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Role of the Hepatic Xanthine Oxidase in Thyroid Dysfunction: Effect of Thyroid Hormones in Oxidative Stress in Rat Liver

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The effect of thyroid hormones on the hepatic xanthine oxidase activity was studied in rats after the intraperitoneal injections of comthyroid (triiodotyronine : thyroxine=1 : 4) at 0.3 mg/kg for 3 consecutive days. The aim of this study was to understand the precise mechanism of hyperthyroidism induced by oxidative stress. The concentration of lipid peroxides determined indirectly by the measurement of thiobarbituric acid reactants was increased in comthyroid treated rats. The hepatic glutathione content was decreased in comthyroid injected rat compared to the euthyroid state. It was also observed that the increment of xanthine oxidase activity has a profound role in oxygen radicals generation system in comthyroid treated rat. These findings suggest that the enhanced xanthine oxidase activity and depleting glutathione content in comthyroid treated rats result in pathophysiological oxidative stress including an increment of hepatic lipid peroxidation.

Key words : Xanthine oxidase, Lipid peroxidation, Comthyroid, Hyperthyroidism

INTRODUCTION

There are many evidence to indicate that hyperthyroid functional state lead to an enhancement of basal metabolic rate, represented by an increase in total oxygen consumption in the animals (Fernandez *et al.*, 1985; Schwartz and Oppenheimer, 1978; Turrens *et al.*, 1985). Also, it has been reported that active oxygen species are generated as by products of oxidative metabolism in hyperthyroidism animal (Asayama *et al.*, 1987). These oxygen radicals lead to lipid peroxidation of biomembrane unless they are removed by free radical-scavenging enzyme (Mano *et al.*, 1995; Pereira *et al.*, 1994). However, the precise mechanism of thyroid hormone-induced oxidative stress was unclear.

Xanthine oxidase in various pathophysiological condition acts as a major enzyme of oxygen free radical generation which induce oxidative stress through increasing lipid peroxidation (Parks *et al.*, 1986; McCord, 1985). It is widely accepted that lipid peroxidation mediated by oxygen free radicals plays an important role in various pathophysiological condition (Asayama *et al.*, 1987; Asayama *et al.*, 1989; Fernandez *et al.*, 1996; Joseph *et al.*, 1990; Parks *et al.*, 1983; Tappel, 1973; Tapia *et al.*, 1997; Videla *et al.*, 1988). It was reported that sulfhydryl compounds including glutathione may be involved in regulation of the xanthine oxidase activity (Bindoli *et al.*, 1988; Della corte and Stirpe, 1972).

Recently, it was also reported that hepatic reduced glutathione (GSH) depletion in hyperthyroidism might represent one of the deternimants of the hepatic oxidative stress observed in experimental animals (Fernandez, 1991; Fernandez *et al.*, 1996; Seven *et al.*, 1996). In this study, the action mechanism of thyroid hormone-induced oxidative stress was investigated with regard to xanthine oxidase activity which is the generation enzyme of reactive oxygen radicals and lipid peroxidation in comthyroid treated rat liver.

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MATERIALS AND METHODS

Materials

Bovine serum albumin (BSA), nicotinamide adenine dinucleotide sodium salt (NAD^+), sodium dodecyl sulfate (SDS), thiobarbituric acid (TBA), 3-3'-5 triiodo-L-thyronine (T_3) sodium salt, L-thyroxine (T_4) sodium salt and xanthine sodium salt were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Other extra pure chemicals were purchased from a reagent commercial company.

Treatment of animals

Male Spraque-Dawley rats (Life Science Co. Korea.)

weighing from 180 to 220 g were fed *ad libitum* and received daily intraperitoneal injections of either comthyroid (triiodithyronine:thyroxine=1:4, 0.3 mg/kg for 3 consecutive days) or equivalent volumes of 0.01 M KOH (controls). Comthyroid was composed of 1 part of 3-3'-5 triiodo-L-thyronine (T_3) sodium salt and 4 parts L-thyroxine (T_4) sodium salt. After treatment, the rectal temperature of comthyroid treated rats (measured with a rectal thermometer) was enhanced comported to controls, suggesting a comthyroid induced thermogenic state and a indicator of hyperthyroidism (Fernandez, 1985).

