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Research report

The role of glutamate release mediated by extrasynaptic P2X7 receptors in animal models of neuropathic pain

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ABSTRACT

Purinergic signaling represents a major non-synaptic signaling mechanism in the normal and pathological nervous system. The expression of the purinergic ligand gated ion channel P2X7 receptor (P2rx7) has been described on nerve terminals as well as in non-neuronal cells, such as astrocytes and microglia. The activation of P2rx7s results in Ca^{2+} influx and increased transmitter release in the brain. P2rx7s previously suggested having a pivotal role in different pain modalities, including neuropathic pain. Here we investigated whether the activation of P2rx7 leads to increased glutamate release from the spinal cord in an experimental model of neuropathic pain (partial nerve ligation of the sciatic nerve, PNL). One week after surgery, we studied the effects of PNL on tactile allodynia using aesthesiometry, in parallel with the in vitro release of [^{3}H]glutamate from lumbar spinal cord slices. The observed allodynia in wild-type (P2rx7+/+) mice one week after PNL surgery was lower that was observed in P2rx7 deficient (P2rx7−/−) animals. Perfusion of spinal cord slices with ATP (10 mM) elicited [^{3}H]glutamate release in both sham operated and neuropathic P2rx7+/+ animals. The ATP-induced [^{3}H]glutamate release was absent in P2rx7−/− mice. Electrically evoked release of [^{3}H]glutamate from spinal cord slices was not significantly altered in PNL animals and in P2rx7−/− mice. The results suggest that activation of P2rx7 by ATP releases glutamate in the spinal cord, which might contribute to mechanical allodynia following PNL. On the other hand, this release does not contribute to glutamate efflux evoked by conventional neuronal activity, which is consistent with the idea that P2X7 receptors are either extrasynaptic or expressed on non-neuronal cells.

This article is part of a Special Issue entitled 'Extrasynaptic ionotropic receptors'.

1. Introduction

Purinergic signaling mediated by ionotropic (P2X1-7) and metabotropic ($P2Y_{1,2,4,6,11,12,13,14}$, A_1, A_{2A}, A_{2B}, A_3) receptors represents a major non-synaptic signaling mechanism in the normal and pathological nervous system (Burnstock, 2012; Cotrina and Nedergaard, 2009; Gao et al., 2011; Li et al., 2012; Lin et al., 2010; Vizi et al., 2010; Xu et al., 2012) and conveys a wide range of actions from conventional neurotransmission and neuromodulation to neuron-glia interactions, cell death and proliferation.

P2X7 receptors (P2rx7) are ligand gated ion channels that are sensitive to ATP and other purine and pyrimidine nucleotides. The homo-oligomeric P2rx7 (Surprenant et al., 1996) belong to the P2X receptors, but there are several properties, whereby it could be distinguished from other members of this receptor family (Jarvis and Khakh, 2009; Song et al., 2006): (1) its intracellular carboxyl-terminal domain is longer than those of other P2X receptor subunits, (2) it has several splice variants that display different functionalities in P2rx7+/+ and knockout (P2rx7−/−) mice lines (Masin et al., 2012; Nicke et al., 2009), (3) its persistent activation elicits the opening of a much larger membrane pore permeable to high molecular weight substances, and (4) it needs high micromolar concentrations of ATP to be activated.

P2rx7s are expressed throughout the nervous system, including the dorsal horn of the spinal cord, examined either by northern blotting (Collo et al., 1997; Rassendren et al., 1997), immunohistochemistry (Deuchars et al., 2001) or receptor-binding assay (Able et al., 2011). In the spinal cord P2rx7s are expressed on microglial cells (He et al., 2012; Kobayashi et al., 2011), whereas other studies found strong P2rx7 immunoreactivity (P2rx7-IR) around the neuropil and astrocytes (Aoyama et al., 2011). Using electronmicroscopic analyses, Deuchars et al. (2001) identified P2rx7 receptors around the presynaptic nerve terminals of asymmetric, probably

Abbreviations: ANOVA, analysis of variance; ATP, adenosine-triphosphate; BBG, Brilliant Blue G; CNS, central nervous system; FRS, stimulation-induced fractional release; GABA, gamma-amino-butyric acid; GLU, glutamate; IL, interleukin; LTP, long-term potentiation; mRNA, messenger ribonucleic acid; PNL, partial nerve ligation; PWT, paw withdrawal threshold.

