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The effect of methyl-lidocaine on the biosynthesis of phospholipids *de novo* in the isolated hamster heart

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Methyl-lidocaine is an amphiphilic agent which has been used as an experimental anti-arrhythmic drug. When hamster hearts were perfused with labelled glycerol, the presence of methyl-lidocaine in the perfusate was found to enhance the labelling in phosphatidylserine, phosphatidylinositol, diacylglycerol and triacylglycerol. However, the labelling of phosphatidylcholine and phosphatidylethanolamine was not significantly changed by methyl-lidocaine treatment. Assays *in vitro* for the enzymes involved in the synthesis of neutral lipids and acidic phospholipids revealed that phosphatidate phosphatase and CTP:phosphatidate cytidylyltransferase activities were stimulated by methyl-lidocaine. The intracellular pool sizes of diacylglycerol and CDP-diacylglycerol were also elevated. We postulate that the enhanced syntheses of the neutral lipids and acidic phospholipids in the methyl-lidocaine-perfused heart were mediated via the direct activation of the key enzymes in the biosynthesis of these lipids *de novo*.

INTRODUCTION

Phospholipids are structural components of the biological membrane, and the compositions of the phospholipids have profound effects on the function of membrane proteins [1]. Certain phospholipids, such as phosphatidylinositol, are actively involved in the transduction of biological signals across the membrane [2]. In the mammalian heart, the conductivity of electrical signals appears to be dependent on the appropriate phospholipid content and composition of the cardiac membrane [3]. The important role of the phospholipids in the maintenance of proper membrane function necessitates the rigid control of their biosynthesis and catabolism in mammalian tissues. Phospholipids are actively synthesized in most mammalian tissues, and the pathways for their biosynthesis have been largely elucidated [4,5]. However, only limited information is available on the control of their biosynthesis.

Methyl-lidocaine is an amphiphilic local anaesthetic which has been used as an experimental anti-arrhythmic drug. It has a methyl group covalently attached to the amino nitrogen of the lidocaine moiety, which causes the molecule to display a permanent cationic charge [6] (Fig. 1). We reported previously that the acyltransferases for the acylation of lysophosphatidylcholine and lysophosphatidylethanolamine in the hamster heart were inhibited by methyl-lidocaine [7]. Unlike the action of other local anaesthetics [8], methyl-lidocaine had no effect on the activities of phospholipase A or lysophospholipase [7]. Although the effect of the drug on phospholipid biosynthesis was not known, several amphiphilic compounds were shown to affect the biosynthesis of the neutral and acidic phospholipids in mammalian tissues [9–13]. In the present study, hamster hearts were perfused with labelled glycerol in the presence and absence of methyl-lidocaine. Our results showed that methyl-lidocaine stimulated the biosynthesis of phosphatidylserine and phosphatidylinositol, but did not affect the biosynthesis of phosphatidylcholine or phosphatidylethanolamine.

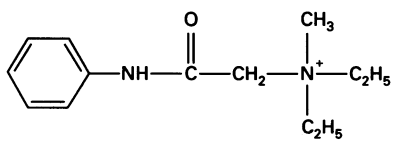

Fig. 1. Structure of methyl-lidocaine

MATERIALS AND METHODS

Materials

Palmitoyl-CoA, oleoyl-CoA, DL-α-glycerophosphate, Tris/HCl, Tris/maleate, EDTA and EGTA were obtained from Sigma Chemical Co. Methyl-lidocaine was obtained from Astra Pharmaceutical Products (Worcester, MA, U.S.A.). The methyl ester standards for g.l.c. were products of Supelco Canada. Phospholipase D (cabbage) was obtained from Boehringer Mannheim Canada. 1-Palmitoyl-2-[^{14}C]oleoyl-phosphatidylcholine, [1,3-^{3}H]glycerol, [1-^{14}C]oleoyl-CoA, [1-^{14}C]palmitoyl-CoA, [5-^{3}H]CTP, *myo*-[2-^{3}H]inositol and [^{3}H]acetic anhydride were obtained from Amersham International. 1-Palmitoylglycerol 3-phosphate, phosphatidic acid (egg lecithin), phosphatidylcholine (pig liver), phosphatidylethanolamine (pig liver), phosphatidylserine (bovine brain), phosphatidylinositol (pig liver), dipalmitoyl-CDP-diacylglycerol, 1,2-diacylglycerol (pig liver), and triacylglycerol (pig liver) were products of Serdary Laboratories, London, Ont., Canada. All other chemicals and solvents were of reagent grade and were obtained locally from the Canlab Division of Baxter Co.

