The Arianna thread: the matching of S-100 family with the RyR's muscle receptor

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Abstract

The functional state of RyR depends on the intracellular calcium concentration and on the oxidation state of its protein components in some particular sites and of some sentinel amino acids. In addition to the regulation of the RyR channel by exogenous substances (caffeine, ryanodine), ions environmental situations (oxidative state), other components, such as some endogenous proteins present in the sarcoplasm and/or in muscle membranes that are able to determine changes in Ca^{2+} channel activity. Among these, calmodulin and S-100A could determine modifications in the status of RyR channel in the skeletal muscle. The currently available data can be justified the use of a simplified S-100/CaM and RyR interaction model for the regulation of Ca^{2+} release in skeletal muscle. Under resting conditions, the CaM/S100A1 binding domain on RyR1 is predominantly dependent on S100A1. Vice versa when the intracellular Ca^{2+} concentration becomes high as well as during repetitive (tetanus) stimulation, the Ca-CaM bond becomes dominant, shifting S100A1 from RyR1 and promoting channel inactivation. This may be one of the mechanism of muscle fatigue.

Key Words: Ca^{2+} release, RyR modulation, S-100 proteins, calmodulin.

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In the mammalian skeletal muscle the contraction occurs because the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) increases from 10^{-7} M until 10^{-5} M. The junctional Sarcoplasmic Reticulum (jSR) is involved in the release of this ion; may contains three isoforms (RyR_1, RyR_2 and RyR_3) of a particular calcium operated channel, the ryanodine Ca-channels called "feet" (juxtaposed to the voltage-dependent channels of type L –DHPR- present in the T tubule). The channels for Ca^{2+} DHPR and RyRs, form groups (4:1) called Ca^{2+} release units[1] As a result of the depolarization brought by the action potential, the voltage-sensitive L-channels cause, with a different mechanism in the skeletal and cardiac muscle, RyR channels to open, causing the release of massive amounts of Ca^{2+} into the sarcoplasm.[2]

The functional state of RyR (closed-open-inactivated) essentially depends on the intracellular calcium concentration and on the oxidation state of its protein components in some particular sites and of some sentinel amino acids.[3]

Many pharmacological substances are able to modify the functional status of the channel and its sensitivity to Ca^{2+}.[4] Among these, the best known are ryanodine and a trimethylxanthine such as caffeine. Ryanodine added in micromolar concentrations, modifies the gating and conductance of RyR in a characteristic way bringing it into a state of subconductivity. Vice versa, if the concentration of the drug reaches the millimolar level, the channel switches into a closed state. A characteristic property of ryanodine-modified channel states is insensitivity to regulation by Ca^{2+}, Mg^{2+}, and ATP.[5]

Caffeine is bound to a specific site of the channel. In contrast to ryanodine, caffeine always has an activating action on the purified channel increasing the probability of opening and without loss of sensitivity to regulation by Ca^{2+}, Mg^{2+} and ATP.[6]

In addition to the regulation of the RYR channel by exogenous substances (caffeine, ryanodine), ions (Ca^{2+}, Mg^{2+}) environmental conditions (oxidative state), other components, such as some endogenous proteins present in the sarcoplasm and/or muscle membranes are able to directly determine changes in the Ca^{2+} channel activity state. Among these, we will consider only calmodulin and the group of proteins belonging to the S-100 family.[7]

