Effects of hyperthyroidism and hypothyroidism on glutamine metabolism by skeletal muscle of the rat

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1. The effects of hyperthyroidism and hypothyroidism on the concentrations of glutamine and other amino acids in the muscle and plasma and on the rates of glutamine and alanine release from incubated isolated stripped soleus muscle of the rat were investigated. 2. Hyperthyroidism decreased the concentration of glutamine in soleus muscle but was without effect on that in the gastrocnemius muscle or in the plasma. Hyperthyroidism also increased markedly the rate of release of glutamine from the incubated soleus muscle. 3. Hypothyroidism decreased the concentrations of glutamine in the gastrocnemius muscle and plasma but was without effect on that in soleus muscle. Hypothyroidism also decreased markedly the rate of glutamine release from the incubated soleus muscle. 4. Thyroid status was found to have marked effects on the rate of glutamine release by skeletal muscle *per se*, and may be important in the control of this process in both physiological and pathological conditions.

INTRODUCTION

Glutamine is utilized at a high rate by a number of tissues, including the liver [1], kidney [2], intestine [3] and cells of the immune system [4,5]. Cells of the immune system are considered to use glutamine at a high rate not only for the provision of energy, but also for provision of optimal conditions for the precise regulation of the rate of synthesis of purine and pyrimidine nucleotides and other compounds necessary for rapid proliferation. Hence mitogen-stimulated proliferation of lymphocytes requires the presence of glutamine [6] and the rate of proliferation is decreased when the concentration of glutamine in the culture medium is decreased below the normal plasma level [7]. Therefore maintenance of the normal plasma level of this amino acid may be necessary to permit a rapid and efficient response to an immune challenge.

Skeletal muscle is known to synthesize glutamine from glutamate and ammonia via glutamine synthetase and the concentration of glutamine in muscle is high [8]. Muscle has been shown to release glutamine into the bloodstream [9,10] and there is evidence that it plays a quantitatively important role in the provision of glutamine via the bloodstream for use by other tissues (e.g. kidney [11] and cells of the immune system [12]). Since glutamine is so important for cells of the immune system, failure of muscle to release glutamine at a sufficient rate could impair the functioning of this important defence system [13].

The available evidence suggests that the process of glutamine release from muscle is the flux-generating step for the pathway of glutamine metabolism in a number of tissues, i.e. the transporter responsible for the outward transport of glutamine catalyses a non-equilibrium process and it approaches saturation with substrate (intracellular glutamine) [14]. In order to change the rate of transport this process must be subject to regulation by factors other than the intracellular glutamine level and its activity must be increased under conditions when more glutamine is required by other tissues. The factor(s) which might increase the rate of glutamine release from skeletal muscle, however, have not been identified. The effect of thyroid status on the rate of muscle

glutamine release has not been systematically studied and the results that are available are conflicting. The effects of thyroid status on the rates of release of glutamine and alanine from muscle have been measured by arterio-venous differences across the limbs of humans. The effluxes of glutamine and alanine were increased in hyperthyroid patients [15] and in normal subjects treated with tri-iodothyronine (T_3) [16], and they were decreased in hypothyroid patients [15]. However, treatment of rats with thyroid hormone did not change the rate of glutamine release from incubated epitrochlearis muscle [17]. These results question the interpretation of arterio-venous difference measurements, since venous blood from the limb drains several tissues. Thus changes in glutamine efflux from limbs could be due to changes in release or uptake by adipose tissue [18], skin [19] or bone marrow [20], in addition to changes in muscle. Consequently it was considered important to study the effects of thyroid status on the rates of release of glutamine and alanine from isolated skeletal muscle. The effects of thyroid status on the concentrations of glutamine and related amino acids in both muscle and plasma have also been examined.

