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Research Report

Postnatal change of GluR5 kainate receptor expression in the substantia gelatinosa neuron of the trigeminal subnucleus caudalis in mice

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ARTICLE INFO

Article history:
Accepted 23 May 2010
Available online 1 June 2010

Keywords:
Kainate
Trigeminal subnucleus caudalis
Substantia gelatinosa
Patch clamp
RT-PCR
Western blot
GluR5

ABSTRACT

The substantia gelatinosa (SG) of the trigeminal subnucleus caudalis (Vc) has been implicated in the processing of nociceptive information from the orofacial region. Kainate receptors (KARs) play an important role in sensory transmission. Five different KAR subunits have been cloned and the expression of the KAR subunits showed developmental changes. In this study, RT-PCR, western blotting, immunohistochemistry and a patch clamp technique were used examine the functional expression of the GluR5 subunit in the SG of the Vc in juvenile, peripubertal and/or adult mice. The levels of mRNA and protein expression of the GluR5 subunit in the SG of the Vc were higher in the juvenile mice than in the peripubertal or adult mice. In addition, the KA and ATPA, a GluR5 KAR agonist, induced membrane depolarization on the SG neurons in both juvenile and adult mice in a concentration-dependent manner. However, the juvenile SG neurons showed a stronger response to KA and ATPA than those of adults. The membrane depolarization by KA was suppressed slightly in the presence of the AMPA receptor antagonist, GYKI 52466. These results show that the GluR5 KAR subunits are expressed functionally on the SG neurons of the Vc in mice, and the expression levels of the GluR5 subunits decrease with postnatal development. These postnatal changes in the GluR5 KAR subunit may be a possible mechanism for age-dependent pain processing.

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doi:10.1016/j.brainres.2010.05.065

1. Introduction

The substantia gelatinosa (SG), the lamina II of the trigeminal subnucleus caudalis (Vc), is a key site for orofacial nociceptive processing because it receives the synaptic inputs from the primary myelinated Aδ and unmyelinated C fibers of the orofacial region (Sessle, 2000). The SG neurons function as excitatory and inhibitory interneurons and regulate the output of the projection neurons in lamina I and IV, which transmits noxious information to a higher center (Gobel et al., 1980; Kumazawa and Perl, 1978; Light et al., 1979, Light and Kavookjian, 1988; Pan and Pan, 2004).

Kainate receptors (KARs) are expressed on the neurons in both the peripheral and central nervous systems. The KARs are expressed in the nociceptive pathways, including the dorsal root ganglion, spinal cord, thalamus and cortex (Wu et al., 2007), particularly at the spinal dorsal horn and Vc involved in pain processing (Chiang et al., 1997; Furuyama et al., 1993; Kalb and Fox, 1997). In addition, KARs can modulate the excitatory and inhibitory synaptic transmission in the spinal SG neurons (Li et al., 1999; Kerchner et al., 2001a,b; Huettner et al., 2002; Youn and Randic, 2004), suggesting that KARs regulates nociceptive processing. The KARs are categorized into five subunits (KA1, KA2, GluR5, GluR6 and GluR7) that are composed of a homomeric or heteromeric assembly (Bettler and Mulle, 1995; Hollmann and Heinemann, 1994; Ullal et al., 2005). Among these subunits, GluR5-containing KAR has been suggested to be involved in nociceptive transmission (Youn and Randic, 2004; Xu et al., 2006; Simmons et al., 1998). For example, the activation of GluR5-containing KARs reduces the nociceptive spinal reflexes in vitro (Procter et al., 1998) as well as the nociceptive behavioral responses in vivo (Mascias et al., 2002). In addition, the systemic administration of GluR5-selective antagonists reduces hyperalgesia (Sang et al., 1998; Simmons et al., 1998). Stegenga and Kalb (2001) reported higher expression of the GluR5 subunit in the spinal cord of rats during development with little or no expression in adult rats according to in situ hybridization.

