CLOTRIMAZOLE INHIBITS THE Ca\textsuperscript{2+}-ATPase (SERCA) 
BY INTERFERING WITH Ca\textsuperscript{2+} BINDING 
AND FAVORING THE E2 CONFORMATION

Gianluca Bartolommei\textsuperscript{1}, Francesco Tadini-Buoninsegni\textsuperscript{1}, Suming Hua\textsuperscript{2}, 
Maria Rosa Moncelli\textsuperscript{1}, Giuseppe Inesi\textsuperscript{2} and Rolando Guidelli\textsuperscript{1}

From the \textsuperscript{1}Department of Chemistry, University of Florence, Sesto Fiorentino, Italy 
and the \textsuperscript{2}Department of Biochemistry and Molecular Biology, 
University of Maryland School of Medicine, Baltimore, Maryland 21201, U.S.A.

Running Title: Clotrimazole effect on SERCA

Address correspondence to: Rolando Guidelli, University of Florence, Department of Chemistry, 
via della Lastruccia 3, 50019 Sesto Fiorentino – Florence (ITALY); tel. +39-055-4573097; fax: +39-055-4573098; e-mail: guidelli@unifi.it

Clotrimazole (CLT) is an antimycotic imidazole derivative that is known to inhibit cytochrome P-450, ergosterol biosynthesis and proliferation of cells in culture, and to interfere with cellular Ca\textsuperscript{2+} homeostasis. We found that CLT inhibits the Ca\textsuperscript{2+}-ATPase of rabbit fast-twitch skeletal muscle (SERCA1), and we characterized in detail the effect of CLT on this calcium transport ATPase. We used biochemical methods for characterization of the ATPase and its partial reactions, and we also performed measurements of charge movements following adsorption of SR vesicles containing the ATPase onto a gold-supported biomimetic membrane. CLT inhibits Ca\textsuperscript{2+}-ATPase and Ca\textsuperscript{2+} transport with a \(K_i\) of 35 \(\mu\)M. Ca\textsuperscript{2+} binding in the absence of ATP, and phosphoenzyme formation by utilization of ATP in the presence of Ca\textsuperscript{2+} are also inhibited within the same CLT concentration range. On the other hand, phosphoenzyme formation by utilization of Pi in the absence of Ca\textsuperscript{2+} is only minimally inhibited. It is concluded that CLT inhibits primarily Ca\textsuperscript{2+} binding and, consequently, the Ca\textsuperscript{2+} dependent reactions of the SERCA cycle. It is suggested that CLT resides within the membrane bound region of the transport ATPase, thereby interfering with binding and conformational effects of the activating cation.

Clotrimazole (CLT) is an antimycotic imidazole derivative widely used for the treatment of yeast infections. Like other drugs belonging to the same imidazole family (e.g. miconazole and ketoconazole), its antimycotic effect is due to inhibition of cytochrome P-450, an enzyme involved in ergosterol biosynthesis by the yeast (1,2). It has also been shown that CLT inhibits cell proliferation of several normal and cancer cell lines \textit{in vitro} (3). Interference with cellular Ca\textsuperscript{2+}-homeostasis contributes to this inhibition: in particular, the action of CLT is thought to involve depletion of intracellular Ca\textsuperscript{2+}-stores, as also confirmed in Madin Darby canine kidney (MDCK) cells, where CLT induces Ca\textsuperscript{2+} release from thapsigargin-sensitive Ca\textsuperscript{2+}-stores (4).

