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The amphiphilic drug-induced tryptophan fluorescence change of ion-transporting ATPases

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Abstract

The tryptophan (Trp) fluorescence of Na^{+},K^{+}-ATPase, Ca^{2+},Mg^{2+}-ATPase, Ca^{2+}-ATPase enriched microsomal membranes have been found to be changed by two amphiphilic drugs, i.e., choroquine (anti-malarial) and chlorpromazine (anti-psychotic) alone or in combination with ligands and/or substrate. The findings suggest that some conformational change possibly in E_1 and E_2 state of the enzymes in presence of these drugs and/or ions and substrate have taken place. The emission maxima of Trp residues were found to be at 335 nm irrespective of experimental conditions. A different level of fluorescence quenching was observed in presence of drugs and in combination with ions and/or substrate. By the use of modified Stern Volmer equation, f_a, the effective fraction of tryptophan most exposed to the drug and effective quenching constant, K_a, have been calculated. The non-linearity of the Stern-Volmer plots indicate that a fraction of Trp residues remain accessible to the quencher, which may correspond to highly hydrophobic regions that are normally buried in the membranes. The differences in f_a and K_a values calculated from the modified Stern-Volmer plots under various conditions indicate the different extent of exposure of Trp residues to the quencher.

Keywords: ATPase, Na^{+}/K^{+}-; ATPase, (Mg^{2+},Ca^{2+})-; ATPase, Ca^{2+}-; Fluorescence; Drug; Microsomal membrane

1. Introduction

The Na^{+},K^{+}-ATPase is an E_1 and E_2 type of enzyme, that is, it oscillates between the two major conformations during the transport cycle: the E_1 state forms in the presence of Na^{+} or ATP, and the E_2 state in the presence of K^{+} [1–4]. Ca^{2+},Mg^{2+}-ATPase is also an E_1 and E_2 type of enzyme [5,6]. The two states differ in that the affinity for Ca^{2+} is high in the E_1 state but low in the E_2 conformation. The Ca^{2+} binding sites are exposed to the outer side of the sarcoplasmic reticulum in E_1 form but exposed to the inside in the E_2 form. [7]. We reported that two amphiphilic drugs chloroquine (CLQ; anti-malarial) and chlorpromazine (CPZ; anti-psychotic) inhibit Na^{+},K^{+}-ATPase, Ca^{2+}-ATPase and Ca^{2+},Mg^{2+}-ATPase in vitro and in vivo [8–11] and that the drug effects are reversible in vivo [12,13]. Many reports are available about the structural change of Na^{+},K^{+}-ATPase but different techniques have yielded conflicting estimates of structural change resulting from the binding of Na^{+} or K^{+} [2–4]. The effect of hydrophobic molecules on the activity of Ca^{2+},Mg^{2+}-ATPase can be explained by the kinetic model of the ATPase [14]. Different probes and/or ligands have been used to monitor the fluorescence pattern of E_1 and E_2 states of Na^{+},K^{+}-ATPase [15–18] and Ca^{2+},Mg^{2+}-ATPase [6,7,19,20].

Previous communications from our laboratory have described mode and mechanism of inhibition of these transport enzyme activities by chloroquine and chlorpromazine in vitro and in vivo in different organs of rat [8–13]. In the present communication, we describe the quenching of tryptophan (Trp) fluorescence of Na^{+},K^{+}-ATPase, Ca^{2+},Mg^{2+}-ATPase and Ca^{2+}-ATPase in various ligand(s), and substrate binding form in the presence of chloroquine or chlorpromazine and degree of exposure of the tryptophan residues of these en-

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zymes in various forms in the presence of these drugs. The study is interesting and important in understanding the correlation between the changes of tryptophan fluorescence of Na^+,K^+-ATPase, Ca^{2+},Mg^{2+}-ATPase and Ca^{2+}-ATPase by CLQ or CPZ alone or in combination with ligand(s) and/or substrate and the alteration of the enzyme activities by these drugs under different experimental conditions as described previously [8–13].

2. Materials and methods

2.1. Materials

Chlorpromazine hydrochloride, chloroquine phosphate, ATP, phenyl methyl sulphonyl fluoride (PMSF), imidazole hydrochloride and histidine hydrochloride were purchased from Sigma Chemical Co., USA. β-Mercaptoethanol (βME) and EDTA were from SISCO Research Laboratory, Bombay, India and all other reagents used were of analytical grade either from E. Merck or BDH, India.

