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Clinico-basic Convergence

Evidence for existence of thyroid hormone inducible semicarbazide-sensitive amine oxidase (SSAO) in rat heart cytosol

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ABSTRACT

Background: Semicarbazide-sensitive amine oxidase (SSAO; EC; 1.4.3.6.) has widespread tissue distribution, and the physiological role of SSAO is quite well known through its involvement in several pathological states.
Aims: The present study examined modulators of SSAO which might be present in the rat heart cytosol and looked for changes in SSAO modulatory activity.
Methods: An endogenous inhibitor of SSAO was separated by gel filtration from 105,000 g supernate of T4-treated rat heart cytosol. SSAO inhibition fraction was referred to as ''endogenous SSAO inhibitor''.
Results: The inhibition by this inhibitor was concentration-dependent. Inhibition of SSAO was not enhanced by varying the time of preincubation of the enzyme, indicating reversible inhibition of SSAO. The molecular weight of this inhibitor was estimated to be 1000–1100 by gel filtration. The isoelectric point (pI) value was determined to be 4.8 isoelectric focusing. This inhibitor was found to be heat-stable and resistant to protease treatment. SSAO inhibition activity was much lower in the cytosol of thyroidectomized, non-T4-treated rats than T4-treated rats, suggesting that this inhibitor was induced by thyroid hormone T4. SSAO activity in rat heart might be regulated by the level of this inhibitor.
Conclusion: These results suggest the presence of SSAO inhibitor in T4-treated rat cytosol and that the level of this inhibitor is regulated by thyroid hormone.

1. Introduction

Semicarbazide-sensitive amine oxidase (SSAO)[1,2] differs from monoamine oxidase (MAO; EC 1.4.3.4) and deaminates various monoamines.[3] Although they are all classified as the amine oxidase (copper-containing) (EC 1.4.3.6), they comprise a large group of enzymes with different substrate specificities and tissue distributions. SSAO is the name used for the benzylamine oxidizing activity that remains after pretreatment with acetylenic MAO inhibitor, clorgyline or deprenyl.[4] SSAO activity is suspected to cause damage, such as diabetes in

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Abbreviations: SSAO, semicarbazide-sensitive amine oxidase; MA, Omonoamine oxidase; ESI, endogenous SSAO inhibitor.
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humans.[5] SSAO has two primary functions, (a) production of metabolites with cytotoxic effects or reactive oxygen species and (b) as an adhesion molecule, in leukocyte trafficking, in regulating glucose uptake, and in adipocyte homeostasis.[6] The role of SSAO is quite well known through its involvement in several pathological states, where its increased serum activity has been found: diabetes mellitus, congestive heart failure, multiple types of cerebral infarction, uremia, and liver cirrhosis. It plays a detrimental role in vascular diseases, particularly atherosclerosis, and its role in the pathophysiology of these conditions has been extensively investigated, from which its role may be deduced.

One of the important actions of thyroid hormone is thought to be the regulation of protein synthesis and enzyme activities.[7] On the contrary, MAO activity in various organs of was shown to be diminished by thyroid hormone[8] or by a pituitary factor,[9] which is known to increase the secretion of thyroid hormones. In the latter case, it is not clear, whether thyroid hormones affect the protein synthesis of MAO or act by another mechanism (*e.g.* induction of specific modulator). There are many reports on the possible existence of the multiple modulators of MAO being present in the cytosol fractions of various organs of animals.[10–12] These studies imply that endogenous MAO modulators might be important in the physiological regulation of MAO activity. None of these investigators, however, provide any information about existence of endogenous SSAO inhibitor (ESI).

In the present study, we found an ESI in rat heart cytosol, and this inhibitor could be induced by thyroid hormone T4.

2. Methods

2.1. Chemicals

Semicarbazide, clorgyline hydrochloride, deprenyl hydrochloride, benzylamine hydrochloride, T4 sodium salt, subtilisin (protease Type III), and protease E (protease Type XIV) were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). The radioactive substrate [7-^{14}C]-benzylamine hydrochloride (1.85–2.29 Gbq/mmol) was obtained from Amersham International (Amersham, England). Servalyte (pH 2–11) was purchased from Serva Fine Biochemical Co. (St. Louis, MO, USA).

