RESEARCH ARTICLE

Age-related responses of the rat cerebral cortex: influence of vitamin E and exercise on the cholinergic system

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Abstract In this study, we have assessed the impact of vitamin E and exercise on acquisition and retention of spatial memory for a given task in aging rats, using a T-maze. Acetylcholine esterase (AChE) and choline-acetyl transferase (ChAT) activities and acetylcholine (ACh) were measured in the cerebral cortex (CC) of male *Wistar* rats of 4- (adult), 12- (middle-aged) and 18-months (old) of age. Animals were categorized into sedentary [(SEC (N)], sedentary supplemented [SEC (+E)], swim trained [SWT (N)] and swim trained supplemented [SWT (+E)]. In the old, ChAT activity increased in the SEC (+E). AChE activity was highest in the adults, irrespective of training or supplementation. By contrast, ACh concentration remained unaltered with age, exercise and supplementation. Middle-aged and old rats were benefited in terms of a better acquisition and retention in the case of those that were trained and supplemented with Vitamin E. Adults showed better retention in all the groups after 7 and 15 days, while in the middle-aged, training was beneficial after 15 days. We observed decreased AChE activity when old rats were trained with the supplement. Our results also suggest that this regimen may be analogous to the AChE inhibitors that are widely advocated to derive positive benefits in up-regulating the possible reduction in ACh and in turn age-associated memory deficits.

Introduction

Cognitive dysfunction one of the most striking impairments in the human brain is often related to increasing vulnerability of the cells to oxidative stress (OS) with age. The cholinergic system plays an important role in cognition with the cholinergic neurons acting upon the cortex by releasing from their terminals acetylcholine (ACh) that excites cortical neurons and stimulates their activation (Perry et al. 2001). However, in the aged brain, an impaired cholinergic neurotransmission contributes to cognitive deficit and behavioral disturbances (Law et al. 2001; Cummings 2000). However, regular physical exercise is known to facilitate plasticity and cognition in rats (Radak et al. 2001). Collectively, an age-related decline in cognitive and motor functions has been evidenced by studies on oxidative molecular damage. Free radical generation and detoxification mechanisms in the cerebral cortex (Asha et al. 2006)

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fluctuate during the different phases of life as observed in in vivo (Asha and Kiran 2004) and in vitro (Kan et al. 1991) studies, implying that free radicals are crucial causative factors in the impairment of learning and memory processes associated with aging. The central cholinergic system is related to learning, with a reduction in the cholinergic activity in normal aging as well as in pathological situations like Alzheimer's disease (Bartus 2000; Kasa et al. 1997). Although the aforementioned studies have reported the effects of age on either endogenous enzymic antioxidants or adjuncts such as vitamin E on memory retention and involvement of cholinergic system in rats (Eidi et al. 2005) in the cortex, it has been difficult to relate a decline in the acquisition and retention capacity for a spatial memory task to the changes in cortical cholinergic system during aging. The present study capitalized on our previous findings on an up-regulated endogenous antioxidant defense in the cortex under the influence of exercise and vitamin E (Jolitha et al. 2006; Asha and Prathima 2005) in aging rats. The prediction based on our earlier studies was that regular exercise and exposure to enriching supplements can improve the acquisition and learning of spatial memory tasks in old rats. The above hypothesis was analyzed through enzymes and substrate related to the cholinergic system, choline acetyl transferase, acetyl choline and acetylcholine esterase in the cerebral cortex of aging rats. We have examined acquisition and learning of spatial memory, using a T-maze task. This study also provides an insight into the importance of a rat model for examining the possible relation between aging and cholinergic dysfunction. The purpose of the study was to determine whether cholinergic system is altered by vitamin E and exercise in the cerebral cortex. We report the possibilities of altering the age related deficiencies in the neurotransmitter system and in turn the memory during normal aging. Cerebral cortex was the region of interest since this is the region that co-ordinates the various pathways that ultimately make an organism to learn a new task and store it as memory in the hippocampus.

Materials and methods

The Institutional Animal Ethics Committee (IAEC), Bangalore University, India, approved the present study.

Animal maintenance and care

Male Wistar albino rats of 4 months of age were obtained from the Central Animal Facility, IISc, Bangalore and maintained until 12-, and 18-months of age in a clean rodent room. Experiments were completed taking in to account the entire age-scale encompassing the adult, middle age and the old. Animals were housed three per cage in polypropylene fitted steel mesh-bottom cages and were maintained at a temperature of $28 \pm 1°C$, relative humidity of $77 \pm 4\%$ and daily exposure of a 12 h-light and 12 h-dark cycle. Animals had free access to standard food (Amruth feeds, India) and tap water ad libitum.

