Biochimica et Biophysica Acta, 687 (1982) 17–26
Elsevier Biomedical Press

BBA 71112

FATTY ACID EFFECTS ON CALCIUM INFLUX AND EFFLUX IN SARCOPLASMIC RETICULUM VESICLES FROM RABBIT SKELETAL MUSCLE *

ARNOLD M. KATZ, PRISCILLA NASH-ADLER, JAMES WATRAS, FRANK C MESSINEO, HITOSHI TAKENAKA and CHARLES F LOUIS **

Cardiology Division, Department of Medicine, University of Connecticut Health Center, Farmington, CT 06032 (U S A)

(Received July 21st, 1981)
(Revised manuscript received December 7th, 1981)

Key words Ca^{2+} *transport, Fatty acid inhibition, Oleic acid, Stearic acid, (Rabbit skeletal muscle)*

Low concentrations of fatty acids inhibited initial Ca uptake by sarcoplasmic reticulum vesicles, the extent of inhibition varying with chain length and unsaturation in a series of C_{14}–C_{20} fatty acids. Oleic acid was a more potent inhibitor of initial Ca uptake than stearic acid at 25°C, whereas at 5°C there was less difference between the inhibitory effects of low concentrations of these fatty acids. When the fatty acids were added later, during the phase of spontaneous Ca release that follows Ca uptake in reactions carried out at 25°C, 1–4 μM oleic and stearic acids caused Ca content to increase. This effect was due to marked inhibition of Ca efflux and slight stimulation of Ca influx. At concentrations of >4 μM, both fatty acids inhibited the Ca influx that occurs during spontaneous Ca release; in the case of oleic acid, this inhibition resembled that of initial Ca uptake at 5°C. The different effects of fatty acids at various times during Ca uptake reactions may be explained in part if alterations in the physical state of the membranes occur during the transition from the phase of initial Ca uptake to that of spontaneous Ca release.

Introduction

Sarcoplasmic reticulum vesicles undergo spontaneous changes in calcium content during calcium uptake reactions carried out in the presence [1,2] or absence [3–6] of a calcium-precipitating anion. In reactions carried out with ATP as substrate and inorganic phosphate as calcium-precipitating anion, phases of initial calcium uptake followed by spontaneous calcium release and renewed calcium uptake *** have been shown to arise from changing rates of calcium influx and efflux across the vesicle membranes [2]. While the mechanisms responsible for these changing flux rates remain unclear, several features of the observed calcium efflux suggest that, like calcium influx, the 'downhill' movement of this ion out of calcium-filled sarcoplasmic reticulum vesicles may be mediated by the calcium pump ATPase [1,2,7].

In an attempt to define further the mechanisms involved in these changing calcium fluxes in the sarcoplasmic reticulum, we investigated the effects

* Preliminary reports of portions of these findings have been reported ((1979) Circulation 59, 11–12; (1980) Circulation 62, 111–112, and (1981) Life Sci 28, 1103

** Present address; Department of Veterinary Biology, College of Veterinary Medicine, University of Minnesota, St Paul, MN 55108, U.S.A.

*** As used in this article, 'calcium uptake' and 'calcium release' refer to the gain or loss of calcium by the vesicles; 'calcium influx' and 'calcium efflux' refer to the unidirectional calcium fluxes into or out of the vesicles, respectively Ca_o, ionized Ca^{2+} concentration outside the vesicles

of various fatty acids. Several investigators have found that oleic acid [8–10] inhibits calcium uptake, and that high concentrations of this fatty acid disrupt these membranes [11]. The effects of fatty acids on the sarcoplasmic reticulum are complex in that palmitic acid can increase calcium accumulation in the absence of a calcium-precipitating anion [12]. Furthermore, high concentrations of palmityl carnitine inhibit this calcium accumulation [13,14], whereas low concentrations of this fatty acid derivative can increase calcium accumulation [14]. These latter effects are reminiscent of those produced by oleic acid on the erythrocyte membrane, where low concentrations exert a 'stabilizing' effect that inhibits osmotic lysis, while higher concentrations promote lysis [15].

The present study examines the effects of a variety of fatty acids on the initial rate of calcium uptake into sarcoplasmic reticulum vesicles, and the response of calcium influx and efflux to oleic and stearic acids added at the time of the spontaneous transition from initial calcium uptake to calcium release.

Materials and Methods

Preparation of sarcoplasmic reticulum vesicles from rabbit fast skeletal muscle, and measurements of calcium uptake and calcium release by the Millipore technique were as described previously [1,16]. Calcium influx was measured by adding tracer amounts of high specific activity carrier-free $^{45}CaCl_2$ to a reaction mixture in which $^{40}CaCl_2$ was used for the initial calcium uptake reaction. A duplicate reaction was started with $^{45}CaCl_2$ instead of $^{40}CaCl_2$ to follow calcium uptake or release throughout the time of the experiment, and to allow calculation of total calcium concentration outside the vesicles at the time of tracer addition [1]. Calcium efflux was calculated by subtracting the rate of calcium uptake from, or adding the rate of calcium release to the calcium influx rate. Unless otherwise specified, all experiments were carried out at 25°C.

ATPase activity was determined by measuring the pyruvate concentration [17] in filtrates obtained after Millipore filtration of reaction mixtures similar to those used for calcium uptake studies except for the inclusion of 5 mM phospho*enol*pyruvate. Pyruvate kinase (0.15 mg/ml), which was added to the filtrate, catalyzed the rapid conversion of phospho*enol*pyruvate to pyruvate as evidenced by complete conversion of 0.1 mM ADP to ATP within 15 s.

