β -Adrenoceptor-agonist and insulin actions on glucose metabolism in rat skeletal muscle in different thyroid states

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1. The actions of the β -adrenoceptor agonist isoprenaline on glucose and glycogen metabolism, in the presence of various concentrations of insulin, were investigated in isolated soleus muscle preparations taken from eu-, hyper- and hypothyroid rats. 2. Hyperthyroidism, induced by 3,3',5-tri-iodo-D-thyronine (T_3) administration for 5 days, increased the rate of lactate formation and suppressed the rate of glycogen synthesis in soleus muscle in response to isoprenaline, even in the presence of physiological or supraphysiological insulin concentrations. 3. Hypothyroidism, induced by administration of 6-n-propyl-2-thiouracil for 4 weeks, decreased the rate of isoprenaline-stimulated lactate formation at all insulin concentrations, but significantly decreased the responsiveness of lactate formation only at low insulin concentrations. In the presence of 100 or 10000 μ -units of insulin/ml, the ability of isoprenaline to suppress the rate of glycogen synthesis was markedly impaired (inhibition at 100 μ -units of insulin/ml and 1 μ M-isoprenaline: eu- $72.6 \pm 2.9\%$; hypo- $41.0 \pm 2.1\%$; $P < 0.001$). 4. Hyperthyroidism had no effect on the number or affinity of β -adrenoceptors, defined by 125 I-pindolol binding, or β -adrenoceptor- or forskolin-stimulated adenylate cyclase activity in membrane preparations of gastrocnemius muscle, whereas hypothyroidism increased the β -adrenoceptor density and decreased the β -adrenoceptor-stimulated adenylate cyclase activity, without affecting the receptor affinity or forskolin-stimulated adenylate cyclase activity. 5. It is concluded that there is a complex interplay between insulin, catecholamines and thyroid hormones to regulate skeletal-muscle glucose metabolism. The changes observed in muscles in hypothyroidism may be explained, at least in part, by changes in β -adrenoceptor–G-protein–adenylate cyclase coupling affecting the generation of cyclic AMP and the regulation of some of the key enzymes of glycogen metabolism; in contrast, the changes observed in muscles in hyperthyroidism do not appear to result from alterations at the level of the receptor-mediated second-messenger generation.

INTRODUCTION

There is considerable evidence that thyroid hormones influence the responses of various tissues to catecholamines, both in man and in laboratory animals. There are clinical similarities between catecholamine and thyroid hormone excess in patients with thyrotoxicosis [1], and mononuclear leucocyte β -adrenoceptor density has been found to be increased in experimental hyperthyroidism in man [2]. Studies in experimentally induced hyperthyroidism in animals have shown enhanced catecholamine-stimulated myocardial contractility [3], phosphorylase activation [4] and catecholamine-stimulated thermogenesis [5], as well as increased β -adrenoceptor number in hearts of hyperthyroid animals [6,7] and enhanced lipolysis in adipose tissue from hyperthyroid rats [8]; all of these findings suggest an increased sensitivity of various tissues to catecholamines. Furthermore, adrenaline and noradrenaline administration has been demonstrated to cause a more pronounced hyperglycaemia in hyperthyroid, compared with euthyroid, rats, owing to a greater increase in the rate of hepatic glucose production [9]. On the other hand, hypothyroidism does not decrease the ability of adrenaline to increase the concentration of cyclic AMP in fat-cells [10], although it may decrease the number of β -adrenoceptors in sarcolemmal membranes prepared from rat skeletal muscle [11].

In contrast with these findings, there is evidence in man which

suggests that the metabolic changes observed in hyperthyroidism are not fully explained by increased sensitivity to catecholamines. Thus administration of the β -adrenoceptor antagonist propranolol to patients with hyperthyroidism does not decrease the rate of glucose turnover [12]; and in experimental hyperthyroidism, although the β -adrenoceptor densities in fat and skeletal muscle are increased, metabolic and haemodynamic sensitivity to adrenaline is not changed *in vivo* [13].

Skeletal muscle is quantitatively the most important tissue for glucose disposal in response to insulin [14]. Although there are numerous studies examining the effects of altered thyroid status on the response of adipose and cardiac tissues and blood cells to catecholamines [2–10,15], there is, to the authors' knowledge, only one study in hypothyroid rats examining the sensitivity of glycogen synthesis to isoprenaline in skeletal muscle [11]. The present studies were therefore undertaken to examine the sensitivity of glucose utilization to the β -adrenoceptor agonist isoprenaline and its interactive effects with insulin in soleus muscles isolated from hyper- and hypo-thyroid rats. In order to assess the β -adrenergic status of skeletal muscle in altered thyroid states, the binding characteristics of the β -adrenoceptors and their interaction with adenylate cyclase via a guanine-nucleotide-binding protein (G-protein) were also determined in sarcolemmal membranes prepared from gastrocnemius muscle. A preliminary communication of part of the studies reported here has been published [16].

