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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 ($V_h = -50$ mV) and were abolished by pretreatment with 30 μ M AP-5 and 10 μ M 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 μ M 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.

Keywords

Electrophysiology; Gastrointestinal; Brainstem

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 observations 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 μ m-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% O₂/5% CO₂) Krebs' solution (see Solutions composition) at 30 °C before use. A single slice was then transferred to a custom-made perfusion chamber (volume 500 μ l), kept in place with a nylon mesh and maintained at 35 $\pm$ 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 M Ω 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 M Ω . 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.

2.3. Morphological reconstructions

Slices were cleared of fixative in PBS-TX and kept at 4 °C until the injected Neurobiotin® was visualized using a cobalt–nickel enhancement of the Avidin _D-horseradish peroxidase (Avidin _D-HRP) technique as described previously [10,24]. Briefly, slices were incubated in Avidin _D-HRP solution (see Solutions composition) for 2 h. Following 15 min rinse in PBS and subsequent incubation for 15–20 min in Avidin _D-HRP and DAB solutions (see Solutions composition), the slice was incubated for 15 additional minutes in the presence of 3% H₂O₂. The slice was then rinsed in PBS, placed on a gelatin-coated coverslip, air dried, cleared in alcohol and xylene, and mounted in Permount®.

Three-dimensional reconstructions of individual Neurobiotin®-labeled neurons, digitized at a final magnification of $\times 600$, were made using Neurolucida® software (Microbrightfield Inc., Williston, VT, USA). Each reconstruction was verified using the software for “mathematical completeness”. The optical and physical compression of the slice that may occur was corrected by rescaling the section to 300 μm (the original thickness at time of sectioning).

The morphological features that were assessed included: soma area and diameter, form factor (a measure of circularity for which a value of one indicates a perfect circle and zero indicates a line; form factor = $4\pi a \times 1/p^2$, where a = soma area and p = the perimeter of the soma in the horizontal plane), whether the cell had bipolar or multipolar soma shape, number of segments (i.e., branching of dendrites), and segments length in the X and Y axes. Data analysis was performed as described previously [10,24].

2.4. Solutions composition

Krebs' (in mM): 126 NaCl, 25 NaHCO₃, 2.5 KCl, 1.2 MgCl₂, 2.4 CaCl₂, 1.2 NaH₂PO₄, and 11 dextrose, maintained at pH 7.4 by bubbling with 95% O₂–5% CO₂.

Intracellular solution (in mM): K gluconate 128, KCl 10, CaCl₂ 0.3, MgCl₂ 1, Hepes 10, EGTA 1, ATP-Na 2, GTP-Na 0.25. Adjusted to pH 7.35 with KOH.

Zamboni's fixative (in mM): 1.6% (w/v) paraformaldehyde, 19 KH₂PO₄, 100 mM Na₂HPO₄·7H₂O, 240 ml saturated picric acid, and 1600 ml H₂O; adjusted to pH 7.4 with HCl. PBS-TX (in mM): 115 NaCl, 75 Na₂HPO₄·7H₂O, 7.5 KH₂PO₄, and 0.3% Triton X-100.

Avidin D-HRP solution: 0.002% avidin D-HRP in PBS containing 1% Triton X-100; 0.05% DAB in PBS containing 0.5% gelatin supplemented with 0.025% CoCl₂ and 0.02% NiNH₄SO₄.

2.5. Statistical analysis

Results are expressed as means $\pm$ SEM with significance defined as $P < 0.05$. Intergroup comparisons were conducted using the Student's grouped t test or the χ^2 test.

3. Results

We conducted electrophysiological studies on 131 neurons of the cNTS; in 19 of these neurons, we obtained complete morphological reconstructions, while in a further 66 neurons, we were able to obtain the reconstruction only of the soma shape. We were also able to analyze 43 of the recorded cNTS neurons for tyrosine hydroxylase immunoreactivity.

3.1. Basic electrophysiological characteristics of cNTS neurons

The basic electrophysiological properties of cNTS neurons ($N = 32$ –69) are summarized in Table 1; representative examples of a single action potential, after-hyperpolarization, and frequency–response curve are shown in Fig. 1. The location of recorded cells is shown in Fig. 2A.

