

Regulation of fructose 2,6-bisphosphate levels in cold-acclimated brown adipose tissue of rat

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The effects of cold exposure and T_4 administration on fructose 2,6-bisphosphate levels, phosphofructokinase-2 and pyruvate kinase activities were examined in rat brown adipose tissue. Cold adaptation (14 days) gave rise to a 2-fold increase in the amount of fructose 2,6-bisphosphate and phosphofructokinase-2 activity, and increased the pyruvate kinase activity 4-fold. If, in addition, the cold-acclimated rats were treated with T_4 , these parameters were again significantly enhanced. The effect on phosphofructokinase-2 was on the V_{max} , without modification of the K_m (for both fructose 6-phosphate and ATP) of the enzyme. In the hypothyroid state, however, the activity of pyruvate kinase remains unchanged. These data support previous observations on stimulation of glycolytic flux during cold adaptation in brown adipose tissue, and a permissive role of thyroid hormones in the process.

Fructose 2,6-bisphosphate; Thyroid hormone; 6-Phosphofructo-2-kinase; Pyruvate kinase; (Brown adipose tissue)

1. INTRODUCTION

Cold adaptation is closely related to an increase in the amount of brown adipose tissue (BAT) and mitochondria [1–3]. In addition to the role of fatty acid as the major fuel during cold exposure, recent studies have indicated that BAT plays an important role in regulating blood glucose concentration after a carbohydrate load, especially in cold-acclimated rats [4,5]. This is supported by the fact that the activities of hexokinase and 6-phosphofructokinase (PFK-1) are increased in BAT during adaptation to low temperature [4]. A relationship between thermogenesis and thyroid hormones has been accepted [6], therefore it appeared to us of interest to relate the functional thyroid state to the

regulation of glucose metabolism during cold adaptation.

Fructose 2,6-bisphosphate (Fru 2,6- P_2) is a newly recognized, positive effector of PFK-1 in all examined tissue [7] and its concentration is greatly increased under conditions in which glycolysis is active. Here, we investigate the effect of cold exposure and thyroid functional state on Fru 2,6- P_2 levels and the kinetic properties of PFK-2 in BAT. We report, for the first time, an increase in Fru 2,6- P_2 in cold-acclimated BAT and also under hyperthyroid conditions. PFK-2 and pyruvate kinase activities were also enhanced in these states.

2. MATERIALS AND METHODS

Male Wistar rats (170–200 g) were used throughout. Hypothyroidism was induced as in [8]. Briefly, rats were made hypothyroid by treatment with $NaClO_4$ in the drinking water for 40 days and hyperthyroid with T_4 (1 mg/ml) added to the drinking water for 7 days. BAT was isolated from the interscapular depot and frozen in liquid N_2 . Fru 2,6- P_2 was assayed as in [9]. The activity of PFK-2 was measured in partially purified preparations with polyethylene glycol as in [10]. For assay of pyruvate kinase activity, BAT and heart muscle (atrial and ventricular muscles, separately) were homogenized in 5 ml buffer containing 100 mM KCl and 50 mM Gly-Gly, pH 7.4.

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Abbreviations: Fru 2,6- P_2 , fructose 2,6-bisphosphate; PFK-1, 6-phosphofructo-1-kinase (EC 2.7.1.11); PFK-2, 6-phosphofructo-2-kinase (EC 2.7.1.105); BAT, brown adipose tissue

The homogenate was centrifuged at $15000 \times g$ for 30 min. Small molecules were eliminated by passing the supernatant through a Sephadex G-25 (fine) column. An aliquot of the eluted fluid was used to determine the pyruvate kinase activity as in [11]. Lactic dehydrogenase activity was assayed as in [12]. All biochemical and purified enzymes were obtained from Sigma and Boehringer. Fructose 2,6-P₂ standard was kindly donated by E.V. Schaftingen and H.G. Hers (Laboratoire de Chimie Physiologique, Université Catholique de Louvain, Belgium).

