Biochimica et Biophysica Acta 897 (1987) 152–158
Elsevier

BBA 73347

Modulation of Na^+-Ca^{2+} exchange and Ca^{2+} permeability in cardiac sarcolemmal vesicles by doxylstearic acids

Kenneth D. Philipson and Robert Ward

Departments of Medicine and Physiology and the American Heart Association Greater Los Angeles Affiliate, Cardiovascular Research Laboratories, University of California, Los Angeles, Center for the Health Sciences, Los Angeles, CA 90024 (U.S.A.)

(Received 5 August 1986)

Key words: Sodium–calcium ion exchange; Sarcolemmal membrane; Calcium ion permeability; Doxylstearic acid

We examine the effects of 5-, 12- and 16-doxylstearic acids on the Na^+-Ca^{2+} exchange and passive Ca^{2+} permeability of cardiac sarcolemmal vesicles. Stearic acid is a weak stimulator of Na^+-Ca^{2+} exchange. A doxyl moiety potentiates stimulation with the order of increasing potency being 5-, 12- and then 16-doxylstearic acid. Stearic acid has little effect on vesicle Ca^{2+} permeability but again the doxylstearates are more effective. The sequence of potency is reversed, however, from that for increasing Na^+-Ca^{2+} exchange. 5-Doxylstearic acid most markedly exchances passive Ca^{2+} flux followed by the 12-, and then 16-doxylstearic acids. Methyl esters of the doxylstearates have no effect on either Na^+-Ca^{2+} exchange or Ca^{2+} permeability. We model the results as follows. For a fatty acid to stimulate Na^+-Ca^{2+} exchange activity, an anionic charge is required to interact with the exchanger protein at the membrane surface. Stimulation is potentiated by a perturbation (such as provided by a doxyl group) within the lipid bilayer. The perturbation is most effective at a location towards the center of the bilayer. To increase passive Ca^{2+} permeability an anionic charge is again essential. Disorder within the bilayer is also important, but now the most important site is near the membrane surface. Results of experiments with linolenic and γ-linolenic acid and previous studies with other fatty acids also support this model.

Introduction

The Na^+-Ca^{2+} exchange system of cardiac sarcolemma catalyzes a highly active countertransport of Na^+ for Ca^{2+}. The exchange is electrogenic with a probable stoichiometry of 3 Na^+ for 1 Ca^{2+} [1–4]. Na^+-Ca^{2+} exchange can be demonstrated in intact cardiac muscle under a variety of experimental conditions, but physiological significance is still poorly defined and controversial (for reviews, see Refs. 5 and 6). Recently, experiments using isolated cardiac sarcolemmal vesicles have enhanced our knowledge of the Na^+-Ca^{2+} exchange process (for reviews, see Refs. 7 and 8).

In a series of studies [9–13], we have reported on sensitive interactions between the Na^+-Ca^{2+} exchange and charged components in the membrane lipid environment. Anionic phopsholipids, fatty acids, and other anionic amphiphiles all stimulate Na^+-Ca^{2+} exchange activity. In contrast, cationic amphiphiles are inhibitors of Na^+-Ca^{2+} exchange. In all cases, the exchanger was

Abbreviations: doxyl, dimethyloxazolidinyl; Mops, 4-morpholinepropanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

Correspondence: K.D. Philipson, Cardiovascular Research Laboratory, A3-381 CHS, UCLA School of Medicine, Los Angeles, CA 90024, U.S.A.

more sensitive to membrane perturbation than other sarcolemmal transporters such as the ($Na^+ + K^+$)-ATPase or the ATP-dependent Ca^{2+} pump.

We found that unsaturated fatty acids stimulated exchange more potently than saturated fatty acids [13]. We speculated that the unsaturation increased the stimulation of Na^+-Ca^{2+} exchange by disordering the lipid bilayer in the exchanger microenvironment. In the present study we further explore this model. We use stearic acid labelled at various positions with a doxyl group. Doxylstearates are commonly used as nonperturbing spin probes to report on lipid structure, but several studies indicate that the doxyl group disturbs its immediate environment (e.g., Refs. 14–16). We use the bulky doxyl group to perturb the exchanger at different depths within the lipid bilayer. We obtain information on which locations within the bilayer are most important for regulating both Na^+-Ca^{2+} exchange and passive Ca^{2+} permeability.