3.2. Morphological and immunocytochemical characteristics of cNTS neurons

The basic morphological properties of cNTS neurons ($N = 19$) are summarized in Table 2; representative examples of neurons with a bi- or multipolar soma are shown in Fig. 2B. Representative examples of neurons TH-IR negative ($N = 28$) and TH-IR positive ($N = 15$) are shown in Fig. 3.

3.3. Correlation between morphological, immunohistochemical, and electrophysiological characteristics of cNTS neurons

Since cNTS neurons could be distinguished based on their morphological (bi- or multipolar somata morphology) or neurochemical phenotype (TH-IR positive or negative) and since their basic electrophysiological properties showed a large range of values (see Table 1), we investigated whether there was a correlation between these subgroups that could have resulted in distinguishing membrane properties.

Sixty of the 85 cNTS neurons in which we were able to reconstruct some morphological characteristics had a bipolar soma, the remaining 25 cNTS neurons had, instead, a multipolar soma. Nineteen of these 85 neurons (12 with bipolar and seven with multipolar soma shape) were sufficiently preserved to provide a complete morphological analysis. The soma area of bipolar neurons ($95.3 \pm 8.82 \mu\text{m}^2$) was significantly smaller than that of multipolar neurons ($152.4 \pm 24.47 \mu\text{m}^2$; $P < 0.05$); however, no significant differences were found in the soma diameter (16.6 ± 1.00 and $17.6 \pm 1.26 \mu\text{m}$ in bi- and multipolar, respectively), in the soma form factor (0.68 ± 0.03 and 0.72 ± 0.03), in the number of segments (5.2 ± 0.48 and 5.42 ± 0.53), or in the segment length (93.5 ± 6.03 and $133.2 \pm 30.02 \mu\text{m}$). When the electrophysiological characteristics of cNTS neurons were compared between neurons with bi- or multipolar shaped soma, however, apart from a smaller cell capacitance in bipolar neurons ($37.7 \pm 1.33 \text{ fF}$) when compared to multipolar cells ($43.7 \pm 3.02 \text{ fF}$; $P < 0.05$), no significant differences were seen in any of the other parameters measured. In fact, the AHP amplitude was 260 ± 28.3 and $195 \pm 37.15 \text{ pA}$, the input resistance was 492 ± 33.1 and $484 \pm 46.9 \text{ M}\Omega$, and the maximal firing rate evoked by injection of 240 pA DC was 52.6 ± 3.85 and $55.3 \pm 6.39 \text{ spikes s}^{-1}$ in neurons with a bi- or multipolar soma shape, respectively.

Fifteen of the 43 cNTS neurons analyzed were TH-IR positive, nine of these neurons had a bipolar soma shape while the six remaining TH-IR positive cells had a multipolar morphology. Similarly, of the 28 TH-IR negative neurons, 18 had a bipolar and 10 had a multipolar soma shape ($P > 0.05$ vs. TH-IR positive neurons; χ^2 test). When the electrophysiological characteristics of cNTS neurons were analyzed, the amplitude of the current underlying the AHP was 225 ± 47.3 and $260 \pm 38.0 \text{ pA}$ and the input resistance was 551 ± 69.8 and $412 \pm 35.1 \text{ M}\Omega$ in TH-IR positive and in TH-IR negative cells, respectively. No significant differences were, however, present.

3.4. Spontaneous synaptic inputs onto cNTS

Excitatory and inhibitory spontaneous synaptic currents were recorded in 61 cNTS neurons. Spontaneous inhibitory currents (sIPSC) were not observed in enough neurons to permit thorough analysis but they were completely antagonized by perfusion with $30\text{--}50 \mu\text{M}$ bicuculline ($N = 4$). The presence of sIPSC did not affect the frequency or amplitude of spontaneous excitatory postsynaptic currents (sEPSC; at $V_h = -50 \text{ mV}$, the sEPSC frequency was 1.36 ± 0.31 and $1.31 \pm 0.45 \text{ events s}^{-1}$ and the amplitude was 20.6 ± 1.63 and $19.2 \pm 0.89 \text{ pA}$ in control and bicuculline, respectively; $N = 4$). The sIPSCs were not further studied.

