Does lindane (gamma-hexachlorocyclohexane) increase the rapid delayed rectifier outward K+ current (IKr) in frog atrial myocytes?

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Research article

Does lindane (gamma-hexachlorocyclohexane) increase the rapid delayed rectifier outward K^+ current (I_{Kr}) in frog atrial myocytes?

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Abstract

Background: The effects of lindane, a gamma-isomer of hexachlorocyclohexane, were studied on transmembrane potentials and currents of frog atrial heart muscle using intracellular microelectrodes and the whole cell voltage-clamp technique.

Results: Lindane (0.34 microM to 6.8 microM) dose-dependently shortened the action potential duration (APD). Under voltage-clamp conditions, lindane (1.7 microM) increased the amplitude of the outward current (I_{out}) which developed in Ringer solution containing TTX (0.6 microM), Cd^{2+} (1 mM) and TEA (10 mM). The lindane-increased I_{out} was not sensitive to Sr^{2+} (5 mM). It was blocked by subsequent addition of quinidine (0.5 mM) or E-4031 (1 microM). E-4031 lengthened the APD; it prevented or blocked the lindane-induced APD shortening.

Conclusions: In conclusion, our data revealed that lindane increased the quinidine and E-4031-sensitive rapid delayed outward K^+ current which contributed to the AP repolarization in frog atrial muscle.

Background

Lindane, a gamma-isomer of hexachlorocyclohexane has largely been used as an insecticide and is widely spread in the environment due to the long life time of the molecule [1]. Absorbed by the respiratory, digestive or cutaneous pathways, it accumulates in tissues in the following order: fat > brain > kidney > muscle > lung > heart > spleen > liver > blood [2]. Lindane stimulates the synaptic transmission of a large number of muscular and nerve preparations, and suppresses the GABA-activated chloride current [3] by interacting with the receptor GABA-chloride channel complex [4]. Due to the similarity between lindane and inositol 1, 4, 5 triphosphate (IP_3) [5], it has been suggested that lindane releases Ca^{2+} from IP_3-sensitive intracellular stores in macrophages [6] and smooth myometrial muscle cells [7]. Lindane transiently depolarizes the membrane, opens Ca^{2+} channels thus increasing the intracellular Ca^{2+} concentration, and subsequently triggers Ca^{2+}-activated K^+ current ($I_{K\text{-}Ca}$) in human sperm [8]. Lindane (1 microM – 100 microM) does not depress the peak of intracellular Ca^{2+} transient in guinea pig myocytes, and does not interact directly with the ryanodine receptor Ca^{2+} release channels from cardiac sarcoplasmic reticulum vesicles [9]. A Ca^{2+} release from the endoplasmic reticulum, mitochondria and other Ca^{2+} stores has been reported in the presence of lindane (0.15 mM) in cat

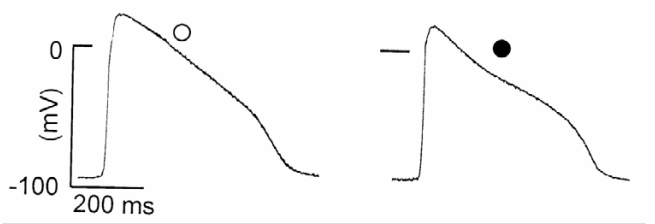

Figure 1
Effect of lindane on spontaneously beating frog auricular action potential (AP) AP recorded intracellularly on the same auricle in the standard Ringer solution (white circle) before and 5 min after application of lindane (3.4 microM; black circle).

kidney cells [10]. Lindane (30 microM) has no effect on the L-type Ca^{2+} current, but suppresses the activity of large conductance Ca^{2+}-activated K^+ channels and increases the firing rate of spontaneous action potentials in rat pituitary GH(3) cells [11].

Little is known about the effect of the pesticide on cardiac tissues. The aim of the present work was to study the effect of lindane on the action potential and transmembrane currents of frog auricular heart muscle.

