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INTERACTION OF LOCAL ANESTHETICS WITH CALMODULIN

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SUMMARY

Dibucaine, tetracaine and lidocaine inhibited selectively Ca^{2+}-calmodulin-induced activation of cyclic nucleotide phosphodiesterase with IC_{50} values of 0.19, 0.44 and 4.80 mM, respectively and were less potent inhibitors in the absence of Ca^{2+}. These drugs also inhibited other Ca^{2+}-calmodulin-dependent enzyme such as myosin light chain kinase from chicken gizzard. [^{3}H]W-7 bound to purified calmodulin was displaced in a concentration-dependent manner by these compounds. Their 50% inhibitory concentrations were linearly related to their octanol-water partition coefficients, implying hydrophobic interaction between these drugs and calmodulin.

INTRODUCTION

Local anesthetics reportedly modify an extraordinary variety of non-neuronal processes. Examples of such processes inhibited by local anesthetics include exocytosis (1), platelet aggregation (2), membrane transport of calcium (3), cell spreading and motility (4-6), and others (7). Moreover, a clear correlation between the inhibitory activity and octanol-water partition coefficients was reported in the case of these compounds (7). Despite the disparate nature of the above phenomena, all have in common the necessity of calcium. The ubiquitous calcium-binding protein, calmodulin, has been shown to play a role in the modulation of a wide variety of Ca^{2+}-dependently cellular functions. It has recently been demonstrated that the calcium-induced exposure of hydrophobic regions of calmodulin may be important for the activation of Ca^{2+}-calmodulin-dependent enzymes (8, 9).

We now report the effect of local anesthetics on Ca^{2+}-calmodulin dependent enzyme activities and their binding to the Ca^{2+}-calmodulin complex. The

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The abbreviation used is: W-7, N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide

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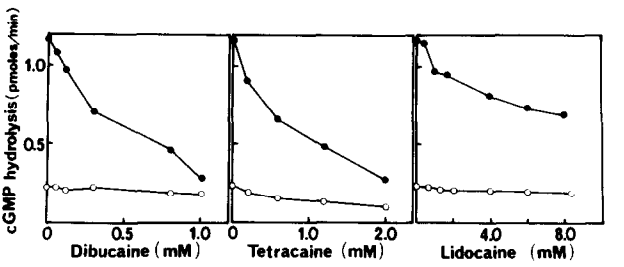

Fig. 1. Inhibition of calcium-dependent phosphodiesterase by local anesthetics. Phosphodiesterase activity was measured in the absence of calmodulin (—○—) and in the presence of 0.03 μM calmodulin (—●—) and varying concentrations of local anesthetics. Each point is the mean of duplicate determinations.

affinity for calmodulin is related to the hydrophobicity of the local anesthetics.

MATERIALS AND METHODS

Protein preparation: Calmodulin-deficient Ca^{2+}-dependent cyclic nucleotide phosphodiesterase from bovine heart was purified to apparent homogeneity, as described by Laporte et al. (10). Phosphodiesterase activity was measured by the method previously reported (11). Bovine brain calmodulin was purified to homogeneity, as described previously (12). Calmodulin deficient myosin light chain kinase and the 20,000-dalton myosin light chain from chicken gizzard were purified according to the method by Adelstein et al. (13) and assays were performed as previously reported (14).

Binding studies: The displacement of [^{3}H]N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) from purified calmodulin in the presence of calcium ion by local anesthetics was investigated by the equilibrium binding technique of Hummel and Dreyer (15) on a Sephadex G-50 gel filtration column, as previously reported (16).

Chemicals: N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) was synthesized by the method of Hidaka et al. (17). The donated drug samples included; dibucaine hydrochloride, Teikoku Chemical Industry; tetracaine hydrochloride, Kyorin Pharmaceutical Co.; lidocaine hydrochloride, Fujisawa Pharmaceutical Co., respectively.

RESULTS

Figure 1 demonstrated the selective inhibition of the activation of phosphodiesterase by dibucaine, tetracaine and lidocaine, respectively. Increasing the concentration of local anesthetics progressively inhibited the activation of phosphodiesterase. The concentrations of dibucaine, tetracaine and lidocaine which inhibited the activation of phosphodiesterase by 50% [IC_{50} (activated)] were 0.19, 0.44 and 4.80 mM, respectively (Table 1).