Abbreviations used: T_3 , 3,3',5-tri-iodo-D-thyronine; G-protein, guanine-nucleotide-binding protein.

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MATERIALS AND METHODS

Materials

All chemicals and enzymes were purchased from the sources given previously [17,18]. Isoprenaline, GTP and forskolin were obtained from Sigma Chemical Co.; Na¹²⁵I (carrier-free) and cyclic [³H]AMP were obtained from Amersham International. Pindolol was generously given by Sandoz Pharmaceuticals, and was iodinated by the method of Barovsky & Brooker [19], to a theoretical specific radioactivity of 2175 Ci/mmol; ¹²⁵I-pindolol was used within 2 weeks of its synthesis.

Animal manipulations

Male Wistar rats (160–180 g) were purchased from Harlan-OLAC, Bicester, Oxon, U.K., and were kept in the Department's animal facility with free access to food and water. Hyperthyroidism was induced by intraperitoneal injection of 0.65 mg of 3,3',5-tri-iodo-L-thyronine (T₃)/kg body wt. daily for 5 days; animals were killed 24 h after the final injection. Hypothyroidism was induced by administration of 6-n-propyl-2-thiouracil for 4–5 weeks. The agent was dissolved in warm ethanol and was added to the drinking water (final concn. 0.5 mg/ml). In all cases, animals were killed by cervical dislocation after a 12 h fast.

Muscle incubations

Soleus muscle strips (25–35 mg) were prepared as described previously [18]. All muscle incubations were carried out in Krebs–Henseleit bicarbonate buffer containing 5.5 mM-glucose, 1.5 % (w/v) defatted BSA, and various concentrations of isoprenaline and/or insulin [17,20]. In experiments where the rate of glycogen synthesis was assessed, [U-¹⁴C]glucose was added to the incubation medium at 0.4 μ Ci/ml.

Metabolite and hormone determinations

The concentration of lactate in the incubation medium was measured enzymically [21]. The rate of incorporation of [¹⁴C]glucose into glycogen was measured as previously described [22].

Blood samples for adrenaline determination were obtained by cardiac puncture of animals anaesthetized with 2 % halothane in N₂O/O₂ (1:1). Samples were collected in heparinized syringes, and 1 mg of ascorbate/ml of plasma was added; samples were centrifuged, and 2 ml of plasma was taken for assay. To each sample was added 0.25 ml of dihydrobenzylamine (12.5 pmol, for standardization of recovery) in 0.1 M-HClO₄, and the pH was adjusted to 7.8–8.2 by adding 1 M-Tris/HCl/30 mM-EDTA, pH 8.6. Alumina (25 mg) was added; samples were vortex-mixed thoroughly and left on ice for 20 min. The plasma was aspirated and the alumina washed with 3 $\times$ 2 ml of distilled water. Catecholamines were eluted from the alumina by addition of 0.25 ml of 0.1 M-HClO₄, and 0.2 ml of the supernatant was used for h.p.l.c. analysis with electrochemical detection [23].

Membrane preparation

Freeze-clamped gastrocnemius muscles were stored at –70 °C. For membrane preparation, muscles were thawed on ice for 15 min, trimmed of fat and tendon and finely chopped with scissors. The muscle was weighed and homogenized in 20 vol. of 50 mM-Tris/HCl, pH 7.4, at 4 °C with a Polytron (setting 5–6; 3 $\times$ 15 s). Homogenates were passed through three layers of muslin, and the filtrate was diluted with a further 10 vol. of homogenization buffer and centrifuged at 40000 g for 15 min at 4 °C. The pellet was resuspended in 20 vol. of 50 mM-Tris/HCl/1 M-KCl, pH 7.4, and left on ice for 30 min. The membrane

fraction was harvested by centrifugation at 40000 g for 15 min at 4 °C, and the pellet was then washed twice by repeating the homogenization/centrifugation steps. The final sarcolemmal pellet was resuspended in 50 mM-Tris/HCl/2 mM-MgCl₂ at a protein concentration of 3 mg/ml and stored at 4 °C overnight before use, or frozen in liquid nitrogen and stored at –70 °C.

