Life Sciences 224 (2019) 128–137

Contents lists available at ScienceDirect

Life Sciences

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

Beta-blockers and salbutamol limited emotional memory disturbance and damage induced by orchiectomy in the rat hippocampus

Fabiola J. Ciprés-Flores[a], Julia J. Segura-Uribe[a,b], Sandra Orozco-Suárez[b], Christian Guerra-Araiza[c], Juan A. Guevara-Salazar[a], Emily L. Castillo-García[a], Marvin A. Soriano-Ursúa[a,*], Eunice D. Farfán-García[a,*]

[a] Departamento de Fisiología y Sección de Estudios de Posgrado e Investigación, Escuela Superior de Medicina, Instituto Politécnico Nacional, Plan de San Luis y Díaz Mirón, 11340 Mexico City, Mexico

[b] Unidad de Investigación Médica en Enfermedades Neurológicas, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, Mexico City, Mexico

[c] Unidad de Investigación Médica en Farmacología, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, Mexico City, Mexico

ARTICLE INFO

Keywords:
Alzheimer's disease
Emotional memory
Beta-adrenergic receptors
Hormone deprivation
Fear memory

ABSTRACT

Aim: To evaluate the therapeutic potential of ligands of beta-adrenoceptors in cognitive disorders. Testosterone and adrenergic pathways are involved in hippocampal and emotional memory. Moreover, is strongly suggested that androgen diminishing in aging is involved in cognitive deficit, as well as beta-adrenoceptors, particularly beta2-adrenoceptor, participate in the adrenergic modulation of memory. In this regard, some animal models of memory disruption have shown improved performance after beta-drug administration.

Material and methods: In this work, we evaluated the effects of agonists (isoproterenol and salbutamol) and antagonists (propranolol and carvedilol) on beta-adrenoceptors in orchiectomized rats, as well as their effects in the performance on avoidance task and damage in hippocampal neurons by immunohistochemistry assays.

Key findings: Surprisingly, we found that both antagonists and salbutamol (but not isoproterenol) modulate the effects of hormone deprivation, improving memory and decreasing neuronal death and amyloid-beta related changes in some regions (particularly CA1-3 and dentate gyrus) of rat hippocampus.

Significance: Two β-antagonists and one β2-agonist modulated the effects of hormone deprivation on memory and damage in brain. The mechanisms of signaling of these drugs for beneficial effects remain unclear, even if used β-ARs ligands share a weak activity on β-arrestin/ERK-pathway activation which can be involved in these effects as we proposed in this manuscript. Our observations could be useful for understanding effects suggested of adrenergic drugs to modulate emotional memory. But also, our results could be related to other pathologies involving neuronal death and Aβ accumulation.

1. Introduction

The incidence and prevalence of diseases with cognitive deficit is increasing, as well as the global burden of these pathologies [1,2]. Among these, Alzheimer's disease (AD) is a relevant malady [2,3]. AD major features are the progressive memory lacking and the gradual and irreversible disruption of cognitive functions that eventually lead to dementia [4,5].

In several of these pathologies, cognitive deficit is related to a loss of cholinergic neurons and a progressive decrease of acetylcholine (ACh) [6]. However, other neurotransmitters and their neuronal systems have also been involved in the etiology and pathogeny of cognitive deficit [7,8]. Among these are the steroid hormones-dependent mechanisms [9], particularly testosterone limitation by orchiectomy [10,11]. In the central nervous system (CNS), hormone deprivation induces changes in some neurotransmitters and their receptors, which are related to the processes observed in cognitive disruption [12].

On the other hand, increasing innovative evidence exists about the role of adrenergic pathways in memory modulation [13,14]. In this sense, both adrenoceptors (ARs) agonists and antagonists have been identified as modulators of cognitive processes. Ni et al. [15] reported an increased amyloid-beta (Aβ) production involved in memory disruption after β2-adrenoceptor activation. In contrast, Valizadegan et al. [16] reported an improved memory performance with salbutamol, and

* Corresponding authors at: Departamento de Fisiología Escuela Superior de Medicina, Instituto Politécnico Nacional, Plan de San Luis y Díaz Mirón, Ciudad de México City 11340, México.

E-mail addresses: msoriano@ipn.mx (M.A. Soriano-Ursúa), efarfang@ipn.mx (E.D. Farfán-García).

https://doi.org/10.1016/j.lfs.2019.03.043
Received 25 September 2018; Received in revised form 12 March 2019; Accepted 18 March 2019
Available online 21 March 2019

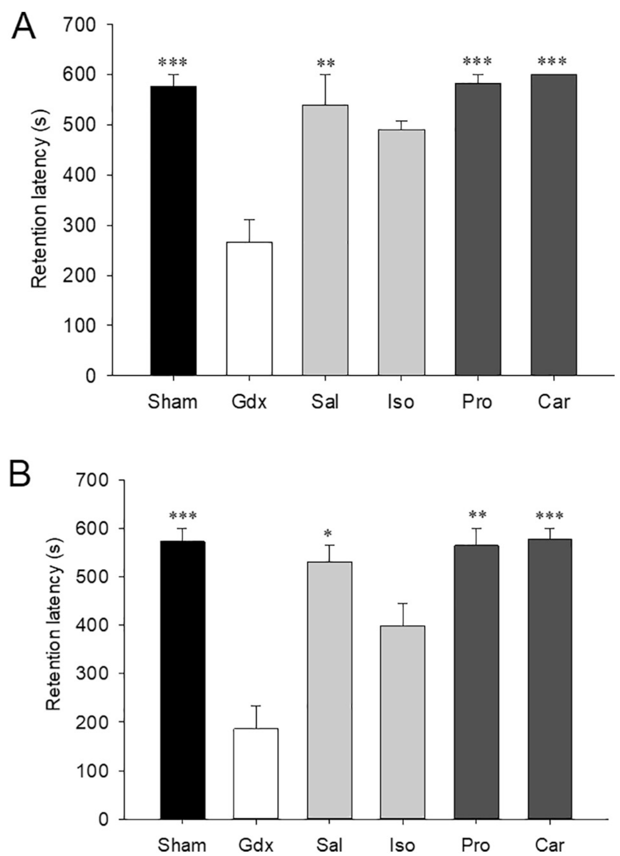

Fig. 1. Post-treatment short- (A) and long-term (B) memory evaluation. Results are expressed as mean ± Standard Error (n = 8). $^{*}p \leq 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to orchiectomized control group (Gdx); Sal, salbutamol; Iso, isoproterenol; Pro, propranolol; Car, carvedilol.

Dobarro et al. [17,18] found attenuated cognitive impairments by propranolol administration linked to an improved profile of neuropathology markers. Thus, the modulation of adrenergic pathways as a therapeutic strategy is controversial [19]. Some researchers support the use of agonists to decrease cognitive impairment [13,20–23], whereas other support the use of antagonists for the same purpose [17,18,24,25]. However, the classification of agonist and antagonist on adrenoceptors remains judged by the reported action in the classical cAMP-dependent pathway albeit increasing reports are regarding the action on additional pathways as β-arrestin(β-arr)/ERK-pathways [26].

To the extent of our knowledge, this is the first study with the aim to investigate the effects of β-ARs agonists and antagonists in the same model of cognitive impairment. In this model, the action of β-ARs agonists and antagonists mentioned as potential treatment for neurodegenerative diseases in murine models, and with the capacity to reach the CNS after subcutaneous (s.c.) administration due to its lipophilicity and ability to cross the blood-brain barrier [27–29], was evaluated to assess neuroprotection. The observed behavior in the passive avoidance task —a test involving emotional memory, strongly linked to the action of catecholamine pathways, conversely to other memory tests and disrupted by androgen deprivation [30,31]— was analyzed and associated with neuronal damage and Aβ changes in the main regions of rat hippocampus. The possible mechanisms as well as the putative involved pathways for these effects are discussed further.

2. Material and methods

2.1. Animals

All experiments were performed according to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. Male Wistar rats (10-week-old and weighing 200–220 g) from the laboratory animal house of Escuela Superior de Medicina (ESM), Instituto Politécnico Nacional (IPN), Mexico City, were kept under standard conditions with access to food and water ad libitum. They were free of known viral, bacterial and parasitic pathogens, and housed in polystyrene cages in a room under controlled humidity (50 ± 5%), temperature (20–25 °C) and lighting (12 h light/dark cycle, lights on at 7 am). All the experiments were carried out between 09:00 and 15:00 h. The current protocol was approved by the Ethics Committee and Institutional Animal Care and Use Committee of the ESM, IPN, and in accordance with the guidelines established by the Mexican Secretary of Agriculture and Animal Breeding (NOM-062-ZOO-1999, SAGARPA) and the guides for the Care and Use of Laboratory Animals of the National Research Council. Animals were randomly assigned into six groups of study (n = 8, N = 48). Sample size calculations were performed online using Biomath (http://www.biomath.info/power/index.html).

