Effects of lysophospholipids on Ca^{2+} transport in rat liver mitochondria incubated at physiological Ca^{2+} concentrations in the presence of Mg^{2+} , phosphate and ATP at 37°C

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1. Lysophospholipids caused the release of $^{45}\text{Ca}^{2+}$ from isolated rat liver mitochondria incubated at 37°C in the presence of low concentrations of free Ca^{2+} , ATP, Mg^{2+} , and phosphate ions. The concentrations of lysophosphatidylethanolamine, lysophosphatidylcholine, lysophosphatidic acid and lysophosphatidylinositol which gave half-maximal effects were 5, 26, 40 and 56 μM , respectively. The effects of lysophosphatidylethanolamine were not associated with a significant impairment of the integrity of the mitochondria as monitored by measurement of membrane potential and the rate of respiration. 2. Lysophosphatidylethanolamine did not induce the release of Ca^{2+} from a microsomal fraction, or enhance Ca^{2+} inflow across the plasma membrane of intact cells, but did release Ca^{2+} from an homogenate prepared from isolated hepatocytes and incubated under the same conditions as isolated mitochondria. 3. The proportion of mitochondrial $^{45}\text{Ca}^{2+}$ released by lysophosphatidylethanolamine was not markedly affected by altering the total amount of Ca^{2+} in the mitochondria, the concentration of extramitochondrial Mg^{2+} , by the addition of Ruthenium Red, or when oleoyl lysophosphatidylethanolamine was employed instead of the palmitoyl derivative. 4. The effects of 5 μM -lysophosphatidylethanolamine were reversed by washing the mitochondria. 5. The possibility that lysophosphatidylethanolamine acts to release Ca^{2+} from mitochondria in intact hepatocytes following the binding of Ca^{2+} -dependent hormones to the plasma membrane is briefly discussed.

The actions of the Ca^{2+} -dependent hormones, α -adrenergic agonists, vasopressin and angiotensin on hepatic metabolism are dependent on an increase in the concentration of free Ca^{2+} in the cytoplasm (Assimakopoulos-Jeannet *et al.*, 1977; Keppens *et al.*, 1977; Murphy *et al.*, 1980; Joseph & Williamson, 1983; Charest *et al.*, 1983). This Ca^{2+} is derived from intracellular stores, including the mitochondria (Chen *et al.*, 1978; Babcock *et al.*, 1979; Blackmore *et al.*, 1979; Murphy *et al.*, 1980; Berthon *et al.*, 1981; Reinhart *et al.*, 1982; Kimura *et al.*, 1982; Joseph & Williamson, 1983), endoplasmic reticulum (Blackmore *et al.*, 1979; Reinhart *et al.*, 1982; Joseph & Williamson, 1983) and possibly other membranes (Althaus-Salzman *et al.*, 1980; Berthon *et al.*, 1981; Kimura *et al.*, 1982) as well as the extracellular medium (Keppens *et al.*, 1977; Assimakopoulos-Jeannet *et al.*, 1977; Foden & Randle, 1978; Barritt *et al.*, 1981; Parker *et al.*,

1983; De Witt & Putney, 1984; Reinhart *et al.*, 1984). Evidence that substantial quantities of Ca^{2+} are released from the mitochondria within 30–60 s after the addition of a Ca^{2+} -dependent hormone has been presented (Reinhart *et al.*, 1982).

The mechanisms by which Ca^{2+} -dependent hormones alter cellular Ca^{2+} transport have not been clearly defined. A number of metabolites have been proposed to catalyse agonist-induced release of Ca^{2+} from intracellular organelles (Barritt *et al.*, 1981; Barritt, 1981a; Whiting & Barritt, 1982; Chong *et al.*, 1983; Creba *et al.*, 1983; Thomas *et al.*, 1983; Litosch *et al.*, 1983; Streib *et al.*, 1983; Joseph *et al.*, 1984) but data presently available indicate that none of these compounds releases Ca^{2+} from mitochondria under physiological conditions. Lysophospholipids, generated by mitochondrial phospholipase A_2 , have been shown to catalyse the release of Ca^{2+} from mito-

