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STIMULATORY AND INHIBITORY EFFECTS OF DIMETHYLSULFOXIDE, PROPRANOLOL AND CHLORPROMAZINE ON THE PARTIAL REACTIONS OF ATPase OF SARCOPLASMIC RETICULUM

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Summary

The effects of dimethylsulfoxide, propranolol and chlorpromazine on the partial reactions of the ATPase of sarcoplasmic reticulum were investigated. When analyzed according to a reaction scheme in which the ADP-sensitive (E_1P) and ADP-insensitive (E_2P) phosphoenzymes occur sequentially and P_i is derived from the latter, dimethylsulfoxide decreased the rate of E_2P hydrolysis whereas it stimulated the rate of the E_1P to E_2P conversion. Propranolol increased the rate of E_2P hydrolysis while it decreased the rate of the E_1P to E_2P conversion. Propranolol exerted an additional effect, presumably inhibition of the phosphoenzyme formation. These effects of dimethylsulfoxide and propranolol can account for both the stimulatory and inhibitory effects of these drugs on the overall rate of ATP hydrolysis observed in the presence and absence of added alkali metal salts.

Chlorpromazine accelerated E_2P hydrolysis whereas it appeared to inhibit the E_1P to E_2P conversion. These effects of chlorpromazine appear able to account for its stimulatory and inhibitory effects on the overall rate of ATP hydrolysis in the presence and absence of alkali metal salts. In the presence of chlorpromazine, however, the rate of P_i liberation during the steady state ATP hydrolysis was found to be greater than the hydrolysis rate of E_2P. This finding suggests that under these conditions P_i is derived not only from E_2P but also from source(s) other than E_2P.

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Abbreviation: EGTA, ethylene glycol-bis-(β-amino-ethylether)-*N*,*N*′-tetraacetic acid.

Introduction

Sarcoplasmic reticulum vesicles actively transport calcium using the energy derived from the hydrolysis of ATP that is catalized by the membrane-bound Ca^{2+} and Mg^{2+}-dependent ATPase [1—4]. The mechanism of this ATP hydrolysis has been studied extensively and elaborate reaction schemes involving several forms of the phosphorylated intermediate have been proposed [5—15]. The minimum reaction sequence may be summarized as follows [14,16];

Scheme 1.

$$E + ATP \overset{ADP}{\swarrow} E_1P \rightharpoonup E_2P \rightharpoonup E + P_i$$

Two distinct forms of the acid-stable phosphoenzyme intermediate are included in this reaction sequence; E_1P is ADP-sensitive as it can donate its phosphate group to added ADP to form ATP, whereas E_2P is ADP-insensitive as it does not donate its phosphate group to added ADP. The ATPase (E) reacts with MgATP and Ca^{2+} on the outer surface of the vesicles and the terminal phosphate of ATP is transferred to the enzyme to form E_1P, ADP being liberated on the outer surface of the membranes. The E_1P to E_2P conversion, which involves the translocation and release of Ca^{2+} on the inner surface of the vesicles, is inhibited by high Ca^{2+} and appears to be stimulated by low Mg^{2+} concentrations. E_2P hydrolysis is accelerated by MgATP, Mg^{2+}, Ca^{2+} or alkali metal salts, and the products, P_i and the enzyme appear to be released on the outer surface of the vesicles.

In the absence of added alkali metal salts E_2P accumulates as the major steady state intermediate and the E_2P hydrolysis appears to be the slowest step in the ATP hydrolysis [16,17]. In the presence of high KCl concentrations, however, the rate of E_2P hydrolysis is much greater than the overall rate of ATP hydrolysis and the rate of the E_1P to E_2P conversion [16,17]. In agreement with these findings, E_1P accumulates as the major steady state intermediate under these conditions [10,17,18]. Thus, the E_1P to E_2P conversion is considered to be the rate-limiting step of ATP hydrolysis in high KCl concentrations.

It was reported previously that dimethylsulfoxide, propranolol or chlorpromazine inhibit calcium transport and concomitant ATP hydrolysis by sarcoplasmic reticulum vesicles [19—24]. In the present study, the effects of these drugs on the partial reactions of ATP hydrolysis by the ATPase of sarcoplasmic reticulum was investigated. The results indicate that these drugs exert both stimulatory and inhibitory effects on the partial reactions of the calcium pump ATPase.

Experimental Procedures

Sarcoplasmic reticulum vesicles were prepared from rabbit skeletal muscle as described previously [25]. Partially purified ATPase protein was prepared according to Meissner and Fleischer [26] with slight modification [27], and freed of alkali metal salts [25]. ATPase activity and phosphoenzyme levels

