Neuropharmacology 95 (2015) 37–49

Contents lists available at ScienceDirect

Neuropharmacology

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

Role of α5-containing nicotinic receptors in neuropathic pain and response to nicotine

Dimitris N. Xanthos [a], Johannes W. Beiersdorf [b], Ariane Thrun [b], Bogdan Ianosi [b], Avi Orr-Urtreger [c, d], Sigismund Huck [b], Petra Scholze [b, *]

[a] Department of Neurophysiology, Center for Brain Research, Medical University of Vienna, Spitalgasse 4, 1090 Austria
[b] Department of Pathobiology of the Nervous System, Center for Brain Research, Medical University of Vienna, Spitalgasse 4, 1090 Austria
[c] The Genetic Institute, Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel
[d] The Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel

ARTICLE INFO

Article history:
Received 30 July 2014
Received in revised form
3 February 2015
Accepted 10 February 2015
Available online 25 February 2015

Keywords:
Nicotinic receptor
α5
Accessory subunit
Neuropathic
Nicotine
Central nervous system
Pain
Tolerance

ABSTRACT

Nicotinic receptors in the central nervous system (nAChRs) are known to play important roles in pain processing and modulate behavioral responses to analgesic drugs, including nicotine. The presence of the α5-neuronal nicotinic accessory subunit in the nicotinic receptor complex is increasingly understood to modulate reward and aversive states, addiction, and possibly pathological pain. In the current study, using α5-knockout (KO) mice and subunit-specific antibodies, we assess the role of α5-containing neuronal nicotinic receptors in neuropathic pain and in the analgesic response to nicotine. After chronic constriction injury (CCI) or partial sciatic nerve ligation (PSNL), no differences in mechanical, heat, or cold hyperalgesia were found in wild-type (WT) *versus* α5-KO littermate mice. The number of α5-containing nAChRs was decreased (rather than increased) after CCI in the spinal cord and in the thalamus. Nevertheless, thermal analgesic response to nicotine was marginally reduced in CCI α5-KO mice at 4 days after CCI, but not at later timepoints or after PSNL. Interestingly, upon daily intermittent nicotine injections in unoperated mice, WT animals developed tolerance to nicotine-induced analgesia to a larger extent than α5-KO mice. Our results suggest that α5-containing nAChRs mediate analgesic tolerance to nicotine but do not play a major role in neuropathic pain.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Neuronal nicotinic acetylcholine receptors (nAChRs) are pentameric ligand-gated channels formed from multiple α (α2–α10) and β subunits (β2–β4) in various combinations that are widely but not uniformly distributed in the peripheral and central nervous system. Heteropentameric nAChRs with an α3β4 backbone prevail in the PNS, whereas α4β2 receptors are more numerous in most parts of the CNS. Both the pharmacological and biochemical properties of nAChRs are critically determined by their subunit composition. Multiple neurobehavioral changes and effects have been attributed to nicotinic receptors in the CNS (Jacob et al., 2013; Dani and Bertrand, 2007; Hurst et al., 2013), including analgesia, allodynia, and pathological pain (Lawand et al., 1999; Bartolini et al., 2011; Umana et al., 2013; Hurst et al., 2013).

Various nicotinic agonists, e.g. epibatidine and related compounds, are potent analgesics acting at the spinal and supraspinal level (Khan et al., 1998, 2001; Bannon et al., 1998; Damaj et al., 1998). Substances such as epibatidine and ABT-594 have been known for quite some time to be equally or more potent analgesics than morphine, depending on the assay (Bannon and Jarboe, 1978). Nicotinic agonist antinociceptive effects have also been shown in animal models of postoperative (Rowley et al., 2008) and of neuropathic pain (Di Cesare et al., 2013; Abdin et al., 2006; Pacini et al., 2010). To date, several types of nAChRs have been implicated in mediating these effects, namely receptors containing the subunits α4 and β2 (Marubio et al., 1999; Khan et al., 2001), α3 (Young et al., 2008; Albers et al., 2014), α5 (Jackson et al., 2010) and α7 (Feuerbach et al., 2009). *In vivo* evidence for receptors containing the above subunits has been provided by the use of receptor-selective agonists and antagonists, as well as with mice carrying deletions of distinct nAChR subunit genes. Based on

* Corresponding author. Center for Brain Research, Medical University of Vienna, Spitalgasse 4, 1090 Vienna, Austria. Tel.: +43 1 40160 34092.
E-mail addresses: dimitris.xanthos@meduniwien.ac.at (D.N. Xanthos), petra.scholze@meduniwien.ac.at (P. Scholze).

