Stimulation of the Ca^{2+}-ATPase of sarcoplasmic reticulum by disulfiram

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Disulfiram [bis(diethylthiocarbamoyl)disulphide] has been found to stimulate reversibly the Ca^{2+}-ATPase of skeletal muscle sarcoplasmic reticulum. At pH 7.2, 2.1 mM ATP and 25 °C, ATPase activity was found to double on addition of 120 μM disulfiram. Stimulation fitted to binding of disulfiram at a single site with a K_d of 61 μM. Disulfiram had no effect on the Ca^{2+} affinity of the ATPase or on the rate of phosphorylation of the ATPase by ATP, but increased the rate of dissociation of Ca^{2+} from the phosphorylated ATPase (the transport step) and increased the rate of dephosphorylation of the phosphorylated ATPase. It also decreased the level of phosphorylation of the ATPase by P_i, consistent with a 7.5-fold decrease in the equilibrium constant of the phosphorylated to non-phosphorylated forms ($E2PMg/E2P_iMg$) at 80 μM disulfiram. Disulfiram had no significant effect on the concentration of ATP resulting in stimulation of ATPase activity, suggesting that it does not bind to the empty nucleotide-binding site on the phosphorylated ATPase. Studies of the effects of mixtures of disulfiram and jasmone (another molecule that stimulates the ATPase) suggest that they bind to separate sites on the ATPase.

INTRODUCTION

The endoplasmic reticulum (ER) has a central role in cell signalling, acting as a Ca^{2+} store within the cell, accumulating and releasing Ca^{2+} in response to a variety of signals. Accumulation of Ca^{2+} by the ER is mediated by the Ca^{2+}-ATPase in the ER membrane. The SERCA2b and SERCA3 isoforms of the Ca^{2+}-ATPase found in the ER of non-muscle cells have close sequence similarity to the SERCA1 isoform found in skeletal muscle sarcoplasmic reticulum (SR) [1]. This is particularly convenient because approx. 70% of the protein in the SR membrane is Ca^{2+}-ATPase, and Ca^{2+}-ATPase can be readily purified from the SR membrane, allowing detailed studies of its structure and function.

The structure proposed for the Ca^{2+}-ATPase consists of two main cytoplasmic regions connected by a narrow stalk to 10 transmembrane α-helices [2–7]. The largest of the cytoplasmic regions, between transmembrane helices 4 and 5, contains three domains: a phosphorylation domain, containing the residue (Asp-351) phosphorylated by ATP, a nucleotide-binding domain, containing the residue (Lys-515) labelled by fluorescein isothiocyanate, and a smaller domain referred to as the hinge domain because it is thought that it might be involved in the relative movement of the phosphorylation and nucleotide-binding domains, as in several kinases.

Covalent modification of Cys residues in the Ca^{2+}-ATPase generally leads to a decrease in ATPase activity. For example, labelling with *N*-ethylmaleimide leads to a total loss of ATPase activity [8], and labelling with 4-(bromomethyl)-6,7-dimethoxycoumarin leads to a 25% reduction in activity [9]. Disulfiram [bis(diethylthiocarbamoyl) disulphide; see Figure 1] has been reported to inhibit the synaptosomal calmodulin-sensitive Ca^{2+}-ATPase, probably by covalent modification of a Cys residue [10]. Clinically, disulfiram is used in aversion therapy for the treatment of alcoholism, effects following from the inhibition of hepatic aldehyde dehydrogenase. *In vitro*, inhibition follows from the formation of a mixed disulphide with an essential thiol on the dehydrogenase [11]. However, covalent modification shows marked specificity, and many thiol-containing enzymes do not react with disulfiram [11]. We show here that disulfiram does not covalently modify the Ca^{2+}-ATPase of SR but that, unexpectedly, it increases ATPase activity.

MATERIALS AND METHODS

Disulfiram and diethylthiocarbamoyl sulphide were obtained from Aldrich. The Ca^{2+}-ATPase was purified from rabbit skeletal muscle sarcoplasmic reticulum as described by East and Lee [12]. ATPase activities were measured at 25 °C by using a coupled-enzyme assay in a medium containing 40 mM Hepes/KOH, pH 7.2, 100 mM KCl, 5 mM $MgSO_4$, 2.1 mM ATP, 1.1 mM EGTA, 0.41 mM phosphoenolpyruvate, 0.15 mM NADH, pyruvate kinase (7.5 units) and lactate dehydrogenase (18 units) in a total volume of 2.5 ml. The reaction was initiated by addition of an aliquot of a 25 mM $CaCl_2$ solution to a cuvette containing the ATPase and the other reagents, to give 10 μM free Ca^{2+}. Disulfiram was added from a stock solution (20 mM) in DMSO.