Radioligand-binding studies

Sarcolemmal membranes (300 μ g of protein) were incubated with 0.1–15 nM-¹²⁵I-pindolol in a total volume of 250 μ l for 30 min at 37 °C [24]. Non-specific binding was determined in the presence of 100 μ M-isoprenaline. Assays were terminated by addition of 4 ml of 50 mM-Tris/HCl/154 mM-NaCl, pH 7.4, followed by immediate vacuum filtration through GF/B filter discs and washing with 3 $\times$ 4 ml of the same wash-buffer. Radioactivity bound to filters was determined with a γ -radiation counter.

Adenylate cyclase assay

Assays were performed by a modification of the method in [25], and were initiated by addition of sarcolemmal membranes (150 μ g of protein) to medium containing 40 mM-Tris/HCl, 2 mM-MgCl₂, 1 mM-EDTA, 10 mM-phosphocreatine, 25 μ g of creatine kinase, 10 μ g of adenylate kinase, 20 μ g of BSA, 0.2 mM-ATP, 100 μ M-Ro 20 1724, pH 7.4, and the various concentrations of GTP, isoprenaline or forskolin stated in the Results section. Incubations were continued for 10 min at 37 °C and terminated by addition of 20 μ l of HCl. After standing on ice for 15 min, samples were centrifuged (4000 g, 20 min, 4 °C), the supernatant was neutralized by addition of 1 M-NaOH, and samples were taken for analysis of cyclic AMP concentration by the method of Brown *et al.* [26].

Data analysis

For each experiment, the dissociation constant (K_d) and maximal binding capacity (B_{max}) were determined by computer-assisted Scatchard analysis. Results are presented as means $\pm$ S.E.M. Statistical analysis was carried out by Student's *t* test for unpaired observations [27].

RESULTS

The effects of altered thyroid status on plasma T₃, insulin, glucose and fatty acid concentrations have been reported previously [17,28]. In common with previous studies, 5 days T₃ administration caused approx. 15-fold increases, and propylthiouracil administration caused a greater than 90 % decrease, in plasma T₃ levels. The concentration of adrenaline in plasma [3.7 ± 0.6 (9) pmol/ml] was not significantly altered after induction of hypothyroidism [2.2 ± 0.5 (6) pmol/ml], but was increased by T₃ administration for 5 days [9.2 ± 1.2 (5) pmol/ml; $P < 0.02$].

The density of β -adrenoceptor sites, defined by ¹²⁵I-pindolol binding, in sarcolemmal membranes prepared from gastrocnemius muscle was increased by 26 % after 4–5 weeks treatment *in vivo* with propylthiouracil, and was unaffected after 5 days treatment of animals with T₃ (Table 1). The affinity of β -adrenoceptors for ¹²⁵I-pindolol was unaffected by both manipulations of thyroid status.

β -Adrenoceptor-dependent and -independent regulation of adenylate cyclase activity in gastrocnemius-muscle sarcolemmal membranes was also investigated. Altered thyroid status had no effect on either basal or forskolin-stimulated adenylate cyclase activity (Table 1). However, the effect of the β -adrenoceptor

Table 1. Effects of altered thyroid status on β -adrenoceptor ^{125}I -pindolol binding and adenylate cyclase activity in gastrocnemius sarcolemmal membranes

Values are means $\pm$ S.E.M. for at least 4 separate gastrocnemius-muscle sarcolemmal-membrane preparations. B_{max} and K_D values were obtained by incubating membranes with at least 8 concentrations of ^{125}I -pindolol (0.1–15 nM) in the absence and presence of 100 μM -isoprenaline to define non-specific binding. Saturation data were analysed by Scatchard transformation. Adenylate cyclase activity was assayed as described in the Materials and methods section. Statistical significance (Student's t test for unpaired observations) is indicated as $^aP < 0.05$ for hypothyroid versus control, and $^bP < 0.01$ for hypothyroid versus control and hyperthyroid groups.

	Thyroid status		
	Control	Hypothyroid	Hyperthyroid
^{125}I -pindolol binding			
B_{max} (fmol/mg of protein)	14.1 $\pm$ 0.7	17.7 $\pm$ 1.2 ^a	15.5 $\pm$ 1.6
K_D (pM)	128 $\pm$ 20	92 $\pm$ 10	130 $\pm$ 22
Adenylate cyclase activity (pmol/min per mg of protein)			
Basal	35.8 $\pm$ 5.7	31.7 $\pm$ 3.4	40.3 $\pm$ 6.9
+ 10 μM -forskolin	186 $\pm$ 23	169 $\pm$ 31	181 $\pm$ 17
+ 10 μM -isoprenaline	81.7 $\pm$ 6.4	50.0 $\pm$ 7.0 ^b	87.3 $\pm$ 5.1
+ 100 μM -GTP			

Table 2. Effects of altered thyroid status on the rate of lactate formation in response to isoprenaline in stripped soleus muscles of the rat in the presence of different concentrations of insulin

Values are means $\pm$ S.E.M. of 5–16 separate incubations. Statistical significance for differences from control values is indicated as $^aP < 0.001$.