Preparation of subcellular fractions

Syrian Golden hamsters weighing 120 ± 20 g were used throughout the study. The animal was killed by decapitation and the heart was rapidly removed and homogenized in 0.25 M-sucrose/10 mM-Tris/HCl, pH 7.5. Microsomes were prepared from the tissue homogenate by differential centrifugation as previously described [7]. Protein concentrations were determined by the method of Lowry *et al.* [14].

Uptake of [^{3}H]glycerol by the isolated hamster heart

The isolated hamster heart was perfused in the Langendorff mode in Krebs–Henseleit buffer containing 1 mM-[1,3-^{3}H]glycerol

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(3 μCi/μmol) and 0.5 mg of methyl-lidocaine/ml for 20–60 min. Hamster hearts perfused in the absence of methyl-lidocaine were used as controls. After perfusion, 10 ml of Krebs–Henseleit buffer was forced through the cannula and the heart was cut open, blotted dry, weighed, and homogenized in chloroform/methanol (1:1 v/v). The homogenate was centrifuged at 1000 *g* for 10 min, and the precipitate was washed twice with chloroform/methanol (1:1, v/v). The supernatants were pooled, and water and chloroform were added to cause phase separation. Samples of the aqueous and organic phases were taken for scintillation counting. The phospholipid and neutral lipid classes in the organic phase were analysed by t.l.c. The solvent for the separation of phospholipids contained chloroform/methanol/15 M-NH_3/water (35:15:2:1, by vol.), whereas the solvent for the separation of neutral lipids contained light petroleum (b.p. 35–60 °C)/diethyl ether/acetic acid (60:40:1, by vol.). The lipid fractions on the thin-layer chromatogram were detected by iodine staining, and the radioactivity in each fraction was determined by scintillation counting.

Preparation of [^{14}C]phosphatidic acid and phosphatidate phosphatase assay

Phosphatidic acid was obtained from 1-palmitoyl-2-[^{14}C]oleoylphosphatidylcholine by phospholipase D treatment [15]. The substrate (50 μmol, 2000 d.p.m./nmol) was suspended in 2 ml of diethyl ether. Phospholipase D (1.33 mg) was dissolved in 2 ml of 100 mM-sodium acetate (pH 5.6)/100 mM-$CaCl_2$ and the solution was mixed with the phosphatidylcholine suspension in a sealed tube. After 30 min of incubation at 42 °C, the reaction mixture was cooled and 1.33 mg of fresh enzyme was added to the mixture. The reaction mixture was re-incubated at 42 °C for another 30 min and the reaction was terminated by removing the diethyl ether under N_2. A chloroform/methanol (2:1, v/v) mixture (6 ml) was added to cause phase separation. The lower phase was analysed by t.l.c. with a solvent system of chloroform/methanol/water/acetic acid (50:37:2:3, by vol.). The silica gel containing phosphatidic acid fraction was removed, and the phospholipid was eluted from the silica gel by the method of Arvidson [16].

