Critical Amino Acid Residues Determine the Binding Affinity and the Ca\(^{2+}\) Release Efficacy of Maurocalcine in Skeletal Muscle Cells*

Maurocalcine (MCa) is a 33 amino acid residue peptide toxin isolated from the scorpion Scorpio maurus palmatus. MCa and mutated analogues were chemically synthesized, and their interaction with the skeletal muscle ryanodine receptor (RyR1) was studied on purified RyR1, sarcoplasmic reticulum (SR) vesicles, and cultured myotubes. MCa strongly potentiates \(^{3}H\)ryanodine binding on SR vesicles (7-fold at pCa 5) with an apparent EC\(_{50}\) of 12 nM. MCa decreases the sensitivity of \(^{3}H\)ryanodine binding to inhibitory high Ca\(^{2+}\) concentrations and increases it to the stimulatory low Ca\(^{2+}\) concentrations. In the presence of MCa, purified RyR1 channels show long-lasting openings characterized by a conductance equivalent to 60% of the full conductance. This effect correlates with a global increase in Ca\(^{2+}\) efflux as demonstrated by MCa effects on Ca\(^{2+}\) release from SR vesicles. In addition, we show for the first time that external application of MCa to cultured myotubes produces a cytosolic Ca\(^{2+}\) increase due to Ca\(^{2+}\) release from 4-chloro-m-cresol-sensitve intracellular stores.

Using various MCa mutants, we identified a critical role of Arg\(^{-24}\) for MCa binding onto RyR1. All of the other MCa mutants are still able to modify \(^{3}H\)ryanodine binding although with a decreased EC\(_{50}\) and a lower stimulation efficacy. All of the active mutants produce both the appearance of a subconductance state and Ca\(^{2+}\) release from SR vesicles. Overall, these data identify some amino acid residues of MCa that support the effect of this toxin on ryanodine binding, RyR1 biophysical properties, and Ca\(^{2+}\) release from SR.

In skeletal muscles, contraction is triggered by the massive release of Ca\(^{2+}\) from sarcoplasmic reticulum (SR). The channel responsible for this release is the type-1 ryanodine receptor (RyR1). RyR1 has been intensively studied because of its unique structural and functional organization. It forms part of a calcium mobilization complex in which RyR1 is apoposed to the l-type voltage-dependent calcium channel (dihydropyridine receptor, DHPR) along many other structural and regulatory components (1). The activation of RyR1 requires a chain of events that starts with plasma membrane depolarization inducing a change in the conformation of DHPR itself transmitted to RyR1. The entire set of events is called excitation-contraction coupling (EC coupling).

In vitro, the activity of RyR1 can be modulated by a number of different effectors such as Ca\(^{2+}\), ryanodine, ATP, caffeine, and 4-chloro-m-cresol (CMC) (2, 3). Among these effectors, only few present high selectivity and affinity for RyR1. More specific pharmacological agents for RyR1 have been discovered in scorpion venoms (4, 5). One such peptide has been isolated from the venom of the chactid scorpion Scorpio maurus palmatus and has been termed maurocalcine (MCa). It is a 33-mer basic peptide reticulated by three disulfide bridges. MCa can be chemically synthesized without any loss of activity (5). It is potently active on RyR1 as it alters channel properties in the low nanomolar range (5, 6). MCa presents a strong sequence homology (82% amino acid sequence identity) with imperatorin A (IpTxA), a toxin active on RyR1 and isolated from another scorpion venom (7–10). Besides the fact that MCa and IpTxA represent two of the most high affinity effectors of RyR1, they also share some amino acid sequence homology with a specific domain (domain A) of the II–III loop of Cav\(_{1.1}\), the subunit that carries the voltage sensor of DHPR (Fig. 1). Although the exact role of the domain A in the EC-coupling process is highly debated (11–13), we have recently shown by using plasmon resonance measurements that it is the single II–III loop sequence interacting with RyR1 (14). Nevertheless, this exceptional form of homology between a channel sequence and a toxin could be indicative that domain A possesses some kind of yet unresolved function in RyR1 regulation. Therefore, studying the MCa effects on RyR1 may produce several interesting hints on how to proceed further on investigating the role of domain A in EC coupling.