http://dx.doi.org/10.1016/j.neuropharm.2015.02.012
0028-3908/© 2015 Elsevier Ltd. All rights reserved.

molecular modeling, desensitization of α4β2α5 receptors has recently been proposed as the mechanism which mediates the analgesic effect of nicotinic agonists (Zhang et al., 2012). Paradoxically, positive allosteric modulation using novel compounds acting on various nAChRs have also been shown to have potent effects in animal behavioral studies (Uteshev, 2014; Pandya and Yakel, 2013; Rode et al., 2012). For example, the positive α4β2 allosteric modulator NS-9283 can potentiate the analgesic efficacy of the epibatidine analogue ABT-594 (Zhu et al., 2011). Although analgesic effects have to date most often been reported to be due to action at α4β2 containing receptors, recent studies suggest that this subunit combination can be deemed as necessary but not necessarily sufficient to produce analgesia (Gao et al., 2010).

A number of studies have furthermore suggested that nAChRs are directly involved in the pain processing of noxious stimuli and in neuropathic pain. Hence, deletion of the β2 subunit lowers the mechanical and thermal nociceptive thresholds in β2-KO mice (Yalcin et al., 2011), knockdown of α5-containing receptors by intrathecal antisense oligonucleotides moderately reduces allodynia (Vincler and Eisenach, 2005), and hyperalgesia in a nicotine withdrawal model is lost in α7-KO mice (Jackson et al., 2008). After spinal nerve ligation in rats, spinal α5 receptor upregulation has also been reported (Vincler and Eisenach, 2004; Young et al., 2008).

Our work focuses on further studying the role of α5-containing receptors in neuropathic pain and in mediating the analgesic effects of nicotine. α5 is considered an accessory subunit as it can only form functional receptors when co-expressed with a principal subunit (such as α2, α3, or α4) and one complementary subunit (β2 or β4, e.g. as α4β2α5 or α3β4α5 receptors) (Wang et al., 1996; Gerzanich et al., 1998; Ramirez-Latorre et al., 1996). Recent studies using specific antibodies have localized the α5 subunit in various CNS regions, including the substantia nigra pars compacta, medial habenula, interpeduncular nucleus (IPN), striatum, thalamus, prefrontal cortex, hippocampus, and the spinal cord in both rats and mice (Mao et al., 2008; David et al., 2010; Grady et al., 2009; Scholze et al., 2012; Beiranvand et al., 2014). α5 assembles into α3β4 receptors in the superior cervical ganglion (SCG) (Mao et al., 2006; David et al., 2010), whereas in CNS regions such as the hippocampus, the striatum, the cerebral cortex, or the thalamus, α5 is found in combination with the subunits α4 and β2 (Mao et al., 2008). In the habenula, α5 co-assembles with both β2 and β4 to form the α3α5β4β2 complex (Grady et al., 2009; Scholze et al., 2012), while in the IPN α5 subunits co-assemble with β2, but not β4 (Grady et al., 2009; Beiranvand et al., 2014). The presence of α5 can profoundly impact the overall pharmacological and physiological properties of the receptor complex. Effects include altered calcium permeability, increased sensitivity to allosteric modulators, altered receptor desensitization, altered single-channel properties, or altered agonist-mediated responses such as effects on the potency and efficacy of agonists (Ciuraszkiewicz et al., 2013; Tapia et al., 2007; Kuryatov et al., 2008). Two tests for thermal sensitivity testing involving spinal and supraspinal mechanisms show that effects of nicotine are largely reduced in α5-KO mice (Jackson et al., 2010).

In the current study, we test whether α5-KO mice differ from their WT littermates in two well-established models of neuropathic pain and in their responses to analgesic doses of nicotine. We furthermore measure the overall number of hetero-pentameric nAChRs and the expression of distinct receptors containing the subunits β2-, β4-, and α5 by means of immunoprecipitation in the lumbar spinal cord, thalamus, hippocampus, habenula, striatum, and the IPN after peripheral nerve injury. We found no differences in the development of neuropathic pain between WT and α5-KO mice, and only minor changes in the expression of nicotinic receptors after peripheral nerve injury. The thermal analgesic effects of acute nicotine administration were also only marginally different. However, when tested in unoperated mice, WT animals developed tolerance to nicotine-induced analgesia to a larger extent than α5-KO mice.

