Ann. Rev. Pharmacol. Toxicol. 1982. 22:413–34

THE INTERACTION OF DRUGS WITH THE SARCOPLASMIC RETICULUM[1]

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INTRODUCTION

The sarcoplasmic reticulum (SR) is the principal regulator of the contraction-relaxation cycle in muscle. This membrane network plays a primary role in the regulation of sarcoplasmic calcium concentration and so participates in controlling the initiation, termination, and intensity of the contractile process. The ATP-dependent transport of calcium into the SR lowers ionized Ca^{2+} concentration in the sarcoplasm to below 10^{-7} M, thereby causing muscle relaxation (1, 2). This process has been extensively studied in fragmented vesicles composed of SR membranes, which can accumulate calcium against a concentration gradient in the presence of ATP (3, 4). The release of calcium from the SR in response to depolarization of the sarcolemma leads to muscle contraction. Much less is known about the mechanism of calcium release, but it is generally agreed that this is a passive process in which Ca^{2+} moves down its electrochemical gradient in response to an increase in the calcium permeability of the SR membrane.

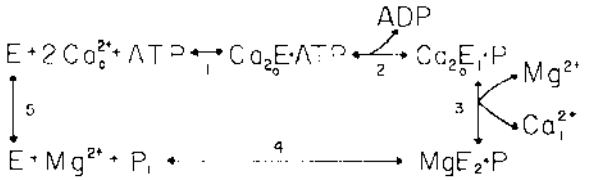
The SR has been an attractive membrane system to study because it has been well purified and characterized (5–7) and its active transport function can be readily measured by a number of investigative techniques. In addition, SR vesicles are composed of a relatively small number of protein and lipid components. The major protein is a Ca^{2+}- and Mg^{2+}-dependent ATP-

[1]Supported by Research Grants from the National Institutes of Health (HL-22135, HL-21812, HL-26903, HL-27630, HL-00911, and GM-04725), and by a grant from Merck, Sharpe and Dohme.

0362-1642/82/0415-0413$02.00

ase of mol wt 119,000 (8) that forms an acyl phosphate complex responsible for the active transport of calcium. This protein comprises about 60% of the total protein present in unfractionated SR vesicles, and more than 90% in light buoyant density SR vesicles fractionated by density gradient ultracentrifugation (6, 9). This simple composition, coupled with the ability of the membrane to orient into fully functional hydrated membrane multilayers, has allowed the detailed structure of the SR membrane to be examined (10–12).

The reaction mechanism of the calcium pump involves the formation of a series of phosphorylated ATPase intermediates during the transport of calcium (1, 2). According to one generally accepted scheme 1 mol of the ATPase protein (E) binds 1 mol of ATP and 2 mol of Ca^{2+} on the outside (cystolic) surface of the vesicles to form a Michaelis complex ($Ca_{2_o}E{\cdot}ATP$) that then liberates ADP to the cytosolic space and forms a high energy phosphate compound ($Ca_{2_o}E_1P$) that faces the outside of the vesicles. The high energy intermediate is subsequently transformed into a lower energy MgE_2P, that faces inside the vesicles, and calcium is liberated into the region of high Ca^{2+} concentration within the intravesicular lumen. During the overall reaction, which is completely reversible, the transport of 2 mol Ca^{2+} is coupled to the hydrolysis of 1 mol ATP.



In this review, the effects of several pharmacological and related molecules on the structure and function of the SR membrane are discussed. Section II, the major section of this review, concentrates primarily on changes in the functional properties of the SR membrane caused by various pharmacological agents. Some attempt is made to distinguish between the various sources of the SR membrane (cardiac muscle, skeletal muscle) where significant differences caused by a particular drug have been observed. Section III summarizes the effects of various fatty acids on SR vesicles, as many of the complex responses to drugs described in Section II can be elicited by these simple amphiphilic substances. The effects of alcohols, especially ethanol, on the properties of the SR membrane are discussed in Section IV. It is postulated that the effects of drugs, fatty acids, and ethanol on the SR membrane are mediated either directly, by an interaction with the calcium pump protein of the SR membrane, or indirectly, by an

alteration of the physico-chemical properties of the bulk lipid phase or lipid-protein interface of the SR lipid bilayer. This review concludes with a brief discussion of the possible mechanisms by which amphiphiles can interact with the SR membrane to alter its structure and function. This is of particular importance since the sites of interaction of these substances with the SR membrane may provide models for drug actions in other membrane systems.

II. EFFECTS OF PHARMACOLOGICAL AGENTS ON THE SARCOPLASMIC RETICULUM

The effects of a variety of pharmacological agents on the functional behavior of sarcoplasmic reticulum are described in this section. No attempt is made to provide a comprehensive review; instead, several categories of pharmacological and nonpharmacological substances (such as hormones) are discussed to illustrate the variety of interactions these substances can have with the relatively simple SR membrane. Distinctions between specific and nonspecific membrane effects will be made and tentative mechanisms regarding the potential nature of the interaction of these agents with SR membranes will be examined.

Local Anesthetics

Local anesthetic agents have complex effects on the functional behavior of the SR. Factors such as the pH of the incubation medium, the concentration of the local anesthetic, and the functional state of the Ca^{2+} pump in the SR membrane at the time a drug is added are important factors that can influence the drug effect.

