PHYSIOLOGICAL REGULATION OF RAT LIVER PHOSPHOENOLPYRUVATE CARBOXYKINASE (GTP) BY INSULIN
INSIGNIFICANCE OF A CYCLIC AMP-INDEPENDENT MECHANISM

By

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ABSTRACT

The effect of re-feeding glucose, protein or fat and the effect of insulin injection on the activity of hepatic phosphoenolpyruvate carboxykinase (GTP: oxaloacetate carboxy-lyase (transphosphorylating) EC 4.1.1.32), the concentration of hepatic cyclic AMP and the level of serum insulin was investigated in starved rats.

Under all conditions examined the concentration of serum insulin was elevated to a high degree. However, only rats re-fed with glucose responded to the increase in serum insulin with a decrease in PEP carboxykinase activity, while the activity of the enzyme remained unchanged or was elevated after re-feeding protein or fat or after insulin injection, respectively.

Since under all conditions there was a close correlation between cyclic AMP concentration and PEP carboxykinase activity, but not between the insulin level and enzyme activity, it is concluded that the hormone physiologically regulates PEP carboxykinase activity by decreasing the intrahepatic cyclic AMP concentration rather than by the postulated cyclic AMP-independent inhibition of specific mRNA translation.

1) Abbreviations used are: cyclic AMP, adenosine 3',5'-monophosphate; PEP, phosphoenolpyruvate; dibutyryl cyclic AMP, N6, O2'-dibutyryl adenosine 3',5'-monophosphate.
It has been shown in several rat liver systems that cyclic AMP\(^1\) increases the activity of phosphoenolpyruvate carboxykinase (GTP: oxaloacetate carboxylase, transphosphorylating, EC 4.1.1.32) by enhancing the synthesis rate of the enzyme (for review see Wicks 1974). In contrast to that, insulin, when administered into alloxan diabetic rats, causes a rapid decline in PEP carboxykinase activity (Shrago et al. 1968) resulting from a suppression of enzyme synthesis (Tilghman et al. 1974). The antagonistic interaction between cyclic AMP and insulin has been visualized in cultured Reuber H-35 hepatoma cells: The increase in the synthesis of PEP carboxykinase produced by dibutyryl cyclic AMP was effectively inhibited by insulin depending on the concentration of the hormone (Gunn et al. 1975).

The mechanism whereby insulin suppresses the synthesis of rat liver PEP carboxykinase is still uncertain. On the one hand it has been shown that the hormone (i) reduces the elevated level of hepatic cyclic AMP when injected into alloxan diabetic rats (Jefferson et al. 1968; Park et al. 1972), (ii) lowers hepatic cyclic AMP concentration in the isolated perfused liver of normal rats (Jefferson et al. 1968), and (iii) antagonizes the glucagon-mediated elevation of cyclic AMP in the isolated perfused rat liver (Jefferson et al. 1968; Exton et al. 1970, 1971) and in isolated rat hepatocytes (Pilkis et al. 1975). Considered as a whole these results have led to the suggestion that insulin inhibits the synthesis of hepatic PEP carboxykinase by lowering the intrahepatic concentration of the established inducer of the enzyme, cyclic AMP.

On the other hand more recent reports argue that insulin affects PEP carboxykinase synthesis without altering the level of cyclic AMP in the liver. The latter hypothesis is particularly supported by the demonstration that the hormone – at a concentration of \(10^{-10}\) M – is capable of completely suppressing the effect of dibutyryl cyclic AMP on PEP carboxykinase induction in Reuber H-35 hepatoma cells although the cyclic nucleotide was offered at a concentration as high as 0.4 mm (Barnett & Wicks 1971). Under these conditions probably no considerable fall in the intracellular level of cyclic AMP occurred as can be deduced from the observation that in the same experiment insulin and dibutyryl cyclic AMP, each inducing tyrosine aminotransferase, produced an additive effect on this enzyme. Moreover, in dibutyryl cyclic AMP-induced Reuber H-35 cells deinduction of PEP carboxykinase by insulin was not impailed by the addition of the inhibitor of cyclic AMP phosphodiesterase, theophylline (Tilghman et al. 1975).

In the present study it was examined whether the postulated cyclic AMP-independent inhibition of PEP carboxykinase mRNA translation by insulin does significantly contribute to the regulation of enzyme activity under appropriate in vivo conditions. Our results argue against this assumption, yet demonstrate a close correlation between hepatic cyclic AMP concentration and PEP carboxykinase activity.

