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Effects of lindane on fluidity and lipid composition in rat renal cortex membranes

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The influence of lindane upon dynamic properties of plasma membranes from rat renal cortex has been investigated using a fluorescence polarization technique. Preincubation with lindane increased membrane fluidity in a manner that is dose-dependent. This increase was higher in brush border membranes than in basolateral membranes. However, a significant decrease of the membrane fluidity was found in brush border membranes when rats were injected with lindane for 12 days. A possible solution to this difference could involve a resistance to membrane disordering by lindane through a regulatory mechanism that would balance the amount of cholesterol and phospholipid classes in the renal cortex membranes of lindane-injected rats.

Introduction

Lindane (1,3,4,6/2,5-hexachlorocyclohexane), as a purified isomer, is widely used both as a less hazardous agricultural insecticide and in human and veterinary medicine to treat ectoparasites and pediculosis [1,2]. However, this organochlorine insecticide induces neurotoxic effects, the most important of which is the production of convulsions in acute toxicity [1,3]. Chronic toxicity studies have provided evidence of cell degeneration in the male rat kidney [4] and the severity of these lesions have been significantly correlated to the amount of lindane accumulated in this organ [4].

Cholesterol is a major component of the plasma membrane of eukaryotic cells and modulate bilayer fluidity through interaction with phospholipids in the membrane [5,6]. Our group has recently reported that a lindane-containing diet induced an increase of renal cortex phospholipid content in rat, but choline glycerophospholipids (PC) and ethanolamine glycerophospholipids (PE) were not equally augmented, because PE increased much more than PC. Therefore, the PC-to-PE ratio decreased in the intoxicated rats [7]. This parameter is an important determinant of lipid fluidity in membranes [8] and considerable evidence now exists that alterations in the physical state of membrane lipids can influence a number of important cell-surface functions, including the activities of certain enzymes [9,10].

Lindane is a highly lipophylic compound that can also oxidize to lipids since free radicals are involved in its metabolism [11]. In the same way, previous studies have indicated [12] that lindane is accommodated in lipid bilayers and this accommodation induces an increase in membrane fluidity.

The present studies were undertaken to determine lindane influence on membrane fluidity and relate it to possible changes in phospholipid and cholesterol composition in brush border (BBM) and basolateral (BLM) membranes of the male rat kidney. Changes in the membrane fluidity were measured by the fluorescence polarization of DPH. Experiments were carried out both in incubations with different lindane concentrations and studies of lindane-injected rats.

Abbreviations: Lindane, 1,3,4,6/2,5-hexachlorocyclohexane or γ-hexachlorocyclohexane; BBM, brush border membranes; BLM, basolateral membranes; DMSO, dimethylsulphoxide; DPH, 1,6-diphenyl-1,3,5-hexatriene; PC, choline glycerophospholipids; PE, ethanolamine glycerophospholipids.

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Materials and Methods

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) and washed three times.

BBM and BLM preparation. The proximal tubules were prepared according to Vinay et al. [13] by means of collagenase digestion. Membranes from proximal tubules were preparated using a $CaCl_2$ precipitation method [14]. The proximal tubules were homogenized in 50 mM mannitol, 2 mM Tris-HCl (pH 7.4). The homogenate was centrifuged at $3500 \times g$ for 5 min and the supernatant was treated with 10 mM $CaCl_2$ during 15 min in the cold. After the second centrifugation ($4000 \times g$, 15 min) the supernatant contained the BBM and the pelleted material the BLM fraction. The supernatant was removed and recentrifuged at $18000 \times g$ for 10 min to obtained the BBM. The pelleted material, which contained the BLM was resuspended in 0.15 M NaCl, 0.05 M Tris-HCl, 0.01 M mercaptoethanol (pH 7.4). After dilution and addition of Percoll (Pharmacia) to a final concentration of 28% (v/v), the crude BLM suspension was centrifuged at $30000 \times g$ for 35 min. The purified BLM were recovered in the zone corresponding to Percoll density 1.030–1.035 g/ml. Since the Percoll material at high concentrations interferes with the method of Lowry et al. [15] that we used for protein determination, it was necessary to remove the Percoll particles by centrifugation.

Alkaline phosphatase (determined by the method of Hubscher and West [16]) and Na^+,K^+-ATPase (determined by the method of Serrano [17]) were measured in order to estimate the purity of the BBM and BLM preparations.

