507

Regulation of Phosphatidylcholine Synthesis in Rat Liver Endoplasmic Reticulum

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The biosynthesis of phosphatidylcholine in rat liver microsomal preparations catalysed by CDP-choline–1,2-diacylglycerol cholinephosphotransferase (EC 2.7.8.2) was inhibited by a combination of ATP and CoA or ATP and pantetheine. ATP alone at high concentrations (20mM) inhibits phosphatidylcholine formation to the extent of 70%. In the presence of 0.1mM-CoA, ATP (2mM) inhibits to the extent of 80% and in the presence of 1mM-pantetheine to the extent of 90%. ADP and other nucleotide triphosphates in combination with either CoA or pantetheine are only 10–30% as effective in inhibiting phosphatidylcholine synthesis. $AMP(CH_2)PP$ [adenosine 5'-($\alpha\beta$-methylene)triphosphate] together with CoA inhibits to the extent of 59% and with pantetheine by 48%. AMP-$P(CH_2)P$ [adenosine 5'-($\beta\gamma$-methylene)triphosphate] together with either CoA or pantetheine had no significant effect on phosphatidylcholine formation. Other closely related derivatives of pantothenic acid were without effect either alone or in the presence of ATP, as were thiol compounds such as cysteine, homocysteine, cysteamine, dithiothreitol and glutathione. Several mechanisms by which this inhibition might take place were ruled out and it is concluded that ATP together with either CoA or pantetheine interacts reversibly with phosphatidylcholine synthetase to cause temporarily the inhibition of phosphatidylcholine formation.

Although phospholipids play many important structural and functional roles in living organisms, virtually nothing is known about the mechanisms involved in the control of their metabolism. Phosphatidic acid and 1,2-diacylglycerols are at branchpoints in the biosynthesis of various phospholipids (Kennedy, 1961). Since phosphatidylcholine, phosphatidylethanolamine and triacylglycerols all arise from 1,2-diacylglycerols it would be reasonable to assume that some form of regulation is exerted at this point in the synthesis of each of these lipids.

It has been shown (Kennedy & Weiss, 1956; Weiss *et al.*, 1958) that 1mM-Ca^{2+} almost totally inhibits phosphatidylcholine synthesis, but the physiological significance of this finding is as yet unclear. It is noteworthy, however, that phospholipases, which are responsible for the degradation of phospholipids, usually require Ca^{2+} for optimal activity (Gatt & Barenholz, 1973), as do enzymes that catalyse phospholipid base-exchange reactions (Dils & Hübscher, 1961; Borkenhagen *et al.*, 1961).

Fiscus & Schneider (1966) have reported that lysophosphatidylcholine stimulates phosphorylcholine cytidyltransferase, suggesting that control of CDP-choline formation may be modulated by the

breakdown products of phosphatidylcholine. It has also been shown (Possmayer *et al.*, 1973) that in brain, cytidine nucleotides inhibit the first step in phospholipid synthesis, namely the acylation of glycerol 3-phosphate.

We have found in our laboratory that, in rat liver microsomal preparations, ATP and CoA or ATP and pantetheine stimulate the synthesis of sphingomyelin approx. 20-fold (Lyman *et al.*, 1976). When these compounds were tested for their effect on phosphatidylcholine it was found that its synthesis was very significantly inhibited. These experiments are described in the present paper and the probable physiological significance of these findings is discussed.

Materials and Methods

Chemicals

ATP, CoA, palmitoyl-CoA, acetyl-CoA, CMP, UTP, GTP, dCMP, ADP, ITP, AMP, cyclic AMP, carnitine, $AMP(CH_2)PP$† and AMP-$P(CH_2)P$ were purchased from P–L Biochemicals, Milwaukee,

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† Abbreviations: $AMP(CH_2)PP$, adenosine 5'-($\alpha\beta$-methylene)triphosphate; AMP-$P(CH_2)P$, adenosine 5'-($\beta\gamma$-methylene)triphosphate.

WI, U.S.A.; pantethine, dithiothreitol, pantothenic acid, pantothenyl alcohol, cysteine, cystamine, cystine, N-acetylcysteine, homocysteine, glucose, hexokinase and sn-glycerol 3-phosphate were from Sigma Chemical Co., St. Louis, MO, U.S.A.; [1,2-^{14}C]choline, [γ-^{32}P]ATP, [^{14}C]ATP and Aquasol were from New England Nuclear, Montreal, Que., Canada. 4'-Phosphopantetheine and 4',4''-diphospho-pantethine were generously supplied by Dr. Yasushi Abiko of The Daiichi Seiyaku Research Institute, Tokyo, Japan.

