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Transport of K^+ and Other Cations across Phospholipid Membranes by Nonesterified Fatty Acids

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Abstract. The rate of change of internal pH and transmembrane potential has been monitored in liposomes following the external addition of various cation salts. Oleic acid increases the transmembrane movement of H^+ following the imposition of a K^+ gradient. An initial fast change in internal pH is seen followed by a slower rate of alkalinization. High concentrations of the fatty acid enhance the rate comparable to that seen in the presence of nigericin in contrast to the effect of FCCP (carbonyl cyanide *p*-(tri-fluoromethoxy)phenyl hydrazone) which saturates at an intermediate value. The ability of nonesterified fatty acids to catalyze the movement of cations across the liposome membrane increases with the degree of unsaturation and decreases with increasing chain length. Li and Na salts cause a similar initial fast pH change but have less effect on the subsequent slower rate. Similarly, the main effect of divalent cation salts is on the initial fast change. The membrane potential can enhance or inhibit cation transport depending on its polarity with respect to the cation gradient. It is concluded that nonesterified fatty acids have the capability to complex with, and transport, a variety of cations across phospholipid bilayers. However, they do not act simply as proton/cation exchangers analogous to nigericin nor as protonophores analogous to FCCP. The full cycle of ionophoric action involves a combination of both functions.

Key words: Fatty acids — Liposomes — Cation transport — Membrane transport

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Introduction

The toxic effects of nonesterified fatty acids are well recognized at the physiological level. At high concentrations, they can cause platelet aggregation, damage to cell membranes and can interfere with electrical conduction in nerves and muscle (Newsholme & Leech, 1983). For example, oleic acid rapidly and reversibly decreases electrical coupling between gap junctions in neonatal rat heart cells (Burt, Massey & Minnich, 1991). The rapid increase in free fatty acids following cerebral ischemic insult (Kidooka, Matsuda & Handa, 1987) may have consequences for subsequent brain damage. Nonesterified fatty acids also act as uncouplers of oxidative phosphorylation but the mechanism is still unclear. Some effect appears to be due to an increase in the proton conductivity of the mitochondrial membrane (Macri et al., 1991) but not all of the uncoupling effect can be explained in this way (Labonia, Muller & Azzi, 1988; Thiel & Kadenbach, 1989; Brown & Brand, 1991).

The mechanism of action of fatty acids at the molecular level in these events is not well understood. Nonesterified fatty acids are known to increase the proton conductance of planar lipid membranes (Gutknecht, 1988) despite being relatively inefficient H^+ carriers compared to classical protonophores such as FCCP (McLaughlin & Dilger, 1980). Their presence in trace amounts has even been proposed to explain the very much higher values (by an order of magnitude of 10^6) for the H^+/OH^- membrane permeability compared to other cations (Gutknecht, 1988). Results from planar lipid membranes, however, are not in agreement with those from liposome systems (Deamer & Nichols, 1989).

An extra complication at the molecular level is that nonesterified fatty acids have been shown to be capa-

ble of translocating various cations across artificial and biological membranes, presumably as fatty acid cation soaps (Serhan et al., 1981; Simpson, Moore & Peters, 1988). It has been suggested that some of the effects of fatty acids on proton electrochemical gradients result from their ability to catalyze an electroneutral K^+/H^+ exchange (Cooper, Wrigglesworth & Nicholls, 1990; Wrigglesworth et al., 1990). In the present paper, we report the results of experiments that directly demonstrate the ability of nonesterified fatty acids to catalyze an electroneutral cation/proton exchange across lipid bilayers. This is in addition to their ability to act as simple protonophores. The resulting mixed-transfer capability is sensitive to cation gradients and to the magnitude and polarity of membrane potential. The results explain some of the apparent anomalies of fatty acid action on biological membranes.

Materials and Methods

Liposomes containing the entrapped pH probe pyranine (8-hydroxy-1,3,6-pyrene trisulfonate, trisodium salt; Molecular Probes, Eugene OR) were prepared by detergent dialysis, essentially according to Wrigglesworth et al. (1987). Basically, 0.25 g of dry asolectin (L-α-phosphatidylcholine, Sigma type IV-S) was dispersed by mixing in 5 ml of 5 mM pyranine (8-hydroxy-1,3,6-pyrene trisulfonate, trisodium salt, Molecular Probes, Eugene, OR) in 100 mM K^+-HEPES buffer, pH 7.0. Cholic acid (0.1 g) was added and, after mixing, the sample dialyzed against 2 × 500 ml of 5 mM pyranine in 100 mM HEPES buffer, pH 7.0 for 4 hr periods and then overnight against 500 ml of 100 mM HEPES buffer pH 7.0. Sonicated vesicles of the same lipid composition were prepared using a pulse sonicator (Dawe Ultrasonics model 250/450, Dawe Ultrasonics, Hayes, Middlesex, UK) at 30% duty cycle for 5 min according to Wrigglesworth et al. (1990). Excess dye was removed from the vesicle preparations by passing the samples through a column of G25 Sephadex equilibrated with 100 mM HEPES (K^+ or Na^+ as cation where appropriate) at pH 7.