2.2. Surgical and pharmacological treatments

For surgical procedure, rats were anesthetized with pentobarbital (40 mg/kg/i.p.). Orchiectomy was performed through ventral incision in the scrotum as described elsewhere [32]. Rats were randomly divided into six experimental groups (n = 8). Group 1 (sham) and Group 2 (orchiectomized control) were treated with vehicle (saline solution 0.9%) daily for 35 days. The animals from the additional groups were orchiectomized and treated by s.c. injection with salbutamol (a β2-AR agonist, 4 mg/kg, Group 3), isoproterenol (a β-AR agonist, 0.5 mg/kg, Group 4), propranolol (a β-AR antagonist, 10 mg/kg, Group 5) and carvedilol (a β-AR antagonist, 5 mg/kg, Group 6) daily for 35 days.

2.3. Behavioral tests

For evaluating memory, the passive avoidance training (PAT) was performed for every group. The PAT test was applied as reported previously [33] due to the relevant participation of hippocampal and adrenergic processes [30,31]. In brief, we used a PAT-apparatus (OMNIALVA®, Mexico) with two chambers: the safe (white) and the shock (black) compartments. For training, each animal was placed in the safety compartment for 10 s, and then the sliding door was lifted. The latency to cross the threshold to the shock compartment was recorded (acquisition latency). Animals which spent > 100 s to cross to the other side were excluded from the experiment. Once the animal crossed with all four paws into the next compartment, the door was closed and a 3 mA foot-shock was delivered for 5 s. The door was opened and the time that the animal took to return to the safe compartment was measured (escape latency). For memory evaluation, the animal remained there for 30 s before being returned to its individual cage. Either 10 min (short-term memory) or 24 h (long-term memory) after this training procedure, a retention test was performed. The animal was again placed in the safety compartment for 10 s, and after the door was opened, the time the animal remained in the safety compartment before entering the shock compartment was recorded (retention latency). The test ended when the animal either entered the shock compartment or remained in the safety compartment for 600 s [34]. Memory evaluation was performed twice, one day before the surgical procedure and after the treatment as previously reported (three weeks after surgical procedure; [35,36]). An open field test was performed for 5 min to discard any motor disturbance affecting memory evaluation (see supplementary material). In brief, rats were placed into motor activity measuring cages (50x50x50 cm, with detectors each 2.5 cm, OA-BioMed OMNIALVA®, Mexico) for determining the total number of movements, distance traveled, highest speed, and vertical movements.

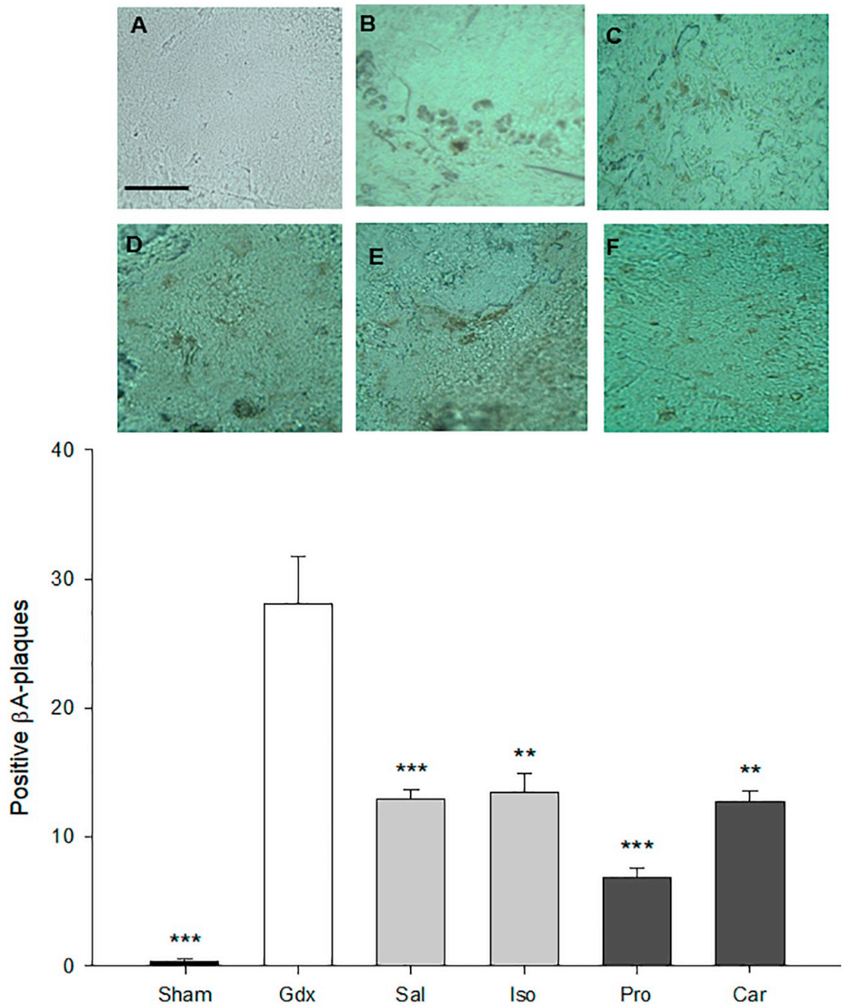

Fig. 2. Photomicrographs displaying Aβ plaques positive labeling seen in the CA1 (A to F): Sham (A), Gdx (B), salbutamol (C), isoproterenol (D), propranolol (E), carvedilol (F). Magnification 40×. Scale bar represents 100 μm. Bars represent the number of positive Aβ-plaques in the hippocampus. Significant differences of groups compared with Gdx group (± SEM, n = 4, **$p < 0.01$, ***$p < 0.001$).

2.4. Immunohistochemistry assays

After the behavioral evaluation, rats were anesthetized with pentobarbital and perfused transcardially with 200 ml of ice-cold (4 °C) sodium phosphate buffer (PBS) 0.1 M, pH 7.4, and a freshly prepared fixative consisting of 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The brain was removed immediately after perfusion and post-fixed in ice-cold (4 °C) 4% paraformaldehyde, followed by 48 h in ice-cold (4 °C) 30% sucrose in 0.1 M phosphate buffer for cryoprotection. Coronal slices of brain (30 μm thick) were cut on freezing microtome and stored at −20 °C in a solution containing 30% ethylene glycol, 30% glycerol and PBS until immunohistochemical analysis.

Free-floating sections were incubated in PBS containing 3% H2O2 for 10 min and rinsed three times with PBS for 5 min. Subsequently, sections were incubated in 0.1% Tween 20 in PBS (T-PBS) for 10 min, then incubated in 0.5% bovine serum albumin (BSA, GE Health Care, UK) in T-PBS for 2 h. Finally, sections were incubated with the primary antibody anti-βA1-24 (1:1000, ABBIOTEC, CA, USA) or anti-NeuN (1:1000, Millipore, MA, USA) at 4 °C for 48 h. The following day, sections were rinsed three times in T-PBS for 5 min and incubated with a biotinylated secondary antibody and a peroxidase-labeled streptavidin reagent (LSAB System-HRP Kit; DAKO, CA, USA) for 30 min each. Antibody labeling was visualized by exposure to 3–3′-diaminobenzidine (DAKO, CA, USA). Omission of primary or secondary antibodies as negative controls resulted in the absence of immunolabeling. Finally, sections were rinsed twice in PBS and mounted in Entellan solution (Millipore, MA, USA). The number of βA1-24 or NeuN-positive neurons was counted through random sampling bilaterally in two sections per animal under a light microscope (magnification 40×) by two independent trained observers who were blind to the treatment for avoiding biased appraisal. Cell counts from the right and left hemispheres of each of the two sections were averaged to provide a single value for each animal. From these data, the mean of these labeled neurons in Cornu Ammonis (CA)1, CA2, CA3 and DG (Dentate Gyrus) hippocampal areas (n = 4) was calculated for each experimental group.

