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Altered hippocampal synaptic transmission in transgenic mice with astrocyte-targeted enhanced CCL2 expression

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ABSTRACT

Elevated expression of neuroinflammatory factors in the central nervous system (CNS) contributes to the cognitive impairment in CNS disorders such as injury, disease and neurodegenerative disorders. However, information on the role of specific neuroimmune factors in normal and abnormal CNS function is limited. In this study, we investigated the effects of chronic exposure to the chemokine CCL2 on hippocampal synaptic function at the Schaffer collateral–CA1 synapse, a synapse that is known to play an important role in cognitive functions such as memory and learning. Synaptic function was measured in vitro using hippocampal slices obtained from transgenic mice that express elevated levels of CCL2 in the CNS through astrocyte expression and their non-transgenic littermate controls. Extracellular field potential electrophysiological recordings showed a significant reduction in the magnitude of synaptic responses in hippocampal slices from the CCL2 transgenic mice compared with slices from non-transgenic littermate controls. Two forms of short-term synaptic plasticity (post-tetanic potentiation and short-term potentiation) thought to be important cellular mechanisms of short-term memory were enhanced in hippocampal slices from CCL2 transgenic mice compared to non-transgenic hippocampal slices, whereas long-term synaptic plasticity (LTP), which is critical to long-term memory formation, was not altered. Western blot analysis of hippocampus from the CCL2 transgenic mice and non-transgenic mice showed no change in level of neuronal specific enolase, a neuronal specific protein, GFAP, an astrocyte specific protein, and several synaptic proteins compared with non-transgenic littermate controls. These results show that CCL2, which is known to be chronically produced at elevated levels within the CNS in a number of CNS disorders, can significantly alter hippocampal function and implicate a role for CCL2 in the cognitive dysfunction associated with these CNS disorders.

1. Introduction

It is now recognized that chemokines, a group of small signaling proteins that are members of the cytokine family of inflammatory factors, are produced within the CNS and can play an important role in normal CNS function and development as well as in CNS disease and injury (Cartier et al., 2005; Ubogu et al., 2006). The primary CNS cell types that produce chemokines are astrocytes and microglia, although neurons also produce chemokines under some conditions (Flugel et al., 2001; Rock et al., 2004). Chemokines are classified into four basic subfamilies, primarily based on the position of specific conserved cysteine residues in the N-terminal structure: α-(or CXC) chemokines, β-(or CC) chemokines (e.g., CCL2), γ-(or C) chemokines and δ-(or CX_3C) (Murphy et al., 2000). Our studies focus on the β-chemokine CCL2 (CC chemokine ligand 2, previously known as monocyte chemoattractant protein-1 or MCP-1), a small secreted protein.

Chemokines were first described in the immune system where they play a role in host immune surveillance, directing leukocyte traffic to sites of inflammation or injury, a role that they also play in the CNS (Miller et al., 2008). Additional roles for chemokines as signaling molecules in the CNS are now emerging, although information is still limited. For example, recent studies show a physiological role for CXCL12 in CNS development. In these studies, selective deletion of either CXCL12 (SDF-1) or its receptor CXCR4, which have a monogamous interaction, disrupts the normal migration of cerebellar granule neurons and leads to abnormal formation of the cerebellum (Ma et al., 1998; Tran and Miller, 2003; Zhu et al., 2002). The CXCR4 deficient mice show numerous deficiencies (Ma et al., 1998). A similar role for CXCL12/CXCR4 was demonstrated for the migratory process occurring during morphogenesis of the dentate gyrus of the hippocampus (Lu et al., 2002). Evidence for a role of CXCL12 in memory function has also appeared. Thus, in

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studies of a mouse model of Alzheimer's disease, CXCL12 levels were down-regulated, coincident with the expression of cognitive deficits (Parachikova and Cotman, 2007).

In contrast to CXCL12, relatively little is known about the actions of CCL2 in the CNS. CCL2 is expressed in the healthy CNS (Foresti et al., 2009; Little et al., 2006; Madrigal et al., 2010; Meng et al., 1999) but a physiological role for CCL2 in the CNS has yet to be established. However, recent studies show that elevated levels of CCL2 occur in the CNS parenchyma or cerebral spinal fluid (CSF) in CNS disease, injury, and neurological and behavioral disorders suggesting a role for CCL2 in these conditions. For example, elevated levels of CCL2 in the CNS were shown to occur in multiple sclerosis (Mahad and Ransohoff, 2003; Sorensen et al., 1999), CNS trauma (Little et al., 2002; Muessel et al., 2002; Rhodes et al., 2009; Stefini et al., 2008), stroke (Losy and Zaremba, 2001), epilepsy (Foresti et al., 2009; Wu et al., 2008), depression (Sutcigil et al., 2007), Alzheimer's disease (Ishizuka et al., 1997; Sokolova et al., 2009), viral and bacterial infection (Dhillon et al., 2008; Klein et al., 2006; Ramesh et al., 2009; Tribouillard-Tanvier et al., 2009) and cancer (Kielian et al., 2002; Sato et al., 1995). Correlative studies indicate that CCL2 is an important factor in the cognitive dysfunction associated with several of these disorders. The increased levels of CCL2 in the CSF of HIV-infected individuals correlate with the level of viral load and severity of dementia (Kelder et al., 1998). Increased levels of CCL2 in the CSF also correlate with cognitive deficits in older Alzheimer's patients (Galimberti et al., 2006). CSF levels of CCL2 were found to significantly increase with the age of patients with and without neuropsychiatric disease (Blasko et al., 2006), suggesting that CCL2 plays an important role in the detrimental effects of aging on the CNS. A correlation of CSF levels of CCL2 and neuropsychiatric syndromes has also been reported for conditions where the primary insult occurs outside of the CNS. For example, CSF levels of CCL2 were significantly higher in patients with systemic lupus erythematosus showing neuropsychiatric symptoms than those without neuropsychiatric symptoms (Iikuni et al., 2006; Okamoto et al., 2010).