were assayed as described previously [27] under the standard conditions unless otherwise stated. The standard conditions were 0.20 to 0.25 mg/ml of partially purified ATPase protein, 15 mM imidazole/HCl (pH 7.0), 2.0 mM $MgCl_2$, 20 μM $CaCl_2$ and 20 μM [γ-^{32}P]ATP at 0°C. ATPase reactions were started by addition of [γ-^{32}P]ATP to the reaction medium and terminated by addition of trichloroacetic acid solution (final concentration; 5 to 6.5% (w/v)) containing 0.1 mM P_i and 1.0 mM ATP as carriers. The Ca^{2+}-dependent ATPase activity and Ca^{2+}-dependent phosphoenzyme levels were estimated by subtracting the ATPase activity and the phosphoenzyme levels in 0.5 to 1.0 mM EGTA from those obtained in the presence of Ca^{2+}. The steady state rate of ATP hydrolysis was calculated from the linear time course of P_i liberation between 15 to 40 s after the start of the reaction. The steady state level of the total phosphoenzyme was measured 15 s or 30 s after the start of phosphorylation. The steady state levels of the ADP-sensitive and ADP-insensitive phosphoenzymes were determined as shown below by the procedure described previously [16]. The phosphoenzyme decomposition induced by addition of excess EGTA and MgADP after steady states were reached in the presence of either dimethylsulfoxide, propranolol or chlorpromazine, and various KCl concentrations, exhibited rapid phases followed by slow phases (cf. Fig. 3). The slow phases obeyed first order kinetics, allowing the amounts of the slowly-decomposing phosphoenzyme fractions at the time of addition of EGTA and MgADP to be determined by extrapolation. The rapidly-decomposing phosphoenzymes could then be estimated by subtracting the amounts of the slowly-decomposing phosphoenzyme from the total amounts of phosphoenzyme present at the time of addition of EGTA and MgADP. The decompositions of the slowly-decomposing fractions of phosphoenzymes observed after addition of EGTA and MgADP were accompanied by almost stoichiometric amounts of P_i liberation. Thus, as in the previous study [16], it was concluded that the slow late phases of phosphoenzyme decomposition represent the hydrolysis of the ADP-insensitive phosphoenzyme, while the rapidly-decomposing fractions represent the ADP-sensitive phosphoenzyme that reacts with added ADP to form ATP [10,14,18]. The rate constants for the hydrolysis of the ADP-insensitive phosphoenzyme (k_d) estimated from the time courses of the phosphoenzyme decomposition after addition of EGTA and MgADP were almost identical to those (k'_d) of the ADP-insensitive phosphoenzyme obtained in the presence of EGTA alone (compare values of k_d and k'_d in Table I). Thus, it was concluded that excess ADP does not affect the hydrolysis rate of the ADP-insensitive phosphoenzyme significantly under the conditions used in the present study.

The Na^+ and K^+ contents of the drug solutions were determined as described previously [27]. The Na^+ or K^+ concentrations contaminating 5 mM propranolol solution were less than 7 and 1 μM, respectively whereas those in 0.2 mM chlorpromazine solution were less than 3 and 1 μM, respectively. The Mg^{2+} content of the drug solutions was determined with Hitachi atomic absorption spectrophotometer (Model 170-30). Free Mg^{2+} concentrations were calculated from the total concentrations of ATP, ADP, calcium and magnesium as described previously [14]. Protein concentrations were determined by the method of Lowry et al. [28] with bovine serum albumin as a standard.

ATP was purchased from Boehringer Mannheim GmbH, Biochemica. ADP,

Tris, EGTA, dimethylsulfoxide and (±)-propranolol · HCl were purchased from Sigma. Chlorpromazine · HCl was a generous gift from Dr. D.T. Walz of Smith Kline French Laboratories. [γ-^{32}P]ATP tetra(triethylammonium) salts (25 to 30 mCi/μmol) was purchased from ICN Pharmaceuticals, Inc. Na_2ATP and Na_2ADP were converted to Tris salts by passage through a Dowex 50W-X8 (Tris) column. All reagents used were of analytical grade. Distilled water was deionized by passage through ion exchange resins prior to use. Glassware was pretreated with chromium-sulfuric acid to remove contaminating cations.

Results

Effect of dimethylsulfoxide concentration on rate of ATP hydrolysis and phosphoenzyme level

The steady state rates of ATP hydrolysis and the steady state levels of phosphoenzyme were studied as a function of dimethylsulfoxide concentration in the presence and absence of KCl (Fig. 1). In the absence of added alkali metal salts, the rate of ATP hydrolysis decreased with increasing concentrations of dimethylsulfoxide. In 100 mM KCl, however, the rate of ATP hydrolysis increased significantly at low dimethylsulfoxide concentrations while it

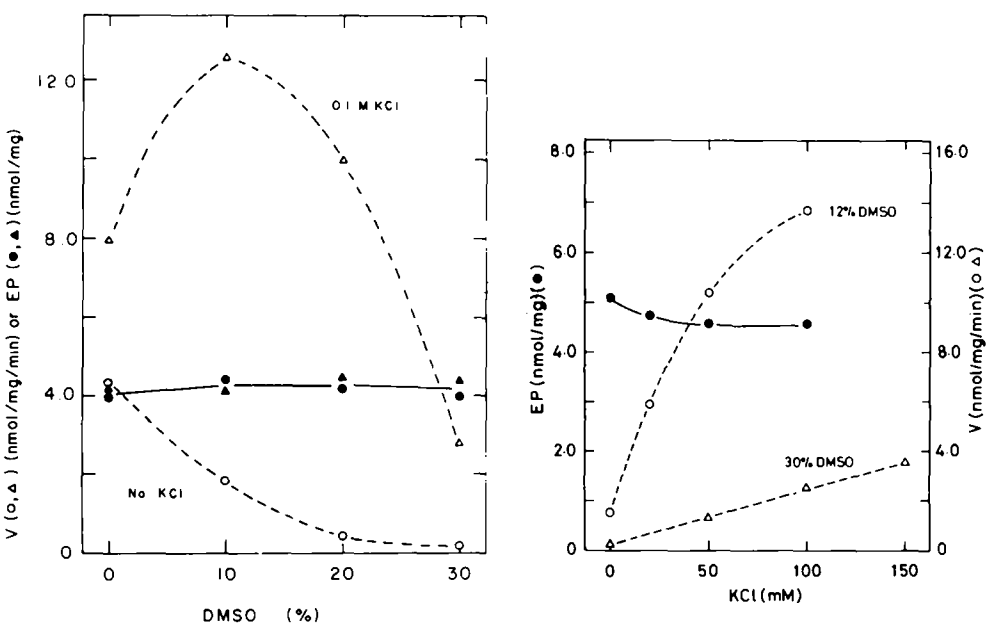

Fig. 1. Effect of dimethylsulfoxide concentration on the steady state rate of ATP hydrolysis and the steady state level of phosphoenzyme. The reactions were carried out under standard conditions in the presence of the dimethylsulfoxide concentrations indicated in the abscissa and in the presence of 100 mM KCl (△,▲) or in the absence of added alkali metal salts (○,●).