In vitro experiments. BBM and BLM (25–30 μg protein/ml) were incubated at 25°C for different times with 154 mM NaCl in 100 mM sodium phosphate buffer (pH 7.4) (β-buffer). When samples were treated with lindane, this was dissolved in DMSO (0.3% in the final incubation mixture). Samples with DMSO without lindane were used as controls.

Fluorescence polarization of DPH. Fluorescence polarization was measured with a Perkin-Elmer Model LS-3B spectrofluorometer. DPH dissolved in tetrahydrofuran was added (final concentration 0.6 μM) into both the incubation mixtures and the membranes (dissolved in β-buffer as above) from lindane-injected rats. Samples were incubated at 37°C for 30 min. DPH was excited at 357 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. The data were corrected for unequal transmission of differently polarized light and for intrinsic fluorescence.

Lipid analysis. Total lipids were extracted and purified according to the method of Bligh and Dyer [18]. The different classes of lipids were separated by thin-layer chromatography on silica gel G plates (Merck) as previously described [19,20]. Briefly, total phospholipids and cholesterol were separated employing hexane/diethyl ether/acetic acid (70:30:1, v/v). Different classes of phospholipids were separated using propionic acid/propanol/chloroform/water (2:2:1:1, v/v). Standard phospholipids and cholesterol were used as comparison. Quantitative analysis of phospholipids was carried out by thin-layer chromatography and phosphorus colorimetric analysis of spots according to Rouser et al. [21]. The colorimetric method from Rudel and Morris [22] was used for quantitative cholesterol determination.

Lindane extraction and quantification. Lindane was extracted and quantified by gas chromatography as previously described [7].

Statistical treatment of the results. Results are expressed as the mean ± SE. The statistical significance of the differences between groups was determined by

TABLE I

Enzyme activities in whole homogenates (H), brush border membranes (BBM), and basolateral membranes (BLM), from rat renal proximal tubules

Results are expressed as mean ± S.E. of six independent determinations. All enzyme activities are given in μmol min^{-1} (mg protein)$^{-1}$.

Enzyme	Specific activity			Enrichment factor	
	H	BBM	BLM	BBM/H	BLM/H
Alkaline phosphatase	0.071 ± 0.001	0.460 ± 0.002	0.093 ± 0.002	6.48	1.30
Na^+,K^+-ATPase	0.069 ± 0.001	0.256 ± 0.002	0.678 ± 0.001	3.71	9.83

the Student's t-test. Differences were considered significant when $P < 0.05$.

Results

Table I summarizes the specific activities and the enrichment factors of the marker enzymes for BBM (alkaline phosphatase) and BLM (Na^+,K^+-ATPase). The purity of the BBM preparations was assessed by the value of the enrichment factor (specific activity in the membrane preparation/specific activity in the homogenate), which was 6.48 for alkaline phosphatase as compared with 3.71 for Na^+,K^+-ATPase. BLM preparations were enriched 9.83-fold in Na^+,K^+-ATPasa activity as compared with 1.30 for alkaline phosphatase.

The absorption and emission spectra of DPH in membranes from rat renal cortex were performed (data not shown). The absorption spectrum is centered at approx. 357 nm and the emission spectrum is centered at 430 nm. Both spectra are typical of those reported for DPH in other membrane systems [23–25]. Upon addition of lindane, the fluorescence spectra of DPH no decreased in intensity and no additional fluorescence band could be detected.

Table II shows the results obtained in a time-course experiment on the influence of 0.3 mM lindane upon the fluorescence polarization of DPH in BBM and BLM from rat renal cortex at four different time-periods (1, 5, 15 and 30 min) and 25°C. Lindane presence decreased the polarization of the fluorescence in the two materials. An incubation time of 5 min was chosen for subsequent experiments.

Fig. 1 shows that the fluorescence polarization of DPH decreased when the lindane concentration in the incubation mixture was increased. This decrease was higher in renal BBM than in BLM. Therefore, lindane increased the membrane fluidity in a way that is clearly dose-dependent, and this lindane effect was different for the two kinds of renal cortex membranes. DMSO has no influence on the fluorescence polarization of DPH (data not shown).

