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Superoxide dismutase activation in thyroid and suppression in adrenal

Novel pituitary regulatory routes

Saguna Verma, Pradeep Kumar G., Malini Laloraya, Manish Nivsarkar and Aparna Singh

School of Life Sciences, Devi Ahilya Vishwavidyalaya, Vigyan Bhawan, Khandwa Road, Indore, M.P. 452 001, India

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This study reports that administration of TSH in young female mice results in a concomittant augmentation of SOD activity in the thyroid gland. A strong thyroid–adrenal interdependence was also evident in the form of a marked loss of SOD activity in the adrenal gland in response to TSH administration. Very recently SOD/O_2^- system had been identified as a potent H_2O_2 generator which provides substrate for the action of key enzyme in thyroxine and progesterone biosynthesis, viz. the peroxidase. Thus, these results strongly suggest that trophic hormones tonically stimulate hormone biosynthesis by modulating activation/suppression of specific enzymes, which could be the basis of the tuning sequence.

TSH; SOD activity; Adrenal gland; Thyroglobulin iodination

1. INTRODUCTION

Data have accumulated concerning the effect of thyroxin stimulating hormone (TSH) on specific chemical and enzymatic events in the thyroid [1–6]. Out of the thyroid hormone synthesizing enzymes, iodide peroxidase is found to be augmented by chronic administration of TSH regimens [7,8]. TSH depletion by hypophysectomy induced a decrease in the enzyme activity which could be reversed by TSH treatments and these changes in the enzyme activity required only a short interval [9]. It has also been shown that the modulation of thyroidal iodide peroxidase by TSH is possibily produced by an effect on the biosynthesis of the enzyme protein [10].

Even though the peroxidation of iodine was identified as a crucial biochemical event in thyroid functioning, the source of H_2O_2 (the substrate for peroxidase action) remained an enigma since long despite an isolated work on glucose oxidase activity in the thyroid cells. In one of our recent reports, we demonstrated a superoxide radical-superoxide dismutase system as a potent H_2O_2-generator in these cells [11]. A non enzymic iodine-activation also could be identified [12]. But the enzymic peroxidation of iodine, directly under the control of TSH, could be regulated by modulating the activities of SOD or peroxidase at the thyroid level. The latter is well documented leaving the SOD modulation in the thyroid gland by TSH absolutely unresolved. This aspect is addressed in this report. Also, a complex interplay between the TSH and adrenal functioning in mammals is demonstrated.

Abbreviations: TSH, thyroid stimulating hormone; SOD, superoxide dismutase; ACTH, adrenocorticotropin hormone; H_2O_2, hydrogen peroxide; O_2^-, superoxide anion radical

Correspondence address: S. Verma, School of Life Sciences, Devi Ahilya Vishwavidyalaya, Vigyan Bhawan, Khandwa Road, Indore, M.P. 452 001, India

2. MATERIALS AND METHODS

2.1. *Reagents*

Trizma base, Trizma HCl, Triton X-100, diethylenetriaminepentacetic acid were obtained from Sigma chemical company Inc., USA. Pyrogallol was from Loba Chemie, India, while TSH was obtained from National Hormone and Pituitary Programe, Baltimore, Maryland. Tris-HCl buffer (pH 8.2, 50 mM) was made by mixing 50 mM Trizma base and 50 mM Trizma HCl in the ratio of 2:1 and 1 mM DTPA was added to this solution and the pH was adjusted to 8.2 at 27°C.

2.2. *Animals*

Young immature female (40–50 days) *Mus musculus* (Swiss strain) bred in our institute colony and housed at temperature (27 ± 1°C) and light (14 h light/10 h dark) controlled rooms were used for the study.

2.3. *Methods*

Immature female mice were used for study and 20 μg of TSH was administered by a subcutaneous injection as a single dose. The females were sacrificed by cervical dislocation at different time periods of TSH injection, viz. 15, 30, 45 and 60 min. The thyroid and adrenal glands were dissected, freed of adhering fat and blood was removed by washing several times with chilled physiological saline and were then used for SOD assays.

2.4. *SOD activity assay in thyroid/adrenal*

SOD from the target tissues was extracted as described elsewhere [11,12] and the enzyme was assayed by the method of Marklund and Marklund [13] using the ability of the enzyme to inhibit the autoxidation of pyrogallol. The enzyme kinetics were carried out on an LKB Ultraspec 4050 spectrophotometer hooked to a peripheral Apple 2e PC and Epson FX800 printer using the Software-Program Enzyme

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Kinetics (LKB Biochrom Inc., Cambridge). All calculations were made as per milligram weight.

2.5. *Statistical analysis*

Statistical analysis was conducted utilizing Introductory Statistics Software Package (ISSP), Version 1.0 [14]. The degree of variance of the results of each group was compared with that of the preceding group by subjecting them to a one-way ANOVA. The correlation between the levels of SOD in thyroid and adrenal was calculated using the above mentioned program.

