Regulation of fatty acid and carbohydrate metabolism by insulin, growth hormone and tri-iodothyronine in hepatocyte cultures from normal and hypophysectomized rats

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The interactions of insulin, growth hormone (somatotropin) and tri-iodothyronine (T_3) in the long-term (24 h) regulation of fatty acid and carbohydrate metabolism were studied in hepatocyte primary cultures isolated from normal or hypophysectomized Sprague–Dawley rats. Hepatocytes from hypophysectomized rats had similar rates of palmitate metabolism, but lower rates of ketogenesis, than hepatocytes from normal rats. They also had a lower endogenous triacylglycerol content and lower activities of NADP-linked dehydrogenases than did cells from normal rats. The inhibitions of ketogenesis and gluconeogenesis by insulin were more marked in hepatocytes from hypophysectomized than from normal rats. Insulin caused a 7–10-fold increase in cellular glycogen in hepatocytes from hypophysectomized rats, compared with a 2–3-fold increase in cells from normal rats, and it increased cellular triacylglycerol by 65% in cells from hypophysectomized rats, compared with 11% in cells from normal rats. In hepatocytes from hypophysectomized rats, growth hormone and T_3 increased ketogenesis both separately and in combination (12% and 23% respectively; $P < 0.05$), whereas in hepatocytes from normal rats only the combination of growth hormone and T_3 caused a significant increase in ketogenesis. In cells from hypophysectomized rats, T_3 and growth hormone had different effects on carbohydrate metabolism: T_3 , but not growth hormone, potentiated the anti-gluconeogenic and glycogenic effects of insulin. It is concluded that hypophysectomy increases the responsiveness of hepatocytes to insulin, growth hormone and T_3 , and that growth hormone and T_3 regulate fatty acid and carbohydrate metabolism by different mechanisms.

INTRODUCTION

Poorly controlled diabetes mellitus and episodes of ketoacidosis are frequently associated with high plasma concentrations of growth hormone (somatotropin) [1–3]. It is not certain, however, whether growth hormone contributes to the metabolic imbalance [4,5]. Growth-hormone infusions or pretreatment in normal subjects and diabetics cause stimulation of lipolysis in adipose tissue and production of ketone bodies in liver [6–9]. Since the latter does not always parallel the increase in plasma fatty acid, there may be regulation of ketogenesis at the hepatic level independent of substrate delivery [8,9]. However, it cannot be deduced from studies *in vivo* whether the increased hepatic ketogenesis results from a direct effect of growth hormone on the liver or from secondary endocrine changes.

Few studies have reported direct effects of growth hormone *in vitro* in hepatocytes. They include: acute inactivation of acetyl-CoA carboxylase in hepatocyte suspensions [10]; an increased activity of phosphatidate phosphohydrolase after 20 h culture of hepatocytes [11]; decreased activities of various lipogenic enzymes, but an increased activity of glucose-6-phosphate dehydrogenase, after 4–6 days culture [12]; increased glucose transport in hepatocytes from hypophysectomized, but not from normal, rats [13]; and increased production of insulin-like growth factor I (IGF I) in hepatocytes from normal, but not from hypophysectomized, rats [14]. Although

there have been several studies on the effects of growth hormone on carbohydrate and lipid metabolism in isolated adipocytes (for a review see [15]), the effects of growth hormone on fatty acid metabolism in hepatocytes have not been studied. In the present study we investigated the interactions of growth hormone, insulin and tri-iodothyronine (T_3) in regulating ketogenesis and carbohydrate metabolism in hepatocytes isolated from normal or hypophysectomized rats and maintained in monolayer culture.

MATERIALS AND METHODS

Materials

Human recombinant growth hormone (Somatorm) was from Kabivitrum (Stockholm, Sweden). Insulin (pig) and T_3 were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Sources of culture media and other biochemicals were as in [16].