Exercise training protocol

Swimming has been used in our lab for exercising rats to study physiological and biochemical responses since the animals are not likely to suffer from foot injuries and physical trauma. An additional advantage is that swimming provides a uniform type of physical activity with the use of ankle and flexor muscles. Rats of different age groups were assigned to two swim exercise-trained groups ($n = 10$) comprising swim-trained without supplementation [SWT(N)] and swim trained with vitamin E supplementation [SWT(+E)] and two sedentary groups that consisted of sedentary control without supplementation [SEC(N)] and sedentary that received vitamin E [SEC(+E)]. Swim training was similar to our earlier protocols (Kiran et al. 2004). In brief, rats were made to swim in groups of three at $28 \pm 1°C$ in a glass tank with 3% of their body weight tied to their tails. The training protocol included a pre-training regimen where the animals were allowed to swim for 5 min/day with a gradual increase in duration and for a total period of 40 days. However, 18-mo-old animals were restricted to 20 min of training/day. Animals were trained daily between 10.00 and 12.30 h. At the completion of the exercise, rats were towel-dried and returned to their respective cages. Supplemented groups received an oral supplementation of 50 IU of vitamin E/kg body weight.

Chemicals

Acetyl thiocholine iodide, vitamin E, choline, choline chloride, acetyl CoA, eserine (physostigmine),

5-dinitro benzoate (DNB) and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO, USA). Solvents were of spectral grade and all other reagents were of analytical grade. All reagents were stored at 4°C and were equilibrated at room temperature for 20 min before using them for various assays.

Tissue preparation

The rats were etherized mildly, decapitated and the brain was immediately removed. This was followed by a rinse in ice-cold phosphate buffered saline (PBS) prior to weighing. The complete cerebral cortex was isolated and a 2.5% homogenate was prepared in ice-cold sodium phosphate buffer (pH 7.0). 1 mM physostigmine (12 μg/ml) was added to the buffer for the following assays.

Cholinergic system

Choline acetyl transferase (ChAT, EC.2.3.1.9)

Choline acetyl transferase was assayed by the method of Nachmansohn and Wilson (1955). The reaction mixture (pH 7.0) containing 0.1 mM acetyl S COA, 10 mM choline chloride, 0.2 mM KCl, and 1 mM EDTA and 10 mM potassium phosphate buffer was equilibrated, followed by the addition of the sample and eserine. This was followed by a dilution of the reaction mixture twice. Incubation was continued for 30 min at room temperature and the reaction was stopped by the addition of 12% TCA. The sample was centrifuged at 1,500g, and to the supernatant 9 mM DNB in 1 mM phosphate buffer (pH 7.0) was added. The solution was mixed with a plastic spatula and the decrease in absorbency was read at intervals of 30 s for a total period of 90 s at 412 nm. A molar absorptivity of 1.36×10^{-4}/cm at 412 nm was used to calculate enzyme activity which was expressed as μM of ACh hydrolyzed $h^{-1}100^{-1}$ mg protein.

Acetyl choline (ACh)

To the homogenate 2 M hydroxylamine hydrochloride and 3.5 M sodium hydroxide were added in equal volumes and mixed well to arrest the reaction. 4 N HCl was added followed by the addition of 0.37 M ferric chloride in 0.1 N HCl (Hestrin 1949). After 2 min, the absorbency was read at 540 nm. A standard graph was

prepared from 0.004 M stock of acetylcholine chloride. ACh content was expressed as μM g^{-1} tissue. 1 mM physostigmine (12 μg/ml) was added to the reaction mixture to inhibit the activity of AChE.

Acetyl choline esterase (AChE, EC 3.1.1.1.7)

AChE was measured by the method of Hestrin (1949). To the sodium phosphate buffered substrate containing 0.004 M acetylcholine chloride in 0.1 M HCl solution of pH 4.0, tissue extract was added without the inhibitor. After 1 min, 2 M hydroxylamine hydrochloride and 3.5 M sodium hydroxide mixed in equal proportions were added. This was followed by the addition of 4 N HCl and of 0.37 M ferric chloride in 0.1 N HCl. After 2 min the absorbency was read at 540 nm against distilled water. AChE activity was expressed as μM acetylcholine hydrolyzed min^{-1} mg^{-1} protein.

Vitamin E

Tissue vitamin E

Vitamin E concentration in the tissues was determined as described by Desai (1984). The tissues were homogenized in five volumes of isotonic KCl. Extraction of vit E and saponification of the sample was conducted by a modified method of Kayden and Traber (1993). In brief, the saponification mixture consisting of the respective tissue homogenate, absolute ethanol and 25% ascorbic acid, as an antioxidant was pre-incubated for 5 min at 70°C. To this 10 N KOH was added and further incubated for 30 min at 70°C. Tubes were cooled in an ice-bath and purified hexane was added to the extract. The non-saponifiable material was vortexed for 1 min. The hexane layer was separated by centrifuging at 600g for 10 min and was evaporated to dryness; the residue was dissolved in pure chloroform and spotted on a silica gel glass plate. Standard alpha-tocopherol was also spotted and the plates were developed using 2:1 benzene-ethyl acetate as solvent system. Reference plates were sprayed with 0.0015 M of rhodamine 6G in purified methanol to identify alpha-tocopherol.