Results

Intracellular recordings of transmembrane potentials show that the addition of lindane (3.4 microM) to the Ringer solution did not alter the RP, decreased the amplitude of the OS and shortened the plateau duration (Fig. 1). The effects of lindane on the AP were dose-dependent. Table 1 shows that lindane (0.34 microM to 6.8 microM) did not significantly modify RP; lindane (0.34 microM) slightly but significantly ($P < 0.05$) shortened APD_{40} and APD_{10} by 6% and 3%, respectively. APD_{40} and APD_{10} shortening was not significantly increased by increasing the lindane concentration to 6.8 microM. APD_0 was only significantly shortened ($P < 0.05$) in the presence of lindane 3.4 microM in the Ringer solution (Table 1). Under voltage-clamp conditions, the remaining currents recorded in the Ringer solution containing TTX (0.6 microM), Cd^{2+} (1 mM) and TEA (10 mM) (control solution) mainly corresponded to the leak current and to the background inward rectifier K^+ current (I_{K1}) (Fig. 2A). Current-voltage relationships plotted for the current measured at the end of the clamp step potential (V) show that the current was inward (I_{in}) at V more negative than HP and outward (I_{out}) at V more positive than HP (Fig. 2B). The addition of lindane (1.7 microM) to the control solution increased I_{out} but did not alter the tail current (Fig. 2A). Current-voltage relationships of Fig. 2B show that lindane (1.7 microM) increased the amplitude of I_{out} which developed at membrane potentials more positive than -70 mV. Subsequent addition of Sr^{2+} (5 mM) to the control solution containing lindane (1.7 μM) decreased the amplitude of I_{out} in the membrane potential range of -120 mV to +30 mV (Fig. 2B), whereas further addition of quinidine (0.5 mM) to the solution containing both, lindane and Sr^{2+}, suppressed the remaining I_{out} whatever the membrane potential tested (Fig. 2B). Lindane (1.7 microM) increased the magnitude of I_{out} which developed when I_{K1} was blocked by the addition of Ba^{2+} (2 mM) to the control solution (Fig. 3A). Current-voltage relationships show that the lindane-increased I_{out} developed at membrane potentials more positive than -20 mV (Fig. 3B). Subsequent addition of E-4031 (1 microM) to the control solution containing lindane blocked the lindane-increased I_{out} (Fig. 2A) whatever the membrane potential studied (Fig. 3B). The addition of E-4031 (2 microM) to the Ringer solution did not modify RP but prolonged APD (Fig. 4Aa) and further addition of lindane (3.4 microM) to the solution containing E-4031 (2 microM) did not modify the APD (Fig. 4Ab). Conversely, the addition of E-4031 (2 microM) to the Ringer solution containing lindane (3.4 microM) lengthened APD_0, APD_{40} and APD_{10} (Fig. 4B).

Discussion

The present study shows that micromolar concentrations of lindane shortened the action potential duration APD and increases a quinidine and E-4031-sensitive outward current in frog auricle.

Our data show that the shortening of the duration of the repolarizing phase (APD_{40} and APD_{10}) of the AP is the first significant event occurring in response to the application of a lindane concentration as low as 0.34 microM. This effect is then followed by a shortening of the plateau duration APD_0 which is clearly visible only at a ten times higher concentration.

Voltage-clamp experiments indicate that lindane increases an outward current (I_{out}). This current develops in the presence of TEA, known to block the delayed K^+ current, in the control solution and under conditions where Ca^{2+} current has previously been blocked by Cd^{2+}, suggesting that a lindane-increased Ca^{2+} influx may not be directly involved in the development of I_{out}. The lindane-increased I_{out} cannot be attributed to the opening of lindane-induced ionic channels since lindane has been shown to be devoid of ionophoretic properties in planar lipid bilayers [9]. Our data show that the lindane-increased I_{out} still persists in the presence of Sr^{2+} which is known to block the background I_{K1}[12] and $I_{K\text{-}Ca}$[13] currents in cardiac tissues. Our findings reveal that quinidine inhibits the effect of lindane on I_{out}. Quinidine is an open channel blocker of the cardiac rapid delayed rectifier K^+ current (I_{Kr}) [14–17]. In addition, they show that micromolar concentrations of E-4031, a specific blocker of I_{Kr}

