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

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INTRODUCTION

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.

Propranolol (20 mg/kg), a β-adrenergic blocking agent, effectively blocks the nocturnal rise in melatonin production in the mammalian pineal gland14. Also, previous studies have shown that (-)-propranolol suppresses the circadian rhythms of pineal NAT20, adenylate cyclase25, cyclic AMP10, 5-HT7 and N-acetylserotonin8. 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 melatonin16.
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-[1-14C]coenzyme A (specific activity: 52.3 mCi/mmol) and S-adenosyl-L-[methyl-14C]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 LD50. 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 synthesis11. Trunk blood and pineal glands were collected from each animal. The blood was collected in 12 x 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 CO2 and stored at -70 °C until assayed.

Pineal NAT and HIOMT activity were assayed according to the methods of Deguchi and Axelrod11 and Axelrod and Weissbach3, 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 radioimmunoassay13,24. Remaining pineal tissue was prepared for chromatography by alumina extraction22, 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 Barchas35.

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 elsewhere5,12,17. NAT is controlled transsynaptically by...
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.

Previously, lindane was found to stimulate rat pineal NAT activity above that in the control animals, thereby causing enhanced pineal and serum melatonin levels4. 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-
factors\textsuperscript{19}, typically decrease following the activation of \textit{NAT}\textsuperscript{12}. The reduction in serotonin would be expected to lead to a drop in 5-HIAA as well\textsuperscript{19} 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 \textit{NAT} activity was not stimulated by lindane when the $\beta$-adrenergic receptors were blocked by propranolol. Thus, since \textit{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\textsuperscript{19}.

Taken together the data reported herein are consistent with the idea that lindane modifies pineal 5-HT metabolism due to an action on either the sympathetic innervation to the pineal gland or on the $\beta$-adrenergic receptors in the pinealocyte membrane. When the $\beta$-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.

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\textbf{REFERENCES}


