Proc. Natl. Acad. Sci. USA
Vol. 78, No. 2, pp. 795–799, February 1981
Biochemistry

Local anesthetics, mepacrine, and propranolol are antagonists of calmodulin

(erythrocyte membrane calcium ATPase/cyclic nucleotide phosphodiesterase/calcium)

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Communicated by M. J. Osborn, October 20, 1980

ABSTRACT **Local anesthetics such as dibucaine, QX572, tetracaine, and phenacaine, as well as other drugs with local anesthetic-like properties (e.g., mepacrine, propranolol, and SKF 525A) inhibit the specific calmodulin-dependent stimulation of erythrocyte Ca^{2+}-ATPase (ATP phosphohydrolase, EC 3.6.1.3) and cyclic nucleotide phosphodiesterases (3',5'-cyclic-nucleotide 5'-nucleotidohydrolase, EC 3.1.4.17) from brain and heart. Basal activities of these enzymes in the absence of calmodulin are relatively unaffected by concentrations of local anesthetics that strongly inhibit the specific stimulation by calmodulin. Increasing calmodulin, but not Ca^{2+}, overcomes the inhibitory action of the local anesthetics on brain phosphodiesterase. However, excess calmodulin does not fully restore activity of erythrocyte Ca^{2+}-stimulated ATPase. Although the mechanism(s) by which the local anesthetics act is unclear, they inhibit binding of ^{125}I-labeled calmodulin to the erythrocyte membrane. Antagonism of calmodulin provides a molecular mechanism that may explain the inhibition of many Ca^{2+}-dependent cellular processes by local anesthetics—e.g., Ca^{2+} transport, exocytosis, excitation-contraction coupling, non-muscle-cell motility, and aggregation.**

Local anesthetics are classified as such by their ability to block conduction of impulses in nerves reversibly. However, these drugs can also affect a wide range of cellular processes in nonnerve tissue, such as excitation-contraction coupling in various types of muscle (1, 2), exocytosis (3–6), membrane transport or permeability of calcium (7), glucose (8), and anions (9), cellular adhesion (10) or aggregation (11), osmotic fragility (12), and ligand-induced Ig receptor capping (13). Several enzymatic activities are also affected by local anesthetics, notably response of adenylate cyclase to catecholamines (14), Mg^{2+},Ca^{2+}-dependent ATPases of brain (15), sarcoplasmic reticulum (16), and erythrocytes (17), and phospholipase A_2 (18). Many of the effects of local anesthetics are manifested on calcium-dependent processes and have usually been attributed to influences on membrane calcium permeability or binding (19).

In addition to effects of local anesthetics mediated by membrane pertubations we considered that antagonism of Ca^{2+}-mediated processes might be due to effects on the actions of calmodulin. Calmodulin, a ubiquitous Ca^{2+}-binding protein in eukaryotes, is one of the most important mediators of calcium's role as an intracellular messenger, affecting contractile proteins, cyclic nucleotide metabolism, glycogenolysis, phospholipid metabolism, membrane phosphorylation, and Ca^{2+} transport (20, 21). We therefore undertook a study of the influence of local anesthetics on several calmodulin-dependent enzymatic reactions. In this paper we report on two such enzymes; Ca^{2+}-ATPase (ATP phosphohydrolase, EC 3.6.1.3) of the erythrocyte membrane and cyclic nucleotide phosphodiesterase (3',5'-cyclic nucleotide 5'-nucleotidohydrolase, EC 3.1.4.17) from brain and cardiac muscle. Preliminary reports of these findings have been published (22, 23).

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METHODS AND MATERIALS

Preparation of Human Erythrocyte Membranes. Erythrocyte membranes were prepared by the method of Gopinath and Vincenzi (24) modified by carrying out hemolysis in 10 mM imidazole·HCl, pH 7.4/1 mM EDTA. Aliquots (2.0 ml) of the membrane suspensions containing 1.0–1.7 mg of protein per ml were frozen in dry ice/acetone and stored at −80°C.

Assay of Calcium ATPase. Calcium-stimulated ATPase activity in the membranes was measured by the radioisotope assay of Seals *et al.* (25) as described in the legend to Fig. 1.

Purification of Erythrocyte Calmodulin. Calmodulin was purified according to the method of Jarrett and Penniston (26) through the Sephadex G-100 chromatography step. Active fractions were dialyzed against water, lyophilized, and redissolved in the minimal volume of 10 mM 1,4-piperazinediethanesulfonic acid at pH 6.5. Pure calmodulin was isolated by preparative discontinuous pH/polyacrylamide gel electrophoresis (27). In 10.5% polyacrylamide, calmodulin migrated with the bromophenol blue dye front and was eluted from the gels into 0.1 M potassium phosphate, pH 6.6/1 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA). Purity of the calmodulin was verified by $NaDodSO_4$/polyacrylamide gel electrophoresis and comparison of activity with a calmodulin standard obtained from D. M. Watterson.

