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Enzyme Activities in Liver and Muscle Biopsy Specimens from Thyrotoxic and Hypothyroid Patients

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Abstract

Diagnosis of thyroid disorders was based on clinical status, radioiodine studies, $PB^{127}I$ and T_3 in vitro test. Needle biopsy samples from livers were immediately frozen (liquid N_2) and enzyme activities were determined in supernatant and sediment fractions of total homogenates of each biopsy sample.

Phosphoglucomutase (E.C.2.7.5.1) is diminished in thyrotoxicosis (N = 10), as compared to controls (N = 19), indicating low glycogenolysis, and glyceraldehyde DH (E.C.1.2.1.12) was increased (augmented glycolytic rate). Hexokinase (E.C.2.7.1.1) and glucose-6-phosphate DH (E.C.1.1.1.49) remained unchanged by thyrotoxicosis or hypothyroidism (N = 5).

The activity of PEP-carboxykinase (4.1.1.31) is elevated in thyrotoxicosis and lowered in hypothyroidism, presumably with equivalent alterations of gluconeogenesis. The latter result has to be discussed with respect to the demonstration of an increased level of free cortisol in serum, resp. of diminished insulin efficiency in our thyrotoxis patients.

Enzymes of citric acid cycle and connected pathways—condensing enzyme (E.C.4.1.3.7), NADP-isocitrate DH (E.C.1.1.1.42), glutamate DH (E.C.1.4.1.2), aspartate amino transferase (E.C.2.6.1.1), malate DH (E.C.1.1.1.37)—were unchanged by thyrotoxicosis or hypothyroidism.

Malic enzyme (E.C.1.1.1.40) was increased in livers of thyrotoxic patients, 3-Hydroxyacyl-CoA DH (E.C.1.1.1.35) was not changed, whereas carnitine acetyl-transferase (E.C.2.3.1.7) was elevated in thyrotoxicosis. Mitochondrial α-glycerol phosphate DH (E.C.1.1.99.5) showed no increase in thyrotoxic patients. This was unexpected in view of the known induction of this enzyme in the liver from rats treated with thyroid hormones (LARDY).

These results are discussed with respect to species differences and to mechanism of action of thyroid hormones.

Extrait

Le diagnostic des troubles thyroïdiens était basé sur l'état clinique, l'exploration par le radioiode, le $PB^{127}I$ et le test à la T_3 in vitro. Les échantillons biopsiques des foies ont immédiatement été congelés (N_2 liquide) et les activités enzymatiques déterminées dans le surnageant et les fractions de sédimentation des homogénats totaux de chaque échantillon biopsique.

La phosphoglucomutase (E.C.2.7.5.1) est diminuée dans la thyrotoxicose (N = 10), en comparaison aux témoins (N = 19), ce qui indique une basse glycogénolyse, et glyceraldehydephosphate DH (E.C.1.2.1.12) était augmentée (taux glycolytique accru). L'hexokinase (E.C.2.7.1.1) et le glucose-6-phosphate DH (E.C.1.1.1.49) n'ont pas été modifiés par la thyrotoxicose ou par l'hypothyroïdie (N = 5).

L'activité de la PEP-carboxykinase (4.1.1.31) est élevée dans la thyrotoxicose et abaissée dans l'hypothyroïdie, probablement avec des altérations équivalentes de la gluconéo-

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génèse. Ce dernier résultat doit être discuté en rapport avec la manifestation d'un taux accru de cortisol libre dans le sérum et d'une efficacité insulinique diminuée chez nos malades thyrotoxiques.

Les enzymes du cycle de l'acide citrique et les voies connectées — citrate synthase (E.C.4.1.3.7), NADP-isocitrate DH (E.C.1.1.1.42), glutamate DH (E.C.1.4.1.2), aspartate amino-transférase (E.C.2.6.1.1), malate DH (E.C.1.1.1.37) — n'ont pas été changés par la thyrotoxicose ou l'hypothyroïdie.

