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BRAIN RESEARCH

Research Report

Involvement of cellular prion protein in the nociceptive response in mice

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ARTICLE INFO

Article history:
Accepted 8 March 2007
Available online 13 March 2007

Keywords:
Cellular prion protein
Thermal and chemical nociception
Inflammation

ABSTRACT

The role of the cellular prion protein (PrP^c) in neuronal functioning includes neuronal excitability, cellular adhesion, neurite outgrowth and maintenance. Here we investigated the putative involvement of the PrP^c function on the nociceptive response using PrP^c null ($Prnp^{0/0}$) and wild-type ($Prnp^{+/+}$) mice submitted to thermal and chemical models of nociception. PrP^c null mice were more resistant than wild-type mice to thermal nociception of the tail-flick test. However, no significant difference was found on the hot plate test. In the acetic acid-induced visceral nociception, PrP^c null mice showed an enhanced response when compared to wild-type mice. However, there was no difference between $Prnp^{0/0}$ and wild-type mice on glutamate- and formalin-induced licking behaviour and Freund's Complete Adjuvant (FCA)-induced mechanical allodynia. PrP^c null mice developed significantly lower paw edema than wild-type mice. In addition, the visceral conditioning stimuli produced by a previous injection of acetic acid (20 days before testing) significantly reduced early and late phases of formalin-induced nociception in wild-type mice. In contrast, the same pre-treatment did not alter the formalin response in PrP^c null mice. These results indicate a role of PrP^c in the nociceptive transmission, including the thermal tail-flick test and visceral inflammatory nociception (acetic acid-induced abdominal constriction). Our findings show that PrP^c is involved with a response mediated by inflammation (paw edema) and by visceral conditioning stimuli.

1. Introduction

Cellular prion protein (PrP^c) is a glycosyl-phosphatidylinositol anchored cell surface glycoprotein that is mainly expressed in neurons and, to a lesser extent, in other types of cells. An alteration in the PrP^c secondary structure, with a much higher proportion of beta-sheet conformational domains, leads to an accumulation of the abnormal protein prion scrapie. This

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doi:10.1016/j.brainres.2007.03.024

results in spongiform encephalopathy, in humans and animals (Prusiner, 1998).

Recent studies have investigated the physiological function of PrP^c (Martins et al., 2002; Walz et al., 2002). Animals in which the PrP^c gene (*Prnp*) was constitutively ablated presented an enhance in the neuronal excitability *in vitro*, either in constitutively or post-natal PrP^c null mice (Walz et al., 2002; Colling et al., 1996; Collinge et al., 1994; Maglio et al., 2004; Mallucci et al., 2002). These animals also showed a higher sensitivity to seizures *in vivo* (Walz et al., 1999). The clearance of extracellular glutamate through astrocytic uptake was decreased in PrP^c null mice (Brown and Mohn, 1999). However, PrP^c is not directly related to neuronal glutamate uptake or release (Thais et al., 2006). PrP^c is also implicated in the protection against oxidative stress (Brown, 2001; Klamt et al., 2001), modulation of neuronal apoptosis (Chiarini et al., 2002; Lopes et al., 2005; Zanata et al., 2002), cellular adhesion, neurite outgrowth (Lopes et al., 2005; Graner et al., 2000a) and maintenance (Walz et al., 2002; Graner et al., 2000b).

Although most findings concerning PrP^c physiology are related to the nervous system, studies have found a role for PrP^c in antioxidant protection not only in the brain, but also in the liver, muscle and heart (Klamt et al., 2001). It has recently been shown that PrP^c modulates phagocytosis and the inflammatory response *in vitro* and *in vivo* (Almeida et al., 2005).

Related cases of spongiform encephalopathy in humans reported that patients presented with a progressive increase in pain (Lundberg, 1998; Sugai et al., 2000). Although a higher pain sensibility was attributed to nerve degeneration (Sugai et al., 2000), how the alteration in PrP^c structure affects responsiveness to pain is still unclear. In an attempt to approach the PrP^c role in pain transmission, the present study investigated the nociceptive response in PrP^c null and wild-type mice that were submitted to thermal and chemical models of nociception.