Radioactive CDP-choline and dCDP-choline were prepared as described by Kennedy (1956) and Kennedy et al. (1959) respectively. 1,2-Diacylglycerol was prepared from egg phosphatidylcholine by treatment with phospholipase C after it had been purified twice by chromatography on silicic acid. Rat liver microsomal fractions were prepared as described previously (Sribney, 1968), and pantethine was converted into pantetheine by the addition of dithiothreitol.

Assay of phosphatidylcholine synthesis

This was done essentially as described by Kennedy & Weiss (1956). Each tube contained Tris/HCl buffer, pH 7.7 (100 mM), Mg^{2+} (20 mM), CDP-[1,2-^{14}C]-choline (1 mM), 1,2-diacylglycerol (4 mM), Tween-20 (0.5 mg) and 0.25–0.50 mg of microsomal protein in a total volume of 0.5 ml. Incubation was at 37°C for 20 min. When glucose and hexokinase were used, they were added at a concentration of 10 mM and 1.0 mg respectively. Dithiothreitol was added as a routine to all tubes at a concentration of 4 mM, except to those containing oxidized thiol reagents. ATP and other additions were made as described in the text or in the legends to the Tables and Figures. After incubation, the lipids were extracted and counted for radioactivity as described previously (Sribney & Lyman, 1973).

Determination of protein

Protein was determined by the method of Lowry et al. (1951), with bovine serum albumin as the standard.

Identification of reaction product

The product obtained after incubation was identified as phosphatidylcholine in a number of ways. The radioactive product co-chromatographed with authentic phosphatidylcholine in two solvent systems on t.l.c. (chloroform/methanol/water, 65:25:4, by vol., and chloroform/methanol/acetic acid/water, 25:15:4:2, by vol.). On treatment with phospholipase C (Clostridium perfringens) radioactive phosphoryl-choline was obtained as well as 1,2-diacylglycerol. On treatment with methanolic 0.5 M-KOH the radioactive product was completely destroyed.

Studies with labelled ATP

In experiments using [γ-^{32}P]ATP and [^{14}C]ATP, microsomal preparations were incubated in the complete system described above, except that unlabelled CDP-choline was utilized. The labelled ATP (2 mM) was incubated in the presence or absence of CoA (0.5 mM) or pantetheine (2 mM). The reaction was stopped by the addition of 1.0 ml of ice-cold 10% (w/v) trichloroacetic acid, and after being left for 10 min at 0°C, the mixture was centrifuged. The precipitate was alternately dissolved in 1 M-NaOH and reprecipitated with trichloroacetic acid. This procedure was done twice before finally washing twice more with trichloroacetic acid. The microsomal precipitate was then solublized in Aquasol and assayed for radioactivity in a Beckman liquid-scintillation counter.

Statistical determinations

The data were analysed by the standard t test, and values of $P < 0.01$ were considered significant. These are given in the Tables with the numbers of determinations in parentheses. The points in all the Figures are means of four determinations and all had errors of less than ±10%.

Results

Effect of varying ATP, CoA and pantetheine concentrations on phosphatidylcholine synthesis

Fig. 1 shows the effect of increasing ATP concentration on phosphatidylcholine synthesis. As the ATP concentration was increased, the amount of phosphatidylcholine synthesized decreased, until at 20 mM-ATP the inhibition was approx. 70% that of the control. In the presence of 1 mM-CoA, however, inhibition
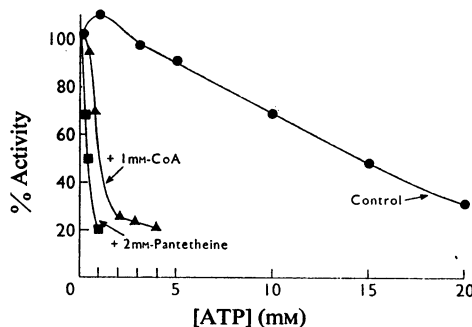

Fig. 1. *Effect of varying ATP concentration in the absence and presence of CoA and pantetheine on phosphatidylcholine synthesis*

The assay was done as described in the Materials and Methods section. The concentration of CoA was 1 mM and that of pantetheine 2 mM.

by 1 mM-ATP was 25% and by 2 mM it was approx. 70%. Similarly, in the presence of 2 mM-pantetheine, inhibition by 1 mM-ATP was about 80%.

