Mechanism of Inhibition of the Plasma Membrane Ca^{2+}−ATPase by Barbiturates†

Danuta Kosk-Kosicka,* Ioulia Fomitcheva, and Maria M. Lopez

Department of Anesthesiology/CCM, The Johns Hopkins University School of Medicine, Baltimore, Maryland

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ABSTRACT: We have demonstrated that sodium pentobarbital inhibited the activation of the human red blood cell plasma membrane Ca^{2+}−ATPase produced by dimerization of enzyme monomers or by calmodulin binding to enzyme monomers. The effects of the barbiturate were dose-dependent. Both V_{max} and Ca^{2+} affinity were reduced. The Ca^{2+}−ATPase activity of the dimeric enzyme was distinctly less sensitive with respect to the effective inhibitory concentrations of pentobarbital and to the rate of onset of inhibition than was the calmodulin-dependent activation of enzyme monomers. Temperature dependence of the inhibition was in agreement with direct, nonpolar interactions of pentobarbital with a water-exposed nonpolar patch on the surface of this transmembrane protein. The barbiturate prevented the increase of intrinsic tryptophan fluorescence associated with substrate Ca^{2+} binding to the enzyme dimer. On the basis of the barbiturate effects we propose a model for the action of detergent-like compounds on the enzyme. They inhibit Ca^{2+}−ATPase activity by binding to a nonpolar patch on the water-exposed dimerization surface of the enzyme monomer, part of which is also the binding site for calmodulin. The model assumes that their binding to the nonpolar patch on the monomer interferes with dimerization and weakens but does not prohibit calmodulin binding, whose activation of the enzyme is then submaximal. The model should be applicable to other proteins as the two activation pathways studied have been demonstrated for various enzymes.

The Ca^{2+}−ATPase of the human red blood cell plasma membrane is solely responsible for Ca^{2+} traffic across the membrane and the maintenance of the steep Ca^{2+} concentration gradient. The absence of voltage-sensitive and ligand-gated Ca^{2+} channels, the Na^+/Ca^{2+} exchanger, and the intracellular membrane Ca^{2+}−ATPase that contribute substantially to the maintenance of Ca^{2+} homeostasis in other cells makes the red cell enzyme a favorable model for investigation of the mechanism of activation and regulation of the plasma membrane Ca^{2+}−ATPase. The events are affected by changes in solute environment of the enzyme. In blood the red cells are exposed to pharmacological agents of various chemical properties. The potential importance of anesthetic effects on the Ca^{2+}−ATPase and ultimately on the intracellular Ca^{2+} homeostasis is receiving increasing attention.

The two modes of activation of the Ca^{2+}−ATPase which we have examined in this work are by calmodulin binding to enzyme monomers and by the calmodulin-independent self-association of monomers to dimers (Kosk-Kosicka & Bzdega, 1988; Kosk-Kosicka et al., 1989, 1990b; Sackett & Kosk-Kosicka, 1993; D. Sackett and D. Kosk-Kosicka, submitted). Both calmodulin binding and dimerization are important activation modes that have been demonstrated for various proteins. For example, activation by dimerization of integral membrane proteins which subsequently undergo phosphorylation on their cytoplasmic surface is also a key event in transmembrane signalling (Spivak-Kroizman et al., 1994). Calmodulin binding that results in the removal of endogenous inhibition is common to calmodulin-modulated enzymes such as protein kinases or myosin light chain kinase (Kemp & Pearson, 1991).

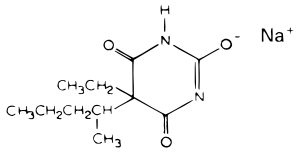

FIGURE 1: Structure of sodium pentobarbital.

We have earlier used the integral membrane protein Ca^{2+}−ATPase as a model target of volatile anesthetics (Kosk-Kosicka & Roszczynska, 1993; Kosk-Kosicka, 1994); integral membrane proteins are plausible *in vivo* pharmacological targets of general anesthetics. We have demonstrated that volatile anesthetics at their clinical concentrations inhibited the normal process of enzyme activation by calmodulin binding or by dimerization with a similar I_{50}. Our findings suggested that the Ca^{2+}−ATPase was a good model target and could also be a functional *in vivo* target for this group of general anesthetics.