Phosphatidate phosphatase activity was assayed by determining the production of [^{14}C]diacylglycerol from [^{14}C]phosphatidic acid [17]. Phosphatidate phosphatase was found to be more active with a phosphatidic acid containing the 1-palmitoyl-2-oleoyl species than the 1,2-dipalmitoyl species. The assay mixture (100 μl) contained 100 mM-Tris/HCl (pH 7.4), 1 mM-dithiothreitol, 0.2 mg of BSA, 0.6 mM-1-palmitoyl-2-[^{14}C]oleoylphosphatidic acid (2000 d.p.m./nmol), 0.4 mM-phosphatidylcholine, 1–20 mM-$MgCl_2$, 1 mM-EGTA, 1 mM-EDTA, 0.75 mM-oleic acid and 0.2–0.3 mg of hamster heart post-mitochondrial fraction. The enzyme source was preincubated for 20 min at 37 °C in the presence of oleic acid. The reaction was initiated by addition of $MgCl_2$ and the mixture was incubated at 37 °C for 60 min. The reaction was terminated by addition of 2 ml of chloroform/methanol (2:1, v/v). Water was added to cause phase separation, and the labelled diacylglycerol in the organic phase was analysed by t.l.c. with a solvent containing light petroleum (b.p. 35–60 °C)/diethyl ether/acetic acid (60:40:1, by vol.).

Acyl-CoA:glycerol-3-phosphate acyltransferase and acyl-CoA:lysophosphatidic acid acyltransferase assays

Enzyme activities were determined by the procedure of Batenburg *et al.* [18] with the hamster heart homogenate. For the acylation of glycerol 3-phosphate, the assay mixture consisted of 100 mM-Tris/HCl (pH 7.5), 1.5 mM-glycerol 3-phosphate, 90 mM-sucrose, 0.5 mg of BSA, 1 mM-dithiothreitol, 40 μM-[1-^{14}C]oleoyl-CoA (4000 d.p.m./nmol) and 0.1–0.2 mg of enzyme in a volume of 500 μl. The reaction was initiated by addition of the enzyme and the mixture was incubated at 30 °C for 30 min. The reaction was terminated by addition of 3 ml of chloroform/methanol (2:1, v/v). Water was added to cause phase separation, and the labelled lysophosphatidic acid in the lower phase was analysed by t.l.c. with a solvent containing chloroform/methanol/15 M-NH_3/water (35:15:2:1, by vol.).

For the acylation of the lysophosphatidic acid, the reaction was carried out under the same conditions, except that glycerol 3-phosphate was replaced with 0.2 mM-1-palmitoylglycerol 3-phosphate [18]. The labelled phosphatidic acid formed was analysed by t.l.c.

Acyl-CoA:1,2-diacylglycerol acyltransferase assay

Enzyme activity was assayed by determining the transfer of [1-^{14}C]palmitoyl-CoA to 1,2-diacylglycerol [19] in hamster heart homogenate. The reaction mixture (0.5 ml) contained 25 mM-Tris/HCl (pH 7.4), 50 μM-[1-^{14}C]palmitoyl-CoA (2000 d.p.m./nmol), 2 mM-1,2-diacylglycerol, 18 mM-$MgCl_2$, 0.5 mg of BSA and 1 mM-dithiothreitol. The reaction was initiated by addition of 0.2–0.3 mg of enzyme protein, and the mixture was incubated at 37 °C for 10 min. The reaction was terminated by addition of 3 ml of chloroform/methanol (2:1, v/v). Water was added to cause phase separation, and the product in the organic phase was analysed by t.l.c. with a solvent containing light petroleum (b.p. 35–60 °C)/diethyl ether/acetic acid (60:40:1, by vol.).

CTP:phosphatidic acid cytidylyltransferase assay

Enzyme activity was determined in hamster heart homogenate. The reaction mixture contained 50 mM-Tris/maleate (pH 6.5), 7.5 mM- or 20 mM-$MgCl_2$, 15 mM-Triton X-100, 1.0 mM-[5-^{3}H]-CTP (10000 d.p.m./nmol) and 0.5 mM-phosphatidic acid [20]. The reaction was initiated by addition of the enzyme protein (0.3 mg) and the mixture was incubated at 37 °C for 15 min. The reaction was terminated by addition of 2.5 ml of 0.1 M-HCl in methanol. After cooling, 5 ml of chloroform was added to the mixture. The chloroform/methanol mixture was washed with 3 × 10 ml of 2 M-$MgCl_2$ [21]. The radioactivity of the labelled CDP-diacylglycerol in the organic phase was analysed by t.l.c. with a solvent containing chloroform/methanol/acetic acid/water (25:14:2:4, by vol.).

