Propranolol reduces cognitive deficits, amyloid and tau pathology in Alzheimer’s transgenic mice

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Abstract

The efficacy of antihypertensive agents in Alzheimer’s disease (AD) is controversial. It has been tested here whether some antihypertensive drugs might influence AD through mechanisms independent of blood pressure-lowering activity. The effects of treatment with the antihypertensive propranolol on cognition and AD-related markers have been studied in the Tg2576 mouse model of AD. Propranolol, at a lower dose than that used as antihypertensive (5 mg/kg, 6 wk), attenuated cognitive impairments shown by Tg2576 mice aged 9 months in the novel object recognition and fear conditioning tests. Propranolol was also able to counteract the increases in hippocampal levels of Aβ42 present in Tg2576 mice. This effect was accompanied by an increased expression of insulin degrading enzyme. Changes in markers of synaptic pathology, as shown by decreases in phosphorylation of Akt and in the expression of BDNF in Tg2676 mice, were also counteracted by propranolol treatment. Tau hyperphosphorylation shown by Tg2576 mice was also decreased in the hippocampus of propranolol-treated mice, an effect probably related to an increase of GSK3β phosphorylation (inactive form) and a decreased JNK1 expression. Overall, these data further strengthen the potential of propranolol as a therapeutic agent for AD.

Received 19 February 2013; Reviewed 14 March 2013; Revised 5 April 2013; Accepted 9 May 2013; First published online 17 June 2013

Key words: Antihypertensive, BACE, BDNF, insulin degrading enzyme, JNK1.

Introduction

Alzheimer’s disease (AD) is the most common neurodegenerative disorder and the first cause of dementia in the elderly (Cummings and Cole, 2002). AD is associated with progressive memory loss and cognitive impairments and at the molecular level by the presence of neurofibrillary tangles, composed of hyperphosphorylated τ fibrils and β-amyloid (Aβ)-containing plaques (Hardy, 2006). Loss of synapses and degeneration of the neurons are also key pathological events in AD (Selkoe, 2002). Over the last few years, several drugs such as Aβ modulators, γ-secretase inhibitors, antioxidants or anti-inflammatory molecules, have emerged as potential therapeutic candidates for the treatment of AD after showing promising results in animal models. However, none of them have proven to be effective.

Identification of risk factors of AD is important for developing therapeutic strategies. Several longitudinal studies suggest that blood pressure levels are increased decades before the onset of AD and higher blood pressure seems to accelerate the rate of cognitive decline in patients with early-onset AD (Bellew et al., 2004; Luchsinger et al., 2005; Skoog and Gustafson, 2006). Furthermore, epidemiological evidence suggests that hypertension may promote the onset and progression of Aβ and τ neuropathology (Sparks et al., 1996; Petrovitch et al., 2000). Antihypertensive treatments have been associated with lower incidence of clinically diagnosed AD and better cognitive function (Hajjar et al., 2005; Khachaturian et al., 2006; Hoffman et al., 2009). For example, Guo et al. (1999) reported that the use of specific cardiovascular agents, especially β-blockers and calcium-receptor antagonists, protected elderly hypertensive subjects from developing AD.
agents in AD dementia (Prince et al., 1998; in’t Veld et al., 2001; Lithell et al., 2004). Thus, at present there is inconsistent evidence regarding the influence of antihypertensive drugs on AD incidence and/or pathogenesis.

This lack of consensus may reflect, in part, the different AD-modifying features among antihypertensive drugs. Wang et al. (2007) performed a high-throughput screening study assessing 55 antihypertensive drugs representing all pharmacological classes of currently available antihypertensives. They found that only a few of the antihypertensive agents were able to significantly reduce the accumulation of Aβ40 and Aβ124 in primary embryonic cortico-hippocampal neuron cultures derived from a transgenic mouse AD model, suggesting that some antihypertensive drugs might influence AD through mechanisms affecting Aβ neuropathology.

Based on this premise, the present work aims to study whether some antihypertensive drugs might influence AD through mechanisms affecting Aβ neuro pathology independent of blood pressure-lowering activity. We have focussed on propranolol, a β-adrenergic antagonist that is commonly used in the treatment of hypertension, cardiac arrhythmia, angina pectoris or acute anxiety. Propranolol has been shown experimentally to reverse cognitive deficits associated with stress (Roozendaal et al., 2004; Aisa et al., 2007) and to decrease Aβ levels in vitro (Wang et al., 2007). Propranolol has been previously used as a therapeutic agent for the treatment of disruptive behaviours (mainly aggression and agitation) in AD (Shankle et al., 1995; Peskind et al., 2005). It is proposed here that propranolol, at lower doses than those used as antihypertensive, could ameliorate memory impairments and Aβ pathology in the Tg2576 transgenic mouse model of AD.

