

Induction of manganese superoxide dismutase by thyroid stimulating hormone in rat thyroid cells

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Abstract Alterations in the superoxide dismutase (SOD) content of thyroid tissues occurring in association with thyroid dysfunction have been reported. In this study, the Mn-SOD content was found to increase in thyroid tissues of rats administered thyroid stimulating hormone (TSH) and in thyrocytes cultured in medium supplemented with TSH. Furthermore, in the thyroid glands of rats whose serum TSH level was elevated by inhibiting the synthesis of T3 and T4 by 6-methyl-2-thiouracil, the Mn-SOD increased as the TSH concentration increased. In the cultured thyrocytes, the increase in Mn-SOD induced by TSH was inhibited by the C-kinase inhibitor H7. These findings suggest the induction of Mn-SOD by TSH in thyroid cells and point to a role of C-kinase in this process, thereby indicating that a close relationship exists between the serum TSH level and the change in Mn-SOD content in thyrocytes with thyroid dysfunction.

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Key words: Superoxide dismutase induction; Thyroid stimulating hormone; Thyroid; Manganese containing superoxide dismutase

1. Introduction

Superoxide dismutase (SOD), which catalyzes the dismutation of superoxide anion (O_2^-), is present as three types of isoenzyme in mammalian tissues, designated CuZn-SOD, Mn-SOD and extracellular SOD [1,2]. The roles of SOD in the thyroid gland have been reported to include not only the protection of cells from radicals, but also involvement in the production of H_2O_2 as a substrate of thyroid peroxidase, a necessary process for the synthesis of thyroid hormone [3]. However, Nakamura et al. reported that the plasma membrane in thyroid cells contained a NADPH-dependent H_2O_2 -generating system which provided H_2O_2 for the thyroid peroxidase-catalyzed biosynthesis of thyroid hormones and that it had no superoxide dismutase activity [4,5]. Thus, no clear description of the role of SOD in thyroid gland cells has yet been offered.

Moreover, a large number of observations have been made on SOD activities associated with various diseases. In particular, changes in Mn-SOD content in association with thyroid

dysfunction have been reported [6,7]. According to Asayama et al. [6], T4-treated rats had an increased Mn-SOD activity and content in smooth muscle and heart. According to Ohno et al. [7], serum Mn-SOD levels were found to be respectively low and high for hyperthyroidism and hypothyroidism, and treatment of hypothyroidism with thyroid hormone resulted in a return of the Mn-SOD content to the normal level. No conclusions have been reached regarding the mechanism by which changes in Mn-SOD content are related to thyroid dysfunction.

We previously reported the immunohistochemical demonstration of a high level of expression of Mn-SOD in thyroid follicular cells of a patient with Hashimoto thyroiditis with a high serum thyroid stimulating hormone (TSH) level [8], and suggested the possibility that Mn-SOD is induced by TSH. The aim of the present study was to determine whether or not Mn-SOD was induced by TSH in thyroid cells.

2. Materials and methods

2.1. Culture of thyrocytes

Male Wistar rats (8 weeks of age) were used for the experiments. The rats were killed after having been anesthetized with ether. The dissected thyroid glands were finely minced and treated with 0.1% collagenase (type I, Sigma)+0.2% dispase (Godo Shusei) for 30–120 min at 37°C. The cells were collected by centrifugation at $90 \times g$ for 3 min and washed several times with Hanks' balanced salt solution. This was followed by centrifugation at $90 \times g$ for 1 min. Cells were cultured in plastic dishes in Dulbecco's modified Eagle medium (Nissui Seiyaku) supplemented with 10% fetal bovine serum (Gibco) or 10% fetal bovine serum+TSH (100 μ U/ml, Sigma), TNF α (100 ng/ml, Genzyme) and forskolin (100 μ M, Funakoshi) in a humidified incubator in an atmosphere containing 5% CO_2 . Thyroid follicle cells were preincubated with the C-kinase inhibitor H7 (50 μ M, Seikagaku), and after 4 h, TSH (100 μ U/ml) was added to the medium. Twenty-four hours after drug treatments, these cells were collected for measurement of Mn-SOD content.

2.2. Exogenous TSH stimulation by subcutaneous injection of TSH

Male Wistar rats were administered TSH by subcutaneous injection at three different doses (0.5, 5, 50 mU/animal). The rats were killed at two different time intervals after TSH injection: 24 and 48 h. The thyroid glands were dissected and freed of adhering connective tissues, and blood was removed by washing several times with chilled physiological saline. The remaining tissue was then used for measurement of Mn-SOD content.

2.3. Endogenous TSH stimulation by administration of 6-methyl-2-thiouracil (MTU)

Male Wistar rats were given drinking water containing 1% MTU (inhibitor of thyroid hormone synthesis, Nakarai). The rats were killed after 0, 1, 2, 5, and 10 days of MTU treatment. Dissected thyroid tissue was used for the measurement of Mn-SOD content and activity. Serum was used for the measurement of T4, T3 and TSH.

