Effects of lindane (γ-hexachlorocyclohexane) on rat heart muscle contraction

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Abstract. The effects of micromolar concentrations of lindane on the mechanical activity of cardiac left ventricular papillary muscles were studied in adult female rats. Lindane decreased the amplitude and duration of the contraction, and slowed down the time course of its ascending phase (i.e. decreased the maximum rate of rise of the initial phase (dC/dt_{max})). Both amplitude and duration of the contraction, but not dC/dt_{max}, were restored by subsequent application of the rapid delayed outward K^+ current (I_{Kr}) blocker E-4031 (10 nmol/l). Increasing the stimulation frequency from 1 to 3.3 Hz in the control solution produced a decrease in the amplitude of the first beat peak contraction while a slow recovery phase (srp) developed, as the result of the Na^+-Ca^{2+} exchanger activity. When the frequency was restored to 1 Hz, a post rest potentiation (prp) with a negative staircase (ns) developed due to the sarcoplasmic reticulum (SR) Ca^{2+} refilling. Lindane increased the amplitude of both srp and prp, but did not affect ns, which indicates that SR Ca^{2+} refilling was not altered by the pesticide. In conclusion, the results strongly suggest that some of the lindane-induced negative inotropic and chronotropic-like effects on the contraction are due to an increased I_{Kr} while the decrease in dC/dt_{max} (i.e. the rate of cross-bridge formation) results from lindane oxidative properties.

Key words: Heart muscle — Peak tension — Lindane — I_{Kr} increase — Oxidative stress

Introduction

Lindane, a γ-isomer of hexachlorocyclohexane used to eradicate insects in agriculture until the mid-1970s to control malaria and to treat lice infection in humans, poultry and livestock, is still widely spread in the environment due to the long life duration of the molecule (Wauchope et al. 1992). The literature on the potential effects of the pesticide on cardiac function is rather limited (Sauviat and Pagès 2002) but recent findings brought new insights. Kolbasin et al. (1993) reported that an oral treatment of male rats by high doses of lindane (1/20 of the lethal dose 50, i.e. 1/20 of the dose required to kill half the members of the tested population) for 30 days, associated with a physical load, produced cardio-vascular dystrophy, contracture, degeneration and necrosis predominantly in the left ventricle (LV) wall. In a subchronic study using low doses of lindane (3 mg/kg/day for 6 weeks), Anand et al. (1995) showed an increased $^{45}Ca^{2+}$ influx in rat atrial trabeculae and papillary muscles while Ca^{2+},K^+-ATPase activity decreased, suggesting that cellular Ca^{2+} homeostatic mechanisms were involved in the cardiovascular effect of the pesticide. However, Buck et al. (1999) reported that lindane concentrations ranging from 1 to 100 μmol/l did not depress the peak of intracellular Ca^{2+} transient in guinea pig myocytes and did not interact directly with the ryanodine receptor Ca^{2+} release channels from cardiac sarcoplasmic reticulum (SR) vesicles. In voltage-clamped frog atrial myocytes, we showed that micromolar concentrations of lindane increased the rapid delayed outward K^+ current (I_{Kr}), sensitive to E-4031 (Sauviat et al. 2002a). Similarly, we reported that lindane acute applications (3.4 to 68 μmol/l) to control rat hearts, *in vitro*, shortened LV papillary muscle (PM) action potential (AP) duration; this shortening, suppressed by quinidine or E-4031, was again

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attributed to a lindane-induced increase in I_{Kr} (Sauviat et al. 2005). We also studied the effect of very low doses of lindane (compatible with human exposure), *in vivo*, in offspring rats chronically exposed during their whole life (i.e. *via* maternal organism during gestation and lactation, then through beverage until sacrifice) to lindane trace concentrations (0.5–2 ppb). We observed that all the concentrations tested modified the heart shape which showed a "cherry-like aspect" (Sauviat and Pagès 2002; Sauviat et al. 2005). In addition, for the higher concentration (2 ppb), both the cardiac LV tissue morphology and PM number were altered (Sauviat et al. 2005). In the 2 ppb-treated group, the repolarization of the LVPM AP was shortened as a result of a I_{Kr} increase (Sauviat et al. 2005). Lindane (2 ppb) increased also the background inward rectifier K^+ current (I_{K1}) sensitivity to decreased temperature but did not affect Na^+,K^+-ATPase activity (Sauviat et al. 2005). By the meantime, Ananya et al. (2005) showed, in the heart of rats fed with higher doses of lindane (1.5 and 7 mg/kg/day for 21 days), that the two doses of pesticide induced both oxidative stress and lipid peroxydation, and decreased the reduced glutathione level, whereas superoxide dismutase and catalase activities were increased only in the 7 mg/kg/day fed group. These authors also reported histopathological changes of cardiac tissues including interstitial oedema in the myocardium at the two doses, and ultrastructural changes consisting in a loss of integrity of myofibrils, Z-band disruption and mitochondrial damage. Finally, we recently reported that cardiovascular morphological and functional alterations were genetically transferred by treated males to the next generation (Boulesteix et al. 2006; Sauviat et al. 2006). Cardiac alterations were associated to basic functional differences in offspring LVPM electrical activity, including altered sensitivity to Ca^{2+} of both Ca^{2+}-induced inactivation of L-type Ca^{2+} channels and of small conductance Ca^{2+}-sensitive K^+ channels (Sauviat et al. 2006).

