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AGE-RELATED CHANGES IN POLYAMINES IN MEMORY-ASSOCIATED BRAIN STRUCTURES IN RATS

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Abstract—Polyamines putrescine, spermidine and spermine are positively charged aliphatic amines and have important roles in maintaining normal cellular function, regulating neurotransmitter receptors and modulating learning and memory. Recent evidence suggests a role of putrescine in hippocampal neurogenesis, that is significantly impaired during aging. The present study measured the polyamine levels in memory-related brain structures in 24- (aged), 12- (middle-aged) and 4- (young) month-old rats using liquid chromatography/mass spectrometry and high performance liquid chromatography. In the hippocampus, the putrescine levels were significantly decreased in the CA1 and dentate gyrus, and increased in the CA2/3 with age. Significant age-related increases in the spermidine levels were found in the CA1 and CA2/3. There was no difference between groups in spermine in any sub-regions examined. In the parahippocampal region, increased putrescine level with age was observed in the entorhinal cortex, and age did not alter the spermidine levels. The spermine level was significantly decreased in the perirhinal cortex and increased in the postrhinal cortex with age. In the prefrontal cortex, there was age-related decrease in putrescine, and the spermidine and spermine levels were significantly increased with age. This study, for the first time, demonstrates age-related region-specific changes in polyamines in memory-associated structures, suggesting that polyamine system dysfunction may potentially contribute to aged-related impairments in hippocampal neurogenesis and learning and memory. **© 2008 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: putrescine, spermidine, spermine, hippocampus, prefrontal cortex, parahippocampal region.

Declined learning and memory ability is a common phenomenon in aged individuals from humans to rodents. Such age-related cognitive decline has been shown to be associated with the dysfunction of the medial temporal lobe, consisting of the hippocampus and its adjacent entorhinal cortex (EC), perirhinal (PRC) and parahippocampal (postrhinal (POR) in rodents) cortices (the parahippocampal region), and the prefrontal cortex (PFC) (for reviews see Gallagher and Rapp, 1997; Tisserand and Jolles, 2003; Burke and Barnes, 2006; Greenwood, 2007). In the adult brain, neurogenesis has been found in the dentate gyrus (DG) of the hippocampus (Altman and Bayer, 1990; Gage, 2002). Newly generated granule cells can mature into functional neurons and the normal rate of neurogenesis in the DG has been shown to be important for maintaining hippocampal function (Markakis and Gage, 1999; Van Praag et al., 2002). During aging, however, the rate of hippocampal neurogenesis is dramatically decreased (Kuhn et al., 1996; Bizon et al., 2004). Several recent studies have demonstrated a link between hippocampal neurogenesis and learning and memory (Driscoll and Sutherland, 2005; Wati et al., 2006).

Polyamines putrescine, spermidine and spermine are positively charged aliphatic amines present in all tissues of almost all species (Wallace, 2000; Wallace et al., 2003). Tissue polyamine levels are mainly regulated by the activities of ornithine decarboxylase (ODC; the rate-limiting enzyme that converts ornithine to putrescine) and spermidine/spermine N^1-acetyltransferase (the key enzyme in polyamine interconversion) (Wallace et al., 2003). It has been well documented that polyamines play important roles in cell proliferation and differentiation, synthesis of DNA, RNA and proteins, protein phosphorylation, signal transduction, as well as the regulation of neurotransmitter receptors (for reviews see Williams, 1997; Wallace, 2000; Oredsson, 2003). Previous studies have reported spermidine-induced memory facilitation and spermine-induced enhancement in synaptic plasticity (Rubin et al., 2000, 2001; Berlese et al., 2005; Camera et al., 2007), and the underlying mechanism may be through the interaction with the polyamine binding site at the *N*-methyl-D-aspartate (NMDA) receptors (Rock and Macdonald, 1995; Williams, 1997). Recently, Malaterre et al. (2004) has demonstrated that the reduction in putrescine levels (induced by difluoromethylornithine, a potent specific and irreversible inhibitor of ODC) significantly impairs adult neurogenesis in the DG in young rats, suggesting an important role of putrescine in hippocampal neurogenesis. Since aging impairs hippocampal neurogenesis dramatically, we hypothesize that the putrescine level decreases with age in the DG.

