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Impaired diffuse noxious inhibitory controls in specific alternation of rhythm in temperature-stressed rats

Yasuo Itomi, Yasuhiro Tsukimi, Toru Kawamura *

Inflammation Drug Discovery Unit, Pharmaceutical Research Division, Takeda Pharmaceutical Company Limited, 26-1 Muraoka Higashi 2-chome Fujisawa, Kanagawa 251-8555, Japan

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ABSTRACT

Fibromyalgia is characterized by chronic widespread musculoskeletal pain. A hypofunction in descending pain inhibitory systems is considered to be involved in the chronic pain of fibromyalgia. We examined functional changes in descending pain inhibitory systems in rats with specific alternation of rhythm in temperature (SART) stress, by measuring the strength of diffuse noxious inhibitory controls (DNIC). Hindpaw withdrawal thresholds to mechanical von Frey filament or fiber-specific electrical stimuli by the Neurometer system were used to measure the pain response. To induce DNIC, capsaicin was injected into the intraplantar of the forepaw. SART-stressed rats were established by exposure to repeated cold stress for 4 days. In the control rats, heterotopic intraplantar capsaicin injection increased withdrawal threshold, indicative of analgesia by DNIC. The strength of DNIC was reduced by naloxone (μ-opioid receptor antagonist, intraperitoneally and intracerebroventricularly), yohimbine (α_2-adrenoceptor antagonist, intrathecally), and WAY-100635 (5-HT_{1A} receptor antagonist, intrathecally) in the von Frey test. In SART-stressed rats, capsaicin injection did not increase withdrawal threshold in the von Frey test, indicating deficits in DNIC. In the Neurometer test, deficient DNIC in SART-stressed rats were observed only for Aδ- and C-fibers, but not Aβ-fibers stimulation. Analgesic effect of intracerebroventricular morphine was markedly reduced in SART-stressed rats compared with the control rats. Taken together, in SART-stressed rats, capsaicin-induced DNIC were deficient, and a hypofunction of opioid-mediated central pain modulation system may cause the DNIC deficit.

1. Introduction

Fibromyalgia is a chronic disorder characterized by widespread musculoskeletal pain, sleep disturbances, and fatigue without obvious organic cause. Although the American College of Rheumatology has published the 2010 Preliminary Diagnostic Criteria for fibromyalgia (Wolfe et al., 2010), it is not widely used in clinical practice (Arnold et al., 2016). Although the etiology of fibromyalgia remains unknown, it is widely believed that physical and mental stressors, accidents, and surgery may lead to fibromyalgia (Bradley, 2009; Theoharides et al., 2015).

Diffuse noxious inhibitory controls (DNIC) are supraspinal mechanism controlling brainstem opioidergic and monoaminergic neurons related to descending pain inhibitory systems, which suppress pain in one region by noxious stimuli to another region (Le Bars et al., 1981b, 2002; Wen et al., 2010; Van Wijk and Veldhuijzen, 2010). The analgesic effect by heterotopic nociception is also mediated by peripheral mechanisms such as endogenous opioids and somatostatin (Stein et al., 2009; Pintér et al., 2006; Szolcsányi et al., 2004) besides supraspinal mechanisms, DNIC. Clinical studies indicate that fibromyalgia patients have attenuated DNIC (Julien et al., 2005; Wood et al., 2007; Normand et al., 2011), suggesting that descending pain inhibitory systems are dysfunctional in fibromyalgia patients and that the function of endogenous pain inhibitory systems can be evaluated by measuring the strength of DNIC.

Several animal models have been created to elucidate fibromyalgia pathophysiology, including the acid injection-induced muscle hyperalgesia model, the vagotomy-induced hyperalgesia model, and the reserpine-induced hyperalgesia model (Khasar et al., 1998; Sluka et al., 2001; Nagakura et al., 2009). In addition to these animal models, stress-induced fibromyalgia-like models are used since stress is an important factor for fibromyalgia pathophysiology (Green et al., 2011; Quintero et al., 2000, 2003). Repeated cold stress such as specific alteration of rhythm in temperature (SART) stress induces chronic

* Corresponding author.
E-mail addresses: yasuo.itomi@takeda.com (Y. Itomi), yasuhiro.tsukimi@takeda.com (Y. Tsukimi), toru.kawamura@takeda.com (T. Kawamura).

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hyperalgesia to both thermal and mechanical stimuli, by suppressing endogenous pain inhibitory systems (Hata et al., 1988, 1991; Ohara et al., 1991; Nasu et al., 2010; Nishiyori et al., 2010). However, it is unclear whether dysfunction of DNIC contributes to the chronic hyperalgesia in the SART-stressed rats.

Afferent somatosensory fibers classically can be divided into non-noxious myelinated Aβ-fibers, noxious myelinated Aδ-fibers, and noxious unmyelinated C-fibers. However, it is well-known that the Aδ- and C-fibers also convey tactile and itching sensations (François et al., 2015; Lou et al., 2015). It is conceivable that each afferent fiber system may have a different mechanism modulated by descending pain inhibitory systems axis. Accordingly, we used the Neurometer system, which can electrically stimulate each afferent fiber separately (Koga et al., 2005; Matsumoto et al., 2006) in addition to the von Frey filaments for pain assessment.

In this study, we first verified that capsaicin-induced DNIC were mediated by descending pain inhibitory systems in control rats with naloxone (μ-opioid receptor antagonist), yohimbine (α_2-adrenoceptor antagonist), and WAY-100635 (5-HT_{1A} receptor antagonist). Subsequently, we investigated drug efficacies on hyperalgesia, dysfunction of DNIC, and analgesic function of central opioid in SART-stressed rats.