Binding of ^{125}I-Labeled Calmodulin to Erythrocyte Membranes. Calmodulin was iodinated as described by Chafouleas *et al.* (28). Binding of ^{125}I-labeled calmodulin to membranes was measured by incubation under the same conditions as described for ATPase assays. After 15 min of incubation, the membrane suspensions were placed onto Sepharose 4B (400 mesh) columns (0.6 × 13 cm) and eluted at 37°C. The elution solution was the same as the incubation medium except that MgATP was deleted and $MgCl_2$ was increased to 2 mM and Ca^{2+} or local anesthetic was included if present in the initial incubation. Fractions (0.3 ml) were collected and assayed for radioactivity in a gamma spectrometer. Total recovery of radioactivity from the column was >98%, indicating negligible binding to the column. The bound and free calmodulin were well separated from each other; membranes and membrane-bound calmodulin eluted in frac-

Abbreviations: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; QX572, *N,N*-bis(phenylcarbamoylmethyl)dimethylammonium chloride; MES, 2-(*N*-morpholino)ethanesulfonic acid; TLCK, N^{α}-*p*-tosyl-L-lysine chloromethyl ketone; SKF 525A, β-diethylaminoethyl-2,2-diphenylpentanoate; cyclic AMP, adenosine 3',5'-cyclic monophosphate; cyclic GMP, guanosine 3',5'-cyclic monophosphate; IC_{50}, concentration for 50% inhibition.

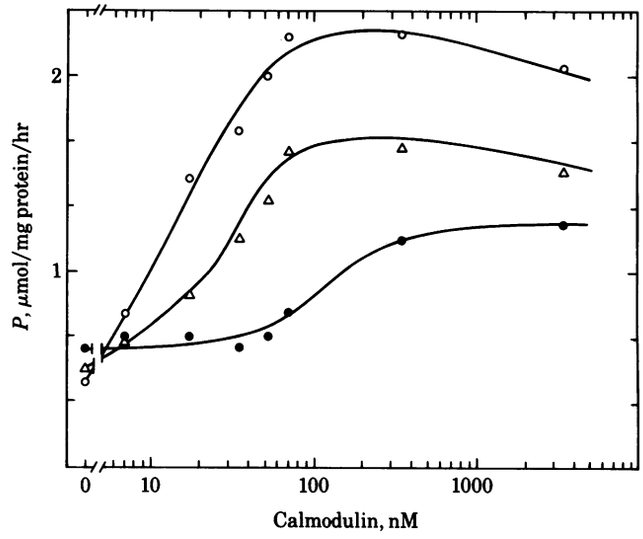

FIG. 1. Effect of dibucaine HCl on the response of erythrocyte membrane Ca^{2+}-stimulated ATPase to calmodulin. Incubation mixtures of 125 μl contained 25 μl of membrane suspension, 80 mM NaCl, 15 mM KCl, 0.1 mM EGTA, 0.1 mM oubain, 1 mM $MgCl_2$, 0.2 mM $CaCl_2$, 1 mM MgATP containing [α-^{32}P]ATP (0.5 μCi/ml), and 18 mM histidine/imidazole, pH 7.1. Calmodulin and dibucaine were preincubated with membranes for 15 min on ice and then incubated at 37°C. Reactions were stopped with 25 μl of 6% $NaDodSO_4$ and 75 μl of molybdate/silicotungstic acid reagent. [^{32}P]Phosphate was separated from [^{32}P]ATP by extraction into 1.25 ml of xylene/isobutanol, and 1.0 ml of the organic phase was taken for scintillation counting. Local anesthetics had no significant effect on the extraction of [^{32}P]phosphate. ○, Control membranes; △, membranes plus 0.25 mM dibucaine; ●, membranes plus 1.0 mM dibucaine. ATPase activity with Mg^{2+} (+ EGTA) has been subtracted.

