INTERACTION OF HYDROCORTISONE AND DIBUTYRYL CYCLIC AMP IN THE INDUCTION OF TYROSINE AMINO-TRANSFERASE AND PHOSPHOENOLPYRUVATE CARBOXY-KINASE IN RAT LIVER

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1. Introduction

It has been reported previously (i) that N^6, $O^{2'}$-dibutyryl cyclic adenosine 3′,5′-monophosphate (DBcAMP) as well as hydrocortisone stimulate the de novo synthesis of tyrosine aminotransferase (TAT, EC 2.6.1.5) [1,2] and phosphoenolpyruvate carboxykinase (PEP-CK, EC 4.1.1.32) [3] in adult rat liver, (ii) that the stimulation by DBcAMP of both enzymes was reduced by adrenalectomy [4] and (iii) that combined application of DBcAMP and glucocorticoids produced additive and sometimes synergistic effects in vivo [1] and in organ culture [5,6]. From the latter two findings the suggestion has been made that the system involved in the regulation of the de novo synthesis of these enzymes is rendered more 'sensitive' to cAMP by glucocorticoids. In contrast to this suggestion the present study supports the view that DBcAMP elevates both enzymes by a mechanism, which, in principle, works independently from glucocorticoids while glucocorticoids stimulate a different process, which is required for the maintenance of the cAMP-mediated enzyme induction.

2. Materials and methods

Male Wistar rats, purchased from E. Jautz, Kisslegg/Allgäu, weighing 200 g, were used. *Intact* animals were kept on a low protein diet (Altromin GmbH, Lage/Lippe, C 1004) for 6 days before the experiments. *Adrenalectomized* rats, receiving 0.9% NaCl solution to drink, were fed a standard chow diet for 4 days and, thereafter, a low protein diet for 6 days. In the beginning of the experiments (8.00 a.m.) food was withdrawn. DBcAMP, theophylline, hydrocortisone and NaCl solutions were injected as indicated in the legend of fig. 1. Rats were killed by decapitation and the livers were homogenized in 7 vol of ice-cold 0.15 M KCl in a Potter–Elvejham homogenizer fitted with a Teflon pestle at 1000 rpm for 30 sec. The homogenate was centrifuged for 30 min at 150 000 g at 0°C and PEP-CK activity was immediately assayed in the supernatant according to Seubert and Huth [7]. Enzyme activity (U) is expressed as μmoles oxaloacetate converted to phosphoenolpyruvate per min at 37°C under the conditions of the assay. TAT activity was determined in the supernatant 24 hr later according to Diamandstone [8], using a modification based on the data of Wurtmann and Lavi [9]. One unit of the enzyme activity is defined as the amount of enzyme required to form 1 μmole of p-hydroxyphenylpyruvate per min at 37°C under the conditions of the assay. Protein was assayed by the biuret method [10]. cAMP was estimated according to Gilman [11] after total alkaline hydrolysis of DBcAMP in 0.1 N NaOH at 70°C for 45 min [12]. Glycogen was determined enzymatically, after decomposition of the tissue in 4 N KOH and hydrolysis with 4 N HCl, by the method of Slein [13]. Glucose was measured according to Schmidt [14]. The significance of differences between means was established by the student t test. Substrates, nucleotides and coupling enzymes were purchased from Boehringer, Mannheim. Hydrocortisone (cristal sus-