Fig. 2. KCl concentration dependence of the steady state rate of ATP hydrolysis and the steady state level of phosphoenzyme obtained under standard conditions in 12% (v/v) (○,●) or 30% (v/v) (△) dimethylsulfoxide.

decreased at high dimethylsulfoxide concentrations. The total amount of phosphoenzyme was not affected significantly by changing concentrations of this agent both in the presence and absence of 100 mM KCl (Fig. 1).

The steady state rate of ATP hydrolysis and the steady state level of phosphoenzyme measured in the presence of 12% (v/v) dimethylsulfoxide are plotted as function of KCl concentration in Fig. 2. The rate of ATP hydrolysis increased markedly with increasing KCl concentration, its value in 100 mM KCl being approximately 9-fold that obtained in the absence of added alkali metal salts. An increase in dimethylsulfoxide concentration from 12 to 30% (v/v) in the reaction medium decreased the rate of ATP hydrolysis markedly at each KCl concentration tested and shifted the KCl-dependence curve of the rate of ATP hydrolysis to much higher KCl concentrations (Fig. 2). Thus, both the stimulatory concentration of KCl and the extent of stimulation were much greater in the presence of dimethylsulfoxide than in the control or in the presence of propranolol (compare Figs. 2 and 6).

Effects of dimethylsulfoxide on ADP-sensitive and ADP-insensitive phosphoenzyme levels and on decomposition rate of ADP-insensitive phosphoenzyme

The phosphoenzyme formed at the steady state in the presence of dimethylsulfoxide and various concentrations of KCl could be resolved into the ADP-sensitive and ADP-insensitive phosphoenzymes (Fig. 3 see also Experimental Procedures). Dimethylsulfoxide, which did not affect the total amount of phosphoenzyme greatly (Fig. 1), increased the steady state levels of the ADP-insensitive fraction of the phosphoenzyme (Tables I and II). The rate constants for decomposition of the ADP-insensitive phosphoenzyme, as measured from

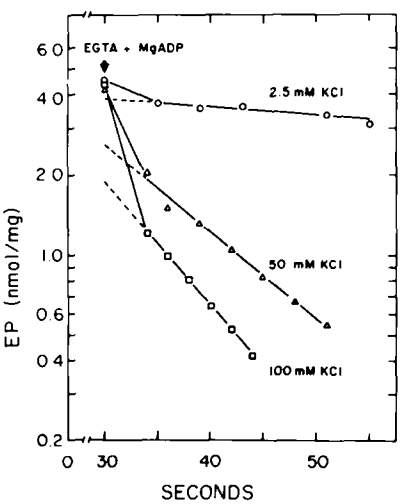

Fig. 3. Time courses of decomposition of the phosphoenzyme formed in various KCl concentrations and 12% (v/v) dimethylsulfoxide after addition of EGTA and MgADP. Enzyme phosphorylation was carried out under standard conditions in the presence of 2.5 mM (○), 50 mM (△) or 100 mM (□) KCl. At 30 s (↓) 0.05 ml of a mixture of 21 mM ADP, 20.9 mM $MgCl_2$ and 145 mM EGTA was added to 1.0 ml of reaction medium and the time courses of the phosphoenzyme decomposition were measured.

TABLE I

EFFECT OF DIMETHYLSULFOXIDE CONCENTRATION ON ADP-INSENSITIVE PHOSPHOENZYME LEVEL AND RATE CONSTANTS FOR PARTIAL REACTIONS OF ATP HYDROLYSIS

The steady state rate of ATP hydrolysis (V), the steady state levels of total (EP), ADP-sensitive (E_1P), and ADP-insensitive (E_2P) phosphoenzymes were measured under standard conditions in the presence of 100 mM KCl and the dimethylsulfoxide concentrations indicated in the table. k_d is the rate constant for decomposition of the ADP-insensitive phosphoenzyme that was estimated from the time course of the phosphoenzyme decomposition observed after addition of EGTA and MgADP (cf. Fig. 3). k'_d is the rate constant for decomposition of the ADP-insensitive phosphoenzyme obtained in the presence of EGTA otherwise under the same conditions. k'_d was measured as described below; the enzyme was phosphorylated under standard conditions in the presence of the dimethylsulfoxide concentrations indicated in the table but in the absence of added alkali metal salts. At 20 s after the start of phosphorylation, excess EGTA was added to the reaction medium to stop further phosphorylation. 10 s thereafter, KCl (final concentration, 100 mM) was added to reaction medium and the time course of phosphoenzyme decomposition followed. The phosphoenzyme obtained 10 s after addition of EGTA under these experimental conditions was completely ADP-insensitive.

Dimethylsulfoxide (%)(v/v)	E_2P/EP (%)	V/E_1P (min^{-1})	V/E_2P (min^{-1})	k_d (min^{-1})	k'_d (min^{-1})
12	46.4	5.58	6.45	6.58	6.04
17	68.8	9.79	4.45	4.57	4.05
30	84.2	4.21	0.80	0.89	0.75

the time courses of the ADP-insensitive phosphoenzyme decomposition after addition of EGTA and MgADP (k_d) or EGTA alone (k'_d) (see the legend to Table I) or as estimated as the ratios between the steady state rate of ATP hydrolysis and the steady state levels of the ADP-insensitive phosphoenzyme, decreased with increasing concentrations of dimethylsulfoxide (Table I). These effects of dimethylsulfoxide on the ADP-insensitive phosphoenzyme level and the rate constant for the decomposition of the ADP-insensitive phosphoenzyme were counteracted by increasing concentrations of KCl (Fig. 3).

