The unsilent majority–TRPV1 drives “spontaneous” transmission of unmyelinated primary afferents within cardiorespiratory NTS

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Andresen MC, Hofmann ME, Fawley JA. The unsilent majority–TRPV1 drives “spontaneous” transmission of unmyelinated primary afferents within cardiorespiratory NTS. Am J Physiol Regul Integr Comp Physiol 303: R1207–R1216, 2012. First published October 17, 2012; doi:10.1152/ajpregu.00398.2012.—Cranial primary afferent sensory neurons figure importantly in homeostatic control of visceral organ systems. Of the two broad classes of visceral afferents, the role of unmyelinated or C-type class remains poorly understood. This review contrasts key aspects of peripheral discharge properties of C-fiber afferents and their glutamate transmission mechanisms within the solitary tract nucleus (NTS). During normal prevailing conditions, most information arrives at the NTS through myelinated A-type nerves. However, most of visceral afferent axons (75–90%) in NTS are unmyelinated, C-type axons. Centrally, C-type solitary tract (ST) afferent terminals have presynaptic transient receptor potential vanilloid type 1 (TRPV1) receptors. Capsaicin activation of TRPV1 blocks phasic or synchronous release of glutamate but facilitates release of glutamate from a separate pool of vesicles. This TRPV1-operated pool of vesicles is active at normal temperatures and is responsible for actively driving a 10-fold higher release of glutamate at TRPV1 compared with TRPV1−terminals even in the absence of afferent action potentials. This novel TRPV1 mechanism is responsible for an additional asynchronous release of glutamate that is not present in myelinated terminals. The NTS is rich with presynaptic G protein-coupled receptors, and the implications of TRPV1-operated glutamate offer unique targets for signaling in C-type sensory afferent terminals from neuropeptides, inflammatory mediators, lipid metabolites, cytokines, and cannabinoids. From a homeostatic view, this combination could have broad implications for integration in chronic pathological disturbances in which the numeric dominance of C-type endings and TRPV1 would broadly disturb multi-system control mechanisms.

Two Classes of Cranial Visceral Afferents: Conduction and Discharge

At the dawn of modern electrophysiology, Lord Adrian recorded impulses traveling along peripheral nerve trunks that included the vagus and aortic depressor nerve (ADN) (2). The flow of afferent impulses is a fundamental building block of organism-wide homeostatic regulation. The central nervous system (CNS) collects moment-to-moment status reports of the ongoing conditions within visceral organ sites and integrates them for homeostatic control of the milieu intérieur. Electrophysiologists discovered early on that nerve fibers were not homogeneous. Recordings showed fast and slowly conducted volleys of action potentials (51, 53, 56). The two major divisions differentiated by conduction velocity are rapidly conducting, myelinated, A-fibers and slowly conducting, unmyelinated, C-fibers (49). Recognition of these two broad classes presaged the discovery of the rich spectrum of pheno-typic heterogeneity of primary afferent neurons, but the consequences of this dichotomy for cranial visceral afferent pathways to a large extent remain poorly understood. Goals of this review include highlighting important aspects of how the characteristics of C-fiber afferents intersect with our understanding of the neural mechanisms of reflex performance and new findings about central afferent synaptic transmission within the solitary tract nucleus (NTS) and the contributions of the TRPV1 receptor in presynaptic control of neurotransmitter release. Many of the specific examples in the review will rely on cardiorespiratory neurons, but the principal conclusions likely apply much more broadly.

Nearly a century ago, Adrian recorded bursts of action potentials with each pulse of arterial pressure from the ADN (1). The ADN contains thousands of aortic baroreceptor axons and generates a blur of action potentials (Fig. 1) with each systolic pressure pulse. Individual signals from active A- and C-fiber baroreceptors are impossible to discern in whole nerve recordings (14). Physically “splitting” the nerve trunk reveals the discharge from single baroreceptor afferents (Fig. 1). Such unit recordings indicated that some afferents discharged at high, regular frequencies (A-fiber), which faithfully encoded...
arterial pressure pulses (Fig. 2), whereas C-fiber baroreceptors generated sparse, irregular patterns of activity with limited fidelity of pressure encoding (107–109, 114). The intuitive appeal of the high fidelity signaling from A-fibers has consequently shaped an overwhelmingly A-fiber centric point of view. Accordingly, this has led to the anticipation that cardiovascular-related central neurons should show a cardiac-related rhythm (3, 35, 55, 83). However, this expectation overlooks the highly variable and often low fidelity encoding of most cardiovascular afferents (i.e., C-fibers, Fig. 2). As a whole, the variation in discharge characteristics across individual primary afferents provides a diverse stream of information into the CNS, but the impact of this C-fiber primary afferent information and its utility to reflex control are poorly understood within the context of an integrated system.

