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The in vivo regulation of hepatic and renal glucose-6-phosphatase by thyroxine

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Abstract

The hepatic and renal microsomal glucose-6-phosphatase enzymes are situated with their active site in the lumen of the endoplasmic reticulum and for normal enzyme activity in vivo transport systems are needed for the substrates and products of the enzyme. We have shown that thyroxine activates the kidney glucose-6-phosphatase enzyme and the liver glucose 6-phosphate transport systems. In contrast, in hypophysectomised and adrenalectomised animals, thyroxine activates the transport systems and the enzyme in both liver and kidney.

Keywords: Glucose-6-phosphatase; Microsome; Liver; Kidney; Thyroid

1. Introduction

Glucose is the primary energy source for most mammalian cells and tissues. Most tissues cannot make significant amounts of glucose, and it is therefore important that blood glucose levels are maintained within a narrow range. The liver (and to a lesser extent, the kidney) plays an important role in blood glucose homeostasis [1,2]. Whenever blood glucose levels fall, e.g., between meals, after exercise and at times of stress or increased metabolic rate, the liver rapidly releases glucose into the bloodstream via the plasma membrane transport protein, GLUT2, for use by other tissues, e.g., [2,3]. The liver can produce glucose via two pathways, gluconeogenesis and glycogenolysis, which have a common terminal step catalysed by microsomal glucose-6-phosphatase (EC 3.1.3.9) [1,2]. The hepatic glucose-6-phosphatase enzyme is on the lumenal side of the endoplasmic reticulum [4–6] which makes in vivo glucose-6-phosphatase activity dependent on endoplasmic reticulum transport systems for the substrate glucose 6-phosphate and the products glucose and phosphate [1,7,8].

Glucose-6-phosphatase activity has been shown to increase under a variety of conditions where hepatic glucose output increases, e.g., starvation and diabetes [1,2,9]. Thyroxine has been reported to increase the overall metabolic rate (e.g., see [10] for review). There is also evidence in the literature of increased hepatic gluconeogenic capacity after thyroid hormone treatment (e.g., see [10–12]) as well as a wide variety of other effects on energy metabolism [10,13]. It seems likely, therefore, that thyroxine might also act on glucose-6-phosphatase. Early work (over 20 years ago) studying the effects of thyroxine and/or triiodothyronine on metabolism, e.g., [9,14–21] indicated that it increased glucose-6-phosphatase activity. However, this early work on the effects of thyroxine on glucose-6-phosphatase is difficult to interpret as it was done before the demonstration that glucose-6-phosphatase is a complex, multicomponent system in the endoplasmic reticulum and because the studies were done under a wide variety of different conditions using different assay techniques. Recently we have demonstrated (Voice et al., submitted for publication) that the liver and kidney glucose-6-phosphatase systems are regulated differently. We have therefore carried out a kinetic analysis of both the liver and kidney glucose-6-phosphatase systems in normal, adrenalectomised and hypophysectomised rats treated with thyroxine. The surgically altered animals were used because they are obvious models to study the effects of thyroxine without counter regulation by the respective endogenous hormones.

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2. Materials and methods

2.1. Materials

Glucose 6-phosphate (monosodium salt), histone 2AS and thyroxine were purchased from Sigma (Poole, UK). All other chemicals (AnalaR grade) were purchased from BDH (Poole, UK).

2.2. Treatment of animals

Adult Wistar male and female rats (approx. 9 weeks of age) were used throughout. All animals had free access to food and water. Adrenalectomised and hypophysectomised animals were obtained from Charles River (Margate, UK) and were allowed five weeks recovery after the surgical procedures. Drinking water was supplemented with 0.9% sodium chloride for the adrenalectomised animals and 0.5% sucrose/0.9% sodium chloride for the hypophysectomised animals.

In hormone treated animals, 4 doses of 0.15 mg/100 g body weight thyroxine were administered at 24 h intervals by intraperitoneal injection. Animals were killed 30 min after the final injection and the livers removed.

2.3. Microsome preparation

Microsomes were prepared from 10% tissue homogenates in 0.25 M sucrose/5 mM Hepes (pH 7.4) as described in [22]. The microsomal pellets were resuspended to a final protein concentration of approx. 20 mg/ml, aliquoted and stored frozen at −70°C until use (within 1 month).

