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INHIBITION OF THE METABOLISM OF H³-MELATONIN BY PHENOTHIAZINES[1]

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ABSTRACT

WURTMAN, RICHARD J., JULIUS AXELROD AND FERNANDO ANTON-TAY: Inhibition of the metabolism of H³-melatonin by phenothiazines. J. Pharmacol. Exp. Therap. **161:** 367–372, 1968. After rats are treated acutely with chlorpromazine, there is an elevation in the level of i.v. administered H³-melatonin in brain and blood. This effect is not the result of the hypothermia produced by chlorpromazine. Other phenothiazines, such as promazine and promethazine, also elevate tissue H³-melatonin. Chlorpromazine has no effect on the level of H³-melatonin when the indole is administered to rats by injection into the lateral cerebral ventricle. Chlorpromazine inhibits the *in vitro* metabolism of H³-melatonin by liver slices, suggesting that the drug alters the tissue levels of the indole by slowing its metabolism. Chronic treatment with phenobarbital causes a decreased H³-melatonin concentration in the brain 30 min after i.v. injection of the indole.

Melatonin (5-methoxy-N-acetyltryptamine) is secreted from the mammalian pineal gland as a hormone (Wurtman *et al.*, 1963; Barchas and Lerner, 1964). It appears to act on the brain (Martini *et al.*, 1968) and perhaps at other loci to produce changes in the functional activity of the gonads (Chu *et al.*, 1964), the pituitary (Clementi *et al.*, 1966) and the thyroid (Baschieri *et al.*, 1963). The physiologic disposition of circulating H³-melatonin has been studied in the cat (Kopin *et al.*, 1961) and the rat (Wurtman *et al.*, 1964b). The indole disappears from the blood with a very short half-life (Kopin *et al.*, 1961). Small amounts are taken up and retained in the tissues, especially in endocrine and nervous structures (Wurtman *et al.*, 1964b); however, most of the circulating H³-melatonin is rapidly transformed in the liver by 6-hydroxylation. The 6-hydroxymelatonin is then conjugated with sulfuric or glucuronic acids (Kopin *et al.*, 1961) and is excreted into the urine.

It has recently been demonstrated that the treatment of rats with chlorpromazine delays the disappearance of isotopically labeled melatonin from their blood and tissues (Wurtman and Axelrod, 1966). This report describes the temporal and dose-response relations of the effects on melatonin metabolism produced by chlorpromazine and related drugs. Evidence is presented that chlorpromazine acts by inhibiting the metabolism of melatonin in the liver.

MATERIALS AND METHODS. Tritiated melatonin with a specific activity of 200 mc/mmol was prepared as described before (Kopin *et al.*, 1961), by reacting methoxytryptamine with H³-acetic anhydride (New England Nuclear Corp., Boston, Mass.) in the presence of ethyl acetate and triethylamine. Sprague-Dawley female rats, weighing 160 to 200 g, received 3 to 6 μc (3.5–7.0 μg; 0.1–0.2 ml) of labeled indole by injection into a tail vein, and were killed 30 min later. In some experiments, 0.2 to 0.5 μc of H³-melatonin was injected into the right lateral cerebral ventricle using the method described by Noble *et al.* (1967), and the animals were killed 1 hr later. Tissues and blood obtained by cardiac or vena caval punc-

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TABLE 1

Effect of time of chlorpromazine injection on H^3-melatonin concentrations in brain and blood

Groups of five rats were given 20 mg/kg of chlorpromazine i.p. and 6.0 μc (7.0 μg) of H^3-melatonin i.v. Control animals received no chlorpromazine. They were killed 30 min after receiving the melatonin. All data are presented as mean ± S.E.

