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ON THE MECHANISM OF INHIBITION OF THE BOVINE HEART F_1-ATPase BY LOCAL ANESTHETICS

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SUMMARY: The rate of inactivation of the mitochondrial F_1-ATPase by dicyclohexylcarbodiimide is slowed by concentrations of chlorpromazine, dibucaine, or tetracaine which have been shown by others (B. Chazotte, G. Vanderkooi, and D. Chignell (1982) Biochim. Biophys. Acta 680, 310-316) to inhibit the hydrolytic reaction catalyzed by the enzyme. The order of effectiveness of the drugs as protectors of the enzyme against inactivation by dicyclohexylcarbodiimide is: chlorpromazine > dibucaine > tetracaine. Examination of the steady state kinetics showed the chlorpromazine inhibits the ATPase competitively at concentrations up to 18.5 μM while complex kinetic behavior is exhibited at chlorpromazine concentrations from 25-50 μM. These results suggest that the drugs inhibit the F_1-ATPase by interacting with the catalytic site of the enzyme and not by promoting its dissociation.

INTRODUCTION: It was reported recently that local anesthetics and other pharmacologically active amphipathic cations inhibit the bovine heart mitochondrial F_1-ATPase (1, 2). Since inhibiting concentrations of tetracaine affect the sedimentation behavior and the electrophoretic mobility of the enzyme, it was suggested that the drugs promote the dissociation of the ATPase, thus inhibiting its activity. However, it was also noted that the inhibition in the presence of tetracaine was relieved by dilution (2). This is atypical behavior if enzyme dissociation is indeed responsible for inhibition of MF_1 by the local anesthetics and related compounds. The imidazolium and anilinium cations have been observed to slow the rate of inactivation of the TF_1-ATPase by DCCD (M. Yoshida and W.S. Allison, unpublished observations, 1981), presumably by interacting with the essential glutamate of the enzyme (3). Since Mg^{2+} and Mg^{2+}-complexes of various nucleotides also slow the inactivation of F_1-ATPases by DCCD (3-7), the function of the DCCD-reactive glutamates in these enzymes appears

Abbreviations: DCCD; dicyclohexylcarbodiimide; MF_1, the F_1-ATPase from bovine heart mitochondria; and TF_1, the F_1-ATPase from the thermophilic bacterium, PS3.

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to be the binding of Mg^{2+}-complexes of adenine nucleotides. Experiments are reported here which test the possibility that the protonated forms of the local anesthetics also interact with the DCCD-reactive glutamate at the catalytic site of MF_1, thus causing its inhibition.

MATERIALS AND METHODS

MF_1 was prepared by the method of Knowles and Penefsky (8) and was assayed by coupling the phosphoenolpyruvate-pyruvate kinase system for regenerating ATP to NADH oxidation by lactate dehydrogenase (9). Sufficient amounts of the coupling enzymes were added to the assay reaction mixtures to insure that MF_1 was rate limiting. Chlorpromazine, dibucaine, tetracaine, and DCCD were purchased from Sigma Chemical Company and were used without further purification. The reagents used in the ATPase assay were also purchased from Sigma Chemical Company.

RESULTS

Protection of MF_1 against inactivation provided by chlorpromazine, dibucaine and tetracaine: The rate of inactivation of MF_1 by DCCD can be determined in the presence of concentrations of chlorpromazine, dibucaine, or tetracaine as high as 1 mM provided that the reaction mixtures are diluted by a factor of 1:500 during enzyme assays. ATPase activity is regained immediately on dilution of the enzyme in these experiments. Fig. 1 shows that the rate of inactivation of MF_1 by 1.0 mM DCCD at pH 7.0 and 32°C is slowed considerably in the presence of 1.0 mM chlorpromazine, 1.0 mM dibucaine, or 1.0 mM tetracaine. The order of effectiveness of these drugs as protectors of the ATPase against DCCD inactivation, chlorpromazine > dibucaine > tetracaine, is the same order of effectiveness observed when Chazotte et al. (2) examined them as inhibitors of the hydrolytic reaction catalyzed by the enzyme. The concentration dependence of the protection of MF_1 against DCCD inactivation afforded by chlorpromazine is illustrated in Fig. 2. Chlorpromazine provides 50% protection at about 70 μM as estimated by graphical analysis of the pseudo-first order rate constant for DCCD inactivation as a function of chlorpromazine concentration. The chlorpromazine concentrations estimated to inhibit the ATPase activity by 50% are estimated to be about 50 μM and 150 μM at pH 7.0 and 8.5, respectively. Since dibucaine and tetracaine are also more effective as inhibitors of the ATPase at pH 7.0 than they are at pH 8.5, the protonated forms of these drugs are responsible for the inhibitions observed. When considered with the evidence that DCCD modifies the catalytic site of MF_1, the similarity of the half saturation concentrations observed at pH 7.0 for