chondria under certain conditions (Pfeiffer *et al.*, 1979; Beatrice *et al.*, 1980, 1982; Schmid *et al.*, 1981) and to enhance Ca^{2+} transport across other membranes (Gerrard *et al.*, 1979; Sedlis *et al.*, 1983). Moreover, thrombin induces the formation of lysophospholipids in blood platelets (Mauco *et al.*, 1978; Kannagi & Koizumi, 1979; Broekman *et al.*, 1980; McKean *et al.*, 1981; Lapetina *et al.*, 1981a; Billah & Lapetina, 1982) and it has been proposed that lysophosphatidic acid is involved in the mechanism by which thrombin increases the cytoplasmic Ca^{2+} concentrations (Gerrard *et al.*, 1979; Lapetina *et al.*, 1981a,b; Billah & Lapetina, 1982). These results suggest that lysophospholipids may catalyse changes in cellular Ca^{2+} transport induced by the action of Ca^{2+} -dependent hormones on the liver.

The aim of the present experiments was to test the effects of lysophospholipids on the transport of Ca^{2+} across the three membrane systems which are known to be affected by Ca^{2+} -dependent hormones in the liver cell, namely, Ca^{2+} outflow from the mitochondria and endoplasmic reticulum, and Ca^{2+} inflow across the plasma membrane. The results indicate that, under ionic conditions which resemble those in the cytoplasm, low concentrations of lysophosphatylethanolamine induce the release of Ca^{2+} from mitochondria without altering the integrity of these organelles, but do not alter Ca^{2+} transport across the microsomal or plasma membranes.

Experimental

Isolation of mitochondria and microsomes

Male hooded Wistar rats, weighing 200–300 g, were obtained from the Institute of Medical and Veterinary Science, Adelaide. The animals were fed *ad libitum*. Mitochondria that sedimented at between 3000 and 22500 g·min ('heavy mitochondria' as defined by Prpić *et al.*, 1978) were isolated as described by Hughes & Barritt (1978) with the modification that the rats were anaesthetized with diethyl ether before decapitation. Fractions enriched in microsomes derived from the endoplasmic reticulum and an 'intermediate' fraction enriched in heavy microsomes were isolated as described by Bygrave & Tranter (1978).

The protein content of subcellular fractions treated with 2% (w/v) sodium deoxycholate was measured by the method of Lowry *et al.* (1951). Mitochondrial respiration was measured as described by Hughes & Barritt (1978). For routine measurements at 25°C the incubation medium (Reed, 1972) contained 100 mM-sucrose, 50 mM-KCl, 15 mM-Tris/HCl, 10 mM-potassium phosphate, 2 mM-MgCl₂, 1 mM-EDTA, 12.5 mM-potassium succinate, adjusted to pH 7.4, and 1.0 mg

of mitochondrial protein/ml. Only mitochondria with values for ADP-stimulated:ADP-depleted rates of O₂ utilization of 4–7 were used. The mean value of the ratio of ADP-stimulated:ADP-depleted O₂ utilization was 4.65 ± 0.21 ($n = 49$). In some experiments respiration was measured at 37°C as described in the legend of Fig. 2(c).

Preparation of homogenates from isolated hepatocytes

Hepatocytes were isolated by the method of Berry & Friend (1969) as described previously (Barritt *et al.*, 1981). Cells (0.3 g wet wt.) were suspended in 10 ml of mitochondrial incubation medium at 0°C, and homogenized by 30 strokes of a glass-on-glass Dounce homogenizer [7 ml capacity, tight ('B') pestle (Kontes Glass Co., Vineland, NJ, U.S.A.)]. The mitochondrial incubation medium contained 150 mM-KCl, 10 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid]/KOH, 10 mM-nitritotriacetic acid, 2 mM-potassium phosphate, 2 mM-potassium succinate, 3 mM-sodium ATP and 2 mM-MgCl₂, adjusted to pH 7.4 at 25°C. Part of the homogenate (3 ml) was mixed with 12 ml of mitochondrial incubation medium and centrifuged for 10 min at 300 g at 4°C. The supernatant was kept at 4°C and used in subsequent experiments. The protein concentration was 11.5 ± 0.5 ($n = 3$) mg/ml.