It was reported that CLT affects the Ca\textsuperscript{2+} pump activity of SR vesicles isolated from rabbit heart muscle, producing inhibition of the Ca\textsuperscript{2+} dependent reactions of the ATPase (SERCA2) obtained from cardiac myocytes (5). With the experiments reported here we found that, in fact, CLT inhibits the Ca\textsuperscript{2+}-ATPase. We investigated the effect of CLT on the Ca\textsuperscript{2+}-ATPase (SERCA1) associated with native membrane vesicles derived from rabbit fast-twitch skeletal muscle. Under optimal coupling conditions, the SERCA pump translocates two calcium ions per ATP molecule from the cytoplasm to the lumen of SR with a turnover rate of about 10s\textsuperscript{-1}, forming a three order of magnitude Ca\textsuperscript{2+} gradient (6-9). Altered SERCA function is relevant to pathogenic mechanisms of several diseases (10-12). Furthermore, SERCA function can be affected by several compounds of possible pharmacological interest, like thapsigargin (TG) (13), cyclopiazonic acid (14), curcumin (15,16), 2,5-di-tert-butyl-1,4-dihydroxybenzene (BHQ) (17) and 1,3-dibromo-2,4,6-tris (methylisothiouronium) benzene (18).
In addition to biochemical characterization of the ATPase and its partial reactions, we performed direct measurements of charge translocation following adsorption of SR vesicles containing ATPase onto a biomimetic membrane supported by a gold electrode and activation by rapid mixing with a suitable substrate (19). This technique has been used successfully in studies of electrogenic transport by several membrane proteins, such as Na\(^+\),K\(^+\)-ATPase (20,21), melibiose permease (22), Na\(^+\)/proline antiporter (23), including the SERCA pump (24,25). We found that CLT interferes specifically with Ca\(^{2+}\) binding to SERCA even in the absence of ATP. For this reason the Ca\(^{2+}\) dependent reactions of this enzyme are also inhibited.

Materials and Methods

**Chemicals.** Calcium and magnesium chlorides and 3-morpholinopropane sulfonic acid (MOPS) were obtained from Merck at analytical grade. Adenosine-5'-triphosphate disodium salt (ATP, ~97%) and dithiothreitol (DTT) purchased from Fluka. Octadecanethiol (98%) from Aldrich was used without further purification. Ethylene glycol-bis[\(\beta\)-aminoethyl ether]-N,N,N',N'-tetraacetic acid (EGTA), calcimycin (calcium ionophore A23187) and 1-[o-Chloro-\(\alpha\),\(\alpha\)-diphenylbenzyl]-imidazole (Clotrimazole) were obtained from Sigma. The lipid solution contained diphanytanoylphosphatidylcholine (Avanti Polar Lipids) and octadecylamine (puriss., Fluka) [60:1] and was prepared at a concentration of 1.5% (w/v) in n-decane (Merck), as described by Bamberg et al. (26).

**ATPase preparation.** Sarcoplasmic reticulum vesicles were obtained by extraction from the fast-twitch hind leg muscle of New Zealand white rabbit, followed by homogenization and differential centrifugation, as described by Eletr and Inesi (27). The vesicles so obtained, derived from longitudinal SR membrane, contained only negligible amounts of the ryanodine receptor Ca\(^{2+}\) channel associated with junctional SR (light vesicles).

Free Ca\(^{2+}\) concentration was calculated with the computer program WinMAXC (28). Unless otherwise stated, 1 \(\mu\)M calcium ionophore A23187 was used to prevent Ca\(^{2+}\) accumulation by the SR vesicles (29).

**ATPase activity.** Ca\(^{2+}\)-ATPase hydrolytic activity was determined following \(P_i\) production by a colorimetric method (30). The reaction mixtures contained 20 mM MOPS (pH 7.0), 80 mM KCl, 3 mM MgCl\(_2\), 0.2 mM EGTA, 0.2 mM CaCl\(_2\), 2 \(\mu\)g of SR protein/ml, 3 \(\mu\)M A23187 ionophore, and were preincubated with various concentrations of CLT for 30 minutes. The reaction was started by addition of 1 mM ATP. The incubation temperature was 37°C.

**\(^{45}\)Ca\(^{2+}\) binding.** Ca\(^{2+}\) binding in the absence of ATP was measured by incubating for 30 minutes SR vesicles (0.1 mg/ml) in a pH 7.0 reaction mixture containing 20 mM MOPS, 80 mM KCl, 5 mM MgCl\(_2\), 10 \(\mu\)M free \(^{45}\)CaCl\(_2\), and 3 \(\mu\)M A23187 ionophore, with different concentrations of CLT. TG (2 \(\mu\)M) was added to half of the samples to provide controls exhibiting no specific Ca\(^{2+}\) binding, because it was demonstrated previously that TG prevents specific Ca\(^{2+}\) binding (13). The reaction temperature was 25°C. The reaction mixture was filtered with 0.45 \(\mu\)m filter and radioactivity was measured by a liquid scintillation counter.

