Neuropharmacology 64 (2013) 137–144

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

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

Propranolol restores cognitive deficits and improves amyloid and Tau pathologies in a senescence-accelerated mouse model

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ARTICLE INFO

Article history:
Received 28 March 2012
Received in revised form
19 June 2012
Accepted 24 June 2012

Keywords:
Ageing
Aβ levels
IDE
BACE1
JNK1
Hippocampus
SAMP8

ABSTRACT

Ageing is associated with a deterioration of cognitive performance and with increased risk of neurodegenerative disorders. Hypertension is the most-prevalent modifiable risk factor for cardiovascular morbidity and mortality worldwide, and clinical data suggest that hypertension is a risk factor for Alzheimer's disease (AD). In the present study we tested whether propranolol, a β-receptor antagonist commonly used as antihypertensive drug, could ameliorate the cognitive impairments and increases in AD-related markers shown by the senescence-accelerated mouse prone-8 (SAMP8). Propranolol administration (5 mg/kg for 3 weeks) to 6-month-old SAMP8 mice attenuated cognitive memory impairments shown by these mice in the novel object recognition test. In the hippocampus of SAMP8 mice it has been found increases in $A\beta_{42}$ levels, the principal constituent of amyloid plaques observed in AD, accompanied by both an increased expression of the cleaving enzyme BACE1 and a decreased expression of the degrading enzyme IDE. All these effects were reversed by propranolol treatment. Tau hyperphosphorylation (PHF-1 epitope) shown by SAMP8 mice at this age was also decreased in the hippocampus of propranolol-treated mice, an effect probably related to a decrease in JNK1 expression. Interestingly, propranolol also phosphorylated Akt in SAMP8 mice, which was associated with an increase of glycogen synthase kinase-3β phosphorylation, contributing therefore to the reductions in Tau hyperphosphorylation. Synaptic pathology in SAMP8 mice, as shown by decreases in synaptophysin and BDNF, was also counteracted by propranolol treatment. Overall, propranolol might be beneficial in age-related brain dysfunction and could be an emerging candidate for the treatment of other neurodegenerative diseases.

This article is part of a Special Issue entitled 'Cognitive Enhancers'.

1. Introduction

Ageing in humans, as well as in experimental animals, is associated with a deterioration of cognitive performance particularly of learning and memory and with increased risk of neurodegenerative disorders (Barzilai et al., 2006; Mehta, 2007). Undoubtedly, identifying the key markers and how they interact to turn benign ageing into pathologic seems a crucial step for the development of therapeutic strategies to prevent or slow down the progression of ageing and neurodegenerative diseases such as Alzheimer's disease (AD).

Besides ageing, which is the most obvious risk factor for the AD, clinical data suggest that hypertension is a risk factor for AD (Luchsinger et al., 2005). Hypertension is the most-prevalent modifiable risk factor for cardiovascular morbidity and mortality worldwide. Hypertension is highly prevalent among older adults (≥65 years), and ageing of the population will substantially increase the prevalence of this condition (Pimenta and Oparil, 2012). Antihypertensive treatments have been associated with lower incidence of clinically diagnosed AD and better cognitive function (Guo et al., 1999; Hajjar et al., 2005; Hoffman et al., 2009; Khachaturian et al., 2006). Propranolol, a β-adrenergic antagonist that is commonly use in the treatment of hypertension, cardiac arrhythmia, angina pectoris or acute anxiety, has been shown experimentally to reverse cognitive deficits associated to stress (Aisa et al., 2007; Roozendaal et al., 2004). Furthermore, recent evidence suggests that propranolol is able to decrease Aβ levels *in vitro* (Wang et al., 2007).

The senescence-accelerated mouse (SAM) is comprised of 14 different strains derived from selective inbreeding of the AKR/J strain (Takeda et al., 1981). One of these substrains, the senescence-

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http://dx.doi.org/10.1016/j.neuropharm.2012.06.047

accelerated mouse prone-8 (SAMP8) strain manifests irreversible advancing senescence and shares similar characteristics with aged humans such as a reduced lifespan, lordosis, hair loss, and reduced physical activity (Hamamoto et al., 1984; Takeda et al., 1994), whereas the senescence-accelerated mice resistant-1 (SAMR1) strain presents a normal ageing pattern (Takeda, 1999). Interestingly, SAMP8 mice also exhibit age-related learning and memory deficits, as well as amyloid-like deposits in the brain (Del Valle et al., 2010; Tomobe and Nomura, 2009) and increased expression of hyperphosphorylated Tau (Canudas et al., 2005; Orejana et al., 2012). Given such features, SAMP8 mice have been proposed as suitable rodent model for studying age-associated pathologies (Tomobe and Nomura, 2009), or even as an AD animal model (Liu et al., 2010; Pallas et al., 2008).

The aim of the present study was to explore whether propranolol could reverse the memory impairments shown by SAMP8 mice as well as the underlying mechanisms. In particular, it is hypothesised here that propranolol treatment may protect from amyloid, Tau and synaptic pathology present in the SAMP8 model of accelerated ageing. Therefore, in the present study it has been checked the effects of propranolol on SAMP8 mice regarding cognitive status in the novel object recognition test, Aβ levels mechanisms of processing (BACE1 levels) and clearance (IDE expression), phosphorylation of Tau and kinases implicated (JNK1 and GSK3β) and markers of synaptic plasticity (synaptophysin and BDNF levels).