Time of Injection of Chlorpromazine	H^3-Melatonin Content	
	Brain	Blood
	mμc/g	
Control	1.14 ± 0.08	1.33 ± 0.75
100 min before H^3-melatonin	3.25 ± 0.62[a]	5.55 ± 0.75[a]
30 min before H^3-melatonin	2.23 ± 0.10[b]	3.45 ± 0.25[a]
Same time as H^3-melatonin	2.70 ± 0.35[b]	3.77 ± 0.25[a]
10 min after H^3-melatonin	1.87 ± 0.24[c]	2.79 ± 0.36[b]

[a] $P < .001$ differs from control.
[b] $P < .01$ differs from control.
[c] $P < .05$ differs from control.

ture were rapidly removed and were homogenized with 3 volumes of cold perchloric acid (0.4 N). The unchanged H^3-melatonin was then extracted into 6 ml of chloroform. The organic phase was washed with 2 ml of water, and a 4-ml aliquot was taken to dryness and its radioactivity measured in a liquid scintillation spectrophotometer (Wurtman *et al.*, 1964b).

The identity of the radioactive material extracted from blood or tissues was confirmed by co-chromatography with authentic melatonin in three systems: butanol–acetic acid–water (8:2:2; ascending paper); isopropanol–ammonium hydroxide–water (8:2:2; ascending paper); and chloroform–methanol (93:7; thin-layer).

All drugs were dissolved in water and injected i.p. in a final volume of 0.2 ml; except where indicated, each experimental treatment group of animals contained six to eight rats. The data were analyzed statistically by means of a *t* test.

RESULTS. *Relation between time that chlorpromazine is administered and its effect on metabolism of H^3-melatonin.* Groups of rats were given chlorpromazine (20 mg/kg) 100 or 30 min before receiving H^3-melatonin, at the same time as the indole or 10 min after its administration. Control animals were injected with an equivalent volume of saline instead of the chlorpromazine. All of the animals were killed 30 min after receiving the H^3-melatonin. At all times examined, the phenothiazine slowed the disappearance of H^3-melatonin from the brain and the blood (table 1). The effect of the chlorpromazine was greatest among the animals which were treated 100 min before the injection of H^3-melatonin. This suggests that the biochemical mechanism by which chlorpromazine acts to delay the disappearance of melatonin is not instantaneous.

Effect of various doses of chlorpromazine on the fate of circulating H^3-melatonin. Groups of animals received a single injection of the phenothiazine in doses ranging from 0.6 to 20 mg/kg 30 min before the H^3-melatonin was administered. They were killed 30 min later, and their brains and blood were assayed for the labeled indole. The smallest dose of chlorpromazine which altered the concentration of the pineal indole in brain or blood was 6.0 mg/kg (fig. 1).

Relation between body temperature and the effect of chlorpromazine on the metabolism of H^3-melatonin. Some of the reported effects of phenothiazines on the metabolism of brain amines appear to be secondary to the hypothermia which these drugs induce (Costa *et al.*, 1962; Gey and Pletscher, 1964). To examine the possibility that a similar mechanism was responsible for the elevated levels of H^3-melatonin in chlorpromazine-treated animals, the effect of the drug was examined in rats kept normothermic by being housed in an incubator heated to 34°C. It had previously been demonstrated that this ambient temperature is adequate to maintain normothermia in rats treated with 20 mg/kg of chlorpromazine (Bartlet, 1965).

Thirty minutes after H^3-melatonin was administered, its content in brain was lower among animals kept at 34°C for the duration of the experiment than in animals kept at room temperature (20°C). This effect was observed both in control animals and in rats treated with chlorpromazine (table 2). However, chlorpromazine treatment continued to exert an action on melatonin metabolism which was independent of temperature: brain H^3-melatonin levels were elevated both in animals kept at room temperature and in rats kept at 34°C (table 2). This indicates that the change in tissue H^3-melatonin concentration which follows chlorpromazine treatment is not the result of hypothermia.

Effects of phenothiazines and other drugs on the levels of H^3-melatonin in blood and tissue. Rats were treated with various phenothiazines, imipramine, desmethylimipramine or amphet-

