Lindane may enhance nocturnal pineal N-acetyltransferase activity via β-adrenergic receptors

A.M. Attia[1,*], B.A. Richardson[1,4], C. Rodriguez[1], M.H. Mostafa[2], S.A. Soliman[2], A.H. El-Sebae[3] and R.J. Reiter[1]

[1]*Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78284-7762 (U.S.A.),* [2]*Department of Environmental Science, Institute of Graduate Studies and Research, Alexandria, (Egypt),* [3]*Department of Pesticide Chemistry, Faculty of Agriculture, University of Alexandria, Alexandria (Egypt) and* [4]*Department of Biology, University of Michigan-Flint, Flint, MI 48502 (U.S.A.)*

Lindane, a chlorinated hydrocarbon pesticide, was previously shown to enhance the nighttime rise in pineal N-acetyltransferase (NAT) activity and melatonin as well as serum melatonin levels. The purpose of the present study was to test whether lindane acts on the pineal gland by means of a β-adrenergic receptor mechanism. Whereas lindane (total dose 17.8 mg/kg b.wt. over 6 days) by itself significantly augmented the nocturnal levels of pineal NAT activity in otherwise untreated rats, the pesticide was ineffective in reference to this enzyme when it was given in conjunction with the β-adrenergic receptor antagonist propranolol (20 mg/kg b.wt., one hour before lights off). The augmentation of NAT activity by lindane also caused significant reductions in pineal serotonin (5-HT) and 5-hydroxyindole acetic acid (5-HIAA); again, both these responses were blocked by propranolol treatment. Neither pineal 5-hydroxytryptophan nor pineal or serum melatonin levels were significantly changed as a result of either lindane or propranolol treatment. The results are consistent with the idea that lindane influences pineal 5-HT metabolism either at the level of the β-adrenergic receptor or via the sympathetic innervation to the pineal gland.

INTRODUCTION

Lindane, an organochlorine pesticide, has been shown to enhance pineal serotonin metabolism by increasing nocturnal levels of serotonin N-acetyltransferase[4]. Another pesticide, DDT, is without a significant effect on pineal melatonin production although both lindane and DDT significantly stimulate circulating catecholamines[4]. Whereas there is strong experimental evidence that pesticides also alter brain neurotransmitter metabolism, there is some disagreement on the specific nature of these changes. Thus, subconvulsant doses of lindane reportedly induce increases in serotonin (5-HT), 5-hydroxyindole acetic acid (5-HIAA) and dopamine (DA) in the dorsal raphe neurons and nerve endings of the frontal cortex and substantia nigra[1,2], while in another report 5-HT and 5-HIAA were decreased in the colliculi, striatum and frontal cortex[23].

Melatonin is synthesized in the pineal gland by the conversion of tryptophan to 5-HT which is then acetylated to form N-acetylserotonin by the enzyme N-acetyl-transferase (NAT, EC 2.3.1.5.)[3]. The activity of this enzyme as well as the melatonin levels in the pineal and in the blood show a clear circadian pattern with highest levels occurring during the night; conversely, the substrate for NAT, 5-HT, as well as 5-HIAA show an inverse concentration pattern in the pineal with highest levels occurring during the dark phase[9]. Nocturnal pineal melatonin synthesis is controlled by the catecholaminergic innervation to the gland via the peripheral sympathetic nervous system[20].

Propranolol (20 mg/kg), a β-adrenergic blocking agent, effectively blocks the nocturnal rise in melatonin production in the mammalian pineal gland[14]. Also, previous studies have shown that (−)-propranolol suppresses the circadian rhythms of pineal NAT[20], adenylate cyclase[25], cyclic AMP[10], 5-HT[7] and N-acetylserotonin[8]. The rhythms of these constituents all relate to the circadian production of melatonin. Catecholamines, especially NE, act primarily via β-adrenergic receptors in the pinealocyte membrane to induce nocturnal rises in NAT and melatonin[16].

