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Research Report

Store-operated calcium entry in vagal sensory nerves is independent of Orai channels

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ABSTRACT

Vagal sensory nerves innervate the majority of visceral organs (e.g., heart, lungs, GI tract, etc) and their activation is critical for defensive and regulatory reflexes. Intracellular Ca^{2+} is a key regulator of neuronal excitability and is largely controlled by the Ca^{2+} stores of the endoplasmic reticulum. In other cell types store-operated channels (SOC) have been shown to contribute to the homeostatic control of intracellular Ca^{2+}. Here, using Ca^{2+} imaging, we have shown that ER depletion in vagal sensory neurons (using thapsigargin or caffeine) in the absence of extracellular Ca^{2+} evoked Ca^{2+} influx upon re-introduction of Ca^{2+} into the extracellular buffer. This store-operated Ca^{2+} entry (SOCE) was observed in approximately 25–40% of vagal neurons, equally distributed among nociceptive and non-nociceptive sensory subtypes. SOCE was blocked by Gd^{3+} but not by the Orai channel blocker SKF96365. We found Orai channel mRNA in extracts from whole vagal ganglia, but when using single cell RT-PCR analysis we found only 3 out of 34 neurons expressed Orai channel mRNA, indicating that Orai channel expression in the vagal ganglia was likely derived from non-neuronal cell types. Confocal microscopy of vagal neurons in 3 day cultures demonstrated rich ER tracker fluorescence throughout axonal and neurite structures and ER store depletion (thapsigargin) evoked Ca^{2+} transients from these structures. However, no SOCE could be detected in the axonal/neurite structures of vagal neurons. We conclude that SOCE occurs in vagal sensory neuronal cell bodies through non-Orai mechanisms but is absent at nerve terminals.

1. Introduction

The mammalian viscera, including the airways, GI tract and heart, are densely innervated by sensory fibers projected by bilateral vagal nerves. These afferent fibers serve to sense the local environment and relay such information to brainstem areas involved in homeostatic control and defensive strategies. The large majority of vagal sensory afferent nerves are slowly conducting fibers (sparse to no myelination) that express proteins involved in the transduction of noxious or potentially noxious stimuli (Taylor-Clark and Undem, 2006). These fibers are called 'nociceptors' and their activation initiates defensive strategies, for example in the airways: cough, reflex bronchospasm, mucus secretion and neurogenic inflammation (Carr and

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Undem, 2003). The sensitivity or excitability of afferent nerves is critical to their function in health and disease.

Intracellular Ca^{2+} ions have significant effects on nerve excitability. Studies of dissociated sensory neurons from vagal, trigeminal and dorsal root ganglia have yielded important details of Ca^{2+} regulation and function (Cordoba-Rodriguez et al., 1999; Gover et al., 2007; Solovyova et al., 2002; Taylor-Clark et al., 2005). In the cell body, there is substantial endoplasmic reticulum (ER) that acts as a controllable sink of free cytosolic Ca^{2+}. Ca^{2+} release from the ER occurs downstream of either G_q protein activation and IP_3 generation or following influx of Ca^{2+} through voltage-gated Ca^{2+} channels (Ca^{2+}-induced Ca^{2+} release) (Cohen and Moore, 1997; Nicolson et al., 2002). Ca^{2+} then modulates excitability, particularly through the modulation of K^+ and Cl^- ion conductances (Gover et al., 2009). For example Ca^{2+} can activate BK and SK channels, thus causing K^+ efflux and hyperpolarization (Bahia et al., 2005; Li et al., 2007). Alternatively, Ca^{2+} can induce the inhibition of KCNQ channels, thus causing a decrease in K^+ leak and depolarization (Liu et al., 2010). In addition, Ca^{2+} activates CLCA channels, thus causing Cl^- efflux (due to depolarized reversal potential set by NKCC1 co-transporter expression) and depolarization (Lee et al., 2005; Liu et al., 2010).

ER Ca^{2+} stores can become depleted following repeated Ca^{2+} mobilization signals. In many cell types store-operated Ca^{2+} entry (SOCE) facilitates the rapid refilling of the ER by extracellular Ca^{2+}. SOCE has principally been described in non-neuronal cells and requires the gating of plasma membrane store-operated channels (SOC) (Parekh and Putney, 2005). Evidence suggests that plasma membrane Ca^{2+}-permeable Orai channels are the major SOC in many cell types, which are activated by the ER protein Stim1 upon loss of Ca^{2+} from the store (Varnai et al., 2009). Evidence supporting SOCE in central and peripheral neurons has been published (Gemes et al., 2011; Gruszczynska-Biegala et al., 2011; Steinbeck et al., 2011; Usachev and Thayer, 1999), but at present there are no published reports of putative SOCE in vagal neurons. It is likely that the presence of SOC would significantly modulate Ca^{2+}-mediated regulation of vagal neuronal excitability (Gover et al., 2009; Parekh and Putney, 2005). In this article we present data demonstrating SOCE in vagal sensory neuronal cell bodies. However, SOCE in vagal neurons is not mediated by Orai channels and SOCE is absent at nerve terminals.