Fig. 2 illustrates the effect of increasing either the concentration of pantetheine or CoA in the presence of 2 mM-ATP. The reaction is inhibited by 50% at 0.2 mM-pantetheine, and by 90% at 1 mM. At 0.02 mM-CoA the synthesis of phosphatidylcholine is inhibited by 50%, and at 0.1 mM-CoA approx. 80% of the activity is lost. The rates of phosphatidylcholine synthesis

Fig. 2. *Effect of varying CoA and pantetheine concentrations in the presence of 2 mM-ATP on phosphatidylcholine synthesis*

The assay was done as described in the Materials and Methods section.

in the systems described in Fig. 2 and in the control tubes were constant with respect to time.

In the above, results were obtained by using 20 mM-Mg^{2+}. Doubling the concentration of Mg^{2+} did not have any effect on the inhibition of phosphatidylcholine synthesis by either ATP and CoA or by ATP and pantetheine.

Effect of various pantothenic acid derivatives and thiol compounds

Various compounds which comprise the CoA molecule were tested together with ATP for their ability to inhibit phosphatidylcholine synthesis in rat liver microsomal preparations. Table 1 shows that CoA and pantetheine in the presence of 2 mM-ATP were the only compounds that together inhibited phosphatidylcholine synthesis. Compounds such as pantethine, 4'-phosphopantetheine, 4',4"-diphosphopantethine, pantothenic acid, pantothenyl alcohol, cystamine and cysteamine at concentrations up to 2 mM had no effect when tested either alone or in the presence of 2 mM-ATP. Other thiol reagents, such as glutathione, cysteine, cystine, N-acetylcysteine, homocysteine and dithiothreitol, also had no effect when tested alone or in the presence of ATP.

Effect of various nucleotides in combination with CoA or pantetheine

The effect of CoA and pantetheine was tested in the presence of a variety of other nucleotides to determine the specificity of the inhibition (Table 2). In the presence of pantetheine, ATP decreased the synthesis of phosphatidylcholine from the control value of 47.9 to 9.6 nmol. In the presence of ADP and pantetheine, phosphatidylcholine synthesis was decreased from 47.9 to 29.0 nmol. When ATP was tested in the pre-
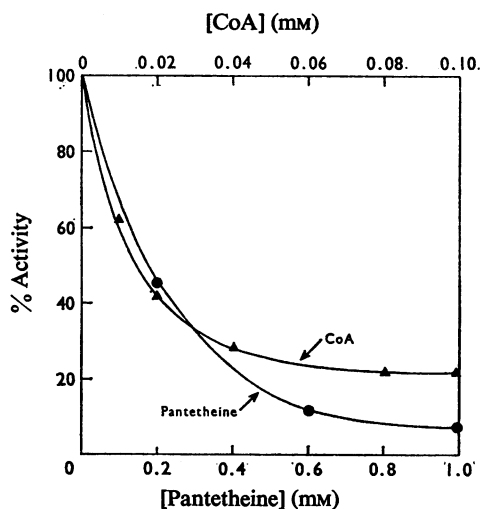
Table 1. *Effect of ATP and pantothenic acid derivatives on phosphorylcholine-glyceride transferase activity*

The assay was carried out as described in the Materials and Methods section. ATP was added at a concentration of 2 mM, CoA and pantetheine were 1 mM and all other compounds were 2 mM. Dithiothreitol (4 mM) was present in all the tubes except those containing pantethine, 4',4"-diphosphopantethine and cystamine. All values are means±S.D. The numbers of determinations are in parentheses.

Additions	Phosphatidylcholine synthesized (nmol/20 min per 0.5 mg of protein)	% Inhibition
None	60.9 ±6.3 (12)	—
CoA	11.0*±1.1 (12)	82
Pantetheine	14.1*±1.3 (12)	77
Pantethine	58.8 ±7.4 (6)	—
4'-Phosphopantetheine	52.2 ±6.8 (6)	—
4',4"-Diphosphopantethine	65.8 ±7.9 (6)	—
Pantothenic acid	62.5 ±7.4 (4)	—
Pantothenyl alcohol	68.0 ±8.3 (4)	—
Cystamine	56.5 ±6.1 (4)	—
Cysteamine	55.3 ±6.7 (4)	—

* $P < 0.001$ versus control.

sence of CoA the synthesis of phosphatidylcholine was decreased from 57.5 to 13.8 nmol, whereas in the presence of ADP and CoA the decrease was less significant, being only 42.9 nmol. When other nucleotides were tested with either pantetheine or CoA all except cyclic AMP were effective, but not to a significant degree.

