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Metabotropic glutamate $mGlu_1$ receptor mRNA expression in dorsal root ganglia of rats after peripheral nerve injury

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Abstract

Although cerebral and spinal metabotropic glutamate $mGlu_1$ receptors are thought to be involved in nociception and in the development/maintenance of chronic pain, it is still unclear to what extent $mGlu_1$ receptors are present in the dorsal root ganglia of peripheral sensory afferents, and whether their expression is affected during development of chronic pain. It was found in the present study that $mGlu_1$ receptor messenger RNA (mRNA) is present in rat L5 dorsal root ganglia and that it is strongly downregulated after unilateral axotomy of the tibial branch of the sciatic nerve, a model of chronic neuropathic pain. However, as sham-operated animals showed a similar downregulation, it is suggested that peripheral tissue damage is sufficient to result in a reduction of peripheral $mGlu_1$ receptor expression.

Keywords: Animal model; Metabotropic glutamate receptor; Pain; Tibial nerve injury

1. Introduction

Among the metabotropic glutamate mGlu receptor subtypes which are thought to be involved in the processing of pain, the $mGlu_1$ receptor subtype appears to play a crucial role in the development and/or maintenance of chronic pain. Thus, compounds with agonist properties at $mGlu_1$ receptors, such as (*R*,*S*)- or (*S*)-3,5-dihydroxyphenylglycine (DHPG), potentiate electrophysiological and behavioral responses to innocuous and noxious stimuli (e.g., Fisher and Coderre, 1998; Neugebauer et al., 1999; Salt and Turner, 1998; Young et al., 1997). On the other hand, it was shown that pharmacological or functional blockade of $mGlu_1$ receptors by means of receptor antagonists, antibodies or antisense oligos reduces sensitization, hyperalgesia and allodynia induced by DHPG, or associated with chronic pain (Bhave et al., 2001; Fisher et al., 1998; Fundytus et al., 1998, 2001; Hargett et al., 2000; Neugebauer et al., 1999; Salt and Turner, 1998; Young et al., 1997, 1998; Zhang et al., 2000). These observations are consistent with the finding that $mGlu_1$ receptors are abundant in central nervous structures involved in the processing of pain, such as the thalamus, the brain stem and the spinal cord (Alvarez et al., 2000; Boxall et al., 1998; Neto et al., 2000, 2001; Shigemoto et al., 1992; Tang and Sim, 1999; Yung, 1998). Moreover, it was demonstrated that peripheral inflammation, leading to chronic pain, induces a downregulation of $mGlu_1$ receptor mRNA in the thalamus (but not in the brain stem, spinal cord or cerebral cortex; Boxall et al., 1998; Neto et al., 2000, 2001).

Although the above-mentioned studies demonstrate that central $mGlu_1$ receptors are involved in nociception and chronic pain, it is less clearly established whether these receptors are also expressed on peripheral sensory afferents, and whether such peripheral $mGlu_1$ receptors are involved in nociception and the development and/or maintenance of chronic pain. Thus, whereas immunohistochemical studies indicated that $mGlu_1$ receptors are abundantly present in the dorsal horn of the rat spinal cord, their localization was suggested to be predominantly postsynaptical to the sensory afferents, on neuronal cell bodies and dendrites (Alvarez et al., 2000; Tang and Sim, 1999; Yung, 1998). This suggestion was supported by the presence of $mGlu_1$ receptor mRNA in the gray matter of the rat spinal cord (Boxall et al., 1998), whereas it could not be detected in dorsal root ganglia of 2-day-old rats (Crawford et al., 2000). Nevertheless, Alvarez et al. (2000)

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reported that a few presynaptic terminals of myelinated afferents contained $mGlu_1$ receptor immunoreactivity, whereas Tang and Sim (1999) described the occurrence of a few $mGlu_1$ receptor-positive axons in the white matter near the lateral horn, suggesting the existence of peripheral $mGlu_1$ receptors. Indeed, in a preliminary report, it was found that $mGlu_1$ receptor immunostaining was decreased in the spinal dorsal horn of rats submitted to dorsal rhizotomy, whereas it was detected in lumbar dorsal root ganglia of intact rats (Hargett et al., 2000). A recent study in mice identified $mGlu_1$ receptor immunoreactivity on unmyelinated peripheral afferents (Bhave et al., 2001), adding further support for the existence of peripheral $mGlu_1$ receptors. Both in rats and mice, it was demonstrated that intraplantar application of $mGlu_1$ receptor antagonists could attenuate the behavioral reaction to intraplantar application of formalin or DHPG (Bhave et al., 2001; Hargett et al., 2000), indicating that peripheral $mGlu_1$ receptors are involved in nociception.

