11β-HSD1 Inhibition Rescues SAMP8 Cognitive Impairment Induced by Metabolic Stress

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Abstract
Ageing and obesity have been shown to increase the risk of cognitive decline and Alzheimer’s disease (AD). Besides, elevated glucocorticoid (GCs) levels cause metabolic stress and have been associated with the neurodegenerative process. Direct pieces of evidence link the reduction of GCs caused by the inhibition of 11β-HSD type 1 (11β-HSD1) with cognitive improvement. In the present study, we investigated the beneficial effects of 11β-HSD1 inhibitor (i) RL-118 after high-fat diet (HFD) treatment in the senescence-accelerated mouse prone 8 (SAMP8). We found an improvement in glucose intolerance induced by HFD in mice treated with RL-118, a significant reduction in 11β-HSD1 and glucocorticoid receptor (GR) protein levels. Furthermore, specific modifications in the FGF21 activation after treatment with 11β-HSD1i, RL-118, which induced changes in SIRT1/PGC1α/AMPKα pathway, were found. Oxidative stress (OS) and reactive oxygen species (ROS), as well as inflammatory markers and microglial activation, were significantly diminished in HFD mice treated with 11β-HSD1i. Remarkably, treatment with 11β-HSD1i altered PERK pathway in both diet groups, increasing autophagy only in HFD mice group. After RL-118 treatment, a decrease in glycogen synthase kinase 3 (GSK3β) activation, Tau hyperphosphorylation, BACE1 protein levels and the product β-CTF were found. Increases in the non-amyloidogenic secretase ADAM10 protein levels and the product sAPPα were found in both treated mice, regardless of the diet. Consequently, beneficial effects on social behaviour and cognitive performance were found in treated mice. Thus, our results support the therapeutic strategy of selective 11β-HSD1i for the treatment of age-related cognitive decline and AD.

Keywords Glucocorticoids · Cognition · Neurodegeneration · High-fat diet · Neuroinflammation · Oxidative stress · Endoplasmic reticulum stress · Ageing · Alzheimer’s disease

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Introduction

Ageing is the most significant risk factor for a majority of chronic diseases, such as age-related cognitive decline and Alzheimer’s disease (AD) [1]. Obesity has recently been considered to play an essential role in mild cognitive impairment (MCI) and dementia [2, 3]. Abundant pieces of evidence suggest that pathological glucocorticoid (GC) excess not only is associated with age-related cognitive decline but also with metabolic stress, obesity and diabetes [4–7]. Thus, it has been described that prolonged exposure to increased levels of GCs exerts deleterious effects on hippocampal electrophysiology, structure and function, both in rodents [8] and humans [9], being part of altered intercellular communication, considered one of the nine hallmarks of ageing [10, 11]. The availability of natural GCs in tissues is regulated by corticosteroid-binding globulin in serum and by locally expressed 11β-hydroxyysteroid dehydrogenase (11β-HSD) enzyme, a microsomal enzyme that catalyses the interconversion of active GCs (corticosterone in rodents but cortisol in humans) and inert 11-keto-forms [11-dehydrocorticosterone (11-DHC), cortisone] [12]. Two isoenzymes have been identified: 11β-HSD type 1 (11β-HSD1) and type 2 (11β-HSD2) [13]. 11β-HSD1 acts as a reductase, thus locally potentiating GC activity, and is the predominant form in the brain, both in rodents and humans. 11β-HSD1 expression in mouse hippocampus and parietal cortex increases with ageing, correlating with impaired spatial memory [14] and its overexpression accelerates age-related cognitive decline [6]. Conversely, 11β-HSD1 knockout mice resist age-dependent cognitive loss [15, 16]. In line with these findings, some preclinical studies demonstrate that 11β-HSD1 inhibition improved cognition and AD hallmarks suggesting a neuroprotective effect [17, 18]. On the other hand, preclinical studies have demonstrated that sub-maximal inhibition of central 11β-HSD1 is able to prevent cognitive impairments in AD and ageing [19, 20] and early preclinical studies demonstrated that administration of brain penetrant 11β-HSD1 (UE2343) is well tolerated [21]. Besides, many selective 11β-HSD1 inhibitors have reached clinical stages for metabolic diseases like type 2 diabetes mellitus (e.g. AZD8329, ABT-384 and BVT.2733).

In addition, several hypotheses argument a link between altered glucose metabolism and dementia, considering an altered metabolic pathway as a potential contributor to persistent oxidative stress (OS) that culminates into neuronal dysfunction and dementia [22]. Increased OS, neuroinflammation, endoplasmic reticulum (ER) stress, misfolded protein removal pathways and autophagy have been identified as components of neuronal metabolic stress, thus developing and aggravating neurological disorders and cognitive impairment, pointing to their implication in the dysregulated energy metabolism characteristic of ageing [5]. Thus, recent studies have focussed on the pathology of cognitive decline and neurodegeneration through high-fat diet (HFD) intervention that leads to metabolic stress [23, 24]. For instance, HFD-fed aged animals showed insulin resistance and increased weight gain among others, both signs of pre-diabetes, obesity, cardiovascular disease, depressive-like behaviour and mental health problems [25–27]. However, the current knowledge about the molecular mechanism responsible for these affections is controversial, even though most of the information is related to reduced insulin sensitivity mediated by HFD. Hence, HFD-induced metabolic stress could be linked with the development of physiopathological conditions, such as AD and other neurodegenerative diseases [28].

