FEBS Letters 471 (2000) 256–260 FEBS 23522

ATP-induced activation of expressed RyR3 at low free calcium

Maria Manunta[a], Daniela Rossi[b], Ilenia Simeoni[b], Eugenio Butelli[c], Christoph Romanin[a], Vincenzo Sorrentino[b,c], Hansgeorg Schindler[a,*]

[a] *Institute for Biophysics, University of Linz, Altenberger Strasse 69, A-4040 Linz, Austria*
[b] *Department of Biomedical Sciences, School of Medicine, University of Siena, Siena, Italy*
[c] *DIBIT, San Raffaele Scientific Institute, Milan, Italy*

Received 29 February 2000

Edited by Maurice Montal

Abstract Ca^{2+} channel properties of the mink ryanodine receptor type 3 (RyR3), expressed in HEK293 cells, were studied in planar lipid bilayers to which RyR3 rich membrane fragments had been fused. RyR3 channels were not active at resting levels of Ca^{2+}_{free} but were gated by an additional 1 mM ATP, exhibiting long open times. The second major finding was the absence of channel inactivation at millimolar Ca^{2+}_{free}. Insertion of a myc tag at the N-terminus of RyR3 did not affect the channel properties. As to skeletal muscle, the observed type 3 channel properties appear physiologically meaningful by assisting type 1 channels in calcium release.

Key words: Ryanodine receptor type 3; Ca^{2+} release channel; Ca^{2+} signaling; Adenosinetriphosphate

1. Introduction

Mobilization of calcium from subcellular compartments, in response to an external stimulus, is carried out via intracellular calcium release channels.

These intracellular Ca^{2+} release channels are arranged in two families [1]: inositol 1,4,5-trisphosphate receptors and ryanodine receptors (RyRs). The former group is characterized by the responsiveness to the second messenger inositol trisphosphate [1,2] while the latter is sensitive to a plant alkaloid, ryanodine [3,4].

For both families at least three isoforms have been identified so far. Ryanodine receptors were found to have a fourfold symmetry [5–8] consisting in 560 kDa monomeric proteins assembled together to form homotetrameric channels [9]. The genes encoding RyR isoforms (RyR1, RyR2 and RyR3) are located in different chromosomes and share a sequence homology of at least 60% [10].

RyR1 is the predominant isoform in skeletal muscle and cerebellar Purkinje cells, RyR2 is expressed in cardiac muscle and all over regions of the brain while RyR3 is detectable in specific regions of the brain and in several peripheral tissues [11–14]. The regulation of ryanodine receptors is not completely understood and still remains to be fully elucidated. However, their role in skeletal and cardiac muscle contraction has been extensively studied [15–18]. Concerning RyR3 much less is known than for type 1 or 2 because it is usually found in lower concentrations than RyR1 and RyR2, and, in skeletal muscle, it is associated with RyR1 [13].

Calcium is well known to be the major activator for RyRs. However, it has been shown that other molecules are able to induce Ca^{2+} release. Fabiato and Fabiato [19] as well as Smith et al. [20] have reported that adenine nucleotides could stimulate Ca^{2+}-induced Ca^{2+} release in cardiac and skeletal muscle. More recently an extensive study of adenosine nucleotide effects on cardiac ryanodine receptor has been published by Kermode et al. [21].

With regard to RyR3, its response to ATP is still unclear and the data reported have led to disagreeing conclusions. Microsomes from diaphragm muscle contain two functionally distinct isoforms: channels with typical properties of type 1 and a second population displaying an open probability (P_o) close to zero in the presence of 1 mM ATP at nanomolar calcium concentrations. The latter population exhibits an increasing P_{od} raising calcium to micromolar levels. This second population was identified as RyR3 [22]. This was confirmed at the level of purified RyR3 from bovine diaphragm, which was not activated by ATP at low calcium concentrations [8]. At variance with the two previous results, Chen et al. [23] demonstrated an activation of the recombinant type 3 channels in the presence of 1.5 mM ATP and 64 nM free Ca^{2+}. Also, when using RyR3 purified from rabbit diaphragm, the P_o value was effectively increased by 1 mM ATP in the presence of 0.25 μM free calcium [24].

Here we approach this controversial issue of RyR3 gating by ATP at the specific level of cloned type 3 isoform, both the wild type and the myc-tagged channels. ATP is found to influence gating properties in specific ways, distinct from observations made so far.