Method

Animals

Experiments were carried out in 16 female Tg2576 AD transgenic mice (23–26 g), that express the human 695-aa isoform of amyloid precursor protein (APP) containing the Swedish double mutation [APPsw; (APP695) Lys670→Asn, Met671→Leu]. The mice were on an inbred C57BL/6SJL genetic background. In the Tg2576 AD mouse model, Aβ peptide content in the brain accumulates exponentially between ages 7 and 12 months (Hsiao et al., 1996). As our hypothesis is that propranolol is able to interfere with Aβ pathology, mice aged 8 months have been used. As a control we used a group of 16 age- and strain-matched non-transgenic mice. Animals were housed (five per cage) in constant conditions of humidity and temperature (22±1°C) with a 12-h/12-h light–dark cycle (lights on 07:00 hours). Food and water were available ad libitum. All the procedures followed in this work were in compliance with the European Community Council Directive of 24 November 1986 (86/609/EEC) and were approved by the Ethical Committee of the University of Navarra. Behavioural experiments were conducted between 09:00 and 13:00 hours. Animals were randomly assigned to control and treatment group.

Drug treatment and experimental design

To study the effect of propranolol (propranolol hydrochloride; Sigma-Aldrich, USA) on cognitive impairment, female Tg2576 mice aged 8 months and their respective controls (non-transgenic mice), were treated once daily with propranolol (5 mg/kg i.p.) or saline for 6 consecutive wk (n=8 per genotype and treatment).

In the final week of treatment the novel object recognition test (NORT) and the fear conditioning test were performed. During the behavioural studies, to avoid any purported effect of an acute drug administration, propranolol was administered after the behavioural testing (20 h before the following behavioural session).

Behavioural tests

Behavioural experiments were conducted between 09:00 and 13:00 hours. Animals were randomly assigned to control and treatment group. Tg2576 mice aged 9 months have shown impairments in recognition memory, but spatial working memory is not altered (Hsiao et al., 1996; Reed et al., 2010). Therefore, the tests chosen to study the effects of propranolol on cognition have been the NORT and fear conditioning.

Locomotor activity

Horizontal locomotor activity was measured for 30 min in an open field, which consisted of four square arenas (40×50×50 cm) using a video tracking system (Ethovision 3.0; Noldus Information Technology BV, The Netherlands), in a softly illuminated room. Total path length (cm) was analysed.

Object recognition

The object recognition test was adapted from Ennaceur and Delacour (1988). The open field consisted of a square open field (40×50×50 cm). During the first trial of the experiment, two objects similar in shape,
size, colour, texture etc., equidistant from the sides (10 cm) were placed within the chamber. The animal was placed into the centre of the open field and allowed to freely explore for 5 min. It was considered that the animal was exploring the object when the head of the mouse was oriented towards the object with its nose within 2 cm of the object. One hour later a second trial took place, in which one object was replaced by a different one and exploration was scored for 5 min. In order to eliminate olfactory stimuli, chamber and objects were cleaned after testing each animal. To avoid preference for one of the objects, the order of the objects was balanced between testing animals. Results were expressed as percentage of time spent with the novel object with respect to the total exploration time (discrimination index).

Fear conditioning test

The conditioning procedure was carried out in a StartFear system (Panlab S.L., Spain) that allows recording and analysis of the signal generated by the animal movement through a high sensitivity weight transducer system. The analogical signal is transmitted to the FREEZING and STARTLE software modules through the load cell unit for recording purposes and posterior analysis in terms of activity/immobility.

The conditioning box is housed inside a soundproof chamber, which minimized external stimulation during the conditioning and retention tests. The box was provided with a house light that supplied dim illumination and with a floor grid through which foot shocks could be administered.

On habituation day, the mice were placed in the conditioning chamber for 5 min. On training day, the mice were placed in the conditioning chamber for 2 min before the onset of a tone at 2800 Hz, 85 dB [conditioned stimulus (CS)], which lasted for 30 s. In order to eliminate olfactory stimulii, chamber and objects were cleaned after testing each animal. To avoid preference for one of the objects, the order of the objects was balanced between testing animals. Results were expressed as percentage of time spent with the novel object with respect to the total exploration time (discrimination index).