For the present study we have selected barbiturates which are widely used to induce general anesthesia, both in humans and in laboratory animals. To study the mechanism of their action we have employed two experimental approaches. First, we measured Ca^{2+}−ATPase activity, and second, we made fluorescence spectroscopy measurements of the Ca^{2+}-dependent increase in the intrinsic tryptophan fluorescence intensity that reflects a conformational change which the enzyme undergoes upon binding the substrate Ca^{2+} in the initial step of its catalytic cycle. We demonstrate that sodium pentobarbital (Figure 1) inhibits both measures in a dose-dependent manner: there is a strong correlation between the attenuation of the Ca^{2+}-dependent conformational change and the inhibition of Ca^{2+}−ATPase activity. Enzyme activation

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* To whom the correspondence should be addressed: Dept. of Anesthesiology/CCM, The Johns Hopkins University, 600 N. Wolfe St., Blalock 1404, Baltimore, MD 21287.

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by dimerization is distinctly less sensitive than enzyme activation by calmodulin binding.

We have characterized the actions of sodium pentobarbital on the two activation pathways of the Ca^{2+}−ATPase in detail. Comparison with the effects exerted by other compounds including volatile anesthetics, alkanols, and diverse organic solutes that we have described previously indicates that the sites of action of volatile anesthetics and barbiturates are separate. We have proposed a model in which sodium pentobarbital and other compounds with structural properties of detergents inhibit the enzyme's function by binding to a nonpolar patch on its water-exposed surface. The model should be useful in analyzing the effects of such compounds on various proteins.

MATERIALS AND METHODS

Egg yolk phosphatidylcholine (P5763) and CNBr-activated Sepharose 4B were purchased from Sigma; octaethylene glycol mono-*n*-dodecyl ether ($C_{12}E_8$) was obtained from Nikko (Tokyo, Japan). Coupling of bovine calmodulin to Sepharose was performed in accordance with Pharmacia LKB Biotechnology instructions as described earlier (Kosk-Kosicka & Bzdega, 1988). Sodium pentobarbital [pK_a = 7.96 (Firestone et al., 1986)] and thiopental were purchased from Anpro Pharmaceutical.

The methods used for enzyme purification, determination of protein concentration, and Ca^{2+} concentration were as described previously (Kosk-Kosicka et al., 1986; Kosk-Kosicka & Bzdega, 1988). Briefly, the enzyme was purified from erythrocyte membrane ghosts by calmodulin affinity column chromatography in the presence of the nonionic detergent $C_{12}E_8$. Protein concentration was measured by Bio-Rad protein microassay. Total calcium was measured by atomic absorption, and free Ca^{2+} concentrations were calculated based on the constants given by Schwartzenbach et al. (1957).

Ca^{2+}−ATPase Activity Assay. Ca^{2+}−ATPase activity was determined by measurements of inorganic phosphate production, generally as described previously (Kosk-Kosicka & Bzdega, 1988). The assay was performed in a reaction mixture containing 50 mM Tris maleate, pH 7.4, 80 or 120 mM KCl, 8 mM $MgCl_2$, 3 mM ATP, 1 mM EGTA, and $CaCl_2$ in concentrations yielding the required free Ca^{2+}.

The concentration of $C_{12}E_8$ was kept constant at 150 μM. The calmodulin-independent activity of dimers was determined in the presence of 120 mM KCl and 17.5 μM free calcium at 30 or 70 nM enzyme concentration (for comparison to fluorescence spectroscopy measurements), as indicated in figure legends. Specific activity was the same for the enzyme at the two concentrations as maximal dimerization and activation occurred at around 30 nM enzyme (Kosk-Kosicka et al., 1989). The effect of barbiturate did not depend on protein concentration. The calmodulin-dependent activation of monomers was determined in the presence of 160 nM calmodulin and 80 mM KCl at 15 nM enzyme concentration. It was determined that at 30 nM enzyme and low KCl and $CaCl_2$ concentrations the effect of barbiturate on the calmodulin-dependent activity was the same as at 15 nM, thus the effect of barbiturate did not depend on protein concentration within the range used. The reaction volume was 100 μL. The enzyme was added after the barbiturate and immediately followed with 3 mM ATP; the reaction was carried out for up to 30 min at either 37 or 25 °C, as specified in figure legends. Aliquots were withdrawn at various times for colorimetric inorganic phosphate measurement. Steady-state velocities were obtained from plots of inorganic phosphate production which were linear with time.