The time-dependences of Ca^{2+} dissociation from the ATPase and of phosphorylation-induced Ca^{2+} release were determined with a Biologic rapid filtration system [13]. A suspension of the ATPase in buffer [20 mM Hepes/Tris (pH 7.2)/100 mM KCl/5 mM $MgSO_4$] containing 100 μM $^{45}Ca^{2+}$ and 500 μM [^{3}H]sucrose, corresponding to 100 μg of ATPase, was loaded on a Millipore HAWP filter (0.45 μm pore size) and then rapidly perfused with the same buffer containing either 2 mM EGTA or 100 μM $^{40}CaCl_2$ and 2 mM ATP. The filters were dried overnight in air and counted in OptiPhase 'HiSafe' 3. The amount of [^{3}H]sucrose trapped on the filter was used to calculate the wetting volume of the filter, and the amount of $^{45}Ca^{2+}$ calculated to be in this volume was subtracted from the total $^{45}Ca^{2+}$ on the filter to give that bound to the ATPase.

Steady-state measurements of phosphorylation by [^{32}P]P_i were conducted in 150 mM Mes/Tris, pH 6.2, containing 5 mM EGTA and the required concentrations of Mg^{2+} and P_i at 25 °C

Abbreviations used: ER, endoplasmic reticulum; SR, sarcoplasmic reticulum.

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and a protein concentration of 0.2 mg/ml [14]. The time-dependences of phosphorylation of the ATPase by [γ-^{32}P]ATP and of dephosphorylation of the ATPase phosphorylated with ATP in the presence of Ca^{2+} were determined at 25 °C as described in Starling et al. [13]. The time-dependence of dephosphorylation of the ATPase phosphorylated with [^{32}P]P_i was determined by first incubating the ATPase (4 mg/ml) in 12.5 mM Mes/Tris, pH 6.0, containing 10 mM EGTA, 1 mM [^{32}P]P_i, 20 mM $MgSO_4$ and 14% (v/v) DMSO. The suspension (1 vol) was mixed in the Biologic QFM-5 system with 16 vol of 100 mM Mes/Tris, pH 7.5, containing 100 mM KCl, 4 mM $MgSO_4$ and 5.3 mM ATP, followed by quenching with 25% (w/v) trichloroacetic acid/0.13 M phosphoric acid. The precipitated protein was filtered, washed and counted.

RESULTS

Steady-state ATPase activity

As shown in Figure 1, the addition of 120 μM disulfiram to the Ca^{2+}-ATPase at 25 °C resulted in a doubling of ATPase activity measured at pH 7.2, 2.1 mM ATP and a saturating concentration of Ca^{2+}. The effect of disulfiram was instantaneous and did not require pre-equilibration of disulfiram with the ATPase. A curve of activity against concentration of disulfiram fits to a single-binding-site equation, with a K_d ($\pm$S.E.) for disulfiram of 61 ± 10 μM and a maximal activity of 7.1 ± 0.5 units/mg of protein. At 37 °C the effects on activity were slightly smaller, with activity increasing from 8 units/mg of protein in the absence of disulfiram to 12.5 units/mg of protein in the presence of 120 μM disulfiram. At pH 6.0 in the presence of 100 mM KCl the level of stimulation was the same as that observed at pH 7.2, but with a slightly higher K_d of 100 μM. Diethylthiocarbamoyl sulphide had no effect on activity at concentrations up to 400 μM. The magnitude of the stimulation by disulfiram was ATP-dependent, with small effects at low concentrations of ATP and larger effects at higher concentrations (Figure 2).

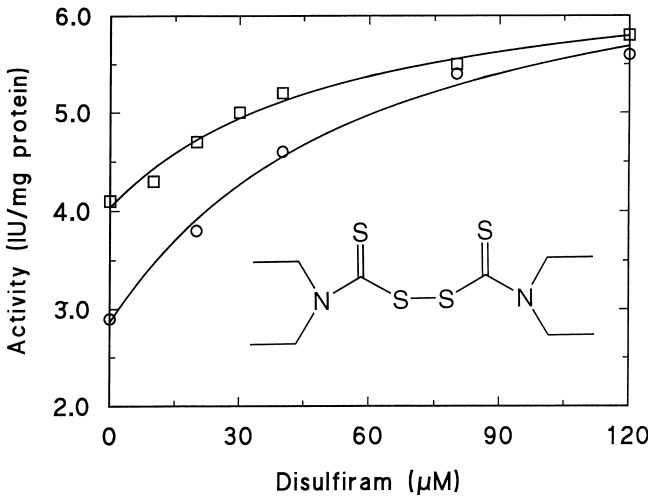


Figure 1 Effect of disulfiram on steady-state ATPase activity

Shown are the effects of disulfiram on ATPase activities [units(IU)/mg of protein] in the absence (○) or presence (□) of 10 μM jasmone, measured at pH 7.2, 2.1 mM ATP, 10 μM Ca^{2+} and 25 °C. The solid lines show fits to a single binding site with K_d values of 61 and 55 μM for disulfiram in the absence and presence of jasmone respectively. The insert shows the structure of disulfiram.