Fluorescence and absorbance changes were monitored using a rotating filter air-turbine spectrophotometer (University of Pennsylvania Biomedical Instrumentation Group, Philadelphia, PA). Excitation of pyranine fluorescence was at 470 nm with emission monitored at 90° to the excitation beam using a 540 nm filter (10 nm band pass). Pyranine absorbance was monitored at 465 nm (2 nm band pass filter). Fluorescence changes of the membrane potential probe Oxonol V (Molecular Probes, Cambridge BioScience) were monitored as described by Cooper, Bruce and Nicholls (1990) using a dye concentration of 3 μM. Oxonol V is membrane permeable and anionic (Bashford, Chance & Prince, 1979) and, as such, can be used to monitor membrane potential, positive inside, in closed vesicles. Aliquots of K^+ sulfate were added to a stirred 3 ml suspension from a 0.5 M stock solution. Freshly made stock solutions (1 N) of H_2SO_4 and KOH were used for acid and alkali additions, respectively. Ionophores and fatty acids were added at the concentrations indicated from appropriately diluted stock solutions in ethanol. The concentration of ethanol in the final mixtures never exceeded 0.03%.

In the present experiments, association of added fatty acids with the liposomes was checked using ^{3}H-oleate and ^{3}H-palmitate. These were added to separate liposome suspensions at a mole fraction concentration (fatty acid:phospholipid) of 15% and the mixtures fractionated on a Sephacryl S-300 column. Greater than 90% of the fatty acids was found to associate with the liposomes within 1 min of mixing. To ensure maximum incorporation for the present experiments, the liposomes were preincubated with the individual fatty acids for a minimum of 10 min before the addition of K^+ sulfate.

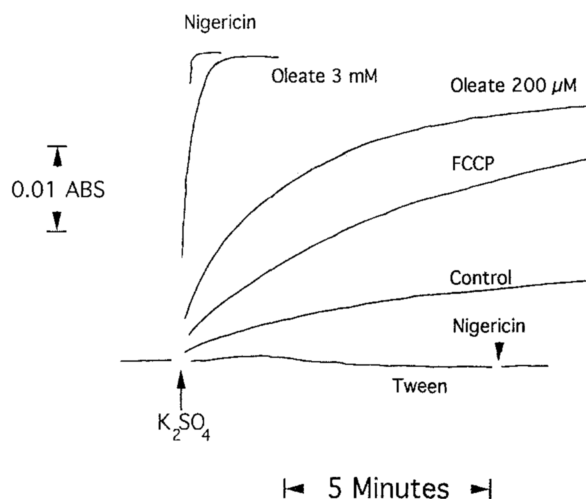

Fig. 1. Changes in the internal pH of liposomes following the external addition of potassium sulfate. Potassium sulfate (45 μmol) was added to 3 ml of a continuously stirred suspension of liposomes (1.2 mg lipid/ml) in 100 mM K^+ HEPES, prepared by dialysis, containing entrapped pyranine at 22°C. Internal pH was monitored by the absorbance change of pyranine as described in Materials and Methods. Addition of standard acid and alkali following nigericin addition indicated that an 0.01 absorbance change was equivalent to 0.077 pH units. Traces are shown for separate experiments done in the presence of Tween-80 (0.1% v/v), FCCP (10 μM), oleic acid (200 μM and 3 mM) and nigericin (100 μM) as indicated.

Results

To determine the effects of nonesterified fatty acids on membrane permeability to cations, a K^+ concentration gradient was created across phospholipid vesicles containing the entrapped pH indicator pyranine. The subsequent rate of change of the internal pH could then be used as a monitor for the transmembrane movement of H^+. Addition of K_2SO_4 to a suspension of the vesicles can be seen to induce a slow change in internal alkalinity (Fig. 1). This rate of change of internal pH increases on the addition of FCCP but only to an intermediate value compared to that seen in the presence of nigericin. Saturating concentrations of FCCP did not affect the rate further. It would appear that the imposition of a K^+ gradient induces a K^+/H^+ exchange across the liposome membrane. In the absence of FCCP, this rate is limited by H^+ counter-ion flux. The permeability of H^+ is known to be many orders of magnitude higher than that for K^+, around 10^{-4} cm × sec^{-1} compared with 10^{-10} cm × sec^{-1} (Deamer & Bramhall, 1986). However, the flux of any ion across a membrane is a function of the

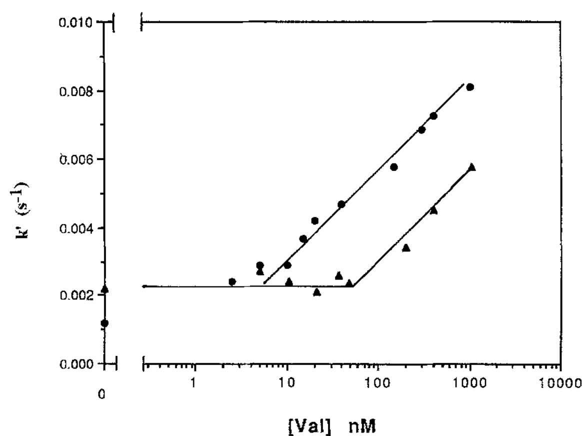

Fig. 2. Effect of valinomycin on the rate of change in internal pH (k') of dialyzed liposomes following the addition of potassium sulfate (●) or sodium hydroxide (▲). The pH changes were monitored by pyranine fluorescence changes using a 3 ml suspension of liposomes (0.4 mg lipid/ml) in the presence of increasing concentrations of valinomycin. The changes were initiated by either the addition to the suspension of potassium sulfate (45 μmol) or sodium hydroxide (26 μmol). Fluorescence changes were used instead of absorbance changes because of the lower liposome concentrations in these experiments. Other conditions as described in the legend to Fig. 1.