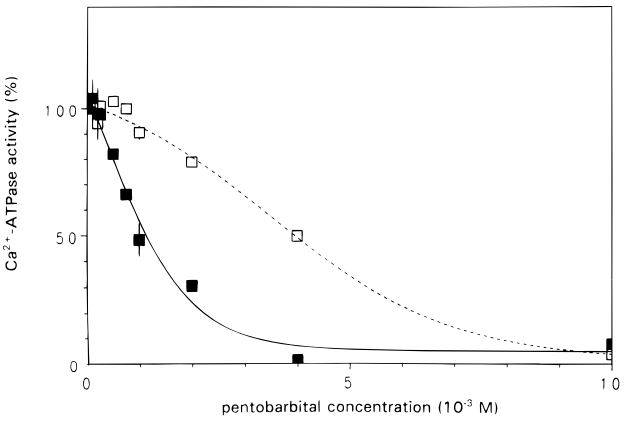

FIGURE 2: Concentration dependence of the inhibitory effect of pentobarbital on the Ca^{2+}−ATPase activity of dimers (□) and calmodulin-activated monomers (■). The activity assays were performed as described in Materials and Methods. The reaction mixture contained 50 mM Tris maleate, pH 7.4, 80 or 120 mM KCl, 8 mM $MgCl_2$, 100 nM or 17.5 μM free Ca^{2+}, 150 μM $C_{12}E_8$, 1 mM EGTA, and 3 mM ATP. The enzyme concentration was 30 nM for dimers and 15 nM for monomers. The calmodulin-dependent activity was calculated as a difference between Ca^{2+}−ATPase activity of the enzyme in the presence and absence of calmodulin. The specific activities (100%) were 100 μmol of P_i/mg of protein/h for the calmodulin-dependent Ca^{2+}−ATPase monomers and 450 ± 40 μmol of P_i/mg of protein/h for the Ca^{2+}−ATPase dimers. The assays were performed at 37 °C.

Fluorescence Measurements. The total tryptophan fluorescence intensity of the Ca^{2+}−ATPase and the change induced by Ca^{2+} binding were measured at equilibrium using a Fluoromax spectrofluorimeter with DM3000F software. Tryptophan fluorescence was excited at 290 nm, and the emission was recorded at 330 nm. The reaction mixture contained 100 mM Tris-HCl, pH 7.4, 0.15 mM $C_{12}E_8$, 120 mM KCl, 8 mM $MgCl_2$, and 1 mM EGTA. Free Ca^{2+} was 17.5 μM, and protein concentration was 70 nM. The measurements were performed at 25 °C in the total volume of 1.1 mL. The solution was gently stirred during the experiment, which lasted 4−5 min. It was determined that the stirring did not perturb the fluorescence signal. Corrections for the dilution effect were made when necessary.

RESULTS

Comparison of Barbiturate Effects on Dimers and Calmodulin-Activated Monomers of the Ca^{2+}−ATPase (at 37 °C). Two investigated barbiturates, sodium pentobarbital and thiopental, reduce calmodulin-dependent Ca^{2+}−ATPase activity in a comparable dose-dependent manner. Pentobarbital has been selected for subsequent detailed studies under various specific experimental conditions. Figure 2 compares the effects of pentobarbital on the Ca^{2+}−ATPase activity of dimers and of calmodulin-activated monomers. The two forms of the Ca^{2+}−ATPase exhibit different sensitivity to the barbiturate: half-maximal inhibition is observed at 3.5 ± 0.4 mM barbiturate for enzyme dimers and at 1.1 ± 0.2 mM for calmodulin-activated monomers.

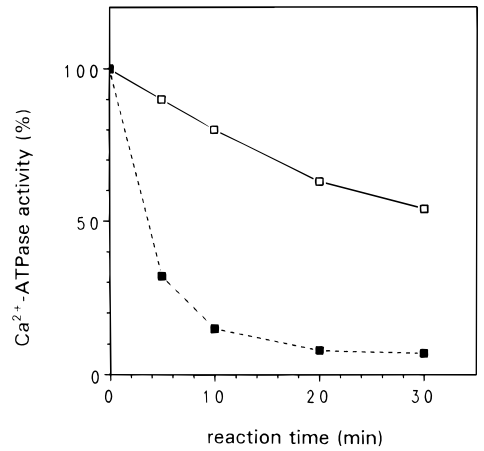

FIGURE 3: Time dependence of the inhibitory effect of pentobarbital on the Ca^{2+}−ATPase activity of dimers (□) and calmodulin-dependent activation of monomers (■). The activity assay was performed as described in Figure 2. Pentobarbital concentration was 4 mM.