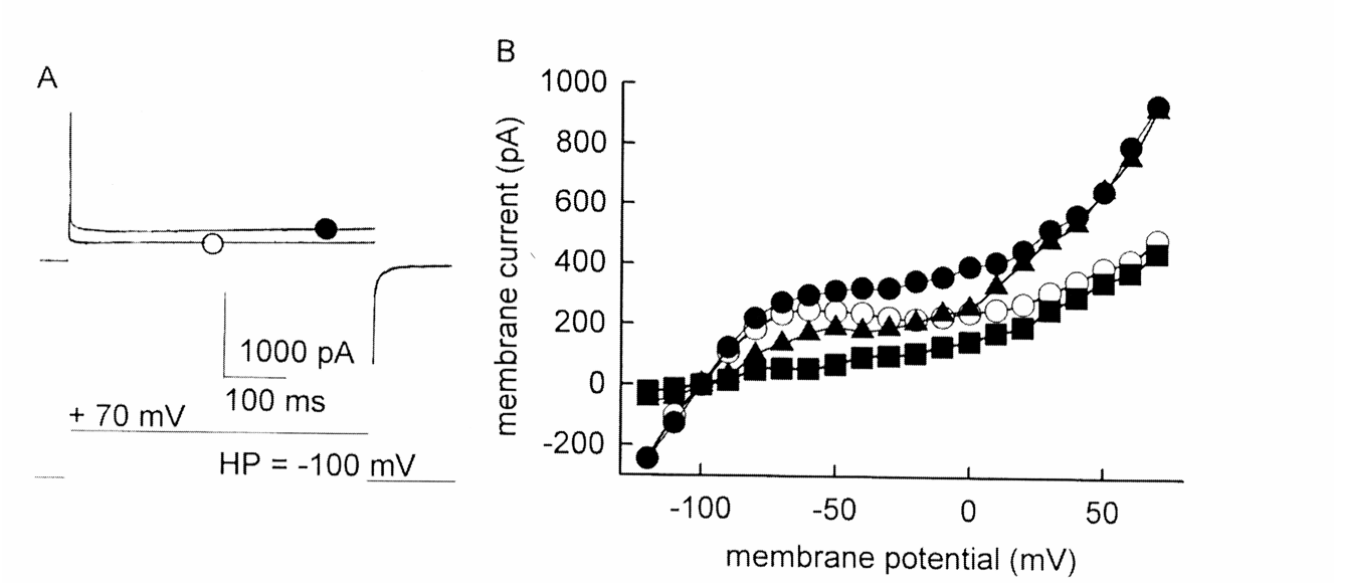

Figure 2
Effects of lindane on frog atrial myocytes membrane current Membrane current was recorded on voltage-clamped frog atrial myocytes bathed in a control Ringer solution containing TTX (0.6 microM), Cd^{2+} (1 mM) and TEA (10 mM). A). Superimposed traces of the current (upper traces) elicited by a 170 mV depolarizing step potential applied from HP = -100 mV (lower trace). (white circle) control solution; (black circle) control solution containing lindane (1.7 microM) B). Current-voltage relationships plotted for the outward current measured at the end (500 ms) of the clamp potential steps, HP = -100 mV. (white circles) control solution; (black circles) control solution containing lindane (1.7 microM); (black triangles) control solution containing lindane and Sr^{2+} (5 mM); (black squares) control solution containing lindane, Sr^{2+} and quinidine (0.5 mM).

[14,15], prolong the APD in frog auricle, are able to prevent or to reverse the APD shortening induced by lindane and in addition suppressed the lindane-increased I_{out}. These observations indicate that I_{Kr} participates to the AP repolarization in frog auricular cells, as in mammalian cardiac tissues [18,19]. This current is sensitive to quinidine and E-4031 but, as reported in rabbit ventricular cells [20], it is not sensitive to Sr^{2+} or Ba^{2+}. The data also reveal that lindane increases I_{Kr}. The mechanism by which lindane increases I_{Kr} is probably not the result of a direct activation of the channel I_{Kr}, believed to be encoded by the human *ether-a-go-go* related gene (HERG) [21–25], and which is involved in long QT syndrome, a cardiac disorder characterized by syncope, seizure and sudden death which can be congenital, idiopathic or iatrogenic [26]. HERG K^+ channel regulation depends on protein-kinase (PK)-dependent pathways. In guinea pig ventricular myocytes, the shift of the activation of HERG K^+ channel induced by phorbol ester involves a PKA-dependent pathway [27]. A PKC-dependent pathway links a G protein-coupled receptor that activates phospholipase C to modulate the Herg channel in Xenopus oocytes co-expressing the channel and tyrotropin releasing hormone receptor [28]. According to Heath and Terrar [29], I_{Kr} is thought to be regulated by PKC which is activated by beta-adrenoceptors stimulation in guinea-pig ventricular myocytes. Lindane activates PKC activity in rat brain and liver tissues [30]. In addition, it has been shown that dynamic regulation of the Herg K^+ channels may be achieved via receptor-mediated changes in phosphatidyl inositol bisphosphate (PIP2) concentrations; elevated PIP2 accelerated activation and slowed inactivation kinetics [31]. But single exposure of rats to lindane (100 mg / kg) did not cause any significant change in phosphoinositide levels in erythrocyte membrane and cerebrum 2 or 24 h after exposure [32].