L'enzyme malique (E.C.1.1.1.40) était augmenté dans les foies des malades thyrotoxiques. 3-hydroxyacyl-CoA DH (E.C.1.1.1.35) n'a pas été modifié, alors que la carnitine acétyl-transférase (E.C.2.3.1.7) était élevée dans la thyrotoxicose. On n'a pas observé une augmentation du α-glycérol-phosphate DH mitochondrial (E.C.1.1.99.5) chez les malades thyrotoxiques. Ce résultat était inattendu étant donné l'induction connue de cet enzyme dans le foie de rats traités par des hormones thyroïdiennes.

Ces résultats sont discutés en ce qui concerne les différences d'espèce et le mécanisme d'action des hormones thyroïdiennes.

Auszug

Die Diagnose von Schilddrüsenstörungen stützte sich auf den klinischen Status, Radiojoduntersuchungen, $PB^{127}J$ und den T_3-*in-vitro*-Test. Nadelbiopsien aus Lebern wurden sofort eingefroren (flüssiger N_2) und die Enzymaktivitäten im Überstand und den Sedimentfraktionen aller Homogenate jeder bioptischen Probe bestimmt.

Die Phosphoglucomutase (E.C.2.7.5.1) ist bei Thyreotoxikose verringert (N = 10), im Vergleich zu den Kontrollen (N = 19), was auf eine niedrige Glykogenolyse hinweist. Glyceraldehydphosphat DH (E.C.1.2.1.12) war erhöht (erhöhte Glykolyserate). Hexokinase (E.C.2.7.1.1) und Glukose-6-Phosphat DH (E.C.1.1.1.49) blieben bei Thyreotoxikose oder Hypothyreose unverändert (N = 5).

Die Aktivität der PEP-Carboxykinase (4.1.1.31) ist bei Thyreotoxikose erhöht und bei Hypothyreose verringert, vermutlich mit äquivalenten Veränderungen der Gluconeogenese. Das letztgenannte Ergebnis muß im Zusammenhang mit dem Nachweis eines erhöhten Titers von freiem Cortisol im Serum bzw. einer verminderten Insulinwirksamkeit bei unseren thyreotoxischen Patienten erörtert werden. Enzyme des Citronensäurezyklus und dessen Nebenwege — Citrat Synthase (E.C.4.1.3.7), NADP Isocitrat DH (E.C.1.1.1.42), Glutamat DH (E.C.1.4.1.2), Aspartataminotransferase (E.C.2.6.1.1), Malat DH (E.C.1.1.1.37) — blieben durch Thyreotoxikose oder Hypothyreose unverändert.

Das Malat Enzym (E.C.1.1.1.40) war in Leberbiopsien von thyreotoxischen Patienten vermehrt. 3-Hydroxyacyl-CoA DH (E.C.1.1.1.35) war nicht verändert, während Carnitin-Acetyltransferase (E.C.2.3.1.7) bei Thyreotoxikose erhöht war. Mitrochondriale α-Glycerolphosphat DH (E.C.1.1.99.5) zeigte keine Erhöhung bei thyreotoxischen Patienten. Dies war im Hinblick auf die bekannte Induktion dieses Enzyms in der Leber von mit Schilddrüsenhormonen behandelten Ratten (LARDY) unerwartet.

Die Ergebnisse werden unter Berücksichtigung der Species-Unterschiede und dem Wirkungsmechanismus der Schilddrüsenhormone besprochen.

The diagnosis of the thyroid status of the patients under study was based on results of tests summarized in Table 1. Only unequivocal cases of thyrotoxicosis or hypothyroidism were selected; however the most severe cases of thyrotoxicosis had to be excluded owing to the need for immediate treatment (SCRIBA et al., 1970). In addition to "typical" values of $PB^{127}I$ and T_3-in-vitro-test, in thyrotoxicosis elevated concentrations of "free" serum cortisol respectively in thyrotoxicosis and in hypothyroidism

Table 1

Clinical data of patients under study (mean + SD).

