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RESEARCH ARTICLE

Dietary restriction regulates brain acetylcholinesterase in female mice as a function of age

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Abstract In the present study, the normal endogenous activity level of acetylcholinesterase (AChE) was investigated in cerebral hemispheres and cerebellum of female mice as a function of age. The effects of 24-h fasting and refeeding, and dietary restriction (DR) on AChE activity and its protein expression patterns were also investigated in young (1-month) and old (18-month) mice. Our results show that the activity (U/mg protein) and level of AChE protein in the cerebral hemispheres of young mice is decreased significantly on 24-h fasting which reverses back on refeeding. On the other hand, DR produces an accumulative effect; thereby it decreases the activity of this enzyme in the cerebral hemispheres of both the young and old mice and the degree of reduction is of different magnitude, dictated in an age- and brain region-specific manner. Our findings suggest that DR regulates the activity of this enzyme which may be useful in related neurodegenerative disease conditions.

Keywords Aging · Acetylcholinesterase · Dietary restriction · Cerebral hemispheres · Cerebellum

Introduction

Neuronal survival, spine density, and the establishment of neuronal circuitry are influenced by experience and environment during nervous system development (Comery et al. 1996). Aging cells in the brain experience increased amounts of oxidative stress (Serrano and Klann 2004; Dkhar and Sharma 2010), perturbed energy homeostasis (Ames 2004), accumulation of damaged proteins (Gray et al. 2003), and lesions in their DNA (Lu et al. 2004). At the cellular level, aging reduces the synaptic contacts, synaptic strength, and plasticity (Burke and Barnes 2006) resulting in diminished brain neurogenesis (Heine et al. 2004). A serious loss of cholinergic function in the central nervous system contributes to cognitive symptoms during aging (Bartus et al. 1982; Petrella et al. 2005). Similarly, changes in multiple aspects of neurotransmitter signalling, for example, declines in the levels of neurotransmitters such as acetylcholine are well documented in the aging brain (Terry and Buccafusco 2003).

Acetylcholinesterase (AChE; 3.1.1.7) hydrolyzes the neurotransmitter acetylcholine (ACh) at the synaptic cleft of cholinergic synapses and neuromuscular junctions (Soreq and Seidman 2001). By degrading ACh in cholinergic synapse, AChE plays a critical role in controlling the effects of ACh, so that the nerve impulse has required duration before the start of the next impulse. In mammals, AChE is

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expressed in a variety of tissues including muscle, nerve, white and grey matter of brain, in amniotic fluid, placenta and hematopoietic cells. In addition to its role in cholinergic transmission, AChE is associated with brain development, learning, memory and neuronal damage (Ballard et al. 2005; Zimmerman and Soreq 2006). It has been used as a marker for cholinergic function in neural tissue because of its implications in synaptogenesis and its involvement in neurodegeneration in adult tissues. Serum AChE evaluation and the checking of different isoforms present in different tissues is being used as an effective marker in detecting several diseases (Voigtländer et al. 1981; Navaratnam et al. 1991).

Dietary restriction (DR), i.e., a reduction in calorie intake without malnutrition, has been known to influence wide variety of physiological processes including neuroendocrinological signalling system (Weindruch 1996; Pahlavani 2000). It changes protein turnover (Scrofano et al. 1998), modifies the expression of many genes (Lee et al. 1999), and decreases glycation of cellular and extracellular macromolecules (Sell et al. 1996). Dietary restriction has been shown to improve synaptic functions such as long-term potentiation (Eckles-Smith et al. 2000) and learning ability (Algeri et al. 1991) as well as to elongate life span (Masaro 2000). Rats maintained on DR perform better on learning and memory tasks than do ad libitum-fed, age-matched rats (Stewart et al. 1989; Pitsikas and Algeri 1992). Importantly, DR induces the expression of neurotrophic factors as well as the expression of specific genes that encode proteins that promote neuronal survival and synaptic plasticity (Yu and Mattson 1999).

As the role of AChE is very crucial in aging and in normal cholinergic systems, in the present study we determined the normal endogenous level of AChE activity in the cerebral hemispheres and cerebellum of mice as a function of age. We assessed the influence of dietary interventions on AChE through the possible modulation by 24 h of fasting and refeeding and also by an alternate day of feeding and non-feeding for 3-months to provide an insight in understanding the involvement of DR as natural intervention in brain aging, with a scope in the improvement and treatment of various related neurodegenerative conditions without using exogenous drugs.

