ENERGY-LINKED REACTIONS IN HYPOTHYROID RAT LIVER SUBMITOCHONDRIAL VESICLES[1]

Timothy C. Evans[2] and Frederic L. Hoch

Departments of Biological Chemistry and Internal Medicine
The University of Michigan Medical Center, Ann Arbor, Michigan

Summary

    Processes in submitochondrial vesicles obtained from the liver mitochon-
dria of hypothyroid rats were compared at 25-30° with those from normal rats.
Hypothyroidism did not alter the rates of oxidation of succinate or β-hydroxy-
butyrate, or energy-independent transhydrogenation of pyridine nucleotides.
Energy-dependent transhydrogenation supported either by added ATP or by suc-
cinate oxidation was doubled in the vesicles of hypothyroid rats, whereas ATP-
supported reversed electron-flow was unchanged. Hormone injection corrected
the abnormal rate. Since submitochondrial vesicles phosphorylate ADP slowly
at 30° in hypothyroidism (5), these data suggest that the thyroid state con-
trols the use rather than the supply of available energy potential.

    Mitochondrial energy-linked processes appear to compete for a common

energy source generated by electron transport (1, 2). The rates of several

of these reactions, when measured at appropriate temperatures, are below nor-

mal levels in hypothyroidism: respiratory stimulation by dinitrophenol (3)

and ATP-driven Ca^{2+} uptake (4) in intact rat liver mitochondria, and phos-

phorylation of external ADP by inner membrane vesicles prepared from such

mitochondria (5). This report examines two other competing reactions in

inner membrane vesicles: the energy-dependent pyridine nucleotide transhy-

drogenase and energy-driven reverse electron-flow through Site I. These data

do not support a concept of generalized decreased enzymatic activity of hypo-

thyroid mitochondria, but suggest instead that the balance of energy conser-

vation is changed in the mitochondrial inner membrane in hypothyroidism,

since this transhydrogenase activity is high.

Abbreviations: LT_4, L-thyroxine; LT_3, L-triiodothyronine; AP-NAD(H), acetyl
pyridine-NAD(H).

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Materials and Methods

Mitochondria were obtained from the livers of normal, hypothyroid and thyroid hormone-injected rats (Spartan, Williamson, Mi.) as described previously (6). Hypothyroid rats were injected once with 0.5 µg of LT_4 per g or 0.25 µg of LT_3 per g body weight, and killed 3 days later. The final mitochondrial suspension was in 0.225 M sucrose, 0.4 mM dibucaine (to inhibit mitochondrial phospholipases (7)) and 2 mM EDTA, pH 8.0. With continuous cooling in an ice water bath, they were sonicated at full power in a Model W185C Branson Sonifier three times for 10 seconds each with 20 seconds between bursts. Following 10 min centrifugation at 6500 g the submitochondrial particles were sedimented from the supernatant at 56,000 g for 30 min. Final suspension was in the sucrose-dibucaine-EDTA medium at 3.0 – 7.2 mg/ml. Protein was measured by a rapid biuret method as modified by (8). Comparisons of rates based on protein contents are valid because the cytochrome a:protein ratios in vesicles and intact mitochondria are not affected by the thyroid state of the rats (9).

The rate of respiration was measured with a Clark electrode in a 1-ml closed chamber with continuous stirring at 25°C and pH 7.5 in a medium containing 0.25 M sucrose, 50 mM Tris, 6 mM $MgCl_2$, 4 mM KH_2PO_4, and 0.6 – 1.4 mg of vesicle protein. Respiration was started with the addition of either 10 mM succinate or 20 mM β-hydroxybutyrate plus 0.6 mM NAD^+.