Local anesthetics such as tetracaine, dibucaine, procaine, and lidocaine, when added prior to the start of the calcium uptake reaction, have been shown to inhibit calcium transport (13–17) and calcium ATPase activity (16, 18) of both cardiac and skeletal SR. Low concentrations of these agents have been shown to inhibit passive calcium efflux from SR vesicles (13), whereas higher concentrations increase calcium efflux and inhibit the calcium pump (13, 15, 16, 18, 19). Balzer (15) showed that 3 mm tetracaine, which inhibited active calcium transport approximately 50%, lowered the expected 2:1 stoichiometry between calcium uptake and ATP hydrolysis without increasing the passive membrane permeability to calcium. It was suggested that drugs like tetracaine might compete with calcium for an active transport site. Tetracaine (0.6 mM) was also reported to interfere with calcium-triggered calcium release (see below) (20). Recently, Trotta et al (21) showed that relatively low concentrations of tetracaine (0.5 to 3 mM), lidocaine, and procaine (5–30 mM) increased the rate of calcium

uptake in a brain microsomal fraction, whereas higher concentrations had an inhibitory effect on calcium uptake. In addition, these three local anesthetics inhibited ATP $\rightleftharpoons$ P_i exchange at the lower concentrations and ATP hydrolysis at the higher concentrations.

The complex effects of dibucaine on calcium transport by skeletal SR were clarified by Nash-Adler et al (22), who found that dibucaine could selectively inhibit calcium efflux from calcium-filled SR vesicles, and that the effects on unidirectional calcium fluxes depend in part on the state of the membrane (i.e. actively pumping calcium, or releasing calcium) at the time that the drug was added. Their findings confirmed in part earlier evidence that dibucaine could reduce membrane permeability at lower concentrations while disrupting the membrane at higher concentrations (23).

In addition to the effects of these agents on passive and active calcium fluxes across the SR membrane, local anesthetics were found to inhibit the release of calcium from calcium-filled SR induced by calcium, caffeine, and halothane [see below; (20, 24, 25)]. The mechanisms responsible for these inhibitory effects of local anesthetics are not known, but the interaction of some of these agents with membranes can be influenced by their charge. Thorpe & Seeman (25) showed that procaine in its charged form (pH 7.4) displaced calcium bound to the sarcolemma, inhibited caffeine-induced release of calcium from the SR, but had no effect on ATP-dependent calcium binding by the SR. At higher pH (8.0), the uncharged form of procaine was found to release a significant amount of actively sequestered calcium from SR.

The concentration- and pH-dependences of the local anesthetic effects on various functional properties of the SR membrane suggest that these agents may have multiple sites of action. The ability of some of these drugs to inhibit passive calcium efflux from the SR could be due to solvation of these agents into the hydrocarbon core of the lipid bilayer (26–30), a surface interaction with calcium-binding sites on the phospholipids (31, 32), or a direct interaction with a putative calcium channel, possibly involving the calcium pump protein. The inhibitory effects of these agents on the calcium pump were correlated with inhibition of calcium-dependent ATP hydrolysis (16, 19), due possibly to interference with the decomposition of the phosphorylated intermediate of the calcium ATPase (16), that could result either from a direct interaction with the calcium pump protein or from modification of the phospholipids that surround this protein within the membrane bilayer (33).

General Anesthetics

General anesthetics such as halothane and enflurane also affect the functional properties of SR, with many of these effects exhibiting a complex concentration-dependence similar to the local anesthetics. Clinical concen-

trations of halothane (1–2 mM) were found to have little effect on calcium sequestration by SR vesicles (34, 35), although these concentrations of both halothane and enflurane have been reported to enhance both calcium-triggered and caffeine-induced calcium release (20). Diamond & Berman (35) found that halothane concentrations below 5 mM at pH 6.8 had no effect on SR function, but that higher concentrations caused an irreversible inhibition of calcium uptake that was accompanied by an increase in ATPase activity. These investigators also reported that halothane did not alter the inulin-accessible space of the membrane vesicles [although such a measurement is subject to centrifugation artefacts (36)], indicating that the inhibitory effect was not due to a nonspecific increase in membrane permeability. Diamond & Berman (35) showed that the inhibitory effect of low pH (6.3) on calcium uptake by SR vesicles was significantly enhanced by halothane, suggesting that protons and halothane act synergistically. On the basis of these studies, they proposed that halothane either interacted with the calcium pump protein directly or the pump protein-lipid interface, but not directly with the bulk lipid matrix of the SR membrane. It is interesting that halothane was shown by crystallographic techniques to bind within a hydrophobic pocket of crystalline adenylate kinase (37). Enflurane was found to have effects similar to those of halothane on calcium-uptake and caffeine-induced calcium release (38).

Complex effects of halothane on the SR over a range of clinical concentrations were observed by Heffron & Gronert (39), who reported that relatively low halothane concentrations ($\leq$ 0.14 mM) had a minimal stimulatory effect on SR calcium sequestration with no effect on calcium release. Higher halothane concentrations ($>$ 0.3 mM) caused moderate inhibition of calcium sequestration which was coincident with an increase in calcium release, suggesting a biphasic concentration-dependent action of halothane on calcium accumulation by the SR. This study also showed that SR loaded passively with calcium was more sensitive to halothane-induced calcium release than was actively loaded SR.