Conclusions

In conclusion, the results presented show for the first time that the rapid delayed outward current I_{Kr}, involved in the repolarization of the cardiac AP, is increased by micromolar concentrations of lindane and may be responsible for the alterations of the AP duration induced by the pesticide. Although the mechanism by which lindane may increase I_{Kr} remains to be elucidated, the consequences of the effect of lindane on I_{Kr} are of toxicological interest since this current is involved in cardiac disorder.

Materials and methods

Experiments were performed at 20–21°C on quiescent whole auricle isolated from frog heart and on myocytes isolated enzymatically from the auricle.

Table 1: Effect of lindane on spontaneously beating frog atrial action potential (AP) AP was recorded using intracellular microelectrodes before and after successive and cumulative addition of lindane to the Ringer solution (control).

Treatments	RP (mV)	OS (mV)	APD_0 (ms)	APD_{40} (ms)	APD_{10} (ms)
control	-90.3 ± 2.0	24.3 ± 1.2	162 ± 9	450 ± 13	532 ± 13
lindane 0.34 microM	-90.0 ± 1.5	23.1 ± 1.3	157 ± 8	422 ± 5*	514 ± 6*
lindane 1.7 microM	-91.9 ± 1.1	26.9 ± 1.4	154 ± 8	420 ± 5	510 ± 5
lindane 3.4 microM	-91.8 ± 1.0	20.7 ± 1.3	128 ± 7+	424 ± 6	507 ± 5
lindane 6.8 microM	-92.7 ± 1.7	18.0 ± 1.7	111 ± 12	423 ± 7	501 ± 9

(RP) resting membrane potential; (OS) amplitude of the overshoot; (APD_0) duration of the AP measured at 0 mV; (APD_{40}) and (APD_{10}) duration of the AP measured at a membrane potential + 40 mV and + 10 mV higher than RP, respectively. The data are mean values ± s. e. mean of 12 AP recorded from 2 different atriums. $P < 0.05$: * lindane 0.34 microM versus control; + lindane 3.4 microM versus lindane 0.34 microM.

Solutions

The composition of the frog standard Ringer solution was (mM): NaCl, 110.5; $CaCl_2$, 2; KCl, 2.5; HEPES-NaOH buffer, 10; pH 7.35. The Ca^{2+}-free solution, used for cells isolation, was obtained by simple Ca^{2+} removal and contained 600 mU / ml type I collagenase (Sigma) and 1.5 mU / ml type XIV protease (Sigma). Tetrodotoxin (TTX; 0.6 microM; Sankyo, Japan) and $CdCl_2$ (1 mM) were added to the standard solution to inhibit the peak Na^+ current (I_{Na}) and L-type Ca^{2+} current (I_{Ca}), respectively. Tetraethylammonium (TEA, Sigma-Aldrich Chimie, Saint Quentin Fallavier, France), quinidine (Sigma-Aldrich Chimie) were used to block delayed K^+ current; E-4031 (Alamone, Jerusalem, Israel) was used to inhibit the rapid delayed outward current; $SrCl_2$ (5 mM) and $BaCl_2$ (2 mM) to block the inward rectifying K^+ current (I_{K1}) and the Ca^{2+}-activated K^+ current ($I_{K\text{-}Ca}$). Lindane (Merck, GmbH) was dissolved in acetone.