Figure 3 shows the time dependence for onset of enzyme inhibition by 4 mM barbiturate. Development of inhibition of the calmodulin-activated monomers is significantly faster than that of enzyme dimers. A fifty-percent reduction of calmodulin-dependent Ca^{2+}−ATPase activity occurs at 3 min and is completed at around 10 min. Dimers are half-maximally inhibited at 25−30 min; the slow onset does not allow us to establish the concentration at which full inhibition occurs.

Calmodulin-Dependent Activation of Monomers. In the presence of pentobarbital the apparent Ca^{2+} affinity of the Ca^{2+}−ATPase activity is shifted toward higher values. At 1 mM barbiturate (*i.e.*, at a concentration that half-maximally inhibits the activation) the $K_{1/2}$ of the calmodulin-dependent activation increases from ∼220 to ∼400 nM free Ca^{2+} (from 6.65 ± 0.05 to 6.40 ± 0.03 pCa) (Figure 4 A). A similar effect was observed for the Ca^{2+}−ATPase activity of dimers (shift from 7.10 ± 0.06 to 6.90 ± 0.05). The effect is most pronounced at the low Ca^{2+} concentrations at which the enzyme is expected to operate under physiological conditions. In addition to increasing the calcium requirement for maximal stimulation by calmodulin, pentobarbital also decreases the extent of the maximal stimulation, from 5.5-fold (at ∼200 nM free Ca^{2+}) down to 3.3-fold (at ∼400 nM free Ca^{2+}) (Figure 4 B).

Examination of the dependence of enzyme activation on calmodulin concentration shows that it is significantly altered by pentobarbital (Figure 5). The effect is on both V_{max} (which decreases 2-fold) and K_{CaM} (which increases from 40 ± 9 nM total calmodulin to 72 ± 4), suggesting noncompetitive inhibition of calmodulin binding to the enzyme by the barbiturate.

Dimeric Enzyme. The effect of pentobarbital on the calcium-dependent conformational change of the dimeric enzyme has been assessed by measurements of the Ca^{2+}-dependent increase in tryptophan fluorescence intensity. The fluorescence titration of the plasma membrane Ca^{2+}−ATPase was previously shown to be indicative of substrate Ca^{2+} binding to specific sites involved in enzyme catalysis in analogy to the sarcoplasmic reticulum Ca^{2+}−ATPase, for which the method was originally developed (Kosk-Kosicka & Inesi, 1985; Inesi et al., 1980; Dupont, 1976). As shown in Figure 6A the Ca^{2+}-dependent fluorescence increase is