⁴⁵Ca²⁺ exchange

⁴⁵Ca²⁺ exchange by isolated mitochondria was measured at 37°C as described by Barritt (1981b) in mitochondrial incubation medium. The concentration of mitochondria was 1.5 mg of protein/ml. Other additions are described in the legends to Figures and in the text. Unless otherwise indicated, the concentration of added CaCl₂ was 50 μM [0.16 μM free Ca²⁺ at equilibrium (Barritt, 1981b)]. The incubation was initiated by addition of the mitochondria (in the presence of the given concentration of ⁴⁰Ca²⁺). After 15 min, tracer amounts of ⁴⁵CaCl₂ (0.24 μCi) were added and samples of the incubation medium subsequently removed for measurement of ⁴⁵Ca²⁺ associated with the mitochondria as described previously (Barritt, 1981b).

⁴⁵Ca²⁺ exchange in isolated microsomes, the intermediate fraction (1.5 mg of protein/ml) and cell homogenates (1.9 mg of protein/ml) was measured by a procedure similar to that employed for the measurement of ⁴⁵Ca²⁺ exchange in isolated mitochondria. In these experiments the particulate fraction was separated from the incubation medium (0.2 ml) by vacuum filtration (Millipore Corp., pore diameter 0.45 μm). The filters were washed with 0.5 ml of 250 mM-sucrose which contained 2 mM-Hepes/KOH, pH 7.4 at 4°C. Lysophospholipids were prepared as stock solu-

tions (4mM) in mitochondrial incubation medium. Fresh solutions were used for each experiment and mixed thoroughly before use.

Amounts of $^{45}\text{Ca}^{2+}$ exchanged by isolated organelles or the particulate fraction of cell homogenates are expressed as a percentage of the initial dose of $^{45}\text{Ca}^{2+}$ added to the incubation mixture (Barritt, 1981b; Baddams *et al.*, 1983). For isolated mitochondria incubated at $50\mu\text{M}$ added Ca^{2+} , a value of 1% initial dose represents approx. 0.76nmol of $^{45}\text{Ca}^{2+}$ exchanged per mg of protein.

Rates of $^{45}\text{Ca}^{2+}$ exchange in isolated hepatocytes were measured at 37°C as described by Barritt *et al.* (1981). Concentrations of free Ca^{2+} and Mg^{2+} were calculated as described previously by Barritt (1981b).

Mitochondrial membrane potential

Changes in mitochondrial membrane potential were determined by measuring the absorbance of Safranine Orange at 25°C as described by Åkerman & Wikström (1976). Absorption was measured at the wavelength pair 511–533nm on a dual-wavelength spectrophotometer (Model UV-3000, Shimadzu Corp., Kyoto, Japan). The incubation medium consisted of mitochondrial incubation medium which contained $50\mu\text{M}$ added Ca^{2+} and 1.5mg of mitochondrial protein/ml. Changes in absorbance induced by a K^+ diffusion potential were correlated with calculated values of membrane potential as described by Åkerman & Wikström (1976).

Treatment of data

Except where indicated otherwise, the data are expressed as the mean and S.E.M. of the number of experiments indicated.

Materials

Lysophosphatidylinositol from pig liver, synthetic palmitoyl and oleoyl lysophosphatidylethanolamine and synthetic palmitoyl lysophosphatidylcholine were purchased from Serdary Research Laboratories, Ontario, Canada. All other phospholipids and Safranine Orange were obtained from Sigma. Other chemicals were obtained from the sources described previously (Whiting & Barritt, 1982).