The techniques used for PB^{127}I (Autoanalyzer^{R}), fluorimetric determination of serum cortisol, assay of "free" T_3-^{125}J and ^{3}H-cortisol by dextran gel filtration, i. v. glucose tolerance test and insulin efficiency coefficient have been published (SCRIBA et al., 1970). Age, body weight, clinical thyroid diagnostic index, data of radioiodine studies and various clinical chemical values of patients, have been reported (SCRIBA et al., 1970). Control patients submitted to liver biopsy were suspect for mild liver disease; the normal ranges reported were derived from healthy control persons

	hypo-thyroidism n = 9	control patients n = 20	thyro-toxicosis n = 18	normal range (mean ± 2 S.D.)
PB ^{127}I × % "free" T_3-^{125}J (μg/100 ml)	0.13 ± 0.06	0.98 ± 0.49	3.92 ± 1.95	0.44–8
serum cortisol × % "free" ^{3}H-cortisol (μg/100 ml)	2.19 ± 0.71	1.13 ± 0.49	2.34 ± 1.53	–
i. v. glucose tolerance test (kG)	1.04 ± 0.24	1.55 ± 0.39	1.13 ± 0.35	1.2 – 2.2
insulin efficiency coefficient (mg glucose/mE IMI)	14.8 ± 13.7	20.6 ± 6.4	11.8 ± 6.9	20 –70

decreased glucose tolerance and diminished insulin sensitivity were observed (Table 1). In thyrotoxicosis the histologic examination of liver biopsy samples revealed an increased width of sinusoids throughout the entire hepatic lobule, well distinguishable from congestive failure (SCRIBA et al., 1970).

Biochemical methods

Biopsy samples from muscles (M. quadriceps, M. erector trunci) and needle biopsy samples from liver were frozen in liquid nitrogen immediately after removal. Extraction of the samples was performed according to (BASS et al., 1969). Soluble and structure-bound enzyme activities representative of glycogen metabolism, glucose oxidation, glycolysis, gluconeogenesis, citric acid cycle and connected pathways, fatty acid oxidation, acetyl transfer and glycerolphosphate oxidation were determined in standardized optical tests (BASS et al., 1969; BÜCHER et al., 1964; BRDICZKA et al., 1969). PEP-carboxikinase (carboxylation reaction) was measured according to CHANG and LANE 1966).

Results

Liver. In thyrotoxicosis (n = 10) (Fig. 1), a distinct decrease ($p < 0.01$) is found in the activity of phosphoglucomutase (PGM, E.C. 2.7.5.1). Hexokinase activity (HK, E.C. 2.7.1.1) is unaffected in thyrotoxicosis (measuring was performed with 2 mM glucose), although it is decreased

significantly in hypothyroidism (n = 5) ($p < 0.02$). No changes are found in the activity level of glucose-6-phosphate dehydrogenase (G6PDH, E.C. 1.1.1.49). Glyceraldehyde-phosphate dehydrogenase activity (GAPDH, E.C. 1.2.1.12) is lower in hypothyroidism and increases in thyrotoxicosis ($p < 0.005$). With regard to enzymes of gluconeogenesis, no changes are observed in the activity of fructosediphosphatase (FDP-ase, E.C. 3.1.3.11); however, a marked increase occurs in the activity level of PEP-carboxykinase (PEP-CK, E.C. 4.1.1.32) in thyrotoxicosis. In hypothyroidism the activity of this enzyme decreases. Also, malic enzyme (ME, E.C. 1.1.1.40), is found at significantly higher activity levels in thyrotoxicosis.

The investigated enzymes of the tricarboxylic acid cycle and connected pathways such as condensing enzyme (CE, E.C. 4.1.3.7), isocitrate de-

