ATP-dependent interaction of propranolol and local anaesthetic with sarcoplasmic reticulum

Stimulation of Ca^{2+} efflux

Varda SHOSHAN-BARMATZ

Department of Biology, Ben Gurion University of the Negev, Beer Sheva 84105, Israel

Preincubation of sarcoplasmic reticulum (SR) with propranolol or tetracaine inhibits Ca^{2+} accumulation and stimulates ATPase activity by more than 2-fold. This effect is obtained only when the preincubation is carried out in the presence of ATP or other nucleoside triphosphates. The (ATP + drug)-induced inhibition of Ca^{2+} accumulation is pH-dependent, increasing as the pH rises above 7.5. The presence of micromolar concentrations of Ca^{2+} or Mg^{2+} during the preincubation prevents the inhibitory effect of ATP plus drug on Ca^{2+} accumulation or ATPase activity. The (ATP + drug) modification of SR vesicles resulted in stimulation of a rapid Ca^{2+} efflux from passively loaded vesicles. The ATP-dependent inhibition of Ca^{2+} accumulation by the drug is obtained with other local anaesthetics. The drug concentration required for 50 % inhibition was 0.15 mM for dibucaine and 0.4 mM for both propranolol and tetracaine, whereas it was 5 mM, 8 mM and > 10 mM for lidocaine, benzoicaine and procaine respectively. The heavy SR vesicles were only slightly affected by the incubation with propranolol or tetracaine in the presence of ATP, but their sensitivity increased markedly after storage at 0 °C for 24–48 h. These results suggest that propranolol and some local anaesthetics, in the presence of ATP, stimulate Ca^{2+} efflux by modifying a protein factor(s) rather than the phospholipid bilayer.

INTRODUCTION

Muscle contraction and relaxation are controlled by the intra-fibre free Ca^{2+} concentration, which in turn is regulated by Ca^{2+} uptake into and Ca^{2+} release from the sarcoplasmic reticulum (SR) (Endo, 1977; De Meis & Vianna, 1979; Martonosi, 1984). The Ca^{2+} uptake mechanism is understood in rather detailed terms (De Meis & Vianna, 1979), but the mechanism by which Ca^{2+} is released is not well understood. Several mechanisms have been proposed for the physiological release of Ca^{2+} , including activation by external Ca^{2+} , 'depolarization' of the membrane, a change in membrane surface charge and/or pH gradient, or by inositol triphosphate (Martonosi, 1984; Ikemoto *et al.*, 1984; Shoshan-Barmatz, 1988; Volpe *et al.*, 1985).

Various studies indicate that there is more than one type of Ca^{2+} release system in the SR, which operate via two or more types of bivalent-cation channels (Palade *et al.*, 1983; Martonosi, 1984; Taguchi & Kasai, 1984; Smith *et al.*, 1986; Suarez-Isla *et al.*, 1986; Shoshan-Barmatz, 1987*a,b*, 1988).

Several investigators have focused their attention on the effects of local anaesthetics and β -blockers on skeletal and cardiac-muscle SR activities (Bianchi & Bolton, 1967; Scales & McIntosh, 1968; de Boland *et al.*, 1975; Yagi & Endo, 1980; Saida & Suzuki, 1981; Herbette *et al.*, 1982; Morii & Tonomura, 1983; Antoniu *et al.*, 1985). However, it has been observed that local-anaesthetic drugs exerted complex effects on the SR. Several local anaesthetics were found to inhibit Ca^{2+} transport coupled to ATP hydrolysis (de Boland *et al.*, 1975; Endo, 1981; Herbette *et al.*, 1982; Morii & Tonomura, 1983).

The drugs were also found to inhibit Ca^{2+} release induced by a variety of methods (Herbette *et al.*, 1982; Morii & Tonomura, 1983; Antoniu *et al.*, 1985). On the other hand, some local anaesthetics are known to cause a contraction by themselves (Bianchi & Bolton, 1967; Yagi & Endo, 1980; Saida & Suzuki, 1981; Herbette *et al.*, 1982). However, whereas dibucaine enhanced the Ca^{2+} -induced Ca^{2+} release (Yagi & Endo, 1980), prilocaine did not facilitate this mechanism, although it increased the release of Ca^{2+} from the SR (Saida & Suzuki, 1981). Thus the complex effects of local anaesthetics on the functional behaviour of the SR suggest that these agents may have multiple sites of action. Their mode of action, however, is still to be determined.