The present study was performed in order to investigate whether $mGlu_1$ receptor mRNA is expressed in rat dorsal root ganglia and whether $mGlu_1$ receptor mRNA in this structure is regulated in a model of chronic neuropathic pain. The model consisted of unilateral transsection of the tibial branch of the sciatic nerve (tibial nerve injury; Hofmann et al., 2000). In this model, thermal hyperalgesia and tactile allodynia develops readily after nerve axotomy, and these symptoms coincide with altered expression of pain-relevant genes such as vasointestinal peptide and galanin in the spinal ganglia and Trk B in the dorsal horn (Hofmann et al., 2000).

2. Materials and methods

Male Wistar rats (180–200 g; strain HsdCpb:WU) were housed in groups of six under standardized conditions and a normal 12-h:12-h light/dark regime. The animals were randomly assigned to control, sham-operated or axotomized groups (control, sham and tibial nerve injury groups, respectively; $n = 7–8$ per group). Distally of the trifurcation of the left sciatic nerve, the tibial branch of the sciatic nerve was transsected, whereas the sural and common peroneal nerves were left uninjured. Sham-operated animals were treated identically to the axotomized animals, except for the tibial nerve cut. The control group did not receive surgery.

Rats were sacrificed 1 or 12 days after surgery. Dorsal root ganglia tissue was harvested and snap frozen with liquid nitrogen. As it was previously reported that $mGlu_1$ receptor mRNA is present in the thalamus and dorsal horn of the spinal cord (Shigemoto et al., 1992), these tissues were also collected from untreated control rats and functioned as a positive control. Isolation of total RNA and cDNA synthesis was performed as previously described (Siegling et al., 1994). Gene expression was quantified using the 7700 Sequence Detector (Taqman) and the SYBR Green PCR Core Reagent Kit, as described in the manufacturers manual (Applied Biosystems, Foster City, CA, USA). Cyclophilin served as an intrinsic control for variations in cDNA amounts. The $mGlu_1$ receptor primers were designed according to the rat $mGlu_1$ receptor mRNA sequence (accession number M61099): 5′-TCA gTg CCC AgT TCC CCC gTA TCT, antisense 5′-TCT TTA Agg CAg CCg CAg CAT TTT, yielding a 270-bp product. Primers for rat cyclophilin were designed as reported by Costigan et al. (1998). Taqman-PCR reactions were performed in 25-μl volumes with a final concentration of 300 nmol for each primer, with 95 °C for 30 s and 61 °C for 90 s for 40 cycles.

3. Results

$mGlu_1$ receptor mRNA was detected in L5 dorsal root ganglia of all tested naive rats. As shown in Fig. 1, the size of the product obtained from dorsal root ganglia appeared to be identical to that obtained from thalamic and spinal dorsal horn tissue. As assessed by real-time polymerase chain reaction (PCR), both unilateral axotomy of the tibial branch of the sciatic nerve and sham-operation resulted in a pronounced downregulation (up to nine-fold) of $mGlu_1$ receptor mRNA in dorsal root ganglia at days 1 and 12 following surgery [$F(4,33) = 12.86$, $P < 0.001$; one-way

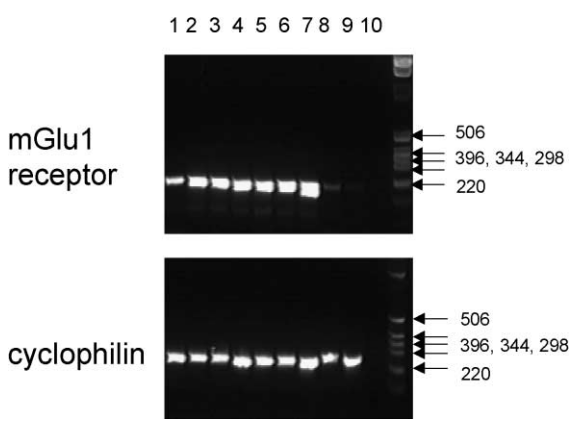

Fig. 1. Expression of $mGlu_1$ receptor mRNA in rat L5 dorsal root ganglia, the dorsal horn of the spinal cord and the thalamus, as assessed by means of RT-PCR and visualized by SYBR green-stained gel electrophoresis. Analysis resulted in a band of 270 bp corresponding to the $mGlu_1$ receptor. In addition, the expression of the housekeeping gene, cyclophilin, was analyzed. Lane 1: dorsal root ganglion, naive; lanes 2–4: dorsal horn, naive; lanes 5–7: contralateral thalamus, naive; lanes 8–9: dorsal root ganglion, axotomy day 12; lane 10: negative control, marker: Ready-Load™ 1 kb DNA Ladder (Invitrogen, Karlsruhe, Germany).

