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Scopolamine impairs behavioural function and arginine metabolism in the rat dentate gyrus

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disorder with progressive memory loss. It has been shown that the cholinergic neurotransmission deficit is one of the neurochemical characteristics of AD, and that L-arginine and its metabolites also play a prominent role in AD pathogenesis. Scopolamine, a non-selective muscarinic receptor antagonist, blocks cholinergic neurotransmission and impairs behavioural function, including learning and memory. This study investigated the effects of scopolamine on animals' behavioural performance and L-arginine metabolism in the hippocampus and prefrontal cortex. Rats were given intraperitoneal injections of scopolamine (0.8 mg/kg) or saline (1 ml/kg) and tested in the Y-maze, open field, water maze and elevated plus maze 30 min post-treatment. After completion of the behavioural testing, the CA1, CA2/3 and dentate gyrus (DG) sub-regions of the hippocampus and the prefrontal cortex were harvested to measure the activity and protein expression of nitric oxide synthase (NOS) and arginase, and the levels of L-arginine, L-citrulline, L-ornithine, agmatine, putrescine, spermidine, spermine, glutamate and GABA. Scopolamine treated rats displayed reduced alternation and exploratory behaviour, increased swimming speed and impaired spatial learning and memory. There were significantly decreased NOS activity, increased arginase activity, and increased L-ornithine and putrescine levels in the DG, but not other regions examined, in the scopolamine treated rats as compared to the controls. These findings suggest that scopolamine impairs behavioural function and alters L-arginine metabolism in the DG sub-region of the hippocampus specifically. The underlying mechanisms of it remain to be explored further.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder with memory loss as one of the earliest symptoms. The pathological hallmarks of this disease are numerous senile plaques composed of beta amyloid (Aβ) peptide, neurofibrillary tangles and cell loss in the affected brain regions, particularly in the areas that are important for learning and memory including the hippocampus and the prefrontal cortex (Hyman et al., 1989). AD is also characterized by altered neurotransmitter systems in the brain. Deficits of the cholinergic system in the brain have been linked to both age- and dementia-related cognitive dysfunction (Perry et al., 1978; Bartus et al., 1982), leading to the so-called "cholinergic hypothesis of AD" (Francis et al., 1999). It has been shown that there are selective and excessive loss of cholinergic neurons, decreased acetylcholine (ACh) levels and reduced number of ACh receptors in AD brains (Davies and Maloney, 1976; Whitehouse et al., 1982; Francis et al., 1999; Guan et al., 2000). The inhibitors of acetylcholinesterase (such as donepezil, rivastigmine and galantamine), which increase ACh level in the synaptic cleft by inhibiting ACh degradation, have therefore been used clinically as first-line therapy in mild-to-moderate AD (for a review see Lleó et al., 2006).

L-arginine is a semi-essential amino acid that is widely distributed in mammalian organs, including brain. It can be metabolized by nitric oxide synthase (NOS) to produce nitric oxide (NO) and L-citrulline, by arginase to form L-ornithine and urea, and by arginine decarboxylase (ADC) to generate agmatine and carbon dioxide

Abbreviations: AD, Alzheimer's disease; Aβ, amyloid beta; Ach, acetylcholine; DG, dentate gyrus; eNOS, endothelial NOS; HPLC, high performance liquid chromatography; iNOS, inducible NOS; i.p., intraperitoneally; LC/MS, liquid chromatography/mass spectrometry; NMDA, N-methyl-D-aspartate; nNOS, neuronal NOS; NO, nitric oxide; NOS, nitric oxide synthase; ODC, ornithine decarboxylase; ONOO, peroxynitrite; PFC, prefrontal cortex; sGC, soluble guanylyl cyclase.

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(for reviews see Zhang and Snyder, 1995; Wu and Morris, 1998). There are three isoforms of NOS – neuronal NOS (nNOS) and endothelial NOS (eNOS) are Ca^{2+}-calmodulin-dependent constitutive forms, whereas inducible NOS (iNOS) is Ca^{2+}-calmodulin-independent and is usually only produced in response to pathological stimuli (Zhang and Snyder, 1995). Although NO serves as a neural messenger and regulates cerebral blood flow at physiological concentrations, excessive amounts of NO can react with superoxide to generate the potent oxidant peroxynitrite ($ONOO^-$), leading to neurotoxicity and neurodegeneration (Law et al., 2001; Malinski, 2007). There is evidence suggesting the presence of two isoforms of arginase (arginase I and II) in mammalian brains (Yu et al., 2001; Colton et al., 2006; Hansmannel et al., 2010). In mammalian cells, the major route to the production of polyamines putrescine, spermidine and spermine appears to be *de novo* synthesis from L-ornithine (the product of arginase) by ornithine decarboxylase (ODC). It has been well documented that physiological concentrations of polyamines are essential for cells to grow and to function in an optimal manner (for reviews see Williams, 1997; Oredsson, 2003; Wallace et al., 2003). The presence of agmatine and its biosynthesis enzyme ADC in mammalian brains was discovered in 1994 (Li et al., 1994). Agmatine is a putative novel neurotransmitter, interacts with a number of receptor subtypes, and regulates the production of NO and polyamines as an endogenous regulator (for reviews see Reis and Regunathan, 2000; Satriano, 2003; Halaris and Piletz, 2007). Because agmatine can be metabolized by agmatinase to form putrescine, it has been considered a member of the polyamine family (Moinard et al., 2005).