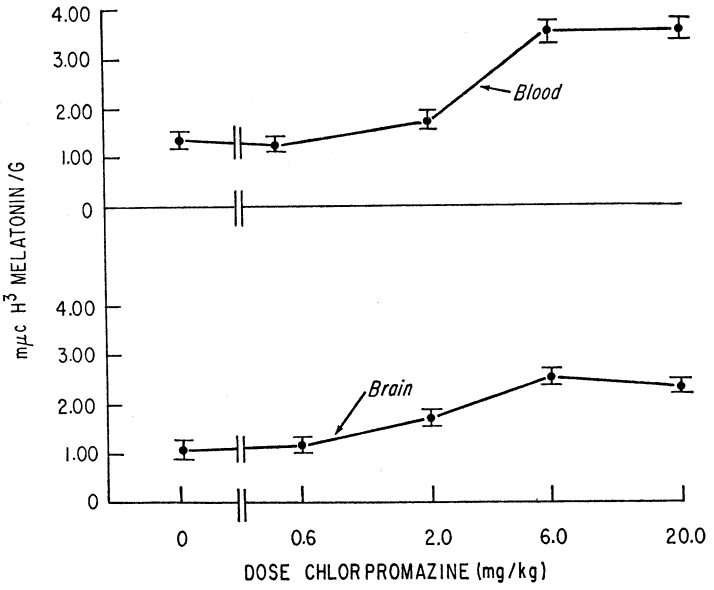

FIG. 1. Groups of six to eight rats were treated with various doses of chlorpromazine 30 min before receiving H^3-melatonin (4.5 μc) i.v. The animals were killed 30 min later, and the brain and blood were assayed for H^3-melatonin. Horizontal bars represent S.E.M.

TABLE 2

Effect of ambient temperature on brain H^3-melatonin content in chlorpromazine-treated rats

Groups of six rats were given chlorpromazine (20 mg/kg) or its diluent and were kept at room temperature or in a chamber heated at 34°C. Thirty minutes later, they were given H^3-melatonin (6 μc) i.v., and were killed 30 min later.

Group	Brain H^3-Melatonin at	
	20°C	34°C
	mμc/g	
Control	3.30 ± 0.55	1.82 ± 0.36[a]
Chlorpromazine-treated	7.60 ± 0.15[b]	5.70 ± 0.40[c]

[a] $P < .05$ differs from untreated control (at 20°C).

[b] $P < .01$ differs from untreated control (at 20°C).

[c] $P < .01$ differs from untreated control (at 34°C), and $P < .05$ differs from chlorpromazine-treated rats kept at 20°C.

amine 30 min before receiving H^3-melatonin. They were killed 30 min later, and the H^3-melatonin contents of brain and blood were determined. All of the phenothiazines tested

TABLE 3

Effect of phenothiazines and other drugs on the levels of H^3-melatonin in blood and tissue

Groups of five or six rats were given drugs i.p. 30 min before receiving 3.5 μc of H^3-melatonin i.v. They were killed 30 min later, and the brain and blood were assayed for H^3-melatonin.

Drug	Dose	H^3-Melatonin Content	
		Brain	Blood
	mg/kg	*mμc/g*	
Control		1.9 ± 0.28	0.9 ± 0.21
Chlorpromazine	20	5.0 ± 0.68[a]	1.6 ± 0.21[b]
Imipramine	20	3.7 ± 0.47[c]	0.5 ± 0.08
Desmethylimipramine	20	1.7 ± 0.35	1.3 ± 0.16
Promethazine	20	3.4 ± 0.33[c]	3.6 ± 0.29[a]
Promazine	20	3.0 ± 0.27[c]	3.2 ± 0.48[c]
Amphetamine	10	2.1 ± 0.19	1.2 ± 0.05

[a] $P < .001$ differs from control.

[b] $P < .05$ differs from control.

[c] $P < .01$ differs from control.

(*i.e.*, chlorpromazine, promethazine and promazine) elevated the concentrations of H^3-melatonin in brain and blood (table 3). Imipramine treatment raised the H^3-melatonin level in brain, but had no effect on blood levels.

