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Effects of alpha 7 positive allosteric modulators in murine inflammatory and chronic neuropathic pain models

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ABSTRACT

Agonists and positive allosteric modulators (PAMs) of α7 nicotinic acetylcholine receptors (nAChRs) are currently being considered as novel therapeutic approaches for managing cognitive deficits in schizophrenia and Alzheimer's disease. Though α7 agonists were recently found to possess antinociceptive and anti-inflammatory properties in rodent models of chronic neuropathic pain and inflammation, the effects of α7 nAChRs PAMs on chronic pain and inflammation remain largely unknown. The present study investigated whether PAMs, by increasing endogenous cholinergic tone, potentiate α7 nAChRs function to attenuate inflammatory and chronic neuropathic pain in mice. We tested two types of PAMS, type I (NS1738) and type II (PNU-120596) in carrageenan-induced inflammatory pain and chronic constriction injury (CCI) neuropathic pain models. We found that both NS1738 and PNU-120596 significantly reduced thermal hyperalgesia, while only PNU-120596 significantly reduced edema caused by a hind paw infusion of carrageenan. Importantly, PNU-120596 reversed established thermal hyperalgesia and edema induced by carrageenan. In the CCI model, PNU-120596 had long-lasting (up to 6 h), dose-dependent anti-hyperalgesic and anti-allodynic effects after a single injection, while NS1738 was inactive. Systemic administration of the α7 nAChR antagonist MLA reversed PNU-120596's effects, suggesting the involvement of central and peripheral α7 nAChRs. Furthermore, PNU-120596 enhanced an ineffective dose of selective agonist PHA-543613 to produce anti-allodynic effects in the CCI model. Our results indicate that the type II α7 nAChRs PAM PNU-120596, but not the type I α7 nAChRs PAM NS1738, shows significant anti-edematous and anti-allodynic effects in inflammatory and CCI pain models in mice.

1. Introduction

Chronic neuropathic pain arguably arises due to long-term plasticity changes in somatosensory pathways from the periphery to the cortex. These plasticity changes often occur after nerve injury and/or dysfunction in the central nervous system (CNS), resulting in significantly enhanced pain sensation (hyperalgesia) or in otherwise non-noxious stimuli to cause pain (allodynia) (Wang et al., 2011; Zhou, 2007; Harden, 2005). Increased pain sensitivity, one of the most common signs of an inflammatory disorder, is mediated by a host of different factors, including enzymes, neuropeptides, eicosanoids, chemokines and cytokines (Dray and Bevan, 1993; Sandkuhler, 2009; Wang et al., 2011). To date, several drugs, such as opioids and anti-inflammatory, anti-seizure and antidepressant agents, used to treat chronic neuropathic pain have major adverse effects and/or incomplete pain relief for patients. Thus, development of drugs possessing increased efficacy and safety is needed.

Previous studies suggest utility of nicotinic acetylcholine receptor (nAChR) agonists to treat chronic pain conditions (Bannon et al., 1998; Khan et al., 2003; Miao et al., 2004; Vincler, 2005; Pacini et al., 2010). Multiple subtypes of nAChRs are expressed in pain transmission pathways (Khan et al., 2003). For example, α4β2* and α7 subtypes are expressed in the spinal cord dorsal horn (Cordero-Erausquin et al., 2004; Cordero-Erausquin and Changeux, 2001; Marubio et al., 1999). Recent work has focused on the role of the α7 nAChRs in modulating inflammation and nociception (Westman et al., 2010; Marrero and Bencherif, 2009; Medhurst et al., 2008; de Jonge and Ulloa, 2007). In addition to their neuronal presence, α7 nAChRs are expressed on macrophages (Tracey, 2002; Wang and Wang, 2003; Ulloa, 2005), which are key immune cells involved in the initiation, maintenance, and

Abbreviations: nAChR, nicotinic acetylcholine receptor(s); s.c., subcutaneous injection; i.p., intraperitoneally; CNS, central nervous system; ACh, acetylcholine; PAMs, positive allosteric modulators; CCI, chronic constriction injury; MIF, migration inhibitory factor.

