INHIBITION OF THYROID IODIDE PEROXIDASE IN VIVO AND IN VITRO BY IPRONIAZID

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SUMMARY

Iproniazid (isopropyl isonicotnyl hydrazine), an irreversible inhibitor of monoamine oxidase, inhibits thyroid iodide peroxidase in vitro and in vivo. As with monoamine oxidase, the peroxidase is inhibited irreversibly by iproniazid which is bound to the enzyme covalently. Spectral changes of iodide peroxidase produced by iproniazid were similar to those with horseradish peroxidase. Inhibition is competitive with KI and of a mixed function type with tyrosine. When rats were given iproniazid 24 hours before injection of tracer amounts of ^{131}I, ^{131}I-incorporation into thyroid protein was inhibited 50%. Pargyline, a non-hydrazine monoamine oxidase inhibitor, did not inhibit thyroid peroxidase in vitro or in vivo.

An enzyme system in the thyroid gland associated with mitochondria or microsomes catalyzes the iodination of free tyrosine or tyrosyl residues in proteins (1). The enzyme is thought to be a peroxidase, but its properties have not yet been elucidated. Thyroid iodide peroxidase has been compared to horseradish peroxidase and is known to have some properties in common with horseradish peroxidase. Recently, horseradish peroxidase was found to be inhibited by iproniazid and other hydrazines which were shown to bind to the nonheme portion of the enzyme irreversibly and stoichiometrically (2). The present studies on the interaction of iproniazid with thyroid iodide peroxidase are an outgrowth of the HRP studies.

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METHODS AND MATERIALS

Soluble enzyme (iodide peroxidase-tyrosine iodinase) from calf thyroid gland were prepared as described previously (1) and further purified by Sephadex G-200 column chromatography (3).

The enzyme assay system contained, in 1.0 ml, 0.05 ml enzyme (5-10 µg protein), 0.5 ml Krebs-Ringer phosphate buffer (pH 7.5), 11 mµmoles KI, 1 µC ^{131}I, 1 µmole tyrosine, 1 µmole glucose and 0.1 mg glucose oxidase (Worthington Biochemical Co.). Incubation was carried out at 37° for 20 min. Following incubation, enzymatically formed iodinated tyrosine was isolated on a cation exchange resin, eluted, and radioactively measured as described previously (4). Enzymatic activities are expressed as percent incorporation of ^{131}I into tyrosine.

Young rats (approximately 200 g) were fed low iodide diets for 7 weeks before being used for *in vivo* experiments. The rats were divided into three groups. The first group received saline, the second group 100 mg/kg of iproniazid and the third group 75 mg/kg pargyline, a non-hydrazine inhibitor of monoamine oxidase. Twenty-four hours after these injections, tracer doses of radioactive iodide (5 µC) were injected into all three groups. The rats were killed 24 hours after injection of radioactive iodide. Thyroid glands were removed, weighed and homogenized in 5 ml of 5% trichloroacetic acid. Following centrifugation, the precipitates were washed three times with 5 ml of 5% trichloroacetic acid and radioactivity associated with the precipitates was measured by sodium iodide crystal scintillation γ-counter.

Inhibition studies (*in vitro*) by iproniazid were carried out in two different ways.

1). Enzyme (500 µg protein) was incubated with ^{14}C-iproniazid (10^{-3}M) in 0.5 ml of Krebs-Ringer buffer (0.02 M, pH 7.4) for periods of time indicated in the legends and 0.1 ml of this solution was diluted with 0.9 ml of 0.02 M Krebs-Ringer buffer pH 7.4. A 0.05 ml aliquot of diluted

solution was used for checking residual enzyme activity. A second aliquot of the solution (0.4 ml) was treated with 0.6 ml of 10% trichloroacetic acid and the suspension was dialyzed against 1000 ml of water for 48 hrs with three changes of solution. Following dialysis, the precipitated protein was collected, dissolved with NCS solution and the ^{14}C which was bound to the enzyme protein was measured in the Brav's solution by the liquid scintillation counter. Control experiments were carried out in such a way that enzyme was incubated without iproniazid and iproniazid was added just before checking activity or adding trichloroacetic acid.

2). Iproniazid, 10^{-5} to 10^{-3}M, was added to the standard assay mixture and inhibition of enzyme by iproniazid was examined.

All chemicals were obtained from commercial sources unless otherwise stated. ^{14}C-iproniazid was prepared by Dr. H. Kaegi and Dr. W. Burger at Hoffmann-La Roche and 1 to 5 μC was used after dilution with unlabelled iproniazid. A low iodide diet (supplemented with vitamins) was obtained from Nutritional Biochemicals Corporation.

