Br. J. Pharmac. (1977), **59**, 157–162

EFFECT OF ANTI-THYROID AGENTS, METHIMAZOLE AND PROPYL-THIOURACIL, ON BRAIN NORADRENALINE CONTENT

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1 Methimazole (1-methyl-2-mercaptoimidazole, MMI) and propylthiouracil (6-propyl-2-thiouracil, PTU) which are used in the therapy of hyperthyroidism were found to reduce brain noradrenaline (NA) content. Endogenous NA levels in rat brain were reduced from 1 to 6 h after intraperitoneal injection of MMI by doses in excess of 25 mg/kg and by PTU at a dose of 50 mg/kg. However, endogenous NA in the rat heart was only slightly reduced after 50 mg/kg of MMI, and was not affected by PTU (50 mg/kg).

2 Both MMI and PTU effectively inhibited the *in vivo* conversion of [^{3}H]-dopamine into [^{3}H]-noradrenaline ([^{3}H]-NA) in the brain of rats after a single intraperitoneal injection of doses above 10 mg/kg (MMI) and 25 mg/kg (PTU). This inhibition by MMI and PTU was dose-dependent over the range of 10 mg/kg to 50 mg/kg, was highest after 2–3 h and continued for at least 6 h after their injection. The conversion rates returned to normal after 24 hours.

3 The results suggest that the reduction of brain NA by these drugs is, at least in part, due to the inhibition of brain dopamine β-hydroxylase.

Introduction

Methimazole (1-methyl-2-mercaptoimidazole, MMI) and 6-propyl-2-thiouracil (PTU) are potent inhibitors of thyroid iodide peroxidase, which catalyzes the initial step of thyroid hormone biosynthesis (Mahoney & Igo, 1966; Coval & Taurog, 1967) and are used clinically in the therapy of hyperthyroidism.

Various sulphydryl compounds such as mercaptoethanol, cysteine, glutathione and coenzyme A are known to be inhibitors of dopamine β-hydroxylase [3,4-dihydroxyphenylethylamine, ascorbate: oxygen oxidoreductase (hydroxylating), EC 1.14.2.1.] (DBH) which catalyzes the biosynthesis of noradrenaline (NA) from dopamine (Nagatsu, Kuzuya & Hidaka, 1967). As MMI has a sulphydryl group, and PTU also contains sulphur, studies on the effect of the two drugs on catecholamine metabolism *in vivo* were carried out. This paper reports that MMI and PTU effectively reduce brain NA content probably by inhibiting brain DBH *in vivo*.

Methods

Male Wistar rats weighing approximately 150 g were kept at a constant temperature in an environment calculated to be stress-free. The animals were exposed for at least ten days to regular light-dark cycles before starting the experiments. Rats were killed by decapitation; the tissues were quickly removed, weighed and stored at −80°C until biochemically analysed. MMI and PTU were suspended in 0.5% carboxymethylcellulose (CMC) and 0.5 ml of these solutions was injected intraperitoneally into rats. Control rats received an intraperitoneal injection of 0.5 ml of 0.5% CMC. Satistical significance was determined by the use of Student's *t* test (Finney, 1967). [^{3}H]-Dopamine (5–10 Ci/mmol) was purchased from New England Nuclear. All other chemicals were reagent grade.

Noradrenaline determination

NA was isolated by alumina adsorption (Anton & Sayre, 1962) and estimated fluorometrically after ferricyanide oxidation of 0.4 ml of the final acid eluate. NA standards were added to aliquots of tissue and processed in the same way as the test samples. All values were corrected for recoveries of 70% ± 10% s.d. Five rats were used per group.

