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RESEARCH NEWS

Reconstituting depolarization-induced calcium release

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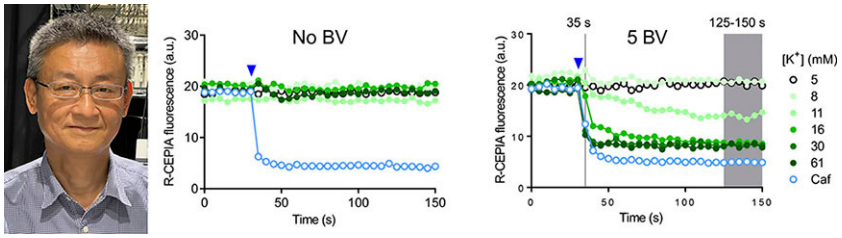
Researchers develop experimental platform that could be used to evaluate mutations and screen drugs for skeletal muscle diseases.

Skeletal muscle contraction is initiated by the release of Ca^{2+} from the sarcoplasmic reticulum upon depolarization of the muscle cell plasma membrane. This depolarization-induced Ca^{2+} release (DICR) is mediated by the type I ryanodine receptor (RyR1), a Ca^{2+} release channel in the sarcoplasmic reticulum that is activated by the voltage-sensitive Cav1.1 subunit of the dihydropyridine receptor. A rapidly expanding number of mutations in the genes encoding the DICR machinery have been linked to skeletal muscle diseases such as congenital myopathy and malignant hyperthermia. But evaluation of these mutations, and the development of drugs to correct defects in DICR, has been hampered by the difficulties associated with culturing and manipulating skeletal muscle cells. In this issue of *JGP*, however, Murayama et al. reconstitute DICR in human embryonic kidney cells, providing a platform to validate mutations and screen drugs in a multiwell format (1).

Working with skeletal muscle cells can be expensive and time consuming but, in 2017, Perni et al. (2) managed to reconstitute DICR in non-muscle cells by patch-clamping tsA201 cells recombinantly expressing RyR1, Cav1.1, and three other proteins required for DICR (the β1a subunit of the dihydropyridine receptor, the adaptor protein Stac3, and the membrane-tethering protein junctophilin-2). "Inspired by their work, we started this study to improve the platform so that it can be applied to multiwell plates,"



Takashi Murayama and colleagues reconstitute DICR in human embryonic kidney cells by using baculovirus to transduce the cells with multiple components of the DICR machinery. Chemical depolarization with high $[K^+]$ solutions elicits no change in ER Ca^{2+} levels in control cells (left) but induces Ca^{2+} release in baculovirus-infected cells (right). The novel platform can be used in multiwell formats and is therefore suitable for validating mutations and drug screening for skeletal muscle diseases.

says Takashi Murayama of the Juntendo University School of Medicine in Tokyo.

Murayama and colleagues had previously generated a human embryonic kidney cell line stably expressing both RyR1 and a fluorescent ER Ca^{2+} reporter called R-CEPIA1er (3). To reconstitute DICR in these cells, Murayama et al. infected them with baculoviruses carrying the genes encoding Cav1.1, β1a, Stac3, and junctophilin-2, achieving widespread expression of the DICR machinery. The researchers also used baculovirus to introduce the inward-rectifying potassium channel Kir2.1, hyperpolarizing the cells so that RyR1 is inactive under resting conditions.

"All the cells can then be simultaneously stimulated by chemical depolarization with a high $[K^+]$ solution, which provides signals high enough to be measured using a microplate reader," Murayama explains. Crucially, the R-CEPIA1er reporter monitors the depolarization-induced loss of Ca^{2+} from the ER, rather than the depolarization-induced increase in cytoplasmic Ca^{2+}, avoiding potential signal contamination arising from the influx of extracellular Ca^{2+}.

After establishing that the platform faithfully reconstitutes DICR, Murayama et al. used it to evaluate several disease-causing mutations in Cav1.1. The malignant hyperthermia-associated mutation R1086H, for example, shifted the voltage-dependence of DICR in kidney cells to more negative potentials, consistent with previous observations in myotubes (4). This suggests that the platform can be used to validate other, novel mutations in DICR components.

Murayama and colleagues also tested several DICR-modulating drugs in their platform, confirming that the RyR1 inhibitors dantrolene and Cpd1 impede

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DICR while the twitch potentiator perchlorate sensitizes the modified kidney cells to DICR.

The researchers hope to further improve the platform by introducing additional proteins that, though not essential for DICR, are known to regulate its activity. Then, they hope to use the technology to validate novel disease-linked mutations and screen for drugs that modulate DICR. "Since the procedure is simple and reproducible, we hope that the reconstituted DICR platform will be widely used for the diagnosis and treatment of muscle diseases," Murayama says.

References

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