INDUCTION OF PHOSPHOENOLPYRUVATE CARBOXYKINASE AND TYROSINE AMINOTRANSFERASE IN THE ISOLATED PERFUSED RAT LIVER: INTERACTION OF DEXAMETHASONE AND DIBUTYRYL CYCLIC AMP

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Received 11 November 1974
Revised version received 17 January 1975

1. Introduction

Recently we have found [1] that injection of N^6, $O^{2'}$-dibutyryl cyclic adenosine 3',5'-monophosphate (DBcAMP) into adrenalectomized rats initially stimulated hepatic phosphoenolpyruvate carboxykinase (PEP-CK, EC 4.1.1.32) and tyrosine aminotransferase (TAT, EC 2.6.1.5) to the same degree as in intact rats. However, despite of repeated DBcAMP administration the raise in the activity of both enzymes ceased after 2 hr, but could be restored when hydrocortisone had been given at zero time. These results suggested that the maintenance of cAMP-mediated induction of PEP-CK and TAT is dependent on the simultaneous presence of glucocorticoids.

Because of the obvious complications associated with whole animal experiments the hypothesis was now re-examined in the isolated perfused rat liver. It could be demonstrated that the interaction between cAMP and glucocorticoids, in fact, occurred as the result of direct participation of the administered agents at the tissue level. Moreover, our in vitro findings support the hypothesis derived from previous in vivo studies [1], that cAMP elevates PEP-CK and TAT by a mechanism which, in principle, works independently from glucocorticoids while glucocorticoids stimulate a different process that is required for the maintenance of cAMP-mediated enzyme induction.

2. Materials and methods

Male Wistar rats, weighing 200 g each, purchased from E. Jautz, Kisslegg/Allgäu (Germany) were used as liver donors. Prior to the experiments *intact* animals, kept on a standard chow diet, were fed ad libitum on a low protein diet (C 1004; Altromin GmbH, Lage/Lippe, Germany) for 3 days. *Adrenalectomized* rats, given 0.9% NaCl solution as drinking water, were fed ad libitum on a standard chow diet for 4 days and, thereafter, on a low protein diet for 3 days.

At the beginning of the perfusion experiments (8.00 a.m.) animals were anaesthetized by intraperitoneal injection of hexobarbital (15 mg/100 g body wt). Livers were isolated and perfused by the technique of Schimassek [2] with a 10% (v/v) emulsion of fluorocarbon 43, made up in the non-ionic detergent Pluronic F 68 by sonication and diluted with a saline buffer. Further details of the preparation of the fluorocarbon medium have been described previously [3]. The perfusate volume was 150 ml containing 135 ml of the fluorocarbon medium, 15 ml of pig plasma and 100 mg of glucose/100 ml.

The plasma was prepared from either intact or adrenalectomized miniature pigs 'Göttingen' (Max-Planck-Institut für Versuchstierzucht, Göttingen, Germany), adrenalectomy being performed 4 days prior to plasma preparation. The pigs were killed by

a striker and exsanguinated by heart puncture. The heparinized blood was centrifuged at 2000 *g* (4°C). Plasma sufficient for all perfusion experiments was pooled from several animals and stored deep-frozen. The absence of glucocorticoids from the plasma of adrenalectomized pigs has been ascertained by the method of Blunck and Willig [4]. In the experiments depicted in figs.1 and 2 plasma obtained from intact pigs was employed for the perfusion of livers from intact rats, and plasma obtained from adrenalectomized pigs was employed for the perfusion of livers from adrenalectomized rats.

After 15 min of perfusion one liver lobe was ligated at its base and excised. Then DBcAMP and/or dexamethasone was added to the reservoir at zero time to give the final concentrations indicated in the legend to fig.1. At 2, 4 and 6 hr further liver lobes were removed.

