Mechanisms of Antithyroidal Activity of Methimazole

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Methimazole produces a significant inhibition of iodide ion absorption by the intact thyroid which may account for the major portion of its antithyroidal action. The principal intrathyroidal effect of methimazole is shown to be due to its reactivity with elemental iodine. It neither inhibits nor serves as a competitive substrate for the hydrogen peroxide–peroxidase enzyme system. Thyroxine is displaced from its serum binding sites by methimazole; however, this mechanism is not considered to be clinically important.

THIOUREA compounds, such as methimazole, are thought to exert their antithyroidal effect by competing with tyrosine for elemental iodine or by inhibiting the oxidative enzyme system which converts iodide ion to iodine. In either case, the formation of diiodotyrosine is prevented and the biosynthetic pathway to the formation of thyroxine is blocked.

In competing with tyrosine for iodine, the thiourea derivative is thought to enolize to form a sulfhydryl group which is easily oxidized by iodine to form a disulfide. Iodine is reduced to iodide ion which is incapable of iodinating tyrosine. Since this reaction proceeds at a rate several hundred times faster than the iodination of tyrosyl groups, competition should be quite favorable for diversion of iodine from organic binding (1, 2).

It has been concluded by Astwood (3) that inhibition of a peroxide–peroxidase enzyme system in the thyroid is the most likely mechanism of antithyroidal activity of the thiourea compounds. Alexander (4, 5) recently demonstrated the existence, in thryoid homogenates, of such an enzyme system which can perform the iodide oxidation. Randall (6) has shown that sulfhydryl compounds are capable of reacting with hydrogen peroxide and that the rates of reaction are accelerated in the presence of peroxidase. It was concluded that sulfhydryl compounds do not inhibit peroxidase, but rather serve as substrates, competing successfully with other substrates. It was not demonstrated that sulfhydryl groups could successfully compete with iodide ion for the peroxide–peroxidase system.

Others have studied the inhibition of secretion or activity of hormonal TSH (7, 8) and the inhibition of the utilization of thyroid hormones at the tissue level (9, 10). The controversial studies of the mechanisms of action of thiourea antithyroid compounds have been reviewed by Pitt-Rivers (11), Astwood (1), and Trotter (12).

The purpose of this study was to determine the extent of contribution to antithyroidal activity by various possible mechanisms of action of methimazole, a typical thiourea derivative. The modes of action investigated included both extrathyroidal and intrathyroidal mechanisms.

EXPERIMENTAL

Reaction of Iodine with Methimazole and Tyrosine.—The course of reaction between iodine and a mixture of methimazole and tyrosine was determined spectrophotometrically. Iodine was allowed to react with methimazole, tyrosine, and a mixture of the two. All reactions were performed at room temperature and completion noted by the discharge of the yellow iodine color. Each of the reactants was prepared in 0.001 *M* concentration in phosphate buffer, pH 7.4.

As shown in Table I, the molar ratio of methimazole–tyrosine–iodine used was 4:1:2 which provides a stoichiometric quantity of iodine to the reactions. In the presence of a mixture of methimazole and tyrosine, iodine was added last to provide a competitive effect and was present in sufficient quantity to react completely with only one component.

After reaction, all samples were diluted to 500 ml. with phosphate buffer and their ultraviolet absorption spectra determined from 275 to 215 mμ using a Beckman model DK-2A recording spectrophotometer. The results are shown in Fig. 1.

The spectrum of the reaction product of iodine with a mixture of methimazole and tyrosine (spectrum *3*) compares very closely with the spectrum of the reaction product of iodine with methimazole to which tyrosine was added after reaction (spectrum *5*). If iodine had reacted with tyrosine to the ex-

TABLE I.—REACTION COMPONENTS

Sample	0.001 *M* Methimazole, ml.	0.001 *M* Tyrosine, ml.	0.001 *M* Iodine, ml.	Buffer, ml.
1	0	2.5	5	10
2	10	0	5	2.5
3	10	2.5	5	0
4	10[a]	2.5	5	0
5	10	2.5[b]	5	0

[a] Added after reaction of tyrosine with iodine was complete. [b] Added after reaction of methimazole with iodine was complete.