Recently, we have developed a brain penetrant 11β-HSD1 inhibitor (RL-118) that has been characterized chemically and pharmacologically in vivo [29]. RL-118 attenuates neuroinflammation, increases the antioxidant defence, promotes autophagy, improves mitochondrial function and reverts memory deficits in senescent mice model [17, 29]. Therefore, the targeted inhibition of 11β-HSD1 may become a potential therapeutic strategy for age-related cognitive decline and AD. Here, we assessed the neuroprotective effects of 11β-HSD1 inhibition on metabolic stress through behaviour, cognitive and molecular changes induced by HFD in a mice model of age-related cognitive decline and late-onset AD (LOAD), the senescence-accelerated mouse prone 8 (SAMP8).

Materials and Methods

Animals

Female SAMP8 mice (n = 48) were used to carry out behavioural, cognitive and molecular analyses. We divided these animals into four groups: normal diet chow (ND Ct, n = 12), ND treated with the RL-118 11β-HSD1 inhibitor (ND + 11β-HSD1i, n = 12), HFD (HFD Ct, n = 12) and HFD treated with RL-118 (HFD + 11β-HSD1i, n = 12). Animals had free access to food and water and were kept under standard temperature conditions (22 ± 2 °C) and 12 h/12 h light-dark cycles (300 lx/0 lx). Animals were fed with both diets since the weaning (1-month-old) up to sacrifice. RL-118 was administered at 21 (mg/kg/day) by oral gavage from 4 months old to end of behavioural test (Fig. 1a). ND provided 3.8 Kcal/g meanwhile HFD 4.7 Kcal/g of 45% fatty acids (D12451 Research Diets, Inc.). The weight of the animals and the ingested food were monitored weekly. Before the performance of the cognitive tests, and the triglycerides determination was conducted.

Studies and procedures involving mice brain dissection and subcellular fractionation were performed following the institutional guidelines for the care and use of laboratory animals established by the Ethical Committee for Animal Experimentation at the University of Barcelona.
Fig. 1 Scheme of experimental design of in vivo experiments (a). Levels of corticosterone in the blood (b) and brain (c) after 11β-HSD1i treatment. Body weight for all mice groups (d). Average caloric intake per mouse for all mice groups (e). Plasma levels of glucose 2 g/kg intraperitoneal (i.p.) administration (f). Blood triglyceride concentration (mg/dL) (g). Representative Western blot and quantification for 11β-HSD1 (h), and GR (i). Values in bar graphs are adjusted to 100% for protein levels of SAMP8 Normal Diet (ND Ct). Values are mean ± standard error of the mean (SEM); (n = 12 for each group). *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. #### Between ND Ct vs HFD Ct; ##### between ND+11β-HSD1i vs HFD+11β-HSD1i, and $$ between HFD Ct vs HFD+11β-HSD1i groups
Glucocorticoid, Glucose Tolerance Test and Triglyceride Determination

Plasma and brain corticosterone concentrations were measured using a commercially available RIA (MP Biomedicals, Irvine, CA). Blood extraction and brain dissection were done among 4 pm and 5 pm in ND + 11β-HSD1i and ND Ct mice.

Intraperitoneal (i.p.) glucose tolerance test was performed following 12 weeks of HFD feeding and 4 weeks of 11β-HSD1i/vehicle treatments, as described previously. In brief, mice fasted overnight for 12 h. The test was performed in a quiet room and 2 g/kg i.p. glucose injection was administered (diluted in H2O) and blood glucose levels were measured at 0, 5, 10, 15, 30, 60 and 120 min after the injection with the Accu-Chek® Aviva blood glucose meter (Accu-Chek® Aviva, Roche, Barcelona, Spain). The determination of triglyceride concentration was performed by using a triglyceride meter device (Accutrend® Plus, Cobas, Roche).

Behavioural and Cognitive Tests

Three-Chamber Test

The Three-Chamber test assesses cognition through sociability and interest in social novelty [30]. Testing occurs in a box with three equally dimensioned rooms. Each test consists of 20 min and is recorded with a camera. The animal is placed in the centre of the box and allowed to explore the three chambers for 10 min (habituation phase). The time spent in each chamber was evaluated. Afterwards, an intruder (same sex and age) was added to one of the rooms in a metal cage and behaviour is recorded for 10 min. In this phase, the time spent in each room is assessed as well as the time interacting with the intruder (e.g. sniffing, grooming).

