Hypophysectomy does not alter the acinar zonation of gluconeogenesis or the mitochondrial redox state in rat liver

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The biochemical and functional heterogeneity of hepatocytes in different zones of the liver acinus may be related to the concentrations of hormones within the liver acinus. We examined the effects of hypophysectomy, which causes marked changes in plasma hormone levels and in activities of hepatic enzymes that are normally heterogeneously distributed, on the degree of metabolic zonation within the liver acinus. In hypophysectomized rats the activity of alanine aminotransferase was increased, but its normal zonation (predominance in the periportal zone) was preserved. The activity in cultured periportal and perivenous hepatocytes was increased by dexamethasone, but not by glucagon. Periportal hepatocytes from hypophysectomized rats expressed higher rates of gluconeogenesis in culture than did perivenous hepatocytes, irrespective of the absence or presence of dexamethasone, glucagon or insulin. Similar differences in rates of ketogenesis and in the mitochondrial redox state in response to glucagon were observed between periportal and perivenous hepatocytes from hypophysectomized rats as between cell populations from normal rats. Although hypophysectomy causes marked changes in hepatic enzyme activities, it does not alter the degree of zonation of alanine aminotransferase, gluconeogenesis or the mitochondrial redox state within the liver acinus.

INTRODUCTION

Hepatocytes in adult mammalian liver express structural and functional heterogeneity relative to their periportal or perivenous location in the liver acinus. Various mechanisms have been proposed for the induction of this acinar zonation, including the concentration gradients along the portal/venous axis of the acinus of substrates, oxygen and hormones, as well as innervation [1–4]. Recent studies have shown that, when hepatocytes are isolated from the periportal (afferent) or perivenous (efferent) zone and maintained in culture in either the absence [5] or the presence of hormones [6,7] differences in rates of gluconeogenesis and urea synthesis between the two cell populations do not converge. This suggests that long-term-acting mechanisms are probably involved in the induction and maintenance of heterogeneity *in vivo*. Few studies have examined the effects of perturbing the endocrine state *in vivo* on the zonation of enzyme activities in the liver acinus [8,9]. In the rat, hypophysectomy results in a marked suppression of the circulating concentrations of glucocorticoids, thyroid hormones and growth hormone [10–12] and is associated with changes in the activities of various hepatic enzymes [13–16] which are now known to be heterogeneously distributed within the acinus [1]. The hypophysectomized rat is therefore a potential model in which to examine whether suppression of the concentrations of long-term-acting hormones alters the degree of metabolic zonation within the liver acinus. The present study shows that, although hypophysectomy causes marked changes in activities of alanine aminotransferase and pyruvate kinase, the degree of heterogeneity within the liver acinus of the activities of these enzymes and of the rates of

gluconeogenesis and of the mitochondrial redox state is preserved.

MATERIALS AND METHODS

Pig insulin was from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Sources of all other reagents were as described previously [7].

Male hypophysectomized rats of the Sprague–Dawley strain were obtained from Charles River U.K. Ltd. (Margate, Kent, U.K.). Hypophysectomy was performed by the parapharyngeal route at a body weight of 150–170 g, and the rats were used between 2 and 4 weeks after surgery, during which time they gained less than 2 g/week, compared with approx. 35 g/week for age-matched controls. Hypophysectomized rats maintained for up to 6 weeks after surgery still showed no increase in body weight. At the time of hepatocyte isolation, the body weights of the hypophysectomized rats were 142 ± 8 g (mean $\pm$ S.E.M., $n = 9$) as compared with 327 ± 18 g ($n = 5$) for the controls. The rats were maintained on standard rat chow *ad libitum*.