Neuronal primary culture

Hippocampal tissues from C57BL/6J mice embryos aged 19 d were homogenized in serum-free Neuro-Basal medium with 2% B27 supplement (Invitrogen, USA). Cells from each embryo were seeded separately in dishes that were pre-coated with poly-d-lysine MW 300000 (0.17 mg/ml; Sigma-Aldrich, USA) in PBS. Cells were grown for 10 d and the culture medium was changed every fourth day. On day 10, 1 μM propranolol was added to a fresh and pre-warm medium and cells were collected 24 h after the treatment.

Cell viability was examined by means of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, which depends on the reduction of the tetrazolium salt MTT to formazan by living cells. Hippocampal/cortical mixed cells were cultured in 48-well plates and treated overnight with 8 μM Aβ25-35 fragments (Sigma-Aldrich, USA) and followed by an overnight incubation with propranolol (1 μM). After treatment, MTT was dissolved in PBS (5 mg/ml) and added (10 μl per 100 μl medium) to all wells of the assay and incubated for 2 h at 37 °C. The MTT solution was aspirated and DMSO was added to the cells. Aliquots were transferred to a 96-well plate and absorbances were measured at 595 nm in a plate reader. Results were expressed as percentages of the respective value obtained for cells treated with 10 mM sodium phosphate buffer at pH 7.25.

Tissue collection

At the end of the behavioural studies, fasting mice were killed between 08:00 and 10:00 hours. Brains were removed and dissected on ice to obtain the hippocampus, or frozen immediately and stored at −80 °C, according to random assignment.

Western blotting

Cytosolic extract preparations from the hippocampus of mice were homogenized in a cold lysis buffer with protease inhibitors (0.2 mM NaCl, 0.1 mM Heps, 10% glycerol, 200 mM NaF, 2 mM Na4P2O7, 5 mM EDTA, 1 mM EGTA, 2 mM DTT, 0.5 mM PMSF, 1 mM Na3VO4, 1 mM benzamidine, 10 μg/ml leupeptin, 400 U/ml aprotinin). Samples (20 μg) were separated by electrophoresis on a sodium dodecyl sulphate–polyacrylamide gel. Membranes were probed overnight at 4 °C with the corresponding primary antibodies (Table 1). Immunopositive bands were visualized using an enhanced
chemiluminescense Western blotting-detection reagent (ECL; UK). The optical density (OD) of reactive bands visible on X-ray film was determined densitometrically. β-Actin or α-tubulin was used as internal control. Results were expressed as percentage of OD values of control (non-transgenic saline) mice.

Aβ levels

For analysis of soluble Aβ40 and Aβ42 burden, the hippocampus was homogenized in a buffer containing 5 M guanidine HCl and 50 mM Tris–HCl, pH 8, protease inhibitors (Complete Protease Inhibitor Cocktail, Roche, Spain) and phosphatase inhibitors (0.1 mM Na3VO4, 1 mM NaF). Aβ levels were measured using a sensitive sandwich ELISA kit (Biosource, USA) following the manufacturer’s instructions.

**Table 1. Conditions used in Western blotting experiments**

<table>
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<th>SDS–polyacrylamide gel</th>
<th>Molecular weight</th>
<th>Primary antibody (dilution)</th>
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<td>100 kDa</td>
<td>Anti-IDE (1:1000)a</td>
</tr>
<tr>
<td>pAkt</td>
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<td>Anti-pAkt (1:1000)b</td>
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<td>Anti-Akt (1:1000)b</td>
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<td>Anti-Synaptophysin (1:500)a</td>
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<td>70 kDa</td>
<td>Anti-BACE 1 (1:1000)a</td>
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<td>46 kDa</td>
<td>Anti-pSAPK/JNK Thr183/Tyr185 (1:1000)b</td>
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<td>46 kDa</td>
<td>Anti-SAPK/JNK (1:1000)b</td>
</tr>
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<td>50 kDa</td>
<td>Anti-pr Ser202/Thr205 AT8 (1:500)c</td>
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SDS, Sodium dodecyl sulphate; IDE, insulin degrading enzyme; p, phosphorylated; SAPK, stress activated protein kinase; m, mature; JNK, c-Jun N-terminal kinase; GSK, glycogen synthase kinase; BDNF, brain-derived neurotrophic factor; APP, amyloid precursor protein.

a Abcam, USA.
b Cell Signalling Technology, USA.
c Pierce, USA.
d Sigma-Aldrich, USA.

