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Transgenic mice with increased astrocyte expression of IL-6 show altered effects of acute ethanol on synaptic function

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ABSTRACT

A growing body of evidence has revealed that resident cells of the central nervous system (CNS), and particularly the glial cells, comprise a neuroimmune system that serves a number of functions in the normal CNS and during adverse conditions. Cells of the neuroimmune system regulate CNS functions through the production of signaling factors, referred to as neuroimmune factors. Recent studies show that ethanol can activate cells of the neuroimmune system, resulting in the elevated production of neuroimmune factors, including the cytokine interleukin-6 (IL-6). Here we analyzed the consequences of this CNS action of ethanol using transgenic mice that express elevated levels of IL-6 through increased astrocyte expression (IL-6-tg) to model the increased IL-6 expression that occurs with ethanol use. Results show that increased IL-6 expression induces neuroadaptive changes that alter the effects of ethanol. In hippocampal slices from non-transgenic (non-tg) littermate control mice, synaptically evoked dendritic field excitatory postsynaptic potential (fEPSP) and somatic population spike (PS) at the Schaffer collateral to CA1 pyramidal neuron synapse were reduced by acute ethanol (20 or 60 mM). In contrast, acute ethanol enhanced the fEPSP and PS in hippocampal slices from IL-6 tg mice. Long-term synaptic plasticity of the fEPSP (i.e., LTP) showed the expected dose-dependent reduction by acute ethanol in non-tg hippocampal slices, whereas LTP in the IL-6 tg hippocampal slices was resistant to this depressive effect of acute ethanol. Consistent with altered effects of acute ethanol on synaptic function in the IL-6 tg mice, EEG recordings showed a higher level of CNS activity in the IL-6 tg mice than in the non-tg mice during the period of withdrawal from an acute high dose of ethanol. These results suggest a potential role for neuroadaptive effects of ethanol-induced astrocyte production of IL-6 as a mediator or modulator of the actions of ethanol on the CNS, including persistent changes in CNS function that contribute to cognitive dysfunction and the development of alcohol dependence.

1. Introduction

Neuroimmune factors play critical roles in homeostatic regulation of CNS function, neuronal development, defense against insult and infection, and repair mechanisms. Abnormal production of neuroimmune factors is considered an important contributing factor to many neuropsychiatric and neurologic condition, such as major depression (Sukoff Rizzo et al., 2012; Young et al., 2014), dementia (Trapero and Cauli, 2014), Alzheimer's disease (Luterman et al., 2000), epilepsy (Li et al., 2011), schizophrenia (Schwieler et al., 2015), autism (Wei et al., 2013), sleep disturbances (Zhu et al., 2012), infection (Jurgens et al., 2012), and trauma (Lloyd et al., 2008). Importantly, recently studies have identified that excessive alcohol (ethanol) exposure, which is known to produce cognitive impairment, induces elevated glial production of IL-6 and other neuroimmune factors (Alfonso-Loeches et al., 2010; Doremus-Fitzwater et al., 2014). Excessive alcohol use is often comorbid with neuropsychiatric and neurologic conditions, and is thought to impact negatively on these conditions (e.g., depression (Briere et al., 2014), epilepsy (Shield et al., 2013), trauma (Kachadourian et al., 2014), HIV infection (Silverstein et al., 2014)). Thus, insight into actions of IL-6 and interactions between IL-6 and ethanol are an important step toward an understanding CNS mechanism involved in the effects of ethanol on the brain and potential interactions with mechanisms underlying

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neuropsychiatric and neurologic conditions co-morbid with alcohol use disorders.