2.4. Enzyme assays

Glucose-6-phosphatase activity was determined as in [23] using concentrations of glucose 6-phosphate of 1, 1.4, 2, 2.6, 5 and 30 mM and 1 mM mannose 6-phosphate. The glucose-6-phosphatase activity of disrupted microsomes was assayed using substrates supplemented with 0.8 mg/ml histone 2AS [24]. Microsomes are isolated as a mixture of intact and disrupted vesicles and the proportion of intact vesicles in each preparation was quantified by measuring mannose-6-phosphatase activity, which is only measurable in disrupted structures [25]. All of the microsomal preparations used were at least 90% intact. The intact values in this paper have been corrected for the contribution by disrupted structures as previously described [23], which eliminates the large errors in activity measurements which occur if even a small proportion of the vesicles are disrupted. Microsomal protein content was determined using the method Lowry as modified by Peterson [26]. Full kinetic analysis of the data was carried out using multiple non-linear regression programs on a BBC microcomputer [27]. Latency is defined in this paper as the percentage of glucose-6-phosphatase activity in disrupted microsomes which is not evident in intact microsomes. Blood glucose levels were determined using a Beckman glucose analyser. Glycogen levels were determined following the method of [28]. Statistical analysis was performed using Student's *t*-test as described in [29].

3. Results

Glucose-6-phosphatase enzyme activity (V_{max}) in disrupted microsomes isolated from normal male rat liver was not significantly changed by thyroxine treatment (Table 1). In contrast, thyroxine more than doubled glucose-6-phosphatase enzyme activity in disrupted microsomes isolated from adrenalectomised and hypophysectomised male rat livers (Table 1). The K_m of the glucose-6-phosphatase enzyme in disrupted liver microsomes was not significantly affected by thyroxine treatment in any of the preparations (Table 1). Similar effects were also seen in disrupted microsomes isolated from female rat livers (e.g., in female hypophysectomised rats ($n = 3$) treated with thy-

Table 1
Effect of thyroxine treatment on normal, adrenalectomised and hypophysectomised male rat liver glucose-6-phosphatase activity

	Disrupted microsomes V_{max} (nmol/min per mg)	Disrupted microsomes K_m (mM)	Intact microsomes V_{max} (nmol/min per mg)	Intact microsomes K_m (mM)	Latency (%)
Normal					
Control ($n = 3$)	241 ± 19	0.5 ± 0.1	152 ± 14	3.4 ± 0.9	37
Thyroxine ($n = 3$)	285 ± 5	0.7 ± 0.1	234 ± 21 *	2.8 ± 0.7	18
Adrenalectomy					
Control ($n = 3$)	171 ± 10	0.4 ± 0.1	109 ± 8	2.4 ± 0.5	36
Thyroxine ($n = 3$)	383 ± 13 **	0.8 ± 0.2	252 ± 8 **	5.5 ± 1.2	34
Hypophysectomy					
Control ($n = 4$)	140 ± 10	0.3 ± 0.1	90 ± 10	3.5 ± 0.4	36
Thyroxine ($n = 4$)	416 ± 44 **	0.5 ± 0.2	227 ± 17 *	3.7 ± 0.1	45

Data are the mean ± S.E. from at least three different microsome preparations. * $P < 0.05$, ** $P < 0.01$ significantly different from corresponding control value.

Table 2
Effect of thyroxine treatment on normal, adrenalectomised and hypophysectomised male rat kidney glucose-6-phosphatase activity

	Disrupted microsomes V_{max} (nmol/min per mg)	Disrupted microsomes K_m (mM)	Intact microsomes V_{max} (nmol/min per mg)	Intact microsomes K_m (mM)	Latency (%)
Normal					
Control ($n = 3$)	210 ± 20	0.4 ± 0.1	150 ± 10	4.2 ± 0.9	29
Thyroxine ($n = 3$)	283 ± 5 *	0.6 ± 0.1	142 ± 18	2.4 ± 0.6	50
Adrenalectomy					
Control ($n = 3$)	256 ± 24	0.5 ± 0.1	160 ± 10	3.4 ± 1.9	38
Thyroxine ($n = 3$)	384 ± 6 **	0.7 ± 0.1	235 ± 8 **	6.6 ± 1.2	39
Hypophysectomy					
Control ($n = 4$)	260 ± 10	0.6 ± 0.1	140 ± 10	4.1 ± 0.4	46
Thyroxine ($n = 4$)	440 ± 59 *	0.3 ± 0.2	228 ± 24 *	4.5 ± 1.1	48

Data are the mean ± S.E. from at least three different microsome preparations. * $P < 0.05$, ** $P < 0.01$ significantly different from corresponding control value.

roxine the glucose-6-phosphatase activity (V_{max}) in disrupted microsomes was increased 2.5-fold without significantly altering the K_m).