Results

**Propranolol restored cognitive function in Tg2576 mice**

Two-way ANOVA indicated a significant genotype×treatment interaction on the measure of discrimination between new and familiar objects (F1,31=9.191, p<0.01). Further analysis revealed that Tg2576 animals showed a significant learning impairment (Tukey’s test, p<0.05) that was reversed by propranolol treatment (Tukey’s test, p<0.05) (Fig. 1a).

The effects observed in NORT do not seem to be associated to differences in locomotor activity, as there was no difference in the total amount of time spent exploring two identical objects between non-transgenic and Tg2576 groups in the NORT (two-way ANOVA, F1,31=0.013, p>0.05; Supplementary Fig. S1). Further supporting this fact, total distance travelled in the open field (F1,31=0.906, p>0.05; Supplementary Fig. S1) was not affected by either genotype or treatment.

Mice were next given the fear conditioning test (contextual learning), another hippocampus-dependent

mean±S.E.M. The level of significance for all analyses testing was set at p<0.05.

Data analysis and statistics

Data were analysed by SPSS for Windows, release 15.0. Normality was checked by Shapiro–Wilks’s test (p>0.05). Behavioural and biochemical data were analysed by Student’s t test or two-way analysis of variance (ANOVA; genotype×treatment). Post hoc comparisons were conducted, if appropriate, using Tukey’s protected least significance test. Data are presented as mean±S.E.M. The level of significance for all analyses testing was set at p<0.05.

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Fig. 1. Chronic treatment with propranolol reverses the learning deficit in Tg2576 transgenic mice. (a) Novel object recognition test, data shown as discrimination index (time exploring new object/total time of exploration×100); (b) Fear conditioning, data shown as percentage of freezing over the 2 min test. Two-way analysis of variance (ANOVA; genotype×treatment) significant interaction: † p<0.05, †† p<0.01 significant differences vs. non-transgenic (Non-tg) saline-treated; ‖ p<0.05, ‖‖ p<0.01 significant differences vs. Tg2576 saline mice.

Learning task, where Tg2576 mice are impaired. As shown in Fig. 1b, Tg2576 mice that received the propranolol showed a freezing response similar to that of the age-matched non-transgenic mice and much more than saline-treated Tg2576 animals (two-way ANOVA, significant interaction $F_{1,31}=6.519$, p<0.05).

All these data confirm that propranolol given chronically ameliorated the memory deficits of this AD mouse model.

**Propranolol decreases Aβ burden and protects against Aβ toxicity**

Tg2576 mice treated with propranolol exhibited significantly lower hippocampal levels of Aβ40 and Aβ42 (Student’s $t$ test, p<0.05, Fig. 2a). No Aβ was detected in non-transgenic littermates. The lowering effects of propranolol on Aβ burden could be related to both a decrease in the production or to an increase in the clearance of Aβ. No differences were found in Tg2576 mice treated with either saline or propranolol in expression of APP or the β-secretase, BACE (Student’s $t$ test, Fig. 2b, c), suggesting that production of Aβ is not affected by propranolol. However, expression of the α-secretase, ADAM17 was increased in propranolol-treated mice (two-way ANOVA, main effect of treatment $F_{1,31}=3.769$, p<0.05; Fig. 2d).

To check clearance, the expression of insulin degrading enzyme (IDE), a metalloprotease that has a crucial part in Aβ clearance in the brain, was measured (Fig. 3a). It was found that propranolol was able to increase IDE expression, independently of genotype (two-way ANOVA, main effect of treatment, $F_{1,31}=19.874$, p<0.001).

To confirm that propranolol was able to directly increase IDE expression, cell culture neurons were exposed to propranolol (1, 2, 4, 8 μM, 24 h) and it was found that IDE expression was significantly enhanced (Student’s $t$ test, p<0.01, Fig. 3b). Under these conditions, these increases in IDE expression seem to lead to decreased Aβ effects (Fig. 3c). In the MTT assay, primary neurons treated with Aβ25-35, 8 μM, showed significantly reduced cell viability as compared with cells treated with sodium phosphate buffer (controls). In the presence of propranolol (1 μM), Aβ-induced cytotoxicity was significantly reversed (two-way ANOVA, $F_{1,19}=19.536$, p<0.01, followed by Tukey’s test).

