The Involvement of Glucocorticoids in Regulating the Activity of Phosphatidate Phosphohydrolase and the Synthesis of Triacylglycerols in the Liver

EFFECTS OF FEEDING RATS WITH GLUCOSE, SORBITOL, FRUCTOSE, GLYCEROL AND ETHANOL

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Feeding rats with sorbitol, fructose, glycerol and ethanol increases the concentration of serum corticosterone without significantly altering the concentration of insulin. This increase appears to be partly responsible for the increases in the hepatic activity of phosphatidate phosphohydrolase (compared with rats fed glucose or 0.9% NaCl) that has been reported [Sturton, Pritchard, Han & Brindley (1978) *Biochem. J.* **174**, 667–670] and the enhanced capacity of the liver to synthesize triacylglycerols. The ethanol-induced increase in phosphohydrolase activity was largely, but not completely, prevented by adrenalectomy.

Phosphatidate phosphohydrolase (EC 3.1.3.4) shows many of the properties of an important regulatory enzyme for the synthesis of triacylglycerols in the liver (Fallon *et al.*, 1977; Brindley, 1978*a,b*). Although the changes in the activity of this enzyme have been documented for a number of physiological conditions, little was known about the stimuli that provoked these changes in enzyme activity. However, when cortisol is injected into rats there is an increase in the hepatic activity of phosphatidate phosphohydrolase and also in the rate at which the liver synthesizes triacylglycerols (Glenny & Brindley, 1978). It was proposed that the increases in phosphohydrolase activity that are seen in starvation, diabetes, after surgical stress and subtotal hepatectomy, in certain types of obesity, and after ethanol feeding (Brindley, 1978*a,b*) could also result from a greater control of metabolism by glucocorticoids. However, it was not clear whether the increases in hepatic phosphohydrolase that are seen after feeding sorbitol (Savolainen & Hassinen, 1977; Sturton *et al.*, 1978), fructose (Lamb & Fallon, 1974; Sturton *et al.*, 1978) or glycerol (Savolainen & Hassinen, 1977; Sturton *et al.*, 1978) could also be explained in this way.

Rats were therefore fed with saline (0.9% NaCl), glucose, sorbitol, fructose, glycerol and ethanol so that the changes in the serum concentrations of corticosterone and of insulin could be related to the changes in hepatic phosphohydrolase activity that

have been reported previously. The results are compatible with the hypothesis that glucocorticoids participate in the long-term control of hepatic triacylglycerol synthesis through phosphatidate phosphohydrolase (Glenny & Brindley, 1978).

Materials and Methods

The source or preparation of most materials used in the present paper have been described previously (Whiting *et al.*, 1977; Sturton & Brindley, 1977). Radioimmunoassay kits for insulin determination and Cortipac kits for corticosterone determinations were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Pooled human serum, taken in the third trimester of pregnancy, was also used as a source of corticosteroid-binding globulin. This was diluted with an equal volume of 10mM-Tris buffer, adjusted to pH 7.4 with HCl, and endogenous steroids were removed by adding 50mg of Norit A per ml of diluted serum, and stirring for 30min. The Norit was removed by centrifugation and the sample stored at –20°C.

Treatment of rats

Rats were purchased and were fed by stomach tube with 5g of ethanol/kg body wt., or with isocaloric glucose, sorbitol, fructose or glycerol, or with 0.15M-NaCl as described by Sturton *et al.* (1978). Rats that were adrenalectomized were given 0.15M-NaCl to drink instead of water and were allowed 10–14 days to recover before the experiments. All rats were subjected to the minimum of stress

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before they were killed by decapitation with a guillotine. Blood was collected from the necks and the serum samples used for insulin assays were frozen in liquid N₂ within 10 min. Samples that showed obvious signs of haemolysis were not used for insulin determination.

Preparation and dialysis of the particle-free supernatant from rat liver

The method was that described by Whiting *et al.* (1977).

Determination of phosphatidate phosphohydrolase activity

Phosphatidate phosphohydrolase activity was measured in the soluble fraction from rat liver by determining the rate of P_i release from a phosphatidate emulsion (Sturton & Brindley, 1978). This method gives similar increases after ethanol feeding to those obtained with membrane-bound phosphatidate as a substrate (Lamb *et al.*, 1979). Each assay contained in a volume of 0.25 ml: 50 mM-Tris/maleate buffer (pH 7.0), 1 mM-dithiothreitol, 0.6 mM-potassium phosphatidate, 0.4 mM-phosphatidylcholine, 1.2 mM-MgCl₂, 0.1 mM-EGTA, and 2 mg of bovine serum albumin/ml. Reactions were started by adding the mixed emulsion of phosphatidate and phosphatidylcholine. Reactions were stopped after incubating for 20 min at 37°C and the concentration of P_i was determined (Ames, 1966).

