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Spatial reference memory deficits precede motor dysfunction in an experimental autoimmune encephalomyelitis model: The role of kallikrein–kinin system

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ABSTRACT

Multiple sclerosis (MS) is a progressive T cell-mediated autoimmune demyelinating inflammatory disease of the central nervous system (CNS). Although it is recognized that cognitive deficits represent a manifestation of the disease, the underlying pathogenic mechanisms remain unknown. Here we provide evidence of spatial reference memory impairments during the pre-motor phase of experimental autoimmune encephalomyelitis (EAE) in mice. Specifically, these cognitive deficits were accompanied by down-regulation of choline acetyltransferase (ChAT) mRNA expression on day 5 and 11 post-immunization, and up-regulation of inflammatory cytokines in the hippocampus and prefrontal cortex. Moreover, a marked increase in B_1R mRNA expression occurred selectively in the hippocampus, whereas protein level was up-regulated in both brain areas. Genetic deletion of kinin B_1R attenuated cognitive deficits and cholinergic dysfunction, and blocked mRNA expression of both IL-17 and IFN-γ in the prefrontal cortex, lymph node and spleen of mice subjected to EAE. The discovery of kinin receptors, mainly B_1R, as a target for controlling neuroinflammatory response, as well as the cognitive deficits induced by EAE may foster the therapeutic exploitation of the kallikrein–kinin system (KKS), in particular for the treatment of autoimmune disorders, such as MS, mainly during pre-symptomatic phase.

1. Introduction

Multiple sclerosis (MS), a chronic inflammatory and demyelinating disease that affects the central nervous system (CNS) (Sospedra and Martin, 2005) is considered to be an autoimmune pathology in which autoaggressive Th1 and Th17 lymphocytes induce a response against components of myelin (Goverman, 2009; Sospedra and Martin, 2005; Steinman, 2007). Th1 and Th17 cells are characterized by their expression of interferon-γ (IFN-γ) and interleukin-17 (IL-17), respectively. Experimental autoimmune encephalomyelitis (EAE), a $CD4^+$ T cell-mediated disease of the CNS, is the best known animal model of MS and can be induced in susceptible rodents and other animals by immunization with myelin antigens such as myelin oligodendrocytes glycoprotein (MOG) (Steinman, 2007). White matter inflammation, loss of myelin and consequent neuronal degeneration, the pathological hallmarks of MS and EAE, are thought to determine the disease severity (Ffrench-Constant, 1994). Clinical symptoms in MS include a progressive decline in motor and sensory functions and permanent disability (Steinman, 2007).

Nonetheless, recent evidence has shown that, in MS, the neuronal compartment of the CNS is affected in parallel to, and even independently of, white matter damage, which has led to a reevaluation of the perceived relationship between inflammation and neurodegeneration in this disease (Centonze et al., 2010; Steinman, 2007). Studies using magnetic resonance imaging (MRI) revealed that the gray matter atrophy, which occurs in cortical and deep sub-cortical brain regions (Filippi et al., 2003), begins early in the disease, continues as the disease progresses (Lisak, 2007) and correlates with motor, sensory and visual disability (Magnano et al., 2006). Moreover, there is few evidence indicating that about 50–70% of MS patients experience important cognitive deficits (Rao, 1995; Shi et al., 2008) which are detectable even before a definitive diagnosis of MS is made. These cognitive and emotional symptoms related to MS strongly affect patients' ability to work, as well as their quality of life (Engel et al., 2007). Although the cause of mem-

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ory dysfunction in MS is currently unknown, information-processing speed and working memory are the most frequently detected cognitive deficits in MS (Rao et al., 1991). In addition, verbal and spatial learning are also affected, reflecting hippocampal dysfunction, especially atrophy of the CA1 region (Sicotte et al., 2008). In this regard, some reports have proposed a linkage between cholinergic system, neuroinflammation and cognitive impairments (Field et al., 2012; Ruan et al., 2010; Ullrich et al., 2010). Particularly, impairment within the cholinergic system has been demonstrated in MS (Antonelli et al., 2013). For instance, Kooi et al. (2011) reported that in MS hippocampus, both activity and protein expression of choline acetyltransferase (ChAT), the acetylcholine synthesizing enzyme, were decreased, whereas the activity and protein expression of acetylcholinesterase (AChE), the acetylcholine degrading enzyme, were found to be unaltered (Kooi et al., 2011). Moreover, cholinergic projections arise from basal forebrain nuclei such as medial septum/vertical limb of the diagonal band to the hippocampus and from nucleus basalis (of Meynert) to the cortex (Wevers, 2011). This anatomical split of the cholinergic system into neocortical and hippocampal divisions underlies cholinergic modulation of working (Croxson et al., 2011) and spatial-reference memories (Deiana et al., 2011), respectively.

The identification of the specific cognitive deficits observed before motor symptoms in MS is of high interest because they may be useful as temporal markers of the disease, and for testing of potential therapeutic treatments even before the onset of motor impairments. Moreover, drugs currently used to treat MS focus mainly on the control of autoimmune and neuroinflammatory responses. Hence, it is incumbent upon novel studies to elucidate a molecular substrate, with the view of discovering therapeutic interventions. In this context, we and others have previously demonstrated that kinin receptors, mainly the B_1 receptor (B_1R), exert a critical role in regulating the early development of experimental autoimmune encephalomyelitis (EAE) progression by modulating the onset of the immune response and affecting the functioning of astrocytes/microglia cells (Dutra et al., 2011).