Dopamine β-hydroxylase assay in vitro

DBH of bovine adrenal gland was purified by ammonium sulphate precipitation, charcoal treatment, calcium phosphate gel treatment, and DEAE-cellulose column chromatography as described by Friedman & Kaufman (1965). The activity of bovine adrenal DBH was determined spectrophotometrically using tyramine as the substrate according to Creveling, Daly, Witkop & Udenfriend (1962), and the activity of rat brain DBH was measured radioisotopically according to Molinoff, Weinshilboum & Axelrod (1971). Rat brains were homogenized with glass homogenizers in 10 volumes of cold tris (hydroxymethyl)aminomethane (Tris) buffer, 0.005 M, pH 7.4, containing Triton X-100, 0.1%. Homogenates were centrifuged at 15,000 *g* for 10 min and portions of the supernatants were used for assay of DBH. DBH activity was not detectable in the pellets. The two stage assay for DBH is based on the conversion of tyramine to octopamine by DBH, followed by N-methylation of the β-hydroxylated product with a ^{14}C-labelled methyl group from S-adenosylmethionine. The second reaction was catalyzed by bovine adrenaal phenylethanolamine *N*-methyltransferase (PNMT), partially purified by the method of Axelrod (1962) by means of ammonium sulphate precipitation and Sephadex G-200 to separate PNMT from DBH. The reaction mixture contained: 200 µl of tissue preparation corresponding to 5 to 50 mg of wet tissue weight, 0.12 µmol of tyramine, and 10 µmol of Tris buffer, pH 6.0. Sufficient catalase (1500 units) and $CuSO_4$ (30 to 80 nmol per tube) were added to give maximal activity.

Conversion of [^{3}H]-dopamine to [^{3}H]-noradrenaline in rat brain

Conversion of [^{3}H]-dopamine to [^{3}H]-NA in rat brain was determined by giving [^{3}H]-dopamine intracisternally as a precursor (Hidaka, Hara, Harada, Hashizume & Yano, 1974). Twenty-five µl of radioisotope solution ([^{3}H]-dopamine, 5 µCi per rat) was given intracisternally; CMC, PTU or MMI were injected (i.p.) at 1, 2, 5, 6 or 24 h before the injection of [^{3}H]-dopamine. Animals were killed 10 min after the administration of [^{3}H]-dopamine. Whole brains were disintegrated with a glass tissue homogenizer in 7.5 ml of 0.4 N perchloric acid solution containing 50 µg of dopamine, NA, normetanephrine, homovanillic acid and 3-methoxytyramine. The tissue extracts were passed over an AG-50 (H^+) column and amines were adsorbed to the resin and were thus separated from the acid and neutral catabolites which passed through in the effluent. The method of Carlsson & Waldeck (1963) was used with an 80 by 5 mm column and a modified elution procedure (Hidaka *et al.*, 1974). When the column was eluted with 15 ml of 0.4 N HCl, both acid and neutral catabolites were found in the first 5 ml eluate as well as in the fractions which immediately followed (i.e., fractionated eluates), each containing 5 ml. The adsorbed amines were eluted with 60 ml of 1 N HCl and the following 20 ml of 2 N HCl. When [^{3}H]-dopamine was used as the precursor, NA was collected in fractions 4 to 6 and dopamine was collected in fractions 8 to 12 (each fraction representing 5 ml). A portion (0.25 ml) of each fraction was transferred to scintillation vials containing 10 ml scintillation fluid, Bray's solution (120 g naphthalene, 0.4 g POPOP, 8 g PPO, 200 ml methanol, 40 ml ethylene glycol, 2 l dioxane). Radioactivity was determined in a liquid scintillation spectrometer (Beckman model LS 233). The efficiency ($24.2 \pm 0.6\%$ tritium) was determined by the use of internal standards. The amounts (nCi) of isolated amines were calculated from determined radioactivity (ct/min) using this efficiency.

Assay of noradrenaline release

The method of Daly, Creveling & Witkop (1966) was employed for this study. Isotonic NaCl solution (0.2 ml) containing 12.5 µCi of [^{3}H]-NA was injected into the tail vein of mice. Drugs were administered intraperitoneally one hour after the radioisotope injection. The mice were killed by a blow on the head 3 h after the radioisotope injection. Hearts were immediately weighed and homogenized in 10 volumes (w/v) of 0.4 N perchloric acid. After centrifugation, 0.25 ml of the supernatant solution was added to 10 ml of Bray's solution, and radioactivity was determined by liquid scintillation counting. At least six mice were used for each group.