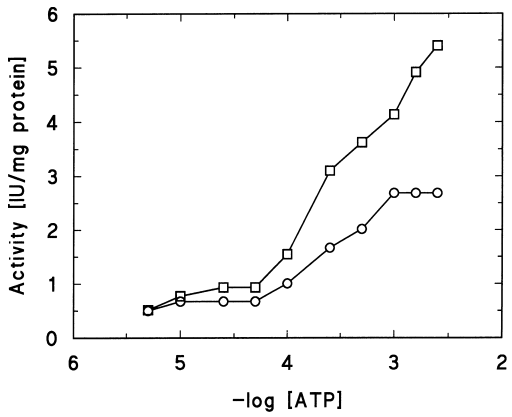


Figure 2 Effect of disulfiram on the ATP-dependence of ATPase activity

Shown are ATPase activities [units(IU)/mg of protein] measured at pH 7.2, 10 μM Ca^{2+} and 25 °C as a function of ATP concentration, in the absence (○) or presence (□) of 80 μM disulfiram.

We have shown that jasmone also increases ATPase activity, half-maximal effects being observed at 14 μM jasmone [13]. Addition of jasmone to the ATPase in the presence of 80 μM disulfiram was found to have no further effect on activity. Figure 1 shows an experiment in which 10 μM jasmone was added to the ATPase resulting in a 30% increase in activity, followed by addition of disulfiram. Again, the stimulation curve fits to a single-binding-site equation with a K_d ($\pm$S.E.) for disulfiram of 55 ± 16 μM, and a maximal activity of 6.6 ± 0.4 units/mg of protein.

The dependence of ATPase activity on Ca^{2+} concentration is complex, with low concentrations of Ca^{2+} activating the ATPase, attributable to binding of Ca^{2+} to the Ca^{2+}-binding sites on the unphosphorylated ATPase (E1; see Scheme 1), and high concentrations of Ca^{2+} inhibiting the ATPase, attributable both to binding of Ca^{2+} to the phosphorylated ATPase (E2P) with a subsequent decrease in the rate of dephosphorylation, and to the formation of CaATP, which is hydrolysed more slowly by the ATPase than is $MgATP^{2-}$ [15–17]. Addition of disulfiram had no significant effect on the Ca^{2+}-dependence of activity in the low-concentration region; in the high-concentration region the pCa giving half-maximal activity shifted only from 3.28 to 3.14 on addition of 80 μM disulfiram at pH 7.2 (results not shown).

The effects on ATPase activity were fully reversible. Incubation of a sample of the ATPase with 80 μM disulfiram led to an increase in ATPase activity from 2.4 to 4.3 units/mg of protein. When the ATPase (1 mg/ml) was incubated with 80 μM disulfiram for 1 h, followed by pelleting of the ATPase and resuspension in buffer, an activity of 2.0 units/mg of protein was measured. We conclude that addition of disulfiram to the ATPase does not result in covalent modification.

Pre-steady-state effects

Disulfiram had no significant effect on the rate of phosphorylation of the ATPase by ATP; the rate of phosphoenzyme formation ($\pm$S.E.) observed when the ATPase, incubated in the presence of Ca^{2+} at pH 7.2, was mixed with a final concentration of 50 μM ATP was 104 ± 12 s^{-1} in the absence of disulfiram and 120 ± 19 s^{-1} in the presence of 80 μM disulfiram.