A low bouyant density sarcoplasmic reticulum fraction ('light' vesicles), purified on a 20% to 60% (w/v) linear sucrose density gradient [18,19] at 4°C, was used in all experiments. Unless otherwise stated, experiments were carried out in 120 mM KCl, 40 mM histidine buffer (pH 6.8), 50 mM potassium phosphate (pH 6.8) as the calcium-precipitating anion, 5 mM MgATP, and 4–6 μg/ml light sarcoplasmic reticulum vesicles. Ionized calcium concentrations outside the vesicles (Ca_o) were calculated according to the equations of Katz et al. [20], except that, when Ca-EGTA buffers were used, a binding constant for CaEGTA of 10^{11} was used.

Fatty acids were added in ethanol such that the final ethanol concentration in the reaction mixture did not exceed 0.5% (v/v), which was without significant effect on calcium influx or efflux. In the absence of vesicles, the low concentrations of fatty acids used in this study did not trap significant amounts of calcium on the Millipore filters. Unsaturated fatty acids were kept in N_2 to avoid spontaneous oxidation.

The association of fatty acids with sarcoplasmic reticulum vesicles was investigated by sucrose density gradient centrifugation in the presence of ^{14}C-labelled oleic or stearic acids. Sarcoplasmic reticulum vesicles (6 μg/ml) were incubated at 25°C in 0.12 M KCl, 60 μM $CaCl_2$, 5 mM MgATP, 50 mM potassium phosphate (pH 6.8), 40 mM histidine (pH 6.8), with 0.04 μCi/ml ^{14}C-labelled oleic or stearic acids at concentrations between 0.16 and 32 μM. 20 ml of this mixture was overlaid on a 10%/80% sucrose 'step' gradient and centrifuged in a Beckman SW 27 rotor at 115000 $\times g$ for 40 min (25°C). The samples were then fractionated (70 fractions/tube) and fractions assayed for protein concentration and radioactivity by Coomassie blue binding [21] and liquid scintillation counting, respectively. There was no significant loss of radioactivity attributable to adsorption of the fatty acids to the tubes or pipettes used in these studies.

Fatty acids were obtained from Applied Sciences Laboratory and Sigma Chemical Co. Pyruvate

kinase (Type III) was purchased from Sigma Chemical Co., and phospho*enol*pyruvate (monopotassium salt) was obtained from Boehringer-Mannheim Co. All reagents used were reagent grade and deionized water was distilled from glass prior to use. Disodium ATP (Boehringer-Mannheim) was desalted and neutralized with Tris or KOH and $MgCl_2$ as described previously [16].

All data are representative of series of at least three replicate experiments unless otherwise stated.

Results

Effect of initial calcium uptake velocity

When added to sarcoplasmic reticulum vesicles at the onset of a calcium uptake reaction, each of a

