Ca^{2+} channel agonist BAY-k 8644 does not elicit Ca^{2+} release from skeletal muscle sarcoplasmic reticulum

Francesco Zorzato, Pompeo Volpe+, Giovanni Salviati and Alfredo Margreth

Centro di Studio per la Biologia e la Fisiopatologia Musculare del CNR, Istituto di Patologia Generale dell'Universita' di Padova, via Loredan 16, 35131 Padova, Italy

Received 25 April 1985

BAY-k 8644, a nifedipine analogue, promotes Ca^{2+} influx into excitable cells via plasma membrane voltage-sensitive Ca^{2+} channels. We report here that sarcoplasmic reticulum (SR) Ca^{2+} release channels are insensitive to BAY-k 8644, as studied in highly purified isolated fractions and in chemically skinned fibers of rabbit skeletal muscle This result suggests that a subcellular heterogeneity exists among Ca^{2+} channels, at least with respect to drug-receptor sites In the course of this study, however we found that BAY-k 8644 reversibly inhibits the SR Ca^{2+} pump, i e , it decreases Ca^{2+} influx into the SR lumen, although at concentrations ($IC_{50} = 3–5 \times 10^{-5}$ M) much higher than those effective on voltage-sensitive Ca^{2+} channels

BAY-k 8644 *Sarcoplasmic reticulum* *Ca^{2+} release* *Ca^{2+} pump*

1. INTRODUCTION

During the past few years, specific, voltage-sensitive Ca^{2+} channels (VSCC) have been discovered in the plasma membrane of excitable cells (review [1]), and in transverse tubules (T-tubules) of skeletal muscle [2]. Transmembrane Ca^{2+} influx via VSCC can be specifically blocked by a group of compounds known as Ca^{2+} antagonists [3], e.g., verapamil, nifedipine and diltiazem. Recently, BAY-k 8644, a nifedipine analogue, has been found to have Ca^{2+} agonist properties, i.e., to increase the contractility of cardiac and smooth muscle, following stimulation of Ca^{2+} influx via VSCC [4], and to increase several-fold Ca^{2+} currents in skeletal muscle T-tubules [5].

Skeletal muscle SR is an intracellular network of membranes which controls the contraction-relaxation cycle by raising and lowering myoplasmic Ca^{2+} concentration. Morphological studies [6] have shown that the SR membrane system consists of two different portions, i.e., longitudinal tubules (LSR) and terminal cisternae (TC). Ca^{2+} efflux pathways (Ca^{2+} channels) for Ca^{2+} release following muscle excitation are selectively localized in TC [7,8]. On the other hand, the ATP-dependent Ca^{2+} pump [9] is uniformly distributed in the SR membrane, with the exception of the junctional area with T-tubules [10].

The results reported here indicate that Ca^{2+} channel agonist BAY-k 8644 has no effect on Ca^{2+} efflux from SR, and are consistent with the hypothesis that SR and plasma membrane Ca^{2+} channels of skeletal muscle are different [11,12]. On the other hand, BAY-k 8644 appears to be a potent, reversible inhibitor of the SR Ca^{2+} pump.

2. EXPERIMENTAL

2.1. *Materials*

BAY-k 8644 was a gift of Dr B. Garthoff (Bayer AG, Wuppertal, FRG). Antipyrylazo III was obtained from Sigma, pyruvate kinase and lactate dehydrogenase from Boehringer, and A23187 from Calbiochem.

+ To whom correspondence should be addressed

Published by Elsevier Science Publishers B V (Biomedical Division)
00145793/85/$3 30

2 2 *SR isolation*

SR was isolated from the fast-twitch skeletal muscles of New Zealand White male rabbits, and further fractionated into LSR and TC by density gradient centrifugation as described [3,14] SR fractions were resuspended in 0.3 M sucrose, 5 mM imidazole, pH 7.4, and stored at −70°C until used. Protein concentration was determined according to Lowry et al. [15], using bovine serum albumin as standard

2 3 *Ca^{2+} loading and Ca^{2+}-dependent ATPase activity of SR fractions*

Ca^{2+} loading, in the presence of precipitating anions, was measured using the Ca^{2+} indicator antipyrylazo III, by following A(710–790 nm) in a Perkin Elmer 356 spectrophotometer The assay was carried out at room temperature (about 22°C) in a medium containing, in a final volume of 1 ml, 92 mM K-phosphate, pH 7 0, 200 μM antipyrylazo III, 1 mM $MgSO_4$, 1 mM Na_2ATP and 30 μg SR protein The reaction was started by adding 50 μM $CaCl_2$ [16]

