Biogenesis of liver δ-aminolevulinate synthase

The role of cAMP in the induction

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Received 22 July 1985

In primary cultures of chick embryo hepatocytes pulse labeled with [^{35}S]methionine, immunochemical analyses indicated that adenosine 3′:5′-cyclic monophosphate (cAMP) did not affect either the rate of production or the maturation of δ-aminolevulinate synthase (ALA synthase). In addition, allylisopropylacetamide caused a slight drop in intracellular cAMP while testosterone caused the levels of cAMP to rise to 260% of the basal levels measured in hepatocytes in culture. Thus the results of this study did not indicate a direct short-term role for cAMP in the regulation of production of ALA synthase.

δ-Aminolevulinate synthase *cAMP* *Hepatocyte culture*

1. INTRODUCTION

Liver δ-aminolevulinate synthase (ALA synthase) catalyzes the rate-limiting step of the pathway of heme biosynthesis [1]. The results of several studies have suggested that adenosine 3′:5′-cyclic monophosphate (cAMP) might play a role in regulating the biogenesis of the enzyme. Dibutyryl cAMP potentiated the capacity of the porphyrogenic agent allylisopropylacetamide (AIA) to induce ALA synthase in suspension cultures of hepatocytes from both rat [2] and chick embryo [3]. In addition, cAMP in the medium of primary cultures of chick embryo hepatocytes enhanced the induction of ALA synthase and of cytochrome P-450 by phenobarbital [4]. Thus, cAMP either induced ALA synthase or played a permissive role in the induction of the enzyme by porphyrogenic agents. The work of Yamamoto et al. [5], on the other hand, suggested that cAMP may repress the induction of liver ALA synthase by blocking the maturation of the enzyme either by acting directly or by causing an increase of the intracellular free heme pool. Here, we determined the effects of 2 porphyrogenic agents on the levels of cAMP in the hepatocytes. This was to test the possibility that cAMP might mediate the induction of the enzyme. We also determined whether exogenously added cAMP affected the maturation of ALA synthase. The results did not indicate a direct short-term role for cAMP in the regulation of production of the enzyme.

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2. EXPERIMENTAL

2.1. *Primary cultures of hepatocytes*

Primary cultures of hepatocytes were prepared from White Leghorn chick embryos and maintained in serum-free medium as described [6].

2.2. *Determinations of cAMP*

Determinations of cAMP levels in hepatocytes were carried out on cells maintained in primary cultures in 35-mm culture dishes. For each determination, the culture medium was removed, and the cells scraped in 2.0 ml ethanol at 0°C. The resulting suspension was sonicated for 30 s in a bath sonicator (Laboratory Supplies, Hicksville, NY). Insoluble material was removed by centrifugation, and the supernatant dried under

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00145793/85/$3.30

vacuum using a SpeedVac concentrator (Savant Instruments). The dried cell extract was suspended in 1.25 ml double-distilled water and the suspension extracted 3 times with equal volumes of chloroform. The aqueous sample was saved for the analysis. cAMP was determined using the radioimmunoassay procedure of Brooker et al. [7]. A typical standard curve used in the determinations is shown in fig.1.

2.3. *ALA synthase activity*

ALA synthase activity was measured in cultures of hepatocytes using the procedures in [6]. Protein was determined by the method of Lowry et al. [8] using bovine serum albumin as a standard.

2.4. *Immunoprecipitation of ALA synthase*

ALA synthase was immunoprecipitated from hepatocytes pulse labeled with [^{35}S]methionine as described [6].

2.5. *Protein analysis*

Proteins were analyzed by gel electrophoresis in the presence of SDS using slabs of 11% polyacrylamide and radiolabeled proteins were detected by fluorography using Kodak SB-5 X-ray film (Eastman Kodak, Rochester, NY) as described [6].

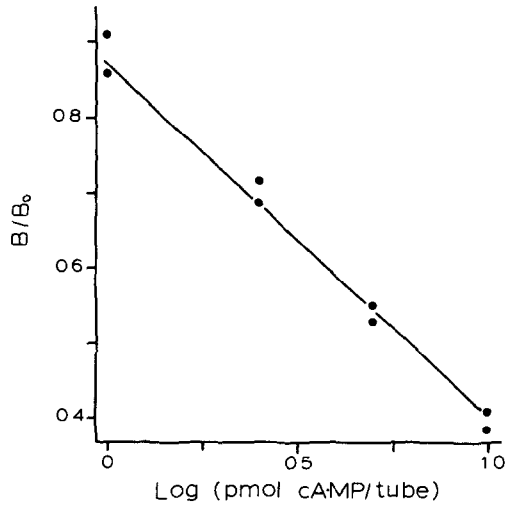

Fig.1. Typical standard curve used in the cAMP radioimmunoassay. The values for the variables were calculated from the definitions given by Brooker et al. [7]. The ratio B/B_0 represents the amount of radioactivity bound to the antibody in the presence of a given concentration of non-radioactive cAMP (B) divided by the amount of radioactivity bound in the absence of cAMP (B_0).