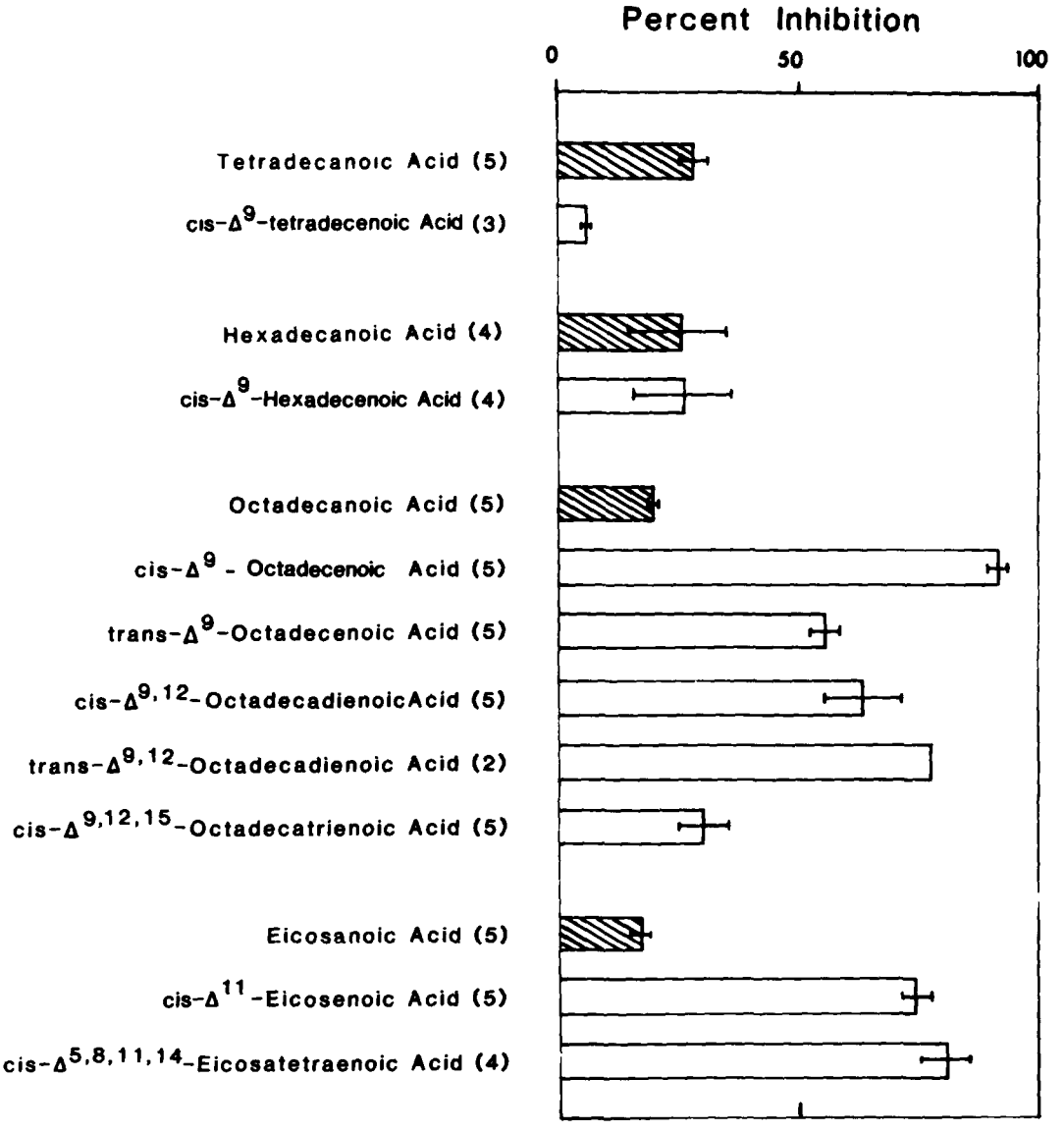

Fig 1 Effect of various fatty acids on initial calcium uptake velocity at 25°C Fatty acids were present at a concentration of 8 μM and total $CaCl_2$ was 48 μM All other reagents were as described in Materials and Methods Error bars represent the standard error of the mean for experiments in which $n>2$. Numbers in parentheses represent the number of individual experiments Data obtained with fatty acids from Applied Sciences Lab and Sigma Chemical Co were pooled, there having been no significant differences between the effects of fatty acids from the two sources Shaded bars represent data from studies with saturated fatty acids

series of long-chain fatty acids at a concentration of 8 μM inhibited initial calcium uptake velocity at 25°C (Fig. 1). In the case of the unsaturated fatty acids, greater inhibition was seen with the C_{18} and C_{20} than with the C_{14} and C_{16} fatty acids. The number of hydrocarbons had less influence on the extent of the inhibitory effect than did the presence of one or more double bonds in the hydrocarbon chain. The saturated C_{18} and C_{20} fatty acids caused much less inhibition than did unsaturated fatty acids of the same chain length, whereas the saturated C_{14} fatty acid was a more potent inhibitor than the corresponding fatty acid with one *cis* double bond. In the C_{18} series, a single *cis* double bond potentiated the inhibitory effect more than a single *trans* double bond, while *cis*-$\Delta^{9,12,15}$-octadecatrienoic acid was a relatively weak inhibitor. A single *cis* double bond in the C_{16} series did not alter significantly the inhibitory effect. The fatty acids with the fewest hydrocarbons that displayed the largest difference in potency between saturated and *cis* unsaturated fatty acids (C_{18}) were chosen for further study.

At concentrations greater than 1 μM, oleic acid (*cis*-Δ^9-octanedecenoic acid) was a more potent inhibitor of initial calcium uptake velocity at 25°C than stearic acid (octadecanoic acid) (Table I). Half-maximal inhibition was observed at oleic acid concentrations less than 4 μM, whereas concentrations of stearic acid up to 16 μM inhibited calcium uptake by only 20%. Stearic acid concentrations above 30 μM, which inhibited initial calcium uptake velocity by less than 30%, formed visibly turbid suspensions so that its half-maximal inhibitory concentration could not be determined.

TABLE I

INHIBITION OF INITIAL CALCIUM UPTAKE BY OLEIC AND STEARIC ACIDS AT 25°C

Values are mean ± S E , numbers in parentheses are the number of individual determinations $CaCl_2$ concentration was 48 μM, vesicles were incubated for 8 min prior to the start of reactions with MgATP Other conditions as described in Materials and Methods

Fatty acid concn (μM)	Initial calcium uptake velocity (μmol/mg per min)	
	Oleic acid	Stearic acid
0	1 52±0 08 (7)	1 47±0 10 (5)
1 0	1 38±0 07 (5)	1 39±0 10 (3)
2 0	1 20±0 09 (5) [a]	1 29±0 10 (3)
4 0	0 66±0 05 (5) [c]	1 26±0 12 (3)
8 0	0 08±0 02 (7) [c]	1 18±0 06 (5) [b]
16 0	0 (1)	1 21 (1)

[a] $P<0.05$ vs control
[b] $P<0.01$ vs control
[c] $P<0.001$ vs control

Preincubation of the vesicles for various times with oleic or stearic acids did not demonstrate a time-dependent increase in the inhibitory effect of a given concentration of either fatty acid, nor was the inhibitory effect reduced when the reaction was started by adding the vesicles to a complete reaction mixture containing the fatty acid. In the range between 0.6 and 9 μM, Ca_o had no significant effect on the potency of either fatty acid.