Results

Inhibition of dopamine β-hydroxylase in vitro

Bovine adrenal and rat brain DBH were inhibited by MMI and PTU *in vitro*. The inhibitory effect of these drugs is summarized in Table 1. Unlike the sulphydryl compounds reported previously to inhibit DBH (Nagatsu *et al.*, 1967), the inhibition of DBH by MMI and PTU was not reversed by the addition of N-ethylmaleimide (NEM). These results might suggest that inhibition of DBH by MMI and PTU is not due to simple chelation between these compounds and copper in the enzyme.

Effects of methimazole and propylthiouracil on noradrenaline content of rat brain and heart

In Table 2 the effects of MMI on rat brain and heart NA content are shown at various times after

Table 1 Effects of methimazole (MMI) and propylthiouracil (PTU) on bovine adrenal and rat brain dopamine β-hydroxylase (DBH) *in vitro*

Compounds	I_{50}* (m)			
	Without NEM		*With 10 mM NEM***	
	Adrenal	Brain	Adrenal	Brain
MMI	3.7	1.2	3.5	—
PTU	1.5	1.8	1.8	—

* Concentration of drug producing 50% inhibition of DBH activity, as determined graphically. ** N-ethylmaleimide (NEM) was not added to the brain DBH assay system because the compound inhibited phenylethylamine-*N*-methyltransferase (PNMT) activity. PTU and MMI did not affect the activity of PNMT.

Table 2 Effects of methimazole (i.p.) on noradrenaline content (μg/g) of rat brain and heart

Tissue	*Dose (mg/kg)*	*Time post drug (h)* 1	2	6	24
			Noradrenaline (μg/g)		
Brain	Control	0.40 ± 0.02	0.41 ± 0.01	0.40 ± 0.02	0.43 ± 0.03
	10	0.34 ± 0.01	0.36 ± 0.02	0.37 ± 0.01	0.40 ± 0.01
	25	0.30 ± 0.01*,**	0.31 ± 0.01*,**	0.35 ± 0.01	0.44 ± 0.02
	50	0.27 ± 0.01*,**	0.27 ± 0.01*,**	0.30 ± 0.01*,**	0.43 ± 0.02
Heart	Control	1.10 ± 0.07	1.05 ± 0.09	1.11 ± 0.08	1.24 ± 0.12
	10	0.92 ± 0.05	1.00 ± 0.05	1.14 ± 0.09	1.13 ± 0.03
	25	0.96 ± 0.07	0.80 ± 0.06	0.97 ± 0.05	1.04 ± 0.05
	50	0.79 ± 0.05*	0.89 ± 0.11	0.93 ± 0.05	0.89 ± 0.05

Five rats were used for each experiment and values are shown as mean ± s.e.
* Significantly different from control ($P < 0.05$); ** Significantly different from the value at 24 h ($P < 0.05$).

Table 3 Effects of propylthiouracil i.p. on noradrenaline content (μg/g) of rat brain and heart

Tissue	*Dose (mg/kg)*	*Time post drug (h)* 2	6	24
			Noradrenaline (μg/g)	
Brain	Control	0.46 ± 0.01	0.47 ± 0.01	0.44 ± 0.01
	25	0.42 ± 0.01	0.44 ± 0.01	0.43 ± 0.02
	50	0.35 ± 0.01*,**	0.38 ± 0.01*	0.43 ± 0.01
Heart	Control	0.96 ± 0.05	0.99 ± 0.09	0.97 ± 0.04
	25	0.89 ± 0.04	0.99 ± 0.05	0.98 ± 0.06
	50	0.99 ± 0.03	0.97 ± 0.08	0.96 ± 0.05

Five rats were used for each experiment and values are shown as mean ± s.e.
* Significantly different from control ($P < 0.05$); ** Significantly different from the value at 24 h ($P < 0.05$).