In this work, we synthesized several MCa analogues in which amino acid residues, also present in homologous position in domain A, were substituted by alanine residues. We then analyzed the effect of MCa and the analogues on \(^{3}H\)ryanodine binding on RyR1. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
binding, Ca\(^{2+}\)-dependent activation of RyR1, channel activity, and Ca\(^{2+}\) release from SR vesicles. We identified an amino acid residue critical for the interaction of MCa with RyR1 and for the induction of a long-lasting subconduction state. The data also demonstrate a clear relationship in the ability of MCa to potentiate \(^3\)Hryanodine binding, induce a subconduction state, and produce Ca\(^{2+}\) release from heavy SR vesicles. To provide a physiological relevance of RyR1 as the major target of MCa, we tested the effect of the peptide on internal Ca\(^{2+}\) release in intact myotubes. We show that the addition of 100 nM MCa in Ca\(^{2+}\)-free extracellular medium induces an increase in cytosolic Ca\(^{2+}\) concentration and a complete inhibition of CMI-induced Ca\(^{2+}\) release.

**EXPERIMENTAL PROCEDURES**

**Chemical Synthesis**—N-α-Fmoc-t-amino acids, 4-hydroxymethylphenylalanine, and reagents used for peptide synthesis were obtained from PerkinElmer Life Sciences. The MCa and analogues were obtained by the solid-phase peptide synthesis (15) using an automated peptide synthesizer (Model 433A, Applied Biosystems Inc.). Analogues were obtained by point mutation (Aila instead of one amino acid in the sequence of macrolactine native-like) and named Aila/Lys\(^\text{a}\), Aila/Lys\(^\text{b}\), Aila/Lys\(^\text{c}\), Aila/Lys\(^\text{d}\), Aila/Lys\(^\text{e}\), Aila/Lys\(^\text{f}\), Aila/Lys\(^\text{g}\), Aila/Lys\(^\text{h}\), Aila/Lys\(^\text{i}\), Aila/Lys\(^\text{j}\), Aila/Lys\(^\text{k}\), and Aila/Thr\(^\text{a}\). Peptide chains were assembled stepwise on \(0.25\) meq of hydroxymethylphenylalanine resin (1% cross-linked; 0.89 meq of amino group) using 1 mmol of \(N\)-Fmoc amino acid derivatives. The side chain-protecting groups were as follows: trityl for Cys and Asn; tert-butylyl for Ser, Thr, Glu, and Asp; pentamethylylcarbonyl for Arg; and tert-butylxoyacarbonyl for Lys. N-α-Amino groups were deprotected by treatment with 18 and 20% (v/v) piperidine/N-methylpyrrolidone for 3 and 8 min, respectively. The Fmoc-amino acid derivatives were coupled (20 min) as their hydroxybenzotriazole active esters in N-methylpyrrolidone (4-fold excess). After peptide chain assembly, the peptide resin (approximately 1.8 mg) was treated between 2 and 3 h at room temperature in constant shaking with a mixture of trifluoroacetic acid/H\(_2\)O/thionisoothantane/ethanedithiol (89: 5/5/2, v/v) in the presence of crystaline phenol (2.25 g). The peptide mixture was then filtered, and the filtrate was precipitated by adding 5/5/2, v/v) in the presence of crystalline phenol (2.25 g). The peptide mixture was then filtered, and the filtrate was precipitated by adding 5/5/2, v/v) in the presence of crystalline phenol (2.25 g). The peptide mixture was then filtered, and the filtrate was precipitated by adding