Table 2. *Effect of various nucleotides in combination with CoA or pantetheine on phosphorylcholine-glyceride transferase activity*

The assay was carried out as described in the Materials and Methods section. Pantetheine, CoA and cyclic AMP were added at a concentration of 1 mM and all other nucleotides were 2 mM. All values are the means±S.D. of six determinations.

Additions	Phosphatidylcholine synthesized (nmol/20 min per 0.5 mg of protein)	
	Pantetheine	CoA
None	47.9 ± 5.2	57.5 ± 6.7
ATP	9.6* ± 0.8	13.8* ± 1.1
CTP	44.1 ± 4.7	44.3 ± 5.1
GTP	44.3 ± 5.1	46.2 ± 5.7
UTP	42.0 ± 3.8	52.3 ± 6.3
ITP	48.5 ± 5.9	47.0 ± 5.6
ADP	29.0† ± 3.0	42.9 ± 4.7
AMP	44.0 ± 4.1	50.1 ± 6.9
CMP	52.1 ± 6.2	45.7 ± 6.2
Cyclic AMP	52.6 ± 8.8	66.2 ± 8.1

* $P < 0.001$.
† $P < 0.01$ with respect to control.

It was possible that the inhibitory activity of ADP in combination with either CoA or pantetheine was due to adenylate kinase (EC 2.7.4.3) activity in the microsomal preparations, converting the ADP into ATP. In a separate experiment (not shown) glucose and hexokinase were included in the incubation system along with ADP, CoA or pantetheine. The inhibition due to ADP and pantetheine was decreased by 11% (from 39 to 28%), but was not significantly decreased when ADP and CoA were tested along with glucose-hexokinase. Addition of glucose and hexokinase to incubation tubes containing ATP and CoA caused a 53% decrease in inhibition (87 to 34%), and in the presence of ATP and pantetheine the inhibition was decreased by 42% (76 to 34%). Since the addition of glucose and hexokinase failed to reverse totally the inhibition by either ADP or ATP when they were incubated in combination with either CoA or pantetheine, it indicates that the inhibition is not totally specific for nucleotide triphosphates.

Effect of acyl-CoA derivatives

ATP and CoA participate in a variety of metabolic reactions, one of which is the activation of fatty acids in the microsomal and mitochondrial compartments of the liver cell (van Tol, 1975). It was possible, therefore, that the inhibitory factor was an acyl-CoA or related derivative which was generated during the incubation with either ATP and CoA or ATP and pantetheine. When palmitoyl-CoA was added to the incubation system at a concentration of 0.4 mM no inhibition could be detected, nor did acetyl-CoA

Table 3. *Effect of acyl-CoA derivatives and other compounds on phosphatidylcholine synthesis*

The assay was carried out as described in the Materials and Methods section. The concentration of ATP was 2 mM; CoA, 0.5 mM; pantetheine, 1 mM; acetyl-CoA, 1 mM; palmitoyl-CoA, 0.4 mM; palmitoyl-S-pantetheine, 1 mM; L-carnitine, 1 mM; sn-glycerol 3-phosphate, 2 mM; bovine serum albumin, 0.5 mg. All values are means±S.D. with the numbers of determinations in parentheses.

Additions	Phosphatidylcholine synthesized (nmol/20 min per 0.5 mg of protein)	% Activity
None	46.5 ± 4.9 (6)	100
ATP+CoA	11.3* ± 1.2 (6)	24
ATP+pantetheine	14.7* ± 1.7 (6)	31
Palmitoyl-CoA	45.5 ± 6.3 (6)	98
Palmitoyl-CoA+ATP	11.5* ± 0.8 (6)	25
Acetyl-CoA	47.1 ± 3.9 (4)	101
Acetyl-CoA+ATP	15.6* ± 2.1 (4)	34
ATP+CoA+serum albumin	11.6* ± 1.4 (6)	25
ATP+pantetheine+serum albumin	14.4* ± 1.1 (6)	30
ATP+CoA+L-carnitine	13.8* ± 1.5 (4)	30
ATP+pantetheine+L-carnitine	15.5* ± 1.7 (4)	33
ATP+CoA+sn-glycerol 3-phosphate	13.2* ± 2.2 (4)	28
ATP+pantetheine+sn-glycerol 3-phosphate	11.6* ± 1.0 (4)	25
Palmitoyl-S-pantetheine	46.6 ± 4.8 (4)	100