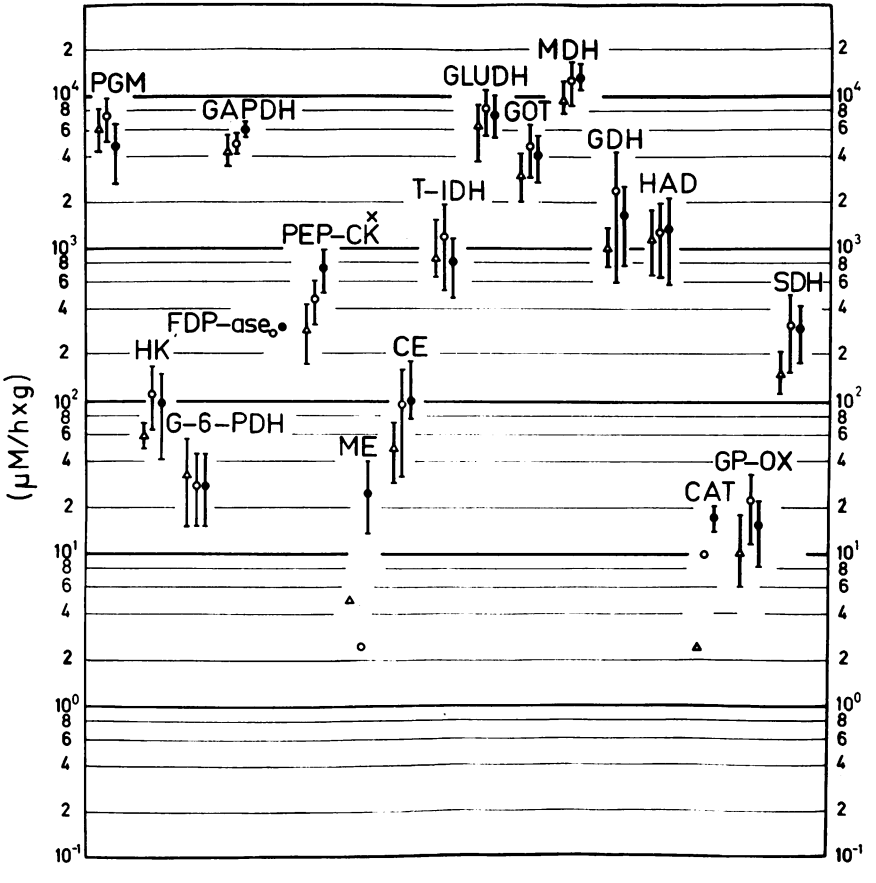

Fig. 1. Enzyme activity pattern of human livers. Vertical bars indicate the standard error of the mean of 5 hypothyroid (△), 15 control (O), 10 thyrotoxic (●) patients. *PEP-CK activity was determined at 37° C. All other enzymes were measured at 25° C.

hydrogenase (IDH, NADP specific, E.C. 1.1.1.42), succinate dehydrogenase (SDH, E.C. 1.3.99.1), malate dehydrogenase (MDH, E.C. 1.1.1.37), glutamate dehydrogenase (GLUDH, E.C. 1.4.1.2), and glutamate oxaloacetate transaminase (GOT, E.C. 2.6.1.1), reveal little or no significant changes of their activity levels. This is also true for 3-hydroxyacyl-CoA dehydrogenase (HAD, E.C. 1.1.1.35). Carnitine acetyltransferase (CAT, E.C. 2.3.1.7) is markedly increased in thyrotoxicosis, whereas it is decreased in hypothyroidism. In patients with thyrotoxicosis or hypothyroidism the activities of glycerol-3-phosphate dehydrogenase (GDH, E.C. 1.1.1.8) and glycerolphosphate dehydrogenase (GP-OX, E.C. 1.1.99.5) remain normal.

These results are valid in terms of absolute as well as of specific activities. The amount of soluble protein shows only minor changes. The following values (mean ± S.D.) were determined in hypothyroid, control and thyrotoxic liver samples: 139 ±57, 106 ±41, 118 ±23 mg of soluble protein/g of fresh weight.

Muscle. The data given in Table 2, refer to results obtained from muscle

Table 2

Activities and activity ratios of enzymes representative of different metabolic systems in normal and thyrotoxic human skeletal muscle

	M. erector trunci control[1])	M. quadriceps thyrotoxic	
PH	514	436	292
HK	34	98	66
GAPDH	12 150	8 748	7 056
LDH	5 750	8 850	5 510
GP-OX	21	21	14
HAD	364	167	212
CE	230	127	92
HK/GAPDH	2.8×10^{-3}	11.2×10^{-3}	9×10^{-3}
HK/CE	1.4×10^{-1}	7.7×10^{-1}	7.2×10^{-1}
PH/GAPDH	4.2×10^{-2}	5×10^{-2}	4.1×10^{-2}
GAPDH/GP-OX	5.8×10^{2}	4.2×10^{2}	5×10^{2}
HAD/CE	1.5	1.3	2.3

[1]) Control values according to SCHIMRIGK et al., 1967.

biopsies of the two thyrotoxic patients examined so far. As is evident, thyrotoxicosis causes a marked increase in the activity level of HK. Corresponding increases in muscle HK activity have been observed in experimental thyrotoxicosis of rats and guinea pigs (SMITH and WILLIAMS-ASHMAN, 1951; BARGONI et al., 1967; KUBISTA et al., 1971). The increased activity level of HK is also obvious from the activity ratios listed at the bottom of Table 2. As has been pointed out elsewhere, the activity ratio

HK/CE is a more or less constant numerical value when different types of vertebrate muscles are compared (PETTE, 1966; BASS et al., 1969). This ratio, however, is elevated in thyrotoxicosis (KUBISTA et al., 1971), and consequently the activity ratio HK/GAPDH is also found to be increased.

