Available online at www.sciencedirect.com
ScienceDirect
Neuropharmacology 54 (2008) 445–453
NEURO PHARMACOLOGY
www.elsevier.com/locate/neuropharm

Heterogeneity of nicotinic acetylcholine receptor expression in the caudal nucleus of the solitary tract

David V. Smith [a], Victor V. Uteshev [a,b,*]

[a] Department of Anatomy and Neurobiology, University of Tennessee Health Science Center, Memphis, TN, USA
[b] Department of Pharmacology, Southern Illinois University School of Medicine, Springfield, IL, USA

Received 8 October 2006; received in revised form 26 September 2007; accepted 22 October 2007

Abstract

The nucleus of the solitary tract (NTS) is the principal integrating relay in the processing of visceral sensory and gustatory information. In the present study, patch-clamp electrophysiological experiments were conducted using rat horizontal brainstem sections. Pre-synaptic and somatic/dendritic nicotinic acetylcholine receptors (nAChRs) expressed in neurons of the caudal NTS (cNTS) were found to be randomly distributed between pre-synaptic and somatic/dendritic sites ($\chi^2 = 0.72$, df = 3, $p > 0.87$, $n = 200$). Pre-synaptic nAChRs were detected by their facilitating effects on glutamatergic neurotransmission of a sub-population of cNTS neurons (categorized as "effect-positive") upon brief picospritzer applications of 0.1–0.5 mM nicotine. These effects were resistant to inhibition by 20 nM methyllycaconitine (MLA) and 4 μM dihydro-β-erythroidine (DHβE), and were replicated by brief picospritzer applications of 0.2–1 mM cytisine. Picospritzer applications of 0.2 mM RJR-2403, a potent agonist of α4β2 nAChRs, did not facilitate synaptic release of glutamate in effect-positive cNTS neurons. The population of somatic/dendritic nAChRs has been found to be heterogeneous and included nAChRs that were activated by RJR-2403 and/or cytisine, or insensitive to cytisine, or inhibited by MLA. The presented results are consistent with the expression of β4-containing (i.e., β4*) nAChRs, likely α3β4*, in pre-synaptic terminals of effect-positive cNTS neurons. Somatic/dendritic nAChRs appear to involve both α7 and non-α7 subunits. Heterogeneity in the subunit composition of pre-synaptic and somatic/dendritic nAChRs may underlie diverse roles that these receptors play in regulation of behavioral and visceral reflexes, and may reflect specific targeting by endogenous nicotinic agents and nicotine.
Published by Elsevier Ltd.

Keywords: Nicotine; RJR-2403; Cytisine; Brainstem; Solitary tract; Pre-synaptic

1. Introduction

The nucleus of the solitary tract (NTS) is a functionally and anatomically heterogeneous group of neurons that acts as a key integrating relay in the processing of visceral sensory and gustatory information (Lawrence and Jarrott, 1996; Contreras et al., 1982; Hamilton and Norgren, 1984). The caudal portion of NTS (cNTS) receives baroreceptor and chemoreceptor afferents from the heart and blood vessels via cranial nerves IX and X to control cardiac output. The NTS also receives visceral afferents from various regions of the gastrointestinal tract (Sun et al., 2005; Zittel et al., 1994; Wang et al., 1999), second-order neurons of the dorsal horn, and other components of the central autonomic system, such as respiratory afferents (Boscan et al., 2002). The rostral portion of NTS (rNTS) receives mostly gustatory information directly from the oral cavity via cranial nerves VII, IX, and X (Ashworth-Preece et al., 1998; Lawrence and Jarrott, 1996; Torvik, 1956).

The presence of choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) immunostaining in the NTS suggests that acetylcholine (ACh) is involved in modulation of visceral sensory and gustatory information (Armstrong et al., 1988; Ueno et al., 1993; Helke et al., 1983; Barry et al., 1993). In the rNTS region, the location of cholinergic neurons is consistent with the distribution of preganglionic parasympathetic

* Corresponding author: Department of Pharmacology MC #9629, SIU School of Medicine, PO Box 19629, Springfield, IL 62794, USA. Tel.: +1 217 545 7351; fax: +1 217 545 0145.
E-mail address: vuteshev@siumed.edu (V.V. Uteshev).