Materials and Methods

Highly purified sarcolemmal vesicles were isolated from canine ventricles as previously described [17] with the following modifications: (1) the DNAase treatment uses DNAase I (type DP) from Cooper Biomedical (Malvern, PA) (approximately 100 mg/dog heart); (2) the centrifugation after polytron treatment has been increased to $17\,000 \times g$ for 15 min; (3) the 34% sucrose layer has been eliminated from the discontinuous sucrose gradient and the volume of the 32% sucrose layer has been increased to 8 ml. The sarcolemmal fraction was collected as described, diluted with 140 mM NaCl, 10 mM Mops/Tris (pH 7.4), spun down ($160\,000 \times g$, 75 min), resuspended in 140 mM NaCl, 10 mM Mops/Tris (pH 7.4), and stored in liquid nitrogen in small aliquots.

Na^+-Ca^{2+} exchange was determined as Na_i^+-dependent Ca^{2+} uptake using methods developed previously for use in the presence of fatty acids [13]. Na^+ (140 mM) -loaded sarcolemmal vesicles (1.5 mg/ml) and $CaCl_2$ ($^{40}Ca^{2+}$ plus $^{45}Ca^{2+}$) were suspended on the side of a polystyrene tube as separate small droplets (0.005 ml) above the reaction medium (0.24 ml). The uptake solution contained 140 mM KCl (or NaCl for blanks), fatty acid as described, and 0.4 μM valinomycin. All solutions also contained 10 mM Mops/Tris buffer (pH 7.4 at 37°C) and were at 37°C. Ca^{2+} uptake was initiated by rapid mixing and stopped by the automated addition of quench solution (0.03 ml of 140 mM KCl plus either 10 mM EGTA or 1 mM $LaCl_3$). If the quench solution continued EGTA, then 1 ml of cold 140 mM KCl, 1 mM EGTA was added immediately after quenching, and 1 ml was taken for filtration (Sartorius, 0.45 μm). When La^{3+} was in the quench solution, 0.22 ml were taken for immediate filtration. Filters were washed with 2×3 ml cold 140 mM KCl, 1 mM EGTA. By mixing both the vesicles and the Ca^{2+} into the uptake medium at the initiation of the reaction, we avoid preincubation of fatty acids with Ca^{2+} for extended periods. This allows measurements of Na^+ gradient-dependent Ca^{2+} uptake without artifacts due to formation of Ca^{2+}-fatty acid aggregates. For further details see Ref. 13.

Passive Ca^{2+} efflux from sarcolemmal vesicles was determined as described previously [13].

All fatty acids were obtained from Sigma and were stored as stock solutions in ethanol at −20°C. The final ethanol concentration during Ca^{2+} flux measurements was less than 0.5% and did not affect measurements. Equal amounts of ethanol were included in tubes to which no fatty acid was added.

Data are expressed as mean ± S.E.

Results

Stimulation of Na^+-Ca^{2+} exchange by doxylstearates

Fig. 1 shows Na_i^+-dependent Ca^{2+} influx in cardiac sarcolemmal vesicles as a function of stearic acid and doxyl derivatives of stearic acid. Stearic acid is a weak stimulator of Na^+-Ca^{2+} exchange activity as described previously [13]. When a doxyl group is included on the molecule, however, stimulation is potentiated and increases as the label is placed further from the hydrophilic head group of the fatty acid. The stimulation decreases at higher concentrations of the fatty acids, most notably for 5-doxylstearic acid. This may be due to inhibitory effects of the fatty acids

