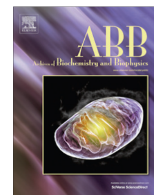
Archives of Biochemistry and Biophysics 540 (2013) 145–159

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi



RyR3 *in situ* regulation by Ca^{2+} and quercetin and the RyR3-mediated Ca^{2+} release flux in intact Jurkat cells

Irina Baran *, Constanta Ganea

"Carol Davila" University of Medicine and Pharmacy, Dept. of Biophysics, 8 Eroii Sanitari, 050474 Bucharest, Romania

ARTICLE INFO

Article history:
Received 28 June 2013
and in revised form 19 October 2013
Available online 5 November 2013

Keywords:
Ryanodine receptor
Open probability
Binding site
Quercetin
Fluorescence
Jurkat cells

ABSTRACT

Ryanodine receptors are generally thought to possess a high-affinity activating cytosolic Ca^{2+} site and a low-affinity inhibitory cytosolic Ca^{2+} site. By performing conformation selective measurements in which quercetin was used as a fluorescent marker for RyR3 (ryanodine receptor type 3) in Jurkat cells, we now find that the rectified RyR3 channel in open conformation may be regulated *in situ* by two cytosolic activating Ca^{2+} sites, of low and high affinity, respectively, whereas no inhibitory Ca^{2+} effect could be delineated. In the closed rectified channel, as well as in the open hindered channel, only the high affinity activating Ca^{2+} site and the inhibitory Ca^{2+} site were functional, whereas in the closed hindered channel all three regulatory Ca^{2+} sites appeared to be operational. RyR3 also seems to possess one activating and two inhibitory quercetin sites. Corresponding Hill coefficients and affinities of these regulatory sites were estimated. Quercetin cellular uptake exhibited an initial rapid phase (~1.04 min), followed by slow accumulation of free quercetin inside the cytosol (~34.5 min). The RyR3-mediated Ca^{2+} release current increased from a baseline of 247 to 287 pA in 1 min. after addition of 50 μM quercetin and then declined slowly to a plateau of 265 pA.

© 2013 Elsevier Inc. All rights reserved.

Introduction

Mobilisation of Ca^{2+} ions from intracellular organelles such as the endo/sarcoplasmic reticulum (ER/SR)[1] constitutes a key step in a wide variety of cellular processes involved in cell proliferation and division, cell death, fertilisation, secretion, memory or contraction [1]. Calcium release from intracellular stores is mediated by activation of inositol 1,4,5-trisphosphate (IP_3) receptors (IP_3Rs) and/or ryanodine receptors (RyRs) [1–8] which are found in the ER/SR membrane. It is generally recognised that IP_3 and ryanodine receptors display remarkably similar structural and functional properties. Each of these two related families of homotetrameric receptors comprise three different isoforms, which are denoted as the type 1, type 2 and type 3 IP_3R/RyR.

Cryoelectron microscopy studies have shown that all RyR isoforms have a similar 4-fold symmetrical, mushroom-like form presenting a large cytoplasmic head which extends 120 Å away from the ER/SR membrane, and a transmembrane assembly, the carboxyl-terminal hydrophobic domain forming a central conducting pore, which is rotated at ~40° with respect to the head [5]. Upon channel opening, the receptor protein experiences some major rearrangements that involve long-range conformational changes in the distal zone of the head, as well as the rotation of ~4° between the cytoplasmic and the transmembrane regions, and the reconfiguration of the transmembrane domain associated with a twisting of part of the transmembrane assembly [5], which probably does not affect the structure of the selectivity filter [9].

To date, the sole means to assess directly RyR3 activity has been to record the ionic current transported by individual receptors incorporated into artificial lipid bilayers, under voltage-clamp conditions. As with the other two RyR isoforms or the IP_3 receptors, these measurements demonstrated a biphasic effect of cytosolic Ca^{2+} on the open probability [10–13], which is generally associated with the existence of two functionally distinct Ca^{2+} regulatory sites situated on the cytoplasmic side of the receptor, namely a high-affinity activating site (with apparent dissociation constant K_d ~ 1 μM), and a low-affinity inhibitory site (with apparent K_d ~ 1–10 mM), respectively. Bilayer experiments and measurements of global Ca^{2+} release in skeletal and cardiac muscle also provided compelling evidence for a luminal Ca^{2+} activating site and a cytosolic inactivating site, with apparent affinities K_d of about 40 and 1 μM, respectively [8,14]. Stimulation of ryanodine receptors by luminal Ca^{2+} has been found to depend on the luminal to cytosolic Ca^{2+} flux. The data pointed to a "luminal-triggered Ca^{2+} feed-through" mechanism of RyR regulation by Ca^{2+} [8,14] whereby the channel opens upon Ca^{2+} binding to the luminal or to the cytosolic Ca^{2+} activating site. The luminal Ca^{2+} flowing through the open channel can further stabilise the open state via an increase in the open state duration induced by prolonged binding