Results

Isolated mitochondria

The effects of 1-acyl-lysophospholipids on the release of Ca^{2+} from isolated mitochondria were studied in organelles incubated in the presence of $0.16\mu\text{M}$ free Ca^{2+} ($50\mu\text{M}$ added Ca^{2+}), ATP, Mg^{2+} , and P_i and labelled for 16min with $^{45}\text{Ca}^{2+}$. At this time, exchange of extramitochondrial $^{45}\text{Ca}^{2+}$ with

Fig. 1. Effects of lysophospholipids on $^{45}\text{Ca}^{2+}$ release from isolated mitochondria

(a) Incubation of the mitochondria and measurement of the amount of $^{45}\text{Ca}^{2+}$ associated with the mitochondria were performed in the presence of $50\mu\text{M}$ added Ca^{2+} ($0.16\mu\text{M}$ free Ca^{2+} at equilibrium) as described in the Experimental section. $^{45}\text{Ca}^{2+}$ was added at 0min. At 16min additions of 1 ($\circ$), 5 ($\blacksquare$), 10 ($\square$), 25 ($\blacktriangle$), 50 ($\triangle$), 75 ($\boxplus$) and $100\mu\text{M}$ ($\ominus$) palmitoyl lysophosphatidylethanolamine or $125\mu\text{l}$ of incubation medium ($\bullet$) were made (indicated by the arrow). The results are the means $\pm$ S.E.M. of four or five experiments. (b) Dose-response curves for the effect of increasing concentrations of lysophosphatidylethanolamine ($\circ$), lysophosphatidylcholine ($\triangle$), lysophosphatidic acid ($\bullet$) and lysophosphatidylinositol ($\blacktriangle$) on the amount of $^{45}\text{Ca}^{2+}$ released from the mitochondria. The results are expressed as a fraction of the amount of $^{45}\text{Ca}^{2+}$ associated with the mitochondria in the absence of lysophospholipid. For each lysophospholipid, experiments similar to those described in (a) were performed. The amount of $^{45}\text{Ca}^{2+}$ associated with the mitochondria following the addition of a given concentration of lysophospholipid was obtained by determining the mean $\pm$ S.E.M. of the values obtained at times between 18 and 28 min after addition of $^{45}\text{Ca}^{2+}$ to the incubation medium in two to five separate experiments.

rapidly-exchangeable mitochondrial Ca^{2+} approaches equilibrium (Barritt, 1981*b*). The amount of Ca^{2+} exchanged at 16 min, 4% of the initial dose of $^{45}\text{Ca}^{2+}$, is approx. 3.0 nmol of Ca^{2+} /mg of protein.

The addition of palmitoyl lysophosphatidylethanolamine induced a rapid release of $^{45}\text{Ca}^{2+}$ (Fig. 1*a*). The new plateau was sustained for at least 12 min. The Ca^{2+} -selective ionophore A23187 caused a complete release of $^{45}\text{Ca}^{2+}$ (results not shown). The concentration of lysophosphatidylethanolamine which gave half-maximal effect was about $5\text{ }\mu\text{M}$ (Fig. 1*b*). Addition of the synthetic palmitoyl derivatives of lysophosphatidylcholine and lysophosphatidic acid, and of lysophosphatidylinositol from pig liver, gave results similar to those shown in Fig. 1*a*), although lysophosphatidic acid and lysophosphatidylinositol were considerably less effective (results not shown). The concentrations of these agents which gave half-maximal effect were 26, 40 and $56\text{ }\mu\text{M}$, respectively (Fig. 1*b*). Lysophosphatidylglycerol at concentrations of 5 and $100\text{ }\mu\text{M}$ released 4 and 70%, respectively, of the $^{45}\text{Ca}^{2+}$ associated with the mitochondria at 16 min. Lysophosphatidylserine was less effective (0 and 50% of the $^{45}\text{Ca}^{2+}$ associated with the mitochondria was released at 5

and $100\text{ }\mu\text{M}$, respectively). Since lysophosphatidylethanolamine was the most effective lysophospholipid tested, further experiments were conducted with this compound.