In view of the previous results, the aim of the present work was to study the effect of acute lindane-treatment on the evoked peak contraction of rat LVPM. It is worth noting that the contraction strongly depends on the Ca^{2+}-induced Ca^{2+} release by the SR (Fabiato and Fabiato 1978) whereas the relaxation is markedly dependent (13-fold *vs.* 2.5-fold for the Na^+-Ca^{2+} exchanger) on SR refilling *via* the Ca^{2+}-ATPase (Bers 1997). An integrative hypothesis for the mechanism of action of lindane is proposed.

Materials and Methods

Animals

Experiments were performed at 32°C on cardiac LVPM of 6 weeks old female Sprague Dawley rats (Charles River Laboratory, L'Arbresle, France) anesthetized by intra-peritoneal injection of sodium pentobarbital (100 mg/kg). In conducting this study, investigators adhered to the Guide for the Care and Use of Laboratory Animals, 1993.

Solutions

The composition of the standard Tyrode solution was (in mmol/l): NaCl 137; $CaCl_2$ 2.5; KCl 5.4; $MgCl_2$ 1; NaH_2PO_4 0.33; HEPES buffered to pH 7.35 with NaOH 10; glucose 10; bubbled with 100% O_2. E-4031 (Alomone Labs, Jerusalem, Israel) was used to selectively block I_{Kr} (Sanguinetti and Jurkiewicz 1990; Snyders 1999; Nerbonne 2000). Lindane (Merck GmbH) was dissolved in acetone.

Mechanical activity recordings

Mechanical activity was recorded by a glass lever using a natural silk thread fixed at one end to a transducer system (Pixie 8101, Endevco) and attached by the other end to the LVPM tendon. LVPM were electrically stimulated (at 1 or 3.3 Hz) by square pulses (5 ms duration) delivered through bipolar earth-isolated platinum electrodes, using an opto-electric coupling device. The following parameters of the contraction were measured: dC/dt_{max} (maximum rate of rise of the initial phase of its ascending phase); C_{50} (duration at 50% of its maximal amplitude); τ_{relax} (time constant of the decay of the relaxation phase) measured by exponential fit of the tension traces (Sauviat et al. 2002b). Contraction forces were displayed and stored on Nicolet 310 oscilloscope (Nicolet, Madison, WI, USA), and entered on the mass storage of a desk computer using Acquis 1 software (G. Sadoc, CNRS, Gif/Yvette, France).

Statistical analysis

Data are expressed as mean values ± S.E.M.; n corresponds to the number of LVPM tested. Comparison between groups was made using paired Student's t-test delivered by the software Sigmaplot (Jandel, Erkrath, Germany); $p < 0.05$ was considered statistically significant.

Results

Effects of lindane on the contraction

Lindane (6.8 μmol/l) addition to the Tyrode solution produced a decrease of the amplitude of the LVPM contraction evoked at 1 Hz (Fig. 1A). Lindane concentrations of 3.4 and 6.8 μmol/l reduced the maximal amplitude by 26 ± 7% ($n = 4$) and 35.6 ± 4.0% ($n = 8$), respectively, within 5 min. Table 1 summarizes the effects of lindane on the different

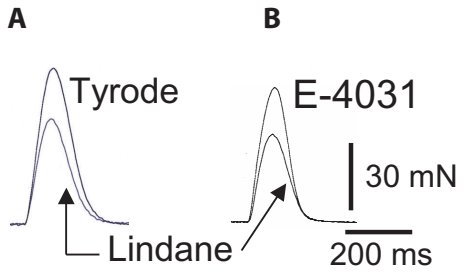

Figure 1. Effects of lindane (6.8 μmol/l) on the contraction of rat left ventricular papillary muscle. Traces recorded (32°C, 1 Hz) in the Tyrode solution before and after 5 min lindane application (A), and in the Tyrode solution containing lindane before and 5 min after subsequent addition of 10 nmol/l E-4031 (B).

