Pflügers Arch – Eur J Physiol (1996) 432:155–157

SHORT COMMUNICATION

A. Herrmann-Frank · F. Lehmann-Horn

Regulation of the purified Ca^{2+} release channel/ryanodine receptor complex of skeletal muscle sarcoplasmic reticulum by luminal calcium

Received: 23 November 1995 / Received after revision: 4 March 1996 / Accepted: 15 March 1996

Abstract. $^{45}Ca^{2+}$ flux and single channel measurements have revealed that the Ca^{2+} release channel/ryanodine receptor complex of striated muscle is regulated by micromolar cytoplasmic Ca^{2+}. The effect of luminal Ca^{2+}, however, remains controversial. In the experiments presented here, we reconstituted the isolated Ca^{2+} release channel of rabbit skeletal muscle sarcoplasmic reticulum into planar lipid bilayers in the presence of symmetrical K^+ solutions. Using K^+ as the charge carrier, we were able to examine the effect of changes in luminal calcium in the micro- to millimolar range. In the presence of activating cytoplasmic Ca^{2+}, the release channel was activated and inactivated in a concentration-dependent manner by luminal Ca^{2+}. Since increasing cytoplasmic EGTA concentrations shifted the dependence of channel open probability on luminal Ca^{2+} to higher Ca^{2+} concentrations, it is suggested that luminal Ca^{2+} exerts its regulating effect by acting on Ca^{2+} binding sites accessible from the cytoplasmic side of the channel.

Key Words: ryanodine receptor - sarcoplasmic reticulum - calcium channel - Ca^{2+}-induced Ca^{2+} release - skeletal muscle

Introduction

In skeletal and cardiac muscle, Ca^{2+} release from sarcoplasmic reticulum (SR) is mediated by the high conductance, ligand-gated ryanodine receptors/Ca^{2+} release channels. SR Ca^{2+} release is activated by μM Ca^{2+}, mM ATP, nM calmodulin, and inhibited by Ca^{2+} in higher concentrations (>100 μM), mM Mg^{2+}, μM calmodulin, and ruthenium red (for reviews see [1, 7]). All these effectors regulate the release channel activity by interacting with cytoplasmic binding domains of the receptor. On the other hand, the kinetics of Ca^{2+}- and caffeine-induced Ca^{2+} release from isolated SR membrane fragments has been shown to depend on the intraluminal SR loading state [2, 5]. However, data about the regulation of the isolated ryanodine receptor by luminal Ca^{2+} on the single channel level are controversial. Depending on the mechanism of activation, both activating and inhibiting effects have been reported [5, 6, 8, 9]. In the present study, we investigated the effect of luminal Ca^{2+} on the gating properties of the Ca^{2+}-activated isolated Ca^{2+} release channel reconstituted into planar lipid bilayers. Our data show, that luminal Ca^{2+} regulates the isolated channel in a concentration-dependent manner when the driving force for Ca^{2+} is directed from the luminal to the cytoplasmic side of the receptor.

Materials and Methods

Preparation of SR membrane vesicles and purification of the Ca^{2+} release channel/ryanodine receptor complex. 'Heavy' SR (HSR) vesicles were isolated from rabbit back muscle by differential centrifugation as described by [10]. Membrane vesicles were rapidly frozen in liquid nitrogen and stored at -80°C or immediately used for receptor purification. HSR vesicles were suspended in 1 M NaCl, 100 μM EGTA, 150 μM $CaCl_2$, 5 mM AMP, 20 mM Na-PIPES, pH 7.2 in the presence of 1.6 % CHAPS (3-[(3-cholamido-propyl)dimethyl-ammonio]-1-propanesulfonate) (Sigma, Deisenhofen, FRG) and 0.5 % phosphatidylcholine (Avanti Polar Lipids, Alabaster, AL, USA). Unsolubilized material was separated by centrifugation at 59,000 x g. The supernatant was layered on top of 10-28 % sucrose gradients containing 1-0 % CHAPS, 0.7 M NaCl, 3.3 mM AMP, 70 μM EGTA, 100 μM $CaCl_2$, 0.5 % phosphatidylcholine, 1 mM DTE, 13 mM Na-PIPES, pH 7.2. Gradients were centrifuged for 16 h at 136,000 x g. Gradient fractions containing the solubilized ryanodine receptor protein as determined by SDS-PAGE were rapidly frozen in liquid nitrogen and stored at -80°C.

