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EFFECT OF RIBOFLAVIN DEFICIENCY ON METABOLISM OF THE RAT IN HYPERTHYROID AND EUTHYROID STATE

J. NOLTE*, D. BRDICZKA AND H. W. STAUDTE
Fachbereich Biologie der Universität Konstanz, D-775 Konstanz (Germany)

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SUMMARY

The basal metabolic rate in riboflavin-deficient rats is lowered. Upon application of triiodothyronine, oxygen consumption is raised to the level of the thyrotoxic controls. Activity levels of flavin enzymes [α-glycerolphosphate dehydrogenase (L-glycerol-3-phosphate:(acceptor) oxidoreductase, EC 1.1.99.5), succinate dehydrogenase (succinate:(acceptor) oxidoreductase, EC 1.3.99.1), monoamine oxidase (monoamines:O_2 oxidoreductase (deaminating), EC 1.4.3.4)] were determined in liver, *musculus rectus femoris*, *musculus soleus*, heart muscle, and *cortex cerebri*. Riboflavin deficiency causes a decrease of α-glycerolphosphate dehydrogenase in liver, *musculus rectus femoris*, and heart muscle and a decrease of succinate dehydrogenase and monoamine oxidase primarily in liver. The induction of α-glycerolphosphate dehydrogenase by triiodothyronine is abolished in deficient animals. It is proposed that under riboflavin deficiency a lower level of thyroid hormones is responsible for the decreased metabolic rate and that the decreased activities of flavin enzymes are not rate limiting.

INTRODUCTION

Lee and Lardy[1] reported that after administration of thyroid hormones α-glycerolphosphate dehydrogenase (L-glycerol-3-phosphate:(acceptor) oxidoreductase, EC 1.1.99.5) activity increases 3 to 20 times in all organs or tissues of the rat which also exhibit elevated oxygen consumption. Considering that the mitochondrial α-glycerolphosphate dehydrogenase together with the extramitochondrial enzyme is involved in hydrogen transfer across mitochondrial membranes (α-glycerolphosphate cycle)[2,3], Lee and Lardy[1] suggested that the increase of activity of this enzyme is responsible for the elevated metabolic rate in thyrotoxicosis. However, the finding that in most animals α-glycerolphosphate dehydrogenase is not or is only slightly induced by thyroid hormones[4–7] is not in line with this hypothesis. Wolf and Rivlin[8] recently reported that in rat liver induction of α-glycerolphosphate dehydrogenase

* Present adress: Medizinische Universitätsklinik Freibrug, D-78 Freiburg, Germany.

by thyroid hormones is abolished during riboflavin deficiency. The present study was performed to determine whether the above postulated correlation between α-glycerolphosphate dehydrogenase activity and basal metabolic rate in rats holds also in riboflavin deficiency.

According to Lee and Lardy[1] the influence of thyroid hormones on metabolic rate might be related to the extra-/intramitochondrial hydrogen transfer. If that is so it must be considered that other mechanisms beside the α-glycerolphosphate cycle, such as the malate–aspartate shuttle[9] may also take part in the extra-/intramitochondrial hydrogen transport. Since heart and skeletal muscle have a high glycolytic activity, oxidation of cytosolic hydrogen by the mitochondria plays a main role in the energy supplying metabolism. Thus, in these tissues the malate–aspartate shuttle alternatively may be active in riboflavin deficiency and may then be induced by thyroid hormones.

MATERIALS AND METHODS

Male animals: FP-49 rats (Thomae, Biberach, Germany) weighing 200–240 g were used in all experiments. Controls were maintained on a commercial standard rat diet (Altromin, Altrogge, Lage, Germany). Riboflavin-deficient diet was obtained from Nutritional Biochemical Corp., Cleveland, Ohio, U.S.A. 3,3',5-triiodo-L-thyronine (Fluka, Buchs, Switzerland), 25 μg/100 g body weight, was administered intraperitoneally for 5–10 days.

In our studies of replacement of riboflavin, 0.1 ml/100 g body weight, Polybion (Merck, Darmstadt, Germany) was applied intraperitoneally for 5 days. Polybion contains the following mixture of substances per ml: 2 m griboflavin 5'-phosphate, 5 mg vitamin B_1, 2 mg vitamin B_6, 4 μg vitamin B_{12}, 3 mg panthenoic acid, 20 mg nicotinamide, 0.25 mg biotin. Determination of basal metabolic rate was achieved according to Pace *et al.*[10]. Measurements were performed for each animal separately.