Phosphatidylinositol synthase and serine base-exchange enzyme assays

Phosphatidylinositol synthase activity was assayed with hamster heart post-mitochondrial fraction by the procedure of Imai & Gershengorn [22]. The reaction mixture contained 100 mM-Tris/HCl (pH 7.5), 1 mM-EGTA, 3 mM-$MgCl_2$, 3 mM-$MnCl_2$, 0.2% Triton X-100, 5 mM-CDP-dipalmitoylglycerol and 5 mM-*myo*-[^{3}H]inositol. The reaction mixture was incubated at 37 °C for 30 min and the reaction was terminated by addition of 1 ml of chloroform/methanol/HCl (100:100:1, by vol.) and 0.4 ml of 0.9% KCl. After phase separation, the labelled phosphatidylinositol in the organic phase was analysed by t.l.c. with a solvent containing chloroform/methanol/acetic acid/water (50:30:8:4, by vol.). The serine base-exchange reaction was assayed with the hamster heart post-mitochondrial fraction with labelled serine [23].

Long-chain acyl-CoA and lipid quantification

Long-chain acyl-CoA levels were determined by the method of Tardi *et al.* [24]. Briefly, long-chain acyl-CoAs were isolated by solvent partition, and quantified by g.l.c. The diacylglycerol pool size in the hamster heart was determined by acetylating the

diacylglycerol with labelled acetic anhydride by the method of Ishidate & Weinhold [25]. CDP-diacylglycerol was isolated from the hamster heart by the method of Kelley & Carman [20]. Triacylglycerol was quantified by g.l.c. by the modified method of Groener *et al.* [26], and all other phospholipids were quantified by the procedure of Bartlett [27].

RESULTS

The effect of methyl-lidocaine on lipid biosynthesis in the hamster heart was investigated. Isolated hamster hearts were perfused with [^{3}H]glycerol for 20–60 min in the absence or presence of 0.5 mg of methyl-lidocaine/ml. After perfusion, the heart was homogenized in chloroform/methanol (1:1, v/v) and the homogenate was centrifuged (1000 *g*-min) to obtain a clear supernatant. The pellet was re-extracted with the same solvent, and the radioactivity in the pooled supernatant (tissue extract) was determined. The uptake of labelled glycerol was elevated in hearts perfused with methyl-lidocaine (Table 1) and the elevation was most prominent at 60 min of perfusion. The tissue extract was separated into the aqueous and organic phases by addition of chloroform and water, and samples of these two phases were taken for radioactivity determination. Perfusion with methyl-lidocaine did not cause any significant change in radioactivity associated with the aqueous phase, but the radioactivity in the organic phase was increased at every time point of perfusion (Table 1). It appears that the elevated amount of radioactivity found in the methyl-lidocaine-perfused heart was localized in the organic phase of the tissue extract.

The lipid fractions in the organic phase were analysed by t.l.c. A portion of the sample was used for analysis of the phospholipid fractions, and an identical fraction was used for analysis of the neutral lipids. In the analysis of the phospholipid fractions, the labelling of phosphatidylserine was elevated only at 60 min of methyl-lidocaine perfusion, whereas the labelling of phosphatidylinositol was elevated at all time points of perfusion (Table 2). No significant change in labelling was detected in the other phospholipid fractions. In the analysis of the neutral-lipid fractions, the labelling of diacylglycerol and triacylglycerol was found to be substantially increased by methyl-lidocaine treatment at all time points (Table 2). Analysis of the pool size of the lipids in the heart groups revealed that there was no significant change in the contents of the phospholipid fractions (Table 3), but the levels of diacylglycerol and triacylglycerol in the heart were increased by methyl-lidocaine treatment.