The rate of dissociation of Ca^{2+} from the ATPase can be

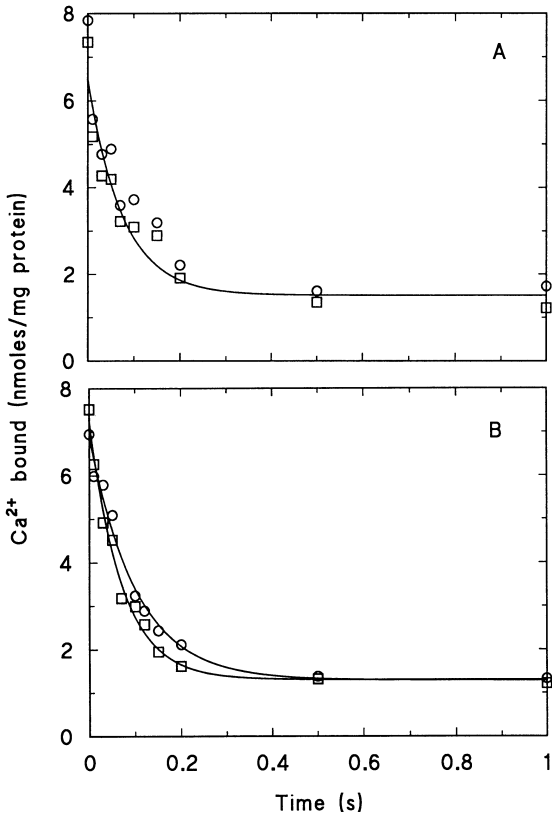


Figure 3 Dissociation of $^{45}Ca^{2+}$ from the ATPase

(**A**) The ATPase was incubated with 100 μM $^{45}Ca^{2+}$ and 0.5 mM [^{3}H]sucrose in buffer (150 mM Mops/Tris (pH 7.2)/100 mM KCl/20 mM Mg^{2+}) in the absence (○) or presence (□) of 80 μM disulfiram. The ATPase (0.1 mg) was then adsorbed on Millipore filters and the loaded filter was perfused for the given periods with the same buffer containing 2 mM EGTA and, for (□), 80 μM disulfiram. The line shows the best fit to a single exponential for the data in the presence of disulfiram. (**B**) ATP-induced release of $^{45}Ca^{2+}$ from the ATPase. The experiment above was repeated but with perfusion of the loaded filter with buffer containing 100 μM $^{40}Ca^{2+}$ and 2 mM ATP. The lines show the best fits to single-exponential decays with the parameters given in the text.

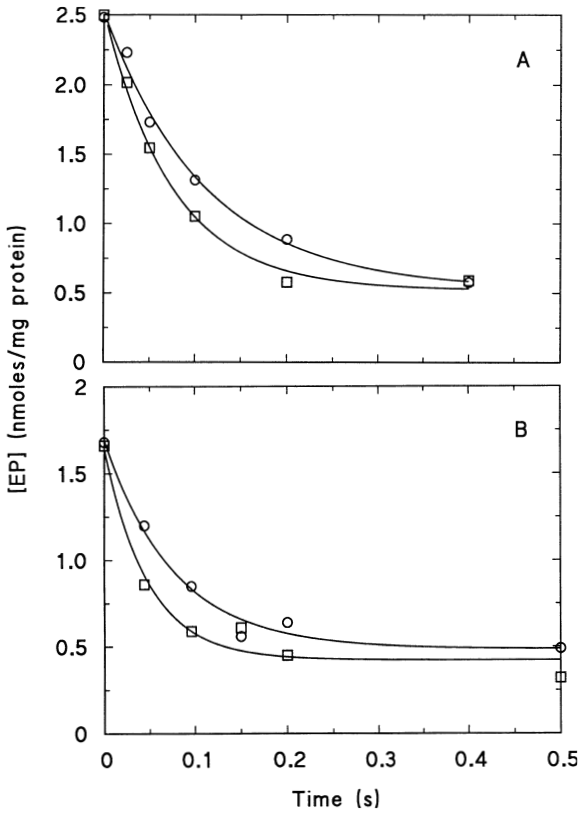


Figure 4 Effect of disulfiram on the rate of dephosphorylation of the ATPase in the absence (A) or presence (B) of Ca^{2+}

(**A**) The enzyme syringe contained ATPase (0.2 mg/ml) in 150 mM Mops/Tris (pH 7.2)/5 mM Mg^{2+}/100 mM KCl/100 μM Ca^{2+}. This was mixed in a 1:1 ratio with a solution containing 100 μM [γ-^{32}P]ATP in the same buffer. The mixture was incubated for 200 ms and then mixed in a 1:1 ratio with the same buffer containing 5.0 mM unlabelled ATP. The syringes also contained either no (○) or 80 μM (□) disulfiram. The reaction was quenched at the given times with 25% (w/v) trichloroacetic acid/0.2 M phosphoric acid. (**B**) The enzyme syringe contained ATPase (4.0 mg/ml) in 12.5 mM Mes/Tris (pH 6.0)/10 mM EGTA/1 mM [^{32}P]P_i/20 mM Mg^{2+}/14% (v/v) DMSO. The second syringe contained 100 mM Mes/Tris (pH 7.5)/100 mM KCl/4 mM Mg^{2+}/5.3 mM ATP. Syringes also contained either no (○) or 80 μM (□) disulfiram. The contents of the enzyme syringe were mixed in a 1:16 volume ratio with the dephosphorylation mixture (the pH after mixing was 7.3) and the reaction was quenched at the given times with 25% (w/v) trichloroacetic acid/0.2 M phosphoric acid. The solid lines represent fits to single exponentials with the parameters given in the text.