The purpose of the present study was to examine whether lindane stimulates pineal function by acting via pineal β-adrenergic receptors or via the sympathetic innervation to the gland.

MATERIALS AND METHODS

Adult (100–125 g) male albino rats (*Rattus rattus*) were obtained from Harlan Sprague–Dawley, Houston, Texas. The animals were housed (4 per plexiglass cage) in a windowless environmentally controlled (22 ± 2 °C) room with a 14:10 (LD) photoperiod (light on at 07.00 h). The rats received standard laboratory chow and water *ad libitum*.

1,2,3,4,5,6,-Hexachlorocyclohexane (lindane) (97%) was purchased from Aldrich Chemical Company, and (±)-propranolol HCl from Sigma Chemical Company. Acetyl-[1-^{14}C]coenzyme A (specific activity: 52.3 mCi/mmol) and S-adenosyl-L-[methyl-^{14}C]methionine (specific activity: 56.2 mCi/mmol) were purchased from New England Nuclear (Boston, MA).

The insecticide was dissolved in corn oil and groups of rats received either the pesticide in corn oil or oil only by gastric intubation for 6 successive days at 09.00 h. The total dose of lindane administered (17.8 mg/kg) represents 1/5 of the LD$_{50}$. The control rats received an equal volume of the vehicle for the same experimental period. The animals were killed by decapitation at either 11 (20.00 h), 14 (23.00 h), or 16 h (01.00 h) after the last application of either the insecticide or vehicle.

After one week of acclimation, the animals were divided into 4 groups of 9 each and were treated as follows: group 1 (control) received corn oil daily for 6 days and a single injection of saline one hour before lights off on the day of sacrifice; group 2 received an i.p. injection of (±)-propranolol (20 mg/kg) in saline (0.85%) one hour before lights off (20.00 h) on the day of sacrifice; group 3 received lindane (2.75 mg/kg/day) daily in corn oil; group 4 received lindane plus an i.p. injection of (±)-propranolol (20 mg/kg) in saline one hour prior to lights off. After 6 consecutive days of treatment with corn oil or corn oil plus lindane, the animals were killed by decapitation at 01.00 h under dim red-light; this wavelength and light intensity are not capable of influencing nighttime pineal melatonin synthesis[17]. Trunk blood and pineal glands were collected from each animal. The blood was collected in 12 × 75 mm tubes and centrifuged at 3000 rpm for 30 min to obtain serum. After collection, pineal glands and sera were frozen immediately on solid CO$_2$ and stored at –70 °C until assayed.

Pineal NAT and HIOMT activity were assayed according to the methods of Deguchi and Axelrod[11] and Axelrod and Weissbach[3], respectively, using the modifications of Champney et al.[9] for determination of both enzymes in the same tissue. Pineal and serum concentrations of melatonin were estimated by means of a direct radioimmunoassay[13,24]. Remaining pineal tissue was prepared for chromatography by alumina extraction[22], and pineal 5-hydroxytryptophan (5-HTP), 5-HT and 5-hydroxyindole acetic acid (5-HIAA) were estimated by high performance liquid chromatography with electrochemical detection (HPLC-EC) using the method of Mefford and Barchas[15].

Data are expressed as the means ± S.E.M. The data were analyzed using a one-way ANOVA and the statistical significance between specific means was determined using the Newman–Keuls multiple range test.

RESULTS

Lindane, when administered alone, augmented the nocturnal rise in pineal NAT activity above levels found in control animals (Fig. 1). When propranolol was used to prevent the nighttime increase in pineal NAT, lindane

was incapable of stimulating the activity of the acetylating enzyme. Pineal HIOMT, the melatonin forming enzyme, was not influenced by any of the treatments (Fig. 1). Pineal and serum melatonin levels were suppressed by propranolol administration ($P < 0.02$ in each case) and lindane was without effect on either parameter when administered to propranolol-injected rats (Fig. 2). Likewise, lindane by itself did not change pineal or blood levels of melatonin. Pineal 5-HTP levels were unaffected by either propranolol or lindane (Fig. 3). Conversely, lindane treatment by itself depressed pineal 5-HT ($P < 0.005$) and 5-HIAA ($P < 0.05$) levels. The combined treatment of propranolol and lindane led to significant rises in both 5-HT and 5-HIAA above levels found in rats treated with lindane alone (Fig. 3).

