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Channeling Satiation: a primer on the role of TRP channels in the control of glutamate release from vagal afferent neurons

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Abstract

Obesity results from the chronic imbalance between food intake and energy expenditure. To maintain homeostasis, the brainstem nucleus of the solitary tract (NTS) integrates peripheral information from visceral organs and initiates reflex pathways that control food intake and other autonomic functions. This peripheral-to-central neural communication occurs through activation of vagal afferent neurons which converge to form the solitary tract (ST) and synapse with strong glutamatergic contacts onto NTS neurons. Vagal afferents release glutamate containing vesicles via three distinct pathways (synchronous, asynchronous, and spontaneous) providing multiple levels of control through fast synaptic neurotransmission at ST-NTS synapses. While temperature at the NTS is relatively constant, vagal afferent neurons express an array of thermosensitive ion channels named transient receptor potential (TRP) channels. Here we review the evidence that TRP channels pre-synaptically control quantal glutamate release and examine the potential roles of TRP channels in vagally mediated satiety signaling. We summarize the current literature that TRP channels contribute to asynchronous and spontaneous release of glutamate which can distinctly influence the transfer of information across the ST-NTS synapse. In other words, multiple glutamate vesicle release pathways, guided by afferent TRP channels, provide for robust while adaptive neurotransmission and expand our understanding of vagal afferent signaling.

Keywords

vagus; solitary tract; vesicle release; synaptic; calcium; autonomic reflexes; TRPV3; TRPM3; spontaneous; asynchronous; synchronous; temperature

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INTRODUCTION

Primary vagal afferent neurons provide a direct neural pathway through which the status of peripheral organ systems (including the heart, lungs, and gastrointestinal tract) and satiety signals are relayed to the brain [1-3]. Vagal afferent neurons convey a broad range of stimuli which vary by information types, time-frames, and relative physiological urgencies. As a result, information transfer must be at once reliable and precise while maintaining plasticity to match autonomic function to the physiological state. To control information transfer, vagal afferents release the fast excitatory neurotransmitter glutamate via three distinct pathways: synchronous, asynchronous, and spontaneous (Figure 1) [4]. The interplay between these multiple complementary vesicle release pathways allows for robust and precise information transfer which is also adaptable and plastic to changing physiological states.

Vagal afferent neurons are broadly divided into myelinated A-fibers, lightly myelinated A δ -fibers, and unmyelinated C-fibers based off conduction velocity and histological analysis. C-fibers compose the majority of neurons in the vagus and robustly express the transient receptor potential vanilloid type 1 (TRPV1) ion channel thus conveying their sensitivity to capsaicin lesion [5]. Centrally vagal afferents converge with the facial (VII) and glossopharyngeal (IX) nerves to form the solitary tract (ST) bundle and innervate second-order NTS neurons [2]. Afferent innervation onto second-order NTS neurons is limited relative to central synapses; with as few as 1-5 discrete primary afferent inputs converging [6]. Further, convergent inputs are completely segregated based on their expression of TRPV1 or not [7]. This organizational schema allowed for the observation that the presence of TRPV1 in the central terminals predicts facilitated forms of quantal glutamate release; including higher frequencies of spontaneous and asynchronous quantal release compared to afferents lacking TRPV1 [4]. This activity-dependent asynchronous glutamate release prolongs the period of action potential driven release (from milliseconds to seconds) and extends the postsynaptic excitatory period despite frequency dependent depression (FDD) of synchronous glutamate release [4]. Furthermore, TRPV1, and likely other thermosensitive TRP channels, provide temperature sensitive calcium conductances which largely determine the rate of spontaneous vesicle fusion [8, 9]. We speculate these additional vesicle release pathways contribute to the relative strength of C-fibers in activating autonomic reflex pathways, including those that control food intake and energy balance.