2. Results

2.1. Ca^{2+} store in dissociated vagal sensory neurons

We first investigated the presence of functional Ca^{2+} stores in the soma of dissociated vagal sensory neurons using thapsigargin, an inhibitor of the sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) pump. Thapsigargin (10 μM) evoked a Ca^{2+} transient in vagal neurons (Fig. 1), indicating the presence of ER stores in these cells: 14.4±2.2% of ionomycin (n=111). Previous studies have suggested that the thapsigargin-induced increase in $[Ca^{2+}]_{cyt}$ can lead to the activation of the sensory nerve plasmalemmal non-selective ion channel TRPA1 (Jordt et al., 2004). As such, the thapsigargin-induced Ca^{2+} transient observed in these neurons may be secondary to TRPA1 activation. Nevertheless, thapsigargin (10 μM) evoked Ca^{2+} transients in vagal neurons derived from TRPA1−/− mice (13.8±2.2% of ionomycin (n=74)), suggesting the vast majority of the Ca^{2+} transient was directly as a result of Ca^{2+} store mobilization.

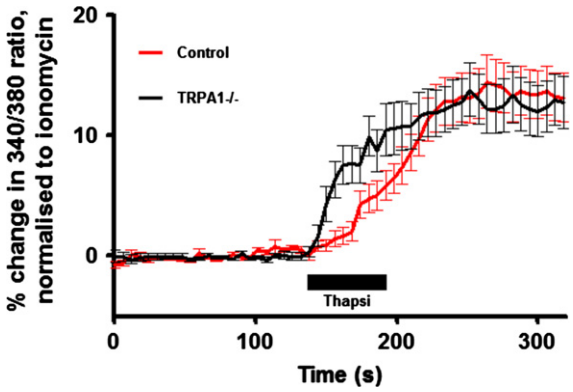

Fig. 1 – Mean +/− SEM Ca^{2+} responses of WT (n=111, red) and TRPA1−/− (n=74, black) vagal neurons to thapsigargin (10 μM). Blocked line denotes the 60-s application of agonist. Data are presented as mean change in 340/380 ratio as a % of ionomycin peak. All neurons responded to KCl (75 mM) applied immediately before ionomycin (not shown).

2.2. Store-operated Ca^{2+} entry in vagal sensory neurons

As part of intracellular Ca^{2+} homeostatic machinery in many cells, SOC allow for the refilling of Ca^{2+} stores (e.g., ER) following physiological mobilization or pathophysiological depletion. SOC activation is responsible for SOCE. To investigate SOCE, we studied the effect of removal and re-introduction of extracellular Ca^{2+} on the $[Ca^{2+}]_{cyt}$ of dissociated vagal neurons. The removal of Ca^{2+} for 3 min produced a small decrease in $[Ca^{2+}]_{cyt}$ in 75 of 88 neurons (−4.9±0.5% of ionomycin). Following the re-introduction of 2.2 mM Ca^{2+}, the $[Ca^{2+}]_{cyt}$ returned to baseline. In 4 of 88 neurons, the re-introduction of Ca^{2+} evoked a significant Ca^{2+} influx above the baseline (Fig. 2). Thus, Ca^{2+} removal alone is unable to elicit a classic SOCE or 'Ca^{2+} addback response' in vagal neurons.

Ca^{2+} stores can be depleted using thapsigargin (inhibits SERCA pump) or caffeine (activates ryanodine receptors). We treated vagal neurons with the store depletors in the absence of extracellular Ca^{2+}, then after 3 min, Ca^{2+} was re-introduced. Under these conditions, caffeine (10 mM) caused a greater decrease in $[Ca^{2+}]_{cyt}$ than Ca^{2+} removal alone (−8.6±0.9% of ionomycin) and upon re-introduction of Ca^{2+} evoked a small increase above baseline (4.6±0.9% of ionomycin) (n=233). Thapsigargin (10 μM) also caused a decrease in $[Ca^{2+}]_{cyt}$ (−11.4±0.5% of ionomycin) and upon re-introduction of Ca^{2+} evoked a small increase above baseline (9.6±1.2% of ionomycin)(n=310) (Fig. 2). Thapsigargin (1 μM) caused similar responses (n=108, data not shown). Vagal neurons are a heterogeneous population of sensory nerve subtypes, thus we analyzed these data to determine if