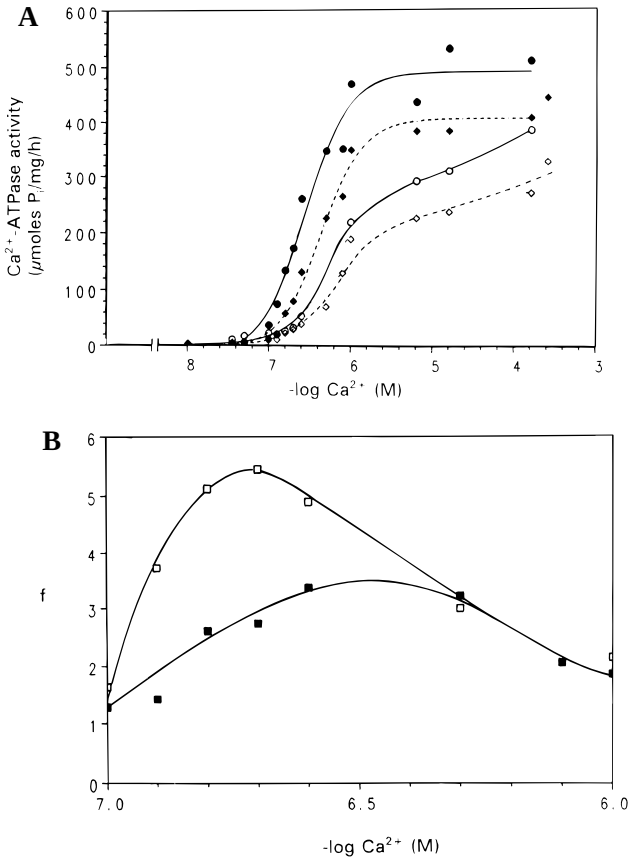

FIGURE 4: (A) Ca^{2+}-dependence of the Ca^{2+}−ATPase activity of monomers in the presence (filled symbols) and absence (open symbols) of calmodulin was measured at up to 200 μM free Ca^{2+}. Diamonds (◇, ◆) are for measurements in the presence of pentobarbital. (B) Calcium-dependence of the calmodulin-dependent activation of Ca^{2+}−ATPase monomers in the presence (■) and absence (□) of 1 mM pentobarbital. The effect of pentobarbital on calmodulin ability to stimulate the enzyme is expressed as an activation factor (*f*), $f = V_c/V_o$, where V_c is the maximal activity in the presence of CaM and V_o is the maximal activity in the absence of CaM. The activities were assayed as described in Figure 2.

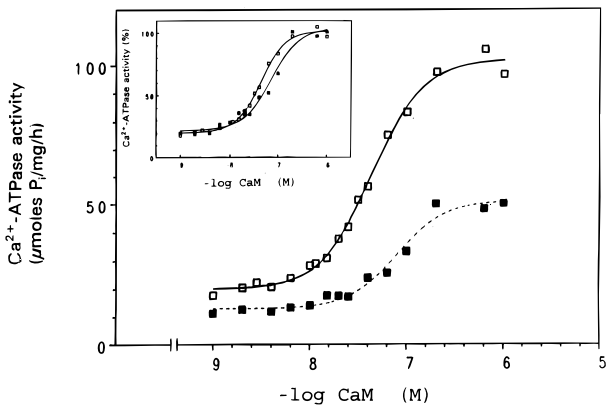

FIGURE 5: Calmodulin dependence of the Ca^{2+}−ATPase activity in the presence (■) and absence (□) of pentobarbital. Experimental conditions were as described in Figure 2. Inset shows the normalized data, where 100% is the maximal activity either with (■) or without (□) 1 mM pentobarbital.

reduced by pentobarbital in a dose-dependent manner with a half-maximal effect at ∼5 mM concentration. No Ca^{2+}-dependent increase in fluorescence intensity is observed at ≥7 mM pentobarbital. The Ca^{2+}-induced fluorescence increase is readily reversed by addition of EGTA both in the presence and in the absence of pentobarbital.