The association of oleic and stearic acids with sarcoplasmic reticulum vesicles was examined to determine whether the different potencies of these fatty acids were related to different degrees of association with the membranes. The vesicles concentrated at the 10%/80% sucrose interface after centrifugation were collected and analyzed for protein and ^{14}C-labelled fatty acid as described in Materials and Methods. Less than 10% of the labelled fatty acids were collected at the interface in the absence of the vesicles, so that most of the radioactivity that appeared at this interface in the presence of the vesicles could be attributed to association of fatty acids with the sarcoplasmic reticulum. The amounts of oleic and stearic acids collected at the interface increased when the fatty acid concentrations were increased from 0.16 to 32 μM in the presence of 6 μg/ml vesicles in a complete reaction mixture. Approx 80% of both fatty acids were associated with the sarcoplasmic reticulum vesicles under the conditions of these experiments, and no significant differences were found between oleic and stearic acids.

To examine the possibility that the different inhibitory potencies of these fatty acids on initial calcium uptake velocity at 25°C (Table I) could be explained by an interaction of the hydrocarbon chains with specific physical states of the membrane, the effect of lowering temperature on the inhibition of initial calcium uptake velocity by oleic and steartic acids was investigated. Oleic acid

was a less potent inhibitor at 5°C than at 25°C in that half-maximal inhibition by oleic acid increased from 4 μM at 25°C to approx. 8 μM at 5°C, and the extent of inhibition by 4 μM oleic acid decreased from 57% to 10% when temperature was lowered (Table II). In contrast, the slight inhibitory effect of stearic acid was not significantly affected by lowering the temperature from 25 to 5°C. A statistically significant stimulation of initial calcium uptake velocity by low oleic acid concentrations appeared at the lower temperature (Table II), whereas at 25°C there was no evidence for a stimulatory effect of 1–2 μM (Table I) or lower (0.1–1.0 μM, data not shown) oleic acid concentrations.

Effect on calcium content at the time of initial maximum of calcium content

To evaluate the possibility that the previously reported time-dependent changes in calcium influx and efflux rates that occur spontaneously during the calcium uptake reaction [2] are accompanied by changes in the physical state of the membrane phospholipids, we examined the effects of oleic and stearic acids added at the initial maximum of calcium content, where calcium influx rate had slowed and calcium efflux was rapid [2]. Low concentrations of oleic and stearic acids when added at this time affected the calcium uptake reaction differently than when the fatty acids were added at the start of the reaction (Fig. 2). A low concentration (2 μM) of either fatty acid added at the time of initial maximum of calcium content caused renewed calcium uptake (Fig. 2). At concentrations between 1 and 4 μM, oleic and stearic acids added at this time caused calcium content to increase, whereas 8 and 16 μM concentrations caused net calcium release (Fig. 3).

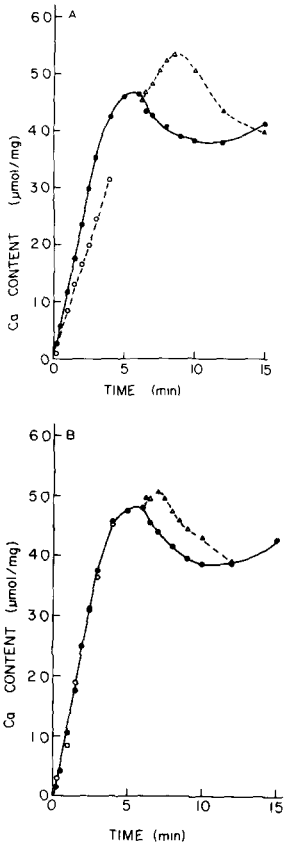

Fig. 2 Effect of addition of 2 μM oleic (A) or stearic (B) acid on calcium content. Reaction conditions were as described in Materials and Methods. $CaCl_2$ concentrations were 60 μM. Control (●); fatty acid added at zero time (○); fatty acid added at $t=6$ min, at the time of the initial maximum of calcium content (△)

TABLE II

INHIBITION OF INITIAL CALCIUM UPTAKE VELOCITY BY OLEIC AND STEARIC ACIDS AT 5°C

Values are mean ± S.E., numbers in parentheses are the number of individual determinations. $CaCl_2$ concentration was 30 μM, vesicles were preincubated for 8 min prior to the start of the reactions with MgATP. Other conditions are described in Materials and Methods.

Fatty acid concn (μM)	Initial calcium uptake velocity (μmol/mg per min)	
	Oleic acid	Stearic acid
0	0.050 ± 0.010 (6)	0.050 ± 0.010 (6)
1.0	0.054 ± 0.005 (6)	0.051 ± 0.007 (6)
2.0	0.066 ± 0.008 (6) [a]	0.045 ± 0.005 (6)
4.0	0.051 ± 0.008 (6)	0.038 ± 0.005 (6) [a]
8.0	0.018 ± 0.007 (6) [a]	0.050 ± 0.005 (6)
16.0	0.001 ± 0.001 (4) [a]	0.032 ± 0.007 (4)

[a] $P<0.05$ vs. control

Effect on unidirectional calcium fluxes at the time of the initial maximum of calcium content

Calcium influx and efflux rates at 25°C were measured to determine whether the ability of low