The silica gel corresponding to the reference plates was scraped and eluted in purified absolute ethanol and centrifuged at 2,500 rpm for 5 min. 0.2%

bathophenanthroline reagent was added to the eluate and this was followed by the addition of 0.001 M ferric chloride and orthophosphoric acid. The absorbency of the samples and the standard were obtained at 536 nm against distilled water. Concentration of vitamin E was expressed as mg/g tissue.

Serum vitamin E

The sample was obtained by the method of Desai (1984). Fresh venous blood was drawn with a syringe and transferred into conical plastic centrifuge tubes containing 0.2 ml of 1% solution of EDTA. To 1 ml of serum in a glass-stoppered centrifuge, 2 ml of 2% solution of pyragallol in purified ethanol was added and mixed thoroughly. The mixture was heated at 70°C for 2 min after which 0.3 ml of saturated KOH was added and mixed again. The mixture was further incubated at 70°C for 30 min. The tubes were cooled in an ice-bath and 1 ml of distilled water was added followed by 4 ml of purified hexane. Samples were shaken vigorously for 2 min and centrifuged at 1,500 rpm for 10 min to separate the phases. The hexane extract was subjected to TLC to separate small amounts of hexane—soluble non-tocopherol compounds. Separation was similar to that of the tissue vitamin E by TLC. Serum vitamin E was quantitated and expressed in terms of mg/ml.

Protein measurement

Total protein content of tissues was measured by the method of Lowry et al. (1951) using BSA as a standard.

Learning and memory

Learning through simultaneous or successive brightness—discrimination task

The experiments were performed in a dimly lit room. In this task, the goal (food pellet) was systematically connected with the discriminida (light). The correct arm of the T-maze was dimly illuminated with a zero candle bulb. Rats were initially allowed to explore the apparatus for 5 min with this experimental set up (Bures et al. 1976).

During the learning task, the animal was placed in the start position, brightly illuminated (as an alternative for foot shock). The lights of both the goal areas were switched on. The start compartment door was raised, the animal was allowed to make a choice, if it entered, of the correct arm; the animal had an access to the food pellet. If a wrong choice was made, the animal was made to stay for 3 min as a punishment. Each animal was given a time of 3–5 min and an inter trial interval of 15–20 min. This task was continued until the animal learnt to perform at least nine correct choices out of 10 trials day^{-1}. Therefore the total number of trials was 30 in number.

Memory

Memory was tested after a period of 7 d and 15 d following the last learning trial as a memory retention test. Here the goal was randomly shifted from right to left and vice versa and tested for the number of accurate choices.

Statistical evaluation

All data are expressed as mean ± SEM of 5–10 animals. Changes in acetylcholine level, acetylcholine esterase, choline acetyltransferase activities and tissue vitamin E, between the groups of same age were analyzed by two way-analysis of variance (ANOVA). Comparison of serum vitamin E, between group and age were done by two-way analysis. Duncan's multiple range test (DMRT) was performed and significance was considered at $P < 0.05$. Correlation between ACh and AChE with age, in the supplemented and trained groups was analyzed by Karl Pearson's test. Learning and memory tests were analyzed by two-way ANOVA between the trials and groups followed by Tukey Kramer's multiple comparison test and considered significant at $P < 0.05$ using GraphPad Instat 3 software. Only statistically significant ($P < 0.05$) data are described in the Results section.

Results

Acetyl choline

Acetyl choline concentration did not reveal any significant change either between the groups or the ages (Fig. 1a–c).

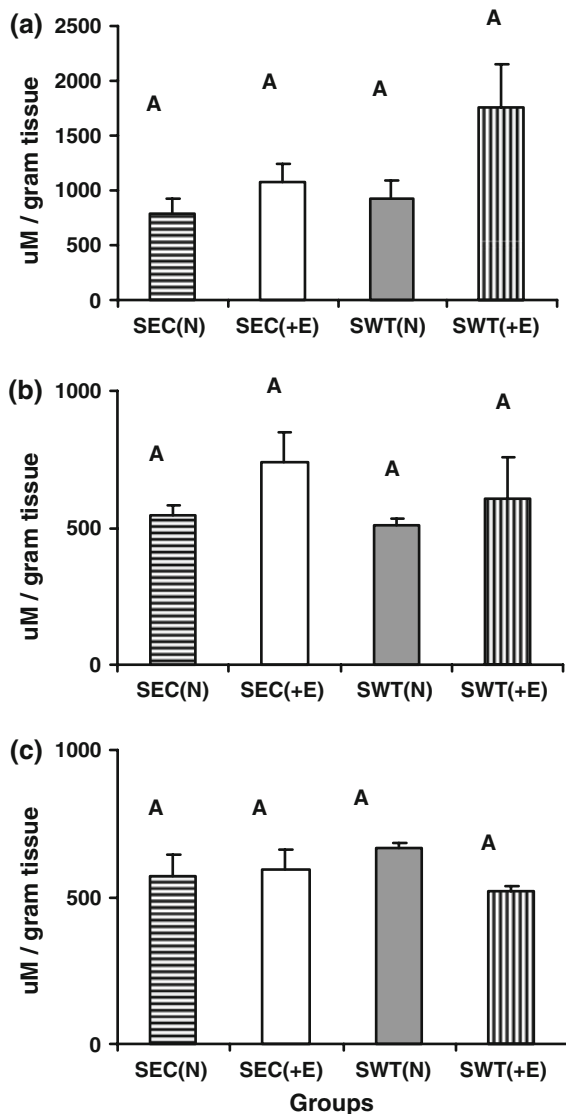**Fig. 1** Effect of age on acetylcholine level in the cerebral cortex of (**a**) 4-mo-, (**b**) 12-mo- and (**c**) 18-mo-old rat. SEC(N) sedentary control; SEC(+E), sedentary rats supplemented with vitamin E; SWT(N), trained swimmers; SWT(+E), trained swimmers supplemented with vitamin E. Significance between group means of these ages were analyzed by DMRT is at $P < 0.05$ and is represented in the upper case and between experimental groups in lower case. Those not sharing the same letters are significantly different. Mean ± SEM of five animals/ group

