Int J Pept Res Ther (2016) 22:471–480
DOI 10.1007/s10989-016-9527-7

Can Carnosine Prevent the Aging-Induced Changes of Blood Platelet and Brain Regional Monoamine Oxidase-A mRNA in Relation to its Activity?

Soumyabrata Banerjee[1] · Mrinal K. Poddar[1]

Accepted: 18 April 2016 / Published online: 27 April 2016

Abstract It is well known that the monoamine oxidase (MAO) activity deregulates during aging along with anti-oxidant activity. Carnosine (β-Ala-L-His) is an endogenous dipeptide biomolecule, having both anti-oxidant and anti-glycating properties. The present study deals with the effect of carnosine on aging-induced changes in MAO-A mRNA expression of brain regions and blood platelets in relation to their MAO-A activity. Results showed that aging significantly and characteristically increased the brain regional MAO-A mRNA whereas, in blood platelets it was significantly reduced with an increase in blood platelet counts. Carnosine attenuated both aging-induced (i) increase in brain regional MAO-A mRNA expression and blood platelet count, (ii) decrease in blood platelet MAO-A mRNA expression and its (platelet MAO-A) activity without affecting the young rats' brain regions and platelet. The present results thus suggest that carnosine attenuated and restored the aging-induced (a) increase of platelet count and (b) changes in brain regional and blood platelet MAO-A mRNA expression and (c) decrease in platelet MAO-A activity, towards their respective basal level that were observed in young rats.

Keywords Blood platelet · Brain regions · MAO-A activity · MAO-A mRNA · Aging · Carnosine

✉ Mrinal K. Poddar
mrinalkp@yahoo.com

[1] Department of Biochemistry, University of Calcutta, 35, B.C. Road, Kolkata 700 019, India

Introduction

Monoamine oxidase (MAO, E.C. 1.4.3.4.) is a mitochondrial membrane bound monoamine catalyzing enzyme (Tipton et al. 2004). This MAO catalyzes the monoamine reaction: $RCH_2NH_2 + H_2O + O_2 = RCHO + NH_3 + H_2O_2$. This enzyme has been found in different tissues (Johnston 1968) including brain (Johnston 1968) and blood platelets (Bond and Cundall 1977). It has been also found that the platelet MAO activity is reduced in neurodegenerative disorders like schizophrenia (Del et al. 1983). Pharmacologically, the MAO enzyme exists in two different isoforms MAO-A and MAO-B depending on their substrate specificities and inhibitor sensitivities (Johnston 1968). The structural and functional aspects of MAO-A have been studied by using cDNA cloning, cloning of genomic DNA and genetic engineering (Shih 2004). The MAO-A gene is known to be located on X chromosome (Xp11.23) (Zeirmans et al. 2012). The knowledge about the MAO-A activity in brain and blood platelets is very limited. In fact, no such knowledge about the blood platelet MAO-A activity is known (Bortolato and Shih 2011) till the article has been published from our laboratory (Banerjee and Poddar 2013). Since the mitochondrial MAO-A activity with aging has been found to be increased in brain regions and decreased in blood platelets (Banerjee and Poddar 2013, 2015) and Gokhan and Xandra (1991) have shown a correlation between MAO-A activity and its gene expression, it is not unreasonable to pay an attention on brain regional and blood platelets mitochondrial MAO-A mRNA expression in relation to the change in their MAO-A activity during aging and also with carnosine.

Carnosine, an endogenous (Kohen et al. 1988) dipeptide having two amino acids (β-Ala-L-His) is an anti-glycating and anti-oxidant molecule (Bellia et al. 2011). This

endogenous biomolecule is present in several tissues including brain and muscle tissues (Kohen et al. 1988). It is synthesized by the enzyme carnosine synthase and degraded by carnosinase (Boldyrev et al. 2013). It also acts as a reactive oxygen species scavenger and has a protective effect in oxidative and nitrosative driven neuro diseases (Bellia et al. 2011; Boldyrev et al. 2013), anti-senescence activity (Yuneva et al. 1999), and also involves in gene regulation (Quinn et al. 1992).

Since blood platelet count is one of the important physiological biomarker and hence its (platelet) MAO-A activity and no study has yet been done to characterize the MAO-A mRNA expression in blood platelet and brain regions during aging, the aim of the present study is to analyse quantitatively the MAO-A activity and its mRNA expression in both blood platelet and different brain regions during aging and also with the treatment of carnosine. The quantification of the tissue specific mRNA expression levels of MAO-A during aging using the most promising gene expression tool as real-time reverse transcriptase-polymerase chain reaction (RT-PCR) analysis and its correlation with their MAO-A activity in the present study, first time provide insight into the normal biological and pathological cellular processes at the molecular level.

Material and Methods

Materials

L-Carnosine, 5-Hydroxytryptamine-HCl, Triton X-100, Primers (Forward and Reverse of 18 s r-RNA and MAO-A), SYBR Green Master Mix were purchased from Sigma chemicals (St. Louis, MO, USA). TRIzol® reagent and Ultra pure DEPC Treated water 750023 were bought from Ambion and Invitrogen, BIOTAQ™ respectively. The DNA polymerase, magnesium ion, 10X NH_4 Reaction buffer were purchased from BIOLINE. The dNTP Mix, 5X Reaction buffer, RevertAid Reverse Transcriptase, Ribolock RNase inhibitor, Random hexamer were supplied by Fermentus, Thermo Fischer Scientific Inc. (NYSE:TMO) (MA, USA). All other chemicals including agarose, tris-(hydroxymethyl)-aminomethane, trisodium citrate, formaldehyde, brilliant blue, and ethidium bromide of analytical grade were purchased from Merck-India (Worli-Mumbai), India. Liquid nitrogen was obtained from University Science Instrumentation Centre, University of Calcutta, Kolkata, India.

Animals and Animal Care

In the present study the male albino rats of Wistar strain were used as an experimental animal. The animals were kept in a room having constant relative humidity (80 ± 5 %) and temperature 28 ± 0.5 °C with a 12 h light–dark cycle. Animals were maintained with standard laboratory diet and water ad libitum. In the present study to minimize the animals used and their sufferings were taken care following the guidelines of the Institutional animal ethical committee (Department of Biochemistry, University of Calcutta).

