Control of Hepatic Triacylglycerol Synthesis

DIURNAL VARIATIONS IN HEPATIC PHOSPHATIDATE PHOSPHOHYDROLASE ACTIVITY AND IN THE CONCENTRATIONS OF CIRCULATING INSULIN AND CORTICOSTERONE IN RATS

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Male rats were kept for 14 days with alternating 12 h periods of light and darkness. The hepatic activity of soluble phosphatidate phosphohydrolase and the concentration of serum insulin were maximum at about 2 h after dark. The peak concentration of serum corticosterone occurred 2 h before the dark period. It is proposed that corticosterone is partly responsible for the increased phosphohydrolase activity, and that this enables the liver to increase its capacity to synthesize triacylglycerols during the period of maximum feeding.

Evidence has accumulated that phosphatidate phosphohydrolase (EC 3.1.3.4) is important in controlling the rate at which different types of glycerolipids are synthesized in the liver (Fallon *et al.*, 1977; Brindley, 1978; Brindley *et al.*, 1979b). The activity of the enzyme in liver changes within a few hours of an alteration in the physiological state of an animal so as to parallel the observed rate of triacylglycerol synthesis, or the capacity of the liver to synthesize triacylglycerols. Other enzymes in the pathway do not change at all in activity, or the changes are normally smaller than those observed for the phosphohydrolase.

Injection of cortisol into rats increased the activity of the phosphohydrolase in liver and it was proposed that many of the increases observed in phosphohydrolase activity in other conditions could also be produced by the increased importance of glucocorticoids in controlling metabolism (Glenny & Brindley, 1978). These conditions include starvation, diabetes, surgical stress, subtotal hepatectomy, liver damage, ethanol feeding and obesity. This relationship can also be extended to the acute feeding of rats with fructose, glycerol, or sorbitol, which increase both the activity of the phosphohydrolase (Savolainen & Hassinen, 1977; Sturton *et al.*, 1978) and the concentration of circulating corticosterone (Brindley *et al.*, 1979a,b). Similarly, rats that are fed with saturated fats show an increased release of corticosterone in response to stress (Hülsmann, 1978), and an increased specific activity of the phosphohydrolase in their livers (Glenny *et al.*, 1978).

The direct involvement of glucocorticoids in regulating the activity of the phosphatidate phosphohydrolase in the liver is demonstrated by the observation that this activity increases when livers are

perfused with cortisol (Lehtonen *et al.*, 1979). Adrenalectomy of rats abolishes over 85% of the ethanol-induced increase in phosphohydrolase activity (Brindley *et al.*, 1979a). The effects of ethanol on the phosphohydrolase activity, and on the rate of synthesis and of accumulation of triacylglycerols in the liver can also be largely prevented by pretreating rats with benfluorex (Pritchard & Brindley, 1977; Pritchard *et al.*, 1977). This treatment also diminishes the ethanol-induced increase in the concentration of circulating corticosterone (Brindley *et al.*, 1979c; Pritchard *et al.*, 1979).

It therefore seems likely that phosphatidate phosphohydrolase activity should undergo a diurnal variation in response to changes in the concentrations of circulating glucocorticoids. The present work investigates this prediction and examines the relationship of the changes in phosphohydrolase activity to alterations in the concentrations of circulating insulin and of corticosterone and to the known feeding patterns of rats.

Materials and Methods

The source or preparation of the materials used in the present paper have been described previously (Sturton & Brindley, 1977; Whiting *et al.*, 1977). Male Wistar rats were purchased and maintained for 14 days on a 41B diet (Whiting *et al.*, 1977) at a temperature of 22°C and in a room that was lit from 15:00 h to 03:00 h. The rats were handled daily for the 4 days before they were killed by decapitation with a guillotine. Blood samples were collected and the concentrations of insulin and of corticosterone were measured in the sera (Brindley *et al.*, 1979a). For the latter determination the assay based on the

displacement of [^3H]corticosterone from the binding globulin was used. The livers were taken from the rats without perfusion and the soluble fraction was prepared and dialysed (Whiting *et al.*, 1977). Phosphatidate phosphohydrolase activity was determined by measuring the rate of P_i release from phosphatidate (Brindley *et al.*, 1979a), except that the final composition of the mixed emulsion used as a substrate was 0.4 mM-potassium phosphatidate and 0.27 mM-phosphatidylcholine.

