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Magnesium-Dependent Inhibition of Beef Heart Soluble Mitochondrial Adenosine Triphosphatase by Tricyclic Antipsychotics

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SUMMARY

The effect of fluphenazine and related phenothiazine and thioxanthene derivatives on beef heart soluble mitochondrial ATPase (EC 3.6.1.3) was studied under a precise control of the Mg^{2+}-ATP equilibrium. These drugs were shown to be reversible, noncompetitive inhibitors with respect to the substrate (the Mg·ATP complex). The inhibition was found to be dependent on the concentration of free magnesium ions, although free Mg^{2+} was not essential for the interaction of the inhibitors with the enzymatic protein. Bicarbonate anions, which are known to antagonize the effect of free Mg^{2+} on the enzyme kinetics, also antagonized the drug-induced inhibition. Concentrations giving 50% inhibition of enzyme activity were in the micromolar range. Inhibitory potencies increased when the pH of the reaction mixture was lowered from 8.2 to 6.9. Cleland [*The Enzymes* (P. D. Boyer, ed.), Vol. II. Academic Press, New York, 1–65 (1970)] analysis of the inhibition, by means of slope and intercept replots, indicated that the inhibition was the result of the interaction with more than one drug molecule. All drugs tested afforded complete protection against the cold-induced inactivation of soluble mitochondrial ATPase. These results point to a specific mode of inhibition that mimics, in some respects, the action of the natural inhibitor protein of mitochondrial ATPase.

INTRODUCTION

Phenothiazine and thioxanthene derivatives, currently known as tricyclic antipsychotics, are able to interfere with the activity of various ATPases (1, 2). However, only for the sodium-potassium-stimulated ATPase (3, 4) and the calcium-stimulated ATPase (5, 6) has the mechanism of inhibition been studied in detail. Little information is instead available on the molecular interaction between such drugs and mitochondrial ATPase, although the inhibitory effect of chlorpromazine on this enzyme has long been reported (1). A better understanding of the inhibition process may provide further insight into the general area of the interaction between basic amphiphilic drugs and membrane proteins and may, therefore, help to elucidate the molecular basis of local anesthesia. Recent studies using mitochondrial ATPase and functionally linked enzymes as model systems have in fact indicated that the membrane actions of tertiary amine local anesthetics, including chlorpromazine, are primarily due to direct interaction with membrane proteins (7–9).

Preliminary experiments reported elsewhere (10) have indicated that tricyclic antipsychotics inhibit mitochondrial ATPase by interacting with the F_1 component of the enzyme. These experiments have also shown that the degree of inhibition increases upon increasing the concentrations of ATP and $MgCl_2$ in equimolar ratio. However, varying ATP and Mg^{2+} in constant ratio causes simultaneous changes in the concentration of each of the three major ionic species at equilibrium at physiological pH, namely Mg·ATP[1] (the true substrate for the enzyme reaction), ATP_f, and Mg_f. F_1-ATPase possesses "tight" as well as "rapidly reversible" binding sites for adenine nucleotides and magnesium ions. The role of these ligands in modulating the activity of the enzyme, although not precisely established, is amply documented (e.g., see refs. 11–13). Each of the aforementioned ligands (Mg·ATP, ATP_f, and Mg_f) might thus be responsible for the observed variation in inhibition. Discriminating among these possibilities requires a precise knowledge of the association constant for the Mg·ATP complex so that the concentration of each species present in the reaction mixture can be carefully controlled. To this purpose, the apparent stability constant for the Mg·ATP complex was determined under the experimental conditions used for the kinetic assay. Three drugs were tested for their effect on F_1-ATPase: the phenothiazine derivatives chlorpromazine and fluphenazine, and flupenthixol, the thioxanthene analogue of fluphenazine. Since the three drugs

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[1] The abbreviations used are: Mg·ATP, $Mg \cdot ATP^{2-}$ complex; ATP_f, free ATP^{4-}; Mg_f, free magnesium ions; F_1-ATPase, soluble mitochondrial ATPase; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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exhibited qualitatively identical patterns of inhibition, only the experiments with fluphenazine are presented.