2.5. Statistical analysis

Data were expressed as the mean ± S.E.M. One-way ANOVA followed by Tukey's post hoc test was used in the case of parametric data. For the data found in behavioral evaluation (Fig. 1), Kruskal–Wallis test was employed as the data are not normally distributed, and comparisons were done by Dunn's post hoc test. Differences among data in immunohistochemistry assays (Figs. 2 to 7) were identified by one-way ANOVA followed by Tukey's post hoc test. Spearman's rho rank correlation test was used to compare between NeuN neuron counts and Aβ

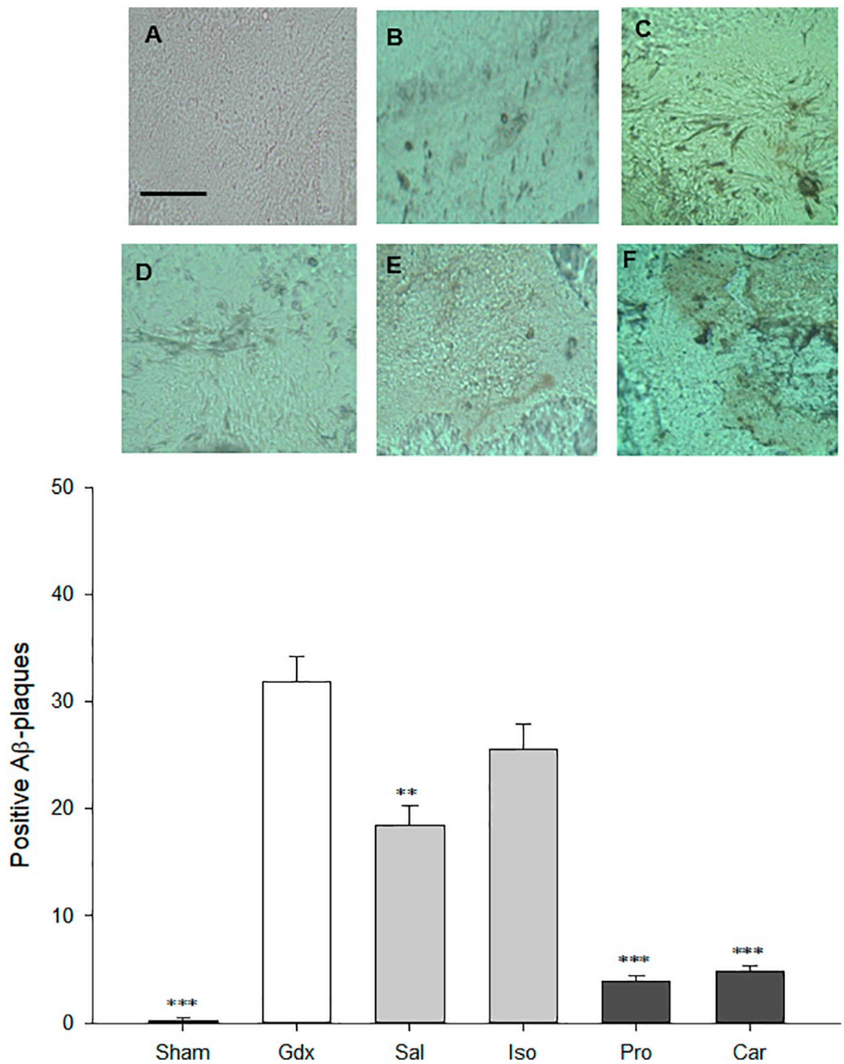

Fig. 3. Photomicrographs displaying Aβ plaques positive labeling seen in the CA2/CA3 (A to F): Sham (A), Gdx (B), salbutamol (C), isoproterenol (D), propranolol (E), carvedilol (F). Magnification 40×. Scale bar represents 100 μm. Bars represent the number of positive Aβ-plaques in the hippocampus. Significant differences of groups compared with Gdx group (± SEM, n = 4, $^{**}p < 0.01$, $^{***}p < 0.001$).

plaque counts. All statistical analyses were performed using Sigma Plot 12.0. Statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1. Behavioral changes by hormone deprivation and the effect of early treatment with beta-agonists or antagonists

The diminished cognitive performance in PAT was observed after three weeks of orchiectomy, as previously reported [37,38]. Thus, a significant decrease in latency [Fig. 1a; H = 34.669, DR = 25.25, df = 5, $p < 0.05$ and Fig. 1b; H = 29.378, DR = 25.75, df = 5, $p < 0.05$] was registered in comparison with homogeneous pre-surgical gonadectomy behavior [Suppl. Fig. 1, all $p > 0.09$]. These shorter latencies have been attributed to the decreased levels of androgens in rats [39,71] as some memory deficits have been related to androgens decreasing in humans [40,41]. In rats, testosterone decreasing causes effects in the synthesis and degradation of key neurotransmitters such as acetylcholine and catecholamines [42], as well as disrupted expression of the receptors and proteins of signaling pathways related to their effects on the CNS [43].

The administration of the β-ARs antagonists, propranolol or carvedilol, but also the β2-ARs agonist salbutamol increased the retention latency in the evaluation of short-[Fig. 1a; H = 34.669, DR ≥ 24.375, df = 5, $p < 0.05$] and long-term [Fig. 1b; H = 29.378, DR ≥ 21.625, df = 5, $p < 0.05$] memory. However, the administration of β-ARs agonist isoproterenol did not improve significantly the performance in PAT evaluations [Fig. 1; DR ≤ 9.625, df = 5, $p > 0.05$]. These results are noteworthy, since other cases of biological effects of antagonists shared with agonists, specifically with salbutamol, have been reported. For example, the shared effect of antagonists and salbutamol in the decrease of cell migration in a breast cancer cell-line [44]. Moreover, the improved performance induced by propranolol and salbutamol in an avoidance learning task has been reported previously [45,46].

No significant differences were observed in the motor performance open field test in the pre-surgical, pre-treatment or post-treatment evaluations [Suppl. Fig. 2, all $p > 0.08$], which is important to discard motor disruption affecting avoidance performance. It should be mentioned that due to limitations of the used PAT test to evaluate spatial and non-emotional memory, which are linked to AD and other cognitive disorders in humans, the use of additional behavioral test is desirable to evaluate the effects of adrenergic drugs.

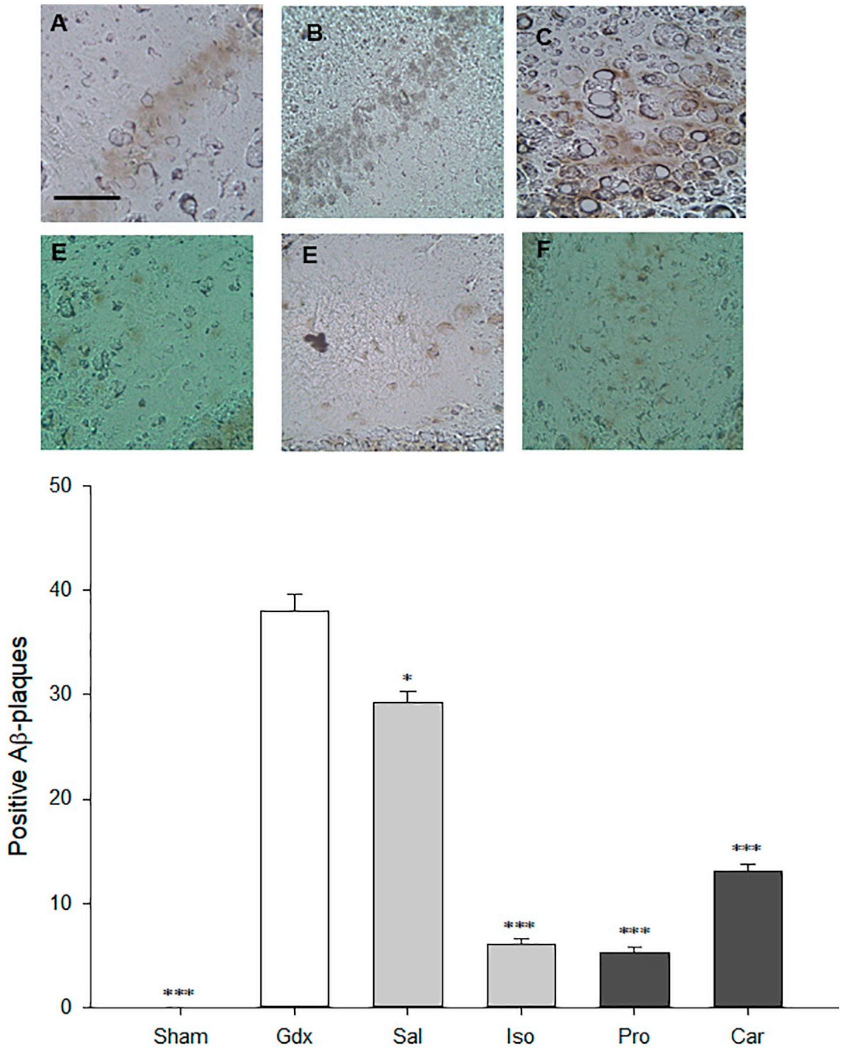

Fig. 4. Photomicrographs displaying Aβ plaques positive labeling seen in the DG (A to F): Sham (A), Gdx (B), salbutamol (C), isoproterenol (D), propranolol (E), carvedilol (F). Magnification 40×. Scale bar represents 100 μm. Bars represent the number of positive Aβ-plaques in the hippocampus. Significant differences of groups compared with Gdx group (± SEM, n = 4, *p < 0.05, ***p < 0.001).