Aortic baroreceptors are fairly representative of differences in discharge (high fidelity vs. sparse) and physiological thresholds (low vs. high) across A- and C-fiber axons, respectively, regardless of organ origin. Thus cranial visceral afferents repeat similar patterns whether from the heart, blood vessels, airways, lungs, or gastrointestinal visceral regions, and all send their information to the caudal NTS (17, 29, 37, 106). Conduction velocity and differences in discharge characteristics are two functional aspects that subdivide primary afferent neurons, but phenotypic cellular and molecular differences also segregate across these two classes. Such divisions in cranial visceral afferent neurons resemble the broad phenotypic separation of somatosensory primary afferents located in the spinal dorsal root ganglia that includes the expression of key ion channels and receptors (73, 96, 97). C-type neurons often express a different mixture of particular ion channels than myelinated afferents (e.g., tetrodotoxin (TTX)-resistant sodium channels (5)), and these differences extend to ligand gated channels (e.g., TRPV1).

Cranial Visceral Afferents: Mostly Physiologically Quiet and C-Fibers

The C-fiber class is overwhelmingly the dominant phenotype of primary afferents from any given organ, a fact that is perhaps under appreciated, despite the starkly contrasting numbers. The relationship or ratios of the numbers of myelinated and unmyelinated afferents varies with species and organ. For example, aortic baroreceptors (10) and lung afferents (70, 94) are ~90% unmyelinated. Physiologically, however, if one considers normal prevailing conditions that might activate afferents, then the functional activity of these groups flips this relationship to A-fibers dominating transduction of ongoing local conditions. In somatosensory neurons (8, 11, 12, 71), A-fibers are associated with fine discriminating sensation (e.g., knee flexion), whereas somatic C-fibers tend to be associated with nociception and near damaging stimuli (e.g., noxious heat). In cranial visceral afferents, C-fibers often have supra-physiological thresholds and sparse, irregular discharge. A-fiber afferents in contrast regularly discharge during basal physiological circumstances (e.g., normal ventilation or ambient resting blood pressure). Since visceral thresholds are so high, the stimuli required to activate C-fiber afferents correspond to harsh organ-level conditions (e.g., high distending pressures) and may thus correspond to potentially damaging stimuli, paralleling spinal cord afferents in the dorsal root ganglia (4, 17, 29, 30, 73). Visceral C-fibers are also, on average, less sensitive to mechanical, chemical, or thermal stimuli, and their discharge remains sporadic even with intense stimuli. The relatively mild stimuli that activate myelinated cranial visceral afferents has resulted in a greater knowledge of A-fibers and their pathways, again contributing to an A-fiber centric view (69). One ramification is that C-type afferents and their role in homeostatic regulation may be under recognized.

An illustrative example of how a common set of prevailing conditions differently activates A- and C-fiber afferent subtypes is aortic baroreceptors. Nearly all myelinated baroreceptors (90% of the A-type baroreceptor population) actively discharge at resting blood pressures (i.e., suprathreshold), and this information is conducted into the CNS (Fig. 2). At the same time, C-fiber baroreceptors are physiologically silent at these same prevailing blood pressures (114). Since most cranial afferents are C-type and they are likely to send little activity to the CNS, this prompts us to reconsider our understanding of their function in homeostatic control under normal conditions. In the arterial baroreflex for example, experimental lesions using the ultra-potent TRPV1 agonist resiniferatoxin (RTX) resulted in loss of neural structures: TRPV1 labeling in the aortic arch, nodose ganglion, and solitary tract (ST). RTX had no effect on...
blood pressure challenges below 125 mmHg, but RTX reduced the baroreflex responses above 125 mmHg, a deficit corresponding to the minimal pressure range for C-type aortic baroreceptors (102). Thus, TRPV1 and C-fibers had no measurable contribution unless pressure was forced well above normal prevailing pressures, effectively a silent deficit that required a specific stimulus to reveal. Afferent cellular phenotype strongly impacts the physiological activation thresholds and sensitivity above threshold, and these in turn determine the number of afferent action potentials sent to the CNS for reflex action. This example represents an interesting corollary that points out that very different adequate stimuli are required to assess A- and C-type functional contributions. Such phenotypic distinctions are an integral aspect of assessing the fate of their excitatory signals within the central “wiring,” and these pathway distinctions may well strongly influence their functional contributions to CNS integration.