**Fluorescence Measurements**—Cytosolic Ca\(^{2+}\) release from SR vesicles was measured using the Ca\(^{2+}\)-sensitive dye, antipyrylazo III. The absorbance was monitored at 710 nm by a diode array spectrophotometer (MOS-200 Optical System, Biologic, Claix, France). Heavy SR vesicles (50 μg) were actively loaded with Ca\(^{2+}\) at 37°C in a 2-ml buffer containing 100 mM KCl, 7.5 mM sodium phosphopyruvate, 20 mM potassium MOPS, pH 7.0, supplemented with 250 μM ATP/MgCl\(_2\), 5 mM phosphocreatine, and 12 μM creatine phosphokinase. Ca\(^{2+}\) loading was started by sequential additions of 50 and 20 μM of CaCl\(_2\). These loading conditions, no calcium-induced calcium release interferes with the observations. After the end of each experiment, Ca\(^{2+}\) remaining in the vesicles was determined by the addition of Ca\(^{2+}\) ionophore A23187 (4 μM) and the absorption signal calibrated by two consecutive additions of 20 μM CaCl\(_2\).

**RESULTS**

**Synthesis of MCa and Analogues**—Fig. 1 presents the primary structure of the different MCa analogues that we synthesized. The structural determination of MCa shows that the stretch of basic residues (from Lys\(^\text{a}\) to Arg\(^\text{f}\)) forms a single basic-rich surface (22). We will define the residues forming this basic surface as residues belonging to the “basic class.” The opposite surface of the toxin contains four acidic residues (Asp\(^\text{a}\), Asp\(^\text{b}\), Asp\(^\text{c}\), Asp\(^\text{d}\)).
Glu$^{12}$, Asp$^{15}$, and Glu$^{29}$) and one basic residue (Lys$^8$). We will define this acidic surface as the “acidic face.” This electrostatic charge distribution creates a marked anisotropy in which the role in Mca pharmacology is unknown. We choose to substitute one by one all of the Mca amino acid residues common between MCa and the domain A of the II–III loop of Ca,α$_{1,1}$ subunit. Most of these residues belong to the basic class with the exception of Thr$^{26}$ and therefore were replaced by neutral alanine residues. We also synthesized the mutant [Ala$^8$]Mca because Lys$^8$ is not conserved in the II–III loop but is located on the toxin face opposite to the basic surface (23). All of the peptides were folded/oxidized in a 200 mM Tris-HCl buffer, pH 8.3, by a 72-h exposure to air and purified to homogeneity by preparative C$_{18}$ reversed-phase high pressure liquid chromatography. Both analysis of elution profiles and circular dichroism spectra indicate that all of the peptides have proper secondary structures (data not shown).

Effects of Mca on $[^3]$Hryanodine Binding to RyR1—We recently described that Mca stimulates the binding of $[^3]$Hryanodine onto heavy SR vesicles that contain RyR1 (6). Fig. 2A confirms this initial finding as we observe that 200 nM Mca increases the binding of $[^3]$Hryanodine by 7.4-fold with an EC$_{50}$ of 199 nM. These data show that Mca has sequence similarities with domain A of the II–III loop of Ca,α$_{1,1}$ subunit. MCa has sequence similarities with domain A of the II–III loop from the Ca,α$_{1,1}$, subunit of DHPR and with IpTxA, another scorpion toxin. The homology stretches from Lys$^{19}$ to Thr$^{26}$ of Mca. The primary structure of the mutated Mca analogues is also shown.