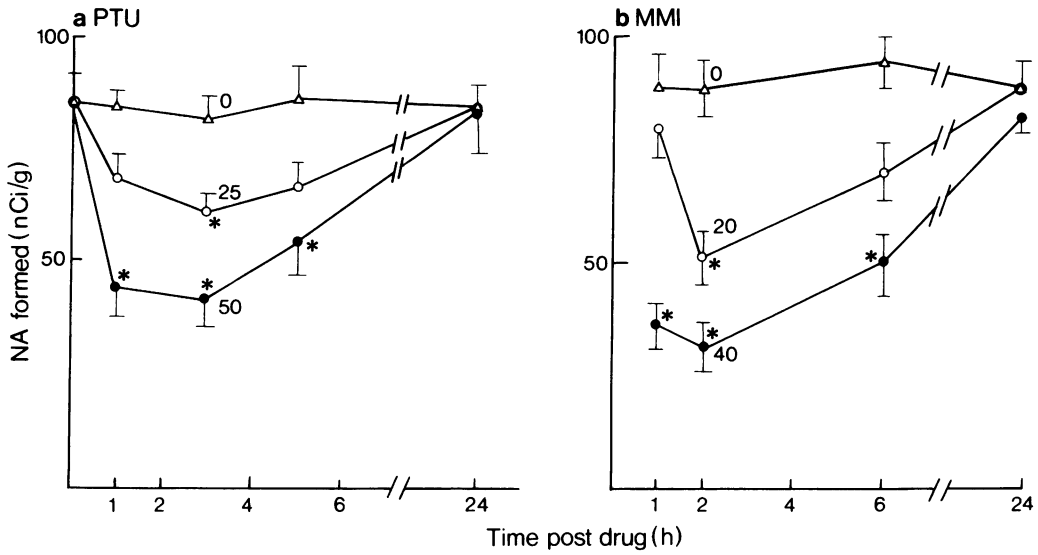

Figure 1 Effects of methimazole (MMI) and propylthiouracil (PTU) on the conversion of [^{3}H]-dopamine into [^{3}H]-noradrenaline ([^{3}H]-NA) in rat brain as a function of time. Five rats were used for each experiment. Drugs were injected intraperitoneally; 5 μCi of [^{3}H]-dopamine per rat (25 μl) was injected intracisternally at the time indicated after drug injection. The brain was removed 10 min after [^{3}H]-dopamine injection. NA was isolated as described in the Methods section. Indicated values (nCi) were calculated from ct/min of NA isolated (efficiency 24%). Five determinations were averaged. Vertical bars indicated standard errors. (△) Control; (a) PTU: (○) 25 and (●) 50 mg/kg; (b) MMI (○) 20 and (●) 40 mg/kg. * Significantly different from the control and the value at 24 h ($P < 0.05$).

intraperitoneal injection of different doses of MMI. Brain NA was significantly reduced at doses of 25 and 50 mg/kg. Maximum reduction of brain NA occurred 1–2 h after the drug was given. Heart NA decreased significantly 1 h after MMI only at a dose of 50 mg/kg and the effect of MMI on heart NA was significantly weaker than that of the drug on brain NA. Table 3 summarizes the effects of PTU on NA in rat brain and heart. Maximum reduction of brain NA was observed 2 h after this drug. Heart NA did not decrease significantly. PTU proved to be more toxic than MMI, the intraperitoneal injection of more than 100 mg/kg of PTU killing most of the animals. Accordingly experiments with PTU were performed using doses of

Table 4 Effects of methimazole and propylthiouracil on the release of [^{3}H]-noradrenaline ([^{3}H]-NA) from mouse heart

Drug	*Dose (mg/kg)*	*No. of animals*	ct min^{-1} g^{-1} x 10^{-4}
Methimazole	25	7	22.1 ± 1.4
	50	7	19.2 ± 1.0
	100	7	17.6 ± 2.0
Polythiouracil	25	7	16.8 ± 2.6
	50	7	17.1 ± 2.2
Reserpine	1	6	4.1 ± 0.3*
	5	6	1.8 ± 0.2*
Control (saline)		10	20.8 ± 0.9

[^{3}H]-NA (12.5 μCi) was injected into the tail vein of mice. Drugs were administered (i.p.) 1 h after radioisotope injection. The mice were killed 3 h after the radioisotope injection. The hearts were homogenized with 10 volumes of 0.4 N perchloric acid. Values are presented as ct min^{-1} g^{-1} ± s.e.
* Significantly different from control ($P < 0.01$)