A number of studies have investigated age-related changes in ODC activity and polyamine levels in the CNS in humans and experimental animals. Morrison et al. (1995), for example, reported decreased spermine, but not putrescine or spermidine, level in the aged occipital cortex. Vivo et al. (2001) investigated the distribution of polyamines in the human basal ganglia and observed a consistent negative correlation between spermidine and

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Abbreviations: ANOVA, analysis of variance; DG, dentate gyrus; EC, entorhinal cortex; HPLC, high performance liquid chromatography; LC/MS/MS, liquid chromatography/mass spectrometry; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; NOS, nitric oxide synthase; ODC, ornithine decarboxylase; PFC, prefrontal cortex; POR, postrhinal cortex; PRC, perirhinal cortex; SD, Sprague–Dawley; SpdS, spermidine synthase; TE, temporal cortex.

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spermine content and age in several brain areas. In rats, Das and Kanungo (1982) reported age-related decrease in ODC activity and spermidine and spermine, but not putrescine, content in the rat cortex. Virgili et al. (2001) demonstrated significantly increased ODC activity and putrescine and spermidine content in the spinal cord, but not cerebellum, cortex or hippocampus, in aged rats relative to the young adult controls. Collectively, this earlier research suggests that the ODC/polyamine system is potentially involved in the normal process of aging.

To the best of our knowledge, there is no systematic investigation of the effects of aging on polyamine levels in memory-associated brain structures. In the present study, we measured the putrescine, spermidine and spermine levels in the hippocampus and the EC, PRC, POR and PFC, as well as the temporal cortex (TE) (an auditory cortex), in young, middle-aged and aged rats. Because the CA1, CA3 and DG areas of the hippocampus have differential contributions to memory processing (Rolls and Kesner, 2006), age-related changes in polyamines in the hippocampus were investigated at the sub-regional level.

EXPERIMENTAL PROCEDURES

Subjects

In the present study, 24-month-old Sprague–Dawley (SD) rats were termed aged rats (Cha et al., 1998, 2000; La Porta and Comolli, 1999; Liu et al., 2003a,b,c, 2004a,b, 2005, 2008a,b; Kollen et al., 2008). Significant behavioral and electrophysiological impairments were observed in 24-month-old SD rats as compared with the young adults (4-month-old) (Liu et al., 2004b, 2005; Kollen et al., 2008). Groups of aged (24-month-old, $n=9$), middle-aged (12-month-old, $n=9$) and young (4-month-old, $n=9$) male SD rats were housed three to five per cage ($53\times33\times26$ cm^3), maintained on a 12-h light/dark cycle (lights on 7 a.m.) and provided *ad libitum* access to food and water. The health condition (e.g. body weight, eyes, teeth, fur, skin, feet, urine and general behavior) of aged and middle-aged animals was regularly monitored by animal technicians and a consultant veterinarian. Only animals showing good health were used for the study. All experimental procedures were carried out in accordance with the regulations of the University of Otago Committee on Ethics in the Care and Use of Laboratory Animals. Every attempt was made to limit the number of animals used and to minimize their suffering.

Tissue preparation

All rats were killed by decapitation without anesthesia. The brains were rapidly removed and left in cold saline (4 °C) for 45 s. The sub-regions of the hippocampus (CA1, CA2/3 and DG), and the PFC, EC, PRC, POR and TE were dissected freshly on ice. There has been some debate as to whether the rat has an area of cortex that could be considered analogous to the PFC of primates. In the present study, we treated the anterior cingulate cortex (Zilles's Cg1, Cg2, and Cg3; 9) as equivalent to at least a portion of the primate PFC that receives afferents from the medialis dorsalis of the thalamus. The frontal association cortex, prelimbic cortex, cingulate cortex (area 1) and the most anterior part of the secondary motor cortex were freshly dissected out as defined in Paxinos and Watson (1998) on ice (see Fig. 1 of Liu et al., 2004a). After the PFC was dissected out, the anterior portion of the brain, cerebellum and brain stem were cut off and the remaining parts of the brain were separated by making a cut along the longitudinal fissure. The ventro-medially located white matter was then carefully removed to expose the hippocampus. The whole hippocampus was flipped up and out by inserting a spatula into the lateral ventricle along its free edge, and then separated from the cortex. The CA1, CA2/3 and DG sub-regions of the hippocampus were dissected out as described by Hortnagl et al. (1991). The EC, PRC (areas 35 and 36), POR and TE (Te2 and Te 3) were then dissected from the remaining cortex based on Fig. 2B of Burwell et al. (1995). All of the dissected tissues were weighed, homogenized in ice-cold 10% perchloric acid (~50 mg wet weight per milliliter) and centrifuged at 10,000 rpm for 10 min to precipitate protein. The supernatants (the perchloric acid extracts) were frozen immediately and stored at −80 °C until analysis.