Characterization of the Effects of Mca Mutants on $[^3]$Hryanodine Binding to RyR1—All of the Mca analogues also stimulate $[^3]$Hryanodine binding (Fig. 3A) with the exception of [Ala$^{23}$]Mca (up to 5 μM). For all of the functional analogues, the EC$_{50}$ of the stimulatory effect is reduced with values ranging between 25 ± 4 nM for [Ala$^{3}$]Mca and 432 ± 168 nM for [Ala$^{23}$]Mca, which would correspond to a reduction of affinity between 4- and 35-fold. [Ala$^{3}$]Mca also appeared less effective than Mca with an EC$_{50}$ of 199 ± 19 nM. These data show that the amino acid residues of Mca can be classified in three different groups: (i) residues belonging to the basic class that are important for interaction with RyR1 (Lys$^{22}$, Arg$^{23}$, and Arg$^{29}$); (ii) residues belonging or not to the basic class that appear less important for the interaction with RyR1 (Lys$^{25}$, Lys$^{29}$, and Thr$^{28}$); and (iii) a residue of the acidic face that appears to mildly affect the interaction with RyR1 (Lys$^{8}$). Besides various effects on EC$_{50}$, all of the active analogues also present a significant decrease on the stimulation efficacy on $[^3]$Hryanodine binding. The stimulation efficacies of the analogues are on average 1.9-fold less than Mca itself and ranged between 3.2 ± 0.4-fold for [Ala$^{23}$]Mca and 4.7 ± 0.1-fold for [Ala$^{3}$]Mca. [Ala$^{23}$]Mca combines two negative changes com-
pared with wild-type MCa, a decrease in the apparent affinity and the lowest [3H]ryanodine binding stimulation efficacy. Two explanations can be put forward to explain why [Ala24]MCa is ineffective on [3H]ryanodine binding. Firstly, it may not bind to RyR1, which is the simplest hypothesis. Secondly, it may bind onto RyR1 but cannot produce allosteric changes required to enhance [3H]ryanodine binding. In this latest case, [Ala24]MCa may not bind to RyR1 and cannot alter the binding affinity of [3H]ryanodine, suggesting that this analogue is not able to bind onto RyR1. We also checked whether or not the absence of the effect of [Ala24]MCa on [3H]ryanodine binding is related to experimental pCa conditions. Indeed, no [3H]ryanodine binding stimulation was observed at pCa values ranging between 2 and 7 (Fig. 3C).

Characterization of the Effects of MCa and MCa Mutants on Ca2+ Release from SR Vesicles—To investigate the effect of MCa on Ca2+ release from heavy SR vesicles, we first actively loaded the vesicles by two consecutive additions of Ca2+ (50 μM and 20 μM) in the presence of ATP-Mg2+, pyrophosphate, and ATP-regenerating system. After Ca2+ loading reaches equilibrium, the addition of 1 μM MCa to the external medium induces Ca2+ release as shown in Fig. 4A. Similar results were obtained with 100 nM MCa (data not shown), but 1 μM MCa was chosen to allow a comparison with the effects of the lower affinity MCa analogues. External Ca2+ level reaches a plateau that corresponds to a new equilibrium between Ca2+ release and Ca2+ uptake rates as evidenced by the additional change produced by 4 μM A23187 calcium ionophore. The release of Ca2+ induced by 1 μM MCa was prevented by preincubating the SR vesicles with 10 μM ruthenium red, demonstrating that MCa-induced calcium release occurs through RyR (Fig. 4B). Similarly, the application of 1 μM MCa prevents the release of Ca2+ induced by the addition of 500 μM CMC, and conversely, the application of 500 μM CMC prevents an additional release of Ca2+ by 1 μM MCa (Fig. 4C). These data again demonstrate that MCa re-
leases Ca\(^{2+}\) from SR vesicles by acting on RyR. Fig. 4D shows that the application of 1 \(\mu\)M of any one of the mutated MCa analogues induces Ca\(^{2+}\) release from SR vesicles with the exception of [Ala\(^{23}\)]MCa and [Ala\(^{24}\)]MCa. However, these two last MCa analogues are not equivalent since application of 10 \(\mu\)M [Ala\(^{23}\)]MCa resulted in Ca\(^{2+}\) release, whereas 10 \(\mu\)M [Ala\(^{24}\)]MCa remained inactive (Fig. 4E). The present observation made for [Ala\(^{24}\)]MCa is in complete agreement with its lack of effect on \([\text{H}]\)ryanodine binding (Fig. 3B). The results obtained with [Ala\(^{23}\)]MCa are also coherent with the data on \([\text{H}]\)ryanodine binding (lower affinity and lower stimulation efficacy), although a discrepancy exists between the [Ala\(^{23}\)]MCa concentration needed for Ca\(^{2+}\) release and those inducing an increase in \([\text{H}]\)ryanodine binding.