Effect of dimethylsulfoxide on conversion of ADP-sensitive to ADP-insensitive phosphoenzymes

The relative amounts of the ADP-sensitive and ADP-insensitive phosphoenzymes formed during the initial transient phase of ATP hydrolysis in the absence of added alkali metal salts, are dependent on free Mg^{2+} concentrations [14]. The change in the ratio of these two types of phosphoenzyme observed

TABLE II

EFFECT OF DIMETHYLSULFOXIDE ON ATP HYDROLYSIS RATES, LEVELS OF PHOSPHOENZYME AND ITS COMPONENTS, AND THEIR RATIOS

The steady state rate of ATP hydrolysis (V) and the steady state levels of total (EP), ADP-sensitive (E_1P) and ADP-insensitive (E_2P) phosphoenzymes were measured under standard conditions in 30 mM KCl and in the presence or absence of 10% dimethylsulfoxide.

	V (nmol/mg/min)	EP (nmol/mg)	E_2P/EP (%)	V/E_1P (min^{-1})	V/E_2P (min^{-1})
Control	8.77	3.48	28.2	3.51	8.93
10% dimethylsulfoxide	8.52	3.83	70.3	7.50	3.16

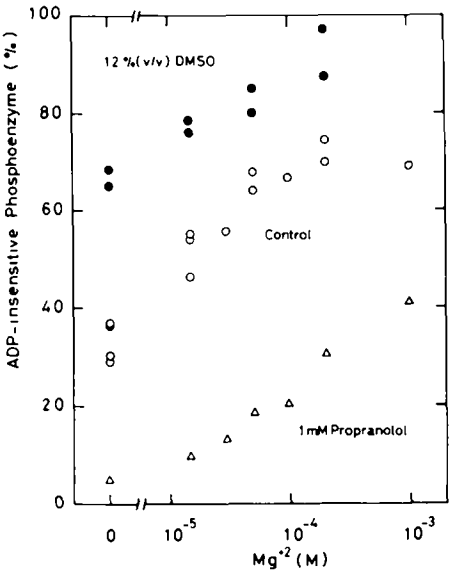

Fig. 4. Mg^{2+} concentration dependence of the percentage of the ADP-insensitive phosphoenzyme formed at 5 s after the start of phosphorylation. Reactions were carried out at 0°C with 0.25 mg/ml of partially purified ATPase protein in 15 mM imidazole/HCl (pH 7.0), 5.0 μM [γ-^{32}P]ATP, 20 μM $CaCl_2$ and various concentrations of $MgCl_2$, and in the presence of 12% (v/v) dimethylsulfoxide (●) or 1.0 mM propranolol (△), or in their absence (○). The amounts of the total phosphoenzyme and the ADP-sensitive and ADP-insensitive phosphoenzyme present at 5 s after the start of phosphorylation were determined as described in Experimental Procedures. Free Mg^{2+} concentrations that are shown on the abscissa, were calculated as described in Experimental Procedures assuming that the amount of Mg^{2+} contaminating the reaction medium is negligibly small.

under these conditions can be interpreted to reflect the change in the rate of conversion of the ADP-sensitive to ADP-insensitive phosphoenzymes as evidence has been presented for the sequential appearance of these two types of phosphoenzyme intermediate [14]. In the experiment shown in Fig. 4, the amounts of the ADP-sensitive and ADP-insensitive phosphoenzymes were measured at 5 s after the start of phosphorylation in 5.0 μM [γ-^{32}P]ATP, 20 μM $CaCl_2$ and various concentrations of Mg^{2+} at 0°C. In agreement with our previous report [14], the percentage of the ADP-insensitive fraction of phosphoenzyme in the control experiment increased with increasing concentrations of Mg^{2+} in the range between 0 and 100 μM (Fig. 4). In the presence of 12% (v/v) dimethylsulfoxide, the percentages of the ADP-insensitive phosphoenzyme fraction were already high even at low Mg^{2+} concentrations (Fig. 4). Thus, dimethylsulfoxide appears to stimulate the rate of conversion of the ADP-sensitive to ADP-insensitive phosphoenzymes. This stimulatory effect of dimethylsulfoxide is pronounced at low Mg^{2+} concentrations where the conversion is slow and the turnover of the ATPase is minimal [14]. This effect of dimethylsulfoxide cannot be attributed to the contamination of the drug solution by high concentrations of Mg^{2+} because the Mg^{2+} contamination in 12% (v/v) dimethylsulfoxide was less than 1.0 μM. In agreement with the result of the experiment of Fig. 4, the ratio between the steady state rate of ATP hydrolysis and the steady state level of the ADP-sensitive phosphoenzyme