The mechanism for the enhancement of labelling of the cardiac lipids by methyl-lidocaine was investigated. The changes in labelling of the cardiac lipids might be caused by direct activations of the key enzymes involved in the production of these lipids. In order to test this hypothesis, the activities of the enzymes

Table 1. Effect of methyl-lidocaine on the uptake of labelled glycerol in the isolated perfused hamster heart

Isolated hamster hearts were perfused in the Langendorff mode for 20–60 min in Krebs–Henseleit buffer containing 1 mM labelled glycerol (3 μCi/μmol) in the presence or absence of 0.5 mg of methyl-lidocaine/ml. After perfusion, the hearts were homogenized in chloroform/methanol (1:1, v/v) to obtain the tissue extracts. The tissue extracts were separated into aqueous and organic phases by addition of chloroform and water. Samples of the tissue extract, aqueous and organic phases were used for radioactivity determination. The results are expressed as means ± S.D. for *n* individual experiments in each set (*$P < 0.05$).

Fraction	Time (min)...	10^{-3} × Uptake (d.p.m./g wet wt. of heart) 20 ($n = 3$)	40 ($n = 3$)	60 ($n = 5$)
Total uptake	(control)	2282±144	2400±514	3233±960
	(drug)	2828±82*	3315±41*	5423±1019*
Aqueous phase	(control)	1552±194	1203±131	1425±500
	(drug)	1634±82	1387±299	1625±367
Organic phase	(control)	546±58	881±29	1752±534
	(drug)	974±89*	1568±244*	3363±430*

Table 2. Effect of methyl-lidocaine on the labelling of neutral lipids and phospholipids in the hamster heart

Hamster hearts were perfused with labelled glycerol in the presence or absence of methyl-lidocaine as described in Table 1. After perfusion, the lipid fractions were separated by t.l.c. and the radioactivity in each lipid fraction was determined. The results are expressed as means ± S.D. for *n* individual experiments in each set (*$P < 0.05$).

Fraction	Time (min)...	10^{-3} × Labelling (d.p.m./g wet wt. of heart) 20 ($n = 3$)	40 ($n = 3$)	60 ($n = 5$)
Lysophosphatidic acid	(control)	6.3±1.7	10.1±2.8	22.8±3.9
	(drug)	7.5±1.7	10.2±1.8	29.5±5.6
Phosphatidic acid	(control)	6.8±1.8	3.9±0.2	69±1.0
	(drug)	6.6±2.7	3.4±1.0	8.1±2.1
Phosphatidylcholine	(control)	37.2±2.2	78.5±12.7	219.0±46.4
	(drug)	40.7±4.7	79.2±24.8	219.9±81.2
Phosphatidylethanolamine	(control)	27.8±2.6	104.1±9.0	217.7±63.9
	(drug)	37.7±8.7	94.6±22.8	258.6±70.7
Phosphatidylserine	(control)	10.0±1.8	11.5±2.5	20.8±3.9
	(drug)	13.0±2.4	15.7±2.6	27.2±2.3*
Phosphatidylinositol	(control)	14.8±0.8	38.0±1.2	58.1±11.0
	(drug)	23.5±5.4*	60.9±1.8*	90.7±14.5*
Diacylglycerol	(control)	86.6±8.1	114.9±22.4	139.9±19.4
	(drug)	159.0±7.5*	162.3±11.9*	246.7±57.9*
Triacylglycerol	(control)	213.6±40.6	795.7±39.4	1549.1±354
	(drug)	455.8±103.8*	1518.9±228*	3427.4±506*

Table 3. Effect of methyl-lidocaine on the phospholipid and neutral-lipid contents in the hamster heart

Hamster hearts were perfused with labelled glycerol in the presence or absence of 0.5 mg of methyl-lidocaine/ml for 60 min as described in Table 1. After perfusion, the lipid fractions were separated by t.l.c. The amount of lipid in each fraction was determined. The results are expressed as means ± S.D. from three separate experiments (*$P < 0.05$).