MATERIALS AND METHODS

Enzyme preparation. F_1-ATPase was obtained from beef heart mitochondria by the procedure of Horstman and Racker (14), and stored at 4° in a 50% saturated solution of $(NH_4)_2SO_4$ containing 125 mM sucrose, 1 mM EDTA, and 5 mM Tris·HCl (ph 7.4). The purity of each preparation was checked by polyacrylamide gel electrophoresis as previously indicated (12). The enzyme was in the 5-subunit form described in the literature (11). Protein concentration was determined according to the method of Lowry *et al.* (15), using crystalline bovine serum albumin as a standard.

Determination of stability constants. Apparent stability constants for the Mg·ATP complex were determined according to the method of Burton (16), in a Perkin-Elmer spectrophotometer equipped with a temperature-controlled cell holder. The spectral measurements were carried out at 30° and pH 7.4 in cells of 4-cm light path containing 10 ml of solution, with 8-hydroxyquinoline concentrations of 0.7, 1, or 1.2 mM. The molar extinction coefficient of the Mg^{2+}·8-hydroxyquinoline complex was determined to be 2000, in good agreement with previous determinations (17). The concentration of ATP was determined by optical density at 259 nm, assuming a millimolar extinction coefficient of 15.4 (18). The solutions of $MgCl_2 \cdot 6H_2O$ were standardized by means of atomic absorption spectrophotometry, as previously indicated (12).

Enzyme assay. ATPase activity was measured at 30° by coupling the hydrolysis of ATP to the oxidation of NADH via the pyruvate kinase and lactic dehydrogenase reactions (19) in an Eppendorf spectrophotometer equipped with a Sargent recording system. Initial rates of ATP hydrolysis, as deduced from the slope of the velocity progress curves (20), were used in all calculations. The reaction mixture contained, in a final volume of 1 ml, 10 mM Hepes-KOH (pH 7.4), 2 mM phosphoenolpyruvate, 0.2 μmole of NADH, 10 units each of pyruvate kinase and lactate dehydrogenase (in glycerol solution), 1 mg of bovine serum albumin, and the indicated concentrations of ATP and $MgCl_2$. Control experiments showed that the inclusion of albumin had no effect on the enzyme or inhibition kinetics.

Phosphoenolpyruvate and ATP solutions were brought to pH 7.4 with KOH. Hepes was chosen as buffer because it does not appreciably bind magnesium ions (20). At 10 mM, Hepes had no significant stimulating effect on the activity of F_1-ATPase. The reaction was started by the addition of 0.2–0.3 unit of enzyme which had been previously centrifuged to remove $(NH_4)_2SO_4$, and then resuspended in a solution of 10 mM Hepes-KOH (pH 7.4) containing bovine serum albumin (1 mg/ml) to prevent enzyme inactivation. The activity of the various enzyme preparations, at 3 mM ATP·Mg, varied from 100 to 130 μmoles of ATP hydrolized per minute per milligram of protein. It was verified that, under all experimental conditions used, large variations in the concentration of the coupling enzymes had no effect on the ATPase kinetics. All of the experiments were performed under conditions preventing the formation of the free radicals of tricyclic antipsychotics (3) and were repeated at least four times with different enzyme preparations. Regression lines were determined by the least-squares method. IC_{50} values and equilibrium and kinetic constants are the means ± standard error of the mean of four determinations.

Materials. ATP, NADH, phosphoenolpyruvate, Hepes, and bovine serum albumin were purchased from Sigma Chemical Company (St. Louis, Mo.). 8-Hydroxyquinoline was obtained from E. Merck (Darmstadt, Federal Republic of Germany); pyruvate kinase and lactate dehydrogenase were obtained from Boehringer Mannheim (Mannheim, Federal Republic of Germany). Fluphenazine was a generous gift from Squibb (Rome), and flupenthixol and chlorpromazine were gifts from Carlo Erba (Milan).

RESULTS

Determination of stability constants. Although stability constants for magnesium complexes with adenine nucleotides have already been determined in Hepes buffer (20), other variables such as temperature, pH, ionic strength, and the nature of the supporting medium can greatly affect the apparent values of these constants (21). The association constant for Mg^{2+} and ATP was determined to be 120,000 ± 5,000 M^{-1} in 10 mM Hepes KOH (pH 7.4) a value close to that obtained by Rudolph and Fromm under similar conditions (see ref. 20). However, when the determination was carried out in the presence of the other ionic species present in the coupled assay system, i.e., 2 mM phosphoenolpyruvate and albumin (1 mg/ml), a value of 42,000 ± 2,000 M^{-1} was obtained. This value was used for the present experiments, except when pH was varied or $KHCO_3$ was added to the incubation mixture. For these experiments, the association constant was corrected by means of described normalization procedures (17, 21). The binding of Mg^{2+} to the inhibitory drugs was also investigated. Although such determinations were hampered by the limited solubility of phenotiazine and thioxanthene derivatives, it could be estimated that these compounds had a very weak tendency to form magnesium complexes in 10 mM Hepes (pH 7.4). Considering the drug concentrations employed, it was concluded that the presence of the inhibitors did not appreciably interfere with the formation of the Mg·ATP complex.