The rat renal cortex accumulated 96.15 ± 9 ppm lindane (expressed per wet tissue weight) when animals

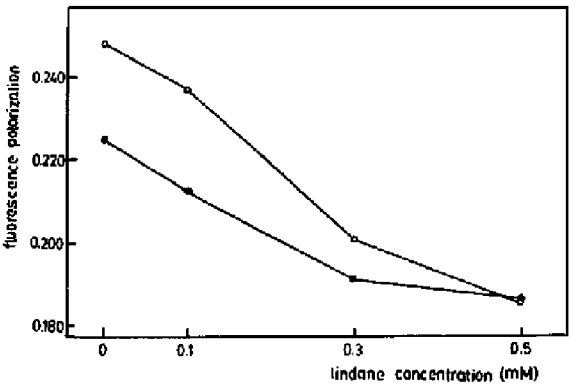

Fig. 1. Concentration influence of lindane on the fluidity of brush border membranes (○) and basolateral membranes (●) from rat renal cortex. Samples were preincubated with lindane for 5 min at 25°C. Samples were assayed for membrane fluidity using DPH as a fluorescence probe. The standard error of each point was less than 0.001 ($n = 20$).

were injected subcutaneously with this insecticide for 12 days. The corresponding effect on membrane fluidity is indicated in Fig. 2. As this figure shows, no significant differences existed when comparing BLM from control and lindane-treated rats. However, a significant decrease of membrane fluidity was found in BBM. Interestingly, the changes in membrane fluidity induced by lindane in the incubation samples (Fig. 1) were different from the changes induced when the insecticide was injected subcutaneously (Fig. 2).

The total lipid phosphorus and cholesterol contents of BBM and BLM isolated from renal proximal tubules of rats injected with lindane are shown in Table III. The treatment with lindane increased both the total lipid phosphorus content and the cholesterol content above control values. The effect was more marked in BBM than in BLM, with the increase of cholesterol being very important. consequently, the cholesterol-to-phospholipid ratio increased when rats were injected with lindane, and an increase in this ratio would predict a decrease in membrane fluidity [26,27].

But, even though the total lipid phosphorus content

TABLE II

Fluorescence polarization measures of brush border membranes (BBM) and basolateral membranes (BLM), in relation to preincubation time (1, 5, 15 and 30 minutes) with lindane

Results are expressed as mean ± S.E. of six independent determinations.

Time	BBM		BLM	
	control	0.3 mM lindane	control	0.3 mM lindane
1 min	–	0.206 ± 0.001	–	0.224 ± 0.002
5 min	0.246 ± 0.001	0.203 ± 0.001	0.228 ± 0.001	0.191 ± 0.001
15 min	–	0.207 ± 0.001	–	0.193 ± 0.002
30 min	0.243 ± 0.001	0.214 ± 0.001	0.231 ± 0.004	0.195 ± 0.002

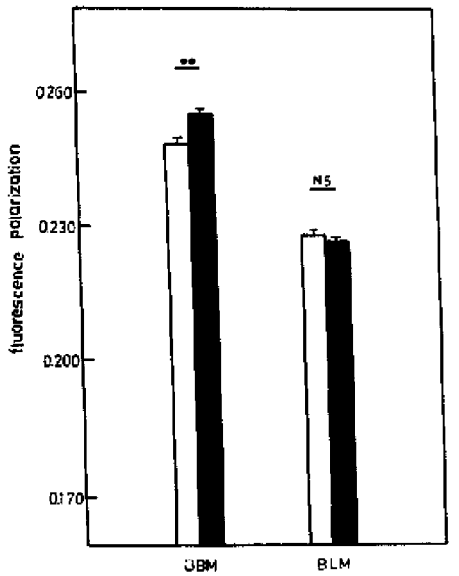

Fig. 2. Influence of lindane treatment on the fluorescence polarization of renal brush border membranes (BBM) and basolateral membranes (BLM) in control rats (open bars) and in rats injected subcutaneously with 1 mg/100 g body weight of lindane (solid bars). The results are expressed as the mean ± SE of eight independent determinations. Differences between groups were considered significant for $P < 0.01$ (**); NS, not significant.

in renal proximal tubule membranes from lindane-injected rats increased, all the phospholipid classes were not equally augmented (Table IV). Sphingomyelin and PE increased much more than others, and this resulted in an increase in the molar ratio of sphingomyelin to PC and a decrease in the PC-to-PE ratio. The changes in these lipid ratios, which would be expected to cause a decrease in membrane fluidity [26,27], were more important in BBM than in BLM.

