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The Effect of Thyroxine Status on Hepatic Levels of 10-Formyltetrahydrofolate Dehydrogenase

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ABSTRACT

The effect of thyroid hormone on hepatic levels of 10-formyltetrahydrofolate dehydrogenase (10-formyltetrahydrofolate : NADP oxidoreductase, E.C. 1.5.1.6.) was studied using Sprague-Dawley rat. Hypothyroidism increased histidine oxidation by 5 fold and increased 10-formyltetrahydrofolate dehydrogenase activity by 142%, and also decreased methylenetetrahydrofolate reductase activity by 52%. Decreased methylenetetrahydrofolate reductase acts by decreasing synthesis of 5-methyl folate, thereby increasing the proportion of non-methyl folate required for folate-dependent reactions.

Increased histidine oxidation produced by hypothyroidism may be attributed to its effect in decreasing 10-formyltetrahydrofolate dehydrogenase.

KEY WORDS : 10-formyltetrahydrofolate dehydrogenase · thyroxine.

Introduction

Tht production of hyperthyroidism by feeding of thyroid powder has long been shown to produce symptoms of folate impairment similar to those produced by vitamin B_{12} deficiency[1)2)3)]. This is evidenced by an increased excretion of formiminoglutamic acid (FIGLU) ane decreased oxidation of 2-carbon of histidine to carbon dioxide[4)5)6)].

Inhibition of thyroid function, on the other hand, by thyroidectomy or feeding a thyroid inhibitor produced the opposite effect by decreasing FIGLU excretion and increasing the oxidation of histidine to CO_2[6)]. Thyroidectomy has decreased the proportion of 5-methyl-tetrahydrofolate and decreased the level of methylenetetrahydrofolate reductase(E.C. 6.3.3.2.) which is responsible for the observed decrease in the proportion of 5-methyl folate coenzymes[6)]. It has been shown that the elevation of endogenous S-adenosyl methionine(SAM) levels inhibits methylenetetrahydrofolate reductase allosterically[7)].

In the mammalian cells, histidine is catabolized primarily to glutamic acid via the formation of FIGLU. The degradation of FIGLU to glutamate in the liver cell is brought about by the transfer of the formimino group to tetrahydrofolate(THF) in the generation of 10-formyl-THF. Three enzymatic steps catalyze the conversion of FIGLU and THF to glutamate, ammonia and 10-formyl-THF. Kutzbach and Stokstad[8)] decribed that 10-formyltetrahydrofolate dehydrogenase (E.C. 1.5.1.6.) could convert 10-formyl-THF to THF and $C0_2$.

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They suggested that the *in vivo* activity of this enzyme might explain the earlier observations a reduced oxidation of formate to CO_2 in folate deficient animals and tissues.

In view of the symptoms ot folate impairment observed in the feeding of thyroid powder, it seemed of interest to determine the effect of thyroid status on the levels of 10-formyl-THF dehydrogenase.

Materials and Methods

1. Animal and diet

Intact and thyroidectomized male Sprague-Dawley weanlings weighing 50 to 60g were individually caged in a screen-bottomed cages in a constant temperature- and light-controlled room during the course of experiment. Both diet and water were allowed *ad livbitum.*

In the first experiment, the intact animals were maintained for 28 days. The intact animals in group 1 to group 5 were placed on a basal diet supplemented with 100 μg vitamin B_{12} and 5 mg folic acid per kg diet. The composition of the basal diet containing marginal amounts (0.2%) of DL-methionine were presented in the previous paper[9]. The intact animals in group 2 and 3 received additionally 3g of thyroid powder(Sigma) per kg diet and 1g of 2-thiouracil (4-hydroxy-2-thiopyrimidine, Sigma) per kg diet respectively. The intact animals in group 4 received additonal 7.5g of DL-methionine per kg diet. The intact animals in group 5 were placed on basal diet for 28 days and then injected with 1 μmole of methionine per g body weight intraperitoneally 1 hr, 4 hr and 24 hr prior to sacrifice.