0028-3908/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.neuropharm.2007.10.018

neurons labeled by horseradish peroxidase applied to the chorda tympani branch of the VIIth nerve and the lingual-tonsillar branch of the IXth nerve (Contreras et al., 1980). Furthermore, both low- and high-affinity nicotinic binding sites have been demonstrated in the NTS of cats (Maley and Seybold, 1993); and electrophysiological recordings from the NTS in brainstem slices revealed a functionally heterogeneous population of nicotinic (nACh) and muscarinic (mACh) receptors (Uteshev and Smith, 2006; Ernsberger et al., 1988; Shihara et al., 1999). For the most part, nicotinic and muscarinic receptors were expressed by different cells (Shihara et al., 1999; Uteshev and Smith, 2006).

Although the main neurotransmitter released from the solitary tract appears to be glutamate (Shihara et al., 1999; Li and Smith, 1997; Wang and Bradley, 1995), some solitary tract afferents may be cholinergic, providing extrinsic cholinergic inputs to the NTS. Moreover, glutamate has been demonstrated to be the main neurotransmitter of baroreceptor afferents terminating in the NTS (Talman et al., 1980; Reis et al., 1981). However, microinjections of nicotine into the NTS elicited hypotension and bradycardia similar to that elicited by activation of baroreceptors (Kubo and Misu, 1981; Talman and Lewis, 1991). Local cholinergic interneurons may also provide cholinergic inputs within the NTS region (Kobayashi et al., 1978). Therefore, both glutamatergic and cholinergic systems seem to be involved in processing visceral sensory and gustatory information by the NTS. Additional extrinsic cholinergic inputs may arrive to the NTS from the adjacent dorsal motor nucleus of the vagus (Maley, 1996; Farkas et al., 1997) or nucleus ambiguus (Farkas et al., 1997).

2. Materials and methods

Electrophysiological patch-clamp experiments have been conducted in the Department of Anatomy and Neurobiology at the University of Tennessee Health Science Center in Memphis, TN, and in the Department of Pharmacology at the Southern Illinois University School of Medicine in Springfield, IL. The experimental results obtained in both institutions have resulted in identical conclusions.

2.1. Animals

Sprague–Dawley male or female rats (P18-25, ~100 g) were used in experiments. Animal care was in accordance with the Guide for the Care and Use of Laboratory Animals (NIH 865-23, Bethesda, MD) and was approved by the Animal Care and Use Committee of the University of Tennessee and Southern Illinois University.

2.2. Electrophysiology

Rat brains were rapidly removed following decapitation and placed for 1 min in ice-cold oxygenated sucrose-based solution of the following composition (in mM): sucrose 250, KCl 2.5, NaH_2PO_4 1.23, $MgCl_2$ 5, $CaCl_2$ 0.5, $NaHCO_3$ 26, glucose 10 (pH 7.4), when bubbled with carbogen (95% O_2 and 5% CO_2). The brainstem was then transferred to the brain slicer chamber (Vibratom-3000 (UT, Memphis, TN) or Vibratom-1000 (SIU, Springfield, IL), Vibratome, St. Louis, MO) and two or three horizontal brainstem slices (250 μm thick) were cut. Slices were transferred to a storage chamber, where they were perfused at 30 °C for 30 min in an oxygenated artificial cerebrospinal fluid (ACSF) solution of the following composition (in mM): NaCl 125, KCl 2.5, NaH_2PO_4 1.23, $MgCl_2$ 1, $CaCl_2$ 2, $NaHCO_3$ 26, glucose 10 (pH 7.4). In experiments conducted at SIU, Springfield, IL, the ACSF contained 35 mM $NaHCO_3$ to maintain pH 7.4. Slices were then perfused with an identical oxygenated ACSF at 24 °C for up to 10 h.