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MATERIALS AND METHODS

Male Wistar rats, weighing 190–210 g, purchased from E. Jautz, Kisslegg, Allgäu, FRG, were kept at 23°C in a room illuminated from 6:00 a.m. to 6:00 p.m. All animals were fed ad libitum on a standard chow diet, starved for 48 h and then treated as described in the legend to Fig. 1.

For the determination of hormones and glucose from the blood and of PEP carboxykinase activity in the liver rats were killed by decapitation. Serum insulin was measured according to Meade & Klitgaard (1962), serum glucagon according to Faloona & Unger (1974), blood glucose according to Schmidt (1961). Hepatic PEP carboxykinase was assayed by the method of Seubert & Huth (1965), enzyme activity (U) being expressed as μmoles oxaloacetate converted to PEP per min at 37°C under the conditions of the assay. Hepatic cyclic AMP was determined according to Gilman (1970) after killing the animals by the double hatchet method (Faupel et al. 1972). All determination procedures have been described in detail previously (Seitz et al. 1976).

The concentrations of hepatic cyclic AMP and the activity of PEP carboxykinase have been tested for alterations by the method of analysis of variance (oneway layout). In addition, test for trend has been performed and the data have been examined for homogeneity of the variances using Barlett’s test.

Substrates, cosubstrates, nucleotides and coupling enzymes were obtained from C. F. Boehringer/Söhne, Mannheim, FRG. Insulin was purchased from Hoechst AG, Frankfurt/Main-Hoechst, FRG.

RESULTS

The effect of re-feeding glucose, protein or fat and the effect of a single insulin injection on hepatic PEP carboxykinase activity, hepatic cyclic AMP concentration, serum insulin concentration and blood glucose level of 48 h starved rats are shown in Fig. 1.

When the animals were re-fed with glucose there was a sharp increase in the blood glucose level and in serum insulin concentration within 20 min. Under this condition the concentration of hepatic cyclic AMP dramatically decreased, reaching minimum values after 2 h (Fig. 1, A), while the activity of hepatic PEP carboxykinase coordinately fell at a rate approximating the known half-life of the enzyme of about 6 h (Hopgood et al. 1973).

Re-feeding starved rats with casein resulted in an only slight elevation of the blood glucose level, but provoked the same response of serum insulin as re-feeding glucose (Fig. 1, B). In these animals both the concentration of hepatic cyclic AMP (after a slight decline within the first hour) and the activity of PEP carboxykinase remained almost unchanged throughout the experiment.

When the rats were re-fed with bacon fat the blood glucose level increased slightly during the first hour, yet returned to normal thereafter (Fig. 1, C). Initially, the response of serum insulin was less pronounced, but within 2 to 4 h approximately the same hormone concentration was reached as compared
Fig. 1.
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Fig. 2.
Effect of insulin injection on serum glucagon concentration of 48 h starved rats. For further details see legend to Fig. 1, D. Values are given as means ± SEM; n = 5–7.

Fig. 1.
Effect of re-feeding glucose (A), protein (B), triglycerides (C) or insulin injection (D) on hepatic phosphoenolpyruvate carboxykinase activity, hepatic cyclic AMP concentration, serum insulin concentration and blood glucose level of 48 h starved rats. In the re-feeding experiments animals were re-fed ad libitum with purified glucose, casein or bacon fat. Insulin-treated animals were injected intramuscularly with bovine insulin (Alt-Insulin-Hoechst, chromatographically purified, 1 unit/100 g body wt.) once at zero time (●—●). Controls were administered 0.9 % NaCl (○——○). At the various intervals indicated the rats were killed and the assays were performed as described under “Materials and Methods”. Values are given as means ± SEM; n = 5–6.

Statistical analysis:
PEP carboxykinase activity: re-feeding glucose: P < 0.0001; re-feeding protein: n.s.; re-feeding fat: n.s. and insulin injection: P < 0.0001.

Cyclic AMP: re-feeding glucose: P < 0.0001; re-feeding protein: the temporary slight decrease (P < 0.002) at 40 and 60 min is followed by a return to the initial values at 2 and 4 h; re-feeding fat: n.s. and insulin injection: P < 0.0005.
with re-feeding glucose or protein. Throughout the whole experiment there was no marked change either in the concentration of hepatic cyclic AMP or in PEP carboxykinase activity.

Insulin injection into starved rats once at zero time resulted in a maximum elevation in serum insulin concentration followed by strong hypoglycaemia (Fig. 1, D). At the same time the serum glucagon level was increased maximally (Fig. 2) while the concentration of hepatic cyclic AMP as well as the activity of PEP carboxykinase were coordinately elevated (Fig. 1, D).