Sarcoplasmic CaM is a 16.7 kD protein that regulates the

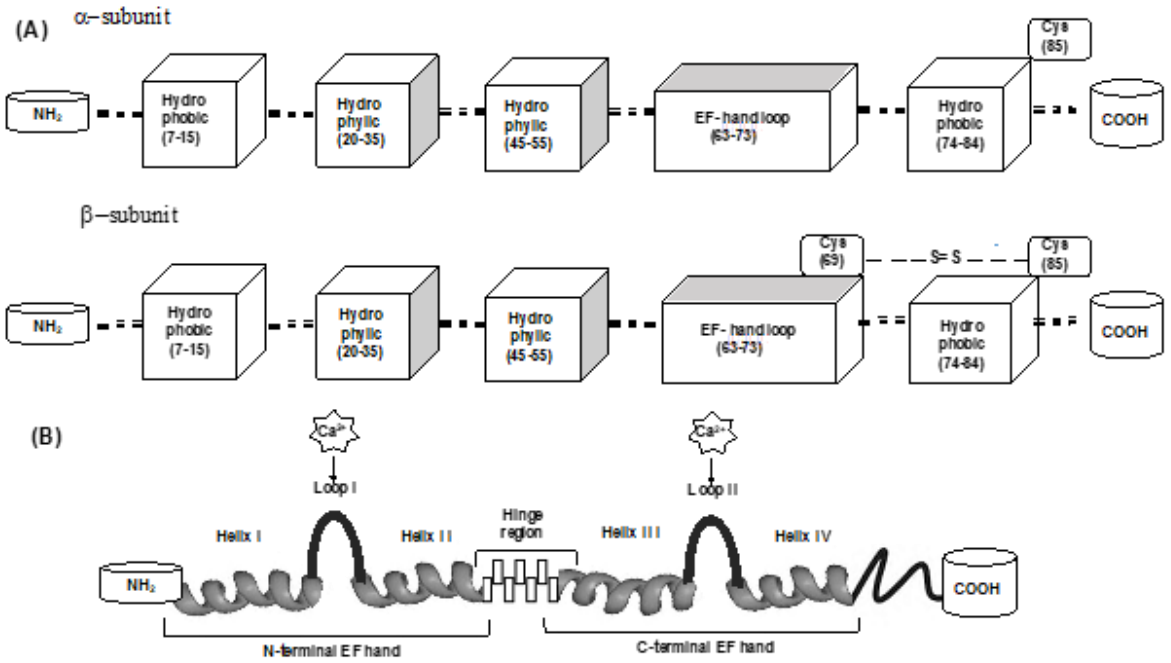

Fig 1. ***A****, S-100 protein domains. The AA sequence and subunits (alfa and beta) of the protein share a large degree of homology. Some differences, which are used as immunological markers, are present in the hydrophilic region. The presence of two cysteine residues in the subunit can confer to this subunit some particular feature (data derived from Fanò al.1995).*[7]
B*, The monomeric structure (MW 10.4 Kd) consists of a repetitive EF-hand motif, whereas eachCa2+-binding Loop (Loop I and II) is flanked by α-helices. The N-terminal and the C-terminal EF hands are connected by a linker region (hinge region). The data reported derived from Völkers.*[12]

release of SR Ca^{2+} through direct binding to RyRs or through other proteins that interact with RyRs and bind CaM. As with ryanodine, the effect of CaM on the release of Ca by the RyR channel depends on the concentration of Ca-free. For [Ca^{2+}] >1 µM, CaM inhibits all three mammalian RyR isoforms. Vice versa, for Ca-free concentrations < 1 µM, the activity of RyR_1 and RyR_3 channels is increased, while RyR_2 is inhibited. From this comes the hypothesis of a physiological role for CaM which, by prolonging the channel closure state at [Ca^{2+}] greater than 10^{-6} M, would allow the ion to spread in the sarcomere, away from the release sites.[8]
Studies with CaM-free mutants indicate that if CaM's binding to RyR_2 is altered, cardiac function is impaired.[9]
The S100 protein was named S100 because of its solubility in a 100% saturated solution with ammonium sulphate.[10] The term S100 is used to embrace a multigenic family of mostly dimeric calcium-binding proteins.[11] In nature can be found both as a low molecular weight monomer (alpha or beta) and as an alpha-alpha (S100A) and beta-beta (S-100B) homodimer. Each chain has a molecular weight of 10-12 kDa.
The S100 proteins are highly conserved in amino acid composition among vertebrate species, suggesting that they may have crucially conserved biological roles.[7]
S100 proteins are small, acidic proteins and contain two distinct EF-hands, 4 α-helical segments, a central hinge region of variable length and the N- and C- terminal variable domains (Figure 1). At present, at least 25 proteins have been identified as belonging to the S100 protein family, 21 of them having genes clustered at chromosome locus 1q21.[12] S100 proteins do not have intrinsic catalytic activity. They are generally thought to be that, in a similar manner to calmodulin and troponin C, undergo conformational changes and modulate biological activity via calcium binding. The two subunits have many aspects in common and each of them is characterized by two Ca^{2+} site binding: one at high affinity (kD= 10^{-5} M) on the C-terminal portion and another unconventional with low affinity for the ion.[13]
Even though the members of the S100 family share a remarkable sequence homology, they express themselves in specific tissue patterns. S100A is the most highly expressed member of the family in striated muscle, where the protein shows the highest presence in the heart muscle, followed by slow contraction red fibers and finally white, fast fibers.[14]
To date, a considerable amount of data has accumulated to hypothesize a functional role for S100A in cardiac muscle, but research on a possible role for S100 in skeletal muscle is still incomplete.[15] The first experiments involving protein intervention in the