Experimental Design

Male albino rats of Wistar strain of three different age groups (4, 18 and 24 months) were taken and divided into three groups Group-1, Group-2 and Group-3 respectively. The rats of each group was divided into three subgroups subgroup-1a, 1b and 1c; subgroup-2a, 2b and 2c; subgroup-3a, 3b and 3c. Each subgroup contained 4-6 animals. Subgroups 1a, 2a and 3a were treated with carnosine (2.0 μg/kg/day) intrathecally (i.t.) for 21 consecutive days to (i) use the minimum quantity of drug and (ii) ensure an easy direct administration to the central nervous system (CNS). The control groups (Subgroups 1b, 2b and 3b) of corresponding experimental groups (Subgroups 1a, 2a and 3a respectively) were treated with same volume (20 μl) of saline as vehicle of carnosine through the same route under similar conditions. The dosage and duration response studies (Banerjee and Poddar 2013, 2015) with carnosine (2.0 μg/kg/day, i.t.) treatment for 21 consecutive days have previously shown a maximum effect than any other dosages and durations of treatment. So, in this study the authors consider the particular dosage and duration of treatment as mentioned in the present methodology. Subgroups 1b, 2b and 3b were considered as the control group of the corresponding experimental group of subgroups 1a, 2a and 3a respectively. Subgroups 1c, 2c and 3c were without vehicle treated. The rats of all these groups were sacrificed between 9:00 and 10:00 a.m. to avoid the circadian effect, if any after 4 h of the last administration of carnosine or its vehicle (Banerjee and Poddar 2013).

Collection of Brain

Immediately after sacrifice of all the above mentioned subgroups of rat of three different age groups, the brains were collected in an ice cold condition (0–4 °C) and different regions (cerebral cortex, hippocampus, hypothalamus and pons-medulla) were dissected out according to the method as previously described (Poddar and Dewey 1980). The brain regions were stored in liquid nitrogen for RNA isolation.

Preparation of Platelet Rich Plasma (PRP)

Immediately after sacrifice of all the above mentioned subgroups of rats of three different age groups, blood was

collected with 1 % EDTA solution under ice cold (0–4 °C) condition and PRP was prepared following the method as previously described (Banerji et al. 1977). These PRP preparations were used as an enzyme source and the source for platelet count.

Measurement of Platelet Count

The platelet was counted in a neubauer chamber using the compound microscope (Mukherjee 1990). Briefly, the PRP sample (0.02 ml) was added into 3.98 ml solution containing 3.84 % tri-sodium citrate, 5 % neutral formaldehyde in deionised water and a pinch of brilliant blue. Mixed well and transfer it into the neubauer chamber for counting. Placed the chamber in a moist chamber for 15 min and then counted the 25 big squares. The count was expressed as $\times 10^9$ platelets/L.

Assay of Blood Platelet Monoamine Oxidase-A (MAO-A) Activity

The MAO-A activity of blood PRP preparation was measured using 0.24 mM 5-HT (Serotonin) as its (MAO-A) substrate with 100 µg enzymes following the method of Banerji et al. (1977). The standard incubation volume of a mixture of 1.0 ml contains phosphate buffer (0.1 M, pH 7.0), enzyme source (100 µg), triton-X 100 (0.01 %), semicarbazide (0.125 M) and serotonin (0.24 mM). This mixture was incubated for 30 min at 37 °C. After the incubation, 0.1 M 2,4 dinitrophenyl hydrazine (DNPH) in 2 N HCl was added to stop the reaction. The corresponding enzyme blank was prepared by adding 2,4 DNPH before the addition of serotonin. Immediately after termination of the reaction the 4.0 ml benzene was added. The products of the reaction was extracted in the organic layer and added into NaOH (0.4 %), mixed well, and centrifuged at 2800×g for 5 min. The benzene layer was removed and the aqueous layer was kept at 80 °C for 10 min cooled at room temperature. The color developed was measured spectrophotometrically (Hitachi U-2010) at 450 nm against a reagent blank. The kinetics study of MAO-A was measured with varying concentrations (0.05-0.4 mM per 100 µg enzyme) of serotonin. The activity of blood platelet MAO-A was expressed as $\Delta OD/10^8$ platelets/h.

Total RNA Isolation from PRP as Well as Brain Tissues and Reverse Transcription (RT)

Total RNA of both PRP and brain tissue preparations (as mentioned earlier) were isolated individually following the protocol provided by Life Technologies™ using TRIzol reagent (Hummon et al. 2007). Aliquots of the RNA samples were then subjected to electrophoresis on 2 % agarose gel to confirm the integrity of the RNA bands and the RNA quantity and quality was measured with 260:280 nm absorbance ratios by NanoDrop spectrophotometer (Thermo Scientific). The cDNA synthesis was performed in a PCR thermal cycler (Applied Biosystems Gene Amp PCR System 9700). The thermal cycle used for cDNA synthesis was at 25 °C for 10 min, 42 °C for 60 min, and 70 °C for 10 min and then held at 4 °C and finally stored at −20 °C until assay. Following analysis, aliquots of the RT products (single-stranded DNA, ssDNA) were ten folds diluted with nuclease-free water and used for real-time PCR (RT-PCR) analysis, as described below.