* $P < 0.001$ versus control.

significantly cause any inhibition when it was tested at a concentration of 1 mM (Table 3). Addition of ATP together with either palmitoyl-CoA or acetyl-CoA caused a very significant inhibition of phosphatidylcholine synthesis. This inhibition was probably due to the fact that CoA is released by an acyl-CoA hydrolase (EC 3.1.2.2) (Barden & Cleland, 1969), by lysophosphatidylcholine acyltransferase (EC 2.3.1.23) (Lands, 1960) and by diacylglycerol acyltransferase (EC 2.3.1.20) (Weiss *et al.*, 1960), which are known to occur in microsomal preparations. If indeed ATP and CoA inhibit by way of acyl-CoA formation, then addition of L-carnitine or *sn*-glycerol 3-phosphate might result in reversal of inhibition. Carnitine is known to accept acyl-CoA to form acylcarnitine derivatives (Fritz, 1963) and *sn*-glycerol 3-phosphate forms 1-acylglycerol 3-phosphate and phosphatidic acid (Kornberg & Pricer, 1953). When these two acyl acceptors were incubated together with ATP and CoA or ATP and pantetheine, no reversal of inhibition in the presence of these compounds was apparent, as can be seen in Table 3.

It was possible that ATP and pantetheine might also react in our microsomal preparations to form palmitoyl-*S*-pantetheine or a related derivative. It has been shown that palmitoyl-CoA is converted into palmitoyl-*S*-pantetheine phosphate and then into palmitoyl-*S*-pantetheine by rat liver plasma-membrane preparations (Trams *et al.*, 1968). We have synthesized the latter compound and tested it in the incubation system, but it had no inhibitory effect on phosphatidylcholine synthesis (Table 3).

Acyl-CoA derivatives have been shown to inhibit numerous enzymes (Taketa & Pogell, 1966), and it has been assumed that this is due to the detergent properties of these derivatives. Addition of bovine serum albumin along with acyl-CoA derivatives usually reverses their inhibitory activity (Tubbs & Garland, 1964; Taketa & Pogell, 1966). Shown in Table 3 are results indicating that bovine serum albumin does not reverse the inhibitory effect of either ATP and CoA or of ATP and pantetheine.

Pande & Mead (1968) have found that 5 mM-ADP or -AMP inhibit the formation of palmitoyl-CoA

catalysed by rat liver microsomal acyl-CoA synthetase (EC 6.2.1.3). Table 2 showed that 2 mM-ADP, in the presence of either CoA or pantetheine, slightly inhibits (by 30%) phosphatidylcholine synthesis, whereas 2 mM-AMP has little significant effect. When ADP was tested at a concentration of 5 mM in the presence of CoA, the synthesis of phosphatidylcholine was inhibited to the extent of 44% and in the presence of pantetheine to the extent of 55%. When ATP (2 mM) and CoA (0.5 mM) were incubated with 5 mM-ADP, there was no reversal of the inhibition of phosphatidylcholine synthesis, as might be expected if ATP and CoA action were through formation of palmitoyl-CoA. Similar results were obtained with 5 mM-AMP, indicating that it is unlikely that palmitoyl-CoA is the cause of inhibition of phosphatidylcholine synthesis.

It was also decided to determine whether the addition of fatty acids, such as oleate or palmitate, might increase the amount of acyl-CoA formed and thus act to inhibit phosphatidylcholine synthesis further through its well-known detergent effect or by reacting with the diacylglycerol, in effect decreasing its concentration. The results of these experiments are shown in Table 4. Oleate addition at a concentration of 0.4 mM reversed the inhibitory effect of ATP and CoA from 73% inhibition to only 57%. Palmitate on the other hand neither enhanced nor lessened the inhibition. These results indicate that, if any quantity of acyl-CoA is formed, it is not responsible for inhibiting phosphatidylcholine synthesis. We have shown in our laboratory (Sribney & Lyman, 1973) that oleate significantly stimulates the synthesis of phosphatidylcholine in the presence of low concentrations of diacylglycerol. It is unlikely, however, that any appreciable amount of acyl-CoA is formed, because under our experimental conditions no exogenous fatty acid is added and the amount of ATP being utilized (2 mM) is well below the K_m of 4 mM reported for the microsomal acyl-CoA synthetase (Pande & Mead, 1968; Bar-Tana *et al.*, 1971). In the experimental systems described above, Tween-20 was present in all incubation tubes, and we have found (M. Sribney, unpublished work) that Tween-20

