Neurochemistry International 90 (2015) 166–172

Contents lists available at ScienceDirect

Neurochemistry International

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

Nicotinic receptors modulate the function of presynaptic AMPA receptors on glutamatergic nerve terminals in the trigeminal caudal nucleus

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ARTICLE INFO

Article history:
Received 23 March 2015
Received in revised form
13 July 2015
Accepted 7 August 2015
Available online 12 August 2015

Keywords:
Nicotinic receptors
AMPA receptors
glutamate release
Isolated nerve endings
Trigeminal caudal nucleus

ABSTRACT

In this study, we demonstrate the existence on trigeminal caudal nucleus (TCN) glutamatergic terminals of α4β2 nicotinic receptors (nAChRs) capable of enhancing the terminals' spontaneous release of [^{3}H]D-aspartate ([^{3}H]D-Asp). In rat TCN synaptosomes, spontaneous [^{3}H]D-Asp release was increased by nicotine and the nicotinic receptor agonists (±)epibatidine and RJR2403. The increase was potentiated by the positive allosteric modulator of nAChRs LY2087101, inhibited by the nicotinic antagonists mecamylamine (MEC) and dihydro-β-erythroidine hydrobromide (DHβE), and unaffected by α-bungarotoxin (α-BgTx) and methyllycaconitine (MLA). Evidence of functional interaction was observed between the α4β2 nAChRs and cyclothiazide-sensitive, alfa-amino-3-hydroxy-5-methyl-4-isoxazolone propionate (AMPA) receptors co-localized on the TCN synaptosomes. Brief pre-exposure of synaptosomes to 30 μM nicotine or 10 μM RJR2403 abolished the AMPA (100 μM) -induced potentiation of $[K^+]_e$-evoked [^{3}H]D-Asp release, an effect that seems to be caused by nicotine-induced increases in the internalization of AMPA receptors. Indeed, the effects of nicotine-pretreatment were not seen in synaptosomes containing pre-entrapped pep2-SVKI, a peptide known to compete for the binding of GluA2 subunit to scaffolding proteins involved in AMPA endocytosis, while entrapment of pep2-SVKE, an inactive peptide used as negative control, was inefficacious. These findings show that nicotine can negatively modulate the function of AMPA receptors present on glutamatergic nerve terminals in the rat TCN. Dynamic control of AMPA receptors by the nicotinic cholinergic system has been observed under other experimental conditions, and it can contribute to the control of synaptic plasticity such as long-term depression and potentiation. Nicotine's ability to reduce the functionality of presynaptic AMPA receptors could contribute to its analgesic effects by diminishing glutamatergic transmission from the primary afferent terminals that convey nociceptive input to TCN.

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1. Introduction

The current therapies for chronic pain have limited efficacy and are associated with dose-limiting side effects. Compounds that act at nicotinic acetylcholine receptors (nAChRs) in the CNS and periphery have been reported to show antinociceptive activity in several rodent acute and chronic pain models (Decker et al., 2001). nAChRs are ligand-gated ion channels composed of α and β subunits that assemble to form receptors subtypes with a wide range of physiological and pharmacological profiles (Le Novère et al., 2002; Gotti et al., 2006). nAChRs are preferentially located at the preterminal and presynaptic sites regulating neurotransmitter release in several brain regions (Wonnacott, 1997).

Multiple subtypes of nAChRs are expressed in pain transmission pathways (Wada et al., 1989; Khan et al., 2003; Umana et al., 2013). 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). The dorsal spinal cord plays important roles in receiving, integrating, and transmitting peripheral nociceptive information. nAChRs subunits are found mainly on primary afferent terminals (Khan et al., 2003) and intrinsic spinal interneurons (Cordero-Erausquin et al., 2000). Research on the effects of nicotine and (±)epibatidine on

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http://dx.doi.org/10.1016/j.neuint.2015.08.008
0197-0186/Published by Elsevier Ltd.

nociceptive transmission in the spinal cord suggests that cholinergic antinociception depend on facilitation of inhibitory GABAergic and glycinergic neurotransmission (Cordero-Erausquin et al., 2004; Genzen and McGehee, 2005; Kiyosawa et al., 2001; Rashid et al., 2006) and activation of descending pain-inhibitory pathways (Christensen and Smith, 1990).

In the present study, we have studied presynaptic nAChRs involved in the regulation of [^{3}H]D-aspartate ([^{3}H]D-Asp) release from nerve terminals isolated from trigeminal caudal nucleus (TCN). The TCN is an integrative relay station that links peripheral and central pain pathways, and its neurons share many features (structural and functional) with those of the dorsal spinal cord (Sessle, 2000). Spinal cord neurons process pain stimuli convoy from the body, while TCN neurons receive and process sensory input from the orofacial tissues (Sessle, 2000). The presence of nAChRs subunits in sensory neurons of trigeminal ganglia (TG) that extend their central projections to TNC, has been well documented by binding, expression and functional studies (Wada et al., 1989, 1990; Liu and Simon, 1996).

The primary afferents terminals that relay nociceptive information to the TCN neurons contain several transmitters, including glutamate (Glu) (Lazarov, 2002), which plays major roles in the transmission and modulation of sensory information in the trigeminal sensory system (Vikelis and Mitsikostas, 2007). Glutamate not only conveys pain information: it is also an important mediator of the processes leading to the sensitization of second-order neurons that transmit nociceptive information from the TCN to higher brain areas (Bleakman et al., 2006). TCN neuron sensitization appears to worsen headache pain and increase sensitivity to nociceptive stimuli (Silberstein, 2004).

