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STUDIES ON THE MECHANISM OF ACTION OF LOCAL ANESTHETICS ON PROTON TRANSLOCATING ATPase FROM *MYCOBACTERIUM PHLEI*

NEERAJ AGARWAL and VIJAY K. KALRA *

Department of Biochemistry, University of Southern California, School of Medicine, 2025 Zonal Avenue, Los Angeles, CA 90033 (U.S.A.)

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We have measured the inhibitory potencies of local anesthetics (procaine, lidocaine, tetracaine and dibucaine) on ATP-mediated H^+-translocation, Ca^{2+}-transport and ATPase activity in membrane vesicles from *Mycobacterium phlei*. Procaine and lidocaine up to 1 mM concentration did not inhibit ATP-dependent H^+-translocation, Ca^{2+}-transport and ATPase activity. However, tetracaine and dibucaine at 0.2 mM concentration caused dissipation of the proton gradient, measured by the reversal of the quenching of fluorescence of quinacrine, and inhibition of active Ca^{2+}-transport. Tetracaine (1 mM) inhibited membrane-bound ATPase activity without affecting solubilized F_1-ATPase activity. Studies show that these local anesthetics do not prevent the inactivation of F_0-F_1 ATPase by dicyclohexylcarbodiimide (DCCD). Binding of [^{14}C]DCCD to F_0-proteolipid component remained unchanged in the presence of tetracaine indicating that DCCD and tetracaine do not share common binding sites on the F_0-proteolipid sector. The inhibition of H^+-translocation and membrane-bound ATPase activity by tetracaine was substantially additive in the presence of vanadate.

Introduction

Local anesthetics are well known as blockers of nerve conduction at a concentration which does not significantly alter the resting potential [1]. The potency of local anesthetics have been correlated to their ability to interact with phospholipid model membranes [2,3] and red blood cell membrane [4]. It has been proposed that local anesthetics mediate their action by directly interacting with proteins [5] as well as perturb the membrane lipids environment, which alters the activities of membrane enzymes. Recent studies have shown that local anesthetics inhibit mitochondrial F_1-ATPase [6,7]. Moreover, it has been observed that these local anesthetics protect mitochondrial F_1-ATPase against inactivation by dicyclohexylcarbodiimide [8]. However, thermophilic ATPase (TF_1) exhibited resistance to inhibition by local anesthetics [9] which was interpreted as due to the extreme stability of the conformation of TF_1, even though high degree of homology in amino acid sequence exists among catalytic β subunit of various ATPases including thermophilic ATPase [10,11].

These reports prompted us to investigate the interaction of local anesthetics with membrane-bound and solubilized ATPase from *Mycobacterium phlei*. Our studies show that dibucaine and tetracaine inhibit ATP-mediated H^+-translocation and Ca^{2+}-transport while procaine and lidocaine were ineffective. Moreover, tetracaine inhibits membrane-bound ATPase activity without

* To whom request for reprints should be addressed.
Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; DCCD, *N,N'*-dicyclohexylcarbodiimide.

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affecting solubilized F_1-ATPase activity. To our knowledge, this is the first report describing inhibition of proton-translocating ATPase by tetracaine. The inhibition of H^+-translocation by tetracaine occures presumably by interacting with the F_0 sector of the F_0-F_1 complex.

Materials and Methods

Preparation of membrane vesicles and F_1-ATPase. *Mycobacterium phlei* ATCC 354 was grown and harvested as described by Brodie and Gray [12]. Membrane vesicles were prepared by sonication of cells as described by Brodie [13]. Membrane vesicles were suspended in 50 mM Tris-acetate buffer (pH 8.0) containing 0.15 M KCl and 4 mM $MgCl_2$. F_1-ATPase was solubilized from membranes as described by Higashi et al. [14].

Trypsin treatment to activate ATPase in membrane vesicles. Unmasking of ATPase by trypsin treatment was carried out in a reaction mixture containing membrane vesicles or solubilized F_1-ATPase (1–2 mg/ml) in 50 mM Tris-acetate buffer (pH 7.0) containing 0.15 M KCl and 4 mM $MgCl_2$ and bovine pancreas trypsin (50 μg/mg membrane protein) for 10 min at 30°C. After 10 min, the reaction was terminated by the addition of soybean trypsin inhibitor (100 μg/mg protein).