Modification of RyR1 Calcium Channel Conductance by MCa and Analogues—Application of 200 nM MCa to the cis face of the bilayer-recording chamber (cytoplasmic side of RyR1) induces a characteristic long-lasting subconductance state (LLSS) (Fig. 5A). This subconductance state represents 60% of the full conductance state, and the channel spends 54.8 ± 6.2% of its time in this subconductance level. A much less frequent smaller LLSS (48% of the full conductance) was described in earlier reports (5, 6), but the experimental conditions were slightly different (purified RyR1 versus junctional SR vesicles and K\(^{+}\) current versus Cs\(^{+}\) current in previous reports (5, 6)).

Next, we examined whether the LLSS induced by MCa could be correlated to Ca\(^{2+}\) release from heavy SR vesicles and stimulation of \([\text{H}]\)ryanodine binding. Therefore, we tested the effect of [Ala\(^{24}\)]MCa on the conductance level of RyR1 in lipid bilayers (Fig. 5B). Indeed, this mutant peptide has no effect on \([\text{H}]\)ryanodine binding (Fig. 3A) nor on the Ca\(^{2+}\) release from SR vesicles (Fig. 4D). As expected, we show that the application of 200 nM [Ala\(^{24}\)]MCa does not induce any LLSS of RyR1. Applications of higher concentrations (up to 750 nM) also were without any effect (data not shown). Conversely, the application of 200 nM of an active MCa analogue, [Ala\(^{20}\)]MCa, that conserves MCa-like properties (for \([\text{H}]\)ryanodine binding and Ca\(^{2+}\) release from SR vesicles) produces the appearance of the charac-

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**Fig. 3. Effect of MCa analogues on \([\text{H}]\)ryanodine binding to heavy SR vesicles.** A, \([\text{H}]\)ryanodine binding was measured as described in Fig. 2A in the presence of various concentrations of each MCa analogue (open circles). For comparison, the solid line represents the fit of the data shown in Fig. 2A for MCa. The EC\(_{50}\) values for each analogue were calculated as in Fig. 2A and reported in insert. B, effect of varying [Ala\(^{24}\)]MCa concentration on \([\text{H}]\)ryanodine binding measured in the absence (filled circles) and presence of 20 (gray circles) and 100 nM MCa (open circles). C, extravesicular Ca\(^{2+}\) concentration dependence of \([\text{H}]\)ryanodine binding in the absence (filled triangles) and presence of 300 nM [Ala\(^{24}\)]MCa (gray triangles). The experiments were performed with 5 nM [\text{H}]ryanodine for 2.5 h at 37 °C.
Fig. 4. Ca\(^{2+}\) release from heavy SR vesicles induced by MCA and MCA analogues. A, heavy SR vesicles were actively loaded with Ca\(^{2+}\) by sequential addition of 50 and 20 µM CaCl\(_2\) in the monitoring chamber. After each increment, the absorbance was monitored until the added Ca\(^{2+}\) was taken up by SR and the trace relaxed close to its original base line (CaCl\(_2\) additions constituting ~70–80% of their loading capacity). In these conditions, the addition of 1 µM MCA produces Ca\(^{2+}\) release. The addition of 4 µM A23187 empties the SR vesicles (open arrow). Calibration of Ca\(^{2+}\) release is performed by two consecutive additions of 20 µM Ca\(^{2+}\) (data not shown). B, similar experiment as in A but with a preliminary application of 10 µM ruthenium red (RR). RR produces a deflection of absorbance due to its optical properties. C, traces illustrating the lack of effect of 500 µM CMC after MCA application (left) and, conversely, the lack of effect of 1 µM MCA after application of 500 µM CMC (right). D, similar experiments as in A but with 1 µM of various MCA analogues. Two consecutive applications were performed for [Ala\(^{23}\)]MCA. E, traces illustrating the maintenance of effect of MCA on Ca\(^{2+}\) release despite the prior application of [Ala\(^{23}\)]MCA (left) or [Ala\(^{23}\)]MCA (right).
FIG. 5. MCa but not [Ala²⁴]MCA induces long-lasting openings of RyR1. Single channel recordings were taken as described under “Experimental Procedures.” The holding potential was −80 mV, the conductance of the RyR was approximately 500 pS. The time duration of each record is 20 s. Channel openings are downward deflections, and C and O designate the closed and opened state of the channel, respectively. Each toxin was applied at a concentration of 200 nM in the cis chamber. Free Ca²⁺ concentration was 238 nM in the cis chamber. A, control and MCa-induced RyR1 channel activity. Left panel, representative current traces. Right panel, current amplitude histograms. B, as in A but for [Ala²⁴]MCA. C, as in A but for [Ala²⁰]MCA.
teristic LLSS. With this analogue, the LLSS are characterized by a conductance corresponding to 54% of the full-conductance state. Under this condition, the channel spends on average 28 ± 8.6% of its time in this state (data not shown). The lesser probability for RyR1 to develop in the LLSS in the presence of transmitted light image (differential interference contrast).

