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Research Report

Characterization of neurons of the nucleus tractus solitarius pars centralis

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Abstract

Esophageal sensory afferent inputs terminate principally in the central subnucleus of the tractus solitarius (cNTS). Neurons of the cNTS comprise two major neurochemical subpopulations. One contains neurons that are nitric oxide synthase (NOS) immunoreactive (-IR) while the other comprises neurons that are tyrosine hydroxylase (TH)-IR. We have shown recently that TH-IR neurons are involved in esophageal-distention induced gastric relaxation. We used whole cell patch clamp techniques in rat brainstem slices combined with immunohistochemical and morphological reconstructions to characterize cNTS neurons. Postrecording reconstruction of cNTS neurons revealed two morphological neuronal subtypes; one group of cells (41 out of 131 neurons, i.e., 31%) had a multipolar soma, while the other group (87 out of 131 neurons, i.e., 66%) had a bipolar soma. Of the 43 cells in which we conducted a neurochemical examination, 15 displayed TH-IR (9 with bipolar morphology, 6 with multipolar morphology) while the remaining 28 neurons did not display TH-IR (18 with bipolar morphology, 10 with multipolar morphology). Even though the range of electrophysiological properties varied significantly, morphological or neurochemical distinctions did not reveal characteristics peculiar to the subgroups. Spontaneous excitatory postsynaptic currents (sEPSC) recorded in cNTS neurons had a frequency of 1.5 ± 0.15 events s^{-1} and an amplitude of 27 ± 1.2 pA (Vh = −50 mV) and were abolished by pretreatment with 30 μM AP-5 and 10 μM CNQX, indicating the involvement of both NMDA and non-NMDA receptors. Some cNTS neurons also received a GABAergic input that was abolished by perfusion with 30–50 μM bicuculline. In conclusion, our data show that despite the heterogeneity of morphological and neurochemical membrane properties, the electrophysiological characteristics of cNTS neurons are not a distinguishing feature.
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1. Introduction

Vagal sensory afferent fibers enter the brainstem via the tractus solitarius and terminate in a viscerotopically organized manner in the subnuclei of the nucleus tractus solitarius (NTS) [1,2,5]. Although sensory inputs from distinct peripheral organs, such as, for example, the aortic branch and the stomach, do not converge on single NTS neurons [26], the same subnucleus may receive sensory information from more than one peripheral organ. For example, neurons involved in arterial baroreflex circuits as well as neurons part of vago-vagal gastric reflexes are similarly located in the medial subnucleus of the NTS [1,5–7,11,16,22,25,30]. The overlap of these viscerotopically organized areas makes the distinction of cells devoted to a particular function quite problematic, even though a recent report suggested that innovative techniques may be used to distinguish NTS neurons receiving discrete projections in a brainstem slice preparation [13]. The subnucleus centralis (cNTS), which is located adjacent to the tractus solitarius [5], receives inputs from vagal afferent fibers originating almost exclusively from the esophagus [1,6,8,16,27], making it an excellent model for the study of a population of second order neurons controlling esophageal-mediated reflexes.

Various groups have reported recently that NTS neurons display a certain degree of morphological and electrophysiological diversity [12,14,18–20,26,34]. These obser-

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vations suggest that it may be possible to differentiate between neurons subserving similar functions from adjacent neurons devoted to different roles.

We have shown that esophageal distension induces gastric relaxation and increases the firing rate and cFos expression in cNTS neurons [27,28]. Furthermore, the majority of neurons that express induced cFos activity contain tyrosine-hydroxylase immunoreactivity (TH-IR) and are more likely located in the outer, rather than the inner, core of cNTS [28]. These findings are suggestive of an organization of cNTS neurons, similar to that described for vagal motoneurons, whereas cells projecting to defined areas display common membrane and pharmacological characteristics [10]. In a recent report on cNTS neurons in vitro [23], however, the authors did not report the basic membrane properties of these neurons. Hence, one of the aims of this work was to characterize and correlate the electrophysiological, morphological, and neurochemical phenotype of cNTS neurons.

A vast array of studies have shown that sensory vagal information to NTS neurons is excitatory and uses, principally, excitatory amino acids such as glutamate [3,14–17,21,23,29,33]. Lu and Bieger recently reported that glutamate was the neurotransmitter mediating fast excitatory neurotransmission from vagal afferent fibers to cNTS neurons [23]; however, they did not characterize the glutamatergic receptors subtype(s) involved. The second aim of this work was to characterize the receptor subtype(s) used by the glutamatergic input onto cNTS neurons.

