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Action of insulin and triiodothyronine on energy-controlled pathways of hydrogen

Stimulation of biological oxidations by triiodothyronine has been shown by many investigators[1–3] and formerly was deduced from uncoupling effects[4,5], but during recent years was formulated on the basis of alterations of mitochondrial enzyme patterns[6,7]. However, as shown previously in our laboratory[8], the respiratory capacity of liver mitochondria was also markedly increased by insulin treatment of rats. A concomitant increase of several mitochondrial enzymes was observed but no change of P/O ratios as reported elsewhere[9–12]. The present report deals with major changes of mitochondrial contents of cytochromes and pyridine nucleotides produced by insulin or triiodothyronine. The contrary effects of both hormones on the respiratory system may operate as a switch for energy-controlled hydrogen flux.

Hyperthyreotic rats were obtained by daily treatment with 100 μg triiodothyronine per 100 g body weight. Insulin was injected in doses of 2×2 to 2×4 I.U. per day. All animals were kept under standard conditions and sacrificed after 4 days. Liver mitochondria were prepared and incubated as described previously[13]. Respiration, P/O ratios, and respiratory control were measured polarographically[14]. Enzyme activities were measured according to standard methods[15–17]. Mitochondrial pyridine nucleotides were determined enzymatically[18,19] and cytochrome content was measured by the method of KLINGENBERG[20,21], using a Phoenix dual-wavelength scanning spectrophotometer. All results are given on a protein basis.

As shown in Fig. 1, respiration with various substrates is stimulated by triiodothyronine and insulin as well. Respiratory rates are increased unspecifically by triiodothyronine; whereas, insulin, with a group of substrates, produced an increase of respiration in constant proportions. The rate of oxidation of α-glycerophosphate was not influenced by insulin, but rose to the 30-fold after triiodothyronine treatment[22].

The stimulation of respiratory capacity resembles increases of enzyme activities,

which in part also occur in constant proportions following insulin treatment (isocitrate dehydrogenase (EC 1.1.1.41), isocitrate dehydrogenase (NADP) (EC 1.1.1.42), aspartate transaminase (EC 2.6.1.10), glutamate dehydrogenase (NAD(P)) (EC 1.4.1.3), hydroxybutyrate dehydrogenase (EC 1.1.1.30)).

In Figs. 2a, 2b these results are shown together with data obtained after simultaneous treatment with insulin and triiodothyronine, which leads to additive effects in most cases. Neither insulin nor triiodothyronine cause any changes of P/O ratios,

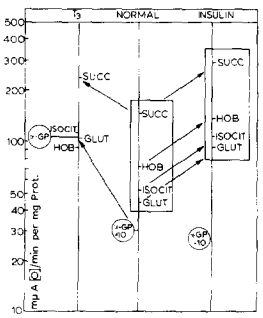
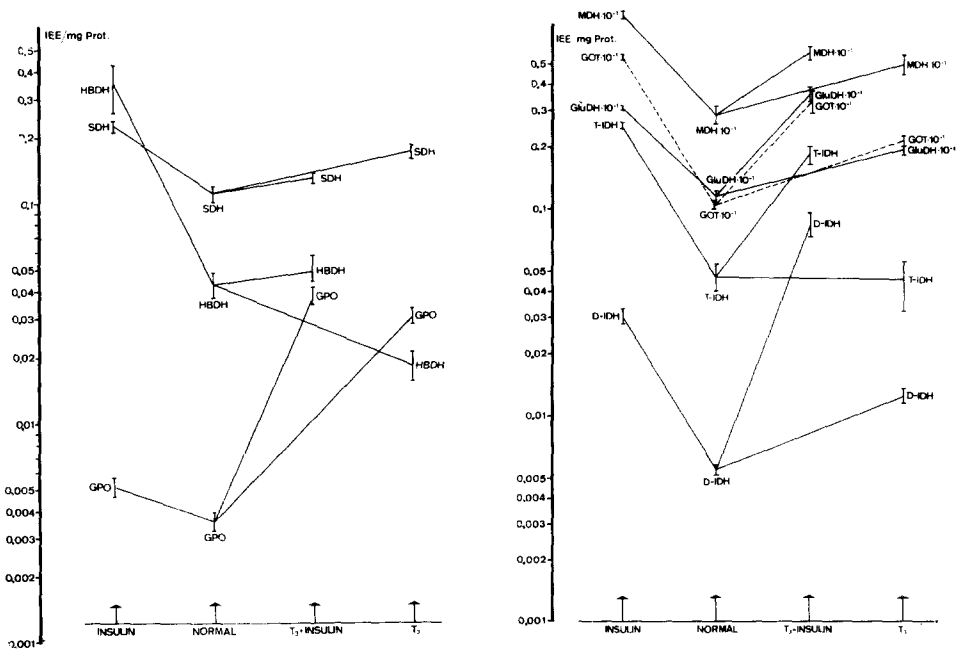


Fig. 1. Maximum respiration of rat liver mitochondria after a 4-day treatment with insulin or triiodothyronine. Conditions described in the text. Abbreviations: ISOCIT, isocitrate; HOB, hydroxybutyrate; GLUT, glutamate; SUCC, succinate; α-GP, α-glycerophosphate.



