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Role of Lindane in Membranes. Effects on Membrane Fluidity and Activity of Membrane-Bound Proteins

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The influence of lindane (gamma-hexachlorocyclohexane) on fluidity of plasma membranes from rat renal cortical tubules has been investigated. Preincubation with lindane increased membrane fluidity. This effect was accompanied by (i) a decrease in the transport of glucose with regard to the controls and (ii) an inhibition of the β-adrenergic stimulatory activity upon cyclic AMP accumulation. However, a significant decrease of the membrane fluidity was found when rats were injected with lindane for 12 days. The injection of lindane exerted the opposite effect on the membrane proteins, the glucose transporter and the β-adrenergic receptor, enhancing the glucose uptake and increasing the isoproterenol-stimulated cycle AMP accumulation. A possible explanation of the difference could involve a resistance to membrane disordering by lindane through a regulatory mechanism that would balance the activity of many lindane-sensitive proteins in insecticide-injected rats.

KEY WORDS: lindane; membrane fluidity; adenylate cyclase; glucose transport.

INTRODUCTION

Lindane, the gamma isomer of hexachlorocylcohexane, is an important organochlorine insecticide extensively used in developing countries as a household, agricultural and gardening pesticide and in human and veterinary medicine as an ectoparasiticide to treat lice and scabies infestation (1, 2). Lindane gains entry into the body system as a food toxicant, by inhalation into lungs or by diffusion through the skin (3, 4). Even though lindane is rapidly metabolized in the body and most of the metabolites are excreted in water-soluble form (5, 6), this insecticide is accumulated in the adipose tissue and in the membrane lipid bilayer of the cells (7).

The adverse health effects of lindane have been studied extensively. The

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principal responses of acute exposure to lindane in animals appear to be neurological and behavioral alterations (8, 9), although changes in hepatic and kidney functions have also been documented in chronic exposure (10). Lindane incorporates in membrane lipid moieties and this effect perturbs membrane permeability (7). In this way, previous *in vitro* toxicity studies from our group with lindane have demonstrated that this toxicant increased the membrane fluidity in a way that is clearly dose-dependent (11, 12). Therefore, many cellular events in which membranes are involved would be affected by lindane. In this regard, lindane interacts with (a) the modulation of calcium levels (13–15); (b) the inositol phospholipid turnover (16–19); (c) the γ-aminobutyric acid (GABA)-activated chloride channels (20–22); (d) the stimulatory activity of several agonists on cyclic AMP accumulation (23–27); and (e) the glucose transport (18, 28). However, the changes in membrane fluidity induced by lindane, when the toxicity studies were carried out *in vitro,* were different from the changes induced when the insecticide was injected subcutaneously in chronic toxicity studies (12), suggesting a resistance to membrane disordering by lindane through a regulatory mechanism that would balance the membrane lipid composition (12, 29).

These features prompted us to study the possibility that renal adaptation to lindane intoxication could affect the activity of membrane-bound proteins. For this purpose we tested in isolated renal cortical tubules the effect of lindane treatment on: (i) β-adrenergic stimulation of cyclic AMP accumulation and (ii) glucose uptake.

MATERIALS AND METHODS

Chemicals

Diphenylhexatriene (DPH) was obtained from Sigma (St. Louis, MO, U.S.A.); dimethylsulphoxide (DMSO) from Merck (Darmstad, Germany); lindane (99%) from Chem. Service (West Chester, PA, U.S.A.) and 3-O-methyl-D-(U-^{14}C)glucose (357 mCi/mmol) from Du Pont (Boston, Mass, U.S.A.). All other chemicals were of analytical grade.

Animals and Treatment

Male Wistar rats aged 75–80 days were separated into two groups of six rats each. The animals from one of the groups were injected subcutaneously with 1 mg/100 g body weight of lindane dissolved in sesame oil (10 mg/ml) at the same time every day for 12 days. The animals from the other group (control rats) were injected at the same times with sesame oil without lindane. The animals were killed by decapitation. The kidneys were removed and the medulla was carefully dissected out. Miniprisms of the resultant chips of kidney cortex were made by use of a McIlwain tissue chopper. The miniprisms were pooled in 30 ml of ice-cold Hank's solution-Hepes buffer, pH 7.4 containing 6 mM glucose (α-buffer) and washed three times.