3. RESULTS AND DISCUSSION

This study has been undertaken to verify the hypothesis that SOD could be induced by TSH in the thyroid gland of mammals, which could provide a substrate for peroxidase action. Experiments were designed using immature female Swiss mice which received a shot of 20 μg TSH in 1 ml physiological saline. Controls received an infection of 1 ml physiological saline alone.

Even before the external administration of TSH, the thyroid gland homogenates exhibited a fairly good SOD activity. But, after the administration of 20 μg TSH, there was an immediate surge in SOD activity in the thyroid gland noticeable at 15 min after injection. The activity of this enzyme reached its crescendo ($P<0.01$) at 30 min after injection which dropped down significantly ($P<0.01$) from 45 min onwards. The results are represented graphically in Fig. 1.

The adminstration of TSH affected the SOD activity in the adrenal gland in a very interesting fashion. Before TSH administration, the SOD activity in the adrenal gland was found to be very high (Fig. 1). But after the TSH infection, it could be seen that there was a dramatic loss ($P<0.01$) of SOD in the adrenal. The activity reached its lowest levels at 30 min after the injection. But at 45 min after the injection, we could find a significant ($P<0.01$) reversal of the TSH-induced SOD depletion. No significant reversal could be noted thereafter (Fig. 1).

Peroxidation of iodine and organification of active iodine by incorporation into the tyrosine moiety of thyroglobulin are the 2 important steps in the biosynthesis of thyroid hormones. The enzymes catalysing the reaction have been demonstrated in vitro [15–21]. A controversy exists whether both the reactions are involved for these 2 separate half reactions [22]. We recently demonstrated that the former half of this reaction could be free-radical mediated [12]. The latter half of the reaction seems to be mediated by a thyroid peroxidase, which is a membrane bound, glycosylated hemoprotein enzyme [23]. In an attempt to trace back the H_2O_2-generator in the mammalian thyroid, we could recently identify a very efficient superoxide–superoxide dismutase activity that operates at the thyroid level [11]. Data documenting the induction of thyroid peroxidase by TSH [7,8] thereby introducing regulatory functions in thyroid physiology have thus left research opportunities to resolve the modulation of

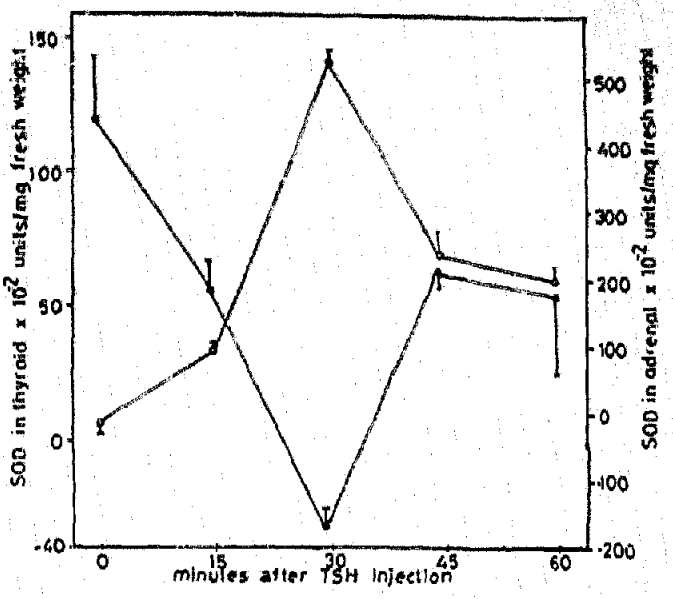

Fig. 1. Levels of SOD enzyme activity at different time intervals after TSH administration in thyroid gland (○) and in adrenal gland (●). Data represented here are mean values ± standard error of the mean (SEM).

H_2O_2 synthesis. In this report, we provide convincing experimental evidences for the induction of SOD by TSH in the thyroid. Probably, the induction of SOD activity is a primary effect of TSH that may trigger a cascade of oxidase-peroxidase enzyme systems leading to the iodination events.

There are reasonable grounds to believe that there is a strong thyroid-adrenal interdependence. Adrenocortical function is decreased in pituitary hypothyroidism and increased in hyperthyroidism [24]. It has also been shown that the thyroid hormones influence pituitary adrenal function by increasing ACTH secretion and consequently corticosterone production [24]. We have witnessed a very interesting observation in connection with the thyroid-adrenal functions. TSH has displayed a striking potency to inhibit SOD activity in the adrenals of immature mice. Even though, we cannot decipher why TSH should inhibit the SOD activity in this organ. This effect could have manifold effects since the SOD-mediated oxidation of pregnenolone to progesterone in the adrenal is plausible. However, this study has revealed novel and fascinating regulatory mechanisms exerted by the mammalian pituitary gland.

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