EFFECT OF DIBUTYRYL cAMP ON THE ENZYMES OF FATTY ACID SYNTHESIS AND OF GLYCOGEN METABOLISM

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1. Introduction

Inhibition of fatty acid synthesis by 3′,5′-cAMP or its dibutyryl derivative (db cAMP) has been described in liver [1–5], but no inhibitory action was detectable in adipose tissue [3, 4]. A stimulation of insulin release by this nucleotide has been suggested to explain this last result. An other explanation could be that the hepatic fatty acids synthesis inhibition was only apparent and due to a dilution effect caused by glycogenolysis induced by this nucleotide [3]. It was of interest, therefore, to determine whether 3′,5′-cAMP acts directly on the liver fatty acid synthesis either by repression of the synthesis of the corresponding enzymes or by decreasing their activity. To test the role of a dilution of the radioactive precursor by endogenous glucose, we studied in parallel the db cAMP action on fatty acid synthesizing enzymes and on glycogen metabolism in presence and absence of an inhibitor of the protein synthesis. A decrease of the incorporation of various precursors by db cAMP into the liver fatty acids does not occur *in vitro*, and the inhibitory effect observed *in vivo* disappears if the animals have been deprived of their hepatic glycogen. These facts indicate that 3′,5′-cAMP exerts no inhibitory action on fatty acid synthesis, as such and that a dilution by hepatic glycogenolysis is responsible for the appearent decrease of the fatty acid synthesis caused by 3′,5′-cAMP *in vivo*.

2. Experimental

2.1. *General methods*

Liver glycogen and radioactivity were measured after extraction of the organ with boiling KOH, precipitation with ethanol and transformation into acetylglycogen [6]. The acetyl glycogen was dissolved in acetic acid and counted in a three-channel Packard Tri-Carb liquid scintillation spectrometer (5 g of PPO and 300 of POPOP per litre of toluene).

Fatty acids were extracted from the saponified mixture and counted using the same scintillation mixture. Corrections for quenching were made according to Hendler [7].

To determine the activities of the fatty acid synthesis enzymes, portions of liver were homogenized in cold 0.25 M sucrose in a Potter–Elvehjem homogenizer with a Teflon pestle. The homogenates were centrifuged at 2600 rpm for 15 min at 4°. The supernatants were used for the assay of fatty acid synthetase [8] and recentrifuged in a Spinco model L ultracentrifuge at 30,000 rpm for 30 min for the assay of acetyl-CoA carboxylase [9].

2.2. *Treatment of animals*

Experiment 1: Mice (Swiss strain, 30 g) were divided into 3 equal groups: mice of the first group received puromycin (6 mg, i.p.) and twelve minutes later dibutyryl cAMP (6 mg, i.p.). The mice of the second group received only dibutyryl cAMP. 48 min later, mice of the three groups were given glucose-6-^{3}H intravenously. Twelve minutes after the administration of tracer the animals were killed.

Experiment 2: The treatment of animals was the same as in the previous experiment except that the radioactive precursor was L-alanine-^{3}H (G).

Experiment 3: This experiment is divided in two parts: (a) In the first part, the mice received db cAMP (6 mg, i.p.) or NaCl (0.9%) (controls) followed 60 min later by an intravenous injection of acetate-1-^{14}C. They were killed 12 min after the administration of the radioactive presursor. (b) In the second part, the mice received two successive injections of db cAMP (4 mg/ml, i.p.) or NaCl 0.9% (controls) at 30 min intervals. 30 min after the second injection they were given acetate-1-^{14}C intravenously and were killed 12 min later. The data were evaluated statistically by means of the Student t test. Values for $p>0.05$ were taken to indicate non-significant changes.

3. Results

Experiment 1 (see table 1): The glycogen concentration is strongly decreased by db cAMP in presence or absence of puromycin. The specific radioactivity is about 2 fold higher in the db cAMP-treated animals than in the controls. The total radioactivity of glycogen is several fold lower in db cAMP-treated animals than in the controls. Puromycin exerts no significant effect on the action of db cAMP.

Experiment 2 (see table 2): *In vivo*, db cAMP significantly inhibits the incorporation of L-alanine-^{3}H (G) into liver fatty acids. Puromycin at doses which inhibit protein synthesis, does not prevent this inhibition and also has no action upon the controls. *In vitro*, in the same animals, the activities of acetyl-CoA carboxylase and fatty acid synthetase are not modified after the administration of db cAMP. Puromycin administered *in vivo* decreases the liver glycogen concentration and does not suppress the lowering action of db cAMP on the liver glycogen concentration.

Experiment 3 (see table 3): The administration of two successive injections of bd cAMP shows that under these conditions, i.e. when liver glycogen concentration is low, this nucleotide does not decrease the incorporation of acetate-1-^{14}C into liver fatty acid *in vivo*. A single injection of db cAMP, in contrast, decreases the acetate incorporation into liver fatty acids.

4. Discussion

The present results indicate that db cAMP has no direct inhibitory action on the fatty acid synthesis. In fact, it does not inhibit the fatty acid synthesis in *in vitro* experiments, i.e. under conditions where concentrations of substrates and cofactors were optimum

Table 1

In vivo effect of db cAMP on the glycogen hepatic concentration and synthesis in presence or absence of puromycin.