MATERIALS AND METHODS

Animals

Male Wistar rats (160–180 g) were purchased from Harlan-Olac, Bicester, Oxon., U.K. and were kept in the Department's animal house for at least 7 days with access to food and water *ad libitum*. Hyperthyroidism was induced in the rats by intraperitoneal injection of T_3 (0.65 $\mu\text{g/g}$ body weight) for 5 or 10 consecutive days. Experiments were performed 24 h after the final injection. Hypothyroidism was induced by administration of 6-n-propylthiouracil for 3–4 weeks. The drug was dissolved in warm ethanol and was added to the drinking water at a final concentration of 0.5 mg/ml. Ethanol was also added to the drinking water of the control rats at the same final concentration (1.2%, v/v). Animals were fasted for 12–14 h before they were killed, which was done by cervical dislocation.

Abbreviation used: T_3 , tri-iodothyronine.

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Chemicals and enzymes

All chemicals, biochemicals and enzymes were obtained from sources given previously [21]. In addition, T_3 , propylthiouracil and aprotonin were obtained from Sigma Chemical Co., Poole, Dorset, U.K. Sagatal (pentobarbitone) was obtained from May & Baker, Dagenham, Essex, U.K., and the radioimmunoassay kit for measurement of T_3 concentration was obtained from Amersham International, Amersham, Bucks., U.K.

Incubation of muscles

Stripped soleus muscle preparations were prepared and incubated as previously described [21]. All muscles were incubated for 60 min in Krebs-Henseleit bicarbonate buffer containing 5.5 mM-glucose, 1.5% defatted BSA and insulin (10 μ units/ml). This preparation is known to provide a viable 'in vitro' muscle preparation with which to study rates of glutamine release [22], especially since the concentration of glutamine in muscle is unchanged during the incubation procedure (pre-incubation 4536 ± 412 nmol/g; post-incubation 4814 ± 174 nmol/g; means $\pm$ S.E.M. for at least 3 separate preparations).

Metabolite and hormone determinations

The rates of release of amino acids from incubated muscle strips were determined by the enzymic analysis of the concentrations of glutamine [3] and alanine [23] in the incubation medium.

Soleus muscles were freeze-clamped in liquid N_2 immediately after the incubation period. Gastrocnemius muscles were freeze-clamped and blood samples were taken from the heart immediately after the animals were killed. Muscle and plasma samples were prepared and extracted as described previously [21], and concentrations of glutamine [3], alanine [23], glutamate [24] and branched-chain amino acids [25] were subsequently measured.

For the determination of plasma levels of T_3 , rats were anaesthetized with pentobarbitone (40 μ g/g body weight) and blood was taken from the iliac artery in heparinized syringes. Blood was immediately centrifuged and plasma was stored at -20°C with aprotonin (1000 i.u./ml). The plasma T_3 concentration was determined using a radioimmunoassay kit.

RESULTS

The level of T_3 in the plasma of control rats was 0.51 ± 0.03 ng/ml (mean $\pm$ S.E.M.), and this was increased to 7.50 ± 0.47 and 7.45 ± 0.43 ng/ml after 5 and 10 days of treatment with T_3 respectively. In hypothyroid rats the level was decreased from 0.80 ± 0.04 to 0.05 ± 0.01 ng/ml.

The concentration of glutamine in the soleus muscle was decreased in hyperthyroidism, but it was not changed in the gastrocnemius muscle (Tables 1 and 2). The concentrations of alanine in both gastrocnemius and soleus muscles were increased in hyperthyroidism. The glutamine concentration in the plasma

Table 1. Effects of hyperthyroidism and hypothyroidism on concentrations of glutamine, glutamate, alanine and branched-chain amino acids (BCAA) in gastrocnemius muscle and plasma

Values are means $\pm$ S.E.M. for the numbers of measurements given in parentheses. The statistical significance of the differences between treated and control groups is denoted by ^a ($P < 0.05$), ^b ($P < 0.01$), ^c ($P < 0.002$) or ^d ($P < 0.0001$). The statistical significance of the differences between the two hyperthyroid groups is denoted by ^A ($P < 0.05$) or ^B ($P < 0.02$).