Table 4. *Effect of addition of oleate or palmitate on the inhibition of synthesis by ATP and CoA*

The assay was carried out as described in the Materials and Methods section. The concentration of ATP was 2 mM, CoA 0.5 mM, oleate 0.4 mM and palmitate 0.2 mM. All values are means of eight determinations ± s.D.

Additions	Phosphatidylcholine synthesized (nmol/20 min per 0.5 mg of protein)	% Inhibition
None	68.7 ± 4.4	0
ATP+CoA	18.5 ± 1.7	73
ATP+CoA+oleate	29.4* ± 1.1	57
ATP+CoA+palmitate	19.4 ± 2.2	72

* $P < 0.001$ versus experimental result shown in second line.

Table 5. *Effect of phosphonic acid analogues of ATP in combination with CoA and pantetheine on phosphatidylcholine synthesis*

The assay was carried out as described in the Materials and Methods section. The concentration of CoA was 0.5 mM, pantetheine 1 mM; ATP and the phosphonic acid analogues of ATP were added at 2 mM. All values are means of eight determinations $\pm$ s.D.

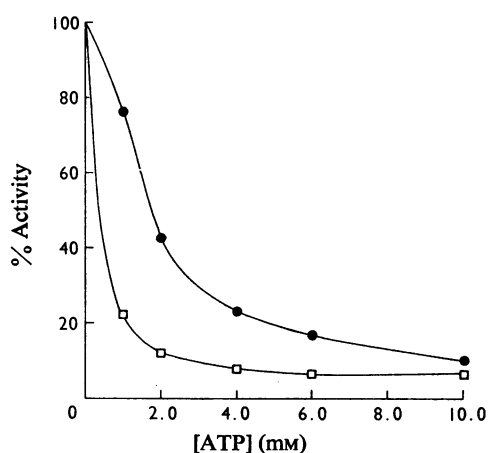| Additions	Phosphatidylcholine synthesized (nmol/20min per 0.5mg of protein)	% Inhibition
None	88.9 ±7.6	—
ATP+CoA	18.7*±2.1	79
AMP(CH_2)PP+CoA	36.0*±3.6	59
AMP-P(CH_2)P+CoA	81.1 ±6.7	9
ATP+AMP(CH_2)PP+CoA	10.8*±0.8	88
ATP+AMP-P(CH_2)P+CoA	15.3*±1.2	83
ATP+pantetheine	22.3*±2.7	75
AMP(CH_2)PP+pantetheine	46.5*±3.5	48
AMP-P(CH_2)P+pantetheine	81.3 ±6.9	9
ATP+AMP(CH_2)PP+pantetheine	21.9*±2.0	75
ATP+AMP-P(CH_2)P+pantetheine	15.0*±0.9	83

* $P<0.001$ versus control.

Fig. 3. *Effect of varying ATP concentration in the absence and presence of pantetheine, in the presence of* 1 mM-Mn^{2+}

●, No additions; □, +2 mM-pantetheine. All other conditions are described in the Materials and Methods section.
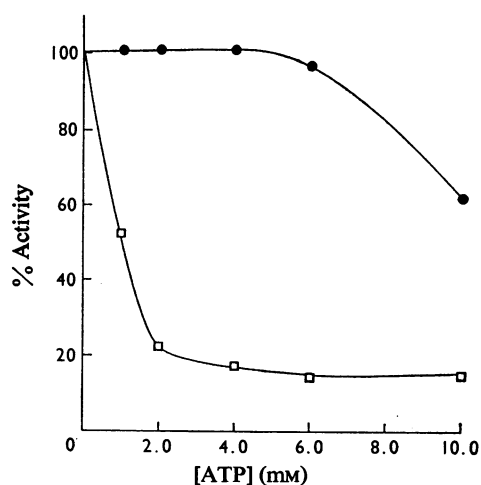

Fig. 4. *Effect of varying ATP concentration in the absence and presence of pantetheine, in the presence of* 5 mM-Mn^{2+}

●, No additions; □, +2 mM-pantetheine. All other conditions are described in the Materials and Methods section.

inhibits the synthesis of acyl-CoA by approx. 50% under the optimal conditions described by Pande & Mead (1968).