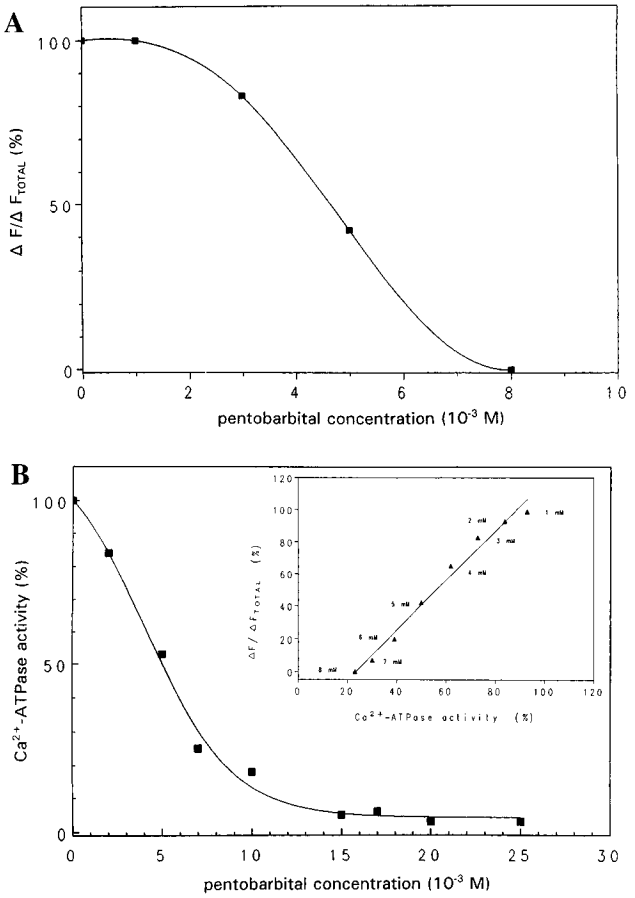

FIGURE 6: Concentration dependence of the suppressing effect of pentobarbital on the Ca^{2+}-dependent increase in the intrinsic fluorescence (A) and Ca^{2+}−ATPase activity (B) of dimers. Ca^{2+}−ATPase activity assay was performed as described under Material and Methods and in Figure 2, except the assay temperature was 25 °C. The fluorescence measurements were performed at 25 °C as described under Material and Methods. Enzyme concentration was 70 nM. Fluorescence increase was induced upon addition of 1.2 μM free Ca^{2+}. The Ca^{2+}-dependent change in tryptophan fluorescence was 1.2%−2% of the total tryptophan fluorescence. The increase was reversible upon addition of EGTA. The specific activity (100% activity) was 220 ± 9 μmol of P_i/mg of protein/h. Inset: Correlation between barbiturate effect on Ca^{2+}−ATPase activity and Ca^{2+}-dependent fluorescence change.

To assess whether the conformational change in the enzyme revealed by attenuation of tryptophan fluorescence intensity is related to the inhibition of its activity, we have compared the concentration dependence of the pentobarbital effect on both fluorescence and Ca^{2+}−ATPase activity at the same temperature, *i.e.*, 25 °C (Figure 6 A,B). The inhibition of activity at 25 °C is dose-dependent, similar to the inhibition at 37 °C (compare Figures 6B and 2), except significantly higher barbiturate concentrations are required (I_{50} = 5 mM). There is a linear correlation between the effect of pentobarbital on the Ca^{2+}-induced conformational change and the Ca^{2+}−ATPase activity (Figure 6B, inset).

Effect of Pentobarbital on the Ca^{2+}−ATPase in Red Cell Membranes. Figure 7 shows the effects of pentobarbital on ATPase activities in red cell membrane ghosts. In addition to the calmodulin-independent and calmodulin-dependent Ca^{2+}−ATPase (both of which are Mg^{2+}-dependent) investigated in a purified enzyme, a Ca^{2+}-independent Mg^{2+}-ATPase activity also needs to be considered in the membranous preparation. The membranous enzyme shows greater sensitivity of the calmodulin-dependent than the

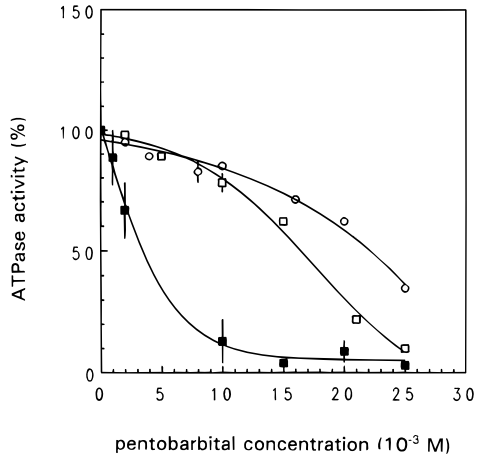

FIGURE 7: Concentration dependence of the inhibitory effect of pentobarbital on the calmodulin-dependent Ca^{2+}−ATPase activity (■), calmodulin-independent Ca^{2+}−ATPase (□), and Mg^{2+}-ATPase activity in erythrocyte ghost membranes. The activity assays were performed as described in Material and Methods. The reaction mixture contained 50 mM Tris maleate, pH 7.4, 120 mM KCl, 8 mM $MgCl_2$, and 1 mM EGTA for determination of the Ca^{2+}-independent Mg^{2+}-ATPase activity. The Ca^{2+}−ATPase activities were assayed at 17.5 μM free Ca^{2+}, and the Mg^{2+}-ATPase activity was subtracted. Protein concentration was 160 μg/mL and calmodulin when present was at 10 μg/mL. The calmodulin-dependent activity was calculated as the difference between Ca^{2+}−ATPase activity in the presence and absence of calmodulin. The specific activities (100% activity) were 0.3 μmol of P_i/mg of protein/h for the Mg^{2+}-ATPase, 0.6 μmol of P_i/mg of protein/h for the calmodulin-independent Ca^{2+}−ATPase, and 1.5 μmol of P_i/mg of protein/h for the calmodulin-dependent Ca^{2+}−ATPase activity.

calmodulin-independent Ca^{2+}−ATPase activity to pentobarbital, which is similar to the purified enzyme. The I_{50} values are 5 and 15 mM pentobarbital, respectively. The Mg^{2+}-ATPase is not involved in Ca^{2+} transport and requires ~22 mM barbiturate for half-maximal inhibition.