Renal Cortical Tubules Preparation

The cortical tubules were prepared according to Vinay *et al.* (30). Briefly, kidney cortex miniprisms from treated and control rats were resuspended in 10 ml of α-buffer containing 0.15 g/100 ml collagenase (obtained from Chlostridium histolyticum, grade II, Boehringer, Germany). At the end of the digestion procedure, which lasted 60 min at 37°C, approximately 30 ml of ice-cold α-buffer was added and the suspension was gently shaken to disperse the fragments of tissue. The whole suspension was filtered through a tea strainer to remove collagen fibers. The tubule suspension was then centrifuged, the supernatant discarded and the cortical tubules rapidly resuspended in 30 ml of ice cold α-buffer. This washing procedure was repeated three times. Cell viability was above 90% as measured by exclusion of trypan blue dye. Protein concentration was determined by the method of Lowry *et al.* (31) using BSA as a standard.

In vitro Experiments

(i) For fluorescence polarization determination: Cortical tubules (0.4 mg protein/ml) were resuspended in 154 mM NaCl in 100 mM sodium phosphate buffer, pH 7.4 (β-buffer) and preincubated with lindane (0.1, 0.3 and 0.5 mM) at 25°C for 15 min. Lindane was dissolved in DMSO (0.3% in the final incubation mixture). Samples with DMSO without lindane were used as control.

(ii) For glucose uptake study: Cortical tubules (8 mg protein/ml) were resuspended with α-buffer without glucose and preincubated with lindane (0.1, 0.3 and 0.5 mM) at 25°C for 15 min. Lindane was dissolved in DMSO as above.

Fluorescence Polarization

Fluorescence polarization was measured with a Perkin-Elmer Model LS-B3 spectrofluorimeter. DPH dissolved in tetrahydrofuran was added (final concentration 0.6 μM) to cortical tubules from *in vitro* experiments. DPH dissolved in tetrahydrofuran was also added to β-buffer resuspended cortical tubules (0.4 mg protein/ml) from lindane-injected and control rats. Samples were incubated at 37°C for 30 min. DPH was excited at 345 nm and emission was measured at 430 nm. Steady-state fluorescence polarization was computed according to the relationship: $P = (I_{vv} - I_{vh})/(I_{vv} + I_{vh})$ where I_{vv} and I_{vh} are the fluorescence intensities observed with the analyzing polarizer parallel and perpendicular to the polarized excitation beam, respectively. Data were corrected for unequal transmission of differently polarized light and for intrinsic fluorescence.

Cyclic AMP Study

Cortical tubules from control and lindane treated rats were incubated as previously described (32). In short, renal cortical tubules (1 mg protein/ml) were incubated with increasing concentrations (up to 100 nM) of isoproterenol in 0.5 ml of 35 mM Tris-HCl buffer, pH 7.5, containing 1.4% BSA and 0.2 mM isobutylmethylxantine as a phosphodiesterase inhibitor. After 60 min at 15°C, 2.5 ml

methanol was added and the mixture was centrifuged. The supernatant was evaporated for cyclic AMP determination by a protein-binding assay (33).

Glucose Uptake Study

A radioactively-labelled glucose analog 3-O-methyl-D-(U-^{14}C)glucose was used and the glucose uptake was measured according to Sakhrani *et al.* (34) with some modifications. Cortical tubules from *in vitro* experiments and lindane treated rats (resuspended in α-buffer without glucose, 8 mg protein/ml) were used in the study. Uptake was initiated by the rapid addition of 100 μl of 2.5 mM 3-O-methyl-D-glucose with radioactive tracer (0.5 μCi) in α-buffer without glucose. The incubations were carried out at 37°C at a constant shaking speed. At the appropriate time the transport was terminated by addition of 2 ml of ice-cold stopper solution containing 300 mM D-Mannitol, 10 mM Hepes, pH 7.4. The cortical tubules were rapidly separated from the medium by centrifugation and the supernatant removed by aspiration. The cell pellet was resuspended with gently shaking in 2.5 ml of fresh ice-cold stopper solution, pelleted by centrifugation and the radioactivity in the cells counted in 2 ml of scintillation cocktail. Non specific uptake measurement was obtained by the addition of the labelled 3-O-methyl-D-glucose solution to cortical tubules previously well mixed with stopper solution. The net uptake was obtained by subtracting the non-specific uptake from the total uptake.

