Improving effect of chronic resveratrol treatment on central monoamine synthesis and cognition in aged rats

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Abstract Resveratrol is a polyphenol exhibiting anti-oxidant and neuroprotective effects in neurodegenerative diseases. However, neuroprotective properties during normal aging have not been clearly demonstrated. We analyzed the in vivo effects of chronic administration of resveratrol (20 mg/kg/day for 4 weeks) in old male rats (Wistar, 20 months), on tryptophan hydroxylase (TPH) and tyrosine hydroxylase (TH) activities which mediate central monoaminergic neurotransmitters synthesis, and besides, on hippocampal-dependent working memory test (radial maze). Our results show an age-related decline in neurochemical parameters that were reversed by resveratrol administration. The resveratrol treatment enhances serotonin (5-HT) levels in pineal gland, in hippocampus, and in striatum, and those of noradrenaline (NA) in hippocampus and also dopamine (DA) in striatum. These changes were largely due to an increased activity of TPH-1 (463 % in pineal gland), TPH-2 (70–51 % in hippocampus and striatum), and TH (150–36 % in hippocampus and striatum).

Additionally, the observed hippocampal effects correlate with a resveratrol-induced restorative effect on working memory (radial maze). In conclusion, this study suggests resveratrol treatment as a restoring therapy for the impaired cognitive functions occurring along normal aging process, by preventing 5-HT, DA, and NA neurotransmission decline.

Keywords Resveratrol · Aging · Brain · Monoamine synthesis · Cognition

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Introduction

Resveratrol is a polyphenol found in grape skins, red wine, raspberries, blueberries, peanuts, and medicinal plants, such as *Polygonum cuspidatum* (Chen et al. 2007) with different beneficial effects on health, including prevention of cancer, cardiovascular diseases or ischemic injuries, and even extend the lifespan (for review, see Baur and Sinclair 2006). Resveratrol effects include antioxidant and anti-inflammatory effects but also has been reported to regulate the expression level and activity of different proteins associated with cell survival and ion channel regulation, between others (Bai et al. 2010; Baur and Sinclair 2006; Chen et al. 2007; Guarente and Franklin 2011). Evidences suggest that resveratrol also regulates neuronal function via a SIRT1-dependent mechanism (Araki et al. 2004; Baur 2010; Chuang et al. 2009), and mechanisms involving monoamine neurotransmission facilitation since resveratrol inhibits monoamine (MAO) catabolism and also

monoamine reuptake into presynaptic neurons (Xu et al. 2010; Yañez et al. 2006). Furthermore, resveratrol protective properties have been reported in neurodegenerative disease models (Li et al. 2014; Blanchet et al. 2008; Donmez et al. 2010; Lofrumento et al. 2014; Albani et al. 2010; Wang et al. 2011) but have not been clearly demonstrated during normal aging (for review, see Baur 2010).

Nowadays, population aging is a major problem in developed countries, which has led to increase the prevalence of age-dependent diseases, such as Alzheimer's disease, depression, and other dementias. Biochemical changes occurring in aged brain are responsible of this age-associated cognitive impairment and are also crucial in the further development of these neurodegenerative diseases (Gareri et al. 2002; and for review, see Hedden and Gabrieli 2004). Central monoamines exhibit a marked decline in different brain regions as part of normal aging process (Esteban et al. 2010a, b; Luine et al. 1990; Míguez et al. 1999; Venero et al. 1991) which is responsible, at least partially, for the age-related cognitive impairment (Collier et al. 2004; Koprowska et al. 2004; Lemon and Manahan-Vaughan 2006; Meneses 1999; Murchison et al. 2004). Working memory declines in late adulthood (Payer et al. 2006; Borella et al. 2008) and is considered to be one of the main contributing factors of cognitive impairment in old age (Park et al. 2002). Therefore, it is not surprising that a number of studies have been investigating the trainability of working memory across the lifespan (Takeuchi et al. 2010; Morrison and Chein 2011; Shipstead et al. 2010).

SIRT1 activators (e.g., resveratrol) have emerged as a promising new treatment for age-related brain disorders since it has been reported to play a relevant role promoting learning (Chuang et al. 2009; Gao et al. 2010). Thus, the aim of this study was to examine the effects of chronic resveratrol administration in old rats on in vivo synthesis and metabolism of monoamines (5-HT, NA, and DA) in crucial brain regions for regulation of memory processing, in parallel to behavioral performance evaluation on working memory test.