For patch-clamp experiments, slices were transferred to the recording chamber and perfused with an oxygenated ACSF at a rate of 1.5 ml/min using a Dynamax peristaltic pump (Rainin Instrument CO, Emeryville, CA) at UT, Memphis, TN; or 1 ml/min using a 2232 Microperpex S peristaltic pump (LK.B, Upsalla, Sweden) at SIU, Springfield, IL. Typically, cNTS neurons selected for patch-clamp recordings were smaller than 25 μm in diameter. Whole-cell recordings were conducted at 24 °C. The intracellular electrode solution contained (in mM): K-gluconate 125, KCl 1, $MgCl_2$ 2, EGTA 1, and K–HEPES 10 (pH 7.3). In some experiments, the intracellular solution did not contain $MgCl_2$ and EGTA. The electrophysiological data were recorded using: a HEKA-9/2 patch-clamp amplifier (HEKA Elektronik, Lambrecht, Germany) at UT, Memphis, TN; or a MultiClamp-700B patch-clamp amplifier at SIU, Springfield, IL (Molecular Devices, Sunyvale, CA). The seal resistance was >2 GΩ; the access resistance was between 10 and 30 MΩ, and typically, was not compensated. Patches with access resistances higher than 30 MΩ were corrected by applying an additional negative suction or discarded. Data were sampled at 10 or 20 kHz and filtered at 3.33 or 6.67 kHz, respectively. Occasionally, low frequency electrical noise (60 Hz) was filtered out during off-line analysis.

Syringe pumps Pump-33 (Harvard Apparatus, Holliston, MA) at UT, Memphis, TN, or Genie Plus (Kent Scientific Corporation, Torrington, CT) at SIU, Springfield, IL, were used to add experimental drugs to the ACSF just before they entered the recording chamber. The final drug concentrations in the bath were arithmetically calculated based on the known concentrations of stock solutions and adjustable rates of all pumps. Peristaltic and syringe pumps used in this study provide exceptional stability of fluid flow and are routinely calibrated. The high degree of stability of flows generated by pumps translates into a high stability of final drug concentrations that are easily calculated. Solutions are mixed in a ~0.3 ml reservoir, ~10 s before entering the first "entrance" chamber (~1 ml), where additional mixing occurs. After the "entrance" chamber, solutions enter the main recording chamber (~2 ml), where the final mixing and application takes place. This 3-step process guarantees a complete mixture of solutions.

A picospritzer (Parker Hannifin Instrumentation, Cleveland, OH) was used for agonist applications via a pipette (4–7 MΩ) identical to that used for patch-clamp recordings. The bath solution always contained 0.1–0.5 μM tetrodotoxin (TTX) to block Na^+ channels. The absence of sodium action potentials was routinely tested in the current-clamp mode by applying 10–50 pA depolarizing steps from the resting potential of ~−60 mV.

In some experiments, an iontophoretic pump (Union-40, Kation Scientific, Minneapolis, MN) was used to apply nicotine or RJR-2403. The retention and ejection currents were set around −20 nA and +180 nA, respectively. The output current was monitored during experiments via a built-in current monitor. Iontophoretic pipettes that did not provide specified current amplitudes were replaced. Patch-clamp pipettes with resistances 20–60 MΩ (if filled with the K-gluconate-based internal solution) were used for iontophoretic applications of nicotine (100 mM) and RJR-2403 (50 mM). Application pipettes for picospritzer and iontophoretic applications were mounted on two Sutter 285-3 manipulators allowing applications of at least two nicotinic agents to the same neuron during the course of a single experiment. In experiments with iontophoretic applications of nicotine, 4 μM DHβE was also added to the ACSF. In all tests involving iontophoresis, antagonists (i.e., TTX, MLA and DHβE) were added directly to the ACSF and therefore, syringe pumps were not used.

2.3. Biocytin labeling

In some experiments, the recording pipette contained 0.2% biocytin (Sigma Chemical Co., St. Louis, MO) and the neurons were filled by diffusion during patch recording. Slices were fixed in 4% buffered paraformaldehyde for at least 24 h. After fixation, slices were rinsed in phosphate-buffered saline for 15 min and then incubated for 4 h with a Vectastain ABC kit (Vector Laboratories, Burlingame, CA) at room temperature. The avidin/biotin was diluted 1:100 in PBS containing 0.5% Triton-X100 (Sigma). The slices were rinsed for 15 min and reacted with 3,3′ diaminobenzidine (DAB) from a DAB substrate kit (Vector). After rinsing, the sections were mounted on gelatin-coated