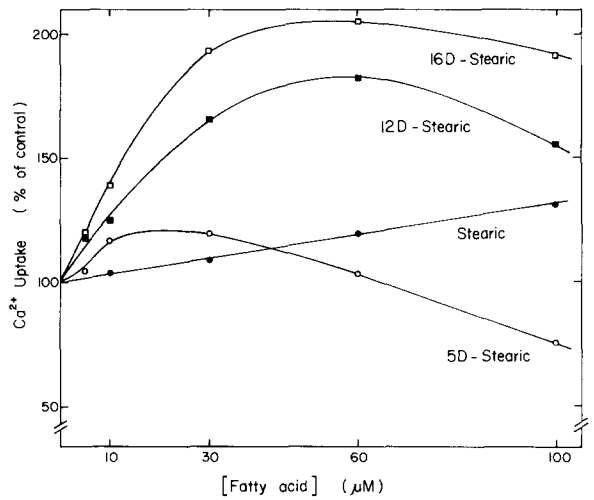

Fig. 1. Dependence of Na^+-Ca^{2+} exchange on doxylstearic acid (D-Stearic) concentration. Na_i^+-dependent Ca^{2+} influx was measured over 1.5 s in the presence of 10 μM Ca^{2+}. Control Na^+-Ca^{2+} exchange activity was 10.6 ± 2.1 nmol/mg protein per s. $n = 3$ or 4. S.E. bars are omitted for clarity but averaged $6.2 \pm 1.3\%$ of mean values.

on Na^+-Ca^{2+} exchange or to increased vesicle Ca^{2+} permeability (see below).

Experiments with methyl esters of doxylstearates were also performed. None of the doxylstearate methyl esters had significant effects on Na^+-Ca^{2+} exchange or passive Ca^{2+} permeability at concentrations up to 100 μM (not shown). This is consistent with our earlier studies [13] which showed that the anionic carboxyl group on a fatty acid was essential for perturbing the sarcolemmal membrane.

Fatty acids will rapidly partition into biological membranes. We used the same amount of sarcolemmal protein in all Ca^{2+} uptake experiments so that fatty acid-to-protein ratios would not vary (at a particular fatty acid concentration) and different experiments could be compared.

For all Ca^{2+} uptake experiments, blanks were performed by diluting the Na^+-loaded vesicles into Ca^{2+} uptake media containing NaCl instead of KCl. The small amount of Ca^{2+} uptake (typically, less than 3% of Ca^{2+} uptake in KCl media) was subtracted, and this corrected for any Ca^{2+} movements which were not Na^+-gradient dependent.

Time course of Na^+-Ca^{2+} exchange in the presence of doxylstearates

Na_i^+-dependent Ca^{2+} uptake was approximately linear for about 2.5 s in the presence and absence of doxylstearic acids. After early times, however, the stimulatory effect of the doxylstearates became less apparent. For example, in one set of experiments, 30 μM 16-doxylstearic acid stimulated Na^+-Ca^{2+} exchange by $123 \pm 23\%$ after 1.5 s of uptake, but the stimulation declined to $39 \pm 3\%$ and $14 \pm 11\%$ after 6 and 20 s of Ca^{2+} uptake, respectively ($n = 3$).

Ca^{2+} concentration dependence of Na^+-Ca^{2+} exchange in the presence of 16-doxylstearic acid

Both the V_{max} and the Ca^{2+} affinity of Na^+-Ca^{2+} exchange were increased by 16-doxylstearic acid (30 μM). Initial rates of Na_i^+-dependent Ca^{2+} uptake were measured at $[Ca^{2+}] = 4$, 7.5, 15, 25, 40, and 60 μM. The V_{max} increased from 27.5 ± 2.8 to 39.4 ± 3.0 nmol/mg protein per s, and apparent K_m (Ca^{2+}) declined from 20.4 ± 2.5 to 13.7 ± 1.3 μM ($n = 3$).

Effects of doxylstearic acids on passive Ca^{2+} permeability

Ca^{2+}-loaded sarcolemmal vesicles were exposed to doxylstearic acids and the subsequent loss of Ca^{2+} was measured (Table I). As noted previously [13], stearic acid has no effect on vesicle leakiness. Doxyl-labelled stearates, however, induce passive

TABLE I

EFFECTS OF DOXYLSTEARIC ACIDS ON PASSIVE Ca^{2+} EFFLUX

Vesicles were first preloaded with Ca^{2+} by Na_i^+-dependent Ca^{2+} uptake as described in Materials and Methods. Passive Ca^{2+} loss from the vesicles was measured over a 2.0 min period. Fatty acids were present only during the efflux period at a concentration of 20 μM. The Ca^{2+} load, prior to initiation of Ca^{2+} efflux, was 41.3 ± 2.5 nmol/mg protein ($n = 3$).

