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PERSPECTIVE

Regulation of Orai channels by intracellular pH: one step closer to understanding CRAC channel biology?

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Store-operated Ca^{2+} entry (SOCE) is a unique mechanism in the intracellular Ca^{2+} signalling that is essential both in excitable and in non-excitable cells for refilling the intracellular Ca^{2+} stores. During receptor-evoked Ca^{2+} signalling, release of the endoplasmic reticulum (ER) Ca^{2+} stores via inositol 1,4,5-trisphosphate receptors is followed by the influx of extracellular Ca^{2+} via store-operated Ca^{2+} channels, also called Ca^{2+} release-activated channels (CRAC). SOCE is initiated by the ER Ca^{2+} sensor stromal interaction molecule 1 (Stim1), which changes conformation upon diffusion of the Ca^{2+} ions from the N-terminal EF hands, thus leading to multimerization and puncta formation of Stim1 at the sites of ER/plasma membrane (PM) contact (Liou *et al.* 2005). This triggers the assembly and activation of the PM Ca^{2+} channel Orai1 leading to extracellular Ca^{2+} influx (Prakriya *et al.* 2006). In general, SOCE maintains Ca^{2+} signalling by providing the necessary Ca^{2+} to refill the ER stores via the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pumps. In eukaryotic cells, three different Orai proteins are expressed (Orai1–3). Although all three Orai proteins are ubiquitously expressed, only the function of Orai1 has been widely studied; Orai2 and Orai3 are usually associated with Ca^{2+} influx in cancer cells. On the other hand, the different Orai proteins have different properties, such as the regulation of channel activity by reactive oxygen species (ROS) (Frisch *et al.* 2019). While Orai1 and 2 are blocked by ROS due to oxidation of the cysteine residue at amino acid position 195, the substitution of glycine at this position within Orai3 renders it ROS-insensitive.

In a previous issue of *The Journal of Physiology*, Rychkov *et al.* (2021) describe another remarkable difference between the pH_i regulation of the three Orai proteins (Fig. 1). In an earlier publication, the authors found that pH_i regulates the amplitude of the Orai1-mediated I_{CRAC} current and described the Ca^{2+}-dependent gating of Orai1 (Gavriliouk *et al.* 2017). The fast Ca^{2+}-dependent inactivation (FCDI) of Orai1 was abolished at pH_i 6.3, but the current amplitude was significantly smaller. Notably, they were able to measure I_{CRAC} with similar kinetics at physiological pH_i if the transfection level of Orai1 was increased, relative to Stim1 (Stim1:Orai1 at 1:1 instead of 1:4). Mutations of the negatively charged residues in the auto-inhibitory domain of Stim1 abolished the pH_i dependence of I_{CRAC} kinetics, whereas the pH_i dependence of the current amplitude was unaffected. In the current paper, the authors used whole-cell patch clamping to compare the pH_i dependence of Orai1-, Orai2-, and Orai3-mediated I_{CRAC} in transiently transfected Hek293T cells expressing Orai and Stim1 at different ratios. pH_i was manipulated with the application of sodium propionate and NH_4Cl in the bath solution, which changed the pH_i in a range of ~6.0–8.0. Using this experimental setup, they demonstrated that the pH_i-dependent regulation of the three Orai proteins are different from each other. Orai2 current amplitude was changed by pH_i similarly to Orai1, whereas FCDI remained unchanged resulting in the lack of Ca^{2+}-dependent re-activation at acidic pH_i. In contrast, Orai3 displayed no sensitivity to pH_i changes. Generating Orai1 clones with different point mutations and Orai1–Orai3

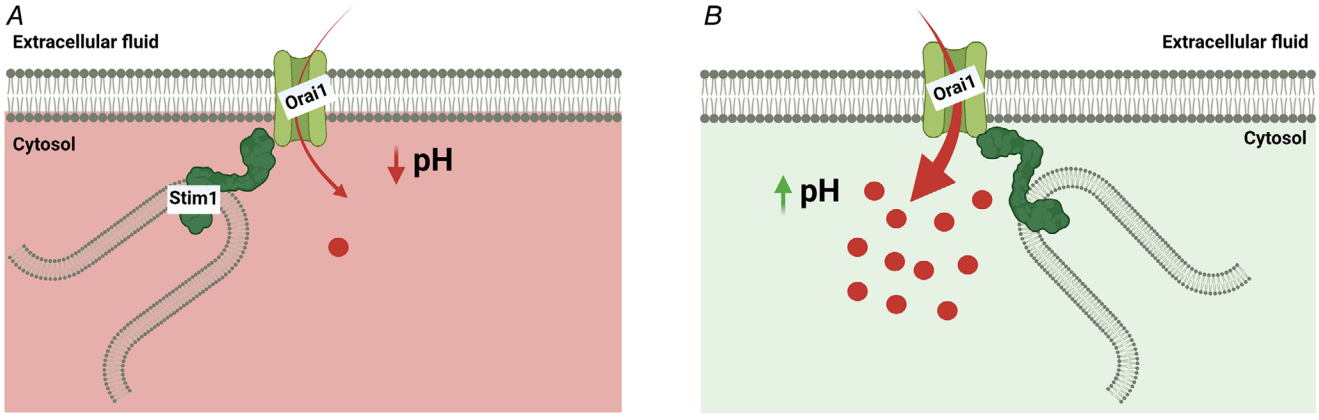

Figure 1. Regulation of Orai1 by intracellular pH
A, acidic intracellular pH decreases the Orai1-mediated Ca^{2+} current, resulting in lower extracellular Ca^{2+} influx. *B*, in contrast, alkaline intracellular pH enhances the Orai-mediated Ca^{2+} influx.

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chimeras suggested the interaction between intracellular loop 2 and N-terminus and their interaction with Stim1 play a critical role in Orai1 dependence on pH_i.

The physiological and pathophysiological aspects of these findings remain unknown. The proper regulation of neutral pH_i is crucial to cell function and survival. However, future studies will be needed to determine whether regulation of Orai-mediated I_{CRAC} by pH_i is part of a regulatory mechanism or not. Acidic pH, especially pH 6.0 used in these studies, is usually associated with pathological events, such as anoxia, disturbed mitochondrial function or the presence of toxins. Thus, decreasing the Orai1-mediated extracellular Ca^{2+} influx may protect the cells from further damage or cell death. On the other hand, cancer cells display neutral to relatively alkaline pH_i, and therefore the increased extracellular Ca^{2+} influx via Orai1 may promote tumour progression by increasing the rate of cell proliferation and metastasis. This raises the possibility that inhibition of Orai1 may be a potential therapeutic strategy in cancer treatment, an idea that will require evaluation in different *in vitro* and *in vivo* disease models. The physiological or pathological impact of the different behaviours of Orai proteins at different pH_i also remains elusive and should be investigated further in the future.

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Additional information

Competing interests

The author has no conflict of interest to declare. CalciMedica supplied CM5480 for the study.

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Sole author.

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Keywords

Ca^{2+} signalling, intracellular pH, Orai1, store operated Ca^{2+} entry

Supporting information

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