Another general anesthetic, diethyl ether, was found by the Millipore filtration method to abolish calcium transport at relatively high concentrations (40–42). However, Salama & Scarpa (43), using a spectrophotometric technique, found that diethyl ether increased initial calcium uptake rate, total calcium accumulation, and Ca^{2+}-activated ATPase activity. These investigators suggested that the previously reported inhibition of calcium transport was an artefact arising from effects of diethyl ether on the Millipore filters used in the earlier studies. The stimulatory effects observed by Salama & Scarpa (43) may be due to an action of diethyl ether to increase the intravesicular space of the SR vesicles, thereby allowing a greater accumulation of total calcium with a concomitant reduction of the inhibitory effect of high intravesicular Ca^{2+}-concentration. Alternatively, the almost

two-fold increase in initial calcium transport rate could be related to a fluidizing effect of ether on the SR membrane that accelerates calcium pump turnover (44). Diamond & Berman (35) proposed that halothane might interact with the ATP binding site of the calcium pump protein because the inhibitory effects of this anaesthetic agent could be reduced by calcium and ATP; alternatively, halothane might alter protein-lipid interactions that depended on the conformational state of the calcium pump protein.

The clinical syndrome of malignant hyperthermia, in which susceptible individuals experience a rapid and often lethal rise in body temperature during inhalation anaesthesia, has been attributed to an effect of the anesthetic agents—notably halothane—to inhibit calcium uptake and/or induce calcium release from a genetically abnormal SR. Studies of muscle from both halothane-sensitive patients and inbred strains of swine that are susceptible to malignant hyperthermia suggest that halothane impairs calcium transport by the SR (45, 46), although other reports have not confirmed a selective sensitivity of the SR from susceptible animals or man to volatile anaesthetic agents (47–50). These discrepancies have been attributed to a temperature dependence of the response of the SR to these anesthetic agents (51).

Calcium Channel Blockers and β-Adrenergic Receptor Blocking Agents

Calcium channel and β-adrenergic receptor blocking agents also have complex effects on the SR. To a large extent, these drugs appear to act on the SR nonspecifically rather than through mechanisms related to their calcium channel or β-adrenergic blocking actions. At concentrations ($< 10^{-5}$ M) where these agents act as calcium channel blockers in whole hearts, verapamil and D-600 were found to have no effect on active calcium transport and passive calcium release of cardiac SR (52, 53). Inhibition of calcium transport by verapamil and D-600 was observed only at concentrations in the range 10^{-5} to 10^{-3} M (15, 54), although 10^{-3} M concentrations of either compound had no effect on calcium release by cardiac SR. Balzer (15), who showed that 50% inhibition of calcium transport by cardiac SR occurred at 10^{-3} M verapamil, suggested that this effect might be due to inhibition of calcium uptake rather than an increase in SR calcium permeability.

Recently our laboratory has investigated the effects of several calcium channel blockers, verapamil, nifedipine, nimodipine, and nitrendipine on calcium transport and ATPase activity of both skeletal and cardiac SR (R. Colvin, N. Pearson, F. C. Messineo, and A. M. Katz, unpublished data). Both nimodipine and nitrendipine (10^{-4} M) stimulated calcium transport; nitrendipine, the more potent stimulator, increased Ca^{2+}-activated ATPase activity and calcium transport to a similar extent. This effect depended

largely on the drug:membrane-phospholipid-mole ratio as well as absolute drug concentration, suggesting that the calcium channel blocker exerted its effect through an interaction with the phospholipid component of the SR membrane. The high concentrations of these agents necessary to affect SR function also support the view that these amphiphilic drugs act by partitioning into the lipid matrix of the SR membrane.

β-adrenergic receptor blocking drugs such as propranolol, timolol, pronethalol, MJ-1999, INPEA, and Ro-03-3528 have been shown by several investigators to inhibit calcium transport and total ATPase activity of both cardiac and skeletal SR (55–59). Propranolol and pronethalol were also found to inhibit calcium efflux from SR vesicles (56). Acebutolol and practolol were found to cause a slight inhibition of calcium uptake and ATPase activity (but not total calcium sequestration) by SR (57), whereas sotalol and oxprenolol apparently did not modify the functional behavior of the SR (56).

Studies of the mechanism of interaction of β-adrenergic receptor blockers on SR function have centered primarily around the actions of propranolol. Katz et al (59) showed that calcium, but not isoproterenol, could reverse partially the inhibitory effect of propranolol on cardiac SR. These investigators concluded that the inhibition of calcium transport by propranolol was not due to a direct β-blocking action, although propranolol could prevent stimulation of cardiac microsomal adenylate cyclase by epinephrine. Noack et al (60) reported that concentrations of propranolol that inhibited the Ca^{2+}-activated ATPase of skeletal SR did not interfere with formation of the phosphorylated ATPase intermediate, suggesting that propranolol acted primarily on a step in the reaction sequence subsequent to phosphorylation and so prevented hydrolysis of the phosphorylated enzyme. Shigekawa et al (61) showed that propranolol inhibited phosphoenzyme formation in skeletal SR with a concomitant increase in the rate of E_2P hydrolysis and decrease in the rate of conversion of the high energy phosphorylated enzyme to the low energy intermediate.