In this report, an ATP-dependent propranolol- or local-anaesthetic-induced Ca^{2+} efflux from SR membranes is described.

EXPERIMENTAL

Materials

ATP, ADP, p[NH]ppA, CTP, UTP, GTP, ITP, EGTA, Tricine, propranolol, procaine, benzocaine, dibucaine, lidocaine and tetracaine were obtained from Sigma Chemical Co. ϵ ATP and ϵ CTP were generously given by N. Shavit of this Department. $^{45}\text{CaCl}_2$ was obtained from The Radiochemical Centre, Amersham, Bucks., U.K., and ^{32}P P_i was from Nuclear Research Center, Negev, Israel. $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ was synthesized from ^{32}P P_i and ADP by photophosphorylation with lettuce chloroplasts and was purified on Dowex 1-X8 with NaCl as the eluent, as described previously (Shoshan-Barmatz, 1986).

Abbreviations used: SR, sarcoplasmic reticulum; p[NH]ppA, 5'-adenylyl imidodiphosphate; ϵ ATP, 1,*N*⁶-ethenoadenosine triphosphate; ϵ CTP, 3,*N*⁴-ethenocytidine triphosphate.

Membrane preparations

SR vesicles were prepared from rabbit fast-twitch skeletal muscle as described by MacLennan (1970). This preparation contained a mixture of light, heavy and intermediate vesicles. Heavy SR membranes were isolated as described by Meissner (1984). Protein concentration was determined by the method of Lowry *et al.* (1951).

Modification of SR with propranolol or local anaesthetics

SR membranes (1 mg/ml) were incubated with the indicated drugs in 0.1 M-NaCl and 20 mM-Mops, pH 7.8, and other reagents as indicated. The mixture was incubated for 5 min at 30 °C, and the samples were then transferred to ice. Portions were diluted 11-fold with the reaction mixture for measuring Ca^{2+} transport or ATPase activity and assayed as described below.

In some experiments, as indicated in the Table and Figure legends, the modified membranes were separated from the unreacted reagent by centrifugation-chromatography on a column of Sephadex G-50 (fine grade) equilibrated with 20 mM-Tricine and 100 mM-NaCl, pH 7.2 (Penefsky, 1977). The protein concentration in each sample was determined.

Assays

Active $^{45}\text{CaCl}_2$ uptake by SR vesicles was determined by Millipore filtration. The basic reaction mixture contained 20 mM-Mops, pH 6.8, 100 mM-KCl, 3 mM- MgCl_2 , 3 mM-ATP, 0.5 mM- CaCl_2 (containing ^{45}Ca , about 10^6 c.p.m./ μmol), 0.5 mM-EGTA and 50 mM-potassium phosphate. The uptake was initiated by the addition of SR (90 $\mu\text{g}/\text{ml}$). After incubation for 1 min at 24 °C, 0.18 ml samples were filtered through 0.45 μm -pore-size nitrocellulose filters and washed with 5 ml of 0.15 M-KCl. Radioactivity on the filters was measured with a liquid scintillation counter. ATPase activity was assayed under conditions similar to those used for Ca^{2+} uptake, except that $^{45}\text{CaCl}_2$ was omitted, $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ was added to the reaction mixture, and the $[\text{P}^{32}]\text{P}_i$ released from ATP was determined as described by Shoshan-Barmatz (1986). Ca^{2+} efflux from passively or actively loaded vesicles was assayed as described in the Table and Figure legends. The results represented in the Tables and Figures demonstrate a representative experiment of four to six similar experiments.

RESULTS

Preincubation of SR vesicles with propranolol (0.4 mM) resulted in about 90% inhibition of Ca^{2+} accumulation and 2-fold stimulation of ATPase activity only when ATP was present during the preincubation (Fig. 1). The ATP concentration required for half-maximal inhibition of Ca^{2+} accumulation or stimulation of ATPase activity by propranolol was about 0.4 mM, and the maximal effect of propranolol was obtained in the presence of 1.5 mM-ATP. The ATP analogues, ϵATP and p[NH]ppA, but not ADP, could replace ATP in this propranolol inhibition of Ca^{2+} accumulation (Table 1). Other nucleoside triphosphates such as ITP, UTP, CTP and GTP were able to act in the same way as ATP (Table 1).