Silent Majority and NTS Neurons

Afferent discharge activates central terminals which begins the translation and integration of that information. Cranial visceral primary afferents send their axons via the IXth and Xth cranial nerves directly to the brain stem and synapse on second-order sensory neurons within the caudal portions of the NTS (11, 12). These transmission lines have an interesting substructure: the peripheral nerve trunks are composed of smaller bundles of primary visceral afferents that contain a mix of 1–3 myelinated axons with 5 or more C-fiber axons. The peripheral and central nerve endings share the pattern that as the nerve fascicle approaches the target, e.g., the visceral organ, the A-fiber primary afferent axon loses its myelin and branches into final sensory arbors. The accompanying C-fiber cohort spreads across the same general region as the A-fibers [e.g., aortic baroreceptors (67, 105, 111) or pulmonary afferents (68)]. At the central ends within the NTS, A- and C-fiber synaptic terminal distributions share similar subregions (6, 59–61) and A-type axons become “postmyelinated” (60). Central mapping efforts detailed single afferent branching and terminal fields and yielded remarkably similar maps: whether for A- or C-type, or for baroreceptor or chemoreceptor, or for pulmonary or carotid sinus afferents (42, 46–48). Since single cranial visceral afferent axons commonly branch to cover similar NTS topologies, the proximity creates the potential for overlap and convergence (42, 47, 48, 69). This concept of convergence is an important one, but precisely where and how afferent information is combined was less clear. Tests on the same or different nerves suggest that convergence at NTS neurons was remarkably low (25, 45, 82, 84). In vivo data demonstrated that even maximal stimulation (i.e., activating perhaps >50,000 axons) failed to activate more than one input in ~85% of single NTS neurons. Thus, contrary to anatomical impressions, each second-order neuron directly received limited afferent input. Even among seemingly similar modalities (e.g., cardiovascular), arterial baroreceptors rarely (<13%)
activated single NTS neurons that were also activated by cardiac mechanoreceptors (85, 98). The centralis region and its gastrointestinal inputs, however, may present a more mixed profile with respect to TRPV1 and other presynaptic markers (27). Most single second-order NTS neurons receive a stream of primary afferent excitation that is limited and focused and thus they resemble “labeled lines” dedicated to parsing select information.

**Synaptic Transmission From Cranial Visceral Afferents in Brain Stem Slices**

Some of the highest experimental resolution is afforded by in vitro approaches. Slices that are cut along the alignment of the visceral afferent inflow tract, the ST, produce horizontal brain stem slices for study. This preserves contiguous segments of cranial visceral afferents (i.e., the ST) that course caudally and medially from cranial nerve rootlets to second-order neurons in the caudal one-third of the NTS (11, 12). In these slices, minimal intensity shocks to ST axons reliably activate excitatory postsynaptic currents (EPSCs) through non-N-methyl-D-aspartate (NMDA) glutamate receptors (15, 50) (Fig. 3). The EPSCs activate with a nearly invariant latency from the ST shock and the standard deviation of the latency (jitter < 200 μs) serves as a metric to discriminate monosynaptic pathways to the neuron from those activated by an indirect, polysynaptic route. Anatomical tracers applied to peripheral afferent nerves are transported centrally to fluorescently mark ST terminals located on the soma and proximal dendrites of NTS neurons and anatomically confirm that direct contacts correlate with low jitter ST-EPSCs (13, 50, 66, 99). Small increments in intensity outline a recruitment profile with a sharp, minimum intensity shock that almost always excites an action potential in the postsynaptic neuron with a high safety factor for transmission of afferent activity (16, 21).