Thyroid status	Amino acid concentration (nmol/g or nmol/ml)							
	Glutamine		Glutamate		Alanine		BCAA	
	Muscle	Plasma	Muscle	Plasma	Muscle	Plasma	Muscle	Plasma
Control	3412 $\pm$ 77 (28)	929 $\pm$ 23 (28)	1869 $\pm$ 78 (24)	281 $\pm$ 21 (25)	1419 $\pm$ 63 (26)	375 $\pm$ 18 (22)	414 $\pm$ 21 (24)	565 $\pm$ 17 (13)
Hyperthyroid (5 days)	3188 $\pm$ 91 (17)	851 $\pm$ 31 (15)	817 $\pm$ 60 (14) ^d	359 $\pm$ 22 (23)	1744 $\pm$ 114 (14) ^a	388 $\pm$ 23 (15)	402 $\pm$ 35 (4)	566 $\pm$ 11 (12)
Hyperthyroid (10 days)	3173 $\pm$ 129 (19)	902 $\pm$ 36 (17)	800 $\pm$ 61 (15) ^d	341 $\pm$ 25 (15)	1943 $\pm$ 130 (13) ^b	488 $\pm$ 23 (10) ^{ba}	571 $\pm$ 34 (5) ^{cb}	643 $\pm$ 24 (14) ^{aa}
Control	3671 $\pm$ 127 (15)	983 $\pm$ 39 (15)	1790 $\pm$ 102 (15)	272 $\pm$ 31 (15)	1597 $\pm$ 81 (15)	346 $\pm$ 22 (10)	437 $\pm$ 24 (15)	546 $\pm$ 22 (12)
Hypothyroid	2866 $\pm$ 139 (15) ^c	627 $\pm$ 22 (15) ^d	1157 $\pm$ 78 (15) ^c	150 $\pm$ 10 (18) ^c	1415 $\pm$ 70 (14)	278 $\pm$ 29 (10)	330 $\pm$ 27 (15) ^a	452 $\pm$ 15 (13) ^a

Table 2. Effects of hyperthyroidism and hypothyroidism on the concentrations and rates of release of glutamine and alanine from isolated incubated soleus muscles

Values are means $\pm$ S.E.M. for numbers of measurements in parentheses. The statistical significance of the differences between treated and control groups is denoted by ^a ($P < 0.05$), ^b ($P < 0.01$) or ^c ($P < 0.0001$). The statistical significance of the difference between hyperthyroid groups is denoted by ^A ($P < 0.01$).

Thyroid status	Glutamine		Alanine	
	Muscle concentration (nmol/g)	Rate of release (nmol/min per g)	Muscle concentration (nmol/g)	Rate of release (nmol/min per g)
Control	4382 $\pm$ 269 (12)	28.1 $\pm$ 1.8 (32)	1869 $\pm$ 44 (7)	21.7 $\pm$ 0.9 (18)
Hyperthyroid (5 days)	3538 $\pm$ 142 (10) ^a	47.1 $\pm$ 3.0 (25) ^c	2247 $\pm$ 92 (10) ^b	32.9 $\pm$ 1.4 (12) ^c
Hyperthyroid (10 days)	3302 $\pm$ 414 (10) ^a	64.9 $\pm$ 5.4 (16) ^{ca}	2202 $\pm$ 99 (6) ^a	29.6 $\pm$ 1.1 (10) ^c
Control	3892 $\pm$ 107 (9)	32.5 $\pm$ 2.4 (17)	2046 $\pm$ 227 (9)	23.7 $\pm$ 2.1 (15)
Hypothyroid	4329 $\pm$ 174 (11)	21.1 $\pm$ 1.5 (18) ^a	1236 $\pm$ 98 (11) ^b	11.4 $\pm$ 0.9 (19) ^c

was unchanged, and that of alanine was increased after 10 days of hyperthyroidism.