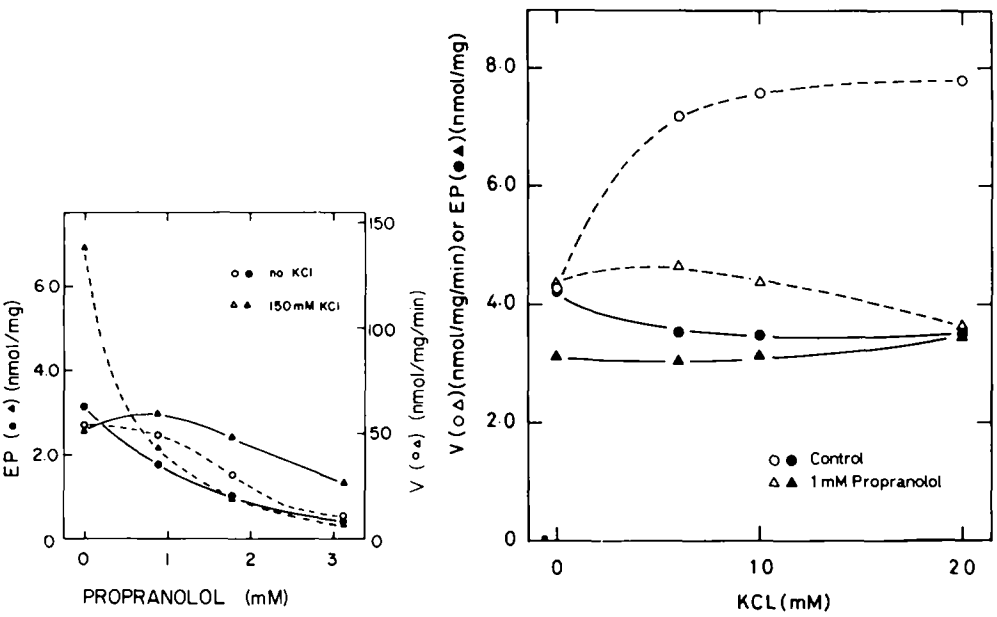

Fig. 5. Effect of propranolol concentration on the steady state rate of ATP hydrolysis and the steady state level of phosphoenzyme. The reactions were carried out at 10°C with 0.207 mg/ml of partially purified ATPase protein in 15 mM imidazole/HCl (pH 7.0), 50 μM [γ-^{32}P]ATP, 0.8 mM $MgCl_2$ and 15 μM $CaCl_2$ in the presence of the propranolol concentrations indicated in the abscissa and in the presence of 150 mM KCl (△,▲) or in the absence of added alkali metal salts (○,●).

Fig. 6. KCl concentration dependence of the steady state rate of ATP hydrolysis and the steady state level of phosphoenzyme obtained under standard conditions in the presence (△,▲) or absence (○,●) of 1.0 mM propranolol.

obtained in high Mg^{2+} and 30 mM KCl under standard conditions was 2-fold greater in 10% (v/v) dimethylsulfoxide than in the absence of the drug (Table II).

Effect of propranolol on rate of ATP hydrolysis and phosphoenzyme level

The steady state rate of ATP hydrolysis and the steady state level of phosphoenzyme were studied at 10°C as a function of propranolol concentration (Fig. 5). In the presence of 150 mM KCl, the rate of ATP hydrolysis decreased markedly with increasing propranolol concentrations whereas the total amount of phosphoenzyme decreased significantly only at high concentrations of the drug. In the absence of added alkali metal salts, propranolol decreased the rate of ATP hydrolysis to a much less extent than in 150 mM KCl. However, the drug decreased the total amount of phosphoenzyme to a greater extent in the absence of added alkali metal salts (Fig. 5). In Fig. 6, the steady state rate of ATP hydrolysis and the steady state level of phosphoenzyme measured in the presence of 1.0 mM propranolol are shown as a function of KCl concentration. The stimulation of the rate of ATP hydrolysis by KCl was not significant in the presence of 1.0 mM propranolol.

TABLE III

EFFECT OF PROPRANOLOL ON ADP-INSENSITIVE PHOSPHOENZYME LEVEL AND RATE CONSTANTS FOR PARTIAL REACTIONS OF ATP HYDROLYSIS

The steady state rate of ATP hydrolysis (V) and the steady state levels of total (EP), ADP-sensitive (E_1P) and ADP-insensitive (E_2P) phosphoenzymes were measured under standard conditions in the presence of the KCl and propanolol concentrations indicated in the table. k_d is the rate constant for decomposition of the ADP-insensitive phosphoenzyme directly estimated from the time course of the phosphoenzyme decomposition after addition of EGTA and MgADP. The experiment was performed as described in the legend to Fig. 3.

KCl (mM)	Propranolol (mM)	E_2P/EP (%)	V/E_1P (min^{-1})	V/E_2P (min^{-1})	k_d (min^{-1})
0	0	78.4	4.76	1.30	0.93
	1.0	61.7	3.66	2.27	1.62
6	0	56.1	4.62	3.62	3.28
	1.0	30.4	2.20	5.06	4.25
20	0	34.8	3.39	6.37	6.92
	1.0	15.2	1.23	6.86	7.80

Effect of propranolol on ADP-sensitive and ADP-insensitive phosphoenzyme levels

As in the presence of dimethylsulfoxide, the phosphoenzyme formed in the presence of propranolol and various concentrations of KCl could also be resolved into ADP-sensitive and ADP-insensitive fractions (data not shown; cf. Fig. 3). Unlike dimethylsulfoxide, however, propranolol decreased the percentage of the ADP-insensitive fraction of phosphoenzyme significantly (Table III). This effect of propranolol became pronounced when KCl concentration in the reaction medium was increased (Table III).

Effects of propranolol on conversion of ADP-sensitive to ADP-insensitive phosphoenzymes and on decomposition rate of ADP-insensitive phosphoenzyme

The percentage of the ADP-insensitive fraction of phosphoenzyme was measured at 5 s after the start of the phosphorylation as a function of Mg^{2+} concentration (Fig. 4). When 1.0 mM propranolol was present in the reaction medium, the percentage of the ADP-insensitive fraction of phosphoenzyme was significantly lowered at each Mg^{2+} concentration tested. This effect of propranolol was pronounced at low concentrations of Mg^{2+}, the ADP-insensitive fraction obtained at 15 μM Mg^{2+} in 1.0 mM propranolol being approximately 1/5 of the corresponding fraction obtained in the control experiment (Fig. 4). In agreement with this finding, the values of the ratio between the rate of ATP hydrolysis and the ADP-sensitive phosphoenzyme level obtained at the steady state in high Mg^{2+} and in 0 to 20 mM KCl were 1.3 to 2.8-fold less in the presence of 1.0 mM propranolol (Table III). Thus, this effect of propranolol was pronounced with higher concentrations of KCl in the reaction medium.