Recordings of membrane potentials

Spontaneously beating action potentials (AP) were recorded on quiescent whole auricle, by means of intracellular microelectrodes used in the "floating mode". The tip length (less than 5 mm) of conventional glass microelectrodes (filled with 3 M KCl, 25–30 Mohms resistance, tip potential less than ± 3 mV) was connected to the input stage of the differential voltage follower by means of a thin Ag / AgCl wire. The following AP parameters were measured: RP: resting membrane potential; OS: overshoot, AP duration (APD): APD_0 duration of the plateau measured at 0 mV; APD_{40} and APD_{10}: duration of the AP at the end of the plateau and of the repolarization phase were measured at a membrane potential + 40 mV and + 10 mV higher than RP, respectively [33].

Recordings of membrane currents

Membrane currents were recorded on single myocytes dispersed by enzymatic digestion of the auricle of frog heart [34]. After isolation of the auricle from the heart, the external epithelial sheet surrounding the auricular tissue was carefully detached and removed. The epithelial-free auricle was then pinned at the bottom of an isolating chamber in which the solutions used for the dissociation were maintained at 30°C and gently stirred with a small magnet. The auricle was successively bathed for 30 min : i) in a Ca^{2+}-free Ringer solution, ii) in a Ca^{2+}-free Ringer solution containing ethylene glycol tetra acetic acid (EGTA) neutralised with NaOH (0.1 mM), iii) in a Ca^{2+}-free Ringer solution then, iv) in a Ca^{2+}-free solution containing collagenase and protease. All solutions were filtered and oxygenated. When the tissue was digested, the auricle was rinsed twice (10 min) with a Ca^{2+}-free Ringer solution and then bathed in a standard Ringer solution and kept at 4°C. Before experimentation, cells were dispersed in a Petri dish (outer diameter 33 mm, depth 10 mm, Corning, New-York, USA) filled with Ringer solution (1 ml) by gently shaking the digested auricle. Patch clamp pipettes (Propper Manufacturing glass, id 1.2 mm, wall 0.2 mm, resistance 1.5 to 2.5 Mohms) were filled with a solution containing (mM); KCl, 150; Na2-creatine phosphate, 5; ATP, 5; EGTA neutralised with KOH, 5; HEPES (KOH) buffer, 10; pH = 7.3. The cell current was monitored using an Axopatch 220B amplifier feedback amplifier (Axon Instruments, Foster City, USA). Starting from a holding potential (HP) of -100 mV, the membrane potential (V) was displaced in rectangular steps of 10 mV at a rate of 0.2 Hz. Positive potentials correspond to depolarization, positive currents correspond to outward current [34].

Transmembrane potentials and currents were recorded with a Labmaster acquisition card (DMA 100 OEM, Dipsi, Cachan, France), driven by Acquis 1 software linked to the mass storage of a desk computer (AT 80486 DX 33), and displayed on an oscilloscope Nicolet 310 (Nicolet, Madison, WI, USA).