	Glycogen weight (mg/g liver)		Specific radioactivity og glycogen (dpm/mg)		Total radioactivity of hepatic glycogen	
Controls	27.9		237		10,143	
	↑		↑		↑	
	$p<0.001$		$p<0.02$		$p<0.01$	
	↓		↓		↓	
db cAMP	2.1	$p<0.001$	546	$p=0.05$	1,476	$p<0.01$
	↑		↑		↑	
	n.s.		n.s.		n.s.	
db cAPM +	↓		↓		↓	
puromycin	2.6		844		1,490	
Puromycin	db cAMP (6 mg, i.p.)		Glucose-6-^{3}H (10 μCi, i.v.)		Killed	
↓	↓		↓		↓	
0 min	12 min		60 min		72 min	

Table 2
Action of db cAMP on the liver fatty acids synthesis *in vivo* and *in vitro*.

	Total radioactivity of fatty acids synthesized *in vivo*	Activity of the enzymes of fatty acids synthesis		Protein (specific radioactivity)	Glycogen (mg/g liver)
		Acetyl-CoA carboxylase	Fatty acids synthetase		
Precursors	L-Alanine-^{3}H (G)	Acetyl-CoA-1-^{14}C incorporated	Malonyl-CoA-1,3-^{14}C incorporated	L-Alanine-^{3}H (G)	
Controls	17,916	586,100	3,480,000	237.4	27.9
	n.s.	$p < 0.05$	n.s.	$p < 0.001$	$p < 0.05$
Controls + puromycin	14,137	933,400	3,700,000	130.6	6.6
	$p < 0.005$	$p = 0.05$	n.s.	$p < 0.001$	$p < 0.01$
db cAMP	3,446	645,600	3,960,000	235.6	1.45
	n.s.	n.s.	n.s.	$p < 0.001$	n.s.
db cAMP + puromycin	6,720	721,600	4,050,000	131.4	3.63
	$p < 0.001$	n.s.	n.s.	n.s.	$p < 0.001$

Puromycin (6 mg, i.p.)	db cAMP (6 mg, i.p.)	L-Alanine-^{3}H (G) (10 μCi, i.v.)	Killed
0 min	12 min	60 min	72 min

for this process. Hence the two key enzymes of fatty acid synthesis, acetyl-CoA carboxylase and fatty acid synthetase are not affected by db cAMP. A modification of the concentration of a substrate or a cofactor is probably responsible for the inhibition *in vivo*. This inhibition is probably only apparent and due to a dilution effect consecutive to glycogenolysis induced by this nucleotide. The radioactivity of the applied labeled precursor would be diluted by acetyl-CoA through glycogen degradation.

This last assumption is supported by the following facts: first, *in vivo*, the inhibitory effect appears only in liver and not in the carcass or in adipose tissue with cAMP or its dibutyryl derivative [3] or with cGMP [4]; second, the two successive administrations of db cAMP failed to inhibit the liver fatty acid synthesis *in vivo*. Thus when the animals have been deprived of glycogen by a first injection of db cAMP, the inhibitory effect of this compound disappears. It is also interesting that while it is not possible to provoke a dilution effect by exogenous glucose [10], an overload of intracellular glucose can dilute acetyl-CoA. From these results it also appears that not only is glycogenolysis stimulated *in vivo* by 3′,5′-cAMP but also the synthesis of glycogen is inhibited. Indeed, at the time when the synthesis of glycogen from radioactive acetate begins, the glycogenolytic action of cAMP is almost finished (unpublished results). If glycogen synthesis was unimpaired, the specific radioactivity of the remaining glycogen would be expected to be much higher than we have found. This suggests that both glycogen synthetase and phosphorylase are modified *in vivo* as *in vitro* [11–13] under the influence of cAMP. This nucleotide exerts its action not on the synthesis of these enzymes but on their activities, probably by inducing an immediate allosteric modification as puromycin does not prevent its action. It is surprising that *in vivo* as *in vitro*, cAMP exerts an effect similar to glucagon or epinephrine on the metabolism of glycogen [14–17] but does not have the same action on the fatty acid synthesis [18, 19], unless the decrease of fatty acids synthesis provoked by these hormones is due to the same phenomenon of dilution.

Table 3
Comparison of the effect of a single and of two successive injections of db cAMP on the fatty acid synthesis and glycogenolysis in mice liver.

	Total radioactivity of fatty acids	Hepatic glycogen (mg/g of liver)
Controls	69,000	36.6
	↑	↑
	$p < 0.005$	$p < 0.001$
	↓	↓
db cAMP	26,700	1.5

db cAMP (6 mg, i.p.)	Acetate-1-^{14}C (2 μCi, i.v.)	Killed
↓	↓	↓
0 min	60 min	72 min

	Total radioactivity of fatty acids	Hepatic glycogen (mg/g of liver)
Controls	69,300	41.4
	↑	↑
	n.s.	$p < 0.001$
	↓	↓
db cAMP	90,000	2.07

db cAMP (4 mg, i.p.)	db cAMP (4 mg, i.p.)	Acetate-1-^{14}C (2 μCi, i.v.)	Killed
↓	↓	↓	↓
0 min	30 min	60 min	72 min

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