Fig. 2 Effect of age on the activity of choline acetyltransferase in the cerebral cortex of (**a**) 4-mo-old, (**b**) 12-mo-old and (**c**) 18-mo-old rat. SEC(N) sedentary control; SEC(+E), sedentary rats supplemented with vitamin E; SWT(N), trained swimmers; SWT(+E), trained swimmers supplemented with vitamin E. Significance between group means of these ages were analyzed by DMRT is at $P < 0.05$ and is represented in the upper case and between experimental groups in lower case. Those not sharing the same letters are significantly different. Values are Mean ± SEM of five animals/group

ChAT activity

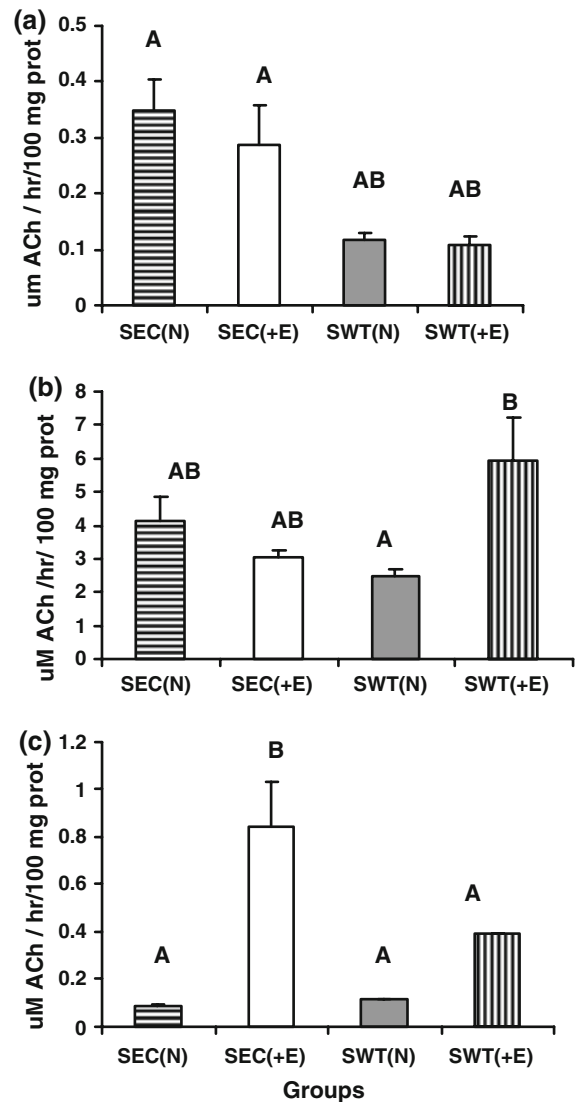ChAT activity decreased by 68% in the 4-mo-old (Fig. 2a) exercised trainees irrespective of supplementation with vitamin E. Middle-aged unsupplemented trainees showed a 58% decrease over their supplemented counterparts (Fig. 2b). In the old, sedentarys when supplemented with vitamin E showed a 78% increase in enzyme activity over the unsupplemented ones (Fig. 2c).

AChE activity

AChE activity increased to 42% in the supplemented as well as unsupplemented trainees over their control group and to 27% over their untrained but supplemented counterparts among the 4-mo-old (Fig. 3a) animals. In the 12-mo-old, AChE activity was reduced by 48% in the supplemented animals over the sedentary (Fig. 3b). However, 18-mo-old supplemented trainees showed a decreased enzyme activity by 25% over their sedentary normals (Fig. 3c).