Addition	Ca^{2+} efflux (nmol/mg per 2 min)
None	10.7 ± 2.8
Stearic acid	9.9 ± 1.3
5-Doxylstearic acid	26.4 ± 2.0
12-Doxylstearic acid	16.8 ± 2.1
16-Doxylstearic acid	14.9 ± 1.1

Ca^{2+} efflux. The 5-doxylstearic acid was most potent, followed by 12- and then 16-doxylstearic acid. This sequence is opposite to the potency sequence for stimulation of Na^+-Ca^{2+} exchange (Fig. 1). Although the standard errors are relatively large in Table I, the same pattern was seen in each experiment and in preliminary experiments performed at other concentrations of the doxylstearic acids.

Effects of linolenic acids on sarcolemmal Ca^{2+} transport

In the experiments described above, we used the doxylstearic acids to perturb the lipid bilayer at different depths. Other molecules appropriate for such studies are linolenic acids which are available in the usual form (*cis* double bonds in the 9, 12, and 15 positions) and as γ-linolenic acid (*cis* double bonds in the 6, 9, and 12 positions). We examined the effects of these two fatty acids on Na^+-Ca^{2+} exchanged and passive Ca^{2+} permeability. Na_i^+-dependent Ca^{2+} influx experiments were performed using the same format as for the experiments shown in Fig. 1. Similar stimulations for the two fatty acids were seen at all concentrations with maxima at 60 μM fatty acid. At 60 μM, linolenic acid stimulated exchange by $87.3 \pm 13.9\%$ whereas γ-linolenic acid stimulated exchange by $74.7 \pm 9.2\%$ ($n = 3$).

Effects of linolenic and γ-linolenic acids on passive Ca^{2+} permeability were measured using the same procedure as for the experiments shown in Table I. Vesicles were preloaded with 52.3 ± 8.2 nmol Ca^{2+}/mg protein, and subsequent Ca^{2+} efflux was followed over 2 min. Control vesicles lost 13.9 ± 2.6 nmol Ca^{2+}. In the presence of linolenic or γ-linolenic acid (30 μM), vesicles lost 23.6 ± 1.6 or 30.4 ± 2.8 nmol Ca^{2+}, respectively ($n = 4$).

Effects of Ca^{2+} ionophore A23187 on Na^+-Ca^{2+} exchange and passive Ca^{2+} permeability

In the experiments described above, Ca^{2+} efflux is measured over 2.0 min, whereas the much more rapid Na^+-Ca^{2+} exchange is usually measured for 1.5 s. Moderate increases in vesicle Ca^{2+} leakiness will have little effect on Na_i^+-dependent Ca^{2+} uptake measurements since the magnitude of the rate of Ca^{2+} flux in the uptake experiments is much greater than that in passive flux studies. Because fatty acids affect both Na^+-Ca^{2+} exchange and passive Ca^{2+} flux, we designed an experiment to demonstrate the insensitivity of Na^+-Ca^{2+} exchange measurements to small increases in passive Ca^{2+} flux.

Sarcolemmal vesicles were exposed to various concentrations of Ca^{2+} ionophore A23187 during both Na^+-Ca^{2+} exchange and Ca^{2+} efflux experiments (Fig. 2). As A23187 concentration is increased, passive Ca^{2+} efflux also markedly increases (Fig. 2, bottom). The effects of these concentrations of ionophore on Na_i^+-dependent Ca^{2+} uptake influx, however, are much less noticeable (Fig. 2, top). Although 0.3 μM A23187 causes over 80% of a Ca^{2+} load to passively leak from vesicles over a 2.0 min period, this concentration of ionophore only causes an apparent inhibition of Na^+-Ca^{2+} exchange of 15%. Lower concentrations of ionophore induce a passive leak of Ca^{2+} from

