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Pannexin 1: A novel participant in neuropathic pain signaling in the rat spinal cord ☆

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ABSTRACT

Pannexin 1 (panx1) is a large-pore membrane channel expressed in many tissues of mammals, including neurons and glial cells. Panx1 channels are highly permeable to calcium and adenosine triphosphatase (ATP); on the other hand, they can be opened by ATP and glutamate, two crucial molecules for acute and chronic pain signaling in the spinal cord dorsal horn, thus suggesting that panx1 could be a key component for the generation of central sensitization during persistent pain. In this study, we examined the effect of three panx1 blockers, namely, 10panx peptide, carbenoxolone, and probenecid, on C-reflex wind-up activity and mechanical nociceptive behavior in a spared nerve injury neuropathic rat model involving sural nerve transection. In addition, the expression of panx1 protein in the dorsal horn of the ipsilateral lumbar spinal cord was measured in sural nerve–transected and sham-operated control rats. Sural nerve transection resulted in a lower threshold for C-reflex activation by electric stimulation of the injured hindpaw, together with persistent mechanical hypersensitivity to pressure stimuli applied to the paw. Intrathecal administration of the panx1 blockers significantly depressed the spinal C-reflex wind-up activity in both neuropathic and sham control rats, and decreased mechanical hyperalgesia in neuropathic rats without affecting the nociceptive threshold in sham animals. Western blotting showed that panx1 was similarly expressed in the dorsal horn of lumbar spinal cord from neuropathic and sham rats. The present results constitute the first evidence that panx1 channels play a significant role in the mechanisms underlying central sensitization in neuropathic pain.

1. Introduction

Pannexin 1 (panx1) is a protein that functions as a large-pore membrane channel with up to 500 pS conductance [2]. It has been widely investigated since it was identified in the mammalian genome in the year 2000 by Panchin et al. [35]. Panx1 can be opened after mechanical stimulation [2], membrane depolarization [2,6], purinergic [27,36], and *N*-methyl-D-aspartate (NMDA) receptor [56] activation, intracellular calcium [27], or elevated extracellular potassium [44,46]. Panx1 mRNA has been found during development and in mature systems in many tissues in rats, mice, and humans [6]. In the central nervous system, panx1 is expressed both in neurons and astrocytes [50]. In neurons, panx1 has been observed in the postsynaptic density of hippocampal pyramidal cells in co-expression with the postsynaptic density 95 protein, suggesting a modulatory role in excitability of postsynaptic neurons [58]. At present, there is growing evidence that supports a role of panx1 channels in some pathologic conditions of the central nervous system, particularly in epilepsy, cerebral ischemia, and neuroinflammation [16,44,46,50,51].

Glutamate and adenosine triphosphatase (ATP) have been described as crucial molecules in acute pain signaling in the dorsal horn of the spinal cord, as well as in the process of developing and maintaining central sensitization underlying chronic pain [5,7,25,30]. Interestingly, panx1 is highly permeable to ATP [2,8,27], the main activator of the purinergic signaling, 1 of the pathways that mediates neuroplastic phenomena occurring in

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neuropathic pain [52]. Besides, ATP and glutamate can open panx1 channels via activation of P2X7 [20,36] and NMDA [50] receptors respectively, thus leading to the question of whether panx1 participates in pain signaling in the spinal cord of either normal animals and/or in neuropathic pain conditions. In this regard, a recent report showed that the gap junction blocker carbenoxolone attenuated mechanical hypersensitivity in a model of pathological pain induced in rat by partial transection of the infraorbital nerve [54]. However, carbenoxolone is a nonselective inhibitor of gap-junctions and hemichannels, and therefore, as pointed out by Wang et al. [54], a variety of different mechanisms (mostly occurring in glial cells) could account for the inhibitory effect of carbenoxolone on central sensitization produced by nerve injury, such as inhibition of intercellular Ca^{2+} waves in astrocytes via gap-junctions, modulation of astrocyte volume–regulated anion channels, inhibition of the expression of interleukin-23 in microglia, and suppression of the release of certain glial mediators via inhibition of hemichannels (for specific references, see Wang et al. [54]). Thus, because the role of panx1 in chronic pain remains largely unknown, we addressed this question in a rat model of neuropathic pain. We hypothesized that panx1 participates in dorsal horn mechanisms of hyperalgesia/allodynia accompanying chronic neuropathic pain, and that pharmacological blockade of panx1 would reduce mechanical hyperalgesia and some neuroplastic processes (eg, spinal wind-up activity) in neuropathic animals.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (225–250 g body weight) were used in this study. All animals were obtained from the facilities of the Faculty of Medicine of the University of Chile, held in a light–dark cycle of 12/12 hours, starting at 8 AM, with food and water ad libitum. The experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH) and adhered to the guidelines of the Committee for Research and Ethical Issues of IASP [56]. Furthermore, the housing conditions and experimental procedures conformed to protocols approved by the Bioethics Committee of the University of Santiago of Chile.