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resolution of inflammation (Fujiwara and Kobayashi, 2005). Previous studies have demonstrated the importance of acetylcholine (ACh) directly interacting with α7 nAChRs expressed on macrophages and other cytokine-producing cells in down-regulating proinflammatory cytokine synthesis and preventing tissue damage (Tracey, 2002; De Rosa et al., 2009; Wang et al., 2009). In addition, Xiao et al. (2002) showed an up-regulation of α7 nAChR subunit expression in the rat dorsal root ganglion fourteen days after sciatic nerve axotomy. Moreover, α7 nAChRs agonists elicited significant anti-inflammatory and antinociceptive effects in rodent models of chronic neuropathic pain and inflammation (Damaj et al., 2000; Wang et al., 2005; Hamurtekin and Gurun, 2006; Medhurst et al., 2008; Gurun et al., 2009; Rowley et al., 2010). Therefore, the α7 nAChR represents a promising target for the development of analgesic and anti-inflammatory agents. However, concerns regarding α7 nAChR agonists as clinical candidates persist. For example, α7 nAChRs desensitize rapidly in response to high agonist concentration in vitro followed by a long period of desensitization (Bertrand et al., 1992). Furthermore, an agonist-based therapeutic approach would disrupt endogenous cholinergic tone (Papke et al., 2009).

One alternative approach to selectively enhance activity of the α7 nAChRs is via positive allosteric modulation. As reported previously (Faghih et al., 2007), positive allosteric modulators (PAMs) facilitate endogenous neurotransmission and/or enhance the efficacy and potency of an agonist without directly stimulating the agonist-binding sites. In principle, PAMs do not exhibit intrinsic activity at the receptor, however they can reinforce endogenous cholinergic neurotransmission without directly activating α7 nAChRs (Albuquerque et al., 2001; Faghih et al., 2007, 2008). PAMs have been classified as either type I, such as NS1738, or type II, such as PNU-120596, on the basis of their distinct effects on desensitization (Bertrand and Gopalakrisshnan, 2007; Bertrand et al., 2008; Timmermann et al., 2007). PNU-120596, but not NS1738, modifies the equilibrium among active and desensitized states resulting in significantly prolonged responses, even promoting the activation of previously desensitized receptors (Grønlien et al., 2007; Hurst et al., 2005; Roncarati et al., 2008). Initially, allosteric modulators of the α7 nAChRs were developed for the treatment of cognitive disorders such as Alzheimer's disease and schizophrenia, however their effects in pain models have not been reported (Ahring et al., 2007; Faghih et al., 2007; Conejero-Goldberg et al., 2008; McLean et al., 2012).

Therefore, in the present study, we evaluated whether potentiating the endogenous α7 cholinergic system through the allosteric modulation of α7 nAChRs produces anti-inflammatory, anti-hyperalgesic and anti-allodynic effects in mouse models of inflammation and chronic neuropathic pain. Accordingly, NS1738 (a type I α7 nAChR PAM) and PNU-120596 (a type II α7 nAChR PAM) were evaluated in the carrageenan short-term inflammatory pain and the chronic constriction injury (CCI) neuropathic pain models. In addition, we evaluated whether PNU-120596 enhances the antinociceptive effects of a selective α7 nAChRs agonist, PHA-543611, in these models.

2. Materials and methods

2.1. Subjects

Naïve male adult ICR (Harlan Laboratories; Indianapolis, IN) mice weighing between 20 and 30 g served as subjects. Mice were housed 4–5 per cage in a temperature-controlled (20–22 °C) environment with a 12-h light–dark cycle and were given unlimited access to food and water in their home cages. All animals were maintained in a facility approved by the American Association for Accreditation of Laboratory Animal Care and the study was approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University. At the end of each experiment, the animals were euthanized by way of CO_2 inhalation. We attest that all efforts made to minimize the number of animals used and their suffering.

2.2. Drugs

Methyllycaconitine citrate (MLA), was purchased from Sigma–Aldrich Inc. (St. Louis, MO). PNU 120596 [1-(5-Chloro-2,4-dimethoxy-phenyl)-3-(5-methyl-isoxazol-3-yl)] and PHA-543613 were obtained from the National Institute on Drug Abuse (NIDA) supply program (Bethesda, MD). NS 1738 [*N*-(5-Chloro-2-hydroxyphenyl)-*N*′-[2-chloro-5-(trifluoromethyl)phenyl] was purchased from Tocris Biosciences (Minneapolis, MN). All drugs except for PNU-120596 and NS1738 were dissolved in physiological saline (0.9% sodium chloride) and injected subcutaneously (s.c.) in a volume of 1 ml/100 g body weight unless noted otherwise. PNU 120596 and NS1738 were dissolved in vehicle consisting of 1 volume ethanol, 1 volume Emulphor-620 (Rhone-Poulenc, Inc., Princeton, NJ) and 18 volumes distilled water, and injected via the (i.p.) route of administration. Doses of NS1738 and PNU-120596 were chosen based on their activity in *in vivo* models of memory and cognition (Timmermann et al., 2007; Christensen et al., 2010). Lambda-carrageenan was purchased from Sigma–Aldrich (St. Louis, MO) and dissolved in saline. All doses are expressed as the free base of the drug.