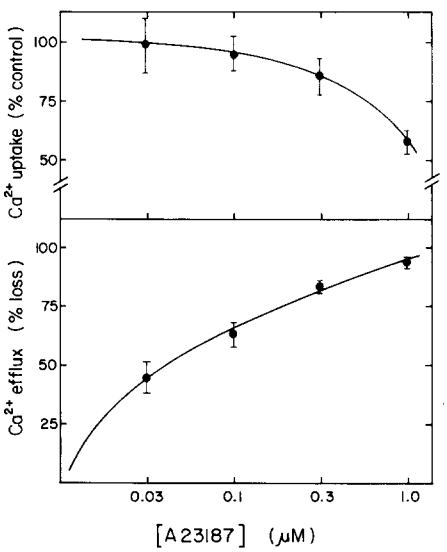

Fig. 2. Effects of Ca^{2+} ionophore on Na^+-Ca^{2+} exchange and passive Ca^{2+} efflux. Na_i^+-dependent Ca^{2+} uptake (top) was measured for 1.5 s at $Ca^{2+} = 10$ μM. Control (100%) activity was 6.5 ± 0.9 nmol/mg protein per s. Passive Ca^{2+} efflux (bottom) was measured as described in Table I. The 100% Ca^{2+} load is that Ca^{2+} remaining within the vesicles after a 2.0 min efflux period in the absence of ionophore (48.7 ± 10.7 nm/mg protein). Data are expressed as the percentage of this Ca^{2+} load which is lost as a result of the presence of ionophore. See Materials and Methods for further experimental details. $n = 3$–5.

vesicles but have no effect on the initial rate of Na^+-Ca^{2+} exchange.

Discussion

Na^+-Ca^{2+} exchange is sensitive to charged lipid components in the sarcolemmal membrane [9–13,18]. Apparently, the charged groups interact with the exchanger at the membrane surface and modulate transport activity. Anionic amphiphilic molecules enhance and cationic amphiphiles diminish exchange activity. We previously noted that unsaturated fatty acids stimulate sarcolemmal Na^+-Ca^{2+} exchange more potently than saturated fatty acids [13]. *Cis* double bonds in the hydrophobic portion of fatty acids will produce bends in the molecule and will induce local disorder. We speculated that local disorder within the bilayer enhanced the stimulation caused by anionic charge at the membrane surface. Methyl esters of fatty acids have no effect on Na^+-Ca^{2+} exchange indicating that stimulation requires an anionic charge.

We use doxylstearic acids to further investigate this model. Doxylstearates are commonly used at nanomolar concentrations in EPR experiments to report on membrane environment. At this low level, doxylstearic acids usually do not cause measurable changes in membrane properties. Nevertheless, the bulky doxyl group is still likely to cause local perturbations [14–16]. We take advantage of the perturbations caused by doxyl groups at different positions on stearic acid. We probe the effect of doxyl groups at different depths in the bilayer on sarcolemmal Na^+-Ca^{2+} exchange and Ca^{2+} permeability.

As previously reported [13], stearic acid, being a saturated fatty acid, has little effect on either Na^+-Ca^{2+} exchange or Ca^{2+} permeability. The perturbing effect of a doxyl group on stearic acid causes stimulation of both Na^+-Ca^{2+} exchange activity and passive Ca^{2+} efflux (Fig. 1 and Table I). The sequences for the ability of the different doxylstearates to increase exchange and permeability, however, are opposite. For Na^+-Ca^{2+} exchange, 16-doxylstearic acid is the most potent stimulator, followed by 12- and 5-doxylstearic acid. In contrast, 5-doxylstearic acid stimulates passive Ca^{2+} flux most potently, and 16-doxylstearic acid increases flux least potently. These results with the doxylstearic acids are consistent with our interpretation of the effects of unsaturated fatty acids. Here, membrane perturbation caused by doxyl groups, instead of unsaturation, produces similar results. The implications of these data are discussed below.

The response of the Na^+-Ca^{2+} exchanger to the doxylstearic acids is qualitatively similar to the response to unsaturated fatty acids [13]. In both cases, there is stimulation of exchange activity at low concentrations of amphiphile but apparent inhibition at high concentrations as vesicle permeability barriers breakdown. We observe an increase in both the Ca^{2+} affinity and maximal velocity of the exchanger after treatment with 16-doxylstearic acid. As seen with other anionic amphiphiles [9,13], only the initial rate of Na^+-Ca^{2+} exchange activity is maximally stimulated by doxylstearic acids. At longer times, Ca^{2+} influx declines and effects of the doxylstearic acids on passive Ca^{2+} permeability become more prominent.