RT-PCR Analyses

The ssDNA products were then used for quantitative PCR (qPCR) analysis, using a RT-PCR system (7500 Fast Real-Time PCR System, Applied Biosystem™). All qPCR reactions were carried out with1 µl template, 0.3 µl primer (forward and reverse), 3.4 µl nuclease free water and 5 µl SYBR Green Master Mix in a 10 µl reaction volume. The thermal cycling was initiated at 94 °C for 5 min for DNA polymerase activation. Forty steps of PCR were performed, each one consisting of heating at 94 °C for 30 s, 58 °C for blood platelet and 59 °C for brain regions for 30 s each, 72 °C for 30 s and 72 °C for 5 min. Each ssDNA sample was assayed in triplicate. The primers sets were designed (Table 1) on *Rattus norvegicus* genome to obtain mRNA sequences for genes of MAO-A. Sequence data were examined with the Basic Local Alignment Search Tool (BLAST) software. The experiment was carried out in triplicate along with 18S rRNA as an endogenous control. The specificity of PCR amplification for each primer was confirmed by melting curve analyses using the ΔΔCT method and the relative quantification (RQ) data were analyzed between the groups (Kenneth 2001). Relative mRNA quantifications based on the CT (Threshold cycle, number of cycles in real time PCR required for the fluorescent signal to cross the threshold) values were carried out by comparing the genes of the present interest (MAO-A) with the reference gene (18S). CT value is inversely proportional to the targeted nucleic acid (here MAO-A) amount. The ΔCT is the difference between the CT value of targeted gene (MAO-A) and the endogenous control (18S rRNA). The fold change or RQ was calculated with the formulae $2^{-\Delta\Delta CT}$ between the groups (vehicle treated and carnosine treated) of rats. The ΔΔCT value was calculated from the difference of ΔCT values with respect to (i) vehicle treated 4 months young rats (for aging-induced study) and (ii) age-matched (4, 18 and 24 months) vehicle treated rats (for carnosine treated study).

Table 1 Sequences of synthetic primers used to assay gene expression by real-time polymerase chain reaction (RT-PCR)

Gene	Forward primer	Reverse primer	Amplicon length (bp)
18S	TCAAGAACGAAAGTCGGAGG	TGCCCTTCCGTCAATTCCTT	189
MAO-A	ACTTCCCTCCTGGTATCATGAC	CATTCAACACCTCTCTAGCTGC	156

Details are same as described in "Materials and Methods"

Table 2 Effect of carnosine on aging-induced blood platelet count (using light microscope) of male albino rats

Blood platelet count (10^9 Platelets/L of blood) (%)			
Conditions of treatment	Age groups of rat		
	Young (4 months)	Aged (18 months)	Aged (24 months)
Control (without vehicle)	100.00 ± 5.23	190.47 ± 19.32*	257.07 ± 27.38*
Vehicle treated	102.31 ± 3.96	189.67 ± 26.29*	253.58 ± 30.86*
Carnosine treated	81.85 ± 6.76	71.86 ± 2.20	135.42 ± 1.03†

Results (in percent) are expressed as mean ± SEM of 4–6 separate observations. Each observation was made from blood of a single rat. Percent value was calculated with respect to the control (without vehicle treated) value of 4 months young rats. The control value of blood platelet count (10^9 Platelets/L of blood) in young (4 months) rat is 232.40 ± 3.96

Significantly different from corresponding without or with vehicle treated (i) young (4 months) rats * $p < 0.001$, (ii) age-matched 24 months groups of rat † $p < 0.001$

Statistical Analyses

A two-way analysis of variance (ANOVA) using post hoc Tukey's test were used for multiple comparisons in case of three or more comparisons between groups. The relations between relative changes of MAO-A mRNA levels were assessed by Mann–Whitney U-test to find out the exact probability for the independent samples by using SPSS (Version 20.0., SPSS Inc., Chicago, IL). Significance was accepted at $p < 0.05$.

Results

Effects of Carnosine on Platelet Count using Light Microscope

The microscopical study presented in Table 2 shows that the blood platelet count (10^9 platelets/L) was significantly increased in both 18 months (90.47 %, $p < 0.001$) and 24 months (157.07 %, $p < 0.001$) aged rats with respect to the counts observed in 4 months young rats. Carnosine (2.0 μg/kg/day, i.t. for 21 consecutive days) significantly attenuated the aging-induced increase of blood platelet counts of 18 months (62.11 %, $p < 0.001$) and 24 months (46.60 %, $p < 0.001$) aged rats with an apparent decrease in platelet counts of 4 months young rats with respect to their corresponding age-matched vehicle treated group. Carnosine didn't show any significant effect in blood platelet count in young (4 months) rats under similar condition.

Changes of Blood Platelet MAO-A Activity of Rats During Aging With Carnosine

Table 3 depicts the effect of carnosine on aging-induced decrease of blood platelet MAO-A enzyme activity (ΔOD/10^8 platelets/h). The blood platelet mitochondrial MAO-A activity was significantly reduced in 18 months (73.46 %, $p < 0.001$) and 24 months (82.35 %, $p < 0.001$) aged rats with respect to the corresponding results of 4 months young rats. There was no significant difference between the blood platelet MAO-A activity of untreated and vehicle treated group of rats of the respective age. In 4 months young rats, the treatment of carnosine (2.0 μg/kg/day, i.t. for 21 consecutive days) though didn't significantly change the blood platelet MAO-A activity in comparison to their corresponding vehicle treated rats, it significantly attenuated the aging-induced decrease in blood platelet MAO-A activity of 18 months (88.35 %, $p < 0.001$) and 24 months (324.19 %, $p < 0.001$) aged rats in comparison to their corresponding age matched control (18 and 24 months) rats.

Carnosine-Induced Changes in Kinetic Parameters of Blood Platelet MAO-A Activity in Rats During Aging

Table 4 and Fig. 1 show that the V_{max} of platelet MAO-A activity (ΔOD/10^8 platelets/h) was significantly reduced with the increase of age from 4 to 18 months (80.31 %, $p < 0.001$) and 24 months (82.84 %, $p < 0.001$) of rats

Table 3 Effect of carnosine on blood platelet MAO-A activity during aging in male albino rats

Blood platelet MAO-A activity ($\Delta OD/10^8$ Platelets/h) (%)			
Conditions of treatment	Age groups of rat		
	Young (4 months)	Aged (18 months)	Aged (24 months)
Control (without vehicle)	100.00 ± 1.67	26.56 ± 0.37*	17.69 ± 0.21*
Vehicle treated	101.78 ± 1.87	27.12 ± 0.29*	17.81 ± 0.14*
Carnosine treated	92.00 ± 1.72	51.08 ± 0.57†	75.47 ± 2.87†