Assay of latent ATPase activity. ATPase activity in trypsin-treated membrane vesicles and solubilized F_1 was carried out in the presence of 4 mM $MgCl_2$ and 10 mM ATP at 30°C, as described previously [15]. The inhibition of membrane-bound ATPase activity by various local anesthetics was assayed by addition of anesthetic prior to the addition of ATP.

Assay of the calcium transport. Uptake of $^{45}Ca^{2+}$ in membrane vesicles and trypsin-treated vesicles was determined by filtration method, utilizing 0.45 μM Millipore filter as described previously [16,17]. Where indicated, local anesthetics were added prior to the initiation of uptake by the addition of $^{45}CaCl_2$ (50 μM).

Measurement of the proton gradient by fluorescence method. Changes in transmembrane proton gradient (ΔpH) were estimated from the energy-linked quenching of quinacrine fluorescence, as described earlier [18,19]. The assay medium comprised trypsin-treated vesicles (0.5 mg protein/ml)/50 mM Hepes-KOH buffer (pH 7.4)/4 mM $MgCl_2$/4 μM quinacrine, in a total volume of 2.5 ml. Unless otherwise indicated, ATP was added at a concentration of 0.8 mM. Fluorescence measurements were carried out at 37°C, using a Perkin-Elmer MPF-4 spectrofluorometer with excitation at 420 nm and emission at 500 nm.

Measurement of respiration. Rates of oxygen consumption in membrane vesicles were measured in the presence of succinate (20 mM), with a Clark-type oxygen electrode (YSI Model 53, oxygen monitor, Yellow Spring Instrument, Yellow Springs, OH). The concentration of all the local anesthetics used was 1 mM.

Estimation of [^{14}C]DCCD binding. The reaction mixture in a total volume of 0.5 ml, comprising membrane vesicles (3–5 mg proteins)/ 50 mM Tris-acetate buffer (pH 8.0)/0.15 M KCl/4 mM $MgCl_2$ was incubated with [^{14}C]DCCD (specific activity, 54.6 Ci/mol) in the presence and absence of tetracaine (1 mM) for a period of 2 h at 37°C. After the incubation, the suspension was centrifuged at $144000 \times g$ for 20 min and the pellet washed twice with the same buffer. The washed pellet was homogenized in 10 mM Tricine-NaOH buffer, pH 8.0 and [^{14}C]DCCD-labeled proteolipid extracted by butanol, and dried under N_2 gas as described [18,20] and counted for radioactivity by liquid scintillation counting.

Protein measurement. Protein was estimated by the method of Lowry et al. [21] using bovine serum albumin as standard.

Materials. [^{14}C]DCCD (54.6 Ci/mol) was purchased from Research Products International Corp., IL; $^{45}CaCl_2$ from New England Nuclear; vanadate (orthovanadate) from Fisher Scientific; ATP (vanadate free), quinacrine, procaine, dibucaine, tetracaine hydrochloride and lidocaine were purchased from Sigma Chemical. All other chemicals were of reagent grade purity.

Results

Effect of local anesthetics on membrane functions mediated by ATP hydrolysis

Studies were carried out to determine whether membrane functions, i.e., formation of proton gradient (ΔpH) and uptake of Ca^{2+}, mediated by ATP hydrolysis, were affected by local anesthetics,

i.e., procaine, lidocaine, tetracaine and dibucaine. The quenching of fluorescence of quinacrine, indicative of proton uptake and ΔpH formation in membrane vesicles, was measured during hydrolysis of ATP. As shown in Fig. 1A, addition of tetracaine and dibucaine at 100 μM concentration produced more than 70% reversal of quenching of fluorescence of quinacrine. Furthermore, addition of tetracaine and dibucaine to membrane prior to the addition of ATP prevented the quenching of fluorescence (Fig. 1B). The addition of bovine serum albumin after the addition of tetracaine caused ATP-induced quenching of fluorescence, indicating that affect of tetracaine is reversible in nature. In contrast lidocaine and procaine at concentration up to 500 μM (Fig. 1A) did not affect the quenching of fluorescence of quinacrine mediated by ATP-hydrolysis, indicating procaine and lidocaine do not dissipate proton gradient formation. Fig. 2 shows the relationship between the percentage reversal of fluorescence quenching as a function of the concentration of dibucaine and tetracaine, after the addition of ATP. The degree of reversal of quenching was dependent upon the concentration of either tetracaine or dibucaine added. The local anesthetic concentration yielding 50% of V_{max} ($S_{0.5}$) was approx. 92 μM and 16 μM for tetracaine and dibucaine, respectively.

