Short Communications

The Regulation of Rat Liver L-Alanine–Glyoxylate Aminotransferase by Glucagon *in vivo*

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A previous report briefly indicated that rat liver L-alanine–glyoxylate aminotransferase activity was increased after glucagon treatment (Rowell, Snell, Carnie & Al-Tai, 1969). Along with other evidence this was taken to signify an involvement of the enzyme in the process of gluconeogenesis. Since evidence has been presented elsewhere that the effects of glucagon *in vivo* on hepatic gluconeogenesis (Exton *et al.* 1970) and on the induction of specific liver enzymes (Yeung & Oliver, 1968; Jost, Hsie & Rickenberg, 1969; Wicks, Kenney & Lee, 1969) are mediated via adenosine 3':5'-cyclic monophosphate (cyclic AMP), it was decided to examine the effects of cyclic AMP on L-alanine–glyoxylate aminotransferase activity *in vivo*. The results obtained indicate that the glucagon stimulation of aminotransferase activity is indeed mediated via cyclic AMP, by a process dependent on protein synthesis but insensitive to inhibition by actinomycin D.

Materials and methods. Cyclic AMP and its 6-N,2'-O-dibutyl derivative were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K.; glucagon was from Eli Lilly and Co. Ltd., Basingstoke, Hants., U.K.; actinomycin D was from Calbiochem, Los Angeles, Calif., U.S.A.; cycloheximide was from Sigma (London) Ltd., London S.W.6, U.K. Male Wistar rats (90–150 g body weight) were injected intraperitoneally without anaesthesia with the appropriate agent in 0.5 ml of 0.9% sodium chloride, except for glucagon, which was dissolved in the vehicle supplied by the manufacturer. The dosages employed were 0.5, 5.0, 5.0, 0.1 and 0.1 mg/100 g body wt. for glucagon, cyclic AMP, dibutyl cyclic AMP, cycloheximide and actinomycin D respectively. Injections were carried out at 11 a.m.; inhibitors, when used, were administered 30 min before the stimulating agent. Liver L-alanine–glyoxylate aminotransferase activity was assayed as described by Rowell *et al.* (1969) 24 h after injection.

Results and discussion. The time-course of glucagon stimulation of L-alanine–glyoxylate

aminotransferase activity was investigated. Activities of 3.57 ± 0.03 (3), 3.93 ± 0.13 (3), 5.32 ± 0.17 (3), 5.43 ± 0.15 (3), 5.00 ± 0.05 (3) and 4.57 ± 0.17 (3) μ mol of pyruvate formed/min per g of liver [means $\pm$ S.E.M. (numbers of observations)] were assayed at 0, 6, 24, 48, 72 and 96 h after a single injection of glucagon. Maximum activity was reached by 24 h and remained elevated, falling only slightly over the next 3 days. The aminotransferase activity assayed at 24 h after glucagon administration was proportional to tissue concentration over the range 0.083–1.667% (w/v) final concentration of liver in the assay system. This suggests that the elevated activity in glucagon-treated rats is the result of an increased amount of enzyme rather than the activation of pre-existing enzyme, since if an activation phenomenon were involved then dilution of the tissue homogenate should dilute any activator present, with a consequent decrease in enzyme activity per g of liver and a deviation of the activity–tissue-concentration plot from linearity. This conclusion is supported by the finding that glucagon (0.1 mm final concn.), cyclic AMP or dibutyl cyclic AMP (3.0 mm) are all without effect on aminotransferase activity *in vitro*.

L-Alanine–glyoxylate aminotransferase activity was stimulated significantly *in vivo* by glucagon, cyclic AMP and dibutyl cyclic AMP as compared with saline-injected controls ($P < 0.001$) (see Table 1). All the agents were equally effective as stimulators of enzyme activity and the stimulation by glucagon and cyclic AMP together was the same as that with either agent by itself. These results suggest that the glucagon effect is being mediated via cyclic AMP and extend the limited list of enzymes known to be induced via cyclic AMP. However, only with tyrosine aminotransferase has this effect previously been demonstrated on normal basal enzyme activity (Wicks *et al.* 1969). For the other enzymes investigated (serine dehydratase and phosphoenolpyruvate carboxykinase) activities were first depressed to low values by feeding the animals on low-protein diets before demonstration

of 'induction' by glucagon or cyclic AMP (Jost *et al.* 1969; Wicks *et al.* 1969). It is worth noting, however, that dietary protein can markedly alter the nature of enzyme responses to various hormones (Freedland, Murad & Hurvitz, 1968).

Rat liver L-alanine-glyoxylate aminotransferase activity has been shown to be increased by 34% after 42h starvation and by 62% 24h after alloxan treatment (Snell, 1971). The present results suggest that these effects may be mediated via increased concentrations of cyclic AMP, since hepatic concentrations of the nucleotide rise during starvation and in diabetes (Jefferson, Exton, Butcher & Sutherland, 1968).

