The effect of glucagon-induced adenosine 3′,5′-monophosphate concentrations on bile acid synthesis in isolated rat liver cells

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The effect of increased intracellular adenosine 3′,5′-monophosphate (cAMP) concentrations on bile acid synthesis in isolated rat hepatocytes was investigated. When the cells were incubated in the presence of glucagon (0.2 μM) and theophylline (1 mM) the observed rise in the level of cAMP was accompanied by an increase in bile acid production. Hepatocyte cAMP concentrations after 1 h of incubation showed a highly significant positive linear correlation with the amounts of bile acid synthesised by the cells during this time. These results suggest that bile acid production is related to the concentration of cAMP in isolated hepatocytes and provide evidence for a role for the cyclic nucleotide in the regulation of bile acid synthesis.

Bile acid synthesis *cAMP* *Isolated hepatocyte* *Glucagon* *Rat liver*

1. INTRODUCTION

Recent experiments in our laboratory have shown that bile acid synthesis in isolated hepatocytes is increased in the presence of Bt_2 cAMP [1]. Authors in [2] reported similar effects. There is also some evidence from in vitro studies that the activity of cholesterol 7α-hydroxylase, the rate-limiting enzyme in bile acid synthesis, can be increased by phosphorylation [3,4]. These findings suggest that cAMP may be involved in the regulation of bile acid synthesis. If this is so then a rise in the endogenous levels of cAMP in isolated hepatocytes should lead to increased production of bile acids. Glucagon is known to cause a large elevation of the cAMP content of isolated liver cells [5,6] and this response is increased and prolonged in the presence of the methylxanthine, theophylline [5,7]. We have here used a combination of glucagon and theophylline to investigate the relationship between intracellular cAMP concentrations and bile acid synthesis in isolated rat hepatocytes.

† Professor G.S. Boyd died on 10th January, 1983

Abbreviations: cAMP, adenosine 3′,5′-monophosphate; Bt_2 cAMP, dibutyryl cAMP

2. MATERIALS AND METHODS

2.1. *Chemicals*

Glucagon and collagenase were obtained from Sigma (London). Bile acids were removed from bovine serum albumin fraction V powder (Sigma, London) as in [8]. Theophylline was supplied by Chas. Zimmerman & Co. (Perivale, Middlesex).

2.2. *Cell preparation*

Female rats of the Wistar strain (150–250 g) were maintained on a diet consisting of 70% wholemeal flour, 25% skimmed milk and 5% dried yeast. Animals were allowed access to food and water ad libitum.

Hepatocytes were prepared and incubated as in [8] except that hyaluronidase was omitted from the appropriate perfusion medium and 0.5 mM calcium was added. Cell viability was >85% as determined by trypan blue exclusion. For bile acid determinations duplicate samples from each in-

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cubation were taken at zero time and after 1 h and prepared for radioimmunoassay as in [1]. Samples for estimation of cAMP content were taken at the appropriate times, treated as in [9], and assayed by a method utilising cAMP binding protein supplied in kit form by Boehringer (London).

2.3. *Radioimmunoassay*

Conjugated cholic, chenodeoxycholic and β-muricholic acids were determined using radioimmunoassays [10–12]. The rabbit antiserum used in the β-muricholic acid assay differed from that in [12] in that it maintained 100% cross-reactivity between the tauro- and glycoconjugates. The amount of each individual bile acid synthesised by the hepatocytes was calculated by subtracting the amount found at 0 h from that found after 1 h incubation.