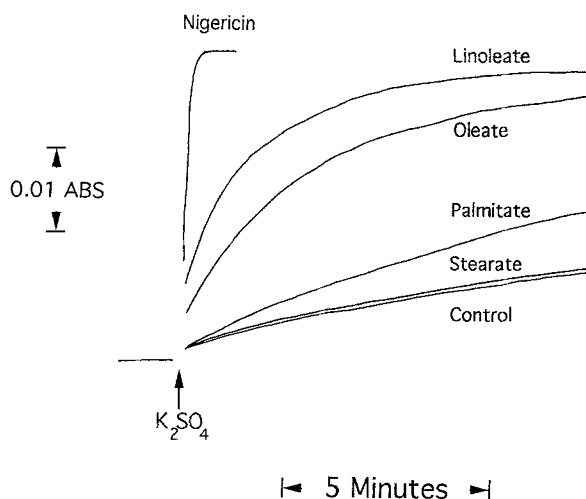

Fig. 3. Relative efficacies of different fatty acids on the internal pH changes of dialyzed liposomes following the external addition of potassium sulfate. Palmitate (16:0), stearate (18:0) oleate (18:1), and linoleate (18:2) were added to the suspension of liposomes to a final concentration of 200 μM at least 15 min before the addition of potassium sulfate to the suspension. Other conditions as in Fig. 1.

permeability and the ion concentration gradient as well as $\Delta\Psi$.

Under the present conditions, a K^+ gradient of approximately 15 mM is sufficient to increase the flux of K^+ to a value greater than that for H^+ (at pH 7). FCCP increases H^+ flux until H^+ counter-ion movement no longer limits the exchange. Under these conditions, H^+ movement is now limited by K^+ movement. This interpretation is supported by the results of adding valinomycin to the system. In the absence of FCCP, small concentrations of valinomycin have no effect on internal alkalinization when an external pulse of OH^- (as sodium salt) is added (Fig. 2). It is only at higher concentrations (>100 nM), where the protonophoric action of valinomycin becomes apparent (Wrigglesworth et al., 1990), that the rate of internal alkalinity increases. This is in contrast to the findings when external K^+ (with sulfate as the anion) is added. Here, the movement of K^+ across the membrane is faster than any compensating H^+ movement and a diffusion potential forms, positive inside, which increases H^+ efflux. Valinomycin, even at low concentrations, enhances the effect.

Oleic acid added to the vesicle suspensions increases the rate of internal pH change following the imposition of a K^+ gradient (Fig. 1). There is a small rapid initial alkalinization immediately following the addition of the cation salt, followed by a slower rate of dye absorbance increase. However, unlike the classical uncoupler FCCP, increasing concentrations of oleic acid do increase the pH changes to a rate commensurate with that seen with nigericin. A detergent-like action of the fatty acid on membrane structure is excluded by pH changes being induced by a potassium sulfate solution at neutral pH. No pH changes are seen in the absence of liposomes (*data not shown*) or in the presence of liposomes disrupted by the detergent Tween 80 (Fig. 1). The latter result also indicates that the internal pH of the liposomes matched that of the external buffer (pH 7.0). It would seem that an intact vesicular system is required for the salt-induced pH changes to occur. The effects of oleate were also seen using liposomes prepared by sonication as well as with liposomes made from phosphatidyl choline plus phosphatidyl ethanolamine (egg phospholipids at a weight ratio of 2:1). The latter preparations showed increased light scattering compared with the asolectin vesicles but an effect of oleate on internal pH change following K^+-sulfate addition could be detected at oleate:phospholipid ratios of 5% and greater.

Other nonesterified fatty acids have the ability to mimic oleate but with differing efficacies (Fig. 3). The ability to affect the rate of internal pH change increases with the degree of unsaturation and decreases with increasing chain length. A possibility exists that the differing abilities of the fatty acids to catalyze internal pH changes is a reflection of their efficiency of incorporation from bulk solution into the bilayer after their addition to the liposomal suspension. However, there are indications that this transfer is rapid. Fast (seconds) rates of transfer of long chain fatty acids have been shown to occur between phospholipid vesicles and albumin (Daniels et al., 1985; Hamilton & Cistola, 1986; Spooner et al., 1990). In the present experiments, added

