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Lindane and DDT-Induced Changes in Rat Harderian N-Acetyltransferase Activity, Melatonin Levels, and Porphyrin Concentration

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The Harderian glands, large lipid-secreting glands present in the orbits of most mammals, are particularly prominent in rodents. Among the interesting characteristics of these glands are presence of the methoxyindole, melatonin, and the enzymes, N-acetyltransferase (NAT) and hydroxyindole-O-methyltransferase (HIOMT), which are required to synthesize melatonin from serotonin (Bubenik et al., 1976; Brammer et al., 1978). As in the pineal gland (Reiter, 1986), Harderian gland melatonin content exhibits a 24 hour rhythm (Reiter et al., 1983).

Porphyrias are metabolic diseases in which there is an accumulation of porphyrins due to enzymatic defects in the pathway of haem biosynthesis. Besides genetic deficiencies, the major factors known to be implicated in the expression of these diseases are steroids, nutritional factors and a variety of drugs including halogenated aromatic hydrocarbons (Akins and Hooper, 1993). Harderian glands often synthesize large amounts of porphyrins. For this reason, these modified lacrimal glands have been increasingly used as a model for porphyrin biosynthesis (Rodriguez et al., 1992).

The organochlorine insecticide 1,2,3,4,5,6-hexachlorocyclohexane (lindane) augments the nocturnal increase in pineal NAT activity and pineal and serum melatonin levels (Attia et al., 1990). Trichloro 2,2-bis(p-chlorophenyl)ethane (DDT) is without a measurable effect on rat pineal melatonin production although both lindane and DDT significantly stimulate circulating catecholamines (Attia et al., 1990). Whereas there is strong experimental evidence that these pesticides also alter brain neurotransmitter metabolism, there is some disagreement as to the specific nature of these changes (Attia et al., 1991). Thus, subconvulsant doses of lindane reportedly induce increases in serotonin (5HT), 5-hydroxyindole acetic acid (5HIAA) and dopamine (DA) in the dorsal raphe neurons and nerve endings of the cortex and substantia nigra (Artigas et al., 1988). In light of the neural effects of lindane and DDT and their effect on melatonin synthesis in the rat pineal,

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hepatic microsomal enzymes and thyroid hormone, the purpose of the present study was to determine the effect of lindane and DDT on Harderian melatonin levels, NAT activity and porphyrin concentration in the Harderian glands.

MATERIALS AND METHODS

Lindane (97%) and DDT (99%+) were purchased from Aldrich Chemical Company, St. Louis, MO. Acetyl-[L-^{14}C]-coenzyme A (specific activity: 52.3 mCi/mmole) was purchased from New England Nuclear, Boston, MA. Other chemicals were purchased from standard commercial sources.

Male albino rats (*Rattus rattus*) used in these experiments were obtained from Harlan Sprague-Dawley, Houston, Texas. The rats weighed 100-125g. After arrival, all animals were allowed to acclimate to the animal facilities for at least 1 week before manipulations were performed. The rats received standard laboratory chow and water *ad libitum*; bedding was changed twice per week. They were housed in Plexiglas cages with 4 animals per cage. The animal rooms were windowless with automatic controls for temperature (22±2°C) and lighting [lights on at 0700 and off at 2100h; 14h light, 10h dark per day (LD, 14:10)].

The insecticides were dissolved in corn oil and groups of rats received either lindane or DDT in oil or corn oil only by gastric gavage at 0900h for 6 successive days. The doses administered (22.6 mg/kg for DDT and 17.8 mg/kg for lindane) represent 1/5 of the LD_{50} of each insecticide. The control groups received an equal volume of the vehicle for the same experimental period. The animals were killed by decapitation at either 11 (2000h), 14 (2300h), or 16 hours (0100h) after the last application of either the insecticide or vehicle. 2000h represented a point near the end of the light phase while the 2300 and 0100h corresponded to 2 and 4 hours after darkness onset, respectively.

To test the effects of the pesticides of melatonin synthesis and porphyrin concentration in the Harderian gland, control (n=6/group), lindane (n=8/group), and DDT-treated (n=8/group) rats were killed by decapitation at 2000, 2300 and 0100h. Harderian glands were collected from each animals and frozen immediately on solid CO_2 and stored at -70°C until assayed. During the night, animals were exposed to a dim red-light (Kodak safe-light, 15 Watt incandescent bulb behind a # 1A red Filter) for several seconds before they were decapitated; this light intensity is incapable of influencing pineal melatonin synthesis.