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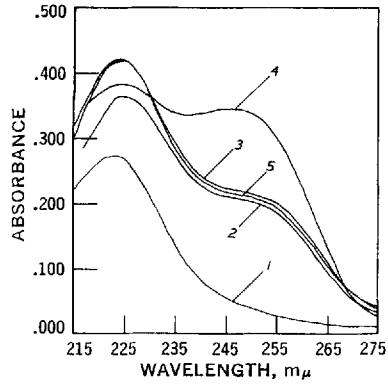

Fig. 1.—Ultraviolet spectra of methimazole and tyrosine reaction products with iodine. Key: *1*, tyrosine–iodine; *2*, methimazole–iodine; *3*, tyrosine and methimazole–iodine; *4*, tyrosine–iodine with added methimazole; *5*, methimazole–iodine with added tyrosine.

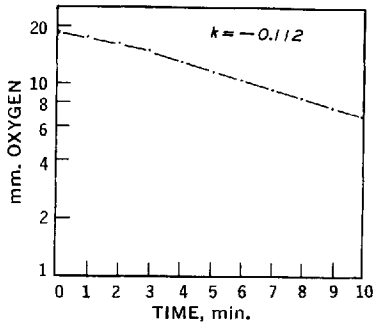

Fig. 2.—Catalytic decomposition of hydrogen peroxide by iodide ion.

clusion of methimazole, an absorption spectrum similar to spectrum *4* would have resulted. A mixture of reaction products would have resulted in an absorption spectrum of intermediate character. It is apparent that iodine in the presence of a mixture of methimazole and tyrosine reacts almost exclusively with methimazole.

To further verify that tyrosine failed to react with iodine in the presence of methimazole, the competitive reaction, containing twice the quantity of tyrosine, was repeated. Ultraviolet spectra indicated that methimazole had completely utilized the available iodine. Chromatography of this sample on Whatman No. 1 paper in a descending *n*-butanol–acetic acid (9:1)–water system failed to separate mono- or diiodotyrosine using 0.2% ninhydrin as detector. It was established that this system was capable of separating and detecting as little as 0.5% of the theoretical quantity of diiodotyrosine which could have been formed.

Interaction of Iodide Ion and Methimazole with Hydrogen Peroxide–Peroxidase Enzyme System.—The oxidation of iodide ion, methimazole, and a mixture of the two with hydrogen peroxide in the presence of peroxidase was studied using the manometric technique of Randall (6). All reactions were performed in the Warburg apparatus at 37.5°. The main chamber of the flask contained 1 ml. of either 0.06 *M* potassium iodide, 0.06 *M* methimazole, or both potassium iodide and methimazole in 0.06 *M* concentrations. To the flask were added 0.6 ml. of peroxidase (1 unit/ml.) and 0.4 ml. phosphate buffer, pH 7.4. After temperature equilibration for 10 min., 0.5 ml. of 0.06 *M* hydrogen peroxide was added from one sidearm and the reaction allowed to continue for the desired period of time. Catalase, 0.075 mg. contained in 0.5 ml., was added from the second sidearm to stop the reaction by decomposing the remaining hydrogen peroxide. Oxygen evolution, directly proportional to the hydrogen peroxide remaining in the flask, was read directly from the manometer after 2 min. All solutions were prepared using phosphate buffer, pH 7.4. Pressures (mm. oxygen) reported are averages of a minimum of four determinations corrected to a single manometer tube and corrected for barometric pressure changes. Reaction rates reported were all calculated using a first-order expression since the data obtained corresponded closely to this order.

In the reaction of iodide ion with hydrogen peroxide–peroxidase, a steady evolution of oxygen was noted before the addition of catalase. This was due to the catalytic decomposition of hydrogen peroxide by iodide ion as described by several investigators (13–15). This effect began after a brief induction period and continued until the hydrogen peroxide was decomposed by the addition of catalase. During the induction period, iodide ion is oxidized to a steady-state concentration of iodine. When this concentration is reached, hydrogen peroxide decomposition begins with no further iodine generation. In measuring oxygen pressures at given time intervals, it was first necessary to determine the catalytic oxygen pressure. The catalase solution was then added and the total oxygen pressure recorded after 2 min.

The difference between the catalytic oxygen pressure at various time intervals and the original peroxide oxygen pressure which was shown to be constant over the time involved is a measure of the total theoretical oxygen remaining if no other reactions are occurring. From the resulting data, as shown in Fig. 2, the rate of the catalytic decomposition of hydrogen peroxide can be calculated. This rate, $k = -0.112$, is in good agreement with the literature value (13).