2.2. Methods

2.2.1. Preparation of the enzyme-enriched membranes

The microsomal membranes enriched with Na^+, K^+-ATPase were isolated from rat brain according to a method published earlier [8]. The α and β subunits of Na^+,K^+-ATPase constituted about 65–70% of the total protein and more than 95% activity was found to be sensitive to ouabain when assayed following a method of Sen et al. [18]. The rat testicular membranes enriched with Ca^{2+},Mg^{2+}-ATPase and Ca^{2+}-ATPase were prepared according to the method of Nag Das et al. [21] with some modification and found to constitute 70% of the total proteins. Protein was estimated according to the method of Lowry et al. [23] using bovine serum albumin as standard.

2.2.2. Fluorescence measurement

The fluorescence spectra were taken in a perkin Elmer MDF-44 fluorescence spectrophotometer. The excitation and emission maxima of Trp residues on Na^+,K^+-ATPase and Ca^{2+}-ATPase in 25 mM Tris-HCl, 0.5 mM EDTA, 25 mM sucrose at pH 7.5 at a protein concentration of 100 μg/ml were found to be 290 and 335 nm, respectively, at 3 nm bandwidth to minimize photobleaching. All the subsequent fluorescence measurements were made at an emission of 335 nm and an excitation of 290 nm to ensure that all the fluorescent emission was due to tryptophan residues. The effect of chlorpromazine and chloroquine on fluorescence intensities were determined by continuous monitoring at 335 nm at a given drug concentration or at different concentrations as specified in the legends. Addition of drugs was made from a concentrated stock of 1 mM. Fluorescence intensities were corrected for dilution. The temperature was maintained at 25°C throughout the study.

2.3. Data analysis

The fluorescence signal obtained due to Trp residues on the ATPase was normalized to 100 (control) and the relative quenching in the presence of either drugs or ligands or substrate were calculated with respect to the control.

The fluorescence quenching in the presence of different concentrations of chlorpromazine was analyzed according to Stern-Volmer relationship: $F_0/F = 1 + K_a[Q]$, where F_0 and F are the fluorescence in the absence and in the presence of millimolar concentration of the quencher [Q] and K_a is the Stern-Volmer quenching constant obtained from the slope of a plot of F_0/F versus [Q] [24]. For multiflurophore proteins, the Stern-Volmer plot would be non-linear when the individual fluorophore is not equally accessible to the quencher. For such heterogeneous systems, a modified Stern-Volmer equation has been proposed by Lehrer and Leavis [25] as $[F_0/(F_0 - F)] = 1/Qf_aK_a + 1/f_a$. From a plot of $[F_0/(F_0 - F)]$ versus [Q], one could get the values of f_a and K_a, the fractional accessible fluorescence and quenching constant respectively. f_a was calculated from the relation $f_a = 1/$intercept on y axis (when the graph was extrapolated linearly to y axis) and K_a was calculated from $K_a = 1/f_a \tan\theta$ (tan θ could be calculated from the slope of the graph). In the case of curved modified plots, extrapolation of measurements at low quencher concentration yields information about the most accessible groups. These values are termed 'effective'. The values shown were the mean of at least two independent determinations. Statistical calculations (standard error, mean, P values etc.) were done from 2–3 separate values for f_a and K_a obtained from 2–3 determinations.

3. Results

The addition of CPZ or CLQ to Na^+,K^+-ATPase, Ca^{2+}-ATPase and Ca^{2+},Mg^{2+}-ATPase-enriched membranes resulting in quenching of tryptophan fluorescence without affecting the emission maxima (Fig. 1A–D). With increase in concentration of drugs, more and more quenching of tryptophan fluorescence were observed. The absorption were shown after necessary corrections due to drugs alone (compared to control, 2–5% absorption at different concentrations of drugs were observed with low extinction coefficient) were made. Thus it is expected that quenching observed was