The high propranolol concentrations ($> 10^{-5}$ M) needed to inhibit calcium transport by the SR suggest that the interaction may be due to actions of the drug on the lipid or lipid/protein interface in the SR membrane. This interpretation is supported by the studies of Fujita (62), who showed that propranolol inhibition of calcium binding in cardiac SR was correlated with an increase in ANS fluorescence. Calorimetric, radioisotope-binding, and neutron diffraction studies recently carried out in our laboratory indicate that propranolol interacts primarily with the bulk lipid matrix of the SR membrane (see Section V). These studies also show that the inhibition of initial calcium uptake rate in SR by propranolol reported by Messineo & Katz (58) is correlated with the amount of propranolol solvated in the bulk

lipid matrix. Timolol, which has a much weaker effect than propranolol on SR calcium transport (58), has a correspondingly lower partition coefficient with the SR membrane (L. Herbette, A. M. Katz, and J. M. Sturtevant, unpublished data).

Data currently available are consistent with the view that the calcium channel and β-adrenergic receptor blocking agents exert their effects on SR vesicles by interacting primarily with the bulk lipid phase of the membrane. Since most of these drugs and many anesthetic agents are amphiphiles, they are able to interact with the phospholipids of the membrane bilayer. Such an interaction would explain the relatively high concentrations of these agents needed to exert presumably nonspecific effects on the functional behavior of SR. In contrast, the specific effects of lower drug concentrations appear to involve a direct, high-affinity interaction of a specific membrane protein, e.g. a β-adrenergic blocker interacting with the β-adrenergic receptors. The nature of the specific site with which calcium channel blockers interact is less clear, but in view of their widely different but amphiphilic structures, it is possible that they may interact with the hydrophobic region of the channel proteins within the sarcolemmal membrane.

Hormones

Relatively low concentrations of several hormones can modify SR function. Luteinizing hormone releasing hormone (LHRH), at concentrations of $3 \times 10^{-8} - 3 \times 10^{-6}$ M (63) decreases calcium accumulation by cardiac SR. Thyroxine has been found to inhibit calcium transport and Ca^{2+}-activated ATPase activity in skeletal SR (64); half-maximal inhibition of enzymatic activity was seen at $1.5–2.0 \times 10^{-5}$ M thyroxine for the membrane-bound ATPase, and 1.0×10^{-5} M thyroxine for the highly purified, solubilized ATPase. Triiodothyronine, but not diiodothyronine (which lacks hormonal activity), had a similar effect. Zamaraeva et al (64) suggested that the active thyroid hormones, like the anaesthetic agents discussed in the previous section, interfere with the dephosphorylation step of the ATP hydrolysis reaction. A role for these effects in producing the hypermetabolic state characteristic of hyperthyroidism is not clear. While inhibition of the calcium pump would have an energy sparing effect, the secondary effect of a high cytosolic calcium concentration would increase ATP utilization by a variety of calcium-activated systems (notably the myofibrils) and so might contribute to the high rate of energy expenditure associated with high circulating levels of thyroid hormones.

Miscellaneous Agents

The effects of caffeine on both cardiac and skeletal SR have been extensively studied and there is a general consensus that at 5–20 mM this methylxanthine inhibits calcium uptake and promotes calcium release (15, 20, 65–72).

Our own experience, however, is that this effect is much less in SR vesicles than in the SR of "skinned" muscle preparations (71). Dantrolene, a skeletal muscle relaxant, was reported to inhibit calcium efflux from heavy SR vesicles, enriched in terminal cisternae, at concentrations of $4–9 \times 10^{-5}$ M (73). At the higher concentration (9×10^{-5} M), dantrolene also was found to inhibit the calcium release induced by 10 mM caffeine (73). We (F. C. Messineo, P. B. Pinto, and A. M. Katz), however, found that dantrolene had little effect on calcium fluxes in unfractionated skeletal SR vesicles.

Thymol has been shown to depress both calcium influx and efflux across the SR membrane (74). While thymol inhibits Ca^{2+}-activated ATPase, it depresses calcium efflux to a greater extent so that the amount of calcium accumulated is increased. Perturbations of unidirectional calcium fluxes may also be responsible for the effects of local anesthetic agents and β-adrenergic receptor blockers on SR function (see above). In addition, a recent spin label study has suggested that thymol and procaine can disorder localized regions of the phospholipid bilayer of the SR (75), possibly secondary to changes in the SR proteins. However, this disorder in the lipid bilayer was not induced by conformational changes in proteins caused by caffeine, providing circumstantial evidence that different substances can interact either with different lipid or protein components of the SR membrane, or at different sites of a particular component.

III. EFFECTS OF FATTY ACIDS ON THE SARCOPLASMIC RETICULUM

The interaction of fatty acids with the SR membrane provides a relatively simple, nonpharmacological model to study the response of this membrane to amphiphilic compounds. In addition to their potential usefulness in studying models of drug-membrane interaction, this class of amphiphilic molecules may be of pathophysiological importance, as they appear to accumulate in the ischemic myocardium (76). Different fatty acids produce a variety of effects on the SR that can either increase or decrease the ability of these membranes to transport calcium (76a).

Fatty acids, and fatty acid derivatives such as palmitylcarnitine, have complex effects on membrane ATPase systems, ranging from complete inhibition to two-fold increases in ATPase activity and other coupled biological reactions (76a–84). In general, fatty acids and their derivatives stimulate SR ATPase activity regardless of their effects on calcium transport (81–84). For example, both oleic and palmitic acids stimulate ATPase activity, whereas oleic acid decreases, and palmitic acid enhances, calcium sequestration [(83), F. C. Messineo, C. Favreau, and A. M. Katz, unpublished data].

