Short Communications

Stimulation by 6-*N*,2'-*O*-Dibutyryladenine 3':5'-Cyclic Monophosphate and Glucocorticoids of Phosphoenolpyruvate Carboxykinase in the Isolated Perfused Rat Liver

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Dibutyryl cyclic AMP stimulated the activity of phosphoenolpyruvate carboxykinase in perfused livers of rats, fed on a low-protein diet, linearly over a 6 h period. The enzyme activity was also significantly elevated by dexamethasone, the effect being considerably lower than that of the cyclic nucleotide. Since the time-course of phosphoenolpyruvate carboxykinase activity in response to dibutyryl cyclic AMP resembled that observed after dibutyryl cyclic AMP injection into intact animals, it is suggested that induction of the enzyme *in vivo* is due to a direct action of the cyclic nucleotide on the liver. Combined administration of dibutyryl cyclic AMP and glucocorticoids did not lead to an additive increase of liver phosphoenolpyruvate carboxykinase activity, either *in vivo* or in the perfused organ.

Phosphoenolpyruvate carboxykinase (EC 4.1.1.32), which is generally assumed to be the rate-limiting enzyme of gluconeogenesis in the liver (for review see Exton, 1972), can be induced by injection of dibutyryl cyclic AMP into intact rats (Wicks *et al.*, 1969). However, until now the induction of phosphoenolpyruvate carboxykinase by the cyclic nucleotide has not been demonstrated in the isolated liver. Menahan & Wieland (1969) reported no alteration of phosphoenolpyruvate carboxykinase activity at the end of an 80 min perfusion period after the addition of glucagon to the perfusate. In contrast with that, the present work demonstrates a linear and effective stimulation of phosphoenolpyruvate carboxykinase in the isolated perfused rat liver by dibutyryl cyclic AMP and a slight increase after the addition of dexamethasone.

Materials and methods

Intact male Wistar rats (weighing 200 g each; purchased from E. Jautz, Kisslegg/Allgäu, Germany) were fed *ad libitum* on a low-protein diet (C1004; Altromin G.m.b.H., Lage/Lippe, Germany) for 3 days.

At the beginning of the experiments on induction of the enzyme *in vivo* (8:00 a.m.) food was withdrawn. Dibutyryl cyclic AMP, cortisol or 0.9% NaCl was administered as indicated in the legend to Fig. 1. Rats were killed by decapitation and their livers were prepared for enzyme determination as described below.

For perfusion experiments animals were anaesthetized by intraperitoneal injection of hexobarbital

(15 mg/100 g body wt.). Livers were isolated and perfused by the technique of Schimassek (1963), with a 10% (v/v) emulsion of fluorocarbon 43 made up in the non-ionic detergent Pluronic F68 by sonication and diluted with Tyrode buffer. For further details of the preparation of the medium see Krone *et al.* (1974). The perfusate volume was 150 ml, containing 120 ml of the fluorocarbon medium, 30 ml of plasma (prepared by centrifugation at 2000 g of heparinized blood from pigs that had been kept without food for 12 h) and 100 mg of glucose/100 ml. The pig plasma was collected from several animals, pooled and stored deep-frozen. After 15 min equilibration one liver lobe was ligated at its base and excised. Then dibutyryl cyclic AMP 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 h further liver lobes were removed.

Liver tissue was immediately homogenized in 7 vol. of 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 phosphoenolpyruvate carboxykinase activity was immediately assayed in the supernatant by the method of Seubert & Huth (1965). Enzyme activity units are expressed as μ mol of oxaloacetate converted into phosphoenolpyruvate/min at 37°C. Protein was assayed by the biuret method (Layne, 1957). The significance of differences between means was established by Student's *t* test. Substrates, nucleotides and coupling enzymes were purchased from C. F. Boehringer und Soehne G.m.b.H. (Mannheim, Germany). Cortisol (hydro-

Fig. 1. Effects of dibutyryl cyclic AMP and glucocorticoids on rat liver phosphoenolpyruvate carboxykinase activity (a) *in vivo* and (b) during perfusion of the isolated organ

(a) Dibutyryl cyclic AMP (20 mg/kg) + theophylline (20 mg/kg) was injected intraperitoneally into intact male rats, fed on a low-protein diet for 3 days, at zero time and applied further every 90 min. Cortisol was injected at zero time followed by further injection of 0.9% NaCl every 90 min. Two groups of controls were performed, the one without injection, the other injected with 0.9% NaCl every 90 min. For both control groups and the cortisol-treated group, $n = 6$; for the groups treated with dibutyryl cyclic AMP and with dibutyryl cyclic AMP + cortisol, $n = 8-11$. At 2 h the value for the cortisol-treated group was significantly elevated above that of the NaCl control ($P < 0.025$), as were all values for the groups treated with dibutyryl cyclic AMP and with dibutyryl cyclic AMP + cortisol at 2, 4 and 6 h ($P < 0.001$). Key: ○, control, without injection; ●, control, NaCl; ◻, dibutyryl cyclic AMP; ▲, cortisol; ◼, dibutyryl cyclic AMP + cortisol. Values are given as means $\pm$ S.E.M.