Intracellular Ca\textsuperscript{2+} Release in Intact Myotubes Induced by MCa—We next analyzed the effect of MCa on cytosolic Ca\textsuperscript{2+} variations by changes in Fluo-4 fluorescence levels in cultured myotubes. Extracellular application of 100 nM MCa on a group of representative myotubes produces a fast increase in intracellular Ca\textsuperscript{2+} level (Fig. 6A). The time course of the change in fluorescence level (\(\Delta F/F\)) shows that Ca\textsuperscript{2+} increase occurs within 3 s followed by a rapid decrease back to the basal level (Fig. 6B). MCa produces a similar elevation in cytosolic Ca\textsuperscript{2+} when extracellular medium is supplemented with 50 \(\mu\)M La\textsuperscript{3+} and deprived of Ca\textsuperscript{2+} by the addition of 1 mM EGTA (Fig. 6B). This observation demonstrates that MCa-induced Ca\textsuperscript{2+} mobilization occurs from internal sources. An analysis of the average change in fluorescence level confirms that there is no significant difference in cytosolic Ca\textsuperscript{2+} elevation in the absence or presence of extracellular Ca\textsuperscript{2+} (Fig. 6C).

DISCUSSION

In skeletal muscles, RyR1 appears to represent the main target of MCa. We have previously shown that this toxin stimulates \(^{3}H\)ryanodine binding, stabilizes RyR1 into a subconductance state, and produces Ca\textsuperscript{2+} release from heavy SR vesicles. In this work, we further investigated the biochemical and functional properties of MCa. Using synthetic mutated analogues of MCa, we defined some critical amino acid residues of MCa that are required for its effects on RyR1. We also show for the first time that MCa is able to induce intracellular Ca\textsuperscript{2+} release in intact myotubes. This effect is conserved in the absence of external Ca\textsuperscript{2+}, demonstrating that the increase in...
cytoplasmic Ca\(^{2+}\) concentration is due to the release of Ca\(^{2+}\) from internal stores. In these conditions, Ca\(^{2+}\) release induced by CMC, a RyR1 agonist, is completely inhibited by the preapplication of 1 \(\mu\)M [Ala\(^{24}\)]MCa. Conversely, the application of the inactive [Ala\(^{24}\)]MCa analogue does not induce any Ca\(^{2+}\) release from internal stores nor does it inhibit CMC-induced Ca\(^{2+}\) release. These data demonstrate that Ca\(^{2+}\) release induced by CMC occurs through RyR1 activation. The cellular effect of MCa is of the greatest importance because it demonstrates that the toxic effect of the toxin can be explained by its action on RyR1 despite the intracellular location of this target. This result also reveals that MCa has the ability to cross the plasma membrane of skeletal muscle cells, a property that is shared by some other conformational analogues, such as [Ala\(^{20}\)]MCa, have in common the ability to induce long-lived subconductance states of RyR1. The appearance of this subconductance state probably explains the release of Ca\(^{2+}\) from SR vesicles and in part the stimulation of \([^{3}\text{H}]\)ryanodine binding. This finding is coherent with the hypothesis that ryanodine preferentially binds onto the open state of RyR1 (26). There are interesting functional similarities between ryanodine and MCa. Both appear able to induce subconductance states, and MCa favors \([^{3}\text{H}]\)ryanodine binding. These data strongly suggest the existence