In most neurons, only a single monosynaptic ST afferent connection can be detected (13, 80), echoing in vivo findings. Our ability to discriminate single axons allowed detailed investigations of the mechanisms responsible for ST synaptic transmission. The variations in EPSC amplitudes across many trials provide an index of the probability of vesicle release. In this variance-mean analysis (V-M), the probability of vesicle release changed with external Ca2+ and V-M estimated that ST-evoked transmission averaged ~90% in 2 mM Ca2+ with remarkably low failure rates to ST shocks (i.e., <1%) (13, 22, 88). Overall, afferent evoked remarkably similar release rates from a readily releasable pool (RRP) of docked vesicles, and we calculated that this arose from an average of about 20 release sites. These synaptic characteristics were similar for A- and C-type afferents and together suggest a uniformity of basic excitatory transmission via synchronous release of glutamate. This is a surprising finding given the substantial differences in ion channels and action potential characteristics between A- and C-type primary visceral afferent neurons (74, 77, 96, 97).

**Active TRPV1 on Primary Afferents in NTS**

Capsaicin has long been associated with activation of unmyelinated afferents including cranial sensory neurons (38, 39). The responsiveness to capsaicin viewed from current knowledge implies the expression of TRPV1 and is correlated with afferent activation under extraordinary conditions such as over-stretch, anoxic conditions or irritating chemicals (38, 39). TRPV1 cloning (26, 31, 79) advanced the close association between TRPV1 and nociceptive primary afferents. TRPV1 receptors are cation-selective ion channels that are activated by a canonical trio of stimuli including thermal (>43°C), H+ (pH < 5.0), or phosholipid (vanilloids). Thus a single receptor complex underwrites multivalent integration (58). TRPV1’s location, selectivity for calcium (~10 μM Ca2+/Na+, and depolarizing action (31) had potential ramifications when extended to afferent synaptic terminals within the NTS.

Shortly before the cloning of TRPV1, we began to study whether capsaicin might alter ST synaptic transmission in...
slices of NTS (9). Early surveys demonstrated TRPV1 at the central terminals of vagal afferents within the NTS (110), and recent genetic approaches confirmed that they are presynaptic (32). Superfusion of brain stem slices with nanomolar concentrations of capsaicin robustly increased “spontaneous” glutamate vesicle release (sEPSCs). Within 1–5 min exposure to capsaicin, ST shocks failed to activate synchronized EPSCs despite continued high frequencies of random sEPSC events. Not all afferents were capsaicin sensitive (TRPV1+) indicating that transmission from single ST axons was either TRPV1+ or completely capsaicin insensitive (TRPV1−). This all-or-nothing finding suggested that ST afferents segregate and single NTS neurons received either only TRPV1+ or only TRPV1− inputs (86). All capsaicin actions were consistent with presynaptic mechanisms controlling glutamate release, and there was no evidence of postsynaptic TRPV1-mediated currents in NTS neurons. Parallel tests in nodose neurons indicated that capsaicin activated inward currents only in cells with C-type conduction velocities and capsaicin-resistant nodose neurons had A-type conduction velocities (57). Thus capsaicin responses in ST-EPSC transmission to NTS neurons indicated that TRPV1+ responses were from C-fibers and TRPV1− from A-fibers. Using this method of separating TRPV1+ from TRPV1− ST inputs, no differences in the detailed characteristics of evoked synchronous glutamate release (ST-EPSCs) could be identified, a finding that suggests that TRPV1 does not participate in synchronous release (13). Likewise, dye-labeled baroreceptive NTS neurons had synaptic machinery equivalent to that of adjacent unlabeled and presumably nonbaroreceptor neurons whether A- or C-type ST afferents (13). Thus synchronous glutamate transmission by all cranial visceral afferents within the medial NTS is remarkably uniform despite different afferent phenotypes and different visceral organ sources.