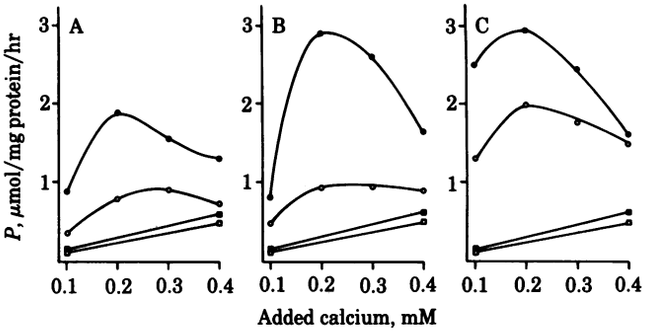

FIG. 2. Erythrocyte membrane Ca^{2+}-stimulated ATPase activity as a function of calmodulin and $CaCl_2$ concentrations. Two lower added $CaCl_2$ concentrations, 0.05 and 0.01 mM, stimulated ATPase activity but are omitted for simplicity. Free Ca^{2+} concentrations in the presence of 0.1 mM EGTA were calculated according to Katz *et al.* (31), neglecting binding to membranes, and are given as micromolar free Ca^{2+} with the respective added $CaCl_2$ (in μM) shown in parentheses: 0.29 (10), 2.0 (32), 12 (100), 76 (200), 159 (300), and 223 (400). Maximal activity was attained at 76 μM free Ca^{2+}. ATPase activity was 80–90% of maximum at 30 μM (by interpolation), the concentration previously reported, by Jarrett and Kyte (33) to be optimal for human erythrocyte calmodulin-stimulated Ca^{2+}-ATPase. Assays were carried out as described in the legend for Fig. 1 except that $CaCl_2$ was varied. The calmodulin concentrations were: 96 nM in *A*, 192 nM in *B*, and 14.6 μM in *C*. Experiments shown are for membranes without calmodulin (□), membranes plus 1.0 mM dibucaine without calmodulin (■), membranes with calmodulin (●), and membranes with calmodulin plus 1.0 mM dibucaine (○). Mg^{2+}-ATPase activity has been subtracted.

tions 6–12, and free ^{125}I-labeled calmodulin appeared in fractions 15–22.

Preparation of Calmodulin-Sensitive Cyclic Nucleotide Phosphodiesterase. Whole rat brains (30 g) were homogenized in 150 ml of 10 mM Tris·HCl, pH 8.0/1 mM EGTA/40 μM N^{α}-*p*-tosyl-L-lysine chloromethyl ketone (TLCK) at 4°C. The homogenate was adjusted to pH 6.0 with acetic acid and centrifuged at 177,400 × *g* (R_{max}) for 60 min. The supernatant was applied, by ascending flow, to a column (2.6 × 20 cm) of DEAE-Sephacel equilibrated in a buffer designed for maximal stability of phosphodiesterase (29): 5 mM 2-(*N*-morpholino)ethanesulfonic acid (MES), 30% ethylene glycol, 1 mM dithiothreitol, 5% (wt/vol) glucose, 10 mM sodium acetate, 10 mM NaF, 20 μM TLCK, 1 mM EGTA (pH 6.5); the column was washed with 8 bed volumes of the same buffer. Phosphodiesterase activity was eluted with a 300-ml linear gradient of sodium acetate (pH 6.5) from 0.01 to 1.2 M in the same buffer readjusted to pH 6.5. The flow rate was 1 ml/min, and 4-ml fractions were collected. Enzyme activity eluting between 15 and 19 mS (0.24–0.34 M sodium acetate) was pooled and used as the source of calmodulin-sensitive phosphodiesterase. Activity was the same when assayed in the presence of $CaCl_2$ (0.1 mM) or EGTA (1 mM), indicating complete separation of the enzyme from endogenous calmodulin. In the absence of calmodulin the enzyme had a V_{max} of 0.071 μmol/min per mg of protein and a K_m of 45 μM cAMP. In the presence of 40 nM calmodulin, the V_{max} increased to 0.26 μmol/min per mg of protein and the K_m was 53 μM cAMP. Activator-deficient cardiac muscle cyclic nucleotide phosphodiesterase was obtained from Sigma.

Assay of Cyclic Nucleotide Phosphodiesterase Activity. Enzyme activity was assayed by the two-step radioisotope procedure using Dowex 1-X2 columns to separate reaction product from unreacted substrate (30) as described in the legend to Fig. 5.

RESULTS

Inhibition of Calcium ATPase. The mean (± SEM) ATPase activity of erythrocyte membranes prepared as described was 0.23 ± 0.015 μmol of phosphate per mg of protein per hr in the presence of Mg^{2+} and 0.1 mM EGTA; it increased to 0.63 ± 0.03 after addition of 0.2 mM $CaCl_2$. This "basal" Ca^{2+}-stimulated ATPase activity was completely inhibited by trifluoperazine and by calcineurin (kindly donated by C. Klee), indicating the presence of some residual endogenous calmodulin. Nevertheless, the enzyme could be markedly stimulated by the addition of exogenous calmodulin (Fig. 1).