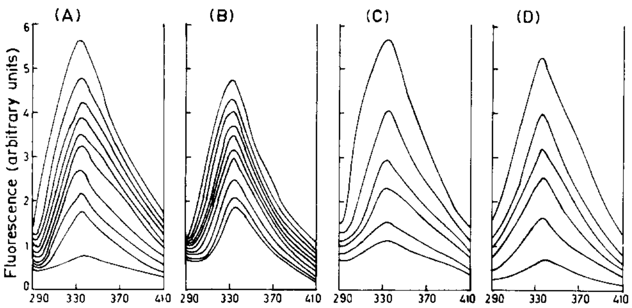

Fig. 1. Emission spectra of (A) Na^+,K^+-ATPase, (B) Ca^{2+},Mg^{2+}-ATPase and Ca^{2+}-ATPase-enriched microsomal membranes in the presence of different concentrations of chlorpromazine, and (C) Na^+,K^+-ATPase, (D) Ca^{2+},Mg^{2+}-ATPase and Ca^{2+}-ATPase in the presence of different concentrations of chloroquine. 100 μg of Na^+,K^+-ATPase-enriched rat brain or Ca^{2+},Mg^{2+}- and Ca^{2+}-ATPase-enriched rat testicular microsomal membranes in 1 ml of Tris-HCl buffer containing 25 mM sucrose, 0.5 mM EDTA, pH 7.5 at 25°C were recorded for Trp emission spectra with increasing concentration of drugs. At zero concentration of drugs maximum fluorescence was observed. Lowest to highest concentrations of CPZ were 0, 5, 10, 15, 25, 30, 40, 70, 100 and 125 μM and that of CLQ 0, 10, 25, 50, 100 and 125 μM. Excitation wavelength was at 290 nm and emission was scanned from 290 nm to 410 nm. Fluorescence intensity was plotted against emission wavelength. Fluorescence intensities were corrected for different concentration of drugs at 290 nm as described in the text.

due solely to the effect of drugs on tryptophan fluorescence.

The quenching of tryptophan fluorescence was observed in the presence of these drugs and under a variety of conditions. A typical fluorescence tracing with Na^+,K^+-ATPase-enriched microsomal membranes from rat brain is shown in Fig. 2. An increase in quenching of the fluorescence was observed when Mg^{2+}, Na^+, K^+ and ATP were added sequentially

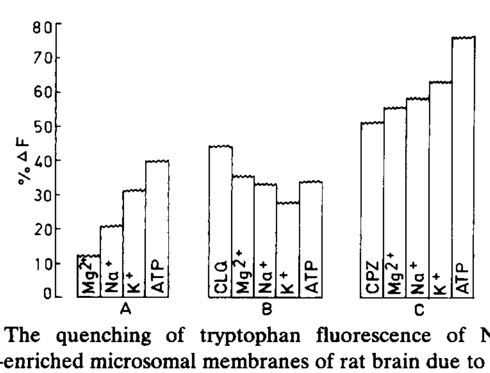

Fig. 2. The quenching of tryptophan fluorescence of Na^+,K^+-ATPase-enriched microsomal membranes of rat brain due to sequential addition of ligand/substrate (A). In (B) and (C) 50 μM CLQ or 30 μM CPZ respectively was added followed by the same sequence of addition of ligand and substrate as in (A). 100 μg of the enzyme-enriched microsomal membranes in 1 ml of Tris-HCl buffer (pH 7.5) containing 25 mM sucrose, 0.5 mM EDTA at 25°C was taken. To this sequentially 1.8 mM Mg^{2+}, 130 mM Na^+, 20 mM K^+ and 2.5 mM ATP at final concentration were added. The Trp fluorescence of the control was taken as 100 (zero quenching) and percent quenching (%ΔF) was calculated with respect to the control.

(Fig. 2A). However, different patterns of fluorescence were observed in the presence of CLQ or CPZ, thus CLQ led to about 45% quenching which was reduced on sequential addition of Mg^{2+}, Na^+ and K^+ followed by a slight increase with the addition of ATP (Fig. 2B). With CPZ, on the other hand, about 50% quenching could be seen which was further enhanced on sequential addition of Mg^{2+}, Na^+, K^+ and ATP (Fig. 2C). Similarly, with Ca^{2+},Mg^{2+}-ATPase and Ca^{2+}-ATPase-enriched membranes from rat testis, addition of CLQ or CPZ and/or ions, substrate led to different level of of Trp quenching (data not shown).

The quenching of tryptophan residue due to CLQ and CPZ in different forms of the enzymes were calculated from Stern-Volmer plots. The plot at different concentrations of CLQ with Na^+,K^+-ATPase-enriched membranes under different conditions are shown in Fig. 3a–g. In the presence of optimal concentration of Na^+ or ATP, both f_a, the 'effective' value, and K_a, the Stern-Volmer constant, were same, indicating that in Na^+ or ATP binding form, the accessible Trp residues were the same. A summary of the quenching parameters obtained from Fig. 3a–g for Na^+,K^+-ATPase and that calculated for CPZ under the above conditions (plots not shown) is shown in Table 1. The enzyme in the presence of CLQ alone has an f_a value of 1, indicating that most of the Trp residues were quenchable by the drug. In the presence of K^+ and Na^+, K^+ and ATP, the f_a values were 0.88 and 0.83 respectively, indicating that about 88% and 83% Trp residues were accessible respectively in these forms. In full ion and substrate binding form, i.e. $Mg^{2+}+Na^{+}+K^{+}+$ ATP, about 80% Trp residues were available for quenching by CLQ having f_a of 0.79. With CPZ, on the other hand, an f_a value of 0.91 indicates that 91% of the Trp residues were accessible which is comparable to the full ion and substrate binding form, i.e. $Mg^{2+}+Na^{+}+K^{+}+$ATP (98%). In the presence of Na^+ or ATP about 25% accessibility has been noticed, whereas in the presence of Mg^{2+} or K^+ or $Na^{+}+K^{+}$ + ATP about 63%, 48% and 83%, respectively, could be seen.