In brief, there was no significant change in ACh levels as shown in Fig. 1. However, AChE activity decreased with aging. There was no significant correlation between AChE and ACh with aging i.e. among SEC (N) ($r = -0.3822$). However, in the trained supplemented, trained unsupplemented and untrained supplemented a positive correlation was found with age [(SWT(+E) $r = 0.87$; SWT(N) $r = 0.82$; SEC(+E) $r = 0.9418$].

Cerebral cortical vitamin E

Cerebral cortical vitamin E concentration in the 4-mo-old increased in the supplemented sedentary by 71% over the unsupplemented sedentary. Between the experimental groups an average of 55% reduction in the vitamin E concentration was seen in the supplemented as well as unsupplemented trainees, and these decreases were with respect to the supplemented sedentary (Fig. 4a). A similar trend was noticeable in the middle-aged animals wherein the supplemented sedentary group showed an increase in the vitamin E concentration by 80% over the unsupplemented sedentary. Supplemented trainees revealed a higher vitamin E content over their unsupplemented trainees (Fig. 4b).

In the 18-mo-old, vitamin E level was much lower compared to that seen in the cortex of middle-aged animals. In the supplemented sedentary, an increase of 36% over their unsupplemented sedentary was noticeable along with increases of 49% over their unsupplemented trainees and of 29% over their supplemented trainees, respectively (Fig. 4c).

Serum vitamin E

Serum vitamin E levels decreased in the 12- and 18-mo-old sedentary when compared to their adults.
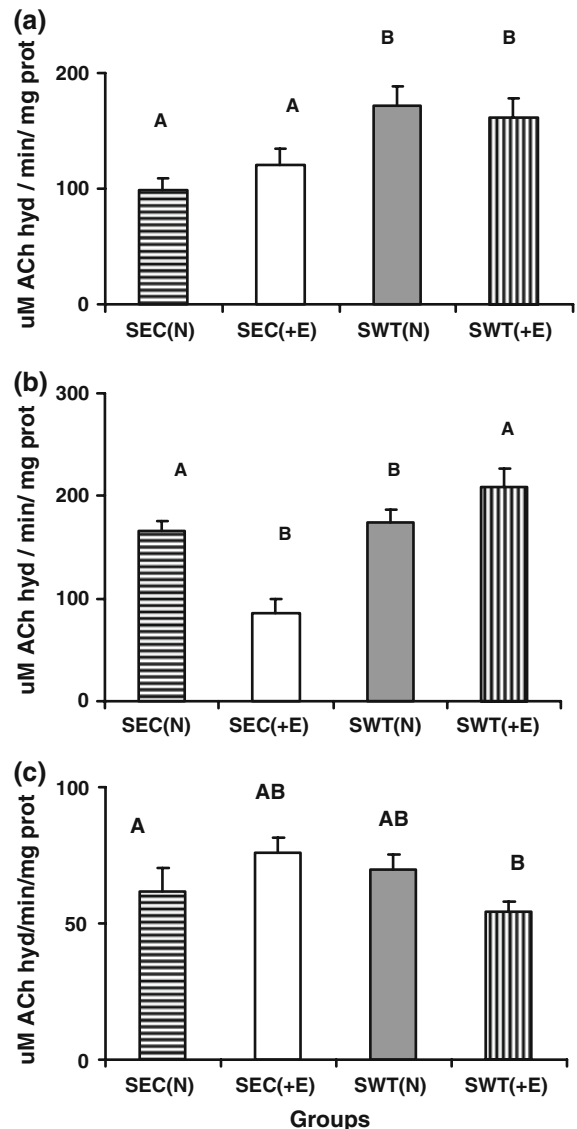

Fig. 3 Effect of age on the activity of acetylcholine esterase activity in the cerebral cortex of (a) 4-mo-old, (b) 12-mo-old and (c) 18-mo-old rat. SEC(N) sedentary control; SEC(+E), sedentary rats supplemented with vitamin E; SWT(N), trained swimmers; SWT(+E), trained swimmers supplemented with vitamin E. Significance between group means of these ages were analyzed by DMRT at $P < 0.05$ and is represented in the upper case and between experimental groups in lower case. Those not sharing the same letters are significantly different. Values are Mean ± SEM of five animals/group

However, vitamin E increased in the supplemented trainees over their unsupplemented counterparts irrespective of age (Fig. 4d).