3. RESULTS AND DISCUSSION

3.1. *Effect of cold adaptation in brown adipose tissue*

The levels of Fru 2,6-P₂ and activities of PFK-2 and pyruvate kinase in BAT are listed in table 1. Maintenance of rats at 4°C for 13 days doubled the concentration of Fru 2,6-P₂ and the PFK-2 activity. Since Fru 2,6-P₂ is the major factor responsible for the regulation of glycolysis, the increased levels of this metabolite during cold acclimatization suggest that the overall glycolysis flux is stimulated at the level of the PFK-1 step. This assumption is reinforced by the fact that pyruvate kinase activity was also enhanced (table 1). An increase of hexokinase and PFK-1 has also been reported [4]. The increased content of Fru 2,6-P₂ could also enhance the rate of the Fru 6-P/Fru 1,6-P₂ cycle during cold adaptation, in order to generate heat as is known to occur in muscle [13]. The amount of Fru 2,6-P₂ in this tissue (0.32 pmol/g wet tissue) is of the same order as that previously found in white adipose tissue [9]. The increased activities of the cited enzymes provide new data to explain the enhanced capacity for oxidizing glucose during cold adaptation, and suggest that in this state the synthesis of enzymatic proteins is stimulated. Table 1 also shows the effect of T₄ on the studied parameters. It is shown that in BAT the hyperthyroid state was associated with a clear stimulation of PFK-2 and pyruvate kinase activities and an increase in the tissue levels of Fru 2,6-P₂. These data may be of interest in the light of the increased rate of energy consumption and stimulation of oxidative metabolism after the addition of T₄ [15,16], events associated with a greater requirement of substrate.

3.2. *Kinetic parameters of PFK-2 from brown adipose tissue*

Because the adaptation to cold produces an in-

Table 1

Effect of cold exposure and T₄ treatment on Fru 2,6-P₂ levels, and activities of PFK-2 and pyruvate kinase in brown adipose tissue

	Fru 2,6-P ₂ (nmol/g tissue)	PFK-2 (μ U/unit LDH)	Pyruvate kinase (U/unit LDH)
Normal (6)	0.327 \pm 0.05	1.37 \pm 0.05	7.00 \pm 1.0
Cold-acclimated (8)	0.739 \pm 0.05	2.86 \pm 0.16	29.49 \pm 1.2
Cold-acclimated + T ₄ treatment (5)	1.320 \pm 0.12	4.08 \pm 0.18	43.97 \pm 2.2

Brown adipose tissue was homogenized and treated as indicated in section 2. Values are means \pm SE. The number of rats is indicated in parentheses

crease in both maximal activity of PFK-2 and Fru 2,6-P₂ concentration, it is of interest to study whether other kinetic properties of the enzyme were modified in this state. Fig.1 shows the dependence of PFK-2 activity on Fru 6-P concentration. The effect of adaptation to cold was on the V_{max} of the enzyme, while the K_m remained unchanged regarding control conditions. Similar results were found when the effect of ATP, another substrate for the enzyme, was studied (not shown). Since the enzyme activity was measured after precipitation with polyethylene glycol, the observed effect was not due to the presence of (a) putative effector(s) present in the homogenate. Recently, the existence of isoenzymes of PFK-2 has been postulated [17]. Fig.1B illustrates that PFK-2 activity from brown adipose tissue is progressively inhibited by citrate, whereas glycerol 3-P shows scarcely any capacity for inhibition of the enzyme activity. These properties resemble those of the cardiac isoenzyme of PFK-2 [17].

3.3. *Pyruvate kinase activity in BAT and heart*

It is accepted that thyroid hormones induce the stimulation of glucose utilization in muscle and in adipose tissue [14]. In heart muscle, hypothyroidism produces a decrease in Fru 2,6-P₂ levels and PFK-1 activity, whereas the hyperthyroid state is without effect [8]. Table 2 shows the activity of pyruvate kinase under these conditions. In BAT, hypothyroidism was without effect, whereas the augmentation of T₄, enhanced the activity of pyruvate kinase. This last result agrees with the in-

