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Review Article

Cellular Heterogeneity Within the Solitary Tract Nucleus and Visceral Afferent Processing—Electrophysiological Approaches to Discerning Pathway Performance

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Abstract

Many homeostatic reflexes depend on autonomic central nervous system mechanisms to systemically coordinate visceral organ function. The nucleus of the solitary tract (NTS) is the common entry of cranial visceral afferents into these regulatory pathways. Such NTS neurons initiate adjustments in cardiovascular, respiratory, gastrointestinal and other visceral systems. Diversity of neurons within the NTS appears integral to such processing but is daunting to approach experimentally. This review outlines three experimental approaches to understanding cellular heterogeneity within NTS and its relation to function. Brainstem slice preparations coupled with patch recordings afford cellular–molecular resolution with substantial links to the more intact system. Pharmacological approaches based on visceral afferent phenotype have helped identify myelinated and unmyelinated solitary tract inputs to NTS neurons. An interesting outcome has been the robust association of A-type potassium currents with NTS neurons receiving unmyelinated afferents. Neuroanatomical tracers offer a second, complementary approach. Anterograde transport of fluorescent dye identifies cranial visceral afferent terminals on second order neurons that cluster on or proximal to the soma—a highly unusual distribution in the central nervous system. Thus, second order baroreceptive neurons can be identified neuroanatomically *in vitro*. Equally helpful has been identification of NTS projection neurons by retrograde tracers injected into target regions of the hypothalamus or brainstem and this approach indicates substantial specialization—relative homogeneous neurons within the overall heterogeneity of NTS. Lastly, transgenic mouse strains, particularly those expressing marker chromophores, have identified phenotypic subtypes such as GABAergic inhibitory neurons within NTS. Combined methodologies are forging new understanding of NTS and autonomic regulation. (*Tzu Chi Med J* 2007;19(4):181–185)

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1. Introduction

Homeostatic reflexes regulate visceral organ function. Autonomic effectors target the cardiovascular, respiratory, gastrointestinal and other visceral systems [1–4]. These organs function across very different regulatory time frames ranging from cycles within single beat-to-beat intervals of the heart (hundreds of milliseconds) to coordinated cycles that span hours for smooth muscle contraction within the gastrointestinal tract. In each of these visceral systems, primary afferent nerves encode local conditions and this information is transmitted to the central nervous system. Cranial visceral primary afferent neurons extend central processes to make synaptic contact within the brainstem at the nucleus of the solitary tract (NTS). Thus, neurons within the NTS initiate the reflex regulatory process.

A hallmark of NTS is frank cellular heterogeneity across neurons even within local sub regions and this has long been a challenge for our overall understanding of NTS and the processing of afferent information. Understanding results from neuroanatomical and pharmacological approaches is confounded by different types of neurons lying within the close proximity and considerable overlap in common cellular features such as glutamate receptors. This means that many interventions act broadly across neurons and cross organ related systems to influence multiple pathways with different functions. These interactions may confound interpretations based for example on reflex outcome.

2. Systems integration with a cellular perspective

Many "systems" issues have been addressed using relatively broad techniques. Neuroanatomical tracers provided point-to-point long distance information about routing of connections between brain areas. The NTS is broadly interconnected with other brainstem regions as well as major forebrain cell groups [5]. Indicators of "activity" such as cFos expression light up cohorts of neurons in which activity becomes elevated to a system challenge such as increased blood pressure [6] or intraperitoneal injection of satiety signaling compounds (e.g. cholecystokinin) [7]. Immunocytochemical labels identify neuron classes linked by common phenotypic markers such as tyrosine hydroxylase for catecholaminergic neurons [8]. Each of these approaches offers a different glimpse of NTS and its relation to a greater whole. The limitation of such global views in terms of function, however, is often a lack of sufficient detail. The global resolution is bounded by the singular focus and the presence of markers does not distinguish functional interactions rigorously. Particularly important in NTS, these global or system level views alone cannot distinguish among various neurons for other critical clues as to their identity and their relation to local pathways.