Morris Water Maze

This test evaluates both learning and spatial memory [31]. An open circular pool (100 cm in diameter, 50 cm in height) filled with water was used. Water was painted white with latex in order to make it opaque and its temperature was 22 ± 1 °C. Two main perpendicular axes were established (North-South and East-West), thus configuring four equal quadrants (NE, NW, SE and SW). Four visual clues (N, S, E and W) were placed on the walls of the tank so that the animal could orientate and could fulfil the objective. The test consists of training a mouse to find a submerged platform (learning phase) and assess whether the animal has learned and remembered where was the platform the day that it is removed (test). The training lasts five consecutive days, and every day, five trials are performed, which have a different starting point (NE, E, SE, S and SW), with the aim that the animal recognizes the visual clues and learns how to locate the platform, avoiding learning the same path. At each trial, the mouse was placed gently into the water, facing the wall of the pool, allowed to swim for 60 s and there was not a resting time between trials. If the animal was not able to locate the platform, the investigator guided it to the platform and was allowed to rest and orientate for 30 s. The platform was placed approximately in the middle of one of the quadrants, 1.5 cm below the water level. Above the pool, there was a camera that recorded the animals’ swimming paths and the data was analysed with the statistical program SMART® ver.3.0. During the learning phase, a learning curve was drawn, in which the latency to find the platform every training day is represented. On the day test, more parameters were measured, such as the target crossings and the swim distance in the platform zone.

Novel Object Recognition Test

The Novel Object Recognition Test (NORT) protocol employed was a modification of [32]. In brief, mice were placed in a 90°, two-arms, 25-cm-long, 20-cm-high, 5-cm-wide black maze. Before performing the test, the mice were individually habituated to the apparatus for 10 min for 3 days. On day 4, the animals were submitted to a 10-min acquisition trial (first trial), during which they were placed in the maze in the presence of two identical, novel objects at the end of each arm. After a delay (2 h and 24 h), the animal was exposed to two objects, one old object and one novel object. The time that mice explored the novel object (TN) and time that mice explored the old object (TO) were measured. A discrimination index (DI) was defined as (TN – TO)/(TN + TO). To avoid object preference biases, objects were counterbalanced. The maze, the surface and the objects were cleaned with 70% ethanol between the animals’ trials to eliminate olfactory cues.

Immunodetection Experiments

Brain Processing

Three days after the behavioural and cognitive tests, eight animals per group were euthanized for protein extraction, RNA and DNA isolation, and four animals per group were euthanized for immunohistochemistry (IHHIC).

When the animals were for protein extraction, RNA and DNA isolation, the brains were immediately removed and the hippocampus was isolated, frozen on powdered dry ice and maintained at -80 °C until procedures. When the animals were for IHHIC, mice were intracardially perfused with 4% paraformaldehyde (PFA) diluted in 0.1 M phosphate buffer solution after being anaesthetised by intraperitoneal injection of ketamine 100 mg/kg and xylazine 10 mg/kg. Afterwards, the brains were removed and post-fixed in 4% PFA overnight 4 °C; then, the solution was changed into PFA + 15% sucrose.
Finally, the brains were frozen burying directly on powdered dry ice (around 5 min) and stored at −80 °C until sectioned.

Western Blotting

Tissue samples were homogenized in lysis buffer (Tris HCl pH 7.4 50 mM, NaCl 150 mM, EDTA 5 mM and 1X-Triton X-100) containing phosphatase and protease inhibitors (Cocktail II, Sigma-Aldrich) to obtain total protein homogenates.

For Western blotting (WB), aliquots of 15 μg of hippocampal protein extraction per sample were used. Protein samples were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (8–14%) and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore). Afterwards, membranes were blocked in 5% non-fat milk in Tris-buffered saline (TBS) solution containing 0.1% Tween 20 TBS (TBS-T) for 1 h at room temperature, followed by overnight incubation at a 4 °C with the primary antibodies listed in Supplementary Table 1. Then, the membranes were washed and incubated with secondary antibodies listed in Supplementary Table 1 for 1 h at room temperature. Immunoreactive proteins were viewed with the chemiluminescence-based ChemiLucent™ detection kit, following the manufacturer’s protocol (ECL Kit, Millipore), and digital images were acquired using ChemiDoc XRS + System (BioRad). Semi-quantitative analyses were done using ImageLab software (BioRad), and results were expressed in arbitrary units (AU), considering control protein levels as 100%. Protein loading was routinely monitored by immunodetection of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or β-tubulin.

Immunofluorescence

Coronal section of 30 μm was obtained by a cryostat (Leica Microsystems CM 3050S, Wetzlar, Germany) and kept in a cryoprotectant solution at −20 °C.