Materials and methods

Animals and diet

Female Swiss albino (Balb/C strain) mice were subjects of this study. They were maintained under normal laboratory conditions (25 ± 2°C; 12 h light/dark cycle). The animals were caged in polycarbonate cage and fed with a standard pellet diet and water as per experimental schedule. For determination of the normal endogenous activity of AChE as a function of age, three different age groups (1-, 6-, and 18- month) were chosen. For study of the possible effects of 24 h fasting, refeeding and DR on AChE, mice of two different age- groups i.e., 1- and 18- month were used. All animals were treated humanely and Institutional Ethics Committee (IEC) guidelines on use of animals were followed during experimentation.

Fasting regimen

Young (1-) and old (18-month) mice under study were divided into three different groups separately. Group I includes the ad libitum fed control group that have access to continuous supply of food. Group II animals were subjected to a defined period of fasting (24 h-Fasted), while group III animals were subjected to 24 h of fasting followed by 24 h of refeeding (24 h-Refed). However, water was supplied regularly to all the above three groups.

Dietary restriction regimen

Young (1-) and old (18-month) mice under study were divided into three different groups separately. Group I includes the ad libitum fed control group that have access to continuous supply of food. Mice subjected to DR regimen were fed on alternate days for a period of 3 months (Merry 1999; Goyary and Sharma 2005). Mice maintained on such regimen are known to consume 30% less food over a period of time and live up to 30% longer, compared to ad libitum animals (Lee et al. 2000). Based on the day of sacrificing the animals, group II consists of animals that were sacrificed at the end of a non-feeding day (DR-Fasted), while the animals that were sacrificed at the end of the feeding day (DR-Fed) constitute the group III. Water was supplied regularly to all the above three groups.

Chemicals

A rabbit anti-AChE polyclonal antibody was purchased from Santa Cruz Biotechnology, Inc., USA. Goat-antirabbit-IgG-HRP conjugate was obtained from Bangalore Genei, India. Acetylthiocholineiodide and 5, 5′ -Dithiobis (2-nitro-benzoic acid) were purchased from Sigma Chemical Co., USA. All other chemicals used were of analytical grade. Millipore distilled water was used in the preparation of buffers.

Buffers

Following buffers were used: (A) 0.32 M sucrose; (B) 100 mM phosphate buffer, pH 8.0; (C) Tris buffer saline (TBS; 20 mM Tris–HCl, pH 7.5/50 mM NaCl); (D) Tween Tris buffer saline (TTBS; 20 mM Tris–HCl, pH 7.5/50 mM NaCl/0.5% Tween 20); (E) Towbin buffer (25 mM Tris, 192 mM glycine, 20% w/v methanol, pH 8.3); (F) Phosphate buffer saline (PBS; 0.14 M NaCl/3 mM KCl/10 mM Na_2HPO_4/2 mM KH_2PO_4, pH 7.3).

Tissue preparation

Mice were sacrificed by cervical dislocation at a fixed time of the day (13:00 h) and their cerebral hemispheres and cerebellum were excised out immediately, washed in chilled physiological saline and blotted dry. A 10% (w/v) homogenate of these tissues was prepared in ice-cold 0.32 M sucrose solution. The homogenate was centrifuged at 20,000×*g* for 30 min at 4°C. The supernatant thus obtained were used for the assay of AChE activity and protein estimation.

AChE activity assay

AChE activity was determined by method of Ellman et al. (1961) with some modifications of our own. Hydrolysis rate was measured at acetylthiocholine (S) concentrations of 0.8 mM with 100 mM phosphate buffer, pH 8 and 1.0 mM 5,5′ -Dithiobis (2-nitro-benzoic acid) (DTNB). Fifty microliters of enzyme preparation was added to the reaction mixture making a 3 ml assay mixture. The hydrolysis was monitored by formation of the thiolate dianion of DTNB at 412 nm for 3 min (intervals of 30 s) at 25°C.

Western blot analysis

An aliquot of sample proteins (30 µl) containing 60 µg protein was loaded in each lane. The sample proteins were separated by electrophoresis performed on 10% sodium dodecyl sulphate–polyacrylamide gels (SDS–PAGE) at room temperature. Following electrophoresis, the proteins in the gel were transferred to a nitrocellulose membrane using Bio-Rad Mini trans-blot® electrophoretic unit at 100 V (constant) for 60 min. The electroblotted membrane was transferred in blocking solution containing 5% nonfat dry milk in Tris buffer saline (TBS) for 1 h at room temperature. After washing in Tween Tris buffer saline (TTBS), the membrane was incubated overnight with anti-AChE antibody solution (1:500). The membrane was washed twice in TTBS to remove unbound antibodies and transferred to a solution containing Goat-antirabbit-IgG conjugated to horseradish peroxidase (1:1000) for 3 h, and the color developer substrate (TMB/H_2O_2) was added till the development of color. The reaction was stopped by washing the membrane in ddH_2O and photographed using an hp scanjet 7400C scanner.