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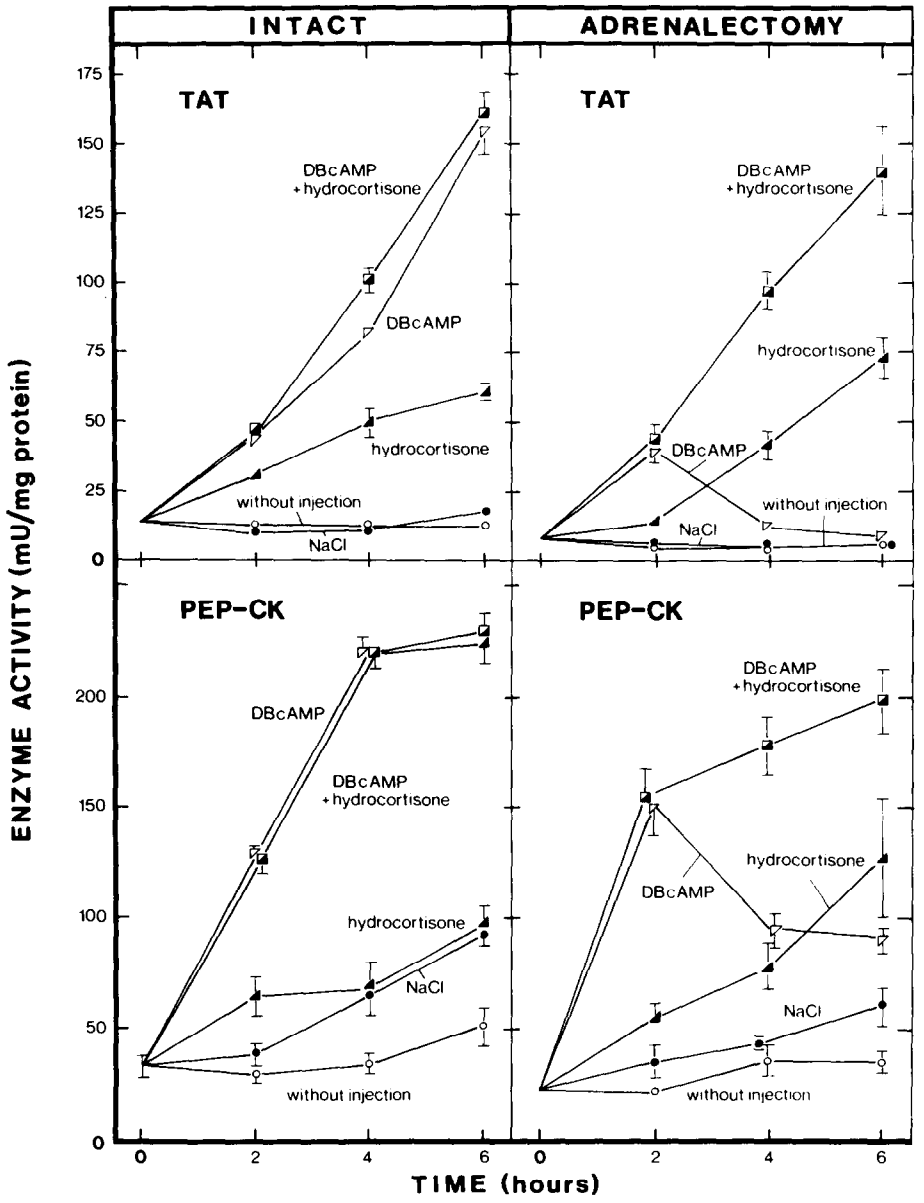

Fig. 1. Time course of effects of dibutyryl cyclic AMP and/or hydrocortisone on hepatic tyrosine aminotransferase (TAT) and PEP carboxykinase (PEP-CK) activities of intact and adrenalectomized rats. DBcAMP (20 mg/kg) + theophylline (20 mg/kg) was injected intraperitoneally at zero time and again every 90 min. Hydrocortisone (25 mg/kg) was injected at zero time followed by further application of 0.9% NaCl every 90 min. Two groups of controls were performed, one without injection, the other injected with 0.9% NaCl every 90 min. For both control groups, $n = 6$; for hydrocortisone, $n = 6–8$; for DBcAMP and DBcAMP + hydrocortisone, $n = 8–11$. Values are given as means ± SEM. SEM was determined for all data points, but in several cases the brackets were too small to be conveniently included in the drawing. Statistical analysis TAT: *Intact:* All hormone or DBcAMP values versus NaCl controls: $p < 0.0005$. *Adrenalectomy:* All hydrocortisone values versus NaCl controls; $p < 0.0005$. DBcAMP, 2,4 and 6 hr versus NaCl controls: $p < 0.0005$, $p < 0.005$ and non significant (= n.s.). Statistical analysis PEP-CK: *Intact:* All DBcAMP and DBcAMP + hydrocortisone values versus NaCl controls: $p < 0.0005$. Hydrocortisone, 2 hr versus NaCl control: $p < 0.025$. NaCl controls, 2, 4 and 6 hr versus 'without injection': n.s., $p < 0.005$ and $p < 0.0025$. *Adrenalectomy:* DBcAMP + hydrocortisone, 2, 4 and 6 hr versus NaCl controls: $p < 0.0005$. DBcAMP, 2, 4 and 6 hr versus NaCl controls: $p < 0.0005$, $p < 0.0005$ and $p < 0.005$. Hydrocortisone, 2, 4 and 6 hr versus NaCl controls: $p < 0.05$, $p < 0.025$ and $p < 0.025$. NaCl controls, 2, 4 and 6 hr versus 'without injection': $p < 0.05$, n.s. and $p < 0.01$.

pension, 25 mg/ml) was obtained from Hoechst AG, Frankfurt/Main-Hoechst.

3. Results

The time courses of effects of DBcAMP, hydrocortisone and DBcAMP + hydrocortisone on hepatic TAT and PEP-CK activities of intact and adrenalectomized rats are shown in fig. 1.

A single injection of hydrocortisone into intact rats fed a low protein diet elevated TAT activity linearly up to 6 hr. About twice this increase occurred when DBcAMP was injected repeatedly every 90 min. The combination of DBcAMP and hydrocortisone produced no greater response at 2 and 6 hr than did DBcAMP alone, but was more effective at 4 hr ($p < 0.0005$).

