

 LETTER

Maurocalcin phosphorylated at threonine 26 maintains its activity on ryanodine receptor-mediated Ca^{2+} release in intact muscle fibers

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Ronjat et al. (1) report that a phosphorylated form of the scorpion venom toxin maurocalcin (MCA) loses its ability to activate its preferred target, the type 1 ryanodine receptor (RYR1). RYR1 is the sarcoplasmic reticulum (SR) Ca^{2+} release channel responsible for the rise in cytosolic Ca^{2+} , which activates contraction during excitation–contraction coupling of skeletal muscle. Ronjat et al. report that phosphorylation of MCA threonine 26 (MCAThr²⁶) completely reprograms the toxin, converting it into a RYR1 blocker. To assess the function of MCA and of MCAThr²⁶-Phospho, the authors relied on several *in vitro* assays, including measurements of current through RYR1 channels reconstituted into lipid bilayer and [³H]-ryanodine binding and Ca^{2+} release from SR vesicles. However, Ronjat et al. did not obtain data from intact muscle fibers where RYR1 functions in its native physiological environment, being activated by its molecular partner, the voltage-sensing protein CAV1.1 in the plasma membrane. This is unfortunate because MCA has a unique effect on RYR1-mediated SR Ca^{2+} release in intact muscle fibers, making this assay both reliable and physiologically relevant: indeed, under these conditions, MCA transiently keeps RYR1 channels from closing following membrane repolarization. This process manifests in a remarkable prolongation of the voltage-activated cytosolic Ca^{2+} transient (2, 3) after the end of voltage activation.

We had the chance to test the effect of MCAThr²⁶-Phospho, together with MCAThr²⁶-E, where threonine is replaced with a glutamic acid residue, in intact muscle fibers. We believe that the results are interesting in regard to the issue raised. Fig. 1A shows examples of voltage-clamp activated cytosolic Ca^{2+} transients in muscle fibers equilibrated with either MCA (10 μM) or MCAThr²⁶-Phospho (10 μM) or MCAThr²⁶-E (100 μM) in

the voltage-clamp pipette. Experiments were conducted under conditions routinely used in our laboratory (4). For each fiber, we show the change in rhod-2 fluorescence in response to a 10- and 100-ms-long pulse, from -80 to $+20$ mV. As reported previously (2), the effect of MCA is use-dependent (i.e., the longer the depolarization, the larger the effect). Thus, the 100-ms pulse in the presence of MCA generates a prominent, sustained elevation of cytosolic Ca^{2+} after the pulse. The exact same effect was observed with MCAThr²⁶-Phospho but not with MCAThr²⁶-E. Fig. 1B shows mean values for the peak (Fig. 1B, *Upper*) and for the end of record (~ 11 s following repolarization) (Fig. 1B, *Lower*) Ca^{2+} levels, in the different conditions. There was no effect of MCA or of either derivative on the peak; however, Ca^{2+} levels remained elevated after repolarization in a use-dependent manner for both MCA and MCAThr²⁶-Phospho.

Altogether, we believe that the above results are an important complement to the data presented by Ronjat et al. (1) and there are reasons worth discussing as to why MCAThr²⁶-Phospho becomes inefficient in *in vitro* assays but remains active in intact muscle fibers. One possibility could be that high phosphatase activity inside the muscle fibers so completely dephosphorylates intracellular MCAThr²⁶-Phospho as to release active MCA. If so, one may reasonably doubt that intracellular PKA activity would have the potential to inactivate native MCA in the fibers.

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