First, free-floating slices were selected and placed on a 24-well plaque, after that, were washed five times with PBS 0.01 M + 1% Triton X-100. Then, free-floating sections were blocked with a solution containing 5% fetal bovine serum (FBS), 1% Triton X-100, PBS 0.01 M + gelatine 0.2% for 2 h at room temperature. Afterwards, slices were washed with PBST (PBS 0.1 M, 1% Triton X-100) five times for 5 min each and were incubated with the primary antibodies overnight at 4 °C (Supplementary Table 2). On the following day, coronal slices were washed with PBST 6 times for 5 min and then incubated with the secondary antibodies (Supplementary Table 2) at room temperature for 2 h. Later, sections were co-incubated with 1 ng/mL DAPI staining solution (Sigma-Aldrich, St. Louis, MI) for 5 min in the dark at room temperature and washed with PBS 0.01 M. Finally, the slices were mounted using Fluoromount G (EMS, USA), and image acquisition was performed with a fluorescence laser microscope (Olympus BX41, Germany). At least four images from four different individuals by the group were analysed with ImageJ/Fiji software available online from the National Institutes of Health.

RNA Extraction and Gene Expression Determination by q-PCR

Total RNA isolation was carried out using TRIzol™ reagent according to the manufacturer’s instructions (Bioline Reagent, UK). The yield, purity and quality of RNA were determined spectrophotometrically with a NanoDrop™ ND-1000 (Thermo Scientific) apparatus and an Agilent 2100B Bioanalyzer (Agilent Technologies). RNAs with 260/280 ratios and RIN higher than 1.9 and 7.5, respectively, were selected. Reverse transcription-polymerase chain reaction (RT-PCR) was performed as follows: 2 μg of messenger RNA (mRNA) was reverse-transcribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Real-time quantitative PCR (qPCR) was used to quantify mRNA expression of oxidative stress and inflammatory genes listed in Supplementary Table 3. SYBR® Green real-time PCR was performed in a Step One Plus Detection System (Applied-Biosystems) employing SYBR® Green PCR Master Mix (Applied-Biosystems). Each reaction mixture contained 6.75 μL of complementary DNA (cDNA) (in which concentration was 2 μg), 0.75 μL of each primer (in which concentration was 100 nM) and 6.75 μL of SYBR® Green PCR Master Mix (2×).

Data were analysed utilizing the comparative cycle threshold (ΔΔCt) method, where the housekeeping gene level was used to normalize differences in sample loading and preparation [30]. Normalization of expression levels was performed with β-actin for SYBR® Green-based real-time PCR results. Each sample was analysed in duplicate, and the results represent the n-fold difference of the transcript levels among different groups.

Oxidative Stress Determination

Hydrogen peroxide was measured in hippocampus protein homogenates as an indicator of oxidative stress, and it was quantified using the Hydrogen Peroxide Assay Kit (Sigma-Aldrich, St. Louis, MI) according to the manufacturer’s instructions.

Data Analysis

Data analysis was conducted using GraphPad Prism ver. 7 statistical software. Data are expressed as the mean ± standard error of the mean (SEM) of at least six samples per group. Diet
and treatment effects were assessed by the two-way ANOVA analysis of variance, followed by Tukey post hoc analysis or two-tailed Student’s t test when it was necessary. Statistical significance was considered when p values were < 0.05. The statistical outliers were determined with Grubbs’ test and subsequently removed from the analysis.

Results

Treatment with 11β-HSD1i Decreases GC Levels and Improves Glucose Intolerance Induced by HFD

As expected, corticosterone levels were significantly reduced in blood and brain tissue after 11β-HSD1i treatment (Fig. 1b, c). Body weight was measured weekly during the intervention. All animal groups significantly increased body weight over time (Fig. 1d). Furthermore, both HFD-fed mice groups exhibited increase in body weight from 12 weeks to the end time point (Fig. 1d), correlating with the higher caloric intake (Fig. 1e). However, 11β-HSD1i treatment did not significantly modify those parameters. Likewise, significant higher glucose levels between 15 and 90 min were found in HFD Ct (Fig. 1f). Noteworthy, 11β-HSD1i treatment reduced significantly glucose levels in HFD-fed mice (Fig. 1f). On the other hand, triglyceride concentration was higher in HFD mice groups but did not differ from 11β-HSD1i-treated groups (Fig. 1g). In conjunction, 11β-HSD1i ameliorated glucose metabolism only after metabolic stress induced by HFD feeding.

Finally, WB analysis revealed a significant reduction in 11β-HSD1 protein levels in treated mice, both in ND- and HFD-fed mice (Fig. 1h). Additionally, the inhibition of 11β-HSD1 diminished GR protein levels significantly regardless of the diet (Fig. 1i).