Polyamine analysis

For each brain region, samples from all three age groups were assayed under one set of conditions. High purity putrescine spermidine, spermine and internal standard (1, 7-diaminoheptane) were used (Sigma, Sydney, Australia). All other chemicals were of analytical grade.

Determination of spermidine and spermine was carried out according to a published method (Marce et al., 1995) with some modifications. Briefly after adding internal standard (1, 7-diaminoheptane) to 20 μl of the perchloric acid extracts, the samples were alkalized with saturated sodium carbonate and derivatized with dansyl chloride. Spermidine, spermine and internal standard were extracted with toluene. The toluene phase was evaporated to dryness, reconstituted and injected onto the high performance liquid chromatography (HPLC) system. The HPLC system consisting of a programmed solvent delivery system at a flow rate of 1.5 ml/min, an autosampler, a reversed-phase C_{18} column, and a fluorescence detector set at the excitation wavelength of 252 nm and emission wavelength of 515 nm. Identifications of spermidine and spermine were accomplished by comparing the retention times of samples with the known standard. Assay validation showed that the analytical method was sensitive and reliable with acceptable accuracy (88–112% of true values) and precision (intra- and inter-assay CV $<$15%). The concentrations of spermidine and spermine in tissue were calculated with reference to the peak area of external standards and values were expressed as μg/g wet tissue.

Because of its low level in the brain tissues, putrescine concentrations in brain tissue samples were measured by a highly sensitive liquid chromatography/mass spectrometric (LC/MS/MS) method (Zhang et al., 2007). After adding internal standard to 20 μl of the perchloric acid extracts, the samples were alkalized with saturated sodium carbonate and derivatized with dansyl chloride. Putrescine and its internal standard were extracted with toluene. The toluene phase was evaporated to dryness, reconstituted and injected onto the LC/MS/MS system. The samples were analyzed by a reversed-phase C_{18} column (150×2.0 mm, 5 μm, Phenomenex) with 80% acetonitrile: 20% water containing 0.1% formic acid as mobile phase at a flow rate of 0.2 ml/min. The retention time of putrescine and the internal standard were 4.0 and 4.8 min, respectively. The total run-time was 15 min. Detection by MS/MS used an electrospray interface (ESI) in positive ion mode. The standard curves for putrescine were linear up to 1000 ng/ml ($r^2>0.99$). The intra- and inter-day coefficients of variation were $<$15%. The concentrations of putrescine in tissue were calculated with reference to the peak area of external standards and values were expressed as μg/g wet tissue.

Statistical analysis

The data were analyzed using a one-way analysis of variance (ANOVA) followed by Newman-Keuls (or Bonferroni in the case of inhomogeneity of variances) post hoc tests. The significance level was set at 0.05 for all comparisons.

RESULTS

Putrescine

Putrescine levels in the sub-regions of the hippocampus and the PFC across three age groups are presented in Fig. 1A. A one-way ANOVA revealed significant differences between groups in CA1 ($F(2,24)=5.43$; $P=0.01$), CA2/3 ($F(2,24)=5.50$; $P=0.01$), DG ($F(2,24)=5.23$; $P=0.01$), and PFC ($F(2,24)=4.18$; $P=0.028$). Post hoc tests revealed significantly decreased putrescine levels in the aged, but not the middle-aged, CA1 ($P<0.05$), the aged and middle aged DG (all $P<0.05$), and the aged, but not the middle-aged, PFC ($P<0.05$). There was also a significant difference in the putrescine level between the aged and middle-aged PFC ($P<0.05$). In the CA2/3 region of the hippocampus, the putrescine levels were significantly increased in the aged and middle-aged rats relative to the young adults (all $P<0.05$) with no difference between the former two.

Fig. 1B illustrates the putrescine levels in the EC, PRC, POR and TE across the three age groups. A one-way ANOVA revealed a significant difference between groups in EC ($F(2,24)=6.82$; $P=0.005$), but not PRC ($F(2,24)=1.72$; $P=0.20$), POR ($F(2,24)=2.13$; $P=0.14$) or TE ($F(2,24)=0.58$; $P=0.57$). Post hoc test revealed a significant increase in putrescine in EC in the aged, but not middle-aged, group relative to the young one ($P<0.01$).