Discussion

Fluorescence polarization techniques have used DPH to investigate the dynamic properties of rat renal plasma membranes. Recently several different laboratories have shown that BBM and BLM differ markedly with respect to both fluorescence polarization and lipid composition [14,28–33]. Our results showing that BLM fluidity is higher than BBM fluidity concur with others [14,29,30,33]. The differences in lipid composition between BBM and BLM provide a biochemical basis for the observed differences in BBM and BLM fluidity. In this regard, the factors that may contribute to the more fluid state of the BLM include: (1) the lipid-to-protein ratio; (2) the cholesterol-to-phospholipid ratio; (3) the PC-to-PE ratio; (4) the sphingomyelin-to-PC ratio and

TABLE III

Influence of lindane treatment on total lipids, phospholipids and cholesterol content of brush border membranes (BBM) and basolateral membranes (BLM) from rat renal proximal tubules

Results are expressed as mean ± S.E. of six independent determinations. [a], expressed as mg/mg protein; [b], expressed as nmol/mg protein. Asterisks indicate significant differences from control values (*, $P < 0.05$; ***, $P < 0.001$). CL, cholesterol; PL, phospholipids.

Membranes	Total lipids [a]		Phospholipids [b]		Cholesterol [b]		CL/PL ratio	
	control	+ lindane	control	+ lindane	control	+ lindane	control	+ lindane
BBM	0.55 ± 0.01	0.67 ± 0.01 ***	557 ± 25	653 ± 29 *	302 ± 7	403 ± 12 ***	0.54 ± 0.02	0.62 ± 0.02
BLM	0.60 ± 0.01	0.62 ± 0.02	546 ± 20	570 ± 24	279 ± 13	310 ± 16	0.51 ± 0.02	0.54 ± 0.02

TABLE IV

Phospholipid class content (nmol/mg protein) of brush border membranes (BBM) and basolateral membranes (BLM) from rat renal proximal tubules

Results are expressed as mean ± S.E. of six independent determinations. Asterisks indicate significant differences from control values (*, $P < 0.05$; ***, $P < 0.001$). PS, serine glycerophospholipids; PI inositol glycerophospholipids; SM, sphingomyelin.

Phospholipids	BBM		BLM	
	control	+ lindane	control	+ lindane
PE	106 ± 6	148 ± 14 *	133 ± 11	148 ± 13
PS	60 ± 5	65 ± 6	42 ± 3	41 ± 2
PI	18 ± 2	20 ± 2	23 ± 3	24 ± 3
PC	154 ± 7	174 ± 8	186 ± 15	200 ± 10
SM	160 ± 8	192 ± 10 *	116 ± 6	128 ± 8
PC/PE ratio	1.45 ± 0.03	1.17 ± 0.03 ***	1.40 ± 0.02	1.35 ± 0.03
SN/PC ratio	1.04 ± 0.01	1.10 ± 0.02 *	0.62 ± 0.02	0.64 ± 0.01

(5) the length and degree of unsaturation of fatty acyl chains. Therefore, BBM and BLM should differ in one or more of these parameters. However, investigation of the contribution of these parameters to the differences in fluidity between the BBM and BLM, has led to different conclusions. Hise et al. [30] and our Table III (first column) in the present paper showed that the lipid-to-protein ratio was significantly higher in BLM than in BBM. The ratio of lipid-to-protein has been described as being of major importance, suggesting that the proteins themselves have a rigidizing effect on these membranes.

The relative content of phospholipids and cholesterol and the PC-to-PE ratio have an important influence on the physical state of model and natural membranes. In the studies of Bode et al. [28], Hise et al. [30] and the corresponding studies of this paper with control rats there were no differences in the cholesterol-to-phospholipid and PC-to-PE ratios of these membranes. However, a difference in these ratios in BBM and BLM have been found by others [31,32], and the reasons for the discrepancy are unclear.

Also, the ratio of sphingomyelin to PC is an important determinant for the physical state of membranes. Decreased ratios are associated with more fluid membranes. The smaller ratio of sphingomyelin to PC in the BLM has been found by us and others [30,31].