The aim of the present study was to use nerve terminals isolated from the TCN to assess the characteristics and mechanisms underlying nAChRs regulation of excitatory glutamatergic transmission at level of primary central afferents that extend to the spinal trigeminal neurons.

Exposure of TCN synaptosomes prelabelled with [^{3}H]D-Asp to three selective nAChR agonists, epibatidine, nicotine, and RJR2403 stimulated [^{3}H]D-Asp overflow suggesting that nAChRs are localized on TCN nerve endings. Presynaptic nAChRs in the CNS may interact with other metabotropic or ionotropic receptors producing an integrated response which, in turn, generates antagonistic or synergistic effects (Marchi and Grilli, 2010). We have therefore investigated the possible cross-talk between presynaptic nAChRs and AMPA autoreceptors localized on isolated TCN nerve endings (D'Amico et al., 2010). Our results demonstrate that nicotine causes a rapid decrease in the number of surface located coexisting cyclothiazide-sensitive AMPA receptors on TCN glutamatergic terminals. These observations could be relevant to the comprehension of the molecular mechanisms at the basis of nicotine antinociceptive effects.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (weights 200–250 g) were used in the experiments. Animals were housed under constant conditions of temperature (22 ± 1 °C) and relative humidity (50%) with lights on from 7 am to 7 pm and free access to food and water. All animal experiments performed in this study were carried out in accordance with the European Community Council Directive of 1986 (86/609/EEC) and were approved by the Ethics Committee of the Catholic University of the Sacred Heart. Special care was taken to minimize suffering and the number of animals used.

2.2. Preparation of synaptosomes

Animals were killed by decapitation, and the brain and upper portion of the spinal cord were rapidly removed and immersed in cold normal saline (0.9%). We then dissected out the brain and cut a slice of tissue at the level of the caudal brainstem. The tissue block containing the brainstem was sectioned at the level of the obex and 0.4 cm below the obex to isolate the segment containing the TCN (Han et al., 2008). The lateralmost portions (right and left) of this segment were dissected out and used to prepare crude TCN synaptosomes, as previously described (Martire et al., 2004). In brief, the tissues were placed in 40 volumes of 0.32 M sucrose that had been buffered to a pH of 7.2 with phosphate and homogenized (12 strokes at 900 rpm in ~1 min) with a glass-Teflon tissue grinder (clearance 0.25 mm). The homogenate was centrifuged at 1000 g for 10 min and the supernatant re-centrifuged (12,000 g for 20 min) to isolate the synaptosomes. All the above procedures were performed at 0 °C–4 °C.

2.3. Release experiments

The synaptosome pellet was resuspended in a physiological medium (standard medium) containing (in mM) NaCl 125, KCl 3, $MgSO_4$ 1.2, $CaCl_2$ 1.2, NaH_2PO_4 1.0, $NaHCO_3$ 22, and glucose 10 (pH 7.40) and oxygenated with 95% O_2/5% CO_2. The synaptosomes were incubated in an atmosphere of 95% O_2/5% CO_2 for 15 min at 37 °C with D-[2,3-^{3}H]aspartic acid ([^{3}H]D-Aspartate) (an unmetabolizable Glu analog routinely used in release studies as a marker of the endogenous excitatory amino acid transmitter). To prevent glial uptake of the tracer, we added 500 μM dihydrokainic acid. Identical aliquots of the synaptosome suspension (containing from 0.8 to 1.2 mg of protein, depending on the experiment) were placed on 0.8-μm Millipore filters positioned at the bottom of a set of parallel superfusion chambers, which were maintained at 37 °C (Raiteri and Raiteri, 2000). The synaptosome suspension was then washed with 3 × 10 ml of standard medium at 37 °C under moderate vacuum filtration and superfused at a rate of 0.5 ml/min with standard medium aerated with 95% O_2/5% CO_2. After a 30-min equilibration period, the synaptosomes were perfused for 8 min with standard medium, with or without test substances. At this point (i.e., after the first 38 min of perfusion), the synaptosomes were exposed to agonists or to the depolarizing stimulus (15 mM KCl, equimolar substitution of NaCl) (from $t = 38$ min till the end of superfusion). When used, antagonists were added 8 min before the agonists (from t = 30) and remained in the medium till the end of superfusion. When indicated, synaptosomes were exposed in superfusion for 8 min to nicotine (30 μM) or to RJR2403 (10 μM) in the absence or in the presence of DHβE. Certain experiments were conducted with synaptosomes containing entrapped peptide pep2-SVKI or pep2-SVKE. These synaptosomes were obtained by homogenizing the rat TCN tissue in buffered sucrose containing 20 mM of the peptide. Based on experiments conducted with [^{3}H] sucrose, the estimated intrasynaptosomal concentration of the entrapped compound is roughly 5% of the original concentration in the homogenization medium (Raiteri et al., 2000). Detailed descriptions of specific experiments are provided in the figure legends. Fractions of the superfusate were collected every 2 min, starting from minute 35 of the pre-stimulation phase, and radioactivity was counted in each fraction and in the superfused synaptosomes themselves.

2.4. Calculations

Liquid scintillation counting was used to measure radioactivity in each superfusate fraction and in the superfused filters containing