Planar lipid bilayer measurements. The CHAPS-solubilized ryanodine receptor was incorporated into planar lipid bilayers formed across a 250 μm

A. Herrmann-Frank (✉) · F. Lehmann-Horn
Department of Applied Physiology, University of Ulm,
D-89069 Ulm, Germany

aperture from a mixture of phosphatidylethanolamine, phosphatidylserine and L-α-phosphatidylcholine (Avanti Polar Lipids) in the ratio 5:4:1 dissolved in decane (20 mg/ml) (Fluka, Neu-Ulm, FRG). Reconstitution was started in symmetric buffer solution (250 mM KCl, 100 μM EGTA, 150 μM $CaCl_2$, 20 mM PIPES, pH 7.1), using K^+ as the charge carrier. Small aliquots of the solubilized receptor (< 0.5 μg) were added to one side of the bilayer chamber defined as the cytoplasmic (cis) side. Successful incorporation was detected as steplike increase in current. Only channels exhibiting the full conductance of 600-650 pS were used for further analysis. Recordings containing more than one channel were excluded. Electrical signals were filtered at 1 kHz through an 8-pole low-pass Bessel filter and digitised at 3 kHz. Applied voltages are defined with reference to the trans side of the bilayer chamber corresponding to the luminal side of the channel. Open probability values (Po) were calculated from representative data segments of 20-60 s duration. Channel open events are indicated as downward deflections. The experimental temperature was 20°C.

The data were analysed, using pClamp 6.0 (Axon Instruments) and Sigma Plot 5.0 (Jandel Scientific) software. Free concentrations of Ca^{2+} were calculated by a computer program using binding constants published by [3]. Error bars represent SEM.

Results

Ca^{2+} release from skeletal muscle SR is regulated in a concentration-dependent manner by cytoplasmic Ca^{2+} [1, 7]. Fig. 1A shows the effect of changes in the cis (cytoplasmic) Ca^{2+} concentration on the open probability (Po) of the isolated Ca^{2+} release channel. Typically, the channel is activated by cis Ca^{2+} up to 100 μM while higher concentrations are inhibitory. For comparison, Fig. 1B shows the modulatory effect by trans (luminal) Ca^{2+}. When the channel was preactivated by cis Ca^{2+}, the Po was more than 2-fold increased if trans Ca^{2+} was raised to 600 μM. Similar to cis Ca^{2+}, higher concentrations of trans Ca^{2+} decreased the Po of the channel. The regulatory effect of trans Ca^{2+} was only observed at negative holding potentials when the driving force favoured the flux of Ca^{2+} from the trans to the cis side of the bilayer chamber (Fig. 1C). Accordingly, the conductance of the channel decreased in the presence of trans Ca^{2+} concentrations higher than 1 mM (Fig. 2, inset). The trans Ca^{2+}-inhibited channel is further modulated by its endogenous ligands. The channel could be reactivated e.g. by addition of mM ATP to the cis chamber (data not shown). The voltage dependence of the effect of trans Ca^{2+} suggested that Ca^{2+} had to pass the channel pore in order to exert its regulatory action. If this hypothesis is correct, the modulation by trans Ca^{2+} should depend on the cis Ca^{2+} buffer concentration. For this purpose, we increased the EGTA concentration on the cis side of the channel. Fig. 2 shows the correlation between $[EGTA]_{cis}$ and $[Ca^{2+}]_{trans}$ necessary to maximally activate the release channel. In the presence of 100 μM EGTA on the cis side of the channel, maximal activation is achieved at 600 μM trans Ca^{2+}. For comparison, if the cis EGTA concentration is raised to about 5 mM, 4 mM trans Ca^{2+} was necessary. These data suggest that trans Ca^{2+} acts on Ca^{2+} binding sites that are located on the cytoplasmic side of the Ca^{2+} release channel or inside the channel pore.

