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Research Report

Microinjection of L-arginine into corpus callosum cause reduction in myelin concentration and neuroinflammation

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ABSTRACT

Role of nitric oxide (NO) in inflammationary diseases such as multiple sclerosis (MS) has been proposed previously. We sought to examine if NO plays centrally a key role in MS related phenomena; demyelination or neuroinflammation. Female Wistar rats (weighing 200–250 g) were mounted in a stereotaxic apparatus and received injections of L-arginine aimed at corpus callosum (AP: 1.2, L: ±1.8, V: 3.2). The drug (50–200 μg/rat) was microinjected intra-corpus callosum repeatedly (3–5 times/each per day). Control groups solely received saline (1 μg/rat) into the corpus callosum. The animals were tested for the novelty seeking behavior using the conditioning task. Memory impairment was examined using the shuttle box and Y-maze. L-NAME was pre-injected to L-arginine to involve the NO. All animals' brains were also processed for histological evaluation. L-arginine produced significant changes in the novelty seeking behavior but not in the memory formation, evidenced by passive avoidance and alternation behaviors. Pre-injection of L-NAME reversed the response to L-arginine. Present study further revealed a prominent inflammation as well as myelin elimination in the L-arginine treated rats' brains. These data suggest that the NO infusion in the myelin rich areas such as corpus callosum may lead to MS signs centrally.

1. Introduction

Multiple sclerosis (MS) is identified an autoimmune, inflammatory, demyelinating, and neurodegenerative disease of the central nervous system affecting over 2 million people worldwide (Kurnellas et al., 2007). The main histopathological hallmarks of MS are listed as inflammation, demyelination, oligodendrocyte death, gliosis, axonal damage and neurodegeneration (Bruck and Stadelmann, 2005; Prat and Antel, 2005). The disease, though the pathogenesis of MS is unknown, represents an autoimmune disorder directed against nervous system antigens (Hemmer et al., 2002; Noseworthy et al., 2000; Wekerke, 1998). The underlying mechanism of neural damage in MS has often been addressed by use of animal model called experimental autoimmune encephalomyelitis (EAE) (Hohlfeld, 2009; Kurnellas et al., 2007). EAE shows similarity in pathological,

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histological and clinical features to MS and covers different aspects of the disorder (Gold et al., 2006; Subramaniam, 2005). EAE is induced in some animal species and strains either by immunization with myelin components or by passive transfer of encephalotigenic T-cells.

Nitric oxide (NO) is a free-radical produced from the oxidation of terminal guanidino nitrogen of arginine, and this reaction is catalyzed by a NADPH-dependent enzyme, NO synthase (NOS) (Lowenstein and Snyder, 1992). This diffusible neuronal second messenger, NO, is centrally formed by a neuronal isoform of the NOS (nNOS) (Knowles and Mocada, 1994), the enzyme that is blocked by N^G-nitro-L-arginine methyl ester, L-NAME (Moncada et al., 1991). The molecule NO is involved in MS (Bishop et al., 2009; David et al., 2006; Gold et al., 1997); a continuous and high concentration of NO metabolites in CSF and serum of MS patients in relapsing phase is suggested to cause damages to myelin and oligodendroglia (Acar and Idiman, 2003). A level of NO is considered to be a useful marker of the MS disease (Danilov and Andersson, 2003).

Although evidence (Encinas et al., 2005) points to the contribution of NO in various aspects of the disorder such as inflammation, oligodendrocyte injury, changes in synaptic transmission, axonal degeneration and neuronal death (Encinas et al., 2005), the pathogenic role of NO in MS, however, remains controversial. The present study discovers the importance of NO in a highly myelinated area corpus callosum (CC) by investigating the disease process in the L-arginine injected female Wistar rats both behaviorally and histologically.

The female rats were preferred based on the previous data showing that the MS occurs more commonly in females than males and that the prevalence of the disease is much greater in the gender (Pozzilli et al., 2003; Whitacre et al., 1999). It should be notified that no significant differences in brain lesions of MS has been identified during ovarian cycle or change in ratio of progesterone/estradiol levels in women (Bansil et al., 1999; Holmqvist et al., 2006).