Enzyme preparation

Enzymes were prepared from liver tissue in rats killed by decapitation. The livers were removed and kept on ice throughout the preparation. After weighing, the pieces of livers were homogenized with 4 volumes of ice cold 0.1 M potassium phosphate buffer (pH 7.5) solution containing 1 mM phenyl-methyl-sulfonylfluoride, a protease inhibitor. The homogenate was centrifuged at 600×g for 10 min at 4°C. The pellet was discarded and the supernatant was centrifuged at 10,000×g for 20 min at 4°C. The supernatant fraction was further ultracentrifuged at 105,000×g for 60 min at 4°C. The resultant cytosolic fraction was used as the enzyme source for the xanthine dehydrogenase or xanthine oxidase assays.

Enzyme assay

Xanthine dehydrogenase activity was assayed by measuring, spectrophotometrically, the amount of uric acid formed from xanthine sodium with NAD^+ as a cofactor in the reaction mixture according to the method of Della Corte and Stirpe (1972). Xanthine oxidase activity was aerobically determined by measuring the rates of uric acid fromation without NAD+ in the reaction mixture from xanthine sodium as substrate. The reaction mixture contained 0.1 M potassium phosphate buffer (pH 7.4), 0.1 ml of enzyme source, 0.06 mM of the substrate and distilled water in a final volume of 4 ml. The reaction was carried out at 37°C for 10 min.

Measurement of lipid peroxides (LPO) in rat hepatic homogenates

The level of hepatic lipid peroxide was determined using the method of Ohkawa *et al.* (1979). A portion of homogenates (0.2 ml) which were prepared the same method as in enzyme preparation was mixed with 8.1% SDS, 20% acetate buffer (pH 3.5) and 0.8 % TBA solution for 3 min and incubated for 60 min at 95°C. Malondialdehyde (MDA), a TBA reactive substance, was extracted with a butanol-pyridine mixture solution. The absorbance measured at 532 nm was expressed as nanomoles of MDA.

Determination of hepatic glutathione content

The content of hepatic glutathione (GSH) was measured by the method of Griffith *et al.* (1980). This assay was performed in a medium containing 125 mM sodium phosphat buffer, pH 7.5, 6.3 mM Na-EDTA, 6 mM 5,5'-dithiobis-(nitrobenzoate), 0.3 mM NADPH sodium salt, 50 U/ml GSH reductase and 100 µl liver extract, at 30°C. The absorbance measured at 412 nm was expressed as µmoles of glutathione. For determination hepatic glutathione content, the homogenate was mixed with 8% sulfosalicylic acid and centrifuged at 2,000×g for 10 min.

Protein assay and statistical analysis

Protein content was determined by the method of Lowry *et al.* (1951) using BSA as the standard. The differences between the experimental groups were analyzed with Student's t-test.

RESULTS

Time course of comthyroid on the rectal temperature of the rats

Because thyroid hormone induced calorigenesis led to a drastic enhancement in metabolic rate, thyroid hormone induced oxidative stress status was indirectly determined by measuring rectal temperature. Fig. 1 shows the time course of comthyroid effects on the rectal temperature of the rats measured with a rectal thermometer. Treatment of rats with comthyroid (0.3

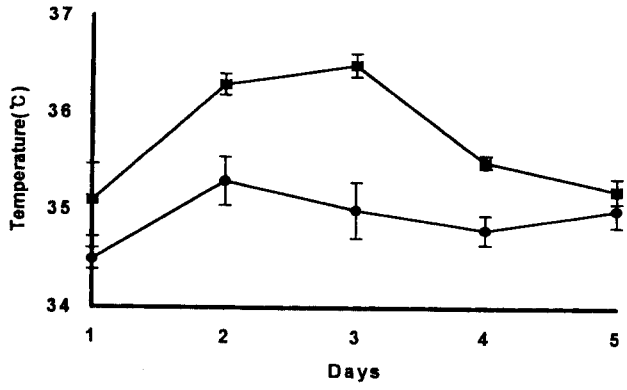

Fig. 1. Time course of comthyroid on the rectal temperature of the rats. The assay procedure was described in the materials and methods. In comthyroid treated rats (■) 0.3 mg comthyroid/kg body weight, was given i.p. for 3 consecutive days. Control animals (●) received eqivalent amounts of comthyroid diluent. Results represent the means±SEM for nine animals per group. Significance for the effects of comthyroid treatment compared to control. $P<0.05$ for all parameters shown.

