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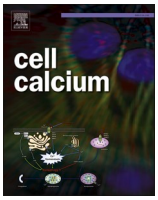

Commentary

TRPM4 keeps up the pace

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ABSTRACT

The study by Li et al., provides a detailed pharmacological characterization of the ionic mechanisms that underlie rhythmic activity of retrotrapezoid nucleus neurons that control breathing. Specifically, the authors demonstrate a role of the transient receptor potential melastatin 4 (TRPM4) ion channel in the generation of subthreshold excitatory oscillations. Additionally, they propose that the ion channel contributes to tonic action potential (AP) firing – referred to as "pacemaking" – of these brainstem neurons with relevance for respiratory breathing and homeostasis *in vivo*.

1. Commentary

Sustained rhythmic activity of specific cellular assemblies underlies the proper functioning of diverse basic physiological processes including motor activity, circadian rhythms, thermoregulation and breathing [1–5]. Determination of the mechanisms driving these processes is key not only for broadening our fundamental knowledge but also to formulate new therapeutic strategies.

No universal mechanism has been found that is utilized ubiquitously by excitable cells to generate rhythmic intrinsically-driven activity. Pacemaking can be achieved by different ion channel combinations being present at the cell membrane. Traditionally, the term "pacemaker current" refers to the currents produced by hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. While HCN channels play a prominent role in stabilizing rhythmic activity in sinoatrial node cells and thalamic neurons (reviewed in [6]), their ionic currents appear not to contribute majorly to other tonically firing neurons.

Interestingly, intrinsic cell autonomous pacemaking in neurons oftentimes coincides with subthreshold depolarizing potential changes (oscillations) that can be revealed in the presence of tetrodotoxin (TTX), which blocks AP firing. Such subthreshold TTX-resistant membrane potential oscillations have been identified in hypothalamic suprachiasmatic nucleus (SCN) neurons relevant for maintenance of diurnal rhythms [1,7], midbrain dopaminergic neurons [8], spontaneously active neurons in the locus coeruleus [9] and striatal interneurons [10]. In these examples, oscillations are usually slower in frequency compared to the superimposed tonic AP firing and dependent on calcium influx. However, the contribution of these oscillations to pacemaking is less clear and appears to be cell-type (and context) dependent.

Li and colleagues in their recent paper [11] identify subthreshold oscillations in chemoreceptor retrotrapezoid nucleus Phox2b-expressing (RTN^{Phox2b}) neurons that regulate respiratory homeostasis. The authors identify TRPM4 as a new component of the molecular machinery that drives these oscillations in RTN^{Phox2b} neurons. TRPM4 belongs to an extended family of TRP ion channels that are widely known to serve as sensors for a diverse spectrum of stimuli [12]; however, their involvement in subthreshold oscillations (and/or pacemaking) is not well established. Through elegant experiments, combining electrophysiology and pharmacology, Li and colleagues demonstrate that TRPM4 can play an important role in generating subthreshold membrane voltage oscillations that likely contribute to intrinsic excitability of RTN^{Phox2b} neurons.

Most TRP channels are non-specific cationic channels with high permeability to calcium; TRPM4 and TRPM5 are exceptional in this regard as these two channels are activated by intracellular calcium but themselves are only permeable to monovalent cations [12]. Consistent with this, experiments done by Li et al. demonstrate that subthreshold oscillations in RTN^{Phox2b} neurons can be suppressed by the application of TRPM4 antagonists; the oscillations are also absent in the presence of voltage-gated calcium (CaV) channel blockers. Mechanistically, and given its calcium-dependence, TRPM4 would be expected to act downstream of CaV channels in the molecular cascade (although a hierarchical relationship between the two channels has not formally been established). Nonetheless, both TRPM4 and CaVs are required for RTN^{Phox2b} neuron subthreshold oscillations that have a substantial impact on the membrane potential when AP firing is blocked by TTX.

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Additionally, the authors implicate the channel also in modulating induced AP firing of RTN^{Phox2b} neurons and demonstrate that low pH- and 5 H T-stimulated firing is inhibited by pharmacologically antagonizing TRPM4. If and how TRPM4-mediated subthreshold oscillations are causally linked to these induced firing rate changes or whether the two are separate phenomena altogether remains unresolved. The observation that the TRPM4 antagonist does not inhibit pacemaking in RTN^{Phox2b} neurons argues for the latter possibility and suggests that the two phenomena, subthreshold oscillations and tonic firing/pacemaking, are not directly linked. On the other hand, TRPM4 blockers, when injected into the RTN, indeed suppress respiratory frequency *in vivo*, consistent with the hypothesis that subthreshold membrane oscillations and pacemaking/tonic firing are linked.