**Phosphorylation by \([\gamma\-\)P\]ATP.** Enzyme phosphorylation by ATP was measured in a pH 7.0 ice-cold reaction mixture containing 20 mM MOPS, 80 mM KCl, 2 mM MgCl\(_2\), 10, 50 or 200 \(\mu\)M CaCl\(_2\), 0.1 mg of SR protein/ml, and 3 \(\mu\)M A23187 ionophore, after a preincubation with various concentrations of CLT for 30 minutes. Individual samples (1 ml) were started by the addition of 20 \(\mu\)M \([\gamma\-\)P\]ATP and quenched at 1 second with 1 M perchloric acid. After filtration and washing of the sample, radioactivity of the sample was measured with a liquid scintillation counter.

**Phosphorylation by \([\gamma\-\)P\]Pi.** Enzyme phosphorylation with \(P_i\) was measured at 25°C in a pH 6.2 reaction mixture containing 50 mM MES, 10 mM MgCl\(_2\), 2 mM EGTA, 20% of DMSO, after a 30 minutes preincubation with various concentrations of CLT, in the presence
of 1 mM \[^{32}\text{P}i\] and 0.5 mg of SR protein/ml. The reaction was acid-quenched after 10 minutes incubation by the addition of 1 M perchloric acid. The EP measurements were conducted with a filtration method.

**Measurement of charge movements.** Charge movements were measured by adsorbing the SR vesicles containing the Ca\(^{2+}\)-ATPase onto a biomimetic membrane supported by a gold electrode (the so-called solid supported membrane, or SSM). The SSM consisted of an octadecanethiol monolayer covalently bound to a gold surface via the sulfur atom, with a phospholipid monolayer on top of it (31). After adsorption, usually carried out at an applied potential of +0.1V, the protein was activated by a rapid injection of a solution containing the appropriate substrate, e.g. ATP or Ca\(^{2+}\) ions. If at least one electrogenic step is involved in the relaxation process, a current transient (pre-steady state) can be recorded along the external circuit. The acquisition of transients obtained under different experimental conditions, together with their subsequent elaboration, can provide important kinetic information about protein function and/or its modulation by drugs, peptides and small soluble proteins. In particular, from a current transient three main data can be extracted: the peak current, the translocated charge and the decay time constants. The charge is obtained by numerical integration of the current transient, while the decay time constants are provided by a multiexponential fitting of the transient (19, 20, 24, 25). The time constants are strictly correlated with the rate constants of the reaction steps involved in the activation of the pump (32).

The preparation of the gold electrodes, the whole experimental set-up, as well as the solution exchange technique are described in detail in Tadini-Buoninsegni et al. (25).

For the activation experiments, the composition of the solutions was the following:

a. **for Ca\(^{2+}\) concentration jumps:** the non-activating solution contained 150 mM choline chloride, 25 mM MOPS (pH 7.0), 0.25 mM EGTA, 1 mM MgCl\(_2\), 0.2 mM DTT. The activating solution had the same composition plus the required CaCl\(_2\) amount added from a 5, 50 or 500 mM stock solution;

b. **for ATP concentration jumps:** the non-activating solution contained 150 mM choline chloride, 25 mM MOPS (pH 7.0), 1 mM EGTA, 1 mM MgCl\(_2\), 1.1 mM CaCl\(_2\) (100 \(\mu\)M free Ca\(^{2+}\)), 0.2 mM DTT. The activating solution had the same composition plus 100 \(\mu\)M ATP.

CLT was added from a 10 mM stock solution in DMSO to the buffer solution, when needed.