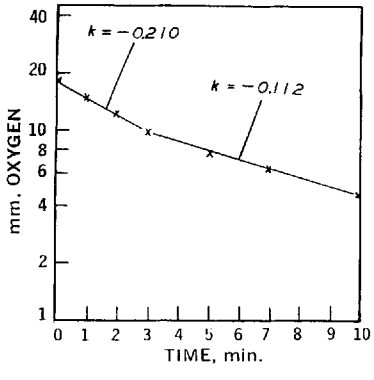

Fig. 3.—Oxidation of iodide ion and catalytic decomposition of hydrogen peroxide.

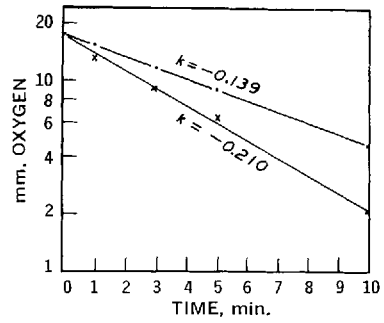

Fig. 4.—Reaction of hydrogen peroxide–peroxidase with methimazole and methimazole–iodide ion. Key: —·—·, methimazole and hydrogen peroxide–peroxidase; —×—×, methimazole, iodide ion, and hydrogen peroxide–peroxidase.

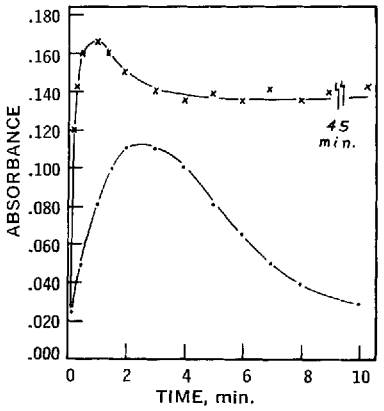

Fig. 5.—Iodine generation by hydrogen peroxide–peroxidase. Key: —×—×, iodide ion and hydrogen peroxide–peroxidase; —·—·, iodide ion, methimazole, and hydrogen peroxide–peroxidase.

The difference between the catalytic oxygen pressure and the total measured oxygen pressure after addition of catalase is equivalent to the actual peroxide oxygen remaining. These data (Fig. 3) show the initial oxidation of iodide ion to iodine during the induction period ($k = -0.210$) and the catalytic decomposition effect ($k = -0.112$). Beyond the induction period, only catalytic decomposition is occurring as evidenced from the rate constant.

Methimazole and iodide–methimazole substrates did not produce a catalytic peroxide decomposition. The rates of these reactions could be followed by direct measurement of residual oxygen pressure at a given time as shown in Fig. 4. Methimazole reacts with the hydrogen peroxide–peroxidase system at a slower rate ($k = -0.139$) than a mixed iodide–methimazole substrate. The rate constant of the latter reaction is the same as that calculated for the oxidation of iodide ion to a steady-state concentration of iodine. Since no catalytic decomposition was observed and the reaction rate was uniform over the time period measured, it would seem likely that the primary reaction is the oxidation of iodide ion in an attempt to gain a steady-state concentration of iodine which, in the presence of methimazole, cannot be attained.

The same ratio of reactants (methimazole–iodide ion–peroxide = 2:2:1) at 6 times the previous concentration also failed to display the catalytic peroxide decomposition effect. The same result was obtained at ratios of 2:2:2 and 2:2:3. At ratios of 2:2:4, 2:2:5, and 2:2:6, catalytic oxygen evolution began after a 6 to 8-min. induction period.

Further verification of iodine generation in a mixed iodide–methimazole system was obtained spectrophotometrically. During the manometric studies, a transient pale yellow color was noted in the first few minutes of reaction. The iodide ion reaction and the iodide–methimazole reaction with hydrogen peroxide–peroxidase were repeated as described and the absorbance at 400 mμ measured using a Bausch & Lomb Spectronic 20 spectrophotometer. As shown in Fig. 5, in the absence of methimazole, a steady-state concentration of iodine is reached within 2 to 3 min. and remains throughout the period of observation. In the presence of methimazole, iodine is produced; however, it never reaches a steady-state concentration. It gradually decreases in concentration probably by reaction with methimazole.

Inhibition of Thyroidal Absorption of Iodide Ion.—Methimazole has been observed to inhibit iodide ion accumulation in euthyroidal humans in doses of 0.5 mg. (16). Williams and Coker (17) have demonstrated that iodide ion absorption by surviving thyroid slices is completely inhibited upon incubation for 2 hr. in a solution containing 5 mcg. of methimazole. The following study was performed to quantitate this effect in the intact thyroid.

