INFLUENCE OF INSULIN AND GLUCAGON ON THE SYNTHESIS OF GLYCEROLIPIDS IN RAT HEPATOCYTES

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1. Introduction

Although the pathways involved in the formation of hepatic glycerolipids have been largely established more than a decade ago [1], very little information is available on the possible role of hormones in the regulation of glycerolipid synthesis in the liver [2]. Progress in this field was hampered by the lack of an in vitro system which will maintain hepatic glycerolipid synthesis for extended periods of time and will exhibit hormone induced alterations in hepatic lipid metabolism. This problem could be overcome with the non-proliferating monolayer cultures of adult rat hepatocytes [3]. These preparations exhibit many functional [3–8] and morphological [9,10] features of normal adult liver for extended periods of time.

We observed [11] that in rat liver the rates of synthesis of triacylglycerols (TG), phosphatidylcholines (PC) and phosphatidylethanolamines (PE), which share 1,2-diacyl-*sn*-glycerols as common precursor, could be changed by dietary alterations. This observation suggested the possible involvement of insulin and glucagon in the regulation of the biosynthesis of these glycerolipids since the ratio of insulin and glucagon is determined largely by the dietary state of the animal [12]. Recently it could be demonstrated that physiological concentrations of insulin accelerated the synthesis of fatty acids by hepatocytes maintained as monolayers, whereas low levels of glucagon inhibited this process [8,10].

This paper reports the effects of insulin and glucagon on the synthesis of TG, PC and PE in adult hepatocytes in monolayer culture using [U-^{14}C]glucose or [2-^{14}C]glycerol as precursors for the glycerol backbone and either [1-^{14}C]palmitic acid or [1-^{14}C]-acetate as precursors for the fatty-acyl moieties of the glycerolipids. The results indicate that under conditions of increased glucagon concentrations, diacylglycerols may be utilized preferentially for phospholipid synthesis.

2. Methods and materials

Male Wistar rats, 250–300 g, were meal-fed a standard laboratory chow. Hepatocytes were isolated essentially according to [13] as described [14]. After isolation the cells were suspended in the medium described [7], except that insulin was omitted. Hepatocyte preparations were employed only if more than 90% of the cells excluded trypan blue in fresh smears. Cell counting was performed with a Bürker chamber. Cell suspensions were diluted with medium to give approx. 5.0×10^5 cells/ml. Cell suspension, 4 ml, was added to vented plastic Petri dishes (Falcon, 60 mm) for plating [7]. The dishes were kept in equilibrium with a 95% oxygen and 5% carbon dioxide gas mixture. Temperature was maintained at 37°C throughout the isolation, plating and incubation periods. Full details of the procedure for cell isolation and culturing have been described [10].

After 3 h plating, the medium was removed by aspiration and 4.0 ml fresh medium were added with or without insulin (85 nM) or glucagon (10 nM). After another 3 h [1-^{14}C]acetate (10 mM, spec. act. 0.125 Ci/mol), L-[U-^{14}C]lactate (10 mM, spec. act. 0.1 Ci/mol), [2-^{14}C]glycerol (0.5 mM, spec. act. 1 Ci/mol), D-[U-^{14}C]glucose (a trace was added to

the unlabelled glucose, already present in the medium; final conc. 11.64 mM, spec. act. 0.43 Ci/mol) or [1-^{14}C]palmitate (0.52 mM, spec. act. 0.1 Ci/mol) were added to the incubation mixture to estimate the rate of lipid synthesis. All additions of hormones and substrates were in 50 μl quantities. After incubation for an additional hour, the medium was removed by aspiration and the cells were washed 3 times with ice-cold 0.15 M KCl. Subsequently the cells were scraped off with a rubber policeman and transferred to a test tube for total lipid extraction according to Sundler et al. [15]. Separation and analysis of lipids were carried out as in [16]. Protein was estimated according to [17].

Collagenase, type II, was obtained from Worthington (Freehold, NJ), and components of the culture media from Flow (Irvine, Scotland). Radioactive compounds were purchased from The Radiochemical Centre (Amersham, England). Insulin and glucagon were gifts from Dr Walter Shaw (Eli Lilly Labs., Indianapolis, IN).

