JGP Journal of General Physiology

RESEARCH NEWS

It's time to look more closely at RYR3

Caitlin Sedwick

***JGP* study shows that ryanodine receptor 3 is important for extraocular muscle function.**

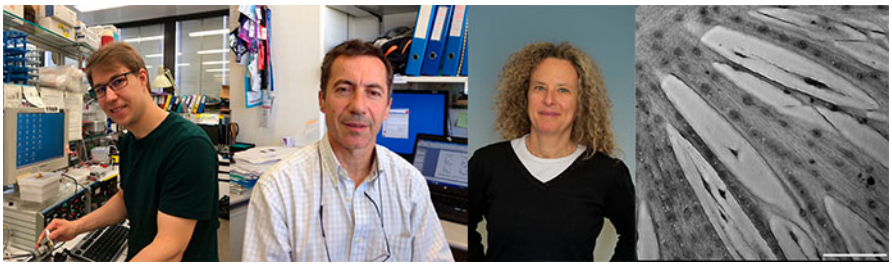
Ryanodine receptors (RYRs) are calcium (Ca^{2+}) channels that reside on the endoplasmic reticulum (ER). Type 1 and type 2 RYRs (in skeletal and cardiac muscle, respectively) release Ca^{2+} from the ER in response to excitation of muscle membranes to promote muscle contraction. Type 3 RYR (RYR3) is ubiquitously expressed but is less well studied. A new *JGP* paper by Eckhardt et al. sheds more light on this mysterious protein (1).

"Nobody really knows what RYR3 does," explains Dr. Susan Treves, senior author on the paper. "It seems to increase in expression in developing muscle cells, called myotubes, but in mature skeletal muscle its level of expression is very low."

Accordingly, RYR3 knockout mice don't show any strong phenotype changes in mature muscle (2, 3). RYR3-deficient mice are slower than wild-type mice at completing a behavioral task called the water maze, but this was attributed to a neuronal deficit because RYR3 is strongly expressed in neurons (4). For this reason, neither Treves nor co-senior author Francesco Zorzato was initially focused on RYR3. That is, until Maya Sekulic-Jablanovic and Jan Eckhardt, PhD students in Treves and Zozato's laboratory in Basel, Switzerland, began studying how some congenital myopathies affect the muscles that surround the eye (called extraocular muscles, or EOM). They observed strong RYR3 expression in human and murine EOM (5).

"RYR3 is such a large protein that if you don't need it you wouldn't usually be expressing it, so we thought it might be important in eye muscles," says Treves. Graduate student Jan Eckhardt headed the group's efforts to test this hypothesis.

EOM control the ability of the eye to focus, so functional defects in these muscles may result in poor vision. The authors therefore investigated the vision of RYR3-deficient mice using two behavioral tests: the water maze and the optokinetic reflex test. In both tests, the mice behaved as if they had trouble tracking their surroundings, but their problems could stem from either poor EOM function or from neuronal or retinal defects. To determine whether RYR3-deficient mice have impaired EOM function, the researchers needed to examine the extraocular muscle fibers themselves—a challenging task because the eye muscles are complex and tiny.



Left to right: Jan Eckhardt, Francesco Zorzato, Susan Treves, and colleagues (not pictured) demonstrate that RYR3 is involved in force production and calcium handling in extraocular muscle (see micrograph).

After perfecting methods to isolate EOM fibers, Eckhardt et al. found that RYR3-deficient EOM produce much less force than their wild-type counterparts, and do so at a slower velocity. These flaws were not due to muscle atrophy or large-scale morphological changes.

"We also excluded a change in expression of excitation–contraction coupling proteins," says Treves. "There could have been downregulation of RYR1 in EOM, for example, but all the coupling proteins except for parvalbumin were the same in control and knockout mice."

RYR3-deficient muscle fibers did, however, show differences in how they handle Ca^{2+}.

"The peak Ca^{2+} level during muscle contraction was the same in the wild type and the knockout, but the knockout's half-relaxation time was much slower," notes Treves.

Prior studies in amphibians and birds showed that RYR3 is involved in Ca^{2+} sparks—rapid, tiny releases of Ca^{2+} from the ER that help regulate cytoplasmic Ca^{2+} levels. Surprisingly, Eckhardt et al. found no evidence for this in single isolated fibers from mouse EOM. Instead, they observed that RYR3 was needed for rapid Ca^{2+} oscillations that take place in EOM-derived myotubes. That's interesting because EOM are under constant use, and the abundant myotubes are constantly fusing into adult extraocular muscles.

"Our conclusion was that RYR3 is involved in something like Ca^{2+}-induced Ca^{2+} release to amplify the Ca^{2+} signal in myotubes of EOM," explains Treves. She suspects this may also be the case in adult EOM fibers. "It just might be so fast that we don't have the systems to see it, and we're missing it."

1. Eckhardt, J., et al. 2019. *J. Gen. Physiol.* https://doi.org/10.1085/jgp.201912333
2. Bertocchini, F., et al 1997. *EMBO J.* 16:6956–6963.
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4. Balschun, D., et al 1999. *EMBO J.* 18:5264–5273.
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sedwick@gmail.com.