3. RESULTS AND DISCUSSION

In the experiment shown in fig.1 hepatocytes were incubated in the presence and absence of glucagon (0.2 μM) and theophylline (1 mM). The amount of conjugated cholic acid produced by the cells during 1 h incubation was increased significantly when the drugs were present (fig.1A). Conjugated chenodeoxycholic acid synthesis, however, was unchanged (not shown). We have shown that much of the chenodeoxycholic acid produced by hepatocytes in vitro is metabolised to β-muricholic acid [13]. The sum of the amounts of these two bile acids formed by the cells, therefore, represents a truer measure of chenodeoxycholic acid synthesis than the amount of chenodeoxycholic acid alone. The sum showed a significant rise in the presence of glucagon and theophylline (fig.1B). Conjugated cholic, chenodeoxycholic and β-muricholic acids together probably represent about 90% of the bile acid synthesised by isolated hepatocytes [1,13]. The sum of the amounts of these 3 bile acids (referred to as total bile acid synthesis for clarity) was increased significantly when glucagon and theophylline were included in the incubations (fig.1). These results show that glucagon in the presence of theophylline stimulates bile acid synthesis in isolated hepatocytes.

As expected cAMP levels in the cells were elevated when the drugs were present (fig.1D). Previous work has shown that glucagon induces a

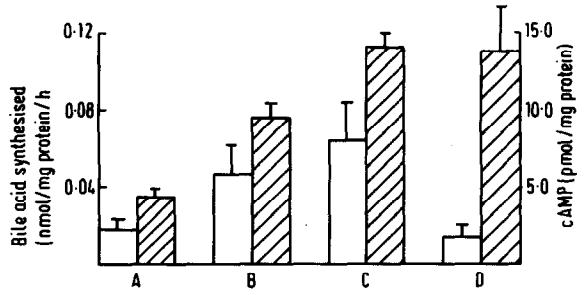

Fig.1. The effect of glucagon and theophylline on bile acid synthesis and cAMP concentrations in isolated hepatocytes. (A) Conjugated cholic acid synthesis; (B) conjugated chenodeoxycholic + β-muricholic acid synthesis; (C) conjugated cholic + chenodeoxycholic + β-muricholic acid (total bile acid) synthesis and (D) cAMP concentrations in isolated hepatocytes incubated in the presence (unshaded bars) and absence (shaded bars) of glucagon (0.2 μM) and theophylline (1 mM). Values given are the mean from 3 separate incubations. Error bars show the SD. The results shown represent a typical experiment from 8 performed. Significance limits (+ glucagon and theophylline *vs* controls): conjugated cholic acid, $p < 0.01$; conjugated chenodeoxycholic + β-muricholic acid, $p < 0.05$; total bile acid, $p < 0.025$; cAMP, $p < 0.0025$.

rapid rise in cAMP concentrations in isolated hepatocytes which reaches a peak after a few minutes [5–7]. The duration of this elevation can be prolonged by increasing the concentration of glucagon and by including theophylline in the incubations [7]. In our experiments, using a concentration of 0.2 μM glucagon and 1 mM theophylline, the total amount of cAMP found in the cells after 1 h incubation was not significantly different from the maximum attained during this time (fig.2). This is consistent with the results of authors in [7], who used 75 nM glucagon and 1 mM theophylline and found that the level of cAMP in isolated hepatocytes had not declined from its peak value after 40 min of incubation. Our results, then, show that the glucagon-induced rise in cAMP concentrations in isolated liver cells is accompanied by a rise in bile acid synthesis. It is possible, however, that the two effects are independent of each other. To test this the data from 8 separate experiments were used to estimate the correlation between cAMP levels and the amounts of bile acid synthesised by the cells in 1 h incubation (fig.3). The concentrations of cAMP showed