Discussion

Thyrotoxicosis causes certain changes in the enzyme patterns of liver and muscle. Obviously the changes observed in human liver differ qualitatively and quantitatively from those observed in experimental thyrotoxicosis, especially of the rat (LEE and LARDY, 1965; KADENBACH, 1966; NIKKILÄ and PITKÄNEN, 1959). Quantitative differences are probably due to differences existing between the levels of thyroid hormones in "physiological" and experimental thyrotoxicosis. Nevertheless, the decrease observed in the activity level of PGM as well as the increase in the activity levels of GAPDH, PEP-CK, ME and CAT correspond to similar changes found in experimental thyrotoxicosis.

The behaviour of GP-OX is of special interest. This enzyme is involved in extra-intramitochondrial hydrogen transfer by means of the glycerin-1-phosphate cycle. As first shown by LEE and LARDY (1965), experimental thyrotoxicosis causes an increase of GP-OX in rat liver by the factor of 10. As demonstrated by our findings, the human liver enzyme remains unaffected. This result is important with regard to the mechanism of action of thyroid hormones. It is generally believed that the increase of GP-OX assumes a predominant role in thyrotoxicosis. This increase holds for rat liver but not for human liver.

The differences are probably due to a species-specific response to thyroid hormones. This suggestion is also supported by the fact that differences exist between the changes observed in livers of thyrotoxic rats and guinea pigs. Thus, thyrotoxicosis does not cause an increase in activity levels of G6PDH, GP-OX and ME in the liver of the guinea pig, as this is typical for the rat liver. Species-specific differences may also be concluded from the data represented in Table 3. Table 3 lists activities of GAPDH and GP-OX as well as the activity ratios GAPDH/GP-OX (glycolysis/glycerolphosphate oxidation) in livers and skeletal muscles of different species. With regard to absolute activities of the two enzymes as well as their activity ratios, great differences exist in livers of different species. In the case of skeletal muscle, the activity ratio GAPDH/GP-OX varies to a much smaller degree (BASS et al., 1969; PETTE, 1966) than in the liver. In thyrotoxicosis, no changes are found in humanmuscle. In the rat and the guinea pig, the ratio GAPDH/GP-OX is shifted. These changes, however, occur only in red muscles (e.g. m. soleus), and are due to the fact that thyrotoxicosis induces an increase of GP-OX in this type of muscle only (KUBISTA et al., 1971). The most important change in the enzyme activity pattern of thyrotoxic human muscle is the high elevation of the HK activity. This change appears to be one of the few common and constant changes found in "physiological" and experimental thyrotoxicosis.

Table 3

Activities and activity ratios of glyceraldehydephosphate dehydrogenase and glycerolphosphate oxidase in normal and thyrotoxic livers and muscles of various species. Activities are given as μM/hxg w.w. Thyrotoxic animals received 15μg 3,3,'5-triiodothyronine per 100 g body weight daily for 6 days.

liver

	rat		guinea pig		pig	man	
	control	thyrotoxic	control	thyrotoxic	control	control	thyrotoxic
GAPDH	7 139	10 000	5 980	11 272	5 380	4 851	6 080
GP-OX	56	368	27	27	28	22	18
$\frac{\text{GAPDH}}{\text{GP-OX}}$	130	25	218	417	270	220	340

muscle

	rat				guinea pig				man	
	M. rectus f.		M. soleus		M. rectus f.		M. soleus		M. erector trunci	M. quadriceps
	control	thyrotoxic	control	thyrotoxic	control	thyrotoxic	control	thyrotoxic	control	thyrotoxic
$\frac{\text{GAPDH}}{\text{GP-OX}}$	200	210	300	140	280	166	234	155	580	455

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