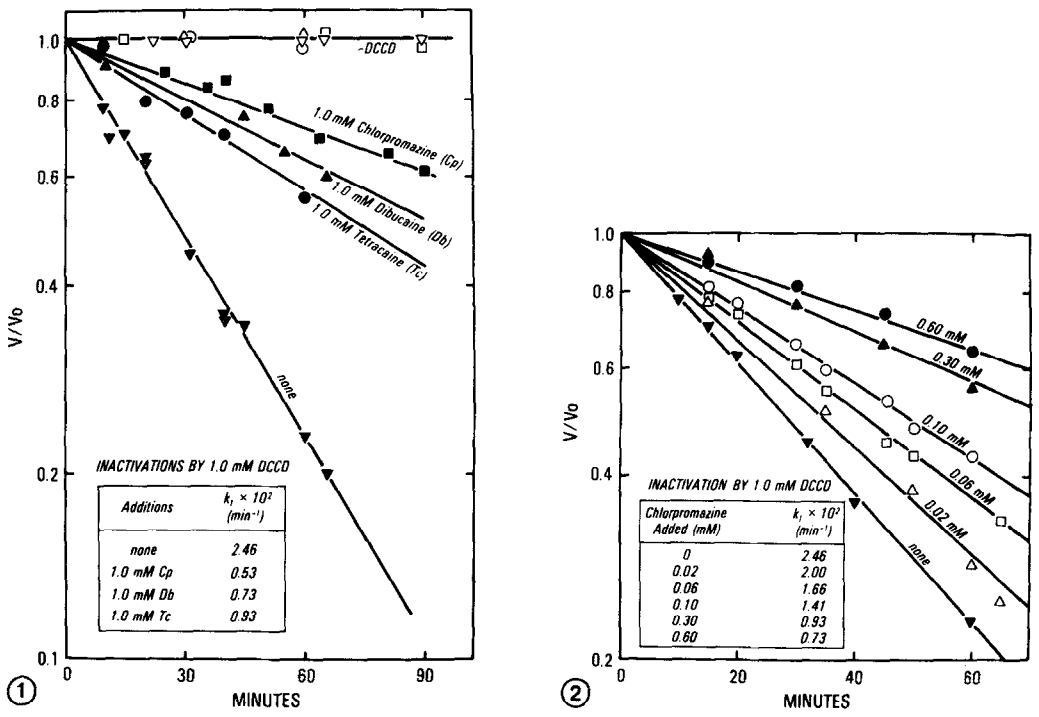

Figure 1. Protection against DCCD inactivation of MF_1 provided by chlorpromazine, dibucaine, and tetracaine. The reaction mixtures were incubated at 32°C and contained in 210 µl of 50 mM triethanolamine·HCl, pH 7.0; 1.0 mM DCCD added as a 100 mM ethanolic solution, 210 µg of MF_1, and the drugs at the following concentrations: none, (▼); 1.0 mM chlorpromazine, (■); 1.0 mM dibucaine, (▲); and 1.0 mM tetracaine, (●). Additions to controls that did not contain DCCD: none, (▽); 1.0 mM chlorpromazine, (□); 1.0 mM dibucaine, (△); and 1.0 mM tetracaine, (○).

Figure 2. Dependence of protection against DCCD inactivation on chlorpromazine concentration. The reaction mixtures were prepared and assayed as described in the legend to Fig. 1. The chlorpromazine concentrations were: none, (▼); 0.02 mM, (△); 0.06 mM, (□); 0.10 mM, (○); 0.30 mM, (△); and 0.60 mM, (●).

chlorpromazine, acting both as an inhibitor of the ATPase and as a protector against DCCD inactivation, suggests that chlorpromazine binds to the catalytic site of the enzyme. With this in mind the steady state kinetics of the ATPase were examined in the presence of chlorpromazine.

