Calcium Modulates the Lipid Dynamics of Rat Hepatocyte Plasma Membranes by Direct and Indirect Mechanisms†

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ABSTRACT: Calcium ion decreases the motional freedom of lipid molecules in isolated rat hepatocyte plasma membranes and in sonicated dispersions (liposomes) of the membrane lipid. The decrease in lipid fluidity was monitored by estimation of the fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene. At least two processes are involved in the mode of action of the cation. The first is direct, i.e., observed on addition of calcium to the liposomes, relatively rapid, with a half-time of 10–15 min at 37 °C, proportional to the calcium concentration in the range 0–4 mM, and readily reversed on addition of excess EDTA. The second mechanism is indirect and requires the presence of the membrane proteins. It occurs relatively slowly, with a half-time of 75 min at 37 °C, tends to plateau, with a calcium half-saturation concentration of approximately 1 mM, is of greater magnitude than the direct effect, and cannot be reversed on chelation of calcium by EDTA. Moreover, the indirect effect is specific for Ca^{2+} as compared to other divalent cations and it results in changes in the lipid composition. Stimulation of phospholipase A activity is likely but does not account for the change in fluidity. The direct action of calcium is ascribed to binding to the lipid bilayer, whereas the indirect action probably results from modulation of membrane-bound enzymes which can alter the lipid composition. The effects of calcium on the membrane lipid fluidity may underly certain of its regulatory actions on membrane functions.

There is considerable evidence that calcium ion can interact directly with phospholipids arranged in monolayers (Shah & Schulman, 1967; Papahadjopoulos, 1968) or model bilayers (Traüble & Eibl, 1974; Ohnishi & Ito, 1974; Jacobson & Papahadjopoulos, 1975; Papahadjopoulos & Poste, 1975; Galla & Sackman, 1975; Hartmann et al., 1977) and thereby restrict the motional freedom or "lipid fluidity"[1] of the arrays. Calcium appears to act similarly on a number of biological membranes. Addition of the cation to rat intestinal microvillus membranes, for example, increased the polarization of fluorescence of retinol and 12-anthroyl stearate in the membrane bilayer, indicating a decrease in the motional freedom of the lipid probes (Schachter & Shinitzky, 1977). Similarly, electron spin resonance studies (Gordon et al., 1978) demonstrated that calcium restricts the motion of the spin probe 5-nitrostearic acid in rat hepatocyte plasma membranes. In the course of recent studies on the lipid dynamics of isolated rat hepatocyte plasma membranes (Livingstone & Schachter, 1980), it was of interest to determine whether the foregoing effect of calcium could be observed via changes in the steady-state fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH),[2] a hydrocarbon fluorophore used widely as a membrane lipid probe (Shinitzky & Barenholz, 1978). The results described below support the conclusion that the cation decreases the motional freedom of lipid molecules in these isolated membranes. However, the mode of action of the cation is complex and involves at least two mechanisms: a direct effect, which is observed on addition of calcium to sonicated dispersions of the membrane lipid (liposomes) and can be reversed subsequently upon addition of EDTA, and an indirect effect, which is found on treatment only of the intact membranes and persists despite the subsequent removal of calcium by EDTA.

Experimental Procedures

Membrane Preparations. Suspensions of isolated rat hepatocyte plasma membranes were prepared from Albino male rats of the Sherman strain, as described previously (Livingstone & Schachter, 1980). The final purification step consisted of centrifugation in a discontinuous sucrose density gradient, and the purified membrane fraction was harvested at the 39–51% interface and washed twice with 0.9% NaCl. Membrane aliquots not used immediately were stored in 0.9% NaCl at −18 °C for up to 3 months. Purity of the preparations was assessed by estimating the marker enzyme 5′-nucleotidase, and the specific activity ratio [purified membrane]/[homogenate] was 9.1 ± 0.6 (mean ± SE). In addition, succinic dehydrogenase activity was quantified as an index of mitochondrial contamination and the specific activity ratio [purified membrane]/[homogenate] was 0.18 ± 0.04 in these preparations.

