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IN VITRO INHIBITION OF PROSTAGLANDIN SYNTHESIS BY ANTITHYROID DRUGS

J. M. BOEYNAEMS,* N. GALAND and J. E. DUMONT

Institute of Interdisciplinary Research, School of Medicine, Free University of Brussels, Belgium

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Abstract—Methimazole inhibited the release of PGE_2† and $PGF_{2\alpha}$ by dog thyroid slices: at 1 mM, the inhibition was 70% for PGE_2 and 63% for $PGF_{2\alpha}$. The concentrations required to inhibit prostaglandin synthesis (0.1–1 mM) were much higher than those which depressed protein iodination (1–10 μM). A similar inhibition of prostaglandin synthesis was obtained with carbimazole, propylthiouracil and aminotriazole, but not with imidazole. Basal release and release stimulated by ionophore A_{23187}, carbamylcholine and epinephrine were decreased to the same extent. Methimazole also inhibited the release of PGE_2 and $PGF_{2\alpha}$ by slices of dog renal cortex and inner medulla. The conversion of arachidonic acid to $PGF_{2\alpha}$ and PGE_2 by a dog kidney medulla homogenate was depressed by methimazole. The antithyroid drugs constitute a new class of prostaglandin synthesis inhibitors.

It has been proposed that a peroxidase mechanism is involved in the oxygenation step(s) of prostaglandin biosynthesis [1]. Drugs used in the treatment of thyrotoxicosis, such as methimazole and carbimazole, inhibit the iodination of tyrosyl residues in thyroglobulin and their coupling in iodothyronines by blocking thyroid peroxidase [2]. However, the action of these antithyroid drugs is not specific to the thyroid, since they can inhibit other peroxidases (lactoperoxidase, chloroperoxidase, etc.) [3]. We report here that these drugs are able to inhibit prostaglandin synthesis in various organs.

MATERIALS AND METHODS

Slices of dog thyroid, kidney cortex and kidney inner medulla were prepared with a Stadie–Riggs microtome after the organs were removed from mongrel animals of either sex anesthetized with nembutal. Between 20 and 50 mg tissue (wet wt) were incubated in 2 ml of the following medium: NaCl, 124 mM; KCl, 5 mM; $MgSO_4$, 1.25 mM; $CaCl_2$, 1.45 mM; $NaHCO_3$, 10 mM; KH_2PO_4, 1.25 mM; HEPES, 20 mM, pH 7.4; glucose, 5.6 mM. Incubations were performed at 37°, under an air atmosphere and with constant shaking (80 r.p.m.). The slices were preincubated 1 hr before the test incubation, at the end of which prostaglandins were measured. Antithyroid drugs, when added, were present during both incubations; in the case of inner medulla, the first incubation lasted 90 min in order to reach a steady-state output of prostaglandins [4]. In each experiment, the various experimental conditions were tested in triplicate: results are expressed as the mean ± S.D. PGE_2, $PGF_{2\alpha}$ and 15-keto-13, 14-dihydro-$PGF_{2\alpha}$ were assayed directly in the incubation medium without prior extraction and chromatography. Assays were performed in polypropylene tubes to which the following reagents were added: 0.1 ml of tritium labeled prostaglandin (22,000 d.p.m.) in 50 mM Tris buffer, pH 7.4; 0.1 ml of a 1% solution of bovine gamma globulins in 50 mM Tris buffer, pH 7.4; 0.1 ml of incubation medium samples or prostaglandin standards, prepared in the same medium (0.4 ml in the case of 15-keto-13, 14-dihydro-$PGF_{2\alpha}$ assay); 0.1 ml of an antiserum dilution in 50 mM Tris buffer, pH 7.4, which bound about 30% of the tracer. This reaction mixture was left for 1 hr at room temperature; then 0.4 ml (or 0.7 ml) of a cold 25% (w/w) solution of polyethylene glycol 6000 was added. After vigorous shaking the tubes were centrifuged for 20 min at 3000 *g*. The supernatant was aspirated and the pellet dissolved in 0.1 ml 0.1 N NaOH; 2.5 ml of RIALUMA PEG was added and the tubes were counted in a Packard liquid scintillation counter. Prostaglandin concentrations were computed with a Wang calculator, after smoothing of the standard curves by a linear interpolation method (Swillens, unpublished method).