Hepatocyte isolation and monolayer culture

Parenchymal hepatocytes were isolated either from male Sprague–Dawley rats (body wt. 323 ± 38 g for normal rats and 171 ± 11 g for hypophysectomized rats) obtained from Charles River U.K. Ltd. (Margate, Kent, U.K.) or from male Wistar rats (284 ± 64 g) obtained from Bantin and Kingman (Aldborough, Hull, U.K.) by collagenase perfusion of the liver [16]. The rats were fed

Abbreviations used: T_3 , 3,3',5-tri-iodo-L-thyronine; IGF I, insulin-like growth factor I.

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on standard rat chow *ad libitum*. Hypophysectomized rats were used between 1 and 3 weeks after surgery, during which time they gained less than 2 g/week, compared with approx. 35 g/week for normal Sprague-Dawley rats. The hepatocytes were suspended in Minimum Essential Medium supplemented with 5% (v/v) fetal bovine serum and plated in 25 cm² culture flasks as in [16].

After cell attachment (4–7 h) the medium was replaced with 4 ml of serum-free Minimum Essential Medium containing dexamethasone (10 nM) and the test hormones, each in duplicate flasks. Dexamethasone was used throughout this study because it helps preserve cell viability and potentiates the effects of insulin on lipid and carbohydrate metabolism [16]. The change to serum-free medium was designated zero time. The flasks were equilibrated with 5% CO₂ in air and incubated at 37°C, with a change of medium after 12 h, containing the same hormone concentrations as at time zero. Measurements of the rate of metabolic flux were made between 24 and 26 h in fresh hormone-free medium.

Determination of palmitate metabolism, ketogenesis and gluconeogenesis

After 24 h the medium was replaced with 2 ml of Minimum Essential Medium (containing 5 mM-glucose) and supplemented with 0.75 mM-palmitate, 1 mM-[3-¹⁴C]-pyruvate (0.2 Ci/mol), 0.5 mM-L-carnitine and 7.5 mg of defatted bovine serum albumin/ml, and the flasks were incubated at 37°C for a further 2 h. The medium was then decanted and a 1 ml portion was rapidly deproteinized with HClO₄ (0.33 M) for determination of acetoacetate, D-3-hydroxybutyrate [17] and [¹⁴C]glucose [18]. Fatty acid was measured in the untreated medium [19].

Determination of cellular glycogen, triacylglycerol and enzyme activities

The hepatocyte monolayers were rapidly rinsed with 2 × 5 ml of 150 mM-NaCl, extracted in buffer containing 50 mM-Tris, 100 mM-KCl, 20 mM-EDTA, 25 mM-KF and 0.05% (w/v) Lubrol PX, pH 7.9, and membranes were disrupted by sonication (4–6 μm, 20 s). Cellular triacylglycerol [20] and glycogen [17] were determined as previously described. Activities of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) [21], 6-phosphogluconate dehydrogenase (EC 1.1.1.44) [17] and NADP-linked malic enzyme (EC 1.1.1.40) [17] were determined at 30°C. Cellular protein was determined [22] with bovine serum albumin as standard.

Calculations and expression of results

Rates of uptake of palmitate and formation of ketone bodies (acetoacetate + D-3-hydroxybutyrate) were linear during the 2 h incubation and are expressed as nmol of substrate utilized or product formed/h per mg of protein. The rate of ketogenesis is expressed as a percentage of the maximal rate of ketogenesis from the palmitate taken up by the cells, assuming that 1 mol of palmitate can form a maximum of 4 mol of ketone bodies. Gluconeogenesis was determined from the incorporation of [3-¹⁴C]pyruvate into glucose, and is expressed as nmol of pyruvate incorporated/h per mg of protein. Cellular glycogen is expressed as nmol of glucosyl units/mg of protein. Enzyme activities are expressed as munits/mg of protein, where 1 munit is the amount converting 1 nmol of substrate/min at 30°C.