3.2. Effects on beta amyloid presence and neuronal survival in hippocampus

Significative differences were observed between sham and orchiectomized groups in all measured immunohistochemistry assays [Figs. 2–7; $F(5,19) \geq 13.08$, $p < 0.001$]. Orchiectomy reduced neuronal survival and increased the changes associated to amyloid presence after hormonal deprivation, surgical procedures or drugs administration [47–49]. Figs. 2–7). In the analysis of both Aβ presence and cells marked with NeuN, our results showed that the administration of antagonists and agonists of β-ARs exert neuroprotection in CA1, CA2 and CA3 and DG hippocampal areas (Suppl. Fig. 3, Figs. 2–7). Particularly, all administered drugs reduced Aβ presence in all the analyzed areas [Figs. 2–4; $F(5,19) \geq 35.23$, all $p < 0.05$], except for isoproterenol in CA2/CA3 [Fig. 3; $F(5,19) = 0.44$, $p = 0.89$].

In fact, our results support that antagonists like propranolol [17,18] or carvedilol [50–52] diminish the changes related to Aβ presence; in addition, one of the two β-agonists tested (salbutamol) also decreased such changes. To the best of our knowledge, these results have not been reported previously, though other authors have reported improved performance in memory with the administration of β-agonists [16,45].

Regarding NeuN-positive cells, the administration of β-antagonists reduced the loss of neurons by androgen deprivation [53,54] in all the analyzed areas ($p < 0.05$); salbutamol reduced the loss of neurons in CA1 and DG [Fig. 5; $F(5,19) = 42.89$, $p < 0.001$ and Fig. 7; $F(5,19) = 61.87$, $p < 0.001$] but not in CA2/CA3 [Fig. 6; $F(5,19) = 0.31$, $p = 0.71$], while isoproterenol did not induce significant changes [all $p > 0.05$] in the number of cells in any area when compared with the group of orchiectomized rats. These results agree with the reported neuroprotective effect of propranolol, an antagonist [51], and clenbuterol, a selective β2-agonist like salbutamol, on the number of NeuN-positive neurons in DG hippocampal area [20].

Regarding correlation, the Spearman's rho value of −0.686 was obtained suggesting an inverse relationship between Aβ presence and the total number of cells marked with NeuN. These results on Aβ presence and neuronal survival could be associated with behavior since isoproterenol was the one compound which failed to improve performance in PAT, and it did not diminish Aβ presence or increase neuronal survival. Regarding specific hippocampal areas related with improved performance in behavioral tests, Ji et al. [55] suggested a key role of β-ARs in CA1, area on which propranolol, carvedilol, and salbutamol seemed to exert neuroprotective effects.

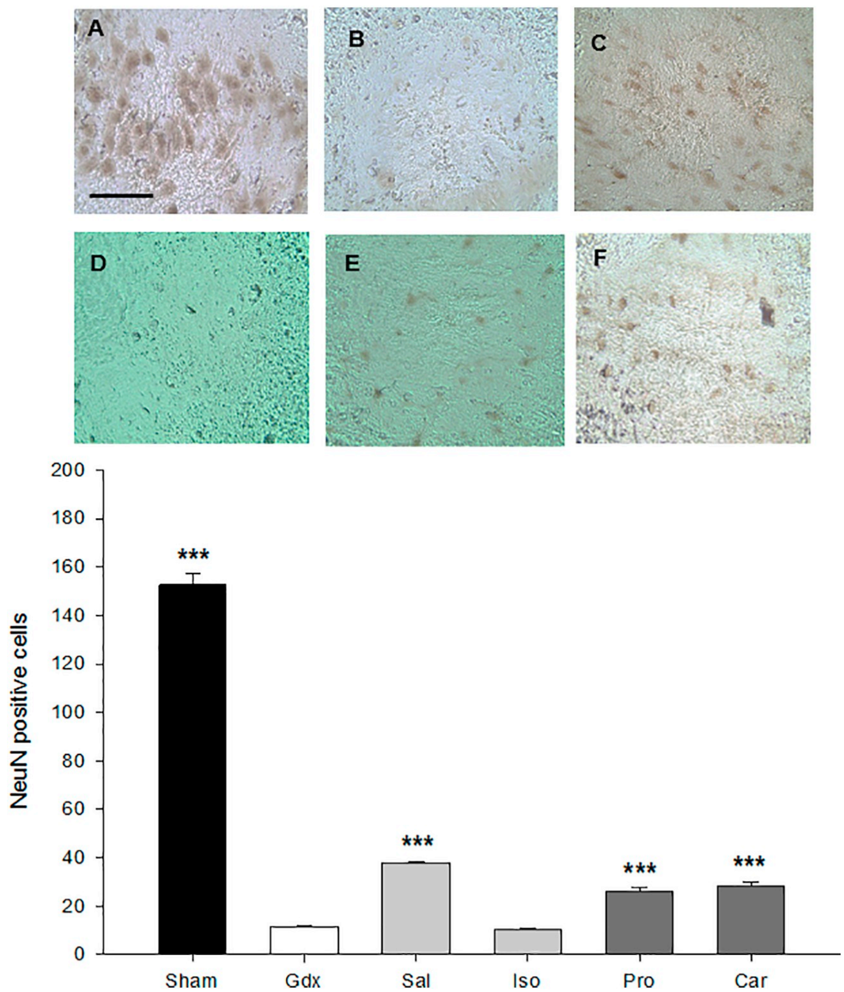

Fig. 5. Photomicrographs of NeuN positive labeling seen in the CA1 (A to F): Sham (A), Gdx (B), salbutamol (C), isoproterenol (D), propranolol (E), carvedilol (F). Magnification 40×. Scale bar represents 100 μm. Bars represent the number of NeuN-positive neurons. Significant differences of groups compared with Gdx group (±SEM, n = 4, ***$p < 0.001$).

3.3. Mechanisms involved in behavior and neuronal changes induced by adrenoceptor ligands

Multiple neural pathways may be modulated by hormone deprivation; among these, testosterone deprivation modulates adrenergic pathways [56]. Adrenergic signaling in CNS is important for the retrieval of fear, intermediate-term contextual and spatial memories, and the role of adrenergic pathways is relevant in some cerebral areas as locus coeruleus, hippocampus and cortex [56]. Moreover, it is well-known that locus coeruleus is the first area to develop neurofibrillary changes [57] and its diminishing noradrenaline output could be inducing changes in adrenoceptors on target areas, which agrees with the observed improved memory of rodents administered with β-agonists [16,20,58,59].

Previously reported data [15,17,18,24,25], as well as the results in the present contribution support the preferred use of antagonist in the modulation of both histopathological changes and behavior performance. In this sense, Fitzgerald [60] has reported an increasing amount of data supporting the increased release of norepinephrine before and during Alzheimer's disease progression by compensatory mechanisms after damage in the locus coeruleus. Therefore, some reports suggest the neuroprotective effects of β-adrenergic antagonists, alone or in combination with cholinergic agents as agents in AD therapy [60,61].

Albeit the role of some subtype of adrenoceptor, the areas in which they are expressed and the effects of castration on these processes are poorly understood; regarding the key role of androgen activity modulating adrenergic pathways, Jarzab et al. [62] suggested that βARs activation or blockage during postnatal development impairs the gonadotropin release to gonadal steroids in female rats and the expression of ejaculatory behavior in male rats. Recently, it was described that noradrenaline, a non-selective agonist, and clonidine, a non-selective αAR agonist, enhanced hippocampal-prefrontal long-term potentiation while isoprenaline, a non-selective beta agonist, decreased it [63]. These results agree with our observations, as isoproterenol lacked neuroprotective effects or improvement in memory evaluation.