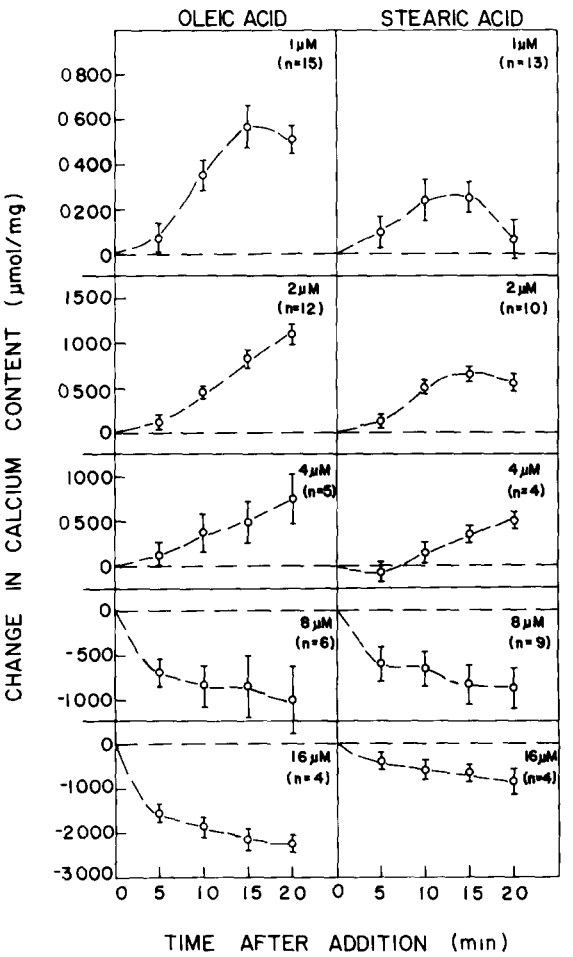

Fig 3 Effect of oleic and stearic acids added at the initial maximum of calcium content The control calcium contents were normalized and plotted as zero on the abcissa (dashed line) The change in calcium content after fatty acid addition is plotted as the mean ± S E of the net change from control The number of determinations is in parentheses Zero time represents the time of fatty acid addition Reaction conditions as in Fig 2 except initial $CaCl_2$ concentrations ranged between 48 and 72 μM

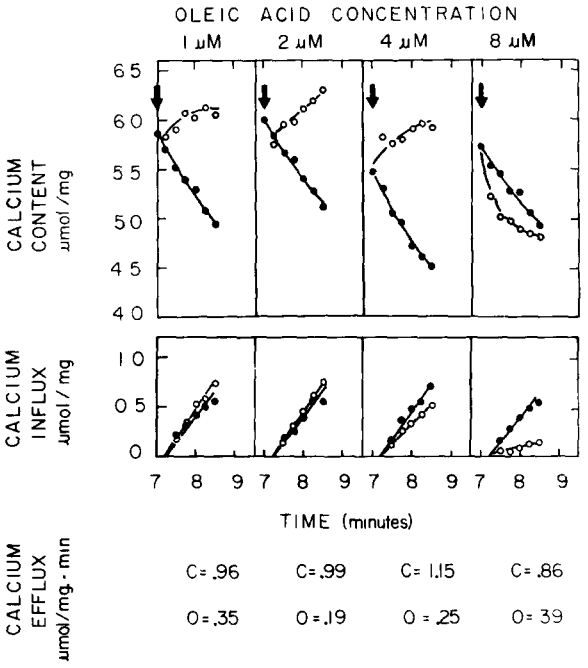

Fig 4 Effect of oleic acid on calcium content, calcium influx and calcium efflux when the fatty acid was added at the initial maximum of calcium content Calcium content (upper curves) was measured at $t = 7$ min as described in Materials and Methods in reactions containing 60 μM $^{45}CaCl_2$ (●), portions of which were transferred into oleic acid (○) Calcium influx (lower curves) was measured in identical reaction mixtures as described in Materials and Methods in the presence (○) or absence (●) of oleic acid added at $t = 7$ min Calcium content and calcium influx were measured every 15 s for 1 5 min after addition of the ^{45}Ca tracer Calcium efflux rates were calculated from calcium content and calcium influx data (see Materials and Methods) (C control, O oleic acid)

fatty acid concentrations to increase calcium content at the time of the initial maximum of calcium content (Figs. 2 and 3) was due to inhibition of calcium efflux, stimulation of calcium influx, or both. In a typical experiment, 1 and 2 μM oleic acid increased calcium influx rate slightly,whereas concentrations above 4 μM inhibited calcium influx (Fig. 4). Calcium efflux was markedly inhibited by 1–8 μM oleic acid concentrations (Fig. 4).

When data from all of at least four replicate experiments were analyzed, 1 and 2 μM oleic acid, and 1, 2, and 4 μM stearic acid caused a 10–20% stimulation of calcium influx rate when added at the time of the initial maximum of calcium content (Fig. 5C). These low fatty acid concentrations, however, induced a more pronounced inhibition (up to 80%) of calcium efflux rate at this time (Fig. 5D).