Fig. 2 depicts the manner in which preincubation of SR vesicles with different concentrations of tetracaine or procaine in the absence or the presence of ATP affects

Table 1. Effect of nucleotides on the modification of Ca^{2+} accumulation by propranolol

SR membranes were incubated with propranolol (0.4 mM) in the absence or the presence of the indicated nucleotide and were assayed for Ca^{2+} accumulation capacity as described in the Experimental section.

Incubation conditions	Ca^{2+} accumulation ($\mu\text{mol}/\text{min}$ per mg of protein)	
	– propranolol	+ propranolol
ATP (0.2 mM)	1.98	1.29
ATP (1 mM)	1.52	0.76
p[NH]ppA (0.2 mM)	2.02	1.35
p[NH]ppA (1 mM)	1.54	0.56
ADP (0.2 mM)	1.67	1.56
ADP (1 mM)	1.32	1.16
ATP (2 mM)	1.50	0.63
ϵATP (1 mM)	1.92	0.80
CTP (2 mM)	1.91	1.01
ϵCTP (2 mM)	1.69	0.74
ITP (2 mM)	1.65	0.47
UTP (2 mM)	1.71	0.49

Ca^{2+} accumulation. In the presence of ATP, an increase in concentration of tetracaine, but not of procaine, leads to a progressive decrease in Ca^{2+} accumulation. Thus tetracaine and propranolol, but not procaine, inhibited Ca^{2+} accumulation when incubated with the SR vesicles in the presence of ATP. Since the pK_a of procaine is 9.0, compared with 8.2 for tetracaine, we tested the effect of preincubation of SR vesicles with procaine in the presence of ATP, at pH 8.6. However, even under these conditions, only about 15% inhibition of Ca^{2+} accumulation was obtained (results not shown).

We tested the effect of preincubation of SR vesicles with different local anaesthetics, in the absence and presence of ATP, on their Ca^{2+} -accumulation capacity. Table 2 summarizes these experiments by presenting the concentration of the drug required for 50% inhibition of Ca^{2+} accumulation (I_{50}); the presence of ATP during preincubation decreases the I_{50} by several-fold. Dibucaine and tetracaine are the most effective, whereas procaine, lidocaine and benzocaine are much less effective.

The effect of treatment of SR vesicles with propranolol and tetracaine (in the presence of ATP) on their Ca^{2+} accumulation capacity remained after the removal of free ATP and propranolol or tetracaine by sedimentation of the membranes by ultracentrifugation or chromatography on Sephadex G-50 (Penefsky, 1977) (results not shown).

Fig. 3 shows that the pH of the preincubation medium had a marked influence on the degree of inhibition of Ca^{2+} accumulation by propranolol or tetracaine in the presence of ATP. The inhibition was more pronounced at alkaline pH. Half-maximal inhibition was obtained at around pH 7.8.

The effect of the bivalent cations Ca^{2+} and Mg^{2+} on the inhibition of Ca^{2+} accumulation by preincubation of the membranes with propranolol or tetracaine in the presence of p[NH]ppA is shown in Fig. 4; the inhibition of Ca^{2+} accumulation is prevented by both Ca^{2+} and Mg^{2+} .

Fig. 1. Effect of preincubation of SR vesicles with propranolol, in the absence or presence of ATP, on their Ca²⁺-accumulation and ATP-hydrolysis activities

SR vesicles were incubated with the indicated concentrations of ATP in the absence (○) or the presence (●) of 0.4 mM-propranolol, as described in the Experimental section. After incubation for 5 min, samples were assayed for Ca²⁺ accumulation and ATP-hydrolysis activities, also as described in the Experimental section. The final propranolol concentration in the assay medium was one-eleventh of that present during the preincubation.

Fig. 2. Effect of preincubation of SR vesicles with tetracaine and procaine, in the absence or presence of ATP, on Ca²⁺ accumulation

SR vesicles were incubated with different concentrations of tetracaine or procaine in the absence (○) or the presence (●) of 1 mM-ATP and were assayed for Ca²⁺-accumulation capacity, as described in the Experimental section.