The present results showed a shared improvement of memory-performance for two beta-blockers (propranolol and carvedilol are considered as such agents, even though they have lower affinity to alpha adrenoceptors in some assays) and at least a selective β2-agonist. However, future studies are required to investigate if the neuroprotective effects after androgen deprivation are carried out by direct action of βARs, and to observe shared or different effects of each receptor subtype, since blockage of α- and β1-adrenoceptors has been also involved, [64,65], and the specific signaling pathways related to its activation. This knowledge would be important for our results; interestingly, it has been observed that propranolol, carvedilol and salbutamol

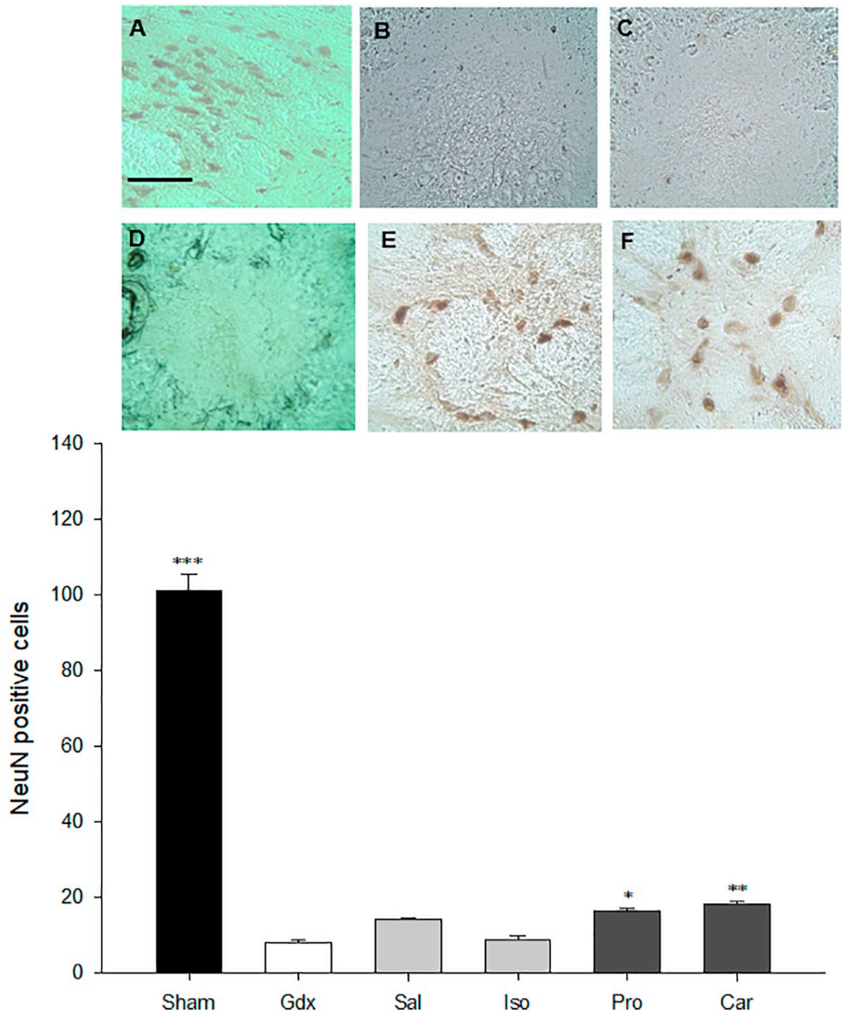

Fig. 6. Photomicrographs of NeuN positive labeling seen in the CA2/CA3 (A to F): Sham (A), Gdx (B), salbutamol (C), isoproterenol (D), propranolol (E), carvedilol (F). Magnification 40×. Scale bar represents 100 μm. Bars represent the number of NeuN-positive neurons. Significant differences of groups compared with Gdx group (± SEM, n = 4, *p ≤ 0.05, **p < 0.01, ***p < 0.001).

share a weak activity to β-arrestin (β-arr)/ERK-pathways by β2-activation [26], and ERK signaling has been related to improved memory function, even if molecular consequences are unclear [65] and some mechanisms as dendritic spine plasticity, ubiquitination, or signaling by several kinases have been related to this β-arr signaling [66]. Then, we propose the weak activity pathway as relevant to induce the observed changes in our behavioral evaluation. Thus, weak β-arr activation results in poor internalization of adrenoceptors and γ-secretase complexes, where γ-secretase activated by β-arr cleaves Aβ peptide from APP C-terminal fragment. Subsequently, Aβ is poorly secreted by exocytosis, where it accumulates producing Aβ plaques, which induce inflammatory processes that cause injurious local changes (Fig. 8). Moreover, it was observed that selective blockade of the Gs/cAMP/PKA signaling but not the β-arrestin/ERK signaling by the biased β2-adrenergic ligands does not inhibit reconsolidation; thus, some authors suggested a potential role for β-arrestin-biased ligands in the treatment of memory-related disorders [65]. However, unbiased ligands with high activity on β-arr/ERK-pathways (as isoproterenol and adrenaline) lack the ability to induce memory improvement [63,65]. Several experiments are required in order to support or discard the participation of these pathways and the relevance of the grade of β-arr/ERK-pathway activation.

Additionally, other mechanisms can be related to the observed results by β-antagonists and salbutamol, such as the effects of these compounds in the blood brain barrier and other vascular effects [27,67]. Also, some direct effects on the formation of Aβ plaques, as it has been suggested that anthracyclines and carbazoles (as the β-antagonists used in this study) can inhibit Aβ formation [50] and they have inhibitory effects on acetylcholinesterase as well [68]. No macroscopic changes induced by gonadectomy were observed in pineal gland as previously was described [69]. Finally, it should be mentioned that albeit in other tissues, such as the prostate, the down-regulation of adrenoceptors after orchiectomy was observed [70], further studies on the details of the expression of adrenoceptors in the hippocampus after androgen deprivation are required, since the current data are insufficient to support relevance of their participation.

4. Conclusions

In the present study, two β-antagonists and one β2-agonist modulated the effects of hormone deprivation: memory improvement and a decrease neuronal death and Aβ presence in the hippocampus were observed. However, the mechanisms of action of these drugs are unclear, even if used β-ARs ligands share a weak activity on β-arrestin/ERK-pathway activation which can be involved in these effects as we proposed in this manuscript.

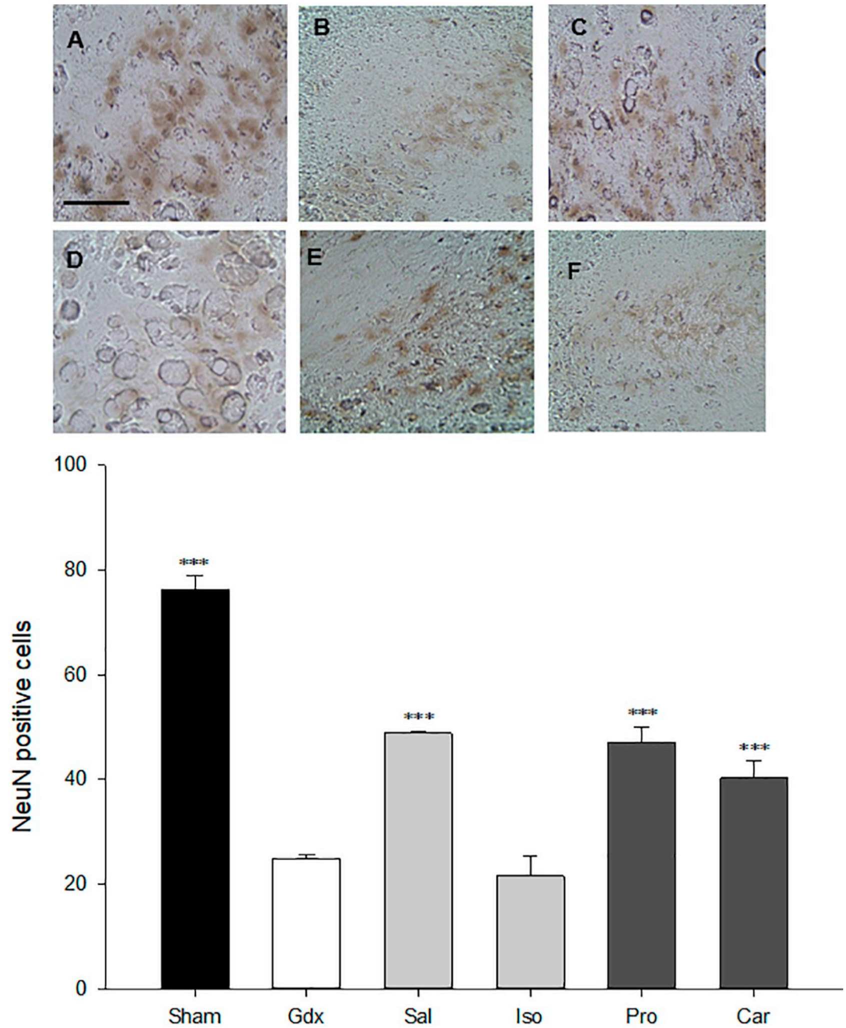

Fig. 7. Photomicrographs of NeuN positive labeling seen in the DG (A to F): Sham (A), Gdx (B), salbutamol (C), isoproterenol (D), propranolol (E), carvedilol (F). Magnification 40×. Scale bar represents 100 μm. Bars represent the number of NeuN-positive neurons. Significant differences of groups compared with Gdx group (± SEM, n = 4, ***$p < 0.001$).

