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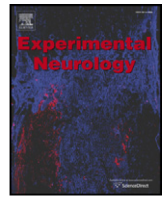

Research Paper

The interaction between alpha 7 nicotinic acetylcholine receptor and nuclear peroxisome proliferator-activated receptor-α represents a new antinociceptive signaling pathway in mice

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ABSTRACT

Recently, α7 nicotinic acetylcholine receptors (nAChRs), primarily activated by binding of orthosteric agonists, represent a target for anti-inflammatory and analgesic drug development. These receptors may also be modulated by positive allosteric modulators (PAMs), ago-allosteric ligands (ago-PAMs), and α7-silent agonists. Activation of α7 nAChRs has been reported to increase the brain levels of endogenous ligands for nuclear peroxisome proliferator-activated receptors type-α (PPAR-α), palmitoylethanolamide (PEA) and oleoylethanolamide (OEA), in a Ca^{2+}-dependent manner. Here, we investigated potential crosstalk between α7 nAChR and PPAR-α, using the formalin test, a mouse model of tonic pain. Using pharmacological and genetic approaches, we found that PNU282987, a full α7 agonist, attenuated formalin-induced nociceptive behavior in α7-dependent manner. Interestingly, the selective PPAR-α antagonist GW6471 blocked the antinociceptive effects of PNU282987, but did not alter the antinociceptive responses evoked by the α7 nAChR PAM PNU120596, ago-PAM GAT107, and silent agonist NS6740. Moreover, GW6471 administered systemically or spinally, but not via the intraplantar surface of the formalin-injected paw blocked PNU282987-induced antinociception. Conversely, exogenous administration of the naturally occurring PPAR-α agonist PEA potentiated the antinociceptive effects of PNU282987. In contrast, the cannabinoid CB_1 antagonist rimonabant and the CB_2 antagonist SR144528 failed to reverse the antinociceptive effects of PNU282987. These findings suggest that PPAR-α plays a key role in a putative antinociceptive α7 nicotinic signaling pathway.

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1. Introduction

Nicotinic acetylcholine receptors (nAChRs) play an active role in modulating pain transmission pathways (Khan et al., 2003), in which their stimulation produces antinociception in pre-clinical and clinical pain models (Umana et al., 2013). Functional nAChRs are pentameric structures that may be homomeric, containing only α subunits, or heteromeric, containing α and β subunits (Jensen et al., 2005). Homomeric α7 nAChRs are abundantly expressed in the central and peripheral nervous systems, including neuronal and non-neuronal cells (Girod et al., 1999). These receptors represent viable drug targets for cognitive and neurodegenerative disorders, and may have potential to treat inflammatory and pain disorders.

Activation of the α7 nAChR ion channel is primarily controlled by the binding of agonists at orthosteric sites, and may also be regulated by allosteric conformational stabilization (Horenstein et al., 2016). Full and partial α7 nAChR agonists elicit significant anti-inflammatory and antinociceptive effects in several experimental models of tonic and

Abbreviations: nAChRs, α7 nicotinic acetylcholine receptors; PPAR-α, nuclear peroxisome proliferator-activated receptors type-α; PAMs, positive allosteric modulators; ago-PAMs, ago-allosteric ligands; PEA, palmitoylethanolamide; OEA, oleoylethanolamide; CB_1 and CB_2, cannabinoid receptors 1 and 2; CNS, central nervous system; AEA, anandamide; 2-AG, 2-arachidonoylglycerol.

