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Studies on the inhibition a fatty acid synthesis in the chicken liver by adenine compounds *in vitro*

N. R. BHAT, G. R. KULKARNI, A. MADHAVA RAO and S. K. MURTHY

Department of Biochemistry, Indian Institute of Science, Bangalore 560 012

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Abstract. The biosynthesis of fatty acids from [1-^{14}C]-acetate in the chicken liver slices *in vitro* was inhibited by cAMP, adenosine, 5'-AMP, 3'-AMP, ATP, NAD and FAD but not by adenine, guanine or inosine. The minimum structural requirement for inhibition appears to be adenosine. The inhibitory action of adenosine, 5'-AMP and NAD on fatty acid synthesis is likely to be mediated by adenosine or its metabolites since adenosine deaminase reverses the inhibition while it has no effect on the inhibition by cAMP; thus, the inhibitory effect of cAMP is probably not mediated through its hydrolysis products, 5'-AMP, or adenosine.

Keywords. cAMP; adenosine; fatty acid synthesis; chicken liver; adenosine deaminase.

1. Introduction

Following the postulation of Sutherland's second messenger hypothesis of hormonal action (Robinson *et al.,* 1968), cAMP was shown to mimic the hormonal effects in a variety of systems. In all such studies the concentration of cAMP required to elicit physiological effects was generally, several orders of magnitude more than the intracellular concentration and this was attributed to limited permeability of the plasma membrane to cAMP. However, the possibility of rapid extracellular degradation of cAMP by the target tissue was never ruled out. Since the demonstration by Levine *et al.* (1969) that rat liver rapidly destroyed cAMP during perfusion, a number of tissues/cells such as thymic lymphocytes (Macmanus *et al.,* 1971), slime mould (Riedel and Gerisch, 1971), avian erythrocytes (Aurbach *et al.* 1975), frog skeletal muscle (Woo and Manery, 1973), adrenal tumor cells (Wolff and Hope-Cook, 1977), isolated rat kidney (Coulson, 1976), and cultured hepatoma cells (Grenner *et al.,* 1975), were shown to degrade exogenously added cAMP extracellularly. More recently, Gorin and Brenner (1976) showed that in the rat, intravenously administered cAMP was metabolised to ATP, ADP, AMP and IMP by kidney, liver and muscles. Since it is well known that plasma membrane is permeable to adenosine (Murray, 1971) it was suggested that the physiological effects attributed to exogenously added cAMP in *in vitro* systems, may well be due to the adenosine formed from cAMP or some of its metabolites.

Following the demonstration by Tepperman and Tepperman (1972) that lipogenesis in animal liver is tinder hormonal regulation, hepatic synthesis of fatty acids was shown to be inhibited by cAMP or dibutyryl cAMP *in vivo* (Tous, 1970) or *in vitro* with tissue slices (Allred and Roehrig, 1973; Akhtar and Bloxham, 1970; Bricker and Levey, 1972; Bhat *et al.,* 1978) or isolated cells (Goodridge, 1973; Capuzzi *et al.,* 1974; Harris, 1975). Again in such studies mM concentrations of cAMP were found to be required. In view of the suggestion that cAMP effects may be effected through its degradation product adenosine, it was decided to examine the effect of adenosine and a number of other compounds having the adenosine moiety in their structure on fatty acid synthesis from [1-^{14}C]-acetate by chicken liver. The results presented here show that even though adenosine and a number of other compounds can inhibit fatty acid synthesis, the inhibitory action of cAMP cannot be attributed to its degradation products.

Materials and methods

Adenine, adenosine, 5'-AMP, 3'-AMP, ADP, ATP, NAD, FAD, inosine, guanosine and adenosine deaminase (EC 3.5.4.4) were obtained from Sigma Chemical Company, St. Louis, Missouri, USA. The sources of all other chemicals have already been described (Bhat *et al.,* 1978).

Handling of chicken and preparation of liver slices for incubation are as described before (Bhat *et al.,* 1978). Wherever necessary, the test compounds were neutralised to pH 7·4 with 0·2 M sodium bicarbonate. Isolation of lipids from the incubation mixtures and estimation of the incorporated radioactivity were according to Bhat *et al* (1978). In the values presented here, radioactivity incorporated into total lipids represents the changes in the fatty acid synthesis from acetate. The validity of this is borne out by the observation that saponification of total lipids showed that fatty acids accounted for 96% of the total radioactivity, while only 3–4% was present in cholesterol.