Determination of DNA concentration

This was determined by the method of Burton (1956).

Determination of the concentrations of insulin and corticosterone

Insulin concentrations were determined by using a commercially available kit according to the manufacturer's instructions. The corticosterone concentrations in Fig. 1 were measured by displacing ⁷⁵Se-labelled cortisol from corticosteroid-binding globulin by using a Cortipac kit according to the manufacturer's instructions for cortisol determination with cortisol standards. Those in Table 1 were obtained by displacing [³H]corticosterone from the binding globulin essentially as described by De Jong & van der Molen (1972). These two methods gave equivalent results ($r = 0.97$ for 16 estimations), but corticosterone was 1.5-fold less effective at displacing cortisol than was cortisol itself. This factor was used to express the results from the Cortipac assays in terms of corticosterone. The same conversion factor was also derived when selected samples from Fig. 1 were assayed for corticosterone by using the fluorimetric method of Mattingly (1962).

Results and Discussion

The concentration of corticosterone in the sera of rats fed a variety of compounds is shown in Fig. 1. Feeding ethanol results in an increased concentration of serum corticosteroids (Mendelson, 1971), and in the present work an increased concentration of serum corticosterone was seen at 0.75, 1.5, 3 and 4 h after feeding (Fig. 1). This may result both from an increased secretion of corticotropin, and from the hepatotoxic effects of ethanol, which could delay the removal of glucocorticoids from the blood. It is also known that an intact pituitary-adrenal axis is necessary for the appearance of the ethanol-induced fatty liver (Brodie & Maickel, 1963; Maickel & Brodie, 1963) and for the maximum stimulation of phosphohydrolase activity by ethanol (Table 1). The adrenalectomized rats that were not fed with ethanol had a lower ($P < 0.05$) phosphatidate phosphohydrolase activity in their livers than did the controls. However, since the former activity is relatively high, it seems unlikely that the phosphohydrolase activity is maintained entirely by glucocorticoids. Treatment of the adrenalectomized rats with ethanol increased the phosphohydrolase activity by 1.4- and 1.7-fold respectively ($P < 0.02$ and $P < 0.005$) at 4 h and 7 h after feeding compared with 3- and 6.9-fold for the control rats (Table 1). Although the increased concentration of circulating corticosterone is probably largely responsible for the subsequent increase in phosphohydrolase activity in the control rats, this does not appear to be the only mechanism for increasing this enzyme's activity.

We also have evidence that treating rats for 5 days with 50 mg of benfluorex/kg body wt. partially prevents the ethanol-induced increase in circulating corticosterone. It is known that this treatment with benfluorex also partly prevents the appearance of the ethanol-induced increases in the liver of: (a) phosphatidate phosphohydrolase activity (Pritchard *et al.*, 1977); (b) the rate of triacylglycerol synthesis (Pritchard & Brindley, 1977); and (c) the accumulation of triacylglycerols (Pritchard & Brindley, 1977).

The consumption of sorbitol (Lederer *et al.*, 1978), fructose (Lamb & Fallon, 1974) or glycerol (Nikkilä & Ojala, 1964; Narayan & McMullen, 1978) can also promote the synthesis and secretion of triacylglycerols by the liver, and these increases may also be facilitated by the enhanced activity of phosphatidate phosphohydrolase (Sturton *et al.*, 1978). However, much less is known about the effects of these nutrients on the concentration of serum glucocorticoids. Fig. 1 shows that sorbitol, fructose or glycerol also increase the concentration of serum corticosterone at 0.75 and 1.5 h after feeding. These increases were less prolonged than that produced by ethanol and at 3 h after feeding these concentrations were similar to those of rats fed glucose or saline.

Fig. 1. Effect of feeding rats with glucose, sorbitol, fructose, glycerol and ethanol on the concentrations of insulin and corticosterone in their sera

Rats were fed by stomach tube with the various nutrients (Sturton *et al.*, 1978) and the concentrations of insulin (○) and corticosterone (●) were measured in the sera at various times after feeding. Results are given as means ± S.E.M. There were four rats in each group except for the glucose group at 45 min, the ethanol group at 4 h and the rats that were not fed, for which there were eight, three and ten animals respectively.