The concentration of glutamine in the gastrocnemius muscle was decreased in hypothyroidism but it was not changed in soleus muscle (Tables 1 and 2). The concentration of alanine was unchanged in gastrocnemius muscle, but decreased in soleus muscle. The concentration of glutamine in plasma was decreased and that of alanine was unchanged by hypothyroidism.

The rates of release of both glutamine and alanine from soleus muscles were increased in hyperthyroidism (Table 2). After 5 days of T_3 treatment the percentage increase in the rate of glutamine release was 68 %, and after 10 days treatment it was 131 %; the increases in alanine release were similar after 5 and 10 days. The rates of release of both amino acids were decreased in hypothyroidism (Table 2).

DISCUSSION

This is, to our knowledge, the first report of effects of changes in thyroid status on the rates of release of glutamine and alanine from skeletal muscle *per se*. The rates of release of glutamine and alanine were increased from incubated soleus muscle isolated from hyperthyroid rats and they were decreased from muscle of hypothyroid rats. These changes in the rates of release are in agreement with those calculated from arterio-venous difference measurements in hyper- and hypo-thyroid patients [15], so that the present results suggest that the changes *in vivo* are due to effects of thyroid hormone on skeletal muscle. Changes in the plasma level of this hormone may, therefore, be an important means for controlling the rate of release of glutamine in different conditions and, in addition, may provide a useful tool to study the mechanism by which the rate of glutamine release from muscle is controlled.

This systematic study of the effects of thyroid hormone excess and insufficiency on the concentration of glutamine in skeletal muscle is of interest, since it has been suggested that the concentration of this amino acid may play some part in the control of the rate of protein synthesis in muscle [10,26]. The changes in the concentration of glutamine in soleus muscle in hypo- and hyper-thyroidism reported in this work are consistent with this suggested role of glutamine: the rate of protein synthesis is reported to be decreased in muscle of hyperthyroid patients and is unchanged in muscle of hypothyroid patients [15]. However, this relationship does not appear to hold for muscle that has a greater proportion of type II fibres than the soleus (i.e. gastrocnemius muscle). That changes in glutamine concentration may differ in different muscle fibre types in response to a given stimulus has also been recently reported for the response to glucocorticoids [27]. This raises an interesting question of whether muscle must be considered differently in relation to glutamine metabolism according to its fibre type composition.

Glutamine and alanine account for 35 % and 25 % respectively of all amino acids released from muscle [28], but they constitute only 3 % and 7 % respectively of muscle protein [29]. This indicates that the rates of glutamine and alanine release do not merely reflect degradation of muscle protein. Furthermore, although glutamine release is generally increased in conditions associated with increased rates of net protein breakdown, this is not always the case [30]. In addition, the muscle concentration of glutamine, even in the hypothyroid state, is well in excess of the K_m value for the intracellular glutamine of the release process ([14]; see the Introduction section), so that changes in the intracellular level of glutamine reported in the present work would only be expected to have minor effects on the rate of release of glutamine. Consequently, the marked increase in the rate of release of glutamine by soleus muscle in hyperthyroidism

must be due to an effect on the transporter responsible for the efflux of glutamine. This effect is likely to be mediated via an increase in the number or activity of the transporters in the membrane.

The precise role of T_3 in the control of amino acid metabolism in both physiological and pathological conditions is not clear (see [31,32]). The present work suggests that T_3 increases the rate of glutamine release from skeletal muscle, which may be important in providing more glutamine for a number of different tissues, including liver, kidney, intestine and cells of the immune system. Since an adequate supply of glutamine may be essential for the functioning of these tissues, one means by which T_3 may influence the function of, for example, cells of the immune system is via an effect on the rate of glutamine release from muscle. Indeed, there is some evidence that T_3 is required for an effective immune response. Thus administration of thiourea, an inhibitor of thyroid function, prevented elevation of serum antibody titres following antigenic stimulation in birds [33]. This effect could be explained by a decrease in the plasma glutamine concentration caused by a decrease in the rate of glutamine release by muscle.

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