Vol. 94, No. 1, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 14, 1980 Pages 174-181

PHOSPHOLIPIDS BIOSYNTHESIS BY METHYLATIONS AND CHOLINE INCORPORATION: EFFECT OF 3-DEAZAADENOSINE

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Received March 19,1980

SUMMARY

Methylation of phospholipids or phosphatidylethanolamine *in vivo* was greatly reduced in livers of rats or hamsters treated with 3-deazaadenosine. However, it was found that the absolute amounts of phopholipids and phosphatidylcholine remained constant between the livers of control or treated animals. Concomitant with the inhibition of phospholipid methylation, there was a drastic increase in the incorporation of choline into phosphatidylcholine, or phospholipids fraction. In rat liver, the rate of uptake of choline may be determined by its utilization for phosphatidylcholine biosynthesis. These observations suggest intricate control mechanisms regulating phospholipids biosynthesis between methylation pathway and incorporation from choline. In the hamster liver, where 3-deazaadenosylhomocysteine is the major nucleosidylhomocysteine present after administration of 3-deazaadenosine, it is apparent that 3-deazaadenosylhomocysteine exerts an inhibitory potency similar to that of adenosylhomocysteine.

INTRODUCTION

It was observed earlier that administration of 3-deazaadenosine (3-deaza-Ado) to rats results in perturbation of a number of methylation reactions *in vivo* (1). The most sensitive target so far appeared to be the methylation of phospholipids and we have observed a 10-fold reduction in the incorporation of the methyl-^{3}H group of methionine into liver phospholipids, in spite of an apparent doubling of the specific radioactivity of [methyl-^{3}H]AdoMet (1). The inhibition of phospholipid methylation was attributed to an alteration of the ratio of AdoMet/nucleosidylhomocysteine, brought about by an increase in intracellular concentration of AdoHcy and the formation *in vivo* of a novel congener, 3-deaza-AdoHcy (2, 3). The increase in the concentration of AdoHcy can be ascribed to the inhibition of AdoHcy hydrolase by 3-deaza-Ado (2), and

ABBREVIATIONS: AdoMet, adenosylmethionine; AdoHcy, adenosylhomocysteine, 3-deaza-Ado, 3-deazaadenosine.

0006-291X/80/090174-08$01.00/0

the *in vivo* formation of 3-deaza-AdoHcy due to the ability of the AdoHcy hydrolase to catalyze the formation of novel congener of AdoHcy from 3-deaza-Ado (2-6).

In our earlier work we reported that, in spite of the drastic inhibition of the methylation of phospholipids in the liver, the total amounts of phospholipids in the livers of control and 3-deaza-Ado treated rats remained unchanged, about 30 mg/g wet weight. It is well established that in liver and some other tissues, phosphatidylcholine can be made enzymatically not only by successive methylation of phosphatidylethanolamine but also from free choline, after conversion to CDP-choline and transfer of the phospho-base to 1,2-diacylglycerol (7-13). We concluded therefore, as a working hypothesis, that inhibition of the synthesis of phosphatidylcholine via successive methyltransfer from AdoMet to phosphatidylethanolamines might be coupled in some way to the synthesis of this compound via the CDP-choline pathway. We report here experiments supporting the hypothesis that *in vivo* when the metabolic flow through the methylation pathway of phospholipids is inhibited, more choline is incorporated into phosphatidylcholine.

MATERIALS AND METHODS

Radioisotopes were purchased from New England Nuclear. Male Wistar rats (200 g) and female golden Syrian hamsters (90 g) from Charles River (Wilmington, MA) were used. All chemicals or isotopes were dissolved in 0.9% NaCl, and control animals were injected intraperitoneally with the 0.9% NaCl vehicle. Thirty to 45 min after injection, the animals were killed by decapitation. The procedure of Folch *et al.* (14) was followed for the extraction of lipids from livers. The isotope-labelled phospholipids were quantified and separated by thin layer chromatography using cellulose H plates (Analabs, North Haven, CT), with the following solvent system: $CHCl_3$/propanol/propionic acid/water (2:3:2:1 by volume). Specific activity of [methyl-^{3}H]AdoMet was determined after separation by high performance liquid chromatography as described previously (1, 15). Quantitative determination of phospholipids was performed according to the method of Zilversmith and Davis (16).