Results are expressed as means ± S.E.M. for duplicate flasks from the numbers of cell preparations indicated in parentheses. Statistical analysis was by Student's paired *t* test for comparisons of different hormonal treatments within the same group of cell preparations, and by the unpaired *t* test for comparisons between cells from hypophysectomized and normal (age-matched) rats or between cells from Sprague-Dawley and Wistar rats.

RESULTS

Palmitate uptake and ketogenesis in hepatocytes from Sprague-Dawley rats

Hepatocytes isolated from age-matched normal or hypophysectomized rats were cultured for 24 h with 10 nM-dexamethasone and the hormones shown in Table 1. Rates of palmitate uptake were similar in hepatocytes from normal and hypophysectomized rats and were unaffected by insulin, growth hormone or T₃, but hepatocytes from hypophysectomized rats had lower rates of ketogenesis (*P* < 0.005) and consequently, also, lower partitioning of palmitate towards ketogenesis than did hepatocytes from normal rats (Table 1). In hepatocytes from normal rats, insulin suppressed ketogenesis by 24–27% or by 16–17% in the absence or the presence of growth hormone respectively, and in hepatocytes from hypophysectomized rats the inhibition by insulin was 33–36% and 27–34% respectively (Table 1).

Growth hormone (1 μg/ml) caused small but significant (9–19%, *P* < 0.05) increases in ketogenesis in the presence of insulin and/or T₃ in hepatocytes from normal rats. In hepatocytes from hypophysectomized rats, growth hormone increased ketogenesis in the absence of insulin or T₃ (12%, *P* < 0.05) as well as in the presence of these hormones (25%, *P* < 0.005; Table 1). When the effects of growth hormone in hepatocytes from hypophysectomized rats were examined at different dexamethasone concentrations (0, 1, 10, 100 nM), growth hormone (1 μg/ml) increased ketogenesis by 9% in the absence of dexamethasone and by 27–33% in the presence of 1–100 nM-dexamethasone. Dexamethasone (100 nM) increased ketogenesis by 27% in the absence of growth hormone and by 41–54% in the presence of 10–1000 ng of growth hormone/ml (results not shown). T₃ (100 nM) increased ketogenesis in hepatocytes from normal rats only in the presence of growth hormone (11–14%, *P* < 0.05; Table 1), whereas in hepatocytes from hypophysectomized rats T₃ increased ketogenesis in the presence of dexamethasone alone (11–14%, *P* < 0.05) and also in the presence of growth hormone (12%, *P* < 0.05). The T₃ concentration half-maximally effective on ketogenesis was 6.5 nM (results not shown). The increase in ketogenesis by the combined presence of growth hormone and T₃ was higher in hepatocytes from hypophysectomized (35 and 23% increase in the presence and the absence of insulin respectively) than from normal rats (24 and 9% respectively; Table 1).

D-3-Hydroxybutyrate/acetoacetate production ratio

The 3-hydroxybutyrate/acetoacetate ratio reflects the mitochondrial NADH/NAD⁺ redox state if the activity of 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) catalyses near equilibrium [23]. Studies using freshly isolated hepatocyte suspensions [24] and also hepatocyte cultures (D. Tosh & L. Agius, unpublished work) have

Table 1. Effects of insulin, growth hormone (GH) and T_3 on fatty acid metabolism in hepatocyte cultures isolated from normal or hypophysectomized (Hypox) Sprague-Dawley rats