sEPSCs with a frequency of $1.5 \pm 0.27 \text{ events s}^{-1}$ and amplitude of $26.3 \pm 1.53 \text{ pA}$ ($V_{\text{hold}} = -50 \text{ mV}$) were recorded in 52 cNTS neurons in the presence of $30 \mu\text{M}$ bicuculline. Perfusion with a solution containing the non-selective glutamate antagonist kynurenic acid (1 mM) completely abolished the sEPSC ($N = 8$), indicating that glutamate was the underlying neurotransmitter. When measured at $V_{\text{hold}} = -70 \text{ mV}$, the amplitude of the sEPSC was $36.2 \pm 4.32 \text{ pA}$ and the total charge was $235.3 \pm 30.77 \text{ fC}$ ($N = 9$). As expected (because of Mg^{2+} block of the NMDA receptor), perfusion with the selective NMDA antagonist AP-5 ($30 \mu\text{M}$)

did not affect the sEPSC amplitude or the total charge (35 ± 3.86 pA and 216 ± 17.03 fC, respectively; $P > 0.05$ vs. control), whereas perfusion with the non-NMDA selective antagonist CNQX ($10 \mu\text{M}$) completely abolished the sEPSC. When measured at $V_{\text{hold}} = -30$ mV, where the Mg^{2+} block of the NMDA receptor is released, the amplitude of the sEPSC was 31.1 ± 4.2 pA and the total charge 300.7 ± 39.38 fC in control conditions ($N = 9$). Perfusion with $30 \mu\text{M}$ AP-5 did not affect the sEPSC amplitude significantly (32.5 ± 4.67 pA; $P > 0.05$ vs. control) but reduced the total charge to 210 ± 21.9 fC ($P < 0.05$ vs. control), while perfusion with a solution containing both AP-5 and $10 \mu\text{M}$ CNQX completely abolished the sEPSC (Fig. 4). These data suggest the involvement of both NMDA and non-NMDA receptors.

Five cNTS neurons were tested at $V_{\text{hold}} = -50$ mV in the presence of the synaptic transmission inhibitor tetrodotoxin (TTX, $1 \mu\text{M}$). Perfusion with TTX did not alter the frequency and amplitude of the miniature EPSC (at $V_h = -50$ mV, the mEPSC frequency was 1.8 ± 0.87 and 1.7 ± 1.10 events s^{-1} and the amplitude was 28 ± 1.5 and 29 ± 2.1 pA in control and TTX, respectively; $N = 5$), indicating that most of the glutamate was released from primary afferent fibers. In another four cNTS neurons, tested at $V_{\text{hold}} = -30$ mV, perfusion with the NMDA antagonist AP-5 ($30 \mu\text{M}$) reduced the total charge from 293 ± 63.3 in TTX to 172 ± 33.3 fC in TTX + AP-5 ($P < 0.05$); however, the amplitude of the mEPSC was unaffected by AP-5 (data not shown).

4. Discussion

In the present manuscript, we have shown that: (1) cNTS neurons can be distinguished into subgroups based either on the morphological characteristics of the soma, i.e., bi- or multipolar, or on their neurochemical phenotype, i.e., TH-IR positive or TH-IR negative. Despite having distinguishing morphological and neurochemical properties, however, the electrical properties of cNTS neurons are not a distinctive feature; (2) spontaneous excitatory currents onto cNTS neurons are mediated by glutamate interaction with both NMDA and non-NMDA receptors; and, (3) some cNTS neurons also receive GABAergic sIPSC.

Neurons of the nucleus tractus solitarius (NTS) receive inputs from fibers carrying sensory information from the gastrointestinal, gustatory, cardiovascular, and respiratory tracts. The subnuclei of the NTS are viscerotopically organized [1,2,5]; however, the same subnucleus might receive sensory information from more than one peripheral organ [1,5–7,11,16,22,25,30]. The subnucleus centralis (cNTS), however, receives sensory inputs originating almost exclusively from esophageal afferent fibers [1,8,9,16,23], thus, this nucleus may be a possible model for the study of NTS neurons devoted to the control of esophagus-mediated reflexes.