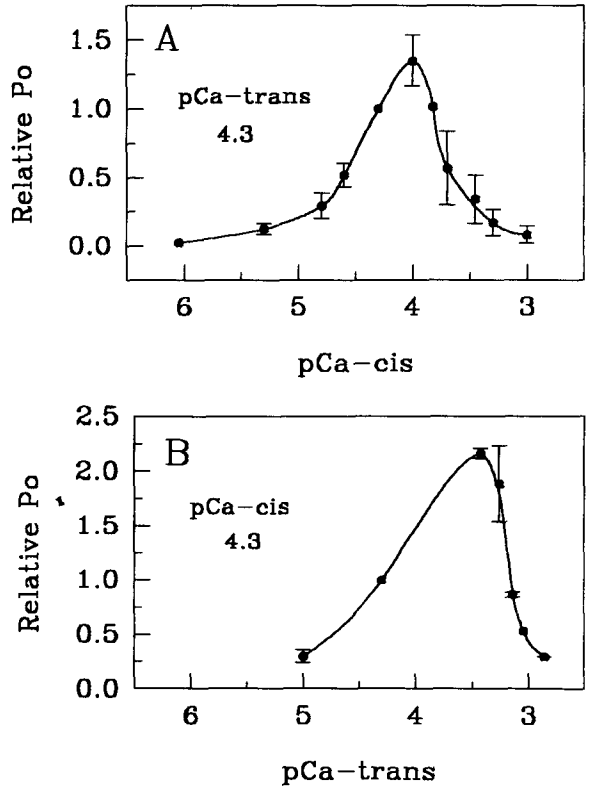
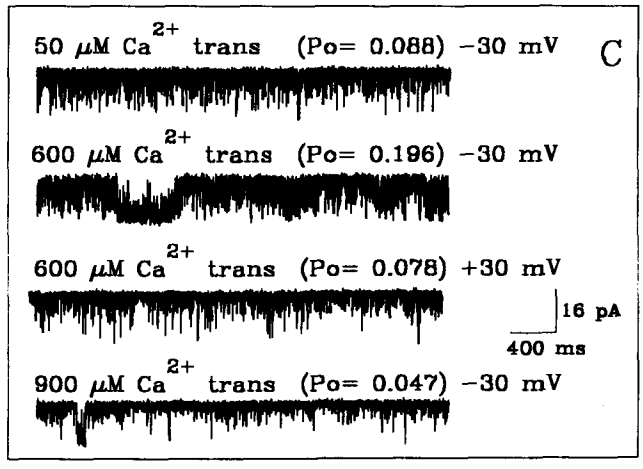


Fig. 1. Regulation of the isolated Ca^{2+} release channel by cytoplasmic and luminal Ca^{2+}. **A** Dependence of channel open probability (Po) on cis (cytoplasmic) Ca^{2+}. Data were taken from single channel recordings (n=9) in the presence of 50 μM free Ca^{2+} trans and indicated concentrations of free cis Ca^{2+}. **B** Dependence of open probability on trans (luminal) Ca^{2+}. Channels (n=5) were preactivated by 50 μM free cis Ca^{2+}. Data in **A** and **B** were normalised to the Po calculated at 50 μM Ca^{2+} cis/trans. **C** Single channel traces of the K^+-conducting Ca^{2+} release channel in the presence of 50 μM free Ca^{2+} cis. The indicated concentrations of trans Ca^{2+} were successively added to the trans bilayer chamber.



Discussion

In skinned skeletal muscle fibres and isolated membrane systems, cytoplasmic Ca^{2+} has been shown to induce SR Ca^{2+} release. Single channel experiments with isolated SR vesicles or with the purified ryanodine receptor, however, revealed that Ca^{2+} by itself is not able to fully activate the Ca^{2+} release channel. Other activating ligands, among them ATP and caffeine, have to be included to achieve maximal activation [1, 7]. In the experiments presented here, we have demonstrated that potentiation of the release channel activity can be mediated by trans (luminal) Ca^{2+}. The data reporting the effect of luminal Ca^{2+} on the single channel level are controversial. While Fill et al. [4] and Ma et al. [6] described an inhibition of the Ca^{2+}-activated channel by higher concentrations of luminal Ca^{2+}, Sitsapesan & Williams [8, 9] observed a regulatory effect by luminal Ca^{2+} only when the channel was activated by other ligands than Ca^{2+}, i.e. ATP and cADPR. In our own experiments, we examined the effect of luminal Ca^{2+} on the cis (cytoplasmic) Ca^{2+}-activated isolated ryanodine receptor and analysed the voltage dependence of this effect. In a similar way to cis Ca^{2+}, the open probability of the release channel was increased or decreased by trans Ca^{2+} in a concentration-dependent manner. This regulation appears to be Ca^{2+} specific. Ba^{2+} in the same concentration range was not able to replace Ca^{2+} (data not shown), indicating that the effects induced by trans Ca^{2+} are not due to surface potential changes. Two findings support the hypothesis that trans Ca^{2+} acts on binding sites located on the cytoplasmic side of the release channel or inside the ion-conducting pathway: 1. Trans Ca^{2+} affected the channel activity only at negative holding potentials, i.e. when the Ca^{2+} flux was directed to the cis side of the channel. 2. When the Ca^{2+} buffer concentration was increased in the cis bilayer chamber, the Po - [trans Ca^{2+}] dependence was shifted to higher Ca^{2+} concentrations. Regulation by cis Ca^{2+} has been suggested to be due to binding of Ca^{2+} to high-affinity activating and low-affinity inhibiting Ca^{2+} binding sites [1, 7]. The biphasic regulation by trans Ca^{2+} indicates that luminal Ca^{2+} interacts with both types of binding sites. An efflux of Ca^{2+} from the SR into the cytoplasm, as it occurs at physiological activation of a muscle fibre, could in this way regulate the open state of the Ca^{2+} release channel. These results favour the idea of a Ca^{2+}-dependent component in EC-coupling of skeletal muscle.