Figs. 2a and 2b. Enzyme activities in rat liver mitochondria of normal, triiodothyronine-, and insulin-treated rats. The activities are given in international units. Abbreviations: GPO = α-glycerophosphate dehydrogenase (EC 1.1.2.1), GOT = aspartate transaminase (EC 2.6.1.15), MDH = malate dehydrogenase (EC 1.1.1.37), GluDH = glutamate dehydrogenase (EC 1.4.1.3), T-IDH = isocitrate dehydrogenase (NADP) (EC 1.1.1.42), D-IDH = isocitrate dehydrogenase (NAD) (EC 1.1.1.41), HBDH = β-hydroxybutyrate dehydrogenase (EC 1.1.1.30), SDH = succinate dehydrogenase (EC 1.3.99.1).

TABLE I

RESPIRATORY CONTROL AND P/O RATIOS OF LIVER MITOCHONDRIA FROM NORMAL, TRIIODOTHYRONINE- AND INSULIN-TREATED RATS

All figures represent mean values from 15–20 mitochondrial preparations and 10–15 single measurements with each preparation. Average standard deviation ± 0.14. Animals were treated with the hormones for 4 days. R.C. = respiratory control.

Particle state	*α-Glycerophosphate*		*Succinate*		*β-Hydroxybutyrate*		*Glutamate*		*Isocitrate*	
	R.C.	*P/O*	*R.C.*	*P/O*	*R.C.*	*P/O*	*R.C.*	*P/O*	*R.C.*	*P/O*
Normal	—	—	5.9	1.9	3.7	2.7	4.4	2.8	3.6	2.7
T_3	2.9	1.9	3.9	1.8	3.4	2.8	6.4	2.7	4.6	2.8
Insulin	—	—	6.6	1.9	5.5	2.9	4.3	2.8	5.3	2.6

TABLE II

PYRIDINE NUCLEOTIDE AND CYTOCHROME CONTENTS OF LIVER MITOCHONDRIA FROM NORMAL, TRIIODOTHYRONINE-, AND INSULIN-TREATED RATS

All data in μmoles/g protein ± standard deviation. All figures represent means of 15–20 experiments in each group. Hormone treatment as described in Table I.

	Insulin	*Normal*	*Triiodothyronine*
$\Sigma NAD^+ + NADH$	3.60 ± 0.1	3.86 ± 0.2	3.10 ± 0.09
$\Sigma NADP^+ + NADPH$	4.76 ± 0.1	4.84 ± 0.2	2.89 ± 0.07
$\Sigma NADP/\Sigma NAD$	1.32	1.25	0.93
Cytochrome *c*	0.17 ± 0.02	0.25 ± 0.01	0.31 ± 0.01
Cytochrome *a*	0.16 ± 0.01	0.20 ± 0.01	0.27 ± 0.01
ΣNAD/cytochrome *c*	21.2	15.3	9.9
$\Sigma NADP$/cytochrome *c*	27.6	19.3	9.3

i.e., of the basic efficiency of oxidative phosphorylation, as shown in Table I. Therefore, any uncoupling effects can be excluded. However, the kinetics of electron flux through the respiratory chain are influenced in an opposite manner. Respiratory control increases under insulin and decreases below normal under triiodothyronine. These findings may be explained by concomitant changes of cytochrome and pyridine nucleotide contents, which are demonstrated in Table II. Insulin causes minor changes of only mitochondrial pyridine nucleotides; whereas, triiodothyronine drastically diminishes them. The ratio of total NADP/NAD clearly indicates a relative increase of NADP (the coenzyme of most synthetic pathways) by insulin and a decrease by triiodothyronine. The changes are consistent with the anabolic and catabolic roles of these hormones. Since contrary effects of insulin and triiodothyronine were observed on mitochondrial contents of cytochromes *a* and *c*, which are increased by triiodothyronine (as already shown by other investigators[23,24]) and are decreased by insulin treatment, two conclusions may be drawn:

1. The respiratory rate under optimum conditions is limited by the activity of the dehydrogenases rather than by cytochromes.

2. The ratio of pyridine nucleotides to cytochrome *c* is changed to above normal by insulin and to below normal by triiodothyronine, respectively (Table II). This

ratio can, to a certain extent, be taken as a measure of electron pressure put on the respiratory chain, if there is a maximum turnover of the respiratory chain-linked NAD. Thus, saturation of cytochromes with electrons (cytochrome turnover) is enhanced in mitochondria from insulin-treated rats and diminished in those from triiodothyronine-treated rats, according to Table II.

From the results outlined in Tables I, II, it is evident, that respiratory control is a function of the ratio of pyridine nucleotides to cytochromes. Further support is given to this statement by the results of MACLENNAN AND TZAGOLOFF[25], which demonstrate the loss of respiratory control by successive depletion of mitochondrial NAD. Therefore, our results may allow more insight into the anabolic or catabolic actions of insulin or triiodothyronine by suggesting that both hormones switch the energy-controlled pathways of hydrogen, since the relative rates of energy-controlled hydrogen flux from the flavoprotein region to NAD^+ or from NADH to $NADP^+$ may be enhanced by insulin and diminished by triiodothyronine in accordance with the degree of respiratory control.

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