Here, we discuss the plasticity of vagal afferent signaling within the context of food intake and energy balance. We will discuss the different forms of glutamate release which define the adaptive nature of vagal afferent signaling and summarize evidence that thermosensitive TRP channels guide glutamate release pathways. Consideration of peripheral and central peptides and their effects on satiation through these glutamate release pathways will be given. In short, complementary vesicle release pathways, segregated innervation of vagal afferents, and the presence of highly adaptable temperature sensitive TRP ion channels allow vagal afferent signaling to be predictive and reactive in order to efficiently maintain energy homeostasis. By elucidating the cellular mechanisms that enable the adaptive nature of vagal afferent, we hope to gain a better understanding of vagal afferent control of food intake and energy balance.

GLUTAMATE RELEASE PATHWAYS

Spontaneous vesicle fusion and release

Spontaneous vesicle fusion is a stochastic but regulated process of neurotransmitter release which occurs independently of action-potential depolarization of the terminal (Figure 1B, top panel) [10]. Individual vesicles fuse and are released probabilistically over time from pre-synaptic active zones. The average peak current amplitude generated by the glutamate released from single vesicles acting at the postsynaptic receptors represents the fundamental unit or quantum (q) of neurotransmission. Because the charge transfers of discrete quanta are small they are often recorded as a surrogate for action-potential release pathways and in the past not considered important signaling events by themselves [11]. However, it is now generally appreciated that spontaneous neurotransmission can be important for neuronal communication during development, for setting synapse gain sensitivity, and in the trophic maintenance of the synaptic contacts [11]. The frequency of spontaneous release from vagal afferent endings is robust enough to be determinant of postsynaptic membrane excitability by itself and directly under the control of thermo-sensitive TRP channels [9].

At ST-NTS synapses the frequency of spontaneous vesicle release is largely driven by calcium influx via thermosensitive TRP channels [9]. The presence of TRPV1 in ST primary afferents predicts relatively high spontaneous frequency, while pharmacological antagonism of TRPV1 reduces overall temperature sensitivity of the spontaneous release pathway particularly at the highest temperatures recorded (~40°C) [4, 9]. This phenomenon has also been observed in the dorsal motor nucleus of the vagus (DMV) [12]. In TRPV1 KO animals we found that frequency of spontaneous release was indistinguishable from control animals including during temperature ramps from 33-37°C [8]. The observation that spontaneous frequency is maintained at warm temperatures and is still reactive to temperature steps indicates that additional temperature sensitive mechanisms are participating in addition to TRPV1. In our recent paper we report functional expression of TRPV3 and TRPM3 ion channels; plausible candidates for the maintenance of temperature driven spontaneous release at warm physiological temperatures. These temperature driven changes may be relevant in detecting a number of physiological processes that alter internal temperatures such as post prandial thermogenesis [13], circadian temperature changes [14], or fever.

Synchronous vesicle fusion and release

Synchronous glutamate release is a tightly regulated process by which an action potential evokes the coordinated release of multiple glutamate vesicles into the synaptic cleft [15]. At ST-NTS synapses this precise timing is accomplished by the close approximation of N-type voltage activated calcium channels with primed vesicles positioned at the membrane [16]. As a result synchronous vesicle release provides a large excitatory postsynaptic current (EPSC) from the afferent to the postsynaptic neuron and ensures high fidelity synaptic throughput [17].

The parameters of vesicle release that affect EPSC can be demonstrated through the equation:

$$EPSC = q P_r N$$

where 'N' represents the number of available release sites, 'q' the quantal size of single vesicles as represented by spontaneous release (see above), and 'P_r' the probability of release [18]. The probability of release (P_r) is the probability that a vesicle will fuse and release neurotransmitters for a given presynaptic action potential. Compared to most central synapses, vagal afferents exhibit a high 'P_r' under physiological conditions (70-90%), resulting in nearly complete release of docked/primed vesicles following an action-potential [19, 20]. This helps to ensure a high ratio of presynaptic to postsynaptic action-potential throughput, particularly at relatively low frequencies of firing. In contrast, high frequency activation results in a pronounced decrease in afferent glutamate release, a process known as frequency dependent depression (FDD) (Figure 1B, middle panel) [21]. FDD is the result of vesicle release out pacing the vesicle mobilization to the membrane. This produces a temporary, functional, loss of vesicle release sites 'N' because fewer vesicles are docked and primed at the presynaptic membrane [18]. While synchronous release is steeply calcium dependent, including both 'Pr' and vesicle mobilization, calcium influx via TRP channels does not appear to impact these parameters; consistent with localized micro- and nanodomain calcium determined by voltage activated calcium channels. However, TRP channel derived calcium is determinant of quantal vesicle release as described above for spontaneous release and contributes to asynchronous vesicle mobilization reviewed next.