DISCUSSION

Circadian rhythms in pineal indole metabolism, especially 5-HT, N-acetylserotonin, and melatonin, are regulated primarily by the activity of the enzyme NAT. The evidence supporting this contention has been summarized elsewhere[5,12,17]. NAT is controlled transsynaptically by
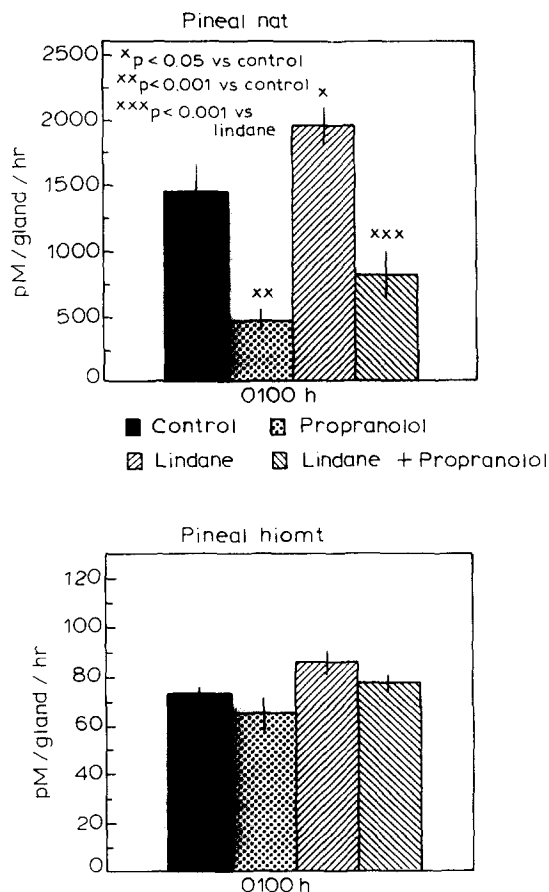

Fig. 1. Effect of lindane and propranolol on nocturnal NAT and HIOMT activities. Animals were killed at 4 h (01.00 h) after lights off. Data are means ± S.E.M..

Pineal melatonin

14
12 ×× p < 0.01 vs lindane
10
8
6
4
2
0

ng / gland

0100 h

■ Control   ▨ Propranolol
▨ Lindane   ◪ Lindane + Propranolol
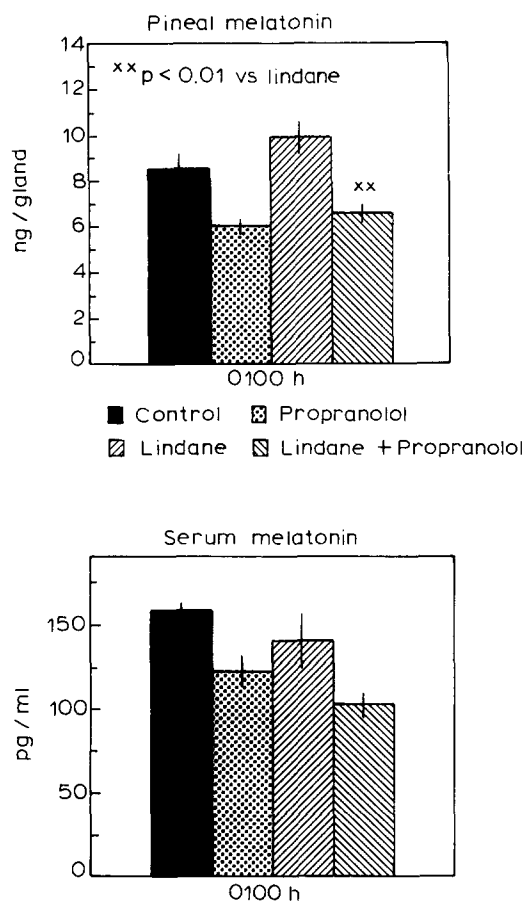
Serum melatonin

150
100
50
0

pg / ml

0100 h

Fig. 2. Effect of lindane and propranolol on nocturnal pineal and serum melatonin levels. Animals were killed at 4 h (01.00 h) after lights off. Data are means ± S.E.M..