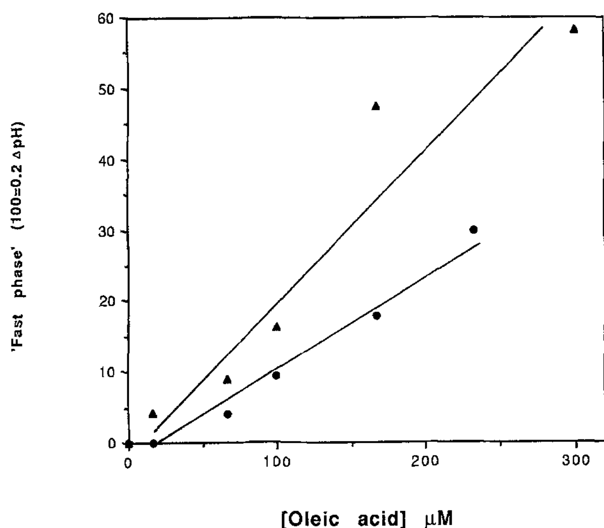

Fig. 4. Effect of oleate concentration on the initial fast change in internal pH in dialyzed liposomes (0.4 mg lipid/ml) following the addition of potassium sulfate (45 μM) (●) or sodium hydroxide (26 μM) (▲). pH changes were monitored by fluorescence as described in Materials and Methods. Other conditions as in Fig. 1.

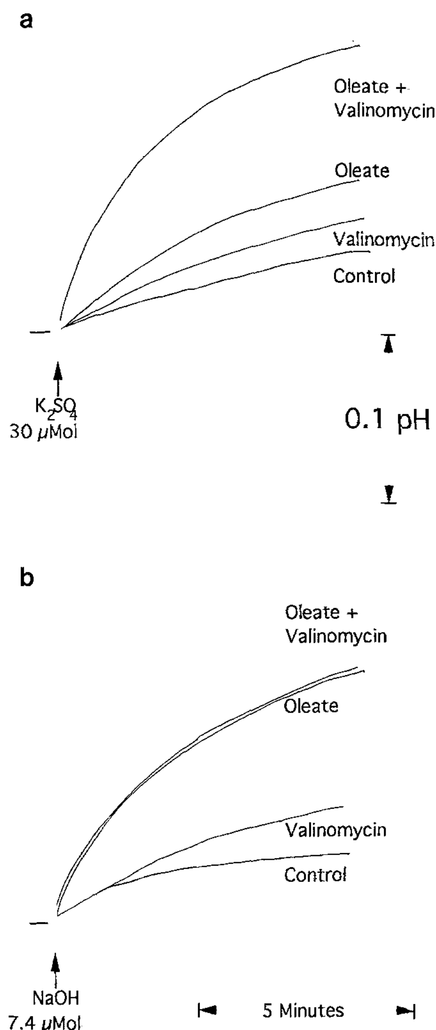

Fig. 5. Effect of valinomycin on oleic acid-induced pH changes in dialyzed liposomes (0.3 mg lipid/ml) following the external addition of (*a*) potassium sulfate (30 μmol) or (*b*) sodium hydroxide (17.5 μmol). Oleate concentration was 50 μM and valinomycin was added to a final concentration of 10 nM. pH changes were monitored by pyranine fluorescence. Other conditions of measurement as in Fig. 1.

oleate and palmitate associated with the vesicles within 1 min. To maximize fatty acid incorporation, the fatty acids were added and incubated with the liposomes for a minimum of 10 min before the imposition of the ion gradients. The possibility remains, however, that the lack of effect with stearate could still be a result of nonincorporation into the bilayer.

As reported previously (Wrigglesworth et al., 1990) and noted in the present experiments, there is a small but fast initial jump in internal alkalinity on the addition of potassium sulfate before the slower changes in internal pH occur. This initial change is not affected by repeated passing of the liposomes through a gel column but does increase as the concentration of oleate increases. The magnitude of the change is directly proportional to the amount of oleate added (Fig. 4) and increases with increasing oleate/phospholipid ratio.

Valinomycin concentrations that have little effect on internal pH in the absence of oleate, enhance the rate of internal pH change when oleate is present (Fig. 5*a*). This effect is observed when external K^+ is added to the suspension to initiate the changes but not when a OH^- pulse is used (Fig. 5*b*). As expected, a combination of valinomycin and FCCP, in the absence of fatty acid, enhances the rate of the internal pH change commensurate to that seen with nigericin (*results not shown*).

The influence of cation type on the ability of oleic acid to catalyze cation/H^+ exchange is shown in the Table. Both Li^+ and Na^+ salts cause a similar initial fast change in alkalinity but have less effect than K^+ on the subsequent rate of internal alkalinity change. Similarly, the main effect of divalent cation salts is on the initial fast pH change. Ni^{2+} and Mn^{2+} in particular cause large changes in the initial pH jump with significant changes in the control liposomes also. It is known that negatively charged phospholipids in liposomes have the ability to bind divalent cations (Eisenberg et al., 1979; McLaughlin, 1982), including Ni^{2+}. Binding of cations to the fraction of negatively charged lipid in asolectin [around 20% (Casey, 1984)] would appear to occur in the present case even in the absence of added fatty acid. However, changing the cation, with the exception of K^+ and Mg^{2+}, has little effect on the slow rate of pH change.