Insulin	Iso-prenaline (M)	Rate of lactate formation ($\mu\text{mol/h per g}$)		
		Control	Hypothyroid	5 days T ₃
(i) 1 $\mu\text{-unit/ml}$	0	7.03 $\pm$ 0.39	4.64 $\pm$ 0.59 ^a	15.65 $\pm$ 1.06 ^a
	10 ⁻⁹	8.67 $\pm$ 0.42	5.01 $\pm$ 0.37 ^a	17.95 $\pm$ 1.37 ^a
	10 ⁻⁸	9.77 $\pm$ 0.60	5.35 $\pm$ 0.32 ^a	18.35 $\pm$ 0.30 ^a
	10 ⁻⁷	11.76 $\pm$ 0.72	5.83 $\pm$ 0.65 ^a	23.75 $\pm$ 0.64 ^a
	10 ⁻⁶	11.94 $\pm$ 0.72	6.73 $\pm$ 0.27 ^a	22.96 $\pm$ 1.30 ^a
(ii) 100 $\mu\text{-units/ml}$	0	11.16 $\pm$ 0.51	5.24 $\pm$ 0.46 ^a	21.56 $\pm$ 1.45 ^a
	10 ⁻⁹	13.45 $\pm$ 0.62	6.82 $\pm$ 0.57 ^a	29.93 $\pm$ 0.60 ^a
	10 ⁻⁸	14.87 $\pm$ 0.81	7.84 $\pm$ 0.28 ^a	33.21 $\pm$ 1.12 ^a
	10 ⁻⁷	15.67 $\pm$ 0.73	8.61 $\pm$ 0.49 ^a	35.86 $\pm$ 1.01 ^a
	10 ⁻⁶	16.53 $\pm$ 0.81	7.98 $\pm$ 0.09 ^a	32.90 $\pm$ 0.99 ^a
(iii) 10000 $\mu\text{-units/ml}$	0	14.80 $\pm$ 0.44	6.14 $\pm$ 0.54 ^a	28.03 $\pm$ 1.24 ^a
	10 ⁻⁹	13.82 $\pm$ 0.76	7.56 $\pm$ 0.56 ^a	31.85 $\pm$ 0.68 ^a
	10 ⁻⁸	15.19 $\pm$ 1.87	7.38 $\pm$ 0.48 ^a	34.98 $\pm$ 1.65 ^a
	10 ⁻⁷	16.72 $\pm$ 1.25	8.02 $\pm$ 0.58 ^a	36.04 $\pm$ 1.24 ^a
	10 ⁻⁶	18.00 $\pm$ 1.32	9.40 $\pm$ 0.75 ^a	42.72 $\pm$ 1.31 ^a

agonist isoprenaline on adenylate cyclase activity (in the presence of 100 μM -GTP) was significantly decreased in sarcolemma isolated from hypothyroid rats. The concentration-response relationship for isoprenaline-stimulated adenylate cyclase activity is shown in Fig. 1(a). No differences were observed between the

Fig. 1. Effects of altered thyroid status on (a) isoprenaline-stimulated and (b) GTP-stimulated adenylate cyclase activity in gastrocnemius sarcolemmal membranes

Values are means $\pm$ S.E.M. for euthyroid ($\bullet$), hypothyroid (Δ) and 5-days- T_3 -treated ($\blacktriangle$) animals for at least 3 separate experiments performed in triplicate. Cyclic AMP concentration was measured in neutralized acid extracts as described in the Materials and methods section. Incubations were initiated by addition of 150 μg of membrane protein to medium containing (a) the indicated concentrations of isoprenaline + 100 μM -GTP or (b) the indicated concentrations of GTP + 10 μM -isoprenaline. All incubations were terminated after 10 min at 37 $^\circ\text{C}$. Statistical significance between the membranes isolated from hypothyroid compared with control animals is indicated as $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

membranes prepared from hyperthyroid and euthyroid groups, but adenylate cyclase activity was decreased at 0.1–10 μM -isoprenaline in membranes from hypothyroid rats. These data suggest, but by no means prove, that both the sensitivity and responsiveness of skeletal-muscle adenylate cyclase to isoprenaline are affected in hypothyroidism. The effect of GTP on isoprenaline-stimulated adenylate cyclase was also investigated (Fig. 1b); in the presence of 10 μM -isoprenaline, GTP caused a concentration-related increase in activity in sarcolemmal membranes isolated from control and hyperthyroid rats (control, 97 $\pm$ 12%; hyperthyroid, 85 $\pm$ 10%; for percentage changes shown in Fig. 1b), but this effect of GTP was significantly decreased in membranes from hypothyroid rats (59 $\pm$ 5%; $P < 0.05$). Taken together, these results suggest that the efficiency of β -adrenoceptor-G-protein-adenylate cyclase coupling is