Liposome Preparations. For preparation of sonicated lipid dispersions (liposomes), lipids were extracted from the hepatocyte plasma membranes by the method of Folch et al. (1957). Approximately 1–2 mg of dried lipid was suspended in 1 mL of an isotonic buffer consisting of 145 mM NaCl, 4 mM KCl, 5 mM sodium phosphate of pH 7.4, 0.1 mM NaEDTA, and from 0 to 4 mM $CaCl_2$, added as required. The suspension was sonicated under N_2, in an ice bath, for approximately 8 min, using a Branson Model 350 sonifier (Branson Sonic Power Co., Danbury, CT), and subsequently centrifuged for 10 min at 5000*g*, and the supernatant liposome suspension was used.

Fluorescence Polarization Studies. Membranes or liposomes were loaded with DPH as previously described (Schachter & Shinitzky, 1977) and molar ratios of probe/lipid varied from 0.001 to 0.002. In a typical experiment membranes equivalent to 0.25 mg of protein (and approximately 0.25 mg of lipid) were incubated for 2 h at 37 °C in 0.5 mL of the isotonic buffer described above containing 50 μM DPH. Thereafter, 1.5 mL of the isotonic buffer was added and the suspension was examined in a polarization spectrofluorometer

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[1] The term "lipid fluidity" as applied to bilayer membranes is used to express the relative motional freedom of the lipid molecules or substituents thereof. The sense in which we use the term and the method of quantification of lipid fluidity by means of steady-state fluorescence polarization are discussed further by Livingstone & Schachter (1980).

[2] Abbreviation used: DPH, 1,6-diphenyl-1,3,5-hexatriene.

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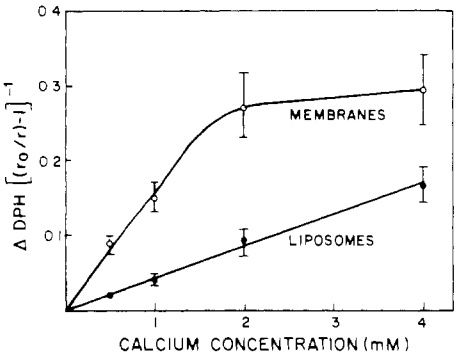

FIGURE 1: Effects of calcium on the anisotropy parameter of diphenylhexatriene in hepatocyte plasma membranes and in liposomes of the membrane lipid. Values shown are means ± SE of 12 experiments with 8 preparations of membranes and 7 experiments with 5 preparations of liposomes. The increments in $[(r_0/r) - 1]^{-1}$ with respect to the values observed in the absence of calcium, i.e., 2.13 ± 0.15 for the membranes and 1.58 ± 0.06 for the liposomes, are plotted. Suspensions were incubated with calcium for 2 h at 37 °C. The $[(r_0/r) - 1]^{-1}$ values were estimated at 25 °C.

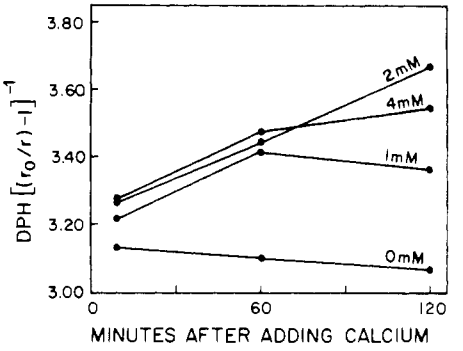

FIGURE 2: Time course of calcium effect on the anisotropy parameter of diphenylhexatriene (25 °C) in hepatocyte plasma membranes. Values are for one experiment and illustrate the net changes in $[(r_0/r) - 1]^{-1}$ with time of incubation at 37 °C after addition of the indicated concentrations of the cation.