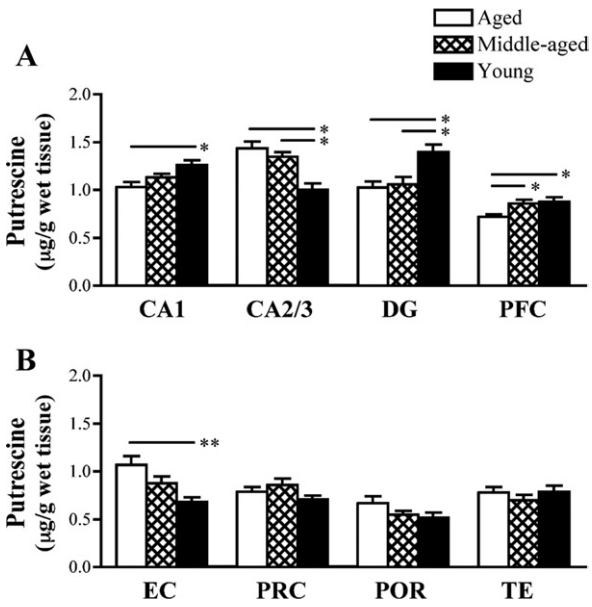

Fig. 1. Putrescine levels (mean±S.E.M.) in the CA1, CA2/3 and DG sub-regions of the hippocampus and the PFC (A) and the EC, PRC, POR and TE (B) in the aged, middle-aged and young rats ($n=9$ for each group). Significantly decreased levels of putrescine with age were found in the CA1, DG and PFC, whereas markedly increased putrescine levels with age were seen in the CA2/3 and EC. There were no significant differences between the three age groups in the PRC, POR and TE. * Indicates a significant difference between groups: * $P<0.05$; ** $P<0.01$.

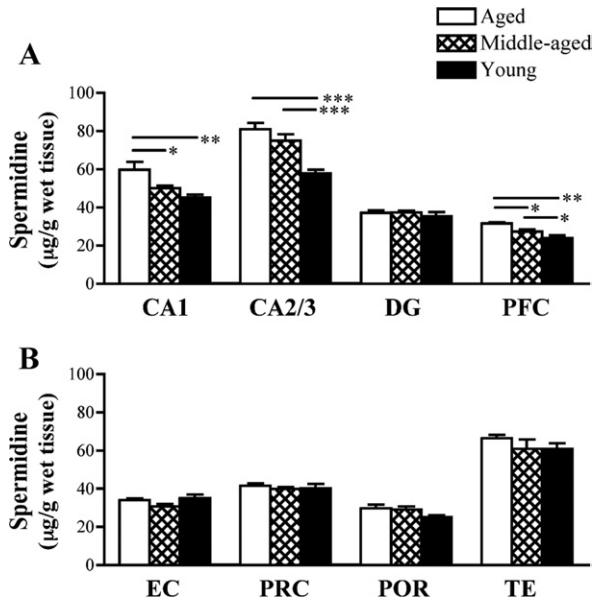

Fig. 2. Spermidine levels (mean±S.E.M.) in the CA1, CA2/3 and DG sub-regions of the hippocampus and the PFC (A) and the EC, PRC, POR and TE (B) in the aged, middle-aged and young rats ($n=9$ for each group). Significantly increased levels of spermidine with age were observed in the CA1, CA2/3 and PFC. No significant differences between groups were found in other brain regions examined. * Indicates a significant difference between groups: * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Spermidine

Spermidine levels in the sub-regions of the hippocampus and the PFC across three age groups are presented in Fig. 2A. A one-way ANOVA revealed significant differences between groups in CA1 ($F(2,24)=8.21$; $P=0.0019$), CA2/3 ($F(2,24)=15.62$; $P<0.0001$) and PFC ($F(2,24)=11.92$; $P=0.0002$), but not DG ($F(2,24)=0.45$; $P=0.64$). Post hoc tests revealed a significant increase in the spermidine level in CA1 in the aged group relative to the middle-aged ($P<0.05$) and young ($P<0.01$) groups with no difference between the latter two. In the CA2/3 region of the hippocampus, the spermidine levels were markedly increased in the aged and middle-aged groups relative to the young one (all $P<0.001$), and there was no significant difference between the former two. In the PFC, the highest level of spermidine was observed in the aged group followed by the middle-aged and young groups. Post hoc test revealed significant differences between the aged and young groups ($P<0.01$), the aged and middle-aged groups ($P<0.05$), and the middle-aged and young groups ($P<0.05$).

Fig. 2B illustrates the spermidine levels in the EC, PRC, POR and TE across the three age groups. A one-way ANOVA revealed that there were no significant differences between groups in EC ($F(2,24)=3.03$; $P=0.07$), PRC ($F(2,24)=0.38$; $P=0.69$), POR ($F(2,24)=2.88$; $P=0.08$) and TE ($F(2,24)=0.90$; $P=0.42$).