Pineal 5-HTP

2
1.5
1
0.5
0

ng / pineal

0100

Pineal 5-HT

60
×p < 0.001 vs
50   lindane
×× p < 0.005 vs
40   control
30
20   ××
10
0

ng / pineal

×

0100

Pineal 5-HIAA

5
×× p < 0.001 vs lindane &
4 p < 0.005 vs con-   ××
        trol
3
2
1
0

ng / pineal

0100
Time

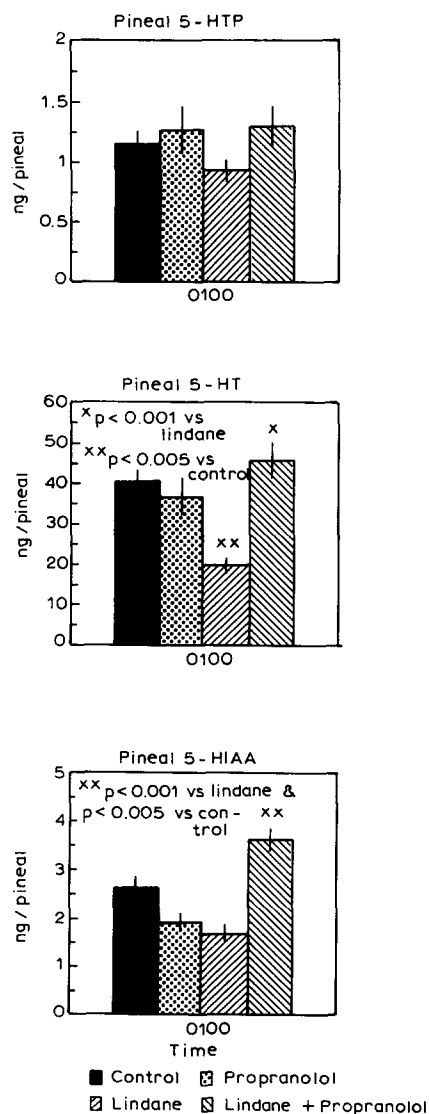■ Control   ▨ Propranolol
▨ Lindane   ◪ Lindane + Propranolol

Fig. 3. Effect of lindane and propranolol on nocturnal pineal 5-HTP, 5-HT and 5-HIAA levels. Animals were killed at 4 h (01.00 h) after lights off. Data are means ± S.E.M..

the release of NE from intrapineal postganglionic sympathetic nerve endings; after its release from the sympathetic fibers, NE interacts especially with β-adrenergic and to a lesser extent with α-adrenergic receptors in pinealocyte membranes. The former interaction results in the activation of membrane-bound adenylate cyclase, the production of the intracellular second messenger cyclic AMP, and the increased synthesis of a specific protein which, in turn, may be directly or indirectly involved in the induction of NAT. α-Adrenergic stimulation by NE augments, by roughly 15%, the NAT rise induced by the action of NE on the β-adrenoceptor. The newly synthesized NAT then catalyzes the acetylation of 5-HT to N-acetylserotonin which is subsequently methylated to melatonin by HIOMT. Unlike with NAT, the activity of the melatonin forming enzyme, HIOMT, typically exhibits no 24-h rhythm[19].