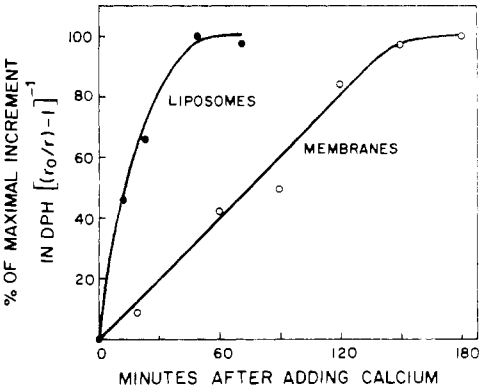

FIGURE 3: Comparison of the time course of calcium effect on diphenylhexatriene $[(r_0/r) - 1]^{-1}$ in hepatocyte plasma membranes and in liposomes of the membrane lipid. Values are means of two preparations of membranes and liposomes. Suspensions were incubated with 4 mM Ca^{2+} at 37 °C. The $[(r_0/r) - 1]^{-1}$ values were estimated at 25 °C. The maximal increments in $[(r_0/r) - 1]^{-1}$ for the membranes and liposomes respectively were 0.43 and 0.46.

(SLM Instruments, Champaign, IL) as previously described (Schachter & Shinitzky, 1977), using an exciting wavelength of 365 nm. Controls consisting of either membranes, liposomes, or probe suspension alone were examined, and the background fluorescence, amounting respectively to 9, 25, and 5% of the total fluorescence, was subtracted. Fluorescence polarization was expressed as the fluorescence anisotropy, r, and as the anisotropy parameter, $[(r_0/r) - 1]^{-1}$, which is proportional to the apparent rotational relaxation time of the probe, as previously discussed (Brasitus & Schachter, 1980). The value of r_0, the maximal limiting anisotropy of DPH, was taken as 0.362 (Shinitzky & Barenholz, 1974). Possible changes in the excited state lifetime of the probe were monitored by calculation of the fluorescence intensity, $F = I_{\parallel} + 2I_{\perp}$, where $I_{\parallel}$ and $I_{\perp}$ are the fluorescence intensities oriented respectively parallel and perpendicular to the direction of polarization of the exciting light (Shinitzky & Inbar, 1974). The changes in fluorescence anisotropy described below are not accounted for by changes in the excited state lifetime assessed by F.

Other Methods. The activity of 5′-nucleotidase was quantified by the method of Bodansky & Schwartz (1963) and that of succinic dehydrogenase by the procedure of Sottocasa et al. (1967). The composition of total lipid extracts of membrane preparations was examined by thin-layer chromatography according to the procedure of Katz et al. (1976). Protein was estimated by the method of Lowry et al. (1951), using bovine serum albumin as standard.

Results

Effects of Calcium Concentration. Rat hepatocyte plasma membranes (13 preparations) and liposomes of the membrane lipid (6 preparations) were loaded with DPH and simultaneously incubated in the presence of 0–4 mM Ca^{2+} for 2 h at 37 °C. The $[(r_0/r) - 1]^{-1}$ values were then estimated, and the results are shown in Figure 1. Although calcium increased DPH $[(r_0/r) - 1]^{-1}$ in both membranes and liposomes, the magnitude and pattern of the responses differed considerably. The liposome values rose linearly with Ca^{2+} concentration to 4 mM, at which point the relative increment was 10.1 ± 2.5% (mean ± SE). The increase in membrane $[(r_0/r) - 1]^{-1}$, by contrast, was 2–4 times that of the liposomes in the range 0.5–2.0 mM Ca^{2+} ($P < 0.01$), and the membrane values tended to plateau between 2.0 and 4.0 mM Ca^{2+}. The apparent half-saturation concentration for the Ca^{2+} effect on membranes was approximately 1 mM, and the relative increase in $[(r_0/r) - 1]^{-1}$ observed at 4 mM Ca^{2+} was 14.7 ± 2.0%.

Time Course of Calcium Effects. The time course of DPH $[(r_0/r) - 1]^{-1}$ after addition of calcium to membranes preloaded with the probe is illustrated by the results in Figure 2. Whereas in the absence of calcium the anisotropy parameter decreased slightly on incubation at 37 °C for 2 h, addition of 1–4 mM Ca^{2+} yielded increases over the zero time value. The time course was examined further with two preparations of intact membranes and two preparations of liposomes, and the relative increments with time are compared in Figure 3. Addition of 4 mM Ca^{2+} to the liposomes increased the anisotropy parameter relatively rapidly, with 50% of the total increment in approximately 10–15 min. In contrast, the membrane values rose more slowly and required approximately 75 min to reach the 50% level.