**Effects of propranolol on markers of synaptic plasticity**

The hippocampus is essential for the formation and storage of memories, which is in turn mediated, at least in part, by hippocampal synaptic plasticity. Reversal of memory dysfunction by propranolol in Tg2576 mice might be attributable to changes in synaptic plasticity. Several different markers of synaptic plasticity were selected to be analysed: Akt, as part of one of the most critical pathways in regulating cell survival; synaptophysin, as a widely used marker to estimate synaptic density; brain-derived neurotrophic factor (BDNF), which helps the survival of existing neurons and facilitates the growth of new neurons and synapses.

There was a main effect of genotype and treatment regarding phosphorylated (p) Akt levels normalized to total Akt (main effect of genotype, $F_{1,31}=58.399$, p<0.01; and main effect of treatment $F_{1,31}=20.031$, p<0.01; Fig. 4a). Synaptophysin levels were also increased by propranolol (main effect of treatment, $F_{1,31}=5.445$, p<0.05, Fig. 4b).
BDNF is secreted to the extracellular matrix in a proBDNF form, which negatively affects survival and synaptic plasticity. However, proBDNF is mainly cleaved to mature (m) BDNF, which eventually has positive effects on survival, growth, synaptic plasticity and memory formation. Therefore, the proBDNF: mBDNF ratio has been measured (Fig. 4c) and a decrease in the pro-BDNF: mBDNF ratio has been found in Tg2576 mice (main effect of genotype, $F_{1.31}=9.930, p<0.01$) while propranolol treatment was able to increase these levels (main effect of treatment $F_{1.31}=5.855, p<0.05$).

**Effects of propranolol on τ pathology**

Although Tg2576 mice do not suffer from real τ pathology in terms of neurofibrillary tangles, these animals manifest age-related hyperphosphorylated τ-containing neurites and higher activities of various τ kinases. Lately, it has been suggested that abnormal

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**Fig. 2.** Propranolol (Prop) decreases β-amyloid (Aβ) burden. Aβ levels (a) and amyloid precursor protein (APP; b), BACE1 (c) and ADAM17 (d) expression in the hippocampus of Tg2576 transgenic mice. In (b), (c) and (d), representative Western blot bands from hippocampal tissues are shown. The histograms represent the quantification of the immunochemically reactive bands in the Western blot. Data are expressed as mean percentage of the results from the non-transgenic (Non-tg) mice receiving saline (Sal; Non-tg Sal, 100%). In (a) * significant effect, Student’s t test. In (b) two-way analysis of variance (ANOVA; genotype×treatment), §§ $p<0.01$ main effect of genotype. In (d) two-way ANOVA (genotype×treatment), # $p<0.05$ main effect of treatment.
Propranolol in AD transgenic mice

Fig. 3. Propranolol (Prop) increases β-amyloid (Aβ) clearance and toxicity. In (a) increased levels of insulin degrading enzyme (IDE) expression in the hippocampus of propranolol treated mice might reflect an increased clearance of Aβ in the hippocampus of Tg2576 transgenic mice. In neuronal primary cultures IDE expression was also increased after propranolol treatment (b) and this treatment protected against Aβ25-35 toxicity (c). In all cases representative Western blot bands from hippocampal tissues are shown. The histograms represent the quantification of the immunochemically reactive bands in the Western blot. Data are expressed as mean percentage of the results from the non-transgenic (Non-tg) mice receiving saline (Sal; Non-tg Sal, 100%). In (a), two-way analysis of variance (ANOVA; genotype×treatment), **p<0.01 main effect of treatment. In cell culture experiments, (b) Student’s t test, ** p<0.01, significant differences vs. control. In (c) two-way ANOVA (Aβ×Prop), significant interaction, †† p<0.01, significant differences vs. control; ^^^ p<0.01, significant differences vs. Aβ25-35 toxicity.

Discussion

The main finding in this study is the reversal of memory deficits in the Tg2576 mouse model of AD using propranolol, a brain penetrating non selective τ phosphorylation is a secondary event to Aβ pathology in AD. In addition, considering the significance of the Akt–glycogen synthase kinase (GSK)3β–τ pathway and given the results found measuring Akt, we analysed next the levels of τ phosphorylation in the mice hippocampi. Immunoblot analysis showed a significant genotype×treatment interaction regarding pr at pSer202/Thr205 (pr normalized to total τ levels; two-way ANOVA, F1.31 = 6.869, p<0.05). Further analysis revealed that Tg2576 animals showed significant hyperphosphorylation of τ that was reversed by propranolol treatment (Tukey’s test, p<0.05, Fig. 5a). Similarly, pr at Ser396/404, using the PHF1 antibody (Fig. 5b), was also altered in Tg2576 mice (two-way ANOVA, significant genotype×treatment interaction, F1.31 = 4.651, p<0.05) and the increases in pr levels showed by Tg2576 were reverted by propranolol (Tukey’s test, p<0.05).