Elegant studies by Paton's group characterized both electrophysiological as well as morphological properties of NTS neurons responsive to subdiaphragmatic vagal stimulation [26]. Only four of the neurons investigated, however, could have been classified as cNTS by their response to esophageal distention. Their study, however, did not further investigate the characteristics of cNTS neurons. More recently, Smith's group reported a study of gastrointestinal related NTS neurons, some of which were identified using the GFP-conjugated transynaptic tracer PRV-152 [14], but their study was restricted to NTS neurons innervating the corpus of the stomach only. In both studies, the authors reported that sensory gastrointestinal second order neurons of the NTS comprise neuronal subpopulations with distinguishing morphological features. In particular, Smith's group reported that NTS neurons receiving sensory information from the corpus can be classified into three subgroups [14]. The vast heterogeneity of morphological features of gastrointestinal NTS

neurons was reinforced further by studies of Fogel's group, which classified NTS neurons into three subpopulations based upon their different responses to gastrointestinal stimulations [34].

In the present study, we observed that cNTS neurons, identified as per their location in close proximity to the tractus solitarius, can be grouped into two morphologically distinct subpopulations distinguishable only by their soma shape. This reduced heterogeneity of morphological features may be ascribed to the relatively homogeneous sensory input from esophagus impinging on the cNTS [1,6,8,16,27].

Some membrane properties of cNTS neurons, however, seem to indicate that these neurons have characteristics that distinguish them from other NTS neurons, most noticeably the smaller soma area and the higher input resistance [12,14,17,19,26,32,34]. These electrophysiological membrane features would seem to be best suited to signaling the fast dynamic changes in esophageal pressure observed during the passage of a bolus in the esophagus, since even minute changes in neurotransmitter release would induce large changes in the membrane potential. Despite the relative homogeneous esophageal input, cNTS neurons still show a certain degree of heterogeneity with regard to their morphological, i.e., bi- or multipolar soma, and neurochemical, i.e., TH-IR positive or negative, characteristics, indicating the presence of different types of cells. These anatomically defined groups of cells, however, do not show discrete electrophysiological properties.

Characteristics such as the soma area (range 62–254 μM^2) and input resistance (range 211–999 M Ω) show large variability and suggest different levels of excitability of cNTS neurons. The experiments testing the action potential firing rate support this suggestion, since injection of 240 pA DC, for example, induced a frequency response ranging from 18 to 105 action potentials s^{-1} . It is, thus, possible that the response to neurotransmitters or to pharmacological agents reveals distinguishing characteristics correlated to the cell type.

The processing of autonomic sensory signals entering the CNS first occurs at the level of the synapse between the vagal afferent fibers and neurons of the NTS. As is the case for NTS neurons in the other subnuclei [3,14,17,21,23,29,33], cNTS neurons also use glutamate as the main fast excitatory neurotransmitter; in fact, cNTS neurons receive on average 1.5 glutamate-mediated excitatory events s^{-1} . Similar to the response in identified neurons in the subnucleus medialis receiving corpus-originating afferent fibers [14] or cardiovascular inputs [4] but contrary to the response of putative cardiovascular NTS neurons [3], the EPSCs evoked by glutamate release from primary afferent fibers use both NMDA and non-NMDA receptors. The different glutamate receptor subtypes may underlie different means for NTS neurons to integrate information originating in the periphery. It is possible that neurotransmitters or circulating hormones interact and modulate differently the afferent vagal input because of the diverse complement of glutamate receptors on either gastrointestinal or cardiovascular NTS neurons.

In summary, our results clearly demonstrate that, when considering either the morphological or neurochemical characteristics, cNTS neurons are heterogeneous, but these anatomical characteristics are not predictive of differences in electrical properties. Future studies correlating the response to neurotransmitters/neuromodulators to membrane characteristics are needed to further elucidate the neuroanatomical organization of cNTS neurons.

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Fig. 1.

(A) Current clamp recording trace showing a single action potential evoked upon injection of DC. Holding potential = -60 mV. (B) Voltage clamp recording trace showing the afterhyperpolarization current evoked upon stepping the membrane from -60 to $+10$ mV. (C) Current clamp recording trace showing the action potentials frequency response to injection of 15 and 240 pA DC. Holding potential = -60 mV. Traces from panels A–C are from the same cNTS neuron. (D) Graphic summarizing the action potentials frequency response to injection of graded intensities of DC (15–240 pA; $N = 32$). Holding potential = -60 mV.

Fig. 2.