2.2. Induction of neuropathy

Neuropathy was induced by using a modification of the spared nerve injury rat model described by Decosterd and Woolf [16], which results in early, prolonged, and robust changes in mechanical sensitivity and thermal responsiveness that closely mimic many features of clinical neuropathic pain. In the original description [16], 2 of the 3 terminal distal branches of the sciatic nerve were axotomized (tibial and common peroneal nerves), sparing 1 (sural nerve), whereas in the present version of the model, only the sural nerve was transected, sparing the tibial and common peroneal nerves. This procedure allowed us to generate a neuropathic pain model in which nociceptive reflexes to be recorded are preserved (eg, C-reflex activity), as the sural nerve contains almost no motor fibers [38].

Animals were anesthetized with 400 mg/kg i.p. of 7% chloral hydrate solution (w/v). After shaving the right hindpaw at the level of sciatic nerve, a skin incision approximately 10 mm long was made. The subcutaneous tissue was dissected, and the biceps femoris muscle was freed from the pelvic and vertebral heads to expose the sciatic nerve. The nerve path was then followed until its split into 3 branches: the sural, common peroneal, and tibial nerves. The sural nerve was cut 2 mm from its emergence, and the overlying tissues were sutured in layers. During the 2 days after surgery, animals were daily given 3 mg/kg s.c. of the analgesic ketoprofen and 5 mg/kg s.c. of the antimicrobial agent enrofloxacin. The neural lesion described above resulted in mechanical hyperalgesia of the hindpaw that persisted for at least 28 days (data not shown). Control (sham) rats received similar surgery but without severing the sural nerve.

2.3. Pharmacological blockage of panx1 in spinal cord

For studying the effect of panx1 channel blockade on mechanical hyperalgesia induced by neuropathy, panx1 channels of the lumbar spinal cord were challenged with the 10panx peptide (Trp-Arg-Gln-Ala-Ala-Phe-Val-Asp-Ser-Tyr), a mimetic peptide of the first extracellular loop domain of panx1 [36] that seems to act through a steric interference with channel function, obtained from Tocris (St Louis, MO). Neuropathic and sham rats received a single 10-μL i.t. injection of 10, 30, 100, or 300 μmol/L 10panx, whereas the respective control groups received 10 μL saline solution i.t. In addition, 2 other drugs described in the literature as pharmacological blockers of panx1 were also used: carbenoxolone (Cbx), a synthetic drug that blocks panx1 channels expressed in Xenopus oocytes [7], which was i.t. injected as 100 μmol/L solution; and probenecid (Prb), an agent that exhibit inhibitory activity against panx1 [45], which was i.t. injected as 150 μmol/L solution. Both Cbx and Prb were obtained from Sigma (St Louis, MO).

For the electrophysiological study of the effect of panx1 channel blockade on C-reflex wind-up activity, a single 10-μL i.t. injection of 300 μmol/L 10panx, 100 μmol/L Cbx, 150 μmol/L Prb, or saline solution was administered to neuropathic and sham control animals. Single doses of 10panx, Cbx and Prb were adapted from the studies of Thompson et al. [50], Bruzzone et al. [7] and Ma et al. [28], respectively. Injections into the subarachnoid space were made under brief isoflurane anesthesia (2 minutes), by direct percutaneous injection between lumbar vertebrae L5 and L6, and evidenced by the slight movement of the rat's tail that results from mechanical stimulation when the needle penetrates the meninges of the spinal cord [29]. At the end of the behavioral and electrophysiological experiments, rats were euthanized with an overdose of chloral hydrate (1 g/kg i.p.).

2.4. Electrophysiological assessment of the C-fiber–evoked nociceptive reflex and wind-up activity

The C-reflex was elicited in the right hindlimb of rats anesthetized with 1.5% to 1.8% isoflurane in oxygen using a latex diaphragm-modified rodent facemask, as described previously by Smith and Bolon [47]. Briefly, rectangular electric pulses of supramaximal strength and 2-millisecond duration were applied every 10 seconds to the common peroneal and tibial nerve receptive field by means of 2 stainless steel needles inserted into the skin of the second and third toes (Grass S11 stimulator equipped with a Grass SIU 5 stimulus isolation unit and a Grass CCU 1A constant current unit; Astro-Med, West Warwick, RI). The C-fiber–evoked reflex response was recorded from the ipsilateral biceps femoris muscle by using another pair of stainless steel needles. After amplification (Grass P511 preamplifier; Astro-Med, West Warwick, RI), the electromyographic responses were digitized at 100 KHz and integrated into a time-window from 150 to 450 ms after the stimulus (Powerlab ML 820, ADInstruments, Castle Hill, NSW, Australia). Once stable C-reflex responses were obtained, the stimulus strength was lowered, and the current required for threshold activation of the C-reflex was determined. Integrated C-reflex responses, evoked by single stimuli with twice the intensity of the threshold stimulating current, were then recorded. Afterward, trains of 15 stimuli each, at 1 Hz and twice the threshold intensity, were delivered to the toes to develop wind-up activity. In the C-reflex paradigm,