Current data suggest the use of antagonists and some agonists to improve the cognitive deficit related to hormone deprivation. However, additional studies are required to obtain insights regarding the mechanism(s) involved in the observed effects, and the effects of the same compounds in advanced stages of hormonal deprivation. Moreover, the evaluation in other types of memory is also necessary to generate a profile of benefits and risks regarding the therapeutics of adrenergic-drugs in cognitive deficit processes commonly associated to aging.

Therefore, our observations could be useful for understanding the suggested effects of adrenergic drugs to modulate emotional memory (as it has been observed in clinical studies, for example the modulation of post-traumatic stress disorder by propranolol). In addition, our results could be related to other pathologies where neuronal death and Aβ accumulation are key events, such as Alzheimer's disease or cognitive deficit in Down syndrome; in this regard, increasing of clinical correlation is required.

Author contributions

CFFJ, SUMA and FGED conceived the protocol and experiments. CFFJ participated in all experiments, FGED and SUMA supervised the assays and concerted the analysis and writing; CGEL, SUJJ, GSJA, OS and GAC participated and analyzed the surgical and behavioral approaches. CFFJ, CGEL and FGED carried out the immunohistochemistry assays. All authors participated in the manuscript preparation and all they approved the final version of this manuscript.

Conflict of interest

Authors declare they have not conflict of interest on the content of this manuscript.

Compliance with ethical standards

No conflict of interest exists regarding the content of this manuscript, funding from institutions is included in cover letter. This article does not contain any studies with human participants performed by any of the authors.

All experiments with animals were performed according to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines as is stated in the Material and methods section.

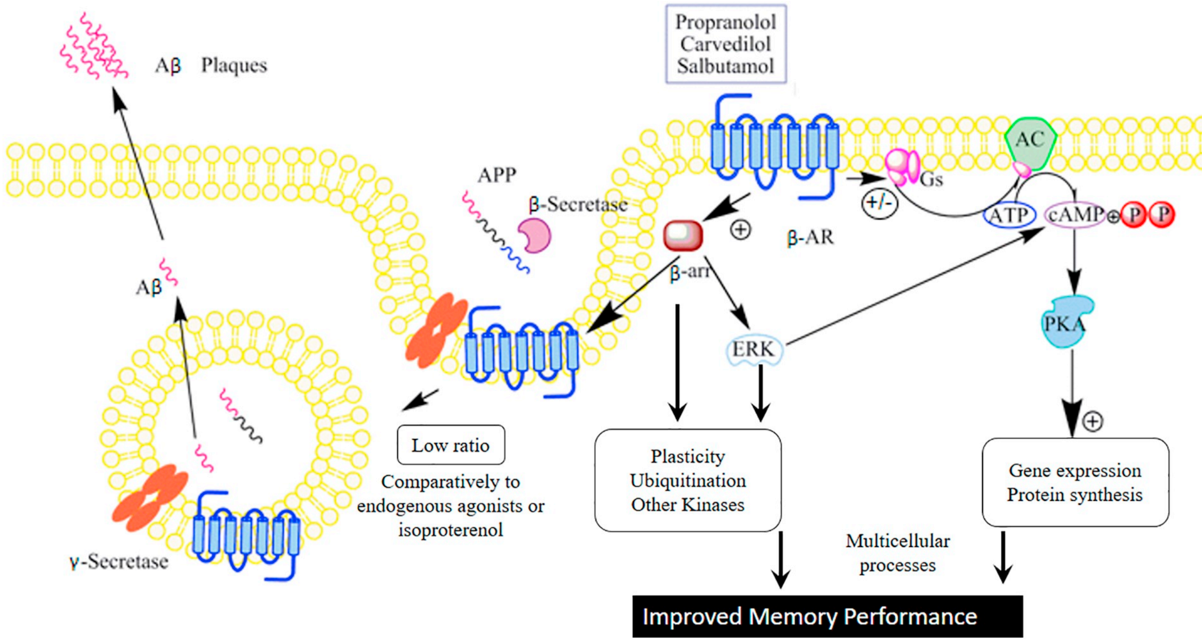

Fig. 8. Putative signaling pathways modulated by β-ARs through G-protein and/or β-arr by the interaction of the agonist salbutamol and the antagonists carvedilol and propranolol, which could be related to the observed behavioral performance. In addition, to those effects mediated trough G-protein coupled pathway and ERK modulation, the poorly β-arrestin (β-arr) activation (comparatively to endogenous β-ARs agonists) results in the diminished internalization of adrenoceptor and γ-secretase complexes, where γ-secretase activated by β-arr cleaves Aβ peptide from APP C-terminal fragment. Subsequently, Aβ is poorly secreted by exocytosis, where it accumulates producing Aβ plaques, which induce inflammatory processes that cause injurious local changes.

Acknowledgements

The authors thank Dr. Trujillo-Ferrara for the academic contribution through suggestions on this manuscript. GAC thanks Fundación IMSS A.C. for recognizing him as participant in the Excelencia en Investigación Program.

Funding

We would like to thank the Comisión de Operación y Fomento de Actividades Académicas, Instituto Politécnico Nacional, the Secretaría de Investigación y Posgrado, Instituto Politécnico Nacional (SIP M1930 and 20181043), and the Consejo Nacional de Ciencia y Tecnología (CONACyT- CB235785), for financial support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2019.03.043.

References

[1] V. Boccardi, M. Baroni, L. Paolacci, S. Ercolani, A. Longo, M. Giordano, C. Ruggiero, P. Mecocci, Anticholinergic burden and functional status in older people with cognitive impairment: results from the ReGAl project, J. Nutr. Health Aging 21 (2017) 389–396.
[2] H. Shah, E. Albanese, C. Duggan, I. Rudan, K.M. Langa, M.C. Carrillo, K.Y. Chan, Y. Joanette, M. Prince, M. Rossor, S. Saxena, H.M. Snyder, R. Sperling, M. Varghese, H. Wang, M. Wortmann, T. Dua, Research priorities to reduce the global burden of dementia by 2025, Lancet Neurol. 15 (2016) 1285–1294.
[3] J. Cummings, P. Aisen, R. Barton, J. Bork, R. Doody, J. Dwyer, J.C. Egan, H. Feldman, D. Lappin, L. Truyen, S. Salloway, R. Sperling, G. Vradenburg, Re-engineering Alzheimer clinical trials: global Alzheimer's platform network, J. Prev. Alzheimers Dis. 3 (2016) 114–120.
[4] C. Borrás Blasco, J. Viña Ribes, Neurophysiology and ageing. Definition and pathophysiological foundations of cognitive impairment, Rev. Esp. Geriatr. Gerontol. 51 (2016) 3–6.
[5] B. Dubois, H. Hampel, H.H. Feldman, P. Scheltens, P. Aisen, S. Andrieu, H. Bakardjian, H. Benali, L. Bertram, K. Blennow, K. Broich, E. Cavedo, S. Crutch, J.F. Dartigues, C. Duyckaerts, S. Epelbaum, G.B. Frisoni, S. Gauthier, R. Genthon, A.A. Gouw, M.O. Habert, D.M. Holtzman, M. Kivipelto, S. Lista, J.L. Molinuevo, S.E. O'Bryant, G.D. Rabinovici, C. Rowe, S. Salloway, L.S. Schneider, R. Sperling, M. Teichmann, M.C. Carrillo, J. Cummings, C.R. Jack Jr., Proceedings of the Meeting of the International Working Group (IWG) and the American Alzheimer's Association on "The Preclinical State of AD"; July 23, (2015); Washington DC, USA. Preclinical Alzheimer's disease: definition, natural history, and diagnostic criteria, Alzheimers Dement. 12 (2016) 292–323.
[6] A.J. Wesson, Treatment of Alzheimer's disease: the legacy of the cholinergic hypothesis, neuroplasticity, and future directions, J. Alzheimers Dis. 47 (2015) 149–156.
[7] P. Lewczuk, B. Mroczko, A. Fagan, J. Kornhuber, Biomarkers of Alzheimer's disease and mild cognitive impairment: a current perspective, Adv. Med. Sci. 60 (2015) 76–82.
[8] P. Scheltens, K. Blennow, M.M. Breteler, B. de Strooper, G.B. Frisoni, S. Salloway, W.M. Van der Flier, Alzheimer's disease, Lancet 388 (2016) 505–517.
[9] C.J. Pike, Sex and the development of Alzheimer's disease, J. Neurosci. Res. 95 (2017) 671–680.
[10] S.C. Papasozomenos, The heat shock-induced hyperphosphorylation of tau is estrogen-independent and prevented by androgens: implications for Alzheimer disease, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 6612–6617.
[11] M. Ramsden, A.C. Nyborg, M.P. Murphy, L. Chang, F.Z. Stanczyk, T.E. Golde, C.J. Pike, Androgens modulate beta-amyloid levels in male rat brain, J. Neurochem. 87 (2003) 1052–1055.
[12] N.N. Dygalo, T.S. Kalinina, N.Y. Sournina, G.T. Shishkina, Effects of testosterone on alpha 2A-adrenergic receptor expression in the rat brain, Psychoneuroendocrinology 27 (2002) 585–592.
[13] V. Gao, A. Suzuki, P.J. Magistretti, S. Lengacher, G. Pollonini, M.Q. Steinman, C.M. Alberini, Astrocytic β2-adrenergic receptors mediate hippocampal long-term memory consolidation, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) 8526–8531.
[14] J. Zheng, F. Luo, N.N. Guo, Z.Y. Cheng, B.M. Li, β1-and β2-adrenoceptors in hippocampal CA3 region are required for long-term memory consolidation in rats, Brain Res. 1627 (2015) 109–118.
[15] Y. Ni, X. Zhao, G. Bao, L. Zou, L. Teng, Z. Wang, M. Song, J. Xiong, Y. Bai, G. Pei, Activation of beta2-adrenergic receptor stimulates gamma-secretase activity and accelerates amyloid plaque formation, Nat. Med. 12 (2006) 1390–1396.
[16] F. Valizadegan, S. Oryan, M. Nasehi, M.R. Zarrindast, Interaction between morphine and noradrenergic system of basolateral amygdala on anxiety and memory in the elevated plus-maze test based on a test-retest paradigm, Arch. Iran Med. 16 (2013) 281–287.
[17] M. Dobarro, L. Orejana, N. Aguirre, M.J. Ramírez, Propranolol reduces cognitive deficits, amyloid β levels, tau phosphorylation and insulin resistance in response to chronic corticosterone administration, Int. J. Neuropsychopharmacol. 16 (2013) 1351–1360.
[18] M. Dobarro, L. Orejana, N. Aguirre, M.J. Ramírez, Propranolol restores cognitive deficits and improves amyloid and tau pathologies in a senescence-accelerated mouse model, Neuropharmacology 64 (2013) 137–144.
[19] Nguyen L.T. Luong Kv, The role of Beta-adrenergic receptor blockers in Alzheimer's