Preparation of tissues

Rats were sacrificed by a blow to the head followed by decapitation and exsanguination. Organs were promptly removed, weighed and placed in cold preparation media. In liver and heart tissue extra- and intramitochondrial enzymes were separated by fractional extraction according to Pette[11]. From *musculus soleus* and *musculus rectus femoris* no fractional extraction but rather total homogenates were made[12]. Mitochondria from the livers were prepared in 0.3 M sucrose[13].

Enzyme determinations

Hexokinase (EC 2.7.1.1), glycogen phosphorylase (*a* + *b*) (EC 2.4.1.1), triosephosphate dehydrogenase (EC 1.2.1.12), 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35), citrate synthase (EC 4.1.3.7), α-glycerolphosphate dehydrogenase were determined according to ref. 12. Succinate dehydrogenase (EC 1.3.99.1) and monoamine oxidase (EC 1.4.3.4) were determined as described in ref. 13, riboflavinkinase (EC 2.7.1.26) was measured by the method of ref. 14, malate dehydrogenase (EC 1.1.1.37) and aspartate aminotransferase (EC 2.6.1.1) by the method described in ref. 15.

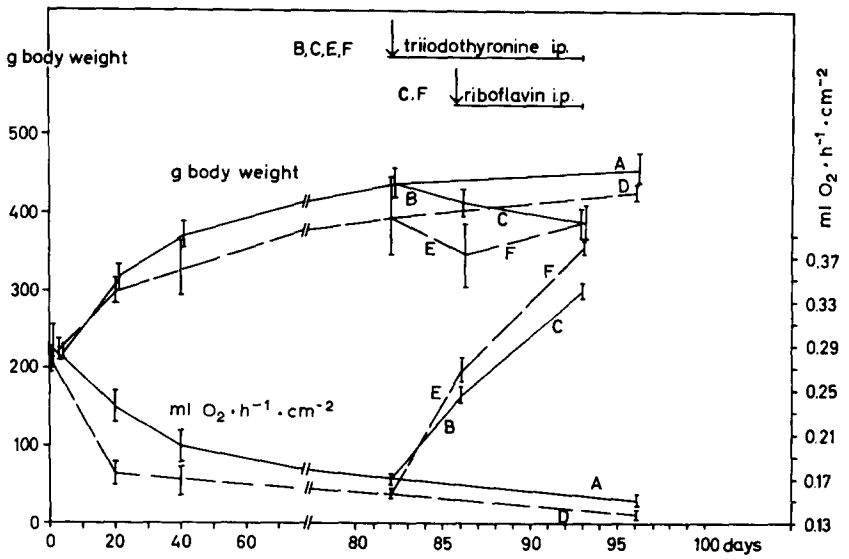

Fig. 1. Basic metabolic rates and body weights of normal and riboflavin-deficient rats. Six groups composed of three animals were formed: Group A was held on standard diet, Group D on riboflavin-deficient diet for 96 days, Group B was placed on standard diet and received 25 μg triiodothyronine per 100 g body weight for 5 days (from 82nd to 86th day), Group E held on riboflavin deficient diet for 96 days was treated with triiodothyronine from the 82nd to 86rh day, Group C received standard diet together with triiodothyronine injection for 10 days (from 82nd to 93th day) and additionally from the 82nd up to the 93rd day injections of Polybion (0.15 mg riboflavin per 100 g body weight), Group F was maintained on riboflavin deficient diet for 93 days and was treated with triiodothyronine and Polybion in the same way as C. Left ordinate indicates body weight, right ordinate indicates basal metabolic rate given as ml $O_2 \cdot h^{-1} \cdot cm^{-2}$ body surface. Means $\pm$ S.D. are shown in bars. O_2 consumption of each animal was determined 3 times. ———, controls; — — —, riboflavin-deficient animals. Arrows indicate the beginning of triiodothyromine and riboflavin application, respectively. i.p. = intraperitoneally.

RESULTS AND DISCUSSION

Changes in body weight

Fig. 1 shows the course of changes in body weight and basal metabolic rate of normal and riboflavin-deficient rats.

The body weight of riboflavin-deficient animals (D) increased at a slower rate compared to the controls (A). After application of triiodothyronine the body weight of normal (B) and riboflavin-deficient animals (E) decreased. When in deficient animals riboflavin was replaced (F) the body weight increased up to the level of those animals treated with thyroid hormone (C). These findings are in agreement with ref. 8.