Reversibility of Calcium Effects. Inasmuch as the preceding experiments indicate that the effects of calcium on the membranes, as compared to the liposomes, occur relatively slowly, we hypothesized that the membrane action involves a protein-dependent chemical alteration of the membrane lipids, a change which should not be reversed on removal of the cation. To test the hypothesis, we incubated whole membranes (two preparations) and liposomes (three preparations) with various concentrations of Ca^{2+} (0–4 mM) and in the presence of DPH for 2 h at 37 °C, as described above. Thereafter, EDTA to a final concentration of 8 mM was added to each sample, the suspensions were incubated at 37 °C for 2–4 h more, and the DPH $[(r_0/r) - 1]^{-1}$ values were deter-

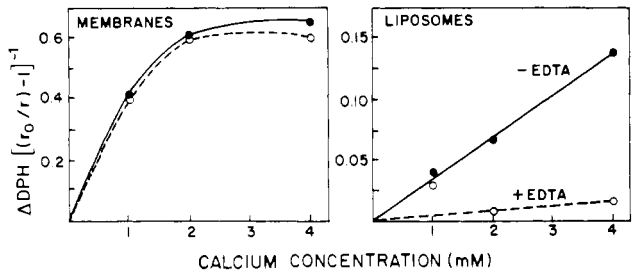

FIGURE 4: Effects of removing calcium with EDTA on the diphenylhexatriene anisotropy parameter. Values are means for three preparations of liposomes and two preparations of hepatocyte plasma membranes. The calcium-dependent increments in DPH $[(r_o/r) - 1]^{-1}$ observed before (●) and after (○) treatment with 8 mM EDTA are shown. The suspensions were incubated at 37 °C. The $[(r_o/r) - 1]^{-1}$ values were estimated at 25 °C.

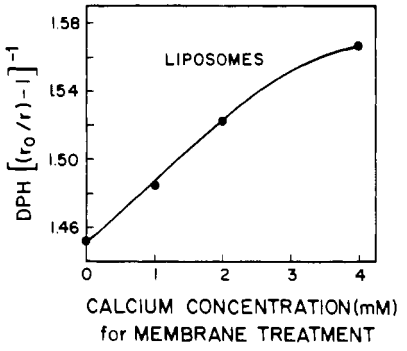

FIGURE 5: Effects of calcium treatment of hepatocyte plasma membranes on the membrane lipid isolated subsequently. Mean values are shown for four preparations of hepatocyte plasma membranes incubated for 2 h at 37 °C with various concentrations of Ca^{2+}. After incubation, the membrane lipids were extracted, liposomes prepared, and the DPH $[(r_o/r) - 1]^{-1}$ values estimated at 25 °C.

mined. The results illustrated in Figure 4 indicate that the Ca-dependent increment in the anisotropy parameter was decreased by approximately 90% on treatment of liposomes with EDTA. The corresponding increment in the intact membranes, however, was not affected significantly by EDTA. For determination of how completely the 8 mM EDTA removed the membrane calcium in these experiments, tracer quantities of $^{45}CaCl_2$ were added to the initial incubation mixtures with the nonradioactive calcium. After the final treatment with EDTA, the membranes were harvested by centrifugation and the residual membrane ^{45}Ca was less than 0.2% of the amount added initially.