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Abbreviations: SOD, superoxide dismutase; Mn-SOD, manganese containing superoxide dismutase; TSH, thyroid stimulating hormone; TNF α , tumor necrosis factor- α ; MTU, 6-methyl-2-thiouracil; ELISA, enzyme-linked immunosorbent assay; IL-1, interleukin-1

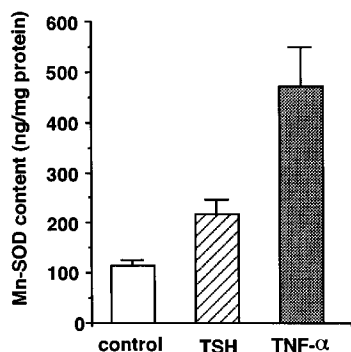


Fig. 1. Induction of Mn-SOD by TSH and TNF α in cultured thyrocytes. The increase in Mn-SOD content was observed after the addition of TNF α (100 μ U/ml) or TSH (100 ng/ml). Data are the mean \pm S.E.M. ($n=5$).

2.4. Preparation of materials

The cultured cells or the tissues were immediately frozen in liquid N₂ and stored at -80°C until the Mn-SOD content was measured. The frozen samples were placed in 20 mM Tris-HCl buffer (pH 7.4) and homogenized in a Polytron homogenizer, then the homogenate was centrifuged at $500\times g$ for 10 min. The supernatants were used for the ELISA. The protein content of the homogenate was determined using a BCA protein assay kit (Pierce).

2.5. Serum T4, T3 and TSH

The serum concentrations of T4, T3 and TSH were measured by radioimmunoassay: T4 with the Amerlex-MAB-T4 radioimmunoassay kit (Amersham), T3 with the Amerlex-MAB-T3 radioimmunoassay kit (Amersham), and TSH by radioimmunoassay with the rat TSH-¹²⁵I assay system (Amersham).

2.6. Mn-SOD content

ELISA was performed essentially as described by Kawaguchi et al. [9,10]. Measurement of Mn-SOD was carried out using polyclonal antibody raised against rat liver Mn-SOD [9].

2.7. Mn-SOD activity

The assay of SOD activity was basically as described by Beachap and Fridovich [11]. Briefly, the assay was based on the inhibition of nitroblue tetrazolium conversion by SOD into a blue tetrazolium salt, mediated by superoxide radicals which had been generated by xanthine oxidase. The amount required to inhibit the rate of reduction of nitroblue tetrazolium by 50% was defined as 1 unit of activity. For inhibition of the CuZn-SOD activity, the assay was conducted in the presence of 2 mM NaCN after preincubation for 30 min.

3. Results and discussion

In our experiments, cultured thyroid cells were positive to anti-thyroglobulin antibody when tested immunohistologi-

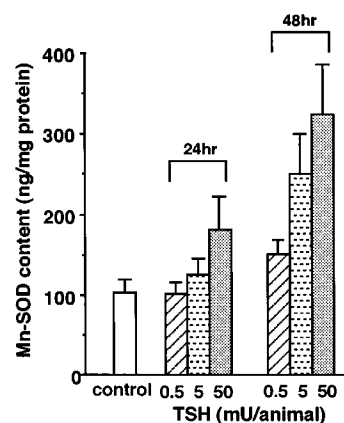


Fig. 2. Induction of Mn-SOD content in TSH-treated rat thyroid tissues. Administration of exogenous TSH (0.5, 5 and 50 mU/animal) produced dose- and time-dependent increases in Mn-SOD in the thyroid gland. Data are the mean \pm S.E.M. ($n=4$).

cally, and manifested distinct cytoplasmic dilatation 24 h after the addition of TSH. In thyroid follicle cells, the induction of Mn-SOD by TNF α has been reported. Similarly, in our experiments, the addition of TNF α resulted in a significant increase in Mn-SOD content, to approximately four times that in the control. Similarly, the addition of TSH resulted in an increase in Mn-SOD content, to approximately twice that in the control; this rate of induction was less than that for TNF α (Fig. 1).

In TSH-treated rats (exogenous TSH stimulation), increases in Mn-SOD content in thyroid tissues depending upon the dose of TSH were noted at 24 and 48 h after TSH administration (Fig. 2). The increase in the ratio of Mn-SOD content was higher at 48 h than at 24 h after TSH administration (Fig. 2).

In order to promote endogenous stimulation of TSH secretion, MTU in doses known to inhibit thyroid hormone synthesis was administered to rats. The serum TSH level was elevated by MTU treatment, and Mn-SOD activity and content in thyrocytes increased as well depending on the increase in the serum TSH level (Fig. 3).