The validity of these assays was established by the following criteria: specificity of the antisera—in particular, the anti-PGE_2 antiserum exhibits only 3.1% cross-reaction with PGE_1 and less than 0.15% with a variety of other prostaglandins (16); accurate recovery of known amounts of prostaglandins added to incubation media samples; superimposability between standard curves and displacement curves obtained by serial dilutions of samples containing a high amount of prostaglandins; 85% inhibition by indomethacin (10 μg/ml) of the basal release of immunoreactive PGE_2- and $PGF_{2\alpha}$-like material by dog thyroid slices; none of the drug tested in this study interfered with these assays.

For protein iodination measurement the dog thyroid

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* Aspirant of the Fonds National de la Recherche Scientifique. Present address: Department of Pharmacology, Vanderbilt University, Nashville, TN 37232, U.S.A.

† Abbreviations used are: PG, prostaglandin; TxB_2, thromboxane B_2; BSA, bovine serum albumin; PTU, propylthiouracil.

slices were incubated in the same conditions as described above, but the incubation medium also contained $NaClO_4$ (1 mM) and $K^{131}I$ (5 μM; 2 μCi/ml). At the end of the incubation, the slices were homogenized in a 1 mM methimazole solution; proteins were precipitated with 10% TCA and the pellets washed once, as previously described [5].

Dog inner renal medulla was homogenized in 0.1 M potassium phosphate buffer, (pH 7.4), 1 mM EDTA, with a Potter-driven glass homogenizer. After addition of [^{3}H]arachidonic acid (135 Ci/mmole; 0.5 μCi/ml), these homogenates were incubated for 30 min at 37°. They were then extracted with 3 vol of ethylacetate–isopropanol–0.1 N HCl (3:3:1, by vol), followed by the addition of 3 vol of 0.9% NaCl and 2 vol of ethylacetate. After centrifugation, the organic phase was evaporated under reduced pressure and the residue spotted on a silica gel plate. Elution was performed with a mixture of ethylacetate and acetic acid (99:1, v/v) [6]. One centimetre large zones were scraped from the plate and counted in 10 ml of LIPOLUMA in a liquid scintillation counter. Alternately, the products of the incubation of [^{14}C]arachidonic acid (58 mCi/mmole; 0.2 μCi/ml) with a kidney medulla homogenate were separated by high performance liquid chromatography on a microporasil column (Waters), as previously described [17]; the PGE_2 and $PGF_{2\alpha}$ peaks were identified by the co-migration of ^{3}H-standards.

Labeled prostaglandins and arachidonic acid were obtained from Amersham. Anti PGE_2-BSA and anti $PGF_{2\alpha}$-BSA antisera were purchased, respectively, from Institut Pasteur (Paris) and Clinical Assays; the anti 15-keto-13, 14-dihydro $PGF_{2\alpha}$-BSA antiserum was a generous gift from Dr. B. A. Peskar (University of Freiburg, Germany). Bovine gamma globulins grade II were from Sigma; polyethylene glycol 6000 and the various organic solvents were from Merck. Polypropylene tubes, RIALUMA PEG and LIPOLUMA scintillation liquids were purchased from Lumac (Meise, Belgium). Methimazole was obtained from U.C.B. and propylthiouracil from Sanders. Carbimazole was a gift from Nicholas Laboratory. Aminotriazole was a gift of Dr. J. Nunez (Paris). Ionophore A_{23187} was a gift of Dr. Hamill (Eli Lilly). All the prostaglandins were given by Dr. J. Pike (Upjohn).

RESULTS

Methimazole (1 mM) inhibited the release of prostaglandins by dog thyroid slices: the inhibition was 70 ± 17 per cent for PGE_2 (mean ± S.D., 12 experiments) and 63 ± 19 per cent for $PGF_{2\alpha}$ (mean ± S.D., 7 experiments). When this prostaglandin release was stimulated by carbamycholine (50 μM) [7], the extent of the methimazole inhibition was similar: 56 ± 16 per cent for PGE_2 (mean ± S.D., 8 experiments) and 60 ± 16 per cent for $PGF_{2\alpha}$ (mean ± S.D., 6 experiments). The release of PGE_2 and $PGF_{2\alpha}$ by dog thyroid slices stimulated by ionophore A_{23187} and epinephrine were also inhibited (2 experiments) (Fig. 1). Methimazole also depressed the release of PGE_2 and $PGF_{2\alpha}$ by horse and human thyroid slices (not shown).