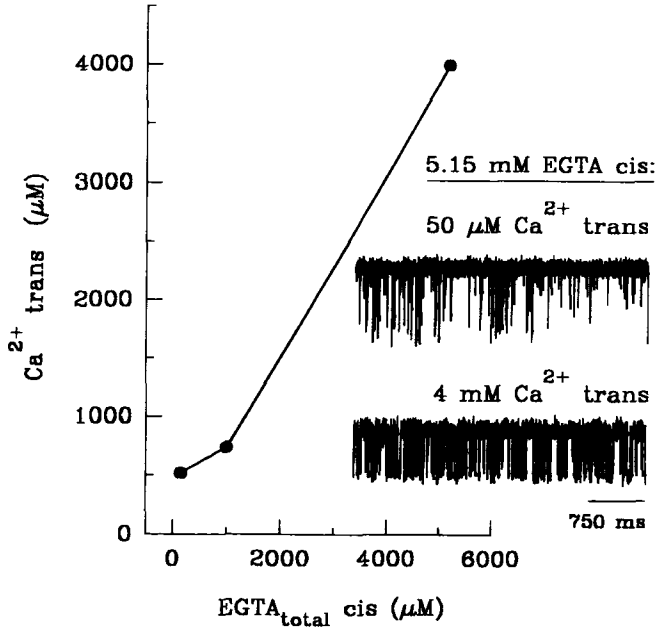


Fig. 2. Dependence of the activating effect of luminal Ca^{2+} on the cytoplasmic Ca^{2+} buffer concentration. The trans (luminal) Ca^{2+} concentration necessary for maximal channel activation has been plotted against the cis (cytoplasmic) EGTA concentration. Inset: Representative single channel recordings in symmetric 250 mM K^+ solutions containing the indicated concentrations of trans Ca^{2+} at 5.15 mM EGTA cis. The channel was activated by 50 μM free cis Ca^{2+}. At the applied holding potential of -31 mV, the unit conductance was 635 pS for 50 μM Ca^{2+} trans and 462 pS for 4 mM Ca^{2+} trans.

Acknowledgements. The authors wish to thank Prof. Dr. H. Ch. Lüttgau for his continuous support. Supported by Deutsche Forschungsgemeinschaft (FG Konzell, Ruhr-University Bochum, FRG) and by a Young Investigator Award from the Ministerium für Wissenschaft und Forschung des Landes Nordrhein-Westfalen to A.H.F..

References

1. Coronado R, Morrissette J, Sukhareva M, Vaughan DM (1994) Structure and function of ryanodine receptors. Am J Physiol 266: C1485-C1504.
2. Donoso P, Prieto H, Hidalgo C (1995) Luminal calcium regulates calcium release in triads isolated from frog and rabbit skeletal muscle. Biophys J 68, 507-515.
3. Fabiato A. (1988) Computer programs for calculating total from specified free or free from specified total ionic concentrations in aqueous solutions containing multiple metals and ligands. Meth Enzymol 157, 378-417.
4. Fill M, Coronado R, Mickelson JR, Vilven J, Ma J, Jacobson BA, Louis CF (1990) Abnormal ryanodine receptor channels in malignant hyperthermia. Biophys J 50, 471-475.
5. Ikemoto N, Ronjat M, Meszaros LG, Koshita M (1989) Postulated role of calsequestrin in the regulation of calcium release from sarcoplasmic reticulum. Biochem 28, 6764-6771.
6. Ma J, Fill M, Knudson M, Campbell KP, Coronado R (1988) Ryanodine receptor of skeletal muscle is a gap junction-type channel. Science 242, 99-102.
7. Meissner G (1994) Ryanodine receptor/Ca^{2+} release channels and their regulation by endogenous effectors. Annu Rev Physiol 56, 485-508.
8. Sitsapesan R, Williams AJ (1995) The gating of the sheep skeletal sarcoplasmic reticulum Ca^{2+}-release channel is regulated by luminal Ca^{2+}. J Membrane Biol 146, 133-144.
9. Sitsapesan R, Williams AJ (1995) Cyclic ADP-ribose and related compounds activate sheep skeletal sarcoplasmic reticulum Ca^{2+} release channel. Am J Physiol 268, C1235-C1240.
10. Herrmann-Frank A, Varsanyi M (1993) Enhancement of Ca^{2+} release channel activity by phosphorylation of the skeletal muscle ryanodine receptor. FEBS Lett 332, 237-242.