A number of in vivo and in vitro studies have established that both acute and chronic ethanol alter CNS expression of IL-6 and other neuroimmune factors, primarily due to an action of ethanol on astrocytes and microglia. These neuroimmune factors include the proinflammatory cytokines IL-6, IL-1β and TNF-α and the chemokine CCL2 (Alfonso-Loeches et al., 2010; Blanco et al., 2005; He and Crews, 2008; Kane et al., 2013, 2014; Lippai et al., 2013; Qin and Crews, 2012; Tiwari et al., 2009; Valles et al., 2004; Vongvatcharanon et al., 2010; Ward et al., 2009; Zhang et al., 2014; Zhao et al., 2013). Ethanol-induced CNS expression of these neuroimmune factors appears to depend on brain region, cell type, and/or dose, duration and method of ethanol exposure. In recent studies acute ethanol (4 g/kg, i.p.) was shown to produce a prominent and prolonged increase (3–9 h) in the level of IL-6 mRNA in the hippocampus of rats, whereas the level of TNF-α mRNA was reduced, and the level of IL-1 β mRNA was unaffected (Doremus-Fitzwater et al., 2014). Chronic ethanol (6 g/kg per day for 10 days, by gavage) increased IL-6 mRNA levels in the CNS of adult mice, but only in cerebellum, whereas increased levels of CCL2 mRNA were observed in hippocampus, cerebellum and cortex, and TNF-α mRNA levels were not altered in any of these three CNS regions (Kane et al., 2014). Chronic ethanol consumption (13 g/kg per day for 5 months) induced a toll-like receptor 4 (TLR4) response in the cortex of mice that was associated with astrocyte activation and production of IL-6 mRNA, and IL-1 and TNF-α protein, an effect that was not observed in TLR4 knockout mice (Alfonso-Loeches et al., 2010). Increased levels of IL-6, IL-1β and TNF-α protein in the CNS of mice were also observed following three weeks of ethanol consumption using a two-bottle choice drinking paradigm (Zhang et al., 2014). Both acute (100 mM, 24 h) and chronic (50 mM, 7 day) ethanol increased IL-6 production in primary cultures of cortical astrocytes, whereas there was no effect of ethanol on TNF-α production (Sarc et al., 2011). Ethanol (50–100 mM, 24 h) also increased secretion of cytokines in primary cultures of cerebral microglia, including IL-6, TNF-α, MIP-1-α, and MIP-2 (Boyadjieva and Sarkar, 2010).

While these and other studies have established that both acute and chronic ethanol alter CNS expression of neuroimmune factors, little is known about the consequences of this action of ethanol and underlying mechanisms. Fundamental to this issue is an understanding of how neuroimmune factors affect basic neuronal functions such synaptic transmission and cell excitability, which are target sites of ethanol action, and if or how the actions of neuroimmune factors and ethanol interact.

In the hippocampus, ethanol has been shown to alter hippocampal function through modifications of cellular mechanisms mediating synaptic transmission and plasticity (Peris et al., 1997; Weiner and Valenzuela, 2006; White and Swartzwelder, 2004; Zorumski et al., 2014). IL-6 and other proinflammatory cytokines (e.g. IL-1β and TNF-α) also alter hippocampal synaptic transmission and plasticity (Beattie et al., 2002; Bellinger et al., 1993; Coogan and O'Connor, 1997; Ikegaya et al., 2003; Katsuki et al., 1990; Li et al., 1997; Nelson et al., 2012; O'Connor and Coogan, 1999; Pribiag and Stellwagen, 2013; Steffensen et al., 1994; Stellwagen and Malenka, 2006; Tancredi et al., 2000, 1992; Wheeler et al., 2009; Yang et al., 2005; Zhang et al., 2010). This commonality of ethanol and cytokine action raises the possibility that increased levels of proinflammatory cytokines produced by ethanol could result, either directly or indirectly, in transient or long-term neuroadaptive changes that then alter the effects of ethanol. Our studies focus on this possibility.