ATPase activities were measured by a coupled-enzyme assay [17], following NADH oxidation at 340 nm in a Perkin Elmer 551S spectrophotometer The assay was carried out at 37°C in a medium containing, in a final volume of 3 ml, 20 mM histidine, pH 7.2, 0.1 M KCl, 5 mM $MgSO_4$, 2 mM ATP, 0 15 mM NADH, 0 5 mM phosphoenolpyruvate, 5 U pyruvate kinase, 5 U lactate dehydrogenase and 5 or 15 μg of LSR and TC, protein, respectively Mg^{2+}-dependent ATPase (basal ATPase) activity was measured in the presence of 0.2 mM EGTA. Ca^{2+}-dependent ATPase was determined as the difference between total ATPase, measured in the presence of 0 2 mM $CaCl_2$ (estimated free Ca^{2+} = 10 μM), and the basal ATPase. Maximal Ca^{2+}-dependent ATPase activity was measured in the presence of Ca^{2+} ionophore A23187 (1 5 μM)

2.4. *Skinned fiber experiments*

Chemically skinned fibers were prepared from the adductor muscle of New Zealand White male rabbits, by exposure to a 'skinning solution' containing 5 mM K_2-EGTA, 0.17 M K-propionate, 2.5 mM K_2Na_2ATP, 2.5 mM Mg-propionate and 10 mM imidazole-propionate, pH 7.0 [13,18] After 24 h at 0°C, muscle bundles were transferred to skinning solution made up in 50% glycerol and stored at −20°C until used.

Ca^{2+} loading in the presence of oxalate was measured by a light scattering method [19] Ca^{2+} accumulation into the SR lumen elicits precipitation of Ca-oxalate crystals and increases fiber light scattering, which, after a lag phase, becomes linear and proportional to the amount of calcium accumulated. Segments of skinned fibers were placed in a 0 6 ml chamber, attached to stainless-steel clamps and stretched to 130% of slack length in solution R (5 mM K_2-EGTA, 0 17 M K-propionate, 2.5 mM Mg-propionate, 5 mM Na_2K_2ATP and 10 mM imidazole propionate, pH 7 0) Fibers were then exposed to a p*Ca* 6.4 solution (0 17 M K-propionate, 2.5 mM Mg-propionate, 5 mM Na_2K_2ATP, 2.15 mM $CaCl_2$, 5 mM K_2-EGTA and 10 mM imidazole propionate, pH 7 0), and Ca^{2+} loading was started by adding 5 mM oxalate

3. RESULTS

3 1. *BAY-k 8644 inhibits Ca^{2+} loading by SR of skinned fibers*

Fig.1A shows, as monitored by a light scattering method, that BAY-k 8644 inhibited oxalate-facilitated Ca^{2+} loading by the SR of single skinned skeletal muscle fibers (IC_{50} 33 μM). The effect appeared to be reversible (fig 1B), since the

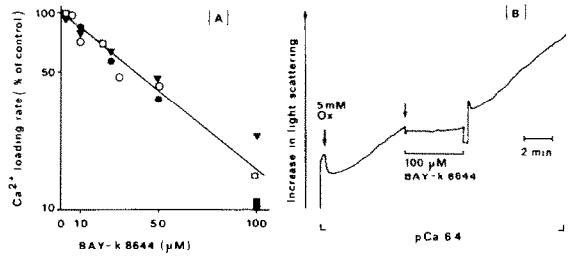

Fig 1 BAY-k 8644 inhibits Ca^{2+} loading by SR of chemically skinned fibers Panel B shows a typical trace of Ca^{2+} loading by SR of single skinned fiber monitored by a light scattering method The fiber was exposed to a p*Ca* 6 4 solution, that, after 60 s, was supplemented with 5 mM oxalate Addition and removal of BAY-k 8644 caused inhibition and restoration of Ca^{2+} loading, respectively Panel A summarizes data obtained from several experiments, as shown in B, on 5 different fibers Data are expressed as percentage of control rates, and fitted by linear regression analysis (r = −0 95)

removal of BAY-k 8644, after Ca^{2+} loading had been completely blocked by the drug (100 μM), restored the initial rate of Ca^{2+} loading.

To determine whether the inhibition was due to decrease of Ca^{2+} influx into the SR lumen, mediated by the Ca^{2+} pump, enhancement of Ca^{2+} efflux from the SR via specific Ca^{2+} channels, or both, we investigated the effect of BAY-k 8644 on isolated SR fractions.

3.2. *BAY-k 8644 inhibits Ca^{2+} loading and Ca^{2+}-dependent ATPase activity of LSR and TC fractions*

Fig.2 shows that BAY-k 8644 inhibited Ca^{2+} loading in both LSR and TC. LSR was more sensitive than TC fractions, the IC_{50} being 31 μM (fig. 2B). Dose-response curves were similar to that observed with skinned fibers (fig 1A), and indicated that Ca^{2+} loading decayed exponentially with first-order kinetics.

Fig.3 shows that BAY-k 8644, at concentrations above 5 μM, likewise inhibited Ca^{2+}-dependent ATPase activity of TC and LSR fractions, to a similar extent, in the absence of Ca^{2+} ionophore A23187 (fig.3A; IC_{50} 32 and 30 μM, respectively), as well as in its presence (fig.3B; IC_{50} 47 and 53 μM, respectively). Since SR Ca^{2+}-ATPase couples the hydrolysis of ATP to active Ca^{2+} transport [9], these results imply that the inhibition of Ca^{2+} loading by BAY-k 8644 is due to a direct effect on the Ca^{2+} pump and the consequent reduced rate of Ca^{2+} influx into the SR lumen.