The degree of enhancement of ATPase activity depended upon the concentrations of both Ca^{2+} and calmodulin (Fig. 2). Increasing calmodulin concentration increased the ATPase activity at the optimal Ca^{2+} level. At high concentrations of calmodulin the sensitivity to calcium was increased so that activity was nearly maximal even at the lower calcium concentrations (Fig. 2*C*). A similar effect of high calmodulin concentration on calcium sensitivity has been observed with heart phosphodiesterase (34). Increasing calcium beyond the optimum resulted in a decreased ATPase activity independent of the calmodulin concentration, as also observed by Luthra and Kim (35).

Several local anesthetics inhibited the stimulatory effect of calmodulin. The basal Mg^{2+},Ca^{2+}-ATPase activity was not inhibited (or was only slightly inhibited, ≤15%) by anesthetic concentrations that strongly (>80%) antagonized the stimulation by calmodulin. Dibucaine shifted the concentration–response curves for the modulator protein (Fig. 1). Increasing calmodulin tended to overcome the local anesthetic inhibition (Fig. 1), but even a 60-fold increase in calmodulin (over that required for 0.9

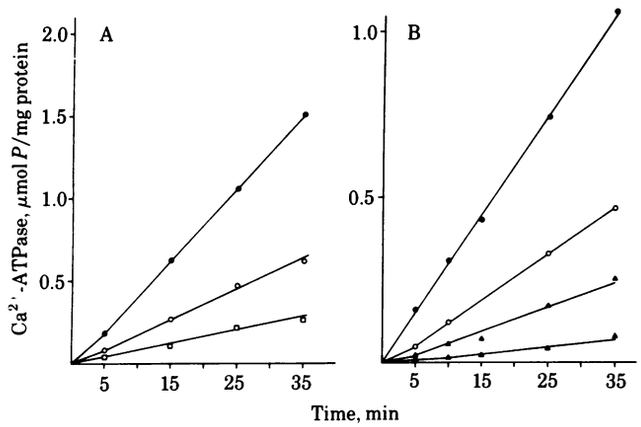

FIG. 3. Effect of dibucaine HCl on the time course of erythrocyte membrane Ca^{2+}-stimulated ATPase activity. Assays were performed as described in the legend to Fig. 1. (*A*) All experiments contained membranes plus 28 nM calmodulin. ●, Controls; ○, plus 0.5 mM dibucaine; □, plus 1.0 mM dibucaine. (*B*) ●, Membranes with 28 nM calmodulin; ○, membranes with 28 nM calmodulin plus 0.5 mM dibucaine; ▲, membranes with 3.5 nM calmodulin; △, membranes with 3.5 nM calmodulin plus 0.5 mM dibucaine. The Mg^{2+}-ATPase activity has been subtracted.

V_{max}) produced only an incomplete reversal of the local anesthetic effect (Figs. 1 and 2). Increasing Ca^{2+} could not restore the normal calmodulin stimulation of ATPase activity in the presence of local anesthetics (Fig. 2). Inhibition of the rate of ATP hydrolysis was a function of dibucaine concentration (Fig. 3*A*) and occurred at different concentrations of calmodulin (Fig. 3*B*). Inhibition by dibucaine also was evident at the approximate physiological concentration of calmodulin (≈1 μM) in the intact erythrocyte (Figs. 1 and 2*C*).

In addition to dibucaine, other local anesthetics inhibited the stimulation of Ca^{2+}-ATPase by calmodulin; the approximate concentrations for 50% inhibition (IC_{50}) determined at 66 nM calmodulin (0.9–1.0 V_{max}) were: mepacrine, 0.15 mM; dibucaine, 0.26 mM; tetracaine, 1.0 mM; phenacaine, 1.0 mM; and QX572, 1.2 mM.

Inhibition of ^{125}I-Labeled Calmodulin Binding to Erythrocyte Membranes by Local Anesthetics. Binding of ^{125}I-labeled calmodulin to erythrocyte membranes was low in the absence of Ca^{2+} but increased markedly in the presence of 0.2 mM $CaCl_2$ plus 0.1 mM EGTA (Fig. 4). Dibucaine produced a concentration-dependent inhibition of ^{125}I-labeled calmodulin binding (Fig. 4) similar to the inhibition of Ca^{2+}-ATPase activity under the same conditions (Fig. 3). Other local anesthetics also inhibited binding (the details of these experiments will be presented elsewhere).

Inhibition of Cyclic Nucleotide Phosphodiesterase. Cyclic nucleotide phosphodiesterase prepared from rat brain was stimulated by calmodulin in a dose-dependent manner (Fig. 5). At 1 μM cyclic GMP (K_m cGMP = 5 μM), calmodulin maximally stimulated enzyme activity 5.3-fold with half-maximal stimulation at 6 nM calmodulin. At 0.25 μM cyclic AMP (K_m cAMP = 45 μM), calmodulin maximally stimulated enzyme activity 3.7-fold with half-maximal stimulation at 3.5 nM calmodulin.