Restoration of FGF21 Levels After Treatment with 11β-HSD1i Is Accompanied by Changes in the Nutrient Sensor SIRT1/PGC1α/AMPKα Pathway

Fibroblast growth factor 21 (FGF21) and SIRT1/PGC1α/AMPKα axis were evaluated by immunoblot. A significant reduction in FGF21 in HFD Ct group compared to the ND Ct group (Fig. 2a) was observed, demonstrating that HFD reduced FGF21 protein levels. Of note, a significant increase in FGF21 was found in the HFD + 11β-HSD1i group in comparison with the HFD Ct group (Fig. 2a). No significant changes in FGF21 protein levels were found in ND + 11β-HSD1i compared to ND Ct group, although there was a slight increase. Likewise, analysis of the nutrient sensor axis showed a significant reduction in SIRT1 protein levels in the HFD group, but RL-118 was unable to prevent it. Conversely, ND + 11β-HSD1i showed a significant increase in SIRT1 compared to ND Ct group (Fig. 2b). Importantly, liver kinase B1 (LKB1) was activated in 11β-HSD1i-treated groups compared to the control mice regardless of the diet (Fig. 2c). A significant increase in the phosphorylated activated protein kinase (p-AMPKα) ratio levels in the HFD + 11β-HSD1i group was observed compared to the HFD Ct mice (Fig. 2d). Finally, peroxisome proliferator-activated receptor-gamma coactivator 1-α (PGC1α) protein levels were increased in a significant way only in the ND + 11β-HSD1i compared to the ND Ct group, but not to HFD groups (Fig. 2e).

By contrast, 11β-HSD1 pharmacological inhibition through RL-118 was only able to increase SIRT1, LKB1 and PGC1α in ND-fed animals. Albeit RL-118 did not cause apparent changes in SIRT1 and PGC1α in HFD-fed mice, the increase in AMPKα phosphorylation indicated that improvements in nutrient sensing and mitochondrial function after 11β-HSD1i inhibition also occur in HFD. In whole, our findings pinpoint the beneficial effect of reducing GC signaling by 11β-HSD1 inhibition in SAMP8 under metabolic stress.

Treatment with 11β-HSD1i Reduced OS Markers

HFD induced a significant increase in GPX1 and a moderate increase in SOD1 protein levels that were prevented by RL-118 treatment (Fig. 3a, b). In addition, iNOS gene expression was reduced in 11β-HSD1i-treated animals in comparison with the control groups that reached significance in HFD Ct mice (Fig. 3c). Likewise, analysis of hydrogen peroxide levels showed that 11β-HSD1i can reduce although in a not significant way (Fig. 3d).

Reduction of Inflammatory Markers and Microglial Activation After Treatment with 11β-HSD1i

HFD did not modify NF-κB protein levels. However, 11β-HSD1i treatment induced a significant diminution in NF-κB protein levels both in ND and HFD mice (Fig. 4a). Il-1β, Il-4, Il-6 and Tnf-α gene expression was reduced after 11β-HSD1i treatment, being significant in Il-6 and Tnf-α, regardless of the diet (Fig. 4b). By last, immunostaining quantification of Iba1 fluorescence intensity demonstrated that 11β-HSD1i treatment reduced Iba1 staining, especially in the dentate gyrus (DG) and CA1 regions (Fig. 4c–f).

Treatment with 11β-HSD1i Increased Autophagy Through PERK Pathway

Next, we evaluated the ER stress response. PERK pathway revealed changes in phosphorylated PKR-like endoplasmic reticulum kinase (p-PERK) and phosphorylated eukaryotic translation-initiation factor 2 (p-eIF2α) activating transcription factor 4 (ATF4), but not in and C/EBP homologous
Fig. 2 Representative Western blot and protein level quantification for FGF21 (a), SIRT1 (b), LKB1 (c), ratio of p-AMPKα (d), and PGC-1α (e). Values in bar graphs are adjusted to 100% for protein levels of SAMP8 Normal Diet (ND Ct). Values are mean ± standard error of the mean (SEM); (n = 6 for each group). *p < 0.05; **p < 0.01
protein (CHOP). All these protein levels were higher in 11β-HSD1i-treated groups compared to its Ct group, regardless of the diet (Fig. 5a–d). Moreover, the HFD Ct group showed less phosphorylated PERK and eIF2α protein levels compared to ND Ct (Fig. 5a, b). Regarding BCL-2 protein levels, no changes were found among groups (Fig. 5e). However, Beclin 1 protein levels were slightly increased in HFD + 11β-HSD1i compared to the HFD Ct group (Fig. 5f).

**Reduction of AD Hallmarks After Treatment with 11β-HSD1i Are Associated with Glycogen Synthase Kinase 3 Signalling Pathway Induced by HFD**

HFD did not alter phosphorylation in Tyr217 of glycogen synthase kinase 3 beta (p-GSK3β) and Tau hyperphosphorylation. However, p-GSK3β (Tyr217) was significantly diminished in the HFD + 11β-HSD1i group compared to HFD Ct (Fig. 6a). In parallel, a reduction in p-Tau (Ser202, Thr205), as well as p-Tau (Ser404) protein levels after the 11β-HSD1i treatment were found in HFD Ct mice (Fig. 6b).