3. RESULTS AND DISCUSSION

The effect of cAMP on the maturation of ALA synthase was investigated in hepatocytes maintained as primary cultures in the presence of AIA. The cultures were pulse labeled with [^{35}S]methionine for 45 min in the presence and absence of cAMP, then ALA synthase was immunoprecipitated from the labeled cells. Electrophoretic analyses of the immune precipitates indicated that cAMP did not affect the maturation of ALA synthase since only the processed form of

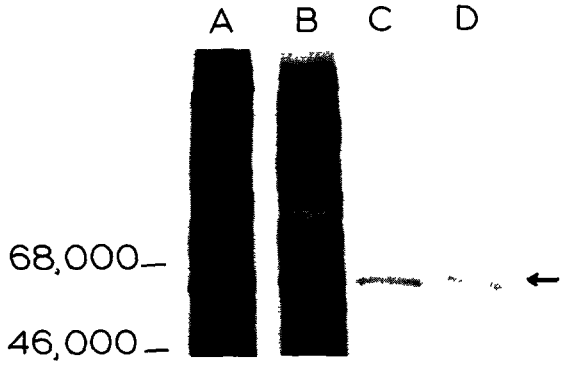

Fig.2. Effect of cAMP on the processing of pre-ALA synthase. Primary cultures of hepatocytes were incubated for 5 h in the presence of 0.075 mg/ml AIA. At the end of the 5 h, the medium was exchanged with identical medium containing AIA but lacking methionine. Dibutyryl cAMP was added to the medium of some of the cultures to a final concentration of 0.050 mM, and the cultures incubated for 10 min. [^{35}S]Methionine was then added to the cultures to a final concentration of 20 μCi/ml (spec. act. 1200 Ci/mmol). The cells were incubated for 45 min, and then ALA synthase was immunoprecipitated from the cells. Portions from both the immunoprecipitates and cell extracts were analyzed by electrophoresis, and the labeled proteins detected by fluorography. Cells from two 35-mm culture dishes (0.2 mg protein/dish) were pooled for each determination. The positions of M_r markers are indicated, and the arrow indicates the position of mature ALA synthase. Slots A and B show profiles of extracts from cells labeled in the absence (A) and presence (B) of cAMP; slots C and D show the profiles of material immunoprecipitated from cells labeled in the absence (C) or presence (D) of cAMP.

the enzyme was recovered from the cells (fig.2). In addition, our studies indicated that cAMP did not significantly affect the rate of production of ALA synthase as demonstrated by the incorporation of the labeled amino acid into the protein during the pulse labeling (not shown). Similar results were obtained when 8-bromo-cAMP was added to the medium instead of dibutyryl cAMP. These findings are in apparent conflict with the suggestion of Yamamoto et al. [8] that the nucleotide, when injected into rats, may block the maturation of the enzyme in liver. Of course, long-term metabolic alterations in hepatocytes may be triggered by cAMP and thus could possibly affect the production of ALA synthase indirectly.

To test the possibility that the induction of ALA synthase by porphyrogenic agents might be mediated through changes in the intracellular levels of cAMP, a radioimmunoassay was used to measure intracellular cAMP. In preliminary studies, we determined the changes in intracellular cAMP during a period of 15 min following the addition of forskolin, an activator of adenylate cyclase [9], to the culture medium of the hepatocytes. Following the addition of forskolin (40 μM), intracellular cAMP increased rapidly during the first 10 min and remained elevated by the end of 15 min. This established that embryonic chick hepatocytes were capable of responding to a common activator of adenylate cyclase and provided us with a time frame for examining the possible effects of porphyrogenic agents on cAMP. First we examined the effect of AIA on the intracellular levels of cAMP, measured 12 min following the addition of AIA to the culture medium. The results in table 1 indicate that no significant change (or possibly a slight drop) was detected in the cells following the addition of AIA. Next we examined the effect of testosterone, an agent relatively weaker than AIA in its effect on ALA synthase induction (table 1). As seen in table 1, testosterone caused a significant increase in the intracellular content of cAMP within 12 min. Thus, it did not appear that the inductions of ALA synthase by the 2 porphyrogenic agents were the primary results of directed changes in the intracellular concentrations of cAMP since the agents had opposite effects on the intracellular concentrations of the nucleotide. The requirements for exogenous cAMP for the induction of ALA synthase, as reported in some studies [2–4], may have been due to non-specific physiological changes occurring in hepatocytes maintained under different culture conditions and may be of no direct relevance to the mechanism of induction of ALA synthase.

Table 1

Effects of porphyrogenic agents on ALA synthase activity and on cAMP content in primary cultures of hepatocytes

Addition	ALA synthase[a] (nmol/h per mg protein)	cAMP[b] (pmol/mg protein)
None	0.115 ± 0.010 (5)	8.13 ± 2.97 (3)
AIA (75 μg/ml)	2.93 ± 0.72 (6)[c]	5.23 ± 2.00 (3)
Testosterone (5.0 μg/ml)	0.542 ± 0.170 (4)[c]	21.7 ± 3.1 (3)[c]

[a] Primary cultures of hepatocytes were prepared as described in section 2 and incubated for 19 h in either the absence or presence of the indicated agent, being added to the culture medium at the given final concentration. Cells from four 10-cm culture dishes (2–3 mg protein/dish) were pooled and used for each determination

[b] Primary cultures of hepatocytes were prepared and incubated in the serum-free medium for 5 h as described in section 2. The culture medium was then substituted with identical fresh medium either lacking or containing the indicated agent. The cells were incubated for 12 min then processed for cAMP determinations. Two 35-mm culture dishes (0.2 mg protein/dish) were pooled for each determination

[c] Value significantly different from its corresponding control at $P < 0.05$ as determined by the Student's t-test

Numbers in parentheses indicate the number of independent determinations carried out for each condition

ACKNOWLEDGEMENTS

We are grateful to Dr William Higgins for providing us with antiserum against cAMP and to Dr Samuel Edwards for his help in the development of the radioimmunoassay for cAMP. This work was supported by grant GM31672 from the National Institutes of Health.

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