Hepatocyte monolayers were cultured with 10 nM-dexamethasone and other hormones as indicated: insulin (100 nM), T_3 (100 nM) and GH (μ g/ml), with a change of medium after 12 h, containing the same hormone concentrations as at zero time. Fatty acid utilization and the formation of ketone bodies (acetoacetate + 3-hydroxybutyrate) were determined between 24 and 26 h in hormone-free medium. Rates are expressed as nmol of substrate utilized or product formed/h per mg of protein. The formation of ketone bodies, expressed as a percentage of the rate of fatty acid uptake (KB/FA %), assuming that 1 mol of palmitate can form a maximum of 4 mol of ketone bodies, and the ratio of production rates of 3-hydroxybutyrate/acetoacetate are shown. Cellular triacylglycerol was determined at 26 h and is expressed as nmol/mg of protein. Values are means $\pm$ S.E.M. for duplicate flasks from the numbers shown in parentheses. Statistics (paired analysis): * $P < 0.05$, ** $P < 0.005$, insulin versus respective control; † $P < 0.05$, †† $P < 0.005$, GH versus respective control; ‡ $P < 0.05$, ‡‡ $P < 0.005$, T_3 versus respective control.

Additions	Fatty acid uptake (nmol/h per mg of protein)		Ketone-body formation (nmol/h per mg of protein)		KB/FA (%)		Hydroxybutyrate acetoacetate		Cellular triacylglycerol (nmol/mg of protein)	
	Normal (7)	Hypox (6)	Normal (7)	Hypox (6)	Normal (7)	Hypox (6)	Normal (7)	Hypox (6)	Normal (7)	Hypox (6)
None	204 $\pm$ 7	195 $\pm$ 24	291 $\pm$ 15	216 $\pm$ 7	38 $\pm$ 3	28 $\pm$ 4	1.4 $\pm$ 0.1	1.5 $\pm$ 0.2	82 $\pm$ 8	49 $\pm$ 7
GH	203 $\pm$ 16	225 $\pm$ 35	283 $\pm$ 9	241 $\pm$ 6†	38 $\pm$ 3	29 $\pm$ 5	1.4 $\pm$ 0.1	1.2 $\pm$ 0.1†	81 $\pm$ 8	59 $\pm$ 4
Insulin	201 $\pm$ 6	203 $\pm$ 22	214 $\pm$ 9**	144 $\pm$ 6**	27 $\pm$ 1**	17 $\pm$ 2*	1.7 $\pm$ 0.2**	1.2 $\pm$ 0.1*	91 $\pm$ 10*	81 $\pm$ 6**
Insulin + GH	190 $\pm$ 10	219 $\pm$ 25	234 $\pm$ 9**†	160 $\pm$ 7**††	32 $\pm$ 2*†	19 $\pm$ 2*	1.6 $\pm$ 0.2*	1.1 $\pm$ 0.1	86 $\pm$ 9	85 $\pm$ 6**
T_3	205 $\pm$ 9	224 $\pm$ 42	294 $\pm$ 14	241 $\pm$ 10†	38 $\pm$ 3	29 $\pm$ 6	1.2 $\pm$ 0.2†	0.9 $\pm$ 0.1††	83 $\pm$ 8	59 $\pm$ 4
T_3 + GH	209 $\pm$ 6	184 $\pm$ 26	316 $\pm$ 17†††	265 $\pm$ 13	40 $\pm$ 3	36 $\pm$ 5	1.2 $\pm$ 0.1	1.0 $\pm$ 0.9††	82 $\pm$ 8	61 $\pm$ 4
Insulin + T_3	200 $\pm$ 6	239 $\pm$ 37	223 $\pm$ 9**	155 $\pm$ 8**	29 $\pm$ 1**	17 $\pm$ 3*	1.5 $\pm$ 0.1**††	1.1 $\pm$ 0.1*	88 $\pm$ 9	84 $\pm$ 7**
Insulin + T_3 + GH	214 $\pm$ 12	220 $\pm$ 35	266 $\pm$ 9**††††	194 $\pm$ 10**††††	33 $\pm$ 1**††	22 $\pm$ 3**††	1.4 $\pm$ 0.1*†	1.1 $\pm$ 0.1*	83 $\pm$ 9	76 $\pm$ 6**