The calmodulin stimulation of cyclic nucleotide phosphodiesterase from both rat brain and bovine heart was inhibited by local anesthetics and drugs exhibiting local anesthetic properties. In the presence of 0.5 mM dibucaine, the apparent K_m of phosphodiesterase for calmodulin increased from 3.5 nM to about 170 nM at 0.25 μM cyclic AMP and from 6 nM to about 400 nM at 1 μM cyclic GMP (Fig. 5). Lineweaver–Burk analysis indicated that inhibition by dibucaine was competitive with respect to calmodulin with a calculated K_i of 8–10 μM. In a separate experiment using higher concentrations of calmodulin, the inhibition of cyclic AMP hydrolysis by 0.5 mM dibucaine was completely overcome at 1500 nM calmodulin. This is somewhat

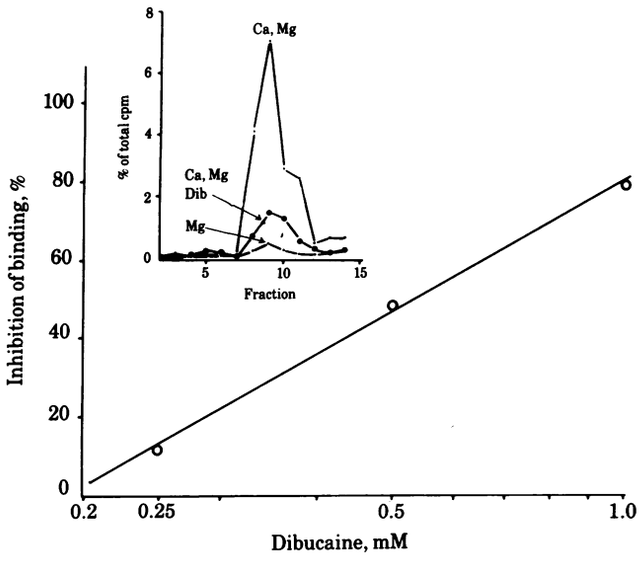

FIG. 4. Inhibition of binding of ^{125}I-labeled calmodulin to erythrocyte membranes by dibucaine HCl (Dib). Inhibition of binding is expressed as a percentage of the specific Ca^{2+}-dependent binding in the absence of dibucaine. (*Inset*) Sepharose gel elution pattern of membrane-bound ^{125}I-labeled calmodulin under varying conditions—in the presence of: 2 mM $MgCl_2$; 0.2 mM $CaCl_2$ plus 2 mM $MgCl_2$; or 1.0 mM dibucaine plus 0.2 mM $CaCl_2$ and 2 mM $MgCl_2$. EGTA (0.1 mM) was present in all experiments. The ^{125}I-labeled calmodulin concentration was 7 nM.

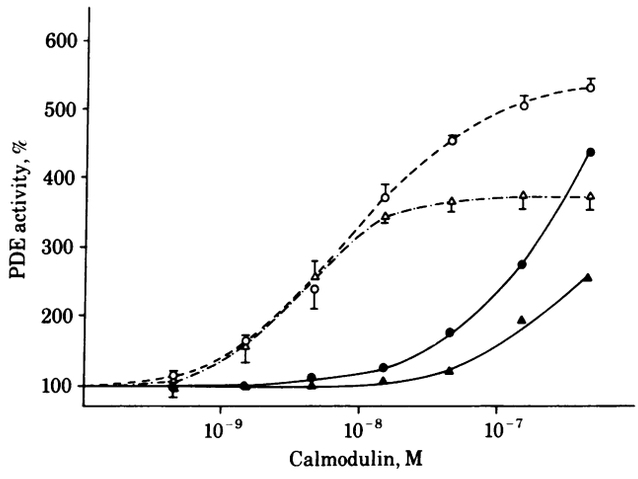

FIG. 5. Inhibition of calmodulin-stimulated brain cyclic nucleotide phosphodiesterase (PDE) activity by dibucaine HCl. The reaction mixture (0.4 ml) contained 50 mM Tris·HCl (pH 8.0), 10 mM $MgCl_2$, 2.5 mM 2-mercaptoethanol, 0.2 mM $CaCl_2$, 1 × 10^5 cpm of either cyclic [^{3}H]AMP (0.25 μM) or cyclic [^{3}H]GMP (1 μM), 50 μg of bovine serum albumin, enzyme (1.26 μg for cyclic AMP hydrolysis; 0.32 μg for cyclic GMP hydrolysis), and, where applicable, local anesthetic and purified brain calmodulin (generously supplied by D. M. Watterson). Reactions were run for 10 min at 30°C. Protein was determined by the method of Udenfriend *et al.* (32) with bovine serum albumin as standard. Phosphodiesterase activity with cyclic GMP (○, ●) or cyclic AMP (△,▲) as substrates. ○, △, Controls; ●, ▲, with 0.5 mM dibucaine. Dibucaine, calmodulin, and the enzyme were added together on ice, and reactions were initiated by the addition of substrate.