Table 2. Effects of frequency of stimulation changes on contraction parameters of rat LVPM recorded in the Tyrode solution before (control) and after (5 min) lindane (6.8 μmol/l) application

Treatments	Control (n = 7)	Lindane (n = 7)
% maxi peak reduction [b/a]	50.2 ± 7.1	47.9 ± 5.8
srp [c-b/a]	1.52 ± 0.27	2.17 ± 0.41*
τ_{srp} (s)	4.64 ± 0.59	5.44 ± 0.46*
prp [d-e/e]	3.14 ± 0.65	4.24 ± 0.62*
τ_{ns} (s)	6.21 ± 0.52	6.43 ± 0.50

a, b, c, d, e are the amplitude of the different parameters shown in Fig. 2A; srp, slow recovery phase; prp, post rest potentiation; τ_{srp} and τ_{ns}, time constants of the srp and of the negative staircase, respectively; * $p < 0.05$, paired values for lindane *vs.* control.

Table 1. Effects of lindane (3.4 and 6.8 μmol/l) on rat LVPM contraction parameters recorded in the control Tyrode solution (32°C; 1 Hz) before and after (5 min) cumulative addition of lindane (6.8 μmol/l) and subsequent addition of E-4031 (10 nmol/l) to the solution containing lindane

Treatments	Control (n = 8)	Lindane (3.4 μmol/l) (n = 5)	Lindane (6.8 μmol/l) (n = 8)	E-4031 (10 nmol/l) (n = 5)
dC/dt_{max} (N/s)	1.57 ± 0.17	1.44 ± 0.47	1.37 ± 0.15	0.95 ± 0.22
C_{50} (ms)	91.1 ± 6.6	69.0 ± 13.6*	69.2 ± 6.2*	97.9 ± 10.5#
τ_{relax} (ms)	60.3 ± 3.9	46.3 ± 7.6*	47.7 ± 4.1*	69.6 ± 8.5#

dC/dt_{max}, maximum rising rate of the contraction ascending phase; C_{50}, duration of the contraction measured at 50% of its maximal amplitude; τ_{relax}, time constant of the relaxation phase decay. Mean values ± S.E.M. of n muscles. * $p < 0.05$, lindane (3.4 and 6.8 μmol/l) *vs.* control; # $p < 0.05$, E-4031 *vs.* lindane (6.8 μmol/l).

parameters of the contraction. It shows that, compared to controls, lindane (3.4 μmol/l) slowed dC/dt_{max} by 8% and significantly decreased both C_{50} and τ_{relax} ($p < 0.05$). The presence of 6.8 μmol/l lindane in the Tyrode solution further slowed dC/dt_{max} by 13%. Subsequent addition of the I_{Kr} blocker E-4031 (10 nmol/l) to the Tyrode solution containing lindane (6.8 μmol/l) increased the amplitude of the contraction (Fig. 1B). In five muscles, E-4031 application restored the amplitude of the peak tension by 38.5 ± 9.5%. Table 1 shows that after subsequent E-4031-treatment, both C_{50} and τ_{relax} significantly ($p < 0.05$) recovered while dC/dt_{max} did not.

Effect of stimulation frequency changes

In the Tyrode solution, increasing the stimulation frequency from 1 to 3.3 Hz (Fig. 2A,B) decreased the contraction amplitude of the first beat. This decrease was followed by a slow recovery phase (srp), attributed to an activation of the Na^+-Ca^{2+} exchanger in the reverse mode, which reached a steady-state with a time constant (τ_{srp}). After returning to a 1 Hz frequency, a positive post rest potentiation (prp) developed, attributed to SR Ca^{2+} refilling ability, and was followed by a negative staircase (ns), meaning that the amplitude of the peak contraction declined gradually with a time constant (τ_{ns}) (Fig. 2A,B). Increasing the stimulation frequency from 1 to 3.3 Hz after the addition of 6.8 μmol/l lindane to the Tyrode solution produced similar effects (Fig. 2C). Table 2 shows that, compared to the Tyrode solution, lindane affected similarly the contraction amplitude of the first beat, but significantly ($p < 0.05$) increased srp (43%), τ_{srp} (17%), and prp (35%) while τ_{ns} was not modified.

Discussion

In the present study, we demonstrate that acute lindane-treatment of rat LVPM exerts a negative inotropic effect and a negative chronotropic-like effect on the evoked contraction, i.e. decreases the contraction amplitude and duration, respectively.