In the above studies assessing the relationship between serum and cortical vitamin E concentrations,

Fig. 4 Effect of age on the cerebral cortex of (**a**) 4-mo-old, (**b**) 12-mo-old and (**c**) 18-mo-old rat (**d**) Serum vitamin E as a function of age. SEC(N) sedentary control; SEC(+E), sedentary rats supplemented with vitamin E; SWT(N), trained swimmers; SWT(+E), trained swimmers supplemented with vitamin E. Significance between group means of these ages were analyzed by DMRT is at $P < 0.05$ and is represented in the upper case and between experimental groups in lower case. Those not sharing the same letters are significantly different. Values are Mean ± SEM of five animals/group
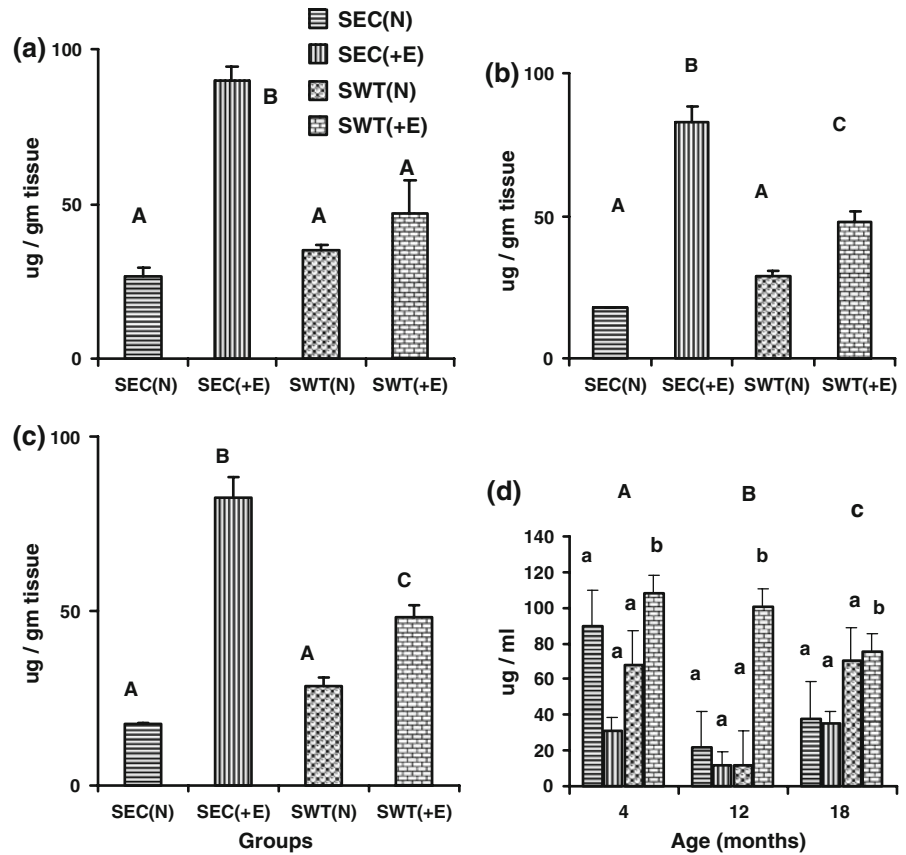
no significant differences were noticed between serum concentrations in the two groups of sedentary in any of the age groups, and was in contrast to the trained animals wherein trained animals showed significant increases in all the age groups. Tissue vitamin E concentrations were significantly elevated in the sedentary as well as trained ones over their unsupplemented ones in all the age groups. In the adults, supplemented with vitamin E, increases of 3.6- and 1.2-fold in the serum concentrations of vitamin E resulted in increases of 246 and 34% in the cortex of sedentary and trainees. Among the middle-aged sedentary, a 4.5-fold increase in serum vitamin E resulted in a 368% increase in vitamin E of the cortex. Among the old, sedentary, a 2-fold and 5-fold increases in serum vitamin E increased cortical vitamin E by 55 and 39% in the sedentary and trainees, respectively.

Learning

Learning in terms of the acquisition of a defined task showed an overall significance between the groups

and number of trials conducted, between all the age groups. Among the 4-mo-old animals, training enhanced the learning potential in the unsupplemented trainees over the sedentary controls and the supplemented sedentarys in the last trial. However during the initial three trials of the learning task, supplemented sedentary (40%) performed better than the unsupplemented trainees. In 12-mo-old, unsupplemented trainees showed a better acquisition potential of 35% over the sedentary group in the first two trials of learning task. Similarly supplemented trainees showed an enhanced learning capacity of 32 and 42% over their unsupplemented sedentary during the first and the second trials, respectively. In the 18-mo-old, significant learning performance was noticeable only in the supplemented groups and supplemented trainees showed increased learning of 30, 10 and 12% over their unsupplemented sedentarys, supplemented sedentarys and unsupplemented trainees, respectively, during the second trial of the learning period (Table 1).