Fraction	Content (μmol of lipid P/g wet wt. of heart)	
	Control	Methyl-lidocaine-treated
Lysophosphatidic acid	0.27 ± 0.10	0.25 ± 0.07
Phosphatidic acid	0.33 ± 0.09	0.42 ± 0.13
Phosphatidylcholine	11.71 ± 0.80	10.94 ± 1.00
Phosphatidylethanolamine	9.81 ± 0.80	9.09 ± 1.15
Phosphatidylserine	1.84 ± 0.38	1.41 ± 0.27
Phosphatidylinositol	1.65 ± 0.25	1.48 ± 0.36
Diacylglycerol†	6.54 ± 0.37	9.71 ± 1.25*
Triacylglycerol†	3.88 ± 0.93	8.74 ± 0.97*

† μmol of glycerol/g wet wt. of heart.

Table 4. Effects of methyl-lidocaine and chlorpromazine on hamster heart phosphatidate phosphatase

Phosphatidate phosphatase activity was determined in the hamster heart post-mitochondrial fraction in the presence of 0–2 mg of methyl-lidocaine/ml or 1–5 mM-chlorpromazine. The results are expressed as means ± S.D. of four separate experiments, each of which was performed in duplicate (*$P < 0.05$).

Drug concn. [$MgCl_2$]...	Phosphatidate phosphatase (nmol/h per mg of protein)		
	1 mM	7.5 mM	20 mM
Methyl-lidocaine			
0 (control)	12.41 ± 1.9	13.23 ± 4.6	12.35 ± 1.89
0.2 mg/ml	20.36 ± 1.9*	26.88 ± 6.1*	15.11 ± 1.54
0.5 mg/ml	23.25 ± 3.48*	30.87 ± 8.5*	18.39 ± 6.78
1.0 mg/ml	23.56 ± 6.59*	24.57 ± 4.2*	13.69 ± 3.63
2.0 mg/ml	18.16 ± 1.23*	32.41 ± 5.0*	9.82 ± 1.80
Chlorpromazine			
1 mM	18.17 ± 1.07*		
2 mM	3.70 ± 1.81*		
3 mM	2.89 ± 1.16*		
4 mM	3.03 ± 0.63*		
5 mM	2.09 ± 0.90*		

responsible for synthesis of the acidic phospholipids as well as diacylglycerol and triacylglycerol were determined in the presence of methyl-lidocaine. Since the subcellular localizations of some lipid-biosynthetic enzymes were not well defined [28], our initial approach was to use the tissue homogenate as the enzyme source, in order to encompass enzyme activity associated with mitochondrial, microsomal and/or cytosolic fractions.

The effects of methyl-lidocaine on the enzymes involved in the biosynthesis of lipids were studied. The activities of acyl-CoA:glycerol-3-phosphate acyltransferase and acyl-CoA:lysophosphatidate acyltransferase in hamster homogenate were assayed in the presence of 0–2 mg of methyl-lidocaine/ml. Methyl-lidocaine did not cause any changes in the activities of these enzymes (results not shown). The activity of acyl-

Table 5. Hamster heart CTP:phosphatidic acid cytidylyltransferase and phosphatidylinositol synthase activities

CTP:phosphatidic acid cytidylyltransferase and phosphatidylinositol synthase activities were assayed with hamster heart homogenate and the post-mitochondrial fraction, respectively, in the presence of 0–2 mg of methyl-lidocaine/ml. The results are expressed as means ± S.D. of three separate sets of experiments, each of which was performed in duplicate (*$P < 0.05$).

Methyl-lidocaine (mg/ml)	Activity (nmol/h per mg of protein)	
	CTP:phosphatidic acid cytidylyltransferase	Phosphatidylinositol synthase
0 (control)	0.648 ± 0.09	0.090 ± 0.006
0.2	0.972 ± 0.16*	0.089 ± 0.008
0.5	1.032 ± 0.24*	0.093 ± 0.013
1.0	1.422 ± 0.16*	0.085 ± 0.007
2.0	1.434 ± 0.19*	0.084 ± 0.004

Table 6. Effect of methyl-lidocaine on the long-chain acyl-CoA and CDP-diacylglycerol pools in hamster hearts

Hamster hearts were perfused with glycerol in the presence or absence of 0.5 mg of methyl-lidocaine/ml for 60 min. The pool sizes of diacylglycerol and long-chain acyl-CoA were determined. The results are expressed as means ± S.D. of three separate sets of experiments, each of which was performed in duplicate (*$P < 0.05$).