Harderian glands were thawed, divided into small pieces ranging from 25 to 50 mg, weighed and homogenized with a Polytron (Kinematica) in chilled phosphate buffer, pH 7.4. The concentration of tissue was adjusted to 25 mg/ml. The samples were centrifuged for 15 min at 3000 rpm. The lipid layer was aspirated and the supernatant was used for the measurement of NAT activity using a radioenzymatic

method; melatonin was measured by radioimmunoassay (Champney et al., 1984). Protein content was measured using the method of Lowry et al (1951). NAT activity and melatonin levels were expressed as pmol product/mg protein/h and pg/mg protein, respectively. Porphyrin concentration was measured by fluorescence spectroscopy.

Data are expressed as means ± standard errors. Statistical significance among groups was determined by data analysis using an ANOVA followed by the Student-Newman Keuls test.

RESULTS AND DISCUSSION

Lindane treatment led to a substantial reduction ($p < 0.001$) in NAT activity 1 hour before lights off (2000h) (Fig. 1). This reduction was accompanied by lower ($p < 0.001$) Harderian melatonin levels at the same time (Fig. 1). At 2300h, lindane-treated rats exhibited no change in NAT activity although melatonin levels were increased ($p < 0.001$). At 0100h, neither NAT activity nor melatonin levels in lindane-treated rats varied from controls (Fig. 1).

DDT induced a dramatic reduction ($p < 0.001$) in NAT activity one hour before lights off (2000h) (Fig. 2). This reduction was accompanied by a concomitant decrease ($p < 0.005$) in rat Harderian melatonin levels at the same time point (2000h) (Fig. 2). Two hours after darkness onset, DDT-treated animals still had lower ($p < 0.001$) Harderian NAT activity (Fig. 1). At 0100h both Harderian NAT and melatonin levels were compared in DDT-treated rats compared to those of controls (Fig. 2).

A substantial reduction ($p < 0.005$, $p < 0.01$, respectively) in Harderian gland porphyrin levels occurred at both 2 and 4 hours after lights off (Fig. 3) compared to one hour before lights off. At 2000h, lindane-treated rats had a lower ($p < 0.005$) porphyrin concentration when compared to untreated rats at the same time. Two and four hours after darkness onset, neither DDT nor lindane-treated rats exhibited a change in porphyrin concentrations (Fig. 3). One hour before the lights off DDT-treated rats did not vary from controls in terms of their Harderian gland porphyrin levels (Fig. 3).

In this study lindane reduced Harderian NAT activity at 2000h. This reduction was accompanied by an obvious drop in Harderian melatonin levels at the same time. This contrasts with observations made in the pineal where at 2000h lindane had no effect of NAT activity (Attia et al., 1990). The lower NAT levels in the Harderian glands seen in lindane-treated animals was not maintained at 2300h. However, at this time melatonin levels increased. This may explain the increase in serum melatonin levels at 2300h caused by lindane as seen in an earlier study (Attia et al., 1990). The lower NAT activity caused by lindane at 2000h is equal to the reduction caused by castration. It is known that a marked testicular atrophy with

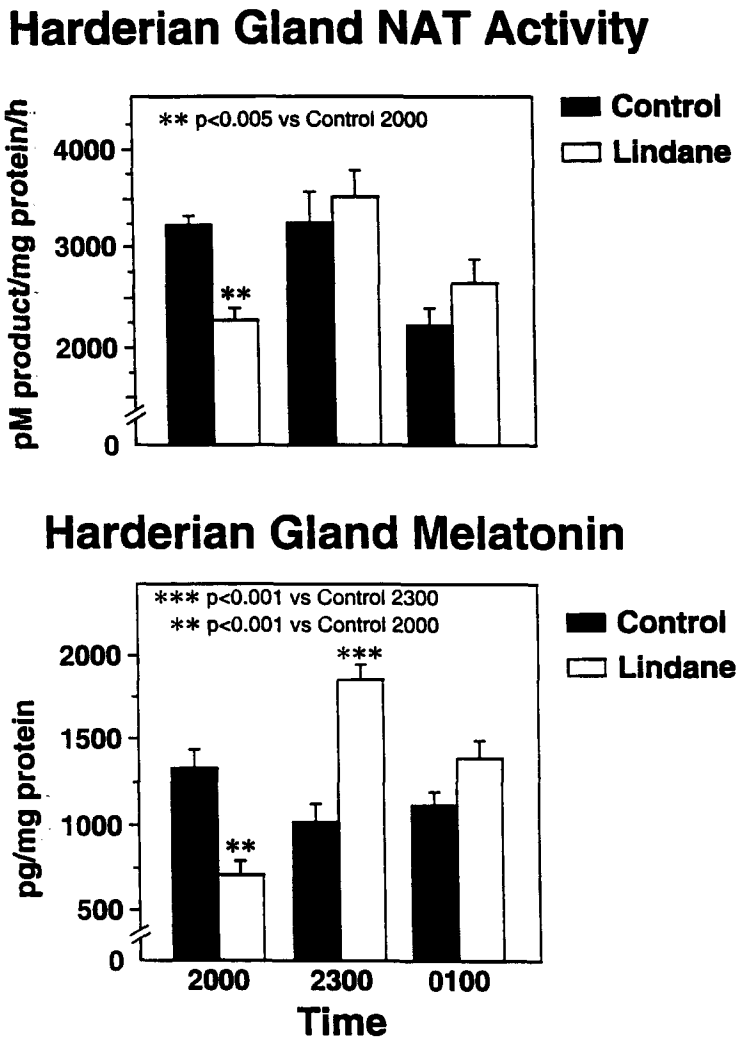

Figure 1. Effect of lindane on Harderian NAT activity and melatonin levels at 1h before light out (2000h) or at either 2 (2300h) or 4 (0100h) hours after lights off. Data are means ± SEM.