mg/kg) for 3 consecutive days progressively enhanced their metabolic rates, the rectal temperature of the rats were significantly increased as compared to those of the control rats [controls; 35.0±0.36 (n=9); comthyroid treatment rats; 36.5±0.57 (n=9); P<0.05].

Changes of the hepatic lipid peroxide levels on the comthyroid treated rats

The hepatic lipid peroxide levels on the comthyroid treated rats were measured by the amount of thiobarbituric acid reactants. The effect of comthyroid treatment on the hepatic lipid peroxidation were shown in Fig. 2. MDA levels in the comthyroid treated rats were significlantly increased as compared to that of control rats by 27% [controls, 3.77±0.48 (n=9); comthyroid treated rats, 4.78±0.27 (n=9); P<0.001].

Effect of comthyroid on hepatic xanthine dehydrogenase and oxidase activities in rats

Xanthine oxidizing enzymes exist mainly as xanthine

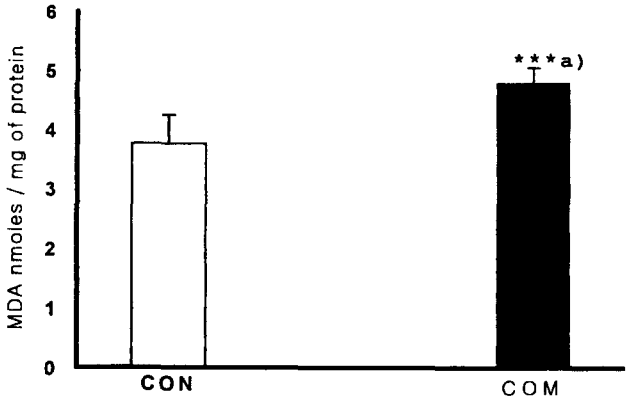

Fig. 2. Hepatic levels of lipid peroxide on the comthyroid treated rats. The assay procedure was described in the materials and methods. In comthyroid treated rats (COM) 0.3 mg comthyroid/kg body weight, was given i.p. for 3 consecutive days. Control animals (CON, day 1) received equivalent amounts of comthyroid diluent. Results represent the means±SEM for nine animals per group. a) represent significantly difference compared to the control group (***P<0.001)

Table I. The change of hepatic xanthine oxidase activity in comthyroid treated rats

	Specific Activity (Uric acid n moles/mg protein/min)	
	Xanthine oxidase	Xanthine Dehydrogenase
Control	0.584±0.01	1.756±0.11
Comthyroid	0.665±0.08*[b)]	2.035±0.14***[a)]

The assay procedure was described in the materials and methods. In comthyroid treated rats 0.3 mg comthyroid/kg body weight, was given i.p. for 3 consecutive days. Control animals (day 1) received equivalent amounts of comthyroid diluent. Results represent the means±SEM for nine animals per group. a) represent significantly difference compared to the control group (***P<0.001) b) represent significantly difference compared to the control group (*P<0.05)

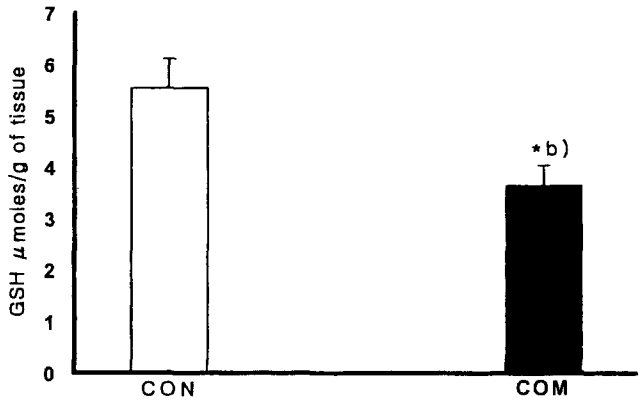

Fig. 3. Hepatic glutathione content on the comthyroid treatment rats. The assay procedure was described in materials and methods. In comthyroid treated rats (COM) 0.3 mg comthyroid/kg body weight, was given i.p. for 3 consecutive days. Control animals (CON, day 1) received eqivalent amounts of comthyroid diluent. Results represent the means ±SEM for nine animals per group. b) represent significantly difference compared to the control group (*P<0.05)