Table 1. Effect of adrenalectomy on the activity of the soluble phosphatidate phosphohydrolase in the livers of rats after ethanol feeding

The treatment of the rats and the methods of measurement are described in the Materials and Methods section. Results are given as means ± S.E.M. and the numbers of animals are shown in parentheses.

Treatment	Phosphatidate hydrolysed (nmol/min per μmol of DNA phosphorus)		[Corticosterone] (μg/litre)	
	Control	Adrenalectomized	Control	Adrenalectomized
No ethanol	131 ± 14 (6)	98 ± 6 (6)	65 ± 13 (6)	4.3 ± 1.7 (6)*
4 h after ethanol	397 ± 21 (3)	136 ± 12 (6)	158 ± 21 (3)	8.3 ± 8.0 (6)*
7 h after ethanol	900 ± 38 (3)	170 ± 18 (6)	68 ± 25 (3)	8.3 ± 4.4 (6)*

* The corticosterone concentrations for the adrenalectomized rats are probably overestimates since other steroids can displace corticosterone from the binding globulin (Murphy, 1967).

This may explain why the increased activity of phosphatidate phosphohydrolase at 6 h is greater in those rats fed ethanol than that seen in the rats fed with sorbitol, fructose or glycerol.

Although all of these four compounds probably stimulate hepatic triacylglycerol synthesis through the increased concentration of circulating glucocorticoids, they also have acute effects. These

probably involve the increased production of glycerol phosphate and of dihydroxyacetone phosphate, and in the case of sorbitol, glycerol or ethanol there may be additional effects due to the changes in the redox state of the liver.

The rats fed glucose did not show an increase in phosphohydrolase activity 6h after feeding (Sturton *et al.*, 1978), nor at a variety of other times (Savolainen, 1977) when compared with rats fed saline. However, a transient increase ($P < 0.05$ compared with rats fed saline) in serum corticosterone concentration occurred at 0.75h after feeding glucose, but this was paralleled by an increase ($P < 0.05$) in the concentration of circulating insulin (Fig. 1). This response is therefore different to that seen after feeding sorbitol, fructose, glycerol or ethanol.

The results in Fig. 1 and in Table 1 are therefore compatible with the hypothesis that increases in the concentration of circulating glucocorticoids are partly responsible for the increases in phosphatidate phosphohydrolase activity in the liver. This hypothesis arose from work in which rats were injected with cortisol or corticotropin (Glenny & Brindley, 1978), and the parallelism between increased control of metabolism by glucocorticoids and increased phosphohydrolase activity was identified. This parallelism has now been extended to include the feeding of sorbitol, glycerol or fructose. It is also noteworthy that the prolonged consumption of diets rich in fructose can lead to hypercortisolism (Yudkin, 1978), and to an increased phosphatidate phosphohydrolase activity and triacylglycerol synthesis in the liver (Lamb & Fallon, 1974). A similar relationship may exist for the saturated and monounsaturated fat in the diet. Feeding rats with diets rich in lard or in the C_{22:1} fatty acids derived from rapeseed oil or from hardened fish oil makes them more responsive to stress, and this is manifested by an increased concentration of circulating glucocorticoids (Hülsmann, 1978). Also, feeding diets enriched with lard increases the activity of phosphatidate phosphohydrolase and the rate of triacylglycerol synthesis in the liver (Glenny *et al.*, 1978).

A further relationship between glucocorticoids and hepatic triacylglycerol synthesis is provided by the unpublished work of Lehtonen *et al.* (1979), who have demonstrated that perfusion of rat livers with cortisol increases phosphatidate phosphohydrolase activity and we already know that treatment of livers with glucocorticoids stimulates triacylglycerol synthesis and lipoprotein secretion (Klausner & Heimberg, 1967; Reaven *et al.*, 1974). The mechanism by which glucocorticoids increase the phosphohydrolase activity is not yet established, but it is likely that they stimulate the synthesis of this enzyme. This suggestion is supported by the observation that actinomycin D can prevent the increase in phosphohydrolase activity that is normally observed in rats that have been sub-

jected to subtotal hepatectomy (Mangiapane *et al.*, 1973).

The relationship between circulating glucocorticoids, increased phosphatidate phosphohydrolase activity and triacylglycerol synthesis in the liver may provide clues for a better understanding of lipoprotein metabolism and the development of atherosclerosis. This relationship appears to exist after consuming diets rich in saturated fat and sucrose, in diabetes and in stress conditions. It may be significant that these are all potentially atherogenic conditions and that they represent risk factors in ischaemic heart disease.

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