Effect of fluphenazine on ATP hydrolysis. The concentration of Mg_f optimal for enzyme activity was determined, as suggested by Fromm (20), at the highest and lowest levels of substrate used. In either case the optimal Mg_f concentration was found to be 0.1 mM. The effect of fluphenazine on the kinetics of ATP hydrolysis is shown in Fig. 1 in the form of a double-reciprocal plot. As previously reported (22), the control line showed a downward curvature. The Hill coefficient (0.81 ± 0.06) was not appreciably modified in the presence of the inhibitor (0.75 ± 0.04). Although a curved plot does not allow an easy evaluation of the type of inhibition, it can be shown by making the approximation of drawing tangents to the curves, that the inhibition was essentially noncompetitive with respect to the substrate.

The fact that $K_{0.5}$, the substrate concentration giving half-maximal velocity, was not significantly changed

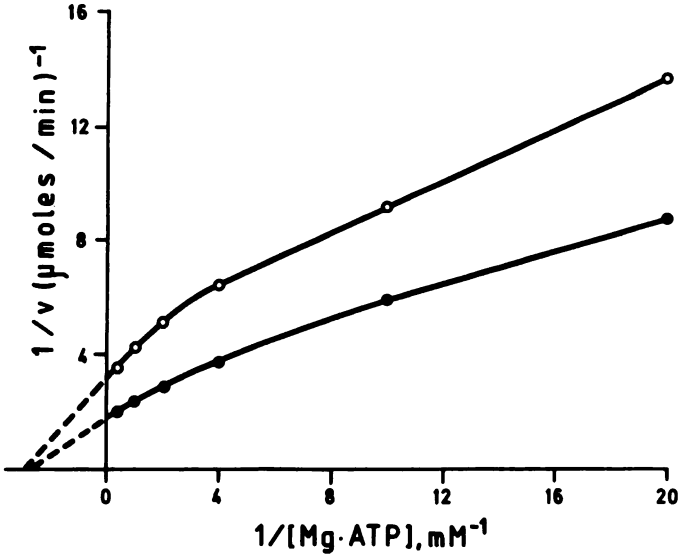

FIG. 1. *Double-reciprocal plot of ATP hydrolysis*

●, Control; ○, 30 μM fluphenazine. Experimental conditions were the same as described under Materials and Methods, except that the concentration of Mg·ATP was varied from 0.05 to 3 mM. Mg_f was held at a constant level of 0.1 mM. The ATP_f to Mg·ATP ratio was 0.24. Values for n (Hill coefficient) and $K_{0.5}$ (substrate concentration at half-maximal velocity) were calculated from Hill plots. The values for such parameters were as follows: $n = 0.81 \pm 0.06$ and $K_{0.5} = 0.37 \pm 0.05$ mM in the absence of fluphenazine, and $n = 0.75 \pm 0.04$ and $K_{0.5} = 0.30 \pm 0.02$ mM in the presence of 30 μM fluphenazine.