India ink stain

To ascertain that an equal amount of protein was loaded in each lane, a parallel set was run and stained overnight with 0.2% India ink in Phosphate buffer saline (PBS) solution containing 0.3% Tween-20. Destaining was performed with PBS solution, and the blot was photographed using an hp scanjet 7400C scanner.

Protein determination

Protein content of the enzyme preparation was measured according to the dye-binding method of Bradford (1976) using bovine serum albumin (BSA) as standard.

Statistical analysis

All values reported are mean ± SD for five mice in each group. Data obtained from different sets were analyzed using Student's *t* test for paired data. The level of significance ($P < 0.05$) was considered to be statistically significant.

Results

Normal endogenous level of AChE activity as a function of age

Among the regions of the brain studied, the normal endogenous level of AChE activity (U/mg protein) was found to be the highest in the cerebral hemispheres of 1-month old mice and declined significantly in 18-month old mice. The percentage of decrease was 45% ($P < 0.001$) as compared to the activity in the 1-month old mice (Fig. 1a)). In the cerebellum, AChE activity remained fairly constant and no significant changes was observed in all the three different ages studied. Immunoblots analyses of cerebral hemispheres proteins of the selected ages confirmed that the decline was at the level of AChE protein (Fig. 1b (a)). India ink stain indicated that an equal amount of protein was loaded in each lane (Fig. 1b (b)) during experimentation.

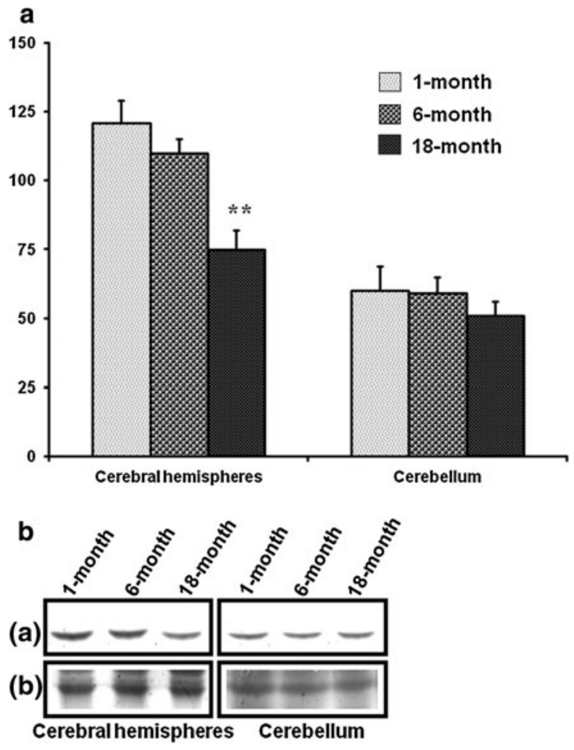

Fig. 1 **a** Normal endogenous level of acetylcholinesterase activity in the cerebral hemispheres and cerebellum of young (1-month), adult (6-month) and old (18-month) mice. Values are expressed as mean ± SD of five mice at each group. *Asterisks* (**) indicate statistically significant ($P < 0.001$) values as compared to young mice. **b** Western blot analysis of AChE protein from the cerebral hemispheres and cerebellum of young (1-month), adult (6-month) and old (18-month) mice (*a*). The India ink stained Western blots of proteins from cerebral hemispheres and cerebellum of the same ages (*b*)

Effect of 24 h fasting and refeeding on AChE activity

After 24 h of fasting, the level of AChE activity was significantly decreased in the cerebral hemispheres of 1-month-old mice in comparison with the control group. The percentage of decrease was 30% ($P < 0.001$). However, no significant changes observed in the cerebral hemispheres of 18-month-old mice. Refeeding the mice for 24 h after fasting reversed the above decrease and no significant change was observed in comparison with the age-matched control mice (Fig. 2a). Western blot analysis corroborated the decrease in AChE activity at the protein expression level (Fig. 2b (a)). India ink stain indicated that an equal amount of protein was loaded in each lane (Fig. 2b (b)) during experimentation. In the cerebellum of both 1- and 18-month old mice, no significant changes are observed in comparison with their age-matched control mice (Fig. 3a). Immunoblot analyses of cerebellum AChE confirmed that there are indeed no changes at the level of AChE protein (Fig. 3b (a)) India ink stain indicated that an equal amount of protein was loaded in each lane (Fig. 3b (b)) during experimentation.

Effect of DR on body weight

In our experimental schedule, it has been observed that the total body weight (g) of mice during alternate days of feeding and non-feeding for 3 months exhibited a significantly decrease in both the 1- and 18 month old mice. The percentage of decrease is 30% ($P < 0.05$) in the case of 1-month and 16% in the case of 18-month old mice, as compared to the weight of the age-matched ad libitum fed control mice (Fig. 4).