A striking example of the complexity of fatty acid-SR membrane interaction is afforded by the effects of palmitylcarnitine on cardiac SR. Pitts et al (81) reported that palmitylcarnitine inhibits calcium-activated ATPase activity, the extent of inhibition depending on the ratio of palmitylcarnitine to SR protein. In addition, calcium uptake could be completely abolished at a very low (2.5 μM) concentration of palmitylcarnitine; this concentration of palmitylcarnitine also stimulated calcium release. Some, but not all, of these results were confirmed by Adams et al (82), who found that relatively low palmitylcarnitine concentrations (5–50 μM) enhanced calcium sequestration and ATPase activity, whereas 50–200 μM palmitylcarnitine concentration inhibited both measurements. Adams et al (82) proposed that a "detergent-like" interaction of pamitylcarnitine with the membrane altered the function of the SR and this interaction occurred in three stages. At the lower concentrations, monomeric palmitylcarnitine may be incorporated into the sarcoplasmic reticulum membrane, resulting in submolecular and molecular alterations that increase the turnover of the calcium pump ATPase, along with macroscopic structural changes that increase permeability. As the critical micellar concentration of palmitylcarnitine is approached, excessive incorporation of the amphiphile leads to membrane disruption. At still higher concentrations the enzyme could be delipidated of its native phospholipids. This proposed continuum of structural events in the SR membrane would correlate with the functional changes in ATPase and calcium transport activities. Whether such a mechanism of progressive physicochemical interactions of palmitylcarnitine with cardiac membranes is, in part, responsible for the decline in contractile force during myocardial ischemia is not known (84, 85); however, such a mechanism might produce membrane disruption and contribute to irreversible membrane damage in the ischemic myocardium (76).

IV. EFFECTS OF ALCOHOLS ON THE SARCOPLASMIC RETICULUM

Alcohols, especially ethanol, have been shown to affect the functional and presumably structural states of several biological membranes (86–93), including the SR (34, 35, 94–96). Ethanol is able to partition directly into the membranes (97–99), where it exerts a fluidizing effect (88, 89, 91), whereas the development of cellular tolerance to ethanol may arise from a decrease in membrane fluidity (90, 92, 93), due possibly to an increase in the ratio of saturated:unsaturated fatty acyl chains (92, 93). In some of these studies (86, 87, 91) a direct effect of ethanol on membrane ATPase enzymes was observed; both stimulation (91) and inhibition (87) were reported. Effects of ethanol on SR ATPase activity and calcium transport were demonstrated

(34, 35, 94–96) in which two proposed sites of interaction, descriptively referred to as the leakage and denaturation sites, were implicated (94). Solvation of ethanol into, and partial solubilization of, the lipid bilayer may be the cause of the increase in ATPase activity (leakage site), whereas inhibition of calcium transport may be caused by a direct interaction with the calcium pump protein (denaturation site).

Recently, we measured the association of ethanol with a light SR membrane fraction using a radioisotope centrifugation procedure. Preliminary data show that relatively low volume percentages of ethanol yield significant molar association ratios when correlated with the amount of calcium pump protein present (see Table 1); a partition coefficient of ethanol in SR of approximately equal to 3.12 was obtained. Our findings are consistent with those of Rottenberg et al (100), who measured the partitioning of ethanol in mitochondrial and synaptosomal membranes. This association of ethanol with SR membranes may be relevant to the findings of Ohnishi (20) that ethanol increases calcium- and caffeine-induced calcium release from SR. Ethanol may also enhance the solvation of other agents into the SR membrane, especially substances that have relatively smaller partition coefficients. For these reasons, caution must be exercised when water insoluble agents dissolved in ethanol or other organic solvents are added to membranes.

V. AMPHIPHILE-SARCOPLASMIC RETICULUM MEMBRANE INTERACTIONS

The structure of the SR membrane provides a basis for understanding the nature of its interaction with various amphiphilic molecules. A combination of electron microscopy and x-ray and neutron diffraction has provided some

Table 1 Association of ethanol with sarcoplasmic reticulum membrane via radiochemical technique[a]

	Percent ethanol			
	0.1	0.25	0.5	1.0
Number	3	1	1	3
Moles ethanol/mole lipid	0.07 ± 0.02[b]	0.11	0.22	0.42 ± 0.13
Moles ethanol/mole Ca^{2+} pump protein	7.54 ± 1.85	12.47	24.55	45.91 ± 16.38
Moles ethanol/milligram protein	63.37 ± 15.65	104.80	206.32	385.78 ± 137.68

[a] Experimental results have been corrected for possible ethanol partitioning into the intravesicular and/or extravesicular water spaces of the sarcoplasmic reticulum vesicles.
[b] Each value represents the mean or the mean ± SEM.

detail of the overall structural organization of the SR membrane and the static conformation of the calcium pump ATPase protein in this membrane (Figure 1). The calcium pump ATPase molecules are embedded in the membrane bilayer, probably arrayed in a weak hexagonal-rectangular lattice (C. Napolitano, P. Cooke, and L. Herbette, unpublished data) when the membranes are not engaged in calcium transport. This protein molecule spans the membrane, with a substantial portion projecting into the extravesicular space, and contacts both the extravesicular and intravesicular water spaces, thereby providing a potential pathway for calcium transport from one side of the impermeable lipid bilayer to the other. Data obtained so far indicate that the conformational states of the sarcoplasmic reticulum membrane and the calcium pump ATPase protein change during ATP-induced calcium transport (101), indicating that the static structural picture shown in Figure 1 can be extended to provide a dynamic structural mechanism of ion transport in this biological membrane.