The effect of cycloheximide and actinomycin D on the stimulation of the aminotransferase activity is also shown in Table 1. In each case stimulation was abolished by cycloheximide ($P < 0.001$), suggesting that the increase is dependent on continued protein synthesis. Actinomycin D did not prevent the stimulation at a concentration and under conditions known to be effective in blocking the induction of other enzymes by glucagon or cyclic AMP (Csanyi, Greengard & Knox, 1967; Jost *et al.* 1969; Pitot & Peraino, 1964; Holten & Kenney, 1967); the lack of inhibition by actinomycin D suggests that cyclic AMP (or glucagon) may be exerting its effect at the level of translation (where translation is defined as all reactions subsequent to the transcription of DNA into RNA). The sensitivity of other enzymes to actinomycin D inhibition during induction by glucagon or cyclic AMP would appear to rule out a general translational mechanism for the nucleotide. Kenney (1970) has argued, however, that the inhibition observed for these enzymes may be a consequence of labile mRNA templates, which would rapidly become deficient after actinomycin D inhibition, thereby negating any translational control effects. If this interpretation is correct then the results presented here suggest that the mRNA coding for L-alanine-glyoxylate aminotransferase is relatively stable. Direct determination of this would require a knowledge of actual rates of enzyme synthesis after administration of actinomycin D, rather than measurements of enzyme activity, to take account of possible effects of actinomycin on enzyme turnover. Such measurements are not possible at the present time, since procedures for the purification of rat liver L-alanine-glyoxylate aminotransferase have not been devised and the purified enzyme is a prerequisite for measuring synthesis by specific immunoprecipitation techniques (Kenney, Reel, Hager & Wittliff, 1968).

One other case of the involvement of cyclic AMP in an actinomycin-insensitive induction in the intact animal has been reported by Garren, Ney & Davis (1965), who observed that actinomycin D

Table 1. *Stimulation of rat liver L-alanine-glyoxylate aminotransferase activity in vivo*

Animals were injected intraperitoneally with glucagon (0.5 mg/100 g body wt.), cyclic AMP (5.0 mg/100 g body wt.), dibutyryl cyclic AMP (5.0 mg/100 g body wt.) or glucagon + cyclic AMP (0.5 mg and 5.0 mg/100 g body wt. respectively). Actinomycin D (0.1 mg/100 g body wt.) or cycloheximide (0.1 mg/100 g body wt.) was injected 30 min before the stimulating agent. Liver L-alanine-glyoxylate aminotransferase was assayed by the method of Rowsell *et al.* (1969) 24 h after injection. Activities are the means $\pm$ S.E.M. with the numbers of observations in parentheses. The results are taken from a series of experiments. Each experiment on any one day involved animals injected with saline alone, animals injected with each of the stimulating agents and animals injected with each of the stimulating agents together with one of the inhibitors.

Treatment	L-Alanine-glyoxylate aminotransferase activity (μ mol of pyruvate formed/min per g of liver)
Controls (0.9% NaCl)	3.48 $\pm$ 0.02 (12)
+ actinomycin D	3.40 $\pm$ 0.07 (3)
+ cycloheximide	3.02 $\pm$ 0.15 (3)
Glucagon	5.40 $\pm$ 0.20 (4)
+ actinomycin D	5.50 $\pm$ 0.17 (4)
+ cycloheximide	3.37 $\pm$ 0.25 (4)
Cyclic AMP	5.15 $\pm$ 0.13 (4)
+ actinomycin D	5.38 $\pm$ 0.22 (4)
+ cycloheximide	3.28 $\pm$ 0.18 (3)
Dibutyryl cyclic AMP	6.73 $\pm$ 0.38 (3)
+ actinomycin D	6.07 $\pm$ 0.15 (3)
+ cycloheximide	3.25 $\pm$ 0.13 (3)
Glucagon + cyclic AMP	5.33 $\pm$ 0.17 (3)

failed to prevent the adrenocorticotrophin-induced steroid production by the adrenals, and they interpreted this in terms of a translational control mediated via cyclic AMP, which is involved in the induction of adrenocorticotrophin-inducible adrenal steroidogenic enzymes (Garren, 1968).

The mechanism by which cyclic AMP might control enzyme synthesis at the translational level is not known. Khairallah & Pitot (1967) have presented observations implicating the nucleotide in the release of nascent polypeptide chains from polyribosomes, but how cyclic AMP could confer specificity of enzyme induction by this process is not clear. The actinomycin D-insensitive response of L-alanine-glyoxylate aminotransferase to cyclic AMP induction suggests that this may be a useful model system for elucidating the mechanisms by which the nucleotide exerts its control at the translational level.

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