A deazaadenosine-insensitive methylation of phosphatidylethanolamine is involved in lipoprotein secretion

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We have examined the effect of inhibitors of methylation of phosphatidylethanolamine on lipoprotein secretion from cultured rat hepatocytes. The incorporation of [1-^{3}H]ethanolamine into phosphatidylcholine of hepatocytes and secreted lipoproteins was inhibited by greater than 90% by the methylation inhibitors 3-deazaadenosine and Neplanocin. In addition, these compounds strongly inhibited the incorporation of [3-^{3}H]serine into the choline moiety of phosphatidylcholine of the hepatocytes, but had no effect on incorporation of [3-^{3}H]serine into secreted phosphatidylcholine. The results suggest that a pool of phosphatidylcholine targeted for lipoprotein secretion originates from phosphatidylethanolamine made from serine and this methylation reaction has the unique property of being insensitive to 3-deazaadenosine.

Phosphatidylethanolamine methylation *Lipoprotein secretion* *Phosphatidylserine* *3-Deazaadenosine* *Neplanocin*

1. INTRODUCTION

DZA is a well-established and potent inhibitor of the methylation of proteins, DNA and other compounds [1]. In addition, DZA (10 μM) inhibits by greater than 90% the methylation of PE (made from ethanolamine) in cultured rat hepatocytes [2]. However, when hepatocytes are incubated with DZA for 18 h, there is no inhibition of lipoprotein secretion [3]. We have recently demonstrated that PC made from PE derived from phosphatidylserine, rather than from ethanolamine, is preferentially utilized for lipoprotein secretion [4]. Apparently, there is a biosynthetic compartmentation for PC and PE synthesis which had not been previously recognized [4]. We now report another unexpected result: DZA does not inhibit the synthesis of PC made via the methylation of phosphatidylserine-derived PE, and recovered in secreted lipoproteins. This is the first report, to our knowledge, of a transmethylation reaction which requires *S*-adenosylmethionine and which is insensitive to DZA.

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Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; DZA, 3-deazaadenosine

2. MATERIALS AND METHODS

The methylation inhibitors DZA and Neplanocin were kindly provided by Drs Peter Chiang and R.T. Borchardt, respectively. Methotrexate was purchased from Sigma. Cultured rat hepatocytes (2×10^6 cells/60 mm dish) were incubated in serum-free Dulbecco's modified Eagle's medium for up to 24 h in the presence of [1-^{3}H]ethanolamine (10 μCi/dish),

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[3-^{3}H]serine (20 μCi/dish) or [*methyl*-^{3}H]methionine (10 μCi/dish) as described [3,4]. For experiments with [^{3}H]serine, the tracer was added in medium that contained no serine. All radioactive precursors were obtained from Amersham International.

The lipoproteins were concentrated from either the culture medium or lipoprotein fractions (separated from the medium on the basis of density) by the addition of Cab-*O*-Sil (a colloidal form of silica purchased from Sigma) [5]. The Cab-*O*-Sil was pelleted by centrifugation and the phospholipids were extracted and purified by thin-layer chromatography [3,5].

Radioactivity from [3-^{3}H]serine was incorporated into all portions of PC and PE (head group, glycerol moiety and fatty acyl chains). Hence, the purified PC was degraded (greater than 95%) with phospholipase C from *B. cereus* so that incorporation of radioactivity into the choline moiety of PC could be measured as previously described [4]. For incubations with [^{3}H]ethanolamine the PC was not routinely degraded since greater than 98% of the dpm in the PC molecules was in the choline moiety.

3. RESULTS AND DISCUSSION

The incorporation of [^{3}H]ethanolamine via the methylation pathway into PC of the cells (fig.1A) and of the culture medium (fig.1B) was dramatically inhibited by DZA. However, when [^{3}H]serine was used as a precursor of PC (via phosphatidylserine and the methylation of PE), the inhibition by DZA was unexpectedly different. DZA inhibited the incorporation of serine into PC of the cells (fig.1C) but had only a small effect on the incorporation of serine into PC in the culture medium, even after 20 h (fig.1D).

The lack of inhibition by DZA of serine incorporation into PC of the medium was confirmed (table 1) by separation of the culture medium into lipoprotein fractions on the basis of density. DZA inhibited the incorporation of ethanolamine into PC of the cells and the VLDL and HDL fractions by greater than 90%; the significance of the smaller effect of DZA on the fraction of density greater than 1.18 g/ml is not understood. The amount of radioactivity from ethanolamine in the PE in the cells was increased by DZA (table 1).