Membrane Lipids after Calcium Treatment. The foregoing results support the hypothesis that calcium treatment of whole membranes, but not of liposomes, effects a chemical alteration of the membrane lipids which persists following removal of the cation. For a demonstration of the action more directly, four preparations of the hepatocyte membranes were incubated with 0–4 mM Ca^{2+} plus tracer quantities of $^{45}CaCl_2$ for 2 h at 37 °C. Thereafter, the membranes were harvested by centrifugation and total lipids extracted by the method of Folch et al. (1957). Liposomes prepared from these lipid extracts were found to contain less than 0.002% of the ^{45}Ca added initially, and these suspensions were loaded with DPH and the anisotropy parameter was estimated. The results shown in Figure 5 demonstrate clearly that the initial calcium treatment of the whole membrane increased the DPH $[(r_o/r) - 1]^{-1}$ value observed subsequently in liposomes virtually free of calcium. As a further control, the final liposome samples above were treated directly with 8 mM EDTA, and this did not change significantly the values shown in Figure 5. Finally, it is noteworthy that the increment in DPH $[(r_o/r) - 1]^{-1}$ observed

Table I: Effects of Divalent Cations on the DPH Anisotropy Parameters in Hepatocyte Membranes

	before EDTA[a]			after EDTA[b]		
divalent cation (4 mM)	no.	DPH $[(r_o/r) - 1]^{-1}$ (mean ± SE)	P[c]	no.	DPH $[(r_o/r) - 1]^{-1}$ (mean ± SE)	P[c]
none	5	1.48 ± 0.02		3	1.22 ± 0.01	
Ca^{2+}	4	1.68 ± 0.01	<0.001	2	1.37, 1.42	<0.01
Sr^{2+}	4	1.57 ± 0.02	<0.005	2	1.24, 1.24	ns[d]
Ba^{2+}	4	1.50 ± 0.01	ns	2	1.12, 1.23	ns
Mg^{2+}	4	1.55 ± 0.02	ns	2	1.20, 1.25	ns
Zn^{2+}	4	1.48 ± 0.02	ns	2	1.27, 1.29	<0.05

[a] Membranes were incubated with the appropriate cation (chloride salt) for 2 h at 37 °C, and DPH $[(r_o/r) - 1]^{-1}$ was then estimated at 25 °C. [b] Membranes were treated subsequently with 8 mM EDTA for 35 min at 37 °C. DPH $[(r_o/r) - 1]^{-1}$ was estimated at 25 °C. [c] P values are for the differences between the cation-treated and untreated membranes. [d] ns = not significant.

for liposomes from membranes treated initially with 4 mM calcium amounted to 12.6 ± 3.9%, similar to the corresponding increment of 14.7 ± 2.0% noted previously in the whole membranes.

Specificity of Divalent Cation. For an examination of the relative specificity of the effects of calcium, whole membranes were incubated for 2 h at 37 °C with different divalent cations at a final concentration of 4 mM. The DPH $[(r_o/r) - 1]^{-1}$ values were estimated, and thereafter portions of each sample were shaken in the presence of 8 mM EDTA for 35 min and the anisotropy parameter was determined again. As shown in Table I, Ca^{2+} increased $[(r_o/r) - 1]^{-1}$ by 13.5% ($P < 0.001$), Sr^{2+} appeared to increase the value by 6.1% ($P < 0.05$), and Ba^{2+}, Mg^{2+}, and Zn^{2+} had no significant effect. After the subsequent incubation with EDTA, only the calcium-treated samples retained the increment in $[(r_o/r) - 1]^{-1}$ relative to the controls, 14.8% ($P < 0.01$), and the strontium-treated samples were no longer significantly different from the controls. The results indicate that the increase in $[(r_o/r) - 1]^{-1}$ which is not reversible by EDTA is highly specific for calcium as compared to other divalent cations. It is also noteworthy in Table I that treatment of the control preparations with 8 mM EDTA lowered the $[(r_o/r) - 1]^{-1}$ values, an observation we have made repeatedly in these studies. Although the mechanism of this decrease is unknown, it may result from the removal of endogenous membrane calcium and a reversal of the usual effects of the cation.