Our results show that lindane decreased the amplitude of the peak contraction, dC/dt_{max}, C_{50}, and τ_{relax}. These

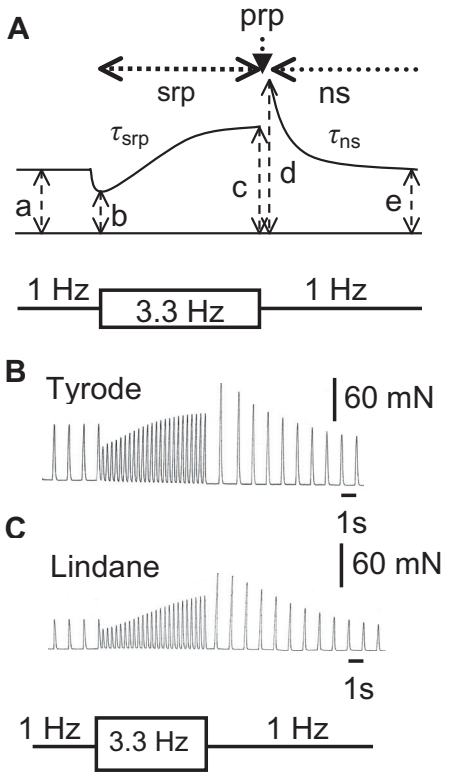

Figure 2. Effects of changes in the frequency of stimulation on the contraction recorded in the absence and in the presence of lindane (6.8 μmol/l) in the Tyrode solution (32°C). **A.** Schematic representation of the contraction decay (upper traces) in response to the experimental protocol (lower traces). Starting from 1 Hz, the stimulation frequency was increased to 3.3 Hz until the peak contraction reached a steady-state value and was then lowered back to 1 Hz. Increasing the frequency to 3.3 Hz produced an initial fast decrease in the amplitude (b) of the evoked twitch contraction (a) which reached a maximum value at the first beat and was followed by the development of a srp reaching a steady-state (c) with a time constant τ_{srp}. Returning back to 1 Hz triggered a positive prp measured at the first beat (d) followed by ns phenomenon which reached a steady amplitude (e) with a time constant τ_{ns}. **B.** Tyrode solution. **C.** Tyrode solution containing lindane.
srp, slow recovery phase; prp, post rest potentiation; ns, negative staircase.

effects, except for dC/dt_{max} which did not recover, were reversed by a subsequent application of E-4031, a blocker of I_{Kr}. E-4031 has already been reported to restore the AP duration previously shortened by lindane-treatment (Sauviat et al. 2002a, 2005). Therefore, it is likely that some of the negative inotropic and chronotropic-like effects induced by acute lindane on the contraction are concomitant to the pesticide-induced AP duration shortening, as the result of an increased I_{Kr}. Although the molecular mechanism by which lindane increases I_{Kr} remains to be elucidated in rat (see Sauviat et al. 2005), our previous results obtained by a transmembrane current analysis in frog strongly suggest that proteinkinase-dependent pathways, rather than direct activation of K^+ channels, are involved (Sauviat et al. 2002a).