Changes in basal metabolic rate

It is well known that there exists a reciprocal correlation between body weight and oxygen consumption. As already demonstrated in Fig. 1 the body weight of the different experimental groups showed marked differences during the experiment. Therefore oxygen consumption was based on body surface area[16]. If this calculation was applied, the basal metabolic rate of the control and the riboflavin-deficient

animals decreased, but O_2 consumption of deficient animals in all stages of the experiment was lower (A,D). After addition of triiodothyronine the basal metabolic rate of deficient and control animals increased to the same level (E, B). This suggests that the influence of thyroid hormones on the basal metabolic rate is independent of the supply of riboflavin, as may be seen also from the fact that replacement of riboflavin in deficient thyrotoxic animals (F) leads to the same further increase of the basal metabolic rate as compared to normal thyrotoxic rats.

Changes of enzyme activities in riboflavin deficiency

As shown in Table I activities of all flavoenzymes decreased in riboflavin deficiency. While activity of monoamine oxidase in liver and of α-glycerolphosphate dehydrogenase in all tissues listed (except in brain and *musculus rectus*) decreased by a factor of 2 and more, the activity of succinate dehydrogenase was diminished by almost less than a factor of 1.5. This suggests that enzymes which are active in basic metabolic pathways such as succinate dehydrogenase in the citric acid cycle are slightly reduced, whereas enzymes which catalyze alternative metabolic pathways in some tissues show much higher deviations from normal activity levels. It is interesting that the activity of α-glycerolphosphate dehydrogenase is much less reduced in the brain during riboflavin deficiency. In this tissue hydrogen transfer to the mitochondria is essential because glycolysis is the main source of energy supply.

Changes in enzyme activities in thyrotoxicosis

Application of triiodothyronine to normal animals causes the well-known induction of α-glycerolphosphate dehydrogenase in liver with an increase of activity by a factor of 10 and in heart and red muscle by a factor of 2 (ref. 17). In white muscle[17] and brain where the activity levels of glycerolphosphate dehydrogenase are normally high no further induction of the enzyme by triiodothyronine was observed. In contrast to this finding succinate dehydrogenase in *musculus rectus* (normally exhibiting a low activity level of this enzyme) was induced by thyroid hormone whereas in liver, heart and red muscle no significant induction could be detected.

Changes in enzyme activities in thyrotoxicosis and riboflavin deficiency

In riboflavin-deficient animals induction of α-glycerolphosphate dehydrogenase by triiodothyronine was almost abolished in red and white muscle and *cortex cerebri* whereas in liver and heart the enzyme was induced by a factor of 2.5 to 3.0. When riboflavin in deficient thyrotoxic animals was replaced, the activity of α-glycerolphosphate dehydrogenase increased to the level found in control thyrotoxic rats. In red and white muscle upon addition of riboflavin, the activity of α-glycerolphosphate dehydrogenase exceeds that of the control thyrotoxic animals. In contrast to that finding, the activity of succinate dehydrogenase in red and white muscle was not increased up to the level of thyrotoxic control animals when riboflavin was replaced. On the other hand, in liver and heart muscle (where activity to succinate dehydrogenase slightly decreased in riboflavin deficiency) there was observed an activity level which was normally found in thyrotoxic controls.

As described recently by Wolf and Rivlin[8] activity of riboflavinkinase is induced by thyroid hormones. This induction is also observed in riboflavin deficiency

TABLE I

ACTIVITIES OF FLAVIN ENZYMES AND RIBOFLAVINKINASE IN TISSUES OF CONTROL AND RIBOFLAVIN-DEFICIENT RATS

Experimental groups are described in the legend of Fig. 1. The data were obtained from pooled organs of three animals.