	Content (nmol/g wet wt.)	
	Control	Methyl-lidocaine treated
Long-chain acyl-CoA	61.2 ± 9.2	59.6 ± 7.9
CDP-diacylglycerol	12.1 ± 0.1	15.9 ± 0.2*

CoA:diacylglycerol acyltransferase in the hamster heart homogenate was also determined. No enzyme activity was detected when the assay was performed without exogenous diacylglycerol. In the presence of exogenous diacylglycerol, enzyme activity was not affected by methyl-lidocaine (results not shown). The enzyme responsible for the production of diacylglycerol was also analysed. Owing to the low phosphatidate phosphatase activity in the tissue homogenate, enzyme activity was determined in the post-mitochondrial fraction. Enhancement of phosphatidate phosphatase activity by methyl-lidocaine (0.2–2.0 mg/ml) was observed at low or near-physiological concentrations (1 and 7.5 mM) of $MgCl_2$ (Table 4). However, this enhancement was abolished at a higher $MgCl_2$ concentration (20 mM). As a positive control, the effect of chlorpromazine on the phosphatase activity was examined. The biphasic effect of chlorpromazine on the enzyme activity was in agreement with previous studies [29].

The effects of methyl-lidocaine on the enzymes involved in the biosynthesis of the acidic phospholipids were investigated. These enzymes included CTP:phosphatidate cytidylyltransferase, phosphatidylinositol synthase and the serine base-exchange enzyme. Methyl-lidocaine caused a direct stimulation of CTP:phosphatidate cytidylyltransferase in a dose-dependent manner at 20 mM-$MgCl_2$ (Table 5). Similar results were obtained at 7.5 mM-$MgCl_2$ (results not shown). The presence of methyl-lidocaine had no effect on the phosphatidylinositol synthase activity (Table 5). The activity of the serine base-exchange enzyme was not affected by the presence of methyl-lidocaine (results not shown).

The enhancement in the labelling of the lipid groups may be accounted for by changes in the pool size of the acyl donor. Hence, the levels of long-chain acyl-CoA in the isolated perfused heart were determined. Perfusion with methyl-lidocaine did not alter the acyl-CoA content in the heart (Table 6). Since the biosynthesis of the acidic phospholipids might be affected by the level of CDP-diacylglycerol, the labelling and the pool size of this intermediate were also determined. A 30% increase in the labelling (results not shown) together with a 32% increase in the pool size of CDP-diacylglycerol (Table 6) were detected in the heart after 60 min perfusion with methyl-lidocaine.

DISCUSSION

In mammalian tissues, phospholipids are formed *de novo* via the progressive acylation of glycerol 3-phosphate. Since glycerol 3-phosphate is not readily transported across the membrane, labelled glycerol has been routinely used as a general precursor for the study of phospholipid biosynthesis. The ability of the isolated mammalian tissue to utilize exogenous glycerol for phospholipid biosynthesis has been well documented [9,19,30,31]. In the present study, perfusion with labelled glycerol in the presence of methyl-lidocaine caused the enhancement of glycerol uptake and subsequent increases in labelling of the neutral lipids and the acidic phospholipids. The increase in labelling of these lipids did not result from a general increase in the specific radioactivities of their precursors, since the specific radioactivities of lysophosphatidic acid and phosphatidic acid were not changed between the control and the methyl-lidocaine-perfused hearts.

Phosphatidate phosphatase and CTP:phosphatidate cytidylyltransferase are regarded as key enzymes in the production of diacylglycerol and acidic phospholipids respectively [32–34]. The direct activation of these two key enzymes by methyl-lidocaine provided us with a viable explanation of the observed increase in the labellings of diacylglycerol and phosphatidylinositol. Interestingly, the labelling of triacylglycerol was enhanced, but the activity of acyl-CoA:diacylglycerol acyltransferase was not affected by methyl-lidocaine. Since the level of long-chain acyl-CoA was not elevated, the increase in labelling of triacylglycerol in the methyl-lidocaine-perfused heart probably resulted from the enhanced labelling and pool size of diacylglycerol [26]. Similarly, the increase in labelling of phosphatidylinositol in the methyl-lidocaine-perfused heart was caused by an increase in the turnover of CDP-diacylglycerol and not by an increase in the specific radioactivity of the precursor or the activation of phosphatidylinositol synthase.