Accumulating evidence suggests that L-arginine and its metabolites play a prominent role in AD pathogenesis (for reviews see Law et al., 2001; Malinski, 2007; Yi et al., 2009). Aβ stimulates microglial and astrocytic NO production, and both NO- and $ONOO^-$-mediated neuronal damage have been found in AD brains (Law et al., 2001; Malinski, 2007). NO derived from eNOS is a key factor for the stabilization and regulation of the vascular microenvironment (de la Torre, 2009). In AD brains, senile plaques and neurofibrillary tangles are associated with reduced capillary expression of eNOS (Provias and Jeynes, 2008; Jeynes and Provias, 2009). Increased arginase I and arginase II mRNA levels have been found in the AD brains (Colton et al., 2006; Hansmannel et al., 2010), and the presence of the rare arginase II allele rs742869 appears to be associated with an increase in the risk of AD and an earlier age-at-onset (Hansmannel et al., 2010). There are also elevated ODC protein levels and altered sub-cellular localization of ODC and polyamine content in the AD brains (Morrison and Kish, 1995; Morrison et al., 1998; Nilsson et al., 2006).

Scopolamine is a non-selective muscarinic receptor antagonist, and impairs behavioural function (including learning and memory) due to a blockage of cholinergic signalling. It has been reported, for example, that scopolamine treatment at various doses through different delivery routes alters animals' spontaneous alternation behaviour, exploratory and locomotor activity, anxiety level, and spatial learning and memory (for a review see Klinkenberg and Blokland, 2010). Hence, scopolamine has been used as a pharmacological tool to model AD-related cognitive decline by addressing the cholinergic system dysfunction aspect of the disease. In the central nervous system, activation of cholinergic muscarinic receptor subtypes and the N-methyl-D-aspartate (NMDA) subtype of excitatory amino acid receptors activates NOS, and NO leads to an elevation of tissue cGMP levels through the activation of soluble guanylyl cyclase (sGC) (Bredt and Snyder, 1990; Garthwaite et al., 1989; Tonnaer et al., 1991). However, the interaction between scopolamine and arginine metabolic pathways has not been investigated previously.

The present study was designed to investigate the effects of scopolamine on animals' behavioural performance in the Y-maze, open field, water maze, and elevated plus maze, as well as L-arginine metabolism in a single study. After completion of the behavioural tests, the hippocampus and the prefrontal cortex were harvested to measure the activities and protein levels of NOS and arginase, and the tissue concentrations of L-arginine and its metabolites (L-citrulline, L-ornithine, agmatine, putrescine, spermidine and spermine). We also measured the tissue concentrations of glutamate and γ-aminobutyric acid (GABA), the major excitatory and inhibitory neurotransmitters in the central nervous system respectively, since L-ornithine can be converted to L-Glutamyl-*c*-semialdehyde that is further metabolized to glutamate by P5C dehydrogenase (Wu and Morris, 1998). Because of the functional dissociation across the CA1, CA3 and dentate gyrus (DG) sub-region of the hippocampus (for a review see Kesner et al., 2004), neurochemical changes induced by scopolamine in the hippocampus were examined at the sub-regional level.

2. Materials and methods

2.1. Subjects

Nineteen male Sprague-Dawley rats, weighing between 280 and 350 g, were housed one per cage ($33 \times 21.5 \times 17.5$ cm^3) with free access to water and food, and maintained on a 12-h light/dark cycle (lights on 8 am). Treatments and behavioural procedures were conducted during the light period of the light–dark cycle. 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 minimise their suffering.

2.2. Drug and treatment

The rats were randomly allocated to the saline ($n = 9$) and scopolamine ($n = 10$) groups. The variation in animal's body weight was considered and counterbalanced between groups (Saline: 325.4 ± 4.2 g, Scopolamine: 325.8 ± 4.9 g; $t(17) = 0.05$, $p = 0.96$). Scopolamine was purchased from Sigma Chemicals, USA, and was freshly dissolved in saline to a final concentration of 0.8 mg/ml. On days 1, 3, and 5, animals received either saline (1 ml/kg, i.p.) or scopolamine (0.8 mg/kg, i.p.) 30 min prior to the behavioural testing. There were no treatments and behavioural testing on days 2 and 4. Animal's body weight was monitored during the experimental period.

2.3. Behavioural apparatus

All behavioural tests were conducted in a windowless room with three clear and one red 75 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 extramaze cues (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.

The Y-maze was shaped like a Y and made of black painted wood with an angle of 120° between each of the three arms ($40 \times 10 \times 18.5$ cm^3). The positions of the arms were kept constant between animals and groups during the test, with each arm being labelled either A, B or C. The maze was elevated approximately 80 cm above the floor.

The open field apparatus consisted of a 60×60 cm^2 wooden box with identical walls 20 cm high. All four of the chamber walls and the floor of the box were painted black, and the floor was divided into 36 equal sized grid squares. The box was elevated approximately 60 cm above the floor.

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).

The elevated plus maze was shaped like a plus sign, made of black painted wood, with two (open) arms (50×13.5 cm^2) surrounded by 4 cm clear Plexiglas and two walled (enclosed) arms ($50 \times 13.5 \times 29$ cm^3). The central area of the maze measured 13.5×13.5 cm^2. The maze was elevated approximately 60 cm above the floor, and the arm locations were kept constant with north and south being the enclosed arms.

2.4. Behavioural procedures

2.4.1. Y-maze and open field (day 1)

Animals were placed at the centre of the Y-maze facing the south arm 'B' and allowed to explore the maze freely for a period of 8 min. The open field chamber was set up immediately after the Y-maze test. Animals were placed into the chamber and allowed to explore the apparatus for 5 min.

Animals' behavioural performance was videotaped and analysed offline by a computerised tracking system (HVS 2020). For the Y-maze, the number and the