Active transport of Ca^{2+} driven by ATP hydrolysis as well as respiratory-linked substrate oxidation has been demonstrated previously [16,17] in membrane vesicles from *M. Phlei*. As shown in Fig. 3 tetracaine and dibucaine at 200 μM concentration inhibited (90%) active transport driven by ATP. Procaine and lidocaine did not affect the

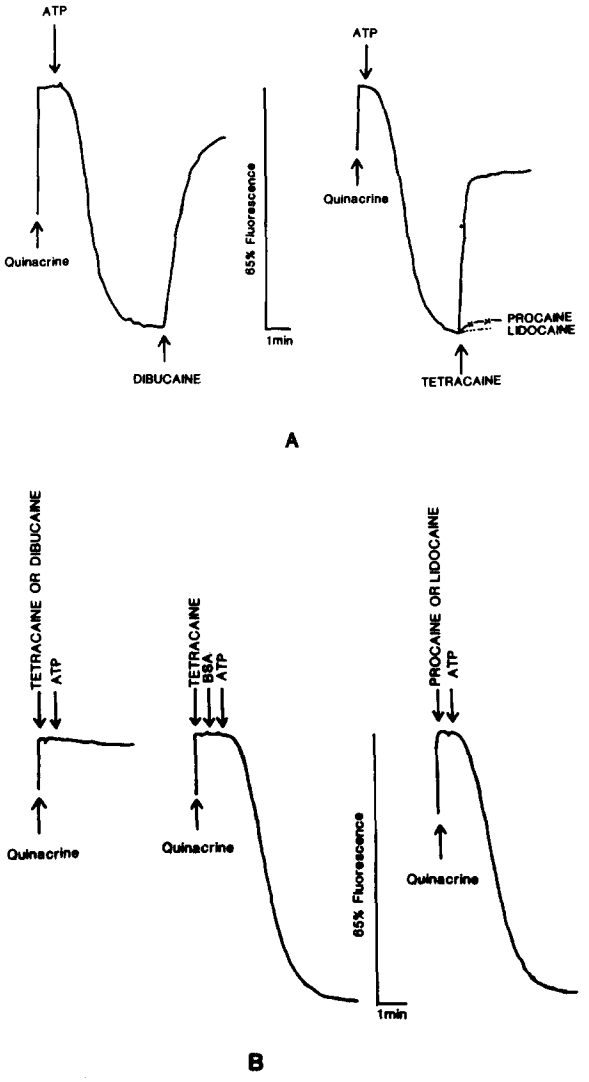

Fig. 1. Effect of local anesthetics on the formation of a proton gradient, driven by ATP hydrolyses. The change in fluorescence of quinacrine was measured in 2.5 ml of reaction mixture comprising 50 mM Hepes-KOH buffer (pH 7.4), 10 mM $MgCl_2$, 4 μM quinacrine and membrane vesicles (1–2 mg protein). ATP (0.8 mM) was added where indicated. (A) Dibucaine (100 μM), tetracaine (100 μM), procaine (500 μM) and lidocaine (500 μM) were added at time intervals as indicated by the arrows. (B) Tetracaine (100 μM) or dibucaine (100 μM) was added before the addition of ATP (0.8 mM). Bovine serum albumin (100 mg) was added before adding ATP (0.8 mM) in the middle curve of Fig. 1(B) Procaine (500 μM) or lidocaine (500 μM) was added before the addition of ATP.