Previously, lindane was found to stimulate rat pineal NAT activity above that in the control animals, thereby causing enhanced pineal and serum melatonin levels[4]. In the present study lindane also increased nocturnal NAT activity but failed when lindane-treated animals were injected with propranolol, a β-adrenergic receptor antag-

onist, an hour before lights off. The failure of lindane to enhance pineal NAT activity after propranolol treatment indicates that the lindane-induced increase in pineal NAT activity may be through the action of the pesticide on β-adrenergic receptors since their blockade obviously prevented the action of lindane. Besides the inability of lindane to modify NAT activity in propranolol-treated rats, the pesticide also had no statistically significant action on either pineal or serum melatonin when it was administered to propranolol-injected rats. Whereas neither propranolol nor lindane, alone or in combination, altered pineal 5-HTP levels, lindane by itself significantly reduced pineal 5-HT and 5-HIAA values. These reductions were presumably due to the augmented activity of NAT as a result of pesticide treatment. Pineal 5-HT levels, although probably being controlled by several

factors[19], typically decrease following the activation of NAT[12]. The reduction in serotonin would be expected to lead to a drop in 5-HIAA as well[9] since the latter compound is a metabolic product of 5-HT.

The most dramatic findings reported herein are the observations that propranolol treatment prevented lindane from inducing reductions in both 5-HT and 5-HIAA (Fig. 3); this is consistent with the observation that NAT activity was not stimulated by lindane when the β-adrenergic receptors were blocked by propranolol. Thus, since NAT activity was not augmented in these pineal glands, 5-HT was allowed to accumulate and its conversion to 5-HIAA restored the levels of this constituent in the pineal gland as well[9].

Taken together the data reported herein are consistent with the idea that lindane modifies pineal 5-HT metab-

olism due to an action on either the sympathetic innervation to the pineal gland or on the β-adrenergic receptors in the pinealocyte membrane. When the β-receptors are blocked, e.g., in the present experiment with propranolol, the pesticide is no longer capable of either modifying the conversion of 5-HT to melatonin or changing the pineal concentrations of either 5-HT or 5-HIAA.

REFERENCES

1 Aldegunde, M., Parafita, M. and Fernandez Otero, M.P., Effect of 1,2,3,4,5,6-hexachlorocyclohexane on serotonin metabolism in rat brain, *Gen. Pharmacol.* 14 (1983) 303–305.

2 Artigas, F., Martinez, E., Camon, L. and Rodriguez Farre, E., Synthesis and utilization of neurotransmitters: actions of subconvulsant doses of hexachlorocyclohexane isomers on brain monoamines, *Toxicology*, 49 (1988) 49–55.

3 Axelrod, J. and Weissbach, H., Purification and properties of hydroxyindole-O-methyltransferase, *J. Biol. Chem.*, 236 (1961) 211–213.

4 Attia, A.M., Mostafa, M.H., Soliman, S.A., El-Sebae, A.H., Nonaka, K.O., Withyachumnarnkul, B. and Reiter, R.J., The organochlorine insecticide 1,2,3,4,5,6,-hexachlorocyclohexane (lindane) but not 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) augments the nocturnal increase in pineal N-acetyltransferase activity and pineal and serum melatonin levels, *Neurochem. Res.*, 15 (1990) 673–680.

5 Binkley, S., Pineal biochemistry: comparative aspects and circadian rhythms. In R.J. Reiter (Ed.), *The Pineal Gland, Vol. 1, Anatomy and Biochemistry*, CRC Press, Boca Raton, FL, 1981, pp. 155–172.

6 Blask, D.E., The pineal: an oncostatic gland. In R.J. Reiter (Ed.), *The Pineal Gland*, Raven, New York, 1984, pp. 253–284.

7 Brownstein, M., Holz, R. and Axelrod, J., The regulation of pineal serotonin by a beta adrenergic receptor, *J. Pharmacol. Exp. Ther.*, 186 (1973) 109–113.

8 Brownstein, M., Saavedra, J.M. and Axelrod, J., Control of pineal N-acetylserotonin by a beta-adrenergic receptor, *Mol. Pharmacol.*, 9 (1973) 605–611.