2. Material and methods

2.1. Animals

Experiments were carried out in 16 male SAMP8 and 16 SAMR1 mice (30–35 g, 6 months old at the beginning of treatment). A pilot study was also carried out in 16 male SAMP8 and 16 SAMR1 mice (31–35 g, 9 months old at the beginning of treatment). Animals were obtained from Harlan (Harlan Iberica, Barcelona, Spain). Animals were housed (5 per cage) in constant conditions of humidity and temperature (22 ± 1 °C) with a 12 h/12 h light–dark cycle (lights on at 7:00 h). 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. All efforts were made to minimise animal suffering, and to reduce the number of animals. Behavioural experiments were conducted between 9:00 a.m. and 1:00 p.m. Animals were randomly assigned to control and treatment groups ($n = 8$ per group age, strain and treatment).

2.2. Drug treatment and experimental design

To study the effect of propranolol on cognitive impairment, SAMP8 and SAMR1 mice were treated once daily with propranolol (5 mg/kg i.p.) or saline for 3 consecutive weeks.

Based on the fact that the recommended dose of propranolol for the treatment of hypertension in humans is 160–320 mg/day, and using FDA 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 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.

2.3. Behavioural experiments

After three weeks of treatment, and during two consecutive days, behavioural experiments were conducted between 9:00 a.m. and 1:00 p.m. Propranolol was administered 20 h before any behavioural test.

2.3.1. Locomotor activity

Horizontal locomotor activity was measured for 30 min in an open field, which consisted of four square arenas (65 × 65 × 45 cm^3) made of black wood, using a video tracking system (Ethovision 3.0, Noldus Information Technology B.V., The Netherlands), in a softly illuminated room. Total path length (cm) was analysed.

2.3.2. Novel object recognition test

The novel object recognition test (NORT) was adapted from Ennanceur and Delacour (1988). The open field consisted of a square open field (65 × 65 × 45 cm^3) made of black wood. During the first trial of the experiment (sample trial), two objects (prisms, conical tubes weighted with coloured sand or small objects were constructed using Lego® blocks) 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 mice was oriented toward the object with its nose within 2 cm of the object. One hour later a second trial (test 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 balance between testing animals. Results were expressed as percentage of time spent with the novel object with respect to the total exploration time (discrimination index).

2.4. Biochemical measurements

2.4.1. Tissue and blood collection

Fasting mice were sacrificed by decapitation between 8:00 and 10:00 a.m.. Brains were removed and dissected on dry ice to obtain the hippocampus. Trunk blood was placed in EDTA tubes, centrifuged at 12,500 *g* (15 min, 4 °C), and plasma was frozen until insulin levels were determined.

2.4.2. Insulin levels

Insulin was measured in 10 μl plasma samples using the Sensitive Mouse Insulin Enzyme Immunoassay Kit (EZRMI-13K, Millipore, Billerica, MA).

2.4.3. Western blotting

Cytosolic extract preparations from the hippocampus of mice were homogenized in a Tris buffer (pH 7.2, 4 °C) of the following composition: Tris 50 mM HCl (Trizma® hydrochloride, Sigma, St. Louis, MO, USA), NaCl 150 mM (Sigma, St. Louis, MO, USA), 1% Nonidet P-40 (Roche), EGTA 150 mM (Sigma, St. Louis, MO, USA), 1:100 inhibitor proteases and phosphatases cocktail (Complete™ Protease Inhibitor Cocktail, Roche). After a 13,000 rpm centrifugation (20 min), the pellet was discarded and protein levels were measured by the Bradford method in the supernatant (cytosolic fraction). Samples (20 μg of protein) 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; Amersham, Buckinghamsire, England). The optical density (O.D.) of reactive bands visible on X-ray film was determined densitometrically. β-actin or α-tubulin were used as internal controls. Results were expressed as percentage of O.D. values of control SAMR1 saline-treated mice.

2.4.4. Aβ levels

$A\beta_{42}$ levels were determined using a commercially available high-sensitive ELISA kits (Wako Pure Chemical Industries, Tokyo, Japan) following manufacturer instructions.

Table 1
Conditions used in western blotting experiments.

Protein	SDS-polyacrylamide gel	Molecular weight	Primary antibody (dilution)
IDE	13%	100 kDa	Anti-IDE (1:1000)[a]
pAkt	13%	60 kDa	Anti-pAkt (1:1000)[b]
Total Akt	13%	60 kDa	Anti-Akt (1:1000)[b]
Synaptophysin	13%	38 kDa	Anti-Synaptophysin (1:500)[a]
BACE1	13%	70 kDa	Anti-BACE1 (1:1000)[a]
pJNK	13%	46 kDa	Anti-pSAPK/JNK Thr183/Tyr185 (1:1000)[b]
Total JNK	13%	46 kDa	Anti-SAPK/JNK (1:1000)[b]
pTau	13%	50 kDa	Anti-pTau Ser202/Thr205 AT8 (1:500)[c]
PHF1	13%	62 kDa	Anti-PHF1-Tau (1:500)[a]
Total Tau	13%	50 kDa	Anti-Tau T46 (1:1000)[d]
pGSK3β	13%	46 kDa	Anti-pGSK3β Ser9 (1:1000)[b]
Total GSK3β	13%	46 kDa	Anti-GSK3β (1:1000)[b]
APP C-terminal	7.5%	100 kDa	Anti-APP C-terminal (676–695) (1:2000)[d]
BDNF	10%	28 kDa	Anti-mBDNF (1:1000)[a]
proBDNF	10%	14 kDa	Anti-proBDNF[a]

Abbreviations: APP C-terminal, APP carboxy-terminal fragments (C83 and C99); IDE, insulin degrading enzyme. Source of antibodies: [a]Abcam, Cambridge, MA, USA; [b]Cell Signalling Technology, Beverly, MA, USA; [c]Pierce, Rockford, IL, USA; [d]Sigma-Aldrich, St. Louis, MO, USA.