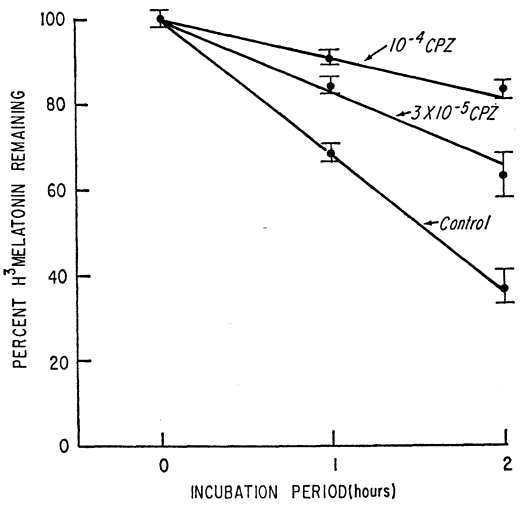

FIG. 2. Effect of chlorpromazine on metabolism of H^3-melatonin by liver slices. Liver slices (200 mg) were incubated at 37°C with 0.5 μc of H^3-melatonin and chlorpromazine (3×10^{-5} M or 10^{-4} M) in 3 ml of Krebs-Ringer phosphate buffer, in an atmosphere of 95% oxygen–5% carbon dioxide. After 1 or 2 hr of incubation, the contents of the flasks were homogenized with 0.4 N perchloric acid, and the H^3-melatonin remaining was extracted with chloroform. Each point represents the mean of five flasks; horizontal bars represent the S.E.M.

TABLE 4

Effect of phenobarbital on the levels of H^3-melatonin in blood and tissue

Groups of five rats were treated with phenobarbital (50 mg/kg/day i.p.) or its diluent for 6 days before receiving H^3-melatonin (3 μc) i.v.

Group	H^3-Melatonin Content	
	Brain	Blood
	mμc/g	
Control	1.08 ± 0.17	1.90 ± 0 49
Phenobarbital-treated	0.52 ± 0.15[a]	1.01 ± 0.77

[a] $P < .05$ differs from control.

Desmethylimipramine and amphetamine caused no changes in H^3-melatonin concentration in either tissue.

Effects of chlorpromazine on metabolism of H^3-melatonin by liver slices. To determine whether the effect of chlorpromazine on the physiologic disposition of H^3-melatonin was the result of inhibition of the metabolism of the indole in the liver, studies were performed on the *in vitro* transformation of H^3-melatonin by liver slices. Sections of fresh rat livers (weighing approximately 200 mg) were prepared with a Stadie Riggs knife. They were incubated in 50-ml Erlenmeyer flasks containing Krebs-Ringer phosphate buffer and 0.5 μc of H^3-melatonin. The flasks were gassed with 95% oxygen–5% carbon dioxide and shaken at 37°C in a Dubnoff metabolic incubator. Chlorpromazine was present in some of the flasks in concentrations of 3×10^{-5} M or 10^{-4} M. After 1 or 2 hr, aliquots of the incubation medium were removed and assayed for their content of unchanged H^3-melatonin by extraction with chloroform.

The disappearance of H^3-melatonin was linear with time in control flasks, as well as in those to which chlorpromazine had been added. The phenothiazine caused a marked decrease in the rate at which melatonin was metabolized *in vitro*; this effect was dose-related (fig. 2).

Effect of phenobarbital treatment on the disappearance of circulating H^3-melatonin. Groups of rats were treated for 6 days with daily doses of phenobarbital (50 mg/kg); control animals received isotonic saline solution. At the end of this period, H^3-melatonin was administered i.v., and its concentration was measured 30 min later in brain and blood from control and treated animals. Rats treated chronically with the barbiturate had significantly lower levels of the radioactive indole in the brain than did control animals (table 4).

Effect of chlorpromazine on disappearance of intraventricularly administered H^3-melatonin from brain. If the action of chlorpromazine in elevating the levels of H^3-melatonin in blood and tissues resulted from an effect on the metabolism of the circulating indole in the liver, it might be anticipated that chlorpromazine would have little or no effect on the disappearance of H^3-melatonin from the brain when the indole was administered intraventricularly, instead of i.v. To examine this possibility, very small amounts of H^3-melatonin were injected into the right lateral ventricles of rats lightly anesthesized with ether (Noble *et al.*, 1967). The distribution of labeled melatonin was found to be similar in the injected and uninjected sides of the brain (table 5); this was taken as evidence that brain H^3-melatonin levels represented uptake into specific sites and were not simply the consequence of nonspecific adsorp-

tion onto the walls of the right lateral ventricle. Pretreatment with chlorpromazine was found to have no effect on the content of H^3-melatonin in brain at 20, 60 (table 6) or 120 min after the indole was administered by the intraventricular route.