TABLE 1

Effect of local anesthetics on calmodulin-induced activation of phosphodiesterase, myosin light chain kinase and [^{3}H]W-7 binding to purified calmodulin.

	concentration (IC_{50}, mM) of local anesthetics producing 50% inhibition			
	phosphodiesterase[a]		myosin light chain kinase[b]	displacement of [^{3}H]W-7 from calmodulin[c]
drugs	unactivated	activated		
Dibucaine	5.4	0.19	0.19	0.22
Tetracaine	1.5	0.44	1.01	0.92
Lidocaine	>10.0	4.80	6.80	5.80

a; Phosphodiesterase activity of a preparation purified from bovine heart was measured in the presence and absence of 0.03 μM calmodulin and various concentrations of the local anesthetics, using 0.4 μM cyclic GMP as substrate (11).

b; Myosin light chain kinase activity was measured as previously described (14).

c; The displacement of [^{3}H]W-7 from purified calmodulin in the presence of calcium ion by local anesthetics was investigated, as described in "Materials and Methods".

The IC_{50} value is defined as the concentration of the drug required to produce a 50% inhibition of each enzyme activity and labeled W-7 binding to purified calmodulin. These values were determined graphically and all experiments run in triplicate.

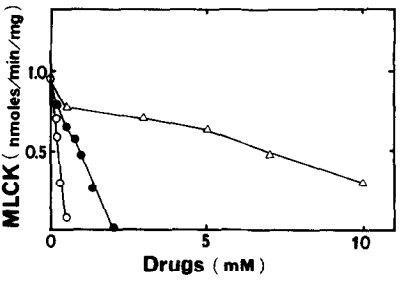

Fig. 2. Inhibition of myosin light chain kinase by local anesthetics. Myosin kinase was assayed in the presence of 0.1 μM calmodulin and varying concentrations of local anesthetics (—○— , dibucaine; —●— , tetracaine; —△— , lidocaine).

In the concentration range examined, dibucaine and lidocaine did not inhibit the activity of phosphodiesterase in the absence of Ca^{2+}-calmodulin (Table 1). Moreover, these compounds produced a concentration-dependent inhibition of myosin light chain kinase from chicken gizzard, as shown in Fig. 2. The IC_{50} values for dibucaine, tetracaine and lidocaine were 0.19, 1.01 and 6.80 mM, respectively (Table 1).

As shown in Fig. 3(A), the [^{3}H]W-7 binding to calmodulin inhibited by dibucaine, in a dose-dependent manner. Similar results were obtained with tetracaine or lidocaine, as shown in Table 1. These agents inhibited [^{3}H]W-7 binding to purified calmodulin at concentrations similar to those which produced inhibition of two kinds of soluble Ca^{2+}-calmodulin dependent enzymes (Table 1) and numerous biological processes (7). As shown in Fig. 3(B), the stoichiometry of interaction between dibucaine and [^{3}H]W-7-calmodulin complex and the inhibitory constant of this agent for the W-7-calmodulin complex were determined from a Dixon plot (18). Dibucaine inhibited the binding of W-7 to calmodulin competitively and the Ki value of this compound against the binding of W-7 was 80 μM. These results suggest the W-7 binding sites of calmodulin are also responsible for binding of dibucaine.

The octanol-water partition coefficient is considered to be an index of hydrophobicity for a molecule (7) and the partition coefficients of these drugs correlated well with IC_{50} values obtained for calmodulin inhibition.