Effect of DR on AChE activity

Our results indicate that the level of AChE activity was significantly decreased in cerebral hemispheres of both 1- and 18-month-old mice. In 1-month-old mice, the percentage of decrease was

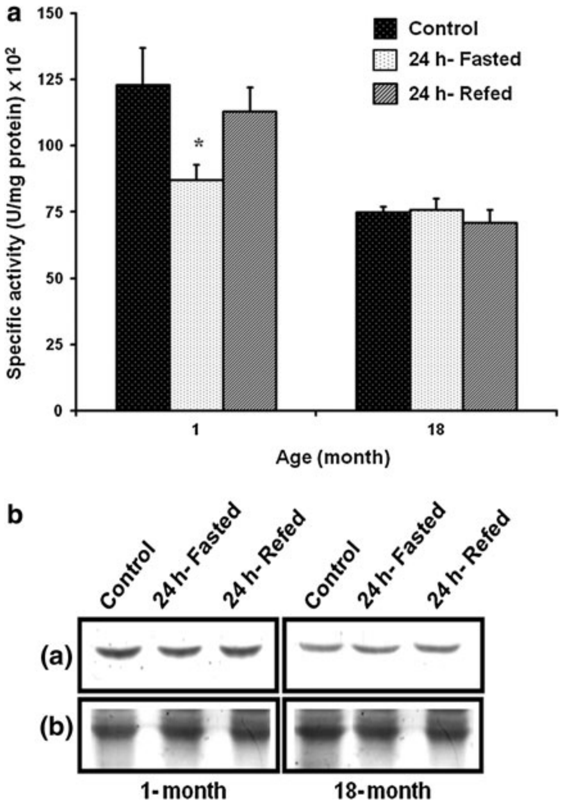

Fig. 2 **a** Effects of 24 h fasting (24 h-Fasted) and refeeding (24 h-Refed) on the activity of AChE in the cerebral hemispheres of young (1-) and old (18-month) mice. *Asterisks* (*) indicates statistically significant ($P < 0.05$) value as compared to their respective age-matched control mice. **b** Western blot analysis of AChE protein from the cerebral hemispheres of young (1-) and old (18-month) mice during 24 h fasting (24 h-Fasted) and refeeding (24 h-Refed) with their respective age matched control mice (*a*). The India ink stained Western blots of proteins from cerebral hemispheres of young (1-) and old (18-month) mice after 24 h fasting (24 h-Fasted) and refeeding (24 h-Refed) with their respective age-matched control (*b*)

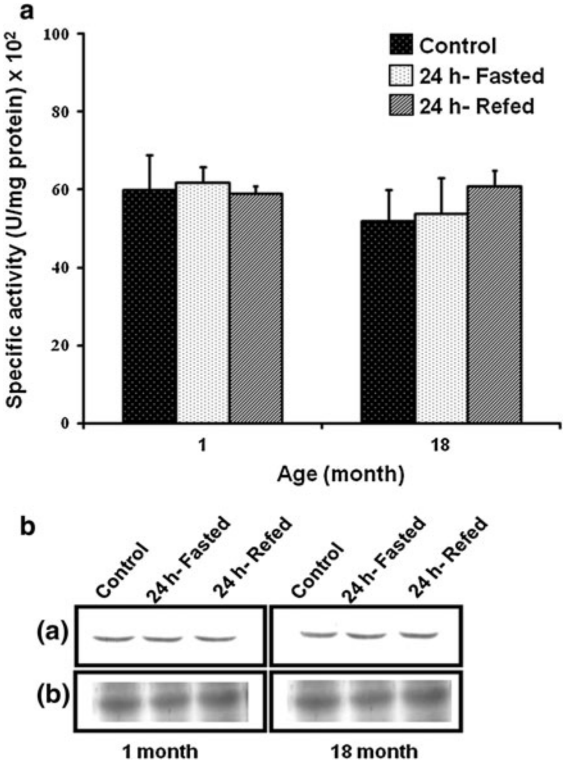

Fig. 3 **a** Effects of 24 h fasting (24 h-Fasted) and refeeding (24 h-Refed) on the activity of AChE in the cerebellum of young (1-) and old (18-month) mice. No significant changes were observed as compared with their respective age-matched control. **b** Western blot analysis of AChE protein from the cerebellum of young (1-) and old (18-month) mice during 24 h fasting (24 h-Fasted) and refeeding (24 h-Refed) with their respective age matched controls (*a*). The India ink stained Western blots of proteins from cerebellum of young (1-) and old (18-month) mice after 24 h fasting (24 h-Fasted) and refeeding (24 h-Refed) with their respective age-matched control mice (*b*)

