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FORMATION OF MOLECULAR IODINE DURING OXIDATION OF IODIDE BY THE PEROXIDASE/H_2O_2 SYSTEM

IMPLICATIONS FOR ANTITHYROID THERAPY

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Abstract—The first step in the biogenesis of thyroid hormones is the oxidation of iodides taken up by the thyroid gland. Oxidation of I^- by the H_2O_2/peroxidase system leads to the formation of iodinium ions I^+ which bond to thyroglobulin by electrophilic substitution. However, it is not clear whether I^- is transformed directly to I^+ or whether it passes through a molecular iodine intermediate. This latter possibility is indicated by the oxidation potentials of the reactions. I_2 can be detected *in vitro* from the formation of I_3^- ions, although this has yet to be confirmed *in vivo*. The present study was designed to determine, albeit indirectly, whether this reaction occurs *in vivo*. If I_2 is produced, it may form charge transfer complexes with numerous drugs. We also investigated the action of various drugs on lactoperoxidase and assessed their antithyroid activity in the rat by assay of plasma levels of T_3, T_4, and TSH. We found a good correlation between the value of K_c, the formation constant of the complex of the drug with molecular iodine, and the antithyroid activity *in vivo*. This correlation was observed in four different classes of compound. The possibility that molecular iodine is produced in the thyroid gland has implications for antithyroid therapy.

The first step in the biogenesis of thyroid hormones is the oxidation of iodides which are taken up by the thyroid gland (trapping). Oxidation of iodide leads to the formation of iodinium ions I^+ [1, 2] which by electrophilic substitution [3] bind to tyrosine residues in thyroglobulin. This oxidation reaction is carried out by the H_2O_2/peroxidase system. It is generally thought [4–9] that thyroid hormone synthesis can be decreased by competition with thyroid peroxidase (TPO†) or blocked by inactivating this enzyme, which forms the basis of the pharmacological treatment of hyperthyroidism. However, it is not clear whether the transformation of I^- into I^+ takes place in a single step or whether there is intermediate formation of molecular iodine. If, in fact, I_2 is produced in the thyroid gland, it could form charge transfer complexes with compounds (electron donors) with an available pair of electrons which can be transferred to a suitable acceptor, in this case iodine. A variety of drugs have this property.

The formation of molecular iodine has been demonstrated in the absence and presence of acceptor [10, 11] but *in vitro* only. It has not yet been possible to demonstrate the formation of I_2 *in vivo*. We present here results which provide indirect evidence for the formation of molecular iodine *in vivo*.

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† Abbreviations: TPO, thyroid peroxidase; LPO, lactoperoxidase; ABTS, 2,2′-azino-bis(3-etylbenzthiazoline-6-sulfonic acid); TSH, thyroid stimulating hormone; SAT, synthethic antithyroid.

MATERIALS AND METHODS

Spectrophotometry. Spectrophotometric methods were performed as reported previously [12]. Briefly, the spectra were recorded on a Perkin–Elmer 554 UV-Visible spectrophotometer equipped with a Peltier effect thermostated sample holder (Oak Brook, IL, U.S.A.). All drugs commercially available were purified by preparative HPLC. Solvents, carbon tetrachloride and chloroform were of spectroscopic grade (Merck, Darmstadt, Germany). None of the donors had significant absorbance in the visible region. Mixing donor and iodine gave rise to a new absorption peak at a shorter wavelength than the iodine peak, the blue-shifted band.

At the concentrations used (10^{-2}–10^{-6} M) all donors absorbed in the UV region. The formation of the donor–acceptor complex led to the appearance of a new band, the charge transfer band. The formation constants of the drug–iodine complexes were evaluated from the blue-shifted band using the method of Lang [13, 14].

ABTS assay. Activity towards peroxidase was tested *in vitro* using LPO. This commercially available peroxidase was employed as it has standardized activity and could, thus, be used to compare the activities of the various compounds. TPO is not easy to isolate and purify without loss of activity but it has been shown that LPO has similar properties to TPO [15, 16]. Furthermore, it has been reported that a monoclonal antibody prepared from patients with Basedow's disease recognizes both human TPO and bovine LPO [17]. Incubation tubes contained 1.33 μg/mL LPO, 1.4 mM ABTS, 0.067 M phosphate buffer tampon pH 7 and 1.15 μM H_2O_2. The

reaction was initiated with H_2O_2 and was read at 411 nm after 20 sec at 20°. Enzyme activity after the addition of various drugs was expressed as the percentage of that observed in the untreated LPO solution.

$$(VO_x - VO)/VO = \text{percentage inhibition}$$

where VO_x = initial rate with drug and VO = initial rate without drug.