Figure 6 shows traces of simultaneous changes in membrane potential ($\Delta\Psi$) and internal pH following the imposition of a K^+ gradient to vesicles prepared and

Table 1. Relative cation specificity of oleic acid-induced pH changes in liposomes following the external addition of cation-sulfate salts

Cation	Control		Plus oleate	
	Initial phase amplitude (%)	Final rate of pH change ($sec^{-1} \times 10^3$)	Initial phase amplitude (%)	Final rate of pH change ($sec^{-1} \times 10^3$)
K^+	0	5	29	21
Li^+	13	5	29	6
Na^+	12	<1	30	8
Ni^{2+}	50	<1	80	8
Co^{2+}	9	<1	43	10
Mn^{2+}	20	<1	58	12
Mg^{2+}	0	10	29	60

The dialyzed liposomes (1.2 mg lipid/ml) contained entrapped pyranine in 100 mM K^+ HEPES. The fast initial change and subsequent slower change in internal pH was monitored by pyranine absorbance change following the addition of the sulfate salt of various cations to a final cation concentration of 15 mM in the absence or presence of oleate (200 μM). The results are expressed as a percentage of the final pH change induced by the addition of nigericin or the divalent-cation ionophore A23187.

suspended in Na^+ HEPES. Preparing the vesicles in Na^+ rather than K^+ allows for the imposition of K^+-induced membrane potential under appropriate conditions when external K^+ sulfate is added. Little change in ΔpH or $\Delta\Psi$ is seen in control vesicles (Fig. 6*a*). The lack of any significant $\Delta\Psi$ generation is in agreement with the earlier conclusion (Fig. 2) that H^+ flux is rate limiting for K^+/H^+ exchange in these vesicles. In the presence of oleate (Fig. 6*b*), a K^+ pulse induces a small but significant potential. This decays slowly at a rate that approximately matches the rate of internal alkalinity increase. The alkalinity change was found to be markedly less in these vesicles compared to vesicles prepared and suspended in K^+ medium. Competition between Na^+ and K^+ for oleate appears to be taking place with the Na^+ associated form of oleate being unable to induce the internal alkalinity changes (*see* the Table). With valinomycin plus oleate present, the magnitude of the potential is enhanced (Fig. 6*c*). In addition, an immediate rise in internal pH occurs, followed by a slow decay (Fig. 6*c*). This effect can be compared to the changes seen with valinomycin in the absence of oleate (Fig. 6*d*). Here, the internal pH rises to a constant level as the imposed diffusion potential rapidly decays.

Discussion

The experiments described above indicate that nonesterified fatty acids have the capability to translocate a variety of cations including protons across phospholipid bilayers. However, they do not act simply as K^+/H^+ exchangers like nigericin nor as protonophores analogous to FCCP. The full cycle of ionophoric action is a combination of both abilities and is sensitive to the membrane potential and cationic gradients across the membrane.

A mechanism for the action of fatty acids on membrane potential and cation gradients is shown in Fig. 7. In the absence of any imposed potential, it is proposed that the fatty acid can translocate a proton or cation as an associated acid (FH) or as a potassium (or other cation)-complexed salt (F^-K^+), respectively. The direction of transport will be determined by the polarity of the ion gradient. The rate of transport will depend on the nature of the fatty acid and the particular cation in the complex (*see* Fig. 6 and Table). For example, the Na^+ complex will translocate much slower than the K^+ complex. It is proposed that the rate of movement of the associated acid is faster than both the cation complex and the dissociated fatty-acid anion. The imposition of a OH^- (Fig. 7*a*) or K^+ (Fig. 7*b*) gradient to the system disturbs the equilibrium between protonated and dissociated fatty acid on the outer side of the membrane. The apparent pK_a of free fatty acids incorporated into phospholipid bilayers is known to be approximately 7–8 (Wilschut et al., 1992). OH^- induces a fast dissociation of fatty acid as does K^+ by forming the cationic salt. The consequence of the drop in protonated fatty acid on the outer surface of the membrane is a fast electroneutral transfer of the lipid-soluble protonated form across the membrane to maintain an equal concentration on both sides. A fast uptake of protons from the liposome interior then follows to restore the equilibrium between the species on the inner surface. The magnitude of the fast phase of pH change will be directly proportional to the molar concentration of fatty acid (*see* Fig. 4). Subsequent (steady-state) changes in