Results and discussion

Effect of different compounds on [l-^{14}C]-*acetate incorporation into lipids*

It is seen from table 1 that fatty acid synthesis from [l-^{14}C]-acetate is inhibited by a large number of compounds. Among the compounds tested, cAMP is the most potent inhibitor (63%), while adenosine, 5'-AMP, 3"AMP, ADP, ATP, NAD and FAD caused about 50% inhibition; in contrast, adenine, inosine and guanosine had no significant effect. An examination of the chemical structure of these compounds show that the minimum structure required for inhibition of fatty acid synthesis is adenosine and also that removal of ribose from adenosine (adenine) or of the 6-amino group (inosine) results in a total loss of the ability to inhibit the synthesis of fatty acids. Guanosine had no effect.

Comparison of the potency of adenosine, ATP and cAMP in inhibiting fatty acid synthesis

It is seen from table 2 that of the three compounds tested, cAMP is the most active. Thus, at a concentration of 0·1 mM, while cAMP inhibited fatty acid synthesis

Table 1. Effect of different derivatives of adenine on fatty acid synthesis from [1-^{14}C] acetate in chicken liver.

Additions (2 mM)	[1-^{14}C]-acetate incorporated into lipids (cpm × 10^{-5}/g/h)
Nil	12·3
Adenosine	6·3
5′-AMP	6·4
3′-AMP	6·2
cAMP	4·5
ADP	6·2
ATP	6·0
NAD	6·8
FAD	5·9
Inosine	11·4
Guanosine	11·4
Adenine	13·1

Liver slices were incubated as described under methods in 2 ml of Krebs-Ringer bicarbonate buffer containing [1-^{14}C]-acetate (1 μCi, 2·5 mM) for 60 min in the presence of various compounds indicated, Total lipids were extracted and the radioactivity determined. The data is an average of three experiments.

by about 30%, adenosine and ATP were totally ineffective. This indicates that cAMP effects could not have been mediated by its hydrolysis products, 5'-AMP or adenosine. This is further substantiated by the following experiment.

Table 2. Comparison of the potencies of adenosine, ATP and cAMP in inhibiting the synthesis of fatty acids.

Nucleotide concentration (mM)	[1-^{14}C]-acetate incorporated into lipids (cpm × 10^{-5}/g/h)		
	Adenosine	ATP	cAMP
0	13·4	13·4	13·4
0·1	13·5	13·2	9·5
0·5	8·6	8·5	6·9
2·0	6·7	6·7	4·7
5·0	6·1	6·0	4·0

The experimental conditions are as described in table 1.

Evidence to show that cAMP action is unrelated to its extracellular hydrolytic products

If it is assumed that adenosine is mediating the effects of cAMP, then, removal of adenosine from the system or its conversion to an inactive product rapidly should result in the abolition of cAMP effect provided the rate of removal of adenosine from the system is faster than its formation. The results of table 1, showed that while the compounds containing the adenosine moiety in thier structure inhibited fatty acid synthesis by 50%, inosine was totally ineffective. It is well known that adenosine is rapidly deaminated to inosine by adenosine deaminase and that it is absolutely specific to adenosine (in separate experiments we found that the deamination of 5'-AMP or cAMP was 0· 01% and 0 ·004% of that of adenosine). Hence the effect of a few active compounds on fatty acid synthesis was examined in the presence and absence of 20 units of adenosine deaminase. (One unit of the enzyme will deaminate 1 μmol of adenosine to inosine per min.) The results of table 3

Table 3. Effect of adenosine deaminase on the inhibition of fatty acid synthesis caused by different compounds.

Additions (2 mM)	[1-^{14}C] acetate incorporated into lipids (cpm × 10^{-5}/g/h)	
	Control	Adenosine deaminase
Nil	12·2	10·7
Adenosine	6·8	10·6
5′-AMP	7·1	10·6
cAMP	5·4	6·6
ATP	6·4	8·7
NAD	7·1	10·5

The experimental conditions are as described in table 1 excepting that 20 units of adenosine deaminase was added to the incubation medium just before the addition of the compounds indicated.