In the second experiment, the intact animals in group 1 were placed on basal diet for 25 days. The thyroidectomized animals in group 2 were injected with 5g of L-thyroxine (3,3'5,5'-tetraiodo-L-thyronine, sodiumpentahydrate, Sigma) every other day for 25 days. The thyroidectomized animals in group 3 were maintained on basal diet for 20 days and given i.p. injections of L-thyroxine at a high dose of 100g/rat per day, beginning at 21 days, to normalize them.

In the third experiment performed to study the effect of thyroid hormone on the hepatic uptake and retention of folate, six intact rats were placed on each of the three experimental diets, basal diet, thyroid powder diet(3g of thyroid powder/kg diet), and thiouracil diet (1g of thiouracil/kg diet) for 28 days. The rats were sacrificed at 6hr, 24 hr, 3 days and 26 days after injection of [^{3}H]folate.

2. Histidine oxidation and formate oxidation

Histidine oxidation was measured for a 2 hour period by injecting 2-[^{14}C]histidine (Amersham) using the procedure of Chan and Stokstad[6]. Formate oxidation was measured for 1 hour by injecting[^{14}C]formate (Amersham) since formate is metabolized more rapidly than histidine.

3. Assay of enzyme activity

10-Formyl-THF dehydrogenase activity was assayed by measuring the increase in absorption at 300 nm produced by the converson of 10-formyl-THF to THF using the procedure of Kutzbach and Stokstad[10]. Animal tissue was homogenized with 3 volumes of 166 mM potassium phosphate buffer(pH 6.3) and centrifuged at 30,000g for 30 min at 4℃.

5,10-Methylene-THF reductase activity was analyzed by the method of Kutzbach and Stokstad[7]. Rat liver was homogenized with 9 volumes of 10 mM potassium phosphate buffer(pH 7.3) and centrifuged for 20 minutes at 20,000g at 4℃.

Result and Discussion

On the first experiment, the effects of feeding

thyroid powder(Th.pd.) and thiouracil(TU) on histidine oxidation and on 10-formyl-THF dehydrogenase were studied(Table 1). The level of histidine oxidation was decreased by the administration of thyroid powder to 35% of normal and markedley increased by thiouracil, which was consistent with the previous observations[7)11)]. Methylene-THF reductase was decreased by feeding thiouracil as previously reported[6)12)].

The level of 10-formyl-THF dehydrogenase are expressed as a percentage of the activity for the intact control rat tissue(Table 1). In the liver, 10-formyl-THF dehydrogenase activity was decreased by feeding thyroid powder to 50% of the control level, while increased by feeding thioruacil to 142% (Table 1).

The activity of 10-formyl-THF dehydrogenase was not changed by either supplementation or injection(i.p.) of methionine. Krebs et al.[13)] demonstrated that the rate of histidine and formate oxidation in the isolated hepatocyte preparations was accelerated by the supplementation. The authors suggested that these effects could be due to the activation of 10-formyl-THF dehydrogenase by methionine or SAM.

Formate has been known to be oxidized by THF-dependent pathway including 10-formyl-THF synthetase and 10-formyl-THF dehydrogenase and peroxidation pathway catalyzed by catalase[14)15)]. Formate oxidation was not altered by thyroid status (Table 2) although formate oxidation shares the metabolic step involving 10-formyl-THF dehydrogenase with histidine oxidation. This observation suggested that formate oxidation might be less susceptible to functional folate activity than histidine oxidation because most of formate might be oxidized by the alternative pathway.