Calcium influx rate at the time that calcium content reached its initial maximum at 25°C was half-maximally inhibited by 7–8 μM concentrations of both fatty acids, whereas calcium efflux was inhibited 50% by 1 μM oleic acid and 2 μM stearic acid (Fig. 5C). At a higher concentration of

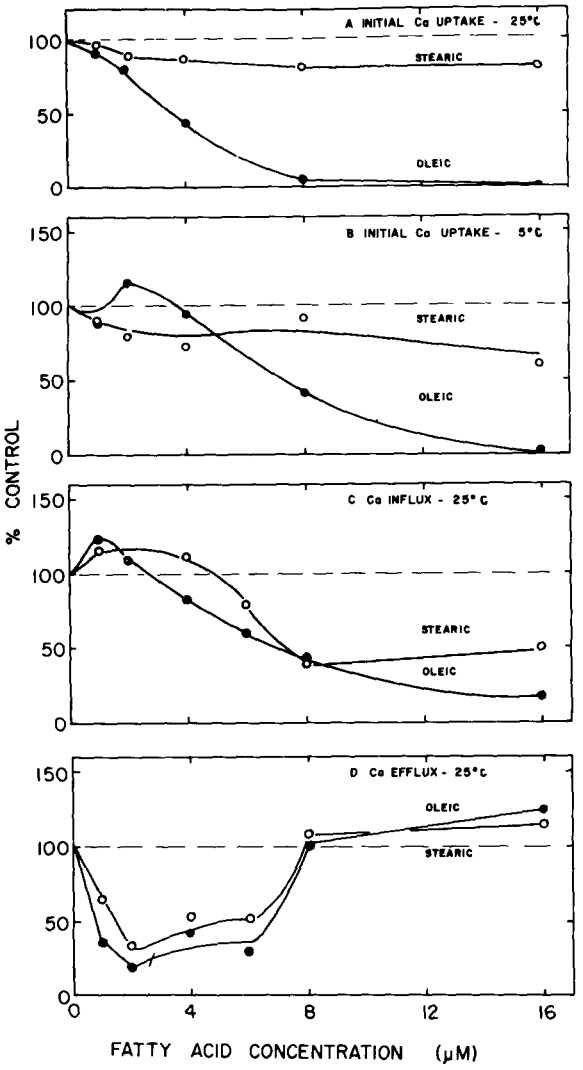

Fig 5 Effects of oleic (●) and stearic (○) acids on initial calcium uptake velocity at 25°C (A) and 5°C (B), and calcium influx (C) and calcium efflux (D) measured at the time of maximum calcium content Data were normalized by setting control values as 100% In C, the stimulation of calcium influx by 1 μM oleic acid was statistically significant ($P<0.05$) as was that by 1 and 4 μM stearic acid ($P<0.05$ and $P<0.01$, respectively) In D, inhibition of calcium efflux by 1–6 μM oleic and stearic acids was also statistically significant Normalized data from Tables I and II are presented in A and B, respectively

16 μM, both fatty acids significantly inhibited calcium influx and tended to increase calcium efflux.

Effects on adenosine triphosphatase activity

At concentrations of 2 μM, oleic and stearic acids slightly inhibited ATPase activity during initial calcium uptake at 25°C (Table III). This inhibitory effect did not increase significantly at fatty acid concentrations up to 8 μM. When added later during the reaction, at the time of the initial maximum of calcium content, both fatty acids stimulated ATPase activity (Table III).

TABLE III

EFFECTS OF OLEIC AND STEARIC ACIDS ON ATPase ACTIVITY AT 25°C

Values are mean±S.E of the difference in ATPase activity compared to a control reaction Numbers in parentheses are the number of individual determinations $CaCl_2$ concentration was 60 μM, other reaction conditions as described in Materials and Methods

Fatty acid	Percent of control	
	Addition at $t=0$	Addition at $t=7$ min
2 μM stearic	80± 8 (6)	117±4 (6)
2 μM oleic	91± 3 (4)	131±3 (4)
8 μM stearic	93±10 (3)	233±1 (3)
8 μM oleic	72± 7 (3)	113±7 (3)

Discussion

The present findings confirm and extend previous reports that oleic acid inhibits calcium uptake by sarcoplasmic reticulum vesicles [8–10]. A possible artefact, that the fatty acid caused the Millipore filters to become leaky as recently described in the case of diethyl ether [22], is excluded by the finding that depending on conditions, fatty acids could either increase or decrease measured calcium content (Figs. 2 and 3), and by the absence of detectable protein in the filtrate following Millipore filtration of vesicles (12 μg/ml) in the presence of these fatty acids. There was no significant binding of calcium to the filters either in the presence or absence of the fatty acids.

The fatty acid concentrations in the present study were well below the critical micelle concentration of approx. 100 μM in pure solution [23]. The ability of higher fatty acid concentrations (>8 μM) to cause calcium release and inhibition of calcium influx could reflect formation of mixed

micelles containing phospholipids removed from the sarcoplasmic reticulum membrane by a 'detergent' action of the fatty acids. However, even at concentrations just below that at which pure stearic acid formed turbid solutions, this fatty acid had relatively little inhibitory effect on initial calcium uptake velocity.

The sarcoplasmic reticulum preparations used in these studies have a phospholipid:protein ratio of approximately 1 μmol/mg (Watras, J., unpublished data) so that a protein concentration of 6 μg/ml corresponds to a membrane phospholipid concentration of approx. 6 μM. As most of the added fatty acid becomes associated with the vesicles, the effects of 1 μM fatty acid on 6 μg/ml vesicular protein occur at a fatty acid:membrane phospholipid molar ratio of 1:6, and a fatty acid:calcium pump ATPase protein ratio of 100:6, assuming the latter to have a molecular weight of 10^5.