Other Studies. Two preparations of hepatocyte plasma membranes were incubated in the presence and absence of 4 mM Ca^{2+} for 2 h at 37 °C. Thereafter, lipid extracts were prepared and their composition was determined by thin-layer chromatography. The results shown in Table II demonstrate at least three alterations in the lipid following calcium treatment of the membranes: increases in lysolecithin ($P < 0.01$) and fatty acids ($P < 0.001$) and a decrease in phosphatidylethanolamine ($P < 0.01$). The content of sphingomyelin and cholesterol was not changed significantly. The molar ratios of cholesterol to phospholipid, 0.84 and 0.76 in the control and calcium-treated preparations, respectively, were not significantly different, and the values correspond to the upper level of the range reported by prior investigators, 0.4–0.7 (Dod & Gray, 1968; Chandrasekhara & Narayan, 1970;

Table II: Lipid Composition following Incubation of Hepatocyte Membranes in the Presence or Absence of Calcium[a]

constituent	% wt of total lipid (mean ± SE) no Ca^{2+}	4 mM Ca^{2+}	P[b]
cholesterol	21.2 ± 1.8	19.6 ± 1.6	ns[c]
cholesterol esters	17.8 ± 2.1	14.9 ± 1.3	ns
triglycerides	3.0 ± 0.4	2.7 ± 0.4	ns
fatty acids	6.0 ± 0.4	9.9 ± 0.4	<0.001
lecithin	20.9 ± 3.1	21.9 ± 0.6	ns
lysolecithin	3.4 ± 1.0	8.3 ± 1.1	<0.01
sphingomyelin	14.2 ± 1.3	12.4 ± 1.3	ns
phosphatidylethanolamine	13.6 ± 1.0	10.4 ± 0.8	<0.01

[a] Values are for four determinations of the lipid from two hepatocyte plasma membrane preparations. Each membrane preparation was incubated with and without 4 mM Ca^{2+} for 2 h at 37 °C, and the total lipids were then extracted. [b] *P* values are for the differences between the calcium-treated and untreated preparations. [c] ns = not significant.

Emmelot & van Hoeven, 1975; Kremmer et al., 1976; Fleischer & Fleischer, 1977).

Discussion

The foregoing results support the conclusion that calcium decreases the motional freedom of lipid molecules, i.e., the lipid fluidity, in isolated rat hepatocyte plasma membranes. This action of the cation, moreover, involves at least two distinct mechanisms. The first is *direct*, i.e., observed on addition of Ca^{2+} to sonicated dispersions of the membrane lipid and reversed by subsequent treatment with excess EDTA. The second mechanism is *indirect* in that it requires exposure of the intact membrane to the cation and cannot be reversed on subsequent removal of Ca^{2+} (Figures 4 and 5). Our experimental results provide further distinctions between the two processes. The direct action on liposomes is relatively rapid, with a half-time of approximately 10–15 min, whereas the half-time of the indirect effect on the membranes is approximately 75 min (Figure 3). At ambient Ca^{2+} concentrations of 2 mM or less, the decrease in lipid fluidity of the membranes considerably exceeds that of the treated liposomes (Figure 1). The magnitude of the direct effect on the liposomes varies linearly with Ca^{2+} concentration in the range 0–4 mM, whereas the effect on the membranes tends to plateau, with an apparent half-saturation concentration of approximately 1 mM (Figure 1). Finally, it is significant that the indirect effect on the membranes is relatively specific for Ca^{2+} as compared to Sr^{2+}, Ba^{2+}, Mg^{2+}, and Zn^{2+} (Table I) and that it involves changes in biochemical composition of the membrane lipids (Table II).

It is reasonable to propose that the direct effect represents the binding of Ca^{2+} to anionic sites of the lipid bilayer, probably to phospholipid polar head groups and to sialic acid residues (Shlatz & Marinetti, 1972). Binding of this type was proposed by Gordon et al. (1978) to explain the decrease in fluidity of hepatocyte plasma membranes studied with a stearic acid spin probe. Calcium binding to anionic phospholipids in model bilayers has been shown to aggregate the molecules and to trigger phase separations (Traüble & Eibl, 1974; Ohnishi & Ito, 1974; Jacobson & Papahadjopoulos, 1975; Hartmann et al., 1977).