Treatment	*Enzyme*	*Enzyme activity (units/g fresh wt)*				
		Liver	*M. rectus*	*M. soleus*	*Heart*	*Cortex cerebri*
(A) Standard diet	α-Glycerolphosphate dehydrogenase	1.1	1.0	0.5	1.2	1.7
	Succinate dehydrogenase	16.2	1.3	5.5	22.8	—
	Riboflavinkinase	$5 \cdot 10^{-6}$	—	—	—	—
	Monoamine oxidase	5*	—	—	—	—
(B) Standard diet	α-Glycerolphosphate dehydrogenase	9.7	1.0	1.0	2.8	1.9
+ triiodothyronine	Succinate dehydrogenase	18	2.4	6.3	25.2	—
	Riboflavinkinase	$14 \cdot 10^{-6}$	—	—	—	—
	Monoamine oxidase	—	—	—	—	—
(C) Standard diet	α-Glycerolphosphate dehydrogenase	9.3	0.9	0.9	2.6	—
+ triiodothyronine	Succinate dehydrogenase	19.5	2.2	6.7	27.5	—
+ riboflavin	Riboflavinkinase	$16 \cdot 10^{-6}$	—	—	—	—
	Monoamine oxidase	4.5*	—	—	—	—
(D) Riboflavin-deficient diet	α-Glycerolphosphate dehydrogenase	0.6	0.5	0.3	0.4	1.3
	Succinate dehydrogenase	12.6	0.7	4.8	15	—
	Riboflavinkinase	$1 \cdot 10^{-6}$	—	—	—	—
	Monoamine oxidase	1.4*	—	—	—	—
(E) Riboflavin-deficient diet	α-Glycerolphosphate dehydrogenase	1.5	0.6	0.4	1.2	1.7
+ triiodothyronine	Succinate dehydrogenase	11	0.4	2.2	13.3	—
	Riboflavinkinase	$38 \cdot 10^{-6}$	—	—	—	—
	Monoamine oxidase	1.2*	—	—	—	—
(F) Riboflavin-deficient diet	α-Glycerolphosphate dehydrogenase	8.3	1.3	1.7	2.3	—
+ triiodothyronine	Succinate dehydrogenase	15.8	1.0	4.5	25	—
+ riboflavin	Riboflavin kinase	$18 \cdot 10^{-6}$	—	—	—	—
	Monoamine oxidase	2.5*	—	—	—	—

* munits/mg mitochondrial protein.

TABLE II

EXTRA- AND INTRAMITOCHONDRIAL ACTIVITIES OF MALATE DEHYDROGENASE AND ASPARTATE AMINOTRANSFERASE IN LIVER AND HEART OF NORMAL AND RIBOFLAVIN-DEFICIENT RATS

The data were obtained from pooled organs of three animals. Units are referred to g fresh wt, percentage distribution in different compartments is given in parentheses.

Treatment	*Enzyme*	*Enzyme activity [units/g fresh wt (%)]*			
		Liver		*Heart*	
		Extramitochondrial	*Intramitochondrial*	*Extramitochondrial*	*Intramitochondrial*
(A) Standard diet	Malate dehydrogenase	277 (47)	309 (53)	750 (49)	790 (51)
	Aspartate aminotransferase	26 (12)	189 (88)	75 (24)	236 (76)
(B) Standard diet + triiodothyronine	Malate dehydrogenase	319 (41)	452 (59)	957 (43)	1278 (57)
	Aspartate aminotransferase	35 (15)	198 (85)	123 (30)	291 (70)
(C) Standard diet + triiodothyronine + riboflavin	Malate dehydrogenase	350 (46)	420 (54)	1100 (58)	840 (42)
	Aspartate aminotransferase	44 (18)	204 (82)	124 (31)	279 (69)
(D) Riboflavin-deficient diet	Malate dehydrogenase	340 (49)	360 (51)	850 (49)	875 (51)
	Aspartate aminotransferase	23 (9)	226 (91)	92 (26)	266 (74)
(E) Riboflavin-deficient diet + triiodothyronine	Malate dehydrogenase	341 (31)	727 (69)	877 (48)	932 (52)
	Aspartate aminotransferase	51 (18)	229 (82)	112 (24)	346 (76)
(F) Riboflavin-deficient diet + triiodothyronine + riboflavin	Malate dehydrogenase	459 (43)	604 (57)	780 (48)	866 (52)
	Aspartate aminotransferase	45 (15)	250 (85)	129 (29)	308 (71)

suggesting that the effect of thyroid hormones on enzymes containing no flavin is present under these conditions as well. Activity levels of monoamine oxidase are not influenced by the action of thyroid hormones. This flavin-containing enzyme depends solely on flavin supply. It does not reach normal activity levels after 9 days of replacement of riboflavin. These results clearly indicate that in riboflavin deficiency the increase of basal metabolic rate is not linked to the activity levels of α-glycerolphosphate dehydrogenase. This is in line with the observation that the nonphysiological D-thyronine in rat liver and in adipose tissue causes an increase of α-glycerolphosphate dehydrogenase activity but no increase of metabolic rate[18]. When induction of basal metabolic rate by thyroid hormones is linked to an increased hydrogen transfer in the mitochondria in riboflavin deficiency the glycerolphosphate shuttle[1,2] is not likely to be involved in this transfer. However, other mechanisms, such as the malate–aspartate shuttle[9], may account for elevated hydrogen transfer into the mitochondria. Absolute activities as well as percentage distribution of malate dehydrogenase and aspartate aminotransferase in the extra- and intramitochondrial compartment showed no significant variations in riboflavin deficiency, thyrotoxicosis and after application of thyroid hormone in riboflavin deficiency (Table II). This finding, however, does not exclude an increased flux rate through the malate–aspartate shuttle if both enzymes are not rate limiting.