To better parse the heterogeneity within sub regions of NTS, one promising approach is to combine system level indicators with cellular functional assays. Electrophysiology in brainstem slices offers information at multicellular, cellular and subcellular resolution that when coupled with systems level strategies helps bridge information between macro- and microscopic levels. This review will highlight several examples of utilizing different macroscopic markers coupled with slice patch intracellular recordings. This work provides new information about the patterns of interneuronal relationships and the transformation of information within particular neural circuits (macroscopic organization).

Our lab approaches the study of NTS using thin-slice, cellular electrophysiology coupled with several strategies to offer a macroscopic context for cellular investigations. Sectioning along the rostral-caudal course of solitary tract (ST) afferent axons, we cut the brain horizontally to preserve a long afferent axon trajectory. By preserving a lengthy section of afferent axon, electrical shocks to ST distant from NTS provide discrete axon activation of multiple axons simultaneously. Stimulus strength can be used to discriminate unique inputs from these activated afferent axons to reach single neurons. This approach allows us to assess in detail the local intra-NTS circuits affecting these neurons.

3. Pharmacological approaches

One orienting feature of NTS within autonomic regulatory control is its relation to diverse afferents from the viscera. Various afferents from different organs sort roughly to different sub regions of NTS and produce a "viscerotopic" map of NTS, but overlap is considerable [5]. Irrespective of the organ of origin, cranial primary afferents entering the central nervous system at NTS may be divided into two major phenotypic cellular classes—the myelinated and the unmyelinated neurons. Associated with these two axon classes of conduction velocity is a rich context of cellular features that include ion channels and receptors that echo to a certain extent a similar dichotomy in somatic and visceral afferents entering the spinal cord at the dorsal horn [9,10]. For example, the expression of the vanilloid receptor TRPV1 (formerly VR1) in nodose neurons was noted in early TRPV1 cloning reports [11], although interestingly, the association of TRPV1 with non-nociceptive primary afferents [12,13] is rarely acknowledged.

Using the family of vanilloid compounds, we conducted a series of studies on the actions of TRPV1 receptors on synaptic transmission in NTS. The results were both interesting with somewhat surprising conclusions for NTS organization [14–20]. First, capsaicin (200 nM) triggers an initial enhancement of glutamate release from ST terminals that over time leads to synaptic depression and block of ST-activated transmission. These compounds have no direct effects on γ-aminobutyric acid (GABA) transmission or on higher order NTS neurons. Second, capsaicin-resistant ST responses correspond to transmission mediated by myelinated conducting nodose afferent neurons. Third, at the level of NTS, capsaicin sensitive and resistant fibers do not directly contact the same second order neurons in medial NTS. This latter finding suggests that myelinated and unmyelinated afferents are segregated to different neurons. This finding offers the interesting possibility that primary afferent segregation fosters trophic interactions between afferent and central neurons that contribute to the phenotypic differentiation of the central neuron (e.g. potassium channel expression) and that this process helps to underwrite multifeature pathway tuning [15,21]. Despite an apparent lack of direct convergence of cranial primary afferent contacts, single NTS second order neurons receive indirect convergence via polysynaptic excitatory and inhibitory inputs which convey capsaicin sensitive and resistant information.

Similar pharmacological strategies suggest remarkable differentiation of afferent pathways through NTS. Interestingly, these differences are predominantly presynaptic, i.e. ST afferent terminal differences. Thus, within medial NTS in the rat or the mouse, subclasses of afferent fibers are sensitive to candidate peptide transmitter/modulators. Subpopulations of medial NTS neurons receive angiotensin [22], vasopressin [14], mu opioid [23] or cholecystokinin sensitive ST afferents. These presynaptic modulators tune up or down the probability of glutamate release from primary afferent terminals and thus transform the relation of afferent activity to the entire reflex pathway response.