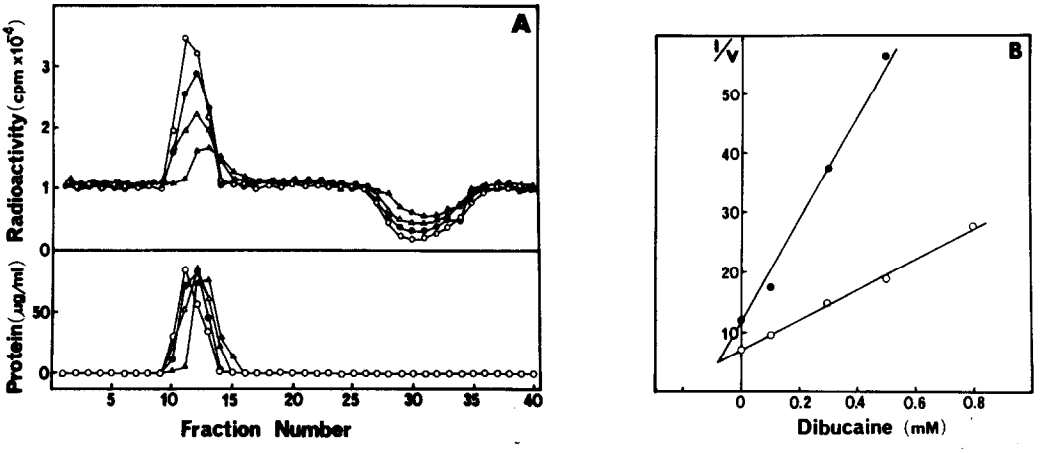

Fig. 3. Inhibition of [^{3}H]W-7 binding to purified calmodulin by dibucaine. (A) Elution profile for measurement of W-7 binding to calmodulin. Sephadex G-50 (0.9 X 27.0 cm) was preequilibrated with buffer containing 20 mM Tris-HCl, pH=7.5, 20 mM imidazole, 3 mM magnesium acetate, 0.5 μM [^{3}H]W-7, 100 μM $CaCl_2$ and various concentrations of dibucaine (—○— , none; —●— , 0.1 mM; —△— , 0.5 mM; —▲— , 10 mM). Purified bovine brain calmodulin (270 μg) was used for each experiment. (B) Kinetic analysis of dibucaine-induced inhibition of W-7 binding to calmodulin. The binding of [^{3}H]W-7 (—●— , 0.25 μM; —○— , 0.70 μM) to calmodulin was measured in the absence or presence of various concentrations of dibucaine. Velocity is expressed as moles of W-7 bound per mole of calmodulin.

Log P was obtained from references (7, 19). Calculation was made by the method of least squares for local anesthetics and chlorpromazine and correlation coefficients ranged between 0.97 and 0.98 (Fig. 4).

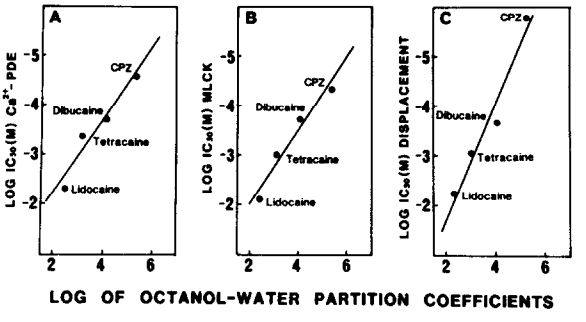

Fig. 4. Correlation between inhibition of calmodulin-dependent enzymes and [^{3}H]W-7 binding to calmodulin by the local anesthetics and by chlorpromazine and the octanol-water partition coefficients for these drugs. Ordinate: The logarithm of the IC_{50} values for inhibition of Ca^{2+}-dependent phosphodiesterase (A), myosin light chain kinase (B), or [^{3}H]W-7 binding to calmodulin (C). Abscissa: The logarithm of the partition coefficients for lidocaine, tetracaine, dibucaine and chlorpromazine (log P).

DISCUSSION

The results indicate local anesthetics interact with the Ca^{2+}-calmodulin complex and selectively inhibit Ca^{2+}-calmodulin dependent enzyme activities. Furthermore, as shown in Fig. 4, there is a significant correlation between affinity for calmodulin and the octanol-water partition coefficients of local anesthetics and one tranquilizer. Using several hydrophobic probes, it has been recently reported that when Ca^{2+} binds to the high affinity sites of calmodulin, there is a conformational change which exposes the hydrophobic regions (8, 9). These hydrophobic regions of Ca^{2+}-calmodulin complex have been demonstrated to be responsible, to some extent, for the Ca^{2+}-dependent function of calmodulin (8, 9, 20). Moreover, N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7), which binds selectively to calmodulin with a dissociation constant of 11 μM and inhibits calmodulin-dependent enzymes (16), has been reported to interact with the hydrophobic regions of calmodulin (8. 20).

Our results suggest that the interactions between local anesthetics and the Ca^{2+}-calmodulin complex may be part of the pharmacological mechanism of action of anesthetics in various biological systems and that their potency as calmodulin antagonists may relate to their hydrophobicity.

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