Table 3. Effects of altered thyroid status on the rate of glycogen synthesis in response to isoprenaline in stripped soleus muscles of the rat in the presence of different concentrations of insulin

Values are means $\pm$ S.E.M. of 5–18 separate incubations. Statistical significance for differences from control values is indicated as $^aP < 0.05$ and $^bP < 0.01$.

Insulin	Iso-prenaline (M)	Rate of glycogen synthesis (μ mol of glucosyl units/h per g)		
		Control	Hypothyroid	5 days T ₃
(i) 1 μ -unit/ml	0	2.39 $\pm$ 0.22	1.90 $\pm$ 0.26	2.32 $\pm$ 0.25
	10 ⁻⁹	2.33 $\pm$ 0.24	1.50 $\pm$ 0.14 ^a	2.33 $\pm$ 0.40
	10 ⁻⁸	1.76 $\pm$ 0.20	1.69 $\pm$ 0.16	1.54 $\pm$ 0.24
	10 ⁻⁷	1.24 $\pm$ 0.17	1.18 $\pm$ 0.12	0.93 $\pm$ 0.08
	10 ⁻⁶	0.89 $\pm$ 0.09	1.06 $\pm$ 0.10	0.91 $\pm$ 0.13
(ii) 100 μ -units/ml	0	4.56 $\pm$ 0.28	4.32 $\pm$ 0.25	1.71 $\pm$ 0.14 ^b
	10 ⁻⁹	3.00 $\pm$ 0.23	4.41 $\pm$ 0.46 ^a	1.60 $\pm$ 0.18 ^b
	10 ⁻⁸	1.89 $\pm$ 0.20	3.36 $\pm$ 0.20 ^b	0.57 $\pm$ 0.08 ^b
	10 ⁻⁷	1.39 $\pm$ 0.14	3.29 $\pm$ 0.37 ^b	0.53 $\pm$ 0.05 ^b
	10 ⁻⁶	1.25 $\pm$ 0.10	2.55 $\pm$ 0.27 ^b	0.38 $\pm$ 0.03 ^b
(iii) 10000 μ -units/ml	0	6.11 $\pm$ 0.09	5.24 $\pm$ 0.11 ^b	5.43 $\pm$ 0.35
	10 ⁻⁹	6.92 $\pm$ 0.64	5.49 $\pm$ 0.65	5.09 $\pm$ 0.28 ^a
	10 ⁻⁸	6.11 $\pm$ 0.17	4.97 $\pm$ 0.57	4.40 $\pm$ 0.51 ^a
	10 ⁻⁷	4.98 $\pm$ 0.44	3.84 $\pm$ 0.15 ^a	2.99 $\pm$ 0.43 ^a
	10 ⁻⁶	2.33 $\pm$ 0.25	2.63 $\pm$ 0.14	0.66 $\pm$ 0.10 ^b

decreased in skeletal-muscle sarcolemmal membranes from hypothyroid rats.

The effects of altered thyroid status on rates of glucose utilization by isolated soleus muscle strips are shown in Tables 2 and 3. In the absence of isoprenaline, increasing the concentration of insulin stimulated the rate of lactate formation by 2-fold and glycogen synthesis 3-fold in muscles from control rats. The rate of lactate formation was decreased in muscles from hypothyroid rats and increased in muscles from hyperthyroid rats at all

concentrations of insulin (Table 2). The rate of glycogen synthesis was decreased only at 100 μ -units of insulin/ml in muscles from hyperthyroid rats, and at 10000 μ -units of insulin/ml in muscles from hypothyroid animals, in accordance with previous observations [17,28,29].