Results and Discussion

The activities of phosphatidate phosphohydrolase and the serum concentrations of insulin and corticosterone are shown in Fig. 1. Phosphatidate phosphohydrolase activity varied significantly throughout the day ($P < 0.01$ from the analysis of variance). The activity at 21:00h was significantly lower ($P < 0.05$ from a Student's *t* test) than that at 01:00, 05:00 and 09:00h. The peak activity at 05:00h was significantly greater ($P < 0.05$) than that at all other times except for that at 09:00h and it was 1.6-fold greater than that at 21:00h.

The concentration of corticosterone in the serum was maximum at 01:00h ($P < 0.02$ for all other times except at 17:00h) and at a minimum at 17:00h (Fig. 1b). This increase in corticosterone just before the dark period is similar to that reported for male rats (Critchlow *et al.*, 1963; Peret *et al.*, 1973). The concentration of circulating insulin was greater at 05:00h ($P < 0.02$ for all time points except 09:00 and 17:00h). A rise in insulin concentrations at the beginning of the dark period was also reported by Peret *et al.* (1973) and Bruckdorfer *et al.* (1974).

Evidence was presented in the introduction that the activity of hepatic phosphatidate phosphohydrolase is partly controlled by the concentration of circulating glucocorticoids. The increased activities of the phosphohydrolase that are seen after rats are fed with ethanol, fructose, glycerol and sorbitol are preceded by increases in serum corticosterone concentrations that are not accompanied by increases in the concentration of insulin in the serum (Sturton *et al.*, 1978; Brindley *et al.*, 1979a). A similar relationship exists in the present experiments in which the peak of phosphohydrolase activity (expressed per liver) occurs 4h after the peak in corticosterone concentration. Corticosterone probably stimulates the synthesis of phosphatidate phosphohydrolase and this suggestion is supported by the observation that the increase in activity that normally results from subtotal hepatectomy is prevented by actinomycin D (Mangiapanne *et al.*, 1973). Factors other than corticosterone must also play a part in regulating the activity of the phosphohydrolase. For instance, this activity in adrenalectomized rats is about 75% of that in the

Fig. 1. The daily variations in the hepatic activity of the soluble phosphatidate phosphohydrolase and in the serum concentrations of corticosterone and insulin in male rats. Results are given as means $\pm$ S.E.M. and the numbers of rats used are indicated in parentheses on the graphs. The activity of the phosphohydrolase is expressed per liver rather than per g of liver, since the liver weight also undergoes a diurnal rhythm (Bruckdorfer *et al.*, 1974). The mean body wt. of the rats used in (a) was 199 g (S.D. $\pm$ 11, $n = 46$), and the profile of the phosphohydrolase activity is the same if the results are expressed per mg of soluble protein.

controls and adrenalectomy does not completely abolish the ethanol-induced increase in activity (Brindley *et al.*, 1979a). Insulin is unlikely to increase the phosphohydrolase activity since the activity of this enzyme is increased in diabetes (Murthy & Shipp, 1976; Whiting *et al.*, 1977), and it is not increased significantly by feeding glucose to rats that have been maintained on a 41B diet (Sturton *et al.*, 1978).

The peak in the phosphatidate phosphohydrolase activity does, however, coincide with that of the insulin concentration at the beginning of the dark

period (Fig. 1). The rise in the concentration of serum insulin also occurs at the time of maximum food consumption (Bruckdorfer *et al.*, 1974). This is when an increase in the rate of synthesis of fatty acids (Fears & Morgan, 1976) and of triacylglycerols is expected in the liver, although fatty acid synthetase activity changes little throughout the day (Bruckdorfer *et al.*, 1974). The capacity of the liver to synthesize cholesterol, as indicated by the activity of β -hydroxy- β -methylglutaryl-CoA reductase (EC 1.1.1.34) normally reached a maximum in the middle of the dark period in rats fed *ad libitum* (Rodwell *et al.*, 1976). However, this change may not necessarily be reflected in an increased rate of cholesterol synthesis (Fears & Morgan, 1976). Part of the cholesterol that is formed in the dark period is likely to be secreted from the liver with newly synthesized triacylglycerol as very-low-density lipoprotein. The increased activity of phosphatidate phosphohydrolase could help to facilitate the increase in triacylglycerol synthesis and this is probably caused in part by the high concentrations of circulating corticosterone that precede this change.

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