It has been reported that the pain threshold was increased by heat stimuli in older adults compared to younger adults (Gibson and Farrell, 2004). Recently, Cole et al. (2010) suggested that the pain-evoked activity may reduce the functional pain-related system according to the developmental age. In their report, younger subjects showed significantly higher levels of pain-related activation than older adults in humans. These previous reports strongly implicate the involvement of GluR5-containing KARs in the processing of nociceptive information as well as an age-dependent pattern of functional expression in the pain pathways. However, there is little information on the postnatal changes in GluR5-containing KAR expression in the SG of the Vc, which is involved in orofacial pain modulation.

In this study, RT-PCR, western blotting and immunohistochemisty were used to examine the mRNA and protein expression of GluR5 KAR in the SG of the Vc in juvenile, peripubertal and/or adult mice. In addition, the changes in the functional expression of GluR5 KAR on the SG neurons of the Vc were also analyzed in juvenile and adult mice using a patch clamp technique.

2. Results

2.1. Postnatal change of GluR5 subunit expression

Fig. 1A gives an example of the expression of the GluR5 subunit mRNA in the SG of Vc in juvenile, peripubertal and adult mice. Fig. 1B compares the mRNA expression level. The expression levels of the GluR5 subunit mRNA were lower in the peripubertal ($n=4$) and adult mice ($n=4$) than in the juvenile mice ($n=4$). Western blot analysis was performed to determine if the protein levels of the GluR5 KAR subunit also show a similar pattern to mRNA expression. Fig. 1C shows the protein expression of the GluR5 subunit. The housekeeping gene encoding α-tubulin was used as a reference to normalize the expression level of each sample. Fig. 1D compares the GluR5 subunit protein expression level in juvenile, peripubertal and adult SG of the Vc. As shown in Fig. 1D, the protein levels were lower in the peripubertal or adult mice than in juveniles indicating a similar pattern to mRNA expression.

Immunohistochemistry was performed to confirm the postnatal changes in GluR5 subunit expression. As shown in Fig. 1, the peripubertal and adult mice showed similar mRNA and protein expression. Therefore, this study mainly compared the GluR5 subunit expression between juveniles and adults. Figs. 2Aa and Ad show the immunoreactivity of the GluR5 subunit in the coronal slices including the SG neurons of the Vc in juvenile and adult males, respectively. In the juvenile males, 64 (75%) out of 85 SG neurons expressed GluR5 containing KAR. However, only 48% (29/60) of the adult SG neurons showed GluR5 immunoreactivity. There was a significant difference between juveniles and adults in the male group (Fig. 2B). Figs. 3Aa and Ad show the immunoreactivity for the GluR5 subunit in juvenile and adult females, respectively. In juvenile females, 69 (78%) out of 89 SG neurons expressed GluR5. However, only 52% (33/64) of adult SG neurons showed GluR5 immunoreactivity. A significant difference was also noted between juveniles and adults in the female group (Fig. 3B). However, there was no significant difference in the expression of the GluR5 subunit between males and females.

2.2. KA-induced membrane depolarization on the postsynaptic SG neurons

As described above, the levels of mRNA and protein expression of the GluR5 subunit were higher in the juvenile mice than in the peripubertal or adult mice (Figs. 1 and 2). The whole-cell recordings were performed to confirm the functional expression of GluR5 KARs. Electrophysiological analysis was carried out on juvenile and adult mice because there was no significant difference in the GluR5 mRNA and protein expression levels in the SG of Vc between peripubertal and adult mice. KA induced membrane depolarization on the SG neurons of the Vc in a concentration-dependent manner (Fig. 4A). Fig 4B shows the mean membrane potential changes in juvenile and adult SG neurons at each KA concentration (0.1 to 10 μM). As shown in Fig. 4B, the juvenile SG neurons showed a stronger response to KA than adults. Some SG are involved in the desensitization by neurotransmitters, inflammation or injury (Han et al., 2009;