4. Neuroanatomical approaches

Within the afferent perspective of NTS, anterogradely transported dyes can reveal the distribution of central contacts within sub nuclei. Since peripheral insults to primary afferent nerves promote frank redistributions of afferent terminals [24,25], the ideal afferent tracer should be as innocuous as possible. Few substances succeed in crossing the soma to transganglionically label central terminals. Carbocyanines enter peripheral axons by diffusion, become endocytosed to endosomes [26] and successfully label terminal fields within NTS. Unfortunately, these dyes are transported both antero- as well as retrogradely and their fluorescence is readily bleached [27]. The aortic baroreceptors in the rat and the rabbit uniquely travel to the nodose ganglion within a separate nerve bundle and offer a unique anatomy by the absence of efferent axons or chemoreceptive afferents. Labeling of the aortic nerve produces punctuate labeling of baroreceptor terminals within limited regions of NTS and a pattern consistent with a concentration on or near the cell soma. All such labeled neurons receive short-latency, low-jitter ST-evoked excitatory postsynaptic currents (EPSCs) that are consistent with direct contacts from either capsaicin sensitive or resistant afferents. Stimulus recruitment curves suggest that such ST-EPSCs arise from a single afferent axon with an exceptionally high probability of glutamate release.

Retrograde dyes can be stereotaxically delivered to limited central nervous system regions and thus disclose projection destinations of dye-positive NTS neurons. Thus, such neurons can be studied under a broader network or systems perspective for such NTS projection neurons. Major questions for study include neurotransmitter interactions, the role of voltage-dependent ion channels, and intra-NTS local circuit connections (the wiring diagram) and their impact on information processing within NTS.

Fluorescent retrograde tracers injected into the hypothalamic paraventricular nucleus (PVN) illuminate cohorts of single NTS neurons with axons projecting to PVN. Likewise, injections within the caudal ventrolateral medulla (CVLM) identify CVLM-projecting NTS neurons. Experiments outlining response patterns to ST activation discerned distinctly and systematically different intra-NTS connectivity to these two different projection neurons [16,21]. ST-evoked synaptic responses indicated that most PVN-projecting medial NTS neurons are contacted via polysynaptic intra-NTS pathways from ST. In contrast, NTS neurons that project to CVLM are nearly exclusively coupled directly to ST afferents. Interestingly, the expression of the A-type, transient potassium channel was quantitatively related to the projection target and thus affected the intrinsic excitability of these neurons and pathway performance. Overall, projection target information identified relatively homogeneous within-projection populations of neurons parsed out of the generic heterogeneity that dominates NTS.

This approach opens up a broad array of questions concerning pathway make up that includes interactions of neurotransmitters/receptors, the role of voltage-dependent ion channels and their modulation, and the mechanisms of synaptic transmission and information processing within NTS. Studies of

neurons identified by projection target offers an additional approach to understanding the relationship between NTS neurons and their properties and distant CNS targets of afferent information.

5. Genetic models

A genetic variation of the identification of classes of neurons is the use of transgenic mouse models in which expression of an introduced marker, e.g. enhanced green fluorescent protein (EGFP), is linked to a phenotypic characteristic of a class of neurons, e.g. GABAergic neurons with glutamic acid decarboxylase-67 (GAD67) (28). In these animals, GABA-synthesizing neurons glow green. Within medial NTS, GAD67 neurons are nearly all second order and receive single, either myelinated (capsaicin resistant) or C-fiber (capsaicin sensitive), ST afferent inputs (29). Thus, in nearly every aspect, patterns of synaptic transmission and other mechanisms are closely similar in the NTS of rats and mice.

The marriage of cellular electrophysiology with multiple other larger scale approaches to studying the brainstem mechanisms within central autonomic areas suggests that the heterogeneities within NTS may represent multiple different signatures of individual sub classes of neurons and circuits. A number of overarching patterns indicate some general constants within these pathways such as a very high release of glutamate with subsequent depression. Multiple mechanisms appear to moderate the full impact of this process and thus, presynaptic modulation of glutamate release is a major mechanism of action by neuromodulators, e.g. peptides. Given the number of interactions between neurotransmitters (heterosynaptic) as well as second and higher order neurons, the cellular information offers a cautionary note. A daunting array of possibilities exists to account for generating outcomes, especially to broadly distributed mechanisms (e.g. glutamate and GABA). Thus, specificity of tools and interventions at the level of NTS will require considered evaluations at multiple levels to provide an adequate or unique understanding.

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