**RESULTS**

We studied the effect of CLT on the SR Ca\(^{2+}\)-ATPase by a series of biochemical measurements including ATPase activity, \[^{45}\text{Ca}i\]Ca\(^{2+}\) binding in the absence of ATP, formation of the phosphorylated enzyme intermediate (E-P) by utilization of \[^{32}\text{P}i\]ATP in the presence of Ca\(^{2+}\) and formation of E-P by utilization of \[^{32}\text{Pi}\] in the absence of Ca\(^{2+}\). In addition, we performed pre-steady state activation experiments with the SSM-based technique (Ca\(^{2+}\) concentration jumps and ATP concentration jumps in the presence of CLT).

**Biochemical experiments.** It is shown in Fig. 1 that the steady state hydrolytic activity of the Ca\(^{2+}\)-ATPase (in the presence of 10 or 200 \(\mu\)M free Ca\(^{2+}\)) is inhibited by CLT within the \(\mu\)M concentration range. It is noteworthy that measurements at two different Ca\(^{2+}\) concentrations (10 and 200 \(\mu\)M) yield the same dependence upon the inhibitor concentration, indicating that no protection is obtained by increasing the Ca\(^{2+}\) concentration above the saturating level.

With the aim of determining whether the effect of CLT is a total inhibition of SERCA activity (as that of TG), or is rather due to interference with a specific partial reaction, we then measured independently \[^{45}\text{Ca}i\]Ca\(^{2+}\) binding in the absence of ATP, E-P formation by utilization of \[^{32}\text{P}i\]ATP in the presence of Ca\(^{2+}\), and E-P formation by utilization of \[^{32}\text{Pi}\] in the absence of Ca\(^{2+}\). It is shown in Fig. 2 that \[^{32}\text{Pi}\] binding and E-P formation from \[^{32}\text{Pi}\] ATP are inhibited with a similar pattern as the
The concentration of CLT is increased from zero up to 50 μM. It should be pointed out that a limit in the solubility of CLT in the aqueous reaction medium renders a concentration increase above 50 μM rather ineffective. It is noteworthy that the same pattern of inhibition with respect to the CLT concentration was observed when enzyme phosphorylation with ATP was measured in the presence of 10, 50 or 200 μM Ca²⁺, indicating that the CLT inhibition is not competitive with respect to Ca²⁺. A most interesting observation is that E-P formation from [³²P]P, (in the absence of Ca²⁺) is only minimally reduced (Fig. 2), indicating that CLT inhibition of the SERCA pump is mainly exerted on Ca²⁺ binding and the Ca²⁺-dependent partial reactions of the enzymatic cycle.

*On and off Ca²⁺ concentration jumps in the presence of CLT in pre-steady state. A Ca²⁺ concentration jump, performed with the SSM-based technique, yields a current transient that can be ascribed to Ca²⁺ binding to the ATPase. An ON signal is obtained following the injection of the Ca²⁺-containing solution (Fig. 3A, step 1), and an OFF signal is obtained upon calcium release from the binding site following injection of a solution containing EGTA and no calcium (Fig. 3A, step 2) (25). We then performed these experiments in the presence of increasing concentrations of CLT to assess the effect of the drug on Ca²⁺ binding in the absence of ATP.

In agreements with direct measurements of Ca²⁺ binding (Fig. 2) we found that charge movements attributed to Ca²⁺ binding are also reduced by CLT.

Fig. 4 compares a saturating calcium concentration jump in the absence and in the presence of 5 μM CLT, showing a clear reduction of the signal in the presence of the drug. Moreover, a titration with increasing Ca²⁺ concentrations (Fig. 5) shows that the signal is reduced to about one third of that recorded in the absence of the inhibitor (25). On the other hand, in spite of the reduction in maximal binding, the Kᵦ and n values (0.53 μM and 1.7, respectively) obtained from fitting to the Hill function are very close to those obtained in the absence of the drug, either by measurements of charge movements (25) or by direct measurements with a radioactive tracer (33). This indicates that non-saturating CLT reduces the amount of Ca²⁺ bound, but does not affect the binding characteristics (i.e., affinity) of the enzyme molecules that are not totally inhibited.