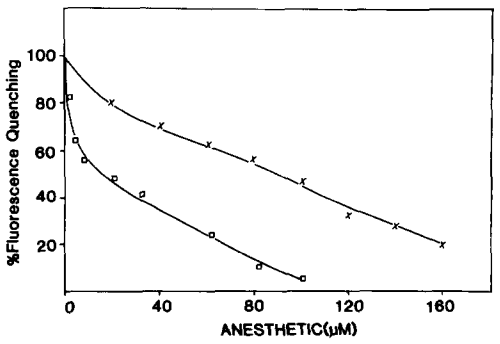

Fig. 2. Effect of tetracaine and dibucaine on the reversal of ATP-linked fluorescence quenching of quinacrine. ATP-induced quenching of fluorescence was measured as described in the legend to Fig. 1. ×——×, tetracaine, □——□, dibucaine.

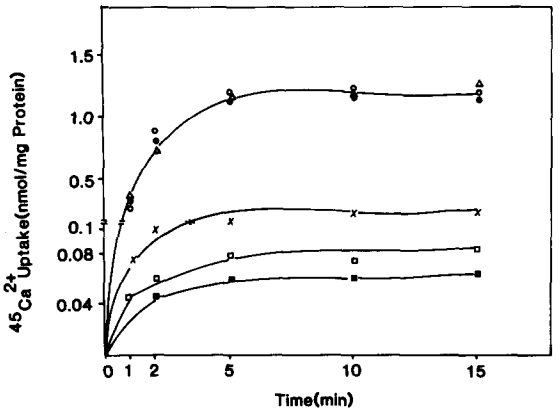

Fig. 3. Effect of local anesthetics on the uptake of $^{45}CaCl_2$. The reaction mixture (0.5 ml) contained trypsin-treated membrane vesicles (2 mg protein), 50 mM Hepes-KOH buffer (pH 7.4) and 4 mM $MgCl_2$. The uptake was initiated by the addition of 1 mM ATP and 50 μM of $^{45}CaCl_2$. Where indicated, local anesthetics were added at various concentrations, prior to the addition of the radioactive calcium in assay system. Samples of 80 μl were withdrawn at the indicated intervals of time and the uptake of calcium was determined as described under Materials and Methods. ○———○, $^{45}Ca^{2+}$ uptake; uptake of $^{45}Ca^{2+}$ in the presence of: ●———●, procaine (1 mM); △———△, lidocaine (1 mM); ×———×, tetracaine (200 μM); □———□, dibucaine (200 μM) and ■———■, *m*-carbonylcyanide phenylhydrazone. The results are shown for a typical experiment. Similar results were obtained in four separate experiments, using different membrane vesicles preparations.

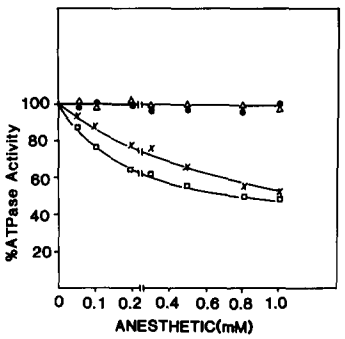

Fig. 4. Effect of various concentrations of local anesthetics on membrane-bound ATPase activity. The percent ATPase activity in the presence of the local anesthetics (1 mM) compared to that in the absence is plotted versus the various concentrations of local anesthetics. The ATPase activity in trypsinized membrane vesicles was assayed by determining the released Pi as described under materials and Methods. ATPase activity in the presence of ●———●; procaine, △———△, lidocaine; ×———×, tetracaine and □———□, dibucaine.

uptake of Ca^{2+} mediated by ATP hydrolysis. None of these local anesthetics (200 μM) inhibited the uptake of Ca^{2+} driven by succinate oxidation (data not shown).

Inhibition of proton-translocating ATPase by tetracaine

As mentioned in the preceeding section, tetracaine and dibucaine inhibited the generation of proton gradient and active transport of Ca^{2+} in membrane vesicles of *M. phlei* only when the process was energized by the hydrolysis of ATP, it was pertinent to determine whether these anesthetics exerted their effect through the inhibition of proton-translocating ATPase. As shown in Table I, tetracaine and dibucaine at 1 mM concentration inhibited membrane-bound ATPase activity, while procaine and lidocaine were ineffective at this

TABLE I

EFFECT OF LOCAL ANESTHETICS ON MEMBRANE-BOUND AND SOLUBLE ATPase ACTIVITY FROM *M. PHLEI*

Membrane vesicles or soluble F_1-ATPase were treated with trypsin, prior to analyzing the effect of various local anesthetics on ATPase activity. The ATPase activity was analyzed by determining the released P_i as described under Materials and Methods.