Lysophosphatidylethanolamine at concentrations between 5 and $100\text{ }\mu\text{M}$ did not significantly alter the rates of O_2 utilization or the ratio of ADP-stimulated:ADP-depleted O_2 utilization for mitochondria incubated in the absence of Ca^{2+} at 25°C under conditions routinely employed to measure mitochondrial integrity (Figs. 2*a* and 2*b*), or the rate of O_2 utilization for mitochondria incubated in the presence of $0.16\text{ }\mu\text{M}$ free Ca^{2+} ($50\text{ }\mu\text{M}$ added Ca^{2+}) at 37°C under the conditions employed for the measurement of $^{45}\text{Ca}^{2+}$ release (Fig. 2*c*). Only a slight decrease in the inner membrane potential was observed within 2 min following the addition of lysophosphatidylethanolamine to mitochondria incubated at 25°C (Fig. 3).

The effects of palmitoyl lysophosphatidylethanolamine on $^{45}\text{Ca}^{2+}$ release were complete within 20 s following the addition of this agent to the incubation medium (Fig. 4). When the fatty acid moiety of lysophosphatidylethanolamine was changed from palmitate to oleate (synthetic oleoyl lysophosphatidylethanolamine) or predominantly stearate (lysophosphatidylethanolamine

Fig. 2. Effects of lysophosphatidylethanolamine on the rate of O_2 utilization by isolated mitochondria incubated at 25°C (a, b), or at 37°C in the presence of ATP and Mg^{2+} (c)

(a, b) Mitochondrial respiration was measured at 25°C in the absence of added Ca^{2+} in the medium of Reed (1972) as described in the Experimental section. Mitochondria (1.0 mg of protein/ml), sodium ADP (125 μM) or 50 μM -2,4-dinitrophenol (DNP), or lysophosphatidylethanolamine (LPE) were added at the times indicated. (c) Mitochondria (1.0 mg of protein/ml) were incubated at 37°C in mitochondrial incubation medium (described in the Experimental section) in the presence of $50\text{ }\mu\text{M}$ added Ca^{2+} . Mitochondria and lysophosphatidylethanolamine (LPE) were added at the times indicated. The traces shown are for one of three (a and c), or four (b) experiments which gave similar results. The values shown (means $\pm$ S.E.M.) are the values of ADP-stimulated:ADP-depleted O_2 utilization (a), or the rates of O_2 utilization (ng-atom of O/min per mg of mitochondrial protein) (b and c).

Fig. 3. Effects of lysophosphatidylethanolamine on the absorbance of Saffranin Orange measured in isolated mitochondria incubated in the presence of ATP and Mg^{2+}

The absorbance of Saffranin Orange was measured at 25°C, and the absorbance values converted to membrane potential, as described in the Experimental section. Palmitoyl lysophosphatidylethanolamine (LPE) or 2,4-dinitrophenol (DNP) were added at the times indicated. The trace shown is that obtained for one of four experiments which gave similar results. The values shown (means $\pm$ S.E.M., $n = 4$) are the estimated values of the membrane potential in mV.

from bovine liver) or stearate plus palmitate (lysophosphatidylethanolamine from egg yolk) the proportion of $^{45}\text{Ca}^{2+}$ released by a given concentration of lysophosphatidylethanolamine was not significantly altered. The concentration of oleoyl lysophosphatidylethanolamine which gave half-maximal effect was $6 \mu\text{M}$ (results not shown).

The proportion of $^{45}\text{Ca}^{2+}$ released by palmitoyl lysophosphatidylethanolamine was not markedly affected by lowering or raising the added Ca^{2+} concentration. Thus at $25 \mu\text{M}$ added Ca^{2+} the amounts of $^{45}\text{Ca}^{2+}$ associated with the mitochondria at 0, 5 and $100 \mu\text{M}$ -lysophosphatidylethanolamine were 2.1, 1.5 and 0.1% of the initial dose of $^{45}\text{Ca}^{2+}$. At $200 \mu\text{M}$ added Ca^{2+} the values were 13.5, 8.3 and 1.6% of initial dose, respectively. The proportion of $^{45}\text{Ca}^{2+}$ released was markedly altered by an increase in the concentration of total Mg^{2+} from 2 mM (0.05 mM free Mg^{2+}) to 5 (0.22 mM free Mg^{2+}) or 10 mM (1.1 mM free Mg^{2+}) (results not shown).