Hepatocytes were isolated from either the periportal or the perivenous zones of the liver by the digitonin/collagenase perfusion technique [17], modified as described previously [7,18]. Cell yield and viability were as reported previously [7]. The hepatocytes were cultured in monolayer in Minimum Essential Medium containing 5% (v/v) fetal-bovine serum either in 25 cm² flasks or in 24-well plates [7]. After cell attachment (4–6 h) the monolayers were cultured in serum-free Minimum Essential Medium either with a range of concentrations of dexamethasone or with 10 nM-dexamethasone and a range of concentrations of either glucagon or insulin as

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Table 1. Enzyme activities in periportal and perivenous hepatocytes from hypophysectomized and normal rats

Enzyme activities were determined in the hepatocyte monolayers after cell attachment and are expressed as munits/mg of cell protein. Values are means $\pm$ S.E.M. for *n* hepatocyte preparations. PP/PV values represent the ratios of activities in periportal and perivenous preparations.

Rats...	Hypophysectomized			Normal		
Hepatocytes ... <i>n</i> ...	Periportal 9	Perivenous 9	PP/PV	Periportal 12	Perivenous 12	PP/PV
Alanine aminotransferase (AAT)	318 $\pm$ 92	168 $\pm$ 45	1.9	159 $\pm$ 33	107 $\pm$ 17	1.5
Fructose-bisphosphatase (FBP)	44 $\pm$ 5	32 $\pm$ 3	1.4	39 $\pm$ 4	33 $\pm$ 4	1.2
Pyruvate kinase (PK)	44 $\pm$ 7	50 $\pm$ 7	0.9	149 $\pm$ 12	200 $\pm$ 18	0.8
AAT/PK	6.6 $\pm$ 1.3	3.3 $\pm$ 0.6	2.0	1.2 $\pm$ 0.1	0.6 $\pm$ 0.1	2.0
FBP/PK	1.1 $\pm$ 0.2	0.7 $\pm$ 0.1	1.6	0.29 $\pm$ 0.04	0.21 $\pm$ 0.03	1.4

Table 2. Effects of dexamethasone and glucagon on alanine aminotransferase activity in hepatocytes from hypophysectomized rats

The activity of alanine aminotransferase was determined after culture of the hepatocyte monolayers for 16 h with the concentrations of hormones indicated. Results are expressed as munits/mg of cell protein, and are means $\pm$ S.E.M. for each of three cell preparations. Values that are significantly different ($P < 0.05$) from the corresponding values without hormone are shown by *a*, and significant differences between periportal (PP) and perivenous (PV) preparations are shown by *b*.

Hormone (nM)	Activity (munits/mg of protein)					
	Dexamethasone			Glucagon		
	PP	PV	PP/PV	PP	PV	PP/PV
0	201 $\pm$ 4	127 $\pm$ 7	1.58 ^b	282 $\pm$ 19	148 $\pm$ 5	1.94 ^b
1	231 $\pm$ 9 ^a	126 $\pm$ 5	1.83 ^b	293 $\pm$ 9	154 $\pm$ 6	1.90 ^b
10	266 $\pm$ 15 ^a	146 $\pm$ 4	1.82 ^b	271 $\pm$ 9	144 $\pm$ 1	1.88 ^b
100	275 $\pm$ 8 ^a	147 $\pm$ 3	1.87 ^b	267 $\pm$ 1	139 $\pm$ 11	1.92 ^b

indicated. After culture for 14 h, rates of gluconeogenesis (incorporation of [3-¹⁴C]pyruvate into glucose) and ketogenesis (formation of acetoacetate and D-3-hydroxybutyrate) were determined during a 2 h incubation with Minimum Essential Medium containing: 0.75 mM-palmitate, 0.5 mM-L-carnitine, 1 mM-[3-¹⁴C]pyruvate (0.1 Ci/mol) and 7.5 mg of bovine serum albumin/ml as described previously [7]. Cell protein was determined by the Lowry method [19], and rates of substrate utilization or product formation were expressed as nmol of substrate or product metabolized/h per mg of protein.

Enzyme activities were determined either immediately after cell attachment [7] or after 16 h culture with hormones. Enzymes were assayed at 30 °C as described previously [7,20], and are expressed as munits per mg of cell protein, where 1 munit is the amount converting 1 nmol of substrate/min.