(A) Schematic diagram depicting the localization of cNTS neurons according to their soma shape (Bipolar = square; multipolar = circle). For purposes of clarity, not all the neurons have been reported in the graphic. Note that there is no discrete localization of any particular type of cNTS neuron. TS = tractus solitarius; AP = area postrema; DMV = dorsal motor nucleus of the vagus; cc = central canal; IV Ventr. = fourth ventricle. (B) Computer-aided reconstructions of two cNTS neurons. Note the distinctive multipolar (left) and bipolar (right) soma shape.

Fig. 3.

Green, FITC filters = tyrosine hydroxylase (TH-IR); red, TRITC filters = Neurobiotin-filled neuron; yellow = merged image with TH-IR and Neurobiotin co-localization. Micrographs depicting two cNTS neurons that were filled with Neurobiotin® following electrophysiological recordings and postfixation immunoreactivity. The neuron depicted in panels A–D did not contain TH-IR; conversely, the neuron depicted in panels E–H co-localized with TH-IR. Panels A and E = low magnification micrograph showing the localization of the Neurobiotin®-labeled cNTS neuron with respect to the tractus solitarius (TS), the dorsal motor nucleus of the vagus (DMV), and neurons in the A2 area (TH-IR positive neurons). Panels B and F = high magnification micrograph (TRITC filters) showing the same neurons as above. Note that the cNTS cell in panel B has a bipolar soma shape, while the cNTS neuron in panel F has a multipolar soma shape. Panels C and G = high magnification micrograph showing the same area as above but taken with FITC filters to visualize TH-IR positive neurons. Panels D and H = merged high magnification micrograph of panels B and C (D) and panels F and G (H). Note that in panel D, the Neurobiotin-filled neuron does not co-localize TH-IR and Neurobiotin®, while in panel H, the Neurobiotin®-filled cNTS neuron co-localizes with TH-IR. Scale bar = 20 μ M.

Fig 4.

(A) Representative traces showing spontaneous excitatory postsynaptic currents (sEPSC) in the presence of 30 μ M bicuculline ($V_{\text{hold}} = -70$ mV, a–b, and -30 mV, c–e). Note that at $V_{\text{hold}} = -70$ mV, perfusion with the non-NMDA antagonist CNQX (10 μ M) abolished completely the sEPSC (b). Conversely, at $V_{\text{hold}} = -30$ mV, perfusion with both CNQX and the NMDA antagonist AP-5 (30 μ M) abolished sEPSCs (d–e). (B) Representative averaged sEPSCs recorded at $V_{\text{hold}} = -30$ mV showing that perfusion with AP-5 decreased the area under the curve (charge transferred) and perfusion with AP-5 and CNQX completely abolished the sEPSCs. Traces in control and in the presence of AP-5 were normalized, conversely, the trace in presence of AP-5 and CNQX was not normalized. (C) Summary graphic depicting the charge transferred at different holding potentials. * $P < 0.05$ vs. control.

Table 1

Basic electrophysiological properties of cNTS neurons

Input resistance (M Ω)	Membrane capacitance (pF)	Action potential duration at threshold (ms)	AHP current (pA)	Frequency of action potentials at 15 pA (action potentials s ⁻¹)	Frequency of action potentials at 240 pA (action potentials s ⁻¹)
571 $\pm$ 29.9 (211–999)	29.7 $\pm$ 0.82 (18–42)	3.5 $\pm$ 0.16 (1.9–7.0)	207.9 $\pm$ 16.60 (55–557)	11.9 $\pm$ 0.91 (2.5–25)	51.0 $\pm$ 3.82 (18–105)

The numbers in bracket represent the range of values in the neuronal population.

Table 2

Basic morphological properties of cNTS neurons

Soma area (μm^2)	Soma diameter (μm)	Form factor (0 = line; 1 = sphere)	Number of segments	Segment length (μm)	Bipolar soma	Multipolar soma	TH-IR negative	TH-IR positive
116.4 ± 12.07 (62–254)	16.9 ± 0.77 (12–23)	0.69 ± 0.02 (0.57–0.86)	5.3 ± 0.35 (2–8)	108 ± 12.05 (56–264)	$N = 60$	$N = 25$	$N = 28$	$N = 15$

The numbers in bracket represent the range of values in the neuronal population.