(b) Intact male rats, fed on a low-protein diet for 3 days, were used as liver donors. Perfusion was performed as described under 'Materials and methods'. Dibutyryl cyclic AMP (0.2 mM) and/or dexamethasone (33 μ M) was added to the medium in the reservoir at zero time. For each condition, $n = 6$. With the exception of the value for the dexamethasone-treated group at 2 h all values for the groups treated with glucocorticoid or dibutyryl cyclic AMP were significantly elevated above the control values ($P < 0.001$). Key: ○, control; ◻, dibutyryl cyclic AMP; ▲, dexamethasone; ◼, dibutyryl cyclic AMP + dexamethasone. Values are given as means $\pm$ S.E.M.

cortisone; crystal suspension) and dexamethasone (Fortecortin) were obtained from Hoechst AG (Frankfurt/Main-Hoechst, Germany).

Results

Injection of dibutyryl cyclic AMP (+theophylline) into intact rats, fed on a low-protein diet for 3 days, led to a rapid increase of phosphoenolpyruvate carboxykinase activity that was linear up to 4 h, reaching

a value of 223 munits/mg of protein after 6 h (Fig. 1a). Cortisol injection also raised phosphoenolpyruvate carboxykinase activity significantly in 2 h but had no further effect during the following 4 h, compared with the NaCl-injected controls. When dibutyryl cyclic AMP (+theophylline) and cortisol were injected together the stimulation of phosphoenolpyruvate carboxykinase activity did not exceed the values obtained with dibutyryl cyclic AMP (+theophylline)

alone. An analogous time-course of phosphoenolpyruvate carboxykinase activity in response to dibutyryl cyclic AMP, dexamethasone and dibutyryl cyclic AMP+dexamethasone was observed in isolated perfused livers from intact rats fed on a low-protein diet as described above (Fig. 1b). However, the absolute increase was considerably lower than that observed *in vivo*. Similar results have been obtained with glucagon instead of dibutyryl cyclic AMP.

Discussion

The rapid increase of phosphoenolpyruvate carboxykinase activity after dibutyryl cyclic AMP injection into intact rats fed on a low-protein diet confirms the similar data reported by Wicks *et al.* (1972). The increase represents synthesis of the enzyme *de novo* (Wicks *et al.*, 1972). The induction of phosphoenolpyruvate carboxykinase by dibutyryl cyclic AMP was also demonstrated in organ cultures of foetal rat liver (Wicks, 1969) and Reuber H35 hepatoma cells (Barnett & Wicks, 1971). However, this direct effect of the cyclic nucleotide on liver target cells was not found for the adult liver by Menahan & Wieland (1969), who reported no significant increase by glucagon of phosphoenolpyruvate carboxykinase activity after 80 min of perfusion. Differing from that negative finding, our experiments demonstrate a direct response of phosphoenolpyruvate carboxykinase to dibutyryl cyclic AMP in the isolated livers of adult rats, which was highly significant as early as 2 h after the addition of the nucleotide. Since the time-course of phosphoenolpyruvate carboxykinase increase in the isolated organ resembles that in the intact animal, it may be assumed that induction of the enzyme *in vivo* by dibutyryl cyclic AMP is due, at least partly, to a direct action of the nucleotide. The only slight increase of phosphoenolpyruvate carboxykinase activity after cortisol injection into adult rats is in accordance with results obtained with intact animals fed normally (Foster *et al.*, 1966) or on a

protein-free diet (Wicks *et al.*, 1969). A similar weak response of the enzyme to glucocorticoids compared with that to dibutyryl cyclic AMP was observed by us in the isolated perfused liver. When dibutyryl cyclic AMP and cortisol were injected simultaneously into intact animals, no addition of the cortisol and dibutyryl cyclic AMP effects was detected. Since it is known that cyclic AMP, as the second messenger of adrenocorticotrophin, releases glucocorticoids from the adrenal cortex, this result does not exclude the possibility that an elevation of cortisol concentration in the blood enhances the effect of the cyclic nucleotide. However, this possibility is not supported by our perfusion experiments: combined administration of dexamethasone and dibutyryl cyclic AMP leads to no addition of the glucocorticoid effect and that of the nucleotide on phosphoenolpyruvate carboxykinase activity.

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