Statistical analysis was by Student's *t* test for differences between the presence and the absence of hormones, and by two-way ANOVA for differences between periportal- and perivenous-cell populations.

RESULTS

Enzyme activities determined after hepatocyte attachment

Hepatocytes from hypophysectomized rats had higher activities of alanine aminotransferase and lower activ-

ities of pyruvate kinase than did hepatocytes from normal rats (Table 1). The ratios of alanine aminotransferase/pyruvate kinase and fructose-bisphosphatase/pyruvate kinase were 4–5 times higher in hepatocytes from hypophysectomized rats compared with normal rats. Despite these differences in enzyme activities between hypophysectomized and normal rats, the differences between periportal and perivenous hepatocyte populations of alanine aminotransferase and pyruvate kinase in hepatocytes from hypophysectomized rats were similar to those in normal rats (Table 1).

Effects of hormones on alanine aminotransferase activity

The ratio of alanine aminotransferase activity between periportal and perivenous hepatocyte preparations persisted during 16 h culture irrespective of the hormonal conditions. The activity was higher in cells cultured with dexamethasone than in the respective controls, but glucagon in the presence of dexamethasone had no effect on the activity (Table 2).

Gluconeogenesis

Rates of gluconeogenesis were higher in periportal than in perivenous hepatocytes from hypophysectomized rats, irrespective of whether the cells were cultured with dexamethasone, glucagon or insulin (Fig. 1). Glucagon caused a greater stimulation ($P < 0.05$) of gluconeogenesis than did dexamethasone, but the effect of either

Fig. 1. Effects of glucagon, dexamethasone and insulin on rates of gluconeogenesis, ketogenesis and the ratio of D-3-hydroxybutyrate/acetoacetate in periportal (PP) and perivenous (PV) hepatocytes from hypophysectomized rats

Periportal (■, PP) and perivenous (▲, PV) hepatocytes isolated from hypophysectomized rats were cultured with the concentrations of hormones indicated for 14 h as described in the Materials and methods section. Rates of gluconeogenesis ($[^{14}\text{C}]$ pyruvate incorporation into glucose), ketogenesis (formation of D-3-hydroxybutyrate and acetoacetate) and the ratio of D-3-hydroxybutyrate/acetoacetate production were determined during a 2 h incubation. Rates of gluconeogenesis and ketogenesis are expressed as nmol of substrate metabolized or product formed/h per mg of cell protein. Values are means $\pm$ S.E.M. (bars). The numbers of hepatocyte preparations were nine, six and eight for periportal and perivenous preparations cultured with glucagon, dexamethasone and insulin respectively. Values for periportal preparations that are significantly different ($P < 0.05$) from the corresponding values of perivenous preparations are shown by *.

hormone was similar in periportal and perivenous hepatocytes. Insulin caused a greater inhibition ($P < 0.05$) of gluconeogenesis in periportal than in perivenous hepatocytes (Fig. 1).

Ketogenesis

Glucagon and dexamethasone increased ($P < 0.05$) ketogenesis in periportal and perivenous hepatocytes from hypophysectomized rats. In the presence of these hormones, rates of ketogenesis were significantly higher in periportal than in perivenous hepatocytes (Fig. 1). These differences are similar to those observed between periportal and perivenous hepatocytes from normal rats

[7]. Insulin caused a greater suppression ($P < 0.05$) of ketogenesis in periportal hepatocytes. The ratio of D-3-hydroxybutyrate/acetoacetate was lower in periportal than in perivenous hepatocytes cultured with dexamethasone, glucagon or insulin, and was decreased by all three hormones relative to the corresponding values in cultures without hormones (Fig. 1).