The second experiment consisted of a time study to determine the rate at which 10-formyl-THF dehydrogenase was normalized after injection of 100μg of thyroxine per day (beginning at 21 day) to thyroidectomized animals (Table 2). Thyroidectomized animals that received 2.5μg of thyroxine per day had the similar histidine oxidation rate and enzyme level to the intact control

Table 1. Effect of thyroid powder(Th. Pd), thiouracil(TU) on the activity of 10-formyl-THF dehydrogenase, and methylene-THF reductase(Exp. 1). (Relative Value Control=100^{+})

Supplement per kg diet	Body wt.(g)[b] (#Animals)	Histidine Oxidation[a] (%in 2 hours)	10-fomyl-THF Dehydrogenase[a] (μmol/hr/g)	Methylene-THF Reductase[a] (μmol/hr/g)
1. None	194 (6)	4.8±3.4	100^{+}±16.0 (16.6)[c]	100^{+}±12.7 (1.37)[c]
2. Th.Pd.3g	189 (6)	1.7±1.1	60.8±9.4	152.0±18.9
3. TU 1g	118 (6)	23.7±8.8	142.1±3.8	51.9±9.9
4. Methionine 7.5g	262 (6)	12.1±10.3	108.0±24.8	158.3±12.0
5. Methionine IP injection[d]				
1hour	206 (3)	ND	90.6±12.6	92.1±11.8
4hour	206 (3)	ND	106.5±12.6	106.6±4.6
24hour	204 (3)	ND	70.5±13.1	103.4±30.3

a Value=Mean±SD
b Weanling rats were fed the diet for 4 weeks
c Absolute enzyme levels in intact animals, enzyme activity per g of liver
d 1 μmol methionine injected per g body wt. prior to sacrifice
ND ; not determined

Table 2. Time study of thyroxine injection to thyroidectimized(Td) rats : effects on hiustidine oxidation, 10-formyl-THF ehydrogenase, and methylene-THF reductase(Exp. 2).

(Relative Value Control=100^{+})

Treatment (Day of Sacrificing)	Bodywt.(g)[b] (#Animals)	Histidine Oxidation[a] (%in 1 hr)	Fomate Oxidation[a] (%in 2 hr)	10-CHO-THF Dehydrogenase[a] (μmol/hr/g)	Methylene-THF Reductase[a] (μmol/hr/g)
1. Intact	171.8±13.0 (4)	10.2±2.8	44.1±8.5	81.6±9.8	103.6±16.3
2. Td+2.5μg T4/day	155.8±15.8 (5) (Control)	8.5±1.7	38.4±11.3	100^{+}±9.9 (10.28)[c]	100^{+}±13.1 (1.154)[c]
3. T4 IP Injection(100μg per day)[b]					
0. day	62.7±6.8 (4)	40.1±1.6	42.3±5.7	196.4±10.7	59.1±9.4
1. day	75.8±20.9 (4)	22.5±14.6	39.9±4.1	125.6±23.9	73.5±9.0
2. days	77.8±19.2 (4)	19.2±8.7	48.4±3.6	87.9±45.0	87.1±16.9
4. days	109.0±37.5 (4)	6.5±4.6	44.3±6.5	7.8±8.9	123.7±17.8
8. days	70.5±13.1 (4)	8.7±2.8	ND	67.3±7.8	118.1±12.5

a Value=Mean±SD

b 5μg of T4 injected every other day for 25 days

c Absolute enzyme levels in normalized euthyroid animals, enzyme activity per gram of wet tissue

ND : Not determined

levels(Table 2) and they were included as a normal control (group 2). In each case histidine oxidation was measured prior to sacrifice for enzyme assay.

10-Formyl-THF dehydrogenase was increased to 141% by thyroidectomy compared to the intact controls. This enzyme became normal after 2 days injection with 100μg thyroxine. Histidine oxidation was normalized after 4 days injection. Methylene-THF reductase was decreased by thyroidectomy as previously reported[6] and was normalized after 4 days of thyroxine injection. The increased histidine oxidation produced by hypothyroidism may be attributed both to its effect in decreasing methylene-THF reductase and also to its effect in increasing 10-formyl-THF dehydrogenase. The former acts by decreasing synthesis of 5-methyl folates, thereby increasing the proportion of non-methyl folates required folate-dependent reactions. Increased levels of 10-formyl-THF dehydrogenase increase the rate at which formate(in the form of 10-formyl-THF) is oxidized to carbon dioxide with regeneration of THF.