Examination of chlorpromazine inhibition of MF_1 by steady state kinetics: Lineweaver-Burk plots which illustrate the effect of increasing fixed concentrations of chlorpromazine on the steady state kinetics of ATP hydrolysis are shown in Fig. 3. Chlorpromazine behaves as a competitive inhibitor at concentrations up to 18.5 µM. However, at chlorpromazine concentrations from 25-50 µM the reciprocal plots are parallel, a pattern resembling that of uncompetitive inhibitors. What causes this deviant kinetic behavior at the high chlorpromazine concentrations is not obvious.

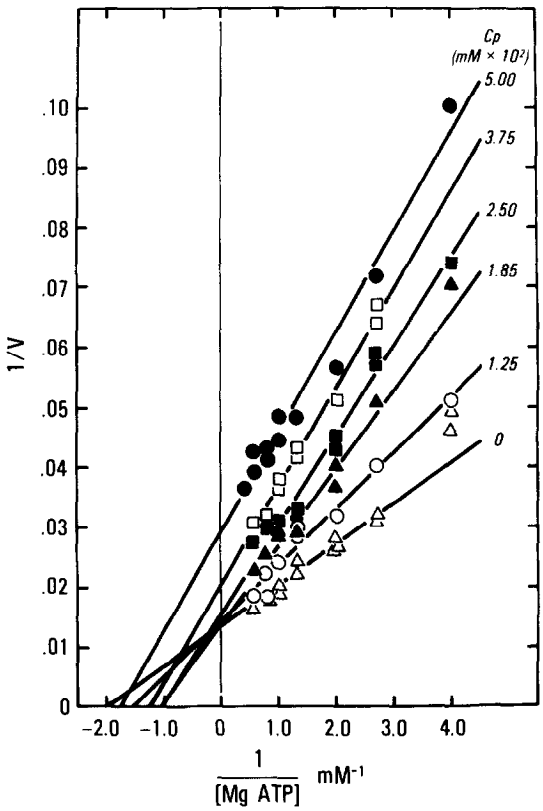

Figure 3. Steady state kinetic analysis of the inhibition of MF_1 by chlorpromazine. To initiate the reactions 5.0 µl of a 930 µg/ml solution of MF_1 in 50 mM triethanolamine·HCl, pH 7.0 was added to 1.0 ml of assay mix which contained: 50 mM Tris·HCl, pH 7.0; Mg·ATP at the concentrations indicated, added by appropriate volumes of a solution containing 25 mM $MgSO_4$ and 25 mM ATP; chlorpromazine at the concentrations indicated; 1.5 mM phosphoenolpyruvate; 0.15 mM NADH; and 7 units each of pyruvate kinase and H_4-lactate dehydrogenase. The initial rates were determined with a recording spectrophotometer. The fixed chlorpromazine concentrations used to obtain the reciprocal plots were: none, (△); 12.5 µM, (○); 18.5 µM, (▲); 25.0 µM, (■); 37.5 µM, (□); and 50.0 µM, (●).

The fact that competitive inhibition is observed at concentrations of chlorpromazine up to 18.5 µM provides further evidence that the amphipathic cations inhibit MF_1 by interacting with its catalytic site.

DISCUSSION

On the basis of changes observed in the sedimentation behavior and electrophoretic mobility of MF_1 in the presence of tetracaine, Chazotte et al. have suggested that local anesthetics and related compounds inhibit the enzyme by promoting its dissociation (2). However, it is not clear that the qualitative changes observed in these experiments are directly related to the inhibition induced by tetracaine. The fact that enzyme activity appears immediately when concentrated solutions of the

enzyme in the presence of inhibiting concentrations of the drugs are diluted to contain non-inhibiting concentrations of the drugs, suggests that enzyme dissociation is not responsible for the inhibition of ATPase activity promoted by the amphipathic cations.

The evidence presented here suggests that the pharmacologically active cations inhibit MF_1 by interacting with the DCCD-reactive glutamic acid side chain at the catalytic site. The structures of chlorpromazine, dibucaine, and tetracaine might be sufficiently similar to that of Mg·ATP to have an affinity for the catalytic site of MF_1. The enzyme site that binds the adenine moiety of Mg·ATP might accommodate the aromatic rings of the inhibitors, thus placing the positively charged tertiary amino group of each inhibitor in a position to interact with the glutamate that normally interacts with the Mg^{2+} moiety of the substrate.

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