Effects of administration of tri-iodothyronine on the response of cardiac and renal pyruvate dehydrogenase complex to starvation for 48 h

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Effects of administration of tri-iodothyronine (T_3) on activities of cardiac and renal pyruvate dehydrogenase complex (active form, PDH_a) were investigated. In fed rats, T_3 treatment did not affect cardiac or renal PDH_a activity, although blood non-esterified fatty acid and ketone-body concentrations were increased. Starvation (48 h) of both control and T_3-treated rats resulted in similar increases in the steady-state concentrations of fatty acids and ketone bodies, but inactivation of cardiac and renal pyruvate dehydrogenase complex activities was diminished by T_3 treatment. Inhibition of lipolysis increased renal and cardiac PDH_a in control but not in T_3-treated 48 h-starved rats, despite decreased fatty acid and ketone-body concentrations in both groups. The results suggest that hyperthyroidism influences the response of cardiac and renal PDH_a activities to starvation through changes in the metabolism of lipid fuels in these tissues.

INTRODUCTION

Glucose is conserved in the rat in starvation by diminished flux through the mitochondrial pyruvate dehydrogenase complex (PDH; EC 1.2.4.1+EC 2.3.1.12+EC 1.6.4.3). This is achieved by increased phosphorylation and inactivation of the complex by PDH kinase (EC 2.7.1.99), the total concentration of the complex (sum of active and inactive forms) being unchanged. The effects of starvation on tissue PDH activities are mediated in part through the increased oxidation of lipid fuels, which, by increasing mitochondrial ratios of [acetyl-CoA]/[CoA] and possibly [NADH]/[NAD^+], not only causes end-product inhibition of the complex, but also activates PDH kinase (Kerbey *et al.*, 1977, 1979). These mechanisms for inactivation of PDH are rapid in onset and are blocked by inhibition of fatty acid oxidation (Caterson *et al.*, 1982). Two further mechanisms have been established which, in heart muscle at least, facilitate prolonged inactivation of PDH. Firstly, the kinase reaction is accelerated by an increased mitochondrial concentration of protein factors that may be PDH kinase or an activator of the kinase (Kerbey & Randle, 1982). Secondly, re-activation of PDH phosphate by PDH-phosphate phosphatase (EC 3.1.3.43) is retarded by phosphorylation of the complex at sites in addition to that concerned with enzyme inactivation (Sugden *et al.*, 1978).

Despite increased mobilization and oxidation of lipid fuels (reviewed by Sestoft, 1980), whole-body glucose utilization is increased in clinical and experimental hyperthyroidism (e.g. Okajima & Ui, 1979; Huang & Lardy, 1981). In view of the importance of changes in PDH activity in the regulation of glucose oxidation, the present work investigated the effects of T_3 administration on cardiac and renal PDH activities in extracts of freeze-clamped tissues. Because a decreased T_3 concentration has been considered to be one of the major metabolic adaptations to starvation (see Goldberg *et al.*, 1978), effects of hyperthyroidism were examined in both fed and 48 h-starved rats. The results indicate that hyperthyroidism attenuates starvation-induced decreases in cardiac and renal PDH activities, and experiments with the lipolysis inhibitor MPCA indicate that this may be due to changes in tissue fat metabolism. A preliminary account of some aspects of this work has been given (Holness *et al.*, 1986).

MATERIALS AND METHODS

Sources of materials were as given in Sugden *et al.* (1982). Results are given as means ± S.E.M., with the numbers of rats in parentheses. Statistical significance of differences was assessed with Student's unpaired *t* test.

Treatment of animals

Female albino Wistar rats (180–220 g) were subjected to a 12 h-light/12 h-dark cycle (light period starting at 08:30 h). Rats were either fed *ad libitum* or starved for the 48 h before sampling (in grid-bottomed cages). In some experiments with 48 h-starved rats, MPCA (1 mg/100 g body wt. in 0.9% NaCl) was injected intraperitoneally at 1 h before rats were killed, to inhibit lipolysis (Sugden *et al.*, 1982; see also Axelrod *et al.*, 1979).