DISCUSSION. These data indicate that the ability of chlorpromazine to elevate the levels of H^3-melatonin in blood and tissues is characteristic of phenothiazines, and is not simply a consequence of the hypothermia which accompanies administration of these compounds. The observations that chlorpromazine requires some time to elapse before it is maximally effective, that the disappearance of intraventricularly administered H^3-melatonin from brain is not prolonged by the phenothiazine and that chlorpromazine also inhibits the metabolism of the pineal indole *in vitro* suggest that the drug elevates H^3-melatonin levels by slowing the rate at which the indole is metabolized in the body. It seems likely that the site at which chlorpromazine acts to produce this effect involves the 6-hydroxylation of melatonin within the liver, since this enzymatic reaction accounts for the metabolism of most of the circulating H^3-melatonin in cats and rats (Kopin *et al.*, 1961; Taborsky *et al.*, 1965). This hypothesis is also supported by the observation that treatment with phenobarbital, a drug which increases the activity of many hepatic microsomal hydroxylases (Remmer, 1959; Conney *et al.*, 1960), decreases the concentration of H^3-melatonin found in the brain. It is possible, of course, that chlorpromazine might also alter the fate of melatonin by additional mechanisms not tested in this study (*i.e.*, by modifying the size of the metabolic compartments in which the indole is distributed).

Melatonin is produced in man (Wurtman *et al.*, 1964a), and is secreted into the blood as a hormone. Hence, the effects of chlorpromazine described here on melatonin metabolism in the rat may have counterparts in the human. It would of interest to determine whether patients who have been treated chronically with phenothiazines store large amounts of melatonin in their tissues, or whether they excrete measurable quantities of the indole into their urine. It is possible, of course, that chronic treatment with chlorpromazine might have effects on melatonin metabolism which are different from those of the single doses used here. Melatonin and chlorpromazine both produce functional changes in a variety of tissues, including the brain (Roldan *et al.*, 1964; Fink, 1960), pigment cells (McGuire and Moller, 1966; Epstein *et al.*, 1957) and certain endocrine organs (Wurtman *et al.*, 1963; Barraclough, 1956; De Wied, 1967). It is possible that some of the pharmacologic effects observed after chronic treatment with chlorpromazine are related to changes in the metabolism of circulating melatonin.

TABLE 5

Distribution of H^3-melatonin administered intraventricularly

Rats received 0.2 μc of H^3-melatonin in the right lateral ventricle. One hour later they were killed and their brains were assayed for H^3-melatonin.

Rat No.	H^3-Melatonin Content of Brain	
	Right side	Left side
	mμc/g	
1	2.9	2.8
2	2.5	2.8
3	2.5	2.5

TABLE 6

Effect of chlorpromazine on the fate of H^3-melatonin administered intraventricularly

Groups of four rats received 0.5 μc of H^3-melatonin in the right lateral ventricle 1 hr after the administration of chlorpromazine (6 mg/kg i.p.) or its diluent. They were killed 1 hr after H^3-melatonin administration and the left side of the brain was assayed for H^3-melatonin.

Group	H^3-Melatonin
	mμc/g
Control	5.1 ± 0.8
Chlorpromazine-treated	5.1 ± 0.8

REFERENCES

BARCHAS, J. D. AND LERNER, A. B.: Localization of melatonin in the nervous system. J. Neurochem. **11:** 489–491, 1964.

BARRACLOUGH, C. A.: Blockade of the release of pituitary gonadotrophin by chlorpromazine. Anat. Rec. **124:** 255, 1956.

BARTLET, A. L.: Influence of chlorpromazine on the metabolism of 5-hydroxytryptamine in the mouse. Brit. J. Pharmacol. Chemotherap. **24:** 497–509, 1965.

BASCHIERI, L., DELUCA, F., CRAMAROSSA, L., DEMARTINO, C., OLIVERIO, A. AND NEGRI, M.: Modifica-

tions of thyroid activity by melatonin. Experientia **19:** 15–17, 1963.