HFD was unable to alter APP processing in SAMP8; neither soluble APP fragment alpha (sAPPα) nor the fragment delivered by β-secretase (β-CTF) protein levels were modified. 11β-HSD1i treatment caused a significant increase in sAPPα in the ND-fed group but not in the HFD-fed mice (Fig. 6c). β-CTF protein levels were decreased after 11β-HSD1i regardless of the diet (Fig. 6d). Finally, a significant increase in ADAM10 protein levels was only found in ND + 11β-HSD1i group compared to the ND Ct group (Fig. 6e), whereas a significant reduction in BACE1 protein levels was found in 11β-HSD1i-treated animals, reaching significance in ND-fed mice (Fig. 6f).

**Beneficial Effects on Social Behaviour and Cognitive Performance After Treatment with 11β-HSD1i**

Social behaviour was investigated by TCT. HFD did not alter the preference for a specific chamber during the habituation,
Fig. 4 Puigoriol-Illamola et al. (2019)
Fig. 5 Representative Western Blot and quantification for the ratio of p-PERK (a), the ratio of p-eIF2α (b), ATF4 (c), CHOP (d), BCL-2 (e), and Beclin1 (f). Values in bar graphs are adjusted to 100% for protein levels of SAMP8 Normal Diet (ND Ct). Values are mean ± standard error of the mean (SEM); (n = 6 for each group). *p < 0.05; **p < 0.01; ***p < 0.001
Fig. 6 Representative Western blot and quantification for the ratio of p-GSK-3β (a), the ratio of p-Tau (Ser202, Thr205, and Ser404) (b), sAPPα (c), the ratio of β-CTF (d), ADAM10 (e), and BACE1 (f). Values in bar graphs are adjusted to 100% for protein levels of SAMP8 Normal Diet (ND Ct). Values are mean ± standard error of the mean (SEM); (n = 6 for each group). *p < 0.05; **p < 0.01; ***p < 0.001
neither the treatment with 11β-HSD1i (Fig. 7a). Moreover, in all the experimental groups, the presence of an intruder increases significantly the time spent in this chamber, regardless of diet or treatment (Fig. 7b). Higher interaction in HFD + 11β-HSD1 group compared to the HFD Ct group was found, although no changes in the interaction between the resident and the intruder in ND groups occurred (Fig. 7c).

Furthermore, cognitive performance was measured by the MWM and NORT tests. Regarding MWM, all mice groups were able to learn through the training period; no differences were found among groups (Fig. 7d). On the test day, 11β-HSD1i-treated mice increased the swim length in the platform zone compared to Ct animals indicating higher cognitive abilities (Fig. 7e). Besides, the number of target crossings was significantly increased in HFD + 11β-HSD1i mice in comparison with the ND Ct (Fig. 7f). Regarding NORT, there were no differences in the exploration time between identical objects during acquisition trial (first trial). In test trial analysis, 11β-HSD1i-treated mice exhibited a significant improvement in cognitive performance both in short-(2 h) and long-(24 h) term recognition memory in comparison with Ct groups, obtaining higher DI values (Fig. 7g).

Discussion

In this study, we provide new evidence that inhibition of 11β-HSD1 improves cognitive impairment after metabolic stress induced by HFD intervention in a model of cognitive decline. To this end, various molecular pathways influenced by HFD and GCs, as well as social and cognitive impairment were evaluated to elucidate new mechanisms by which 11β-HSD1 inhibition exerts neuroprotection.

Our group has previously described that HFD induced metabolic stress in a murine model of accelerated senescence, SAMP8 [33] and in C57BL/6J aged mice [34]. Those metabolic disturbances were prevented by resveratrol [33, 34]. Moreover, in previous reports, the neuroprotective effect of 11β-HSD1 inhibitor (RL-118) was demonstrated in old female SAMP8 [17, 29].

Animals fed with HFD increased body weight in comparison with ND-fed mice showing an alteration in glucose metabolism. The hugest increase in animal weight is not only associated with impaired glucose tolerance but also with an alteration in lipid metabolism. Barroso and co-workers described that the excessive consumption of hypercaloric and high saturated fat food causes an increase in serum triglyceride levels, which is the first step for the development of the atherogenic dyslipidaemia found in obese and diabetic patients [35]. Accordingly, our results showed higher triglyceride concentration in blood in HFD-fed groups.

As aforementioned, there is extensive evidence that HFD impairs glucose metabolism, altering the insulin signalling pathway [36–38]. Recently, a close relationship between insulin insensitivity and neurodegenerative disorders, such as AD, has been extensively reported [39, 40] and it is still being discussed whether to consider AD as a type 3 diabetes mellitus (DM3) [41]. Additionally, high GC levels can mediate insulin resistance response, increasing glucose levels and favouring insulin resistance and obesity [42, 43]. Thus, selective inhibitors of 11β-HSD1 have been postulated as a neuroprotective strategy in several pathological scenarios [44]. RL-118 treatment reduced the 11β-HSD1 enzyme and GC receptor protein levels both in ND- and HFD-fed SAMP8. In concordance, GC levels were reduced both in blood and in plasma of RL-118-treated animals. Results are in line with reports describing a reduction in gene expression of 11β-HSD1 and GR in diet-induced obese mice after treatment with carbenoxolone, a 11β-HSD1 inhibitor [45].