Under the conditions of these experiments (pH 7.8, 1.0 mM-p[NH]ppA and 0.6 mM-propranolol or 0.7 mM-tetracaine), the concentrations of free Ca²⁺ or Mg²⁺ required for preventing 50% of the maximal inhibition were 15 μM and 50 μM respectively. The bivalent cations apparently prevented the effect of propranolol or tetracaine in the presence of p[NH]ppA not by decreasing the free concentration of p[NH]ppA, but rather by a direct interaction with the protein(s) modified by the drugs in the presence of p[NH]ppA. The effect of Ca²⁺ and Mg²⁺ in preventing the (ATP + drug)-induced inhibition of Ca²⁺ accumulation was obtained only when bivalent cations were present during the treatment, but not after (results not shown).

The loss of Ca²⁺-accumulation capacity as a result of propranolol or tetracaine treatment in the presence of ATP is associated with a greatly accelerated passive efflux of Ca²⁺ from the SR vesicles. Fig. 5 compares the Ca²⁺ efflux from vesicles treated with ATP, with ATP and propranolol, with tetracaine or with ATP and tetracaine, and then passively loaded with Ca²⁺ by incubation for 2 h at 22 °C. Pretreatment of SR vesicles with ATP or with propranolol (results not shown) affected the Ca²⁺ efflux from the loaded vesicles only slightly. However, when the vesicles were incubated with both ATP and propranolol, a rapid Ca²⁺ efflux was obtained (Fig. 5a). Fig. 5(b) shows that a rapid Ca²⁺ efflux was obtained when the vesicles were treated with

Table 2. Effect of preincubation of SR vesicles with different drugs in the absence or the presence of ATP on their Ca^{2+} -accumulating capacity

SR vesicles were incubated with different concentrations of the indicated drug, in the absence or the presence of 1 mM-ATP, and were assayed for Ca^{2+} -accumulation capacity, as described in the Experimental section. The drug concentration which produced 50% inhibition of Ca^{2+} accumulation (I_{50}) is given below. Benzocaine and lidocaine were dissolved in ethanol, dibucaine in 50% ethanol, and tetracaine, procaine and propranolol in water. The final ethanol concentration was not more than 4% in the control or in the samples containing the drug.

SR vesicles preincubated with	pK_a^*	$I_{50}(\text{mM})$	
		– ATP	+ ATP
Dibucaine		0.4	0.1
Tetracaine	8.24	1.3	0.4
Propranolol		1.3	0.4
Benzocaine	3.19	30.0	8.0
Lidocaine	7.85	7.0	5.0
Procaine	8.95	> 10.0	> 10.0

* From Bianchi (1968).

tetracaine in the presence of ATP. However, tetracaine alone, at the high concentration used, stimulates a significant Ca^{2+} efflux in the treated vesicles. The Ca^{2+} efflux rate obtained in vesicles treated with tetracaine or propranolol in the presence of ATP was fast, and could not be determined by the filtration method. Since the ATP-hydrolysis activity responsible for Ca^{2+} transport is stimulated by the treatment with tetracaine or propranolol in the presence of ATP, the 'leakiness' of the

treated vesicles is obviously a possible reason for their lack of Ca^{2+} -accumulation capacity.

The effect of preincubation of junctional SR vesicles with tetracaine or propranolol in the presence of ATP is shown in Fig. 6. It is clear that heavy SR membranes (Meissner, 1984) are much less sensitive to either propranolol or tetracaine. In the presence of ATP, preincubation with 0.8 mM-propranolol or 1.0 mM-tetracaine resulted in about 90% inhibition of Ca^{2+} accumulation by SR vesicles isolated by published procedures (MacLennan, 1970; Pickart & Jencks, 1982), but only about 30% inhibition in the heavy SR vesicles. We found, however, that the heavy SR membranes became much more sensitive to the drugs when the membranes were incubated at 0 °C (instead of –70 °C) for 1–2 days before the incubation with tetracaine. For example, the inhibition of Ca^{2+} accumulation by preincubation with tetracaine and ATP increased from 20% to 75% after storage of membranes for 48 h at 0 °C (results not shown). The presence of proteinase inhibitors such as phenylmethanesulphonyl fluoride during the incubation at 0 °C had no effect on the increased sensitivity of these membranes to the drugs (results not shown). Moreover, SDS/polyacrylamide-gel electrophoresis of the membranes incubated for different periods of time at 0 °C or at –70 °C either before or after ultracentrifugation revealed no difference in their protein composition (results not shown). These results suggest that in the junctional (heavy) SR the ATP- and/or the drug-interacting site is not exposed unless some structural changes take place.