Statistical Treatment of the Results

Results are expressed as the mean ± S.E. The statistical significance of the differences between groups was determined by the Student's t-test. Differences were considered significant when $p < 0.05$.

RESULTS

Table 1 shows that the fluorescence polarization of DPH decreased when the lindane concentration in the incubation mixture was increased. Therefore, lindane

Table 1. Fluorescence polarization measures of rat renal cortical tubules in lindane incubations (*in vitro* experiments) and lindane-injected rats

In vitro				Injected rats	
	Lindane (mM)			Control	Lindane
Control	0.1	0.3	0.5		
0.214 ± 0.002	0.204 ± 0.001*	0.194 ± 0.002**	0.182 ± 0.002**	0.209 ± 0.002	0.220 ± 0.003*

Note: Results are expressed as mean ± SE of six independent determinations. Asterisks indicate significant differences from control values (**, $P < 0.01$; *, $P < 0.05$). Control of *in vitro* experiments vs control of injected rats, not significant.

Table 2. Effect of lindane (*in vitro* experiments and lindane-injected rats) on the net uptake of 3-O-methyl-D-(U-^{14}C)glucose into rat renal cortical tubule cells. Samples were incubated with labelled substrate for various time periods (15, 30, 60 and 300 sec.)

	In vitro				Injected rats	
		Lindane (mM)			Control	Lindane
Time (sec)	Control	0.1	0.3	0.5		
15	80 ± 5	73 ± 5	72 ± 8	69 ± 5	76 ± 7	120 ± 13*
30	159 ± 8	145 ± 6	141 ± 7	138 ± 10	129 ± 12	198 ± 10**
60	226 ± 7	208 ± 10	199 ± 7	193 ± 8*	196 ± 12	265 ± 10**
300	341 ± 8	322 ± 8	321 ± 8	300 ± 12*	315 ± 9	363 ± 11**

Note: Values are expressed as mean pmol/mg protein ± SE of six independent determinations. Asterisks indicate significant differences from control values (**, $P < 0.01$; *, $P < 0.05$). Controls *in vitro* experiments vs controls of injected rats, not significant.

increased the membrane fluidity in a dose-dependent way. Previously, we have reported that rat renal cortex accumulated lindane when animals were injected in the present experimental conditions (12). The corresponding effect on membrane fluidity is also indicated in Table 1. As this table shows, a significant decrease of membrane fluidity was found when cortical tubules from control and lindane-treated rats were compared. Surprisingly, membrane fluidity changes induced by lindane in the incubation samples were different from the changes induced when the insecticide was injected subcutaneously.

Renal cortical tubules were incubated with 3-O-methyl-D-(U-^{14}C)glucose for different time periods and the net transport is expressed as pmol of 3-O-methyl-D-glucose uptake/mg protein in Table 2. The preincubation of cortical tubules with lindane (0.5 mM) led to a significant decrease in the transport of this glucose analog, while a significant increase of glucose uptake was detected in the lindane-injected rats (Table 2). DMSO has no influence neither on the fluorescence polarization of DPH nor on the glucose uptake (data not shown).

Concentration-response experiments of isoproterenol-stimulated cyclic AMP accumulation showed an increase of isoproterenol efficiency (about 45% at a maximally active, 100 μM dose of isoproterenol) when determinations were carried out with cortical tubules from rats injected with lindane during 12 days (Fig. 1). The potency of the β-adrenergic agonist was again maintained by the insecticide since half-maximal cyclic AMP response was elicited at about 0.3 μM isoproterenol in both control and lindane-treated rats.