RESULTS AND DISCUSSION

In rats, phosphatidylcholine was doubly labelled *in vivo* through injections of [methyl-^{3}H]methionine and [methyl-^{14}C]choline, or [1,2-^{14}C]choline. In agreement with our earlier findings (1), Table I shows that the administration

TABLE I

Specific Radioactivities of AdoMet and Lipids from Rats*

Treatment	[Methyl-^{3}H]AdoMet (dpm/nmol)	Isotopes in phospholipids (dpm/mg)		Isotopes in phosphatidylcholine (dpm/mg)	
		Methyl-^{3}H	^{14}C	Methyl-^{3}H	^{14}C
Experiment (A)					
Control, 30 min	195 ± 11	6,067 ± 600	——+	12,894 ± 580	168 ± 40
3-Deaza-Ado, 30 min	353 ± 20	1,211 ± 123	3,843 ± 178	2,262 ± 140	5,734 ± 677
Experiment (B)					
Control, 45 min	777 ± 60	33,819 ± 1,558	——+	79,577 ± 9,689	118 ± 84
3-Deaza-Ado, 45 min	1,574 ± 284	3,553 ± 685	3,169 ± 505	13,831 ± 2,005	20,950 ± 2,672

* Rats received injections of 3-deaza-Ado (20 mg) at 0 and 3 hr. In Experiment (A), at 3.5 hr each rat received saline solution containing 60 µCi of [methyl-^{3}H]methionine (200 mCi/mmol) and 13 µCi of [methyl-^{14}C]choline (46 mCi/mmol); at 4.0 hr the rats were sacrificed. In Experiment (B), at 3.5 hr each rat received 118 µCi of [methyl-^{3}H]methionine (200 mCi/mmol) and 6.9 µCi of [1,2-^{14}C]choline (5.4 mCi/mmol); at 4.25 hr, the rats were sacrificed. Results are expressed as means ± S.E.M. for four rats.

\+ Too low to be accurate.

TABLE II

Uptake of [Methyl-^{3}H]Methionine and [Methyl-^{14}C]Choline or [1,2-^{14}C]Choline by Livers of Rats*

Treatment	Uptake of isotopes		Phospholipids
	[methyl-^{3}H]Methionine	[^{14}C]Choline	
	(dpm/10 mg liver)		(mg/g liver)
Experiment (A)			
Control, 30 min	6,378 ± 1,851	712 ± 89	28.3 ± 1.0
3-Deaza-Ado, 30 min	6,381 ± 676	2,282 ± 106	29.4 ± 2.1
Experiment (B)			
Control, 45 min	7,421 ± 794	564 ± 67	31.2 ± 0.9
3-Deaza-Ado, 45 min	7,533 ± 398	1,372 ± 241	32.5 ± 0.8

* Experiment details are the same as described in Table I.

of 3-deaza-Ado to rats results in an 80-90% reduction of the incorporation of the methyl group of methionine into total liver phospholipids and in an equivalent inhibition of the incorporation of methyl group into phosphatidylcholine. In sharp contrast, the incorporation of ^{14}C-choline into total liver phosphatidylcholine was dramatically increased; in experiment A we observed a 30-fold increase in 30 min, and in experiment B a 170-fold increase in 45 min. Total choline uptake into the rat liver tissue was increased 2- to 3-fold by 3-deaza-Ado treatment; but no difference could be found in methionine uptake between treated and control rats (Table II). Total phospholipids and phosphatidylcholine contents were about the same in the treated and control rats, about 30 mg/g and 7-8 mg/g wet weight, respectively. It must be emphasized here that prior to receiving the injection of isotopes, the rats had already been under the influence of 3-deaza-Ado for a total of 3.5 hr.

Whereas the incorporation of the methyl group of methionine into phospholipids declined, the specific radioactivity of AdoMet doubled ($p < 0.01$), thus confirming our earlier observation (1). In such short time intervals, there was no incorporation of the ^{14}C label into the AdoMet from either [methyl-^{14}C]-choline or [1,2-^{14}C]choline.

As reported earlier (1) administration of 3-deaza-Ado results in simultaneous rise in liver level of AdoHcy and in the appearance of 3-deaza-AdoHcy in rat liver, but in the hamster liver, only the formation of 3-deaza-AdoHcy could be seen with negligible effect on the level of AdoHcy (1). Even in the hamster liver, 3-deaza-Ado must also be perturbing biochemical methylations, as can be surmised by a 2-fold rise in the concentration of AdoMet in the hamster liver (1). It was, therefore, of considerable interest to examine the effect of 3-deaza-Ado on phospholipid methylation in hamsters. Table III shows that after treatment with 3-deaza-Ado for 4 hr, incorporation of methyl group of methionine into liver phospholipids was reduced by about 90%. Concomitant with the reduction of the incorporation of methyl-^{3}H label into phospholipids, an increase of 3-fold in the incorporation of [^{14}C]choline into phospholipids can be observed (Table III). The specific radioactivity of [methyl-^{3}H]AdoMet again doubled ($p < 0.05$), in spite of the apparent decreased uptake of [methyl-^{3}H]methionine into the liver ($p < 0.05$). In contrast to the results obtained with the rats, there was no difference in the uptake of [^{14}C]choline between the control and treated hamsters (Table III).

