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COMMENTARY

Conformation State of the Ryanodine Receptor and Functional Effects of Ryanodine on Skeletal Muscle

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ABSTRACT. The ryanodine receptor (RyR) and the dihydropyridine (DHP) receptor (L-channels) comprise the main elements of the functional feet of the triadic element in skeletal muscle. These two main elements have conformational states that are regulated by the membrane potential and the consequent electrical field. The pharmacological action of ryanodine on skeletal muscle depends upon the physiological functional state of the RyR. At a resting potential of −90 m V, ryanodine at very low concentrations, 10^{-11} M, causes the RyR to have a low conductance state which allows calcium to leak from the terminal cisternae of the sarcoplasmic reticulum and to be recycled with ATP utilization, leading to a marked increase in oxygen consumption and aerobic metabolism. At concentrations greater than 10^{-6} M, ryanodine can cause a slowly developing contracture of resting muscle, inhibit the muscle twitch when the RyR complex is formed during stimulation, and, if formed before stimulation, accelerate the development of contracture. Biochemical studies have revealed that the RyR has four binding sites in which the conductance state depends upon the number of sites occupied by ryanodine. Our present understanding of the RyR-operated calcium channel is the result of an interdisciplinary approach in which each discipline (anatomy, physiology, biophysics, and biochemistry) contributes to our knowledge of the pharmacological action of ryanodine. BIOCHEM PHARMACOL 53;7:909–912, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. ryanodine receptor; ryanodine receptor-calcium release channel; L-channel; dihydropyridine receptor; conformational state; functional state

Ryanodine and caffeine were among the first drugs selected for study on the release of calcium from internal muscle stores in relation to muscle contraction. These agents were selected because they cause contraction without causing membrane depolarization and, thus, bypass the electrical field effects of the muscle action potential initiating muscle contraction and act directly on the internal store of calcium. Both of these agents have been shown to cause contraction in conjunction with an increased efflux of calcium [1]. Ryanodine was shown earlier to prevent the resequestration of calcium by the isolated SR† [2, 3].

Since these early studies, there has been a convergence of four disciplinary approaches that have contributed to our understanding of the complexities and functions of the RyR: anatomy, physiology, biophysics, and biochemistry. Clara Franzini-Armstrong and her collaborators [4] have contributed immensely to our understanding of the structural relation of the transverse tubular membrane DHP receptors and the RyR calcium release channel of the terminal cisternae. Together these two structural elements comprise the main elements of the feet that are involved in the transduction of the electrical signal of the transverse tubular membrane to calcium release from the terminal cisternae.

Pharmacological studies have shown that ryanodine at concentrations between 10^{-11} and 10^{-6} M causes a marked increase in oxygen consumption due to calcium leak from the RyR calcium channel and enhanced energy consumption due to recycling of calcium by the SR [5]. At concentrations greater than 10^{-6} M, ryanodine causes an irreversible contracture. Benzocaine, which blocks the release of calcium from the terminal cisternae caused by ryanodine or caffeine, also blocks the increased oxygen consumption. Biophysical and biochemical studies on the calcium release channel of the SR by Smith *et al.* [6–10] and Meissner, [11] have shown the calcium release channel to have a high affinity for [^{3}H]ryanodine and for [^{14}C]doxorubicin [12–14]. As mentioned above, our present understanding of the RyR-operated calcium channel is the result of an interdisciplinary approach in which each discipline contributes to our knowledge. The pharmacology of ryanodine requires the integration of the facts obtained from each of these disciplines.

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† *Abbreviations:* SR, sarcoplasmic reticulum; T, transverse; RyR, ryanodine receptor; L-channel, voltage-dependent slowly activating calcium channel found in transverse tubules, and which contains the receptor for dihydropyridines; and DPH, dihydropyridine.

ULTRASTRUCTURAL STUDIES OF THE FEET BRIDGING THE GAP BETWEEN THE SR AND THE T-TUBULES OR SURFACE MEMBRANE

The feet are the means whereby the depolarization of the surface membrane by the action potential activates voltage sensors of the triadic function. The reversal of the field by 100,000 V allows transduction of the depolarization to cause calcium release from the SR. The calcium release channel is identified as the RyR [15–17]. The isolated RyR/calcium release channel has two components: a large tetrameric assembly corresponding to the foot and a smaller domain that constitutes an intramembrane domain. The RyR calcium release channel and the DHP receptor (voltage-dependent slowly activating L calcium channel present at triadic junctions) are believed to be the main components of tetrads visible in freeze fracture images of functional domains of either T-tubule or surface membranes [14, 16, 17]. The voltage sensor of the cytoplasmic loop between repeats II and III of the DHP receptor causes a conformational change of the RyR by direct or indirect actions leading to calcium release from the terminal cisternae into the sarcoplasm. Pharmacological studies of the RyR, have revealed that there may be a reciprocal opening of the DHP channel of the T-tubule by low concentrations of ryanodine binding to the RyR to allow calcium influx from the lumen of the T-tubule into the sarcoplasm [18].

