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AGEING ALTERS BEHAVIOURAL FUNCTION AND BRAIN ARGININE METABOLISM IN MALE SPRAGUE–DAWLEY RATS

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Abstract—A growing body of evidence suggests the involvement of L-arginine and its metabolites in the ageing and neurodegenerative processes. The present study assessed behavioural performance in 4- (young), 12- (middle-aged) and 24- (aged) month-old male Sprague–Dawley rats, and investigated age-related changes in the activity of two key arginine metabolic enzymes, nitric oxide synthase (NOS) and arginase, and the levels of L-arginine and its downstream metabolites in a number of memory-related brain structures. Aged rats were less anxious and performed poorly in the water maze task relative to the young and middle-aged rats, and both middle-aged and aged rats displayed reduced exploratory activity relative to the young ones. There were significant age-related changes in NOS and arginase activities, and the levels of L-arginine, L-citrulline, L-ornithine, agmatine, putrescine, spermidine, spermine and glutamate, but not γ-aminobutyric acid, in the CA1, CA2/3 and dentate gyrus sub-regions of the hippocampus and the prefrontal, entorhinal, perirhinal, postrhinal and temporal (an auditory cortex) cortices in a region-specific manner. Cluster analyses revealed that the nine related neurochemical variables formed distinct groups, which changed as a function of ageing. Multiple regression analyses revealed a number of significant correlations between the neurochemical and behavioural variables. The present study further supports the involvement of arginine metabolism in the ageing process, and provides further evidence of the effects of animals' behavioural experience on arginine metabolism.

Key words: ageing, L-arginine metabolites, hippocampus, prefrontal cortex, parahippocampal region, memory.

INTRODUCTION

Cognitive decline is common in aged individuals. It has been documented that age-related decline in learning and memory ability is associated with dysfunctions of the medial temporal lobe structures and prefrontal cortex (for reviews see Gallagher and Rapp, 1997; Tisserand and Jolles, 2003; Burke and Barnes, 2006; Greenwood, 2007). The medial temporal lobe memory system consists of the hippocampus and its adjacent entorhinal, perirhinal and parahippocampal (postrhinal in rodents) cortices (the parahippocampal region). The hippocampus contains three major sub-regions, CA1, CA3 and dentate gyrus (DG), and receives both spatial and non-spatial information through the parahippocampal region (Witter et al., 2000; Eichenbaum and Lipton, 2008). A growing body of evidence suggests the functional dissociation among the three sub-regions of the hippocampus (Kesner et al., 2004; Kesner, 2009). Adult neurogenesis exists in the DG, and the normal rate of neurogenesis is important in maintaining hippocampal function (for reviews see Deng et al., 2010; Aimone et al., 2011). It has been well documented that hippocampal neurogenesis is dramatically impaired during ageing (Klempin and Kempermann, 2007; Drapeau and Nora Abrous, 2008; Fabel and Kempermann, 2008). The prefrontal cortex is involved in a variety of memory functions, and is vulnerable to age-related deterioration (Shimamura, 1995; Greenwood, 2007).

L-Arginine is one of the most metabolically versatile amino acids. It can be metabolized, for example, by nitric oxide synthase (NOS) to form nitric oxide (NO) and L-citrulline, by arginase to generate L-ornithine and urea, and by arginine decarboxylase to produce agmatine and carbon dioxide (Zhang and Snyder, 1995; Wu and Morris, 1998; Wiesinger, 2001). NO is a water- and lipid-soluble gas, and plays an important role in synaptic plasticity and learning and memory at physiological concentration (Holscher, 1997; Huang, 1997; Feil and Kleppisch, 2008). However, it can be neurotoxic when present in excessive amount due to its properties as a free radical (Calabrese et al., 2007). It has, therefore, been proposed that NO is critically involved in the ageing process, the so-called NO hypothesis of ageing (McCann, 1997; McCann et al., 1998, 2005), as well as the neurodegenerative process in Alzheimer's disease (Law et al., 2001; Malinski, 2007). Previous research has demonstrated altered NOS activity and expression during ageing in memory-related brain structures and the correlations with age-related cognitive

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

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impairments (Sugaya et al., 1996; Law et al., 2000, 2002; Necchi et al., 2002; Liu et al., 2003a,b, 2004a,b, 2005, 2009b). L-ornithine is the main precursor of polyamines putrescine, spermidine and spermine. Putrescine is mainly formed from L-ornithine by ornithine decarboxylase (ODC). It combines with decarboxylated *S*-adenosylmethionine to produce spermidine and spermine via spermidine synthase and spermine synthase, respectively. It has been well documented that the physiological concentrations of polyamines are essential in maintaining normal cellular function (Williams, 1997; Wallace, 2000; Oredsson, 2003). There is evidence suggesting an important role of polyamines, putrescine in particular, in hippocampal neurogenesis (Malaterre et al., 2004). We have demonstrated that ageing affects polyamine concentrations in the hippocampus, parahippocampal region and prefrontal cortex significantly in a region-specific manner (Liu et al., 2008c).