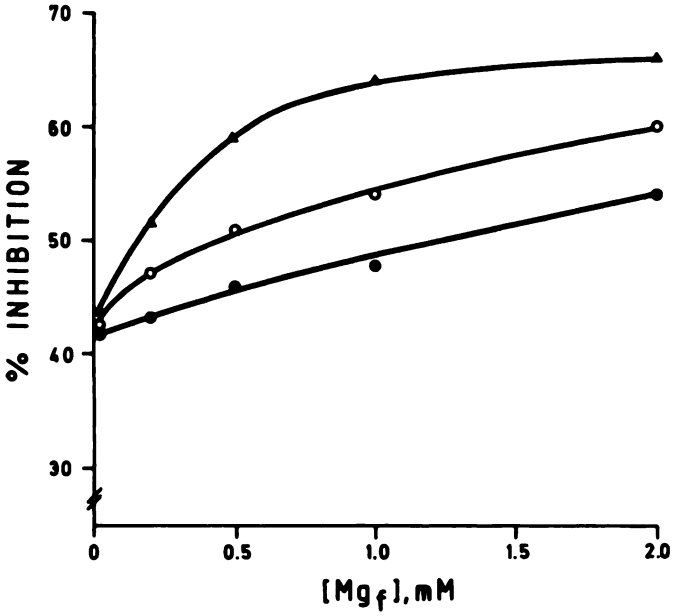

FIG. 2. *Effect of Mg_f on the inhibition of F_1-ATPase activity by 30 μM fluphenazine*

The concentration of Mg_f was varied from 0.025 to 2 mM at different fixed concentrations of Mg·ATP: ●, 0.05 mM; ○, 0.2 mM; △, 1 mM. Higher Mg_f concentrations could not be used because of the inhibitory effect of this cation. The lowest specific ATPase activity recorded was 11 μmoles/mg/min. A comparison of inhibition data was made using Student's t-test. Differences between percentage inhibition values were significant ($p < 0.05$ or lower) at all but the lowest Mg_f concentration.

(0.37 ± 0.05 mM in the absence, and 0.30 ± 0.02 mM in the presence, of fluphenazine) is also suggestive of noncompetitive inhibition. Classical noncompetitive inhibition was obtained when GTP, which gives linear reciprocal plots (22), was substituted for ATP. In the experiment shown in Fig. 1, the concentration of ATP·Mg was varied from 0.05 to 3 mM, while the level of Mg_f was held constant at 0.1 mM. The increase in substrate concentration was therefore accompanied by an increment in the concentration of ATP_f from 0.012 to 0.714 mM. A slight (5–8%) increase in inhibition was constantly observed in such experiments but, as discussed below, it does not appear to be attributable to a direct effect of either ATP·Mg or ATP_f. The foregoing results ruled out the possibility that these two ligands were responsible for the ample variations in inhibition (approximately 25%) previously observed when the total concentrations of ATP and magnesium were varied in equimolar ratio (10). The effect of Mg_f on the inhibition was therefore studied. In these experiments, the concentration of Mg_f was varied at different fixed substrate concentrations. Figure 2 illustrates how the inhibitory effect of fluphenazine varied upon varying the level of Mg_f. It is apparent that the percentage inhibition increased with the concentration of Mg_f. It can also be noted that the higher the substrate concentration, the more effective is Mg_f in enhancing the drug-induced inhibition. The latter observation may offer an explanation for the slight increase in inhibition observed when the concentration of Mg·ATP was varied at a fixed level of Mg_f (Fig. 1). Although it is not apparent from Fig. 2, these experiments showed that also the inhibitory effect of Mg_f on the enzyme activity increased with the substrate concentration; e.g., the decrements in ATPase activity at 2 mM Mg_f, relative to the activity observed at 0.1 mM Mg_f (optimal Mg_f excess), were 35, 48, and 54% at 0.05, 0.2, and 1 mM Mg·ATP, respectively.

Effect of bicarbonate on the inhibition. Bicarbonate and other anions greatly enhance the activity of coupling ATPases (22, 23). Their stimulatory effect has been attributed to either formation of complexes with Mg_f in solution or decrease in the affinity of the enzyme for the divalent cation (23). If the degree of fluphenazine inhibition is solely dependent on the level of Mg_f, then bicarbonate should partially reverse the inhibition in the presence of a significant excess of Mg_f. No appreciable decrease in inhibition should instead be observed at Mg_f levels approaching zero. The results of such a test are presented in Fig. 3. As expected, bicarbonate had a negligible effect on the inhibition when the concentration of Mg_f was 0.01 mM. In the presence of 0.2 mM Mg_f, the inhibition was instead decreased almost to the level observed at 0.01 mM Mg_f. The addition of $KHCO_3$ caused the pH of the incubation medium to increase to 7.65. This elevation of pH was found to cause a 3–4% decrease in inhibition which could not account for the observed effects of bicarbonate. The possibility that the reduction in inhibition was due to potassium ions or ionic strength could be excluded, since KCl at a concentration of up to 50 mM had no effect. As F_1-ATPase yields linear double-reciprocal plots in the presence of bicarbonate (22), the inhibitory effect of fluphenazine could be further analyzed by replotting slopes and intercepts as proposed by Cleland (24). Fig. 4A is a Lineweaver-Burk plot of ATP