The Stern-Volmer plot of Ca^{2+}-ATPase and Ca^{2+},Mg^{2+}-ATPase at different concentrations of CPZ is shown in Fig. 4a–g, and a summary of the quenching parameters obtained from those plots and that for CLQ (plot not shown) is shown in Table 2. In the presence of optimum concentration of Ca^{2+} about 50% Trp residues of the enzyme were quenchable by CPZ with Stern-Volmer constant 2.56 mM^{-1}. The presence of Ca^{2+} and ATP or $Mg^{2+}+Ca^{2+}+$ATP increased the accessibility of Trp residues (f_a 0.90). In the presence of CPZ alone about 80% Trp residues were quenchable with $K_a = 4.82$ mM^{-1} and $f_a = 0.83$. With CLQ an f_a value of 0.81 with a Stern-Volmer constant of 5.20 could be seen. Comparable f_a values are obtained in the presence of Ca^{2+} or Mg^{2+} + ATP or Mg^{2+} + Ca^{2+} + ATP, which is slightly different from either Mg^{2+} or ATP. In the presence of Ca^{2+} about 33% accessibility in Trp fluorescence was seen.

4. Discussion

Fluorescence of organic molecules including proteins is weakened, i.e., quenched in the presence of a certain kind of molecules, called quenchers. This phenomenon is known as 'fluorescence quenching'. The quenching phenomena of fluorescent amino acid residues in proteins and extrinsic fluorescent levels have been used to investigate the extent of exposure of the protein surface or microscopic environment around them. The tertiary structure of a membrane bound protein and its relationship with the lipid bilayer will be the determining factor as to which Trp residues are accessible to quenching. It can also be assumed that changes in protein conformation would lead to changes in relative accessibility which will be reflected in quenching characteristics, since quenching requires contact between the fluorophore and the quencher. Chlorpromazine and chloroquine are two amphiphilic molecules that can penetrate into the biological membrane matrix [26]. The higher level of quenching in the presence of increasing concentration of these drugs (Fig. 1A–D) with respect to the control indicate that a greater portion of tryptophan residue(s) are accessible to these drugs. It may be noted that emission peak (335 nm) does not shift during quenching. This could be explained by efficient energy transfer between the Trp molecules leading to uniform quenching of all the residues [28,29]. It has been reported that denaturation of Na^+,K^+-ATPase with detergent also increased the quenching of fluorescence due to the exposure of more tryptophan residues [27].

Na^+,K^+-ATPase in the E_1 state binds Na^+ and ATP with high affinity [1,31]. In the presence of CLQ, it is evident that accessible Trp residues are quenched at the lowest drug concentration so that the slope of the line at that portion of the graph is extraplotted and the quenching parameters have been determined from it. K^+ preferentially binds to E_2 conformation of Na^+,K^+-ATPase. It seems reasonable to assume that several Trp residues contribute equally to the quenchable fluorescence in E_2 conformation. The quenching parameter, f_a, indicates that most of the Trp fluorescence is quenchable in the presence of CLQ as well as in the presence of either Na^+ or ATP (Table 1). Analysis of data from Table 1 indicates Na^+ or ATP favours E_1 conformation of the enzyme, whereas K^+ favours E_2 conformation. CPZ, on the other hand, seems to favour E_2 conformation. In some cases higher values of K_a have been observed, suggesting that under these conditions, a small fraction of the fluorescence is very accessible to the quencher. Another reason could be that the exposed Trp has reasonably higher quantum yield showing higher K_a [25]. The K_a of free N-acetyl-L-tryptophanamide in aqueous solution was found to be 17.5 [30].