Table 1 Percent correct choices during learning and as a function of age

Age (mo)	Groups	Trial 1[A]	Trial 2[B]	Trial 3[C]	Trial 4
4	SEC (N)	56.7 ± 8.3^a	66.7 ± 6.7	91.2 ± 4.10	106.03 ± 4.44
	SEC (+E)	57.7 ± 6.6^a	82.2 ± 5.2	92.2 ± 2.2	111.87 ± 5.26
	SWT (N)	4.0 ± 6.1^b	72.0 ± 4.9	85.0 ± 3.4	114.68 ± 4.88
	SWT (+E)	65.1 ± 7.7^a	82.0 ± 1.5	93.0 ± 6.2	107.93 ± 3.4
12	SEC (N)	44.0 ± 4.8	48.0 ± 7.4	73.0 ± 3.7	84.0 ± 3.8
	SEC (+E)	61.1 ± 4.8	62.5 ± 6.0	85.0 ± 3.8	93.43 ± 4.4
	SWT (N)	53.0 ± 5.0	72.0 ± 4.7	83.0 ± 2.6	99.33 ± 5.2
	SWT (+E)	64.4 ± 5.8	82.2 ± 3.4	83.3 ± 3.3	95.53 ± 2.3
18	SEC (N)	$45.0 \pm 3.4^{a,A}$	60.0 ± 3.6^B	66.6 ± 4.1^C	73.33 ± 20.6
	SEC (+E)	56.7 ± 5.2^a	63.3 ± 4.6	65.0 ± 4.0	85.00 ± 5.9
	SWT (N)	38.3 ± 14^b	62.5 ± 3.9	67.1 ± 4.2	88.57 ± 3.4
	SWT (+E)	61.7 ± 6.1^a	70.0 ± 5.8	61.7 ± 5.3	90.00 ± 4.9

Values are mean $\pm$ SEM of 10 animals/group. SEC (N), sedentary normal; SEC (+E) sedentary supplemented with vitamin E; SWT(N), normal swim trainees; SWT(+E), swim trainees supplemented with vitamin E. Learning test was analyzed by two-way ANOVA between the trials and groups followed by Tukey Kramer's multiple comparison test and considered significant at $P < 0.05$. Significance between trials is represented by upper case and between groups in lower case. Those not sharing the same letters are significant

Table 2 Age-related changes in per cent latency indicative of memory

Age (mo)	Groups	After 7 days		After 15 days	
		Trial 1	Trial 2	Trial 1	Trial 2
4	SEC (N)	74.4 ± 6.7	75.5 ± 9.3	68.56 ± 3.65	64.25 ± 4.12
	SEC (+E)	71.1 ± 2.3	82.2 ± 9.3	79.25 ± 6.56	71.23 ± 5.88
	SWT (N)	80.0 ± 5.2	79.0 ± 4.3	81.24 ± 4.89	70.01 ± 4.6
	SWT (+E)	93.0 ± 4.2	80.0 ± 3.9	83.77 ± 5.44	69.56 ± 4.5
12	SEC (N)	58.0 ± 7.7^A	$48.0 \pm 17^{a,A}$	52.0 ± 5.3^B	66.0 ± 6.7^C
	SEC (+E)	60.0 ± 8.3	77.5 ± 4.0^b	52.5 ± 5.8	65.0 ± 8.1
	SWT (N)	54.0 ± 8.4	72.0 ± 5.3^a	64.0 ± 5.8	68.0 ± 3.2
	SWT (+E)	57.7 ± 7.4	66.6 ± 6.3^a	57.7 ± 8.6	74.0 ± 5.2
18	SEC (N)[A]	$46.6 \pm 4.1^{a,A}$	$73.3 \pm 4.1^{a,B}$	$46.6 \pm 6.5^{a,C}$	$50.0 \pm 4.4^{a,C}$
	SEC (+E)[B]	50.0 ± 7.1^a	40.0 ± 6.3^b	73.3 ± 7.1^b	66.6 ± 5.9^a
	SWT (N)[C]	71.4 ± 4.0^b	77.1 ± 2.9^a	71.4 ± 4.0^b	70.0 ± 4.0^b
	SWT (+E)[D]	60.0 ± 5.3^a	71.4 ± 8.0^a	54.3 ± 2.9^a	$57.1 \pm 4.6^{a,b}$

Values are mean $\pm$ SEM of 10 animals/group, $P < 0.05$. SEC (N), sedentary normal; SEC (+E) sedentary supplemented with vitamin E; SWT (N), normal swim trainees; SWT(+E), swim trainees supplemented with vitamin E. Learning test was analyzed by two-way ANOVA between the trials and groups followed by Tukey Kramer's multiple comparison test and considered significant at $P < 0.05$. Significance between trials is represented by upper case and between groups in lower case. Those not sharing the same letters are significant

Memory

Memory was measured in terms of % latency, which showed no significant change in the adults between the experimental groups. Hence neither vitamin E nor training was required to recall the task that they had learned. In the middle-aged, considerable retention in the learned task was noticed in the supplemented sedentarys during the second trial after 7 days (Table 2).

After 15 days, all the groups performed better by an average of 69% when compared to the second

trial. In the old, training was effective in enhancing the memory retention irrespective of vitamin E supplement (Table 2).