The indirect effect of calcium, on the other hand, requires the presence of the hepatocyte membrane proteins and probably occurs by stimulation of certain of the membrane-bound enzymes to alter the lipids biochemically. This hypothesis is attractive in view of the well established requirement for calcium for the phospholipase A_1 and A_2 activities of rat hepatocyte plasma membranes (Newkirk & Waite, 1971, 1973; Victoria et al., 1971; Nachbaur et al., 1972). Moreover, a number of authors have pointed to the existence of endogenous plasma membrane reactions for the acylation and deacylation of membrane phospholipids and have proposed the operation of dynamic deacylation–reacylation cycles for regulating the phosphoglycerides (Newkirk & Waite, 1971; van den Bosch et al., 1972; Holub & Kuksis, 1978). To the extent that such regulatory cycles operate in the isolated membrane suspensions studied here, calcium may so modulate the reactions as to result in phospholipids containing more saturated or longer acyl chains, features which decrease the fluidity of the arrays (Oldfield & Chapman, 1971; Shinitzky & Barenholz, 1978). Clearly, the cation could also affect these and other components of the membrane lipid via other membrane enzymes. The changes in lipid composition detected thus far (Table II) suggest the activation of membrane phospholipase A activity. Since the resulting increase in membrane lysophospholipid is expected to enhance membrane fluidity (van Zutphen & van Deenen, 1967; Reman et al., 1969), this effect per se does not account for the net decrease in lipid fluidity demonstrated in our experiments.

There is considerable evidence that calcium ion plays a significant role in the regulation of a variety of functions associated with cell membranes, including the transduction of hormonal signals, neurotransmitter release and action, muscle contraction and relaxation, release of exocrine and endocrine secretory products, transmembrane transport and permeability, and the maintenance of membrane viscoelastic properties and cell shape (Manery, 1966; Rasmussen, 1970; Foreman et al., 1973). Our results underscore the possibility that the effects of the cation on certain functions of the hepatocyte plasma membrane may result from a decrease in lipid fluidity, a hypothesis posed by Sauerheber & Gordon (1975) and Gordon et al. (1978). Three examples of membrane functions which are affected by both calcium and lipid fluidity are of particular interest in this regard. The activity of the adenylate cyclase of hepatocyte membranes is increased by agents, e.g., benzyl alcohol, which increase the lipid fluidity (Dipple & Houslay, 1978), and Arrhenius studies and lipid-substitution experiments confirm the modulating effects of the lipids (Houslay et al., 1976a,b; Dipple & Houslay, 1978). Hepp et al. (1970), moreover, have reported that 1 mM Ca^{2+} inhibits the rat liver adenylate cyclase, whereas EGTA enhances the activity. The $(Na^+ + K^+)$-dependent adenosine triphosphatase of hepatocyte membranes also appears to be affected by the lipid fluidity (Keeffe et al., 1979), and there is additional evidence in a number of membrane types that the enzyme complex requires a relatively fluid lipid environment (Grisham & Barnett, 1972; Kimelberg & Papahadjopoulos, 1974; Brasitus & Schachter, 1980). Correspondingly, Boyer & Reno (1975) noted that 1.25–2.5 mM Ca^{2+} markedly inhibits the enzyme activity in a membrane suspension enriched in bile canaliculi. Finally, Kolb & Adam (1976) observed that the permeability of isolated rat hepatocytes to extracellular K^+ is decreased as the external Ca^{2+} concentration is increased from 10^{-4} to 1 mM. Studies of model bilayer membranes and of erythrocyte membranes provide considerable evidence that passive permeability to neutral molecules and ions can vary concordantly with the lipid fluidity (Papahadjopoulos et al., 1973; van Deenen & de Gier, 1974). In summary, the possibility that certain of the functional effects of calcium may be mediated via changes in membrane lipid metabolism and fluidity should be considered in light of the results reported here.

It is especially noteworthy that the hormone-responsive adenylate cyclase of hepatocyte membranes is sensitive to its lipid microenvironment. Rasmussen (1970) summarized evidence that an increase in intracellular Ca^{2+} concentration frequently accompanies the stimulation of adenylate cyclase by hormones. By decreasing the lipid fluidity of the plasma membrane, the cation could inhibit the enzyme and provide, thereby, a negative feedback loop in the signal transduction system.

Acknowledgments

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