dehydrogenase in natural biologcal conditions. It is postulated that endogenous xanthine dehydrogenase is converted to oxygen radical producing xanthine oxidase during the oxidative stress. As shown in Table I, the hepatic xanthine oxidase activity in comthyroid treated rats were significantly increased as compared to that of control rats [controls, 0.584±0.01 (n=9); comthyroid treated animals, 0.665±0.08 (n=9); P<0.05]. It was also observed that increment of hepatic xanthine dehydrogenase activity in comthyroid treated rats compared to that of control rats [controls, 1.756±0.11 (n=9); comthyroid treated rats, 2.035±0.14 (n=9); P<0.001].

Change of hepatic glutathione content on comthyroid treatment rat

Effect of comthyroid on hepatic glutathione content were observed. The results were shown in Fig. 3. After 3 days of comthyroid treatment, the concentration of GSH was significantly decreased with 34% as compared to that of the control rats [controls, 5.53±0.57 (n=9); comthyroid treated rats, 3.64±0.40 (n=9); P<0.05].

DISCUSSION

It was reported that a hypermetabolic state due to hyperthyroidism accelerates free radicals production in the rat tissue (Asayama *et al.*, 1989; Fernandez *et al.*, 1985) and some investigator tried to explain the relationship between hyperthyrodism with thyroid hormone-induced oxidative stress (Ana *et al.*, 1989; Fernandez *et al.*, 1988; Pereira *et al.*, 1994; Videla *et al.*, 1994). It was reported that active oxygen radicals and lipid peroxidation are closely related to each other in the pathogenesis of oxidative stress-induced hyperthy-

roidism (Asayama *et al.*, 1989; Mano *et al.*, 1995).

Xanthine oxidase is the first documented biological target to produce superoxide radicals and is considered to have an important role in supplying the active oxygen radical. Xanthine oxidase catalyses the reaction in which hypoxanthine is converted to xanthine and then to uric acid. Oxygen can act as the electron acceptor for this reaction and in so doing it can produce superoxide free radicals. Recent studies indicate that an important series of events occur at the onset of hepatic ischemia which may make xanthine oxidase an important source of free radicals which in turn might contribute to ischemia-induced injury. Subsequently, xanthine oxidase used as source of reactive oxygen metabolite in large number of *in vitro* studies (Chamber *et al.*, 1985; Das *et al.*, 1987). It was also reported that direct production of hydroxy radical by xanthine oxidase in cells and tissues could explain the presence of oxidative cellular damage which is not prevented by superoxide dismutase (Kuppusamy *et al.*, 1989). In this investigation, the lipid peroxide content and hepatic xanthine oxidase activity were significantly increased in comthyroid treated rats as compared to those of control rats.

GSH constitutes the major source of low molecular weight thiol in mammalian tissue and functions in detoxification of electrophilic compounds and protection against oxidative injury. Numerous reports show that GSH depletion occurs prior to cell injury for several types of toxic substances (Pessayre *et al.*, 1980; Siegers *et al.*, 1977). It was also observed from this investigation that hepatic GSH depletion in the comthyroid treated rats was associated with an oxidative stress condition and involved with a significant enhancement in the lipid peroxidation.

Based on the previous studies and our experimental results, we conclude that the increasing effect of xanthine oxidase activity may partially contribute to the induction of the oxidative stress which was increasing lipid peroxidation in thyroid hormone treated rats liver. These findings suggest that the enhanced xanthine oxidase activity and depleting glutathione content in comthyroid treated rats result in oxidative stress condition which was closely related with an increment of hepatic lipid peroxidation.

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