reduced tubule size and spermatogenetic activity occurs in rats treated with lindane for 90 days (Shivanadappa and Krishnakumari, 1983). Perhaps the short term administration of lindane (in this case 6 days) also reduced testosterone levels which was followed by a drop in Harderian NAT activity. Melatonin levels have been shown to exhibit a diurnal rhythm in the rat Harderian gland, with peak levels of immunoreactive melatonin detected 2 h after lights on (Reiter et al., 1983). The peak levels of Harderian gland melatonin are augmented by pinealectomy, suggesting that something of pineal origin normally curtails melatonin levels in the Harderian gland. Also, melatonin injection results in increased levels of Harderian gland melatonin, suggesting the possible uptake of this indole from the circulation

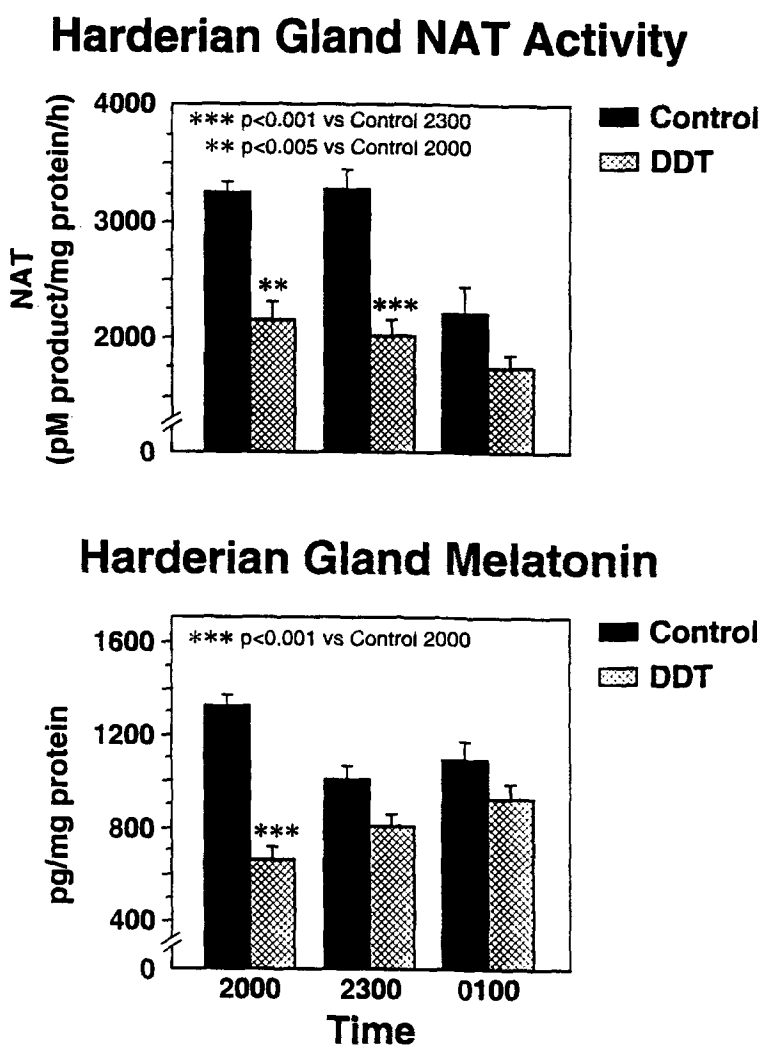

Figure 2. Effect of DDT on Harderian NAT activity and melatonin levels at 1 h before lights out (2000h) or at either 2 (2300h) or 4 (0100h) hours after lights off. Data are means ± SEM.