How could the discrepancy between *ex vivo* and *in vivo* results be explained? It is possible that under native *in vivo* conditions, subthreshold membrane potential oscillations are of higher importance for tonic firing compared to *ex vivo* conditions. Alternatively, under *in vivo* conditions 5 H T, pH (or other factors) may exert subtle (tonic) stimulatory control over RTN neurons and these effects are diminished by blockade of TRPM4 (similar to the *ex vivo* pH/5 H T experiments shown by Li and colleagues). Yet another possible explanation is that even small changes in *in vivo* AP firing frequency of RTN^{Phox2b} neurons have a significant impact on respiratory system function. In this regard, it would be of interest to assess the AP firing frequency of these brainstem neurons *in vivo*, with and without TRPM4 antagonist administration.

Another factor that contributes to excitability in RTN^{Phox2b} neurons is a non-inactivating background sodium current [3] that has also been described before in the SCN [13] and other spontaneously active neurons. Such sodium "leak" current is most commonly ascribed to NALCN channels, but, theoretically, this role could also be fulfilled by TRP channels that are active near resting membrane potentials. In this regard it is interesting that Li and colleagues find other TRPC and TRPV channels expressed in RTN neurons. Additionally, persistent (TTX-sensitive) sodium currents carried by voltage-gated sodium (NaV) channels can also provide a depolarization bias, thereby driving pacemaking/tonic firing [14,15]. Taken together, multiple neuron subtypes that are tonically active without the requirement of synaptic input are characterized by both TTX-resistant membrane oscillations (mediated by CaV and potentially TRPM4/5 channels) and other background Na^+ currents, suggesting that these components may represent widely used (consensus-type) mechanisms that play important supportive roles in generating rhythmic activity (Fig. 1).

The idea that TRPM4 has a significant impact on neuronal excitability is supported by another recent report by Yan et al. [16]. There, the researchers demonstrate that in animal models of neurodegenerative diseases, TRPM4 channels bind to extracellular NMDA receptors thereby amplifying excitotoxic effects; application of a molecule that disrupted this interaction had a neuroprotective effect. This, together with the paper by Li and colleagues shows that TRPM4 has an important role in neurons, both in health and in disease.

Part of the difficulty in our understanding of the role of TRP channels in animal physiology is due to the fact that individual TRP channel knock-out mouse lines frequently fail to yield expected phenotypes, which has been attributed to redundant TRP channel functions and/or compensatory mechanisms. Indeed, Li and colleagues bring up the important point that TRPM4 knock-out animals do not display abnormalities in normal respiratory patterns, putting into question whether the channel is really required for this process. To this end, it would have been advantageous to verify the specificity of the TRPM4 antagonists on RTN^{Phox2b} neuron activity and breathing patterns by including TRPM4-KO mice in these pharmacological experiments similar to what has been by Hof and colleagues [17]. Interestingly, the latter study describes a subtle modulatory role of TRPM4 in pacemaking of the heart.

As studies of TRP channels continue, their versatility and contribution in regulating a multitude of fundamental cellular functions becomes evident. Li and colleagues suggest a role of a TRP channel in neuronal pacemaking. These findings surely are a strong stimulus to further look at TRPs as potential regulators of neuronal rhythmicity, both in normal and pathological conditions.

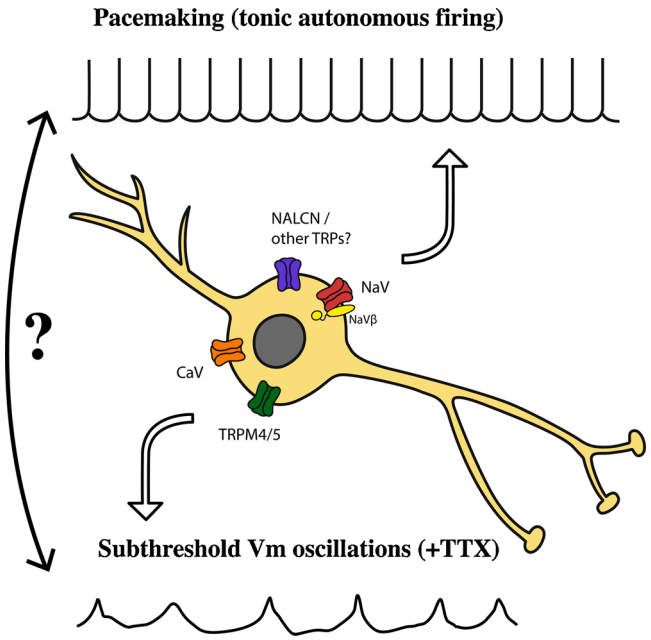

Fig. 1. Cartoon representing common molecular mechanisms utilized by tonically firing central neurons. On one hand, reaching AP threshold is facilitated by a tonic cationic current provided by NALCN; it is possible that TRP channels may also contribute to this current. Depending on the cell type, a depolarization bias promoting AP firing can also be generated by non-inactivating sodium currents (and/or resurgent currents) provided by certain NaV ion channels and their respective beta-subunits (NaVβ). On the other hand, multiple pacemaker neurons are characterized by spontaneous subthreshold membrane voltage oscillations which can further facilitate and modulate firing. These are usually dependent on CaV and potentially TRPM4/5 channels.

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