RESULTS AND DISCUSSION

In vitro experiment: Iodide peroxidase was incubated with ^{14}C-iproniazid for varying periods of time following which residual activity and protein bound ^{14}C were assayed. As shown in Fig. 1, the loss in enzyme activity was paralleled by the amount of ^{14}C-iproniazid bound to enzyme protein. Similar results were obtained previously when horseradish peroxidase was treated with ^{14}C-iproniazid (2). Immediately after addition of iproniazid (10^{-3}M) there was a shift of the 410 mμ peak to about 415 mμ and then a gradual fall in the absorbance at 415 mμ. From 5 to 60 minutes after adding iproniazid, the 415 mμ absorbance dropped from an optical density of 0.40 to 0.25. These spectral changes are comparable to those obtained previously with horseradish peroxidase (2) and suggest that similar sites for interaction with iproniazid occur in iodide peroxidase and horseradish peroxidase. Fifty percent inhibition of the

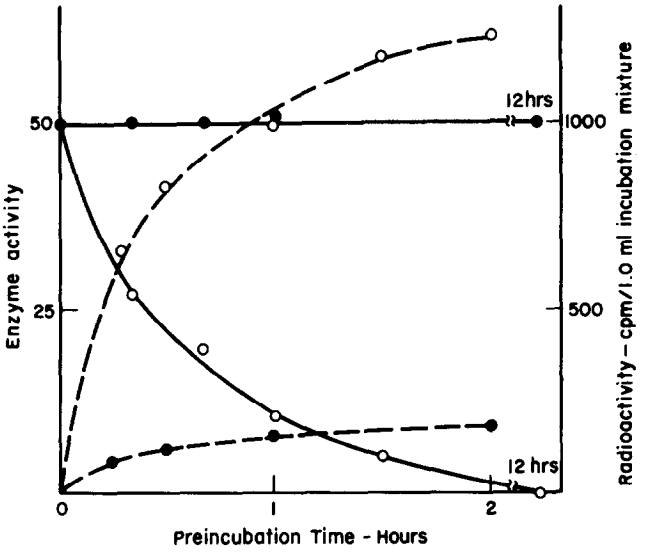

Figure 1. At the times indicated, aliquots were removed from each mixture for measurement of residual enzyme activity and protein bound radioactivity as described under METHODS. In a control experiment the enzyme was incubated without ^{14}C-iproniazid which was then added after reaction was stopped by trichloroacetic acid or dilution with buffer. Residual enzyme activity, O——O; residual enzyme activity in control, ●——●; protein bound radioactivity, O- - -O; control protein bound radioactivity, ●- - -●.

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enzyme was obtained at 3×10^{-4}M iproniazid. Pargyline does not inhibit enzyme activity up to the concentration of 10^{-2}M. The mode of interaction of iproniazid with enzyme was studied by kinetic methods. As shown in Fig. 2, iproniazid inhibition is competitive with KI, implying that iproniazid interacts near the site of the peroxidase function and not near the iodinase function. Iproniazid inhibition with respect to tyrosine is of a mixed type. These findings suggest that the iodination is catalyzed by the enzyme and does not occur non-enzymatically.

In vivo experiment: Rats were injected with radioactive iodide following injection of the drug or saline, as described under METHODS, and protein bound ^{131}I of thyroid glands was measured. As shown in Table 1 ^{131}I incorporation into control thyroids averaged 6432 ± 204 cpm. Iproniazid treatment diminished incorporation to 3113 ± 212 cpm. Since