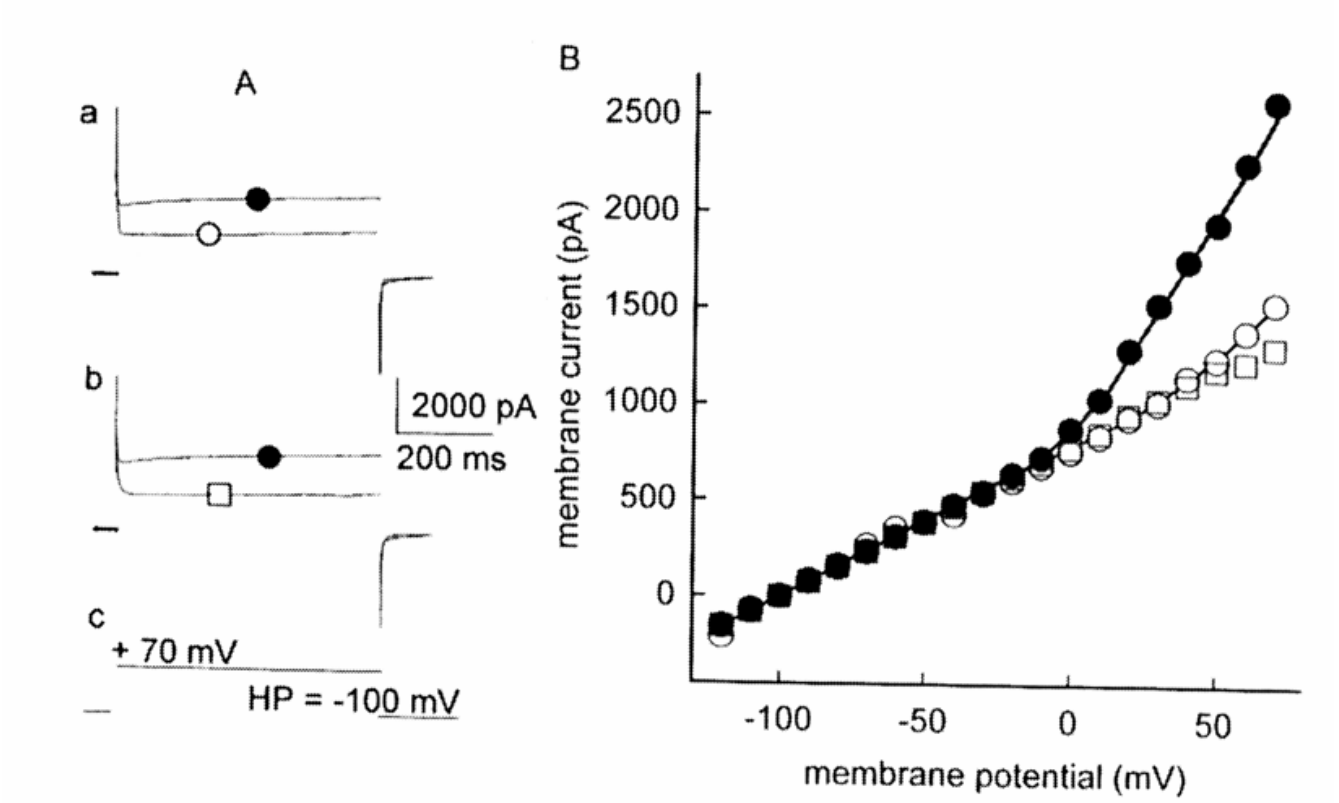

Figure 3
Effects of E-4031 on the lindane-induced outward current Membrane current was recorded on voltage-clamped frog atrial myocytes bathed in a control Ringer solution containing TTX (0.6 microM), Cd^{2+} (1 mM), TEA (10 mM) and Ba^{2+} (2 mM). A). Superimposed traces of the current elicited by a 170 mV depolarizing step potential applied from HP = -100 mV (c) before and after lindane (1.7 microM) application (traces a) and subsequent addition of E-4031 (1 microM) to the solution containing lindane (traces b); (white circle) control solution: (black circle) control solution containing lindane; (white square) control solution containing lindane and E-4031. B). Current-voltage relationships plotted for the outward current measured at the end (500 ms) of the clamp potential steps, HP = -100 mV. (white circles) control solution; (black circles) control solution containing lindane (1.7 microM); (white squares) control solution containing lindane and E-4031 (1 micro M).

Statistical analysis of data

Numerical data are expressed as mean values ± s. e. mean, *n* corresponds to the number of preparations tested. The data were analyzed using the paired Student's *t*-test using Sigmaplot software (Jandel, Erkrath, Germany) and differences were considered significant at $P < 0.05$.

List of abbreviations

AP: action potential

APD: action potential duration

APD_0: duration of the plateau measured at 0 mV

APD_{40}: duration of the AP at the end of the plateau measured at a membrane potential + 40 mV higher than RP

APD_{10}: duration of the AP at the end of the repolarization phase measured at a membrane potential + 10 mV higher than RP