Tissue was immediately homogenized in 7 vol of an ice cold 0.15 M KCl solution in a glass Potter-Elvehjem homogenizer with a Teflon pestle at 1000 rev/min for 30 s. The homogenate was centrifuged at 150 000 *g* for 30 min at 0°C and PEP-CK activity was immediately assayed in the supernatant by the method of Seubert and Huth [5]. Enzyme activity (U) is expressed as μmol 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 [6] using a modification based on the results of Wurtman and Larin [7]. One unit of enzyme activity is defined as the amount of enzyme required to form

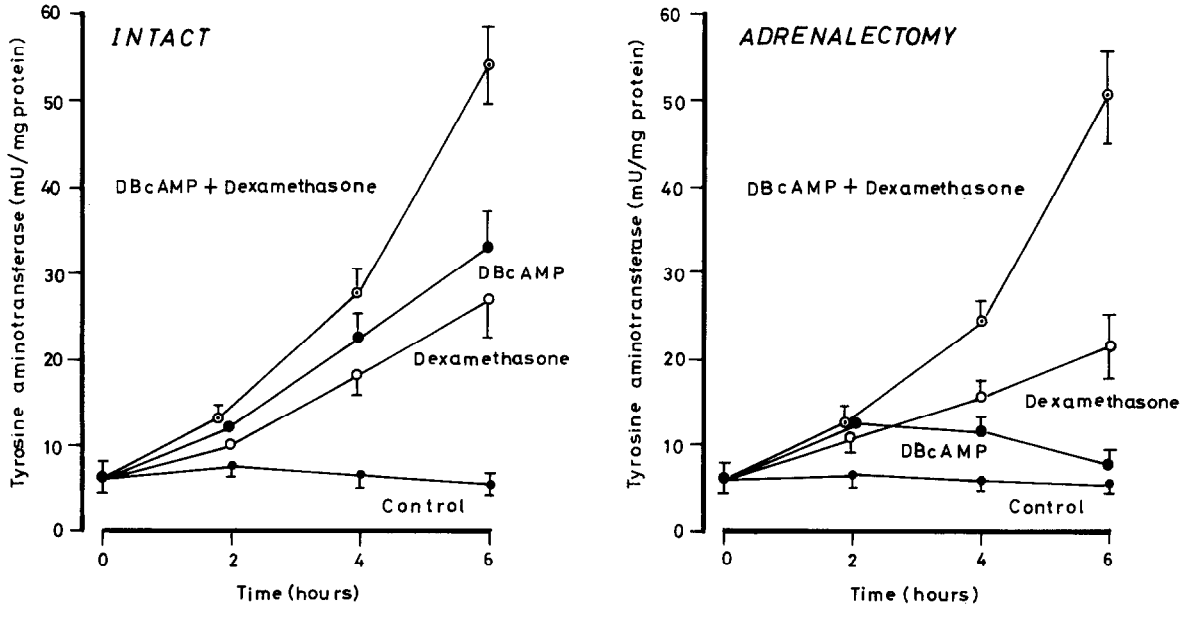

Fig.1. Time course of effects of dibutyryl cyclic AMP and/or dexamethasone on hepatic phosphoenolpyruvate carboxykinase activity in the isolated perfused liver of intact and adrenalectomized rats. Perfusion was performed as described under 'Materials and methods'. Dibutyryl cyclic AMP (DBcAMP, 0.2 mM) and/or dexamethasone (50 μM) was added to the medium in the reservoir at zero time. For control, n = 5; for dexamethasone, n = 4; for DBcAMP, n = 7; for DBcAMP + dexamethasone, n = 7. 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: Intact: Dexamethasone values, 2, 4 and 6 hr versus control: non significant (= n.s.), $p < 0.0005$ and $p < 0.0025$. DBcAMP values, 2, 4 and 6 hr versus control: $p < 0.0025$, $p < 0.0005$ and $p < 0.0005$. DBcAMP + dexamethasone values, 2, 4 and 6 hr versus control: $p < 0.0005$. DBcAMP + dexamethasone values, 2, 4 and 6 hr versus DBcAMP: n.s., n.s. and $p < 0.005$. Adrenalectomy: Dexamethasone values, 2, 4 and 6 hr versus control: n.s., n.s. and p 0.025. DBcAMP values, 2, 4 and 6 hr versus control: $p < 0.005$, $p < 0.0025$ and $p < 0.0025$. DBcAMP + dexamethasone values 2, 4 and 6 hr versus control: $p < 0.025$, $p < 0.0025$ and $p < 0.005$. DBcAMP + dexamethasone values, 2, 4 and 6 hr versus DBcAMP: n.s., $p < 0.0005$ and $p < 0.0005$.

1 μmol of p-hydroxyphenylpyruvate per min at 37°C under the conditions of the assay. Protein was measured by the biuret method [8]. The significance of differences between means was established by Student's t test. Substrates, nucleotides (including DBcAMP) and test enzymes were purchased from C. F. Boehringer and Soehne GmbH (Mannheim, Germany). Dexamethasone (Fortecortin) was obtained from Hoechst AG (Frankfurt/Main-Hoechst, Germany).