Sixteen male, white, Sprague-Dawley rats of the same ages, weighing from 220 to 337 Gm. (average 292 Gm.) were used for tests and controls. Eleven received 0.4 mg./Kg. of methimazole intraperitoneally 12 hr. and again 3 hr. prior to the administration of approximately 7.4 μc. of sodium radioiodide.[1] Five rats used as controls received only sodium radioiodide. Exactly 2 hr. after the administration of sodium radioiodide, the animals were exsanguinated under ether anesthesia. The thyroid glands were immediately removed, transferred to planchets, and counted for 5 min. using a Tracerlab Versamatic II scaler with a Tracerlab scintillation detector, P-20-D, and a 1 × 1.5 in. NaI (Tl) crystal. All counts were corrected for background. The results, shown in Table II, indicate an inhibition of thyroidal iodide ion absorption by methimazole of 80.9%.

Displacement of Thyroxine from Serum Binding Sites.—Diphenylhydantoin has been shown to lower the serum-bound iodine in both epileptic and normal subjects (18). To determine if methimazole exhibits the same property, a dialysis procedure was employed. Test materials were sodium diphenylhydantoin, methimazole, and histamine phosphate. Histamine was used because of its similarity in size and ring structure to methimazole. All were used in equimolar concentrations.

Four 5-ml. portions of fresh dog serum were placed in each of four preboiled 6-in. lengths of cellophane dialysis tubing which were tied at one end. To each was added 1.25 μc. of thyroxine-^{131}I contained in 0.2 ml. The serum control tube was diluted with 5 ml. of phosphate buffer, pH 7.4. To

[1] Oriodide. Abbott Laboratories, Radiopharmaceuticals Division, Oak Ridge, Tenn.

TABLE II.—THYROIDAL IODIDE ION ABSORPTION BY CONTROL AND METHIMAZOLE-TREATED RATS

Rats	^{131}I, c.p.m. Administered	Mean ± σ ^{131}I, c.p.m. Absorption	% ± σ ^{131}I Absorption
Controls	1.68×10^6	57,104 ± 12,941	3.4 ± 0.77
Methimazole-treated	1.68×10^6	10,941 ± 3,270	0.65 ± 0.02

TABLE III.—DISPLACEMENT OF THYROXINE FROM DOG SERUM BINDING SITES

Dialysis Time, hr.	Serum Sample			
	Control	Methimazole	Diphenylhydantoin	Histamine
1	2.0%	2.2%	2.6%	2.0%
4	2.8	3.7	4.0	2.1
8	5.1	29.0	33.7	4.0
12	10.2	75.0	83.2	5.1

the remaining three tubes were added 25 mcg. of methimazole, 60 mcg. of sodium diphenylhydantoin, and the equivalent of 24.3 mcg. of histamine, respectively, each in 5-ml. volume. This quantity of methimazole approximates physiological concentrations. The tubes were tied and each placed in separate capped jars containing 20 ml. of phosphate buffer, pH 7.4. The jars were agitated in a Dubnoff incubator at 37.5°. One hundred-microliter samples of the buffer were removed at 1, 4, 8, and 12-hr. intervals, placed in planchets, and counted using a scintillation detector.

Only if thyroxine is displaced from its protein binding sites will it dialyze into the phosphate buffer medium. The per cent displacement can be calculated from the count rate in the external buffer at a given interval and the known activity added to the serum. The results in Table III indicate that thyroxine is displaced by methimazole and sodium diphenylhydantoin. Histamine did not show this effect, perhaps indicating that a urea or thiourea moiety is required.

DISCUSSION

Methimazole has been shown to compete with tyrosine for elemental iodine so effectively that detectable levels of iodinated tyrosine derivatives are not formed. It neither inhibits nor serves as a preferential substrate for the hydrogen peroxide–peroxidase enzyme system. Iodide ion in the presence of methimazole and the enzyme system is converted to elemental iodine which apparently reacts with methimazole as it is formed. These data indicate that the avidity of methimazole for elemental iodine is the prime basis for its intrathyroidal activity.

It was found that a reduction in the uptake of iodide ion on the order of 80% by the intact thyroid followed the administration of methimazole. Since iodide ion absorption is the first step leading to the biosynthesis of thyroxine, this mechanism of action must be considered very significant and probably more important than the intrathyroidal effects.

In physiological concentrations, methimazole and sodium diphenylhydantoin were shown to displace thyroxine from its serum binding sites. Although sodium diphenylhydantoin causes a reduction in serum-protein bound iodine, it does not produce a clinical hypothyroidism. Therefore, it would appear unlikely that methimazole exerts a significant portion of its antithyroidal effect at this level of thyroid function.

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