Spermine

Spermine levels in the sub-regions of the hippocampus and the PFC across three age groups are presented in

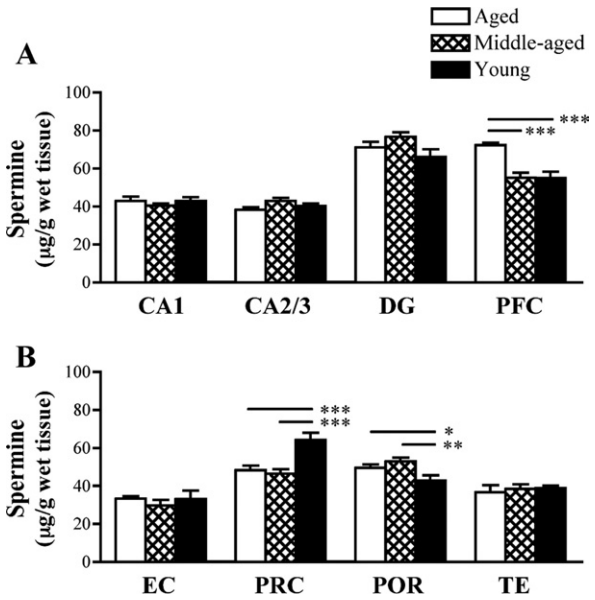

Fig. 3. Spermine levels (mean±S.E.M.) in the CA1, CA2/3 and DG sub-regions of the hippocampus and the PFC (A) and the EC, PRC, POR and TE (B) in the aged, middle-aged and young rats (n=9 for each group). There were significantly increased levels of spermine with age in the PFC and POR, and decreased spermine level with age in the PRC. No significant differences between groups were found in other brain regions examined. * Indicates a significant difference between groups: * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Fig. 3A. A one-way ANOVA revealed a highly significant difference between groups in PFC ($F(2,24)=14.71$; $P<0.0001$), but not CA1 ($F(2,24)=0.61$; $P=0.55$), CA2/3 ($F(2,24)=2.60$; $P=0.09$) or DG ($F(2,24)=1.61$; $P=0.22$). Post hoc test revealed a significant increase in spermine in PFC in the aged group relative to the middle-aged and young groups (all $P<0.001$), and there was no significant difference between the latter two.

Fig. 3B illustrates the spermine levels in the EC, PRC, POR and TE across the three age groups. A one-way ANOVA revealed significant differences between groups in PRC ($F(2,24)=10.57$; $P=0.0005$) and POR ($F(2,24)=5.45$; $P=0.01$), but not EC ($F(2,24)=0.39$; $P=0.69$) and TE ($F(2,24)=0.16$; $P=0.85$). Post hoc tests revealed significantly decreased spermine levels in PRC in the aged and middle-aged groups relative to the young one (all $P<0.001$). In POR, there were increased spermine levels in the aged ($P<0.05$) and middle-aged ($P<0.01$) groups as compared with the young one. There were no significant differences between the aged and middle-aged groups for both PRC and POR.

Putrescine/spermidine and spermidine/spermine molar ratios

The putrescine/spermidine and spermidine/spermine molar ratios in the sub-regions of the hippocampus and the PFC, EC, PRC and POR across three age groups are presented in Table 1. A one-way ANOVA revealed significant differences between groups in the putrescine/spermidine molar ratios in CA1 ($F(2,24)=5.86$; $P=0.0085$), DG ($F(2,24)=6.87$; $P=0.004$), PFC ($F(2,24)=4.28$; $P=0.026$) and EC ($F(2,24)=4.22$; $P=0.027$), but not CA2/3 ($F(2,24)=2.85$; $P=0.75$), PRC ($F(2,24)=1.71$; $P=0.20$), POR ($F(2,24)=2.68$; $P=0.09$) and TE ($F(2,24)=0.49$; $P=0.62$). Post hoc tests revealed that the putrescine/spermidine ratios were significantly decreased in the aged CA1 ($P<0.05$), DG ($P<0.01$) and PFC ($P<0.05$), and the middle-aged CA1 and DG (all $P<0.01$). There was also a significant difference between the aged and middle-aged PFC ($P<0.05$) with the lower ratio in the former. In EC, the putrescine/spermidine ratios were significantly increased in the aged and middle-aged groups relative to the young one (all $P<0.05$) with no difference between the former two.