3. Results

In livers from adrenalectomized rats stimulation of PEPCK and TAT due to dexamethasone or dexamethasone + DBcAMP revealed identical results when plasma from either adrenalectomized or intact pigs was employed for the perfusion experiments. This independence from the choice of plasma donors was also true for the perfusion of livers from intact rats.

Figs.1 and 2 present the effects of dexamethasone, DBcAMP, and dexamethasone + DBcAMP on the activities of both enzymes, which were studied in the isolated liver of intact or adrenalectomized rats perfused with a fluorocarbon medium containing plasma from intact or adrenalectomized pigs, respectively. In livers taken from *intact rats* addition of dexamethasone (50 μM) to the perfusion medium significantly raised PEP-CK activity up to 142% after 6 hr (fig.1). DBcAMP at a concentration of 0.2 mM provoked a more rapid and more effective elevation in enzyme activity which continued to increase linearly throughout the duration of the experiment. Combination of dexamethasone and DBcAMP produced no greater response than did DBcAMP alone. In livers taken from *adrenalectomized rats* dexamethasone elicited the same degree of elevation in PEP-CK activity as in livers from intact rats. Also, adrenalectomy of the liver donors did not reduce the response of the enzyme to DBcAMP during the first 2 hr of

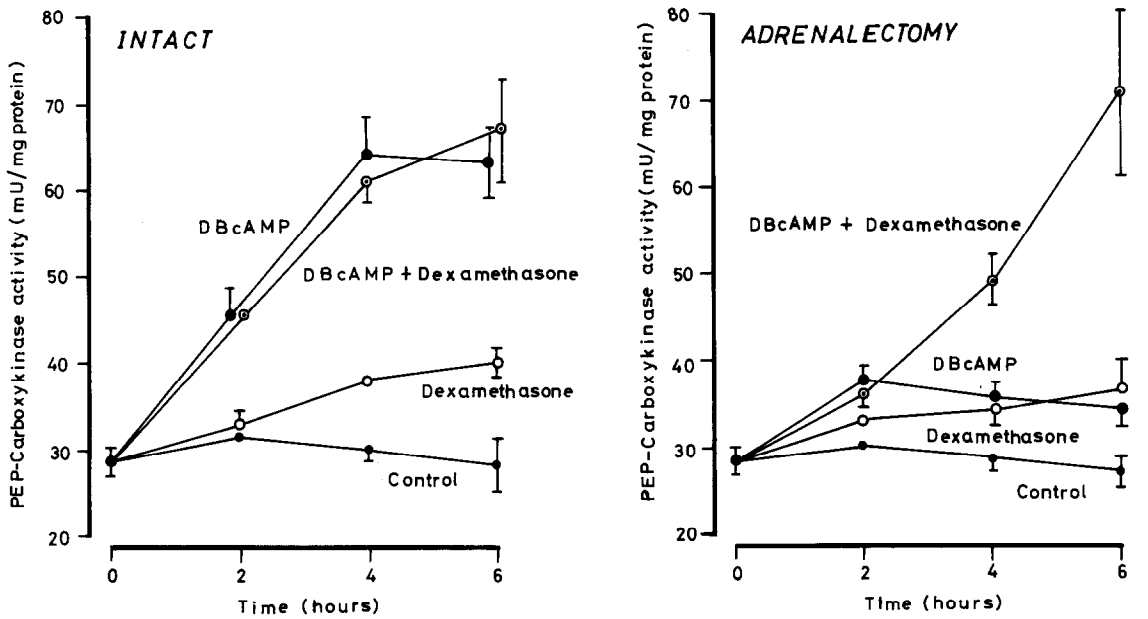

Fig.2. Time course of effects of dibutyryl cyclic AMP and/or dexamethasone on hepatic tyrosine aminotransferase activity in the isolated perfused liver of intact and adrenalectomized rats. For further details see legend to fig.1. Values are given as means ± SEM. Statistical analysis: Intact: Dexamethasone values 2, 4 and 6 hr versus control: n.s., $p < 0.0005$ and $p < 0.0025$. DBcAMP values, 2, 4 and 6 hr versus control: $p < 0.0025$, $p < 0.0005$ and $p < 0.0005$. DBcAMP + dexamethasone values, 2, 4 and 6 hr versus control: $p < 0.05$, $p < 0.0005$ and $p < 0.0005$. DBcAMP + dexamethasone values, 2, 4 and 6 hr versus DBcAMP: n.s. Adrenalectomy: Dexamethasone values, 2, 4 and 6 hr versus control: $p < 0.05$, $p < 0.005$ and $p < 0.005$. DBcAMP values, 2, 4 and 6 hr versus control: $p < 0.025$, $p < 0.0025$ and n.s. DBcAMP + dexamethasone values, 2, 4 and 6 hr versus control: $p < 0.0125$, $p < 0.0005$ and $p < 0.0005$. DBcAMP + dexamethasone values, 2, 4 and 6 hr versus DBcAMP: n.s., $p < 0.005$ and $p < 0.01$.

perfusion. However, after 2 hr the further increase of PEP-CK activity ceased, but replacement of dexamethasone in vitro at zero time restored the DBcAMP effect that was observed with livers from intact rats.

Essentially the same results were obtained for TAT (fig.2). However, there were two differences interesting to note in comparison to PEP-CK: (i) The stimulation of TAT brought about by dexamethasone was more pronounced in both the livers of intact and of adrenalectomized rats. (ii) In intact animals combination of dexamethasone and DBcAMP revealed an additive effect on enzyme activity.

4. Discussion