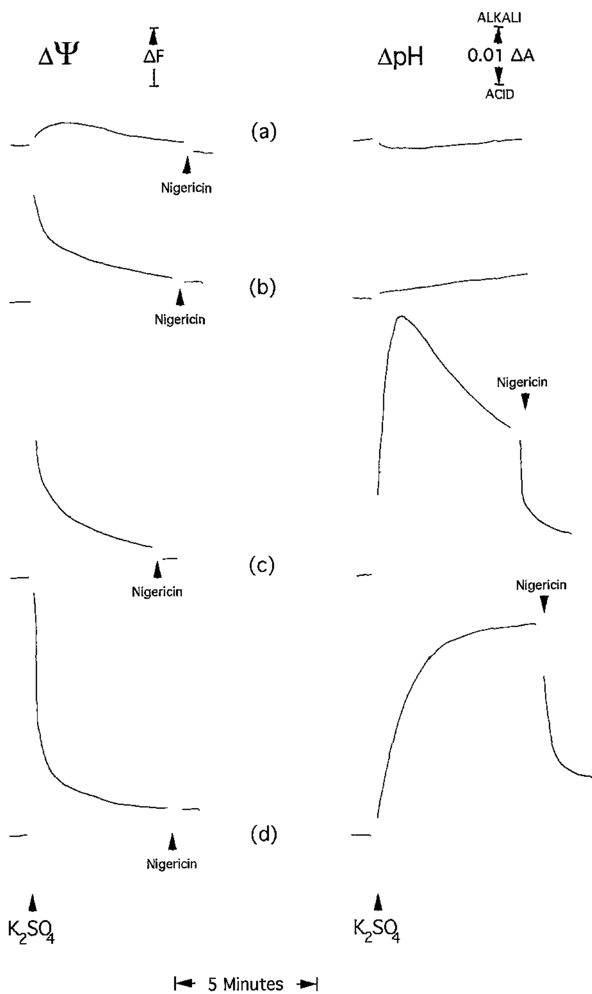

Fig. 6. Simultaneous measurements of $\Delta\Psi$ and ΔpH following the addition of potassium sulfate to a suspension of sonicated liposomes (1.2 mg lipid/ml). $\Delta\Psi$ and ΔpH were monitored by the fluorescence change of Oxonol V and the absorbance change of pyranine, respectively, in a suspension of liposomes (1.2 mg lipid/ml) containing sodium HEPES. The liposomes were prepared by the sonication procedure and suspended in sodium HEPES (100 mM, pH 7.0). (*a*) control; (*b*) plus oleic acid (200 μM); (*c*) plus oleic acid (200 μM) plus valinomycin (10 nM); (d) plus valinomycin (10 nM).

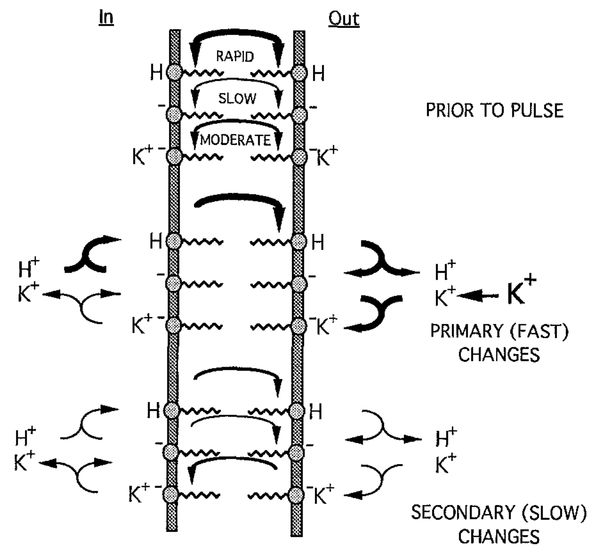

Fig. 7. Proposed molecular mechanisms for fatty acid mediated transfer of cations and protons across phospholipid bilayers. Prior to a pulse of cation salt (for example, potassium sulfate), an equilibrium mixture exists of the three species of fatty acid: the protonated form, the dissociated form and the potassium salt complex. Following the external addition of cation, the equilibrium concentration of the three species is perturbed, resulting in a fast transfer of the protonated form across the membrane and an equally fast uptake of H^+ from the inside phase. The rate of subsequent steady-state changes will be limited by the rate of exchange of cation for H^+ which in turn will be limited by the rate of translocation of the potassium salt complex. The translocation of the dissociated form will be sensitive to the magnitude and polarity of any membrane potential that may be present.

the internal pH will depend on the rate of exchange of cations for protons across the membrane which will be limited by the rate of translocation of the potassium salt. In these studies, unsaturation of the fatty acid makes it a more potent translocator. A similar effect of unsaturation has been reported by Wilschut et al. (1992) for the transmembrane movement of fatty acids induced by pH changes. In the presence of a mixture of cations, transmembrane transport will be affected by the competition between the cations for the fatty acids.

From measurements of NMR and the electrophoretic mobility of liposomes, it has been shown that monovalent and divalent cations can be absorbed to negatively charged lipids (McLaughlin et al., 1978; Eisenberg et al., 1979; Ermakov, 1990). This adsorption is indicated in the present work by the initial fast pH change which also indicates a binding ability of some divalent cations. Fast initial pH changes are also seen in the control liposomes for some cations presumably reflecting the presence of negatively charged phospholipid in the asolectin mixture. Functional groups of lipids that could bind cations are phosphodiester, carboxyl, amine and carbonyl groups (McLaughlin, 1982). Because of the dominance of the cation-carboxylate interaction in soaps, cation complexation strongly affects the packing and order of the hydrocarbon chains (Jandacek & Broering, 1989). However, the ability of a fatty acid to form a soap complex at the surface of a bilayer does not directly correlate with the ability to catalyze the transmembrane movement of the complex. In the present results, only K^+ of the monovalent salts tested was translocated to any significant rate. Liposomes prepared in Na^+ medium showed less capability for H^+/K^+ exchange on the imposition of a K^+ gradient. The competition between Na^+ and K^+ appears to slow down the exchange process. Serhan et al. (1981) report the ability of fatty acids to translocate divalent cations selectively and Simpson et al. (1988) have

demonstrated that Fe^{3+} transport across biological as well as liposomal membranes can be mediated by the formation of Fe^{3+}/(fatty acid)$_3$ complexes.