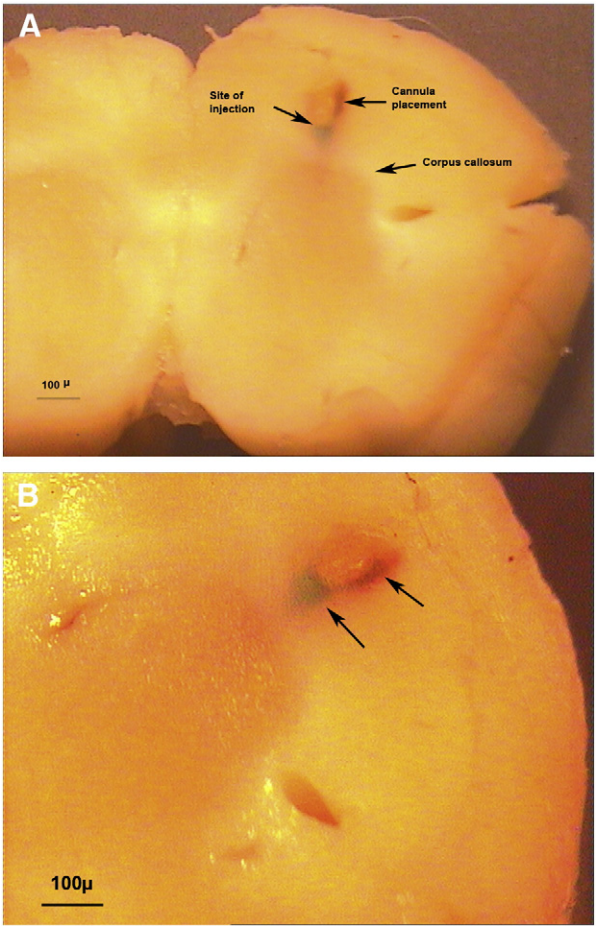

Fig. 1 – (A) Cannulae placements in corpus callosum as evidenced by ink injection in a volume of 1 µl/rat by using the same set up as used for intra-central amygdala injection of drugs (AP: 1.2). Scale shows the magnification and the arrowheads signify the point of microinjection, the cannula placement, and the corpus callosum, (B) The Fig. 1(A) under the magnification.

2. Results

2.1. *Verification of site of microinjection*

Site for microinjections was revealed by injection of 1 µl of a methylene blue solution into the corpus callosum using the same injection set up as used for microinjecting of the drugs (Fig. 1).

2.2. *Effects of microinjection of L-arginine into the corpus callosum on neuroinflammation and demyelination*

Light microscopic observations revealed a decrease ($F_{6,28}$= 9.636, $p<0.001$) in the quantity of cell population in L-arginine treated animals (Fig. 2E–F) in comparison with the controls (Fig. 2A–C). A significant decrease in myelin concentration was observed ($F_{6,28}$=13.611, $p<0.01$) in the samples administered L-arginine (Fig. 2G) compared with that of controls (Fig. 2D). The effect of neuroinflammation at the site of injections was also appeared due to infiltration of the cells (Fig. 2H). Pre-injection of L-NAME (50–200 µg/rat, into the corpus callosum) to the L-arginine caused an attenuation or completely blockade on the effects of L-arginine indicating the NO involvement in the processes (Fig. 2I–J).

2.3. *Dose–response of L-arginine in novelty seeking behavior as evidenced using conditioning place preference paradigm*

Administration of L-arginine at different doses (50–200 µg/rat, into the corpus callosum) after five times resulted in a significant change in behavior response in comparison with the control (saline treated group: 1 µl/rat, into the corpus callosum) ($F_{3,20}$=10.673; $p<0.001$, Fig. 3). More analysis of the data showed that L-arginine at 100 µg/rat potentiated the signs of the behavior when was injected into the corpus callosum for five times. However, the injection of the lower or higher doses of the agent resulted in a decrease in the response signifying the bell shaped response of the NO precursor.