2. Materials and methods

2.1. Cell culture and transfection

HEK293 cells were maintained in αMEM medium supplemented with 2 mM glutamine (Bio-Whittaker), 100 μg/ml streptomycin, 100 U/ml penicillin (Bio-Whittaker), 1 mM sodium pyruvate (Bio-Whittaker), 10% heat-inactivated fetal calf serum (FCS) (Gibco) at 37°C in a humidified atmosphere of 5% CO_2.

DNA transfection was carried out using the calcium phosphate medium. 8×10^5 cells were plated on a 100 mm tissue culture dish 24 h before transfection. One hour before DNA addition medium was changed. 5 μg of RyR3 expression vector and 5 μg of carrier DNA were mixed in a solution containing HBS (5 g/l HEPES, 8 g/l NaCl, pH 7.1), 0.7 mM Na_2HPO_4, 0.7 mM NaH_2PO_4, 120 mM $CaCl_2$ and incubated for 30 min. Calcium phosphate precipitates were added to cells and incubated for 6 h.

For stable transfection, Geneticin sulfate G418 (Boehringer) was added 48 h after transfection to a final concentration of 800 μg/ml.

*Corresponding author. Fax: (43)-732-2468 822.
E-mail: h.schindler@jk.uni-linz.ac.at

PII: S0014-5793(00)01385-5

Single colonies were transferred to a 96 well plate, expanded and tested for RyR3 expression.

2.2. Microsomal protein preparation

Cells were harvested with a rubber scraper, pelleted in 50 ml tubes and homogenized in ice-cold buffer A (in mM: 320 sucrose, 5 Na-HEPES pH 7.4 and 0.1 phenylmethylsulfonyl fluoride) using a Teflon potter. Homogenates were centrifuged at $7000\times g$ for 5 min at 4°C. The supernatant obtained was centrifuged at $100\,000\times g$ for 1 h at 4°C. The microsomes were resuspended in buffer A and stored at −80°C. Protein concentration of the microsomal fraction was quantified using the Bradford protein assay kit (Bio-Rad).

2.3. Western blot analysis

Microsomal proteins were separated by SDS–PAGE, as described [11]. Proteins were then transferred to a nitrocellulose membrane (Schleicher and Schuell) using a transfer buffer containing 192 mM glycine, 25 mM Tris, 0.01% SDS and 10% methanol for 7 h at 398 mA at 4°C. Filters were blocked for 1.5 h in a buffer containing 150 mM NaCl, 50 mM Tris–HCl pH 7.4, 0.2% Tween 20, 5% non-fat milk and incubated overnight at room temperature with specific antibodies. Rabbit polyclonal antibodies, able to distinguish the three RyRs, were prepared as previously described [11].

2.4. Planar bilayer measurement

The bilayer apparatus used was as described previously [25]. In short, planar lipid bilayers were formed across a 100 μm sized hole in a 12 μm thick Teflon sheet which separated two chambers containing 250 mM HEPES/110 mM Tris pH 7.4 at the *cis* side, and 250 mM HEPES/53 mM $Ca(OH)_2$ pH 7.4 at the *trans* side. A lipid mixture was used containing PE:PS:PC (L-α-phosphatidylethanolamine:L-α-phosphatidylserine:L-α-phosphatidylcholine, all from Avanti Polar Lipids, Inc., Alabaster, AL, USA) in weight ratio 5:4:1, dissolved at 50 mg/ml in decane. Vesicle fusion was achieved by addition of 750–1000 mM CsCl and 2 mM $Ca(OH)_2$/250 mM HEPES to the *cis* chamber. Fusion events were monitored by chloride channels, occurring within 10 min after addition of 1–5 μl of microsomes. After fusion events, the *cis* chamber was perfused with 250 mM HEPES/110 mM Tris pH 7.4 for 4 min at 3 ml/min. All experiments were carried out at room temperature (20–25°C). Electrical currents were amplified and low pass filtered at 1 kHz with 12 dB/octade with a preamplifier (SR 560, Stanford Research, CA, USA) at low noise setting. Data were recorded with Axotape (Axon Instruments, CA, USA) and stored in a computer (Pentium, 133 MHz) using an analog–digital interface (TL-1-40, Axon Instruments). Open probabilities as well as dwell time distributions were determined from at least 2 min recordings in 1024 bins based on a 50% criterion using PCLAMP 6.0.3 (Axon Instruments). Open and closed time distributions were fitted by two exponentials using simplex maximum likelihood methods.