**TRPV1 Activity Generates Spontaneous EPSCs**

One key synaptic difference has emerged from the analyses of A- and C-type cranial afferents. The “spontaneous” EPSC rate without any afferent stimulation averaged nearly 10-fold higher at neurons with TRPV1+ afferents compared with those receiving TRPV1− afferents under similar conditions (87). This basal EPSC activity difference persisted when action potentials were blocked by TTX. Together, the pairing of higher spontaneous transmission in TRPV1+ neurons, despite synchronous transmission resembling TRPV1− neurons, was quite surprising. In landmark work, Katz (62) originally discovered that the neurotransmitter released following an action potential (synchronous release) was composed of many small vesicles or quanta and that these same quantal vesicles were contained in the synaptic terminals and vesicles or quanta and that these same quantal vesicles were responsible for basal and asynchronous release enters through TRPV1+ terminals release glutamate synchronously and at a very low proportional threshold for TRPV1 activation (Fig. 4) continuously brain areas together with noncanonical soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins for differential control of release (20, 64, 91, 92). In the NTS, evoked ST-EPSCs from TRPV1+ and TRPV1− sources are quite similar (Fig. 3). The only sign thus far that may reflect the characteristically different excitability, action potentials, and ion channels arising from TRPV1+ and TRPV1− nodose neurons (43, 44, 74–76, 96) may be the increased transmission failures in C-type ST inputs with high frequencies (13). Since evoked EPSCs were indistinguishable and yet the same neurons expressed substantially different basal spontaneous EPSC rates, then glutamate release from ST afferents differs from the conventional view. Bursts of ST shocks depressed synchronous EPSC amplitudes from all afferents in a manner that is consistent primarily with vesicle depletion (87). However, in TRPV1+ afferent neurons, bursts of synchronous EPSCs and depletion of the RRP are followed by an elevated rate of spontaneous EPSCs trailing immediately after the evoked responses, a phenomenon termed asynchronous release (Fig. 3). Thus high basal release and asynchronous release are synaptically diagnostic of TRPV1+ ST afferent transmission and suggested that TRPV1+ ST afferent terminals utilized two distinct pools of glutamate vesicles (54).

**Unconventional Modes of Glutamate Release**

Our studies suggested that myelinated cranial afferent terminals release glutamate synchronously and at a very low spontaneous rate. In contrast, TRPV1+ terminals possess an additional form of release from a separate, TRPV1-operated pool that is responsible for most of spontaneous release in basal conditions. Since vesicular release is promoted by rises in intracellular calcium, changes in external calcium, addition of specific calcium channel blockers or exposure to membrane permeant calcium buffers depressed evoked and asynchronous release similarly (87). However, TRPV1 antagonists decreased both the basal and asynchronous release EPSC rates without affecting the amplitude of the ST-evoked EPSCs (87). In TTX, we found that cadmium, a broad-spectrum blocker of voltage-activated calcium channels, did not affect the rate of mEPSCs in TRPV1+ neurons. This suggests that the calcium influx responsible for basal and asynchronous release enters through TRPV1. Interestingly, this also indicated that TRPV1-related calcium influx did not contribute to synchronous release from TRPV1+ ST terminals. Together, the evidence suggested that TRPV1 controlled or “operated” a distinct pool of glutamate vesicles that were not released by action potentials but rather their release depended on the activity of TRPV1. The independence of ST-EPSCs from TRPV1 activity may mean that TRPV1 and the TRPV1-operated pool of vesicles are localized close together, potentially within a presynaptic-restricted nanodomain such as a lipid raft (28, 95). The high rate of spontaneous EPSCs may thus be due to calcium entry through TRPV1 to release glutamate vesicles not released by action potentials.

Heat can also activate TRPV1+. The canonical thresholds for gating TRPV1 suggested that conditions were inappropriate in our slices for activating TRPV1. Nonetheless, to test whether temperature could be influencing TRPV1 in ST afferent terminals in our slices, we varied bath temperature while monitoring sEPSC rate. Temperatures well below the conventional threshold for TRPV1 activation (Fig. 4) continuously
The issue of localization of central TRPV1 has recently been
investigated. TRPV1 in vasopressin-releasing hypothalamic
donors suggested that TRPV1 was required in hippocampal neurons suggested that TRPV1 was required
for long-term synaptic depression (34, 63). Such actions in
terminal portions, but this presence of TRPV1 within the CNS
arises overwhelmingly from peripheral sensory neurons. A
typical pool appears to avidly hold on to its synaptic vesicles and have little temperature sensitivity (101). These
experiments suggest that at physiological temperatures, C-type afferents have a unique mode of releasing glutamate that actively drives basal release of vesicles and thus is autonomous of afferent action potential activity. This TRPV1-operated mode of release may offer a novel tonic signaling mechanism within those afferent pathways.