show that while the addition of adenosine deaminase to the incubation medium completely blocked the inhibitory effects of adenosine, 5"AMP, and NAD, it was least effective on cAMP inhibition because even in its presence, cAMP continued to inhibit the synthesis of fatty acids, even though the degree of inhibition was slightly lower. Similarly, the inhibitory effects of ATP were also not fully reversed by adenosine deaminase. These results clearly show that cAMP is not exerting its inhibitory action on the synthesis of fatty acids from acetate through its degradation products—5'-AMP or adenosine. On the other hand, the inhibitory effects of NAD and 5'-AMP and probably FAD and 3'-AMP, are brought about by adenosine (or some of its metabolites) since the inhibition caused by them was fully reversed by adenosine deaminase. It is relevant to mention here that adenosine

was shown to be one of the products of degradation of UDP-G (Bischoff *et al.,* 1970). CoA (Domschke *et. al.,* 1971) NAD and ATP (Liersch *et al.,* 1971) and the presence of the hydrolytic enzymes like 5'-nucleotidase, (EC 3.1.3.5) nucleotide pyrophosphatase (EC 3.6.1.9) and ATPases (EC 3.6.1.3), on the cell surface of many cell lines has recently been demonstrated (Trams and Lauter, 1974). It seems possible that enzymes capable of degrading NAD, FAD and AMP are present on the plasma membrane of chicken liver cells also.

Are adenosine effects on fatty acid synthesis mediated by changes in the intracellular levels of cAMP?

While the above results showed that the inhibitory effects of some of the compounds are effected through adenosine, they do not explain as to how adenosine inhibits fatty acid synthesis from acetate. Following the demonstration of Sattin and Rall (1970), it was shown that the adenosine stimulated cAMP levels in cultured human astrotoma cell line (Clark and Gross, 1974) cultures of fetal rat brain (Gilman and Schrier, 1972), platelets (Mills and Smith, 1971), isolated bone cells (Peck *et al* 1974), lymphocytes (Welberg *et al.,* 1975) and cultured adrenal tumor cells (Wolff and Hope-Cook, 1977). On the other hand, in certain other tissues, adenosine was found to inhibit adenylate cyclase (Fain *et al.,* 1972; Schwabe *et al.,* 1975; Weinryb and Michel, 1974; Zenser, 1976). In view of the inhibition of fatty acid synthesis by both adenosine and cAMP, it was of interest to examine whether adenosine was acting by changing the intracellular concentration of cAMP.

The results in table 4 show that the cAMP levels remained unchanged for a 15 min period of incubation in the control samples and addition of aminophylline led to a progressive increase in cAMP levels with time. In contrast, while adenosine by itself had no significant effect on cAMP levels of chicken liver at all time intervals

Table 4. Effect of adenosine on the cAMP levels of chicken liver slices.

Additions	cAMP (*p* mol/mg protein)		
	5 min	10 min	15 min
Nil	3·5±0·42	3·5±0·32	3·3±0·3
Aminophylline (5 mM)	4·7±0·36	5·2±0·4	6·2±0·34
Adenosine (2 mM)	3·5±0·39	3·2±0·3	3·8±0·34
Adenosine (2 mM) + Aminophyline (5 mM)	3·5±0·3	4·6±0·43	5·0±0·28

Liver slices were incubated as described under materials and methods and after incubation for 5, 10,15 min the slices were rapidly frozen in liquid nitrogen. cAMP was then estimated by protein kinase binding assay of Gilman (1970). The results are the mean of three separate incubation ± S.D. Liver protein was estimated by the method of Lowry *et al.* (1951) with crystalline bovine serum albumin as standard.

studied, it partially inhibited aminophylline-induced increase of cAMP. The lack of effect of adenosine on cAMP levels of chicken liver, while at the same time inhibiting fatty acid synthesis from [l-^{14}C]-acetate, clearly shows that adenosine may be acting through mechanisms other than through cAMP.

Since adenosine is known to cross the cell membrane readily, it might exert its effects, probably, through changes in the adenine nucleotide pools. In fact, it has recently been shown that while treatment of different tissues with millimolar concentrations of adenosine resulted in a marked increase in the ATP levels (Plagemann, 1972; de Sanchez *et al.,* 1972; Lund *et al.,* 1975; Wilkening *et al.,* 1975; Grummt and Grummt, 1976; Rapaport and Zamecnik, 1976), adenine, hypoxanthine or inosine were ineffective. It is interesting to note here that Gorin and Brenner (1976) had shown ATP to be one of the main products of the metabolism of exogenously administered cAMP in liver and adipocytes and that adenosine is an intermediate in this process. Further, Lund *et al* (1975) and Rapaport and Zamecnik; (1976) had reported that incubation of cultured rat liver cells with adenosine led to an increase in the ATP/ADP ratio. Since changes in energy charge of cells can influence cellular metabolism, it should be interesting to know whether the inhibition of fatty acid synthesis in chicken liver by the adenine nucleotides or their derivatives is effected through changes in the energy charge.

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