In all experiments Ca^{2+} concentration was buffered at the *cis* side to values of free Ca^{2+} between 0.1 μM and 1.8 mM by adding 1 mM dibromo-BAPTA (Fluka, Switzerland) to $CaCl_2$ levels between 39 and 3500 μM, as calculated from $K_d = 3\times10^5$ M^{-1} [26], assuming an ionic strength of 350 mM and using Ca^{2+} levels measured with a Ca^{2+} electrode (Orion SA 720, Orion, Boston, MA, USA).

ATP was purchased from Sigma and AMP-PCP, used as control, was obtained from Boehringer Mannheim.

If no activity was observed after addition of at least 10 μM Ca^{2+} and 1 mM ATP after fusion, the membrane was considered not to contain activatable RyR3, and the experiment was canceled.

2.5. Microsyringe assay

In order to increase the fusion rate of the microsomes to the planar lipid bilayer, 1–3 μl of the sample was directly applied to the membrane with a microsyringe into the *cis* chamber. The microsyringe, with an inner diameter of 0.85 mm, is provided with a piston that permits direct application to the membrane [27]. The distance between the lipid bilayer and the microsyringe was adjusted to about 0.15 mm.

3. Results

The RyR3 coding sequence from mink was expressed in the human cell line HEK293. Myc-tagged RyR3 protein at the amino-terminus was expressed in the same cell type. Both expressed proteins were verified by Western blot (Fig. 1).

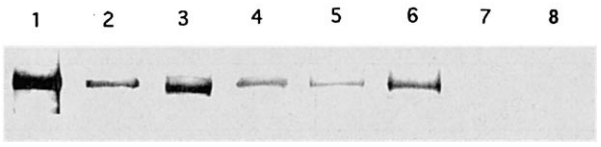

Fig. 1. Expression of wild type and myc-tagged RyR3 transfected in HEK293 cells. Microsomal proteins were obtained from cells transfected with a plasmid expressing cDNA of RyR3 (20 μg, lane 4), the construct of RyR3 myc tag (20 μg, lanes 5, 6), with the expression vector (30 μg, lane 7). Control tissues were: skeletal muscle (10 μg, lane 1), cardiac muscle (5 μg, lane 2) and bovine diaphragm (50 μg, lanes 3, 8). Lane 1 was immunoblotted with polyclonal antibody anti-RyR1, lane 2 with a polyclonal antibody against RyR2, lanes 3, 4, 5, 7 with polyclonal antibody anti-RyR3 and lanes 6, 8 with antibody anti-myc tag.

Microsomes from transfected cells stably expressing wild type (RyR3-wt) and tagged (RyR3-myc) proteins were prepared.

When either RyR3-wt or RyR3-myc was incorporated into planar lipid bilayers, single channel activity was observed (Fig. 2a). In the absence of Ca^{2+} buffering and of ATP, channel P_o varied considerably, between 0.83 and 0.17. This might be caused by varying levels of contaminating calcium in the buffer solutions.

Therefore, in these experiments, the concentration of Ca^{2+}_{free} was first set to a very low value ($Ca^{2+}_{free} < 20$ nM) adding 1 mM dibromo-BAPTA to the *cis* chamber, equivalent to the cytosolic side. In this case, no activity or very low channel activity was detected (Fig. 2b). The P_o was 0.07 and 0.05 for the wild type and the myc-tagged RyR3, respectively (Fig. 3).

Addition of ATP to the *cis* side reactivated both RyR3-wt and RyR3-myc channels (Fig. 2c, left and right traces). Exposure to millimolar ATP, at nanomolar free Ca^{2+} concen-

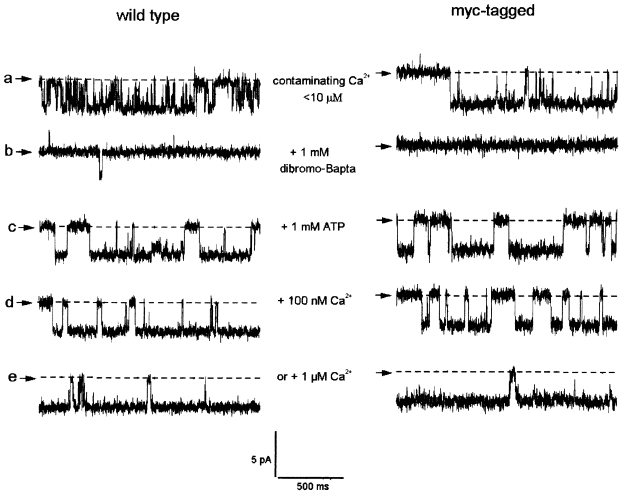

Fig. 2. Channel traces of cloned RyR3. The left and right panels show typical channel traces for single channel experiments with expressed wild type and myc-tagged RyR3. Recordings were made at 0 mV in asymmetric buffer conditions. The spontaneous channel activity (a) was blocked by 1 mM dibromo-BAPTA (b), reducing Ca^{2+}_{free} from <10 μM to <20 nM. In the following step (c), 1 mM ATP at the *cis* side led to reopening of the channel, further enhanced by subsequent addition of 39 or 290 μM $CaCl_2$, respectively (d and e). The channel openings are shown as downward deflections with the baselines indicated by dashed lines and arrows.