Fig. 4. Effect of lysophosphatidylethanolamine on $^{45}\text{Ca}^{2+}$ release from isolated mitochondria at early time periods

Incubation of the mitochondria and measurement of the amount of $^{45}\text{Ca}^{2+}$ associated with the organelles were performed as described in the Experimental section. Palmitoyl lysophosphatidylethanolamine at a concentration of 5 (●) or 100 (○) μM was added at 16 min (indicated by the arrow). The amount of $^{45}\text{Ca}^{2+}$ associated with the mitochondria at times before this addition was determined by taking samples at 14, 14.5, 15 and 15.5 min (data points not shown). This value (indicated by the broken line) was $4.4 \pm 0.09\%$ and $3.8 \pm 0.08\%$ ($n = 12$) of the initial dose of $^{45}\text{Ca}^{2+}$ added to the incubation medium for the experiments conducted at 5 and $100 \mu\text{M}$ -lysophosphatidylethanolamine, respectively. Each data point represents the mean $\pm$ S.E.M. of three experiments.

Moreover, Ruthenium Red ($8 \mu\text{M}$) added 1 min before the lysophosphatidylethanolamine did not alter the proportion of $^{45}\text{Ca}^{2+}$ released by either 5 or $100 \mu\text{M}$ -lysophospholipid (results not shown). This result indicates that the lysophospholipid does not act by inhibiting Ca^{2+} inflow to the mitochondria, since Ruthenium Red has been shown to inhibit Ca^{2+} uptake catalysed by the electrophoretic uniporter (Moore, 1971).

The effect of the removal of lysophosphatidylethanolamine from the incubation medium on the ability of mitochondria to exchange $^{45}\text{Ca}^{2+}$ was tested as follows. Mitochondria previously exposed to 5 or $100 \mu\text{M}$ -palmitoyl lysophosphatidylethanolamine were washed, incubated in the absence of lysophosphatidylethanolamine and the amount of $^{45}\text{Ca}^{2+}$ exchanged measured (Fig. 5a). Fig. 5(b) shows that for mitochondria treated in the same way as those in Fig. 5(a), but not initially exposed to lysophosphatidylethanolamine, the effects of lysophosphatidylethanolamine on $^{45}\text{Ca}^{2+}$ exchange were similar to those observed when the lysophospholipid is added during the exchange curve (Fig. 1a). These results indicate that the effects of $5 \mu\text{M}$ -lysophosphatidylethanolamine are

