Brain, Behavior, and Immunity 69 (2018) 470–479

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Full-length Article

Sustained reversal of central neuropathic pain induced by a single intrathecal injection of adenosine A_{2A} receptor agonists

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ARTICLE INFO

Article history:
Received 28 June 2016
Received in revised form 9 January 2018
Accepted 11 January 2018
Available online 31 January 2018

Keywords:
ATL313
CGS21680
Mechanical allodynia
Rats
Avulsion
Spinal cord injury

ABSTRACT

Central neuropathic pain is a debilitating outcome of spinal cord injury (SCI) and current treatments to alleviate this pain condition are ineffective. A growing body of literature suggests that activating adenosine A_{2A} receptors (A_{2A}Rs) decreases the production of proinflammatory cytokines and increases the production of anti-inflammatory cytokines. Here, the effect of administering intrathecal A_{2A}R agonists on central neuropathic pain was measured using hindpaw mechanical allodynia in a rat model of SCI termed spinal neuropathic avulsion pain (SNAP). Other models of SCI cause extensive damage to the spinal cord, resulting in paralysis and health problems. SNAP rats with unilateral low thoracic (T13)/high lumbar (L1) dorsal root avulsion develop below-level bilateral allodynia, without concomitant motor or health problems. A single intrathecal injection of the A_{2A}R agonist 2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxamido adenosine HCl (CGS21680) reversed SCI-induced allodynia for at least 6 weeks. The reversal is likely in part mediated by interleukin (IL)-10, as intrathecally administering neutralizing IL-10 antibodies 1 week after CGS21680 abolished the anti-allodynic effect of CGS21680. Dorsal spinal cord tissue from the ipsilateral site of SCI (T13/L1) was assayed 1 and 6 weeks after CGS21680 for IL-10, CD11b, and tumor necrosis factor (TNF) gene expression. CGS21680 treatment did not change IL-10 gene expression but did significantly decrease CD11b and TNF gene expression at both timepoints. A second A_{2A}R agonist, 4-(3-(6-amino-9-(5-cyclopropylcarbamoyl-3,4-dihydroxytetrahydrofuran-2-yl)-9*H*-purin-2-yl)prop-2-ynyl)piperidine-1-carboxylic acid methyl ester (ATL313), was also able to significantly prevent and reverse SCI-induced allodynia for several weeks after a single intrathecal injection, providing converging lines of evidence of A_{2A}R involvement. The enduring pain reversal after a single intrathecal injection of A_{2A}R agonists suggests that A_{2A}R agonists could be exciting new candidates for treating SCI-induced central neuropathic pain.

1. Introduction

Central neuropathic pain is a common and debilitating consequence of spinal cord injury (SCI). >66% of people with SCI develop chronic pain (Siddall et al., 1999) and, of that population, ~30% develop below-level central neuropathic pain (Siddall et al., 2003). Below-level pain is defined as pain occurring at least 2 dermatomes away from the original injury. Patients with below-level pain describe their pain as severe (Wrigley et al., 2009), and current pharmacotherapies for treating central neuropathic pain are not effective, with only ~50% pain relief in 1 out of 2–3 people (Beniczkyet al., 2005).

Current animal models of SCI, such as contusion and hemisection, cause extensive damage to the spinal cord and animals develop paralysis, urinary tract infections, and autotomy. This has presented challenges for the study of central neuropathic pain. We have recently developed a rat dorsal root avulsion model, termed spinal neuropathic avulsion pain (SNAP), to study SCI-induced central neuropathic pain. SNAP rats do not have compromised motor function or health problems and unilateral T13/L1 avulsion induces robust, reliable, bilateral below-level mechanical allodynia (Wieseler et al., 2010). These properties of SNAP make this model attractive for studying mechanisms underlying central neuropathic pain.

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https://doi.org/10.1016/j.bbi.2018.01.005
0889-1591/

It is known that spinal glia (microglia, astrocytes) play a key role in facilitating chronic pain states in animal models of peripheral neuropathic pain (Watkins et al., 2007). During an inflammatory event, glia become activated and release neuroexcitatory substances that include proinflammatory cytokines, which drive pain amplification (Milligan and Watkins, 2009). Although less is known about glial mechanisms involved in central pain, recent studies have shown that spinal glia are activated in models of SCI including hemisection (Gwak and Hulsebosch, 2009), contusion, (Tan et al., 2009), and SNAP (Ellis et al., 2014), and persistent activation of microglia and astrocytes can both induce and maintain below-level SCI neuropathic pain (Hulsebosch, 2008). Conversely, glial cells can also release anti-inflammatory mediators that attenuate neuropathic pain (Milligan et al., 2012). Pharmacological therapies that modulate glia in such a way that they express a less proinflammatory phenotype could prove to be an effective treatment for central neuropathic pain in SCI.