tration, led to a P_o value of 0.28 ± 0.09 for the expressed wild type channel and to $P_o = 0.43 \pm 0.29$ for the myc-tagged channel (Fig. 3). This level of open probability for channel gating by 1 mM ATP did not significantly increase by raising the Ca^{2+}_{free} to 0.1 or 1 μM (see Fig. 3), although channel kinetics were influenced considerably (see below).

Channel activation by ATP, for the cloned channels, contrasts with results on native RyR3 (from diaphragm sarcoplasmic reticulum and/or terminal cisternae), which was not activated by 1 mM ATP under the same conditions ([22] and personal observations). In order to ensure that the observed response did not involve phosphorylation of the protein, a non-hydrolyzable ATP analog was used. At 0.5 mM AMP-PCP, the channel was clearly activated to a P_o value of 0.11 ± 0.02 (data not shown).

Single channel traces permitted analysis of the gating kinetics of the expressed type 3 channels. At 1 mM ATP and nanomolar Ca^{2+}_{free} gating kinetics were generally slow as seen in Fig. 2c.

ATP increased the duration of the open time events, affecting the open probability, while its effect on the frequency of channel opening was minor. Open time distributions were well represented by two exponentials (Fig. 4, left), suggesting that RyR3 has at least two open states. At low Ca^{2+} and 1 mM ATP the open time distributions were quite similar for both the expressed wild type and the myc-tagged RyR3 channels. Characteristic values are $\tau_{o1} = 3.22$ ms and $\tau_{o2} = 29.94$ ms for the former and $\tau_{o1} = 3.69$ ms and $\tau_{o2} = 36.38$ ms for the latter. Both characteristic times occurred in comparable proportions for both types of channels.

While the myc tag, inserted at the N-terminus, appeared not to affect the channel open times, the closed time distributions of the myc-tagged channel clearly displayed longer lasting open events, when compared to the wild type channel (Fig. 4, right).

Starting from ATP-activated channels, an increase of the free calcium concentration had drastic effects on channel kinetics. With increasing Ca^{2+}_{free} the mean open times decreased

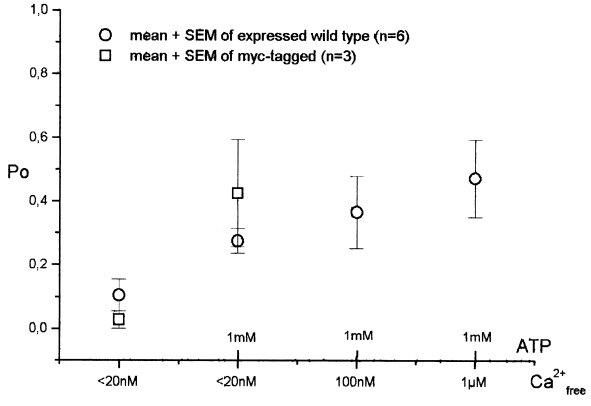

Fig. 3. Open probability of expressed wild type and myc-tagged RyR3 inserted in planar lipid bilayers. Open probability of expressed RyR3-wt (○, $n = 6$) and RyR3-myc (□, $n = 3$) at the indicated buffer conditions, in the presence of ≤20 nM Ca^{2+}_{free} and 1 mM ATP with subsequent addition of 39 or 290 μM $CaCl_2$ to adjust the free Ca^{2+} to 100 nM and 1 μM, respectively. For each condition, the open probabilities were determined from at least 2 min continuous recordings. The symbols and the error bars represent the mean open probability ± S.E.M. P_o from multichannel experiments data was normalized to $n\ P_o/N$ (N = number of levels observed).