Results (%) are expressed as mean ± SEM of 4–6 separate observations. Each observation was made from a single rat. Percent value was calculated with respect to the control (without vehicle treated) value of 4 months young rats. The control value of blood platelet MAO-A activity ($\Delta OD/10^8$ Platelets/h) in young (4 months) rat is 6.97 ± 0.13

Significantly different from corresponding without or with vehicle treated (i) young (4 months) rats * $p < 0.001$, (ii) age-matched groups (18 and 24 months) of rat † $p < 0.001$

Table 4 Effect of carnosine on kinetic parameters of blood platelet MAO-A activity during aging of male rats

Age groups	Age of animals (months)	Kinetic parameters of MAO-A activity			
		Control (vehicle)		Carnosine (2.0 μg/kg/day, i.t. for 21 consecutive days)	
		V_{max} ($\Delta OD/10^8$ platelets/h)	K_m (mM)	V_{max} ($\Delta OD/10^8$ platelets/h)	K_m (mM)
Young	4	9.09 ± 0.40	0.21 ± 0.007	7.94 ± 0.06	0.20 ± 0.009
Aged	18	1.79 ± 0.07*	0.23 ± 0.006	2.74 ± 0.05†	0.22 ± 0.011
	24	1.56 ± 0.08*	0.20 ± 0.012	6.67 ± 0.20‡	0.21 ± 0.007

Results are expressed as Mean ± SEM (n = 4–6). V_{max} ($\Delta OD/10^8$ platelets/h) and K_m (mM) values were calculated from Line–Weaver Burk plot (Fig. 1b). Kinetic studies were carried out using varying concentrations (0.05–0.4 mM) of serotonin

Significantly different from (a) corresponding control rats of (i) young (4 months) * $p < 0.001$, (ii) aged (18 months) † $p < 0.001$, (iii) aged (24 months) ‡ $p < 0.001$

without any significant effect of their corresponding K_m with respect to the corresponding young (4 months) rats. The treatment of carnosine (2.0 μg/kg/day, i.t., for 21 consecutive days) significantly attenuated the aging (18 and 24 months)-induced decrease of corresponding V_{max} (53.07 and 327.56 %, $p < 0.001$ respectively) of platelet MAO-A activity with an apparent change in their corresponding K_m with respect to their corresponding age-matched (18 and 24 months) vehicle treated rats. Carnosine at the same dosage didn't show any significant effect in the V_{max} (-12.65 %, $p > 0.05$) and K_m (5.44 %, $p > 0.05$) of platelet MAO-A activity of 4 months young rats with respect to the corresponding vehicle treated young rats.

Effect of Carnosine on Aging-Induced Blood Platelet MAO-A mRNA Expression

Figure 2 represents that the blood platelet MAO-A mRNA expression was significantly decreased 8.57 fold ($p < 0.05$) in 18 months and 13.45 fold ($p < 0.05$) in 24 months aged rats with respect to the corresponding results of 4 months young vehicle treated rats. Carnosine (2.0 μg/kg/day, i.t. for 21 consecutive days) apparently increased the blood platelet MAO-A mRNA in 4 months young rats with respect to the corresponding 4 months vehicle treated rats. The MAO-A mRNA of 18 and 24 months aged rats, on the other hand, was significantly attenuated with carnosine under similar condition by 5.56 fold ($p < 0.01$) and 25.0 fold ($p < 0.001$) respectively with respect to their corresponding age-matched vehicle treated rats.

Effect of Carnosine on Aging-Induced Brain Regional MAO-A mRNA Expression

Figure 3 depicts that the brain regional mitochondrial MAO-A mRNA expression was significantly increased in cerebral cortex (5.56 fold, $p < 0.05$), hippocampus (1.96 fold, $p < 0.05$), hypothalamus (4.17 fold, $p < 0.05$) and pons-medulla (4.17 fold, $p < 0.05$) of 24 months aged rats; whereas, in 18 months this was apparently increased in all the brain regions studied except in cerebral cortex, where MAO-A mRNA expression was significantly increased (2.86 fold, $p < 0.05$) in comparison to their corresponding 4 months young rats. Carnosine (2.0 μg/kg/day, i.t. for 21