Table 1. Effect of local anesthetics and local anesthetic-like drugs on calmodulin-stimulated and unstimulated brain phosphodiesterase

Drug	IC_{50}, μM*	
	Calmodulin-stimulated	Unstimulated
Trifluoperazine	6	230
Mepacrine	56	>3,000
SKF 525A	130	>3,000
Dibucaine	180	>3,000
Propranolol†	180	>3,000
QX572	310	>3,000
Tetracaine	350	1,200
Phenacaine	930	>10,000

* Concentration of drug that produced 50% inhibition of phosphodiesterase stimulation in the presence of 15 nM calmodulin (calmodulin-stimulated) and 50% inhibition of phosphodiesterase in the absence of calmodulin (unstimulated). Phosphodiesterase activity was determined at 0.25 μM cyclic AMP.

† The *d*-, *l*-, and *d,l*- isomers of propranolol all showed the same potency.

different from the particulate Ca^{2+}-ATPase system in which inhibition by dibucaine was still apparent at this calmodulin concentration (Fig. 1). As observed with the Ca^{2+}-ATPase system, inhibition of phosphodiesterase by dibucaine could not be overcome by increasing calcium up to 1 mM.

Dibucaine had no effect on basal (minus calmodulin) phosphodiesterase activity at a concentration equal to its apparent K_i against calmodulin. Even at 0.5 mM dibucaine, which shifted the calmodulin dose–response curve ≈50-fold, the basal activity was inhibited by only 14%. At a constant calmodulin concentration (15 nM), the inhibition by dibucaine appeared to be noncompetitive with respect to cyclic AMP as substrate when analyzed by Lineweaver–Burk analysis. Inhibition of basal phosphodiesterase activity required much higher concentrations of dibucaine and appeared to be competitive with respect to substrate (K_i ≈5 mM). Similarly, a calmodulin-insensitive, low-K_m phosphodiesterase purified from Kidney (29) was also inhibited by local anesthetics in a competitive manner with respect to substrate. Thus, local anesthetics had two effects: one at low concentrations directed against the effect of calmodulin and independent of substrate and Ca^{2+} concentration, and the second at high concentrations affecting the affinity of the enzyme for substrate.

In addition to dibucaine, calmodulin stimulation of phosphodiesterase was inhibited by other local anesthetics and mepacrine, propranolol, and β diethylaminoethyl-2,2-diphenyl pentanoate (SKF 525A) (Table 1). The action of propranolol was nonstereospecific and therefore was related to its local anesthetic-like properties rather than to its activity as a β-adrenergic antagonist. The relative IC_{50} values for the inhibition of the phosphodiesterase were lower than those obtained for the inhibition of the Ca^{2+}-ATPase (see above); however, the IC_{50} values for the brain enzyme were determined at 15 nM calmodulin (0.9–1.0 V_{max} for phosphodiesterase activation), whereas those for the erythrocyte Ca^{2+}-ATPase were determined at 66 nM calmodulin (0.9–1.0 V_{max} for Ca^{2+}-ATPase activation). Relative IC_{50} values may vary depending upon the concentration of calmodulin used.

DISCUSSION

The present results demonstrate that some local anesthetics and other drugs which act as calcium antagonists (12), such as mepacrine, propranolol, and SKF 525A, inhibit the stimulatory effect of calmodulin on enzymes with little or no effect on the activity of these enzymes in the absence of calmodulin. According to current models for the action of calmodulin, increased concentrations of Ca^{2+} or the modulator protein promote the formation of the Ca^{2+}–calmodulin complex, which in turn shifts the equilibrium for an enzyme from the inactive to the active form (20, 21). Calmodulin appears to be present in cells in excess of the concentration of the enzymes it affects so that cellular calcium fluxes would be most important for regulating enzyme activity. Phenothiazines bind to specific sites on the Ca^{2+}–calmodulin complex with the result that the ternary complex is rendered ineffectual in activating enzymes (36). Similarly, local anesthetics could make the concentration of the modulator protein rather than Ca^{2+} the rate-limiting factor by inactivating calmodulin or by preventing binding of calmodulin to enzymes.