DISCUSSION

Our results showed that DPH fluorescence polarization was significantly lower in cells of renal cortical tubules incubated with lindane than those incubated without the toxicant. In this way, we and others have also reported effects of lindane and other organochlorine insecticides on the fluidity of

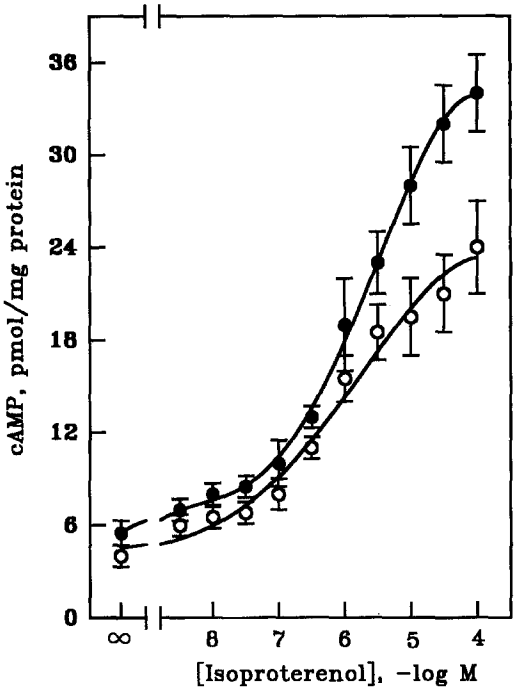

Fig. 1. Influence of lindane on stimulatory effect of isoproterenol upon cyclic AMP accumulation. Renal cortical tubules (1 mg protein/ml) from control (open circles) and lindane-injected rats (solid circles) were incubated with increasing concentrations of isoproterenol (up to 100 nM) at 15°C for 60 min as described in Materials and Methods. Results are the mean ± SE of six triplicate experiments.

well-defined model and native membranes in terms of fluorescence polarization of the probe DPH (12, 25, 35–37). Hence, the decrease of DPH fluorescence polarization is associated with an increase of membrane fluidity.

Studies from our group indicated that lindane chronically administered in the diet (4) or injected subcutaneously (12) accumulates primarily in the renal cortex in agreement with other studies previously reported (3). However, membrane fluidity changes induced by lindane in the incubation samples were different from the changes induced when the insecticide was injected subcutaneously, suggesting the existence of compensatory mechanisms. The origin of the observed fluidity changes is probably the lipid composition. In this way, we have previously described resistance to membrane disordering by lindane through a regulatory mechanism that would balance the amount of cholesterol and phospholipid classes for both renal cortical membranes (12) and prostate gland (29). Similarly, phospholipid changes after exposure to chlorinated hydrocarbons through compensatory remodeling was suggested (38).

The results obtained showed that preincubation of renal tubules with lindane decreases the transport of 3-O-methyl-D-glucose in a time dependent manner.

However the injection of lindane during 12 days induces an increase of glucose uptake probably via membrane fluidity changes. This assumption is in line with the studies of Friedlander *et al.* (39). These authors reported that a decrease of membrane fluidity simultaneously enhances glucose transport in renal epithelial cells. Thus, renal adaptation to lindane intoxication could involve an alteration of the activity of many lindane-sensitive proteins in insecticide-injected rats which compensates for the effects of lindane. In the same way, cellular adaptation to ethanol (40), which is also able to penetrate the lipid bilayer (41), and to other toxicants (42) has been described.

The increase of isoproterenol-stimulated cyclic AMP accumulation in renal cortical tubules from lindane-injected rats (present work), which more than compensates for the observed inhibitory action of lindane upon β-adrenoceptor stimulation of cyclic AMP in *in vitro* experiments (25), could also be interpreted as an example of this response adaptative pattern. Accordingly, it has been inferred that the perturbation of membrane fluidity by lindane may change the coupling of receptor–effector system and be responsible at least in part of the action of the insecticide on isoproterenol-stimulated cyclic AMP accumulation. In the present study, we have not evaluated the effect of the toxicant on G_s protein and adenylate cyclase catalytic subunit. However, previous results of our group demonstrated that G_s protein was unaffected by lindane in rat enterocytes (27). All these results establish a modulatory effect of lindane and other organochlorine compounds on the adenylate cyclase catalytic subunit itself, which may be the consequence of alteration of membrane microdomains surrounding this integral protein.

In conclusion, this work provides support for a model which emphasizes the role of lindane–membrane interaction in the modification of many cellular events in which membranes are involved. Moreover, these results suggest that membrane fluidity might play a physiological role in the acquisition of resistance to membrane disordering by lindane through compensatory mechanisms in the renal tubule cells.

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