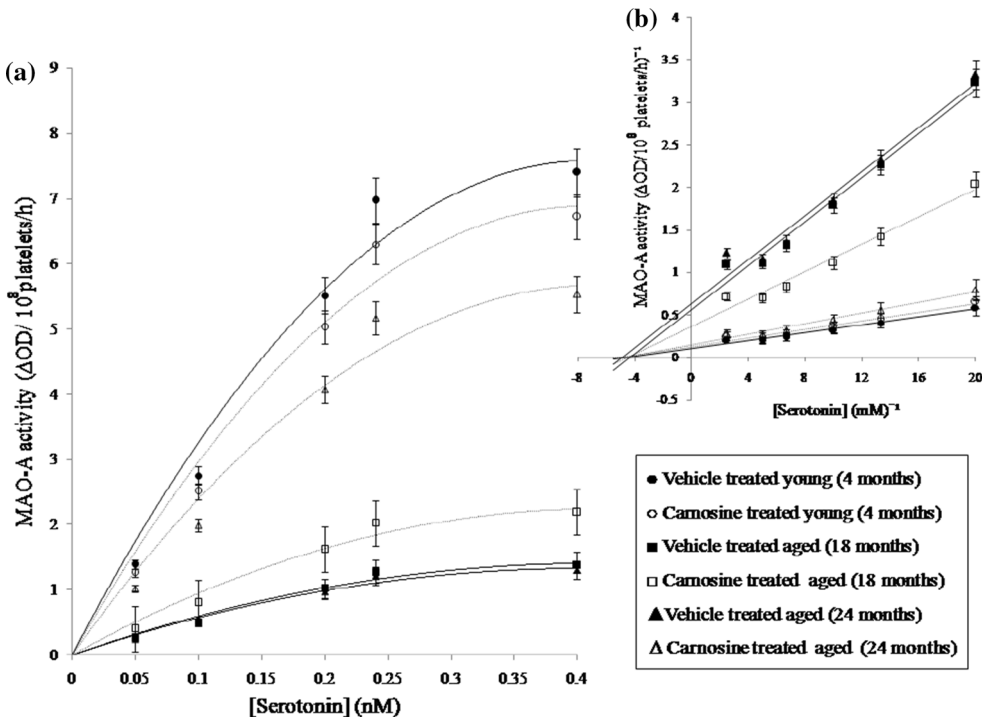

Fig. 1 Effect of carnosine on kinetic parameters (V_{max} and K_m) of blood platelet MAO-A activity during aging. Results are expressed as mean ± SEM of 4–6 separate observations. *Solid line* represents vehicle treated, *dashed lines* indicates carnosine treated conditions. The *vertical lines* (I) of each point in (**a**) and (**b**) indicate ± SEM. V_{max} ($\Delta OD/10^8$ platelets/h) and K_m (mM) values were determined by Line–Weaver Burk plots (**b**) [drawn from the corresponding progress *curves* (**a**)] of platelet MAO-A activity of young (4 months) and aged (18 and 24 months) rats with variable concentration (0.05–0.4 mM) of the serotonin

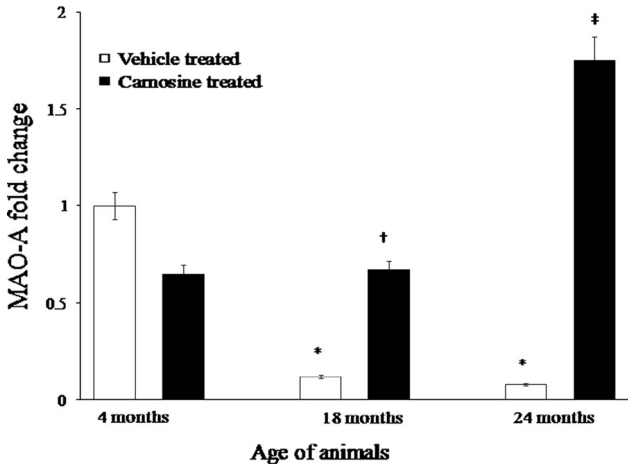

Fig. 2 Effect of carnosine on blood platelet MAO-A mRNA expression during aging in male albino rats. Results are expressed as mean ± SEM of 4–6 separate observations. Each observation was made from a single rat. No significant difference was observed between the results of with and without vehicle treated group of rats. Other details are same as described in "Materials and Methods". The fold changes are significantly different from corresponding vehicle treated (i) young (4 months) rats $^{*}p < 0.05$; (ii) age-matched 18 months; $^{\dagger}p < 0.01$ and 24 months; $^{\ddagger}p < 0.001$ aged rats

consecutive days) in 4 months young rats though apparently decreased the MAO-A mRNA expression in cerebral cortex, hypothalamus and pons-medulla except in hippocampus (2.77 fold, $p < 0.05$), it (carnosine) significantly attenuated the aging-induced increased MAO-A mRNA expression irrespective of the brain regions of 18 months [cerebral cortex (5.17 fold, $p < 0.05$), hippocampus (2.07 fold, $p < 0.05$), hypothalamus (11.63 fold, $p < 0.05$) and pons-medulla (2.62 fold, $p < 0.05$)] and 24 months [cerebral cortex (2.22 fold, $p < 0.05$), hypothalamus (6.31 fold, $p < 0.05$) and pons-medulla (2.23 fold, $p < 0.05$) except hippocampus (1.59 fold, $p > 0.05$)] aged rats in comparison to their corresponding results of vehicle treated age-matched control (18 and 24 months) rats.

Discussion

The present study for the first time has examined the aging-induced tissue specific (blood platelet and brain regional) gene expression of MAO-A and the role of carnosine on it, using RT-PCR analysis. In addition, the present investigation also first time deals with the effect of carnosine on aging-induced blood platelet MAO-A activity in terms of platelet count and its kinetic parameters. The present study has shown that the blood platelet mitochondrial MAO-A activity ($\Delta OD/10^8$ Platelets/h) during aging (18 and 24 months) is significantly decreased (Table 3). Similar trend of observations on platelet MAO-A activity (ΔOD/mg protein/h) were also previously observed by Banerjee and Poddar (2013). In this context it may be mentioned that the changes in enzyme activity expressed in $\Delta OD/10^8$ Platelets/h has been found to be greater (Table 3) than that observed previously in the MAO-A activity expressed in ΔOD/mg protein/h (Banerjee and Poddar 2013). In fact, the