2. Materials and methods

2.1. Experimental animals

Male Sprague–Dawley rats (3 and 5 weeks old) were obtained from Clea Japan Inc. and housed in stainless steel cages under a 12 − h dark–light cycle at 24 ± 1 °C and 55 ± 5% humidity. Rats were used for experiments after a one-week acclimation period. All animal experiments were conducted according to internal Animal Experimentation Ethics Committee guidelines.

Rats were cannulated intracerebroventricularly (i. c. v.) as previously described (Miyamoto et al., 1991; Tortella et al., 1981). Briefly, rats were implanted with a stainless-steel cannula in the lateral cerebral ventricle. After surgery, rats were housed individually and allowed 5 days for recovery. A solution of 10 μl was used for delivery of drugs. Placements of i. c. v. cannula were verified after each experiment, by injecting methylene blue and checking for distribution within the cerebroventricular space. Intrathecal (i. t.) injections were performed previously described (Westin et al., 2010). Briefly, under isoflurane anesthesia, percutaneous i. t. injections were made at the low lumbar level (spinal subarachnoid space L4–5) with a 30-gauge needle perpendicular to the skin. A solution of 10 μl was used for delivery of drugs. Intrathecal placement was verified by a lateral tail flick as the needle entered the subarachnoid space.

2.2. Reagents

Pregabalin (synthesized internally), naproxen (Wako Chemicals), and duloxetine (Luna Chemicals) were dissolved in 0.5% methylcellulose 400 (Wako Chemicals) and administered orally (p. o.) at 10 or 30 mg/kg as indicated. To prepare capsaicin solution stock (50 μg/μl), capsaicin (Wako Chemicals) was first dissolved in ethanol (Wako Chemicals), and then Tween80 (WAKO Chemicals) was added to the solution; the volume of Tween80 was the same as that of ethanol. The stock solution was diluted with saline (Otsuka Pharmaceutical) to 2.5 μg/μl before injection into the intraplantar region of the forepaw (i. pl., 50 μl/rat). Naloxone (Sigma-Aldrich) was dissolved in saline and injected intraperitoneally (i. p., 3 mg/kg) or intracerebroventricularly (i. c. v., 10 μg/rat) just before or 15 min before capsaicin injection, respectively. Yohimbine (Sigma-Aldrich) and WAY-100635 (Sigma-Aldrich) were dissolved in saline and injected intrathecally (i. t.) (Yohimbine: 30 μg/rat, WAY-100635: 3 μg/rat) 15 min before capsaicin injection. Morphine (Takeda Pharmaceutical) was dissolved in saline and injected into the intraplantar region of the hindpaw (i. pl., 100 μg/rat) or intracerebroventricular (i. c. v., 10 μg/rat).

2.3. DNIC assessment

Injection of 125 μg/rat capsaicin (i. pl.) into the forepaw under isoflurane anesthesia was used as a conditioning stimulus to induce DNIC. At 30, 60, 90, and/or 120 min after capsaicin injection, withdrawal thresholds were measured using the von Frey filaments or the Neurometer system as described below. In some experiments, the opioid receptor antagonist naloxone, the α_2-adrenoceptor antagonist yohimbine, or the 5-HT_{1A} receptor antagonist WAY-100635 was administered to investigate the role of descending pain inhibitory systems in the DNIC response. These antagonists were administered at appropriate doses that did not have effects on normal pain thresholds, as stated in previous reports (Wei et al., 2009; Wen et al., 2010).

2.4. Measurement of withdrawal thresholds in response to mechanical or electrical stimulation

Withdrawal thresholds for mechanical stimuli were evaluated using an electronically controlled von Frey filament system (IITC Life Science ALMEMO). Supertip (IITC Life Science) was used as a von Frey filament to apply mechanical stimuli to the plantar surface of the hindpaw. After acclimatization for 20–30 min in a mesh-bottom cage, the rats were stimulated using the Supertip attached to the body of the von Frey apparatus. The pain threshold value (g) was defined as the average of two consecutive avoidance responses. Before SART stress protocol, rats with pain thresholds below 12 g were excluded from subsequent tests because of preexisting hyperalgesia. After the SART stress protocol, withdrawal thresholds were measured in all eligible rats, and then rats were divided into drug treatment groups. For the effects of pregabalin and naproxen, withdrawal thresholds on hindpaw were measured 2 h after oral treatment of drugs on 5th day after the SART stress protocol. For the effect of duloxetine, withdrawal thresholds were measured 3 h after oral treatment of drug on 7th day after the SART stress protocol.

In addition to the von Frey filament system, pain thresholds were measured using the Neurometer system (Neurotron CPT/C) as previously described with slight modifications (Matsumoto et al., 2006). Briefly, rats were wrapped gently in a towel to reduce anxiety, and electrodes made from solder were attached to the sole and back of the right hindpaw with electrode gel (Neurotron) using soft electrode tape (Neurotron). Sine-wave electrical stimulation was applied to selectively activate afferent nerve pathways (2000 Hz for Aβ-fibers, 250 Hz for Aδ-fibers, and 5 Hz for C-fibers). The electric current of the 2000 Hz stimulation was increased by 1 μA/s and that of the 250 and 5 Hz stimulation by 0.4 μA/s until the withdrawal response. Paw withdrawal thresholds to increasing electrical stimuli were measured three times each 30, 90, or 120 min after capsaicin injection, and the averages were calculated. In most cases, the Neurometer tests were performed after completion of the time course study in the von Frey test.

2.5. SART stress

SART stress was induced as previously described (Hata et al., 1988, 1991; Ohara et al., 1991; Nasu et al., 2010; Nishiyori et al., 2010) with slight modifications. Briefly, four-week-old male rats were exposed to alternating one-hour periods of cold (0 °C) and room temperature (24 °C) from 9:30 to 16:30 by repeated transfer