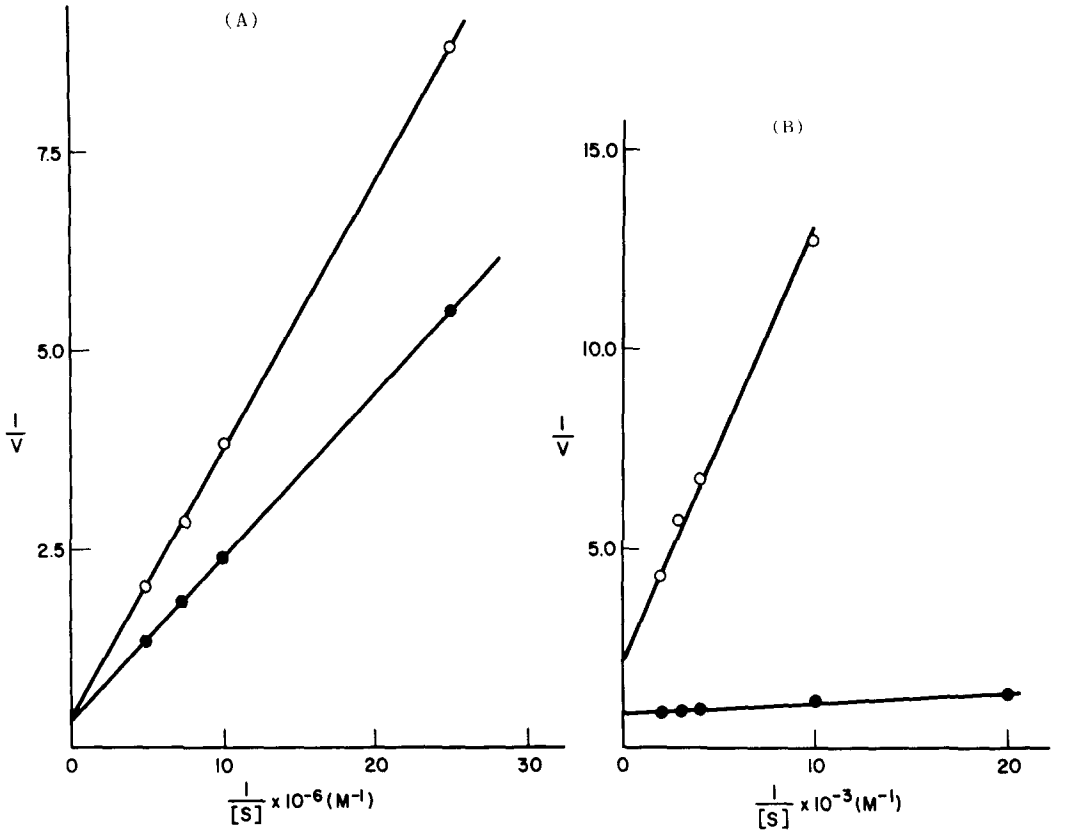

Fig. 2A. Lineweaver-Burk plots of KI against the rate of ^{131}I incorporation to tyrosine, with and without iproniazid. The assay was carried out as described under METHODS and velocity is expressed as percent of ^{131}I bound to tyrosine. Substrate concentration is given as reciprocal molarity. Enzyme alone, ●——●; enzyme plus 1 x 10^{-5}M iproniazid, 0——0.

Figure 2B. Lineweaver-Burk plots of tyrosine concentration versus the rate of ^{131}I binding to tyrosine, with and without iproniazid. Details are the same as Fig. 2A. Enzyme alone, ●——●; enzyme plus 1 x 10^{-5}M iproniazid, 0——0.

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iproniazid also interacts with monoamine oxidase, a non-hydrazine inhibitor of monoamine oxidase (pargyline) was administered to rule out that the effects of ^{131}I incorporation were related to the latter enzyme. As shown in Table 1, pargyline did not lower ^{131}I incorporation.

It had been reported that another hydrazine derivative, isoniazid, inhibits the ^{131}I incorporation catalyzed by dog thyroid peroxidase and that inhibition is competitive with iodide (5). In the case of horseradish

Table 1

Effects of Iproniazid and Pargyline on ^{131}I Incorporation into Protein of Thyroid Gland

No.	Treatment	Weight of Thyroid Glands (mg)	cpm/mg Tissue	Mean Values ± S.E.
1	iproniazid	23	1450	
2	"	22.5	4800	
3	"	26.2	1950	
4	"	19.0	4160	3113 ± 212
5	"	21.5	2980	
6	"	18.0	3340	
7	pargyline	23.0	5750	
8	"	16.5	6600	
9	saline	18.7	4720	
10	"	16.0	8500	
11	"	22.5	5140	6432 ± 204
12	"	19.0	8120	
13	"	22.5	6200	
14	"	23.0	7200	

Details described under METHODS.

peroxidase iproniazid and other hydrazines were shown to combine with the protein portion of the enzyme and to induce specific spectral changes characterized by a shift and gradual decrement of the Soret band (2). By analogy one might expect iproniazid and other hydrazines to interact with the apoenzyme of iodide peroxidase. Verification of this must wait until thyroid iodide peroxidase is purified further. It will be of interest to compare the interaction with iodide peroxidase of iproniazid and other

antithyroid agents such as thiouracil and thiocyanate (6). Conversely it will be of interest to continue studies on the antithyroid activity of iproniazid and other hydrazines and to determine whether any of the reported activities of iproniazid in animals and man are due to this activity.

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