Experiments in vivo. Male Wistar rats were obtained from Janvier (St Berthevin, France). Experimental conditions were as described previously [18]. Thryoid glands were removed, and weighed. Blood samples were taken by cardiac puncture for assay of T_3, T_4 (RIA Gnost Behring, Marburg, Germany) and TSH. Reagents for radioimmunoassay of rat TSH were provided by Amersham International (Amersham, U.K.) (^{125}I assay system code RPA 554).

RESULTS

Formation of drug–I_2 complexes and determination of formation constants K_c

Four types of compound were tested: those with a well-known antithyroid action (KSCN, methimazole, 2-aminothiazole); some with possible antithyroid activity (econazole, trimethoprim, acetiamine); molecules with an NCS group which were devoid of activity (organic thiocyanates, thiazole, 4-methylthiazole, clomethiazole); and molecules with no activity towards LPO but with recognized antithyroid activity (derivatives of phenothiazine and imipramine). Propylthiouracil is not mentioned since, although it complexes iodine, its insolubility in the solvents used meant that we could not determine its K_c. The most commonly encountered complexes formed between donor (drugs) and acceptor (iodine) are of the charge transfer type [19]. Complex formation was analysed in the visible and UV regions. They were of n-6 nature and 1:1 stoichiometry [20, 21]. Table 1 lists the different compounds and the formation constants of the complexes.

ABTS assay

We found that all compounds in the first group (molecules with known antithyroid activity) inhibited LPO (Table 1). Molecules in the third and fourth groups had not activity towards LPO. Only the drugs in the third group were devoid of activity toward both iodine and LPO.

In vivo *experiments*

Plasma levels of T_3, T_4 and TSH were assayed. Thyroid weights were reported and showed goitrigenic effect of drugs (Table 2). The results were correlated with the values of K_c. We found that some drugs without action on LPO had antithyroid activity *in vivo*.

Complex formation and antithyroid activity

For molecules with known antithyroid activity the value of K_c gave a quantitative estimate of the electron donor action. A scale of activity could thus be established and could be related to the antithyroid activity observed *in vivo*. The alkaline thiocyanates had the lowest activity with a K_c of around 100 L/mole. It has been suggested that compounds with with a value of $K_c \geqslant 100$ have potential antithyroid activity. In contrast, methimazole, the strongest SAT agent, had a high value of K_c (23,194 L/mole). The electron donor action could be exerted either on peroxidase by covalent binding with the heme group [22] or on molecular iodine. The reaction is generally an irreversible inactivation [23].

Table 1. Comparison of formation constants (K_c) of drug–I_2 complexes and activities of drugs towards LPO

	K_c (L/mole)	IC_{50} LPO
KSCN	96	3.9×10^{-2} M
Methimazole	23194	2.46×10^{-5} M
2-Aminothiazole	I_3^-	1.2×10^{-4} M
Econazole	575	Insoluble
Acetiamine	216	1.7×10^{-2} M
Trimethoprim	652	5.6×10^{-3} M
RSCN	6–8	0
Thiazole	10	0
4-Methylthiazole	22	11.3×10^{-3} M
Clomethiazole	25	0
Alimemazine	788	0
Etymemazine	731	0
Levomepromazine	738	0
Triflupromazine	2803	0
Trimipramine	1003	0
Desipramine	2087	0

K_c formation constant for complexes determined using Lang's method in CCl_4 at 20°.

I_3^-, complex unstable, formation of I_3^- ions. 0, compound without activity on LPO; insoluble, in the buffer used. RSCN, organic thiocyanates.

Determination of K_c indicated that compounds with a value of K_c above 100 (Group 2) had antithyroid activity. These compounds do not possess a NCS group. Other active drugs such as acetiamine have a different structure. We suspected that compounds with a sulfur or nitrogen heteroatom would possess antithyroid activity due to their electron donor properties. Their activity towards LPO was measured and their activity *in vivo* in the rat was tested in an attempt to establish a structure–activity relationship. Interestingly, we found that certain molecules with a NCS group (Group 3) were devoid of antithyroid activity.