The presence of agmatine in mammalian brains was discovered in 1994 (Li et al., 1994). Agmatine is a putative neurotransmitter and interacts with a number of receptor subtypes, including N-methyl-D-aspartate (NMDA) receptors. It regulates the production of NO and polyamines by influencing the activities of NOS and ODC (for reviews see Reis and Regunathan, 2000; Satriano, 2003; Halaris and Plietz, 2007). Since agmatine can be metabolized by agmatinase to form putrescine, it is considered an alternative precursor of polyamines and a member of the polyamine family (Moinard et al., 2005). Recent research suggests that endogenous agmatine may directly participate in the processes of learning and memory as a neurotransmitter (Liu et al., 2008b, 2009a; Leitch et al., 2011; Seo et al., 2011), and that ageing affects agmatine levels in memory-related structures dramatically in a region-specific manner (Liu et al., 2008a).

As described above, L-arginine can be metabolized to generate a number of bioactive molecules, including glutamate and γ-aminobutyric acid (GABA) due to the alternative source of generation from L-ornithine (Wu and Morris, 1998). Hence, it is important to investigate how the ageing process affects arginine metabolic profile in a single study. Since cognitive decline starts in middle age and continues throughout the ageing process (Finch, 2009; Salthouse, 2009), the present study assessed behavioural performance and arginine metabolic profile changes in memory-related structures in young, middle-aged and aged rats. Specifically, animals were tested in the elevated plus maze, open field and water maze task, and age-related changes in NOS and arginase activity and the levels of L-arginine and its metabolites in the sub-regions of the hippocampus and the prefrontal, entorhinal, perirhinal, postrhinal and temporal (an auditory area as a control) cortices were investigated.

EXPERIMENTAL PROCEDURES

Subjects

Male Sprague–Dawley rats, 4 (young, $n = 10$), 12 (middle-aged, $n = 10$) and 24 (aged, $n = 10$) months old, were housed three to five per cage ($53 \times 33 \times 26$ cm^3), maintained on a 12-h light–dark cycle (lights on at 7 a.m.) and provided *ad lib* access to food and water. The health condition (e.g., body weight, eyes, teeth, fur, skin, feet, urine and general behaviour) 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.

Behavioural procedures

All behavioural experiments were conducted in a windowless room with four 60 W bulbs mounted on the ceiling. A video camera was mounted at ceiling height in the centre of the room and used for recording the performance during the experimental period. A radio speaker was located adjacent to the video camera at ceiling height to provide background masking noise. The extra maze cues (the laboratory furniture, lights and several prominent visual features on the walls, as well as the location of the experimenter) were held constant throughout the entire study.

Elevated plus maze. The elevated plus maze was shaped like a plus sign in black-painted wood, with two unwalled (open) arms (50×13.5 cm^2) surrounded by clear Pleixglass of 4 cm and two walled (closed) arms ($50 \times 13.5 \times 29$ cm^3). The central area of the maze measured 13.5×13.5 cm^2. The arm locations were kept constant with north and south being the closed arms. The maze was elevated approximately 60 cm above the floor. Animals were placed at the centre of the elevated plus maze facing one closed arm, and left in the maze for a period of 5 min. Animal behaviour was videotaped and analysed offline by a computerized tracking system (HVS 2020). The total time spent on each arm during the testing period was analysed. An entry was defined by placing all four paws into an arm, and no time was recorded when the animal was in the centre of the maze (Liu and Bergin, 2009; Liu and Collie, 2009; Bergin and Liu, 2010).

Open field. The open field was an experimental chamber consisting of a 60×60 cm black hardboard box with walls 20 cm high and 36 equal-sized squares on the floor. The box was set up immediately after completion of the elevated plus maze test and elevated approximately 60 cm above the floor. Animals were placed into the chamber, and allowed to explore the apparatus freely for 5 min. Animal behaviour was videotaped and analysed offline by HVS 2020. The duration of wall-supported rearings, the path length generated during the entire 5-min testing period and the percentage of time spent in the outer zone (10 cm from the wall) and the centre (central four 10-cm squares) were analysed (Liu et al., 2008d; Liu and Bergin, 2009; Bergin and Liu, 2010).

Water maze task. The water maze pool was a black circular tank measuring 150 cm in diameter and 45 cm in height. It was filled with water to a depth of approximately 25 cm and maintained at a temperature of 25 ± 1 °C. Four points around the edge of the pool were designated as north (N), south (S), east (E) and west (W), which allowed the apparatus to be divided into four corresponding quadrants (i.e. NE, SW, NW and SE).

Place navigation (days 1–6). The day after the elevated plus maze and open field tests, all rats were trained in the water maze task. During the place navigation trials, a black platform (10 cm in diameter) was located in the centre of the SE quadrant and submerged 2 cm below the water surface. All rats received six