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glutamatergic synapses of the spinal cord (Deuchars et al., 2001). In addition, the expression of P2rx7-IR has also been confirmed in the satellite glia cells of the dorsal root ganglion (Chen et al., 2008). However, the cell-type specific localization of P2rx7 has been the subject of a long-standing debate, and is still elusive. The main sources of ambiguity are the splice variants of P2rx7, which are expressed in the CNS of P2x7 receptor deficient mice of different origins (Masin et al., 2012; Nicke et al., 2009), and have reduced functionality, but give rise pseudo-immunoreactivity in histological sections. Therefore the above observations might be valid only for certain but not all splice variants of the P2rx7.

The primary role of P2rx7s in the brain is the regulation of neurotransmitter release (Cotrina and Nedergaard, 2009; Sperlagh et al., 2006). The activation of P2rx7 results in an inward cationic current and Ca^{2+} influx (Marin-Garcia et al., 2008; Miras-Portugal et al., 2003), which leads to increased excitatory amino acid and GABA release from brain slices (Papp et al., 2004; Sperlagh et al., 2002), nerve terminals (Alloisio et al., 2008; Marcoli et al., 2008; Patti et al., 2006), cultured neurons (Leon et al., 2008) and astrocytes (Duan et al., 2003). The mechanism, whereby the activation of P2rx7 gives rise to extracellular glutamate accumulation includes exocytotic release (Cervetto et al., 2012; Marcoli et al., 2008), non-exocytotic release through the receptor-ion channel complex (Duan and Neary, 2006; Marcoli et al., 2008) and inhibition of amino acid transporters of glia cells (Morioka et al., 2008).

On the other hand P2rx7 also plays an important role in the activation of microglia following pathological insults and in the processing and secretion of mature pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-18, and tumor necrosis factor (TNF)-α, which are produced mainly by non-neuronal cells (Sperlagh and Illes, 2007).

It seems likely, however, that activation level of P2rx7s might not be identical under physiological and pathological conditions: given the low affinity of endogenous ATP, P2rx7s are probably largely silent under normal neuronal activity and their more widespread activation is expected under pathological conditions, when pathological signals such as metabolic stress, inflammation or cell death leads to an ATP-rich extracellular milieu (Cotrina and Nedergaard, 2009; Sperlagh et al., 2006). Therefore both synaptic and extrasynaptic P2rx7s appear to gain significance depending on the actual physiopathological conditions and the level of extracellular ATP at the vicinity of the receptors.

Neuropathic pain syndromes are severe, debilitating and chronic pain states, and are commonly caused by the damage of peripheral or central nerves (Zimmermann, 2001). Unfortunately, pharmacological treatment with conventional analgesics have only limited efficacy and/or efficiency, which underlines the importance of in-depth understanding of the pathophysiology of these etiologically heterogeneous pain states, and to promote the development of adequate and specific anti-neuropathic drugs. A common feature of these pain syndromes is mechanical allodynia, typically persisting even after the significant alleviation or the absence of initial nerve damage, which is related to the long-term sensitization of peripheral and central nerves toward otherwise non-noxious stimuli that therefore become overtly painful.

The role of P2rx7s as well as other P2X receptor subtypes (Gao et al., 2011; Li et al., 2012; Lin et al., 2010; Vizi et al., 2010) have been repeatedly examined in animal models of pathological pain. Genetic deletion of P2rx7 (Chessell et al., 2005) and its specific antagonists has consistently been proved protective in animal models of neuropathic (He et al., 2012; Honore et al., 2006; Kobayashi et al., 2011; McGaraughty et al., 2007; Nelson et al., 2006) and inflammatory (Ando et al., 2010; Dell'Antonio et al., 2002; Honore et al., 2006, 2009) pain, although there are also findings, which show that some antagonists of P2rx7 are ineffective (Ando et al., 2010; Broom et al., 2008), or influence the generation of pain in an opposite direction (Chen et al., 2008). Following the detection of analgesic effect of P2rx7 antagonists in animal experiments, a variety of P2rx7 antagonists are currently under development for the treatment of inflammatory and neuropathic pain, and a few of them have already entered into clinical trial (Donnelly-Roberts and Jarvis, 2007; Gum et al., 2012). Moreover, based on the analysis of physicochemical properties as well as the efficiency of currently available ligands, the P2rx7 receptor has been qualified as a highly druggable target of pain and inflammation, when compared to other members of the P2X receptor family (Gum et al., 2012).