Glucose-6-phosphatase system activity (V_{max}) in intact microsomes isolated from normal male rat liver was significantly increased by thyroxine treatment, without a significant K_m difference (Table 1). Glucose-6-phosphatase activity (V_{max}) in intact microsomes isolated from adrenalectomised and hypophysectomised male rat livers was more than doubled by thyroxine treatment without significant increase in the K_m (Table 1). Similar effects of thyroxine were seen in intact microsomes isolated from female rat livers (e.g., in female hypophysectomised rats ($n = 3$) treated with thyroxine the glucose-6-phosphatase activity (V_{max}) in intact microsomes was increased 1.8-fold without significantly altering the K_m).

In kidney, thyroxine treatment increased the V_{max} of glucose-6-phosphatase activity in disrupted microsomes in normal, adrenalectomised and hypophysectomised animals (Table 2). The K_m of the glucose-6-phosphatase enzyme in disrupted kidney microsomes was not significantly affected by thyroxine treatment in any of the preparations (Table 2). In intact kidney microsomes thyroxine treatment did not increase the glucose-6-phosphatase activity in normal animals but caused a significant increase in both adrenalectomised and hypophysectomised animals (Table 2). In all three cases, thyroxine treatment did not significantly alter the K_m of glucose-6-phosphatase activity in intact kidney microsomes (Table 2).

Thyroxine treatment did not significantly change blood glucose levels of normal, adrenalectomised and hypophysectomised rats (Table 3). Thyroxine dramatically lowered liver glycogen levels in normal and adrenalectomised animals and caused a smaller, not quite statistically significant decrease in liver glycogen in hypophysectomised rats. In kidney, thyroxine significantly decreased glycogen levels in normal rats but not in adrenalectomised and hypophysectomised rats (Table 3).

4. Discussion

The complexity of the glucose-6-phosphatase system, its dependence on microsomal intactness and the problem that individual components of the system are inhibited differently by commonly used buffers and the detergents

Table 3
Effect of thyroxine treatment on blood glucose and liver glycogen levels in normal, adrenalectomised and hypophysectomised rats

	Blood glucose (mM)	Liver glycogen (μg/mg microsomal protein)	Kidney glycogen (μg/mg microsomal protein)
Normal			
Control ($n = 3$)	4 ± 1	532 ± 17	396 ± 19
Thyroxine ($n = 3$)	7 ± 2	69 ± 8 **	128 ± 48 **
Adrenalectomy			
Control ($n = 3$)	9 ± 1	573 ± 73	134 ± 83
Thyroxine ($n = 3$)	9 ± 1	153 ± 56 *	160 ± 12
Hypophysectomy			
Control ($n = 3$)	5 ± 1	704 ± 103	216 ± 102
Thyroxine ($n = 3$)	7 ± 1	306 ± 142	170 ± 109

Data are the mean ± S.E. from at least three different microsome preparations. * $P < 0.05$, ** $P < 0.01$ significantly different from corresponding control value.

used to disrupt the microsomal membrane, means that great care has to be taken in experimental design and choice of assay conditions. Unfortunately, much of the previous work [9,14–21,30,31] on the effects of thyroxine on glucose-6-phosphatase was done before the information needed for optimum assay conditions was widely available, e.g., see [23,32,33]. This unfortunately means that, for a variety of different reasons, it is difficult to interpret whether or not thyroxine does increase glucose-6-phosphatase activity, e.g., [34] and whether, when changes have been reported, they were effects on transport systems or enzyme or whether they were merely due to decreased microsomal intactness.

To ensure that we would detect both regulation of glucose-6-phosphatase expression and short term effects of thyroxine we administered thyroxine for four days and then killed the animals within a short time of the final thyroxine administration. We chose this regime because early work [17] had suggested that the maximal effects of thyroid hormones on liver microsomal enzymes were seen after 60 h. The significant decreases in liver and kidney glycogen content following thyroxine treatment of normal animals (Table 3) indicate that the amounts of thyroxine administered caused substantial effects on both liver and kidney carbohydrate metabolism. The lack of effect of thyroxine treatment on blood glucose levels is presumably caused at least in part by supplementation of the animals' drinking water (freely available) with sucrose.