Some molecules had a high K_c and no action on LPO (Group 4). In this group, we also found a correlation between the value of K_c and antithyroid activity *in vivo* (e.g. phenothiazines and imipramines).

DISCUSSION

Formation of I_2 is readily detected *in vitro*, although *in vivo* its presence has yet to be detected in the thyroid gland. *In vitro*, I_2 is usually determined

Table 2. Thyroid hormone levels and thyroid weights after drug treatments

	Serum hormone level			Thyroid weight (mg/100 g body weight)
	T_3 (ng/dL)	T_4 (μg/dL)	TSH (ng/mL)	
Control	149.0 ± 7.78	4.10 ± 0.33	5.24 ± 0.16	6.20 ± 0.09
KSCN	120.2 ± 1.18*	3.48 ± 0.21*	5.92 ± 0.20*	9.28 ± 1.14
Methimazole	42.4 ± 4.21*	0.51 ± 0.08†	12.16 ± 0.28*	24.75 ± 1.63
2-Aminothiazole	64.2 ± 6.26*	2.84 ± 0.18*	9.14 ± 0.26*	16.18 ± 1.04
Econazole	101.0 ± 5.28*	3.80 ± 0.21*	7.92 ± 0.16*	11.24 ± 1.42
Acetiamine	96.8 ± 9.42†	3.72 ± 0.28†	7.86 ± 0.22*	11.72 ± 1.18
Trimethoprim	68.4 ± 6.40*	2.72 ± 0.21*	8.56 ± 0.23†	12.08 ± 0.92
RSCN	147.0 ± 5.26*	4.00 ± 0.28†	5.12 ± 0.14*	6.06 ± 0.26
Thiazole	144.2 ± 5.62*	3.98 ± 0.23†	5.08 ± 0.16*	6.02 ± 0.34
4-Methylthiazole	144.8 ± 6.22*	4.02 ± 0.24†	5.16 ± 0.12*	5.98 ± 0.18
Clomethiazole	128.6 ± 5.80†	3.88 ± 0.26†	5.44 ± 0.16*	7.44 ± 1.82
Alimemazine	50.8 ± 5.17*	2.44 ± 0.20*	8.96 ± 0.20*	15.04 ± 1.26
Etymemazine	88.2 ± 6.47†	3.36 ± 0.24†	8.44 ± 0.21*	14.62 ± 0.98
Levomepromazine	74.4 ± 4.48*	3.32 ± 0.18*	8.52 ± 0.23*	14.78 ± 1.02
Triflupromazine	56.4 ± 5.24†	1.62 ± 0.14‡	9.02 ± 0.28†	15.54 ± 1.22
Trimipramine	104.0 ± 7.26*	3.64 ± 0.20*	8.66 ± 0.26*	15.32 ± 1.44
Desipramine	117.6 ± 8.7†	3.78 ± 0.18†	8.48 ± 0.22*	11.36 ± 0.86

The administrated doses were 50 mg/kg/day during 3 weeks. Levels of T_3, T_4 and TSH were determined by radioimmunoassay.

Control, untreated rats. Significance of difference was determined by Student's *t*-test. * $P < 0.001$; † $P < 0.01$.

by addition of a solution of KI producing I_3^- ions which absorb strongly at 353 nm (25°). Addition of 50 mM KI leads to a 95% conversion into I_3^- [11]. In addition, TPO activity can be determined from the rate of formation of I_3^- from I^- [24] according to the following equations:

$$2I^- \rightleftharpoons I_2$$

$$I_2 + I^- \rightleftharpoons I_3^-.$$

Thus, *in vitro* it is more convenient to measure the concentration of I_3^- in order to estimate I_2. The formation of I_2 in the absence of receptor (tyrosine) has been reported by Pommier *et al.* [10]. Magnusson *et al.* [11] have proposed the following reaction:

$$E + H_2O_2 \xrightarrow{H_2O} EO + I^- \longrightarrow [EOI]^-$$

where E = enzyme. In the presence of tyrosine (or tyrosine residues in thyroglobulin) EOI^- gives the iodinium ion I^+ either directly or via I_2. I^+ then binds to thyroglobulin. In the absence of tyrosine I_2 must form since I_3^- is produced.