2. Materials and methods

Research reported in the present manuscript conforms fully to National Institute of Health guidelines and was approved by the Pennington Biomedical Research Center-LSU System Animal Care and Use Committee.

2.1. Electrophysiology

The method of slicing the brainstem has already been described [31]. Briefly, 25–35 days old Sprague–Dawley rats of either sex were anesthetized with isoflurane (abolition of the foot pinch withdrawal reflex) before being killed by severing the blood vessels in the chest. The brainstem was removed and glued to the platform of a vibratome, and three coronal slices (300 μm-thick) were cut starting from the posterior end of the area postrema moving rostrally. The slices were stored at least 1 h in oxygenated (95% O_2/5% CO_2) Krebs' solution (see Solutions composition) at 30 °C before use. A single slice was then transferred to a custom-made perfusion chamber (volume 500 μl), kept in place with a nylon mesh and maintained at 35 ± 1 °C by perfusion with warmed Krebs' solution at a rate of 2.5–3.0 ml min^{-1}.

Whole cell recordings were conducted on putative cNTS neurons (identified as per their location in close proximity, within 100 μm, to the tractus solitarius at a level encompassing the mid-rostral area postrema up to approximately 0.5 mm rostral to the anterior portion of the area postrema). Recordings were made with patch pipettes (6–8 MΩ resistance) filled with a potassium gluconate solution (see Solutions composition) by using an Axoclamp 2B amplifier (Axon Instruments, Union City, CA). Data were sampled at 10 kHz and filtered at 2 kHz, digitized via a Digidata 1200C interface (Axon Instr.), acquired, stored, and analyzed on an IBM PC utilizing pClamp 8 software (Axon Instr.). Recordings were accepted only if the series resistance was <15 MΩ. In addition, the action potential evoked after injection of depolarizing current must have had amplitude of at least 50 mV and the membrane potential had to return to the baseline value after the action potential afterhyperpolarization (AHP).

Electrophysiological properties measured included, in voltage clamp configuration: (1) membrane input resistance (measured from the current deflection obtained by stepping the membrane from −50 to −60 mV for 500 ms); (2) membrane capacitance (measured using pClamp software with a 10 mV square pulse); (3) amplitude and decay time of the current underlying the AHP evoked by stepping the membrane from −60 to +10 mV for 50 ms; (4) frequency and amplitude as well as charge transferred of spontaneous excitatory postsynaptic currents. In current clamp configuration we measured: (1) duration of the action potential at the threshold; and, (2) the frequency of action potential firing, expressed as pulses s^{-1}, in response to 400 ms-long DC pulses (15 to 240 pA in step increments).

At the end of recording, Neurobiotin® (2.5% w/v; Vector Labs, Burlingame, CA) was injected into the neuron (0.3 nA, 600 ms duration depolarizing pulse every 2 s) for 15–20 min to permit postfixation morphological or neurochemical reconstruction. Slices were then immersed in Zamboni's fixative (see Solutions composition) and stored at 4 °C until analyzed.

2.2. Immunohistochemistry

The slice was cleared of fixative by washing it repeatedly in PBS before incubation for 18–24 h at 4 °C in PBS-Triton-X (PBS-TX 0.3%, see Solutions composition)-Bovine Serum Albumin (BSA, 0.1%) containing mouse anti-tyrosine hydroxylase (1:1000; Immunostar, Hudson, WI). Slices were then washed in PBS-TX and incubated for 2 h at 37 °C with PBS-TX-BSA containing secondary antibodies (TH staining: goat anti-mouse conjugated with Alexa 488, FITC-1:500, Molecular Probes, Eugene, OR; and, streptavidin-Texas Red 1:100, Vector Labs, Burlingame, CA, to visualize the Neurobiotin®-filled neuron). The slices were then mounted in Fluoromount-G® (Southern Biotechnology Associates, Birmingham, AL) to reduce fading and analyzed for immunofluorescence using a Zeiss 510 confocal scanning laser microscope equipped with a Kr/Ar-ion laser with filters for the selective visualization of Texas Red and FITC.

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