In adrenalectomized rats hydrocortisone elicited almost the same degree of elevation of TAT activity as in intact animals. Also, DBcAMP initially produced the same respone as in intact rats. However, after 2 hr the increase of TAT due to DBcAMP ceased completely and enzyme level fell at a rate approximating the known rate of degradation of this enzyme ($t\frac{1}{2} \approx 90$ min) [15]. Restoration of hydrocortisone to the adrenalectomized rats completely restored the DBcAMP effect which was observed with intact animals.

Essentially the same results were obtained for PEP-CK in intact and adrenalectomized rats. However, there were some differences to be noted in comparison with TAT. (i) PEP-CK activity increased slightly in the non injected controls. This did not represent the circadian rhythm of the enzyme, which is virtually absent in rats on a protein-free diet [16,17], but was probably caused by the beginning of starvation, as could be concluded from the significant decrease of liver glycogen levels. (ii) The repeated injection of NaCl elicited a considerable elevation of PEP-CK activity. Since enzyme activity rose in adrenalectomized rats as well and since glycogen levels were effectively depleted after NaCl injection, compared with the non injected controls, the phenomenon might be attributed to a catecholamine mediated elevation of endogenous cAMP. (iii) In adrenalectomized animals enzyme activity after DBcAMP injection declined after 2 hr less rapidly than did TAT. The smaller rate of decline might be ascribed to the considerably longer half-life of PEP-CK, which has been estimated as 5–6 hr by Shrago et al. [18].

In our experiments DBcAMP was always injected in combination with theophylline. Moreover, we have determined that the decline of TAT and PEP-CK following DBcAMP injection into adrenalectomized rats was not the result of an enhanced degradation of the cyclic nucleotide. At the end of the experiments the concentration of total cAMP in the blood plasma was approximately the same in DBcAMP injected (2.2 ± 1.5 nmoles/100 ml (n=6) and in DBcAMP + hydrocortisone injected (1.9 ± 1.2 nmoles/100 ml (n=6)) adrenalectomized rats.

4. Discussion

The essential finding of the present study is the unexpected time course of TAT and PEP-CK activity after repeated injections of maximal doses of DBcAMP into adrenalectomized rats: in the early phase of the experiments both enzymes were stimulated to the same degree as in intact animals, but, beyond 2 hr, their activity returned towards control values.

These findings might be discussed in view of the hypothesis of Wicks based on the results of studies on fetal rat liver in organ culture and cultured hepatoma cells (for review see ref. [4,19]): Hydrocortisone promotes an increase in the level of TAT and PEP-CK templates and cAMP enhances the expression of preexisting templates. Applying this concept to our results we suggest that adrenalectomy limits the amount of translatable templates by limiting either its synthesis [20] or by enhancing its degradation [21] and, therefore, DBcAMP could stimulate enzyme induction only until preexisting templates were exhausted.

If so, inhibitors of transcription would be expected to prevent de novo synthesis of TAT and PEP-CK induced by glucocorticoids but to be without effect in the early phase of cAMP-mediated enzyme induction. In fact, this has been reported for cultured Reuber H35 hepatoma cells. Actinomycin D completely blocked the response of PEP-CK to dexamethasone, but did not suppress the early increase of the enzyme after addition of DBcAMP [22]. Similarly, in Reuber hepatoma (H–4–II–E) cells, actinomycin D at a dose sufficient to inhibit RNA synthesis 95%, did not alter the elevation of TAT activity in the presence of DBcAMP [23].

In adrenalectomized rats, the inhibitory effect of actinomycin D on the hydrocortisone-induced stimula-

tion of hepatic TAT [24] and PEP-CK [25] has been confirmed. However, conflicting results have been obtained with DBcAMP in vivo. On one hand actinomicin-D was reported to be without effect on the increase of hepatic TAT provoked by DBcAMP injection into neonatal rats [26], and 5-azacytidine, which is believed to promote the synthesis of defective mRNA, did not inhibit the cAMP-mediated induction of TAT in the liver of adrenalectomized rats [27]. Contrary to this, other workers found pretreatment of adrenalectomized rats with actinomycin D to considerably inhibit [28] or even totally block [29] the increase of TAT after glucagon or DBcAMP injection respectively. At this time, therefore, it cannot be excluded that cAMP may regulate the synthesis of TAT and PEP-CK also at the transcriptional level.

Whatever the exact mechanism of interaction between glucocorticoids and cAMP in hepatic enzyme synthesis might be, two conclusions can be drawn from our results: (1) DBcAMP can induce TAT and PEP-CK by a mechanism, which, in principle, works independently of glucocorticoids. (2) The maintenance of DBcAMP-mediated induction of both enzymes requires a process, which is stimulated by glucocorticoids.

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