9 Champney, T.H., Holtorf, A.P., Steger, R.J. and Reiter, R.J., Concurrent determinations of enzymatic activities and substrate concentrations in the melatonin synthetic pathway within the same pineal gland, *J. Neurosci. Res.*, 11 (1984) 59–65.

10 Deguchi, T., Role of the beta-adrenergic receptor in the elevation of adenosine cyclic 3',5'-monophosphate and induction of serotonin N-acetyltransferase in rat pineal glands, *Mol. Pharmacol.*, 9 (1973) 184–190.

11 Deguchi, T. and Axelrod, J., Induction and superinduction of serotonin N-acetyltransferase by adrenergic drugs and denervation in rat pineal organ, *Proc. Natl. Acad. Sci. U.S.A.*, 69 (1972) 2208–2211.

12 Ebadi, M., Regulation of the synthesis of melatonin and its significance to neuroendocrinology. In R.J. Reiter (Ed.), *The Pineal Gland*, 1984, Raven, New York, pp. 1–37.

13 Fraser, S., Cowen, P., Franklin, M., Franey, C. and Arendt, J., Direct radioimmunoassay for melatonin in plasma, *Clin. Chem.*, 29 (1983) 396–397.

14 Lipton, J.S., Petterborg, L.J. and Reiter, R.J., Influence of propranolol, phenoxybenzamine or phentolamine on the *in vivo* nocturnal rise of pineal melatonin levels in the Syrian hamster, *Life Sci.*, 28 (1981) 2377–2382.

15 Mefford, I.N. and Barchas, J., Determination of tryptophan and metabolites in rat brain and pineal tissue by reversed-phase high performance liquid chromatography with electrochemical detection, *J. Chromatogr.*, 181 (1980) 187–193.

16 Pangerl, B., Pangerl, A. and Reiter, R.J., Circadian variations of adrenergic receptors in the mammalian pineal gland: a review, *J. Neural. Transm.* 81 (1990) 17–29.

17 Quay, W.B., Circadian and estrous rhythms in pineal melatonin and 5-hydroxyindole-3-acetic acid, *Proc. Soc. Exp. Biol. Med.*, 115 (1964) 710–713.

18 Reiter, R.J., Action spectra, dose response relationships, and temporal aspects of light's effects on the pineal gland, *Ann. N.Y. Acad. Sci.*, 453 (1985) 215–230.

19 Reiter, R.J., The pineal gland and its indole products: Basic aspects and clinical applications. In M.P. Cohen and P.P. Foa (Eds.), *The Brain as Endocrine Organ*, Springer, New York, 1989, pp. 96–149.

20 Reiter, R.J., Signal transduction mechanisms related to melatonin synthesis in the mammalian pineal gland. In R.J. Reiter and A. Lukaszyk (Eds.), *Advances in Pineal Research, Vol. 4*, John Libbey, London, 1990, pp. 27–37.

21 Romero, J.A., Zatz, M., Kebabian, J.W. and Axelrod, J., Circadian cycles in binding of ³H-alprenolol to β-adrenergic receptor sites in rat pineal, *Nature*, 258 (1975) 435–436.

22 Steger, R.W., Reiter, R.J. and Siler-Khodr, T.M., Interactions of pinealectomy and short photoperiod exposure on the neuroendocrine axis of the male Syrian hamster, *Neuroendocrinology*, 38 (1984) 158–163.

23 Sunol, C., Tusell, J.M., Gelpi, E. and Rodriguez-Farre, E., Regional concentrations of GABA, serotonin and noradrenaline in brain at onset of seizures induced by lindane (γ-hexachlorocyclohexane), *Neuropharmacology*, 27 (1988) 677–681.

24 Webley, G.E., Mehl, H. and Willey, K.P., Validation of a sensitive direct assay for melatonin for investigation of circadian rhythms in different species, *J. Endocrinol.*, 106 (1985) 387–394.

25 Weiss, B. and Costa, E., Selective stimulation of adenyl cyclase of rat pineal gland by pharmacologically active catecholamines, *J. Pharmacol. Exp. Ther.*, 161 (1968) 310–319.