PHYSIOLOGICAL–PHARMACOLOGICAL STUDIES OF RYANODINE ON SKELETAL MUSCLE

The effect of ryanodine on isolated frog sartorius muscle depends upon the physiological functional state of the RyR at the time of binding to the receptor. Ryanodine (10^{-4} M) when added to a solution bathing an isolated sartorius muscle at rest causes a slow rise in tension after a latent period of 30–40 min. A marked increase in calcium efflux is associated with the contraction. The simultaneous addition of both ryanodine (10^{-4} M) and a depolarizing solution containing 80 mM K^+ causes a phasic contracture followed by a slow rise in tension. The binding of ryanodine to the receptor(s) in resting muscle prior to depolarization causes the muscle to go immediately into contracture and rigor upon subsequent depolarization [19]. Ryanodine, when added to a muscle in which the phasic contracture has relaxed but the membrane is still depolarized, has a long latent period for contracture. Ryanodine at 10^{-4} M when added 30 min prior to stimulation of the sartorius muscle at 0.1 Hz causes incomplete relaxation with a full contracture developing after 70 twitches. In contrast to the contracture developed in sartorius muscles when ryanodine is added prior to stimulation, the addition of 10^{-4} M ryanodine to a sartorius muscle during stimulation at 0.1 Hz causes peak tension to decrease after 120 twitches, and complete block of the twitch and relaxation occurs after 150 additional twitches.* Thus, in frog sartorius preparations, ryanodine at 10^{-4} M can cause a contracture after a long latent period or a contracture almost immediately when the ryanodine-treated muscle is stimulated electrically or depolarized by potassium. If the ryanodine is added during stimulation, the muscle twitch becomes blocked [18, 19]. In the intact frog, ryanodine at a dose of 5 mg/kg produces a flaccidity followed by a slowly developing rigor [20].

The action of ryanodine on the receptor depends upon the conformational state of the receptor at the time of binding and the functional state of the receptor. The RyR complex has more than one functional state. The most stable state for the RyR complex appears to be the open state, which at rest has a low probability of occurring, hence a long latent period. When the RyR complex is formed previously, increasing the probability of the open conformation by stimulation accelerates the onset of contracture or rigor. In contrast, the binding of ryanodine during stimulation appears to uncouple the RyR from the DHP receptor-voltage sensor of the foot and prevents the twitch from occurring in response to the muscle action potential. The two opposite responses to ryanodine suggest that ryanodine binds to different sites when the muscle is at rest or is stimulated.

Ryanodine at a low concentration can cause the RyR complex to open at a low conductance state. The slow leak of calcium from the terminal cisternae causes calcium to be recycled, and the utilization of ATP by the sarcoplasmic reticulum CaATPase causes enhanced utilization of O_2 [5, 21, 22]. The increase in oxygen uptake is maximal at 10^{-10} M (27.1 μmol O_2/g · hr) and is decreased to 10.3 μmol/g · hr at 10^{-9} M. Benzocaine, which blocks calcium release from the RyR-operated calcium channel of the terminal cisternae, also blocks the increase in oxygen consumption [22]. The RyR complex formed with 10^{-10} M ryanodine causes an increase in oxygen uptake that peaks after 5 min and then slowly reverts to a basal rate after 20 min.

The triadic junctional feet that bridge the gap between the SR and the T-tubule (triad) or surface membrane (diad) consist of the large RyR calcium release channel(s) linked either directly or indirectly to the DHP receptor or calcium channel (L-channel). These proteins form a complex in which each component may modify the functional state of the other protein. During stimulation of sartorius muscle at 1 Hz, there is a net influx of 5.6 pmol cm^{-2} $twitch^{-1}$ during the first 30 twitches, indicating that the L-channel in the T-tubule can conduct a large calcium current. Tension rises to a maximum after 60 twitches, and net calcium influx declines and reverts to a net efflux of 0.5 pmol cm^{-2} $twitch^{-1}$ during the declining phase of twitch tension. The large initial influx of calcium may be due to the modified L-channel–triadin–RyR complex, allowing the L-channel to initially open for the first 30 to 60 twitches at 1 Hz followed by a closing of the L-channel, which continues to

* Bianchi CP and Narayan S, unpublished results, 1987.

operate as a voltage sensor for the RyR channel, allowing twitches to take place but at a lower twitch tension [23, 24]. The initial large influx of calcium may serve to prime the ryanodine calcium release channel by displacing Mg^{2+} binding site on the calcium release channel [25]. The action of ryanodine at low concentrations (10^{-10} and 10^{-8} M) to increase calcium influx and increase oxygen consumption, while at higher concentrations $>10^{-6}$ M to cause the release of calcium for the terminal cisternae, suggests a bimodal operation of the entire triadic junctional complex (feet) [18].