It should be noted that propranolol had an additional effect on the ATP hydrolysis. The rate constants (k_d) for the decomposition of the ADP-insensitive phosphoenzyme obtained in the absence of added alkali metal salts after addition of EGTA and MgADP increased approximately 1.7-fold when 1.0 mM

propranolol was present in the reaction medium (Table III). In accord with this finding, the value of the ratio between the steady state rate of ATP hydrolysis and the steady state level of the ADP-insensitive phosphoenzyme obtained in the absence of added alkali metal salts was approximately 1.7-fold greater in the presence of 1.0 mM propranolol than in the absence of the drug (Table III). The value of this ratio obtained under these conditions increased with increasing concentrations of propranolol, the value in 3.0 mM propranolol being approximately 3-fold that in the absence of the drug. This stimulatory effect of propranolol, however, was not significant in the presence of 20 mM KCl (Table III).

Effect of chlorpromazine on rate of ATP hydrolysis and phosphoenzyme levels

The steady state rates of ATP hydrolysis and the steady state levels of phosphoenzyme were studied as a function of chlorpromazine concentration in the presence and absence of KCl (Fig. 7A and B). In contrast to the results obtained in the presence of dimethylsulfoxide (Fig. 1), chlorpromazine stimulated the rate of ATP hydrolysis significantly in the absence of added alkali metal salts (Fig. 7A), whereas it inhibited ATP hydrolysis in the presence of 150 mM KCl (Fig. 7B). In the absence of added alkali metal salts, the total amount of phosphoenzyme decreased with increasing concentration of the drug while it decreased to a much less extent in the presence of 150 mM KCl.

The KCl-dependence of the rate of ATP hydrolysis and phosphoenzyme level obtained in the presence and absence of chlorpromazine are shown in Fig. 8. In contrast to the results obtained with dimethylsulfoxide or in the control experiment, increasing concentrations of KCl decreased the rate of ATP hydrolysis in the presence of 0.1 mM chlorpromazine whereas KCl did not affect the total amount of phosphoenzyme significantly under the same conditions (Fig. 8).

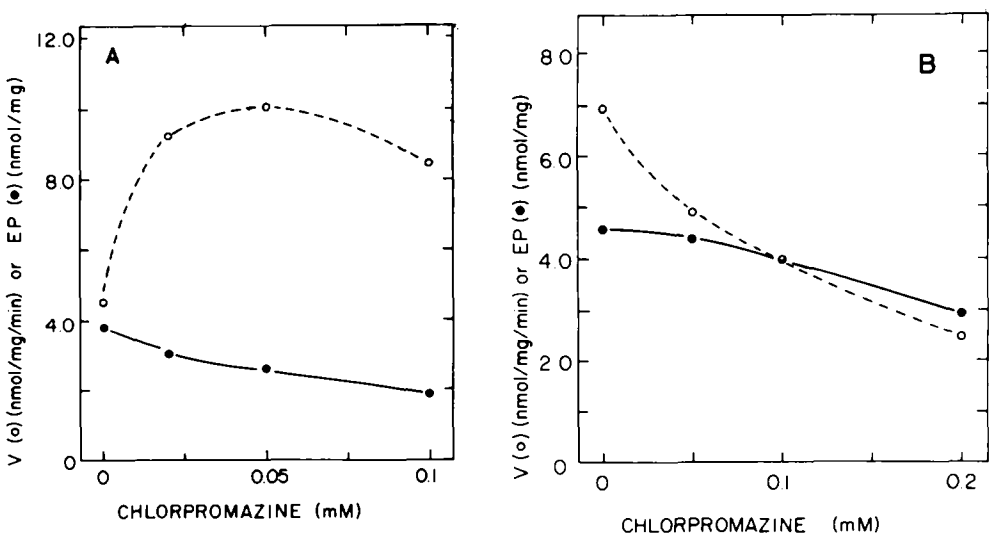

Fig. 7. Effect of chlorpromazine concentration on the steady state rate of ATP hydrolysis and the steady state level of phosphoenzyme. The reactions were carried out under standard conditions in the presence of the chlorpromazine concentrations indicated in the abscissa and in the absence of added alkali metal salts (A) or in 150 mM KCl (B).

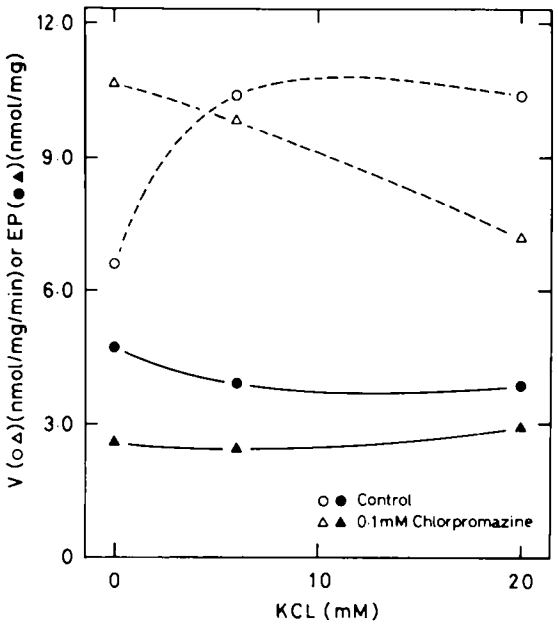

Fig. 8. KCl concentration dependence of the steady state rate of ATP hydrolysis and the steady state level of phosphoenzyme obtained under standard conditions in the presence (△,▲) or absence (○,●) of 0.1 mM chlorpromazine.

