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Research Report

Amino acid transport system A is involved in inflammatory nociception in rats

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ABSTRACT

Previous studies have indicated that central sensitization is a state of increased excitability of nociceptive neurons in the spinal dorsal horn following peripheral tissue injury and/or inflammation and astrocytes play an important role in the central sensitization. The current study investigated the role of amino acid transport system A in central sensitization and hyperalgesia induced by intraplantar injection of formalin in rats. Formalin (5%, 50 μl) injected subcutaneously into the unilateral hindpaw pad induced typical biphase nociceptive behaviors, including licking/biting and flinching of the injected paw and an increase of glial fibrillary acid protein (GFAP, an activated astrocyte marker) expression in spinal dorsal horn, and these effects could be attenuated by intrathecal injection of the competitive inhibitor of amino acid system A transporter, methylaminoisobutyric acid (MeAIB, 0.1, 0.3, 0.5, and 0.7 mmol), in a dose-dependent manner. Intrathecal injection of vehicle (PBS) had no effect on the formalin-induced nociceptive behaviors and increase of the GFAP. These findings suggest that amino acid transport system A is involved in inflammation-induced nociception, and inhibition of this transporter system results in inhibition of the central sensitization and hyperalgesia.

1. Introduction

Previous studies have shown that peripheral injury or inflammation activates both neuronal and non-neuronal (glial) components of the peripheral and central cellular circuitry and that the neuron–glia interactions contribute to pain hypersensitivity in the development of central sensitization and hyperalgesia (Scholz and Woolf, 2007; Watkins et al., 2007). An important function of astrocytes is mediating the glutamate–glutamine cycle, which is involved in the plasticity underlying the generation of central sensitization, hyperalgesia and chronic pain (Hertz et al., 1999; Muscoli et al., 2010; Tsuboi et al., 2011). The glutamate–glutamine cycle converts glutamate to glutamine by the enzyme glutamine synthetase

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after glutamate uptake from the synaptic cleft, and the glutamine is extruded from astrocytes via the system N transporter, and is subsequently taken up by neuronal presynaptic terminals through a system A transporter where it is then converted to glutamate and repackaged into synaptic vesicles for release (Chaudhry et al., 1999, 2002). A recent study has demonstrated that the hyperexcitability of trigeminal nociceptive neurons is attenuated by application of methionine sulfoximine (MSO), an inhibitor of astroglial glutamine synthetase that catalyzes the conversion of glutamate to glutamine (Chiang et al., 2007).

Importantly, in the glutamate–glutamine cycle, amino acid transport system A is responsible for the accumulation of glutamine by neurons (Armano et al., 2002; Varoqui et al., 2000) and is characterized by its ability to bind and transport N-methylated amino acids (Rae et al., 2003), and therefore may play a critical role in the development of central sensitization. The non-metabolized amino acid analog methylaminoisobutyric acid (MeAIB) has thus been used in many studies to identify the system transporter A activity and, indeed, appears to be a specific inhibitor of all isoforms of this transporter (Broer and Brookes, 2001). Chiang et al. (2008) have demonstrated that continuous intrathecal superfusion of MeAIB can suppress mustard oil-induced sensitization of trigeminal caudalis nociceptive neurons, but comparable studies in behavioral models of persistent inflammatory pain are not available. Formalin injected into rat hindpaw induced persistent inflammatory nociceptive behaviors believed to be a result of central sensitization (Bittencourt and Takahashi, 1997; Chen and Koyama, 1998; Malmberg and Yaksh, 1992; Qin et al., 2006; Sweitzer et al., 1999). Therefore, the present study was designed to determine whether intrathecal administration of MeAIB reduces the central sensitization induced in the formalin test, and whether such effects are mediated through astroglia activation.

2. Results

2.1. *Effect of intrathecal MeAIB on formalin-induced nociceptive behavior*

As reported previously (Abbott et al., 1995; Xie et al., 2004), intraplantar injection of 5% formalin induced typical biphasic nociceptive behaviors (flinching and licking/biting of the injected paw). The early phase (phase I) began immediately after injection and lasted 5 min. After a short quiescent period (15 min), a prolonged tonic response ensued, persisting for over 45 min (phase II). The peak response appeared approximately 30 min after injection. Intrathecal administration of the vehicle (PBS, 10 μl) did not alter the formalin-induced nociceptive behaviors; the duration of licking/biting and the number of flinches were not significantly different from animals that received the formalin injection alone ($P>0.05$, $n=6$), either in phase I or phase II (Figs. 1c and d).