of a positive synergy between the binding sites of the two molecules on RyR1. This synergy is further evidenced by the fact that MCa increases by a factor of 6.1-fold the affinity of \([^{3}\text{H}]\)ryanodine for RyR1. In addition, in the presence of MCa, we observed a shift to lower Ca\(^{2+}\) concentrations of the Ca\(^{2+}\) stimulatory effect on \([^{3}\text{H}]\)ryanodine binding and a shift to higher Ca\(^{2+}\) concentrations of the Ca\(^{2+}\) inhibitory effect on \([^{3}\text{H}]\)ryanodine binding. These data are in favor of a model in which the binding of either one of these two molecules, ryanodine or MCa, produces a chain of conformational events, leading to the appearance of subconductance states. All of the active MCa analogues that we tested stimulated \([^{3}\text{H}]\)ryanodine binding with higher EC\(_{50}\) values than MCa itself. We also measured a reduced ability to stimulate \([^{3}\text{H}]\)ryanodine binding. Although the substituted amino acid residues of MCa do not appear decisive for the interaction with RyR1, they seem to contribute to some extent to the binding site recognition. In addition, they also appear to participate to the conformational events that lead to the modifications of the low affinity ryanodine binding sites. We would expect that more drastic substitutions within MCa lead to modifications in the level of RyR1 subconductance states and/or in the time spent in these subconductance states. Indeed, it has been observed that IpTxA, which shares 82% sequence identity with MCa, induces a subconductance state of RyR significantly different from the one triggered by MCa (25% of the full-conductance state for IpTxA versus 58% for MCa) (9, 10). Moreover, in contrast to what is observed with IpTxA, LLSS induced by MCa and its analogues are polarity-independent. Similarly, the domain A of the a\(_1\) subunit of DHPR that shares sequence similarities with both MCa and IpTxA also induces a subconductance state different from those observed with the toxin (65 and 86% of the full-conductance state for domain A) (6). Based on sequence identity, it is probable that MCa and IpTxA bind more than one common site(s) or regions on the RyR1 tetramer. Interestingly, the binding of two structurally related molecules on the same channel can produce highly different conductance states of RyR1. Therefore, it is tempting to postulate that once MCa or IpTxA is bound on RyR1, structural modification of these ligands could produce important changes in the conductance properties of RyR1. This concept of RyR1 conductance modulation could be applied to domain A to provide an explanation for its role in the regulation of RyR1 function. Recently, it was proposed that the three-dimensional structural surface of MCa and IpTxA mimics that of domain A (23). It would be interesting to investigate whether conformational changes in domain A during membrane depolarization are of nature to modify RyR1 channel conductance.

FIG. 7. [Ala\(^{24}\)]MCa does not induce Ca\(^{2+}\) release in intact myotubes. A, variation of the cytoplasmic Ca\(^{2+}\) concentration upon external application of 1 \(\mu\)M [Ala\(^{24}\)]MCa was measured as described in Fig. 6. B, mean peak value of fluorescence variations induced by 250 \(\mu\)M CMC without or with preapplication of 1 \(\mu\)M [Ala\(^{24}\)]MCa.

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