Effect of chlorpromazine on the ADP-sensitive and ADP-insensitive phosphoenzyme levels and on the decomposition rate of the ADP-insensitive phosphoenzyme

The phosphoenzyme formed at the steady state in the presence of chlorpromazine could also be resolved into ADP-sensitive and ADP-insensitive phosphoenzyme (data not shown; cf. Fig. 3). Chlorpromazine, like propranolol, increased the ADP-sensitive fraction of phosphoenzyme both in the presence and absence of KCl (Table IV). The rate constants (k_d) for the decomposition of the ADP-insensitive phosphoenzyme as measured from the time course of the phosphoenzyme decomposition after addition of EGTA and MgADP (cf. Fig. 3) were significantly greater in the presence of chlorpromazine than in its absence; the value of the rate constant (k_d) obtained under standard conditions in the absence of added alkali metal salts but in the presence of 0.02, 0.05 and 0.1 mM chlorpromazine were 2.2-, 3.2-, and 4.1-fold greater than that obtained in the absence of the drug, respectively; the values of k_d obtained in 0.1 mM chlorpromazine and either 0, 6 or 20 mM KCl were 3.5-, 2.2- and 1.8-fold greater than the corresponding values obtained in the absence of the drug (Table IV). In Table IV, the values of k_d are compared with those of the ratio between the steady state rate of ATP hydrolysis and the steady state level of the ADP-insensitive phosphoenzyme obtained under the same conditions. It is interesting to note that the values of the latter are significantly greater than those of the former when 0.1 mM chlorpromazine is present in the reaction medium.

TABLE IV

EFFECT OF CHLORPROMAZINE ON ADP-INSENSITIVE PHOSPHOENZYME LEVEL AND DECOMPOSITION RATE CONSTANT OF ADP-INSENSITIVE PHOSPHOENZYME

The steady state rate of ATP hydrolysis (V) and the steady state levels of total (EP), ADP-sensitive (E_1P) and ADP-insensitive (E_2P) phosphoenzymes were measured under standard conditions in the presence of the KCl and chlorpromazine concentrations indicated in the table. k_d is the rate constant for decomposition of the ADP-insensitive phosphoenzyme measured directly from the time course of the phosphoenzyme decomposition after addition of EGTA and MgADP. The experiment was performed as described in the legend to Fig. 3.

KCl (mM)	Chlorpromazine (mM)	E_2P/EP (%)	V/E_2P (min^{-1})	k_d (min^{-1})
0	0	85.7	1.74	1.34
	0.1	47.5	8.65	4.71
6	0	58.7	4.54	3.86
	0.1	19.6	20.7	8.46
20	0	33.5	8.05	7.46
	0.1	12.3	20.1	13.7
150	0	5.8		
	0.1	2.1		

Discussion

In our previous report [16], it was shown that the rate of P_i liberation during steady state ATP hydrolysis by sarcoplasmic reticulum in the presence and absence of dimethylsulfoxide under the standard conditions of the present study (high Mg^{2+}, low Ca^{2+} and various KCl concentrations at pH 7.0 and 0°C) corresponded to the turnover of the ADP-insensitive fraction of phosphoenzyme. As the ADP-sensitive (E_1P) and ADP-insensitive (E_2P) phosphoenzymes were shown to occur sequentially [14], it was concluded that ATP hydrolysis under these conditions occurs via an obligatory pathway that includes E_1P and E_2P (Scheme 1) [16]. In the present study, the rates of P_i liberation in the presence of 1.0 mM propranolol and 0 to 20 mM KCl were found to be similar to the product of the amounts of E_2P and the rate constants for E_2P hydrolysis observed after addition of EGTA and MgADP (Table III). Thus, it appears that the reaction sequence of Scheme 1 is also applicable to ATP hydrolysis in the presence of propranolol.

Dimethylsulfoxide and propranolol displayed disparate effects on the partial reactions of the ATPase of sarcoplasmic reticulum. Dimethylsulfoxide increased the rate of the E_1P to E_2P conversion (Fig. 4 and Table III) whereas it decreased the rate of E_2P hydrolysis (Table I and II, and ref. 16). Propranolol, however, inhibited the E_1P to E_2P conversion while it stimulated the rate of E_2P hydrolysis (Fig. 4 and Table II). Propranolol appears to have an additional effect on ATP hydrolysis because both the rate of ATP hydrolysis and the total amount of phosphoenzyme decreased significantly at high concentrations of propranolol (Fig. 5). Propranolol which has properties of a local anesthetic, was reported to inhibit calcium uptake and ATP hydrolysis by decreasing the apparent affinity of the calcium pump for Ca^{2+} [21,22]. As other local anesthetics were shown to decrease the phosphoenzyme level also by reducing the

apparent affinity of the ATPase for Ca^{2+} [29], it appears that the phosphoenzyme formation step is inhibited by high concentrations of propranolol. Some of these effects of dimethylsulfoxide and propranolol are consistent with the previous findings reported by other investigators. It was shown that dimethylsulfoxide or propranolol inhibits ATP hydrolysis by decreasing the decomposition rate of the total amount of phosphoenzyme [19,23]. The observation by The and Hasselbach that ATP-ADP exchange catalyzed by sarcoplasmic reticulum vesicles decreased significantly in the presence of high concentrations of dimethylsulfoxide, is consistent with our findings (Tables I and II, and ref. 16) that dimethylsulfoxide decreases the rate of E_2P hydrolysis and that E_2P accumulates as the major steady state intermediate in high concentrations of dimethylsulfoxide even in the presence of high KCl concentrations. The and Hasselbach [19] also reported that dimethylsulfoxide did not affect ATP binding to the ATPase or the activation of the ATPase by ionized Ca^{2+}.