50% ($P < 0.001$) on the non-feeding day and 40% ($P < 0.001$) on the feeding day, respectively. In 18-month-old mice, the level of AChE exhibited a significant decrease of 15% ($P < 0.05$) and 12% ($P < 0.05$) on the non-feeding and feeding day, respectively (Fig. 5a). Western blot analysis supported the above observation that the decrease in AChE activity by DR was at the protein level (Fig. 5b (a)). India ink staining suggested that an equal amount of protein was loaded in each lane (Fig. 5b (b)). However, no significant changes were observed in the activity of AChE in the cerebellum of both 1- and 18-month-old mice in comparison to their age-matched control mice (Fig. 6a). Western blot analysis supported the above observation that there are no changes at the protein level (Fig. 6b (a)). India ink staining suggested that an equal amount of protein was loaded in each lane (Fig. 6b (b)).

Discussion

Our study shows that endogenous activity of AChE is highest in the cerebral hemispheres of young mice with a significant decline in old mice. However, in

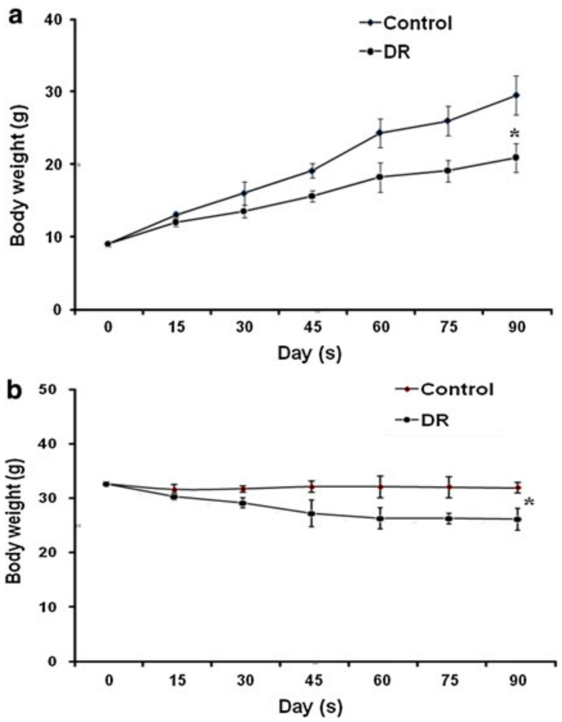

Fig. 4 Changes in the body weight (g) of young (1-month) (**a**) and old (18-month) (**b**) mice during the period of alternate day of feeding for 3-months. *Asterisks* (*) indicates statistically significant ($P < 0.05$) value as compared to their respective ad libitum fed control mice

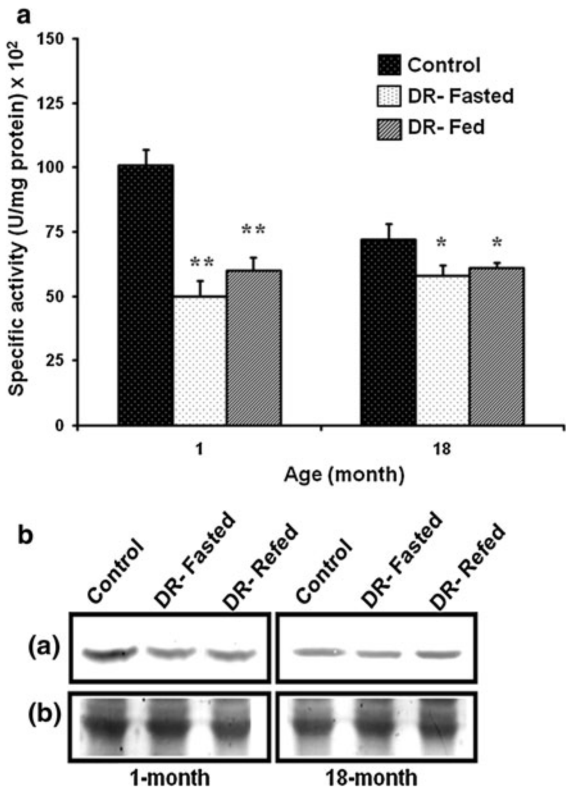

Fig. 5 **a** Effects of dietary restriction (DR) on the activity of AChE in the cerebral hemispheres of young (1-) and old (18-month) mice on the non-feeding (DR-Fasted) and feeding (DR-Fed) day. *Asterisks* (*) and (**) indicate statistically significant at ($P < 0.05$) and ($P < 0.001$), respectively as compared to their respective age-matched control mice. **b** Western blot analysis of AChE protein from the cerebral hemispheres of dietary restricted (DR) young (1-) and old (18-month) mice with respective to their age-matched control (*a*). The India ink stained Western blots of proteins from cerebral hemispheres of the same ages (*b*)