Recently, FGF21 has been demonstrated to modulate energy homeostasis of glucose and lipid through activation of SIRT1/PGC1α/AMPK axis, mainly through LKB1 activation [46]. Consistent with this hypothesis, treatment with 11β-HSD1 inhibitor significantly increased protein levels of FGF21 and LKB1 under both dietary conditions. Albeit RL-118 did not induce direct changes in SIRT1 and PGC1α protein levels in HFD-fed SAMP8, the increase in AMPKα phosphorylation suggests an improvement in nutrient sensing and mitochondrial function. In sum, these results demonstrate the beneficial effects of reducing GC signalling by 11β-HSD1 inhibition in SAMP8 under HFD-induced metabolic stress.

It has been reported that HFD increases OS and inflammation [47]. Accordingly, OS markers, such as GPX1, SOD1 and iNOS, were increased in HFD-treated groups, and 11β-HSD1i treatment was only able to reduce them when animals were fed with HFD, whereas a clear tendency to reduce hippocampal ROS levels in all 11β-HSD1i-treated animals was found. Regarding neuroinflammation, IL-6 and IL-4 gene expression and microglial activation evaluated through Iba-1, increased under HFD confirming the cellular dysfunction induced by impaired energy metabolism. Importantly, 11β-HSD1 inhibition diminished gene expression of both Tnf-α and IL-6, as well as p65 protein levels, inhibiting microglial reactivity. These findings agree with already published evidence describing that 11β-HSD1 inhibition modulates OS and inflammatory processes [17, 29, 48].

11β-HSD1 inhibition by RL-118 reduced autophagy and apoptosis markers in old SAMP8 [17], but in the present work, we did not observe significant changes in Beclin 1 or BCL2, probably because of the young age of mice. Because ER stress response activates proteostatic mechanisms [49, 50], ER stress markers were studied. While HFD did not modify ATF4 protein levels in a significant way, PERK and eIF2α activation were significantly reduced in those animals. Of interest, inhibition of 11β-HSD1 by RL-118 recovered the ratio of phosphorylation of PERK and eIF2α, and in addition, increased ATF4 protein levels. By contrast, in our hands, CHOP showed a narrow but not significant increase after RL-118 treatment [51]. Regarding the accumulation of misfolded proteins, a characteristic of ageing and AD, 11β-HSD1 inhibition reduced GSK3β activation and tau phosphorylation after HFD feeding. Regarding the β-amyloid
Fig. 7 Results of TCT for all mice groups. Habituation phase (a), chamber preference (b), and sociability (c). Results of MWM for all mice groups. Learning curves of MWM during the spatial acquisition phase (d), distance in platform zone during the test (e), and number of entries in the platform zone during the test (f). Results of NORT for all mice groups. Summary of DI from 2 and 24 h after the familiarization phase (g). Values are mean ± standard error of the mean (SEM) (n = 12 for each group). *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.
pathway, RL-118 promoted APP processing by the non-amyloidogenic pathway, decreasing pro-amyloidogenic APP fragments, as well as reducing BACE1 and increasing ADAM10 protein levels. Results suggest that 11β-HSD1 inhibition reduced the negative impact of HFD on cellular and tissue hallmarks of cognitive decline and AD [33, 34, 52].

Of paramount importance, RL-118 promoted changes in sociability behaviour, recognition memory, in both short- and long-term and spatial memory in both dietary conditions. These results point out that the GC levels have a key role in memory and learning processes, influencing mood-like behaviours in mice, both physiological and pathological conditions, supported by the molecular results.

In conclusion, our results demonstrate that HFD induced systemic metabolic dysfunctions and exacerbated cognitive impairment in adult SAMP8 mediated by alterations in insulin signalling, OS, neuroinflammation and aberrant protein processing. Decreasing GC levels with RL-118 reduced global stress and led to beneficial effects in most of the markers evaluated in HFD- and ND-fed mice. Finally, modulation of GC activity by 11β-HSD1 inhibition contributes to enhance cognitive performance in senescent mice regardless of the dietary influence. Because new approaches are needed to fight against cognitive decline and dementia, such as AD, the control of GC levels may open new avenues to prevent these devastating conditions.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

References

1. Guerreiro R, Bras J (2015) The age factor in Alzheimer’s disease. Genome medicine 7:106. https://doi.org/10.1186/s13073-015-0232-5

2. Bischof GN, Park DC (2015) Obesity and aging: Consequences for cognition, brain structure, and brain function. Psychosomatic medicine 77(6):697–709. https://doi.org/10.1097/PSY.0000000000000212

3. Feinikohl I, Lachmann G, Brockhaus WR, Borchers F, Piper SK, Ottens TH, Nathoe HM, Sauer AM et al (2018) Association of obesity, diabetes and hypertension with cognitive impairment in older age. Clinical epidemiology 10:853–862. https://doi.org/10.2147/CLEP.S164793