* Corresponding author. Fax: +40 21 3125955.
E-mail addresses: baran@ifin.nipne.ro, virbaran@yahoo.com (I. Baran).

[1] *Abbreviations used:* ER/SR, endo/sarcoplasmic reticulum; IP_3, 1,4,5-trisphosphate; RyRs, ryanodine receptors.

0003-9861/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.abb.2013.10.024

of Ca^{2+} to the cytosolic activation site. However, at high levels of Ca^{2+} feed-through, the inactivation site causes a reduction in the open dwell time and induces partial inactivation of the channel, reflected in a 20–40% decrease of its open probability (P_{open}).

Despite the identification of several potential regions with affinity for Ca^{2+} within the amino acid sequence of the ryanodine receptor that may be involved in channel gating, the exact number and location of the RyR Ca^{2+} binding sites remains still to be determined [5–8]. In addition, the detailed mechanisms of RyR pore opening/closing by Ca^{2+} binding to antagonist (activating/inhibitory) cytosolic sites have remained unidentified as well. In analogy to other different channels, it has been proposed that Ca^{2+} bound to the cytosolic activating site of the RyR1 receptor induces the expansion of a cytoplasmic gating ring thought to drive the kinking of the four transmembrane inner helices, thereby opening the ion gate [15]. The general location of the cytosolic activating and inactivating sites is thought to reside in the pore-forming region of the receptor, at about 16 and 34 nm from the pore mouth, respectively, hence providing accessibility to luminal Ca^{2+} flowing through the open channel [8,14], whereas the inhibitory site is most likely situated far from the conduction pathway, hence unable to sense directly the permeating Ca^{2+} flux [8].

In a former work [16] we have identified that the flavonoid quercetin (3,3′,4′,5,7-pentahydroxyflavone; QC) can be used as an efficient fluorescent probe to distinguish with high sensitivity between the open and the closed conformation of the RyR3 channel in human leukemia Jurkat T-cells. Thus, we could quantify RyR3 activity in both intact and permeabilised cells and characterise the dependence of the open probability on the cytosolic concentration of Ca^{2+} or QC. The data presented therein also indicated that *in situ*, the RyR3 channel activity under physiological conditions is partially suppressed (the hindered channel, presenting an open probability of 0.29 in intact cells under resting conditions) whereas the channel is nearly fully activated upon exposure to high ($\sim$1 mM) concentrations of bulk cytosolic Ca^{2+} and subsequent chelation of Ca^{2+} (rectified channel). Here we investigate in more detail these two distinct modes of activity by assessing RyR3 regulation by Ca^{2+} in either hindered or rectified channel states, in the open as well as in the closed conformation. Importantly, our current measurements unveil a second activating, low-affinity Ca^{2+} cytosolic site of the RyR3 receptor, which appears to be operational in the rectified but not in the hindered channel state. Nevertheless, the bell-shaped Ca^{2+}-dependence of the open probability of the rectified channel [16] appeared to be remarkably similar to that observed in bilayer experiments [10–13], which underlines the idea that the steady state P_{open} data are not sufficient by themselves to infer the total number of regulatory sites.