Local anesthetics inhibit the ability of calmodulin to stimulate two different types of enzymes; one an integral membrane protein involved in transport of Ca^{2+}, and the other a soluble enzyme concerned with cyclic nucleotide degradation. The action of the anesthetics is therefore not confined to membrane-dependent reactions.

The local anesthetics prevent binding of calmodulin to the erythrocyte membrane, producing a corresponding inhibition of ATPase activity. However, we have not as yet identified the site, or sites, of action of the local anesthetics as being on calmodulin itself [as has been postulated for phenothiazines (36)], on the calmodulin recognition site of the enzyme, or on some other regulatory site.

We propose that many of the pharmacological actions of local anesthetics, which are directed against calcium-dependent cellular processes, may be due to antagonism of calmodulin's effects on enzymes because both the pharmacological effects and the inhibition of calmodulin *in vitro* occur at similar concentrations of these drugs (1–13). For example, inhibition of Ca^{2+} transport in erythrocytes by local anesthetics (37) may be related to the need for calmodulin in the transport process (38). Similarly, inhibition of smooth muscle contraction (2) and non-muscle cellular motility (10) by local anesthetics could be produced by inhibition of calmodulin-dependent myosin light chain phosphorylation (39) as observed with phenothiazines (40). Also, inhibition of the mobilization of arachidonic acid from phospholipids by local anesthetics (41, 42) may be related to the reported stimulation of phospholipase A_2 by calmodulin (43).

Antagonism of calmodulin could also be responsible for some of the actions of local anesthetics on synaptic transmission. Local anesthetics as well as trifluoperazine (44) depress responses of some chemosensitive postsynaptic membranes. The role of calmodulin in the nervous system is not yet understood but it may play an important role in presynaptic and postsynaptic processes. Wood *et al.* (45) and Lin *et al.* (46) have localized calmodulin by immunocytochemical methods to postsynaptic membranes in mouse and rat brain. Grab *et al.* (47) identified calmodulin in postsynaptic densities isolated from canine cerebral cortex. Calmodulin can activate brain synaptic membrane Ca^{2+}-ATPase (48) and Shulman and Greengard (49) have shown that calmodulin stimulates phosphorylation of brain membranes.

The diverse pharmacological effects of local anesthetics are probably due to more than one mechanism of action. The mechanism by which anesthetics inhibit cell processes that are not dependent on Ca^{2+} may not involve calmodulin. For example, effects on membrane permeability appear to be clearly attributable to interactions with specific ionic channels (50). On the other hand, many of the other cellular actions of local anesthetics directed against Ca^{2+}-dependent processes may be due

to a specific molecular action, the antagonism of enzyme stimulation by calmodulin.

This work was supported by U.S.Public Health Service Grant GM-17536 and grants from the Pharmaceutical Manufacturers Association and the University of Connecticut Research Foundation.