shown that for a particular hormonal condition, with increasing fatty acid concentration there is a parallel increase in the rate of ketogenesis (and presumably in the mitochondrial production of NADH by β -oxidation) and in the 3-hydroxybutyrate/acetoacetate ratio, suggesting that the ratio reflects the mitochondrial NADH/NAD⁺ couple [24]. In the present study, with different hormonal treatments the ratio D-3-hydroxybutyrate/acetoacetate did not parallel the changes in ketogenesis. T_3 decreased this ratio, despite the increase in ketogenesis (Table 1). Growth hormone caused a small decrease in this ratio in hepatocytes from hypophysectomized rats in the presence of dexamethasone (Table 1), and a more marked decrease in the absence of dexamethasone (results not shown), which itself decreases the ratio [16]. In hepatocytes from normal, but not from hypophysectomized, rats insulin increased the 3-hydroxybutyrate/acetoacetate ratio despite suppressing ketogenesis (Table 1).

Cellular triacylglycerol

The cellular triacylglycerol content of hepatocytes from hypophysectomized rats maintained without insulin was lower than in cells from normal rats (Table 1). However, when cultured with insulin there was a marked increase in triacylglycerol in cells from hypophysectomized rats (16–32 nmol/mg of protein) compared with cells from normal rats (5–6 nmol/mg of protein). This increase in triacylglycerol in cells from hypophysectomized rats is consistent with the low partitioning of palmitate towards ketogenesis in these cells (Table 1).

Gluconeogenesis and cellular glycogen

Insulin caused a greater percentage inhibition of gluconeogenesis in hepatocytes from hypophysectomized rats (42 or 64 % inhibition in the absence or the presence of T_3 respectively) than in cells from normal rats (31 or 32 %; Table 2). Growth hormone and T_3 had no significant effect on gluconeogenesis in the absence of insulin. However, in hepatocytes from hypophysectomized rats, T_3 potentiated the inhibition of gluconeogenesis by insulin (Table 2). The glycogen content in hepatocytes cultured without insulin was similar in hepatocytes from hypophysectomized and normal rats. However, insulin caused a larger increase in glycogen deposition in hepatocytes from hypophysectomized rats (7–10-fold increase) than in cells from normal rats (2–3-fold increase). In hepatocytes from hypophysectomized rats, T_3 potentiated the increase in glycogen deposition by insulin (Table 2).

NADP-linked dehydrogenases in hepatocytes from Sprague-Dawley rats

Previous studies have shown that growth hormone increases the activity of glucose-6-phosphate dehydrogenase, but decreases the activity of NADP-linked malic enzyme in hepatocytes after 4–6 days culture [12]. We determined the activities of these enzymes on termination of the culture (26 h after the first exposure to hormones). The activities of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and malic enzyme were lower (68 %, 67 % and 71 %; $P < 0.005$) in hepatocytes from hypophysectomized (4.2 ± 0.4 , 7.2 ± 1.3 and 2.7 ± 0.5 munits/mg of protein; $n = 6$) than from normal rats (13.0 ± 1.4 , 22.1 ± 1.8 and 9.2 ± 1.0 munits/mg of protein; $n = 7$). Insulin increased ($P < 0.005$) the

Table 2. Effects of insulin, growth hormone (GH) and T_3 on gluconeogenesis and cellular glycogen in hepatocytes from normal or hypophysectomized (Hypox) Sprague-Dawley rats

Hepatocyte monolayers were cultured with 10 nm-dexamethasone and the test hormones as indicated (concentrations were as described in Table 1). Gluconeogenesis was determined from the incorporation of [14 C]pyruvate into glucose (see the Materials and methods section) and is expressed as nmol incorporated/h per mg of protein. Cellular glycogen was determined at 26 h and is expressed as nmol of glucosyl units/mg of protein. Values are means $\pm$ S.E.M. for duplicate flasks from the numbers of cultures shown in parentheses. Statistics (paired analysis): * $P < 0.05$, ** $P < 0.005$, insulin versus respective controls; ‡ $P < 0.05$, †† $P < 0.005$, T_3 versus respective control.