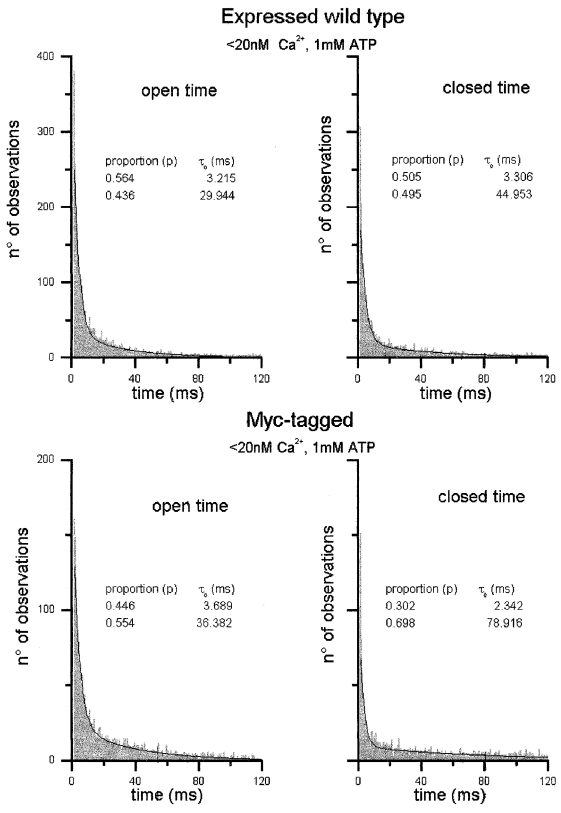

Fig. 4. Kinetics of the cloned RyR3 channels. Open time (left) and closed time (right) distributions of ATP-activated channels are shown for cloned wild type RyR3 (upper panel) and the myc-tagged RyR3 (bottom panel). The potential was held at 0 mV and the *cis* buffer was 250 mM HEPES/110 mM Tris pH 7.4, 1 mM dibromo-BAPTA and 1 mM ATP.

(Fig. 5). The values of 3.22 and 29.94 ms at ≤20 nM Ca^{2+}_{free} (Fig. 4 left, upper panel) decreased to 1.75 and 11.53 ms at 0.1 μM, or to 1.93 and 13.73 ms at 1 μM Ca^{2+}_{free}.

A particular characteristic of these cloned channels is their responsiveness to high calcium levels (Fig. 6). At 1 mM ATP and a Ca^{2+}_{free} level as high as 1.8 mM, channel open probability increased to reach values close to 1 (Fig. 6a,b) with extremely

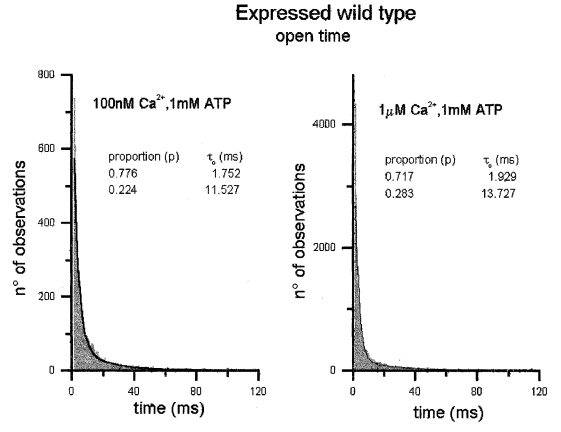

Fig. 5. Effect of Ca^{2+} on the open lifetimes of the ATP-activated RyR3-wt channel. The *cis* buffer contained 250 mM HEPES/110 mM Tris pH 7.4, 1 mM dibromo-BAPTA, 1 mM ATP, 39 and 290 μM $CaCl_2$, respectively.

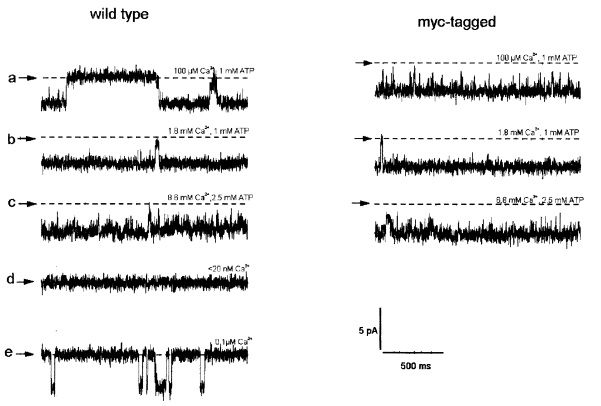

Fig. 6. Absence of RyR3 inactivation at millimolar Ca^{2+}_{free}. The channels were primarily found in the open state both for the wt (left panel) and myc-tagged (right panel) RyR3 channels. The left trace in a shows a rare observation of a long channel closing event. The holding potential was 0 mV. To the normal *cis* buffer (250 mM HEPES/110 mM Tris pH 7.4) 1 mM dibromo-BAPTA, 1 mM ATP, 1076 μM (a) and 3.5 mM (b) $CaCl_2$ were added. The total concentrations were then increased to 2.5 mM ATP and to either 9.5 (c, right trace) or 11.5 (c, left trace) mM $CaCl_2$. For the traces, 1 mM dibromo-BAPTA (d), and 39 μM $CaCl_2$ (e) were added into the *cis* compartment, demonstrating that the channel can be reactivated by changing buffer conditions.