2.3. Pain models

2.3.1. Carrageenan model of short inflammatory pain

These procedures have been previously described by (Lichtman et al., 2004). Briefly, edema was induced by giving an intraplantar injection of 0.5% lambda-carrageenan in a 20 μl volume into the hind right paw using a 30½ gauge needle. Saline (20 μl) was injected into the left hind paw.

2.3.1.1. Measurement of paw edema. The thickness of the carrageenan-treated and control paws were measured both before and after carrageenan injection at the time points indicated in Results section, using digital calipers (Traceable Calipers, Friendswood, TX). Data were recorded to the nearest ±0.01 mm and expressed as change in paw thickness ΔPT = right paw thickness − left paw thickness.

2.3.1.2. Measurement of thermal hyperalgesia. Mice were placed in clear plastic chambers (7 cm × 9 cm × 10 cm) on an elevated glass surface and allowed to acclimatize for at least 30 min before testing. The infrared beam of a radiant heat source was directed at the plantar surface of each hind paw, in the area immediately proximal to the toes. A 20-s cut-off time was used. Three measures of paw withdrawal latency were taken and averaged for each hind-paw using the Hargreaves test (Yalcin et al., 2011). The paw withdrawal latency was defined as the time from the onset of radiant heat to withdrawal of the animal's hind paw (Lichtman et al., 2004). Withdrawal thresholds were measured in each hind paw. Results were expressed either as withdrawal latency for each paw or as ΔPWL (s) = contralateral latency − ipsilateral latency.

NS1738, PNU-120596 or vehicle was administered 15 min prior to an intraplantar injection of carrageenan and then mice were tested 6 h after the injection for paw withdrawal latencies and paw diameters. For the antagonist study, s.c. MLA was injected 10 min prior to a PNU-120596 or NS1738 injection, which was followed 15 min later with carrageenan and then mice were tested 6 h after the last injection.

To determine whether PNU-1209596 can reduce established thermal hyperalgesia and paw edema after carrageenan injection, paw withdrawal latency and paw diameter were measured after the establishment of thermal hyperalgesia or paw edema, respectively (3 h after carrageenan administration). Subsequently, a treatment of either vehicle or PNU-120596 (8 mg/kg, i.p.) was administered 3 h after carrageenan, and paw withdrawal latency and paw diameter were measured 15 min, 1 h, and 3 h after either treatment. The dose of 8 mg/kg of PNU-1209596 was chosen since it is generally thought that reversal of inflammation usually requires higher doses than development.

2.3.2. Chronic constriction injury (CCI)

Mice were anesthetized with pentobarbital (45 mg/kg, i.p.). An incision was made just below the hipbone, parallel to the sciatic nerve. The right common sciatic nerve was exposed at the level proximal to the sciatic trifurcation, and a nerve segment 3–5 mm long was separated from surrounding connective tissue. Two loose ligatures of 6–0 silk suture, spaced 1.0–1.5 mm apart, were made around the nerve. Skin and muscles were closed with suture. This procedure resulted in chronic constrictive injury of the ligated nerve. In sham-operated controls, an identical surgical incision was performed on the same paw, except that the sciatic nerve was not ligated. For the purposes of this paper, the paw that underwent surgery will be referred to as the ipsilateral paw, and the paw that did not undergo surgery will be referred to as the contralateral paw. After surgery, mice were allowed to recover in a warmed cage on clean paper towels and then returned to their home cage after regaining consciousness. Any suture that remained after two weeks was removed from the healed surgical wound. We assessed both thermal hyperalgesia and mechanical allodynia in CCI mice via the Hargreaves test and von Frey filaments test, respectively.

2.3.2.1. Thermal hyperalgesia. Thermal hyperalgesia was measured via the Hargreaves test as described earlier, in the context of carrageenan. In the CCI model of neuropathic pain, mice were pretreated with either vehicle or NS1738, and then