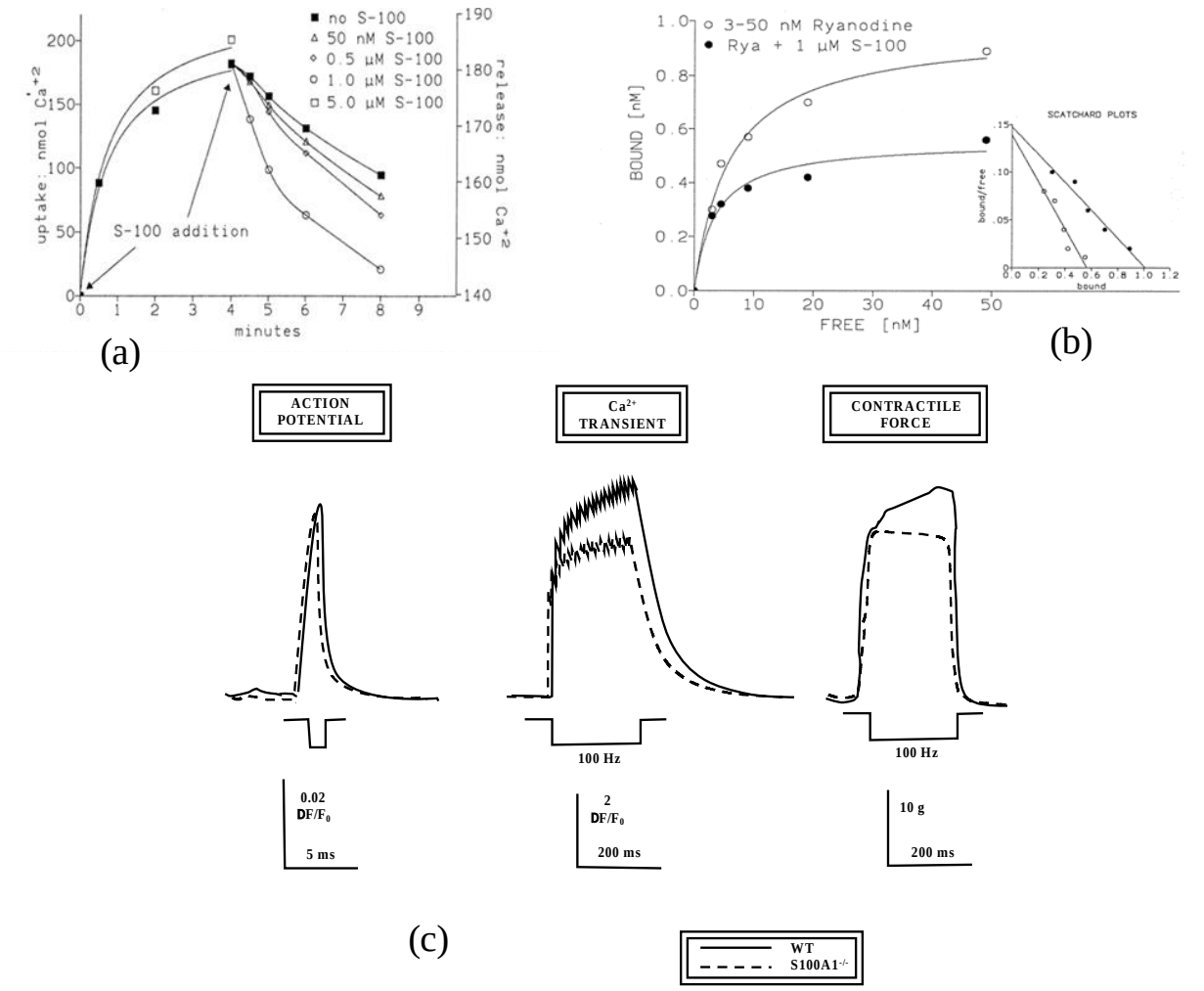

Fig 2. (a) Effect of S-100 on Ca^{2+} uptake and release in vesicles derived from SR fragmentation of skeletal muscle of Rana esculenta. The uptake was induced by 5mM ATP plus 5.0 µM S100; (b) Specific binding of 3-5 nM ryanodine in presence of 1 µM S100. (c) S100A1 modulation of skeletal muscle EC coupling in genetic ($S100^{-/-}$) ablated fibers. Data reported in part (a) and (b) derived from Fanò et al.[17] while part (c) represents the result of observations carried out from Prosser et al.[18]