EGTA: ethylene glycol tetra acetic acid

HP: holding potential

IP_3: inositol 1, 4, 5 triphosphate

$I_{K\text{-}Ca}$: Ca^{2+}-activated K^+ current

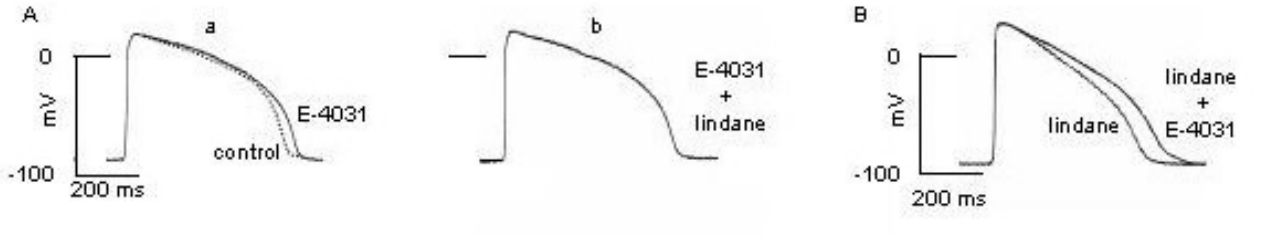

Figure 4
Effects of E-4031 (1 microM) and lindane (3.4 microM) on the action potential (AP). Superimposed traces of the AP recorded on frog auricle using intracellular microelectrodes. A. a): AP recorded before and after addition of E-4031 to the Ringer solution; b) further addition of lindane to the solution containing E-4031. B). AP recorded in the Ringer solution containing lindane before and after further addition of E-4031.

I_{in}: inward current

I_{K1}: inward rectifying K^+ current

I_{out}: outward current

I_{Kr}: rapid delayed outward current

microM: micromolar

mM: millimolar

mm: millimeter

ms: millisecond

mV: millivolt

mU / ml: milliunit per milliliter

OS: overshoot

pA: picoampere

PIP2: phosphatidyl inositol bisphosphate

PK: protein kinase

RP: resting membrane potential

TEA: tetraethylammonium

TTX: tetrodotoxin

V: clamp step potential

References

1. Wauchope RD, Buttler TM, Hornsby AG, Augustijn-Beckers PW, Burt JP: **The SCS/ARS/CES pesticide properties database for environmental decision making.** *Rev. Environ. Contam Toxicol* 1992, **123:**1-155
2. Srinivasan K, Radhakrishnamurty R: **Studies on the distribution of beta- and gamma-isomers of hexachlorocyclohexane in rat tissues.** *J Environ Sci Health B.* 1983, **18:**401-418
3. Narahashi T: **Neuronal ion channels as the target sites of insecticides.** *Pharmacol & Toxicol* 1996, **78:**1-14
4. Pomes A, Rodriguez-Farre E, Sunol C: **Disruption of GABA-dependent chloride flux by cyclodienes and hexachlorocyclohexanes in primary cultures of cortical neurons.** *J Pharmacol Exp Ther* 1994, **271:**1616-1623
5. Parries GS, Hokin-Nevaverson M: **Inhibition of phosphatidylinositol synthase and other membrane-associated enzymes by stereoisomers of hexachlorocyclohexane.** *J Biol Chem* 1985, **260:**2687-2693
6. Pinelli E, Campon C, Tronchere H, Chap H, Teissie J, Pipy B: **Ca(2+)-dependent activation of phospholipases C and D from mouse peritoneal macrophages by a selective trigger of Ca^{2+} influx, gamma-hexachlorocyclohexane.** *Biochem Biophys Res Commun* 1994, **199:**699-705
7. Criswell KA, Stuenkel EL, Loch-Caruso R: **Lindane increases intracellular calcium in rat myometrial smooth muscle cells through modulation of inositol 1, 4, 5-triphosphate-sensitive stores.** *J Pharmacol Exp Ther* 1994, **270:**1015-1024
8. Silvestroni L, Fiorini R, Palleschi S: **Partition of the organochlorine insecticide lindane into human sperm surface induces membrane depolarization and Ca^{2+} influx.** *Biochem J* 1997, **321:**691-698
9. Buck ED, Lachnit WG: **In Pessah: Mechanisms of delta-hexachloracyclohexane toxicity: I. Relationship between altered ventricular myocyte contractility and ryanodine receptor function.** *J Pharmacol Exp Ther* 1999, **289:**477-485
10. Lu CH, Lee KC, Chen YC, Cheng JS, Yu MS, Chen WC, Jan CR: **Lindane (gamma-hexachlorocyclohexane) induces internal Ca^{2+} release and capacitative Ca^{2+} entry in Madin-Darby canine kidney cells.** *Pharmacol Toxicol* 2000, **87:**149-155
11. Wu SN, Li HF, Chiang HT: **Stimulatory effects of delta-hexachlorocyclohexane on Ca^{2+}-activated K^+ currents in GH(3) lactotrophs.** *Mol Pharmacol* 2000, **57:**865-874
12. Shioya T, Matsuda H, Noma A: **Fast and slow blockades of the inward-rectifier K^+ channel by external divalent cations in guinea-pig cardiac myocytes.** *Pflügers Arch* 1993, **422:**427-435
13. Coraboeuf E, Carmeliet E: **Existence of two transient outward currents in sheep cardiac Purkinje fibrers.** *Pflügers Arch* 1982, **392:**352-359