the cerebellum there are no significant changes observed in the activity of AChE in all the ages studied. These findings agree with the earlier findings where the activity of AChE in female rats is found to be the highest in the immature (9 weeks), and decreases by 50% after 29 weeks which further decreases in old age (Moudgil and Kanungo 1973). The higher activity of AChE in young mice might reflect the involvement of AChE in neuronal differentiation or cellular maturation processes of the basal forebrain cholinergic neurons and to higher metabolism of acetylcholine associated with learning and/or exploratory behaviour (Deutsch 1971; Oliverio et al. 1973). A lower level of AChE in the old age as observed, may be attributed to the gradual loss of neurons (Johnson and Erner 1972) and/or decrease in the rate of AChE protein synthesis (Murthy 1966) after the growth period is completed.

In our experimental schedule, it has been observed that long-term DR significantly reduces the body weight of both the young and old mice. However, the magnitude of this reduction is more pronounced in young mice as compared to the older ones. Our observation of body weight reduction confirms that the animals have a reduced food intake which is also consistent with earlier reports (Lee et al. 2000; Dutta and Sharma 2004). A decrease in the activity of AChE in the cerebral hemispheres of 1-month-old mice on 24 h fasting and its restoration after 24 h refeeding suggest that dietary interventions play a role in regulating the activity of this enzyme during maturation process. Similarly, in comparison to the cerebral hemispheres of old mice and the cerebellum of young and old mice where no significant changes were observed indicates the level of high synaptic plasticity of neurons present in cerebral hemispheres of young mice.

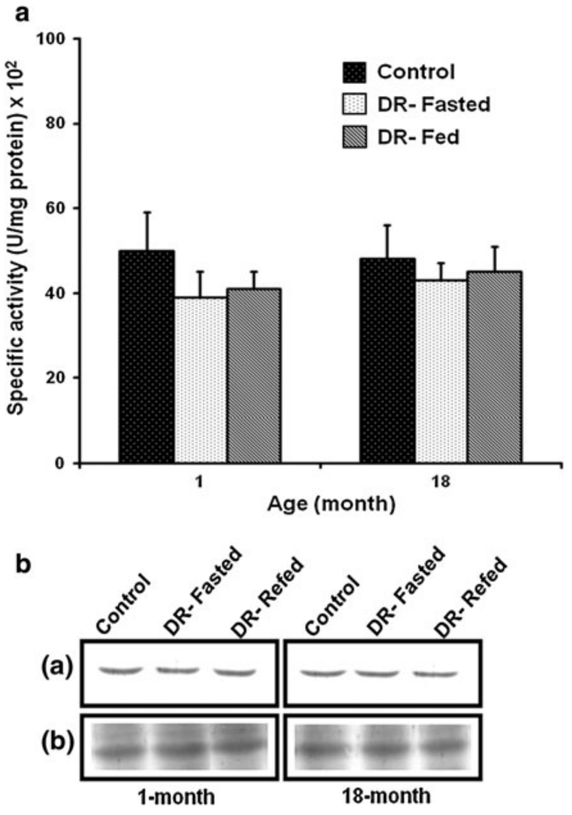

Fig. 6 **a** Effects of dietary restriction (DR) on the activity of AChE in the cerebellum of young (1-) and old (18-month) mice on the non-feeding (DR-Fasted) and feeding (DR-Fed) day. No statistically significant changes were observed as compared to their respective age-matched control mice. **b** Western blot analysis of AChE protein from the cerebellum of dietary restricted (DR) young (1-) and old (18-month) mice with respect to their age-matched control mice (*a*). The India ink stained Western blots of proteins from cerebellum of the same ages (*b*)

According to the hormesis mechanism, repeated mild stress has beneficial effect from the cellular responses and its aging-retardant action results from stimulating the maintenance and repair processes during repeated exposure to stress (Rattan 2004). Dietary restriction is a sustained mild stressor that enhances the ability of rats and mice to cope with acute and intense stressors (Masoro 1998). In comparison with 24 h fasting and refeeding, our studies showed that DR produces a more pronounced and cumulative effects irrespective of the day of sacrificing the mice i.e., on the day of fasting and/or on the non-feeding day. This may be possible because DR produces a hormetic response that increases cellular stress resistance, metabolic adjustments and adaptability (Mattson 2008). Previous studies indicate that there are alterations in AChE activity and ACh metabolism, where there is a higher activity of AChE in the medulla oblongata and striatum as well as lower level of ACh in all regions of the brain of Zucker fatty rat as compared to the lean rat (Seitaro et al. 1990). The decreased activity of AChE in the cerebral hemispheres of young mice during DR suggests the possibility based on the fact of ACh crucial involvement in synaptic plasticity and synaptic scaling with responsive sensitivity of young mice synapse to send a sensory input through its enhanced pre-synaptic and post-synaptic connections during DR period. This enhanced synaptic activity during DR regulates the efficiency of presynaptic ACh release and/or the numbers of postsynaptic receptors to down regulate AChE biosynthesis via a feedback loop mechanism in compensating for the decreased ACh synthesis during this time of limited substrate availability. Thus, an increase in the level of ACh would ensure increase in receptor occupancy and potentiate its action on neuronal excitability and inhibitory interneuron. This is manifested in increased amplitude of synaptic scaling and long term potentiation in many regions of the brain.