determined by adsorbing $^{45}Ca^{2+}$-bound ATPase on Millipore filters and washing with EGTA. Dissociation of $^{45}Ca^{2+}$ from the ATPase fitted to a single-exponential process with rates (±S.E.) of 12.1±3.1 s^{-1} and 13.2±3.4 s^{-1} in the absence and presence of 80 μM disulfiram respectively (Figure 3A); we conclude that disulfiram has no effect on the rate of dissociation of Ca^{2+} from the ATPase. The same experiment was repeated but with washing of the filters with a solution containing a mixture of $^{40}Ca^{2+}$ and ATP; the rate of loss of Ca^{2+} was equivalent to the rate of the Ca^{2+} transport step [13]. As shown in Figure 3B, in this case 80 μM disulfiram caused an increase in rate, from 9.8±1.0 s^{-1} in the absence of disulfiram to 14.2±1.3 s^{-1} in its presence. Disulfiram also caused an increase in the rate of dephosphorylation of the phosphorylated ATPase. For the ATPase phosphorylated with [γ-^{32}P]ATP in the presence of Ca^{2+} and then mixed with a high concentration of unlabelled ATP, rates of dephosphorylation of 9.0±1.3 s^{-1} and 13.4±1.4 s^{-1} were obtained in the absence and presence of 80 μM disulfiram respectively (Figure 4A). For the ATPase phosphorylated with [^{32}P]P_i in the absence of Ca^{2+} at pH 6.0 and presence of DMSO and dephosphorylated by changing the pH to 7.5 in the absence of DMSO, rates of dephosphorylation (±S.E.) were 12.9±2.3 s^{-1} and 20.5±3.1 s^{-1} in the absence and presence of 80 μM disulfiram respectively (Figure 4B).

Phosphorylation by P_i

The ATPase is phosphorylated by P_i at acid pH values in the absence of Ca^{2+} [18]. The level of phosphorylation was decreased in the presence of disulfiram (Figure 5). In the absence of disulfiram, phosphorylation of the ATPase fitted Scheme 1, with dissociation constants k_1, k_2 and k_3 of 100 mM and an equilibrium constant for phosphorylation (k_4) under these conditions of 15.0 [19], the E1–E2 equilibrium being defined by the constants given in Lee et al. [20] (the parameter set fitting the data is not unique). In the presence of 80 μM disulfiram, the data fitted reasonably well to the same model but with a k_4 of 2.0 (Figure 5).

The level of phosphorylation of the ATPase by P_i is increased in the presence of DMSO [18]. The effect of disulfiram on phosphorylation by P_i was not observed in the presence of

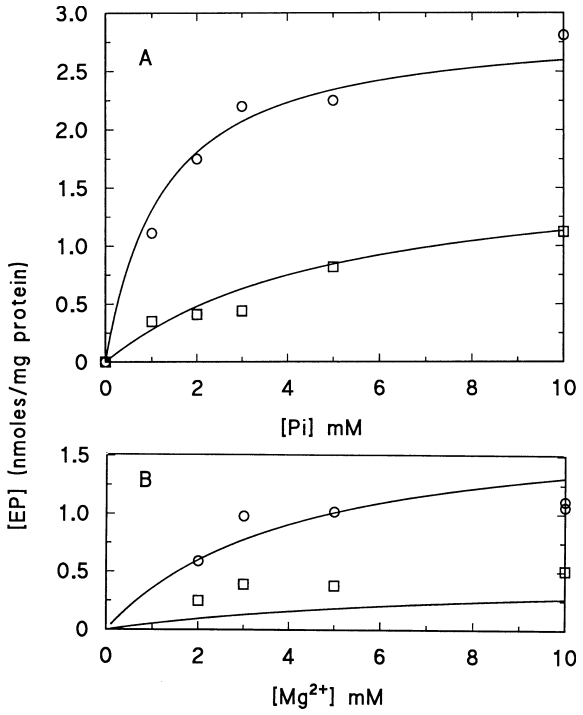
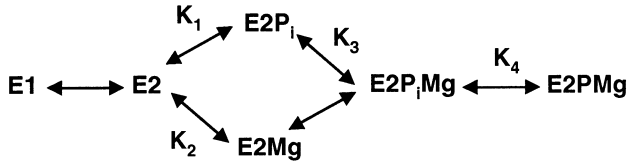