The release of 15-keto-13, 14-dihydro-$PGF_{2\alpha}$ was depressed to the same degree as that of $PGF_{2\alpha}$, indicating that methimazole was not acting through an activation of prostaglandin degradation (not shown). It was

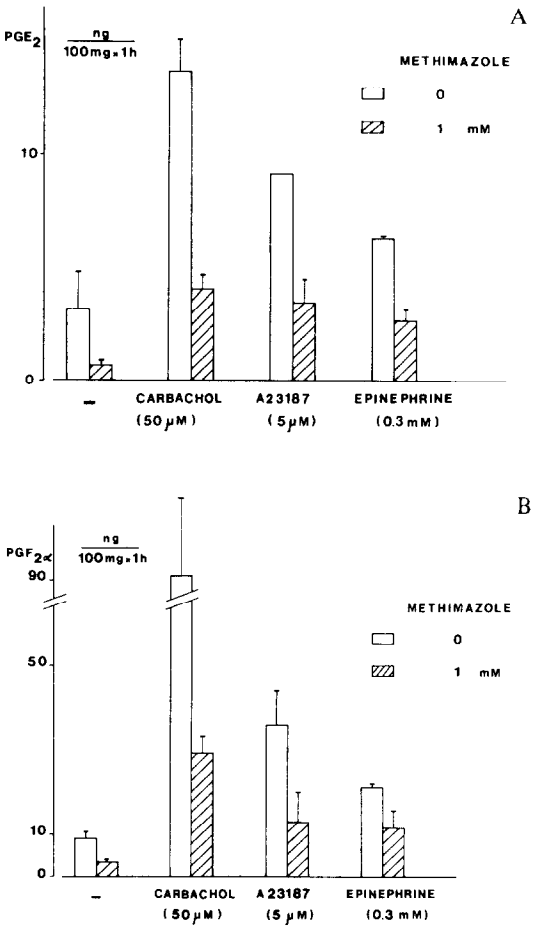

Fig. 1. Inhibition by methimazole of the release of PGF_2 (A) and $PGF_{2\alpha}$ (B) by dog thyroid slices, either in resting conditions or under stimulation by ionophore A_{23187}, carbamylcholine and epinephrine. Results are expressed as the mean ± S.D. of triplicate determinations for one representative experiment.

also confirmed that when known amounts of PGE_2 and $PGF_{2\alpha}$ were dissolved in aliquots of the usual incubation medium with or without methimazole, which were treated in exactly the same conditions as the samples except for the absence of tissue, the recovery was the same, indicating that methimazole *per se* neither chemically modified prostaglandins nor interfered with their assay.

In two experiments the inhibitory effect of methimazole was mimicked by propylthiouracil and carbimazole, but not by imidazole (Table 1). At equimolar concentrations (1 mM), methimazole was the most effective drug and propylthiouracil the least effective. Aminotriazole (1 mM) too was able to inhibit prostaglandin release: inhibitions of 34 and 43 per cent were observed for PGE_2 and $PGF_{2\alpha}$ respectively (3 experiments; not shown).

An inhibitory effect of methimazole was already detectable at 0.1 mM, but the inhibitory potency increased up to 1 mM (4 experiments); higher concentrations were not tested (Fig. 2A). The dose-dependency of the inhibition by methimazole of protein iodination

Table 1. Inhibition by antithyroid drugs of prostaglandin release by dog thyroid slices

ng / 100 mg × 1 hr	PGE_2	$PGF_{2\alpha}$
—	1.9 ± 0.1	4.9 ± 0.6
Methimazole (1 mM)	0.5 ± 0.1	0.5 ± 0.4
PTU (1 mM)	1.1 ± 0.1	2.2 ± 1.0
Carbimazole (1 mM)	0.9 ± 0.1	1.0 ± 0.2
Imidazole (1 mM)	1.6 ± 0.2	4.9 ± 0.5

Mean ± S.D. of triplicate determinations for one representative experiment.

Table 2. Inhibition by methimazole of PGE_2 and $PGF_{2\alpha}$ release by slices of dog renal inner medulla and cortex

ng / 100 mg × 1 hr	PGE_2	$PGF_{2\alpha}$
Dog renal cortex		
—	3.8 ± 0.9	5 ± 1
Methimazole (1 mM)	1.5 ± 0.6	1.0 ± 0.2
A_{23187} (10 μM)	8.1 ± 0.9	9 ± 2
A_{23187} (10 μM) + Methimazole (1mM)	1.8 ± 0.9	2.5 ± 0.5
Dog renal inner medulla		
—	640 ± 230	520 ± 50
Methimazole (1mM)	200 ± 70	130 ± 70
A_{23187} (10 μM)	1330 ± 160	910 ± 130
A_{23187} (10 μM) + Methimazole (1 mM)	290 ± 80	170 ± 80

Mean ± S.D. of triplicate determinations, for one representative experiment.