Activities of enzymes representing basic metabolic pathways (triosephosphate dehydrogenase, citrate synthase, hydroxyacyl-CoA dehydrogenase) are neither decreased in riboflavin deficiency, nor increased in thyrotoxicosis (Table III). Considering that no changes in activity levels of enzymes from the malate–aspartate

TABLE III

ACTIVITIES OF TRIOSEPHOSPHATE DEHYDROGENASE, CITRATE SYNTHASE AND HYDROXYACYL-CoA DEHYDROGENASE IN DIFFERENT ORGANS OF NORMAL AND RIBOFLAVIN-DEFICIENT RATS

Experimental groups are described in the legend of Fig. 1. The data were obtained from pooled organs of three animals.

Treatment	*Enzyme*	*Enzyme activity (units/g fresh wt)*			
		M. rectus	*M. soleus*	*Heart*	*Liver*
(A) Standard diet	Triosephosphate dehydrogenase	450	155	226	100
	Citrate synthase	2.7	15.9	61	7.1
	Hydroxyacyl-CoA dehydrogenase	2.1	22	104	97
(B) Standard diet + triiodothyronine	Triosephosphate dehydrogenase	520	170	—	—
	Citrate synthase	2.7	12	53	12.5
	Hydroxyacyl-CoA dehydrogenase	2.1	17.5	100	100
(C) Standard diet + triiodothyronine + riboflavin	Triosephosphate dehydrogenase	322	127	227	180
	Citrate synthase	1	6.6	57	5.2
	Hydroxyacyl-CoA dehydrogenase	1.0	7.5	78	62
(D) Riboflavin-deficient diet	Triosephosphate dehydrogenase	473	133	200	141
	Citrate synthase	3.5	20	76	6
	Hydroxyacyl-CoA dehydrogenase	—	20	93	110
(E) Riboflavin-deficient diet + triiodothyronine	Triosephosphate dehydrogenase	472	160	—	—
	Citrate synthase	2.2	13.1	47	15
	Hydroxyacyl-CoA dehydrogenase	1.3	15	78	196
(F) Riboflavin-deficient diet + triiodothyronine + riboflavin	Triosephosphate dehydrogenase	440	198	245	175
	Citrate synthase	1.8	9	64	11.6
	Hydroxyacyl-CoA dehydrogenase	0.5	11.4	104	62.7

shuttle and of enzymes catalyzing basic metabolic pathways are observed in riboflavin deficiency, it may be suggested that the decrease of basal metabolic rate is caused by reduced activity of flavin enzymes. However, despite a lower activity of flavin enzymes in deficient animals upon application of triiodothyronine, oxygen consumption is increased to the level of the control thyrotoxic rats. This increase in basal metabolic rate is observed although activities of flavin enzymes are maintained on a level 2–3 times lower than thyrotoxic controls (Table I).

As accomplished in the present experiments it can be concluded that flavin enzymes are not rate limiting in this state of deficiency. It is proposed that the decrease of basal metabolic rate in riboflavin deficiency is due to a lowered level of thyroid hormones (Table IV).

TABLE IV

SERUM THYROXINE OF CONTROL AND RIBOFLAVIN-DEFICIENT RATS

The values are given as mean ± S.D. of μg thyroxine per 100 ml serum ($N = 4$). Experimental groups were described in legend of Fig. 1. The serum thyroxine levels were obtained by determination of iodine content of thyroxine by means of a competitive protein-binding method[19].

Treatment	*μg thyroxine per 100 ml serum*
(A) Standard diet	2.5 ± 0.7
(D) Riboflavin-deficient diet	0.31 ± 0.15

The serum thyroxine values shown in Table IV revealed a marked decrease in riboflavin-deficient rats. This decrease may be produced by a diminished activity level of NADPH–cytochrome *c* reductase which contains flavinadenindinucleotide as prosthetic group. *In vivo* production of H_2O_2 required for the iodide peroxidase reaction during biosynthesis of thyroid hormone may involve NADPH–cytochrome *c* reductase[20].

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