Asynchronous vesicle fusion and release

As with synchronous release, asynchronous neurotransmitter release is action-potential dependent, but distinguishes itself by being only loosely coordinated with depolarization [15]. While synchronous release is a large and immediate release of vesicles, from vagal afferents asynchronous is characterized by a relatively long period (up to a few seconds) of stochastic release of individual quanta which occurs after the action potential is terminated (Figure 1B, lower panel) [4, 7]. Asynchronous release from vagal afferents is dependent a serial transduction of events including terminal depolarization, opening of voltage activated calcium channels with calcium influx, and synchronous vesicle fusion and release [4]. As a consequence, disruption of any of these processes disrupts both synchronous and asynchronous release. However, not all vagal afferent synapses exhibit asynchronous release but do share common synchronous release mechanisms. These findings lead to the observation that reduction of TRPV1 signaling, either pharmacologically or with temperature, selectively diminished asynchronous but not synchronous glutamate release [4].

In the unmyelinated C- and lightly myelinated Aδ-fibers of the ST-NTS synapses, asynchronous release represents an unusually high proportion of neurotransmission, in some cases making up nearly half of total vesicle release [4, 7, 8]. In rats the asynchronous profile decays with a biexponential fit suggestive of perhaps multiple processes determining the prolonged release. However, in mice the asynchronous release decays with a single exponential and as such is much faster. Despite these differences this form a neurotransmission is a major signaling component and drives prolonged postsynaptic

membrane excitability and spiking [4]. Understanding the specific mechanisms common and selective to synchronous and asynchronous release should allow for the modification of one process or the other independently. So far, only attenuation of TRP channel function has allowed us to alter asynchronous release and not synchronous; although it is likely additional mechanism will be revealed with future experiments. At other synapses asynchronous release is the result of differences in presynaptic calcium channels, synaptotagmin subtypes with varying calcium affinities, and expression of specific synapsin proteins [22-24]. Investigation in to these possibilities will likely clarify our understanding of asynchronous glutamate release at vagal afferent neurons.

VAGAL AFFERENT 'TRP' CHANNEL EXPRESSION

In this review, we present evidence to suggest that presence of thermosensitive TRP channels in vagal afferents contributes to the neurocircuitry of feeding by maintaining adaptable synaptic transmission at the ST-NTS synapse. TRPV1 is expressed at the terminals of vagal afferent terminals specifically in C-type fibers and provides a unique form of asynchronous vesicular release as well as temperature sensitive spontaneous vesicular release [4, 9, 25]. Recent findings that involve pharmacological and genetic deletion of TRPV1 also implicate other thermosensitive TRP channels in this process.

We now appreciate there are likely more TRP channels, other than TRPV1, controlling neurotransmission through asynchronous and spontaneous glutamate release [8]. Table 1 reports the extensive expression of TRP channels identified in vagal afferent neurons [26-31]. New functional evidence from our laboratory shows that with pharmacological blockade and genetic deletion of TRPV1, some asynchronous release and temperature driven spontaneous release of glutamate is maintained at ST-NTS synapses [8]. These findings suggest additional thermosensitive TRP channels as potential candidates for further study of glutamatergic neurotransmission at ST-NTS synapses. We highlight two potential candidates TRP channels that may be participating in vagal neurotransmission; TRPV3 and TRPM3.

TRPV3

The ion channel TRPV3 is member of the TRP vanilloid subfamily and highly homologous to TRPV1 [32]. Characterized as a thermosensitive calcium channel found in keratinocytes, TRPV3 is also expressed in discrete brain regions and primary sensory afferents also expressing TRPV1 [33-37] including primary vagal afferents [31]. Because of the sequence homology with TRPV1, co-localization on the same chromosome, and similar expression profiles, work has begun to explore the interactions between TRPV3 and TRPV1.