Additions	State of rat ...	Gluconeogenesis (nmol of [14 C]pyruvate incorporated/h per mg of protein)		Cellular glycogen (nmol/mg of protein)	
		Normal (5)	Hypox (5)	Normal (6)	Hypox (6)
None		249 $\pm$ 41	223 $\pm$ 47	66 $\pm$ 6	42 $\pm$ 5
GH		216 $\pm$ 42	190 $\pm$ 42	67 $\pm$ 8	63 $\pm$ 6
Insulin		171 $\pm$ 36	130 $\pm$ 34**	167 $\pm$ 16**	405 $\pm$ 53**
Insulin + GH		209 $\pm$ 52	109 $\pm$ 20*	157 $\pm$ 17**	420 $\pm$ 51**
T_3		247 $\pm$ 46	188 $\pm$ 26	74 $\pm$ 9	74 $\pm$ 7††
T_3 + GH		255 $\pm$ 51	147 $\pm$ 24	83 $\pm$ 10††	85 $\pm$ 7††
Insulin + T_3		168 $\pm$ 36*	67 $\pm$ 22***†	175 $\pm$ 24**	469 $\pm$ 62**
Insulin + T_3 + GH		183 $\pm$ 42*	73 $\pm$ 19*†	179 $\pm$ 25***†	486 $\pm$ 62***†

Table 3. Fatty acid metabolism and cellular glycogen in hepatocytes from normal Wistar rats

Hepatocyte monolayers isolated from normal male Wistar rats were incubated with 10 nm-dexamethasone and the hormones as indicated (concentrations as described in Table 1). Expression of units are as in Tables 1 and 2. Values are means $\pm$ S.E.M. for duplicate flasks from the numbers of cultures shown in parentheses. Statistics (paired analysis): * $P < 0.05$, ** $P < 0.005$, insulin versus respective control.

Additions	Fatty acid utilization (nmol/h per mg of protein) (4)	Ketone-body formation (nmol/h per mg of protein) (4)	Cellular triacylglycerol (nmol/mg of protein) (4)	Cellular glycogen (nmol/mg of protein) (3)
None	209 $\pm$ 20	411 $\pm$ 26	58 $\pm$ 7	67 $\pm$ 11
GH	200 $\pm$ 21	395 $\pm$ 28	60 $\pm$ 8	77 $\pm$ 12
Insulin	209 $\pm$ 20	315 $\pm$ 25**	76 $\pm$ 9*	156 $\pm$ 20**
Insulin + GH	216 $\pm$ 19	335 $\pm$ 17**	75 $\pm$ 10*	143 $\pm$ 22**
T_3	194 $\pm$ 17	398 $\pm$ 21	60 $\pm$ 7	85 $\pm$ 10
T_3 + GH	207 $\pm$ 11	415 $\pm$ 14	61 $\pm$ 8	89 $\pm$ 9
Insulin + T_3	225 $\pm$ 18	328 $\pm$ 23**	78 $\pm$ 11*	151 $\pm$ 27*
Insulin + T_3 + GH	211 $\pm$ 16	351 $\pm$ 15**	76 $\pm$ 11*	139 $\pm$ 18*

activities of these enzymes in hepatocytes from hypophysectomized (6.5 ± 0.9 , 13.3 ± 0.5 and 3.8 ± 0.6) and normal rats (23.4 ± 1.6 , 30.1 ± 1.8 and 13.3 ± 1.6), but the activities in cells from hypophysectomized rats remained lower (72%, 56% and 71% respectively) than in cells from normal rats. T_3 and growth hormone, alone or in combination, had no effect on the activities of these enzymes after 26 h culture in hepatocytes from either normal ($n = 7$) or hypophysectomized ($n = 6$) rats (results not shown). This agrees with the previous report, which showed significant changes only after 4 days culture with growth hormone [12].