We also found that titrations with increasing CLT concentrations in the presence of 10 μM or 100 μM Ca²⁺ concentrations (Fig. 6) yield the same inhibition curve. These results are in agreement with the experiments on enzyme phosphorylation with ATP (Fig. 2) and ATPase hydrolytic activity (Fig. 1), and imply that increasing the Ca²⁺ concentration above the saturating level does not protect the enzyme from CLT. Therefore, the CLT inhibition is not competitive with respect to Ca²⁺. On the other hand, we found that the effective CLT concentration is lower when the inhibitor is added *before* (Kᵦ = 7 μM, as in Fig. 6) rather than *after* Ca²⁺ (Kᵦ = 35 μM, as in Figs. 1, 2 and 7). A similarly greater resistance of the Ca²⁺ bound conformation of the enzyme (CaₓE₁) as compared with the Ca²⁺ free conformation (E₂), was previously found with respect to TG (13) and miconazole (34).

*ATP concentration jumps in the presence of CLT. An ATP concentration jump in the presence of Ca²⁺, performed on a SSM with SR vesicles containing Ca²⁺-ATPase adsorbed onto it, produces a current transient due to the electrogenicitiy of Ca²⁺ release into the lumen of the SR and, to some extent, to Ca²⁺-re-binding to the protein after the hydrolytic cleavage of P-E₂ intermediate (Fig. 3B, steps 2 and 4). It was previously determined that the formation and hydrolytic cleavage of the phosphorylated intermediate are not electrogenic (35).

Fig. 7 shows the effect of 35 μM CLT on a 100 μM ATP concentration jump in the presence of saturating Ca²⁺ (100 μM). It is clear that the current transient due to Ca²⁺ translocation is affected by CLT. In fact, starting from no CLT and increasing CLT concentration up to 35 μM, peak currents are progressively decreased. Furthermore, integration of the current transients yields values for moved charges that exhibit a behavior very similar to that of peak currents with respect to the effect of CLT (inset of Fig. 7).

It is noteworthy that exponential fitting of the current transients yields two time constants for current decay. The two constants, τ₁ and τ₂, have average values of 27±2ms and
42±3ms respectively, and do not appear to be significantly affected by CLT (not shown), irrespective of the effect of CLT on peak currents and moved charge.

**DISCUSSION**

CLT is an antifungal agent whose effect was attributed to inhibition of cytochrome P-450 and ergosterol biosynthesis in yeast (1,2). It was also shown that CLT inhibits cell proliferation in vitro (3), interferes with calcium homeostasis in cultures cells (3,4), and inhibits the calcium pump (SERCA2) obtained with the microsomal fraction of heart muscle (5). We have characterized in detail the effect of CLT on the Ca²⁺ ATPase (SERCA1) activity associated with microsomal vesicles obtained from skeletal muscle. This enzyme preparation is highly purified and well suited to characterization of the catalytic cycle and its partial reactions (6-9).

In addition, several SERCA inhibitors have been described (13-18), thereby yielding useful grounds for comparison of inhibitory mechanisms. Finally, the simultaneous use of biochemical and biophysical methods in the characterization of CLT inhibitory mechanism, has given us the opportunity to demonstrate clearly the functional relevance of charge transfer measurements and their correspondence to partial reactions of the ATPase cycle.

As outlined in Figure 3B, the ATPase (SERCA) cycle requires initial activation through Ca²⁺ binding from the exterior of the vesicles (step 4), followed by utilization of ATP to form a phosphorylated intermediate (step 1). The bound Ca²⁺ is then dissociated into the lumen of the vesicles (step 2), followed by hydrolytic cleavage of the phosphoenzyme (step 3). Using direct binding essays by a radioactive tracer, as well as measurements of charge movements by the SSM-based technique, we found that the initial Ca²⁺ binding, before utilization of ATP, is inhibited by CLT (Figs. 2, 4 and 6). It is of interest that plots of charge as a function of Ca²⁺ concentration (Fig. 5) yield affinity constants and cooperative behavior identical to that of equilibrium binding isotherms obtained in the absence of CLT by direct measurements with isotopic tracer (33). The correspondence of the two types of measurements is highly satisfactory and demonstrates the specificity of charge movement with regard to Ca²⁺ binding and its inhibition by CLT.