DISCUSSION

As delineated in the introduction it is well established that insulin decreases PEP carboxykinase activity in the liver by decreasing the concentration of the potent inducer of the enzyme, cyclic AMP. In addition, it has recently been postulated that the hormone inhibits the translation of specific mRNA by a cyclic AMP-independent mechanism (Wicks et al. 1974). It appears, however, that the latter hypothesis is inconsistent with our present results obtained in vivo after systematically elevating insulin levels in the blood at unchanged, decreasing or increasing hepatic cyclic AMP concentrations, respectively.

As can be seen from Fig. 1, B and C, neither re-feeding protein nor re-feeding triglycerides caused a considerable change in hepatic cyclic AMP concentration, probably as a consequence of the unchanged insulin/glucagon ratio in the blood (Müller et al. 1970; Böttger et al. 1973). Provided that insulin affected PEP carboxykinase synthesis at the level of translation independently of cyclic AMP one would expect that in both experiments the highly elevated insulin level should result in a decrease in enzyme activity. However, no decline in PEP carboxykinase activity was observed (comp. Fig. 1, B and C).

While in the case of re-feeding triglycerides this line of argument against a cyclic AMP-independent effect of insulin appears to be conclusive, it is less stringent in the case of re-feeding protein. As has been demonstrated previously by Foster et al. (1966) administration of L-tryptophan effectively increased the already elevated levels of PEP carboxykinase in the liver of fasted rats, probably due to a retardation of enzyme degradation (Ballard & Hopgood 1973). Accordingly, the postulated cyclic AMP-independent repressive effect of insulin on PEP carboxykinase synthesis might have been compensated for by the opposing effect of the tryptophan in the protein diet. It is, however, doubtful whether the tryptophan content in the casein diet (1.9 °/o) at a disappearance rate of the food from the stomach of 0.5 g casein/h (Itoh et al. 1974) is sufficient to elicit a significant effect of this amino acid on hepatic PEP carboxykinase.
Moreover, if a decrease in the degradation rate of PEP carboxykinase, which has been demonstrated in rats injected with large doses of tryptophan, actually prevented an insulin-mediated decline in enzyme activity after re-feeding protein, it would be difficult to understand that in rats fed a protein-rich diet PEP carboxykinase activity—in the course of the circadian rhythm of the enzyme—declines with the known half-life of the enzyme protein although the animals still continue eating (Lane & Mavrides 1970).

A cyclic AMP-independent inhibition by insulin of PEP carboxykinase mRNA translation is also unlikely from the results depicted in Fig. 1, D: Injection of insulin into starved rats caused a severe hypoglycaemia and, consequently, a dramatic release of glucagon from the pancreas (comp. Fig. 2), resulting in a considerable elevation of cyclic AMP concentration in the liver. In close correlation to the hepatic cyclic AMP level, PEP carboxykinase activity was increased to maximum values despite the pharmacological concentrations of insulin in the blood. This in vivo result contrasts with the observation by Barnett & Wicks (1971) that insulin totally prevented the increase in PEP carboxykinase activity provoked by dibutyryl cyclic AMP in cultured Reuber H-35 hepatoma cells.

Tilghman et al. (1974) have recently suggested that the suppressive effect of insulin on rat liver PEP carboxykinase synthesis might require the simultaneous presence of a sufficiently high concentration of glucose. This hypothesis has been particularly deduced from the observation that insulin injection into either fasted normal or fasted diabetic rats was ineffective in reducing the synthesis rate of the enzyme. Accordingly, in our experiments solely re-feeding glucose but nor re-feeding protein or fat nor insulin injection, should have resulted in a decline in PEP carboxykinase activity, since only glucose re-feeding led to a hyperglycaemia sufficient to enable the manifestation of the insulin effect on enzyme synthesis (comp. Fig. 1, A). However, such an interpretation of our results is incompatible with two recent findings: In cultured Reuber H-35 cells insulin was found to be equally effective in lowering the synthesis of PEP carboxykinase in the presence or in the absence of glucose (Tilghman et al. 1975). Furthermore, in the isolated perfused liver of fasted rats the hormone produced a decline in PEP carboxykinase activity at glucose levels as low as 50 mg/100 ml (Wimhurst et al. 1974).

Considering the fact that under all conditions examined in this study there was a strong correlation between the concentration of hepatic cyclic AMP and PEP carboxykinase activity, but not between the serum insulin level and enzyme activity, it appears that insulin physiologically regulates PEP carboxykinase activity by decreasing the intrahepatic cyclic AMP concentration rather than by a cyclic AMP-independent inhibition of specific mRNA translation.
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