Hepatocytes from Wistar rats

Previous studies have indicated that there are differences in the plasma concentrations of glucose,

insulin, glucagon and possibly also growth hormone, and differences in glucagon receptors, between the Sprague-Dawley and Wistar strains of rats [25]. Experiments were carried out with hepatocytes from normal Wistar rats (Table 3) under similar conditions to those used above for Sprague-Dawley rats (Tables 1 and 2). Hepatocytes from Wistar rats had similar rates of palmitate uptake, but higher rates of ketogenesis (31–47%, $P < 0.005$), than did cells from Sprague-Dawley rats. Cellular triacylglycerol was slightly (9–29%), but not significantly, lower in hepatocytes from Wistar rats, whereas cellular glycogen was similar in cells from the two strains. The suppression of ketogenesis by insulin (18–23%) was similar to that in Sprague-Dawley rats (Table 1), but growth hormone and T_3 , alone or in combination, had no significant effect

on ketogenesis in hepatocytes from Wistar rats. The activity of glucose-6-phosphate dehydrogenase was lower (41–56 %, $P < 0.005$) than in cells from Sprague–Dawley rats, and was increased by insulin (114–149 %) but unaffected by growth hormone or T_3 (results not shown).

DISCUSSION

Effects of growth hormone on hepatocyte cultures

Although several studies *in vivo* in normal and diabetic subjects have provided evidence for an association between high plasma growth-hormone concentrations and increased ketogenesis [1–5], little is known about the direct effects of growth hormone on the liver. The present study shows that growth hormone causes small increases in ketogenesis in hepatocytes from hypophysectomized rats cultured with dexamethasone and with or without insulin or T_3 , suggesting that high plasma growth-hormone concentrations *in vivo* may contribute to the increased ketogenesis by a direct effect on the liver. The increase in ketogenesis was not associated with an increase in the total clearance of palmitate by the hepatocytes, and therefore suggests increased partitioning of palmitate towards ketogenesis.

Growth hormone has been shown to increase the activity of phosphatidate phosphohydrolase, which is involved in conversion of long-chain fatty acids into glycerolipid [11]. It appears that growth hormone has qualitatively similar effects on hepatocytes to those of glucocorticoids, which also increase phosphatidate phosphohydrolase activity [11], but nevertheless favour an increased partitioning of long-chain fatty acids towards mitochondrial ketogenesis [16] as opposed to glycerolipid formation. Dexamethasone decreases the concentration of glycerol 3-phosphate in hepatocyte cultures [16], and may therefore favour partitioning of fatty acids towards mitochondrial ketogenesis by decreasing the availability of the co-substrate for formation of glycerolipid. Growth hormone, unlike dexamethasone, did not affect the concentration of glycerol 3-phosphate (results not shown), suggesting that other mechanisms may be involved. Growth hormone may lower the malonyl-CoA concentration through inactivation of acetyl-CoA carboxylase [10]. Since malonyl-CoA is a potent inhibitor of carnitine palmitoyltransferase I, which catalyses the transfer of long-chain fatty acids into mitochondria [26], this would result in de-inhibition of the enzyme and thereby increased entry of fatty acids into mitochondria.

Human growth hormone binds to two types of receptors ('somatogenic' and 'lactogenic') in rat liver. The number of somatogenic receptors is increased after hypophysectomy [27] and decreased after a single injection of growth hormone [28]. Previous studies have shown that growth hormone increases glucose transport and oxidation in hepatocytes from hypophysectomized, but not from normal, rats [13], but it increases the production of IGF I in hepatocytes from normal, but not from hypophysectomized, rats [14]. Whether the two types of receptors mediate different effects of growth hormone is not known. In the present study, small effects of growth hormone on ketogenesis in cells cultured with insulin or T_3 were observed in hepatocytes from both normal and hypophysectomized rats, but in the absence of these hormones growth hormone increased ketogenesis

only in hepatocytes from hypophysectomized rats. Studies *in vivo* have suggested that insulin [29] and T_3 [30] increase the number of growth-hormone receptors. Whether these hormones also increase growth-hormone receptors in culture is not known. Differences in responsiveness of hepatocytes from normal and hypophysectomized rats to growth hormone may reflect differences in receptor number.