DISCUSSION

In this study, the effect of local anaesthetics and propranolol on the functional behaviour of SR membranes is described. Several similar studies have been carried out (Bianchi & Bolton, 1967; Scales & McIntosh, 1968; de Boland *et al.*, 1975; Yagi & Endo, 1980; Said

Fig. 3. Dependence of the inhibition of Ca^{2+} accumulation by tetracaine or propranolol on the incubation pH

SR vesicles were incubated at the indicated pH with or without 0.4 mM-tetracaine or 0.4 mM-propranolol and in the absence (○) or the presence (●) of 1 mM-ATP, and were assayed for Ca^{2+} -accumulation activity as described in the Experimental section. The buffers used were 20 mM-Mops for pH 6.0, 6.5, 7.0 and 7.4, and 20 mM-Tricine for pH 7.8, 8.2, 8.4 and 8.6. Ca^{2+} accumulation by vesicles incubated at the different pH values in the absence or the presence of ATP (100%) was 0.89 ± 0.04 and 0.81 ± 0.04 $\mu\text{mol}/\text{min}$ per mg of protein respectively.

Fig. 4. Ca^{2+} and Mg^{2+} both prevent the inhibition of Ca^{2+} accumulation by preincubation of SR vesicles with propranolol or tetracaine in the presence of p[NH]ppA

SR vesicles were incubated with tetracaine (0.4 mM) or propranolol (0.6 mM) in the presence of 1 mM-P[NH]ppA and the indicated free concentrations of Mg^{2+} or Ca^{2+} as described in the Experimental section. The free Ca^{2+} and Mg^{2+} concentrations were calculated with a computer program by using apparent binding constants (K_a) of $8.17 \times 10^3 \text{ M}^{-1}$ and $1.87 \times 10^4 \text{ M}^{-1}$ for Ca-ATP and Mg-ATP respectively at pH 7.8, assuming that they were identical for p[NH]ppA. The total Ca^{2+} concentration was between 20 and 500 μM , and that of Mg^{2+} between 0.1 and 5 mM. Ca^{2+} accumulation by control vesicles in the presence or absence of the drugs and/or Ca^{2+} was $1.5 \pm 0.09 \mu\text{mol}/\text{min}$ per mg of protein, and it was $1.33 \mu\text{mol}/\text{min}$ per mg of protein in the presence of p[NH]ppA.

Fig. 5. Ca^{2+} efflux from control and propranolol- or tetracaine-treated vesicles

In (a), SR vesicles were incubated with 1 mM-ATP ($\circ$) or with ATP+0.2 mM-propranolol ($\bullet$) or with ATP+0.6 mM-propranolol ($\blacktriangle$), and in (b) with 1 mM-tetracaine ($\circ$), with 1 mM-ATP ($\bullet$) or with both ($\triangle$), as described in the Experimental section. The treated vesicles were centrifuged at 80000 g for 30 min, resuspended (1.8 mg/ml) in 20 mM-Mops, pH 6.8, containing 100 mM-KCl and 5 mM- $^{45}\text{CaCl}_2$ (1.5×10^7 c.p.m./ml), and incubated for 2 h at 22 $^{\circ}\text{C}$. For Ca^{2+} efflux, samples (30 μl) were rapidly filtered and washed for the indicated time with a solution identical with the loading solution, except that CaCl_2 was omitted. The washing rate was approx. 1 ml/s.

& Suzuki, 1981; Herbette *et al.*, 1982; Morii & Tonomura, 1983; Antoniu *et al.*, 1985). However, our novel finding is an ATP-dependent drug-induced Ca^{2+} efflux from SR and its inhibition by micromolar concentrations of Ca^{2+} and Mg^{2+} . We found that preincubation of SR vesicles with dibucaine, tetracaine or propranolol in the presence of ATP yielded a stimulated ATPase activity (up to 2-fold) and a lower Ca^{2+} -accumulation capacity.

Preincubation of SR vesicles with ATP+drug also resulted in an increased membrane permeability to Ca^{2+} . This 'leakiness' of the treated vesicles is obviously a possible reason for their lack of Ca^{2+} -accumulation capacity.

The involvement of ATP in the drug-induced increase in membrane Ca^{2+} permeability does not entail its

hydrolysis, since the non-hydrolysable analogue p[NH]-ppA acts as ATP. Therefore it seems that ATP action consist of direct binding to a specific SR protein(s).