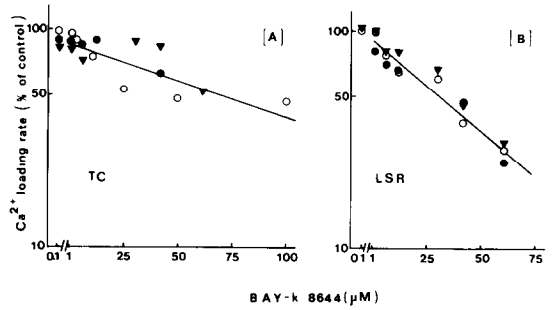

Fig 2. BAY-k 8644 inhibits Ca^{2+} loading by SR fractions. Ca^{2+}-loading rate was measured using antipyrylazo III as Ca^{2+} indicator Data were obtained on 3 different SR preparations, are expressed as percentage of control rates, and fitted by linear regression analysis ($r = -0\,85$ and $-0\,96$ in panel A and B, respectively). Control rates were $0\,49 \pm 0\,22$ and $4\,95 \pm 0\,78$ μmol Ca^{2+}/min per mg protein for TC (A) and LSR (B), respectively

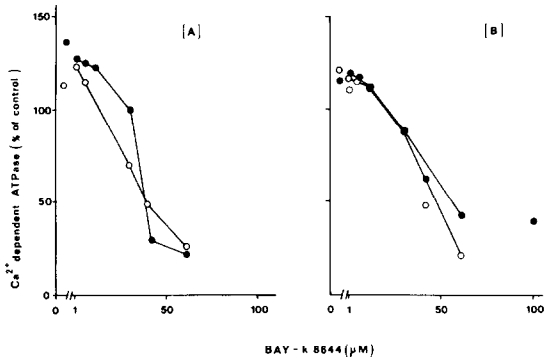

Fig.3. Effect of BAY-k 8644 on Ca^{2+}-dependent ATPase activity of SR fractions ATPase activities were measured using an enzyme-coupled assay as described in section 2. Data were obtained on two different SR preparations, and are expressed as mean percentage of control activities. In the absence of A23187, control activities of LSR (●) and TC (○) were 7.06 and 6 23 μmol P_i/min per mg protein, respectively In the presence of A23187 3(B), control activities of LSR (●) and TC (○) were 16 23 and 9.49 μmol P_i/min per mg protein, respectively Below 5 μM, BAY-k 8644 increased Ca^{2+}-dependent ATPase activity of both LSR and TC (A) by 20–30%, even though it inhibited Ca^{2+} loading (see fig.2) At low concentrations, BAY-k 8644 might uncouple ATP hydrolysis from active Ca^{2+} transport

4. DISCUSSION

Here we provide new evidence that BAY-k 8644 is a reversible inhibitor of the Ca^{2+} pump of rabbit skeletal muscle SR. As compared to other Ca^{2+} pump inhibitors, BAY-k 8644 appears to be as potent as quercetin [20] and vanadate [21], although its mode of action remains to be elucidated.

We also observed that BAY-k 8644 has no effect on SR Ca^{2+} release channels, and this confirms similar findings that Ca^{2+} antagonists have no effect on SR Ca^{2+} channels [11,12,22]. Since the Ca^{2+} agonist BAY-k 8644 and the Ca^{2+} antagonist nifedipine act competitively on VSCC [23], and skeletal muscle T-tubules VSCC are blocked by Ca^{2+} antagonists [24] and activated by BAY-k 8644 [5], it would appear that these channels and the SR Ca^{2+} release channels are different, at least with respect to their drug receptors [12], i e., there

is a Ca^{2+} channel heterogeneity according to the type of subcellular membrane.

In this context it is noteworthy that in skeletal muscles, sarcolemmal and SR Ca^{2+}-ATPases [25] are heterogeneous with respect to: (i) molecular mass (140 vs 105 kDa); (ii) vanadate sensitivity (IC_{50} 1.7 vs $IC_{50} > 50\ \mu M$); (iii) regulation by calmodulin (calmodulin sensitivity vs calmodulin insensitivity), and that the sarcolemmal and T-tubule Ca^{2+}-ATPases also differ by some of these properties [25]. Such a membrane specificity of Ca^{2+}-ATPases, as documented also for pig stomach smooth muscle [26], is in agreement with the relative non-tissue specificity of plasma membrane Ca^{2+}-ATPases from several biological sources [25-28]. By analogy, our findings support the conclusion that Ca^{2+} channels are also specific to the membrane type.

ACKNOWLEDGEMENTS

Work supported by institutional funds from the Consiglio Nazionale delle Ricerche and grant funds to A.M. from the Ministero della Pubblica Istruzione.

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