3. Results

The effect of insulin and glucagon on the synthesis of TG, PC and PE were investigated in monolayers of hepatocytes derived from meal-fed, adult rats.

Table 1 shows the rates of incorporation of label from [U-^{14}C]glucose and [2-^{14}C]glycerol into TG, PC and PE. In separate experiments (not shown) it was demonstrated that the formation of TG, PC and PE proceded at linear rates up to at least 60 min incubation. The radioactivity from both [U-^{14}C]-glucose and [2-^{14}C]glycerol was recovered almost completely in the glycerol portion of these glycerolipids. Addition of insulin did not affect the synthesis of glycerolipids from [U-^{14}C]glucose. Glucagon, however, significantly inhibited the rates of synthesis of the various glycerolipids from [U-^{14}C]glucose, although the formation of TG was suppressed more severely than that of either PC or PE.

Insulin did not exert a significant effect on the rate of formation of glycerolipids from [2-^{14}C]glycerol or on the distribution of this precursor among TG, PC and PE. In contrast to its inhibitory effect on the incorporation of [U-^{14}C]glucose, glucagon enhanced the entry of [2-^{14}C]glycerol into PC and PE but it did not significantly influence the formation of TG.

Table 2 shows the effect of insulin and glucagon on the rate of incorporation of [1-^{14}C]acetate and [1-^{14}C]palmitate into TG, PC and PE. Insulin enhanced total glycerolipid synthesis from [1-^{14}C]-acetate. The % stimulation was about the same for both TG and the phospholipids PC and PE. On the other hand, insulin did not significantly influence the incorporation of exogenously added palmitic acid into the various glycerolipids. Addition of glucagon to the cell cultures resulted in a general decrease in the rate of synthesis from [1-^{14}C]acetate; the syntheses of TG, PC and PE were affected to about the same degree. With [1-^{14}C]palmitic acid as substrate, the

Table 1
The effects of insulin and glucagon on the rates of synthesis of glycerolipids from D-[U-^{14}C]glucose or [2-^{14}C]glycerol in hepatocyte monolayer cultures

Substrate	Hormone	TG	PC	PE
		(nmol substrate incorporated/mg protein.h)		
[U-^{14}C]Glucose	None	0.34 ± 0.09	0.61 ± 0.10	0.14 ± 0.03
	Insulin	0.47 ± 0.19	0.81 ± 0.21	0.16 ± 0.05
	Glucagon	0.09 ± 0.01[a]	0.30 ± 0.03[a]	0.07 ± 0.01[a]
[2-^{14}C]Glycerol	None	0.91 ± 0.03	0.70 ± 0.06	0.21 ± 0.06
	Insulin	0.95 ± 0.15	0.95 ± 0.20	0.29 ± 0.05
	Glucagon	1.01 ± 0.11	1.18 ± 0.12[a]	0.39 ± 0.04[a]

[a] Different from the control with $p < 0.01$

Each value represents the average ± standard deviation of at least 3 incubations

Table 2
Effect of insulin and glucagon on the rates of synthesis of glycerolipids from [1-^{14}C]acetate or [1-^{14}C]palmitate in hepatocyte monolayer cultures

Substrate	Hormone	TG	PC	PE
		(nmol substrate incorporated/mg protein.h)		
[1-^{14}C]Acetate	None	7.09 ± 0.45	6.80 ± 0.10	1.67 ± 0.05
	Insulin	10.85 ± 0.32[a]	9.52 ± 0.57[a]	2.39 ± 0.09[a]
	Glucagon	2.66 ± 0.18[a]	2.32 ± 0.20[a]	0.65 ± 0.06[a]
[1-^{14}C]Palmitate	None	53.23 ± 3.27	15.79 ± 0.61	1.74 ± 0.02
	Insulin	56.12 ± 1.07	16.54 ± 1.37	1.85 ± 0.16
	Glucagon	33.79 ± 3.86[a]	14.92 ± 0.93	1.54 ± 0.13

[a] Different from the control with $p < 0.01$

Each value represents the average ± standard deviation of at least 3 incubations

effect of glucagon was more specific. Whereas glucagon inhibited the formation of TG, it did not affect the formation of either PC or PE.