For the spermidine/spermine molar ratio, a one-way ANOVA revealed highly significant differences between groups in CA1 ($F(2,24)=16.31$; $P<0.0001$), CA2/3 ($F(2,24)=19.49$; $P<0.0001$), PFC ($F(2,24)=13.24$; $P=0.0001$) and

Table 1. Mean (±S.E.M.) putrescine/spermidine and spermidine/spermine molar ratios in the CA1, CA2/3 and DG sub-regions of the hippocampus and the PFC, EC, PRC, POR and TE in aged (A), middle-aged (M) and young (Y) rats (n=9 for each group)

	Putrescine/spermidine	Spermidine/spermine
CA1		
A	0.020±0.0019*	1.927±0.057***,$
M	0.018±0.0013##	1.728±0.073##
Y	0.025±0.0014	1.472±0.031
CA2/3		
A	0.016±0.0007	2.956±0.128***,$$
M	0.016±0.0007	2.443±0.122##
Y	0.017±0.0017	2.012±0.056
DG		
A	0.025±0.0016**	0.732±0.023
M	0.026±0.0022##	0.681±0.014
Y	0.036±0.0028	0.711±0.015
PFC		
A	0.023±0.0014*,$	0.609±0.007$$$
M	0.028±0.0014	0.698±0.022###
Y	0.030±0.0023	0.611±0.006
EC		
A	0.027±0.0029*	1.444±0.067
M	0.026±0.0025#	1.512±0.134
Y	0.018±0.0014	1.333±0.071
PRC		
A	0.017±0.001	1.213±0.039***
M	0.019±0.0014	1.204±0.036###
Y	0.016±0.0013	0.937±0.034
POR		
A	0.020±0.0009	0.876±0.044
M	0.017±0.0011	0.771±0.026
Y	0.017±0.001	0.838±0.043
TE		
A	0.011±0.001	2.349±0.081
M	0.011±0.001	2.250±0.144
Y	0.012±0.001	2.198±0.123

* Indicates a significant difference between the aged and young groups: * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

# Indicates a significant difference between the middle-aged and young groups: # $P<0.05$; ## $P<0.01$; ### $P<0.001$.

$ Indicates a significant difference between the aged and middle-aged groups: $ $P<0.05$; $$ $P<0.01$; $$$ $P<0.001$.

PRC ($F(2,24)=18.39$; $P<0.0001$), but not DG ($F(2,24)=2.05$; $P=0.15$), EC ($F(2,24)=0.90$; $P=0.42$), POR ($F(2,24)=1.90$; $P=0.17$) and TE ($F(2,24)=0.42$; $P=0.66$). Post hoc tests revealed that the spermidine/spermine ratios were significantly increased in the aged CA1, CA2/3 and PRC (all $P<0.001$), and the middle-aged CA1 ($P<0.01$), CA2/3 ($P<0.01$) and PRC ($P<0.001$). There were also significant differences between the aged and middle-aged groups in CA1 ($P<0.05$) and CA2/3 ($P<0.01$). For PFC, a significantly increased spermidine/spermine ratio was found in the middle-aged group relative to the aged and young groups (all $P<0.001$) and there was no difference between the latter two.

DISCUSSION

This study, for the first time, systematically investigated age-related changes in the polyamine levels in several memory-associated brain structures in rats using LC/MS/MS (for putrescine) and HPLC (for spermidine and spermine). The putrescine, spermidine and spermine levels were 0.5–1.4, 25–70, and 30–70 μg/g tissue, respectively, across the hippocampus and cortices, which appear to be consistent with some of the earlier reports in general (Jaenne et al., 1964; Sohn et al., 2002; Adachi et al., 2003; Hayashi et al., 2004). The hippocampus, the key structure of the medial temporal lobe, consists of three major sub-regions, CA1, CA3 and DG. The granule cells in DG receive the cortical inputs from the EC through the perforant path. The pyramidal neurons in CA3 receive the inputs from DG via the mossy fibers and project to CA1 via the Schaffer collateral pathway. CA1 pyramidal neurons project back to the EC either directly or indirectly through the subiculum (Manns and Eichenbaum, 2006). Increasing evidence suggests different roles of the sub-regions of the hippocampus in memory processing (for a review see Rolls and Kesner, 2006). For example, the DG sub-region is characterized by orthogonalization of sensory inputs to create a metric spatial representation and therefore is involved in spatial pattern separation. The CA3 sub-region is important for the integration of spatial information and for associating ongoing events through pattern completion and sequence encoding, whereas the CA1 region supports processes associated with temporal pattern association/completion and intermediate-term memory. It has been shown that aging results in hypofunction in the DG due to reduced synaptic contacts from the EC and hyperfunction in the CA3 due to reduced cholinergic inputs and/or GABA receptor expression (for a review see Wilson et al., 2006). In the present study, spermine did not change with age in any sub-regions examined. There were age-associated decreases in putrescine and the putrescine/spermidine ratios in the CA1 and DG, and increased putrescine level with age in the CA2/3. Significantly increased levels with age in spermidine and spermidine/spermine ratios were noticed in the CA1 and CA2/3, and there were no differences between groups in the DG. Collectively, these results suggest that the aging process affects polyamines differently across the three sub-regions of the hippocampus. Virgili et al. (2001) compared the putrescine, spermidine and spermine levels in the whole hippocampus between aged and young rats, and failed to detect age-related alterations for all three polyamines. Given the differential effects of aging on polyamines across the sub-regions of the hippocampus described above, the examination of the whole hippocampus may contribute greatly to their negative results.