Rats were made hyperthyroid by the subcutaneous injection of T_3 dissolved in 10 mM-NaOH/0.03% bovine serum albumin (100 μg/100 g body wt. per day for 2 consecutive days, as in Sugden *et al.*, 1983). When effects of starvation were investigated, T_3 was administered

Abbreviations used: PDH, pyruvate dehydrogenase complex; PDH_a, its active form; T_3, tri-iodothyronine; NEFA, non-esterified fatty acids; MPCA, 5-methylpyrazole-3-carboxylic acid.

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during the period of starvation. Control rats were injected with an equivalent volume of 10 mM-NaOH/0.03% bovine serum albumin. Rats were killed on day 3. T_3 treatment produced an approx. 4% decrease in body weight in fed rats [control (9), $+1.3\pm0.4$ g/100 g body wt.; T_3-treated (30), -4.1 ± 0.7 g/100 g body wt.; $P < 0.01$] and an increased weight loss in response to 48 h starvation [control (21), -9.0 ± 0.4 g/100 g body wt.; T_3-treated (39), -12.1 ± 0.4 g/100 g body wt.; $P < 0.001$]. Despite the decrease in body weights, T_3 treatment increased the wet weight of both heart and kidney. Heart weights were 0.755 ± 0.014 (27) or 0.818 ± 0.013 (20) g ($P < 0.001$) in fed control or T_3-treated rats, corresponding total kidney weights being 1.512 ± 0.027 (27) and 1.673 ± 0.035 (21) g respectively ($P < 0.01$). Tissue weights in 48 h-starved rats were: heart, control (27), 0.702 ± 0.019 g, T_3-treated (27), 0.826 ± 0.015 g ($P < 0.001$); kidney, control (27), 1.479 ± 0.023 g, T_3-treated (27), 1.645 ± 0.028 g ($P < 0.01$).

Enzyme and metabolite assays

Activities of the active form of PDH (PDH_a), total PDH complex (the sum of active and inactive forms) and citrate synthase (EC 4.1.3.7) were measured in freeze-clamped tissue extracts as described by Caterson *et al.* (1982). Further details are given in Kerbey *et al.* (1976). A unit of enzyme activity converts 1 μmol of substrate into product/min at 30 °C. The results have been expressed both per g wet wt. and also relative to the mitochondrial marker citrate synthase (see Caterson *et al.*, 1982), in order to correct for possible differences in mitochondrial extraction of tissues from the four groups of rats. T_3 treatment had no effect on cardiac total PDH activity, assayed after incubation of tissue extracts with purified PDH-phosphate phosphatase (Whitehouse *et al.*, 1974) [control, 88.4 ± 7.5 (5) munits/unit of citrate synthase; T_3-treated, 84.4 ± 6.3 (5) munits/unit of citrate synthase; $P > 0.5$], but decreased renal total PDH activity by approx. 17% [control, 213.2 ± 4.1 (5) munits/unit of citrate synthase; T_3-treated, 177.8 ± 12.1 (5) munits/unit of citrate synthase; $P < 0.05$]. Citrate synthase activities were similar in control and T_3-treated rats in both kidney and heart (Table 1).

Arterial blood obtained at the time of sampling was assayed for NEFA (plasma) and the ketone bodies, D-3-hydroxybutyrate and acetoacetate (KOH-neutralized $HClO_4$ extracts of whole blood) as described previously (Sugden *et al.*, 1982). Total ketone-body concentration refers to the sum of the concentrations of D-3-hydroxybutyrate and acetoacetate.

RESULTS

Pyruvate dehydrogenase complex activities in hearts and kidneys of rats fed *ad libitum*

Tissue PDH activites are shown in Table 1. The concentrations of active PDH complex, and the proportions of total PDH as active complex in hearts and kidneys of fed rats, were similar to those obtained by others (e.g. Guder & Wieland, 1974; Whitehouse *et al.*, 1974; Caterson *et al.*, 1982) and were unaffected by hyperthyroidism when the results were expressed relative to the mitochondrial marker citrate synthase. The slight (not significant) decrease in renal PDH_a, expressed on a wet-wt. basis (Table 1; see also Holness *et al.*, 1986), may be due to less efficient mitochondrial extraction, possibly owing to changes in mitochondrial morphology (see, e.g., Gross, 1971; Sterling, 1979). The increase in the proportion of renal PDH in the active form in T_3-treated rats is a consequence of the decreased total PDH activity (see the Materials and methods section).