In the presence of isoprenaline (1 nM–1 μ M), the absolute rates of lactate formation were significantly increased at all concentrations of insulin in muscles from hyperthyroid rats (Table 2). However, since there was an increase in the basal rate of lactate formation, the results have also been calculated to show increases over basal values (see Fig. 2): this transformation of the data shows that the responsiveness of the rate of lactate formation (i.e. the change in the rate of lactate formation in the presence of 1 μ M-isoprenaline compared with basal values) was increased in muscle strips from 5-day-T₃-treated animals compared with the other two experimental groups in the presence of 100 and 10000 μ -units of insulin/ml ($P < 0.01$; Fig. 2). In addition, the sensitivity of lactate formation to isoprenaline [defined as the concentration of isoprenaline required to cause a half-maximal increase in the rate of lactate formation (EC₅₀)] was assessed. Unfortunately the response to isoprenaline in the presence of 10000 μ -units of insulin/ml in control muscle incubations was too small to allow accurate assessment of sensitivity; however, in the presence of 1 or 100 μ -units of insulin/ml there were no observed differences between muscles from hyperthyroid or control animals (EC₅₀ 10.2 $\pm$ 2.9 versus 14.0 $\pm$ 3.8 and 15.5 $\pm$ 5.8 versus 4.8 $\pm$ 2.0 nM for hyperthyroid and control animals in the presence of 1 or 100 μ -units of insulin/ml respectively).

In muscle strips from hypothyroid animals, the rates of lactate formation were lower than control values at all concentrations of insulin (Table 2); subtraction of the basal rate of lactate formation revealed a reduced responsiveness to isoprenaline compared to controls in the presence of 1 μ -unit of insulin/ml. However, responsiveness to isoprenaline in the presence of either 100 or 10000 μ -units of insulin/ml was similar to control values (Fig. 2). Because of the small stimulations of lactate formation above basal values by isoprenaline ($< 3 \mu$ mol/h per g), it was not possible to assess EC₅₀ values accurately (Fig. 2).

Fig. 2. Effects of altered thyroid status on the concentration-response relationship for isoprenaline stimulation of lactate formation in isolated soleus-muscle strips incubated in the presence of (a) 1, (b) 100 and (c) 10000 μ -units of insulin/ml

Values are means $\pm$ S.E.M. expressed as increments above the rate of lactate formation observed in the absence of isoprenaline, for muscles isolated from euthyroid ($\bullet$), hypothyroid (Δ) and 5-days-T₃-treated ($\blacktriangle$) animals. The numbers of experiments are given in Table 2. Statistical significance is indicated as $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ for hypothyroid compared with normal muscle incubations.

Table 4. Effects of altered thyroid status on the rate of [^{14}C]lactate formation in response to isoprenaline in rat soleus muscle in the presence of different concentrations of insulin

Values are means $\pm$ S.E.M. of 4–12 separate incubations. Statistical significance for differences from control values is indicated as ^a $P < 0.01$ and ^b $P < 0.001$.

Insulin	Iso-prenaline (M)	Rate of [^{14}C]lactate formation ($\mu\text{mol/h per g}$)		
		Control	Hypothyroid	5 days T_3
(i) 1 $\mu\text{-unit/ml}$	0	7.52 $\pm$ 1.67	4.91 $\pm$ 0.79	6.30 $\pm$ 0.28
	10 ⁻⁹	7.62 $\pm$ 0.24	4.67 $\pm$ 0.68 ^a	6.89 $\pm$ 0.58
	10 ⁻⁸	7.22 $\pm$ 1.47	5.27 $\pm$ 0.48	5.95 $\pm$ 0.22
	10 ⁻⁷	7.43 $\pm$ 1.40	4.40 $\pm$ 0.58	7.29 $\pm$ 0.76
	10 ⁻⁶	7.92 $\pm$ 1.40	5.44 $\pm$ 0.66	8.00 $\pm$ 0.46
(ii) 100 $\mu\text{-units/ml}$	0	11.26 $\pm$ 0.60	5.23 $\pm$ 1.09 ^b	17.58 $\pm$ 1.70 ^a
	10 ⁻⁹	11.13 $\pm$ 0.59	5.98 $\pm$ 1.15 ^a	16.40 $\pm$ 0.73 ^b
	10 ⁻⁸	10.29 $\pm$ 0.40	6.17 $\pm$ 0.65 ^b	16.53 $\pm$ 1.10 ^b
	10 ⁻⁷	10.81 $\pm$ 0.73	7.13 $\pm$ 0.65 ^a	21.29 $\pm$ 1.65 ^b
	10 ⁻⁶	11.18 $\pm$ 0.72	5.77 $\pm$ 0.20 ^b	16.69 $\pm$ 1.53 ^b

An assessment of the glucose-to-lactate flux by specifically measuring the rate of [^{14}C]lactate formation in muscles from animals in different thyroid states is shown in Table 4. In the presence of 1 $\mu\text{-unit}$ of insulin/ml and in the absence of isoprenaline, there is excellent agreement between [^{14}C]lactate and spectrophotometrically determined lactate accumulations for both control and hypothyroid groups (Tables 2 and 4). However, for the hyperthyroid group the rate of lactate formation from glucose is only about 40% of the rate of total lactate formation. Therefore, in agreement with our previous findings [28], there appears to be an increased rate of glycogenolysis at low insulin concentrations, despite a normal rate of [^{14}C]glucose incorporation into glycogen, suggestive of an enhanced rate of glycogen/glucose 1-phosphate cycling [30]. In the presence of 100 $\mu\text{-units}$ of insulin/ml the discrepancy between the rates of [^{14}C]lactate and total lactate formation in muscles from hyperthyroid animals is decreased; in this case glucose-to-lactate flux accounts for > 80% of total lactate formation (Tables 2 and 4). The effective suppression of glycogenolysis by insulin probably accounts for the fact that enhanced glycogen/glucose 1-phosphate cycling was not observed by us in a previous study [30].