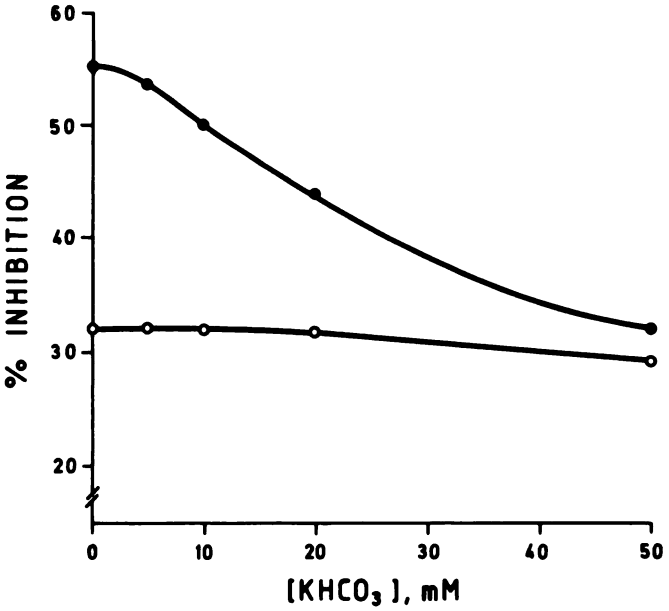

FIG. 3. *Effect of bicarbonate on the inhibition of F_1-ATPase activity by 30 μM fluphenazine*

Mg·ATP concentration was kept constant at 1 mM. Mg_f concentrations were as follows: ○, 0.01 mM; ●, 0.2 mM. The addition of bicarbonate caused the ATPase activity in the absence of fluphenazine to vary from 45 to 65 μmoles/mg/min at 0.01 mM Mg_f and from 75 to 162 μmoles/mg/min at 0.2 mM Mg_f.

hydrolysis in the presence of various inhibitor concentrations. It clearly appears that fluphenazine was a noncompetitive inhibitor of F_1-ATPase according to the definition of Cleland (24). This indicates that bicarbonate decreased the effectiveness of the inhibitor without modifying its mode of interaction with the enzymatic protein.

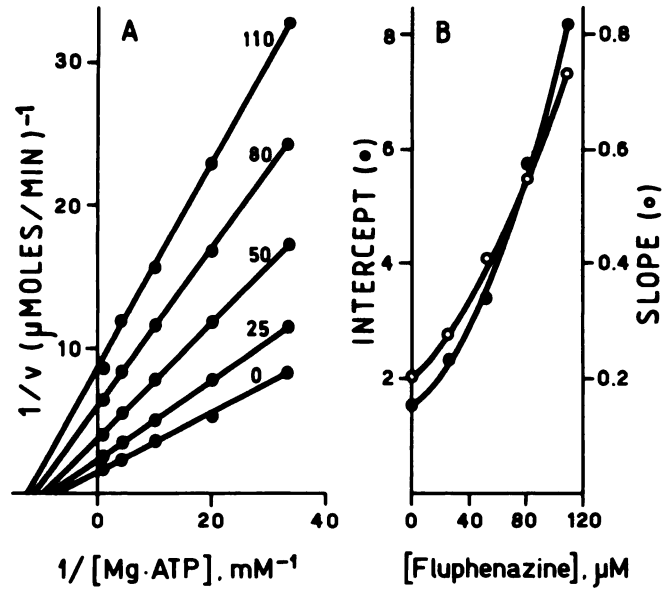

FIG. 4. *Inhibition of F_1-ATPase activity by fluphenazine in the presence of 20 mM $KHCO_3$*

A, Double reciptocal plot; B, slope and intercept replots. The concentrations of fluphenazine are indicated in micromolar units. The Mg·ATP concentration was varied from 0.03 to 1 mM at a constant Mg_f level of 0.1 mM.