rare longer lasting closing events (exemplified in the upper left trace). At even higher Ca^{2+}_{free} concentrations (8.6 and 6.8 mM in Fig. 6c), the channels still showed no inactivation but fast flickering, which was reduced by increasing the level of ATP to 2.5 mM (see traces in Fig. 6c). Thus, both the expressed wild type and the myc-tagged RyR3 are highly active at millimolar Ca^{2+}_{free} concentrations. Traces d and e of Fig. 6 provide evidence that the channel still behaved normally at low Ca^{2+} (d) and reactivated by Ca^{2+} (e), having experienced very high Ca^{2+}_{free} before.

Another interesting observation was that cloned RyR3-wt and RyR3-myc exhibited one pronounced subconductance state (Fig. 7). Although rarely observed (≤1%), its occurrence was highly specific and is, therefore, regarded as a property of these expressed channels. The subconductance state was at 68% of full conductance (O_1 in Fig. 7). It was populated

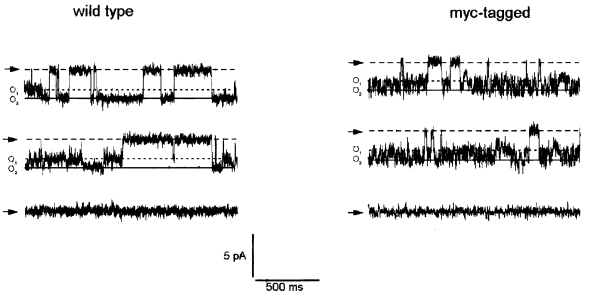

Fig. 7. Evidence for a subconductance state of expressed wild type and myc-tagged RyR3. The subconductance state (O_1) is indicated by a dotted line, the state of normal conductance (O_2) by a straight line. Single channel current was recorded at a holding potential of 0 mV and the calcium concentration was <20 nM. The traces showing subconductances were recorded in the presence of 1 mM dibromo-BAPTA and millimolar ATP. The bottom traces show pure baseline fluctuations, in the presence of 1 mM dibromo-BAPTA and in the absence of ATP.

mostly by fast transitions to and from the full conductance state (O_2); this switching mode between O_1 and O_2 increased the open state lifetime considerably, indicating that channel closure occurs via state O_2.

4. Discussion

Previous studies came to differing conclusions concerning the ATP response of the RyR3 channel [8,22–24]. In this study we analyzed the response to ATP of the recombinant wild type and myc-tagged RyR3 channel in planar lipid bilayers.

When free calcium was set below 20 nM at the *cis* side (cytosolic side), 1 mM ATP clearly activated the channel. More precisely, ATP acts at least as an inducer, enhancing and promoting the gating activity at ≤20 nM Ca^{2+}_{free}. Evidence that this effect is by direct ATP binding, rather than via a secondary process requiring ATP hydrolysis, was obtained using AMP-PCP, a non-hydrolyzable analog of ATP.

The early involvement of RyR3 in the excitation–contraction coupling [28] is in agreement with the ATP effect at low calcium.

The differences in the protein sequence between the three isoforms are likely related to distinctions in functional properties, for which at least the most extensively studied type 1 and type 2 RyR provide examples [29]. Besides such divergence, some common regions of the protein may contain significant binding sites, basic for the function of all isoforms.

The calcium binding site has been resolved to be between amino acid (aa) positions 4478 and 4512, for the skeletal muscle RyR [30,31]. Calcium is well known to be a major stimulating agent for all RyRs, including type 3, as is evident from the effects of Ca^{2+}_{free} on channel kinetics reported here. This is also supported by an increase of P_o, calcium-induced, in the absence of ATP (data not shown).