Energy-independent transhydrogenation was measured in the direction NADPH + $AP-NAD^+$ → AP-NADH + $NADP^+$ (10) at 30°C and pH 6.5, in a 3-ml reaction chamber containing 0.25 M sucrose, 80 mM KH_2PO_4, 5.1 µM rotenone, 1 mM KCN, 0.167 mM NADPH, and 0.3 – 0.7 mg of vesicle protein. The reaction was started with the addition of 0.167 mM $AP-NAD^+$ and continuously recorded in an Aminco-Chance split beam spectrophotometer at 375 nm. The extinction coefficient of AP-NADH minus NADPH was found to be 6.2 A $mM^{-1}cm^{-1}$. The energy-dependent transhydrogenase was measured by the method of (11), and recorded in the dual-wavelength spectrophotometer at 340-374 nm. The difference extinction coefficient of NAD(P)H was 4.2 A $mM^{-1}cm^{-1}$. Reverse electron-flow activity was measured according to (12), with protein amounts of 0.3 – 0.7 mg, and followed at 340-374 nm.

Results

Vesicles obtained from the liver mitochondria of hypothyroid rats oxidize succinate or β-hydroxybutyrate at rates similar to those in vesicles from normal rats (Table I). The addition of 0.5 µg of oligomycin per ml to either group of vesicles inhibits the phosphorylation of ADP but not respiration (not shown in Table I); therefore respiration is loose-coupled and independent of the energy state in vesicles from normal or hypothyroid rats. Injection of either LT_4 or LT_3 significantly increases the rate of succinate oxidation but does not change β-hydroxybutyrate oxidation.

The rate of the energy-independent transhydrogenation is not significantly different in the vesicles obtained from hypothyroid rats. Pretreatment with hormone decreases this transhydrogenase activity slightly but significantly, but to levels that are not significantly different from the normal rates.

In vesicles from hypothyroid rats the rate of energy-driven transhydrogenation, supported by either ATP addition or substrate oxidation, is twice that seen in vesicles from normal rats. This excessive rate is partially

Table I

ENERGY-INDEPENDENT AND ENERGY-LINKED ACTIVITIES IN
RAT LIVER SUCROSE-DIBUCAINE-EDTA PARTICLES

Process	Normals (5-6)	Hypothyroids (4-9)	LT_4-Injected Hypothyroids (4-5)	LT_3-Injected Hypothyroids (3-6)
Respiration				
+ succinate	136.4 $\pm$ 15.2	154.8 $\pm$ 3.6	186.2 $\pm$ 12.0[#]	185.6 $\pm$ 13.0[#]
+ β-hydroxy-butyrate	71.0 $\pm$ 8.6	76.8 $\pm$ 4.8	80.0 $\pm$ 14.8	80.2 $\pm$ 10.8
TH	99.9 $\pm$ 10.0	122.7 $\pm$ 3.4	96.9 $\pm$ 5.5[#]	83.0 $\pm$ 10.2[#]
∿REF	25.8 $\pm$ 7.0	41.0 $\pm$ 4.7	40.1 $\pm$ 4.3	33.6 $\pm$ 5.9
∿TH				
+ ATP	36.9 $\pm$ 4.2	75.0 $\pm$ 3.4[*]	48.7 $\pm$ 1.1[#]	49.9 $\pm$ 7.1[#]
+ succinate	28.0 $\pm$ 4.8	52.8 $\pm$ 2.2[*]	37.9 $\pm$ 1.6[#]	36.0 $\pm$ 5.1[#]

[*] p <.05 vs normal

[#] p <.05 vs hypothyroid

The number of experiments are in parentheses; activities are expressed as n mole (NAD(P)H or 0) $min^{-1}mg^{-1}$.

Abbreviations: ∿REF, ATP-supported reversed electron-flow; TH and ∿TH, energy-independent and energy-dependent transhydrogenase respectively.

corrected by pretreatment with either LT_4 or LT_3.

The rate of the energy-dependent reversed electron-flow is not statistically different in vesicles from normal and hypothyroid rats, and remains unchanged after pretreatment of hypothyroid rats with either thyroid hormone.