The inhibitory effects of different fatty acids on initial calcium uptake velocity appear unlikely to have resulted from changes in overall membrane structure, such as membrane expansion, because the extent of inhibition was dependent on the structure of the fatty acyl chain (Fig. 1). All of the saturated fatty acids tested at a concentration of 8 μM inhibited calcium uptake velocity by less than 30% and showed a tendency for inhibition to decrease as chain length increased from 14 to 20 carbons. A single *cis* double bond decreased the inhibitory effect of the C_{14} fatty acids, had little effect in the case of the C_{16} fatty acids, and markedly potentiated the inhibitory effects of the C_{18} and C_{20} fatty acids. A single *trans* double bond in the C_{18} fatty acid was associated with less inhibition than was seen with the corresponding *cis* unsaturated fatty acid, and increasing the number of *cis* double bonds decreased the inhibitory effects of the C_{18} fatty acids. The wide disparity between the potencies of fatty acids with different degrees of saturation but similar chain length, and between stereoisomers, may be explained if effects on calcium transport resulted from specific interactions of the fatty acids with a region of the phospholipid membrane intimately related to the calcium pump protein. A suggested correlation between melting temperature and the inhibitory potency of a number of fatty acids on acetylcholine receptor function [25] is not confirmed by the data in Fig. 1.

The lack of correlation between the inhibitory effects of 2 μM oleic and stearic acids on initial calcium uptake velocity (Table I) and the concurrent rate of ATP hydrolysis (Table III) indicates that inhibition of initial calcium uptake velocity is not due simply to 'uncoupling' of the calcium pump. Even at fatty acid concentrations above 8 μM, inhibition of calcium uptake cannot be explained by a non-specific increase in membrane permeability as these fatty acid concentrations increased calcium efflux velocity only slightly (Fig. 5) and 8 μM oleic acid did not disrupt vesicle structure examined in electron microphotographs. The greater potency of oleic than stearic acid in causing inhibition of initial calcium uptake velocity (Fig. 5A) is similar to the previously reported sensitivity of the $(Na^+ + K^+)$-activated ATPase activity of rat brain to these fatty acids [26]. These differences may be related to a greater ability of the unsaturated fatty acid to insert into a fluid domain of the membrane [27] that is intimately related to the calcium pump ATPase protein in the sarcoplasmic reticulum. The finding that the sarcoplasmic reticulum calcium pump ATPase is more active when the membrane phospholipids contain unsaturated than saturated fatty acyl chains [28,29] is in accord with the view that the phospholipid structure most favorable for calcium pump function is a fluid rather than a gel structure. The lower inhibitory potency of the saturated fatty acids on initial calcium uptake at 25°C (Figs. 1 and 5) may therefore be explained if they are excluded from such a fluid domain.

The transition from initial calcium uptake, where calcium efflux rate is at or near zero [2], to spontaneous calcium release, where calcium efflux rate exceeds that of calcium influx, is accompanied by changes in the relative sensitivity of calcium influx to oleic and stearic acids (Fig. 5C). A simple explanation for the reduced sensitivity of calcium influx during the phase of calcium release to the inhibitory effects of oleic acid, and the increased sensitivity to stearic acid (compare Fig. 5C with 5A) is that the phospholipid region surrounding the calcium pump assumes a less fluid conformation during calcium release. This explanation would predict that the incorporation of oleic acid in this

region of the membrane would be reduced during calcium release, while that of stearic acid would be increased. This hypothesis was tested by examining the effects of oleic and stearic acids on initial calcium uptake velocity at 5°C (Fig. 5B), assuming that the reduction in temperature would tend to order this region of the phospholipid membrane. It has been reported previously that lowering temperature from 22 to 0°C partially uncouples calcium transport from ATP hydrolysis [30,31], and that the calcium pump assumes a new conformation at the lower temperature [32]. These findings offer some support to the present hypothesis that the phospholipid environment of the calcium pump becomes less fluid during the spontaneous transition from initial calcium uptake to the state of rapid calcium efflux as ATP hydrolysis becomes uncoupled from calcium transport during this transition [2]. As predicted by this hypothesis, the inhibitory effects of oleic acid on initial calcium uptake velocity were significantly reduced at 5°C, the concentration producing half-maximal inhibition increasing from approximately 4 μM to 8 μM (compare Fig. 5B with 5A). However, the reduction in temperature did not significantly increase the sensitivity to stearic acid. The present findings, therefore, provide only partial support for the hypothesis that the transition from initial calcium uptake to the phase of spontaneous calcium release, and the associated increase in calcium efflux rate, is accompanied by an ordering of the phospholipids surrounding the calcium pump.

The appearance of a stimulatory effect of low oleic and stearic acid concentrations on calcium influx at the time of the initial maximum of calcium content (Fig. 5C), an effect not seen in the case of initial calcium uptake velocity at 25°C (Table I, Fig. 5A), might also be partly explained if ordering of the membrane lipids surrounding the calcium pump alters the effects of the fatty acids. This hypothesis is supported by the finding that low oleic acid concentrations stimulated initial calcium uptake at 5°C; however stearic acid did not stimulate initial calcium uptake at the lower temperature (Table II, Fig. 5B). Calcium efflux at the time of the initial maximum of calcium content at 25°C was markedly inhibited by both fatty acids (Fig. 5D), but there was no clear reciprocity between stimulation of calcium influx and inhibition of calcium efflux.