Transmembrane pH gradients have been shown to have a strong controlling effect on the distribution of fatty acids between the two sides of the bilayer (Wilschut et al., 1992). Inward movement of oleic acid is extremely fast (<1 sec) in response to changes in pH gradient (Kamp & Hamilton, 1992). According to Fig. 7, this effect is due to the fast equilibration of the associated acid. The presence of a membrane potential will be expected to speed up the translocation of the anionic species, as is well known for protonophoric uncouplers. The cycle shown in Fig. 7 will then be dominated by the "uncoupler-type" action of the fatty acid with cation transport taking a minor role. Hence, a synergistic effect of valinomycin and oleate is seen for K^+ pulses where a significant diffusion potential is present, but not when a potential is absent as for example with OH^- pulses (*see* Fig. 5). It can easily be predicted that the "uncoupler-type" action of the fatty acid will be inhibited if the imposed potential has the opposite polarity to the cation gradient. In this case, the dissociated species will be trapped on the positive side of the membrane. A "nigericin-type" of action would then predominate with an electroneutral exchange of cations for protons. The predominant effect would be on the cation gradients rather than on the membrane potential. This can be seen in the analogous system of cytochrome oxidase proteoliposomes (Wrigglesworth et al., 1990) where the addition of nigericin allows the internal and external pH to equilibrate and causes a small increase in $\Delta\Psi$ as the enzyme responds to the change in proton gradient. Similarly, in active mitochondria where the internal K^+ concentration can be high and $\Delta\Psi$ is positive at the cytoplasmic surface of the membrane, a nigericin-like activity of fatty acids would lower Δ pH with little change, or even a small increase, in $\Delta\Psi$. Normally, the contribution of ion cycling to mitochondrial uncoupling would be low in isolated mitochondria where fatty acid levels are low (Nobes, Hay & Brand, 1990), but these so-called anomalous effects of added fatty acids on mitochondria have been reported by Rottenberg and Hashimoto (1986) and Nobes et al. (1990). In addition, Klug et al. (1984) have observed that the presence of nonesterified fatty acids increases the magnitude of the negative surface charge of the mitochondrial membrane as might be expected if the charged form of the fatty acid was trapped on the outer surface by the positive potential.

According to the scheme shown in Fig. 7, a sudden switch in potential, for example during depolarization of an action potential, could initiate a fast movement of any fatty acid anion to the positive potential side of the membrane with subsequent disturbances of the proton-associated and cation-complexed forms. The disturbance would be directly proportional to the amount of fatty acid in the membrane and would also depend on the polarity and nature of the cation gradients present. Such an effect may contribute to some of the uncoupling action of fatty acids on cardiac cells during action potential generation (Cowan & Vaughan-Williams, 1977; Burt et al., 1991) when plasma fatty acid concentrations rise to elevated levels under conditions of stress or exercise (Newsholme & Leech, 1983).

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References

Bashford, C.L., Chance, B., Prince, R.C. 1979. Oxonol dyes as monitors of membrane potential: Their behaviour in photosynthetic bacteria. *Biochim. Biophys. Acta* **545:**46–57

Borst, P., Loos, J.A., Christ, E.J., Slater, E.C. 1962. Uncoupling activity of long-chain fatty acids. *Biochim. Biophys. Acta* **62:**509–518

Brown, G.C., Brand, M.D. 1991. On the nature of the mitochondrial proton leak. *Biochim. Biophys. Acta* **1059:**55–62

Burt, J.M., Massey, K.D., Minnich, B.N. 1991. Uncoupling of cardiac cells by fatty acids: structure-activity relationships. *Am. J. Physiol.* **260:**C439–448

Casey, R.P. 1984. Membrane reconstitution of the energy-conserving enzymes of oxidative phosphorylation. *Biochim. Biophys. Acta* **768:**319–347

Cooper, C.E., Bruce, D., Nicholls, P. 1990. Use of oxonol V as a probe of membrane potential in proteoliposomes containing cytochrome oxidase in the submitochondrial orientation. *Biochemistry* **29:** 3859–3865

Cooper, C.E., Wrigglesworth, J.M., Nicholls, P. 1990. The mechanism of potassium movement across the liposomal membrane. *Biochem. Biophys. Res. Commun.* **173:**1008–1012

Cowan, J.C., Vaughan-Williams, R.N. 1977. The effect of palmitate on intracellular potentials recorded from Langendorff-perfused guinea-pig hearts in normoxia and hypoxia and during perfusion at reduced rate of flow. *J. Mol. Cel. C.* **9:**327–342

Deamer, D.W., Bramhall, J. 1986. Permeability of lipid bilayers to water and ionic solutes. *Chem. Phys. Lipids* **40:**167–188

Deamer, D.W., Nichols, J.W. 1983. Proton-hydroxide permeability of liposomes. *Proc. Natl. Acad. Sci. USA* **80:**165–168

Deamer, D.W., Nichols, J.W. 1989. Proton flux mechanisms in model and biological membranes. *J. Membrane Biol.* **107:**91–103

Eisenberg, M., Gresalfi, T., Riccio, T.M., McLaughlin, S. 1979. Adsorption of monovalent cations to membranes containing negative phospholipids. *Biochemistry* **18:**5213–5223

Ermakov, Y.A. 1990. The determination of binding site density and association constants for monovalent cation adsorption onto liposomes made from mixtures of zwitterionic and charged lipids. *Biochim. Biophys. Acta* **1023:**91–97