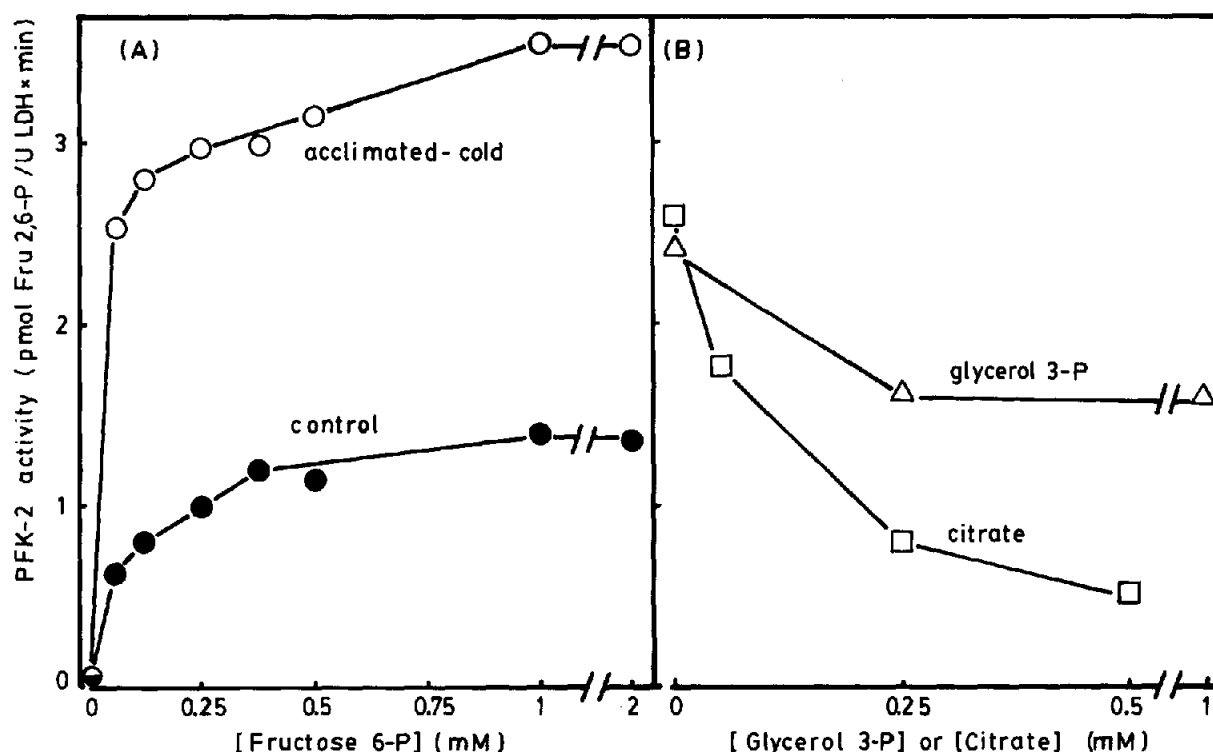


Fig.1. Affinity of PFK-2 for Fru 6-P (A) and effect of glycerol 3-P and citrate on the activity of PFK-2 (B) from brown adipose tissue. The enzyme was partially purified from normal (O) and cold-acclimated rats (○, □, △). Fru 6-P and ATP concentrations in (B) were 0.1 and 0.5 mM, respectively.

crease observed in the pyruvate kinase activity in cold-adapted rats (table 1). A different situation appears in heart muscle where neither hypothyroidism nor hyperthyroidism alters the activity of the enzyme. Recently, regional differences in the insulin regulation of atrial and ventricular PFK-1 activity have been shown [19]. The possibility that thyroid hormones also regulate the cardiac pyruvate kinase in a regional manner has been evaluated. However, the available data indicate that the kinetic parameters of the enzyme were unchanged by the thyroid state in either atrial or ventricular muscle (atrial muscle: $K_m = 0.05$ mM, $V_{max} = 0.4$ U/mg protein; ventricular muscle: $K_m = 0.06$ mM, $V_{max} = 0.5$ U/mg protein).

3.4. General conclusions

This study demonstrates that cold exposure and T_4 administration in vivo can increase several-fold the enzyme activities associated with the glycolytic

pathway in BAT. These changes could be part of a co-ordinated response of glucose catabolism in response to cold, since thermogenesis requires a continuous source of oxidizable substrates. On the basis of the results presented above, it can be con-

Table 2

Effect of thyroid state on pyruvate kinase activity in brown adipose tissue and heart muscle

State	Pyruvate kinase activity (mU/mg protein)	
	BAT	Heart
Normal (6)	64.6 ± 3.4	650 ± 15
Hypothyroid (7)	73.9 ± 2.5	600 ± 11
Hyperthyroid (7)	160.7 ± 5.8 ^a	634 ± 12

Rat brown adipose tissue and heart muscle, in several thyroid states, were homogenized and filtered on Sephadex G-25. Pyruvate kinase activity was assayed in the eluted fractions. The number of rats used is given in parentheses. ^a $p < 0.001$ vs normal rats

cluded that the relative importance of Fru 2,6-P₂ as a regulator of glycolysis is increased during cold exposure and hyperthyroidism in BAT. Recently it has been shown that thyroidectomized rats are unable to survive chronic exposure to cold [20]. In addition, the action of thyroid hormones in the stimulation of thermogenesis is well documented (review [6]). Our results about the permissive role of T₄ treatment in PFK-2 and pyruvate kinase activities in BAT support this suggestion. The different responses among tissues to thyroid hormones are also noteworthy. Thus, cardiac pyruvate kinase does not show the thyroid adaptivity that is seen in BAT during hyperthyroidism. Further work should be directed toward establishing flux rates for glucose oxidation in BAT, and in making further comparisons in normal and pathophysiological states.

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