Alone, or perhaps in heteromeric confirmation with TRPV1, TRPV3 provides a temperature stimulated calcium conductance in the same temperature range that drives spontaneous release independently of TRPV1 (33-39°C) [38, 39]. Preliminary electrophysiological studies using transgenic TRPV1 KO mice suggest that TRPV3 may drive some of the remaining asynchronous release at the ST-NTS synapse independent of TRPV1 and maintain the temperature sensitivity of spontaneous release. Previous reports [31] also support the presence of TRPV3 and TRPV1 co-expression in vagal afferent neurons. Ethyl

vanillin (EVA), a TRPV3 agonist [40], dose-dependently increases calcium in a subpopulation of TRPV1 positive neurons. Furthermore, when TRPV1 and TRPV3 are cloned into HEK cells, the proteins spontaneously assemble as hetero-tetramer subunits [36]. As such, we have reason to predict that TRPV3 will also influence TRPV1-dependent asynchronous and spontaneous glutamate release in native synapses.

TRPM3

TRPM3 is a relatively new and under investigated member of the melastatin family of TRP channels [41]. It is of interest to us because like TRPV1, it passes calcium and is highly thermosensitive in the physiological range for spontaneous glutamate release. In somatosensory neurons TRPM3 is crucial for temperature detection with TRPM3 KO mice showing a clear decrease in their avoidance of noxious heat [42]. Between all the TRP channels, TRPM3 has the largest number of splice variants [43], which results in a range of functionality. However, the majority conduct calcium and are strongly and specifically activated by the steroid pregnenolone sulfate [41]. Additionally, almost all vagal afferent neurons that express TRPM3 co-express TRPV1 in their membrane [8], suggesting that they will likely share some functional role in the control of glutamate release. With this in mind, it is probable that their thermosensitive nature and passage of calcium facilitates part of the asynchronous and synchronous neurotransmitter release in the ST-NTS, along with TRPV1 and TRPV3.

SYNAPTIC PLASTICITY and SATIETY SIGNALING

Vagal afferent to NTS glutamatergic synaptic transmission maintains homeostasis of many physiological processes, including those that control food intake and energy balance. To maintain energy homeostasis, the neuronal circuits that convey peripheral information are matched with hormonal, nutrient, and neuronal signals of the organism. This neurocircuitry is robust and precise in its ability to transmit signals, yet the fidelity of these signals must change from moment to moment in order to adapt to a changing internal environment and the current needs of the organism. Similar considerations are also present in the neurocircuitry of the hypothalamus and may inform NTS processing [44, 45]. Here, we discuss the cellular mechanisms present at the ST-NTS synapse in order to maintain the synaptic plasticity crucial to efficient satiety signaling.

Vagal afferent signaling must be robust and precise in the initiation of autonomic reflex pathways and the control of feeding. These characteristics are seemingly in opposition to synaptic plasticity at the ST-NTS synapse. The ST-NTS synapse undergoes changes in synaptic strength and neurotransmission via presynaptic calcium influx, a key regulator in short-term synaptic plasticity [46]. These rapid functional changes ensure that vagal afferent signaling can quickly adapt to changes in the environment and the internal milieu of the organism. The probabilistic nature of glutamate release coupled with the multiple vesicle release pathway enable vagal afferents to maintain these opposing traits.

Table 2 provides a summary of the peptides secreted peripherally (from the gastrointestinal tract, pancreas, and adipose tissue) and centrally (from the hypothalamus) whose actions converge at the NTS [19, 20, 47-52]. The information from these converging signals is

integrated at the brainstem for control of food intake and energy balance. Some of these peptides contribute to efficient vagal afferent signaling by directly or indirectly modulating glutamate release at the ST-NTS synapse. For instance, peripheral peptides such as cholecystokinin (CCK) and central peptides such as oxytocin act on second order NTS neurons to enhance vagally mediated satiety signaling [20, 47]. At the cellular level, they exert their actions by increasing synchronous, asynchronous, and spontaneous glutamate release from vagal afferent terminals. In doing so, they contribute to the adaptive nature of neurotransmission across the ST-NTS synapse and efficiency of vagal afferent signaling.