The structural organization of the SR membrane provides several possible locations for the incorporation of amphiphilic molecules. Lipid soluble amphiphiles with relatively large partition coefficients, such as propranolol, verapamil, and a variety of fatty acids and alcohols, are likely to be incorporated mainly into the bulk lipid domain of the SR membrane. In Figure 2 *A* a freeze fracture micrograph of the SR membrane reveals the characteristic particle-studded concave fracture plane and relatively smooth convex fracture plane. These particles are generally believed to be composed of monomers or oligomers of the calcium pump ATPase protein, which due to their mass distribution (Figure 1) remain associated with the outer leaf of the bilayer in the concave fracture plane. The addition of micromolar concentrations of palmitic acid (Figure 2*B*) results in the appearance of smooth, particle-free "lakes" that may be composed of palmitic acid and/or phospholipid, indicating that fatty acids are incorporated into bulk lipids of the SR membrane. Recent calorimetric (in collaboration with Dr. J. M. Sturtevant, Yale University) and radioisotope measurements obtained in our laboratory indicate that the partition coefficient for propranolol in a model membrane composed of dimyristoyl lecithin is approximately 90, suggesting that propranolol is localized somewhere in the hydrocarbon core of the lipid bilayer. This localization has been confirmed by neutron diffraction experiments which indicate that propranolol is solvated in the hydrocarbon core of the lipid bilayer nearest the phospholipid headgroup. In the SR membrane and in purified SR lipids, the major interaction of propranolol is with the bulk phospholipids of the SR membrane. Removal of the calcium pump protein increased the mole propranolol/mole phospholipid ratio, suggesting that the calcium pump ATPase protein causes a portion of the lipid matrix

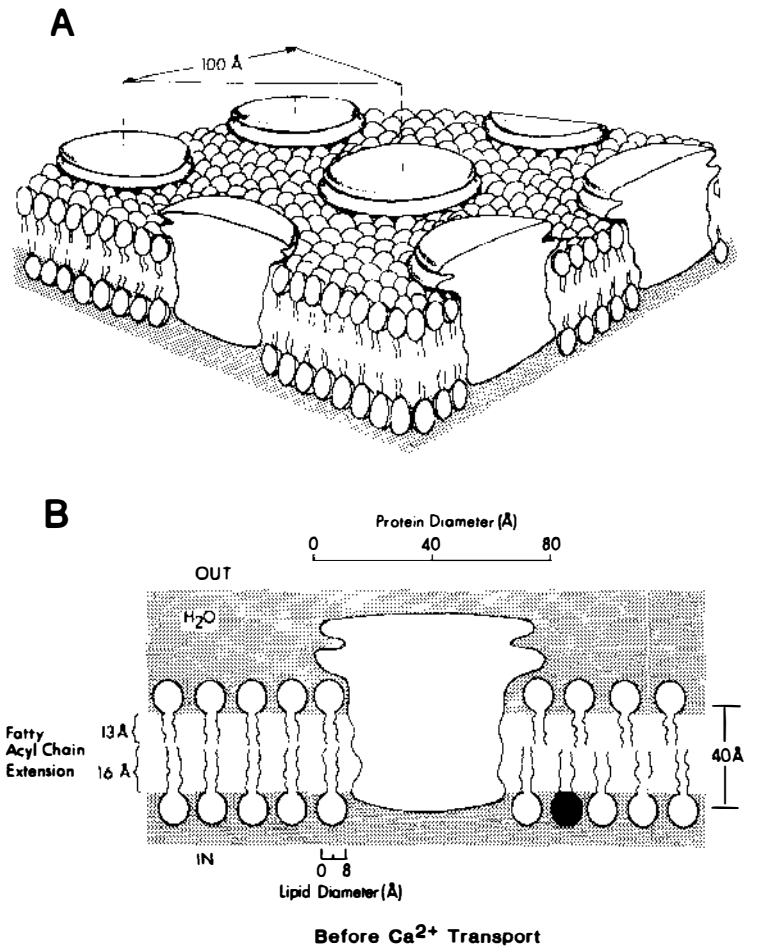


Figure 1 Structural organization of the sarcoplasmic reticulum membrane obtained by x-ray and neutron diffraction studies. In (*A*), a schematic representation depicts the calcium pump protein molecules assembled in the SR lipid bilayer with an average center to center separation of approximately 100 Å. A detailed cross-sectional view of the SR membrane is shown in (*B*), with actual dimensions of the lipid and protein components and the cylindrically averaged protein shape obtained from the structural data. The calcium pump protein molecule is embedded in the lipid bilayer with 52% of its mass located outside the layer of phospholipid headgroups, 30% in the outer monolayer, and 18% in the inner monolayer. Approximately 6–10% more phospholipid molecules are located in the inner than the outer monolayer of the lipid bilayer, consistent with the protein distribution within this region of the bilayer. A darkened phospholipid molecule on the inner monolayer represents the additional (10%) lipid on this side of the membrane. The inner monolayer fatty acyl chains average approximately 3–4 Å longer than those of the outer monolayer. The shaded area indicates the location of water in the SR membrane. (These structural studies are the result of a collaborative effort between L. Herbette, J. K. Blasie, P. De Foor, and S. Fleischer.)