The most important result in our report is that DPH fluorescence polarization was significantly and substantially lower in the membranes (BBM and BLM) of renal proximal tubules incubated with lindane than in the controls incubated without lindane. Several pesticides can act as quenchers of suitable fluorescent molecules solubilized in the membranes [34]. Indeed, lindane has been used to quench the fluorescence of a series of carbazole derivatives in model membranes [35,36]. In our experimental conditions, lindane does not quench DPH fluorescence in native membranes (BBM and BLM). In this way, other authors have also reported effects of lindane and other organochlorine insecticides in the fluidity of well-defined model and native membranes in terms of fluorescence polarization of the probe DPH [37,38]. Therefore, the decrease of DPH fluorescence polarization was associated with an increase of membrane fluidity.

Lindane alters the activity of some membrane-bound proteins [39–42]. In this regard, lindane interaction with the GABA receptor complex [3] and stimulation of Ca^{2+} mobilization by this organochlorine compound have been reported [43,44]. It has also recently been reported that lindane decreased glucose transport in rat brain cortex cells [45]. On the other hand, lindane incorporates in membrane lipid moieties and this effect perturbs membrane permeability [12]. Low BBM fluidity also appears to be an additional requirement for the activation of some of the inserted proteins [46]. In conclusion, with the present data, it is realistic to say that lindane could carry out its toxicological effects in the kidney through the modification of the membrane fluidity.

The epithelial cells of the renal tubules are highly asymmetrical morphologically. This asymmetry, also found in the specific distribution between BBM and BLM of enzyme activities and transport systems, appears to be maintained by the existence of intercellular contacts [14]. The present fluorescence polarization data clearly indicate that the asymmetry also existed at the level of lindane interaction, because the effect caused by the lindane presence was also higher in BBM than in BLM.

Chronic toxicity studies from our group indicated than lindane administrated in the diet [7] or injected subcutaneously (present results) accumulates primarily in the renal cortex in agreement with other studies previously reported [4]. The partition coefficient values clearly indicate that lindane is very soluble in model and native membranes [12,35,36,47]. However, the decreases of DPH fluorescence polarization were not detected in the lindane-injected rats, suggesting the existence of compensatory mechanisms. Thus, renal adaptation to lindane intoxication involves changes in membrane lipid composition, such as an increase in the cholesterol, PE and sphingomyelin contents, which may mediate membrane fluidity. In fact, the experimental animals may endeavor to maintain membrane fluidity by changing the lipid composition of their membranes. In this regard, the increases in cholesterol-to-phospholipid and sphingomyelin-to-PC molar ratios as well as the decrease in the PC-to-PE molar ratio were associated with a decrease of membrane fluidity [5,6,8,30,31]. This conclusion is reinforced by the results of Roux et al. [48] that showed a decline in the inhibitory effects of lindane on lymphocyte growth in relation to the time of addition to the culture, suggesting that with longer exposure times the cells become refractory to inhibition. Moreover, the fact that cholesterol effectively withdraws lindane from the membrane has been reported [12].

Other authors have previously demonstrated a phospholipid alteration in the liver of rats chronically exposed to $CHCl_3$ due to the production of free radicals [49,50]. Since free radicals are involved in lindane metabolism [11], the decrease in PC-to-PE ratio with chronic lindane treatment can be due to free radical damage. However, the organochlorine lipid binding is generally thought to be a measure of free radical production, and the low level of this kind of binding by lindane [11] as compared to halothane [51], DDT [52], CCl_4 [53] and $CHCl_3$ [49,50] casts doubt on this mechanism. This conclusion is reinforced with data previously obtained with lindane in our experimental model: the accumulation of lindane in kidney was high but the

level of lindane metabolite accumulation was very low [7].

Other factors controlling membrane fluidity are the length and degree of unsaturation of the fatty acyl chains [30] and the ethanolamine plasmalogen content [31]. We have previously reported [7] that administration of lindane does not affect the plasmalogen amounts in rat kidney cortex. Also, most of the fatty acids showed little differences between control and lindane treated-rats in rat renal cortex [7].

The adaptation to lindane intoxication through changes in lipid content was more important in BBM than in BLM, which corresponds to the fact that they are two distinct membrane populations [14,31,33,54]. This may explain the absence of lindane-induced effects in the fluidity of BLM preparations.

From the present data, it is possible to conclude that lindane modifies membrane fluidity and, therefore, many cellular events in which membranes are involved would be affected by lindane. The data also suggests that renal adaptation to lindane intoxication occurs via the increase of many membrane lipid contents.

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