Adenosine is an endogenous substance in the central nervous system (CNS) that can bind 4 different G protein-coupled receptors: adenosine 1 receptor (A_1R), $A_{2A}R$, $A_{2B}R$, and A_3R (Sawynok and Liu, 2003). $A_{2A}Rs$ are unique because they are found not only on neurons, but also on immune cells in the peripheral and central nervous systems (Dare et al., 2007). The anti-inflammatory effects of adenosine are generally attributed to occupancy of the A_{2A} receptor (Hasko and Cronstein, 2004), and there is an expanding body of literature that documents the ability of $A_{2A}R$ agonists to decrease inflammation by suppressing proinflammatory cytokines and increasing anti-inflammatory cytokines in peripheral (Hasko and Cronstein, 2004) and central (Loram et al., 2009; Loram et al., 2013) immune cells. Furthermore, a single intrathecal administration of either of two structurally and pharmacologically different $A_{2A}R$ agonists (CGS21680, ATL313) reversed peripheral neuropathy-induced allodynia for >4 weeks, while also decreasing microglial activation and the proinflammatory cytokine tumor necrosis factor-alpha (TNF) (Loram et al., 2009). Whether or not this enduring reversal from a single administration of an $A_{2A}R$ agonist can also be observed in a model of central neuropathic pain, and what the mechanisms might be, has not been previously investigated. The aim of this study was to provide an initial characterization of $A_{2A}R$ agonism and potential underlying mechanisms in central neuropathic pain.

Our hypothesis was that intrathecal $A_{2A}R$ agonists would attenuate SNAP allodynia, in association with a decrease in the microglial activation marker CD11b and decreased gene expression of the proinflammatory cytokine TNF, while also increasing the production/release of the anti-inflammatory cytokine IL-10. These endpoints were chosen for analysis because pilot and published studies from our lab (Loram et al., 2009), as well as in the literature (Duan et al., 2012; Ohta and Sitkovsky, 2001; Vincenzi et al., 2013), identify these analytes as being specifically modulated by $A_{2A}R$ agonists. Therefore, our first goal was to administer CGS21680 at a single timepoint and record a behavioral timecourse and then attempt to block the anti-allodynic effect. The next step was to determine whether there were changes in gene expression in the spinal cord at different timepoints following CGS21680 administration. The last step was to define whether the effects were either agonist or timing-specific. Thus, another $A_{2A}R$ agonist, ATL313, was administered both early and late in the development of SNAP.

2. Materials and methods

2.1. Animals

Pathogen-free male Sprague-Dawley rats (325–350 g; Harlan Laboratories, Madison, WI, USA) were used for all experiments. Rats were pair-housed prior to surgery and then single-housed after surgery with standard rat chow and water available *ad libitum* throughout. Housing was in a temperature-controlled room that was maintained at 23 ± 2°C with a 12 h light/dark cycle (lights on at 0700 h). The rats were allowed a minimum of 1 week to habituate to the colony room before initiating the experiment. All procedures were performed during the light cycle. All protocols were approved by the University of Colorado at Boulder Institutional Animal Care and Use Committee.

2.2. Drugs

The $A_{2A}R$ agonist 2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxamido adenosine HCl (CGS21680) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The $A_{2A}R$ agonist 4-(3-(6-amino-9-(5-cyclopropylcarbamoyl-3,4-dihydroxytetrahydrofuran-2-yl)-9*H*-purin-2-yl)prop-2-ynyl)piperidine-1-carboxylic acid methyl ester (ATL313) was a gift from PGxHealth, a division of Clinical Data, Inc (New Haven, CT, USA). All drugs were dissolved in 100% DMSO to create a 100 mM stock solution for CGS21680 and 10 mM stock solution for ATL313 and were stored at −20 °C. Fresh aliquots were diluted in sterile endotoxin-free isotonic saline (Abbott Laboratories, Abbott Park, IL, USA). The vehicle for all drugs was 0.01% DMSO saline solution given the dilution of the drugs from stock was 1:10,000 to yield a 10 μM dose for CGS2160 and a 1 μM dose for ATL313, based on our past studies of their relative efficacies in reversing pain induced by peripheral neuropathy (Loram et al., 2009; Loram et al., 2013). All vehicle injections were administered equivolume to CGS21680 and ATL313. Rat IL-10 neutralizing antibodies were raised in sheep at the National Institute of Biological Standards and Control (South Mimms, Hertfordshire, UK), purified by Avigen (Alameda, CA, USA), and administered in saline. An equal dose of normal sheep IgG in saline (Sigma-Aldrich, St Louis, MO, USA) was used as the control.

2.3. Spinal neuropathic avulsion pain (SNAP) surgery

Unilateral (left) T13/L1 dorsal root avulsion was performed under isoflurane anesthesia, as previously described (Wieseler et al., 2010). Briefly, laminectomy was performed at the T12 vertebral level and the dura mater was incised over the dorsal root entry zone. The T13 and L1 dorsal rootlets were carefully isolated and then clamped at the dorsal root entry zone and briskly pulled out (avulsed). The dura was then sutured closed with sterile 6–0 silk. Sterile saline-moistened surgical sponge was placed over the exposed spinal cord to protect it, the muscle was sutured in layers with sterile 3–0 silk, and the skin was closed with stainless steel wound clips. Immediately following surgery, rats were single-housed in a cage with foam padding for a few hours to protect their spinal cord from further trauma due to the brief ataxic period that follows recovery from anesthesia. Sham operated animals were treated identically, including dura suturing, except for avulsing of the rootlets. Combi-Pen-48 antibiotic (Bimeda, Inc., Le Sueur, MN, USA) was administered at the time of surgery and daily for 4 days after surgery. Notably, sham-operated animals were only used as controls for behavioral studies, whereas naïve animals were used as controls for tissue studies. Sham SNAP surgery causes significant inflammation which may have altered the effect of $A_{2A}R$ agonist administration (Murphree et al., 2005), masking important and clinically-relevant effects of $A_{2A}R$ agonist treatment for SCI, which encompasses ongoing pain from both nervous system tissue and non-nervous system tissue. It is important to note, however, that the observed mRNA changes in SNAP animals may reflect in part the trauma to non-nervous system tissue associated with SNAP surgery.