The EC, PRC and POR (parahippocampal in primates) (the parahippocampal region) are the gateway to the hippocampus. The hippocampus receives spatial information through the POR (parahippocampal) and medial entorhinal area, and non-spatial information through the PRC and lateral entorhinal area (Manns and Eichenbaum, 2006). It has been shown that the parahippocampal region is critically involved in complex functions, such as memory, object recognition, sensory representation and spatial orientation (Witter and Wouterlood, 2002). Moreover, the EC and PRC are affected initially and severely in Alzheimer's disease, a neurodegenerative disease characterized by the progressive memory loss (Braak and Braak, 1991; Van Hoesen et al., 2000). Our recent research has demonstrated that the parahippocampal region is also affected greatly by aging (Liu et al., 2003a,b, 2004b, 2008a,b, in press). In the present study, increased level of putrescine with age was found in the EC and aging did not alter the spermidine levels in the parahippocampal region structures. For spermine, age-related increase was seen in the POR, whereas decreased level with age was observed in the PRC. Furthermore, significantly increased putrescine/spermidine and spermidine/spermine ratios with age were found in the EC and perirhinal cortices, respectively. The PFC is involved in a variety of memory functions and is vulnerable to age-associated deterioration (Shimamura, 1995; Greenwood, 2007). Interestingly, age-related changes in all three polyamines were found in the PFC with decreased putrescine level and putrescine/spermidine ratio, and increased spermidine and spermine levels and spermidine/spermine ratio. By contrast, all three polyamines in the TE (an auditory cortex) were not affected by aging. These results clearly demonstrate that the aging process has differential effects on polyamines across the five cortical regions examined. Das and Kanungo (1982) reported age-related decrease in spermidine and spermine, but not putrescine, content in the rat cerebral cortex, whereas Virgili et al. (2001) failed to detect age-associated changes in all three polyamines in the cortex. Since the sub-regions of the cortex were not examined in these two studies, a direct comparison of age-related changes in polyamines cannot be made with the present one.

The underlying mechanisms and functional significance of age-related region-specific alterations in polyamines observed in this study are not fully understood at present. It has been well documented that the physiological concentrations of polyamines are essential for cells to grow and/or function in an optimal manner (for reviews see Oredsson, 2003; Wallace et al., 2003). Because of their nature as polycations, putrescine, spermidine and spermine can interact with negatively charged DNA, RNA, pro-

teins and phospholipids to stabilize their structure (Carter, 1994). Therefore, decreased polyamine levels could potentially affect DNA, RNA and protein synthesis and other metabolic function, and lead to the neuronal dysfunction. Previous studies have demonstrated that aging impairs neurogenesis in the DG dramatically and that reduced rate of hippocampal neurogenesis correlates to age-associated memory impairments (Driscoll and Sutherland, 2005; Wati et al., 2006). Given the role of polyamines in neurogenesis (Malaterre et al., 2004), decreased putrescine levels in the DG in both aged and middle-aged rats (see Fig. 1) may contribute significantly to age-related impairments in hippocampal neurogenesis. It has been shown that GABA, the major inhibitory neurotransmitter in vertebrate brain, can be formed from putrescine and there are close regulatory interrelations between GABA and putrescine (Seiler and Al-Therib, 1974; Seiler et al., 1979). Thus, altered putrescine levels could potentially affect the neuronal excitability. Given the hyperactivity of CA3 neurons in the aged rats (for a review see Wilson et al., 2006), it is likely that increased putrescine levels in the CA2/3 with age observed in this study may be a compensatory mechanism to normalize the excitability of CA3 neurons.