Lamb and Stephenson [25], in a series of elegant studies on a skinned muscle fiber preparation that allowed access to the internal cystosolic environment while retaining the intact voltage sensor control of calcium release, showed that 25 μM ryanodine prolongs the open state of the voltage-activated calcium release channel (RyR) and delays deactivation upon repolarization. They suggested that the calcium-release channel remains open but at a low conductance state insufficient to cause tension development of the skinned fiber, but sufficient to deplete the sarcoplasmic reticulum of calcium. In their preparation, 1 mM Mg^{2+} blocked the calcium release channel. The block was removed by depolarization of the voltage sensor, allowing the calcium release channel to open.

Biochemical studies have revealed an additional protein that may modify the function of the RyR calcium release channel. Jayaraman *et al.* [26] reported the identification of a 12 kDa protein that is tightly associated with the purified RyR from skeletal muscle. The 12 kDa protein is the binding protein for the immunosuppressant drug FK506 [27]. The function of the FK506 binding protein appears to be optimizing the opening of the RyR calcium release channel [14].

Biochemical studies also have shown that the relationship between binding of ryanodine to the RyR calcium release channel and the transformation of the channel to an open state or closed state depends upon occupation of high affinity sites to open and of low affinity sites to close. Four molecules of ryanodine bind to each tetrameric RyR with binding constants of K_1 = 1–4 nM, K_2 = 30–50 nM, K_3 = 500–800 nM, and K_4 = 2–4 μM [13]. High affinity binding to the first of four ryanodine binding sites has been suggested by Lai *et al.* [16] to result in a low conductance state with subsequent binding to low affinity sites resulting in channel closure [14]. Buck *et al.* [28] found that ryanodine stabilizes multiconformational states of the skeletal RyR calcium release channel. Conductance studies performed on purified calcium release channels incorporated into preformed planar bilayers showed that ryanodine at 5–40 nM increase the open probability by 300% of control without changing the unit conductance of 468 pS. The increase in probability of open time by ryanodine at 5–40 nM was fully reversible. The effect of ryanodine at this concentration correlated with [^{3}H]ryanodine binding to high affinity binding sites (K_{D_1} = 0.7 nM). Ryanodine at 20–50 nM caused occasional fluctuations to a lower conductance state and corresponded to a binding site with a K_{D_2} = 23 nM. Ryanodine at greater than 50 nM stabilized the channel into a low subconductance state of 234 pS which was not readily reversible [13]. At 200 nM, ryanodine caused complete closure of the channel. The increase in open probability of the high conductance state by ryanodine at 5×10^{-9} M corresponds to the maximal increase in oxygen uptake due to calcium recycling observed at lower concentrations (10^{-12} to 10^{-10} M) in intact isolated frog sartorius muscle [5, 29]. The increase in oxygen consumption peaked after 5 min to 10× the basal levels and then slowly returned to basal level after 20 min, suggesting that ryanodine occupation of the high affinity sites causes an initial marked increase in open probability of the Ry/R calcium release but returns to a basal state after 20 min [5].

At higher concentrations at ryanodine (10^{-8} M) the peak increase in oxygen consumption was less than at 10^{-10} M, which would be consistent with ryanodine binding to the low affinity sites with a K_D of 23 nM. Hasselbach and Migala [30] found that ryanodine at concentrations of 2×10^{-4} M decreases the rate of calcium uptake by heavy sarcoplasmic reticulum residues (rabbit) and prevents the release of calcium by 10 mM caffeine. The block of the caffeine release of calcium does not occur if ryanodine is added 1–5 min before the addition of caffeine. The sensitivity to ryanodine is enhanced when the preparations are incubated in high salt solution (0.6 M KCl). The initial retardation by 3×10^{-4} M (300 μM) ryanodine of calcium uptake is followed by an increase in rate of uptake after 5 min. The time of the increased rate of uptake coincides with the latent period required to block the caffeine release of calcium, suggesting that the increased uptake may be due to the transformation of the ryanodine calcium release channel into a closed state, thus decreasing the leak of calcium from the heavy SR. The closed state would prevent caffeine from opening the calcium release channel.

The studies of ryanodine at these high concentrations in intact muscle are more consistent with the calcium release channel being locked into the open state. In contrast, studies on the isolated SR of rabbit muscle show that ryanodine locks the calcium channel in a closed state. Similar findings for the isolated purified receptor incorporated into a bilayer show a contradiction between the physiological–pharmacological studies on the effect of ryanodine on the intact foot present in isolated sartorius muscle preparations and the isolated heavy SR preparations and the purified RyR channel incorporated into a bilayer. Perhaps these discrepancies will be resolved as we reconstitute the proteins of the foot, DHP receptor channels, triadin, calcium release channel, and FK506 binding protein as well as other possible components. The foot structure involved in signal transduction has been well resolved by ultrastructure and biochemical studies. The complexity of signal transduction as revealed by ryanodine binding studies must conform to the physiological process of signal transduction during the func-

tional process of excitation–contraction coupling in skeletal muscle.

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