The secondary plot (Fig. 4B) shows that both slope and intercept are parabolic functions of the inhibitor concentration. Such a slope-parabolic, intercept-parabolic inhibition is the result of the combination of more than one inhibitor molecule with the enzyme (24).

pH dependence of the inhibition. The influence of pH on the inhibition of F_1-ATPase by tricyclic antipsychotics is shown in Fig. 5. IC_{50} values (concentrations giving half-maximal inhibition) decreased upon lowering the pH of the incubation medium from 8.2 to 6.9. The results obtained with flupenthixol and chlorpromazine are also shown, since a comparison between the pH dependence of the three drugs illustrates more clearly the relationship between effect of pH and pK_a of the drugs. Flupenthixol and fluphenazine, whose pK_a is 7.8, exhibited a marked increase in inhibitory potency with decreasing pH. A considerably smaller variation was observed with chlorpromazine, which has a pK_a of 9.3 (25). This correspondence between variation in inhibition and pK_a of inhibitors suggests that the effect of pH was due to the ionization of the drugs, although concurrent effects related to the ionization of the enzyme molecule cannot be excluded. Correction for the degree of ionization of the drugs revealed that the protonated species alone could not account for the inhibition, thus indicating that the free base was also able to interact with the enzyme.

Effect of fluphenazine on cold-induced inactivation of F_1. F_1-ATPase undergoes a rapid decline in activity when exposed to low temperatures. This cold lability, which results from the dissociation of the enzymatic protein

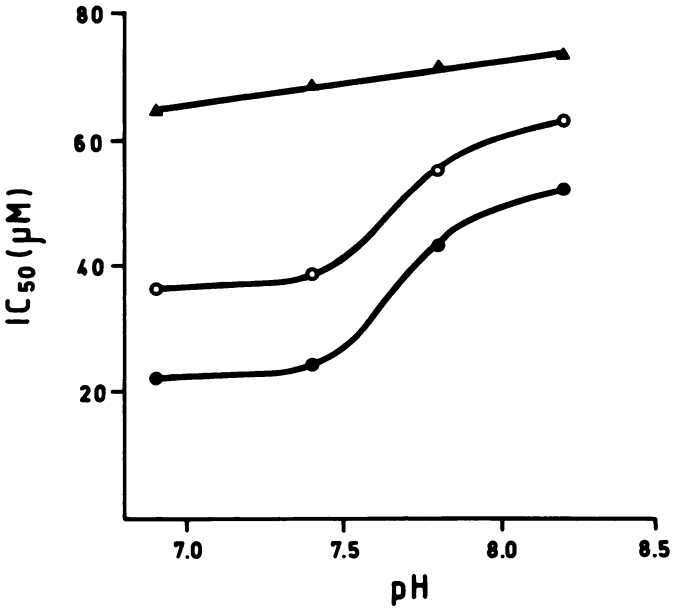

FIG. 5. *Inhibitory potency of tricyclic antipsychotics as a function of pH*

●, Flupenthixol; ○, fluphenazine; △, chlorpromazine. The reaction medium was buffered with 10 mM Hepes-KOH, adjusted to the appropriate pH at 30°. The pH of the final incubation mixture was measured in a duplicate reaction mixture. Other conditions were as described under Materials and Methods. ATPase activity in the absence of drugs varied from 72 (pH 6.9) to 142 (pH 8.2) μmoles/mg/min. IC_{50} values were obtained from inhibition curves at each pH. At pH 7.4, IC_{50} values were as follows: flupenthixol, 25 ± 1 μM; fluphenazine, 38 ± 2 μM, chlorpromazine, 69 ± 2 μM.

into its subunits, is prevented by the specific ATPase inhibitor protein (26). The interaction between F_1-ATPase and its natural inhibitor protein is strengthened by magnesium ions in the presence of ATP and is favored by a decrease in pH (14, 26). The possibility was therefore investigated that, like the natural ATPase inhibitor, fluphenazine could afford a protection against the cold-induced inactivation of F_1-ATPase. To this purpose, the enzyme was incubated for 20 min at 3° in the presence of increasing concentrations of fluphenazine and the initial velocity of ATP hydrolysis was then determined as usual at 30°. Preliminary experiments showed that the inhibitory effect of the drug was fully reversible upon dilution.