According to Magnusson *et al.* [11] there is little production of I_2 in the presence of acceptor (around 5–10 μM) since I_2 is transformed rapidly into I^+ which reacts immediately with tyrosine. In the absence of tyrosine, levels rise to around 25 μM in a small excess of I^- and to around 95 μM when iodide is in charge excess. However, iodine levels are not measured readily due to the low molar extinction coefficient and instability of I_2 in aqueous solution at pH 7. As reported previously [18], on the basis of the oxidation potentials, the oxidation reaction producing I_2 is energetically more favorable than that giving I^+, which explains why I_2 is observed in the absence of receptor. The small amount of I^+ formed will react rapidly with nucleophiles such as I^- to form I_2. Then iodide is oxidized *in vivo* in the absence of receptor (the receptor protein thyroglobulin) by H_2O_2/TPO. Using autoradiography and electron microscopy, Ericson [25], Ofverholm and Ericson [26], and Ekholm [27] have shown that iodide and TPO are located in the apical membrane while tyroglobulin is synthesized in the follicular cells to be transferred, and accumulated in the follicular lumen some distance from the other components required for the synthesis of thyroid hormones. This suggests that iodine is in fact oxidized in the absence of receptor *in vivo* and could thus form significant amounts of I_2.

The agreement between the value of the formation constant of the drug–iodine complex and the antithyroid activity of the drug *in vivo* tends to indicate an action of the drug on molecular iodine, especially for drug without activity on peroxidase. We have proposed a molecular mechanism of action of antithyroid drugs involving I_2 [18]. Iodine as I_2 cannot take part in iodination and must be further oxidized to I^+ by a peroxidase with a sufficient level of oxidation such as compound II [28].

The formation of I_2 means that strong electron donors will tend to form complexes with it. We have shown that SAT have a stronger affinity for molecular iodine than for peroxidase (unpublished results). This can help explain why iodide protects TPO from inactivation by SAT.

The presence of molecular iodine can have implications for the treatment of thyroid pathology. Up until now the activity of SAT agents has been judged with respect to their action on TPO. In some instances, this action may be irreversible leading to severe hypothyroidism. A drug with an action on molecular iodine and preferably without an action on TPO will

not completely block biosynthesis of vital hormones. Complex formation is a reversible reaction liberating iodide which will tend to protect TPO from inactivation. Furthermore, if the drug only acts on iodine and is devoid of activity towards TPO, it will be unlikely to have an action on other peroxides in the organism. We have shown that drugs with an action on TPO also have activity towards the peroxidase in the prostaglandin synthetase system (unpublished results) and will thus influence arachidonic acid metabolism.

The ability to react with molecular iodine to form charge transfer complexes means that drugs with strong electron donor properties will tend to have an antithyroid action. Prolonged treatment with such drugs is thus liable to lead to iatrogenic hypothyroidism. This is in fact observed with phenothiazines, imipramines and a number of other drugs.