Discussion

The role of the mitochondrion in the actions and effects of thyroid hormones has been controversial. Early work on oxidative phosphorylation demonstrated an uncoupling effect of thyroxine, but this required micromolar and higher concentrations of hormone (13-15). Later investigators concluded that liver mitochondria in hypothyroids are depleted both in number per cell and in enzyme and carrier components, secondary to defective protein synthesis

(16-20). Our present studies, however, indicate that this mechanism may re-
quire some reconsideration.

The similarity between the rates of loose-coupled respiration in inner
membrane vesicles prepared from the liver mitochondria of hypothyroid or nor-
mal rats indicates that the electron-transport system has a normal capacity
in hypothyroidism. The cytochrome:protein ratios of vesicles or intact mito-
chondria from our hypothyroid rats are also at normal levels (4, 5, 9) al-
though others have reported decreases (21). Both the pyridine nucleotide (22,
23) and flavin (4, 24) contents are elevated. Thus, the depression in the
rate of translation in the livers of hypothyroid rats (25) does not apparently
deplete the mitochondrial respiratory chain. The normal rate of reversed
electron-flow in hypothyroids (Table I) is further evidence for an unchanged
transport capability through Site I, and shows in addition that the available
energy potential remains normally accessible to at least one energy-linked
process.

The equality of the rates of energy-independent transhydrogenase activity
in vesicles from hypothyroid and normal rats is consistent with the transhy-
drogenase:protein ratio being unaffected by the thyroid state. This enzyme is
identical with that catalyzing the energy-dependent reaction, as shown by ex-
periments on their immunologic (26), kinetic (11, 27) and stereospecificity
(28) properties. The doubling of the rate of energy-dependent transhydrogen-
ation therefore seems ascribable to either an increase in the available energy,
or a redistribution of use of the available potential energy. Since the rate
of succinate-supported phosphorylation of external ADP is depressed in the
vesicles from hypothyroid rats as compared with those from normal rats (5, 29),
and ATP-supported reverse-electron-flow is the same in hypothyroid as normals,
whereas transhydrogenase driven by either ATP or succinate is increased, the
most tenable explanation appears to be a redistribution of the available energy
in hypothyroidism that favors energy-dependent transhydrogenation over phos-
phorylation.

This is the first demonstration of an increased rate of a mitochondrial
enzymatic process in hypothyroidism at a temperature commonly used for assays.
Decreased rates of phosphorylation of added ADP in hypothyroidism are corre-
lated with abnormal Arrhenius profiles and phospholipid fatty acid contents
of the inner membranes of liver mitochondria (5). The selectively increased
rate of energy-dependent transhydrogenation shown here suggests a hypothyroid
mitochondrion which is not defective or depleted but rather which has altered
its energy utilization dynamics as a result of the changed lipid environment
in which the enzymatic and electron-carrier proteins operate.

The energy-driven mitochondrial pyridine nucleotide transhydrogenase

shunts electron pairs away from oxidation, heat production and synthesis of
immediately utilizable high energy phosphate bonds, and in effect either
stores the reducing equivalents as NADPH for subsequent oxidation (a low-
capacity rapidly-available electron pool) or makes them available for reduc-
tive lipid syntheses (a large-capacity slowly-available electron pool). The
net movement of electrons away from the electron-transport chain towards NADPH
would be favored in conditions of cellular rest with its lowered demand for
ATP and the reversed movement would be favored under conditions of cellular
activity and relative ATP insufficiency. An exaggerated differential usage
of the potential energy of the NADH electron pair is seen in hypothyroidism,
where the energy-dependent transhydrogenase competes more effectively for the
driving energy potential than in the euthyroid state. A net shift of reducing
equivalents away from the electron-transport chain towards NADPH occurs, which
is further emphasized by the 50% increase in the mitochondrial content of NADP(H)
in hypothyroidism (22). These observations suggest a metabolic role for the
transhydrogenase in regulating the utilization of the free energy changes of
oxidative reactions.

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