	ATPase activity (μmol P_i/h per mg protein)			
	Membrane-bound	Percentage inhibition	Soluble	Percentage inhibition
Control	6.55	0.0	8.11	0.0
Tetracaine (1 mM)	3.52	46.2	8.28	0.0
Dibucaine (1 mM)	2.90	55.7	5.73	29
Procaine (1 mM)	6.83	0.0	8.10	0.0
Lidocaine (1 mM)	6.57	0.0	8.15	0.0

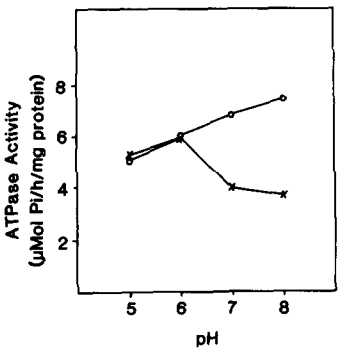

Fig. 5. Effect of pH on the inhibition of membrane-bound ATPase activity by tetracaine (1 mM). Membrane-bound ATPase activity in the presence and absence of tetracaine in trypsinized membrane vesicles (2 mg) was determined as described under Materials and Methods. ATPase activity; ○——○, in the absence of tetracaine and ×——×, in the presence of tetracaine.

concentration. However, tetracaine (7 mM) procaine and lidocaine did not inhibit soluble F_1-ATPase activity. Dibucaine (1 mM) exhibited partially inhibitory (29%) effect on soluble F_1-ATPase activity. Fig. 4 shows the effect of increasing concentration of tetracaine and dibucaine on the inhibition of membrane-bound ATPase activity. It should be mentioned that there was residual (40%)

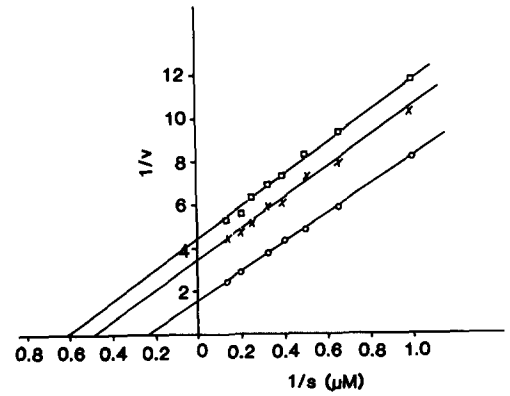

Fig. 6. Lineweaver-Burk plot of membrane-bound ATPase activity in the presence of dibucaine or tetracaine, as a function of various ATP concentration. The reaction mixture and the assay procedures were similar to those described in Fig. 5. Dibucaine (□——□) or tetracaine (×——×) was added at a concentration of 1 mM (○——○, control). The plots were obtained using a programmable Hewlett-Packard computer attached to a plotter. Correlation coefficient R value was 0.99.

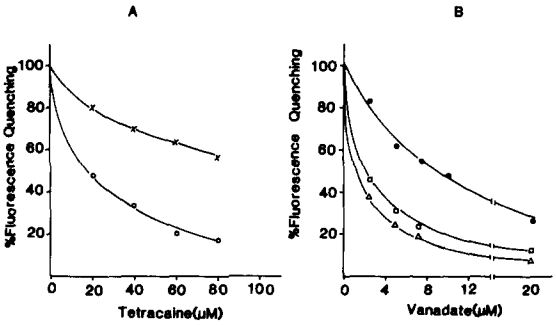

Fig. 7. The effect of the presence of both vanadate and tetracaine on the percentage quenching of fluorescence of quinacrine. Titration curve on the left-hand panel was obtained in the absence of vanadate (×——×), or with 2.5 μM vanadate (○——○). Titration curve on the right-hand panel was obtained in the absence of tetracaine (●——●) or with 20 μM tetracaine (□——□) and 40 μM tetracaine (△——△). Data were obtained by the measurement of the rate of quenching of quinacrine energized by ATP hydrolysis at different concentrations of either tetracaine or vanadate using trypsin treated vesicles (1–2 mg) as described in the legend to Fig. 1.