DISCUSSION

The effect of sodium pentobarbital on the Ca^{2+}−ATPase activity was investigated utilizing two activation pathways: calmodulin binding to monomers (calmodulin-dependent activation) and self-association of inactive monomers to fully active dimers (calmodulin-independent activation) characterized in detail previously (Kosk-Kosicka & Bzdega, 1988, 1990, 1991; Kosk-Kosicka et al., 1989, 1990b, 1992a; Bzdega & Kosk-Kosicka, 1992; Persechini et al., 1993; Sackett & Kosk-Kosicka, 1993; D. Sackett and D. Kosk-Kosicka, submitted). On the basis of our previous findings and the published data of others, we suggest the following simple model to describe the action of sodium pentobarbital and other weakly hydrated compounds on the Ca^{2+}−ATPase (Figure 8). The dark patch on the monomer symbolizes a nonpolar "recognition" region for calmodulin or for a second monomer (Figure 8). Binding of either calmodulin or another monomer activates the enzyme by inducing the proper conformational change.

It has been proposed that the calmodulin binding domain in the inactive enzyme obstructs the active site and its removal by either calmodulin binding or proteolytic digestion is a prerequisite for activation in analogy to other calmodulin-regulated enzymes (Falchetto et al., 1992; Enyedi et al., 1980; Klee, 1980). In the model this is indicated by the alteration in the position of the dark patch upon calmodulin binding.

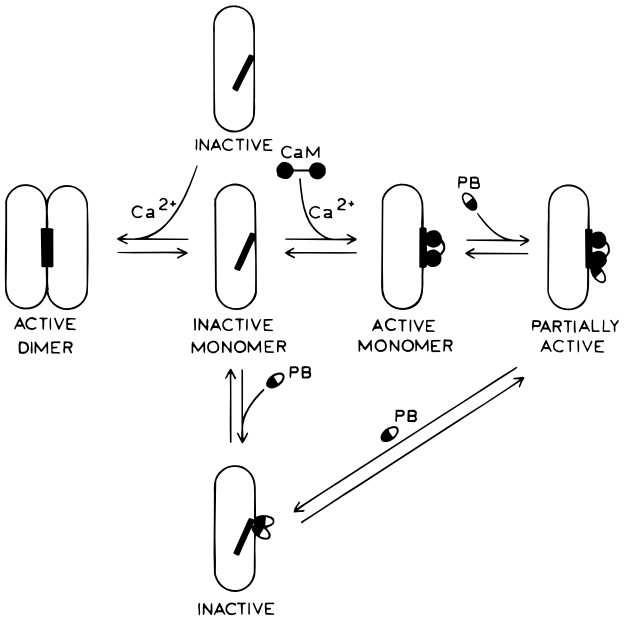

FIGURE 8: A model of monomer activation (by either calmodulin binding or dimerization) and inhibition of the activation by pentobarbital. The dark patch symbolizes a nonpolar recognition site to which either calmodulin (CaM) or another monomer binds in the presence of Ca^{2+} and which results in the activation of the enzyme. In this model, pentobarbital (PB) binds only to monomers both in the presence and absence of calmodulin.

The interaction of calmodulin with different proteins has been studied extensively, and it has been established that both nonpolar and electrostatic interactions are important (Ikura et al., 1992; Meador et al., 1993; Johnson & Miles, 1986). The nonpolar surfaces of calmodulin that become exposed upon binding of Ca^{2+} form a nonpolar channel in which a target peptide fits, as has been recently demonstrated by multidimensional heteronuclear magnetic spectroscopy for a 26-residue synthetic peptide from skeletal muscle myosin light chain kinase which binds to calmodulin (Ikura et al., 1992). Upon complexation there is a decrease in the accessible nonpolar surface area of calmodulin as well as of the target, which accounts for the very tight binding (Ikura et al., 1992). The similarity of the calmodulin binding domain of the Ca^{2+}−ATPase to the studied synthetic peptide and especially the presence of nonpolar amino acids in the Ca^{2+}−ATPase that could interact with nonpolar regions of calmodulin suggests a similar manner of interaction (Ikura et al., 1992; Vorherr et al., 1992). Thus, calmodulin binding to Ca^{2+}−ATPase monomers is expected to induce conformational changes in calmodulin which alter its Ca^{2+} affinity and to induce conformational changes in the Ca^{2+}−ATPase which result in its activation, as discussed previously (Kosk-Kosicka & Bzdega, 1988; Kosk-Kosicka et al., 1990a,b). By analogy we expect that dimerization causes similar conformational changes that result in increased Ca^{2+} affinity of the enzyme, allowing for substrate Ca^{2+} binding at high-affinity sites and subsequently for phosphorylation at Asp 475 upon ATP binding to Lys 609. In the model this is indicated by the alteration in the position of the dark patch upon dimerization.