Figure 5 Effect of disulfiram on the level of phosphorylation of the ATPase by phosphate

The ATPase (0.1 mg/ml) was incubated in 150 mM Mes/Tris, pH 6.2, containing 5 mM EGTA and (**A**) 10 mM Mg^{2+} and the given concentration of P_i, or (**B**) 1 mM P_i and the given concentration of Mg^{2+} in the absence (○) or presence (□) of 80 μM disulfiram. The lines are simulations calculated as described in the text assuming a maximal level of phosphorylation of 3.5 nmol of [EP]/mg of protein, with equilibrium constants for phosphorylation (k_4) of 15.0 and 2.0 in the absence and presence of disulfiram respectively.



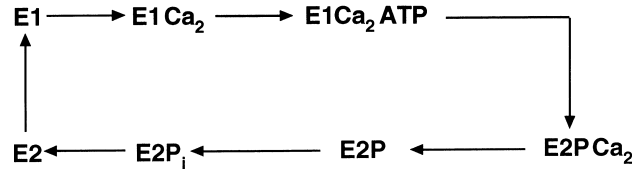
Scheme 1 Phosphorylation by P_i

DMSO, with identical levels of phosphorylation being obtained at pH 6.0, 1 mM [^{32}P]P_i, 20 mM $MgSO_4$ and 14% (v/v) DMSO, in the absence or presence of 80 μM disulfiram (Figure 4B).

DISCUSSION

The addition of 120 μM disulfiram to the Ca^{2+}-ATPase leads to a doubling of ATPase activity at 25 °C, pH 7.2 and 2.1 mM ATP, the half-maximal effect being observed at 61 μM (Figure 1). The effects of disulfiram on the ATPase are fully reversible, showing that covalent modification of Cys residues on the ATPase is not involved. Disulfiram had no significant effect on the Ca^{2+}-dependence of ATPase activity or on the rate of dissociation of Ca^{2+} from the unphosphorylated ATPase, suggesting that disulfiram does not interact with the Ca^{2+}-binding sites on the ATPase.

The mechanism of stimulation of the ATPase was established by a series of rapid-kinetic studies. Effects have been interpreted in terms of the E1–E2 scheme shown in Scheme 2; in the scheme



Scheme 2 E1–E2 scheme for ATPase

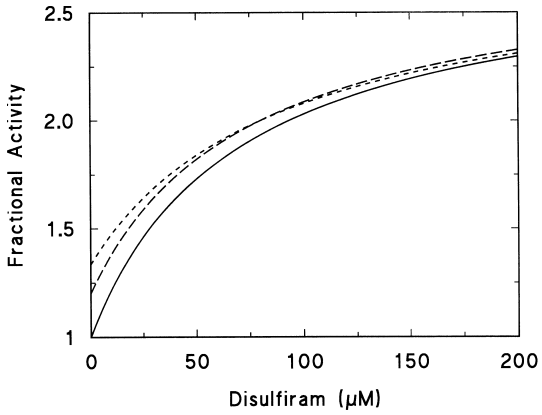


Figure 6 Simulation of the effects of two stimulatory compounds binding at the same site on the ATPase

The solid curve shows the increase in activity expected as a function of disulfiram concentration if disulfiram binds to a single site on the ATPase with a K_d of 60 μM, binding to this site resulting in a maximal activity 2.75 times that in the absence of disulfiram. The broken and dotted curves show the expected effects of disulfiram in the presence of 5 or 10 μM jasmone respectively, if jasmone binds to the same site with a K_d of 20 μM, binding of jasmone resulting in a doubling of maximal activity.

only one phosphorylated, Ca^{2+}-bound form of the ATPase ($E2PCa_2$) is shown, consistent with recent experiments [21]. In the E1 conformation, the ATPase possesses two outward-facing Ca^{2+}-binding sites of high affinity. After binding of Ca^{2+} and ATP the enzyme is phosphorylated and undergoes a conformation change to $E2PCa_2$, from which Ca^{2+} is lost to the lumen of the SR. Dephosphorylation of E2P allows recycling to E1.