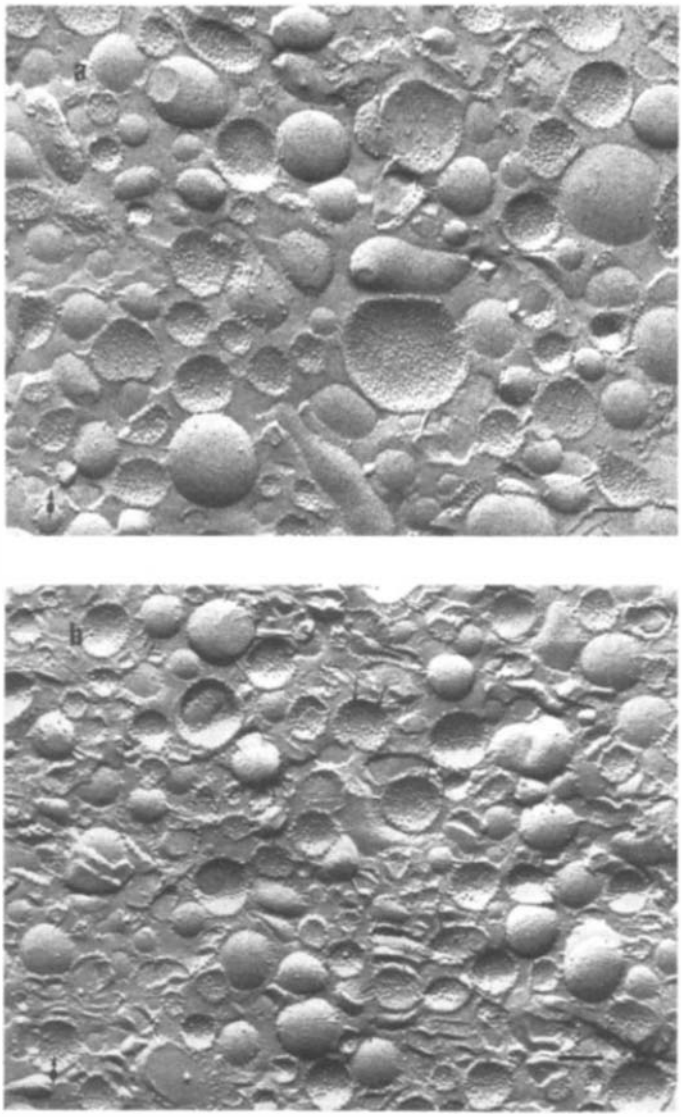


Figure 2 Freeze fracture picture depicting the particle studded concave fracture surfaces and relatively smooth convex surfaces of SR membranes (*A*) and SR treated with added palmitic acid (*B*). In (*B*), arrows point to particle-free segregated "lakes" probably composed of palmitic acid and/or phospholipid. Note the lower particle density in (*B*) vs (*A*). ↕ indicates direction of shadow. The magnification bar equals 0.1 μm.

to become inaccessible to propranolol. The nature of this drug-inaccessible region of the membrane is not known, but may represent the boundary lipids that surround the calcium pump ATPase protein [(102–104), see below].

This lipid-protein interface may itself represent a second potential site of interaction of drugs with the SR membrane. Approximately 30 mol phospholipid have been reported to be required for calcium pump activity (102), and it has been suggested that this represents the boundary lipids that surround this intrinsic membrane protein (103, 104). However, the average cross-sectional area of the calcium pump protein, determined by structural studies, is equal to approximately 3000 $Å^2$ (Figure 1). This value would require that 50–60 phospholipid molecules are needed to surround completely the calcium pump protein in both halves of the lipid bilayer. For this reason, if a boundary lipid annulus of 30 phospholipids indeed exists, it would appear to be discontinuous. Recent ^{31}P NMR measurements, which measure lipid headgroup motions on a time scale of 10^{-6} sec, failed to demonstrate an immobilized boundary lipid annulus in the SR membrane (105). Both these findings suggest that a long-lived annulus appears unlikely, although it remains possible that the hydrophobic region of the calcium pump protein determines a membrane lipid structure at which drugs could act.

A third and potentially important site of drug interaction is with the calcium pump protein itself. This intrinsic membrane protein must possess both hydrophilic and hydrophobic surfaces, so that both regions could be susceptible to interaction with amphiphilic drugs that could directly alter the functional states of the calcium pump protein. A schematic summary of the potential sites of interaction of amphiphiles with the SR membrane discussed in this review is given in Figure 3.

One concept that emerges from a consideration of these amphiphile membrane interactions is the potential for flip-flop of amphiphiles (diffusion) from one monolayer to the other monolayer of a membrane bilayer, thereby allowing amphiphiles to cross biological membranes in the absence of specific channels. For example, Tocco et al (106) measured differences in the concentrations of propranolol and timolol found in plasma and brain following intraocular administration of these drugs. It was found in this study that tissue concentrations of propranolol were at least an order of magnitude higher than those of timolol. This difference correlates with the lipid solubility characteristics of these drugs measured in our laboratory, and may provide some insight into the ability of the drugs to cross the blood-brain barrier.