In the present study, TSH administration was found to increase Mn-SOD content in the thyroid gland both in vivo and in vitro. These findings indicate that TSH induces Mn-SOD in thyroid cells, and agreed well with the findings of our previous study that thyroid follicular cells in a patient with Hashimoto thyroiditis had high serum TSH levels and showed

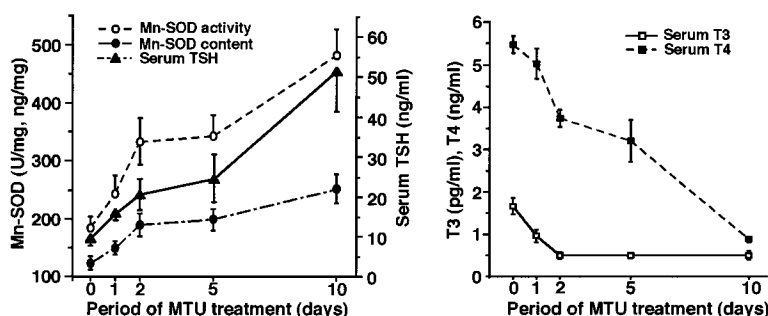


Fig. 3. Relationship between Mn-SOD induction and changes in serum TSH, T3, and T4 levels in MTU-treated rats. Serum levels of endogenous TSH were elevated by MTU treatment, and the content and activity of Mn-SOD increased in association with this elevation. However, the serum T3 and T4 levels showed significant inverse decreases. Data are the mean \pm S.E.M. ($n=4-6$).

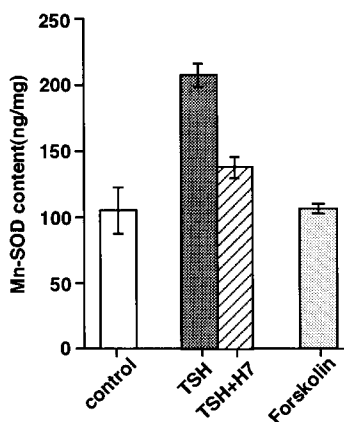


Fig. 4. Inhibitory effect of H7 in Mn-SOD induction by TSH in cultured thyrocytes. Pretreatment of the C-kinase inhibitor H7 (50 μ M) inhibited the increase in Mn-SOD by TSH. Furthermore, the addition of the adenylate cyclase activator forskolin (100 μ M) did not cause any increase in Mn-SOD content. Data are the mean \pm S.E.M. ($n=4-7$).

strongly positive immunohistological staining for anti-Mn-SOD antibody [8].

It has been reported that Mn-SOD was increased in the muscle of rats administered T4 [6,12]. The results of the present experiment also indicate the possibility that Mn-SOD was induced by T4, which was secreted from the thyroid follicle cells in response to the addition of TSH. Accordingly, we administered the thyroid hormone synthesis inhibitor, MTU, and examined changes occurring in Mn-SOD levels when the endogenous T4 level was lowered. In MTU-treated rats, the serum TSH level increased significantly, and the content and activity of Mn-SOD of the thyroid tissue also increased in association with the increase. However, in contrast, the T3 and T4 levels showed marked decreases after treatment (Fig. 3). These results suggest that the induction of Mn-SOD in thyroid cells is not affected by T3 and T4, but, in contrast, is affected by TSH.

As described above, the induction of Mn-SOD by $\text{TNF}\alpha$, IL-1 and LPS has already been reported [13,14]. Although the mechanism by which Mn-SOD induction occurs is largely unknown, it has been suggested that protein kinase C is involved [15], since 12-*O*-tetradecanoylphorbol 13-acetate stimulation can result in the induction of Mn-SOD. The increase in Mn-SOD by TSH was inhibited in part by the C-kinase inhibitor H7, and no increase was seen in the Mn-SOD content of the cells cultured in the presence of forskolin (Fig. 4). TSH is a known activator of the processes of both adenylate cyclase and C-kinase [16,17]. This suggests a close relationship between C-kinase and some part of the mechanism of Mn-SOD induction by TSH confirmed in the present experiment.

Moreover, IL-1, which can induce Mn-SOD, is known to

inhibit thyroid function in a concentration-dependent manner in human thyrocytes [18]. Thus IL-1 appears to have important roles in modulating change in both the Mn-SOD content and thyroid function in patients with thyroid dysfunction. However, the previous finding (Fig. 1) that Mn-SOD was induced by TSH in thyrocytes cultured in medium without change in the IL-1 and $\text{TNF}\alpha$ contents may partly explain the alteration in Mn-SOD content that occurs in association with thyroid dysfunction. However, the biological roles of SOD and the physiological relationship existing between TSH and Mn-SOD are not yet clearly understood, and will require further study.

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