Fig. 5. Effect of removal of lysophosphatidylethanolamine from mitochondria on subsequent $^{45}\text{Ca}^{2+}$ exchange. (a) Effect of treatment of mitochondria with lysophosphatidylethanolamine on $^{45}\text{Ca}^{2+}$ exchange measured after removal of the lysophospholipid. Mitochondria were incubated and treated under the conditions described in the legend of Fig. 1(a) in the absence of $^{45}\text{Ca}^{2+}$ and in the presence of 5 (■) or 100 (▲) μM palmitoyl lysophosphatidylethanolamine added at 16 min or in the absence of lysophospholipid (△). At 26 min, the entire incubation mixture was centrifuged at 4500 g for 5 min at 4°C, the supernatant was removed by aspiration and the resulting pellet suspended in 5 ml of 250 mM-sucrose which contained 2 mM-Hepes, adjusted to pH 7.4 with KOH. The mitochondria were centrifuged at 4500 g for 5 min, the supernatant discarded and the pellet suspended in Ca^{2+} -free mitochondrial incubation medium at 1°C. The suspension was then transferred to an incubation chamber at 37°C. After 2 min 50 μM - CaCl_2 and $^{45}\text{CaCl}_2$ (0.24 μCi) were added to initiate $^{45}\text{Ca}^{2+}$ exchange. Samples were removed at the times indicated and the amounts of $^{45}\text{Ca}^{2+}$ in the mitochondria determined as described in the Experimental section. Each data point represents the mean $\pm$ S.E.M. of three experiments. (b) Effect of lysophosphatidylethanolamine on $^{45}\text{Ca}^{2+}$ exchange by mitochondria incubated at 37°C in the absence of lysophospholipid. Mitochondria were incubated at 37°C in the absence of lysophosphatidylethanolamine, washed, resuspended and incubated in the presence of $^{45}\text{Ca}^{2+}$ as described for (a). The final incubation at 37°C was conducted in the presence of 5 (■) or 100 (▲) μM palmitoyl lysophosphatidylethanolamine, or in the absence of lysophospholipid (△). Each data point represents the mean $\pm$ S.E.M. of three experiments.

completely reversed, while those of 100 μM -lysophosphatidylethanolamine are partially reversed by washing the mitochondria.

Microsomal fractions, cell extract and intact cells

Addition of palmitoyl lysophosphatidylethanolamine (5–100 μM) to a fraction enriched in microsomes derived from the endoplasmic reticulum or to an intermediate fraction composed chiefly of heavy microsomes (Bygrave & Tranter, 1978), incubated under the conditions described in the legend of Fig. 1(a), did not cause a decrease in the amount of $^{45}\text{Ca}^{2+}$ associated with the organelles. By contrast, a complete loss of $^{45}\text{Ca}^{2+}$ was observed following the addition of 10 μM -ionophore A23187 (results not shown).

Lysophosphatidylethanolamine released $^{45}\text{Ca}^{2+}$ from the particulate fraction of an homogenate prepared from isolated hepatocytes incubated under the conditions employed for the measurement of $^{45}\text{Ca}^{2+}$ release from isolated mitochondria. The amounts of $^{45}\text{Ca}^{2+}$ associated with the particulate fraction in the presence of 0, 5 and 100 μM -palmitoyl lysophosphatidylethanolamine, 50 μM -2,4-dinitrophenol, and 50 μM -2,4-dinitrophenol plus 1 μM -ionophore A23187 were 11, 10, 8, 7.6 and 2.8% of the initial dose of $^{45}\text{Ca}^{2+}$, respectively.

Palmitoyl lysophosphatidylethanolamine (100 μM) did not alter the rate of $^{45}\text{Ca}^{2+}$ exchanged at times between 0 and 4 min in intact isolated hepatocytes incubated in the presence of 1.3 mM extracellular Ca^{2+} . By contrast, phospholipase A from bee venom (0.5 $\mu\text{g}/\text{ml}$) caused a 2-fold increase in the amount of $^{45}\text{Ca}^{2+}$ exchanged (results not shown), as described previously (Barritt & Whiting, 1983).

Discussion

The results have shown that, under the conditions employed, low concentrations of lysophosphatidylethanolamine (1–5 μM) induce a reversible release of Ca^{2+} from isolated mitochondria and a new steady-state value of mitochondrial exchangeable Ca^{2+} without significantly altering the integrity of the mitochondrial inner membrane. These observations are consistent with an effect, which is reasonably selective for Ca^{2+} transport, on the permeability of the mitochondrial inner membrane (Beatrice *et al.*, 1980, 1982). The release of Ca^{2+} does not appear to be due to inhibition of Ca^{2+} inflow. Moreover, the failure of lysophosphatidylethanolamine to release Ca^{2+} from the microsomal fraction indicates that the lysophospholipid does not act as a Ca^{2+} -selective ionophore by a mechanism similar to that of ionophore A23187 (compare with Simon *et al.*, 1984).