The lack of effect of hyperthyroidism on PDH activities in kidneys and hearts of fed rats is remarkable in view of the significant increases in plasma NEFA and ketone-body concentrations which are observed (Table 2). Others have also noted increased lipid fuel availability in fed hyperthyroid rats (Laker & Mayes, 1981) and increased concentrations of lipid fuels in the fed euthyroid state

Table 1. Effects of T_3 administration on cardiac and renal PDH activities in fed, 48 h-starved or MPCA-treated rats

For experimental details see the text. A statistically significant difference between fed and 48 h-starved rates is indicated by $^*P < 0.05$ or $^{***}P < 0.001$, and between control and T_3-treated rats by §$P < 0.05$, §§$P < 0.01$ and §§§$P < 0.001$. Significant effects of MPCA are indicated by †$P < 0.05$ or †††$P < 0.001$.

Tissue	Nutritional status	Treatment	PDH complex (active form) (munits/g wet wt.)	PDH complex (active form) (munits/unit of citrate synthase)	PDH complex (active form) (% of total complex)	Citrate synthase (units/g wet wt.)
Heart	Fed	Control	4027±373 (8)	52.5±3.8 (8)	59.4±4.3 (8)	80.2±3.9 (8)
		T_3	4080±232 (7)	52.4±4.2 (8)	62.1±5.0 (8)	77.5±1.7 (8)
	48 h-starved	Control	368±74 (28)***	6.3±1.1 (32)***	7.1±1.2 (32)***	79.3±2.8 (32)
		T_3	2419±116 (15)§§§***	25.8±2.4 (18)§§§***	30.6±2.8 (18)§§§***	83.4±4.1 (18)
		Control+MPCA	2523±153 (12)†††	28.9±2.1 (12)†††	32.7±2.4 (12)†††	88.3±3.6 (12)
		T_3+MPCA	2810±314 (9)	34.7±3.6 (10)	44.2±4.5 (10)†§	88.6±3.6 (10)
Kidney	Fed	Control	1721±100 (17)	78.6±4.2 (16)	36.9±2.0 (16)	18.3±0.6 (18)
		T_3	1567±64 (17)	80.9±4.7 (15)	45.5±2.6 (15)§	18.8±0.8 (15)
	48 h-starved	Control	689±31 (31)***	41.4±1.9 (32)***	19.4±0.9 (32)***	16.5±0.5 (33)*
		T_3	1379±65 (26)§§§*	77.2±5.8 (26)§§§	43.4±3.3 (26)§§§	19.0±0.9 (26)§
		Control+MPCA	1197±61 (12)†††	73.0±4.7 (12)†††	34.2±2.2 (12)†††	17.3±0.4 (12)
		T_3+MPCA	1386±106 (12)	80.4±9.0 (12)	45.2±5.1 (12)	17.4±0.7 (12)

Table 2. Effects of T_3 administration on blood NEFA and ketone-body concentrations in fed, 48 h-starved and MPCA-treated rats

For experimental details see the text. A statistically significant difference between fed and 48 h-starved rats is indicated by ***$P < 0.001$, and between control and T_3-treated rats by §$P < 0.05$ or §§§$P < 0.001$. Significant effects of MPCA are indicated by †††$P < 0.001$.