Of the three doxylstearates tested, 5-doxylstearic acid is the weakest stimulator of Na^+-Ca^{2+} exchange activity (Fig. 1), but 5-doxylstearic acid also potently increases vesicle leakiness (Table I). Possibly the effect on Ca^{2+} leakiness influences the Na^+-Ca^{2+} exchange measurements. To address this question we did experiments with the Ca^{2+} ionophore A23187 under conditions identical to those used for the experiments with the doxylstearates. A23187 will increase vesicle Ca^{2+} permeability, as do the doxylstearates, but without simultaneously stimulating Na^+-Ca^{2+} exchange activity. Low concentrations of A23187 cause substantial effects on passive Ca^{2+} efflux without influence on Na_i^+-dependent Ca^{2+} influx (Fig. 2). The explanation is that the fluxes involved in the passive permeability measurements are relatively small and must be measured over 2 min. Na^+-Ca^{2+} exchange is much more active and substantial Ca^{2+} accumulation occurs within the 1.5 s Ca^{2+} uptake period. Thus, moderate increases in vesicle Ca^{2+} leakiness will not affect Na_i^+-dependent Ca^{2+} uptake measurements. The Ca^{2+} leak induced by 30 μM 5-doxylstearic acid (Table I) may be sufficient to cause a small decrease in Na_i^+-dependent Ca^{2+} influx (Fig. 1). However, 5-doxylstearic acid is the weakest stimulator of

Na^+-Ca^{2+} exchange even at 5 or 10 μM (Fig. 1) where permeability effects will not be significant. Thus, the conclusion that Na^+-Ca^{2+} exchange is stimulated least potently by a doxyl group at the 5 position on stearic acid appears valid.

We model the results as shown schematically in Fig. 3. Stimulation of Na^+-Ca^{2+} exchange by doxylstearic acid (or by any anionic amphiphile) is due to interactions within the exchanger microenvironment. The negative charge on the membrane surface at the interface with the exchanger protein is essential. Disorder within the bilayer accentuates the stimulation. The location where disorder is most effective in stimulating Na^+-Ca^{2+} exchange activity is towards the center of the bilayer. This explains why 16-doxylstearic acid is most potent and 5-doxylstearic acid is least potent in enhancing exchange activity. To facilitate the passive movement of Ca^{2+} across the sarcolemma, again anionic charge is required. The perturbing effect of a doxyl group again increases Ca^{2+} transport, but now perturbation is most effective towards the membrane surface, and 5-doxylstearic acid is most potent in increasing Ca^{2+} permeability. It is curious that disruption of the membrane Ca^{2+} barrier requires an amphiphile to have both an anionic charge (methyl esters are ineffective) and an ability to cause disorder within the bilayer. A possible explanation is that, for Ca^{2+} to passively cross the sarcolemmal membrane, two types of barriers must be overcome. Exogenous anionic charge may perturb an electrostatic barrier at the membrane surface allowing Ca^{2+} to enter the bilayer; further perturbation within the bilayer permits translocation of Ca^{2+} across the bilayer.

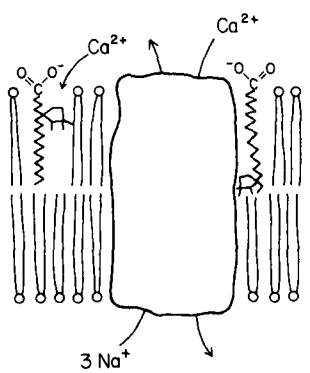

Fig. 3. Model for the regulation of Na^+-Ca^{2+} exchange and passive Ca^{2+} flux by doxylstearic acids. See Discussion for details.

Results obtained from experiments with other fatty acids can also be fit into this model. Linolenic (18 : 3(*cis*-9,12,15)) and γ-linolenic (18 : 3(*cis*-6,9,12)) acids are identical molecules except for the positions of their double bonds. Although the disordering effects of *cis* double bonds will begin closer to the bilayer surface with γ-linolenic acid, both molecules will result in approximately the same perturbation towards the center of the bilayer. That is, in general, *cis* double bonds on fatty acids will introduce some disorder into a membrane at all depths distal to the first double bond. This generalization especially applies to fatty acids with multiple double bonds. Thus, consistent with the experimental results, linolenic and γ-linolenic acids are predicted to have the same stimulatory effects on Na^+-Ca^{2+} exchange. With the initial double bond closer to the membrane surface, however, γ-linolenic acid is predicted to have the larger effect on Ca^{2+} permeability. This prediction is borne out by the experimental result: linolenic acid increases Ca^{2+} permeability by 70%, whereas γ-linolenic acid increases permeability by 119%.