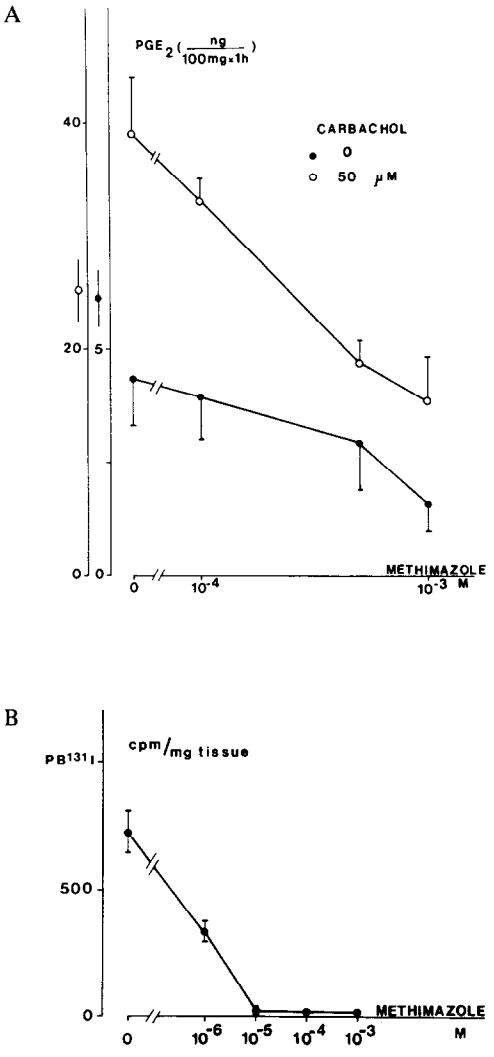

Fig. 2. A. Inhibition by increasing concentrations of methimazole of the release of PGE_2 by dog thyroid slices, either resting or stimulated by carbamylcholine. B. Inhibition by increasing concentrations of methimazole of the incorporation of ^{131}I into proteins of dog thyroid slices. Results are expressed as the mean ± S.D. of triplicates determinations for one representative experiment.

in dog thyroid slices, incubated in similar conditions, was completely different, since a complete inhibition was already observed at 10 μM (3 experiments; Fig. 2B).

The inhibitory effect of methimazole was not restricted to the thyroid tissue. It also depressed the release of both PGE_2 and $PGF_{2\alpha}$ by dog kidney cortex and dog kidney inner medulla (Table 2), either in resting conditions or under stimulation by the ionophore A_{23187} [8] (2 experiments). As shown in Table 2, the kidney cortex released amounts of PGs comparable to the thyroid, whereas this output was much higher in the medulla: the extent of methimazole inhibition was equivalent in both tissues.

The conversion of arachidonic acid in PGE_2 and $PGF_{2\alpha}$ by a dog kidney inner medulla homogenate was inhibited by methimazole (2 experiments; Fig. 3).

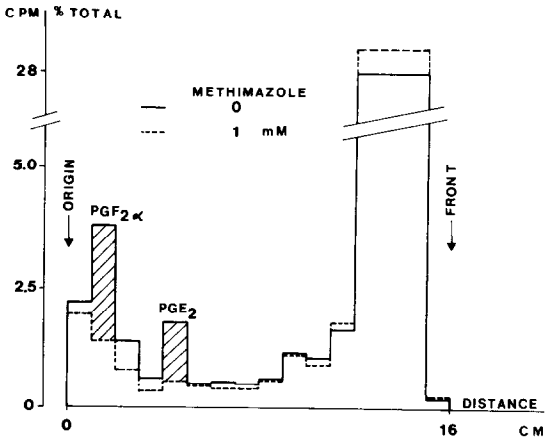

Fig. 3. Thin layer chromatogram obtained after incubation of tritium-labeled arachidonic acid with a dog renal inner medulla homogenate. Results represent the radioactivity in each 1 cm large band as a percentage of the total radioactivity on the plate (mean of duplicate samples). The huge peak near the front corresponds to unreacted arachidonic acid and/or uncharacterized nonpolar derivatives [6]. The shaded areas correspond to peaks migrating in the position of PGE_2 and $PGF_{2\alpha}$, which are depressed in the presence of methimazole.