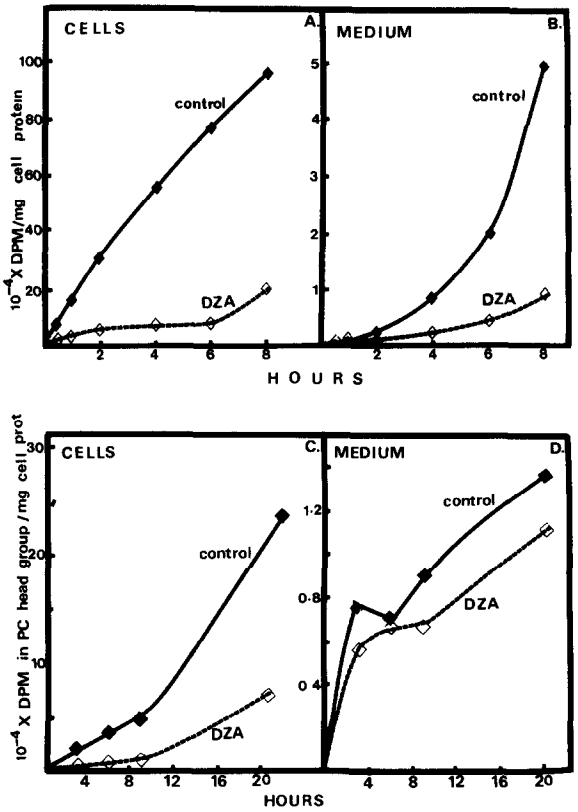

Fig.1. Effect of DZA on the incorporation of [1-^{3}H]ethanolamine and [3-^{3}H]serine into PC of hepatocytes and in the culture medium. (A,B) Incorporation of [^{3}H]ethanolamine into PC. (C,D) dpm incorporated from [^{3}H]serine into the choline moiety of PC, obtained by isolation of the PC from cells and medium, and subsequent degradation of the phospholipid with phospholipase C.

This correlates with an approximate doubling of the size of the cellular pool of PE [3] caused by DZA blocking the conversion of PE to PC, which is the major metabolic fate of ethanolamine-labeled PE in the hepatocyte [6]. [3-^{3}H]Serine incorporation into the choline moiety of PC in the cells was inhibited by 87% by DZA whereas in the VLDL, HDL and the fraction with density greater than 1.18 g/ml there was no significant effect by DZA. There was no effect by DZA on the labeling of PE from [^{3}H]serine.

Hepatocytes were incubated with 2 μM Neplanocin, an alternative inhibitor of methylation reactions [7], and either [1-^{3}H]ethanolamine or [3-^{3}H]serine for 5 h. The results with

Table 1

The effect of DZA treatment (10 μM, for 6 h) on the incorporation of [1-^{3}H]ethanolamine and [3-^{3}H]serine into PC and PE of hepatocytes and into lipoprotein fractions separated from the culture medium

		From [^{3}H]ethanolamine		From [^{3}H]serine	
		PC	PE	PC	PE
Cells	control	310300 ± 27600	291000 ± 20100	7643 ± 1360	70250 ± 4000
	+ DZA	44200 ± 2100	501500 ± 71100	964 ± 247	64730 ± 9970
VLDL	control	32100 ± 400	17600 ± 800	991	62 ± 7
	+ DZA	1500 ± 200	14100 ± 2300	1296	54 ± 6
HDL	control	6040 ± 1270	1620 ± 200	74	49 ± 3
	+ DZA	440 ± 50	1400 ± 100	61	58 ± 24
$d > 1.18$ g/ml	control	27300 ± 4200	8900 ± 1400	474	40 ± 14
	+ DZA	10100 ± 2200	5800 ± 1100	502	49 ± 5

The PC derived from incubation with [3-^{3}H]serine was degraded with phospholipase C, so that only radioactivity in the choline moiety is presented. All values, expressed as dpm/mg cell protein, are means ± SD for 4 determinations, except for PC in the lipoprotein fractions derived from serine, which are the averages of 2 determinations, for which the range is less than 10% of the mean. Low density lipoproteins (LDL) are present at very low levels in the hepatocyte medium [5] and were not analyzed

Table 2

Cultured rat hepatocytes were incubated for 5 h with the adenosine analogue Neplanocin (2 μM) and either [1-^{3}H]ethanolamine or [3-^{3}H]serine