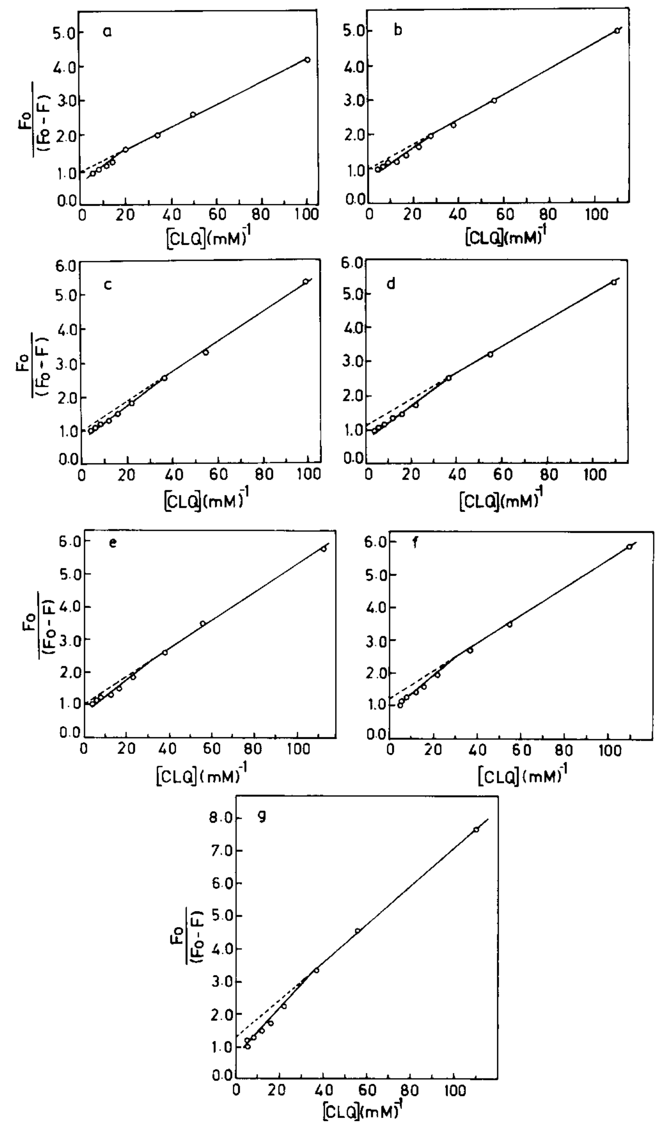

Fig. 3. Modified Stern-Volmer plots of Na^+,K^+-ATPase in different ligands/substrate binding forms in the presence of different concentrations of CLQ. 100 μg of rat brain microsomal membranes were taken in 1 ml of 25 mM Tris-HCl buffer (pH 7.5) containing 25 mM sucrose, 0.5 mM EDTA at 25°C. (a) Fluorescence signal recorded with increasing concentration of CLQ and the result plotted following modified Stern-Volmer equation. (b–g) modified Stern-Volmer plots when fluorescence were measured in the presence of Mg^{2+} (1.8 mM), Na^+ (130 mM), K^+ (20 mM), ATP (2.5 mM), Na^+ (130 mM)+K^+ (20 mM)+ATP (2.5 mM) and Mg^{2+} (1.8 mM)+Na^+ (120 mM)+K^+ (20 mM)+ATP (2.5 mM) respectively, at different concentrations of CLQ.

Like Na^+,K^+-ATPase, Ca^{2+},Mg^{2+}-ATPase also has two conformational states E_1 and E_2 [5]. f_a and K_a values of the enzymes from Stern-Volmer plot in the

Table 1
Summary of the quenching parameters determined from the modified Stern-Volmer plots for Na^+,K^+-ATPase

Conditions	f_a	K_a (mM^{-1})
CLQ	1.00 ± 0.04	1.87 ± 0.15
+ Mg^{2+} (1.8.mM)	0.97 ± 0.03	1.84 ± 0.16
+ Na^+ (130 mM)	1.00 ± 0.03	1.54 ± 0.09
+ K^+ (20 mM)	0.88 ± 0.02 ($P < 0.01$)	2.00 ± 0.14
+ ATP (2.5 mM)	1.00 ± 0.04	1.52 ± 0.11
+ Na^+ (130 mM), K^+ (20 mM), ATP (2.5 mM)	0.83 ± 0.02 ($P < 0.05$)	1.85 ± 0.10
+ Mg^{2+} (1.8 mM), Na^+ (120 mM), K^+ (20 mM), ATP (2.5 mM)	0.79 ± 0.02 ($P < 0.05$)	1.48 ± 0.08
CPZ	0.91 ± 0.05	1.76 ± 0.20
+ Mg^{2+} (1.8.mM)	0.33 ± 0.02 ($P < 0.01$)	1.93 ± 0.31
+ Na^+ (130 mM)	0.25 ± 0.02 ($P < 0.01$)	4.92 ± 0.50
+ K^+ (20 mM)	0.68 ± 0.04 ($P < 0.03$)	0.98 ± 0.10
+ ATP (2.5 mM)	0.25 ± 0.03 ($P < 0.01$)	3.73 ± 0.35
+ Na^+ (130 mM), K^+ (20 mM), Mg^{2+} (1.8 mM)	0.83 ± 0.06	2.11 ± 0.20
+ Mg^{2+} (1.8 mM), Na^+ (130 mM), K^+ (20 mM), ATP (2.5 mM)	0.98 ± 0.05	1.1 ± 0.01