Beatrice *et al.* (1980, 1982) have previously

shown that the release of Ca^{2+} from rat liver mitochondria induced by a number of agents, including P_i and oxaloacetate, is associated with an increase in respiration and swelling, and a marked decrease in membrane potential. Evidence that Ca^{2+} release observed under these conditions is catalysed by lysophospholipids has been presented (Beatrice *et al.*, 1982). The difference between these and the present results is likely to be due to the much higher concentrations of Ca^{2+} employed by Beatrice *et al.* (1980, 1982) and the inclusion of ATP and Mg^{2+} , which increase the stability of the inner membrane (Chappell & Crofts, 1965; Sordahl, 1974; Binet & Volfin, 1975; Jacobus *et al.*, 1975; Harris, 1979), in the present experiments.

A number of agents have been shown to release Ca^{2+} from liver mitochondria. These include phosphoenolpyruvate, compounds (including hydroperoxides) which increase the oxidation state of mitochondrial nicotinamide adenine nucleotides, extramitochondrial pH, cyclic AMP and Na^+ (reviewed by Nicholls & Åkerman, 1982). However, with the exception of Na^+ , the Ca^{2+} -releasing actions of these agents appear to be associated with a collapse of membrane potential, are inhibited by the inclusion of ATP in the incubation medium, require unphysiologically high levels of Ca^{2+} , or require highly selective incubation conditions (reviewed by Nicholls & Åkerman, 1982; Beatrice *et al.*, 1984; but see also Baumhüter & Richter, 1982).

The release by Na^+ of Ca^{2+} from isolated heart mitochondria (Crompton *et al.*, 1976) occurs without damage to the inner membrane (reviewed by Nicholls & Åkerman, 1982). In contrast to the present results with liver mitochondria and lysophosphatidylethanolamine, this effect of Na^+ is associated with a small stimulation of O_2 utilization. This has been attributed to an increase in the rate of Ca^{2+} cycling (Crompton *et al.*, 1976). However, in the present studies the change in Ca^{2+} cycling caused by lysophosphatidylethanolamine appears to be small as judged by similar rates of $^{45}\text{Ca}^{2+}$ exchange for mitochondria in the presence and absence of lysophosphatidylethanolamine (Fig. 5b). Moreover, the contribution of Ca^{2+} cycling to the observed rate of O_2 utilization at 37°C is estimated to be about 0.3%, based on a flux of 1.5 nmol of Ca^{2+} /min per mg of protein at $0.16 \mu\text{M}$ free extramitochondrial Ca^{2+} (Barritt, 1981) and a value of 6g-ions of Ca^{2+} taken up per g-atom of O utilized (Crompton *et al.*, 1976).

The effect of Na^+ on Ca^{2+} efflux from heart mitochondria is potentiated by the presence of Ruthenium Red (Crompton *et al.*, 1976) in contrast with the lack of effect of this inhibitor of Ca^{2+} inflow on Ca^{2+} efflux induced by a suboptimal concentration of lysophosphatidylethanolamine

from liver mitochondria. This difference may be due to differences in the experimental conditions employed, including the concentrations of ATP, Mg^{2+} and Ca^{2+} , inherent differences between Ca^{2+} transport systems in heart and liver mitochondria (reviewed by Nicholls & Åkerman, 1982), and differences between the mechanisms of action of Na^+ and lysophosphatidylethanolamine. In two studies of the effects of Na^+ on Ca^{2+} release from liver mitochondria little or no potentiation by Ruthenium Red of Na^+ -induced Ca^{2+} efflux was observed (Nedergaard & Cannon, 1980; Haworth *et al.*, 1980).

The present results indicate that lysophosphatidylethanolamine is one of the few compounds that will induce the release of Ca^{2+} from mitochondria in a rapid and reversible manner, under conditions which resemble those present in the cytoplasm. These observations suggest that lysophosphatidylethanolamine, generated at either the plasma or mitochondrial membrane, could act to release Ca^{2+} from mitochondria following the interaction of Ca^{2+} -dependent hormones with the liver cell.

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