Studies in experimental animals support a role for CCL2 in the impaired CNS function associated with CNS pathology. For example, neurological impairments such as abnormal gait and diminished righting reflex were reported for transgenic mice (7–15 months of age) that express elevated levels of CCL2 in the CNS under the control of the human GFAP promoter (Huang et al., 2005). Consistent with these results, injection of CCL2 into brain ventricles of adult rats produced altered motor activity (Banisadr et al., 2002). Studies of bigenic mice constructed by crossing an Aβ deposition mouse model (Tf2576) of Alzheimer's disease with a CCL2 overexpressing mouse showed enhanced behavioral deficits, altered hippocampal synaptic transmission, and altered Aβ metabolism in the bigenic mice, suggesting that CCL2 accelerates the detrimental effects of Aβ on the brain (Kiyota et al., 2009a,b).

Several studies have implicated a role for CCL2 in alcohol use disorders. Thus, in behavioral tests both CCL2 and CCR2 (the receptor for CCL2) null mice appear normal but show lower preference for alcohol and lower alcohol consumption than wildtype mice, suggesting a potential role for CCL2 in the motivational aspects of alcohol consumption (Blednov et al., 2005). The levels of mRNA and protein for CCL2 are increased in the CNS of normal mice subjected to either single or repetitive alcohol doses and the elevated levels persist for days after repetitive alcohol exposure, suggesting that mechanisms regulating levels of CCL2 in the CNS are particularly sensitive to alcohol (Qin et al., 2008). Intraperitoneal injections of alcohol increased the levels of CCL2 mRNA in the hippocampus but not the corpus striatum, suggesting regional sensitivity to this effect of alcohol (Breese et al., 2008). Increased levels of CCL2 have been observed in several regions of the brain of alcoholics, including the hippocampus (He and Crews, 2008). Research has identified that the hippocampus is one of several brain regions that play a central role in the cognitive deficits produced by alcohol abuse (Matsumoto et al., 2007; Ryabinin, 1998). Thus, actions of CCL2 in the hippocampus could play an important role in alcohol use disorders. In addition to a role in alcoholism, morphine exposure has been shown to up-regulate the expression of CCL2 in cultures of human brain neurons (Rock et al., 2006) but down-regulate the expression of CCL2 in human astrocytes (Mahajan et al., 2005), results that may forecast a potential role for CCL2 in opiate abuse disorders.

Although these and other studies provide strong evidence for a role of CCL2 in a variety of CNS disorders, relatively little is known about the effects of elevated levels of CCL2 on neuronal function in the CNS. To address this issue, we have investigated the effects of chronically elevated levels of CCL2 on synaptic function in the hippocampus, a CNS region that is essential for cognitive functions such as short- and long-term memory. For these studies we employed transgenic mice that express elevated levels of CCL2 in the CNS through astrocyte expression under the control of the human GFAP promoter (Huang et al., 2005). Results show that chronic in vivo exposure to CCL2 significantly alters synaptic transmission and plasticity in the hippocampus. These results are consistent with an important role for CCL2 in the impaired CNS function observed in CNS disorders associated with increased levels of CCL2 in the CNS.

2. Methods

2.1. Animals

The construction of the CCL2 transgenic mice (CCL2-tg) was carried out as described by Huang et al. (2005). Briefly, the murine CCL2 gene was placed under control of the huGFAP promoter and purified GFAP–CCL2 fusion gene fragment injected into fertilized eggs of the SWXJ (H-21^{g,s}) mice using standard procedures. The transgenic animals and their progeny were identified by analysis of the tail DNA and used to develop huGFAP–CCL2-tg mice on a SJL background. Heterozygous mice of the CCL2-tg line (SJL background) were used for experiments; age matched littermates that did not express the transgene (non-tg) were used as controls.

The CCL2-tg mice have been extensively characterized in the unmanipulated state and in disease models (Bennett et al., 2003; Elhofy et al., 2005; Huang et al., 2002, 2005; Kiyota et al., 2009b). Characterization of the CCL2-tg mice in the unmanipulated state (Huang et al., 2005) showed that transgenic mice younger than 6 months of age appear normal. Transgenic mice from 7 to 15 show mild perivascular infiltrates and impaired blood–brain barrier but no consistent evidence of cell, axon or synapse loss. At these older ages, microglial activation was evident morphologically, but levels of MHC-II, CD11b, CD11c, CD40 and CD45 were not upregulated indicating that CCL2 did not induce the microglia to express features of antigen presenting cells (APCs). Neurological impairment was observed in the older mice and consisted of weight loss, postural changes, difficulty in the righting reflex, limb weakness and hindlimb paralysis.

2.2. Genotyping

Genotyping was carried out as described previously (Huang et al., 2005) using standard protocols. Briefly, DNA was isolated using the Easy DNA Kit (Invitrogen Life Technologies, Carlsbad, CA, USA) from tail samples obtained from individual animals at weaning. Expression of the human GFAP promoter-murine CCL2 transgene was identified by PCR of the genomic DNA with primers