100 μg of Na^+,K^+-ATPase-enriched rat brain microsomal membranes were taken and fluorescence quenching were measured in the presence of different concentrations of CLQ (Fig. 3a–g) or CPZ (plot not shown) in combinations with various ions/substrate. f_a (effective fraction) and K_a (Stern-Volmer constant) were determined as described in Section 2. The results shown are the mean + S.E. ($n = 3$). P was calculated for f_a under different ligands and/or substrate-induced conditions with respect to the effect of drug alone.

presence of CPZ show that in the presence of either Mg^{2+}, Ca^{2+} or ATP they are almost similar (Table 2), indicating that these three ligands favour E_1 conformation, whereas in the presence of Ca^{2+} + ATP or Mg^{2+} + ATP or Mg^{2+} + Ca^{2+} + ATP, they favour E_2 conformation of the enzyme. Ca^{2+}, Mg^{2+} or ATP separately protects quenching, whereas Ca^{2+} + ATP or Mg^{2+} + ATP or Mg^{2+} + Ca^{2+} + ATP potentiate quenching could be due to the hydrolysis of ATP to P_i under these conditions, exerting a different effect. CLQ effect, either alone or in combination of ions and/or substrate (Table 1), could be explained the same way. It has been reported previously that various ions may affect the behaviour of Na^+,K^+-ATPase [32,33] and Ca^{2+}-ATPase [34,35] and that the fluorescence behaviour of the tryptophan on Na^+,K^+-ATPase and Ca^{2+}-ATPase could be different [36–38]. The present findings therefore suggest that with Na^+,K^+-ATPase CLQ favours E_1 conformation whereas in Ca^{2+}-ATPase it favours E_2 conformation. CPZ, on the other hand, favours E_2 conformation in both the enzymes. It is pertinent to mention that the concentration of ions

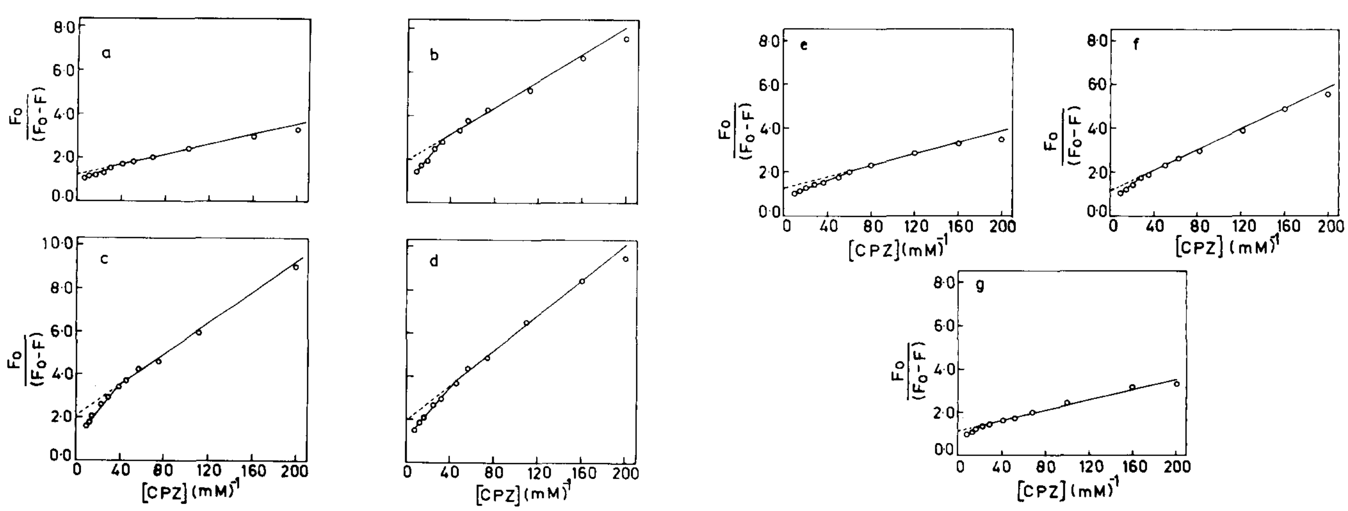

Fig. 4. Modified Stern-Volmer plots of testicular membranes enriched with Ca^{2+},Mg^{2+}- and Ca^{2+}-ATPase in different ligands/substrate binding forms at different concentrations of CPZ. (a) CPZ alone, or in combination with: (b) Mg^{2+} (0.1 mM); (c) Ca^{2+} (2.0 mM); (d) ATP (2.5 mM); (e) Mg^{2+} (0.1 mM) + ATP (2.5 mM); (f) Ca^{2+} (4.0 mM) + ATP (2.5 mM); (g) Mg^{2+} (0.1 mM) + Ca^{2+} (2.0 mM) + ATP (2.5 mM). 100 μg of membrane protein in 1 ml 25 mM Tris-HCl buffer (pH 7.5) containing 25 mM sucrose, 0.5 mM EDTA at 25°C was used.