**TRPV1 in the CNS**

TRPV1 function within the CNS has long been suggested
but is controversial. Certainly, TRPV1 is expressed at primary sensory afferent neurons, and this extends to their central terminal portions, but this presence of TRPV1 within the CNS arises overwhelmingly from peripheral sensory neurons. A number of studies using immunolabeling and autoradiography have suggested that CNS neurons intrinsically express low levels of TRPV1 in cortex, amygdala, thalamus, hypothalamus, and other areas (104). TRPV1 agonists and antagonists acting on hippocampal neurons suggested that TRPV1 was required for long-term synaptic depression (34, 63). Such actions in cortical areas might be related to alterations in cognitive and emotional responses in TRPV1 knockout mice (78). In a different vein, TRPV1 in vasopressin-releasing hypothalamic neurons is linked to central thermosensory transduction (100). The issue of localization of central TRPV1 has recently been revisited, however, using molecular genetics methods incorporating a highly sensitive, TRPV1 reporter mouse, and these results counter the view of widespread TRPV1 expression and

modulated sEPSC rates in TRPV1+ but not in TRPV1− NTS neurons. Blockade of voltage-dependent sodium and calcium channels did not alter this TRPV1-driven release (87, 101). Therefore, at normal temperatures, calcium enters synaptic ST terminals through TRPV1 to activate release of vesicles from the TRPV1-operated pool without affecting conventional synchronous transmission from the same afferents. TRPV1 is absent in myelinated ST afferents and neurons receiving those afferents are therefore remarkably quiet at rest, i.e., their synchronous pool appears to avidly hold on to its synaptic vesicles and have little temperature sensitivity (101). These experiments suggest that at physiological temperatures, C-type afferents have a unique mode of releasing glutamate that actively drives basal release of vesicles and thus is autonomous of afferent action potential activity. This TRPV1-operated mode of release may offer a novel tonic signaling mechanism within those afferent pathways.

**How Could TRPV1 Shape NTS Performance and Impact Reflex Control?**

Several surprising aspects have emerged from our work linking TRPV1 activity and ST synaptic transmission. First, TRPV1 is vigorously active at normal brain temperatures. Second, calcium entry through TRPV1 is coupled to glutamate vesicles that are distinct from those mediating action potential-synchronized EPSCs. Third, terminal depolarization facilitates release from the TRPV1-operated pool of glutamate vesicles but not vice versa. Thus TRPV1 generates a stochastic signal related to afferent endings in the CNS that does not require peripheral sensory activation. At 37°C, TRPV1 channel activity stochastically generates EPSCs from TRPV1+ afferents. This TRPV1-derived, synaptic activity triggered a tonic level of action potential activity in the postsynaptic NTS neurons that was rapidly removed by cooling (87, 101). Recall that ~80–90% of ST terminals are C-type cranial afferents and TRPV1+. Accordingly, most NTS neurons have a tonic basal drive that represents TRPV1 actively driving central activity. Many of these autonomous events trigger action potentials that may contribute a random drive of C-type afferent reflex pathways. This TRPV1 signal may have broad implications but, at this time, it remains largely untested.

Is There a Physiological Role for Central TRPV1 Signaling in the NTS?

Physiological activation of cranial visceral afferents trigger synchronized EPSCs that excite NTS. The function of TRPV1-operated glutamate is uncertain. A recent respiratory reflex example may indicate a physiological impact of TRPV1 actions within the NTS. Laryngeal afferent activation by fluid infused into the larynx of neonatal animals triggers a pronounced airway-protective reflex, the laryngeal chemoreflex (LCR). This reflex is characterized by disrupted respiration, prolonged apnea, coughing, and swallowing (41). Relatively modest elevations in body temperature (2°C) enhanced the LCR in neonatal pigs triggering an abnormally prolonged reflex apnea (41). The LCR may be important in sudden infant death syndrome and hyperthermia may increase this risk (65). Experimentally, the influence of temperature on the LCR is unlikely to reside at the peripheral sensory endings since selectively changing the temperature of the distilled water infused into the larynx failed to alter the LCR (41). In contrast, focal warming of the NTS altered the LCR despite holding body temperature constant and suggested that the NTS regional temperature is critical in the response (113). Furthermore, bilateral injection of the TRPV1 blocker 5′-iodoresiniferatoxin blocked the enhancement of the LCR during body temperature elevation and this result suggested that TRPV1 receptors were essential for the reflex in neonatal pigs (112). Interestingly, respiratory frequency rose following introduction of the
TRPV1 blocker regardless of NTS temperature levels (38.6°C or 40.7°C). Such temperatures are below the canonical TRPV1 threshold (>43°C) but well within the range of temperatures at which TRPV1 strongly promotes glutamate release from C-type afferent endings in rat NTS (87, 101). The evidence suggests that the TRPV1-operated vesicles in C-type primary afferent endings within NTS may be responsible for an important aspect of both basal activity and evoked responses in this respiratory circuit. While this example concerns respiratory afferents, our work in slices suggests that the influence of TRPV1 mechanisms in NTS is unlikely to be limited to the LCR.