However, intrathecal administration of MeAIB (0.1, 0.3, 0.5 and 0.7 mmol; 10 μl for each dose; $n=6$), an inhibitor of the amino acid system A transporter, 10 min prior to the formalin injection significantly depressed the formalin-induced nociceptive behaviors, in a dose-dependent manner ($r=0.992$, $P<0.001$ for the licking/biting response; $r=0.942$, $P=0.017$ for the flinching response), during the 60-min observation period with an ED_{50} of 0.58 mmol (Figs. 1a and b). As shown in Figs. 1c and d, the time course curves for the two different treatments (i.e., for the control (PBS) and the different MeAIB dose groups) were significantly different from each other ($F_{(4,\ 275)}=10.847$, $P<0.001$ for duration of licking/biting; $F_{(4,\ 275)}=11.333$, $P<0.001$ for number of flinches), across time ($F_{(11,\ 275)}=56.438$, $P<0.001$; $F_{(11,\ 275)}=42.822$, $P<0.001$) and in their interactions ($F_{(44,\ 275)}=3.223$, $P<0.001$; $F_{(44,\ 275)}=3.500$, $P<0.001$). Further analyses revealed that a decrease in licking/biting occurred during phase II ($P<0.05$), but not during phase I ($P>0.05$, Figs. 1c and e), while a decrease in flinching occurred in both phases I and II ($P<0.05$, Figs. 1d and f). Detailed comparisons of the individual time points for the different treatments are shown in Figs. 1c and d. In addition, we found that when the dose of MeAIB was 1.0 mmol or higher, the nociceptive behaviors were completely inhibited; and these higher doses were occasionally associated with the appearance of lethargy or convulsions in some rats (data not shown).

2.2. *Effect of intrathecal MeAIB on formalin-induced spinal GFAP expression*

After completion of the behavioral experiments (i.e., 1 h after formalin injection), the expression of GFAP, a marker of activated astrocytes, in the spinal dorsal horn, L4–5, was examined in animals from the various groups. As shown in Fig. 2, GFAP-immunoreactivity was very low in normal control rats, and the astrocytes were evenly distributed in all laminae of the bilateral spinal dorsal horns and possessed small, thin processes (Figs. 2a and a′). However, injection of formalin into the right hindpaw pad significantly increased GFAP expression in the ipsilateral, but not in the contralateral, spinal dorsal horn. GFAP immunoreactivity significantly increased from laminae I to VI, particularly between laminae I and II, and the astrocytes had a significantly hypertrophied morphology, with elongated processes (Figs. 2b and b′). Intrathecal administration of the vehicle (PBS) did not significantly affect the formalin-induced increase in GFAP expression (Fig. 2c). As shown in Fig. 3b, the integrated optical density (IOD) values for the formalin alone and PBS+formalin groups increased to 282.41 and 292.09% of the normal control group value, respectively ($H=12.098$, $P=0.002$, $n=6$ for each group, one-way ANOVA on ranks test).

However, intrathecal administration of MeAIB (0.1, 0.3, 0.5 and 0.7 mmol, in 10 μl for each dose, $n=6$) 10 min prior to intraplantar formalin injection attenuated the formalin-induced increase in GFAP expression, as indicated by the IOD values, in a dose-dependent manner ($r=0.970$, $P=0.006$, $ED_{50}=0.54$ mmol; Fig. 3a). Astrocytes in the spinal dorsal horn displayed a less hypertrophied morphology and had relatively shorter processes. One-way ANOVA indicated that the GFAP immunoreactivity, assessed using IOD value, was significantly different between the various treatment groups ($F_{(4,\ 25)}=78.358$, $P<0.001$). Post hoc comparisons revealed that the 0.3, 0.5 and 0.7 mmol MeAIB groups exhibited significantly lower GFAP immunoreactivity than the PBS+formalin group ($P<0.01$ and $P<0.001$), but the 0.1 mmol MeAIB group was not significantly different from the PBS+formalin group ($P>0.05$), as shown in Fig. 3b.