2.2. Isolation of SSAO inhibitor

Thyroidectomized rats (Male Wistar), weighing 100–150 g, were used for experiments. In the case of T4 administered rats, T4 (dissolved in saline) was injected subcutaneously to the rats after 10 days from operation at a dose of 200 μg/kg per day for 2 weeks. The rats were killed by decapitation and heart quickly removed and homogenized in 10 vol. of 10 mM phosphate buffer, pH 7.4 containing 0.25 M sucrose. The homogenates was centrifuged at 105,000 g for 60 min, and the supernate (cytosol fraction) was applied on a Sephadex G-25 column (1.0 × 60 cm), previously equilibrated with 20 mM phosphate buffer (pH 7.4). The column was eluted with the same buffer at a rate of 10 ml/hr and the fractions were collected in 2.5 ml each. An aliquot of each fraction was assayed for SSAO inhibition activity, and active fractions were combined and used for further characterization. This fraction is referred to as ''ESI''.

2.3. Assay of SSAO activity

Rat heart homogenate fraction was used as a source of SSAO activity. The 10% homogenate of this was prepared in 0.25 M sucrose with 10 mM phosphate buffer, pH 7.4. SSAO activity was assayed radiochemically.[13] Assay mixture contained 20 μl of homogenate fraction (100 μg/ml protein), [^{14}C]-benzylamine (1 μM), in 20 mM phosphate buffer, pH 7.4 (20 μl) in the presence of ESI (0–140 μl). After a 10-min preincubation at 37 °C, the mixture was diluted with the solution of respective unlabeled amines, and the reaction was stopped by adding 2 N HCl (200 μl). The products of the reaction were extracted with 2 ml of benzene-ethyl acetate (1:1, v/v) saturated with water. Triton X-100 toluene scintillation liquid (10 ml) was added to 1.0 ml samples of the extract, and the radioactivity was measured in Beckmann LS-9000 scintillation spectrometer. In inhibition studies, enzyme preparation was preincubated with various concentration of semicarbazude, clorgyline and deprenyl for 30 min at 37 °C before adding [^{14}C]-benzylamine for assay of remaining SSAO activity. The reaction products were extracted with ethyl acetate-benzene (1:l, v/v) and the radioactivity was counted. Protein concentrations of the preparations were measured by the method of Lowry et al. with bovine serum albumin as the standard.[14]

2.4. Isoelectric focusing (IEF)

Gel IEF was performed by the method of Fawcett.[15] The final composition of the gel was 5% acrylamide, 0.2% methylene bisacrylamide, 0.75% Triton X-100, 2% servalyte (pH 2–11), 0.0002% ribofiavin, 0.01% ammonium persulfate, 0.05% TEMED (N,N,N′,N′-tetramethylenediamine). The gel was mounted on a vertical apparatus containing 0.01 M H_3PO_4 in the upper tank (anode) and 0.02 M NaOH in the lower tank (cathode). The current was at 100 V for the first 1 h, 200 V for the next 2 h and then 300 V for 2 h. After electrophoresis, the gel was cut into 4 mm thick slices, and each sliced gel was placed in a test tube and incubated for 1 h at room temperature by adding 1 ml of distilled water and bubbled with N_2 gas. After the measurement of slice pH, a minimum amount of 0.5 M H_3PO_4 was added to adjust the pH to 7.4. An aliquot of each slice suspension was then assayed for SSAO inhibition activity with benzylamine as a substrate.

2.5. Statistical analysis

All values are presented as means ± S.E.M. The significance of difference was determined by using ANOVA with Fisher's *post hoc* test. A p value of less than 0.05 was regarded as being statistically significant.

3. Results

3.1. Inhibition of SSAO activity in rat

As shown in Fig. 1, *in vitro* inhibition of SSAO activity in rat heart homogenate by clorgyline, deprenyl, and semicarbazide