The stimulatory and inhibitory actions of dimethylsulfoxide or propranolol described in the preceding paragraph appear able to account for the complex effects of these drugs on the overall rate of ATP hydrolysis. In the absence of added alkali metal salts, dimethylsulfoxide monotonously decreased the rate of ATP hydrolysis (Fig. 1). This finding can be explained by the inhibitory action of dimethylsulfoxide on the E_2P hydrolysis as this step is the rate-limiting step of ATP hydrolysis under these conditions [16,17]. In the presence of high KCl concentrations, dimethylsulfoxide at low concentration stimulated the rate of ATP hydrolysis significantly whereas it reduced the rate of ATP hydrolysis markedly at high concentrations (Fig. 1). As the E_1P to E_2P conversion in high KCl is significantly slower than E_2P hydrolysis [16,17], the stimulation of the overall rate of ATP hydrolysis by low concentrations of dimethylsulfoxide can be ascribed to the stimulatory action of this drug on the E_1P to E_2P conversion. When dimethylsulfoxide concentration in the reaction medium increased, the rate of E_2P hydrolysis decreased to such an extent that E_2P hydrolysis became slower than the E_1P to E_2P conversion even in the presence of 100 mM KCl (Table I). Thus, the decrease in the overall rate of ATP hydrolysis in the presence of high concentrations of dimethylsulfoxide and KCl appears to reflect the inhibitory action of the drug on E_2P hydrolysis. The finding that E_2P hydrolysis in the presence of high concentrations of dimethylsulfoxide was much slower than the E_1P to E_2P conversion (Table I) can also provide an explanation for the present observations that the rate of ATP hydrolysis under these conditions is markedly stimulated by KCl (Fig. 2) and that its extent of stimulation by KCl appeared comparable to that for E_2P hydrolysis (compare Fig. 2 with Fig. 10 of Ref. 17).

Propranolol at relatively low concentrations reduced the overall rate of ATP hydrolysis markedly in the presence of high KCl (Fig. 5). As the E_1P to E_2P conversion appears to be the rate-limiting step of ATP hydrolysis under these conditions [16,17], this reduction in the rate of ATP hydrolysis can be ascribed mainly to the inhibitory action of propranolol on the E_1P to E_2P conversion. In contrast to this finding, the inhibition of ATP hydrolysis by the relatively low concentration of propranolol was much less in the absence of added alkali metal salts (Fig. 5). This finding can be explained by the observa-

tion that propranolol can stimulate the rate of E_2P hydrolysis (Table III), which is the rate-limiting step under these conditions [16,17]. The absence of stimulation of the rate of the overall ATP hydrolysis by propranolol under these conditions may be ascribed to the following factors. When propranolol concentration in the reaction medium is increased in the absence of added alkali metal salts, the rate of the E_1P to E_2P conversion would become slower than the rate of E_2P hydrolysis as propranolol reduces the rate of the former while stimulating the rate of the latter. Thus, the rate of ATP hydrolysis in the absence of added alkali metal salts would decrease when the inhibitory action of propranolol on the E_1P to E_2P conversion becomes dominant as propranolol concentration increases. In addition, as propranolol concentration increases, the third effect of this drug, presumably the inhibition of phosphoenzyme formation, would become dominant so that both the rate of ATP hydrolysis and phosphoenzyme level would be reduced significantly (cf. Fig. 5). It is interesting to note that this third effect of propranolol appears pronounced in the absence of added alkali metal salts (Fig. 5). When 1.0 mM propranolol was present in the reaction medium, the stimulation of the rate of ATP hydrolysis by KCl was minimal (Fig. 6). This finding can be explained by the observations that the E_1P to E_2P conversion in the presence of propranolol and KCl was significantly slowed and that the values of the rate constant for the rate-limiting step of ATP hydrolysis in the presence of propranolol and 0 to 20 mM KCl did not change greatly (Table III).

Chlorpromazine stimulated the rate of ATP hydrolysis significantly in the absence of added alkali metal salts whereas it decreased the rate of ATP hydrolysis in the presence of high KCl concentrations (Figs. 7A and B, and 8). These effects of chlorpromazine are in good agreement with the previous reports [24,30]. Chlorpromazine accelerated E_2P hydrolysis markedly both in the presence and absence of KCl (Table IV). Thus, this effect of chlorpromazine on E_2P hydrolysis may account for the increased rate of ATP hydrolysis observed in the absence of added alkali metal salts and in the presence of the drug (Fig. 7A). In 150 mM KCl under standard conditions, E_1P constituted 94% of steady state phosphoenzyme (Table IV). As 0.1 mM chlorpromazine increased the percentage of E_1P to 98% of the total phosphoenzyme while decreasing the rate of ATP hydrolysis significantly (Table IV and Fig. 7B), the drug may inhibit the E_1P to E_2P conversion. It should be pointed out, however, that the simple reaction sequence of Scheme 1 does not appear applicable to the ATP hydrolysis in the presence of chlorpromazine. The rate of P_i liberation during steady state ATP hydrolysis in the presence of chlorpromazine were 1.5- to 2.4-fold greater than the products of the amounts of E_2P and the rate constants for E_2P hydrolysis observed after addition of EGTA and MgADP (Table IV). These findings suggest that a significant amount of P_i was derived from source(s) other than E_2P. The existence of the source(s) for P_i liberation other than E_2P was suggested previously when we studied ATP hydrolysis by the calcium pump ATPase in low (approx. 5 μM) Mg^{2+} and low (approx. 5 μM) ATP concentrations and in the absence of added alkali metal salts at 0°C [14]. It should also be noted that in the absence of added alkali metal salts and under the standard conditions of the present study (2 mM $MgCl_2$, 20 μM ATP and 0°C), the rate of P_i liberation was slightly greater than hydrolysis rate of E_2P,

although both agreed well with each other in the presence of KCl (Fig. 3 of Ref. 16 and Tables III and IV of this paper). These findings are consistent with the view that during ATP hydrolysis under certain conditions P_i is formed by parallel pathways resulting from hydrolysis of E_2P and other intermediate(s). In this context, it is interesting to note that Nakamura and Tonomura [31] have recently proposed a reaction scheme for *p*-nitrophenylphosphatase reaction catalyzed by sarcoplasmic reticulum vesicles in which P_i is derived simultaneously from two types of phosphoenzyme intermediate.

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