L-[U-^{14}C]lactate was also investigated as a precursor for TG, PC and PE formation. Although lactate entered predominantly into the fatty-acyl portion of the glycerolipids, a significant proportion was recovered in the glycerol backbone. As could be expected, the incorporation of L-[U-^{14}C]lactate into the fatty-acyl moieties of TG, PC and PE responded to the addition of glucagon and insulin as described for [1-^{14}C]acetate, whereas the entry of lactate into the glycerol portions of the lipids was affected by the hormones as described for [2-^{14}C]glycerol (data not shown).

4. Discussion

The present study concentrates on the possible effects of glucagon and insulin on the synthesis of TG, PC and PE in adult hepatocytes in monolayer cultures. Since it is generally accepted [18] that the glycerol moiety of hepatic glycerolipids may originate either from plasma free glycerol or from glucose, radioactively labelled glucose and glycerol were chosen to monitor the formation of the glycerol backbone of TG, PC and PE. The observed rates of glycerolipid formation from 0.5 mM glycerol compare favourably with those reported for isolated hepatocytes in suspension [15,16] and for normal rat liver in vivo [15]. It is interesting that the rates of incorporation of glucose and glycerol respond differently to the addition of glucagon. The inhibition by glucagon of glycerolipid formation from glucose may be best explained by an inhibition of glycolysis [19]. This will impair the supply of glycerol-3-phosphate and thereby limit the synthesis of glycerolipids. It is intriguing to note, however, that the synthesis of PC and PE is affected much less than that of TG indicating that diacylglycerols are channeled preferentially into phospholipids under a glucagon load.

Glucagon stimulates the entry of labelled glycerol into phospholipids which may be related to the enhanced gluconeogenesis under these conditions [20]. This is also suggested by the increased incorporation of lactate into the glycerol portion of the phospholipids if glucagon is added. In this light it is relevant to mention that recent evidence indicates that triosephosphates produced during gluconeogenesis may be important precursors for the glycerol portion of liver phospholipids [21]. It is of interest to note that glucagon does not enhance the incorporation of glycerol into TG: again the diacylglycerols seem to be utilized preferentially for phospholipid synthesis under these conditions. Although there is increasing evidence that phosphatidate phosphohydrolase plays an important role in the regulation of hepatic glycerolipid synthesis [22,23], the specific effects of glucagon suggest the presence of an additional regulatory point at the level of the final steps of the de novo synthesis of the glycerolipids. In contrast to glucagon, insulin did not significantly affect the formation of glycero-

lipids from either glucose or glycerol.

The observed rates of incorporation of labelled acetate and palmitate as well as their distribution among TG, PC and PE are in good agreement with data reported for isolated hepatocytes in suspension [16,24]. Table 2 shows that insulin stimulates the formation of all 3 glycerolipids about equally when acetate is used as precursor, which is in agreement with studies on perfused liver [25]. Glucagon shows an opposite effect. Whether this is due to an increased, respectively decreased, availability of endogenously synthesized fatty acids or to a direct effect on the utilization of these fatty acids for glycerolipid synthesis, cannot be concluded from the present experiments. In this respect it is of interest to note that insulin does not affect the formation of glycerolipids from exogenously added palmitic acid, although a direct comparison is difficult because of the much higher rates of synthesis in the presence of 0.52 mM palmitate than with 10 mM acetate. The inhibitory action of glucagon on the synthesis of glycerolipids from palmitate is limited to TG, observed with isolated hepatocytes in suspension [24]. This points again to a preferential utilization of diacylglycerols for the synthesis of phospholipids under conditions of increased glucagon.

Glucagon is undoubtedly involved in the regulation of hepatic lipid metabolism. The concentration in the blood increases in fasting [26] corresponding with a decrease in fatty acid synthesis [27] and an increase in fatty acid oxidation [24]. The present paper shows that under this condition the increased glucagon may also be important in maintaining the rate of phospholipid synthesis at the expense of triacylglycerol formation.

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