The labelling of phosphatidylserine was increased only at 60 min of perfusion. This could be explained by the fact that base-exchange was the sole pathway for the formation of phosphatidylserine in mammalian tissues [5]. Since the labelling of phosphatidylserine was dependent on the labelling of the other phospholipids, it was plausible that the increase in labelling of phosphatidylserine would not become apparent until labelling of the other acidic phospholipids were substantially enhanced in the heart.

It has been shown in previous studies that most of the phosphatidylcholine and phosphatidylethanolamine in the heart was synthesized via condensation of the CDP-bases with diacylglycerol [35,36]. The content and specific radioactivity of diacylglycerol were elevated by methyl-lidocaine perfusion, yet the labellings and contents of these two phospholipids were not significantly altered. A similar phenomenon was observed in hepatocyte cultures with labelled palmitate in the presence of glucagon [37]. It appears that the formation of phosphatidylcholine and phosphatidylethanolamine was not dependent on the total intracellular diacylglycerol pool [26]. Indeed, it was suggested that synthesis of phosphatidylcholine and phosphatidylethanolamine was regulated independently from that of diacylglycerol [26]. This postulation is supported by the fact that the total concentration of diacylglycerol in the heart is much higher than the K_m of diacylglycerol for both enzymes [38]. Another factor that might contribute to the observed labelling of the two phospholipids is the possible existence of several intracellular diacylglycerol pools [39]. In addition, the phosphotransferases might display some selective utilization of specific diacylglycerol pools for the synthesis of phosphatidylcholine and phosphatidylethanolamine [31].

It is intriguing to note that methyl-lidocaine also caused the accumulation (increase in pool sizes) of diacylglycerol and triacylglycerol, but not of phosphatidylinositol or phosphatidylserine. One explanation is that the turnover rates of these lipids are different in hamster heart. On the basis of labelling of phosphatidylinositol and phosphatidylserine, and the specific radioactivity of the precursor, only a very small extent of new synthesis occurred. Hence the increase in synthesis of these phospholipids during the perfusion might represent a very small portion of the total cellular pool and might not be detectable. Alternatively, the anti-arrhythmic nature of methyl-lidocaine would decrease the heart rate and, consequently, the requirement for neutral lipids as a source of energy. This might decrease the turnover rates of diacylglycerol and triacylglycerol, whereas the turnover rates of the phospholipids were not affected by methyl-lidocaine treatment.

The effects of several amphiphilic compounds on the synthesis *de novo* of phospholipids in mammalian tissues have been reported [9–13]. In general, these compounds were shown to inhibit phosphatidate phosphatase activity [13,29]. Consequently, the biosynthesis of phosphatidylcholine, phosphatidylethanolamine and diacylglycerol was decreased, with a concomitant increase in synthesis of the acidic phospholipids [9,11]. Methyl-lidocaine is an amphiphilic compound which affects phosphatidate phosphatase and CTP:phosphatidate cytidylyltransferase activities in an unconventional way, resulting in the stimulation of diacylglycerol and phosphatidylinositol biosynthesis. Since the modulation of these enzyme activities is dependent on the Mg^{2+} content, the effect of methyl-lidocaine was studied at different Mg^{2+} concentrations. It is clear that methyl-lidocaine has the ability to stimulate enzyme activities at the physiological concentration (7.5 mM) of Mg^{2+} [34]. The ability to stimulate the synthesis of diacylglycerol and phosphatidylinositol by methyl-lidocaine may provide us and other investigators with an excellent model to study the metabolic consequences of enhanced levels of these second messengers [2].

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