The expression of several kinases implicated in τ pathology was checked. As shown in Fig. 5c, there was also a significant interaction in pGSK3β expression (pGSK3β in Ser9, inactive form) normalized to total GSK3β (two-way ANOVA, F1.31 = 4.349, p<0.05). Further analysis revealed that Tg2576 animals showed decreased levels of pGSK3β (Tukey’s test, p<0.05) that was reversed by propranolol treatment (Tukey’s test, p<0.05). Moreover, there was a genotype×treatment interaction (two-way ANOVA, F1.31 = 6.828, p<0.05) with regard to p-c-Jun N-terminal kinase (JNK)1 levels normalized to total JNK1. Western blot analysis revealed that pJNK1 levels were significantly increased in saline Tg2576 mice compared with non-transgenic mice (Tukey’s test, p<0.05) and propranolol treatment was able to reverse these effects on pJNK1 expression (Tukey’s test, p<0.05; Fig. 5a).

Consistent with a post-transcriptional regulation of these enzymes, total τ, JNK1 and GSK3β levels, normalized using actin, remained unaltered.
β-adrenergic antagonist widely used as an antihypertensive drug. Previous work has reported the ability of propranolol to reverse cognitive deficits associated with deleterious insults such as chronic stress (Aisa et al., 2007). However, to our knowledge, this is one of the first reports describing pro-cognitive actions of propranolol in an experimental model of AD.

Because the overall goal of our study was to test the hypothesis that propranolol may influence AD-type Aβ pathogenesis independent of blood pressure-lowering activity, we treated Tg2576 mice with 5 mg/kg propranolol. Based on the fact that the recommended dose of propranolol for the treatment of hypertension in humans is 160–320 mg/d and using US Food and Drug Administration criteria [human equivalent dose in mg/kg=animal dose in mg/kg×(animal weight in kg/human weight in kg)0.33; http://www.fda.gov/cber/gdlns/dose.htm] and the formula described by Reagan-Shaw et al. (2007) for converting drug equivalent dosages across species area [human equivalent dose in mg/kg animal dose in mg/kg×Km mice (=3)/Km human (=37)], it can be calculated that the dose used in the present study is below the recommended human equivalent dosage range. Therefore, propranolol was used in the present study at a lower dose than that commonly prescribed for the treatment of hypertension in humans (Reagan-Shaw et al., 2007) and the results presented here seem to be independent from the antihypertensive effect of propranolol.

In an attempt to elucidate the mechanisms underlying the beneficial effects of propranolol and based on previously published work (Wang et al., 2007), we focused our study on Aβ and τ pathology, the two main pathological hallmarks in AD and in markers of synaptic plasticity.

A key issue was to determine the optimal age of Tg2576 mice for this study. Tg2576 mice show rapid increases in Aβ levels starting at age 6 months and Aβ peptide content in the brain accumulates exponentially between ages 7 and 12 months. Following our hypothesis that propranolol was able to alter Aβ burden, Tg2576 mice aged 8 months (at the beginning of treatment) were chosen in order to be able to detect significant increases in Aβ content in saline treated mice. Tg2576 mice aged 9 months (at the end of treatment) shown impairments in recognition memory (Mouri et al., 2007; Taglialatela et al., 2009) but spatial working memory is not altered until age 12–15 months (Hsiao et al., 1996; Reed et al., 2010). Therefore, the tests chosen to study the effects of propranolol on cognition have been the NORT, to assess recognition memory, and fear conditioning, to check contextual hippocampal dependent memory. Interestingly, in