Disulfiram was found to have no effect on the rate of phosphorylation of the ATPase by ATP, but it did increase both the rates of the Ca^{2+} transport step ($E2PCa_2 \rightarrow E2P$ in Scheme 2) (Figure 3B) and the rate of dephosphorylation (Figure 4). Jasmone, which caused a similar increase in ATPase activity, was also found to increase the rates of these two steps, with no other effects on the ATPase [13]. However, it seems that the binding sites for jasmone and disulfiram on the ATPase are distinct. If jasmone and disulfiram were to bind at the same site on the ATPase then stimulation of the ATPase by mixtures of the two compounds would show the pattern of effects illustrated in Figure 6, in which the solid curve shows the increase in activity expected as a function of disulfiram concentration if disulfiram binds to a single site on the ATPase with a K_d of 60 μM, binding to this site resulting in a maximal activity 2.75 times that in the absence of disulfiram. The broken and dotted curves in Figure 6 show the expected effects of disulfiram in the presence of 5 or 10 μM jasmone respectively, if jasmone binds to the same site on the ATPase as disulfiram with a K_d of 20 μM, binding of jasmone resulting in a doubling of maximal activity. Half-maximal effects

of disulfiram would be expected at 75 and 90 μM disulfiram in the presence of 5 and 10 μM jasmone respectively (Figure 6). The experimental results do not show this increase in concentration of disulfiram required to produce a half-maximal effect (Figure 1), suggesting that binding of disulfiram and jasmone to the ATPase is non-competitive.

Disulfiram was found to decrease the level of phosphorylation of the ATPase by P_i in the absence of Ca^{2+} (Figure 5). The effect was consistent with disulfiram's having no effect on the affinities of the ATPase for Mg^{2+} or P_i but causing a decrease in the equilibrium constant $E2PMg/E2P_iMg$ from 15 in the absence of disulfiram to 2.0 in the presence of 80 μM disulfiram (Figure 5). Given that the rate of dephosphorylation approximately doubles in the presence of disulfiram (see above), this implies a large decrease in the rate of phosphorylation by P_i.

Plots of ATPase activity against ATP concentration show increases in activity in the μM and mM ranges, corresponding to kinetically distinguishable catalytic and stimulatory sites respectively (Figure 2). It is not clear whether these correspond to two separate sites on the ATPase or whether the stimulation of activity observed at high concentrations of ATP corresponds to binding of ATP to an empty nucleotide-binding site on the phosphorylated ATPase. The stimulation of ATPase activity observed at high concentrations of ATP results from an increase in the rates of a number of the steps after phosphorylation, including the rates of the Ca^{2+} transport step, of dephosphorylation, and of the E2–E1 conformation change (see [16]). It has been shown that eosin can mimic the effects of ATP at the stimulatory site [22]. However, the effect of disulfiram is seen to be greatest at high concentrations of ATP (Figure 2) so that disulfiram cannot be binding to the stimulatory ATP-binding site on the ATPase.

The experiments of Jencks et al. [21,23,24] are consistent with four Ca^{2+}-binding sites on the ATPase, separate pairs being located on the cytoplasmic and luminal sides of the membrane, with the Ca^{2+} ions being transferred from the cytoplasmic to the luminal pair of sites on phosphorylation of the ATPase. Four acidic residues have been identified in the ATPase whose mutation disrupts the high-affinity binding of Ca^{2+} from the cytoplasmic side of the membrane. These residues are located in putative transmembrane helices 4, 5, 6 and 8; the two high-affinity binding sites for Ca^{2+} are presumed to be located in a channel-like structure made up of these four helices [25,26]. Up to four extra acidic residues are then needed for the luminal pair of sites. These extra residues are not within the transmembrane helices because the only acidic residues in the transmembrane helices are the four making up the high-affinity cytoplasmic pair of sites. The extra acidic residues must therefore be located in the luminal part of the ATPase. Electron microscopic studies show that this part of the ATPase comprises only approx. 5% of the total mass of the ATPase [7]; it is predicted to consist of small tight loops between transmembrane helices, except for the loop between helices M7 and M8, which is predicted to be 38 residues long [5]. Release of Ca^{2+} from the phosphorylated ATPase ($E2PCa_2 \rightarrow E2P$ in Scheme 2) thus presumably involves conformational changes in the luminal loops of the ATPase. The observation that both jasmone and disulfiram not only affect the rate of the Ca^{2+} transport step but also alter phosphorylation of the ATPase by P_i (and dephosphorylation) suggests that changes in the phosphorylation domain of the ATPase are linked to the changes in the luminal loops that allow the release of Ca^{2+} from the ATPase.