ATPase activity, which was not inhibited by tetracaine, as has also been observed in F_1-ATPase from submitochondrial particles [6]. Tetracaine inhibited membrane-bound ATPase activity above pH 7.0 (Fig. 5). Lineweaver-Burk plots (Fig. 6) of the effect of tetracaine and dibucaine on the kinetics of ATP hydrolysis showed that the reciprocal plots are parallel, indicating that inhibition is uncompetitive in nature.

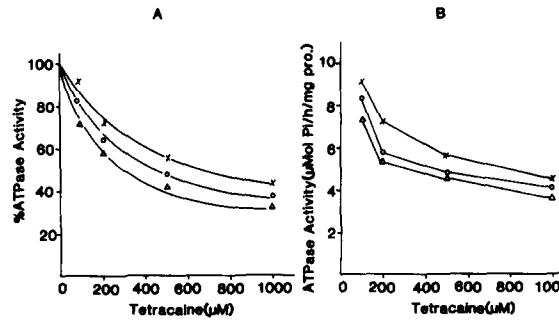

Fig. 8. The effect of the presence of both vanadate and tetracaine on the ATPase activity in membrane vesicles from *M. phlei*. Titration curves were obtained in the absence of vanadate (×——×), or with 20 μM (○——○) or 40 μM (△——△) vanadate. The reaction mixture and assay procedures for determining the ATPase activity were similar to those described in the legends to Fig. 4 except that the concentration of $MgCl_2$ was 15 mM instead of 4 mM.

Effect of local anesthetics on the inhibition of membrane-bound ATPase activity by DCCD

Since tetracaine inhibited membrane-bound ATPase but not solubilized ATPase activity, it was of interest to determine whether the binding of tetracaine to F_0 portion of F_0-F_1 complex influenced the inactivation of ATPase activity brought about by covalent inhibitor, dicyclohexylcarbodiimide [18], which is known to bind at sites on F_0. Studies showed that tetracaine (1 mM) and dibucaine (1 mM) did not prevent the inactivation of ATPase activity by DCCD (data not shown) indicating that these anesthetics do not presumably bind to the same site as DCCD. Incubation of [^{14}C]DCCD with membrane vesicles, in the presence and absence of tetracaine, followed by extraction of DCCD-binding proteolipid [18] revealed that there was no difference in the binding of [^{14}C]DCCD to proteolipid, indicating that tetracaine and DCCD do not share common binding sites (data not shown).

Effect of tetracaine on H^+-ATPase in the presence of vanadate

Previous studies [19] have shown that vanadate (52 μM) inhibited membrane-bound ATPase activity as well as formation of proton gradient in membrane vesicles of *M. Phlei*. We determined whether the effect of tetracaine in the presence of vanadate was additive in nature in the inhibition of ΔpH formation. As shown in Fig. 7, addition of a limiting amount of tetracaine (20–80 μM) caused quenching (18–37%) of fluorescence, which was markedly enhanced by the addition of vanadate. At 20 μM tetracaine concentration, there was 18% reversal of quenching of fluorescence, which was enhanced to 68% by the addition of vanadate 2.5 μM (Fig. 7A). Similarly, the addition of a limiting amount of vanadate (4 μM) caused 30% quenching of fluorescence, which was enhanced to 62% and 72% by the addition of 20 μM and 40 μM tetracaine, respectively, indicating that the effect was independent of the order of addition. As shown in Fig. 8, the inhibition of membrane-bound ATPase activity by tetracaine was partially additive with 20 μM and 40 μM vanadate, respectively.