We postulate that sodium pentobarbital, an amphipathic compound, adsorbs to the nonpolar portions of the "recognition" patch and blocks the mutually exclusive binding of another Ca^{2+}−ATPase monomer or weakens the simultaneous binding of calmodulin. The less extensive inhibition observed at 25 than at 37 °C is consistent with such direct nonpolar interactions of the compound with the protein. We have observed similar effects of sodium pentobarbital on the sarcoplasmic reticulum Ca^{2+}−ATPase (M. M. Lopez & D. Kosk-Kosicka, unpublished) which are in agreement with findings by Fernandez-Salguero et al. (1990); the latter also argued that the order parameter of the membrane is not changed by the barbiturate whereas the conformation of the protein molecule is. In addition, direct binding to the protein rather than to the membrane lipids is suggested by a similar ratio of the sensitivity of monomers and dimers to pentobarbital in the purified enzyme preparations and in the red blood cell membrane. Lifetime fluorescence measurements performed for the purified dimeric enzyme in the presence of 3 mM pentobarbital produced large apparent quenching constants supporting conformational changes related to pentobarbital binding (to monomers) (Lakowicz, 1983; M. M. Lopez, I. Gryczynski, and D. Kosk-Kosicka, unpublished).

The calmodulin-dependent activity of enzyme monomers is significantly more sensitive to pentobarbital than the activity of dimers, both with respect to the concentration dependence and rate of onset of inhibition. Such a difference, although less pronounced, was observed previously in the action of several solutes with structural similarity to detergents, including alkanols (Kosk-Kosicka et al., 1992b, 1994; Kosk-Kosicka & Roszczynska, 1993). In addition to the effects on V_{max} and Ca^{2+} affinity, barbiturates also cause a pronounced alteration in the requirement for calmodulin: both the maximal extent of stimulation by calmodulin and its affinity for calmodulin are decreased. These data suggest that the conformational changes following calmodulin binding to the enzyme that normally result in tighter substrate binding to the enzyme and Ca^{2+} binding to calmodulin are impaired in the presence of the barbiturate.

The lower sensitivity of dimers to pentobarbital could be explained by the fact that the recognition domain is at least partially protected from the compound by being buried in the dimer interface. Since the monomers and dimers are in dynamic equilibrium, the barbiturate could shift the equilibrium toward inactive monomers by binding to the nonpolar patch when it becomes exposed upon dimer dissociation. As a result, sodium pentobarbital would cause the observed attenuation of the Ca^{2+}-induced tryptophan fluorescence increase that occurs in active dimers when they bind substrate Ca^{2+} and undergo a conformational change.

The described differences in pentobarbital action on dimers and monomers resemble the effects of short-chain alcohols and contrast with the action of volatile anesthetics that inhibit the two activation pathways with similar potency and a faster onset rate. In addition, volatile anesthetics inhibit the enzyme at their anesthetic concentrations whereas I_{50} for pentobarbital (and alcohols) appears to be significantly higher than their anesthetic potency (ED_{50} = 0.050−0.080 mM) (Fisher et al., 1948; Richter & Holtman, 1982). The findings suggest that the site of action of volatile anesthetics is separate from the proposed site of action of barbiturates at the nonpolar recognition patch. Its possible location and nature are discussed elsewhere (Lopez & Kosk-Kosicka, 1995).

In conclusion, on the basis of the demonstrated effects of sodium pentobarbital on the plasma membrane Ca^{2+}−ATPase and the current knowledge of its mechanism of activation, we have proposed a working model for the action

of detergent-like compounds on the enzyme. It is a physicochemical model that explains enzyme inactivation by the nature of the compound. It is applicable to other proteins.

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