Effect of T_3 on hepatocyte cultures

Studies on rats with altered thyroid status have indicated a role for thyroid hormones in the regulation of hepatic fatty acid and glucose metabolism. Perfused livers from hyperthyroid rats have increased production rates of ketone bodies and decreased production of triacylglycerol compared with livers of euthyroid rats [31], presumably owing to the higher activity of carnitine palmitoyltransferase I in livers of hyperthyroid rats [32]. A direct effect of T_3 on hepatic fatty acid metabolism *in vitro* has not been demonstrated. The present finding that T_3 in the absence of insulin increases ketogenesis in hepatocytes from hypophysectomized rats indicates that T_3 directly regulates hepatic ketogenesis. The present study also shows that T_3 potentiates the glycogenic and anti-gluconeogenic effects of insulin. This agrees with other studies using cultured hepatocytes, where T_3 potentiated the effects of insulin on the induction of enzymes involved in lipogenesis [33].

Sprague–Dawley and Wistar rats

It is recognized that there are differences in the plasma concentrations of various hormones and also in hormone receptors between the commonly used Wistar and Sprague–Dawley strains of rats [25]. There have been few comparative studies between these two strains, although frequently discrepancies between results from different laboratories are attributed to the different strains of rats used. In this study, the rates of fatty acid uptake were similar in hepatocytes from Wistar and Sprague–Dawley rats; however, rates of ketogenesis were higher in cells from Wistar rats. Since Wistar rats have higher fasting plasma glucagon concentrations, whereas Sprague–Dawley rats have higher non-fasting plasma insulin concentrations [25], a higher glucagon/insulin ratio in Wistar rats may explain the higher rate of ketogenesis and possibly also the lower activities of NADP-linked dehydrogenases in hepatocytes from Wistar rats. Although the effects of growth hormone on ketogenesis in hepatocytes from normal Sprague–Dawley rats were small, they were observed consistently. This was not the case with cells from Wistar rats. In other experiments (D. Tosh, S. Betley & L. Agius, unpublished work) on hepatocytes from normal Wistar rats which were cultured with growth hormone for either a shorter (16 h) or a longer period (48 h), effects of growth hormone similar to those observed in cells from Sprague–Dawley rats were observed only in some experiments (9 out of 19), and consequently were not statistically significant. It has been shown that a single injection of growth hormone causes acute down-regulation of hepatic growth-hormone receptors in hypophysectomized rats [34]. The variability in the effects of growth hormone in hepatocytes from Wistar rats may be related to the variability in plasma growth-hormone concentration in this strain of rats [25].

Effects of hypophysectomy on hepatocyte metabolism and insulin-responsiveness

Hypophysectomy is associated with a decrease in the plasma concentrations of various hormones, including growth hormone, thyroid hormones, glucocorticoids, insulin and glucagon [35,36], and a hypersensitivity to insulin *in vivo* [15]. The number of insulin receptors appears to be unchanged in livers of hypophysectomized rats, although the number of growth-hormone receptors is increased [28]. The present study shows clearly that hepatocytes from hypophysectomized rats are more responsive to insulin in terms of stimulation of glycogen deposition. The effects of insulin on ketogenesis, gluconeogenesis and cellular triacylglycerol deposition were also more marked than in cells from normal rats, although the difference was smaller than for glycogen deposition. The understanding of the action of insulin on metabolic pathways in the liver has lagged behind that in other tissues, because the effects of insulin in most hepatocyte preparations are small. The present findings suggest that hepatocytes from hypophysectomized rats may be a good model for the study of the post-receptor events of insulin in hepatocytes.

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