It is well established that the calcium pump can mediate both an active transport of calcium into sarcoplasmic reticulum vesicles [33,34]; and, when Ca_o is reduced in the presence of low ATP, and high ADP and P_i concentrations, a calcium efflux that is accompanied by ATP resynthesis [35,36]. These vesicles are also capable of a calcium efflux that is not accompanied by ATP resynthesis [7], and which is increased by high Ca_o [1,2,7,37]. This latter process corresponds to the calcium efflux at the time of maximal calcium content described in the present report, and may also be mediated by the calcium pump [1,2,7]. The spontaneous transition from initial calcium uptake, characterized by rapid calcium influx and very slow calcium efflux, to the state when calcium content reaches a maximum, where calcium efflux becomes rapid and calcium influx slow [2], is accompanied by a change in the sensitivity of calcium influx to oleic and stearic acids. As the interactions of these fatty acids with the phospholipid bilayer can be influenced by the fluidity of the membrane [27], the spontaneous change in the functional state of the sarcoplasmic reticulum may be accompanied by a change in the physical state of a critical region of the bilayer that influences the calcium fluxes that are mediated by the calcium pump.

Acknowledgments

This study was supported by Research Grants HL-21812, HL-22135 and HL-26903 from the National Institutes of Health, and the American Heart Association. One of us (J.W.) is a Research Fellow, supported by National Institutes of Health Training Grant HL-07420.

References

1 Katz, A M., Repke, D.I., Dunnett, J and Hasselbach, W (1977) J Biol Chem. 252, 1950–1956
2 Katz, A M, Louis, C.F, Repke, D I, Fudyma, G, Nash-Adler, P, Kupsaw, R. and Shigekawa, M (1980) Biochim Biophys. Acta 596, 94–107
3 Ebashi, S and Ohnishi, T. (1963) J Biochem (Tokyo) 54, 506–511
4 Sreter, F A (1969) Arch Biochem Biophys 134, 24–33
5 Huxtable, R and Bressler, R (1974) J Membrane Biol 17, 189–197
6 Sorenson, M M. and DeMeis, L (1977) Biochim. Biophys Acta 465, 210–223

7 Katz, A M , Repke, D I , Fudyma, G and Shigekawa, M (1977) J. Biol Chem. 252, 4210–4214
8 Hasselbach, W and Makinose, M (1962) Biochem Biophys. Res Commun 7, 132–136
9 Sarzala, M G. and Drabikowski, W (1969) Life Sci 8, 477–483
10 Fiehn, W. and Migala, A (1971) Eur J Biochem 20, 245–248
11 Agostini, B and Drabikowski, W (1969) J Submicrosc Cytol. 1, 207–214
12 Messineo, F C , Pinto, P B and Katz, A M. (1980) J Mol Cell Cardiol. 12, 725–732
13 Barry, J R , Tate, L A , Van Winkle, B , Wood, J M and Entman, M L (1978) Life Sci 23, 391–402
14 Adams, R J , Cohen, D W , Gupte, S , Johnson, J D , Wallick, E.T , Wang, T and Schwartz, A. (1979) J Biol Chem 254, 12404–12410
15 Raz, A. and Livne, A (1973) Biochim Biophys Acta 311, 222–229
16 Katz, A.M , Repke, D I and Hasselbach, W (1977) J Biol Chem 252, 1938–1949
17 Reynard, A M , Hass, L F , Jacobsen, D D. and Boyer, P D (1961) J Biol Chem 236, 2257–2263
18 Meissner, G (1975) Biochim Biophys Acta 389, 51–68
19 Caswell, A H , Lau, Y H and Brunschwig, J -P (1976) Arch Biochem Biophys 176, 417–430
20 Katz, A M , Repke, D I , Upshaw, J E and Polascik, M A (1970) Biochim Biophys Acta 205, 473–490
21 Bradford, M M. (1976) Anal Biochem 72, 248–254
22 Salama, G and Scarpa, A (1980) J Biol Chem 255, 6525–6528
23 Mukerjee, P and Mysels, K J (1971) Critical Micelle Concentrations in Aqueous Surfactant Systems, Natl Bur Standards, NSRDS-NBS 36, Washington, DC
24 Seeman, P (1972) Pharmacol. Rev 24, 583–655
25 Andreasen, T J and McNamee, M G (1980) Biochemistry 19, 4719–4720
26 Ahmed, K and Thomas, B.S (1971) J Biol Chem 246, 103–109
27 Klausner, R D , Kleinfeld, A M , Hoover, R L and Karnovsky, M J (1980) J Biol Chem. 255, 1286–1295
28 Hidalgo, C , Thomas, D D and Ikemoto, N (1978) J Biol Chem 253, 6879–6887
29 Bennett, J , McGill, H and Warren, G B (1980) in Current Topics in Membranes and Transport (Bronner, F and Kleinzeller, A , eds) 128–164, Academic Press, New York
30 Sumida, M and Tonomura, Y (1974) J Biochem (Tokyo) 75, 283–297
31 Ikemoto, N (1975) J Biol Chem 250, 7219–7224
32 Masuda, H and DeMeis, L (1977) J Biol Chem 252, 8567–8571
33 Ebashi, S and Lipmann, F (1962) J Cell Biol 14, 389–400
34 Hasselbach, W and Makinose, M (1961) Biochem Z 333, 518–528
35 Barlogie, B , Hasselbach, W and Makinose, M (1971) FEBS Lett 12, 267–268
36 Kanazawa, T , Yamada, S and Tonomura, Y (1970) J Biochem (Tokyo) 68, 593–595
37 Chiesi, M and Inesi, G (1979) J Biol Chem 254, 10370–10377