cAMP and glucocorticoids induce PEP-CK and TAT in several systems: whole animals, perfused liver, fetal liver in organ culture and cultured hepatoma cells (for review see [9]). Recently we have demonstrated an interaction of both agents in the induction of liver PEP-CK and TAT in vivo by investigating the time course of enzyme activities after administration of DBcAMP and/or hydrocortisone into intact or adrenalectomized rats [1]. In the present study the same concept was applied to the isolated perfused liver.

The essential finding of the experiments presented in this paper is the striking similarity between the results obtained with the isolated perfused liver and those obtained previously with whole animals [1]. (i) In the liver of intact rats PEP-CK and TAT activity was elevated by either DBcAMP or dexamethasone. (ii) The elevation provoked by DBcAMP initially occurred to the same degree in the liver of adrenalectomized animals as well. (iii) However, the maintenance of DBcAMP-mediated stimulation of PEP-CK and TAT in the liver of adrenalectomized rats required the simultaneous presence of glucocorticoids. These findings indicate that the interaction of cAMP and glucocorticoids in the induction of PEP-CK and TAT occurs, in fact, at the tissue level. Moreover, they confirm our preliminary conclusions drawn from the in vivo study that cAMP 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.

At present the mode of interaction between cAMP and glucocorticoids in PEP-CK and TAT synthesis is not clear. An attractive hypothesis consistent with our results has been derived from studies on fetal rat liver in organ culture and cultured hepatoma cells [10]: Glucocorticoids promote an increase in the level of PEP-CK and TAT templates while cAMP stimulates the translation of the preexisting templates.

This hypothesis is in accordance with the results obtained by others in vitro: In Reuber hepatoma cells actinomycin D completely blocked the response of PEP-CK to dexamethasone but did not suppress the early increase of PEP-CK [11] and TAT [12] activity provoked by the addition of DBcAMP. However, conflicting results have been obtained in vivo: as expected from this hypothesis actinomycin D inhibited the hydrocortisone-induced stimulation of hepatic PEP-CK [13] and TAT [14] in the liver of adrenalectomized rats. Also, despite of pretreatment of the animals with actinomycin D or 5-azacytidine, hepatic TAT was found to be stimulated by DBcAMP injection into neonatal [15] and adult [16] rats, respectively. In contradiction to this hypothesis actinomycin D was reported to greatly inhibit [17] or totally block [18] the increase of TAT after glucagon or DBcAMP administration, respectively.

Thus, the exact nature of the interaction between cAMP and glucocorticoids in the regulation of hepatic PEP-CK and TAT remains to be established.

Acknowledgements

We are indebted to Dr H. Dahlke, director of the department of experimental surgery (Ethicon GmbH, Hamburg) for performing adrenalectomy on pigs, and Professor Dr W. P. Blunck, Universitäts-Kinderklinik Hamburg-Eppendorf, for plasma glucocorticoid determination. The skilful technical assistance of Miss B. Dunkelmann and Miss D. Luda is gratefully acknowledged. This work was supported by Deutsche Forschungsgemeinschaft, Sonderforschungsbereich, 34, Endokrinologie.

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