		From [^{3}H]ethanolamine		From [^{3}H]serine	
		PC	PE	PC	PE
Cells	control	12290 ± 1980	42910 ± 3880	8.08 ± 1.82	69.4 ± 3.1
	+ Neplanocin	677 ± 17	53250 ± 5860	1.18 ± 0.31	57.8 ± 4.3
Medium	control	97.7 ± 4.5	137.3 ± 6.5	0.32 ± 0.04	1.09 ± 0.01
	+ Neplanocin	11.0 ± 0.2	105.5 ± 16.5	0.27 ± 0.01	1.03 ± 0.01

The PC and PE were isolated from the cells and the culture medium. From incubations with [^{3}H]serine, the PC was degraded with phospholipase C so that these data include only dpm incorporated into the choline moiety of PC. All values, expressed as dpm/mg cell protein × 10^{-3}, are averages ± SD of 3 determinations

Neplanocin (table 2) were similar to those with DZA. Neplanocin inhibited the incorporation of both [^{3}H]ethanolamine and [^{3}H]serine into cellular PC, and the incorporation of [^{3}H]ethanolamine into secreted PC. However, the incorporation of [^{3}H]serine into the choline moiety of secreted PC was unaffected.

These data (fig.1, tables 1 and 2) strongly indicate different mechanisms for the production of secreted PC by PE methylation, depending on whether the PE originates from ethanolamine or serine. These results also suggest that there is a DZA- and Neplanocin-insensitive PE methyltransferase that preferentially methylates PE derived from serine for the production of PC specifically destined for secretion with lipoproteins.

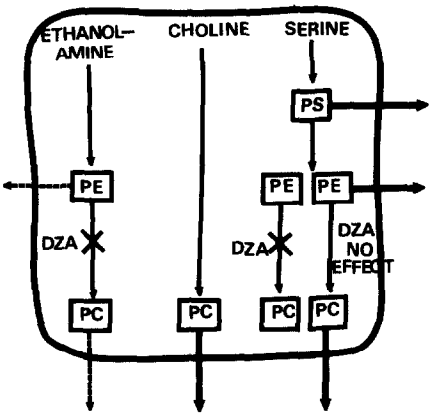

Fig.2. Schematic representation of phospholipid synthesis and secretion by hepatocytes. See text for an explanation. (– – –) Not preferred for secretion, (——) preferred for secretion.

Possibly, *S*-adenosylmethionine is not the methyl donor for this reaction. An alternative methyl donor is a tetrahydrofolate derivative, with C-3 of serine being the source of the methyl group. However, incubation of the hepatocytes with 1 μM methotrexate, a well-established inhibitor of tetrahydrofolate-mediated reactions, had no effect on the incorporation of [3-^{3}H]serine into the choline moiety of either cellular PC (control, 2730 vs treated, 2760 dpm/mg cell protein) or secreted PC (control, 280 vs treated, 250 dpm/mg cell protein). Thus, it appears that tetrahydrofolate is not the methyl group donor for this unusual PE methyltransferase.

A summary scheme for the effect of DZA on the methylation of PE derived from ethanolamine and serine in a hepatocyte is presented in fig.2. Ethanolamine is not an effective precursor for secreted PC or PE [4], and the PE methyltransferase which uses PE made from ethanolamine is strongly inhibited by DZA [2]. PC synthesized from choline, and PE and PC derived from serine are preferred for secretion into lipoproteins [4]. The present study demonstrates that there is a pool of secreted PC made preferentially from serine-derived PE by a methylation which is not inhibited by DZA. Why this PE methyltransferase is insensitive to DZA is not yet explained. The enzyme may simply be insensitive to *S*-adenosylhomocysteine and the DZA analogue, or in a compartment that is accessible to *S*-adenosylmethionine but not the inhibitor.

Another intriguing problem is the origin of the serine-derived PE which is methylated and preferentially secreted. Phosphatidylserine is synthesized on the endoplasmic reticulum by base exchange [8] and is decarboxylated to PE in mitochondria [9]. If this were the sole route for PE synthesis from serine in liver, the data (fig.1) would require this PE to be synthesized, specifically transported, methylated and secreted with lipoproteins within the first hour of the experiment. However, a recent report [10] has suggested that in baby hamster kidney (BHK-21) cells, the half-time for transfer of phosphatidylserine from the endoplasmic reticulum to the mitochondria is greater than 6 h. The origin of the secreted PC derived from serine, presumably via phosphatidylserine, thus requires further investigation.

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