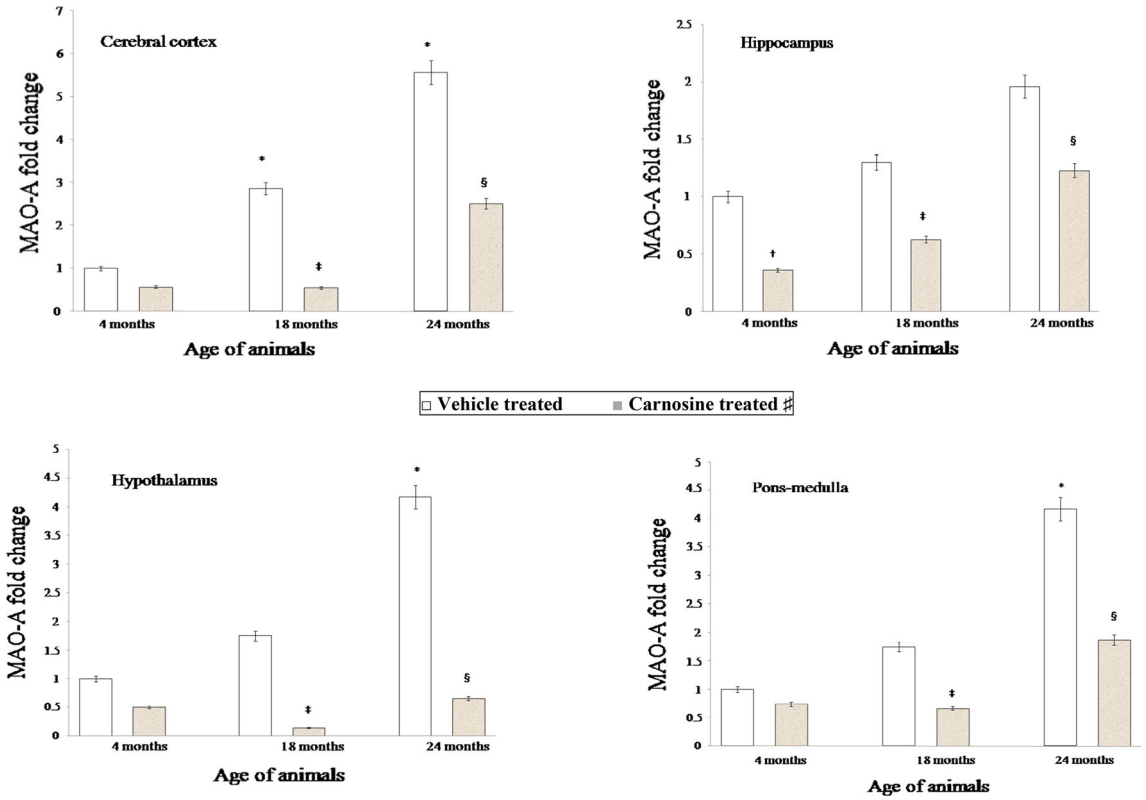

Fig. 3 Effect of carnosine on the brain regional MAO-A mRNA expression during aging in male albino rats. Results are expressed as mean ± SEM of 4–6 separate observations. No significant difference was observed between the results of with and without vehicle treated group of rats. Other details are same as described in "Materials and Methods". The fold changes are significantly different from corresponding vehicle treated (i) young 4 months rats $^{*}p < 0.05$, (ii) age-matched (a) 4 months $^{\dagger}p < 0.05$, (b) 18 months $^{\ddagger}p < 0.05$ and (c) 24 months $^{\S}p < 0.05$ group of rats

unit of expression of MAO-A enzyme activity per platelet count ($\Delta OD/10^8$ Platelets/h) seems to be more accurate than the unit of expression of MAO-A activity per mg protein (ΔOD/mg protein/h) (Banerjee and Poddar 2013). This inhibition of platelet mitochondrial MAO-A activity increases its (platelet) serotonin content (as serotonin is a substrate of MAO-A) which in turn stimulates the megakaryocytopoesis (Meszaros et al. 1998) and hence may enhance the blood platelet counts (Table 2) during aging. As the blood platelet count is increased and its mitochondrial MAO-A activity is reduced during aging, it is not unreasonable to assume that during megakaryocytopoesis, an elevation in platelet count may occur in which all the platelet (including their mitochondria) may not be healthy and functional like normal platelets of young age, though further studies are needed. Further, it may be mentioned that this aging-induced inhibition in platelet mitochondrial MAO-A may be possible due to the erroneous biosynthesis of its mitochondria (Chun et al. 2008) and the reduction of its membrane fluidity by the cascade effect of oxidative stress (Hossain et al. 1999) during aging. It has been also found from the present kinetic data that the aging-induced reduction of the blood platelet mitochondrial MAO-A activity may be due to a significant reduction of their respective V_{max} (Fig. 1; Table 4) only. This significant reduction in V_{max} of MAO-A suggest that there may be a quantitative and/or qualitative alteration(s) in mitochondrial MAO-A activity during aging (Gerhard 1974). These led authors to assume that there may be a change in MAO-A mRNA expression in the aging-induced reduction of blood platelet mitochondrial MAO-A activity at the level of its V_{max} (Fig. 1; Table 4). The present study reveals that in the blood platelet mitochondria has a direct correlation between its enzyme, MAO-A activity (Fig. 1; Tables 3, 4) and their corresponding mRNA expression (Fig. 2) during aging. It has also been revealed that the decrease in blood platelet mitochondrial MAO-A mRNA expression (Fig. 2) during aging may be a cause of aging-induced inhibition of blood platelet mitochondrial MAO-A V_{max} (Fig. 1; Table 4) and hence its activity (Table 3). It is well known that unlike brain mitochondrial MAO-A, enucleated blood platelet mitochondrial MAO-A is not influenced by glucocorticoid (Banerjee and Poddar 2015). This led to suggest that in

enucleated blood platelet, glucocorticoid acts via non-genomic regulation (Ian 2000) possibly by aging-induced increase of ROS mediated reduction in membrane fluidity (Hossain et al. 1999) and erroneous mitochondrial biogenesis (Chun et al. 2008), though it requires further clarification. Ian (2000) has also shown that glucocorticoid acts on nucleated brain tissue through the transcriptional and translational regulation. In this context, it may be mentioned that Zeirmans et al. (2012) have shown that the MAO-A gene is located on X chromosome (Xp11.23). This heterogenic action of glucocorticoid depending on nucleated and enucleated tissues may arise the concept of mitochondrial tissue specificity and suggest a correlation of the aging-induced reduction in blood platelet mitochondrial MAO-A activity (Table 3) with an ROS mediated increase of its mitochondrial membrane steric hindrance or reduction in its membrane fluidity (Banerji et al. 1977; Hossain et al. 1999) which may further strengthen the reduction in platelet mitochondrial MAO-A mRNA expression (Fig. 2).