**Signal Targets of Multimodal Glutamate Release**

In the broad view of excitatory signaling from cranial afferents, glutamate in TRPV1+ terminals has at least three modes of vesicular release depending on calcium source (Fig. 3): 1) synchronous in response to action potentials and voltage dependent calcium channel activation; 2) autonomous in response to calcium entering through TRPV1; and 3) interactive, which results from an unknown mechanism linking action potential-gating of calcium entry to temporary facilitation of the TRPV1-operated release. TRPV1-negative terminals, in contrast, have very low spontaneous release rates (e.g., "reluctant" vesicles) despite comparable numbers of active zones and similar synchronous release. One additional potential role of TRPV1-operated tonic glutamate release might be in maintaining synaptic connections in otherwise generally silent C-type pathways (81, 89, 103).

The NTS is rich with GPCRs that have signal transduction cascades that modify conventional voltage-dependent ion channels. The presence of TRPV1-operated glutamate release adds an additional effector for GPCRs in C-type sensory afferent terminals. C-fiber neurons are strongly associated with neuropeptides and GPCRs (7, 11). GABA_B is a widespread GPCR that inhibits presynaptic glutamate release, and we recently tested whether its actions extended to the TRPV1-operated pool (52, 87, 101). Baclofen, a GABA_B receptor agonist, inhibited synchronous EPSCs in both TRPV1+ and TRPV1– ST afferents and reduced the rates of basal and asynchronous EPSCs associated with TRPV1. In isolation of action potential-mediated release, baclofen strongly suppressed temperature-gated mEPSCs in TRPV1+ neurons. Thus presynaptic GPCRs can act on both the synchronous release mechanism, likely at calcium and/or potassium channels, as well as the TRPV1-operated mechanism. Alternative players in this realm expand beyond amino acid and peptide transmitters to include inflammatory mediators, lipid metabolites, cytokines, and cannabinoids. This raises the question of whether other GPCRs might differentially affect fast synchronous transmission or the TRPV1 mechanism separately. For example, CB1 and TRPV1 are colocalized and have structurally similar endogenous ligands (endocannabinoids and endovanilloids), suggesting that some lipid mediators may affect both receptors (90). A wide variety of mediators modify synchronous EPSCs (18, 19, 22–24, 40, 72, 88, 93), but the modulation of TRPV1-operated release remains largely untested. As in many targeting strategies, it is also possible that particular GPCRs might act to selectively modulate TRPV1-operated release and tonic activation of C-type pathways without affecting phasic transmission. From a homeostatic view, this latter possibility could have broad implications in chronic pathological disturbances in which the numeric dominance of C-type endings and TRPV1 would broadly disturb multisystem control mechanisms.

**Perspectives and Significance**

Two old facts offer a provocative context for a new and intriguing view of afferent transmission in NTS. First, an overwhelming majority of primary visceral afferents in cranial nerves reaching NTS are unmyelinated. This suggests that perhaps most of the glutamate available for excitation in caudal NTS is locked within the terminals of unmyelinated afferents, an idea little appreciated but potentially profound in its implications. Second, the rather harsh physiological stimuli that are “adequate” to activate most C-fiber afferents have invited a conclusion that C-type glutamate terminals may be of less consequence under normal physiological circumstances. Our recent work may help to disconnect these two ideas. We found that TRPV1 on C-type glutamate terminals actively triggers the release of glutamate at a tonic rate corresponding to the local temperature. Thus normal temperature is sufficient to gate calcium entry through TRPV1, and this calcium triggers release from a unique pool of synaptic vesicles, a process that does not require action potentials and is thus intrinsically autonomous. The ramifications of the high rates of spontaneous glutamate release via this TRPV1 mechanism are not well understood, but TRPV1 may be critical in some reflex pathways such as the laryngeal chemoreflex. Since this ongoing TRPV1-mediated release of glutamate can be modulated by GPCRs, the regulation of vesicle release by TRPV1 represents a potential new source of pathway plasticity and complex behaviors (36).

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**DISCLOSURES**

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**AUTHOR CONTRIBUTIONS**


**REFERENCES**