Results from our previous study [13] on the effects of a variety of fatty acids on sarcolemmal Ca^{2+} transport are also consistent with our model. Unsaturated fatty acids with *cis* double bonds generally stimulated Na^+-Ca^{2+} exchange by the same amount. These same molecules also increased passive Ca^{2+} permeability. An apparent anomaly, at the time, was the especially high potency of arachidonic acid to increase passive Ca^{2+} flux. This is now readily understood from the position of the double bonds on arachidonic acid where unsaturation begins at the 5 position. The unsaturation on the other fatty acids with which arachidonic acid was being compared begins at the 9 or 11 position. Consistent with the proposed model, it is disorder near the membrane surface which most effectively increases sarcolemmal Ca^{2+} leak.

Approximations exist in the interpretation of the data. We have no monitor of the quantity of fatty acid which becomes incorporated within the sarcolemma during our measurements. The fatty acids are highly hydrophobic, however, and are

expected to rapidly associate with the membrane. We have previously shown [13] that the effects of fatty acids on sarcolemma are dependent on the ratio of fatty acid to protein. This indicates that a majority of the fatty acid becomes incorporated in the membrane. The results cannot easily be attributed to differential rates of incorporation of the fatty acids. For example, 5-doxylstearic acid is a much weaker stimulator of Na^+-Ca^{2+} exchange than 16-doxylstearic acid. The reason is unlikely to be that 5-doxylstearic acid does not associate with the membrane, since 5-doxylstearic acid so markedly increases passive Ca^{2+} flux. At 5 μM doxylstearic acid, the lowest concentration to stimulate Na^+-Ca^{2+} exchange (Fig. 1), the mole ratio of fatty acid to sarcolemmal phospholipid would be 0.08 if all fatty acid becomes membrane-bound. This indicates that gross changes in the membrane composition are not required to modulate sarcolemmal Ca^{2+} movements. Despite some uncertainties, we are able to fit most data into a simple framework. Our model explains the actions of doxylstearic acids and unsaturated fatty acids on sarcolemmal Na^+-Ca^{2+} exchange and passive Ca^{2+} permeability. Experiments are planned to use this model to further explore the dynamics of sarcolemmal Ca^{2+} transport.

Acknowledgements

This work was supported by Grant HL 27821 from the United States Public Health Service. K.D.P. is an Established Investigator of the American Heart Association with funds contributed in part by the Greater Los Angeles Affiliate.

References

1 Reeves, J.P. and Sutko, J.L. (1980) Science 208, 1461–1464
2 Caroni, P., Reinlib, L. and Carafoli, E. (1980) Proc. Natl. Acad. Sci. USA 77, 6354–6358
3 Philipson, K.D. and Nishimoto, A.Y. (1980) J. Biol. Chem. 255, 6880–6882
4 Reeves, J.P. and Hale, C.C. (1984) J. Biol. Chem. 259, 7733–7739
5 Mullins, L.J. (1979) Am. J. Physiol. 236, C103–C110
6 Langer, G.A. (1982) Annu. Rev. Physiol. 4, 435–449
7 Reeves, J.P. (1985) Curr. Top. Membrane Transp. 25, 77–127
8 Philipson, K.D. (1985) Annu. Rev. Physiol. 47, 561–571
9 Philipson, K.D., Frank, J.S. and Nishimoto, A.Y. (1983) J. Biol. Chem. 258, 5905–5910
10 Philipson, K.D. and Nishimoto, A.Y. (1984) J. Biol. Chem. 259, 16–19
11 Philipson, K.D. (1984) J. Biol. Chem. 259, 13990–14002
12 Philipson, K.D., Langer, G.A. and Rich, T.L. (1985) Am. J. Physiol. 248, H147–H150
13 Philipson, K.D. and Ward, R. (1985) J. Biol. Chem. 260, 9666–9671
14 Bieri, V.G., Wallach, D.F.H. and Lin, P.S. (1974) Proc. Natl. Acad. Sci. USA 71, 4797–4801
15 Butterfield, D.A., Whisnant, C.C. and Chesnut, D.B. (1976) Biochim. Biophys. Acta 426, 697–702
16 Taylor, M.G. and Smith, I.C.P. (1980) Biochim. Biophys. Acta 599, 140–149
17 Frank, J.S., Philipson, K.D. and Beydler, S. (1984) Circ. Res. 54, 414–423
18 Ashavaid, T.F., Colvin, R.A., Messineo, F.C., MacAlister, T. and Katz, A.M. (1985) J. Mol. Cell. Cardiol. 17, 851–861