Material and methods

Animals

Young (3 months, $n=8$) and old (20 months, $n=14$ distributed in control and resveratrol-treated group) male Wistar rats (Harlan, Barcelona, Spain) were individually housed with free access to standard food (Panlab A04) and tap water, under controlled environmental conditions (20 ± 2 °C; 70 % humidity), in a sound-attenuated chamber, and maintained at 12-h light/dark photoperiod (lights on at 0800 h daily) with an average of 300 lux of indirect light provided from fluorescent lamps. Animals were daily handled for several days prior to starting tests to reduce stress during testing. Every 3–4 days during treatments, animals were weighted and the amount of food ingested was registered. All procedures were in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Directive 86/609/EEC) and in agreement with the Bioethical Committee of the University of Balearic Islands.

Drugs and reagents

All drugs and reagents were purchased from Sigma Chemical Company (St. Louis MO, USA). The following drugs were used for animal treatments: resveratrol *trans* isomer (3,4′,5-trihydroxy-*trans*-stilbene), corn oil (delivery vehicle for resveratrol), and NSD-1015 (3-hydroxybenzylhydrazine dihydrochloride).

Drug treatments

Old rats were chronically treated with resveratrol (20 mg/kg/day for 30 days, i.p. at 0730 h; $n=6$) or vehicle (corn oil, 1 ml/kg/day for 30 days, i.p. at 0730 h; $n=8$), which represent the old control group. Young rats also received vehicle as old control ones. Administration route, dose, and administration time were set from a pilot study presented at the XXXVII Congress of the Sociedad Española de Ciencias Fisiológicas (SECF, Granada, Spain 2014). Decision was taken trying to increase the reproducibility and significance of outcomes and to reduce side affects of manipulation during this long treatment. Note that assimilation/dissemination route for i.p. administration is similar to p.o. (Turner et al. 2011), which is the most common route for resveratrol administration.

All animals also received NSD 1015 (100 mg/kg, i.p. at 0730 h) 30 min before being sacrificed by decapitation. This administration was performed to measure the in vivo activity of TPH and TH, through accumulation of 5-HTP and DOPA respectively during these 30 min

(see "Memory behavioral test"). Brains were quickly removed and dissected on an ice-cold plate to separate pineal gland (1.2–1.3 mg), hippocampus (60–65 mg), and striatum (caudate-putamen) (15–20 mg), which were immediately frozen in liquid nitrogen and stored at −80 °C until assays.

Temperature and activity recording during chronic treatments

Body temperature in old rats was continuously recorded to monitor resveratrol treatment effect. For this purpose, a temperature sensor (DS1921H Thermochron® iButtons®, Maxim; sampling rate of 20 min) was implanted in the abdominal cavity of old rats through a simple surgery under isoflurane gas anesthesia (Abbott, Spain). The implantation of the sensor was performed 3 days before starting treatments.

Spontaneous motor activity was also continuously recorded in old rats during chronic treatments. Cages (20×20-cm² diameter) equipped with two perpendicularly crossed infrared beams (placed at 70 mm high) were used. The movements of the animals were digitized by recording the number of beams crossed, which were accumulated during 15-min intervals and automatically stored in a computer for further analysis. Animals were also acclimated for several days before motor activity recording during the different treatments. Motor activity analysis was carried out with the integrated program for chronobiology "El Temps" (©Díez-Noguera, University of Barcelona).

Memory behavioral test

To evaluate the effects of chronic treatment with resveratrol on working memory during normal aging process, the spatial memory eight-arm radial maze test was used. All experiments were performed during the light period between 0900 and 1300 h. Rats were placed in the experimental room 30 min before the beginning of the trials.

The eight-arm radial maze (Panlab, S.L.) consisted of an octagonal central platform (32-cm diameter) with eight equally spaced radial arms (50 cm long, 12 cm wide). The maze was set in an experimental room with several external visual cues. Prior to memory test, animals were submitted to fasting to achieve a convenient motivational level. To test memory on radial maze, rats were free to visit the different arms to obtain food pellets

and trials were judged complete when rats had chosen all eight baited arms or spent 20 min in the trial (maximum time to achieve performance). The sum of nonvisited arms and repeated entry into arms was scored as a working memory error. The apparatus was cleaned with ethanol solution between trials. Rats movements were monitored via a digital video tracking system (LE 8300 with software SEDACOM v 1.3, Panlab, SL, Barcelona, Spain) (for further descriptions, see Esteban et al. 2010a, b; Ramis et al. 2013). Tests were performed before chronic treatments (initial test) and after chronic treatments.