Discussion

A major neuropathological change in the aging brain is synaptic loss followed by neuronal death, particularly in discrete brain regions related to memory and cognition. The hippocampus and sub cortical areas have decreased choline acetyltransferase activity, high-uptake of choline and ACh levels (Auld et al. 2002; Blusztajn and Wurtmen 2000; Kasa et al. 1997). Studies relating to oxidative damage of rat cerebral cortex (Onodera et al. 2003) have indicated deterioration in the age-related cognitive function and degeneration of cholinergic neurons and a loss of cholinergic neurotransmission (Nino-Cabrere et al. 2002).

The present study was conducted to determine the possible effects of vitamin E supplementation and physical exercise on the primary enzymes of the cholinergic neurotransmitter system in the cerebral cortex of the aging brain and as a function of physical exercise. This type of interaction is not well documented in animal models.

The reports on rodent cortical ChAT activity has been inconclusive with ageing since data indicate both decreases and no change in rats (Sarter and Bruno 1998), and increases (Sherman and Friedman 1990) with no change in mice (Bernstein et al. 1985).

Cerebral cortex of adult and middle-aged sedentary rats showed an unaltered ChAT activity. However, middle-aged trainees when given vitamin E showed an increase in enzyme activity. The results reflect an increased acetyl choline synthesis when these rats were challenged with physical activity in conjunction with a daily oral dose of vitamin E compared to their unsupplemented counterparts. Perhaps the elevated ChAT activity in the cortex of these rats may also be suggestive of an increased uptake of choline by the choline transporter 1 since choline uptake from extracellular space serves as a rate-limiting factor (Kar et al. 1998) in the synthesis of ACh, and may also contribute to the observed improvement in learning and memory in these rats. The 18-mo-old supplemented trainees showed a decrease in AChE activity over their controls in

association with lowered ChAT activity. Hence a combination of exercise training and vitamin E could minimize the otherwise hyperactive AChE activity in the old.

In the old animals although, exercise by itself was effective in enhancing the learning ability, a further improvement was noticeable with vitamin E, suggesting that the two components of enrichment, namely, physical training and vitamin E may guard against any excessive free radical reactions to ensure a better acquisition and retention of a learned task. A somewhat similar trend in terms of an improvement in the learning ability has been reported for 22-month-old rats, which were supplemented with L-carnitine and tested in a T-maze (Lohninger et al. 2001).

A notable observation was on the reduced activity of AChE in the middle-aged sedentary and old trainees on supplementation with consistent and unaltered levels of ACh in the respective groups. The reduced enzyme activity and unaltered ACh levels (Fig. 1) in response to swim training and supplementation may be analogous to AChE inhibitors that is conventionally used in pharmacotherapy to improve cognitive ability in the old. Vitamin E which also has antioxidant activity protects the aging brain from LPO. An earlier study on a reduction of H_2O_2 by vitamin E and a free radical in the cortex (Jolitha et al. 2006) may be related to the observed reduction in AChE activity with no appreciable changes in the ACh level in this study. Further, the aforementioned findings support the fact that OH free radical system H_2O_2/Fe^{2+} induces AChE inhibition (Tsakiris et al. 2000) and the observed decrease in cortical AChE in animals treated with vitamin E may be due to its free radical scavenger property on the sites of the AChE molecule. This may be analogous to reports on inhibitors of choline esterase that improve brain function (Canal and Imbimbo 1996; Giacobino and Cuadra 1994). From our results it can be speculated that the substrate concentration but not the enzyme activities remain unaffected by oxidative stress or aging.

Varying results were obtained in all the groups when tested for their memory retention. Except the old, adult and middle-aged sedentary showed better retention capacity in terms of % latency when tested after 7 and 15 days. In adults, swim training was beneficial in terms of retrieval of the learned task after 15 days and was unlike the old wherein vitamin

E was effective in doing so. In the middle-aged, however, exercise in conjunction with vitamin E was effective in enhancing the retention capacity. The improved learning ability in animals support earlier findings on vitamin E as an effective antioxidant in reducing stress and offering better endurance in trained rats subjected to a swim test (Asha et al. 2003). Our results suggest that training imposes an enhanced requirement of vitamin E in the cortex. Further, the results on an increased serum vitamin E concentration in the sedentary and trained animals supplemented with vitamin E are in accordance with those reported on experimental work in animals on vitamin E supplements that increase levels in plasma and in the brain (Vatassery et al. 1988). The increase and decrease in the activities of ChAT and AChE, respectively, may be partially responsible for the enhanced cognition in the older animals which are similar to what was observed in hippocampus and long-term storage of information with increased ChAT activity in rats during a maze- learning (Durkin 1994). Taken together, our findings suggest that the earlier observations on the responses of dietary enrichment through vitamin E supplements on oxidative stress parameters in the cortex (Kiran et al. 2004) interestingly appear to affect the learning and memory in rats when tested with a T-maze. A previous study has demonstrated an interaction of vitamin E with the cholinergic system and memory in rats (Eidi et al. 2005).

These results although represent a limited aspect of cholinergic system, it suggests aged rat can be an appropriate model for screening of antioxidants in pharmacotherapy of cholinergic dysfunction.

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