Rat heart muscle contractile performance strongly depends on the SR Ca^{2+}-induced Ca^{2+} release, and the relaxation on the SR Ca^{2+} reuptake *via* the Ca^{2+}-ATPase rather than the Na^+-Ca^{2+} exchanger. This mechanism is well developed in rat ventricle in which high SR-Ca^{2+}-activity favours SR-Ca^{2+} loading and thus high Ca^{2+} release (Fabiato and Fabiato 2006). In the present study, dC/dt_{max} reflects the net rate of cross-bridges formation. The rapid equilibration of troponin C (TnC) with Ca^{2+} is accompanied by a rapid increase in the rate of cross-bridge formation until a maximum rate is reached, and an equilibrium may be established very early between free cytosolic and TnC-bound Ca^{2+} (Penefsky 1994). Binding of Ca^{2+} to TnC during systole induces conformational changes that relieve the inhibitory influence of cardiac troponin I, the "inhibitory" unit of the troponin complex associated with thin filaments, thereby promoting formation of actomyosin cross-bridges and contraction (Solaro 2001). Our results show that lindane decreased dC/dt_{max} which did not recover after E-4031 treatment. It has been reported that lindane did not depress the peak of intracellular Ca^{2+} transient in guinea-pig myocytes and did not interact directly with the ryanodine receptor Ca^{2+} release channels from rat cardiac SR vesicles (Buck et al. 1999). These observations suggest that the decrease of dC/dt_{max} cannot be attributed to an inhibition of the SR Ca^{2+} release. Since lindane caused oxidative stress and disrupted the Z-bands in the rat heart (Ananya et al. 2005), it can be assumed that the pesticide may affect the equilibration of TnC with Ca^{2+}. This possible mechanism is in agreement with previous works showing that lindane (10 μmol/l) blocked intracellular gap junctions (GJ) by activating protein kinase C and by inducing oxydative stress (Bagchi et al. 1995; Krieger and Loch-Caruso 2001), and not by increasing the intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ (Criswell and Loch-Caruso 1995b). In rat uterine cells, non cytotoxic lindane concentrations (8 to 64 μmol/l) inhibited both GJ and contraction (Criswell and Loch-Caruso 1995a), with a concomitant arachidonic acid release (Wang et al. 2001). In Sertoli cell lines, lindane abolished GJ by decreasing and redistributing membrane connexin C43 towards the perinuclear sarcoplasmic area (Defami et al. 2001). It should be noticed that connexin C43 is present in mammalian heart muscle and that connexins are involved in the synchronization of the different ionic currents involved in cardiac rhythm. GJ disorganization and/or reduction of connexin C43 are associated with arrhythmias in pathological human ventricle (Sever et al. 2001; Van Veen et al. 2001; Van der Velden and Jongsma 2002). Therefore, the oxydative properties (Ananya et al. 2005) of the pesticide may account for the lindane-induced decrease of dC/dt_{max} caused by either disruption of Z-band or GJ inhibition.

Rat LVPM contraction mostly depends on the functional state of the SR at low frequency, whereas at high frequency, the systolic force is more dependent on the Ca^{2+} influx entering the cells *via* Ca^{2+} channels (Stemmer and Akera 1986). The results show that increasing the stimulation frequency of rat LVPM reduced the amplitude of the peak contraction at the first beat by the same value in the absence and in the presence of lindane, suggesting that the pesticide did not change: i) SR-Ca^{2+} release, and ii) the frequency-dependent properties of Ca^{2+} current. The frequency-dependent modulation of rat AP ventricular cells depends on L-type Ca^{2+} channels, a process thought to be related to SR-Ca^{2+} release and to occur through beat to beat adaptation of Ca^{2+}-dependent inactivation of the current (Fauconnier et al. 2003). High frequency stimulation increases internal Na^+ concentration and promotes Ca^{2+} influx *via* activation of the Na^+-Ca^{2+} exchanger in the reverse mode, thus contributing to increase the force (Shattock and Bers 1989). Lindane (6.8 μmol/l) decreased the amplitude of the evoked peak contraction without affecting the Na^+-Ca^{2+} exchanger in frog auricle (unpublished observations). High frequency stimulation shortened the diastole duration, resulting in reduced SR Ca^{2+} reuptake *via* Ca^{2+}-ATPase, leading to an increase in $[Ca^{2+}]_i$ and thus to a progressive increase in the amplitude of the contraction. The result showing that srp amplitude increased in the presence of lindane reveals that an increase in $[Ca^{2+}]_i$ occurred in the vicinity of the contractile elements. Interestingly, several studies have also reported an increase in $[Ca^{2+}]_i$ induced by lindane in various cells that was attributed to: i) Ca^{2+} channel opening in human spermatozoids (Silvestroni et al. 1997), ii) Ca^{2+} release from the endoplasmic reticulum in trout phagocytes (Betoulle et al. 2000), iii) Ca^{2+} release from endoplasmic reticulum, mitochondria and others Ca^{2+} stores in canine kidney cells (Lu et al. 2000), and iv) an initial influx of Ca^{2+} responsible for Ca^{2+}-induced Ca^{2+} release *via* inositol 1,4,5-trisphosphate produced by phospholipase C in mouse peritoneal macrophages (Pinelli et al. 1994). The lengthening of τ_{srp} may be supported by the assumption that lindane affects the equilibration of TnC with Ca^{2+}. However, lindane increased prp magnitude and did not modify τ_{ns}. This indicates that the SR Ca^{2+} reuptake was not affected by the pesticide, and suggests that the SR Ca^{2+}-ATPase activity was not sensitive to lindane.

Conclusion

Our results show that micromolar concentrations of lindane produced negative inotropic and chronotropic-like effects on the contraction of rat LVPM which may be attributed, in part, to the increase in I_{Kr}, as previously shown on LVPM AP duration (Sauviat et al. 2002a; 2005), and to a blockade of the rate of cross-bridge formation which may be related to the oxidative properties (Ananya et al. 2005) of the molecule.

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