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REGIONAL VARIATIONS AND AGE-RELATED CHANGES IN ARGININE METABOLISM IN THE RAT BRAIN STEM AND SPINAL CORD

Y. JING,[a,c] M. S. FLEETE,[a,c] N. D. COLLIE,[a,c] H. ZHANG[b,c] AND P. LIU[a,c]*

[a] *Department of Anatomy, University of Otago, Dunedin, New Zealand*

[b] *School of Pharmacy, University of Otago, Dunedin, New Zealand*

[c] *Brain Health Research Centre, University of Otago, Dunedin, New Zealand*

Abstract—Accumulating evidence suggests that the metabolism of L-arginine, a metabolically versatile amino acid, is critically involved in the aging process. The present study compared the activity and protein expression of nitric oxide synthase (NOS) and arginase, and the levels of L-arginine and its eight down-stream metabolites in the brain stem (pons and medulla) and the cervical spinal cord in 3- (young) and 22- (aged) month-old male Sprague–Dawley rats. Total NOS activity was significantly reduced with age in the spinal cord (but not brain stem), and there were no age-related changes in arginase activity in both regions. Western blot revealed decreased protein expression of endothelial NOS, but not neuronal NOS, with age in both regions. Furthermore, there were significantly decreased L-arginine, glutamate, GABA and spermine levels and increased putrescine and spermidine levels with age in both regions. Although the absolute concentrations of L-arginine and six metabolites were significantly different between the brain stem and spinal cord in both age groups, there were similar clusters between L-arginine and its three main metabolites (L-citrulline, L-ornithine and agmatine) in both regions, which changed as a function of age. These findings, for the first time, demonstrate the regional variations and age-related changes in arginine metabolism in the rat brain stem and spinal cord. Future research is required to understand the functional significance of these changes and the underlying mechanisms.

Key words: aging, L-arginine, nitric oxide synthase, polyamines, brain stem, spinal cord.

INTRODUCTION

Aging is a multifactorial process leading to structural changes and functional decline. It is also a major risk factor for neurodegenerative disorders, such as Alzheimer's disease. L-arginine is a semi-essential amino acid that is widely distributed in mammalian organs, including the brain. A growing body of evidence suggests that arginine metabolism is critically involved in the aging and neurodegenerative processes (for reviews see Law et al., 2001; McCann et al., 2005; Malinski, 2007).

L-Arginine is metabolically versatile with a number of bioactive molecules (Fig. 1; Zhang and Snyder, 1995; Wu and Morris, 1998; Wiesinger, 2001). Nitric oxide (NO), a gaseous signaling molecule, is produced by NO synthase (NOS) with L-citrulline as a by-product. NO derived from neuronal NOS (nNOS) has an important role in regulating synaptic plasticity and neurotransmitter release, whereas endothelial NOS (eNOS)-derived NO is essential in the stabilization and regulation of the vascular microenvironment (Guix et al., 2005). Due to its property as a free radical, however, excessive amounts of NO, especially that derived from inducible NOS (iNOS), can lead to neurotoxicity and neurodegeneration (Law et al., 2001; McCann et al., 2005; Calabrese et al., 2007). L-Ornithine, the product of arginase (arginase I and arginase II), is the main precursor of the polyamines putrescine (PUT), spermidine (SPD) and spermine that are essential in maintaining normal cellular function (Williams, 1997; Wallace, 2000, Wallace et al., 2003). L-Ornithine can also be channeled to generate glutamate and GABA (Wu and Morris, 1998), the main excitatory and inhibitory neurotransmitters in the central nervous system respectively. Agmatine, produced by arginine decarboxylase, is considered a novel putative neurotransmitter and regulates NO production by influencing the catalyzing activity of NOS (Galea et al., 1996; Reis and Regunathan, 2000; Satriano, 2003; Halaris and Piletz, 2007; Santhanam et al., 2007). Because agmatine can be converted to PUT by agmatinase and inhibits ornithine decarboxylase (ODC; the key PUT biosynthesis enzyme), it has an important role in regulating and/or controlling the cellular content of polyamines (Satriano, 2003; Halaris and Piletz, 2007). Previous research has well documented that aging alters arginine metabolism in memory-related brain structures, such as the medial temporal lobe structures and the prefrontal cortex (Sugaya et al.,

*Correspondence to: P. Liu, Department of Anatomy, University of Otago, PO Box 913, Dunedin, New Zealand. Tel: +64-3-479-7536; fax: +64-3-479-7254.
E-mail address: ping.liu@otago.ac.nz (P. Liu).

Abbreviations: ADC, arginine decarboxylase; ANOVA, analysis of variance; ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; eNOS, endothelial nitric oxide synthase; HPLC, high-performance liquid chromatography; iNOS, inducible nitric oxide synthase; LC/MS, liquid chromatography/mass spectrometry; NADHP-d, nicotinamide adenine dinucleotide phosphate-diaphorase; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; ODC, ornithine decarboxylase; PUT, putrescine; SPD, spermidine.

