, like NPY neurons [30, 79].

Collectively, these observations reveal the hypothalamus as an important target tissue for oA β , probably contributing to the metabolic alterations observed in AD [31, 80].

Hypothalamic dysfunction in AD has been previously characterized, and it can be evidenced not only by altered metabolism regulation but also by the dysfunction of other processes regulated by hypothalamic cells, such as circadian rhythmicity and sleep behavior [31, 81]. These noncognitive dysfunctions were initially believed to be consequences of neurodegeneration in the hypothalamus, but it is currently recognized that such symptoms might appear decades before the cognitive symptoms. Our data now provide a molecular mechanism for early dysfunction of hypothalamic-dependent metabolism regulation through soluble oA β impairment of LepR function. Accordingly, our results using the A β -specific accelerated AD model further confirm that leptin-induced increase in P-STAT-3 and P-AKT levels in the hypothalamus is impaired in A β_{25-35} -injected mice compared to mice injected with control scrambled A β peptide. As activation of the STAT-3 pathway by leptin is important for body weight regulation [82, 83] and activation of the AKT/PI3K pathway for glucose homeostasis [84], A β is likely to impact on both leptin-mediated hypothalamic effects. Consistently, both aspects are affected in AD models and patients [31].

Interestingly, A β_{25-35} -injected mice showed significant higher basal level of phosphorylated STAT-3 in both brain areas analyzed, that is, the hypothalamus and hippocampus, and leptin administration was not able to increase further. This effect was not observed in HEK293 cells, most probably because of differences in the responsiveness of the STAT-3 pathway in HEK293 cells versus an integrated systemic response of the hypothalamus and hippocampus. However, we cannot exclude a contribution of differences in the incubation time (2 h *in vitro* vs. 1 day *in vivo*) or the difference between A β_{25-35} and A β_{1-42} . Of note, increased basal levels of phosphorylated STAT-3 in these brain areas are observed in animal models of high-fat diet-induced obesity and are associated with leptin and insulin resistance [85–87]. Thus, we can postulate that A β -induced increase in basal phosphorylated STAT-3 could participate in the leptin and insulin resistance observed in AD patients.

In addition to the hypothalamus, LepRs have been shown to be expressed in other brain areas, such as in the midbrain and hippocampus, where the leptin signal regu-

lates food reward-induced feeding behavior and synaptic plasticity, respectively [88]. It will be interesting to see whether the presence of A β alters also functions in these brain regions. Indeed, leptin-induced AKT activation in the hippocampus was also impaired by oA β , which probably contributes to AD development by counteracting the beneficial cognitive properties of leptin signaling in this brain region [89].

Conclusion and Perspective

Given the extremely low success rate to develop AD therapies, defining specific direct targets of A β , such as LepR, can open new therapeutic paths. Interestingly, improvement of the leptin response has been previously suggested to have a therapeutic value to treat AD by improving neuronal survival and hippocampal function and decreasing the accumulation of pathological forms of A β and tau ([90–92]; reviewed by Maletínská et al. [93]). Therefore, preventing or inhibiting the interaction of A β with LepR is expected to improve the responsiveness of the leptin system, with beneficial effects on metabolic regulation and presumably also on cognitive functions.

Altogether, we demonstrated high-affinity binding of A β to the ECD of LepR, common to all receptor isoforms, and developed an A β binding assay for LepR that might be useful to screen in the future for molecules interrupting this interaction. We show that A β is a NAM of LepR with inhibitory effects visible at the level of leptin binding, LepR signaling through STAT-3, AKT and ERK1/2 pathways, and neuronal function. We expect that the new TR-FRET assay will assist drug development programs and increase the success rate of drug candidates in the AD field.

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Statement of Ethics

All animal procedures were conducted in strict adherence to the European Union Directive 2010/63 and the ARRIVE guidelines [94] and authorized by the National Ethic Committee (Paris).

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

E.C., T.M., V.P., J.D., and R.J. conceptualization. E.C., T.L., T.M., M.L., M.C., A.S., J.W., and L.Z. biochemical investigation. E.C., J.D., and R.J. writing – original draft. E.C., T.M., J.T., J.D., and R.J. writing – review and editing. E.C., M.C., V.P., J.D., and R.J. funding acquisition. E.C., J.T., V.P., J.D., and R.J. supervision.

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