Polyamines modulate learning and memory by interacting with the polyamine binding site at the NMDA receptors (Rock and Macdonald, 1995; Williams, 1997). The NMDA receptor contains at least two families of subunits: NMDAR1 (NR1) and NMDAR2 (NR2A-D). NR1 is necessary and sufficient for the formation of functional channels of the NMDA receptor, whereas NR2 enhances the activity of the receptor when coupled with the NR1 subunit (Magnusson, 1998). Considerable research suggests that aging affects NMDA receptors in the hippocampus (Clayton and Browning, 2001; Magnusson et al., 2006). We have recently observed that NR1 and NR2A protein levels significantly decreased in the aged parahippocampal region and CA2/3 region of the hippocampus (Liu et al., 2008b, in press). The protein expression deficit of NR2B has been shown to be correlated with spatial learning and memory impairments tested in the water maze task and increased expression of NR2B improves learning and memory function in the aged brain (Clayton et al., 2002; Cao et al., 2007). It is of interest to note that NMDA receptors having NR2B subunit are highly sensitive to polyamines (Williams et al., 1994; Williams, 1997) and that polyamines can also be tightly regulated by the NMDA receptor (Fage et al., 1992). Thus, altered polyamine levels could potentially lead to the dysfunction of the NMDA receptors or could be a compensatory mechanism to maintain the NMDA receptor function.

Polyamines are the down-stream metabolites of L-arginine through arginase and ODC. L-Arginine can also be metabolized by nitric oxide synthase (NOS) with the formation of citrulline and nitric oxide (NO), and by arginine decarboxylase with the formation of agmatine (Wu and Morris, 1998). Although ODC is the well-recognized route for polyamine synthesis de novo, agmatine can also serve as a precursor as it is degraded by agmatinase with the formation of putrescine (Hillary and Pegg, 2003). It has been well documented that agmatine plays an important role in regulating intracellular polyamine content through antizyme, an enzyme that increases ODC degradation (for reviews see Satriano, 2003; Pegg, 2006). NO is a gaseous neurotransmitter and is important in regulating physiological functions of the nervous system, including learning and memory (Moncada et al., 1991; Prast and Philippu, 2001). Increasing evidence suggests NO is critically involved in the aging process when present in excessive amounts (McCann et al., 2005). There is evidence suggesting the interactions between polyamines and NO. It has been reported, for example, that NO inhibits ODC through the reaction with Cys360 (for a review see Hillary and Pegg, 2003). Hu et al. (1994) found that all three polyamines could inhibit NOS and therefore led to decreased NO production, although spermidine-induced NO production was also reported (Guerra et al., 2006). We have recently demonstrated age-related region-specific changes in NOS and agmatine in the hippocampus, parahippocampal region and PFC (Liu et al., 2003a,b, 2004a,b, 2005, 2008a, in press). Given the interactions between polyamines and NO and agmatine, a future study needs to be carried out to investigate whether the region-specific changes in polyamines observed in the present study are associated with the age-related alterations in NOS/NO and agmatine.

CONCLUSION

In summary, the present study demonstrates age-related region-specific changes in polyamines in memory-associated structures. Polyamines putrescine, spermidine and spermine have been well documented to play important roles in maintaining normal cellular function (Wallace, 2000; Oredsson, 2003). Although the modulatory role of polyamines in the NMDA receptor function has been long known (Rock and Macdonald, 1995; Williams, 1997), the contributions of endogenous polyamines to learning and memory processing have not been extensively investigated. Krauss et al. (2007) reported the prominent presynaptic localization of spermidine synthase (SpdS; an enzyme that is involved in spermidine synthesis) in cerebellar mossy fiber terminals and the availability of SpdS in hippocampal interneurons and CA1 pyramidal cells, which further supports the involvement of polyamines, particularly spermidine, in synaptic plasticity. Interestingly, we have recently found spatial learning-induced increase in putrescine and spermidine, but not spermine, in the CA1, but not CA2/3 or DG, sub-region of the hippocampus in young adult rats (Liu et al., unpublished observations), indicating the direct involvement of putrescine and spermidine in spatial memory processing. In conjunction with the earlier studies conducted in human and rodents, age-related region-specific changes in polyamines observed in the present study suggest the potential involvement of polyamines in the normal process of brain aging. Given the complex physiological and pathophysiological roles of polyamines in the CNS, as well as the complex interplays with other metabolic pathways of L-arginine, more extensive research needs to be carried out to further understand

the contributions of putrescine, spermidine and spermine to the brain aging process, as well as age-associated cognitive decline.

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