Nutritional status	Treatment	Metabolite concn. (mM) NEFA	Metabolite concn. (mM) D-3-Hydroxybutyrate + acetoacetate
Fed	Control	0.20±0.02 (10)	0.30±0.03 (12)
	T_3	0.48±0.04 (12)§§§	0.45±0.06 (12)§
48 h-starved	Control	0.54±0.04 (10)***	2.01±0.13 (10)***
	T_3	0.59±0.05 (10)	2.11±0.24 (10)***
	Control+MPCA	0.16±0.01 (12)†††	0.38±0.04 (12)†††
	T_3+MPCA	0.24±0.06 (9)†††	0.42±0.05 (9)†††

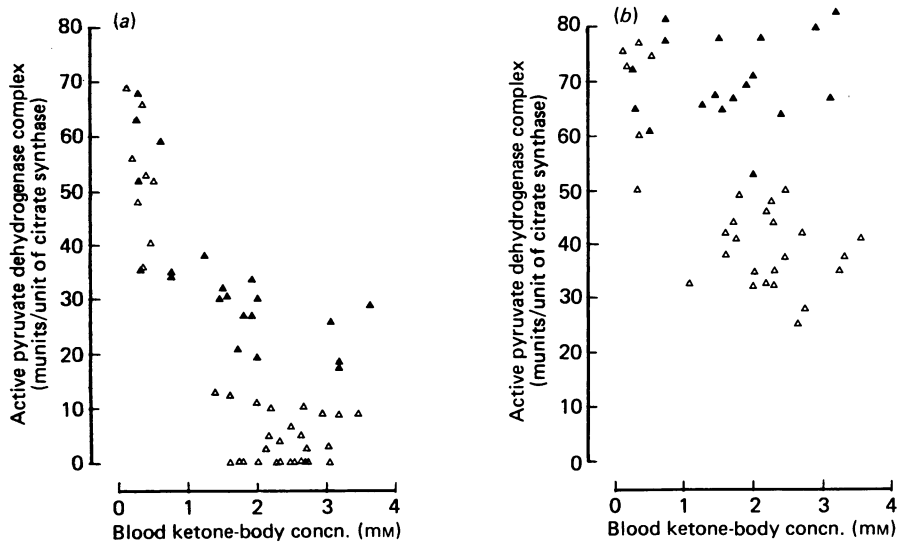

Fig. 1. Relationship between cardiac (*a*) or renal (*b*) active pyruvate dehydrogenase complex activities and blood ketone-body concentrations in control or T_3-treated rats

For experimental details see the text. Tissue PDH_a activities in control (△) or T_3-treated (▲) fed or 48 h-starved rats are plotted as a function of the blood ketone-body concentration for each individual rat. In the heart, when the data were modelled by exponential regression, the line of best fit gave r^2 values of 89% and 72% for control and T_3-treated rats respectively, and in the kidney the r^2 values were 69% and 4% for control and T_3-treated rats respectively. Thus T_3 treatment slightly decreased the correlation between PDH_a activity and blood ketone-body concentration in the heart, and totally removed any correlation between PDH_a activity and blood ketone-body concentration in kidney.

have been associated with decreased tissue PDH activities both *in vitro* and *in vivo* (see, e.g., Newsholme & Randle, 1964; Garland *et al.*, 1964; Randle *et al.*, 1964, 1970; Wieland *et al.*, 1972; Guder & Wieland, 1974).

Hyperthyroidism and the response of cardiac and renal pyruvate dehydrogenase activities to starvation

The preceding results suggested that, contrary to the situation in starvation or alloxan-diabetes, inactivation of tissue PDH by increased oxidation of NEFA or ketone bodies did not occur, or occurred to only a limited extent, in fed hyperthyroid rats. The response of cardiac and renal PDH to 48 h starvation in control and T_3-treated rats is shown in Table 1. As expected from the increases in plasma NEFA and ketone-body concentrations (Table 2), both cardiac and renal PDH_a activities were decreased by starvation in control rats (by 88% and 41% respectively). NEFA and ketone-body concentrations were also increased in response to starvation in T_3-treated rats (Table 2), but, despite similar steady-state concentrations of those metabolites in the control and T_3-treated rats, the response of cardiac PDH_a to starvation was diminished in T_3-treated rats by 37%, whereas no effect of starvation on renal PDH_a was observed (Table 1).