CHU, E. W., WURTMAN, R. J. AND AXELROD, J.: An inhibitory effect of melatonin on the estrous phase of the estrous cycle of the rodent. Endocrinology **75:** 238–242, 1964.

CLEMENTI, F., DE VIRGILLIS, G., FRASCHINI, F. AND MESS, B.: Modifications of pituitary morphology following pinealectomy and the implantation of the pineal body in different areas of the brain. *In* Abstract of Sixth International Congress of Electron Microscopy, 1966.

CONNEY, A. H., DAVIDSON, C., GASTEL, R. AND BURNS, J. J.: Adaptive increases in drug-metabolizing enzymes induced by phenobarbital and other drugs. J. Pharmacol. Exp. Therap. **130:** 1–8, 1960.

COSTA, E., GESSA, G. L. AND BRODIE, B. B.: Influence of hypothermia on chlorpromazine-induced changes in brain amine levels. Life Sci. **1:** 315–319, 1962.

DE WIED, D.: Chlorpromazine and endocrine function. Pharmacol. Rev. **19:** 251–288, 1967.

EPSTEIN, J. H., BRUNSTING, L. A., PETERSEN, M. C. AND SCHWARTZ, B. E.: A study of photosensitivity occurring with chlorpromazine therapy. J. Invest. Dermatol. **28:** 329–338, 1957.

FINK, M.: Effect of anticholinergic compounds on post-conclusive electroencephalogram and behavior of psychiatric patients. Electroencephalogr. Clin. Neurophysiol. **12:** 359–369, 1960.

GEY, R. F. AND PLETSCHER, A.: Effects of chlorpromazine on the metabolism of *dl*-2-C^{14}-dopa in the rat. J. Pharmacol. Exp. Therap. **145:** 337–343, 1964.

KOPIN, I. J., PARE, C. M. B., AXELROD, J. AND WEISSBACH, H.: The fate of melatonin in animals. J. Biol. Chem. **236:** 3072–3075, 1961.

MARTINI, L., FRASCHINI, F. AND MOTTA, M.: Neuroendocrine control of anterior pituitary activity. Recent Progr. Hormone Res., in press, 1968.

MCGUIRE, J. AND MOLLER, H.: Differential responsiveness of dermal and epidermal melanocytes of Rana pipiens to hormones. Endocrinology **78:** 367–372, 1966.

NOBLE, E. P., WURTMAN, R. J. AND AXELROD, J.: A simple and rapid method for injecting H^3-norepinephrine into the lateral ventricle of the rat brain. Life Sci. **6:** 281–291, 1967.

REMMER, H.: Der beschleunigte Abbau von Pharmaka in den Lebermikrosomen unter dem Einfluß von Luminal. Arch. Exp. Pathol. Pharmakol. **235:** 279–290, 1959.

ROLDAN, E., ANTON-TAY, F. AND ESCOBAR, S.: Studies on the pineal gland. IV. Effects on the electroencephalogram. Bol. Inst. Estud. Méd. Biol. Univ. Nac. Méx. **22:** 145–150, 1964.

TABORSKY, R. G., DELVIGS, P. AND PAGE, I. H.: 6-Hydroxyindoles and the metabolism of melatonin J. Med. Chem. **8:** 855–858, 1965.

WURTMAN, R. J. AND AXELROD, J.: Effect of chlorpromazine and other drugs on the disposition of circulating melatonin. Nature (London) **212:** 312, 1966.

WURTMAN, R. J., AXELROD, J. AND BARCHAS, J.: Age and enzyme activity in the human pineal. J. Clin. Endocrinol. Metab. **24:** 299–301, 1964a.

WURTMAN, R. J., AXELROD, J. AND CHU, E. W.: Melatonin, a pineal substance: Effect on the rat ovary. Science (Wash.) **141:** 277–278, 1963.

WURTMAN, R. J., AXELROD, J. AND POTTER, L. T.: The uptake of H^3-melatonin in endocrine and nervous tissues and the effects of constant light exposure. J. Pharmacol. Exp. Therap. **143:** 314–318, 1964b.