Unlike blood platelet, the MAO-A mRNA expression in different brain regions (cerebral cortex, hippocampus, hypothalamus and pons-medulla) during aging is enhanced (Fig. 3). This may be due to the aging-induced elevation of glucocorticoid level by enhancing the transcriptional factor for MAO-A (Ou et al. 2006). This may led to increase brain region specific mitochondrial MAO-A mRNA expression with aging (Fig. 3) and hence increases the brain regional MAO-A activity quantitatively (Banerjee and Poddar 2015). This may led to suggest a direct correlation between the MAO-A mRNA expression and its enzyme (MAO-A) activity as observed in blood platelet (Figs. 1 and 2; Tables 3 and 4). The present study has also revealed that both the hypothalamus and pons-medulla have shown a large amount of mRNA expression followed by cerebral cortex and hippocampus (Fig. 3). Even though, the critical assessment of our present findings may be scrutinized by a comparative analysis between the brain regional MAO-A activity and their corresponding mRNA expression studied (Fig. 3) in aged (24 months) rats. It may be also stated that the aged brain regions exhibit different MAO-A mRNA expression patterns which is not consistently followed the order of the activity of MAO-A (hippocampus > cerebral cortex > hypothalamus > pons-medulla) and its mRNA expression levels (hypothalamus > pons-medulla > cerebral cortex > hippocampus). The region basis order of change of the MAO-A mRNA expression (Fig. 3) and their corresponding enzyme activity (as observed in previous study by Banerjee and Poddar (2015) reveal that among the brain regions studied in aged (24 months) rats the enzyme activity in hypothalamus and pons-medulla has been found to be lesser and their corresponding mRNA expression are higher. This may be explained by the fact that there may be a decrease in nucleated intracellular translational efficiency (Lambertucci et al. 2007) during aging. On the other hand, in other two brain regions (hippocampus and cerebral cortex) an increase in intracellular translational efficiency of the MAO-A mRNA may be suggested as the MAO-A activity was higher in these two (hippocampus and cerebral cortex) brain regions (Banerjee and Poddar 2015) in comparison to the hypothalamic and pons-medullary brain regions, in spite of their less amount of mRNA expression (Fig. 3) in aged rats (24 months), though further studies are yet to confirm. Further the results of the present study suggest that the aging-induced tissue specific changes in mitochondrial MAO-A enzymes, including its activity and mRNA expression may be consider as an index of aging-induced change in mitochondrial structure and function (Seo et al. 2010). The present study also suggests that the aging-induced change in blood platelet counts and in MAO-A activity may be one of the haematological biomarker.

Carnosine (2.0 μg/kg/day, i.t. for 21 consecutive days) attenuates the aging-induced bi-directional change either decrease (Fig. 2) or increase (Fig. 3) depending on the type of tissue either blood platelets or brain regional MAO-A mRNA expression respectively, like the attenuation that have been observed in their corresponding tissue specific MAO-A activity by attenuating the aging-induced tissue specific decrease or increase in their corresponding V_{max} (Fig. 1, Table 4) (Banerjee and Poddar 2015). The gene regulatory property of carnosine (Quinn et al. 1992) may also be suggested in this attenuating effect of carnosine in aging-induced tissue specific increase or decrease in mRNA expression. The results (Fig. 3), in the present study, also suggest that the carnosine (2.0 μg/kg/day, i.t., for 21 consecutive days) during aging may attenuate the aging-induced brain region specific alteration in translational efficiency of MAO-A mRNA (Lambertucci et al. 2007), though it needs further confirmation. In the present results the greater potency in attenuating effect of carnosine on aging-induced decrease in platelet MAO-A activity (Table 3) of 24 months aged rats than that in the 18 months aged rats may be possible due to the down regulation in physiological condition developed during aging as shown by Ahmet et al. (2010), Rani and Panneerselvam (2002). Thus, the physiological as well as biochemical parameters including MAO-A activity of the present age groups of aged rats (18 and 24 months) seems to be gradually more susceptible to the stressful condition in 24 months aged rats than that in 18 months aged rats. In these circumstances, carnosine with this particular dosage and duration may affect greatly the age group which is more susceptible to aging i.e. 24 months aged group than 18 months and attenuates and restores accordingly the aging-induced change in MAO-A enzyme activity towards the activity that has been observed in 4 months young rats.

Further, it has been observed that the tissue specific carnosine-induced attenuation of aging-induced MAO-A activity or its mRNA expression is not significantly altered in comparison to the corresponding results of 4 months young rats (Figs. 2 and 3; Table 3) which may be also strengthened by several evidences with the carnosine and other antioxidants (Ahmet et al. 2010; Rani and Panneerselvam 2002). In this context it may be also mentioned that the carnosine plays a protecting role in attenuating the ROS mediated increase in lipid peroxidation (Hossain et al. 1999), stress-induced elevation in glucocorticoid as well as superoxide level (Ian 2000). Thus, it is not unlikely to assume that carnosine like other antioxidant (Rani and Panneerselvam 2002) may attenuate the aging-induced decrease of membrane fluidity (Rani and Panneerselvam 2002) by reducing oxidative stress (Bellia et al. 2011) and hence may play a tissue specific bi-directional attenuating effect of carnosine on aging-induced decrease in blood platelet (Fig. 2) and increase in brain regional MAO-A mRNA expression (Fig. 3). In addition, carnosine also attenuates (i) the reduction of MAO-A mRNA expression in nucleated progenitor precursor cells (as MAO-A is a X-linked enzyme) in enucleated blood platelet as well as (ii) glucocorticoid-induced induction of MAO-A mRNA expression in nucleated brain regions. The attenuating effect of carnosine on aging-induced decrease in blood platelet mitochondrial MAO-A activity may suggest an attenuation in aging-induced increase of serotonin content in the circulation including platelet. This may lead to suggests that there may be attenuation in the aging-induced increase of megakaryocytopoesis and hence restores the platelet counts (Table 2) towards the counts that are observed in the young rats.

Conclusion

Finally, in agreement with the previous results and the present study, it may be concluded that (1) carnosine attenuates and restores the aging-induced (a) reduction in blood platelets and (b) increase in the brain regional mitochondrial MAO-A activity and its mRNA expression including blood platelets counts characteristically with a positive correlation in their corresponding changes in MAO-A activity towards the results that observed in 4 months young rats; (2) aging-induced changes in blood platelet counts and its MAO-A activity may be considered as one of the haematological biomarker for elder population.

Acknowledgments The authors are thankful to Prof. S. Sengupta, Department of Statistics, University of Calcutta, for helping the statistical analyses of the data. The present investigation was supported by a grant from Indian Council of Medical Research (ICMR), New Delhi, India and University of Calcutta, Kolkata, India. We are also thankful to my colleagues Mrs. Shubhadeepa Majumder and Mrs. Sanmitra Basu, Department of Biochemistry, Mr. Amartya Roy, Department of Physiology, University of Calcutta, for their immense help and support.