Discussion

The results presented in this investigation show that among local anesthetics, dibucaine, tetracaine, lidocaine and procaine, the effect on membrane function is selective in membrane vesicles of *Mycobacterium phlei*. Procaine and lidocaine up to 1 mM concentration do not affect membrane-bound ATPase and solubilized F_1-ATPase activity. In addition, procaine and lidocaine do not affect ATP-dependent quenching of fluorescence of quinacrine or 9-aminoacridine, probes used for the measurement of proton gradient. However, tetracaine and dibucaine exerted potent inhibitory action on the formation of proton gradient (ΔpH), as measured by the quenching of quinacrine fluorescence. Since tertiary amine local anesthetics are weak bases, they might accumulate within the vesicles and dissipate ΔpH. However, this may not be true, since procaine and lidocaine at five times higher concentration than tetracaine and dibucaine did not dissipate ΔpH. It is pertinent to mention that lidocaine, tetracaine and procaine were found not to affect the energy-linked fluorescence response of quinacrine in submitochondrial particles [22], indicating that these anesthetics do not substantially accumulate in these vesicles. Mueller et al. [23] have observed specific binding sites for quinacrine in submitochondrial particles, and thus have indicated that quantitative measurement of proton gradient (ΔpH) by these probes would give an overestimation. Dibucaine and tetracaine both inhibited membrane-bound ATPase activity, while dibucaine (200 μM) inhibited partially (29%) soluble F_1-ATPase activity. It appears that tetracaine exhibits inhibitory action on the H^+-translocation system, which resembles that exerted by dicyclohexylcarbodiimide [21] and vanadate [19] in *Mycobacterium phlei* membranes. The inhibition of the H^+-ATPase by DCCD and vanadate results from specific depression of the proton conduction of F_0 sector of the F_0-F_1-ATPase complex [18]. Since tetracaine does not affect solubilized F_1-ATPase activity, therefore it seems likely that the inhibition of proton conductivity observed may occur as a result of binding of tetracaine to either F_0 sector of F_0-F_1 complex or non-specific hydrophobic binding to the membrane.

Since DCCD and vanadate also bind to the F_0 sector of the F_0-F_1 complex, it was of interest to determine whether tetracaine shared the sites occupied by either DCCD or vanadate on F_0 sector. Studies showed that tetracaine does not prevent the inactivation of ATPase activity by DCCD in *M. phlei* membranes. In submitochondrial particles, Laikind et al. [8] have observed that local anesthetics protect ATPase from inactivation by DCCD by interacting with the catalytic sites of the enzyme. Thus, it appears that in F_0-F_1 ATPAse complex of *M. phlei* membranes binding of tetracaine and DCCD do not occur to the same site or amino acid residues. This is also substantiated by the observation that extent of binding of [^{14}C]DCCD to proteolipid component of F_0-F_1 complex remains the same, even when membranes are preincubated with tetracaine (200 μM).

Studies were carried out to delineate whether vanadate and tetracaine bind to the same or different binding sites in the F_0 sector of F_0-F_1 complex. Our results show that inhibition in ATPase activity and H^+-conductivity were substantially additive in the presence of both inhibitors, namely vanadate and tetracaine. In this respect, the effect of tetracaine resembles to that of *N*-ethylmaleimide, which exhibits partially additive effect with that exerted by oligomycin and triphenyltin in the inhibition of the proton conductivity of the H^+-ATPase in submitochondrial particles [24,25]. However, the effects of oligomycin and dicyclohexylcarbodiimide on the ATPase activity in beef-heart submitochondrial particles have been shown not to be additive indicating different binding sites of these two inhibitors [25]. Since the oligomeric structure of DCCD-binding proteolipid component has not been conclusively established; both trimeric [27–29] and hexameric [30] structures have been proposed, and stoichiometry of binding of either tetracaine or vanadate is not known, therefore it is not clear whether additive inhibition is through binding to the same monomer subunit or oligomeric structure. It is pertinent to mention that Glaser et al. [26] observed that 1 mol oligomycin per mol F_0 could inhibit both H^+-translocation coupled to ATP hydrolysis and passive translocation through F_0 devoid of F_1, suggesting that oligomycin-binding protein may be present only as a monomer in F_0 and may thus be a different subunit than the DCCD-binding oligomer. These local anesthetics do not inhibit respiration (data not shown) and respiratory linked Ca^{2+}-transport, thus the affect observed with these anesthetics cannot be due to non-specific hydrophobic binding to the membrane. The inhibition of membrane-bound ATPase but not solubilized F_1-ATPase by tetracaine may simply be due to the altered configuration of the enzyme in F_0-F_1 complex as opposed to the free F_1 form cannot be ruled out by these experiments. Since the inhibition of proton translocation and ATPase activity in *M. phlei* membrane vesicles by tetracaine did not significantly change with the incubation time, these results suggest that under the influence of tetracaine, F_0 oligomer presumably does not undergo a change in conformational transition state, in contrast to oligomycin and *N*-ethylmaleimide which have been shown to inhibit H^+-translocation in a time-dependent manner [25] as a result of affecting the conformational transition state of the F_0 component.