The relationship between the action of the drug and ATP in the increase in membrane Ca^{2+} permeability is not clear. However, since ATP must be present during the modification with the drug, it seems that ATP, by its binding to a specific protein(s), enables the stimulation of Ca^{2+} efflux to take place by the binding of the drug to a specific exposed site.

The involvement of nucleotides in the Ca^{2+} release activated by different treatments has been reported previously (Endo, 1981; Morii & Tonomura, 1983; Martonosi, 1984; Meissner, 1984; Shoshan-Barmatz, 1987a,b).

Fig. 6. Effect of propranolol and tetracaine on Ca^{2+} accumulation by heavy SR vesicles

Heavy SR (HSR; ●) or mixed SR (○) vesicles were incubated with ATP and different concentrations of tetracaine or propranolol as described in the Experimental section. Ca^{2+} accumulation (100%) by heavy SR and mixed SR was 0.32 and 1.45 $\mu\text{mol}/\text{min}$ per mg of protein respectively.

The experiments described here showed that Mg^{2+} and Ca^{2+} (micromolar concns.), when present during the modification, each prevented the increase in the membrane Ca^{2+} permeability produced by ATP+drug. This effect was probably not due to the formation of a metal-ATP complex and the subsequent decrease in the concentration of free ATP, because the effect of the cation was obtained when the free ATP concentration was high, suggesting a direct binding to a protein(s). This would nullify the effects of ATP and/or the modification by the drug. It is clear that the effect of the bivalent cations occurs during the modification, because their addition after the modification did not affect the properties of the modified membranes. These results may indicate the involvement of a bivalent-cation-binding site in the Ca^{2+} efflux system activated by ATP+drug. A similar suggestion has been made for Ca^{2+} -induced Ca^{2+} release (Martonosi, 1984) and for Ca^{2+} release activated by other methods (Shoshan-Barmatz, 1987a,b; Argaman & Shoshan-Barmatz, 1988).

The mechanisms responsible for the different effects of local anaesthetics (Bianchi & Bolton, 1967; Scales & McIntosh, 1968; de Boland *et al.*, 1975; Yagi & Endo, 1980; Saida & Suzuki, 1981; Herbette *et al.*, 1982; Morii & Tonomura, 1983; Antoniu *et al.*, 1985) are not known. These effects could be due to solution of the drugs into the hydrocarbon core of the lipid bilayer (Feinstein *et al.*, 1977), a surface interaction with Ca^{2+} -binding sites on the phospholipids (Kwant & Seeman, 1969) or a direct interaction with a putative calcium channel(s). Thus, one important question is whether, in our experiments, the drugs act directly on a protein(s), or indirectly by affecting membrane lipids.

The following observations strongly suggest specific interaction of the drugs with Ca^{2+} -release channel(s) or with its regulatory components rather than with the lipid components of the membrane.

(a) The relationship between the molecular structure of the drugs and their effect on the membrane Ca^{2+} permeability does not indicate a correlation between their lipid solubility and their effect. Lidocaine and

procaine were both ineffective compared with tetracaine, although lidocaine, like tetracaine, has no hydrophilic portion (an amino group). Also, there is no correlation with their uncharged or charged form (see pK_a values in Table 2) and their effect on the membrane Ca^{2+} permeability.

(b) The ATP requirement for the drug's action is not consistent with their interaction with phospholipid.

(c) Ca^{2+} and Mg^{2+} each prevented the increase in membrane Ca^{2+} permeability produced by the pre-incubation of the SR membranes with ATP+drug. Mg^{2+} and Ca^{2+} were also found to prevent Ca^{2+} efflux induced by various other treatments (Martonosi, 1984; Shoshan-Barmatz, 1987a,b; Argaman & Shoshan-Barmatz, 1988).

(d) The effect of ATP+drug is much less pronounced in heavy SR membranes (Meissner, 1984) compared with their effect on SR isolated by conventional methods (MacLennan, 1970; Pickart & Jencks, 1982). The sensitivity of heavy SR to the ATP+drug was increased by aging the membranes for 24–48 h, suggesting structural changes in membrane structure rather than in lipid composition.

However, it is possible that tetracaine, dibucaine and propranolol possess a dual mechanism of action: one mechanism is site-specific and the other is non-specific, such as interaction with the lipid bilayer or with the hydrophobic domains within the proteins. It is possible that the ATP-dependent effect of the drug is the specific one.