(Hoffman et al., 1989). The increase in Harderian gland melatonin levels at 2300h in the lindane-treated animals could be a result of increasing pineal and serum melatonin levels at this time (Attia et al., 1990). Another possible explanation for the effects of lindane stems from its known marked effect on the gonads. The Harderian glands of the hamsters are sensitive to changes in circulating gonadal steroids. Castration of male hamsters produces a sharp decrease in Harderian NAT activity and a rapid increase in HIOMT activity (Menendez-Pelaez et al., 1988). Treatment of castrated males with testosterone prevents these changes (Menendez-Pelaez et al., 1988). The reduction of Harderian NAT activity caused by lindane in rats is equal to the reduction that follows castration in hamsters. The observation

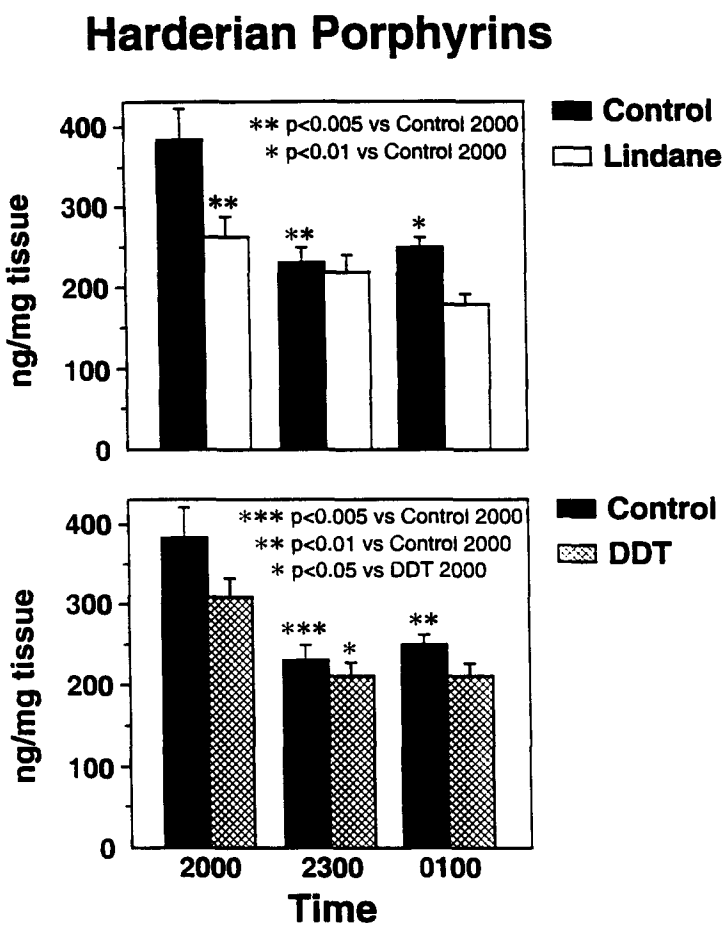

Figure 3. Effect of lindane and DDT on Harderian prophyrin concentrations at 1h before lights out (2000h) or at either 2 (2300h) or 4 (0100h) hours after lights off. Data are means ± SEM.

that lindane can affect the testes, and, presumably testosterone secretion, has been previously observed in rats where there was marked testicular atrophy with reduced tubule size and spermatogenetic arrest after lindane-treatment.

DDT induced a depression of Harderian NAT activity at 2000h concomitant with a fall in melatonin content. By comparison, DDT was not able to change either pineal NAT activity and melatonin levels at 2000h; this could be due to low daytime levels of NAT activity and melatonin at 2000h. Two hours after lights off, DDT reduced Harderian NAT activity. The reduction of Harderian NAT activity in the present study was not shown in the pineal gland (Attia et al., 1990). However,

DDT may change Harderian NAT activity and melatonin levels indirectly through an effect on thyroid hormone. 5'-Deiodinase enzyme (5'D), which converts T_4 to T_3 (the active form of the thyroid hormone), is present in the Harderian glands of male rats (Guerrero et al., 1987). Injection of T_4 or T_3 for 4 weeks led to significantly reduced Harderian gland NAT activity compared with untreated controls (Buzzell et al., 1990). A large group of goitrogenic chemicals, including DDT, disrupts thyriod hormone economy by increasing the peripheral metabolism of thyroid hormones through an induction of hepatic microsomal enzymes.

As in other reports, rat Harderian porphyrin concentration in the present study exhibited highest levels one hour before the lights off. In the present study lindnae significantly reduced porphyrin concentrations at this time. The rodent Harderian gland is remarkable in its ability to synthesize porphyrins, which in some species exceeds that of the liver (Spike et al., 1992). However, it has been generally assumed that the control of haem synthesis in the gland is similar to that of the liver (Spike et al., 1992). Low levels of porphyrin in the Harderian glands of both male and female rats occur after administration of testosterone, indicating that ovarian hormones, like androgens in the male, are necessary for regulation of the Harderian gland porphyrin synthesis. The effects of lindane on the testes may play a role in the reduction of porphyrin levels induced by lindane.

DDT was without measurable effect on Harderian porphyrin when it was given for 6 days only. Alternatively, had Harderian glands been examined after a more prolonged exposure to a high dose of DDT, perhaps Harderian porphyrin production would have changed.

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