As shown in Fig. 6, the 20-min incubation in the cold caused a loss of ATPase activity of the same order as that previously observed by Penefsky and Warner (27). Fluphenazine, at sufficient concentrations, afforded complete protection against the cold-induced inactivation. The effectiveness of flupenthixol, fluphenazine, and chlorpromazine in preventing cold inactivation paralleled their inhibitory potency. A pH dependence similar to that described for the inhibition was also observed; e.g., the concentration of fluphenazine required for half-maximal protection decreased from 160 ± 7 to 85 ± 3 μM when the pH of the medium used for preincubation at 3° was lowered from 8.2 to 6.9. These concentrations were somewhat higher than the concentrations that gave 50% inhibition of ATPase activity. However, the different environmental conditions may afford an explanation for such a diversity. It is known that the strength of hydrophobic bonds, which are the main determinants of the interaction between these lipophilic drugs and biological molecules (1, 2), decreases with temperature. From dose-response curves, a good correlation was found between the effectiveness of a series of tricyclic derivatives as inhibitors of F_1-ATPase (flupenthixol > fluphenazine > chlorpromazine > promazine > promethazine) and their hydrophobic character as measured from the octanol/water partition coefficient (25).

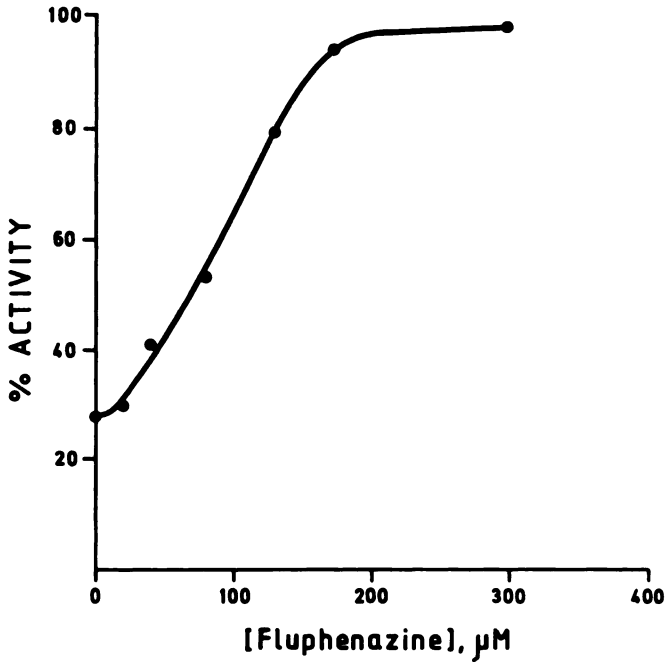

FIG. 6. *Effect of fluphenazine on cold-induced inactivation of F_1-ATPase*

The enzyme (1 mg/ml), was preincubated at 3° in 10 mM Hepes-KOH (pH 7.4) with the indicated concentrations of fluphenazine. At time zero (i.e., before cold preincubation) and after 20 min, 3-μl aliquots were withdrawn from each sample and immediately assayed for ATPase activity at 30°, in the presence of 3 mM Mg·ATP. Final volume (1 ml) and other conditions were as described under Materials and Methods. ATPase activity after the 20-min preincubation at 3° varied from 29 (0 μM fluphenazine) to 103 (300 μM fluphenazine) μmoles/mg/min. The concentration of fluphenazine affording half-maximal protection was 110 ± 4 μM.

DISCUSSION

Tricyclic antipsychotic drugs exert a wide variety of biochemical effects (1, 2). However, as previously pointed out (4), such a large spectrum of actions is not the result of an aspecific detergent-like interaction. When tested at concentrations well below the critical micellar concentration, these drugs exhibit different modes of interaction with different biological systems. The kinetic studies presented above show that fluphenazine inhibits F_1-ATPase in a specific manner that is dependent on Mg_f concentration. The mechanism of inhibition is thus dissimilar to those operating in the inhibition of other ATPases associated with cationic pumps. A true uncompetitive inhibition with respect to the substrate, independent of Mg_f concentration, has been observed with $(Na^+\text{-}K^+)$-ATPase (3). Interaction with calmodulin has been proposed as the mode of inhibition of calcium-stimulated ATPase (6). It is known that these enzymes, although similar in their physiological role, have quite different reaction mechanisms and subunit structures. Figure 2 shows that the inhibition of F_1-ATPase by fluphenazine does not extrapolate to zero when the concentration of Mg_f approaches zero. This indicates that exogenous magnesium is not essential for the interaction of the drug with the enzyme, but only increases the affinity for the inhibitor. When Mg_f is varied at fixed Mg·ATP concentrations, the level of ATP_f changes in the opposite direction. The alternative possibility might thus be considered that the variation in inhibition is due to an antagonistic effect of ATP_f. However, the fact that the inhibition is not decreased when ATP_f is increased at fixed Mg_f concentrations (see Fig. 1 and relevant explanation) rules out this possibility. The finding that bicarbonate anions antagonize the inhibition only in the presence of a significant excess of Mg_f lends further support to the view that Mg_f is the only ligand responsible for the observed variation in inhibition, while confirming previous proposals that bicarbonate acts on coupling ATPases by reversing the effect of Mg_f (13, 23). From Fig. 2 it is also manifest that the higher the concentration of Mg·ATP the lower the concentration of Mg_f at which the fluphenazine-induced inhibition appears to reach a plateau value, as though the binding of the substrate would increase the affinity of Mg_f for the enzyme. The observation that the inhibitory action of Mg_f on ATPase activity also increases with the substrate concentration is consistent with this possibility. Evidence suggesting that Mg·ATP may have an effect on the affinity of F_1 for Mg_f is also provided by some recent findings of Senior and co-workers (28, 29).