Table 2
Summary of the quenching parameters determined from Stern-Volmer plots for Ca^{2+}-ATPase and Ca^{2+},Mg^{2+}-ATPase

Conditions	f_a	K_a (mM^{-1})
CLQ	0.81 ± 0.03	5.20 ± 0.68
+ Ca^{2+} (2.0 mM)	0.33 ± 0.01 ($P < 0.01$)	1.33 ± 0.20
+ Mg^{2+} (0.1 mM)	0.62 ± 0.04	3.90 ± 0.19
+ ATP (2.5 mM)	0.71 ± 0.04	1.84 ± 0.15
+ Ca^{2+} (4.0.mM), ATP (2.5 mM)	0.91 ± 0.05	1.34 ± 0.10
+ Mg^{2+} (0.1 mM), Ca^{2+} (2.0 mM), ATP (2.5 mM)	0.83 ± 0.04	5.00 ± 0.50
+ Mg^{2+} (0.1 mM), ATP (2.5 mM)	0.83 ± 0.04	2.10 ± 0.22
CPZ	0.83 ± 0.03	4.82 ± 0.17
+ Ca^{2+} (2.0 mM)	0.53 ± 0.01 ($P < 0.01$)	2.56 ± 0.05
+ Mg^{2+} (0.1 mM)	0.53 ± 0.01 ($P < 0.01$)	3.02 ± 0.06
+ ATP (2.5 mM)	0.48 ± 0.02 ($P < 0.01$)	2.61 ± 0.09
+ Ca^{2+} (4.0 mM), ATP (2.5 mM)	0.90 ± 0.01	2.39 ± 0.05
+,Mg^{2+} (0.1 mM), Ca^{2+} (2.0 mM), ATP (2.5 mM)	0.91 ± 0.02	4.03 ± 0.70
+ Mg^{2+} (0.1 mM), ATP (2.5 mM)	0.80 ± 0.02	5.00 ± 0.12

100 μg of Ca^{2+}-ATPase and Ca^{2+},Mg^{2+}-ATPase-enriched microsomal membranes from rat testis were taken and fluorescence quenching were measured in the presence of different concentrations of CPZ (Fig. 4a–g) or CLQ (plot not shown) in combinations with various ligands and/or substrate. f_a and K_a were calculated as described in Section 2. The results shown as the mean ± S.E. ($n = 3$). P was calculated for f_a under different ligand and/or substrate-induced conditions with respect to the effect of drug alone.

used here is comparable to the one used in the enzyme activity assay, hence we presume that the effect of ionic strength if any would be minimal. Moreover, Tris at different concentrations did not have any effect on Trp fluorescence change (data not shown). Furthermore, it has been reported by Chetverin et al. [2] that the shape of the Trp fluorescence spectra did not get affected by the ionic strength of the medium.

In conclusion, the present findings could be summarized as, in Na^+,K^+-ATPase, CLQ effects were seen in the presence of $Na^+ + K^+ +$ ATP and $Mg^+ + Na^+ + K^+ +$ ATP, but not when either of the ions was present alone. On the other hand, CPZ effects were observed when Na^+, K^+, Mg^{2+} or ATP were present alone but not in combination. However for Ca^{2+}-ATPase, the effects of CLQ and CPZ were parallel, both preferring conditions when Ca^{2+}, Mg^{2+} or ATP were present alone and not in combination. Furthermore, since both Na^+,K^+-ATPase and Ca^{2+}-ATPase are about 65–70% pure, it is reasonable to believe that most of the tryptophan fluorescence originated from ATPases themselves. Finally, the fact that changes in Trp fluorescence under a variety of experimental conditions could be due to the different conformational states of the enzyme may be correlated with our previous findings that the enzyme activities are altered after treatment with these drugs in vitro and in vivo [8–13]. The finding that the pattern of CLQ/CPZ effect on these two enzymes are different could be due to their different Trp contents and/or the orientation of Trp across the transmembrane segments leading to different effects.