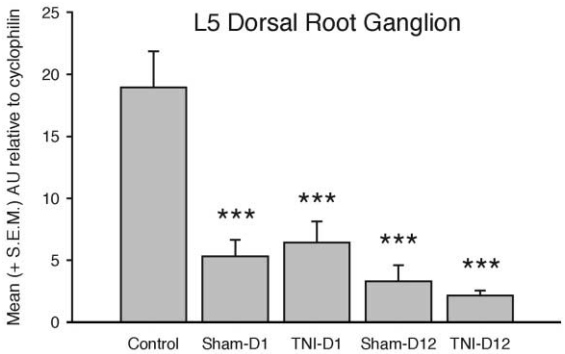

Fig. 2. Metabotropic glutamate $mGlu_1$ receptor mRNA expression in L5 dorsal root ganglia after axotomy of the tibial branch of the sciatic nerve (TNI) of rats. Tissues were harvested from naive (control), sham-operated and axotomized animals ($n = 7–8$ per group) 1 and 12 days after surgery (D1 and D12, respectively). Gene expression of $mGlu_1$ receptor and cyclophilin was measured by quantitative PCR (real time PCR). Values for $mGlu_1$ receptor mRNA expression are shown as the means (+1 S.E.M) of arbitrary units (AU) relative to cyclophilin mRNA expression. $^{***}P < 0.001$ versus control group.

analysis of variance (ANOVA); outcome of Tukey's post hoc *t*-test presented in Fig. 2].

4. Discussion

Although it is well established that metabotropic glutamate $mGlu_1$ receptors located in the central nervous system are involved in nociception and in the development/maintenance of chronic pain (see Section 1), it is still unclear to what extent peripheral $mGlu_1$ receptors may play an additional role. Thus, it remains to be investigated whether $mGlu_1$ receptor mRNA is present in the dorsal root ganglia of peripheral sensory afferents, and whether their expression is affected during development of chronic pain. It was found in the present study that $mGlu_1$ receptor mRNA is present in rat L5 dorsal root ganglia and that it is strongly downregulated after peripheral tissue injury.

The finding that $mGlu_1$ receptor mRNA is expressed in rat dorsal root ganglia is consistent with recent immunohistochemical studies in which it was demonstrated that $mGlu_1$ receptor immunostaining is detectable in lumbar dorsal root ganglia of rats (Hargett et al., 2000), or in unmyelinated peripheral afferents of mice (Bhave et al., 2001). Although Crawford et al. (2000) were unable to detect $mGlu_1$ receptor mRNA in spinal ganglia, it should be mentioned that they used dorsal root ganglia of 2-day-old Wistar rats, whereas the tissue used in the present study was from young adult rats. As Shigemoto et al. (1992) reported that the expression of $mGlu_1$ receptor protein in nervous tissue (dorsal root ganglia apparently not investigated) increases with age, it is possible that the different outcome between the Crawford et al. (2000) and the present study is related to the developmental status of the animal. The finding that $mGlu_1$ receptor mRNA is present in dorsal root ganglia and that $mGlu_1$ receptor protein is expressed in this structure, as well as in sensory afferent nerves, provides a neuroanatomical basis for the observation that intraplantar application of $mGlu_1$ receptor antagonists attenuates the behavioral reaction to intraplantar application of formalin or DHPG (Bhave et al., 2001; Hargett et al., 2000), and suggest that peripheral $mGlu_1$ receptors are involved in nociceptive processing.

Previous studies on the regulation of $mGlu_1$ receptor mRNA have only investigated expression levels in central nervous system tissues and were performed after induction of peripheral inflammation (either by means of ultraviolet irradiation or intraarticular injection of complete Freund's adjuvant; Boxall et al., 1998; Neto et al., 2000, 2001). Thus, it was reported that chronic inflammatory pain coincided with a downregulation of $mGlu_1$ receptor mRNA in the thalamus (Neto et al., 2000). The present finding of a pronounced, similar downregulation after peripheral nerve injury and peripheral tissue injury suggests that damage to peripheral tissue is sufficient to induce a downregulation of peripheral $mGlu_1$ receptors. Further studies are required to investigate whether this finding can be extended to inflammation of peripheral tissue and inflammatory pain. Functionally, the existence of peripheral $mGlu_1$ receptors could imply that these receptors are involved in the control of glutamate release to the dorsal horn of the spinal cord (autoreceptor which negatively controls glutamate release). Downregulation of these receptors by peripheral tissue damage could induce a loss of autoreceptor function and, therefore, result in the hyperglutamatergic condition associated with chronic pain.

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