Following synchronous neurotransmitter release, asynchronous release can persist for many seconds after an action potential and plays an important role in the plasticity of the synapse. At specialized synapses where asynchronous release is prominent, the nature of vesicle release is transformed allowing for more nuanced neurotransmission and coincidence detection [15]. Pre-synaptic asynchronous release contributes to synaptic plasticity in many specialized synapses, including the ST-NTS synapse. Because of asynchronous release, vagal afferent neurons have the ability to transform information transfer by extending the excitatory period after an action potential depolarization via elevation of neurotransmitter release in addition to synchronous release. Thus, facilitation of neurotransmission by asynchronous release at this synapse contributes to plastic changes at this synapse that fine tune the signal from vagal afferent neurons through amplification or sensitization of the original stimulus. In contrast to asynchronous release, pre-synaptic spontaneous release does not depend on action potentials but plays an important role in neurotransmission at the ST-NTS synapse. It does so by setting the tone of neurotransmission. In fact, this form of release has been implicated in synaptic plasticity at many specialized synapses through synaptic stabilization [15].

Thermosensitive TRP channels (particularly TRPV1) are sources of asynchronous and spontaneous release at the ST-NTS synapse. By increasing pre-synaptic calcium influx, TRPV1 dramatically transforms information transfer emanating from vagal afferent neurons by extending the excitatory period and increasing synaptic strength [4]. Without this property, the ST-NTS synapse loses efficiency of information transfer from the gastrointestinal tract to the brain. We postulate that redundancy in TRP channels expression accounts for a large portion of asynchronous and spontaneous release maintaining the multiple vesicle release pathways and fast neurotransmission at the ST-NTS synapse [8]. Thus, the presence of TRP channels endows the ST-NTS synapse with nuance and plasticity so that satiety signaling can adapt efficiently to a changing environment.

CONCLUSIONS and FUTURE DIRECTIONS

To maintain homeostasis of energy stores, vagal afferents carry satiety information to the central nervous system. To do so efficiently, it undergoes continuous change. Vagal afferent to NTS neurotransmission is at once robust and reliable while maintaining the ability to adapt to changing internal environments and physiological states. This balance of traits is a result of the segregation of primary afferent innervation, presence of temperatures sensitive TRP channels, and multiple forms of synaptic vesicles release. Our understanding of how TRP channels signal satiation through control of glutamate release is still in its infancy.

Although other labs have shown that many TRP channels are expressed in vagal afferent neurons, we have yet to elucidate their functional roles at the level of the ST-NTS synapse. We believe that further investigation on TRP channels and their role on glutamate release will lead to a greater understanding of the cellular mechanisms of satiety signaling. We predict that future investigation in this area will reveal the adaptive nature of information transfer through glutamate release from vagal afferent neurons.

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HIGHLIGHTS

1. Vagal afferents initiate homeostatic reflexes via strong glutamatergic synapses.
2. Vagal afferents release glutamate via multiple pathways.
3. Central thermosensitive TRP channels control quantal forms of glutamate release.
4. TRP channels and multiple release pathways allow for robust but plastic signaling.

Figure 1. Summary of NTS slice electrophysiology and forms of glutamate release pathways
A) Schematic representation of a horizontal brainstem slice and location of recording electrode. Horizontal orientation of the slice maintains a long section of the solitary tract (ST) allowing for selective stimulation of primary afferent endings. **B)** Sample post-synaptic electrophysiology recordings representing modes of vesicle release. Spontaneous (*top*) occurs independently of depolarization, synchronous (*middle*) results in depolarization, and subsequent activation causes frequency dependent depression (FDD) in synchronous EPSC

amplitude. Asynchronous (*bottom*) is characterized by an increase frequency of quantal vesicle release following depolarization and synchronous glutamate release.

Table 1

Summary of TRP channel expression in nodose ganglia.