The increases in the rates of lactate formation caused by isoprenaline addition in all cases reflect the increases in the rates of glycogenolysis, since the rates of [^{14}C]lactate formation were unaffected by increasing isoprenaline concentration (Table 4).

In the presence of 1 $\mu\text{-unit}$ of insulin/ml, isoprenaline caused comparable inhibitions of the rates of glycogen synthesis across all experimental groups (Table 3), although the inhibition observed in the presence of 1 μM -isoprenaline in muscles from hypothyroid rats was decreased (control, 63 $\pm$ 6%; hypothyroid, 44 $\pm$ 10%; T_3 -treated, 61 $\pm$ 8%). In the presence of 100 $\mu\text{-units}$ of insulin/ml, this decrease in isoprenaline-responsiveness for muscles isolated from hypothyroid rats was more marked (control, 73 $\pm$ 4%; hypothyroid, 41 $\pm$ 9%; $P < 0.01$), with a decrease in the sensitivity of this flux to isoprenaline also being apparent (Table 3). In contrast, if the difference in basal glycogen-synthesis rates were taken into account, no differences were observed, in terms of either responsiveness (78 $\pm$ 3% inhibition of glycogen-synthesis rate at 1 μM -isoprenaline) or sensitivity to isoprenaline. In the presence of 10000 $\mu\text{-units}$ of insulin/ml, high concentrations of isoprenaline (> 0.1 μM) were necessary to decrease

the rate of glycogen synthesis, which was independent of thyroid status (Table 3). In the presence of 1 μM -isoprenaline and 10000 $\mu\text{-units}$ of insulin/ml, the relative inhibition of the rate of glycogen synthesis was greater in muscles from hyperthyroid rats (88 $\pm$ 3%; $P < 0.01$), but it was decreased in muscles from hypothyroid rats (50 $\pm$ 3%; $P < 0.05$) compared with muscles isolated from control rats (62 $\pm$ 4%).

DISCUSSION

Detailed studies of the effects of changes in thyroid status in the rat induced experimentally *in vivo* have demonstrated that T_3 administration for 3–5 days increases the rate of glucose phosphorylation and glycolysis to lactate in isolated soleus muscle preparations, and may change the concentration–response relationships of these metabolic fluxes to insulin [28,29]. Recent reports that the rate of glucose transport and the expression of the insulin-sensitive glucose transporter (GLUT-4) are increased both in skeletal muscle of hyperthyroid rats [31] and in cultured skeletal-muscle myotubes maintained in the presence of T_3 [32] are in agreement with these findings. In contrast, induction of hypothyroidism does not affect basal rates of glucose phosphorylation, but it appears to increase the proportion of glucose 6-phosphate directed towards glycogen synthesis, leading indirectly to a decreased rate of glycolysis (i.e. lactate formation) [17,33]. Furthermore, the responsiveness of glycolysis in muscles from hypothyroid animals to insulin is decreased, and this may be explained by a decreased rate of glucose phosphorylation at high insulin concentrations [17].

In the present study, we have extended these initial observations by investigating the effects of the β -adrenoceptor agonist isoprenaline on glucose metabolism in muscle from animals in altered thyroid states in the presence of different concentrations of insulin. We have previously reported the interactive effects of catecholamines and insulin on glucose metabolism in isolated muscle preparations taken from euthyroid animals [20,30]. It has been shown that isoprenaline opposes the actions of insulin by increasing the rate of glycogenolysis and thus causing an increase in tissue glucose 6-phosphate concentration, which in turn inhibits the rate of glucose phosphorylation without affecting the rate of glucose transport [20]. In the hypothyroid state, basal and insulin-stimulated rates of glucose metabolism to lactate are decreased, owing to an increased proportion of glucose being incorporated into glycogen; the effect of isoprenaline appears to be dependent on the concentration of insulin present in the incubation medium. At low insulin concentrations (1 $\mu\text{-unit/ml}$), the rate of glycogen synthesis in the presence of increasing concentrations of isoprenaline is similar to that in muscle from euthyroid animals, whereas in the presence of 100 $\mu\text{-units}$ of insulin/ml, isoprenaline appears to be much less effective in decreasing the rate of glycogen synthesis, or, conversely, insulin is more effective in opposing the action of the β -adrenoceptor agonist. A similar conclusion can be made for the rate of lactate formation in the muscles isolated from the hypothyroid rats: although at low concentrations of insulin the responses were lower than those observed in control muscles, in the presence of physiological or supra-physiological concentrations of insulin the responses to isoprenaline were similar to those for muscles from control rats.