TPH activity (synthesis of 5-HT) and TH activity (synthesis of DA and NA)

Transformation of tryptophan into 5-HTP is the limiting step in 5-HT synthesis which requires the TPH-2 isoform enzyme (tryptophan-5-monoxygenase, EC 1.14.16.4) in most of the brain and TPH-1 isoform enzyme in the pineal gland (Walther et al. 2003). Regarding catecholamine synthesis (NA and DA synthesis), the limiting step is the transformation of tyrosine into DOPA which requires the TH enzyme (tyrosine-3-monoxygenase, EC 1.14.16.2). In this manner, the in vivo activity of rate rate-limiting enzymes in synthesis of 5-HT and catecholamines (TPH and TH, respectively) were determined by measuring the accumulation of their precursors (5-HTP and DOPA, respectively) within 30 min after inhibition of the aromatic L-amino acid decarboxylase (EC 4.1.1.28) by a maximally effective dose of NSD 1015 (3-hydroxybenzylhydrazine HCl, 100 mg/kg, i.p.). The administration of L-amino acid decarboxylase inhibitor, shortly before sacrifice, enables to determine tryptophan hydroxylase (TPH) and tyrosine hydroxylase (TH) activity in different brain areas, and quantify the pool of 5-HT, DA, or NA unaffected by recent synthesis and primarily stored within neurons. Furthermore, this method allows to determine levels of some monoaminergic metabolites that can reveal recent use of these neurotransmitters (Moranta et al. 2009).

Brain sample processing and chromatographic (HPLC-ED) analyses

Hippocampus and striatum brain regions were placed individually into cold tubes containing 1 ml of 0.4 M HClO₄, 0.01 % K₂EDTA, and 0.1 % Na₂S₂O₅, and then

homogenized with an Ultra-Turrax homogenizer (Type Tp 18/10). Pineal glands were placed individually into cold tubes, homogenized by sonication for 10 s in 120-µl volume. The homogenate was centrifuged at $40,000 \times g$ for 15 min at 4 °C, and supernatant was filtered through syringe filters 0.45-µm pore size (Spartan-3, Sigma-Aldrich). Aliquots of the purified supernatants (10–20 µl depending on the brain region) were subjected to HPLC on a reversed-phase column (Spherisorb S3 ODS1 C18; 3-µm particle size range; 4.6 mm×10 cm, at 35 °C) coupled to a Tracer ODS2 C18 precolumn (2–5-µm particle size range; Teknokroma, Barcelona, Spain). The mobile phase consisted of 0.1 M KH_2PO_4, 2.1 mM octane sulfonic acid, 0.1 mM K_2EDTA, 2 mM NaCl, and 12 % (v/v) methanol (pH 2.7–2.8, adjusted with 85 % H_3PO_4), which were pumped at a flow rate of 0.8 ml/min with a Waters 600 solvent delivery system. The contents of precursor amino acids (5-HTP and DOPA), monoamines (5-HT, DA, and NA) and metabolites (5-hidroxy-indole acetic acid (5-HIAA) and homovanillic acid (HVA)) were detected electrochemically using a cell with a glassy working carbon electrode and an applied oxidation potential of +0.75 V against an in situ Ag/AgCl reference electrode (ISAAC; Waters 2465 Electrochemical Detector). The current produced was monitored by an interface (Waters busSAT/IN Module) connected to a digital PC. The concentrations of 5-HTP, DOPA, monoamine neurotransmitters, and metabolites in a given sample were calculated by interpolating the corresponding peak height into a parallel standard curve using the software Empower Pro (Waters). For representative chromatographic analyses, see Moranta et al. (2009).

Statistics

Results are normally expressed as means±SEM of the number of determinations. One-way ANOVA followed by Scheffé's test was used for the statistical evaluations (or Fisher's test when indicated). Level of significance was set at $p \leq 0.05$.

Results

Body weight, temperature, and activity during chronic treatments

Weight of young animals increased during experiment (from 327±4.93 g at the beginning to 438±6.45 g at day 30). However, no significant changes were observed in old animal weight through the different treatments (Fig. 1a), neither control animals treated with corn oil (518±38 g at the beginning of treatment) nor resveratrol-treated rats (537±24 g also at the beginning of treatment). In accordance with these effects, resveratrol treatment did not alter the amount of food ingested by animals. The daily average food intake by old control group (14.8±1.5 g) was similar to that of resveratrol-treated rats (15.6±0.5 g).

All animals did suffer weight loss when were subjected to fasting (Fig. 1a). This fasting period was done to achieve a good motivational level for the radial maze test. Weight loss was similar in both groups (6.07±0.37 g in old animals treated with corn oil and 5.96±0.32 g in old rats treated with resveratrol) (Fig. 1a) and in young animals treated with corn oil (5.3±0.3 g; data not shown).

Similar to evolution of weight, the body temperature also showed no significant effect caused by resveratrol treatment in old animals. The daily mean temperature value for all animals was 36.9±0.04 °C (Fig. 1b).