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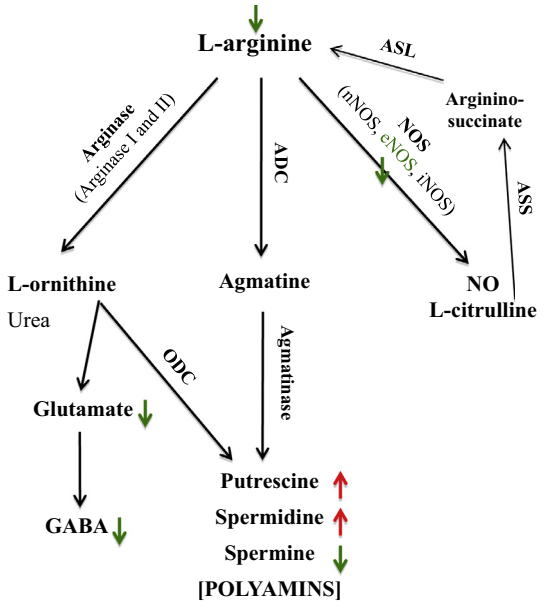

Fig. 1. L-Arginine metabolic pathways. L-Arginine can be metabolized by nitric oxide synthase (NOS), arginase and arginine decarboxylase (ADC) to form a number of bioactive metabolites (see text for detailed description). Red and green arrows indicate the increases or decreases of neurochemical variables in the aged brain stem and spinal cord respectively observed in the present study. ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; eNOS, endothelial NOS; GABA, γ-aminobutyric acid; iNOS, inducible NOS; nNOS, neuronal NOS; NO, nitric oxide; ODC, ornithine decarboxylase. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

1996; La Porta and Comolli, 1999; Law et al., 2000, 2002; Necchi et al., 2002; Liu et al., 2003a,b, 2004a,b, 2008a,c, 2009a,b; Gupta et al., 2012; Rushaidhi et al., 2012), which contributes to age-associated cognitive decline.

The brain stem consists of the midbrain, pons and medulla, and provides a pathway for both the motor and sensory fiber tracts running among the cerebrum, cerebellum and spinal cord. Neurons residing in the brain stem regulate cardiac and respiratory function and body homeostasis, and play a vital role in basic attention, arousal and consciousness. The spinal cord connects the brain and body, acts as a conduit for motor and sensory information transduction, and controls certain reflexes. Chung et al. (2005) reported significantly reduced number of nNOS immunoreactive neurons in the central autonomic nucleus and superficial dorsal horn of spinal cord in aged rats. Using NADHP-d as a histochemical marker for nNOS (Grozdanovic et al., 1995), Kanda (1996) demonstrated a reduced number of NADPH-d-positive neurons with age in the motor nucleus of the spinal cord, and Ma et al. (1997) found decreased NADPH-d activity in the axons and axon terminals, but increased activity in the neuronal bodies, of the gracile nucleus in the medulla. Earlier studies have also reported significantly increased ODC activity and polyamine levels, but decreased glutamate and GABA levels, in the spinal cord in aged rats when compared to the young ones (Virgili et al., 2001b). Collectively, these findings demonstrate that aging also has a significant impact on arginine metabolism in the brain stem and spinal cord.

As L-arginine is metabolically versatile with a number of bioactive metabolites, it is essential to understand how aging affects the metabolic profile of L-arginine in the brain stem and spinal cord. As the spinal cord extends from the brain stem caudally, it would be of interest to compare the arginine metabolic profile between the two structures. In the present study, we harvested the pons and medulla (brain stem) and the cervical portion of the spinal cord (spinal cord) in 3- (young) and 22- (aged) month-old rats to measure the tissue concentrations of L-arginine and its downstream metabolites (L-citrulline, L-ornithine, agmatine, PUT, SPD, spermine, glutamate and GABA; see Fig. 1), as well as the activities and protein levels of NOS and arginase. For the quantifications of enzyme activity and L-arginine metabolites, we processed the brain stem and spinal cord samples from both age groups and conducted the assays under the same experimental conditions. This design would allow us to determine the regional differences in neurochemical variables and the effects of aging on the arginine metabolic profile.

EXPERIMENTAL PROCEDURES

Subjects

Male Sprague–Dawley (SD) rats, 3 (young, $n = 9$) and 22 (aged, $n = 9$) months old, were housed three to five per cage ($53 \times 33 \times 26$ cm^3) under specific pathogen-free environmental conditions, maintained on a 12-h light–dark cycle (lights on 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 behavior) of 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 collection and preparation

All rats were sacrificed by decapitation without anesthesia. The brain and cervical spinal cord (spinal cord: SC) were rapidly removed from each animal and left in cold saline (4 °C). The pons and medulla (brain stem: BS) were then dissected freshly on ice. Both the brain stem and spinal cord were further divided into two parts by making a cut along the midline. For each region, half of the tissue was frozen immediately on dry ice and stored at −80 °C until used for NOS and arginase assays and Western blot. The remaining tissue was then weighed, homogenized in ice-cold 10% perchloric acid (~50 mg wet weight per milliliter) and centrifuged at 10,000 rpm for 10 min at 4 °C to precipitate protein. The supernatants (the perchloric acid extracts) were frozen immediately and stored at −80 °C