In the cerebral hemispheres of older mice where there is lesser reduction in AChE activity during DR, we suggest that DR might act as a natural cholinesterase inhibitor by maintaining the level of the already declining ACh, thus enhancing cholinergic neurotransmission in the cerebral hemispheres of the aged brain. Alternatively, DR may produce free radical scavengers which prevent the binding of free radicals on the sites of the AChE molecule, analogous to reports on inhibitors of AChE that improve brain function (Giacobini and Caudra 1994; Canal and Imbimbo 1996). Our findings thus clearly indicate regulation of AChE expression in a brain region specific manner as a function of age in mice. The dietary restriction attenuation of AChE level may be useful in deducing a beneficial effect of DR in neurodegenerative diseases as better alternatives in future.

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References

Algeri S, Biagini L, Manfridi A, Pitsikas N (1991) Age-related ability of rats kept on a life-long hypocaloric diet in a spatial memory test. Longitudinal observations. Neurobiol Aging 12:277–282

Ames BN (2004) Delaying the mitochondrial decay of aging. Ann NY Acad Sci 1019:406–411

Ballard CG, Greig NH, Guillozet-Bongaarts AL, Enz A, Darvesh S (2005) Cholineesterases: roles in the brain during health and disease. Curr Alzheimer Res 2:307–318

Bartus RT, Dean RL, Beer B, Lippa AS (1982) The cholinergic hypothesis of geriatric memory dysfunction. Science 217:408–417

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem 72:248–254

Burke SN, Barnes CA (2006) Neural plasticity in the ageing brain. Nat Rev Neurosci 7:30–40

Canal N, Imbimbo BP (1996) Eptastigmine study group. Relationship between pharmacodynamic activity and cognitive effects of eptastigmine in patients with Alzheimer's disease. Clin Pharmacol Ther 60:218–228

Comery TA, Stamoudis CX, Irwin SA, Greenough WT (1996) Increased density of multiple-head dendritic spines on medium-sized spiny neurons of the striatum in rats reared in a complex environment. Neurobiol Learn Mem 66:93–96

Deutsch JA (1971) The cholinergic synapse and the site of memory. Science 174:788–794

Dkhar P, Sharma R (2010) Effect of dimethylsulphoxide and curcumin on protein carbonyls and reactive oxygen species of cerebral hemispheres of mice as a function of age. Int J Devl Neurosci 28:351–357

Dutta D, Sharma R (2004) Age-dependent dietary regulation of glucocorticoid receptors in the liver of mice. Biogerontology 5:177–184

Eckles-Smith K, Clayton D, Bickford P, Browning MD (2000) Caloric restriction prevents age-related deficits in LTP and in NMDA receptor expression. Brain Res Mol Brain Res 78:154–162

Ellman GL, Courtney KD, Andress V, Featherstone RM (1961) A new rapid colorimetic determination of acetylcholinesterase activity. Biochem Pharmacol 7:88–95

Giacobini E, Caudra G (1994) Second and third generation cholinesterase inhibitors: from preclinical studies to clinical efficacy. In: Giacobini E, Becker R (eds) Alzheimer's disease: therapeutic strategies. Birkhauser, Cambridge, pp 155–171

Goyary D, Sharma R (2005) Dietary restriction and triiodothyronine (T_3) regulation of malate-aspartate shuttle enzymes in the liver and kidney of mice. Indian J Biochem Biophys 42:345–349

Gray DA, Tsirigotis M, Woulfe J (2003) Ubiquitin, proteasomes, and the aging brain. Sci Aging Knowl Environ 34:RE6

Heine VM, Maslam S, Joels M, Lucassen PJ (2004) Prominent decline of newborn cell proliferation, differentiation, and apoptosis in the aging dentate gyrus, in absence of an age related hypothalamus-pituitary adrenal axis activation. Neurobiol Aging 25:361–375

Johnson HA, Erner S (1972) Neuron survival in the aging mouse. Exp Gerontol 7:111–117