Pyruvate dehydrogenase activities and tissue fatty acid oxidation

Reed *et al.* (1984) have shown that tissue ketone-body oxidation is proportional to the prevailing blood concentration, and in both heart and kidney the ketone bodies may be oxidized in preference to NEFA under conditions where the concentrations of both substrates are increased (reviewed by Robinson & Williamson, 1980). In Fig. 1 the activities of cardiac and renal PDH_a

are plotted as a function of blood ketone-body concentration for each individual rat, showing that the normal response of tissue PDH_a to an increase in ketone-body concentrations is attentuated in the hyperthyroid rat.

Effects of 5-methylpyrazole-3-carboxylic acid on 48 h-starved euthyroid and hyperthyroid rats

Re-activation of PDH *in vivo* in starved rats has been achieved by inhibition of lipolysis (Wieland *et al.*, 1972) or of mitochondrial fatty acid oxidation (Caterson *et al.*, 1982). In the present experiments, lipolysis in 48 h-starved rats was inhibited with MPCA (Table 2). Administration of MPCA to control 48 h-starved rats increased the concentration and the proportion of active PDH complex in kidney to values observed in fed control rats, but in heart re-inactivation was only partial (Table 1). MPCA administration did not significantly affect cardiac or renal PDH activities in T_3-treated rats, despite decreased NEFA and ketone-body concentrations (Table 2). Consequently tissue PDH activities were similar in 48 h-starved MPCA-treated control or hyperthyroid rats.

DISCUSSION

The results demonstrate that hyperthyroidism results in a decreased response of cardiac and renal PDH activities to starvation (Table 1). This may have important implications for glucose conservation, since it implies that in starvation the requirement for net glucose synthesis for maintenance of blood glucose concentrations will be increased. As hyperthyroidism is associated with increased muscle proteolysis (Goldberg *et al.*, 1978; Gardner *et al.*, 1979), the use of the amino acids released from muscle for net production of glucose, which may subsequently be completely oxidized to CO_2, rather than recycled as lactate, may make a major contribution to the negative nitrogen balance associated with thyroid-hormone excess.

The precise mechanism by which the effects of thyroid hormones on cardiac and renal PDH activities are brought about is unclear, but, since effects of T_3 administration are not observed when lipolysis is inhibited, it is implied that the response is secondary to changes in tissue fat metabolism, and it is tempting to speculate that increased rates of oxidation of lipid fuels to CO_2 and/or increased translocation of ATP (Mowbray & Corrigall, 1984) or NADH (see Sestoft, 1980) may influence the steady-state mitochondrial [acetyl-CoA]/[CoA] and/or $[NADH]/[NAD^+]$ ratios such that end-production inhibition of PDH is relieved and/or PDH kinase activity is decreased. Further experiments are required to verify this. It is of intestest that in liver, despite increased rates of fatty acid oxidation (ketogenesis) (Bartels & Sestoft, 1980; Keyes *et al.*, 1981; Laker & Mayes, 1981; Müller *et al.*, 1981), hyperthyroidism is again associated with increased uptake and oxidation of lactate (Laker & Mayes, 1981). The results are of interest in a further connection. Although the response of cardiac PDH to increased ketone-body concentrations was diminished in hyperthyroid rats (Fig. 1), an effect of 48 h starvation to decrease the concentration of active PDH was nevertheless observed (Table 1). This implies that long-term control of PDH activity in the heart by kinase/activator or multisite phosphorylation (see the Introduction) may override control by substrate availability (see also Caterson *et al.*, 1982). The inability of MPCA to cause complete re-activation of cardiac PDH (Table 1) is consistent with effects of multisite phosphorylation of PDH to inhibit re-activation by PDH-phosphate phosphatase (Sugden *et al.*, 1978). Sale & Randle (1982) convincingly demonstrated increased occupancy of phosphorylation sites in PDH-phosphate complex *in vivo* in starved rats, and the ratio of rates of re-activation of partially and fully phosphorylated complexes from heart of fed and starved rats respectively was 2.8. Although Sugden & Simister (1980) were able to demonstrate an effect of multisite phosphorylation to inhibit renal PDH re-activation *in vitro* (see also Teague *et al.*, 1979), the re-activation of renal PDH in 48 h-starved rats to a value comparable with that of a fed rat in the presence of MPCA in the present work suggests that different control mechanisms may operate in heart and kidney *in vivo*.

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