The results presented here confirm and extend our previous findings (1) that methylation of phospholipids *in vivo* is very sensitive to perturbation by 3-deaza-Ado. In the hamster liver, where 3-deaza-AdoHcy is the major nucleosidylhomocysteine present after administration of 3-deaza-Ado (1), the inhibition of phospholipids synthesis via successive methylation of phosphatidylethanolamine is almost certainly due to accumulation of this analog of AdoHcy. In rat liver, where both the levels of AdoHcy and 3-deaza-Ado are elevated after administration of 3-deaza-Ado, it is not possible to determine which of these two compounds is the inhibitor or whether both contribute. A cell-free system will be needed for studies with phosphatidylethanolamine methylation to resolve this question, but it is already established that 3-deaza-AdoHcy is a potent inhibitor of a number of methylases (17, 18). Should it become established that 3-deaza-AdoHcy is significantly more potent

TABLE III

Specific Radioactivities of AdoMet and Lipids from Hamsters[8]

Treatment	Uptake of isotopes		[Methyl-^{3}H]AdoMet	Isotopes in phospholipids	
	[Methyl-^{3}H]Methionine	[1,2-^{14}C]Choline		Methyl-^{3}H	^{14}C
	(dpm/mg liver)		(dpm/nmol)	(dpm/mg)	
Control	2,473 ± 265	379 ± 24	379 ± 52	25,402 ± 1,788	12,827 ± 1,083
3-Deaza-Ado	997 ± 147	357 ± 62	897 ± 94	3,061 ± 183	39,934 ± 1,849

* Female hamsters (90-100 g) were injected with 3-deaza-Ado (6 mg each) at 0 and 2 hr; 0.2 ml of saline containing 70 µCi of [methyl-^{3}H]methionine (200 mCi/mmol) and 4.2 µCi of [1,2-^{14}C]choline (5.4 mCi/mmol) was injected at 3.5 hr; at 4 hr the hamsters were sacrificed. Results are expressed as means ± S.E.M. for four hamsters.

than AdoHcy as an inhibitor of phosphatidylethanolamine methylation, the usefulness of 3-deaza-Ado in the regulation of biological methylations would be greatly enhanced.

In regards to the methylation pathway, we were unable to find any significant accumulation of the monomethyl or dimethyl species of phosphatidylethanolamine labelled with methyl-^{3}H, thus supporting the suggestion first made by Bremer and Greenberg (7) that the incorporation of the first methyl group is the rate limiting step in phosphatidylcholine biosynthesis. Studies by Schneider and Vance (13) with solubilized enzyme system also support such a hypothesis.

The present investigation suggests intricate control mechanisms regulating phospholipids biosynthesis between methylation pathway and incorporation from choline. The contribution of each pathway to the biosynthesis of phosphatidylcholine is probably balanced in a compensatory way; *de novo* synthesis of phosphatidylcholine via the CDP-choline pathway may be accelerated when the methylation pathway is inhibited. Without knowing the pool sizes of the precursors of the choline pathway up to CDP-choline, it is not possible to say what percentage the methylation pathway contributes to the total biosynthesis of phosphatidylcholine under normal physiological conditions, a question that has been a subject of debate (7-12, 19-24). Recently, it was observed that upon infusion of phosphatidylcholine, ^{32}P incorporation into phosphatidylcholine in intestinal mucosa was decreased (25), and in rat liver microsomes, lysophosphatidylcholine acts as a regulator of *de novo* phosphatidylcholine synthesis by being a bimodal effector of 1,2-diacyl-*sn*-glycerol-3-phosphate cholinephosphotransferase (26). In tissues, such as HeLa cells (27) and intestine (25), in which the phosphatidylethanolamine-N-methyltransferase is missing, the control mechanisms involved in the synthesis of phosphatidylcholine is probably less complicated.

It has been suggested that choline is preferentially incorporated into linoleoyl phosphatidylcholine, whereas methylation of phosphatidylethanolamine

preferentially leads to arachidonyl phosphatidylcholine (20, 21). It would be of interest to determine how these two species of phosphatidylcholines are distributed when methylation is inhibited.

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