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REFERENCES

1. Alexander NM, Evidence for the oxidation of iodine by H_2O_2 and peroxidase. *Excerpta medica* (7th international thyroid conference) **361**: 31, 1975.
2. Ohtaki S, Nakagawa H, Kimura S and Yamazaki I, Analysis of catalytic intermediates of hog thyroid peroxidase during its iodinating reaction. *J Biol Chem* **256**: 805–810, 1981.
3. Buxeraud J, Absil AC, Raby C, Catanzano G and Beck C, Anti-thyroidiens: relation structure–activité. Interprétation du mécanisme d'action des ATS par complexation de transfert de charge. *Eur J Med Chem Chim Ther* **20**: 43–51, 1985.
4. Morris DR and Hager P, Mechanism of the inhibition of enzymatic halogenation by antithyroid agents. *J Biol Chem* **241**: 3582–3589, 1966.
5. Maloof F, Smith S and Soodak M, The mechanism of action of the thio carbamide type antithyroid drugs in inhibiting iodination in thyroid tissue. *Mech React Sulfur Compounds* **4**: 61–68, 1962.
6. Taurog A, The mechanism of action of the thioureylene antithyroid drugs. *Endocrinology* **98**: 1031–1046, 1976.
7. Nagashima T, Taurog A and Riesco G, Mechanism of action of thioureylene antithyroid drugs: factors affecting intrathyroidal metabolism of propylthiouracil and methimazole in rat. *Endocrinology* **103**: 2187–2197, 1978.
8. Shiroozu A, Taurog A, Engler H and Dorris ML, Mechanism of action of thioureylene antithyroid drugs in the rat: possible inactivation of thyroid peroxidase by propylthiouracil. *Endocrinology* **113**: 362–370, 1983.
9. Engler H, Taurog A and Nakashima T, Mechanism of inactivation of thyroid peroxidase by thioureylene drugs. *Biochem Pharmacol* **31**: 3801–3806, 1982.
10. Pommier J, Deme D and Nunez J, Effect of iodide concentration on thyroxine synthesis catalysed by thyroid peroxidase. *Eur J Biochem* **37**: 406–414, 1973.
11. Magnusson RP, Taurog A and Dorris ML, Mechanism of thyroid peroxidase and lactoperoxidase-catalyzed reactions involving iodide. *J Biol Chem* **259**: 13783–13790, 1984.
12. Raby C and Buxeraud J, Antithyroidiens: relation structure–activité. I. Formation de complexes moléculaires iodés. *Eur J Med Chem* **15**: 425–430, 1980.
13. Lang RP, Molecular complexes and their spectra. XIV. Iodine complexes with thiourea and thioacetamide. *J Am Chem Soc* **84**: 1185–1192, 1962.
14. Lang RP, Molecular complexes of iodine with tetramethylurea and tetramethylthiourea. *J Phys Chem* **72**: 2129–2135, 1968.
15. Taurog A, Dorris ML and Lamas J, Comparison of lactoperoxidase and thyroid peroxidase catalyzed iodination and coupling. *Endocrinology* **94**: 1286–1294, 1974.
16. Deme D, Pommier J and Nunez J, Specificity of thyroid hormone synthesis. The role of thyroid peroxidase. *Biochim Biophys Acta* **540**: 73–82, 1978.
17. Czarnocka B, Ruf J, Ferrand M and Carrayon P, Parenté antigénique entre la peroxydase thyroïdienne et l'antigène microsomial dans les affections autoimmunes de la thyroïde. *C R Acad Sci (Paris)* **300**: 577–580, 1985.
18. Raby C, Lagorce JF, Jambut-Absil AC, Buxeraud J and Catanzano G, The mechanism of action of synthetic antithyroid drug: iodine complexation during iodide oxidation. *Endocrinology* **126**: 1683–1691, 1990.
19. Mulliken RS, Molecular compounds and their spectra. III The interaction of electron donors and acceptors. *J Phys Chem* **56**: 801–822, 1952.
20. Buxeraud J, Absil AC and Raby C, Secondary antithyroid action of drugs in relation to structure. *J Pharm Sci* **73**: 1687–1690, 1984.
21. Jambut-Absil AC, Buxeraud J, Lagorce JF and Raby C, Charge transfer complexes of drugs with iodine investigation by UV/visible spectroscopy. *Int J Pharm* **35**: 129–137, 1987.
22. Engler H, Taurog A, Luthy C and Dorris ML, Reversible and irreversible inhibition of thyroid peroxidase-catalyzed iodination by thioureylene drugs. *Endocrinology* **112**: 86–95, 1983.
23. Davidson B, Soodak M, Neary JT, Strout HV, Kieffer JD, Mover H and Maloof H, The irreversible inactivation of thyroid peroxidase by methyl mercapto imidazol, thiouracil, and propylthiouracil *in vitro* and its relationship to *in vivo* findings. *Endocrinology* **103**: 871–872, 1978.
24. Alexander NM, A spectrophotometric assay for iodide oxidation by thyroid peroxidase. *Anal Biochem* **4**: 341–345, 1962.
25. Ericson LE, Ultrastructural aspects on iodination and hormone secretion in the thyroid gland. *J Endocrinol Invest* **6**: 311–324, 1983.
26. Ofverholm T and Ericson LE, Intraluminal iodination of thyroglobulin. *Endocrinology* **114**: 827–835, 1984.
27. Ekholm R, Iodination of thyroglobulin. An intracellular or extracellular process. *Mol Cell Endocrinol* **24**: 141–163, 1981.
28. Nunez J, Pommier J, Deme D, Virion A, Michot JL and Courtin F, Acquisitions récentes sur la peroxydase thyroïdienne. *Ann Endocrinol* **42**: 439–445, 1981.