In Table 5 are shown the results obtained by using phosphonic acid analogues of ATP in the presence of either CoA or pantetheine. AMP(CH_2)PP (2 mM), in the presence of CoA, inhibited phosphatidylcholine synthesis by 59% and in the presence of pantetheine the inhibition amounted to 48%. AMP-P(CH_2)P (2 mM), in the presence of either CoA or pantetheine, inhibited to the extent of 9% in both cases. These results indicate that pyrophosphate

cleavage of the ATP is not a prerequisite for inhibition of phosphatidylcholine synthesis to occur.

Effect of ATP and pantetheine in the presence of Mn^{2+}

It has been shown (Weiss *et al.*, 1958) that Mn^{2+} at low concentrations is as effective as 20 mM-Mg^{2+} in promoting the synthesis of phosphatidylcholine by rat liver microsomal preparations. In Figs. 3 and 4, the effect of varying ATP concentration in the absence and presence of 2 mM-pantetheine and with either 1 mM- or 5 mM-Mn^{2+} can be seen. In the pre-

sence of $1mM-Mn^{2+}$, ATP, at $1mM$ concentration, inhibits phosphatidylcholine synthesis to the extent of 15%, but when pantetheine was added, the inhibition was increased to 80%. ATP at $2mM$ inhibited by 58%, and in the presence of pantetheine inhibition was increased to 88%. In the presence of $5mM-Mn^{2+}$, ATP did not show any inhibition until its concentration was increased to $6mM$, and was almost 40% at a concentration of $10mM$. In contrast, $2mM$-ATP inhibited by almost 80% in the presence of pantetheine when $5mM-Mn^{2+}$ was present. Similar results were obtained when ATP concentration was varied in the presence of CoA. These results are probably due to differences in the binding of ATP with Mn^{2+} compared with that with Mg^{2+}.

Possible mechanism of action of ATP and CoA or ATP and pantetheine

It is well established that cellular reactions can be controlled in a number of ways (Segal, 1973). The inhibition of phosphatidylcholine synthesis in rat liver microsomal fractions by ATP and CoA or by ATP and pantetheine could be by phosphorylation or adenylation of the CDP-choline–1,2-diacylglycerol cholinephosphotransferase, converting it into an inactive state. The thiol compounds CoA and pantetheine would in this case act as allosteric effectors in the phosphorylation or adenylation. Experiments were conducted with $[\gamma-^{32}P]ATP$ and $[^{14}C]ATP$ in the presence and absence of either CoA or pantetheine to ascertain if any phosphorylation or adenylation took place. After microsomal proteins had been precipitated and counted for radioactivity, no significant incorporation of either ^{32}P or ^{14}C could be detected over and above the control values. However, a considerable amount of ^{32}P was incorporated into the microsomal fractions, making it difficult to exclude the possibility that phosphorylation did not take place. The results in Table 5 showed that AMP-$P(CH_2)P$ was not effective in the inhibition of phosphatidylcholine synthesis, indicating the possibility that phosphorylation is required for inhibition to occur.

Reversibility of inhibition of phosphatidylcholine synthesis

It was decided to determine whether the addition of ATP and CoA or ATP and pantetheine resulted in irreversible inactivation of phosphatidylcholine synthesis. Microsomal fractions were preincubated with ATP and CoA or ATP and pantetheine under various conditions and for various periods of time, then centrifuged at 0°C and washed with $0.25M$-Tris/HCl buffer, pH 7.7, to assure complete removal of ATP, CoA and pantetheine. In the control tubes, to which no additions were made during the preincubation period, the amount of phosphatidylcholine synthesized was 35.5nmol, whereas those pre-

incubated with ATP and CoA synthesized 38.5nmol and those with ATP and pantetheine 36nmol. Both ATP and CoA or ATP and pantetheine were required for significant inhibition of phosphatidylcholine synthesis to occur, indicating that the effects of preincubation were readily reversible.

Other aspects of inhibition of phosphatidylcholine synthesis

The diacylglycerol used in all the above studies was prepared from egg phosphatidylcholine and consists of various mixtures of diacylglycerols (Hanahan et al., 1960). Using diacylglycerols, such as diolein, 1-palmitoyl-2-oleoyl-*sn*-glycerol and 1-stearoyl-2-oleoyl-*sn*-glycerol, we were able to show that phosphatidylcholine synthesis is inhibited by ATP and CoA or by ATP and pantetheine irrespective of the nature of the diacylglycerol utilized as substrate in the reaction mixture.