In the current studies, we utilized transgenic mice and their non-tg littermate controls to determine if elevated CNS expression of IL-6 can lead to neuroadaptive changes that alter the effects of acute ethanol on hippocampal synaptic function. The transgenic mice model the increased CNS expression of IL-6 that would occur with ethanol use. Expression of elevated levels of IL-6 in the CNS of the IL-6 tg mice was achieved through genetic manipulation of astrocyte expression (Campbell et al., 1993). Astrocytes are the most abundant cell type in the CNS and produce IL-6 both under physiological and pathological conditions (Gruol and Nelson, 1997). Ethanol has been shown to cause increased astrocyte production of IL-6 protein (Alfonso-Loeches et al., 2010; Sarc et al., 2011). Thus, the consequences of the increased astrocyte production of IL-6 in the IL-6 tg mice can inform on potential consequences of ethanol-induced increase in astrocyte production of IL-6.

Expression of IL-6 in the IL-6 tg mice is under control of the GFAP promoter. GFAP mRNA expression, which is regulated by IL-6 acting through STAT3, and GFAP protein do not become prominent until about 1 month postnatal, suggesting that IL-6 production is not prominent until about 1 month postnatal (Sanz et al., 2008). Thus, results from this model are likely to be most relevant to alcohol use that starts in the juvenile or adolescent stage of life, a pattern of alcohol use that has significant risk for developing alcohol dependence and is currently an important societal issue (Foltran et al., 2011; Hingson et al., 2006). Studies in animal models have shown that ethanol exposure increases IL-6 levels in the CNS of adolescents as well as adults (Doremus-Fitzwater et al., 2015).

The highest number of transgene-expressing astrocytes in the forebrain region of the IL-6 tg mice occurs in the hippocampus (Vallieres et al., 2002), making it likely that neuroadaptive effects on synaptic transmission will be evident in this CNS region. The hippocampus expresses one of the highest levels of mRNA for IL-6 receptors (IL-6R), which mediate cellular effects of IL-6, suggesting an important role for IL-6 in this CNS region (Gadient and Otten, 1994; Schobitz et al., 1993). Both neurons and glia cells of the hippocampus express IL-6R (Schobitz et al., 1993; Vollenweider et al., 2003). Astrocytes are a key regulator of synaptic function (Halassa et al., 2007) and astrocyte produced IL-6 or exogenously applied IL-6 has been shown to alter (i.e., depress) hippocampal synaptic plasticity at the Schaffer collateral to CA1 neuron synapse (Balschun et al., 2004; Li et al., 1997; Tancredi et al., 2000). Interestingly, synaptic plasticity at this synapse is also depressed by acute ethanol (Blitzer et al., 1990; Fujii et al., 2008; Izumi et al., 2005; Schummers et al., 1997; Sinclair and Lo, 1986), and these actions of ethanol are thought to contribute to the impairment in memory and learning produced by ethanol.

Our previous studies showed that synaptic transmission at the Schaffer collateral to CA1 pyramidal neuron synapse is enhanced in the IL-6 tg mice, consistent with the expression of persistent neuroadaptive changes in synaptic function in the IL-6 tg mice (Nelson et al., 2012). These neuroadaptive changes in synaptic function may be related to the enhanced susceptibility of the IL-6 tg mice to NMDA- and kainate-induced seizure activity (Samland et al., 2003). Ethanol exposure also results in increased susceptibility to seizure activity, as a consequence of transient or persistent ethanol-induced neuroadaptive changes that render the CNS dependent on the presence of ethanol. When ethanol exposure is terminated, a synaptic imbalance is created that results in hyperexcitability and increased susceptibility to seizure activity (Becker, 2000; Heilig et al., 2010). Thus, neuroadaptive changes in the CNS of the IL-6-tg mice could influence the effects of ethanol on synaptic function and susceptibility to ethanol-induced hyperexcitability/seizure activity, possibilities that we have assessed in the current studies. Results are consistent with interactions between the neuroadaptive changes in the CNS of IL-6 tg mice and the effects of ethanol, and suggest that ethanol-induced astrocyte production of IL-6 may play an important role as a mediator or modulator of CNS actions of