It has been suggested that phosphorylation/dephosphorylation of the ATPase involves relative movement of the phosphorylation and nucleotide-binding domains of the ATPase [27,28]. Furthermore experiments with fluorescent trinitrophenyl nucleotides are consistent with a conformational change on the ATPase after phosphorylation with P_i in which the nucleotide-binding site becomes more hydrophobic, consistent with the closing of a cleft between the phosphorylation and nucleotide-binding domains [29,30]. Cross-linking the ATPase with *o*-phthalaldehyde has been shown to result in a decrease in the rate of dephosphorylation of the ATPase and a decrease in the level of phosphorylation by P_i; it was suggested that this could follow if the ATPase were held in a conformation in which the exposures of the nucleotide-binding site and the phosphorylation domain to water were decreased [28]. Correspondingly, if the exposure of the phosphorylated residue (Asp-351) to water were increased after binding of disulfiram to the ATPase, this could be expected to lead to an increase in the rate of dephosphorylation and a decreased level of phosphorylation by P_i, as observed.

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REFERENCES

1 Green, N. M. (1992) Ann. NY Acad. Sci. **671**, 104–112
2 MacLennan, D. H., Brandl, C. J., Korczak, B. and Green, N. M. (1985) Nature (London) **316**, 696–700
3 Reithmeier, R. A. and MacLennan, D. H. (1981) J. Biol. Chem. **256**, 5957–5960
4 Matthews, I., Sharma, R. P., Lee, A. G. and East, J. M. (1990) J. Biol. Chem. **265**, 18737–18740
5 Mata, A. M., Matthews, I., Tunwell, R. E. A., Sharma, R. P., Lee, A. G. and East, J. M. (1992) Biochem. J. **286**, 567–580
6 Clarke, D. M., Loo, T. W. and MacLennan, D. H. (1990) J. Biol. Chem. **265**, 17405–17408
7 Toyoshima, C., Sasabe, H. and Stokes, D. L. (1993) Nature (London) **362**, 469–471
8 Kawakita, M., Yasuoka, K. and Kaziro, Y. (1980) J. Biochem. (Tokyo) **87**, 609–617
9 Stefanova, H. I., East, J. M., Gore, M. G. and Lee, A. G. (1992) Biochemistry **31**, 6023–6031
10 Nagendra, S. N., Rao, K. M., Subhash, M. N. and Shetty, K. T. (1994) Neurochem. Res. **19**, 1509–1513
11 Vallari, R. C. and Pietruszko, R. (1982) Science **216**, 637–639
12 East, J. M. and Lee, A. G. (1982) Biochemistry **21**, 4144–4151
13 Starling, A. P., Hughes, G., East, J. M. and Lee, A. G. (1994) Biochemistry **33**, 3023–3031
14 Starling, A. P., East, J. M. and Lee, A. G. (1995) Biochem. J. **310**, 875–879
15 Shigekawa, M., Wakabayashi, S. and Nakamura, H. (1983) J. Biol. Chem. **258**, 8698–8707
16 Gould, G. W., East, J. M., Froud, R. J., McWhirter, J. M., Stefanova, H. I. and Lee, A. G. (1986) Biochem. J. **237**, 217–227
17 Orlowski, S., Lund, S., Moller, J. V. and Champeil, P. (1988) J. Biol. Chem. **263**, 17576–17583
18 de Meis, L. (1981) The Sarcoplasmic Reticulum, Wiley, New York
19 Froud, R. J. and Lee, A. G. (1986) Biochem. J. **237**, 207–215
20 Lee, A. G., Baker, K., Khan, Y. M. and East, J. M. (1995) Biochem. J. **305**, 225–231
21 Myung, J. and Jencks, W. P. (1994) Biochemistry **33**, 8775–8785
22 Mignaco, J. A., Lupi, O. H., Santos, F. T., Barrabin, H. and Scofano, H. M. (1996) Biochemistry **35**, 3886–3891
23 Jencks, W. P., Yang, T., Peisach, D. and Myung, J. (1993) Biochemistry **32**, 7030–7034
24 Myung, J. and Jencks, W. P. (1995) Biochemistry **34**, 3077–3083
25 Clarke, D. M., Loo, T. W. and MacLennan, D. H. (1990) J. Biol. Chem. **265**, 6262–6267
26 Lee, A. G., Starling, A. P., Ding, J., East, J. M. and Wictome, M. (1994) Biochem. Soc. Trans. **22**, 821–826
27 Petithory, J. R. and Jencks, W. P. (1986) Biochemistry **25**, 4493–4497
28 Khan, Y. M., Starling, A. P., East, J. M. and Lee, A. G. (1996) Biochem. J. **317**, 439–445
29 Dupont, Y. and Pougeois, R. (1983) FEBS Lett. **156**, 93–98
30 Davidson, G. A. and Berman, M. C. (1987) J. Biol. Chem. **262**, 7041–7046

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