Considering the absolute Ca²⁺ requirement for enzyme activation, it is expected that steady state ATPase activity will be inhibited in parallel with inhibition of Ca²⁺ binding by CLT. In fact, we found that steady state ATPase activity and Ca²⁺ transport were inhibited by the same CLT concentrations producing inhibition of Ca²⁺ binding. This was demonstrated by measuring P₁ by a colorimetric method, after adding ATP to the enzyme in the presence of Ca²⁺ (Fig. 1), as well as by measuring charge translocation following an ATP concentration jump by the SSM-based technique (Fig. 7). Here again, identical results were obtained by the biochemical and biophysical methods. An interesting observation is that while CLT clearly decreases the amount of moved charge (Fig. 7), the decay kinetics of the remaining current transient is not affected by CLT (not shown). Therefore, while an increasing number of enzyme molecules are inactivated by CLT, the remaining enzyme molecules do not bind CLT and continue to function normally. This suggests a specific inhibitory site for CLT within the ATPase molecule, whereby the ATPase molecules are inhibited independent of each other. Furthermore, comparative experiments performed by addition of CLT before or after Ca²⁺, suggest that the Ca²⁺ free conformation of the enzyme is more sensitive to CLT, and is stabilized by the bound inhibitor.

We also found that phosphoenzyme formation by utilization of ATP in the presence of Ca²⁺ is inhibited by CLT within the same concentration range as Ca²⁺ binding (Fig. 2), and ATPase hydrolytic activity (Fig. 1), with a pattern consistent with non competitive inhibition. However, phosphoenzyme formation by utilization of P₁ in the reverse direction of cycle is only minimally affected by CLT (Fig. 2), as also observed by Snajdrova et al. (5). This reverse reaction is not dependent on activation by Ca²⁺, and in fact requires removal of bound Ca²⁺ (36). Therefore, it is apparent that the ATPase catalytic mechanism is not primarily affected by CLT, but only secondarily, through interference with activation by Ca²⁺ binding. Such a specific interference with a partial
reaction (i.e., Ca\(^{2+}\) binding) is of interest, when compared with the global inhibition of the ATPase cycle produced by TG. So far, 1,3-dibromo-2,4,6-tris(methylisothiouronium) benzene is the only other ATPase inhibitor producing specific inhibition of a single partial reaction, i.e. the E1-P ↔ E2-P transition (37).

It should be noted that CLT shows a rather high affinity for SERCA, with a \(K_I\) of 35 \(\mu\)M in the presence of Ca\(^{2+}\), and \(K_I\) of 7 \(\mu\)M in the absence of Ca\(^{2+}\). Considering the hydrophobic character of the CLT molecule and the location of cation binding sites in the membrane bound region of transport ATPases, it is apparent that CLT interferes at this location with the binding of the activating cation and with the conformational change involved in cation binding. In addition to the significance of the CLT effect with regard to the catalytic and possibly pharmacological mechanism, we consider that stabilization of the Ca\(^{2+}\) free, E2 conformation of the enzyme, leaving the catalytic site in a functional state as revealed by the \(P_i\) reaction, may be extremely useful tools in structural and crystallization studies of cation transport ATPases. In fact, ATPase crystal structures of the highest resolution are presently obtained by incorporation of two (rather than one) inhibitors, permitting detection of ordered water molecules and advanced electrostatic calculations yielding estimates of the proton occupancy of acidic residues (38). At the same time, the availability of several inhibitors supports organic synthesis of new, highly specific and potent compounds, guided by the available crystal structure of the ATPase (39, 40).

### ACKNOWLEDGEMENTS

This work was supported by the Ente Cassa di Risparmio di Firenze (PROMELAB project), the Ministero dell’Istruzione, dell’Università e della Ricerca (M.I.U.R.), and by the U.S.A. National Institutes of Health (RO1 HL69830).
REFERENCES

FIGURE LEGENDS

Fig. 1 ATPase activity of Ca\(^{2+}\)-ATPase determined in the presence of increasing concentration of CLT. Two values of calcium concentration were used: 10\(\mu\)M (●) and 200 \(\mu\)M (○).