DISCUSSION

Alanine aminotransferase (EC 2.6.1.2) is the most commonly used 'enzyme marker' in studies on isolated periportal and perivenous hepatocytes [5–7], because

there is a relatively sharp gradient of activity between the portal and venous zones of the liver acinus [1]. Studies *in vivo* suggest that the activity of alanine aminotransferase in whole liver is under complex control by hormones that are suppressed after hypophysectomy [13,15,21–24]. Hypophysectomy is associated with an increase in activity of alanine aminotransferase [13,15] that is partially reversed by growth-hormone replacement [13,15]. Adrenalectomy causes a marked decrease in activity in normal [25] but not in hypophysectomized rats [15], and the former effect is reversed by glucocorticoid replacement [25]. These studies suggest that glucocorticoids either induce or potentiate the induction of alanine aminotransferase by other hormones; and growth hormone (and possibly other hormones that are decreased after hypophysectomy) suppresses the enzyme, either directly or indirectly. Direct evidence for effects of these hormones on alanine aminotransferase activity in primary hepatocyte cultures *in vitro* is lacking. The present study shows that hypophysectomy does not abolish the gradient of alanine aminotransferase between the periportal and the perivenous zones, and that dexamethasone has a direct effect on the activity of alanine aminotransferase *in vitro* in the absence of other hormones in both periportal and perivenous hepatocyte populations, whereas glucagon in the presence of dexamethasone has no additional effect. The regulation of alanine aminotransferase in hepatocyte cultures *in vitro* therefore differs from the regulation of tyrosine aminotransferase (EC 2.6.1.5), which is induced by glucocorticoids and by cyclic AMP or glucagon separately and in combination with glucocorticoids [26–28]. It also differs from the regulation of cytosolic aspartate aminotransferase (EC 2.6.1.1), which is increased by glucagon but not by dexamethasone when present as the sole hormone [29].

The present study also shows that the ratio of fructose biphosphatase/pyruvate kinase was 3-fold higher in hepatocytes from hypophysectomized rats compared with normal rats, but the ratio between periportal and perivenous hepatocyte populations was preserved in hypophysectomized rats. Previous studies showed that freshly isolated periportal hepatocyte suspensions from normal rats have higher rates of gluconeogenic flux than do perivenous hepatocytes [17], and that differences in metabolic flux between the two cell populations do not converge when the hepatocytes are maintained in culture [5]. In the present study, periportal and perivenous hepatocyte cultures from hypophysectomized rats showed similar differences in gluconeogenic flux as did periportal and perivenous hepatocyte cultures from normal rats [7]. This indicates that changes in the total activity of individual enzymes (pyruvate kinase and alanine aminotransferase) were not associated with the loss of acinar zonation (between periportal and perivenous hepatocytes) of either these enzymes or of the rates of gluconeogenic flux.

In normal rats, hepatocytes isolated from the periportal zone have a lower D-3-hydroxybutyrate/acetoacetate ratio during culture with glucagon than do perivenous hepatocytes, suggesting that they maintain a more oxidized mitochondrial redox state despite the higher rate of intramitochondrial production of reducing equivalents by β -oxidation [7]. This difference in redox state may be related to a higher rate of electron transport, which is consistent with the higher activity of cytochrome

oxidase in the periportal zone [30]. Hypophysectomy did not abolish the difference in the redox state between the periportal and perivenous hepatocytes.

Various hormones have a high fractional clearance during circulation through the liver, and it has been proposed that the concentration gradients of hormones across the liver may play an important role in the establishment and maintenance of the zonation of enzyme activities [1–4]. Few studies, however, have examined the effects of perturbation of the endocrine state *in vivo* on metabolic zonation within the acinus. Studies on the zonation of the activities of the gluconeogenic enzymes glucose-6-phosphatase and phosphoenolpyruvate carboxykinase have shown that induction of diabetes with alloxan or streptozotocin results in an increase in the activities of these enzymes without a significant change in their acinar zonation [8,9]. The present study shows that hypophysectomy, and the consequent endocrine perturbations, do not abolish the zonation of alanine aminotransferase, gluconeogenesis or the mitochondrial redox state. Direct evidence for significant changes in hepatic acinar zonation after perturbation of the endocrine state is lacking.

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