Kinins (such as bradykinin and kallidin) are the most potent autacoids involved in inflammatory, vascular and pain processes, and constitute the end-products of the so-called kallikrein–kinin system (KKS). Kinins exert most of their biological effects by the activation of two G-protein coupled receptors, denoted B_1 and B_2 receptors (Calixto et al., 2004; Marceau and Regoli, 2004). In this regard, Wang and Wang demonstrated that injection of bradykinin (BK), a preferential B_2R agonist, is able to induce learning and memory impairment when administered into the rat hippocampus (Wang and Wang, 2002). Extending this idea, Prediger et al. (2008) showed that kinins, acting via activation of B_1R and B_2R in the CNS, exert a critical role in the spatial learning and memory deficits induced by the β-amyloid peptide (Aβ) in mice (Prediger et al., 2008). In addition, up-regulation of both kinin receptors in the brain was related, directly or indirectly, to cognitive processes after infusion of Aβ (Viel et al., 2008). On the other hand, the linkage between KKS and the cholinergic system is currently not fully established. A relevant study conducted by Buccafusco and Serra (1985) showed that the intracerebroventricular (i.c.v.) injection of BK in conscious, freely moving rats evoked dose-related increases in arterial pressure, and pretreatment with hemicholinium-3, which deplete brain acetylcholine levels, produced a choline-reversible blockade of the cardiovascular response to BK (Buccafusco and Serra, 1985). This notion has been extended further by Barnes et al. (1998), who demonstrated that BK-induced bronchoconstrictor response in asthmatics is dependent of cholinergic and sensory nerves (Barnes et al., 1998). However, no consistent evidence for the linkage between KKS and the cholinergic system during neuroinflammatory response, such as MS, has to date been documented.

Because kinin receptors are reported to be involved in the etiology of EAE (Dutra et al., 2011; Gobel et al., 2011; Schulze-Topphoff et al., 2009), and also directly modulate cognitive impairments in experimental models of neurodegenerative disease, such as Alzheimer's disease (Prediger et al., 2008), and some kinin-induced effects depends of cholinergic and sensory nerve stimulation (Abraham et al., 2006), herein we hypothesize that the KKS may play a key role in modulating the development of cognitive impairments in MS by decreasing neuroinflammation and mitigating cholinergic system dysfunction.

2. Materials and methods

2.1. Experimental animals

Experiments were conducted using female C57BL/6 wild-type, kinin B_1R-knockout ($B_1R^{-/-}$), kinin B_2R-knockout ($B_2R^{-/-}$) and kinin B_1B_2R-knockout ($B_1B_2R^{-/-}$) mice (6–10 weeks old). Deletion of the entire coding sequences of the kinin receptors was achieved according to previously described methods (Dutra et al., 2011; Pesquero et al., 2000). $B_1R^{-/-}$-, $B_2R^{-/-}$- and $B_1B_2R^{-/-}$-deficient mice on the 129/SvJ background were backcrossed to C57BL/6 to produce F_{10} offspring. Following the F_{10} offspring the line is 99% genetically identical to the recipient strain (C57BL/6) and is considered congenic with it; therefore we maintained this linage by crossing them with each other. The C57BL/6 animals were used as controls. The mice were kept in groups of six to nine animals per cage, maintained under controlled temperature (22 ± 1 °C), with a 12 h light/dark cycle (lights on at 7:00 a.m.) and were given free access to food and water. All procedures used in the present study followed the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23) and were approved by the Animal Ethics Committee of the Universidade Federal de Santa Catarina (CEUA-UFSC, protocol number 23080038266/2008-43).

2.2. EAE induction

Experimental autoimmune encephalomyelitis (EAE) was induced by subcutaneous (s.c.) immunization into the flanks with 200 μl of an emulsion containing 200 μg MOG_{35-55} peptide and 500 μg *Mycobacterium tuberculosis* extract H37Ra in incomplete Freund's adjuvant oil, as previously described (Stromnes and Goverman, 2006). In addition, the animals received 300 ng of *Pertussis* toxin intraperitoneally (i.p.) on day 0 and day 2 post-immunization. The animals were monitored daily and neurological impairment was quantified using a clinical scale after day 1 post-immunization (Stromnes and Goverman, 2006): 0, no signs of disease; 1, loss of tone in the tail; 2, hindlimb paresis; 3, hindlimb paralysis; 4, tetraplegia and 5, moribund and/or death.

2.3. Behavioral tests

2.3.1. Rotarod test

In order to evaluate motor coordination, the mice were placed on a rotarod apparatus at a fixed rotational speed of 4 rpm, as previously described (Dutra et al., 2011) (Fig. 1A). The maximum time for each trial was set at 60 s. Rotarod training was performed prior to disease induction and consisted of three consecutive trials in which the animals became familiar with the task. After disease induction, the mice were repeatedly tested at different time points until day 25 post-immunization.

2.3.2. Object location task

After seven days of disease induction, the spatial memory of mice was assessed using the object location task (Fig. 1A). The task