Fig. 2 \(^{45}\)Ca\(^{2+}\) binding to the SERCA (●), E-P formation from \([\gamma-\text{\(32\)}^\text{P}]\text{ATP}\) (○, ■, □) and from \([\text{\(32\)}^\text{P}]\text{Pi}\) (▲), as determined at various CLT concentrations. The experiments were conducted as described in Materials and methods. The Ca\(^{2+}\) concentration for enzyme phosphorylation was 10 (○), 50 (■) and 200 \(\mu\)M (□).

Fig. 3 Reaction steps of the enzymatic cycle of SR Ca\(^{2+}\)-ATPase. The grey boxes underline the protein starting conformations for a calcium concentration jump experiment (A) and for an ATP concentration jump experiment (B). Bold characters and arrows indicate an electrogenic event. The circled numbers point out the temporal sequence of the reaction steps during the experiment.

Fig. 4 Comparison of saturating Ca\(^{2+}\) concentration jumps in the absence (solid line) or in the presence (dashed line) of 5 \(\mu\)M CLT. The charges associated with the current transients are respectively 29.0 and 10.5 pC, corresponding to a 36% reduction of the signal in the presence of the drug. The total calcium amount is 0.25 mM, which results in a free concentration of 10.3 \(\mu\)M for the solution composition described in Materials and methods.

Fig. 5 Titration of SERCA with calcium in the presence of 5 \(\mu\)M CLT obtained by Ca\(^{2+}\) concentration jumps experiments. Values are normalized with respect to the bound charge for a saturating Ca\(^{2+}\) concentration jump (10 \(\mu\)M) in the absence of CLT. The solid line represents the fitting to a Hill function \((Q = Q_{\text{norm max}} \ast C^n / (K_{0.5}^n + C^n))\), which yields \(K_{0.5}=0.53 \pm 0.03 \mu\)M and \(n=1.7 \pm 0.2\). The composition of the solutions is indicated in Materials and methods.

Fig. 6 Dependence of the normalized charge following Ca\(^{2+}\) concentration jumps on CLT concentration. Free calcium concentration was 10 \(\mu\)M (● and solid line) or 100 \(\mu\)M (○ and dashed line). The composition of the solutions is indicated in Materials and methods.

Fig. 7 Current transients following 100 \(\mu\)M ATP concentration jumps in the presence of 100 \(\mu\)M free calcium ions and in the absence (solid line) or in the presence (dashed line) of 35 \(\mu\)M CLT. The inset shows the normalized peak currents (●) and the translocated charges (○) obtained from currents transients following 100 \(\mu\)M ATP concentration jumps in the presence of different concentrations of CLT. The normalization was carried out with respect to the current transient obtained in the absence of CLT. The composition of the solutions is indicated in Materials and methods.
Figure 1

A graph showing the relationship between the concentration of CLT and the % ATPase activity.
Figure 2
Figure 3
Figure 4
Figure 5

This figure shows a graph plotting the normalized charge against the free Ca\(^{2+}\) concentration (µM). The data points are represented by black circles, and error bars indicate the variability of the measurements. The x-axis represents the free Ca\(^{2+}\) concentration ranging from 0 to 10 µM, while the y-axis represents the normalized charge ranging from 0 to 0.4.
Figure 6

The figure shows a graph plotting the normalized charge against the concentration of CLT (µM). The x-axis represents the concentration of CLT ranging from 0 to 35 µM, while the y-axis represents the normalized charge ranging from 0.0 to 1.0. The data points are connected by a smooth line, indicating a decrease in normalized charge with increasing concentration of CLT.
Figure 7
Clotrimazole inhibits the Ca\textsuperscript{2+}-ATPase (SERCA) by interfering with Ca\textsuperscript{2+} binding and favoring the E2 conformation

Gianluca Bartolommei, Francesco Tadini-Buoninsegni, Suming Hua, Maria Rosa Moncelli, Giuseppe Inesi and Rolando Guidelli

*J. Biol. Chem.* published online February 1, 2006

Access the most updated version of this article at doi: 10.1074/jbc.M510550200

Alerts:
- When this article is cited
- When a correction for this article is posted

[Click here](https://www.jbc.org/) to choose from all of JBC's e-mail alerts