Nevertheless, the cellular mechanism, whereby the absence or pharmacological blockade of P2rx7 alleviates neuropathic pain is not completely understood. P2rx7s in the dorsal horn of the spinal cord are upregulated following nerve injury on the ipsilateral side both on the mRNA (Kobayashi et al., 2011) and protein level (He et al., 2012; Kobayashi et al., 2011). The P2rx7 antagonist A-438079 reduces the spontaneous and evoked firing of a wide dynamic range of spinal neurons of neuropathic rats in response to both innocuous and noxious stimuli (McGaraughty et al., 2007), which indicates that the spinal cord or supraspinal sites should be the site of action of P2rx7-inhibition mediated analgesia. More recently it has been shown that the P2rx7 antagonist BBG prevented LTP in the spinal cord dorsal horn, the presumed mechanism of central sensitization, and reduced the consequent allodynia induced by tetanic nerve stimulation (Chu et al., 2010). Moreover BBG also prevented the increase in the number of microglial cells and the production of IL-1β (Chu et al., 2010), therefore one potential pathway of P2rx7-mediated analgesia is that it counteracts microglia activation and subsequent production of IL-1β and thereby prevents LTP and the sensitization process. On the other hand, ATP by itself, i.e. without priming stimulus, such as bacterial lipopolysaccharide is unable to trigger the secretion of IL-1β in the spinal cord (Clark et al., 2010), which argues against the role of IL-1β in P2rx7-mediated analgesia, at least in the neuropathic pain. Therefore other mechanisms, such as the direct or indirect facilitation of the release of glutamate, the main excitatory neurotransmitter of the primary sensory neurons, are also worth taking into consideration.

In our study, we investigated how the activation of P2rx7s affects the release of [^{3}H]glutamate from acute spinal cord slices of control and neuropathic, wild-type and P2rx7 deficient mice. Here we report for the first time that P2rx7 activation by ATP leads to elevated extracellular level of glutamate in the spinal cord. On the other hand, despite that animals lacking P2X7 receptors display reduced mechanical allodynia after partial ligation of the sciatic nerve (PNL), we could not observe significant change in the amount of [^{3}H]glutamate efflux evoked by electrical field stimulation.

2. Methods

2.1. Animals

This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the local Animal Care Committee of the IEM HAS approved all experimental procedures (Permission No. 22.1/3671/003/2008). This study used 2- to 3-months old (approx. 30 g) male wild type (P2rx7+/+), and P2rx7 knockout mice (P2rx7−/−). The original breeding pairs of P2rx7−/− mice (C57BL/6J based) were kindly supplied by Christopher Gabel (Pfizer Inc., Groton, CT, USA). The animals contained the DNA construct P2X7-F1 (5′-CGGCGTGCGTTTTGACATCCT-3′) and P2X7-R2 (5′-AGGGCCCTGCGGTTCTC-3′), which have been previously shown to generate the genetic deletion of P2rx7 (Solle et al., 2001). Homozygous P2rx7+/+ mice were bred to C57BL/6 mice. The animals were genotyped using PCR analysis as described earlier (Solle et al., 2001). Mice were bred and kept under standard laboratory conditions (food and water ad libitum, 12 on–off light cycles, 21–23 °C) in the local animal house of the institute (IEM HAS, OGR Unit) during the experiments.

All efforts were made to minimize animal suffering and reduce the number of animals used. The allodynia experiments were carried out between 9:00 and 14:00 in a separate room of the housing facility, while glutamate release experiments took place in investigators' laboratory.