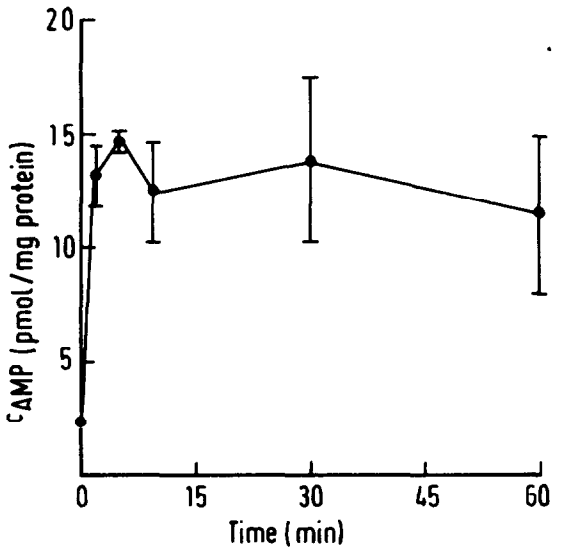

Fig.2. Time course of the effect of glucagon and theophylline on cAMP concentrations in isolated hepatocytes. Triplicate samples were taken from hepatocytes incubated in the presence of glucagon (0.2 μM) and theophylline (1 mM) at the appropriate times and assayed for cAMP. Mean values are shown and error bars indicate the SD. cAMP concentrations in hepatocytes incubated in the absence of glucagon and theophylline did not change significantly from the value found at zero time during 1 h incubation. The results shown represent a typical experiment from 3 performed.

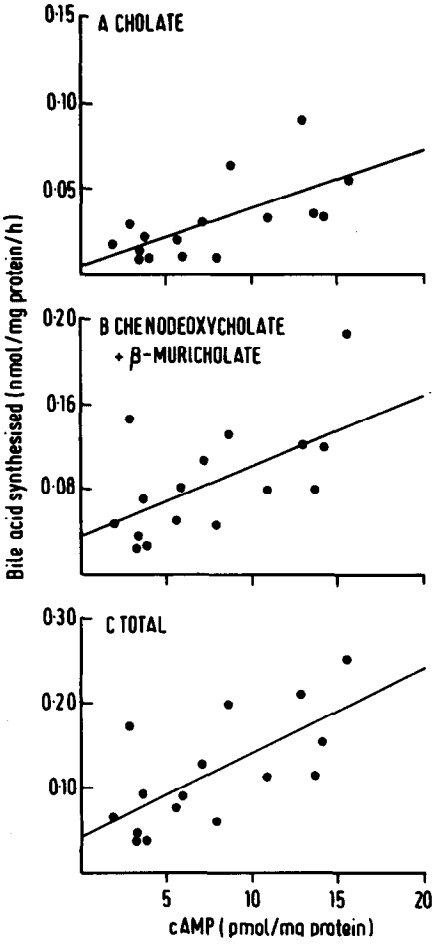

Fig.3. Correlation between cAMP concentrations and bile acid synthesis in isolated hepatocytes. (A) Conjugated cholic acid (Pearsons correlation coefficient $r = 0.65, p < 0.005$); (B) conjugated chenodeoxycholic + β-muricholic acid ($r = 0.62$, $p < 0.01$); (C) conjugated cholic + chenodeoxycholic + β-muricholic acid (total bile acid) ($r = 0.67$, $p < 0.005$). The experiments were performed as described for fig.1 and each point is the mean from 3 incubations. Data from 8 animals were used.

a significant positive linear correlation with conjugated cholic acid synthesis (Pearsons correlation coefficient, $r = 0.65$, $p < 0.005$) conjugated chenodeoxycholic acid + β-muricholic acid synthesis ($r = 0.62$, $p < 0.01$) and total bile acid synthesis ($r = 0.67$, $p < 0.005$). Previous work has shown that the production of bile acids by isolated liver cells is increased in the presence of exogenous Bt_2 cAMP [1,2]. Our findings, therefore, indicate that the amount of bile acid formed is related to the cAMP content of the hepatocytes and provides further evidence for a role for the cyclic nucleotide in the regulation of bile acid synthesis. Recent reports have suggested that the activity of cholesterol 7α-hydroxylase, which is rate-limiting for the bile acid pathway, may be increased when the enzyme is phosphorylated [3,4]. If this were so, then a function for cAMP in stimulating protein kinase activity could be envisaged. A link between the action of cAMP in increasing bile acid synthesis and the phosphorylation of cholesterol 7α-hydroxylase, however, has yet to be established.

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