Quercetin has been found to modulate the activity of the type 1 RyR (the skeletal muscle specific RyR isoform) in lipid bilayers [17] and to promote Ca^{2+} release from purified SR [18]. We determined previously that quercetin induced a strong Ca^{2+} release signal in intact Jurkat cells [16,19] which appears to be mediated by RyR3 receptors [16]. QC can also inhibit reversibly the SR Ca^{2+}-ATP-ase and the uptake of Ca^{2+} ions by the sarcoplasmic reticulum [20–22]. This molecule is poorly fluorescent in aqueous solutions [23,24] but displays a specific fluorescence upon cellular internalisation [23,19], which appears to be connected with binding to mitochondrial proteins [25], ER ryanodine receptors [16,19] or nuclear targets [26]. Previous modeling studies [27] based on steady P_{open} data obtained with the RyR1 isoform incorporated into artificial lipid bilayers [17] suggested that RyR1 has two distinct QC sites which are allosterically coupled with the activating and the inhibitory Ca^{2+} site, respectively. However, while the stimulatory effect of quercetin on RyR opening by Ca^{2+} could be well characterised, the available data [17] were not sufficient to make detailed inferences regarding the effect of quercetin on the inhibitory Ca^{2+} site [27]. Our current investigations suggest that RyR3 possesses three distinct QC binding sites. One of the three sites stimulates RyR3 activation while the other two promote channel closing. Moreover, we show that in the case of the RyR3 receptor the Ca^{2+} and QC binding sites are also allosterically coupled and we confirm some of the QC binding parameters derived for RyR1 [27].

More specifically, in this work we measured the fluorescence of quercetin at two specific excitation wavelengths (380 and 440 nm) which correspond to the flavonoid bound to the RyR3 receptor in the open or the closed conformation [16], and determined in permeabilised Jurkat cells the dependence of the steady state fluorescence emission on the cytosolic concentration of Ca^{2+} and quercetin, respectively. From these measurements we estimated the minimal number of Ca^{2+}- and QC regulatory sites that are functional in the RyR3 receptor *in situ*, as well as their affinities and Hill coefficients. We should recall that in our previous paper [16] we did not analyze individually the fluorescence emitted by the QC–RyR3 complex in the open or the closed conformation, but we focused on the changes in the corresponding fluorescence ratio (Q = F380/F440) under various circumstances. We show here that in addition to relevant information regarding the open probability of the channel (which can be derived from the QC-specific fluorescence ratio Q [16]), this quercetin-based dual-excitation fluorimetric assay can also provide valuable insights into the differential behavior of the receptor in the two specific (open/closed) conformations. Hence, this kind of measurements can deepen considerably our understanding of the RyR3 regulation by its ligands. Most importantly, we can now extend our knowledge by unveiling how large the difference between the receptor's behavior *in situ* and in artificial lipid bilayers can be.

In addition, from kinetic recordings of the dual fluorescence signal emitted by quercetin-bound RyR3 in intact Jurkat cells exposed to a fixed dose of extracellular QC, we could quantify the kinetics of RyR3 recruitment during the cellular uptake of quercetin, which then, combined with the steady state fluorimetric data obtained as mentioned above, allowed us to estimate the cytosolic level of free quercetin and its kinetic profile during cellular uptake.

Results

Discrimination between open and closed channel conformations by dual excitation fluorescence measurements

In a previous paper [16] we have shown that the ratio Q = F380/F440 of the quercetin-specific cellular fluorescence emitted at 540 nm upon excitation at 380/440 nm reflects the open probability of the endoplasmic reticulum RyR3/Ca^{2+} release channel in both intact and permeabilised Jurkat cells. The results presented therein indicate that F380 and F440 quantify the binding of quercetin to the open Ca^{2+} release channels, and to channels found in either open or closed configuration, respectively. Thus, F440 represents the additive contribution of the fluorescence of the QC-liganded closed channels, which displays a specific excitation band at 440 nm, and that of a residual fluorescence emitted by the QC-bound open channels, which is characterised by an excitation maximum centered on 380 nm [16,19].

Some representative images (Fig. 1 and Fig. S1) present, at higher resolution than in our previous work [16], the cellular distribution of these two fluorescent species of quercetin observed by confocal microscopy. Both QC fluorescent signals colocalise with the endoplasmic reticulum (Fig. 1F and G), which appeared as a widely spread thin layer enfolding the mitochondria (Fig. 1A–C and Fig. S1.1A–S1.3A), whereas mitochondria could be distinguished as individual entities of several hundreds of nanometers

Download English Version:

https://daneshyari.com/en/article/8290499

Download Persian Version:

https://daneshyari.com/article/8290499

Daneshyari.com