Meanwhile, spontaneous motor activity of animals was chronobiologically analyzed during treatments to check possible side effects. Figure 1c shows a double-plotted actogram representative of old rats during experimental days. This graph is commonly used in circadian research to plot activity against time and shows the activity rest cycle of animals based upon a definable threshold. Actograms show that both old controls rats and resveratrol-treated ones were well entrained with the L/D cycle. Graphs shown in Fig. 1c are typical of nocturnal animals, with higher activity levels during dark period than during light period. No significant differences were observed between the two groups.

Effects of chronic resveratrol treatment on cognitive capacity

Working memory was assessed by the eight-arm radial maze test. Test performed prior to the chronic treatments showed a marked age-related impairment in working memory. Compared with young animals, old rats lingered more than double time to complete trials (Fig. 1d). After this initial radial maze test, old rats were randomly distributed in the two experimental groups: one received chronically corn oil, and the other one received resveratrol. Note that initial basal results for radial maze test from both goups were similar. Young rats also received
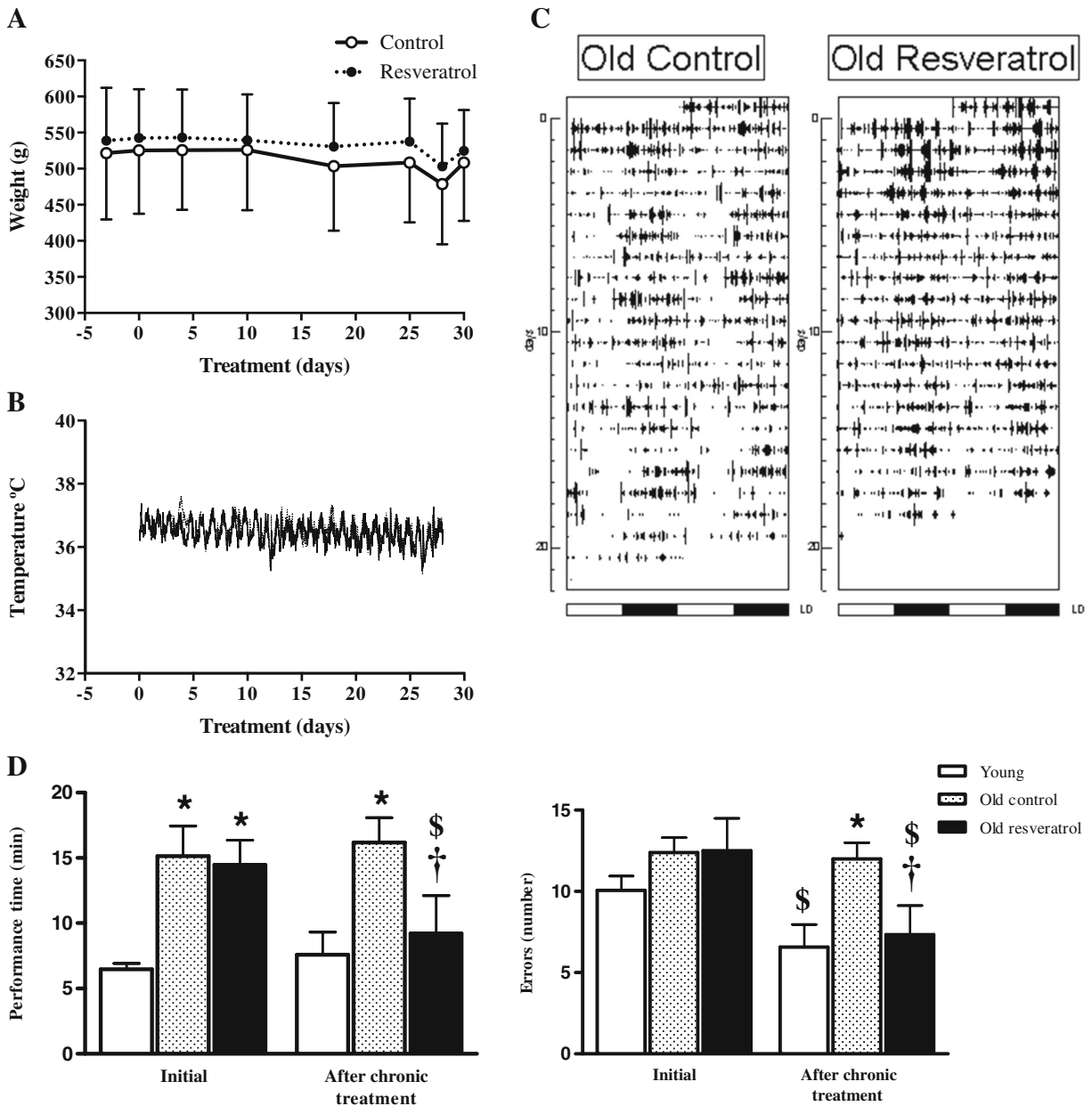
Fig. 1 **a** Body weight during chronic treatments. *Dots* represent body weight (mean±SEM; analyzed at 0900 h) of animals subjected to the different chronic treatments. Body weight reduction observed at day 28 is due to the fasting period performed to get a good motivational level for radial maze test. **b** Body temperature of rats during chronic treatments. *Graph* represents the evolution of the average body temperature in old rats during treatment: corn-oil-treated rats (*solid line*) and resveratrol-treated rats (*broken line*). **c** Synchronization of animals with the LD cycle during chronic treatments. Representative actogram of old animals during chronic treatment with corn oil or resveratrol. Data are presented as a double-plotted actogram. Animals were subjected to a 12/12 L/D photoperiod. **d** Working memory task in aged rats assessed by eight-arm radial maze test. *Bars* represent (mean±SEM) time to complete the task (*left graph*) and total number of errors (sum of unvisited and revisited arms, *right graph*). Tests were performed before chronic treatment (initial) and after chronic treatment (resveratrol or corn oil). After initial radial maze test, old rats were randomly distributed in the two groups of chronic treatments. One-way ANOVA followed by Fisher's test was used for statistical evaluation: *$p \leq 0.05$, when compared with the young control group; †$p \leq 0.05$, compared to the old control group from. And, also *t* test $^{\$}p \leq 0.05$, when compared each experimental group at the beginning and after chronic treatment