Glucose-6-phosphatase activity in intact microsomes is a measure of the combined rate of glucose-6-phosphatase enzyme and the glucose 6-phosphate, phosphate and glucose transport proteins. In disrupted microsomes the substrates have free access to the enzyme, therefore only the activity of the glucose-6-phosphatase enzyme is measured. In isolated control liver and kidney microsomes the transport proteins are normally rate limiting. This means that the glucose-6-phosphatase activity in intact microsomes is usually less than the activity in disrupted microsomes, consequently glucose-6-phosphatase activity in intact microsomes is described as latent. It is therefore necessary to assay the glucose-6-phosphatase activity in both intact and disrupted microsomal vesicles to distinguish between the effects of hormones on the transport capacity and the hydrolytic activity of the glucose-6-phosphatase system.

Thyroxine treatment of adrenalectomised and hypophysectomised male rats resulted in a more than 2-fold increase in the activity of the liver glucose-6-phosphatase enzyme in disrupted liver microsomes (Table 1), clearly demonstrating that thyroxine treatment increases the activity of the hepatic glucose-6-phosphatase enzyme. In contrast, there was not a significant increase in hepatic glucose-6-phosphatase enzyme activity in disrupted microsomes following thyroxine treatment of normal male animals (Table 1). Glucose-6-phosphatase activity in intact microsomes was significantly increased following thyroxine treatment without a significant K_m change in normal, adrenalectomised and hypophysectomised male rat liver (Table 1). In normal animals where thyroxine has no effect on the activity of the glucose-6-phosphatase enzyme, the increased activity of the whole glucose-6-phosphatase system in intact microsomes must be due to increased transport capacity. This is reflected by the decrease in latency (at V_{max}) seen following thyroxine treatment. It has been previously reported that in intact rat liver microsomes the rate-limiting step in G-6-P hydrolysis is the transport of G-6-P across the microsomal membrane via the transport protein T1 [35], therefore the logical target for the effect of thyroxine treatment in normal animals must be T1, although a minor effect on the other transport proteins cannot be ruled out. In disrupted microsomes, the K_m represents the K_m of the G-6-Pase enzyme alone, whereas in intact microsomes the K_m reflects the combined contributions of the enzyme and the various associated transport mechanisms. It has been suggested that in intact rat liver microsomes with glucose 6-phosphate as substrate a major component of the K_m of the system is the K_m of T1 [35]. However the lack of change in K_m of the intact system indicates that thyroxine treatment does not significantly lower the K_m of G-6-P transport.

In contrast, the latency of the glucose-6-phosphatase system is not decreased in adrenalectomised and hypophysectomised male rat liver after thyroxine treatment, indicating that the enzyme is activated together with the transport systems by thyroxine treatment. Studies in female rats gave very similar responses to thyroxine to those seen in male animals, indicating that levels of sex hormones in adult animals do not significantly modulate the effect of thyroxine on the glucose-6-phosphatase system.

Virtually nothing is known about the effects of thyroxine on glucose-6-phosphatase in kidney. We therefore also studied glucose-6-phosphatase activity in the kidney of the animals described above. Thyroxine increased glucose-6-phosphatase enzyme activity in normal adrenalectomised and hypophysectomised rat kidney microsomes although the increases were not as large as those seen in the adrenalectomised and hypophysectomised rat livers (Tables 1 and 2). Glucose-6-phosphatase system activity in intact kidney microsomes was only increased by thyroxine treatment in adrenalectomised and hypophysectomised animals. In thyroxine treated normal rat kidney, there is therefore an increase in the glucose-6-phosphatase enzyme activity without a concomitant increase in the transport capacity, which is reflected in the increased latency of the intact kidney microsomal glucose-6-phosphatase system. In contrast, in adrenalectomised and hypophysectomised rat kidney microsomes, the latency is not altered following thyroxine treatment, reflecting similar increases in both the enzyme activity and transport capacity of the system.

The liver and kidney glucose-6-phosphatase systems were activated similarly by thyroxine in the surgically altered animals. In contrast, in normal rats, the glucose-6-phosphatase enzyme was the major site of thyroxine action

in kidney, while microsomal transport was the major site of action in liver. This suggests that it is the counter regulation of the liver and kidney glucose-6-phosphatase systems that is tissue specific rather than the effect of thyroxine itself. The fact that in different circumstances thyroxine can change either the enzyme, transport systems or both explains the difficulties that are encountered when trying to interpret data from the previous literature, and determine the site of action of thyroxine on the glucose-6-phosphatase system. The different effect of thyroxine in the kidneys and livers of normal rats reinforces our previous findings (Voice et al., submitted for publication) of tissue specific regulation of glucose-6-phosphatase.

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