2. Results

2.1. *Thermal nociceptive response to hot-plate and tail-flick test*

Acute nociceptive response evoked by a noxious heat stimulus in the tail-flick assay displayed different responses between PrP^c null and wild-type mice. Fig. 1A illustrates that PrP^c null mice were more resistant than wild-type mice to the thermal stimulus [$F(1,11)=7.1$, $P<0.05$]. No significant differences were found between PrP^c null and wild-type mice on the hot-plate nociceptive heat stimulus (50.5, 55.0 and 58.0 °C) [$F(1,36)=3.73$, $P>0.05$]. Two-way analysis of variance (ANOVA) revealed no significant interaction between temperature and genotype [$F(2,36)=0.48$, $P>0.05$] (Fig 1B).

2.2. *Abdominal constriction induced by acetic acid*

The response to acetic acid-induced visceral nociception was increased in PrP^c null mice in the first 20 min after acetic acid administration. The number of abdominal constrictions was significantly different among the groups at 5 min [$F(1,10)=20.9$, $P<0.01$], 10 min [$F(1,10)=8.4$, $P<0.05$] and 15 min [$F(1,10)=28.7$, $P<0.001$] (Fig. 2). The total amount of abdominal constrictions in 20 min of observation is shown in the inset of Fig. 2 [$F(1,10)=225.5$, $P<0.001$].

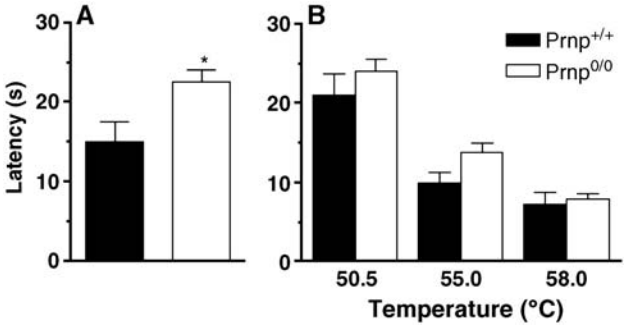

Fig. 1 – Response to thermal noxious stimuli in tail-flick (A) and hot-plate tests (B). The black columns (*Prnp*$^{+/+}$) and white columns (*Prnp*$^{0/0}$) represent the mean ± SE of seven animals. A one-way ANOVA was performed in A and a two-way ANOVA (considering genotype and temperature) was performed in B. The asterisk denotes significant difference from *Prnp*$^{+/+}$ mice by Student's *t*-test (P*<0.05).**

2.3. *Mice nociceptive response induced by intraplantar injection of glutamate and formalin*

The direct activation of peripheral sensorial fibers C by intraplantar injection of glutamate caused a licking behaviour that was similar between PrP^c null and wild-type mice [$F(1,14)=0.21$, $P>0.05$; Fig. 3A]. The intraplantar treatment with formalin exhibited the same profile between both genotype [$F(1,14)=0.47$, $P>0.05$] in the early phase and [$F(1,14)=0.02$, $P>0.05$] in the late phase (Fig. 3B).

2.4. *Paw edema and allodynia induced by FCA*

The intraplantar injection of FCA produced a profound and long-lasting mechanical allodynia in the injected paw of PrP^c null and wild-type mice. The allodynia was initiated 2 h after FCA administration and it was maintained for 8 days [$F(1,196)=52.5$, $P<0.05$, from baseline]. A three-way analysis of variance revealed no interaction between genotype, presence of FCA and time after FCA injection [$F(6,196)=0.04$, $P>0.05$]. No difference was found between PrP^c null and wild-type mice in mechanical allodynia (Fig. 4A).

The FCA injection enhanced paw volume (edema) in PrP^c null and wild-type mice from 2 h after FCA injection and it was maintained for 8 days [$F(1,224)=492.05$, $P<0.05$, from baseline]. A three-way analysis of variance revealed no interaction between genotype, presence of FCA and time after FCA injection [$F(7,224)=0.626$, $P>0.05$]. Edema development was significantly lower in PrP^c null than wild-type mice at 12, 48 and 96 h after FCA administration (Fig. 4B).

2.5. *Visceral conditioning stimuli*

The treatment of animals with acetic acid (AA 0.6%, 10 ml/kg, i.p.) produces a visceral conditioning stimulus, which reduces