The three potential sites of drug-SR membrane interactions discussed above may, however, be oversimplified. Since many drugs possess amphi-

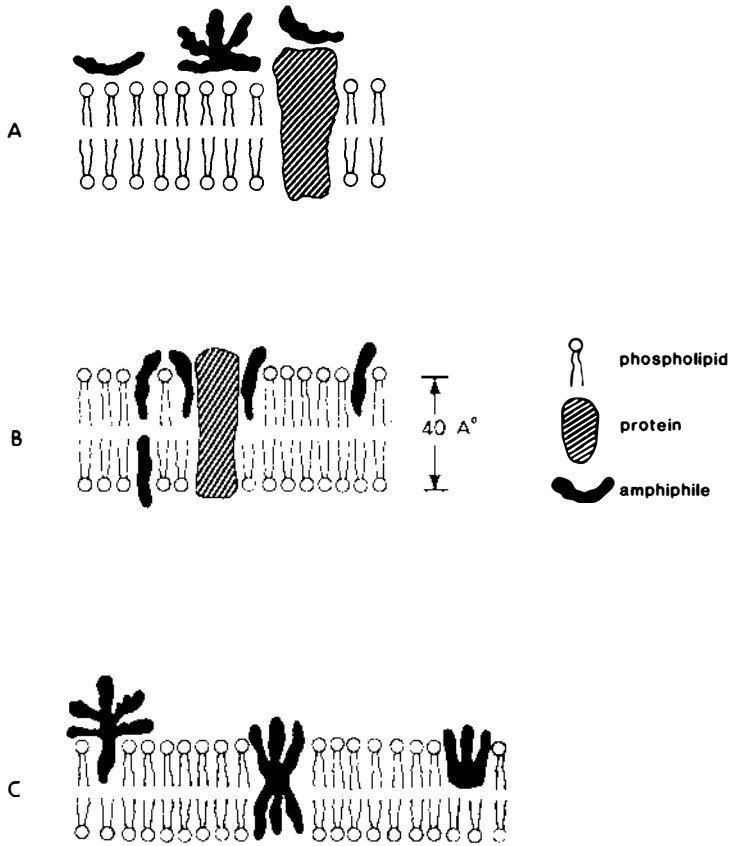


Figure 3 Schematic depiction of the potential sites of interaction of amphiphiles with biological membranes. In (*A*), membrane surface interactions are shown for amphiphilic molecules in monomeric and oligomeric (micellar) structures. Amphiphiles can interact with phospholipid headgroups or membrane proteins (not shown is the alternative micellar interaction with membrane proteins). In (*B*), interactions of monomeric amphiphiles with either or both monolayers of the lipid bilayer or with the lipid/protein interface are shown. Amphiphillic molecules added to one side of a membrane could either solvate in the monolayer on that side or undergo some flip-flop solvation mechanism and be incorporated in both halves of the lipid bilayer. In (*C*), micellar interaction is depicted with all or a portion of the micelle solvated in the hydrocarbon core of the lipid bilayer. Other schemes of interactions, not shown for clarity, are possible.

philic type properties, the study of fatty acid interactions with the SR membrane provides a relatively simple, nonpharmacological model for drug (amphiphile) SR membrane effects (76a). The perturbations of the functional behavior of the SR membrane resulting from the interaction of fatty acids with these membranes are complex, since these amphiphilic molecules can exist either in a monomeric state at low concentrations or as micellar

aggregates at higher concentrations. Another level of complexity may exist, since either of these structural forms of the fatty acids could affect the SR membrane at the molecular and/or ultrastructural levels. At the molecular level, fatty acid interactions (monomeric or micellar) could have a direct (protein-mediated) or indirect (bulk phospholipid-mediated) effect on the molecular mechanism of the calcium pump protein. At the ultrastructural level, fatty acids (monomeric or micellar) could cause morphological changes in the SR membrane (detergent-like) effects and/or micellar formation at either surface of the membrane. Knowledge of the amount and location of fatty acids (or related amphiphiles) associated with the SR membrane can thus provide important clues to aid in understanding these more complex mechanisms of interaction.

VI. SUMMARY

Efforts to determine the mechanisms by which drugs interact with membranes can be facilitated by studies of the SR, which has a relatively simple structure. The fact that the SR lacks most of the specific receptors found on other membranes, notably the sarcolemma, is an advantage, as well as a limitation, in that "nonspecific" drug effects can be evaluated readily in the SR. The presence in the SR of a well-characterized calcium pump protein allows the effects of a given drug on membrane function to be analyzed, and drug effects on the functional properties of the SR can now be correlated with their probable sites of action within the membrane. Equally important is the ability to acquire information regarding the relation between the structure of an amphiphilic drug and its mechanism of interaction with the SR membrane, because these interactions depend on the structural properties of both the membrane and the added agent. The potential sites of interaction of amphiphiles schematically portrayed in Figure 3 may be relevant to membranes other than the SR, as the overall structural properties of this membrane appear not to be unique. For this reason, the interaction of drugs with the sarcoplasmic reticulum membrane may provide structural models that are applicable to other biological membranes.

ACKNOWLEDGMENTS

We would like to thank Dr. Robert Colvin for permitting us to describe some of his recent data regarding the calcium channel blockers—nifedipine, nimodipine, and nitrendipine—and Dr. Charles Napolitano for allowing us to use the ethanol data summarized in Table 1. We are indebted to Ms. Joanne Lamothe for typing the manuscript.

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