2. Materials and methods

2.1. Animals

For behavioral experiments (see exception below) and all biochemical assays, adult male littermate WT mice and mice with a deletion of the α5 nAChR subunit gene (α5-KO) (Wang et al., 2002) were used. Mice used in this study were backcrossed into C57Bl/6J background for at least 7 generations after germ line transmission. For most of the experiments, KO and WT mice were littermates from heterozygous breeding pairs and genotyped at weaning (18 days after birth). When probing for nicotine tolerance, some experiments were, in addition, performed on "cagemate" mice (pooled at weaning from litters of the two homozygous breeding pairs). Experiments were performed within the age range of 2–5 months. All mice were bred in-house and kept in Type IIL cages (~553 cm^2) at a density of 4–6 per cage. Animals were maintained and tested in state-of-the-art temperature and humidity controlled housing facilities and behavioral testing rooms set at 20–24 °C, 40–60% humidity, 12 h light/dark cycle, and food and water provided *ad libitum*. Experiments were always performed during the light cycle between the hours of 10AM–6PM.

Experimental procedures were approved by the Ethics Committee of the Medical University of Vienna and the Austrian Federal Ministry of Science and Research (BMWF). Extra care was taken to minimize animal suffering and to limit the number of animals used for experiments.

2.2. Models of neuropathic pain

For the partial sciatic nerve ligation (PSNL) model, mice received an injury to the left sciatic nerve, according to a standard method previously reported (Malmberg and Basbaum, 1998) which is based on the Seltzer model (Seltzer et al., 1990). Mice were kept anaesthetized using a gaseous mixture of nitrous oxide (~25%), oxygen (~75%), and 1.5% isoflurane (~5% for induction). Under sterile conditions, the sciatic nerve was exposed at thigh level and carefully freed from the surrounding connective tissue. Using a fine curved needle, the nerve was pierced at the midline and one tight ligature using a G-6 (8.0 mm 3/8c) silk suture (Ethicon, Vienna, Austria) was applied to ligate half the nerve. The wound was closed with 7-0 Prolene® polypropylene sutures (Ethicon, Vienna, Austria) which were applied to both muscle and skin. In the sham-operated mice, only the skin and the muscle were carefully freed, while the sciatic nerve was left intact. Nitrofurazone ointment was applied topically on the wound to prevent infection. Animals were housed individually after surgery and monitored for any motor deficits or abnormalities in the days after surgery (less than 3% of animals).

For the chronic constriction injury (CCI) model, a similar procedure as above was followed except that the nerve was not pierced but instead carefully ligated in its entirety with three loose CATGUT® chrome absorbable surgical suture (SMI AG; St. Vith, Belgium) based on the Bennett model (Bennett and Xie, 1988). Double knots were used to prevent knot slippage and ligature tightness was regulated to prevent ischemia. Animals were also housed individually after surgery, and any animal showing severe motor deficits was excluded (less than 5% of animals).

2.3. Behavioral tests

Prior to any behavioral tests, animals were acclimatized to the non-sterile holding rooms for at least two weeks. They were then habituated to the behavioral testing facilities, testing equipment, and to the experimenter for at least three days prior to any experimentation. In the neuropathic pain models, littermate WT and α5-KO mice were tested up to 21 days after PSNL, and up to 29 days after CCI nerve injury or sham surgery at time intervals indicated in the figures ($n = 9$ per group). The order of testing was always first for mechanical, followed by cold, and lastly heat sensitivity, with at least one hour of habituation time between each test modality. The contralateral (unoperated side) hindpaw was tested first, followed by the ipsilateral hindpaw (operated side). WT and α5-KO animals were assigned randomly into treatment and control groups, and these were tested in parallel. All experiments were performed by an experimenter who was blinded to the genotype of the animals. Genotyping was also confirmed at the end of the experiments in randomly chosen animals.

2.3.1. Heat hypersensitivity to measure pathological pain

The Hargreaves plantar test (Hargreaves et al., 1988) was used to test for thermal hyperalgesia in both hindpaws. Mice were placed individually inside Plexiglas cylinders (~7 cm diameter) on the glass floor of an Analgesiometer apparatus (Stoelting Co, Wood Dale, IL, U.S.A.) and habituated for at least 20–30 min prior to testing. Thermal stimulation of the hindpaws was performed by aiming the radiant heat source positioned beneath the glass floor to the center of the plantar hindpaw. The hindpaw withdrawal latency (PWL), i.e. the time until the first clear nociceptive reaction directed to the hindpaw (withdrawal, flinching, licking), was manually

Download English Version:

https://daneshyari.com/en/article/5813647

Download Persian Version:

https://daneshyari.com/article/5813647

Daneshyari.com