Lee CK, Klopp RG, Weindruch R, Prolla TA (1999) Gene expression profile of aging and its retardation by caloric restriction. Science 285:1390–1393

Lee J, Herman PJ, Mattson P (2000) Dietary restriction selectively decreases GR expression in the hippocampus and cerebral cortex of rats. Exp Neurol 166:435–441

Lu T, Pan Y, Kao SY, Li C, Kohane I, Chan J, Yankner BA (2004) Gene regulation and DNA damage in the aging human brain. Nature 429:883–891

Masaro EJ (2000) Mini-review: caloric restriction and aging - an update. Exp Gerontol 35:299–305

Masoro EJ (1998) Food restriction in rodents: an evolution of its role in the study of aging. J Gerontol 43:B59–B64

Mattson MP (2008) Dietary factors, hormesis and health. Aging Res Rev 7:43–48

Merry BJ (1999) Dietary restriction in aging. In: Sternberg H, Timiras PS (eds) Studies of aging—Springer lab manual. Springer, London, pp 143–163

Moudgil VK, Kanungo MS (1973) Effect of age of the rat on induction of acetylcholinesterase of the brain by 17β-estradiol. Biochim Biophys Acta 329:211–220

Murthy MR (1966) Protein synthesis in growing-rat tissues. Polyribosome concentration of brain and liver as a function of age. Biochim Biophys Acta 119:599–613

Navaratnam DS, Priddle JD, Smith AD, Esisri MM, McDonald B, Robinson JR (1991) Anomalous molecular form of acetylcholinesterase in cerebrospinal fluid in histologically diagnosed Alzheimer's disease. Lancet 337:447–450

Oliverio A, Eleftheriou BE, Bailey DW (1973) Exploratory activity: genetic analysis of its modification by scopolamine and amphetamine. Physiol Behav 10:893–899

Pahlavani MA (2000) Caloric restriction and immunosenescence: a current perspective. Front Biosci 5:580–587

Petrella JR, Townsend BA, Jha AP, Ziajko LA, Slavin MJ, Lustig C, Hart SJ, Doraiswamy PM (2005) Increasing memory load modulates regional brain activity in older adults as measured by fMRI. J Neuropsychiatry Clin Neurosci 17:75–83

Pitsikas N, Algeri S (1992) Deterioration of spatial and non-spatial reference and working memory in aged rats: protective effect of life-long calorie restriction. Neurobiol Aging 13:369–373

Rattan SIS (2004) Aging intervention, prevention, and therapy through hormesis. J Gerontol Biol Sci 59:B705–B709

Scrofano MM, Shang F, Nowell Jr TR, Gong X, Smith DE, Kelliher M, Dunning J, Mura CV, Taylor A (1998) Aging, caloric restriction and ubiquitin-dependent proteolysis in the livers of Emory mice. Mech Ageing Dev 101:277–296

Seitaro O, Fu-Hai M, Masataka K, Kinya K (1990) Alteration of acetylcholine metabolism in the brain of zucker fatty rat. Neurochem Int 16:99–103

Sell DR, Lane MA, Johnson WA, Masoro EJ, Mock OB, Reiser KM, Fogarty JF, Cutler RG, Ingram DK, Roth GS, Monnier VM (1996) Longevity and the genetic determination of collagen glycoxidation kinetics in mammalian senescence. Proc Natl Acad Sci USA 93:485–490

Serrano F, Klann E (2004) Reactive oxygen species and synaptic plasticity in the aging hippocampus. Aging Res Rev 3:431–443

Soreq H, Seidman S (2001) Acetylcholinesterase—new roles for an old actor. Nat Rev Neurosci 2:294–302

Stewart J, Mitchell J, Kalant N (1989) The effects of life-long food restriction on spatial memory in young and aged Fischer 344 rats measured in the eight-arm radial and the Morris water mazes. Neurobiol Aging 10:669–675

Terry AV Jr, Buccafusco JJ (2003) The cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: recent challenges and their implications for novel drug development. Pharmacol Exp Ther 306:821–827

Voigtländer T, Friedl W, Cremer M, Schmidt W, Schroeder TM (1981) Quantitative and qualitative assay of amniotic-fluid acetylcholinesterase in the prenatal diagnosis of neural tube defects. Hum Genet 59:227–231

Weindruch R (1996) Oxidative stress, caloric restriction and aging. Science 273:59–63

Yu ZF, Mattson MP (1999) Dietary restriction and 2-deoxyglucose administration reduce focal ischemic brain damage and improve behavioural outcome: evidence for a preconditioning mechanism. J Neurosci Res 57:830–839

Zimmerman G, Soreq H (2006) Termination and beyond: acetylcholinesterase as a modulator of synaptic transmission. Cell Tissue Res 326:655–669