A hypothesis concerning the nature of the binding sites of local anaesthetic molecules to receptors in the SR membranes has been formulated by Bianchi (1968). Specific interactions of local anaesthetics with the acetylcholine receptor (Heidman & Changeux, 1979) or with the Na^+ channel (Hille, 1977) have also been suggested previously.

Further studies of the interaction of the drugs and ATP with the Ca^{2+} release system(s) could provide better understanding of the control mechanism of Ca^{2+} release in the SR membranes.

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REFERENCES

- Antoniou, B., Kim, D. H., Morii, M. & Ikemoto, N. (1985) *Biochim. Biophys. Acta.* **816**, 9–17
- Argaman, A. & Shoshan-Barmatz, V. (1988) *J. Biol. Chem.* **263**, 6315–6321
- Bianchi, C. P. (1968) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **27**, 126–131
- Bianchi, C. P. & Bolton, P. C. (1967) *J. Pharmacol. Exp. Ther.* **157**, 388–405
- de Boland, A. R., Jilka, R. L. & Martonosi, A. (1975) *J. Biol. Chem.* **250**, 7501–7510
- De Meis, L. & Vianna, A. L. (1979) *Annu. Rev. Biochem.* **48**, 275–292
- Endo, M. (1977) *Physiol. Rev.* **57**, 71–108
- Endo, M. (1981) in *The Mechanism of Gated Calcium Transport across Biological Membranes* (Ohnishi, S. T. & Endo, M., eds.), pp. 257–264, Academic Press, New York
- Feinstein, M. B., Volpe, M., Parrie, S., Makriyannis, A. & Shaafi, R. I. (1977) *Mol. Pharmacol.* **13**, 840–851
- Heidman, T. & Changeux, J. P. (1979) *Eur. J. Biochem.* **94**, 281–296
- Herbette, L., Messineo, F. C. & Katz, A. M. (1982). *Annu. Rev. Pharmacol. Toxicol.* **22**, 413–434
- Hille, B. (1977) *J. Gen. Physiol.* **69**, 497–515
- Ikemoto, N., Antoniu, B. & Kim, D. H. (1984) *J. Biol. Chem.* **259**, 13151–13158
- Kwant, W. O. & Seeman, P. (1969) *Biochim. Biophys. Acta* **193**, 338–349
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- MacLennan, D. H. (1970) *J. Biol. Chem.* **245**, 4508–4518
- Martonosi, A. N. (1984) *Physiol. Rev.* **64**, 1240–1319
- Meissner, G. (1984) *J. Biol. Chem.* **259**, 2365–2374
- Morii, H. & Tonomura, Y. (1983) *J. Biochem. (Tokyo)* **80**, 1144–1157
- Palade, P., Mitchell, R. D. & Fleischer, S. (1983) *J. Biol. Chem.* **258**, 8090–8107
- Penefsky, H. S. (1977) *J. Biol. Chem.* **252**, 2891–2899
- Pickart, C. M. & Jencks, W. P. (1982) *J. Biol. Chem.* **257**, 5319–5322
- Saida, K. & Suzuki, A. (1981) *J. Pharmacol. Exp. Ther.* **219**, 815–820
- Scales, B. & McIntosh, D. A. D. (1968) *J. Pharmacol. Exp. Ther.* **160**, 261–267
- Shoshan-Barmatz, V. (1986) *Biochem. J.* **240**, 509–517
- Shoshan-Barmatz, V. (1987a) *Biochem. J.* **243**, 163–175
- Shoshan-Barmatz, V. (1987b) *Biochem. J.* **247**, 497–504
- Shoshan-Barmatz, V. (1988) *J. Membr. Biol.* **103**, 67–77
- Smith, J. S., Coronado, R. & Meissner, G. (1986) *Biophys. J.* **50**, 921–928
- Suarez-Isla, B. J., Crozco, C., Heller, P. F. & Froehlich, J. P. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7741–7745
- Taguchi, T. & Kasai, M. (1984) *J. Biochem. (Tokyo)* **96**, 179–188
- Volpe, P., Salvati, G., Di Virgilio, F. & Pozzan, T. (1985) *Nature (London)* **316**, 347–349
- Yagi, S. & Endo, M. (1980) *Biomed. Res.* **1**, 269–272

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