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chronic pain (Damaj et al., 2000; Feuerbach et al., 2009; Wang et al., 2005). Moreover, previous studies demonstrated that α7 nAChR selective positive allosteric modulators (PAMs) were also active in rodent models of chronic and inflammatory pain (Freitas et al., 2013a, 2013b, 2013c; Freitas et al., 2013b; Munro et al., 2012). PAMs facilitate endogenous neurotransmission and enhance efficacy and potency of α7 nAChR agonists without directly stimulating the orthosteric binding site (Bertrand and Gopalakrishnan, 2007; Faghih et al., 2007). PAMs lack intrinsic agonist activation, but ago-allosteric ligands (ago-PAMs) exhibit dual activity, via allosteric and orthosteric interactions (Gill et al., 2011; Horenstein et al., 2016; Papke et al., 2014a, 2014b; Thakur et al., 2013), eliciting antinociceptive and anti-inflammatory effects in mice (Bagdas et al., 2016). A new class of modulators, α7 nAChR silent agonists, are unique in that they bind the receptor but preferentially induce non-conducting states, which modulate inflammation and nociception in rodents (Papke et al., 2015) via an unknown signaling mechanism.

Several mechanisms may mediate the anti-inflammatory properties of α7 nAChR agonists. In the central nervous system (CNS), α7 nAChRs exhibit rapid activation and desensitization, as well as high calcium permeability, leading to activation of calcium-dependent intracellular phosphatases and kinases (Feuerbach et al., 2009; Williams et al., 2011). While the intracellular pathways following α7 nAChR activation in non-neuronal cells may involve calcium influx through the channel, signaling pathways independent of ion flux were also reported (Papke et al., 2014a, 2014b). Recently, α7 nAChRs pharmacological activation have been found to increase the brain levels of palmitoylethanolamide (PEA) and oleoylethanolamide (OEA), endogenous agonists for nuclear peroxisome proliferator-activated receptor type-α (PPAR-α), in a Ca^{2+}-dependent manner (Melis et al., 2013). These findings suggest a pharmacological crosstalk between PPAR-α and α7 nAChRs in the CNS.

Based on the research outlined above, we hypothesized that PPAR-α activation might represent a novel pathway that mediates analgesic effects of α7 nAChR. To test this hypothesis, we manipulated both receptors in mice using pharmacological and genetic approaches, and then evaluated the mice in the formalin test, a tonic model of pain.

2. Material and methods

2.1. Animals

Male ICR mice (8–10 weeks of age) were obtained from ENVIGO (Indianapolis, IN). Mice null for the α7 (The Jackson Laboratory, Bar Harbor ME) or β2 subunits (Pasteur Institute, Paris, France) on a C57BL/6J background were bred with wild-type (WT) littermates in an Association for Assessment and Accreditation of Laboratory Animal Care approved animal care facility at Virginia Commonwealth University. For all experiments, mice were backcrossed for ≥8 generations. Knockout (KO) and WT mice were obtained by crossing heterozygote mice. Mice were housed in groups of four at 21 °C in a humidity-controlled environment. Animals had ad libitum access to food and water. The rooms were on a 12-h light/dark cycle (lights on at 7:00 AM) with all experiments performed during the light cycle. Unless otherwise noted, animals were promptly euthanized after experiments so as to minimize suffering. The study was approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University. All studies were carried out in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

PNU282987 [*N*-(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl-4-chlorobenzamide] (selective α7 full agonist), PNU120596 [N-(5-chloro-2,4-dimethoxyphenyl)-N′-(5-methyl-3-isoxazolyl)-urea] (α7 nAChR PAM), PHA543613 (selective α7 full agonist), SR144528 [5-(4-chloro-3-methylphenyl)-1-[(4-methylphenyl)methyl]-N-[(1*S*,2*S*,4*R*)-1,3,3-trimethylbicyclo[2.2.1]heptyl-2-yl]-1H–pyrazole-3-carboxamide] (CB_2 antagonist) and rimonabant (CB_1 antagonist) were obtained from the National Institute on Drug Abuse (NIDA) supply program (Rockville, MD). GW6471 [*N*-((2*S*)-2-(((1*Z*)-1-Methyl-3-oxo-3-(4-(trifluoromethyl)phenyl)prop-1-enyl)amino)-3-(4-(2-(5-methyl-2-phenyl-1,3-oxazol-4-yl)ethoxy)phenyl)propyl)propanamide] (selective PPAR-α antagonist), and N-palmitoylethanolamine (PEA) were purchased from Tocris Biosciences (Minneapolis, MN). Methyllycaconitine citrate (MLA) (selective α7 antagonist) was purchased from RBI (Natick, MA). GAT107 ((3a*R*,4*S*,9b*S*)-4-(4-bromophenyl)-3a,4,5,9b–tetrahydro-3*H*-cyclopenta[*c*]quinoline-8-sulfonamide) (α7 ago-PAM) was synthesized as described previously (Kulkarni and Thakur, 2013; Thakur et al., 2013). NS6740 ((1,4-diazabicyclo[3.2.2]nonan-4-yl(5-(3-(trifluoromethyl) phenyl) furan-2-yl) methanone) (α7 silent agonist) was prepared as previously described (Papke et al., 2015).