Tetracaine should be a useful probe for the elucidation of the structure and function of the proton-translocating ATPase, since it is a reversible inhibitor. The precise site of binding of tetracaine in the F_0-F_1 complex will be investigated in future by synthesizing affinity probes of tetracaine and mapping studies.

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References

1 Ritchie, J.M. and Greengard, P. (1966) Annu. Rev. Pharmacol. 6, 405–430
2 Papahadjopoulos, D. (1972) Biochim. Biophys. Acta 265, 169–186
3 Feinstein, M.B. (1964) J. Gen. Physiol. 48, 357–374
4 Koblin, D.D., Pace, W.D. and Wang, H.H. (1975) Arch. Biochem. Biophys. 171, 176–182
5 Eyring, H., Woodbury, J.W. and D'Arrigo, J.S. (1973) Anesthesiology 38, 415–424
6 Vanderkooi, G., Shaw, J., Storms, C., Vennerstorm, R. and Chignell, D. (1981) Biochim. Biophys. Acta 635, 200–203

7 Chazotte, B., Vanderkooi, G. and Chignell, D. (1982) Biochim. Biophys. Acta. 680, 310–316
8 Laikind, P.K., Goldenberg, T.M. and Allison, W.S. (1982) Biochem. Biophys. Res. Commun. 109, 423–427
9 Saishu, T., Kagawa, Y. and Shimizu, R., (1983) Biochem. Biophys. Res. Cummun. 112, 822–826
10 Yoshida, M., Allison, W.S., Esch, F.G. and Futai, M. (1982) J. Biol. Chem. 257, 10033–10037
11 Walker, J.E., Sersate, M., Runswick, M.J. and Gay, N.J. (1982) Eur. Mol. Biol. Org. J. 1, 945–951
12 Brodie, A.F. and Gray, C.T. (1956) J. Biol. Chem. 219, 853–862
13 Brodie, A.F. (1959) J. Biol. Chem. 234, 398–404
14 Higashi, H., Kalra, V.K., Lee, S.H., Bogin, E. and Brodie, A.F. (1975) J. Biol. Chem. 250, 6541–6548
15 Agarwal, N. and Kalra, V.K. (1983) Biochim. Biophys. Acta 727, 285–292
16 Kumar, G., Deves, R. and Brodie, A.F. (1979) Eur. J. Biochem. 100, 365–375
17 Lee, S.H., Kalra, V.K. and Brodie, A.F. (1979) J. Biol. Chem. 254, 6861–6864
18 Agarwal, N. and Kalra, V.K. (1983) Biochim. Biophys. Acta 723, 150–159
19 Yoshimura, F. and Brodie, A.F. (1981) J. Biol. Chem. 256, 12239–12242
20 Kalra, V.K. and Brodie, A.F. (1971) Arch. Biochem. Biophys. 147, 653–659
21 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
22 Mueller, D.M. and Lee, C.P. (1982) FEBS Lett. 137, 45–48
23 Mueller, D.P., Hudson, R.A. and Lee, C.P. (1982) Biochemistry, 21, 1445–1453
24 Papa, S., Guerrieri, F., DeGomez Puyou, M.T., Barranco, J. and Gomez-Puyou, A. (1982) Eur. J. Biochem. 128, 1–7
25 Guerrieri, F. and Papa, S. (1982) Eur. J. Biochem. 128, 9–13
26 Glaser, E., Norling, B., Kopecky, J. and Ernster, L. (1982) Eur. J. Biochem. 121, 525–531
27 Fillingame, R.H. (1976) J. Biol. Chem. 251, 6630–6637
28 Sone, N., Yoshida, M., Hirata, H. and Kagawa, Y. (1979) J. Biochem. (Tokyo) 85, 503–509
29 Kopecky, J., Glaser, E., Norling, B. and Ernster, L. (1981) FEBS Lett. 131, 208–212
30 Sebald, W., and Hoppe, J. (1981) Curr. Top Bioenerg. 12, 1–64