**Fig. 4.** Effects of propranolol (Prop) on markers of synaptic plasticity in the hippocampus. (a) Phosphorylated (p)Akt levels, (b) synaptophysin levels, (c) proBDNF mature (m)BDNF:proBDNF ratio. Representative Western blot bands from hippocampal tissues are shown. The histograms represent the quantification of the immunochemically reactive bands in the Western blot. Data are expressed as mean percentage of the results from the non-transgenic (Non-tg) mice receiving saline (Sal; Non-tg Sal, 100%). Two-way analysis of variance (ANOVA; genotype×treatment): §§ p<0.01, main effect of genotype; † p<0.05, ‡ p<0.01, main effect of treatment.
in our study, a chronic treatment with propranolol has been able to completely reverse memory deficits in both types of cognitive test. The effects of propranolol on memory are complex and, therefore, a note of caution should be exercised regarding the present effects on cognition. Infusion of a β-adrenergic receptor agonist improves memory consolidation (Cahill and McGaugh, 1996) and systemic propranolol impaired memory retrieval on a water maze spatial task (Murchison et al., 2004). Alternatively, propranolol has been shown to reverse cognitive deficits associated with chronic stress (Aisa et al., 2007). In human volunteers, propranolol enhancement of cognitive flexibility is affected by how much difficulty the subject is encountering when searching for the solution (Campbell et al., 2008). In this respect, it has been suggested that the retention-enhancing effects of increased noradrenergic tone may be caused by influences on mechanisms other than

Fig. 5. Effects of propranolol treatment on r pathology in the hippocampus. Propranolol regulates tau phosphorylation [p; (a) and (b)] through glycogen synthase kinase (GSK)3β (c) and c-Jun N-terminal kinase (JNK)1 (d) in Tg2576 transgenic mice. Representative Western blot bands from hippocampal tissues are shown. The histograms represent the quantification of the immunochemically reactive bands in the Western blot. Data are expressed as mean percentage of the results from the non-transgenic (Non-tg) mice receiving saline (Sal; Non-tg Sal, 100%). Two-way analysis of variance (ANOVA; genotype×treatment): significant interaction, † p<0.05, significant differences vs. non-transgenic Sal-treated, ^ p<0.05, significant differences vs. Tg2576 Sal mice. AT8 and PHF1, phosphorylated tau; tot, total.
memory retrieval e.g. nonspecific effects on arousal and attention (Aston-Jones et al., 2000).

Given the genotypic nature of Tg2576 mouse, Aβ should be a major player in the memory dysfunction developed by this transgenic mouse. According to the amyloid cascade hypothesis (Hardy and Selkoe, 2002), Aβ is the starting point for a sequence of pathogenic events, such as τ hyperphosphorylation and neuroinflammation, that contribute to synaptic dysfunction and ultimately cause dementia. Therefore, although lately controversial in literature reports, it could be suggested that the reduction of Aβ burden by propranolol would consequently contribute to ameliorate AD symptoms. Accumulation of Aβ is proposed to result from an imbalance between Aβ production and Aβ clearance. Although changes in the amyloidogenic processing of APP cannot be completely excluded from the present work, the lack of effect of propranolol treatment on BACE1 expression seems to indicate that Aβ production is not altered by propranolol. However, the effects of propranolol on ADAM17 suggest that the non-amyloidogenic processing of APP could be facilitated in propranolol-treated mice. Alternatively, the brain possesses robust intrinsic Aβ clearance mechanisms (Tanzi et al., 2004). Aβ peptides are proteolytically degraded within the brain mainly by neprilysin (Iwata et al., 2000) and IDE (Kurochkin and Goto, 1994). It is unclear whether increased neprilysin levels may be beneficial to Aβ pathology, as this enzyme neither degrades Aβ oligomers nor restores memory function in an AD mouse model (Meilandt et al., 2009). Therefore, we have focused on IDE expression. The results found both in the Tg2576 mice and in cell culture experiments suggest that propranolol, by increasing IDE expression, is able to prevent the cellular damage induced by Aβ.