1. Ford, L. E. & Podolsky, R. J. (1972) *J. Physiol.* **223**, 21–33.
2. Feinstein, M. B. & Paimre, M. (1969) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **28**, 1643–1648.
3. Jaanus, S. D., Miele, E. & Rubin, R. P. (1967) *Br. J. Pharmacol.* **31**, 319–330.
4. Bressler, R. & Brendel, K. (1971) *Diabetes* **20**, 721–728.
5. Eichhorn, J. H. & Peterkofsky, B. (1979) *J. Cell Biol.* **81**, 26–42.
6. Kazimierczak, W., Mieczyslaw, P. & Malinski, C. (1976) *Biochem. Pharmacol.* **25**, 1747–1750.
7. Nash-Adler, P., Louis, C. F., Fudejara, G. & Katz, A. M. (1980) *Mol. Pharmacol.* **17**, 61–65.
8. Lacko, L., Wittke, B. & Lacko, I. (1977) *J. Cell. Physiol.* **92**, 257–264.
9. Deuticke, B. (1970) *Naturwissenschaften* **13**, 172–179.
10. Rabinovitch, M. & DeStefano, M. J. (1975) *J. Cell. Physiol.* **85**, 189–194.
11. Feinstein, M. B., Fiekers, J. & Fraser, C. (1976) *J. Pharmacol. Exp. Ther.* **197**, 215–228.
12. Seeman, P. (1972) *Pharmacol. Rev.* **24**, 583–655.
13. Schreiner, G. & Unanue, E. (1976) *Clin. Immunol. Immunopathol.* **6**, 264–269.
14. Voeikov, V. L. & Lefkowitz, R. J. (1980) *Biochim. Biophys. Acta* **629**, 266–281.
15. Roufogalis, B. D. (1973) *Biochim. Biophys. Acta* **318**, 360–370.
16. Suko, J., Winkler, F., Scharinger, B. & Hellmann, G. (1976) *Biochim. Biophys. Acta* **443**, 571–586.
17. Bond, G. H. & Hudgins, P. M. (1976) *Biochem. Pharmacol.* **25**, 267–270.
18. Waite, M. & Sisson, P. (1972) *Biochemistry* **11**, 3098–3105.
19. Low, P. S., Lloyd, D. H., Stein, T. M. & Rogers, J. A. (1979) *J. Biol. Chem.* **254**, 4119–4125.
20. Cheung, W. Y. (1979) *Science* **207**, 19–27.
21. Klee, C. B., Crouch, T. B. & Richman, P. G. (1980) *Annu. Rev. Biochem.* **49**, 489–515.
22. Volpi, M., Sha'afi, R. I. & Feinstein, M. B. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 958.
23. Epstein, P. M., Andrenyak, D. M. & Feinstein, M. B. (1980) *Adv. Cyclic Nucleotide Res.* **14**, in press.
24. Gopinath, R. M. & Vincenzi, F. F. (1977) *Biochem. Biophys. Res. Commun.* **77**, 1203–1209.
25. Seals, J. R., McDonald, J. M., Bruns, D. & Jarett, L. (1978) *Anal. Biochem.* **90**, 785–795.
26. Jarrett, H. W. & Penniston, J. T. (1978) *J. Biol. Chem.* **253**, 4676–4682.
27. Davis, B. J. (1964) *Ann. N.Y. Acad. Sci.* **121**, 404–427.
28. Chafouleas, J. G., Dedman, J. R., Munjaal, R. P. & Means, A. R. (1979) *J. Biol. Chem.* **254**, 10262–10267.
29. Thompson, W. J., Epstein, P. M. & Strada, S. J. (1979) *Biochemistry* **18**, 5228–5237.
30. Thompson, W. J., Terasaki, W. L., Epstein, P. M. & Strada, S. J. (1979) *Adv. Cyclic Nucleotide Res.* **10**, 69–92.
31. Katz, A. M., Repke, D. L., Upshoux, J. E. & Polascik, M. A. (1970) *Biochim. Biophys. Acta* **205**, 473–490.
32. Udenfriend, S., Stein, S., Bohlen, P., Dairman, W., Leimgruber, W. & Weighe, M. (1972) *Science* **178**, 871–873.
33. Jarrett, H. W. & Kyte, J. (1979) *J. Biol. Chem.* **254**, 8237–8244.
34. Teo, T. S. & Wang, J. H. (1973) *J. Biol. Chem.* **248**, 5952–5955.
35. Luthra, M. G. & Kim, H. D. (1980) *Biochim. Biophys. Acta* **600**, 467–479.
36. Weiss, B. & Levin, R. M. (1978) *Adv. Cyclic Nucleotide Res.* **9**, 285–303.
37. Szasz, I., Sarkadi, B. & Gardos, G. (1977) *J. Membr. Biol.* **35**, 75–93.
38. Larsen, F. L. & Vincenzi, F. F. (1979) *Science* **204**, 306–308.
39. Haslam, R. J. & Lynham, J. A. (1978) *Thrombosis Res.* **12**, 619–628.
40. Kerrick, W. G. L., Hoar, P. E. & Cassidy, P. S. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 1558–1563.
41. Vanderhoek, J. & Feinstein, M. B. (1979) *Mol. Pharmcol.* **16**, 171–180.
42. Vargaftig, B. B. & Dao, Hai, N. (1972) *J. Pharm. Pharmacol.* **24**, 159–161.
43. Wong, P. Y. K. & Cheung, W. Y. (1979) *Biochem. Biophys. Res. Commun.* **90**, 473–480.
44. Cheng, K., Lambert, J. J., Henderson, E. G., Smilowitz, H. & Epstein, P. M. (1981) *J. Pharmacol. Exp. Ther.*, in press.
45. Wood, J. F., Wallace, R. W., Whitaker, J. N. & Cheung, W. Y. (1980) *J. Cell Biol.* **84**, 66–76.
46. Lin, C.-T., Dedman, J. R., Brinkley, B. R. & Means, A. R. (1980) *J. Cell Biol.* **85**, 473–480.
47. Grab, D. J., Berzins, K., Cohen, R. S. & Siekevitz, P. (1979) *J. Biol. Chem.* **254**, 8690–8696.
48. Sobue, K., Ichida, S., Yoshida, H., Yamazaki, R. & Kakiuchi, S. (1979) *FEBS Lett.* **99**, 199–202.
49. Schulman, H. & Greengard, P. (1978) *Nature (London)* **271**, 478–479.
50. Hille, B. (1979) *J. Gen. Physiol.* **69**, 497–515.