The complex interplay between catecholamines and insulin to regulate glycogen metabolism in skeletal muscle is, at least in part, dependent on β -adrenoceptor-stimulated generation of the second messenger cyclic AMP [34,35]. We have shown that, although β -adrenoceptor density is increased in gastrocnemius muscle of hypothyroid rats, the efficiency of coupling for the β -adrenoceptor–G-protein–adenylate cyclase interaction is

decreased. Since a similar effect has been observed in adipocytes isolated from hypothyroid rats [10], it may be a general adaptation in insulin-sensitive tissues [36]. Furthermore, it has been demonstrated in previous studies that isoprenaline-stimulated changes in cyclic AMP concentration are decreased in hypothyroidism in a range of skeletal muscles, including soleus [11]. Therefore, the simplest interpretation of the present results for the hypothyroid state is that an alteration in β -adrenoceptor-G-protein-adenylate cyclase coupling results in a decreased change in tissue cyclic AMP concentration at a given concentration of isoprenaline; this in turn would lead to a shift in the relative interactive effects of β -adrenoceptor agonists and insulin at the level of glycogen metabolism. The precise locus for the alteration in receptor-effector coupling is likely to be at the level of the G-protein, as the changes observed do not correlate with 125 I-pindolol binding, and direct activation of adenylylase by forskolin is unaffected by altered thyroid status. In addition, secondary adaptations in the activities or properties of the enzymes regulating glycogen synthesis and breakdown may also occur: the activity of glycogen synthase *a* has been reported to increase, and that of phosphorylase *a* to decrease, in skeletal muscle of hypothyroid rats [11,37].

For the T_3 -treated experimental group, a clear finding of the present study is that the responsiveness of the rate of lactate formation to isoprenaline is increased, despite the already elevated basal rates of lactate formation. This reflects the increase in the ability of isoprenaline to stimulate glycogenolysis, and to suppress glycogen synthesis in the hyperthyroid state, irrespective of the concentration of insulin present in the incubation medium. In this case, adaptive changes at the metabolic level are implicated, as no correlation with β -adrenoceptor-binding data, adenylylase activity or β -adrenoceptor-G-protein-adenylate cyclase coupling has been demonstrated. The lack of an effect on skeletal-muscle β -adrenoceptor characteristics contrasts with the observation of an increased β -adrenoceptor population in heart in hyperthyroidism [38], and may relate to the proposal that hyperthyroidism causes a preferential increase in β_1 -adrenoceptors, with little effect in tissues expressing a largely β_2 -adrenoceptor population [39].

Studies in spontaneous [12,40] and experimentally induced hyperthyroidism [13,41,42] in man have generally provided evidence for increased rates of glucose uptake and utilization, and have suggested that an increased proportion of glucose metabolism occurs by complete oxidation, whereas that directed towards storage is decreased. The increased rate of glucose uptake is not affected when plasma catecholamine concentration is experimentally increased by infusion of adrenaline [9,40], but the rate of hepatic glucose production is further increased, owing to a stimulation of the rate of gluconeogenesis [9]. Furthermore, although administration of the β -adrenoceptor antagonist propranolol does not affect the rate of glucose turnover in human hyperthyroid subjects [12], or in experimental hyperthyroidism in rats [9], it does lower the plasma levels of lactate and pyruvate, presumably by decreasing their rate of formation [12]. In the hyperthyroid state, the rates of lactate formation and release from skeletal muscle are significantly increased [43]. These data, taken together with the results of the present study, suggest that in hyperthyroidism, although the increase in the rates of hepatic glucose production and peripheral glucose uptake may be due to a direct stimulatory effect of the thyroid hormones, the increase in the rate of lactate formation and its subsequent increase in plasma [12,41,43] are due, at least in part, to an increased responsiveness of glycogenolysis to β -adrenoceptor stimulation in skeletal muscle; the increased rate of glycolysis and lactate release contributes to the observed increase in the activity of the Cori-cycle [42,44] in more severe hyperthyroidism, although this

may not be the case in milder, experimentally induced, hyperthyroid states [13,45].

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