chronic corn oil. The main goal of this study was to analyze the protective effect of exogenous administration of resveratrol on brain decline that normally occurs with aging and to correlate with the age-related cognitive decline.

Corn oil treatment did not impair radial maze test parameters, neither in young animals nor in older ones; only young animals made fewer errors than in the initial test. After the chronic treatments, old control rats showed no differences compared to the results obtained prior the treatment, and they committed almost double errors than young ones (Fig. 1d). However, chronic resveratrol treatment induced a memory enhancement. Old rats treated with resveratrol showed a reduced time to complete the trial (43 % reduction respect old control group), and additionally, they committed a reduced number of errors (unvisited plus repeatedly visited arms; 39 % reduction respect old control group; Fig. 1d).

These results suggest that chronic resveratrol treatment might aid to reduce the cognitive capacity descent that normally occurs as an aging consequence.

Effect of chronic resveratrol treatment on 5-HT synthesis and metabolism

The synthesis and metabolism of 5-HT was measured in regions enriched with serotonergic nerve terminals: hippocampus, striatum, and pineal gland. The synthesis rate of 5-HT was assessed by measuring accumulation of its precursor (5-hydroxytryptamine; 5-HTP) during 30 min after decarboxylase enzyme inhibition with NSD 1015. In this conditions, 5-HTP accumulation reflects the activity of the limiting enzyme TPH. As expected, there was less 5-HT synthesis (THP-2 activity measured as 5-HTP accumulation) in old rats hippocampus and striatum compared with young ones. Consistently, intraneuronal 5-HT and its metabolite 5-HIAA showed an age-dependent reduction (Fig. 2).

However, chronic resveratrol treatment increased 5-HT synthesis in old rats. The 5-HTP accumulation (TPH-2 activity) was higher in hippocampus (70 %) and striatum (51 %) in old rats treated with resveratrol, compared to old control rats treated with corn oil. Furthermore, 5-HT content also increased after resveratrol treatment (55 and 76 % in hippocampus and striatum, respectively), while 5-HIAA levels were reduced after this treatment with resveratrol (22 and 48 % in hippocampus and striatum, respectively) (Fig. 2). Taken together, these results indicate a 5-HT synthesis

acceleration and reduction of its metabolism in these brain regions from old animals treated with resveratrol. Similarly, chronic resveratrol treatment increased significantly 5-HT synthesis in the pineal gland of old rats, but in this case reflecting TPH-1 activity. Resveratrol treatment increased more than fourfold the 5-HTP accumulation in old animals, almost reaching levels of young animals, and also increased more than sixfold the 5-HT content. However, resveratrol treatment did not modify pineal gland 5-HIAA levels (Fig. 2).

Effect of chronic resveratrol treatment on catecholamine synthesis and metabolism

Similarly to the effect on serotonergic systems, NA synthesis rate in hippocampus decreased with age, showing less DOPA accumulation (TH activity) and NA content in old rats (Fig. 3, left graphs). This method did not allow to quantify any noradrenaline metabolite.