Although the Tg2576 mouse is a model of AD amyloidosis and Tg2576 mice do not suffer from real τ pathology in terms of neurofibrillary tangles, the presence of hyperphosphorylated τ epitopes (Ser199, Thr213/Ser235, Ser396 and Ser413) and higher activities of various τ kinases has been consistently reported (Tomidokoro et al., 2001; Sasaki et al., 2002; Ferrer et al., 2005; Cuadrado-Tejedor et al., 2011 and shown in Fig. 4). In fact, Aβ amyloidosis can activate the τ kinase pathway involving GSK3β (via its phosphorylation at Tyr216) and, subsequently, τ is phosphorylated at sites that promote its accumulation and deposition (Tomidokoro et al., 2001; Oth et al., 2002). Among kinases implicated in τ pathology, data on activation of JNK1 seems to be of particular interest. JNK1 is a member of the mitogen-activated protein kinase family that plays a key role in neuronal plasticity, regeneration and cell death (Kögel et al., 2005, Maruyama et al., 2009). Activation of JNK1 phosphorylates τ at sites recognized by the presently used PHF1 antibody (Bellucci et al., 2007). It is of note that JNK1 phosphorylates the insulin receptor substrate-1 at an inhibitory site that can block signal transduction by the insulin receptor (Sabio et al., 2008), therefore purportedly altering insulin-related intracellular pathways, namely the Akt–GSK3β–τ. This Akt–GSK3β route is required for the induction of long-term potentiation and depression, basic processes underlying learning and memory (van der Heide et al., 2006). Accordingly, it has been found, compared to controls, that treatment with propranolol in Tg2576 mice leads to decreased JNK1 activity accompanied by increased pAkt, increased pGSK-3β (inactive form) and decreased pr expression. Together these data suggest that the decrease in the kinase activity of GSK3β and JNK1 due to propranolol was able to prevent the signs of tauopathy observed in the hippocampus of Tg2576 mice, possibly contributing to the reinstatement of cognitive function as data, based on several lines of evidence from transgenic mice and humans, indicating a strong correlation between the extent of τ pathology and cognitive dysfunction (Guilozet et al., 2003; Hanger et al., 2009; Ashe and Zahs, 2010). Interestingly, propranolol seems to restore the GSK3β or JNK1 pathways when they are hyperactivated but not under normal conditions. In fact, these pathways were not affected by propranolol when non-transgenic mice were analysed.

The reversion by propranolol of changes in markers of synaptic pathology in Tg2576 mice should be also considered. BDNF has been described to be highly protective for different neuronal phenotypes and can prevent neuronal cell death in different experimental paradigms in vivo as well as in vitro (Barde, 1994; Siegel and Chauhan, 2000). BDNF levels decline with age and are lower in AD brains that in those without dementia. BDNF delivery ameliorated age-related cognitive impairment in aged primates and mice even when the treatment was initiated after disease onset (Nagahara et al., 2009). Not only that, but synaptophysin seem to be a pre-synaptic vesicle protein partially regulated by BDNF (Tartaglia et al., 2001; Valtorta et al., 2004) and therefore reduced synaptophysin expression may result from a reduced BDNF function. However, hippocampal BDNF is secreted to the extra-cellular matrix in a proBDNF form. ProBDNF is active via p75 neurotrophin receptors and negatively affects survival and synaptic plasticity. ProBDNF is mainly cleaved to mature BDNF to activate TrkB receptors, which eventually has positive effects on survival,
growth, synaptic plasticity and memory formation. Therefore, the proBDNF:mBDNF ratio could be a more accurate form to anticipate BDNF outcomes. It is of note that it could have been expected that β-receptor blockade would have led to decreased BDNF levels (Flores et al., 2010). Therefore, the present increase in the ratio suggests that propranolol could modulate the cleavage of proBDNF to give rise to the mature form. An alternative is to consider the effects of propranolol on ADAM17 expression. Besides APP, many other membrane-bound pro-ligands with trophic or survival properties, such as epidermal growth factor, neuregulin 1/β or transforming growth factor-α, could be the substrate of ADAM17 (Dang et al., 2011). Thus, the possibility is that increases of ADAM17 might be increasing levels of these neurotrophic factors. In this respect, it has been described that cultured neurons from Tg2576 mice showed a reduced viability after treatment with an inhibitor of ADAM17 (Gil-Bea et al., 2012). In addition, it is noted that propranolol was able to increase pAkt, synaptophysin and BDNF also in non-transgenic mice. Even though the mechanism responsible for increases by propranolol in these synaptic markers cannot be unravelled from the present results, it is possible to speculate about the usefulness of this compound for the treatment of any pathology associated with a decreased synaptic function.

Concluding remarks

Collectively, our evidence suggest that some of the drugs, namely propranolol, currently prescribed for the treatment of high blood pressure might also beneficially modulate AD by mechanisms independent of changes in blood pressure (Zhao et al., 2009). Propranolol may have AD-modifying activity and may protect against progressive Aβ/τ-related memory deficits. Although further studies would help to fully understand the mechanisms by which propranolol exert its effects on AD-related pathology, the present preclinical work should foster future clinical studies on the use of propranolol for the treatment of AD.

Acknowledgements

This work has been supported by a grant from FIS (10/01748) and ‘Tu eliges, Tu decides’ projects of CAN.

Conflicts of Interest

None.

Supplementary material

For supplementary material accompanying this paper, visit http://dx.doi.org/10.1017/S1461145713000631.

References