Table 5 Effect of methimazole on the conversion of intracisternally injected [^{3}H]-dopamine into [^{3}H]-noradrenaline ([^{3}H]-NA) in rat brain

Drug	Dose (mg/kg)	No. of animals	Total radioactivity % injected dose	Total radioactivity nCi/g	[^{3}H]-NA nCi/g	[^{3}H]-dopamine nCi/g
Control		10	8.0	399 ± 3.9	88.7 ± 7.0	181 ± 11
Methimazole	10	5	8.0	400 ± 4.4	50.4 ± 5.7*	239 ± 15
	40	5	7.7	384 ± 15	32.9 ± 4.2*	232 ± 11
	100	5	7.6	378 ± 18	27.7 ± 3.5**	231 ± 12
	250	5	8.2	408 ± 17	26.0 ± 3.1**	214 ± 10

Drug was administered (i.p.) to rats 2 h before intracisternal injection of [^{3}H]-dopamine (10 μCi/rat). Rats were killed by decapitation 10 min after [^{3}H]-dopamine and brains were removed. [^{3}H]-NA and [^{3}H]-dopamine were isolated as described in the Methods section. Values are expressed as mean ± s.e. and calculated from ct/min of [^{3}H]-dopamine or NA (efficiency: 24%).

50 mg/kg or less. None of the rats treated with an intraperitoneal injection of 25 or 50 mg/kg of PTU died, at least, not during the first 24 hours. Effects of MMI and PTU on NA release from mouse heart were also examined. Neither MMI nor PTU caused a significant release of NA at doses of 25, 50 or 100 mg/kg. Under the same conditions reserpine was found to release NA (Table 4).

Effects of methimazole and propylthiouracil on the in vivo *conversion of [^{3}H]-dopamine into [^{3}H]-noradrenaline*

As shown in Table 5, the reduced formation of [^{3}H]-NA from [^{3}H]-dopamine in rat brain by MMI was dose-dependent over the range of 10–40 mg/kg. *In vivo* inhibition of DBH by MMI and PTU in rat brain was studied by following the time course of the reduction of [^{3}H]-NA formation at various times after drug administration (Figure 1). The biosynthesis of NA from dopamine decreased rapidly, and maximum inhibition of NA biosynthesis in the brain was attained after 2 hours. This reduced biosynthesis of NA had returned to control levels after 24 hours.

Discussion

MMI and PTU are used clinically in the therapy of hyperthyroidism and are also known to be inhibitors of thyroid iodide peroxidase. Both MMI and PTU inhibit DBH *in vitro*. The concentrations of MMI and PTU producing 50% inhibition of adrenal DBH *in vitro* were 3.7 and 1.5 mM respectively. Hidaka, Asano & Takemoto (1973) have reported that fusaric acid and its derivatives produce 50% inhibition of DBH *in vitro* at a concentration of 0.1 μM. In spite of the relatively weak *in vitro* inhibitory activity of MMI and PTU, they significantly inhibited *in vivo* conversion of [^{3}H]-dopamine to [^{3}H]-NA in doses similar to those for fusaric acid and its derivatives (Hidaka *et al.*, 1974). The *in vivo* inhibition of the conversion of dopamine to NA by MMI and PTU indicates that they inhibited DBH. Intraperitoneal injection of MMI (above 25 mg/kg) and PTU (50 mg/kg) significantly reduced the brain noradrenaline content *in vivo*. However, the results shown in Table 2 indicate that they do not cause this reduction by virtue of a reserpine-like effect. There is a possibility that the decreased biosynthesis of thyroid hormone by the compounds indirectly affects brain NA content, because there are reports that myocardial NA is altered by both hypothyroidism and hyperthyroidism (Goodall, 1951; Goodkind, Farm & Roberts, 1961; Gordon, Reid, Sjoerdsma & Udenfriend, 1966). However, this possibility is unlikely because PTU did not affect myocardial NA and MMI also had a much weaker effect on the myocardium than it did on the brain. This leaves the conclusion that the effect in high doses is exerted by their action on DBH but the problem of why brain NA is affected so very much more than heart NA remains.

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(Received June 16, 1976. Revised August 29, 1976.)