Among the several supposed ATP binding sites, one site appears to be well conserved in mink RyR3 (aa 2237–2242) [31]. It can be speculated that at low Ca^{2+} levels the ATP binding sites are more easily accessible to ATP molecules resulting in an ATP-induced activation of the channel. ATP seems to act synergistically with calcium, and it affects the duration of the openings rather than, as for calcium, their frequency. In all experiments the channels were gated by ATP at nanomolar Ca^{2+} concentrations, and an increase of the open probability as well as the open time has been observed.

While cloned RyR3 are highly susceptible to ATP at low Ca^{2+}_{free}, ATP was less effective at high levels of free Ca^{2+}. At this regime features of Ca^{2+} activation became dominant, i.e. high opening rates and short open times. It remains open whether the reduced ATP effect reflects reduced ATP binding at high Ca^{2+}_{free} or a reduced coupling of its binding energy to the channel structure, due to Ca^{2+}-induced conformational changes, or interactions between neighboring binding sites, or local electrostatic potentials.

On the one hand, the use of cloned RyR3 provided a safer characterization of channel properties, in comparison to mixtures of isoforms in a native environment. On the other hand, its environment is lacking components which may be associated in vivo. Candidates for regulatory associated proteins are FKBP12, calmodulin, sorcin, or other RyRs. Such interactions may well cause the different behaviors of native and

expressed RyR3 channels, with respect to ATP activation and occurrence of substates.

Subconductance states are peculiarities for calcium release channels in skeletal as well as in cardiac muscles that are thought to reflect diversities of the channel structure in response to different possible interactions with associated proteins (other RyRs and/or regulatory proteins). This view is supported by the finding that the binding of regulatory proteins such as FKBP12 [24] to RyR3 has a stabilizing effect on channel gating and on channel open state currents to defined values. The different action of ATP found for RyR3 channels, discussed above, may also be caused by variable interaction with associated components.

Our results on expressed RyR3 concerning the sensitivity to ATP reveal characteristics closer to RyR1 than to RyR2. In contrast, concerning the absence of inactivation at high Ca^{2+}, type 3 resembles type 2 more than type 1, which is inactivated at mM Ca^{2+}_{free}. It is remarkable that at nanomolar Ca^{2+}, in the presence of ATP alone, the gating kinetics are rather slow while shorter and more frequent openings are displayed in the presence of higher calcium.

The behavior of the myc-tagged channel fully corresponds to which has been reported for the wild type. It gives clear evidence that the myc tag at the amino-terminus does not interfere with the gating activity. This evidence strongly suggests that the N-terminal tag is not located close to the pore of the protein and that it does not prevent any conformational change of the receptor that could inhibit its activity. The myc tag does not interact, at least, with the ATP and the calcium binding sites and allows a correct channel function including the absence of Ca^{2+} inactivation, found for the wild type channel.

This absence of inactivation is in agreement with the conclusions of Sonnleitner et al. [22] and with other reports [9,32,33].

The channel activity at high calcium indicates that RyR3 should sustain the calcium release when RyR1 is no longer gating. Thus, the physiological meaning of our findings may be related to the checkerboard organization and colocalization of type 1 and type 3 RyRs in skeletal muscle including cooperation with FKB proteins. These data suggest that the gating of the channel is quite similar to RyR1 at the beginning of the muscle activation, indicating that RyR3 could assist the early rise of the calcium release, and could maintain the release when RyR1 is closed by high calcium.

This would agree with results on spark parameters observed in neonatal muscles, expressing both RyR1 and RyR3, and in the same muscles prepared from RyR3 knock-out mice [34].

Acknowledgements: We thank Vassili Pastushenko for suggestions and help in data analysis and Greg Harms for reading the manuscript. This work was supported by EEC Grant BIO4 CT96 0592 to H.S. and to V.S., and Telethon Grant 1151 to V.S.