4. De Quervain DJ, Poirier R, Wollmer MA, Grimaldi LM, Tsolaki M, Streffer JR, Hock C, Nitsch RM et al (2004) Glucocorticoid-related genetic susceptibility for Alzheimer’s disease. Hum Mol Genet 13(1):47–52

5. Kumar A, Datusalia AK (2018) Metabolic stress and inflammation: Implication in treatment for neurological disorders. CNS Neurol Disord Drug Targets 17(9):642–643. https://doi.org/10.2174/187152731701909026121555

6. Holmes MC, Carter RN, Noble J, Chitnis S, Dutia A, Paterson JM, Mullins JJ, Seckl JR et al (2010) 11β-hydroxysteroid dehydrogenase type 1 expression is increased in the aged mouse hippocampus and parietal cortex and causes memory impairments. J Neurosci 30(20):6916–6920. https://doi.org/10.1523/JNEUROSCI.0731-10.2010

7. Bujańska J, Quinkler M, Tomlinson JW, Montague CT, Smith DM, Stewart PM (2006) Expression profiling of 11β-hydroxysteroid dehydrogenase type-1 and glucocorticoid-target genes in subcutaneous and omental human adipocytes. J Mol Endocrinol 37(2):327–340

8. Canet G, Chevallier N, Zussy C, Desrumaux C, Givalois L (2018) Central role of glucocorticoid receptors in Alzheimer’s disease and depression. Front Neurosci 12:739. https://doi.org/10.3389/fnins.2018.00739

9. Tatomir A, Micu C, Crivii C (2014) The impact of stress and glucocorticoids on memory. Curr Drug Targets 15(1):47–52

10. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G (2013) The hallmarks of aging. Cell 153(6):1194–1217. https://doi.org/10.1016/j.cell.2013.05.039

11. Mattson MP, Arumugam TV (2018) Hallmarks of brain aging: Adaptive and pathological modification by metabolic states. Cell Metab 27(6):1176–1199. https://doi.org/10.1016/j.cmet.2018.05.011

12. Yau JL, Seckl JR (2012) Local amplification of glucocorticoids in the aging brain and impaired spatial memory. Front Aging Neurosci 4:24. https://doi.org/10.3389/fnagi.2012.00024

13. Kadmiel M, Cidlowski JA (2013) Glucocorticoid receptor signaling in health and disease. Trends Pharmacol Sci 34(9):518–530. https://doi.org/10.1016/j.tips.2013.07.003

14. Yau JL, Wheelan N, Noble J, Walker BR, Webster SP, Kenyon CJ, Ludwig M, Seckl JR (2015) Intrahippocampal glucocorticoids generated by 11β-HSD1 affect memory in aged mice. Neurobiol Aging 36(1):334–343. https://doi.org/10.1016/j.neurobiolaging.2014.07.007

15. Yau JL, Noble J, Kenyon CJ, Hibberd C, Kotelevstev Y, Mullins JJ, Seckl JR (2001) Lack of tissue glucocorticoid reactivation in 11beta-hydroxysteroid dehydrogenase type 1 knockout mice ameliorates age-related learning impairments. Proc Natl Acad Sci U S A 98(8):4716–4721

16. Yau JL, McNair KM, Noble J, Brownstein D, Hibberd C, Morton N, Mullins JJ, Morris RG et al (2007) Enhanced hippocampal long-term potentiation and spatial learning in aged 11beta-hydroxysteroid dehydrogenase type 1 knockout mice. J Neurosci 27(39):10487–10496

17. Puigoriol-Illamola D, Girán-Ferré C, Vasilopoulou F, Leiva R, Vázquez S, Pallas M (2018) 11β-HSD1 inhibition by RL-118 promotes autophagy and correlates with reduced oxidative stress and inflammation, enhancing cognitive performance in SAMP8 mouse model. Mol Neurobiol 55(12):8904–8915. https://doi.org/10.1007/s12035-018-1026-8

18. Mohler EG, Brownwee K, Roderwald VA, Cronin EA, Markosyan S, Scott Bittner R, Strakhova MI, Drescher KU et al (2011) Acute inhibition of 11β-hydroxysteroid dehydrogenase type-1 improves memory in rodent models of cognition. J Neurosci 31(14):5406–5413. https://doi.org/10.1523/JNEUROSCI.4046-10.2011

19. Sooy K, Webster SP, Noble J, Binnie A, Mauy JL, Walker BR, Seckl JR, Yau JL (2010) Partial deficiency or short term inhibition of 11β-hydroxysteroid dehydrogenase type 1 improves cognitive function in aging mice. J Neurosci 30:13867–13872

20. Sooy K, Noble J, McBride A, Binnie M, Yau JL, Seckl JR, Walker BR, Webster SP (2015) Cognitive and disease-modifying effects of 11β-hydroxysteroid dehydrogenase type 1 inhibition in male Tg2576 mice, a model of Alzheimer’s disease. Endocrinology 156:4592–4603