disease: potential genetic and cellular signaling mechanisms, Am. J. Alzheimers Dis. Other Dement. 28 (2013) 427–439.
[20] G.S. Chai, Y.Y. Wang, A. Yasheng, P. Zhao, Beta 2-adrenergic receptor activation enhances neurogenesis in Alzheimer's disease mice, Neural Regen. Res. 11 (2016) 1617–1624.
[21] G.S. Chai, Y.Y. Wang, D. Zhu, A. Yasheng, P. Zhao, Activation of β2-adrenergic receptor promotes dendrite ramification and spine generation in APP/PS1 mice, Neurosci. Lett. 636 (2017) 158–164.
[22] V. Dang, B. Medina, D. Das, S. Moghadam, K.J. Martin, B. Lin, P. Naik, D. Patel, R. Nosheny, A.J. Wesson, A. Salehi, Formoterol, a long-acting β2 adrenergic agonist, improves cognitive function and promotes dendritic complexity in a mouse model of Down syndrome, Biol. Psychiatry 75 (2014) 179–188.
[23] H.C. Zhou, Y.Y. Sun, W. Cai, X.T. He, F. Yi, B.M. Li, X.H. Zhang, Activation of β2-adrenoceptor enhances synaptic potentiation and behavioral memory via cAMP-PKA signaling in the medial prefrontal cortex of rats, Learn. Mem. 20 (2013) 274–284.
[24] E.R. Peskind, D.W. Tsuang, L.T. Bonner, M. Pascualy, R.G. Riekse, M.B. Snowden, R. Thomas, M.A. Raskind, Propranolol for disruptive behaviors in nursing home residents with probable or possible Alzheimer disease: a placebo-controlled study, Alzheimer Dis. Assoc. Disord. 19 (2005) 23–28.
[25] K. Schutsky, M. Ouyang, C.B. Castelino, L. Zhang, S.A. Thomas, Stress and glucocorticoids impair memory retrieval via β2-adrenergic, Gi/o-coupled suppression of cAMP signaling, J. Neurosci. 31 (2011) 14172–14181.
[26] E.T. Van der Westhuizen, B. Breton, A. Christopoulos, M. Bouvier, Quantification of ligand bias for clinically relevant β2-adrenergic receptor ligands: implications for drug taxonomy, Mol. Pharmacol. 85 (2014) 492–509.
[27] J. Olesen, K. Hougård, M. Hertz, Isoproterenol and propranolol: ability to cross the blood-brain barrier and effects on cerebral circulation in man, Stroke 9 (1978) 344–349.
[28] Caccia S, Fong MH. Kinetics and distribution of the beta-adrenergic agonist salbutamol in rat brain (1984) J. Pharm. Pharmacol. 36(3):200–202.
[29] G. Neil-Dwyer, J. Bartlett, J. McAinsh, J.M. Cruickshank, Beta-adrenoceptor blockers and the blood-brain barrier, Br. J. Clin. Pharmacol. 11 (1981) 549–553.
[30] G. Bammer, Pharmacological investigations of neurotransmitter involvement in passive avoidance responding: a review and some new results, Neurosci. Biobehav. Rev. 6 (1982) 247–296.
[31] J. Przybyslawski, P. Roullet, S.J. Sara, Attenuation of emotional and nonemotional memories after their reactivation: role of β adrenergic receptors, J. Neurosci. 19 (1999) 6623–6628.
[32] S. Moghadami, M. Jahanshahi, H. Sepehri, H. Amini, Gonadectomy reduces the density of androgen receptor-immunoreactive neurons in male rat's hippocampus: testosterone replacement compensates it, Behav. Brain Funct. 12 (2016) 1–10.
[33] E.D. Farfán-García, M.C. Castillo-Hernández, R. Pinto-Almazán, S. Rivas-Arancibia, J.M. Gallardo, C. Guerra-Araiza, Tibolone prevents oxidation and ameliorates cholinergic deficit induced by ozone exposure in the male rat hippocampus, Neurochem. Res. 39 (2014) 1776–1786.
[34] E.R. Detrait, E. Hanon, B. Dardenne, Y. Lamberty, The inhibitory avoidance test optimized for discovery of cognitive enhancers, Behav. Res. Methods 41 (2009) 805–811.
[35] J. Espinosa-Raya, T. Neri-Gómez, S. Orozco-Suárez, M.G. Campos, C. Guerra-Araiza, Chronic administration of tibolone modulates anxiety-like behavior and enhances cognitive performance in ovariectomized rats, Horm. Behav. 61 (2012) 76–83.
[36] N. Qu, L. Wang, Z.C. Liu, Q. Tian, Q. Zhang, Oestrogen receptor α agonist improved long-term ovariectomy-induced spatial cognition deficit in young rats, Int. J. Neuropsychopharmacol. 16 (2013) 1071–1082.
[37] C.A. Frye, A.M. Seliga, Testosterone increases analgesia, anxiolysis, and cognitive performance of male rats, Cogn. Affect. Behav. Neurosci. 1 (2001) 371–381.
[38] J. Jia, L. Kang, S. Li, D. Geng, P. Fan, L. Wang, H. Cui, Amelioratory effects of testosterone treatment on cognitive performance deficits induced by soluble Aβ1-42 oligomers injected into the hippocampus, Horm. Behav. 64 (2013) 477–486.
[39] S. Mora, A.G. Nasello, M. Mandelli-Lopes, G. Díaz-Véliz, LHRH and rat avoidance behavior: influence of castration and testosterone, Physiol. Behav. 30 (1983) 19–22.
[40] E. Hogervorst, Effects of gonadal hormones on cognitive behaviour in elderly men and women, J. Neuroendocrinol. 25 (2013) 1182–1195.
[41] E.J. Wahjoepramono, P.R. Asih, V. Aniwiyanti, K. Taddei, S.S. Dhaliwal, S.J. Fuller, J. Foster, M. Carruthers, G. Verdile, H.R. Sohrabi, R.N. Martins, The effects of testosterone supplementation on cognitive functioning in older men, CNS Neurol. Disord. Drug Targets 15 (2016) 337–343.
[42] K. Shemisa, V. Kunnathur, B. Liu, T.J. Salvaterra, D.E. Dluzen, Testosterone modulation of striatal dopamine output in orchidectomized mice, Synapse 60 (2006) 347–353.
[43] J. Meitzen, A.N. Perry, C. Westenbroek, V.L. Hedges, J.B. Becker, P.G. Mermelstein, Enhanced striatal β1-adrenergic receptor expression following hormone loss in adulthood is programmed by both early sexual differentiation and puberty: a study of humans and rats, Endocrinology 154 (2013) 1820–1831.
[44] E.M. Rivero, C.P. Piñero, L. Gargiulo, F. Entschladen, K. Zänker, A. Bruzzone, I.A. Lüthy, The β2-adrenergic agonist salbutamol inhibits migration, invasion and metastasis of the human breast cancer MDA-MB-231 cell line, Curr. Cancer Drug Targets 17 (2017) 756–766.
[45] M. Obersztyn, W. Kostowski, Noradrenergic agonists and antagonists: effects on avoidance behaviour in rats, Acta Phys. Pol. A 34 (1983) 401–407.
[46] D. Staneva-Stoytcheva, A. Astrug, D. Ivanov, Effects of some beta-adrenoceptor blockers on avoidance learning in rats, Methods Find. Exp. Clin. Pharmacol. 11 (1989) 535–540.