References

[1] Berridge, M.J. (1993) Nature 361, 315–325.
[2] Berridge, M.J. and Irvine, R.F. (1989) Nature 341, 197–205.
[3] Fleischer, S., Ogunbunmi, E.M., Dixon, M.C. and Fleer, A.M. (1985) Proc. Natl. Acad. Sci. USA 82, 7256–7259.
[4] Coronado, R., Morrissette, J., Sukhareva, M. and Vaughan, D.M. (1994) Am. J. Physiol. 266, C1485–C1504.
[5] Saito, A., Inui, M., Radermacher, M., Frank, J. and Fleischer, S. (1988) J. Cell. Biol. 107, 211–219.
[6] Serysheva, I.I., Orlava, E.V., Chiu, W., Sherman, M.B., Hamilton, S.L. and van Heel, M. (1995) Nature Struct. Biol. 2, 18–23.
[7] Sharma, R.M., Panczek, P., Grassucci, R., Xin, H.B., Fleischer, S. and Wagenknecht, T. (1998) J. Biol. Chem. 273, 18429–18434.
[8] Jeyakumar, L.H., Copello, J.A., O'Malley, A.M., Wu, G.M., Grassucci, R., Wagenknecht, T. and Fleischer, S. (1998) J. Biol. Chem. 273, 16011–16020.
[9] Sutko, J.L. and Airey, J.A. (1996) Physiol. Rev. 76, 1027–1071.
[10] Sorrentino, V. and Volpe, P. (1993) Trends Pharmacol. Sci. 14, 98–103.
[11] Giannini, G., Conti, A., Mammarella, S., Scrobogna, M. and Sorrentino, V. (1995) J. Cell Biol. 128, 893–904.
[12] Conti, A., Gorza, L. and Sorrentino, V. (1996) Biochem. J. 316, 19–23.
[13] Murayama, T. and Ogawa, Y. (1997) J. Biol. Chem. 273, 24030–24037.
[14] Balschun, D., Wolfer, D.P., Bertocchini, F., Barone, V., Conti, A., Zuschratter, W., Missiaen, L., Lipp, H.P., Frey, J.U. and Sorrentino, V. (1999) EMBO J. 18, 5264–5273.
[15] Takeshima, H., Nishimura, S., Matsumoto, T., Ishida, H., Kangawa, K., Minamino, N., Matsuo, H., Ueda, M., Hanaoka, M., Hirose, T. and Numa, S. (1989) Nature 339, 439–445.
[16] Marks, A.R., Tempst, P., Hwang, K.S., Taubman, M.B., Inui, M., Chadwick, C., Fleischer, S. and Nadal-Ginard, B. (1989) Proc. Natl. Acad. Sci. USA 86, 8683–8687.
[17] Otsu, K., Willard, H.F., Khanna, V.K., Zorzato, F., Green, N.M. and MacLennan, D.H. (1990) J. Biol. Chem. 265, 13472–13483.
[18] Zorzato, F., Fujii, J., Otsu, K., Phillips, M., Green, N.M., Lai, F.A., Meissner, G. and MacLennan, D.H. (1990) J. Biol. Chem. 265, 2244–2256.
[19] Fabiato, A. and Fabiato, F. (1978) J. Physiol. 276, 233–255.
[20] Smith, J.S., Coronado, R. and Meissner, G. (1986) J. Gen. Physiol. 88, 573–588.
[21] Kermode, H., Williams, A.J. and Sitsapesan, R. (1998) Biophys. J. 74, 1296–1304.
[22] Sonnleitner, A., Conti, A., Bertocchini, F., Schindler, H. and Sorrentino, V. (1998) EMBO J. 17, 2790–2798.
[23] Chen, S.R.W., Li, X., Ebisawa, K. and Zhang, L. (1997) J. Biol. Chem. 272, 24234–24246.
[24] Murayama, T., Oba, T., Katayama, E., Oyamada, H., Oguschi, K., Kobayashi, M., Otsuka, K. and Ogawa, Y. (1999) J. Biol. Chem. 274, 17297–17308.
[25] Schindler, H. (1989) Methods Enzymol. 171, 225–253.
[26] Harrison, S.M. and Beers, D.M. (1987) Biochim. Biophys. Acta 925, 133–143.
[27] Hain, J., Nath, S., Mayrleitner, M., Fleischer, S. and Schindler, H. (1994) Biophys. J. 67, 1823–1833.
[28] Bertocchini, F., Ovitt, C.E., Conti, A., Barone, V., Schoeler, H.R., Bottinelli, R., Reggiani, C. and Sorrentino, V. (1997) EMBO J. 16, 6956–6963.
[29] Sorrentino, V. and Reggiani, C. (1999) Trends Cardiovasc. Med. 9, 54–61.
[30] Chen, S.R., Zhang, L. and MacLennan, D.H. (1992) J. Biol. Chem. 267, 23318–23326.
[31] Sorrentino, V. (1995) Adv. Pharmacol. 33, 67–90.
[32] Percival, A., Williams, A., Kenyon, J., Grinsell, M., Airey, J.A. and Sutko, J.L. (1994) Biophys. J. 67, 1834–1850.
[33] O'Brien, J., Valdivia, H.H. and Block, B.A. (1995) Biophys. J. 68, 471–482.
[34] Conklin, M., Barone, V., Sorrentino, V. and Coronado, R. (1999) Biophys. J. 77, 1394–1403.