These authors have shown that purified preparations of soluble adenosine triphosphatase from heart mitochondria contain one atom of Mg^{2+} per molecule of enzyme. On incubation with Mg^{2+}, the enzyme takes up one more Mg^{2+} atom, provided that ATP is also present. Depletion of bound nucleotides causes no loss of Mg^{2+} from F_1-ATPase, indicating that the second Mg^{2+} atom is not bound in association with ATP.

It has been reported that phenothiazine derivatives interact, especially at low pH, with certain bi- and trivalent metal ions to form coordination complexes that may be of pharmacological relevance (30). The formation of drug·Mg^{2+} complexes of greater inhibitory potency than the uncomplexed drug might constitute an alternative plausible mechanism for the effect of Mg_f on the inhibition. However, tricyclic antipsychotics exhibited a very weak tendency to form magnesium chelates under the conditions used in the present study. The concentration of free uncomplexed Mg^{2+} is in many tissues of the same order as those used for these experiments (31). This suggests that the modulating effect of Mg_f on the inhibition described here is also operating *in vivo*.

The results of pH studies show that the ionized form of tricyclic antipsychotics is more effective than the free base in inhibiting F_1-ATPase. On the other hand, the inhibitory potency has been found to increase with the lipophilicity of the drugs. Taken together, these findings indicate that both the hydrophobic tricyclic nucleus and the side-chain amino group contribute to the interaction with the enzymatic protein. The protective effect against the cold-induced dissociation of F_1-ATPase would imply that fluphenazine strengthens the hydrophobic bonds that maintain the enzyme in its native conformation. Since a detergent effect would promote dissociation, this is a further indication that the detergent properties of tricyclic antipsychotics are not important in determining their biochemical actions at micromolar concentrations.

Several analogies have emerged from the present study between the action of the natural inhibitor protein and that of tricyclic antipsychotics. Similarly to the endogenous inhibitor, these drugs inhibit ATPase activity in a noncompetitive fashion. This inhibition is favored by the combined action of Mg and Mg·ATP and increases when the pH of the reaction mixture is lowered. In addition, tricyclic antipsychotics share with the naturally occurring inhibitor the ability to protect the enzyme from cold-induced inactivation. Protective effects against the cold lability of F_1-ATPase have been observed with other basic amphiphilic compounds such as alkylguanidines, although at considerably higher concentrations, and it has also been pointed out that the natural inhibitor is a basic molecule (32). This body of evidence suggests that the inhibition of F_1-ATPase by tricyclic antipsychotics is, at least in part, the result of the interaction with the natural inhibitor binding site.

Plasma concentrations of tricyclic antipsychotics after administration of therapeutic doses are generally supposed to lie in the nanomolar range. However, it has been repeatedly shown that there is wide intersubject variability in the plasma levels of these drugs, and micromolar plasma concentrations are not infrequently achieved when large doses are administered (33, 34). Considering the large apparent volume of distribution of tricyclic antipsychotics (10–35 liters/kg; refs. 35 and 36), tissue concentrations are expected to be still higher. It has been found, for instance, that the level of chlorpromazine in brain is up to 10 times as high as that in plasma (36). Since the effects described in this paper occur at micromolar concentrations, the possibility may be taken into consideration that the inhibition of mitochondrial ATPase, and hence of oxidative phosphorylation, contributes to the systemic effects of tricyclic antipsychotics. A reduction in ATP supply is known to cause inhibition of axonal transport (7).

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