Similar age-dependent reduction in TH activity was observed in striatum, but in this case reflecting DA synthesis, with a lower DOPA accumulation and less DA content and metabolite HVA levels in old rats (Fig. 3, right graphs).

The hippocampal noradrenergic system also showed a protective effect by resveratrol treatment. This treatment induced a significant increase in DOPA accumulation (150 %) and NA content (57 %) in hippocampus. Similar effects were observed for dopaminergic system in striatum, since resveratrol treatment in old rats increased DOPA accumulation (36 %) and DA content (53 %) in striatum, while HVA metabolite levels did not change (Fig. 3).

Finally, there were no significant differences between the different groups in total content of DA in hippocampus (total content of DA in hippocampus of young rats was 151.2±24.56 ng/g of wet tissue), and those of NA in striatum (total content of NA in striatum of young rats was 59.69±17.22 ng/g of wet tissue). No significant decrease related with aging was observed in any of this two parameters.

Discussion

Results from the present study in vivo point out that chronic resveratrol administration might aid to delay or prevent deficits in monoamines and memory disabilities associated with aging process. Aging causes an
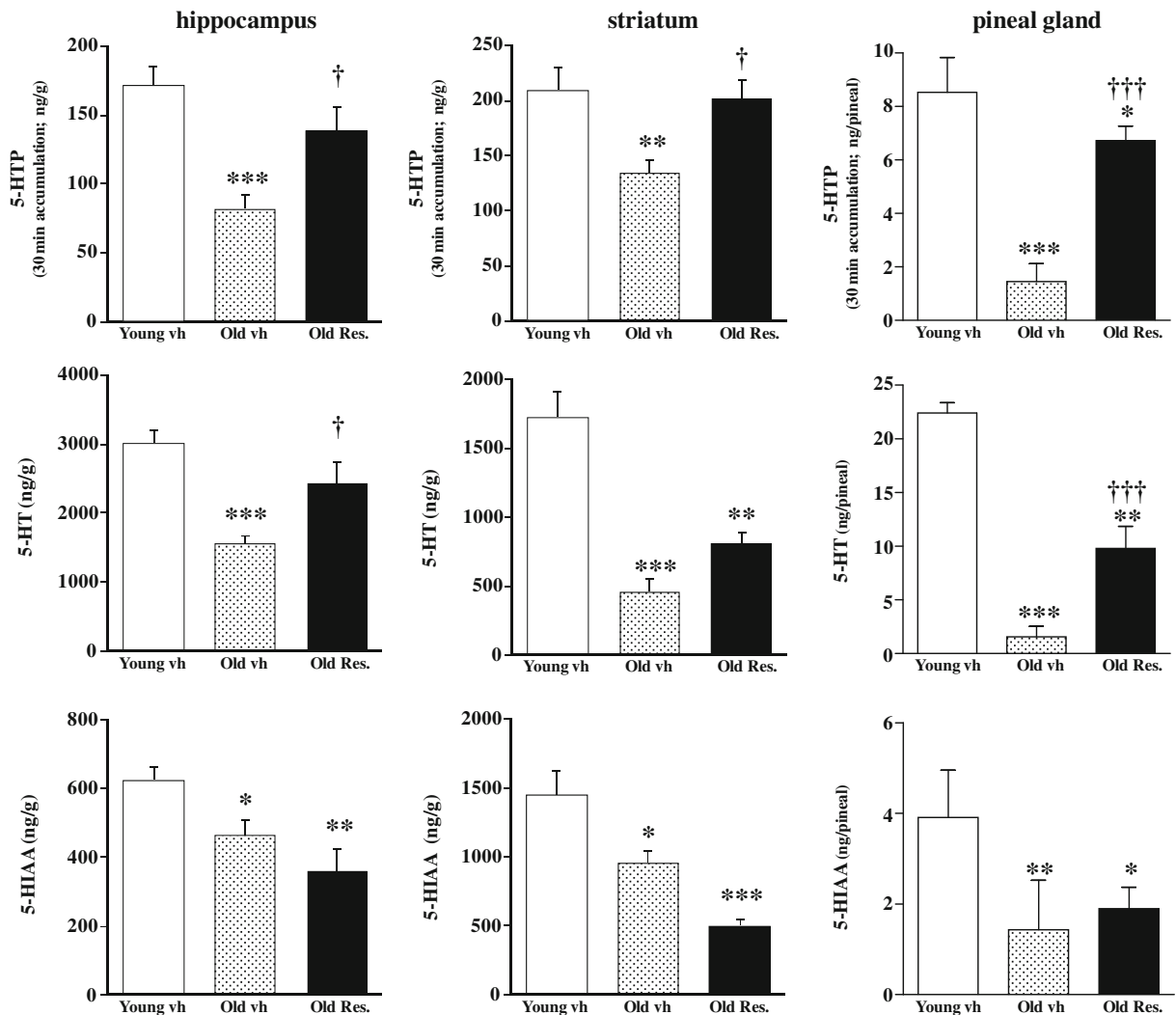
Fig. 2 Effect of chronic resveratrol treatment on serotonergic system in hippocampus, striatum, and pineal gland. *Bars* represent (mean±SEM in ng/g of wet tissue or total pineal gland) 5-HTP accumulation (during 30 min after decarboxylase inhibition), 5-HT tissue content, and 5-HIAA metabolite levels. One-way ANOVA followed by Scheffé's test was used for statistical evaluation: $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$ when compared with the young control group; $\dagger p \leq 0.05$, $\dagger\dagger\dagger p \leq 0.001$ when compared to the old control group