[47] J. Marksteiner, C. Humpel, Beta-amyloid expression, release and extracellular deposition in aged rat brain slices, Mol. Psychiatry 13 (10) (2008) 939–952.
[48] V. Kaliyamurthi, V. Thanigavelan, G.V. Rajamanickam, Effects of diet-induced hypercholesterolemia on amyloid accumulation in ovariectomized mice, J. Biosci. 37 (2012) 1017–1027.
[49] G.D. Silverberg, M.C. Miller, J.T. Machan, C.E. Johanson, I.N. Caralopoulos, C.L. Pascale, A. Heile, P.M. Klinge, Amyloid and Tau accumulate in the brains of aged hydrocephalic rats, Brain Res. 1317 (2010) 286–296.
[50] D.R. Howlett, A.R. George, D.E. Owen, R.V. Ward, R.E. Markwell, Common structural features determine the effectiveness of carvedilol, daunomycin and rolitetracycline as inhibitors of Alzheimer beta-amyloid fibril formation, Biochem. J. 343 (1999) 419–423.
[51] Wang J, Ono K, Dickstein DL, Arrieta-Cruz I, Zhao W, Qian X, Lamparello A, Subnani R, Ferruzzi M, Pavlides C, Ho L, Hof PR, Teplow DB, Pasinetti GM (2011) Carvedilol as a potential novel agent for the treatment of Alzheimer's disease. Neurobiol. Aging 32:2321.e1–2321.e12.
[52] D. Wang, Y.K. Xiang, β-Adrenergic receptor, amyloid β-peptide, and Alzheimer's disease, Curr. Top. Membr. 67 (2011) 205–228.
[53] T.V. Nguyen, A. Jayaraman, A. Quaglino, C.J. Pike, Androgens selectively protect against apoptosis in hippocampal neurones, J. Neuroendocrinol. 22 (2010) 1013–1022.
[54] S.L. Zup, N.S. Edwards, M.M. McCarthy, Sex- and age-dependent effects of androgens on glutamate-induced cell death and intracellular calcium regulation in the developing hippocampus, Neuroscience 281 (2014) 77–87.
[55] J. Ji, X. Zhang, B. Li, Beta-adrenergic modulation of in vivo long-term potentiation in area CA1 and its role in spatial learning in rats, Sci China C Life Sci 46 (2003) 605–614.
[56] N. Oikawa, K. Ogino, T. Masumoto, H. Yamaguchi, K. Yanagisawa, Gender effect on the accumulation of hyperphosphorylated tau in the brain of locus-ceruleus-injured APP-transgenic mouse, Neurosci. Lett. 468 (2010) 243–247.
[57] P. Theofilas, A.J. Ehrenberg, S. Dunlop, A.T. Di Lorenzo Alho, A. Nguy, R.E. Leite, R.D. Rodriguez, M.B. Mejia, C.K. Suemoto, R.E. Ferretti-Rebustini, L. Polichiso, C.F. Nascimento, W.W. Seeley, R. Nitrini, C.A. Pasqualucci, W. Jacob Filho, U. Rueb, J. Neuhaus, H. Heinsen, L.T. Grinberg, Locus coeruleus volume and cell population changes during Alzheimer's disease progression: a stereological study in human postmortem brains with potential implication for early-stage biomarker discovery, Alzheimers Dement. 13 (2017) 236–246.
[58] M.E. Gibbs, D. Maksel, Z. Gibbs, X. Hou, R.J. Summers, D.H. Small, Memory loss caused by beta-amyloid protein is rescued by a beta (3)-adrenoceptor agonist, Neurobiol. Aging 31 (2010) 614–624.
[59] R. Ul Haq, M. Anderson, A. Liotta, M. Shafiq, M.A. Sherkheli, U. Heinemann, Pretreatment with β-adrenergic receptor agonists facilitates induction of LTP and sharp wave ripple complexes in rodent hippocampus, Hippocampus 26 (2016) 1486–1492.
[60] P.J. Fitzgerald, Is elevated norepinephrine an etiological factor in some cases of Alzheimer's disease? Curr. Alzheimer Res. 7 (2010) 506–516.
[61] A.J. Saber, D.P. Cain, Combined beta-adrenergic and cholinergic antagonism produces behavioral and cognitive impairments in the water maze: implications for Alzheimer disease and pharmacotherapy with beta-adrenergic antagonists, Neuropsychopharmacology 28 (2003) 1247–1256.
[62] B. Jarzab, E. Gubala, W. Achtelik, G. Lindner, E. Pogorzelska, K.D. Döhler, Postnatal treatment of rats with beta-adrenergic agonists or antagonists influences differentiation of sexual brain functions, Exp. Clin. Endocrinol. 94 (1989) 61–72.
[63] E.P. Lim, G.S. Dawe, T.M. Jay, Activation of beta- and alpha-2-adrenoceptors in the basolateral amygdala has opposing effects on hippocampal-prefrontal long-term potentiation, Neurobiol. Learn. Mem. 137 (2017) 163–170.
[64] C.H. Kim, I.G. Ko, S.E. Kim, M.S. Shin, Y.H. Kang, J.W. Cho, K.M. Shin, C.J. Kim, B.V. Lim, K.H. Kim, Alpha1-adrenoceptor antagonists improve memory by activating N-methyl-D-aspartate-induced ion currents in the rat hippocampus, Int. Neurourol. J. 19 (4) (2015) 228–236.
[65] X. Liu, L. Ma, H.H. Li, B. Huang, Y.X. Li, Y.Z. Tao, L. Ma, β-Arrestin-biased signaling mediates memory reconsolidation, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 4483–4488.
[66] S.M. Peterson, T.F. Pack, A.D. Wilkins, N.M. Urs, D.J. Urban, C.E. Bass, O. Lichtarge, M.G. Caron, Elucidation of G-protein and β-arrestin functional selectivity at the dopamine D2 receptor, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 7097–7102.
[67] F. Deak, W.M. Freeman, Z. Ungvari, A. Csiszar, W.E. Sonntag, Recent developments in understanding brain aging: implications for Alzheimer's disease and vascular cognitive impairment, J. Gerontol. A Biol. Sci. Med. Sci. 71 (2016) 13–20.
[68] N.K. Dash, M. Azam, G. Gupta, N.Z. Baquer, Effect of chronic administration of propranolol on acetylcholinesterase and 5-hydroxytryptamine levels in rat brain and heart, Biochem. Int. 22 (1990) 475–482.
[69] A. González-Brito, R.J. Reiter, M.G. Tannenbaum, D.J. Jones, Adrenalectomy but not gonadectomy affects rat pineal beta-adrenergic receptor density, Neurosci. Lett. 92 (1988) 330–334.
[70] J.P. Lacey, C.F. Donatucci, D.T. Price, S.O. Page, S.A. Bennett, M.P. Tenniswood, D.A. Schwinn, Effects of androgen deprivation on prostate alpha 1-adrenergic receptors, Urology 48 (1996) 335–341.
[71] J. Fedotova, V. Soultanov, T. Nikitina, V. Roschin, N. Ordyan, L. Hritcu, Cognitive-enhancing activities of the polyprenol preparation Ropren® in gonadectomized β-amyloid (25–35) rat model of Alzheimer's disease, Physiol. Behav. 157 (2016) 55–62.