DISCUSSION

The inhibition by methimazole of prostaglandin release by dog thyroid, kidney cortex and kidney inner medulla could be due either to a blocking of one or the other components of the prostaglandin synthetase or to a decreased availability of precursor arachidonic acid. The fact that methimazole inhibited the conversion to prostaglandins of arachidonic acid by the kidney medulla favours the first hypothesis. The similar inhibition of PGE_2 and $PGF_{2\alpha}$ formation suggests that methimazole acts at an early step in prostaglandin synthesis.

The inhibitory effect of methimazole on prostaglandins E_2 and $F_{2\alpha}$ release is shared by other antithyroid drugs, either chemically related, as carbimazole, or not, as propylthiouracil, but not by the chemical congener imidazole. This action is thus completely different from the selective inhibition by imidazole and derivatives of thromboxane B_2 synthesis [9–12].

O'Brien and Rahimtula [1] have proposed that a peroxidase mechanism is involved in the oxygenation step(s) of prostaglandin biosynthesis. Methimazole and the other antithyroid drugs used in our study are known to inhibit various peroxidases [3]. The large discrepancy between the doses required to inhibit protein iodination and prostaglandin release does not suggest that, in the thyroid, the same peroxidase is involved in iodide oxidation and prostaglandin synthesis, although it has been shown that the potency of peroxidase inhibitors depends on the substrate [2].

Methimazole is often used *in vitro*, as a tool to block iodide oxidation and binding to proteins, in order to selectively study other aspects of thyroid metabolism such as iodide uptake or hormone secretion [13–15]. Millimolar concentrations should no longer be used for this purpose, since they exert an unrelated inhibitory effect on prostaglandin synthesis and 10 μM is sufficient to completely block protein iodination.

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REFERENCES

1. P. J. O'Brien and A. Rahimtula, *Biochem. biophys. Res. Commun.* **70**, 832 (1976).
2. A. Taurog, in *Handbook of Physiology* (Ed. S. R. Geiger) III, 7, 101. Am. Physiol. Soc. (1974).
3. A. Taurog, *Rec. Prog. Horm. Res.* **26**, 189 (1970).
4. A. Danon, L. C. T. Chang, B. J. Sweetman, A. Nies and J. A. Oates, *Biochim. biophys. Acta* **388**, 71 (1975).
5. F. Rodesch, P. Neve, C. Willems and J. E. Dumont, *Eur. J. Biochem* **8**, 26 (1969).
6. F. Sun, *Biochem. biophys. Res. Commun.* **74**, 1432 (1977).
7. M. Waelbroeck and J. M. Boeynaems, *Archs. Int. Physiol. Biochim.* **85**, 1031 (1977).
8. H. W. Knapp, O. Oelz, L. J. Roberts, B. J. Sweetman, J. A. Oates and P. Reed, *Proc. natn. Acad. Sci., U.S.A.* **74**, 4251 (1977).
9. S. Moncada, S. Bunting, K. Mullane, P. Thorogood, J. R. Vane, A. Raz and P. Needleman, *Prostaglandins* **13**, 611 (1977).
10. P. Needleman, A. Raz, J. A. Ferrendelli and M. Minkes, *Proc. natn. Acad. Sci. U.S.A.* **74**, 1716 (1977).
11. V. Diczfalusy and S. Hammarstrom, *FEBS Lett.* **82**, 107 (1977).
12. H. H. Tai and B. Yuan, *Biochem. biophys. Res. Commun.* **80**, 236 (1978).
13. C. Willems, P. A. Rocmans and J. E. Dumont, *Biochim. Biophys. Acta* **222**, 474 (1970).
14. S. Nagataki, in *Handbook of Physiology* (Ed. S. R. Geiger) III, 7, 329. Am. Physiol. Soc. (1974).
15. B. Rapoport, R. J. Adams and M. Rose, *Endocrinology* **100**, 755 (1977).
16. F. Dray, B. Charbonnel and J. Maclouf, *Eur. J. Clin. Invest.* **5**, 311 (1975).
17. A. R. Whorton, M. Smigel, J. A. Oates and J. C. Frölich, *Biochim. biophys. Acta* **529**, 176 (1978).