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References

[1] Glynn, I.M. (1985) in The Enzymes of Biological Membranes (Martnosi, A.N. ed.), 2nd edn., vol. 3, pp. 35–114, Plenum Press, New York.
[2] Chetverin, A.B., Agalarov, S.C., Emelyanenka, V.I. and Burstein, E.A. (1980) Eur. J. Biochem. 108, 157–161.
[3] Gresalfi, T.J. and Wallace, B.A. (1984) J. Biol. Chem. 259, 2622–2628.
[4] Hastings, D.F., Reynolds, J.A. and Tandford, C. (1986) Biochim. Biophys. Acta 860, 566–569.
[5] De Meis, L. and Vianna, A. (1979) Annu. Rev. Biochem. 48, 275–292.
[6] Froud, R.J., East, J.M., Jones, O.T. and Lee, A.G. (1986) Biochemistry 25, 7544–7552.
[7] Michelangeli, F., Orlowski, S., Champeil, P., East, J.M. and Lee, A.G. (1990) Biochemistry 29, 3091–3101.
[8] Mazumder, B., Mukherjee, S. and Sen, P.C. (1988) Biochem. Int. 16, 35–44.
[9] Mazumder, B., Mukherjee, S. and Sen, P.C. (1990) Mol. Cell. Biochem. 95, 13–20.
[10] Mazumder, B., Sikdar, R. and Sen, P.C. (1991) in Biomembranes in Health and Diseases (Kidwai, A.M., Upreti, R.K. and Ray, P.K., eds.), vol. 2, pp. 407–412, Today's and Tomorrow's Printers and Publishers, New Delhi.
[11] Mazumder, B., Sikdar, R. and Sen, P.C. (1991) Ind. J. Biochem. Biophys. 28, 136–139.
[12] Adhikary, G., Nandy, P., Chandra, S., Sikdar, R. and Sen, P.C. (1991) Biochem. Int. 25, 951–961.
[13] Chandra, S., Adhikary, G., Sikdar, R. and Sen, P.C. (1992) Mol. Cell. Biochem. 118, 15–22.
[14] Gould, W.G., East, J.M., Froud, R.J., McWhirter, M.J., Stefanova, I.H. and Lee, G.A. (1986) Biochem. J. 237, 217–227.
[15] Karlish, S.J.D. and Yates, D.W. (1978) Biochim. Biophys. Acta 527,115–130.
[16] Harris, W.E. and Stafl, W.L. (1977) Biochim. Biophys. Acta 485, 203–214.

[17] De Pont, J.J.H.H.M. and Bonting, S.L. (1978) Biochim. Biophys. Acta 522, 602–613.
[18] Sen, P.C., Kapakos, J.G. and Steinberg, M. (1981) Arch. Biochem. Biophys. 211, 652–662.
[19] Pick, U. (1982) J. Biol. Chem. 257, 6111–6119.
[20] Fernandez-Belda, F., Kurzmack, M. and Inesi, G. (1984) J. Biol. Chem 259, 9687–9698.
[21] Nag Das, S.K., Mukherjee, S., Mazumder, B. and Sen, P.C. (1988) Mol. Cell. Biochem. 79, 161–169.
[22] Sikdar, R., Ganguly, U., Pal, P., Mazumder, B. and Sen, P.C. (1991) Mol. Cell. Biochem. 103, 121–130.
[23] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[24] Eftink, M.R. and Ghiram, C.A. (1976) Biochemistry 15, 672–680.
[25] Lehrer, S.H. and Leavis, P.C. (1978) Methods Enzymol. 49, 222–236.
[26] Kodavanti, U.P. and Mahendale, H.M. (1990) Pharmacol. Rev. 42, 327–354.
[27] Tyson, P.A. and Steinberg, M. (1987) J. Biol. Chem. 262, 4644–4648.
[28] Lehrer, S.S. (1971) Biochemistry 10, 3254–3263.
[29] Divita, G., Di Pietro, A., Deleague, G., Roux, B. and Gautheron, D.C. (1991) Biochemistry 30, 3256–3262.
[30] Eftink, M.R. and Ghiron, C.A. (1977) Biochemistry 16, 5546–5551.
[31] Robinson, J.D. and Flashner, M.S. (1979) Biochim. Biophys. Acta 549, 145–176.
[32] Steinberg, S., Kapakos, J.G. and Sen, P.C. (1983) in Current Topics in Membrane Transport, vol. 19 (Bronner, F. and Kleinzether, A. eds.), pp. 457–463, Academic Press, New York.
[33] Skou, J.C. and Esmann, M. (1983) Biochim. Biophys. Acta 746, 101–113.
[34] Murphy, A.J. (1978) J. Biol. Chem. 253, 385–389.
[35] Champeil, P., Le Maire, M., Moller, J.V., Riollet, S., Guillain, F. and Green, N.M. (1986) FEBS Lett. 206, 93–98.
[36] Brandl, C., Green, N.M., Korczak, B. and MacLennan, D.H. (1986) Cell 44, 592–607.
[37] Moutin, M.J. and Dupont, Y. (1991) J. Biol. Chem. 266, 5580–5586.
[38] Henderson, I.M., Khan, Y.M., East, J.M. and Lee, A.G. (1994) Biochem. J. 297, 615–624.