Complicance with Ethical Standards

Conflicts of interest Soumyabrata Banerjee and Mrinal K. Poddar declare that they have no conflict of interest.

References

Ahmet FA, Zeynep KK, Semra DA, Uysal M (2010) Effect of carnosine treatment on oxidative stress in serum, apoB-containing lipoproteins fraction and erythrocytes of aged rats. Pharmacol Rep 62:733–739

Banerjee S, Poddar MK (2013) Platelet monoamine oxidase-A activity and aging: effect of carnosine. J Physiol Sci 63:279–285

Banerjee S, Poddar MK (2015) Carnosine: effect on aging-induced increase in brain regional maonoamine oxidase-A activity. Neurosci Res 92:62–70

Banerji A, Poddar MK, Ghosh JJ (1977) Action of Δ^9-tetrahydrocannabinol on membrane-bound monoamine oxidase activity. Toxicol Appl Pharmacol 40:347–354

Bellia F, Vecchio G, Cuzzocrea S, Calabrese V, Rizzarelli E (2011) Neuroprotective features of carnosine in oxidative driven diseases. Mol Aspects Med 32:258–266

Boldyrev AA, Aldini G, Derave W (2013) Physiology and pathophysiology of carnosine. Physiol Rev 93:1803–1809

Bond BA, Cundall RL (1977) Properties of monoamine oxidase (MAO) in human platelets, plasma, lymphocytes and granulocytes. Clin Chem Acta 80:317–326

Bortolato M, Shih JC (2011) Behavioral outcomes of monoamine oxidase deficiency: preclinical and clinical evidence. Int Rev Neurobiol 100:13–42

Chun S, Kaihua G, David TY, Zhibin Y, Lucy FE, Huaqiao W, Xu J (2008) Effects of aging and Alzheimer's disease on mitochondrial function of human platelets. Exp Gerentol 43:589–594

Del VM, Maj M, D'Ambrosio A, Kemali D (1983) Low platelet MAO activity in chronic schizophrenics: a long-term effect of neuroleptic treatment? Psychopharmacol (Berl) 79:177–179

Gerhard M (1974) Determination of Michaelis constants and inhibitor constants. In: Bergmeyer HU (ed) Methods of enzymatic analysis, 2nd edn. Academic Press Inc, New York, pp 146–151

Gokhan SH, Xandra OB (1991) Human monoamine oxidase A gene determines levels of enzyme activity. Am J Hum Genet 49:383–392

Hossain MS, Hashimoto M, Gamoh S (1999) Association of age-related decrease in platelet membrane fluidity and platelet lipid peroxidation. Life Sci 64:135–143

Hummon AB, Lim SR, Difilippantonio MJ, Ried T (2007) Isolation and solubilization of proteins after TRIzol® extraction of RNA and DNA from patient material following prolonged storage. BioTechn 42:467–472

Ian MA (2000) Review on Molecular mechanisms of glucocorticoid actions. Pulmon Pharmacol Therap 13:115–126

Johnston JP (1968) Some observations upon a new inhibitor of monoamine oxidase in brain tissue. Biochem Pharmacol 17:1285–1297

Kenneth JL (2001) Thomas DS. Analysis of relative gene expression data using real time quantitative PCR and the $2^{-\Delta\Delta C}$T method. Methods 25:402–408

Kohen R, Yamamoto Y, Cundy KC, Ames BN (1988) Antioxidant activity of carnosine, homocarnosine and anserine present in muscle and brain. Proc Natl Acad Sci (USA) 85:3175–3179

Lambertucci RH, Levada-Pires AC, Rossoni LV, Curi R, Pithon-Curi TC (2007) Effects of aerobic exercise training on antioxidant enzyme activities and mRNA levels in soleus muscle from young and aged rats. Mech Ageing Dev 128:267–275

Meszaros Z, Borcsiozky D, Mate M, Tarcali J, Szombathy T, Mekes K, Magyar K (1998) Platelet MAO-B activity and serotonin content in patients with dementia: effect of age, medication and disease. Neurochem Res 23:863–868

Mukherjee KL (1990) Hematology in Medical laboratory technology, vol 1. Tata McGraw-Hill publishing Co Ltd, Noida, pp 228–324

Ou XM, Chen K, Shih JC (2006) Glucocorticoid and androgen activation of monoamine oxidase A is regulated differently by R1 and Sp1. J Biol Chem 281:21512–21525

Poddar MK, Dewey WL (1980) Effect of canabinoids on catecholamine uptake and release in hypothalamus and striatal synaptosomes. J Pharmacol Exp Ther 214:63–67

Quinn PJ, Boldyrev AA, Formazuyk VE (1992) Carnosine, its properties, functions and potential therapeutic applications. Mol Aspects Med 13:379–444

Rani AJA, Panneerselvam C (2002) Effect of L-carnitine on brain lipid peroxidation and antioxidant enzymes in old rats. J Gerentol Biol Sci 57A:B134–B137

Seo AY, Joseph AM, Dutta D, Hwang JCY, Aris JP, Leeuwenburgh C (2010) New insights into the role of mitochondria in aging: mitochondrial dynamics and more. J Cell Sci 123:2533–2542

Shih JC (2004) Cloning, after cloning, knock-out mice and physiological functions of MAO A and B. Neurotoxicol 25:21–30

Tipton KF, Boyce S, O'Sullivan J, Davey GP, Healy J (2004) Monoamine oxidases: certainties and uncertainties. Curr Med Chem 11:1965–1982

Yuneva M, Bulygina E, Gallant S, Kramarenko G, Stvolinsky S, Semyonova M, Boldyrev A (1999) Effect of carnosine on age-induced changes in senescence-accelerated mice. J Anti-aging Med 2:337–342

Zeirmans T, Dumontheil I, Roggeman C, Peyrard-Janvid M, Matsson H, Kere J, Klingberg T (2012) Working memory, brain activity and capacity link MAO-A polymorphism to aggressive behavior during development. Transl Psychi 2:E85. doi:10.1038/TP.2012.7