Kennedy et al. (1959) have shown that phosphatidylcholine synthesis can also occur by the transfer of the phosphorylcholine moiety of dCDP-choline to the free primary hydroxyl group of a diacylglycerol. When this deoxynucleotide was utilized in place of CDP-choline, ATP and CoA as well as ATP and pantetheine inhibited phosphatidylcholine formation by 43 and 62% respectively.

Although phosphatidylcholine is also synthesized from CDP-choline and 1,2-diacylglycerols in other tissues, such as brain, kidney, pancreas and lung, its synthesis in these tissues is not inhibited by either ATP and CoA or ATP and pantetheine.

Discussion

It is an established fact that phosphatidylcholine is required for lipoprotein formation (Skipski, 1972), bile formation (Spitzer et al., 1964) and forms an integral part of the membrane components of the liver cell (van Deenen, 1965). It is also well established that CoA and 4'-phosphopantetheine play an essential role in fatty acid synthesis (Volpe & Vagelos, 1973). It is possible, in the work described above, that the inhibition of phosphatidylcholine synthesis by ATP and CoA or by ATP and pantetheine may be of physiological importance in the regulation of the synthesis of phosphatidylcholine. Increasing ATP, CoA or pantetheine concentrations within the cell may be a signal for temporary inactivation of phosphatidylcholine synthetase (CDP-choline–1,2-diacylglycerol cholinephosphotransferase). If so, this constitutes the first such experimental evidence for the possible regulation of phosphatidylcholine formation at this enzymic level.

The mechanism by which the inhibition by ATP and CoA occurs is difficult to assess at the present time. The possibility remains that an acyl-CoA derivative is formed during incubation with ATP

and CoA and this product by virtue of its detergent-like properties inhibits phosphatidylcholine synthesis. This possibility is difficult to rule out because the incubation system contains 1,2-diacylglycerol and the microsomal preparations contain a very active diacylglycerol acyltransferase (Weiss *et al.*, 1960), as well as an acyl-CoA hydrolase (Barden & Cleland, 1969) and a lysophosphatidylcholine acyltransferase (Lands, 1960), which would immediately remove the exogenously added or endogenously formed acyl-CoA. It has been shown (De Kruyff *et al.*, 1970) that ATP (6.25mM) in the presence of CoA (0.1mM) stimulates triacylglycerol formation while simultaneously decreasing phosphatidylcholine synthesis. The fact that $AMP(CH_2)PP$ together with either CoA or pantetheine significantly inhibits phosphatidylcholine synthesis (Table 5) strongly indicates that inhibition is not the result of acyl-CoA formation.

Likewise it is difficult to assess a mechanism for the inhibition by ATP and pantetheine. It has been shown in several laboratories (Bar-Tana *et al.*, 1971; Pande, 1973; Lyman *et al.*, 1976) that pantetheine does not act as an acyl acceptor in the same manner that CoA does. Pantetheine is formed in mammalian tissues by the action of plasma-membrane pyrophosphatases and phosphatases on palmitoyl-CoA (Trams *et al.*, 1968) and by cytoplasmic pyrophosphatases and phosphatases on CoA (Novelli *et al.*, 1954). The physiological role of the plasma-membrane pyrophosphatases on palmitoyl-CoA is unknown, but it may play some role in controlling membrane formation.

It has been reported (Rossi *et al.*, 1970) that 4'-phosphopantetheine acts in an allosteric fashion to activate the mitochondrial GTP-dependent acyl-CoA synthetase (EC 6.2.1.10). In our laboratory (R. Liteplo & M. Sribney, unpublished work) we have found that the synthesis of phosphatidylethanolamine from CDP-ethanolamine and 1,2-diacylglycerols is inhibited by ATP and pantetheine and not affected by ATP and CoA. In contrast with the effect of ATP and CoA and ATP and pantetheine on phosphatidylcholine and phosphatidylethanolamine synthesis, sphingomyelin formation is stimulated approx. 20-fold by these compounds (Lyman *et al.*, 1976). It is possible that ATP in combination with various pantothenic acid derivatives, serves to regulate the formation of phospholipids, which are required to fulfil functions involved in lipoprotein, bile and membrane formation.

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