excitation-contraction cycle in skeletal muscle, were performed by our group and showed that S100A improved the release of RyR_1 Ca^{2+} in SR terminal cisternae preparations (Figure 2). The most interesting fact of these experiments was to highlight how, despite belonging to the same superfamily as the EF-hand calcium binding protein, CaM and S100A seemed to have contrasting effects on the activation of Ca^{2+} release via RyR_1.[16,17]

Several years later Prosser and collaborators have shown, that the effective concentration of S100A available locally for modulation of RyR_1 is probably higher. However, as has been demonstrated, both ultrastructurally and immunofluorescently, S-100 is located at the overlap area of the A/I band and at the tetrades, where the release of Ca^{2+} into the skeletal muscle occurs.[18] Therefore, it should come as no surprise that S-100 can act as a regulator of the functional status of RyR channels as it has been assumed for another protein with similar characteristics, the calmodulin.[19] In experiments using vesicles derived from the SR fraction containing the RyR channels preloaded with Ca^{2+}, the addition of S-100 in mM concentration increased the release of ion induced by the presence of $[Ca^{2+}]$ mM in the experimental medium. This may be interpreted as an effect of S-100 on the activation time of the channel which becomes, thus, longer. In the same experimental model CaM induces inhibition of Ca release while the use of S100 inactivated is not able to increase the release of the ion from the vesicles previously loaded with Ca in the

presence of ATP.[20]
The currently available data can be justified if one uses a simplified S-100/CaM and RyR interaction model for the regulation of Ca^{2+} release in skeletal muscle. Under this assumption, and in resting conditions, the CaM/S100A1 binding domain on RyR_1 is predominantly dependent on S100A1, which increases Ca^{2+} release and force generation when the muscle is stimulated. When the $[Ca^{2+}]_i$ becomes high during repetitive (tetanus) stimulation, the Ca-CaM bond becomes dominant, shifting S100A1 from RyR_1 and promoting channel inactivation.[18]
In this way, CaM and S100A1 compete to fine tune the release of Ca^{2+} from RyR during normal muscle activation. The interruption of this system leads to a decrease in force production and a reduction in muscle performance. However, the molecular details of these interactions and the consequences of their dysregulation on channel activity are not yet known.[19] Nevertheless, recent studies based on the FRET technique, have suggested that CaM and S100A can simultaneously, but separately, modulate the RyR function without S100A competing for CaM on its specific channel site.[20]
In conclusion, the data that are available today indicate that there is a mechanism, in the skeletal muscle, able to finely modulate the functional state of the RyR channel and therefore the available Ca^{2+} necessary for the contraction. This mechanism is represented by the joint interaction of CaM and S100A on specific common RyR sites. However, fundamental information is still missing to understand the molecular dynamics of this interaction. Furthermore, there are conditions not fully studied, in which the competition between the two calcium binding proteins for the same site changes (during exercise, oxidative stress, fatigue, etc.). Nevertheless, there is strong evidence that the interaction of CaM and S100A can regulate skeletal muscle EC coupling.[20-22] This kind of balanced control could also be at the basis of the onset of the phenomenon of fatigue, which does not yet have a precise determinant because the accumulation of lactate does not seem to be sufficient to explain its occurrence.
If this hypothesis will be confirmed it could represent a turning point in the definition of the functional role for the protein. S100 could finally emerge from the labyrinth in which it has been confined since its discovery. Of course, no one wants it to end up like Theseus.

List of acronyms

ATP - Adenosine TriPhoshate
CaM - Calmodulin
EC - Excitation Contraction
FRET - Fluorescence Resonance Energy Transfer.
RyR - Ryanodine Receptor

Authors contributions

Giorgio Fanò-Illic played a main role in the writing of the manuscript, Silvia Belia in the drafting and finalizing the manuscript, Stefania Fulle in the extensive manuscript revision.

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Conflict of Interest

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