TRP	In Nodose Ganglia (NG)	Methods of detection 'X' indicates present; 'n/a' denotes not known
C1	x	Glazebrook et al., 2005 - immunoreactivity present in soma of rat NG, also distributes to peripheral axons and mechanosensory terminals; Elg et al., 2007 - RT-PCR in mouse showing developmental expression; Zhao et al., 2010 - RT-PCR rat
C2	x	Elg et al., 2007 - RT-PCR shows higher expression in embryonic stages of mouse development compared to postnatal and adult stages
C3	x	Glazebrook et al., 2005 - immunoreactivity present in soma of rat NG, also distributes to peripheral axons and mechanosensory terminals; Elg et al., 2007 - RT-PCR in mouse showing developmental expression; Zhao et al., 2010 - RT-PCR rat
C4	x	Glazebrook et al., 2005 - immunoreactivity present in soma of rat NG, also distributes to peripheral axons and mechanosensory terminals; Elg et al., 2007 - RT-PCR in mouse showing developmental expression
C5	x	Glazebrook et al., 2005 - immunoreactivity present in soma of rat NG, also distributes to peripheral axons and mechanosensory terminals; Zhao et al., 2010 - RT-PCR rat
C6	x	Glazebrook et al., 2005 - immunoreactivity present in soma of rat NG; Elg et al., 2007 - RT-PCR in mouse showing developmental expression; Zhao et al., 2010 - RT-PCR rat
C7	x	Glazebrook et al., 2005 - immunoreactivity present in soma of rat NG; Elg et al., 2007 - RT-PCR in mouse showing developmental expression
V1	x	Zhang et al., 2004 - RT-PCR mouse and immunohistochemistry in mouse and guinea pig; Zhao et al., 2010 - RT-PCR rat
V2	x	Zhang et al., 2004 - RT-PCR mouse and immunohistochemistry in mouse and guinea pig; Zhao et al., 2010 - RT-PCR rat
V3	x	Zhang et al., 2004 - RT-PCR mouse; Zhao et al., 2010 - RT-PCR rat
V4	x	Zhang et al., 2004 - RT-PCR mouse; Zhao et al., 2010 - RT-PCR rat
V5	n/a	
V6	n/a	
M1	n/a	
M2	x	Staaf et al., 2010 - RT-PCR in mouse showing developmental expression
M3	x	Staaf et al., 2010 - RT-PCR in mouse showing developmental expression
M4	x	Staaf et al., 2010 - RT-PCR in mouse showing developmental expression
M5	x	Staaf et al., 2010 - RT-PCR in mouse showing developmental expression with higher expression at embryonic versus adult stages
M6	x	Staaf et al., 2010 - RT-PCR in mouse showing developmental expression
M7	x	Staaf et al., 2010 - RT-PCR in mouse showing developmental expression
M8	x	Staaf et al., 2010 - RT-PCR in mouse showing developmental expression; Zhang et al., 2004 - RT-PCR mouse; Zhao et al., 2010 - RT-PCR rat
A1	x	Zhao et al., 2010 - RT-PCR rat
P1	n/a	
P2	n/a	
P3	n/a	
P5	n/a	
ML1	n/a	
ML2	n/a	
ML3	n/a	

TRP	In Nodose Ganglia (NG)	Methods of detection ' <i>X</i> ' indicates present; ' <i>n/a</i> ' denotes not known
N1	x	Zhang et al., 2004 - RT-PCR mouse

Table 2

Actions of peripheral and central peptides.

Peripheral	Synchronous	Asynchronous	Spontaneous
CCK [47, 48]	↑	↑	↑
Leptin [51]	↓	?	↓
Ghrelin [49]	↓	?	↓
GLP1	?	?	?
Gastrin	?	?	?
PYY	?	?	?
Amylin	?	?	?
Insulin <i>via TRPV1</i>	?	?	↑

Central	Synchronous	Asynchronous	Spontaneous
Oxytocin [20]	↑	↑	↑
Vasopressin [19]	↓	?	↓
α – MSH [50]	↑	?	↑
Somatostatin- <i>unpublished</i>	---	?	↓