GW6471, PNU120596, GAT107, PEA, rimonabant, and SR144528 were dissolved in a mixture of 1:1:18 [1 volume ethanol/1 volume Emulphor-620 (Rhone-Poulenc, Inc., Princeton, NJ)/18 volumes distilled water] and administered intraperitoneally (i.p.) for systemic injections. In addition to i.p. route, GW6471 was also administered intraplantar (i.pl.) and intrathecal (i.t.). PNU282987, PHA54613, NS6740 were dissolved in physiological saline (0.9% sodium chloride) and injected subcutaneously (s.c.), with the exception of NS6740, which was administered i.p. All drugs were injected at a total volume of 1 mL/100 g body weight, unless noted otherwise. All doses are expressed as the free base of the drug.

2.3. Formalin test

The formalin test was carried out in an open, empty Plexiglas cage (29 × 19 × 13 cm). Mice were allowed to acclimate for 15 min in the test cage prior to injection. Each animal was injected with 20 μL of (2.5%) formalin to the right hind paw (i.pl.). Mice were observed from 0 to 5 min (phase I) and 20 to 45 min (phase II) post-formalin injection. The amount of time spent attending to (i.e., licking) the injected paw was recorded with a digital stopwatch. Unless otherwise noted, all experiments were performed on ICR mice. PNU282987 (0.1, 1, 10 and 20 mg/kg, s.c.) or vehicle was administered 15 min prior to formalin injection. In a separate cohort, PNU282987 (10 mg/kg, s.c.) effects in the formalin test were measured in α7 and β2 WT and KO mice. In order to test the involvement of PPAR-α and its site of action in PNU282987-evoked antinociception, i.p., i.t., and i.pl. injections of varying doses of the PPAR-α antagonist GW6471 or vehicle, were injected before PNU282987 (10 mg/kg, s.c.) or vehicle. For systemic experiments, GW6471 (0.2 and 2 mg/kg, i.p.) or vehicle was administered 30 min prior to PNU282987. For CNS experiments, GW6471 (0.2 or 1 μg/5 μL/mouse, i.t.) or its vehicle were administered 5 min before PNU282987 or its vehicle. The i.t. injections were performed free-hand between the fifth and sixth lumbar vertebra in unanesthetized mice according to the method of Hylden and Wilcox (1980). For local experiments, GW6471 (1 μg/20 μL/mouse) or vehicle was administered i.pl. 5 min before PNU282987 or its vehicle. Formalin test was performed 15 min after PNU282987 injection.

To test the effects of GW6471 on various α7 nAChR modulators in the formalin test, we assessed several compounds which preferentially induce different α7 nAChR conformational states (Bagdas et al., 2016; Freitas et al., 2013a; Papke et al., 2015). For these experiments, the α7 nAChR full agonist PHA-543613, silent agonist NS6740, PAM PNU120596, and ago-PAM GAT107 were used. PHA-543613 (6 mg/kg, s.c.), NS6740 (9 mg/kg, i.p.), PNU120596 (10 mg/kg, i.p.), and GAT107 (10 mg/kg, i.p.) or their vehicles were administered 15 min after GW6471 (2 mg/kg, i.p.) or its vehicle. The formalin test began 15 min later.

Additionally, we assessed the pharmacological interaction between the PPAR-α agonist PEA and PNU282987 in the formalin test. We

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