The increased level of 10-formyl-THF observed in hypothyroidism helps to explain the observation that histidine oxidation is higher in thiouracil treated animals than in those fed methionine[12]. Methionine(after conversion to S-adenosylmethionine) decreases the activity of methylene-THF reductase by feedback control[7]. Hypothyroidism works both by decreasing the level of methylene-THF reductase and also by increasing the level of 10-formyl-THF dehydrogenase. Both of these effects work in the direction of increasing histidine oxidation.

The hepatic uptake and retention of folate, which was studied by injecting [^{3}H]folate intraperitoneally, was increased significantly in the liver of hypothyroid rat(Fig. 1). Although initial uptake of labeled folate by liver of hyperthyroid rat does not appear to be impaired, the longterm retention of folate was decreased in rats fed thyroid powder(Fig. 1). Chan et al[6] investigated the effect of thyroxine on 24 hr-period hepatic uptake

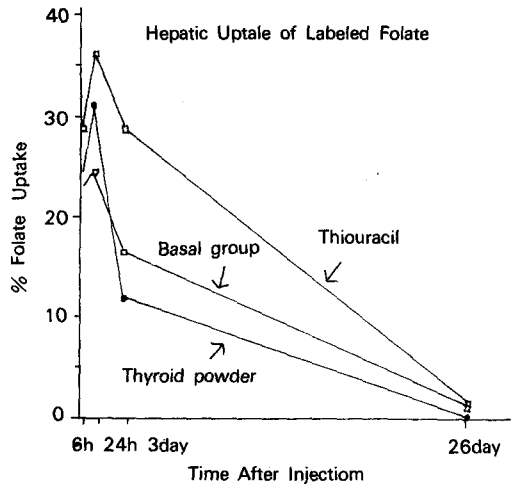

Fig. 1. Time course study of [^{3}H]folate uptake by rat liver (Experiment 3). [^{3}H]Folate (10-25 μCi) was injected to rats intraperitoneally 6 hr, 24 hr, 3 days and 26 days prior to sacrifice.

of [^{3}H]folate in rat and observed that the highest percentage uptake of the injected folate was taken by thyroidectomized animals. When thyroxine was present, the hepatic uptake values were greatly reduced, demonstrating the presence of thyroxine as an important factor regardeless of the level of the hormone[1]. In hyperthyroid animals, the proportion of folate derivatives appearing as methyl folate is increased[6]. Methyl folate is a poor substrate for folylpolyglutamate synthetase[16][17]. In this study, the decreased level of [^{3}H]folate retention in hyperthyroid rats for a longterm period appeared to be directly correlated with the decreased synthesis of polygutamate forms of folate, which led to a failure of the liver to retain the absorbed folate rather than a diminished hepatic uptake of the vitamin.

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갑상선 호르몬이 흰 쥐의 간에 있는 10-Formyltetrahydrofolate Dehydrogenase에 미치는 영향

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갑상선 호르몬이 10-formyltetrahydrofolate dehydrogenase에 미치는 영향을 흰 쥐의 간을 이용하여 조사 하였다. 흰 쥐에게 갑상선 호르몬의 형성을 저지하는 thiouracil을 먹였을 때 히스티딘의 산화속도가 5배 증가 되었으며, 10-formyltetrahydrofolate dehydrogenase의 활성도가 142%로 증가 되었고, 또한 methylenetetrahydrofolate reductase의 활성도는 정상 쥐의 52% 수준으로 저하되었다. thyroid powder를 먹여 갑상선 기능이 항진된 상태에서는 이와 반대 경향을 보였다.

갑상선의 기능이 저하되었을 때 히스티딘의 산화속도가 상승되는 것은 methylenetretrahydrofolate reductase의 활성도의 감소로 인해, folate를 필요로 하는 반응에서 주로 이용되는 non-methyl folate의 비율이 증가되었기 때문이며, 10-formyltetrahydrofolate dehydrogenase의 활성도의 상승으로 인해 히스티딘의 산화속도는 더욱 증가한 것으로 보인다.