Gutknecht, J. 1988. Proton conductance caused by long-chain fatty acids in phospholipid bilayer membranes. *J. Membrane Biol.* **106:**83–93

Jandacek, R.J., Broering, W.B. 1989. X-ray diffraction study of sodi-

um soaps of monounsaturated and polyunsaturated fatty acids. *Lipids* **24:**1008–1013

Kamp, F., Hamilton, J.A. 1992. pH gradients across phospholipid membranes caused by fast flip-flop on unionized fatty acids. *Proc. Natl. Acad. Sci. USA* **89:**11367–11370

Kidooka, M., Matsuda, M., Handa, J. 1987. Ca^{2+} antagonist and protection of the brain against ischemia. Effects of nicardipine on free fatty acid liberation in the ischemic brain in rats. *Surg. Neurol.* **28:**437–444

Klug, G.A., Krause, J., Ostlund, A.-K., Knoll, G., Brdiczka, D. 1984. Alterations in liver mitochondrial function as a result of fasting and exhausive exercise. *Biochim. Biophys. Acta* **764:**272–282

Krishnamoorthy, G., Hinkle, P.C. 1984. Non-ohmic proton conductance of mitochondria and liposomes. *Biochemistry* **23:**1640–1645

Labonia, N., Muller, M., Azzi, A. 1988. The effect of non-esterified fatty acids on the proton-pumping cytochrome c oxidase reconstituted into liposomes. *Biochem. J.* **254:**139–145

Macri, F., Vianello, A., Braidot, E., Zancani, M. 1991. Free fatty acids dissipate proton electrochemical gradients in pea stem microsomes and submitochondrial particles. *Biochim. Biophys. Acta* **1050:**249–255

McLaughlin, A.C. 1982. Phosphorus-31 and carbon-13 nuclear magnetic resonance studies of divalent cation binding to phosphatidylserine membranes: Use of cobalt as a paramagnetic probe. *Biochemistry* **21:**4879–4885

McLaughlin, S.G.A., Dilger, J.P. 1980. Transport of protons across membranes by weak acids. *Physiol. Rev.* **60:**825–863

Newsholme, E.A., Leech, A.R. 1983. Biochemistry for the Medical Sciences. John Wiley & Sons, Chichester

Nichols, J.W., Hill, M.W., Bangham, A.D., Deamer, D.W. 1980. Measurement of net proton-hydroxyl permeability of large unilamellar liposomes with the fluorescent pH probe, 9-aminoacridine. *Biochim. Biophys. Acta* **596:**393–403

Nobes, C.D., Hay, W.W., Brand, M.D. 1990. The mechanism of stimulation of respiration by fatty acids in isolated hepatocytes. *J. Biol. Chem.* **265:**12910–12915

Rossignol, M., Thomas, P., Grignon, C. 1982. Proton permeability of liposomes from natural phospholipid mixtures. *Biochim. Biophys. Acta* **684:**195–199

Rottenberg, H., Hashimoto, K. 1986. Fatty acid uncoupling of oxidative phosphorylation in rat liver mitochondria. *Biochemistry* **25:**1747–1755

Serhan, C., Andersop, P., Goodman, E., Dunham, P., Weissmann, G. 1981. Phosphatidate and oxidized fatty acids are calcium ionophores. *J. Biol. Chem.* **256:**2736–2741

Simpson, R.J., Peters, T.J. 1987. Transport of Fe^{+} across lipid bilayers: Possible role of free fatty acids. *Biochim. Biophys. Acta* **898:**187–195

Simpson, R.J., Moore, R., Peters, T.J. 1988. Significance of non-esterified fatty acids in iron uptake by intestinal brush-border membrane vesicles. *Biochim. Biophys. Acta* **941:**39–47

Spooner, P.J.R., Gantz, D.L., Hamilton, J.A., Small, D.M. 1990. The distribution of oleic acid between chylomicron-like emulsions, phospholipid bilayers and serum albumin. *J. Biol. Chem.* **265:** 12650–12655

Thiel, C., Kadenbach, B. 1989. Influence of non-esterified fatty acids on respiratory control of reconstituted cytochrome-c oxidase. *FEBS Lett.* **251:**270–274

Wilschut, J., Scholma, J., Eastman, S.J., Hope, M.J., Cullis, P.R. 1992. Ca^{2+}-induced fusion of phospholipid vesicles containing free fatty acids: Modulation by transmembrane pH gradients. *Biochemistry* **31:**2629–2636

Wojtczak, L. 1976. Effect of long-chain fatty acids and acyl-CoA on mitochondrial permeability, transport and energy coupling processes. *J. Bioenerg. Biomembr.* **8:**293–311

Wrigglesworth, J.M., Cooper, C.E., Sharpe, M.A., Nicholls, P. 1990. The proteoliposomal steady state: Effect of size, capacitance and membrane permeability on cytochrome-oxidase induced ion gradients. *Biochem. J.* **270:**109–118

Wrigglesworth, J.M., Wooster, M.S., Elsden, J., Danneel, H.-J. 1987. Dynamics of proteoliposome formation: Intermediate states during detergent dialysis. *Biochem. J.* **246:**737–744