impairment of brain monoaminergic systems. Resveratrol treatment did not alter physiological parameters in rats, such as food intake, body weight, temperature, or locomotor activity. However, this resveratrol treatment increased the brain 5-HT levels, in hippocampus, striatum, and pineal gland, and catecholamine levels in hippocampus and striatum in aged rats. These effects were largely due to an increased activity of the limiting enzymes in monoamines syntheses, TPH and TH. The 5-HTP and DOPA accumulation method is the most commonly used assay system to monitor the in vivo rate of tryptophan and tyrosine hydroxylation (TPH and TH activity, respectively) in the brain (Moranta et al. 2009). TPH is known to exist in two isoforms: TPH-1 is mainly expressed in the pineal gland and in gut enterochromaffin cells, and TPH-2 is preferentially expressed in the rest of the brain where it plays a fundamental role in 5-HT synthesis (Walther et al. 2003). Our data refers to the activity of both isoforms on the synthesis of 5-HTP.

Monoamines have been linked to cognitive processes such as memory and learning. Memory processes are partially regulated by neuromodulatory activity of 5-HT, DA, and NA (González-Burgos and Feria-Velasco 2008; Kemp and Manahan-Vaughan 2008; Cools

Fig. 3 Effect of chronic resveratrol treatment on catecholaminergic system in hippocampus and striatum. *Bars* represent DOPA accumulation (during 30 min after decarboxylase inhibition), DA or NA tissue content, and HVA metabolites levels in striatum. One-way ANOVA followed by Scheffé's test was used for statistical evaluation: $*p \leq 0.05$, $***p \leq 0.001$ when compared with the young control group; $\dagger p \leq 0.05$, $\dagger\dagger p \leq 0.01$ compared to the old control group
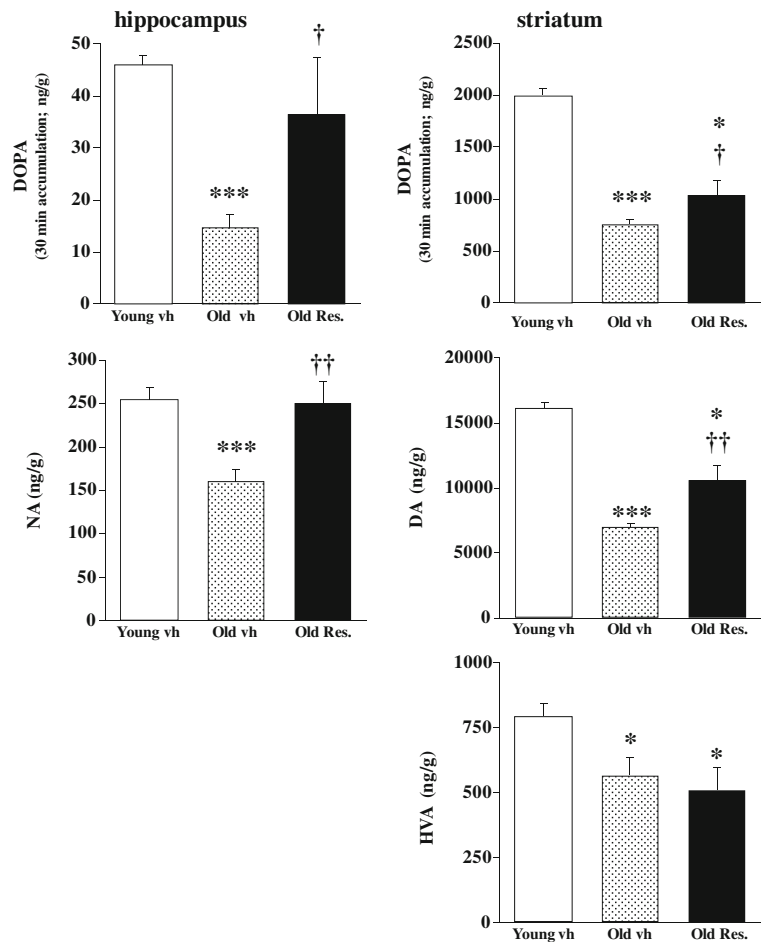
2011), being the hippocampus and striatum two brain regions involved in the regulation of the memory processing (Adams et al. 2008; González-Burgos and Feria-Velasco 2008; Cools 2011). Neurochemical results from the present work are in good correlation with the improving effect of resveratrol treatment on working memory in the radial maze observed in the current work.

Our data confirm that during aging process occur a significant decrease in brain monoamines (Esteban et al. 2010a, b; Koprowska et al. 2004; Luine et al. 1990; Míguez et al. 1999; Moranta et al. 2014; Tsunemi et al. 2005; Venero et al. 1991). Old rats showed a significant decrease in 5-HT synthesis (in hippocampus and striatum), DA synthesis (in striatum), and NA synthesis (in hippocampus). These effects were also accompanied by a lower content of 5-HT, DA, and NA, and by reduced levels of metabolites (5-HIAA and HVA). The age-related decline in monoaminergic function in hippocampus and striatum is believed to be partially responsible

for memory disorders and for the prevalence of neurodegenerative diseases during senescence (Collier et al. 2004; Cools et al. 2011; Esteban et al. 2010a, b; Haider et al. 2014; Hussain and Mitra 2000; Luine et al. 1990; Míguez et al. 1999), which is in agreement with the age-related impairment in working memory that is found in this work (assessed by an hippocampal-dependent test, the eight-arm radial maze test).

The brain is the body tissue with the largest oxygen consumption, being extremely sensitive to oxidative damage. Furthermore, the activity of antioxidant enzymes undergo a significant age-related decrease in brain (Keller et al. 2005). Indeed, in both aged humans and rodents, age-related cognitive impairment and neurodegenerative changes have been correlated to oxidative damage accumulation in lipids, proteins, and nucleic acids, which could lead to the increased neurotransmitter systems' vulnerability to oxidative stress (Dias et al. 2007; Haider et al. 2014; see Butterfield

et al. 2006 for review). In this regard, it has been reported a reduced TPH and TH activities during normal aging brain that has been related to an inefficient phosphorylation and/or an oxidative damage of these enzymes (Cash et al. 1998; De la Cruz et al. 1996; Hussain and Mitra 2000). Considering these previous works, we suggest that the observed effects of resveratrol on monoamines could be due to a protective effect on these limiting enzymes (TPH and TH) against oxidizing agents, which could counteract the effect of aging (Rose et al. 2014; Wang et al. 2011). Similar protective effects of resveratrol on dopaminergic system has been reported against injuries caused by LPS, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), or 6-hydroxyl dopamine (Rose et al. 2014; Blanchet et al. 2008; Lofrumento et al. 2014; Wang et al. 2011). Additionally, some works describe an increased TH expression due to resveratrol treatment, pointing out a SIRT1-mediated effect, which, in turn, could also imply histone deacetylation-mediated effects (Chen et al. 2007; Kumar et al. 2007; Ranney and Petro 2009; Tredici et al. 1999). Thus, SIRT1 activation could be part of the action mechanism for resveratrol pharmacological activities, including a cellular ROS scavenging effect (Araki et al. 2004; Li et al. 2014).

Finally, the observed increase in 5-HT content after resveratrol treatment could also be related to a reduced monoamine metabolization rate, by MAO-A inhibition and/or inhibition of monoamine reuptake (Xu et al. 2010; Yañez et al. 2006), which could be consistent with the observed reduction in 5-HIAA metabolite levels after resveratrol treatment. In contrats, HVA metabolite levels did not change after resveratrol treatment, reflecting the reported greater inhibition on MAO-A respect to the MAO-B (Yañez et al. 2006).

In summary, the observed effects by resveratrol treatment in the present work could be due to a combined effect that involves several mechanisms, pointing out the need to discriminate the significance of the different resveratrol effects.

Conclusion

Results from the present study demonstrate that treatment with resveratrol might aid to prevent the descent in 5-HT, DA and NA neurotransmission that occurs normally as a consequence of aging, and it might aid to delay some aging-related diseases, which are emerging as the greatest health threats of the twenty-first century.

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

Compliance with Ethical Standards All procedures performed on animals were in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Directive 86/609/EEC) and in agreement with the Bioethical Committee of the University of Balearic Islands. This article does not contain any studies with human participants performed by any of the authors.

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