14. Snyders DJ, Knoth KM, Roberts SL, Tamkun HH: **Time-, voltage- and state-dependent block by quinidine of a cloned human cardiac potassium channel.** *Mol Pharmacol* 1992, **41:**322-330
15. Snyders DJ: **Functional and pharmacological diversity of mammalian potassium channels. A molecular approach:** *In "Potassium channels in normal and pathological conditions". (Edited by: J. Verreecke, PP. van Bogaert, F. Verdonck) L University Press, Leuven* 1995, 81-101
16. Snyders DJ: **Structure and function of cardiac potassium channels.** *Cardiovasc Res* 1999, **42:**377-390
17. Tseng GN: **I(Kr): the hERG channel.** *J Mol Cell Cardiol* 2001, **33:**838-849
18. Sanguinetti MC, Jurkiewicz NK: **Two components of cardiac delayed rectifier K^+ current. Differential sensitivity to block by class III antiarrhythmic agents.** *J Gen Physiol* 1990, **96:**195-215
19. Carmeliet E: **Voltage- and time-dependent block of the delayed K^+ current in cardiac myocytes by dofetilide.** *J Pharmacol Exp Ther* 1992, **262:**809-817
20. Paquette T, Clay JR, Ogbaghebriel A, Shier A: **Effects of divalent cations on the E-4031-sensitive repolarization current, I_{Kr}, in rabbit ventricular myocytes.** *Biophys J* 1998, **74:**1278-1285
21. Wang Q, Shen J, Li Z, Timothy K, Vincent GM, Priori SG, Schwartz PJ, Keating MT: **Cardiac sodium channel mutations in patients with long QT syndrome, an inherited cardiac arrhythmia.** *Hum Mol Genet* 1995, **4:**1603-1607
22. Wang Q, Curran ME, Splawski I, Burn TC, Millholland JM, Van Raay TJ, Shen J, Timothy KW, Vincent GM, de Jager T, Schwartz PJ, Toubin JA, Moss AJ, Atkinson DL, Landes GM, Connors TD, Keating MT: **Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias.** *Nat Genet* 1996, **12:**17-23
23. Barhanin J, Lesage F, Guillemare E, Fink M, Lazdunski M, Romey G: **K(V)LQT1 and IsK (minK) proteins associate to form the I(Ks) cardiac potassium current.** *Nature* 1996, **384:**78-80
24. Sanguinetti MC, Jiang C, Curran ME, Keating MT: **A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the IKr potassium channel.** *Cell* 1995, **81:**299-307
25. Sanguinetti MC, Curran ME, Zou A, Shen J, Spector PS, Atkinson DL, Keating MT: **Coassembly of K(V)LQT1 and minK (IsK) proteins to form cardiac I(Ks) potassium channel.** *Nature* 1996, **384:**80-83
26. Witchel HJ, Hancox JC: **Familial and acquired long QT syndrome and the cardiac rapid delayed rectifier potassium current.** *Clin Exp Pharmacol Physiol* 2000, **27:**753-766
27. Kiehn J, Karle C, Thomas D, Yao X, Brachmann J, Kübler W: **HERG potassium channel activation is shifted by phorbol esters via protein kinase A-dependent pathways.** *J Biol Chem* 1998, **273:**25285-25291
28. Barros F, Gomez-Varela D, Viloria CG, Palomero T, Giraldez T, de la Pena P: **Modulation of human erg K^+ channel gating by activation of a G protein-coupled receptor and protein kinase C.** *J Physiol* 1998, **511:**333-346
29. Heath BM, Terrar DA: **Proteine kinase C enhances the rapidly activating delayed rectifier potassium current, IKr, through a reduction in C-type inactivation in guinea-pig ventricular myocytes.** *J Physiol* 2000, **522:**391-402
30. Bagchi D, Bagchi L, Stohs SJ: **Comparative *in vitro* and *in vivo* protein kinase C activation by selected pesticides and transition metal salts.** *Toxicol Let* 1997, **91:**31-37
31. Bian J, Cui J, McDonald TV: **HERG K(+) channel activity is regulated by changes in phosphatidyl inositol 4,5-bisphosphate.** *Circ Res* 2001, **89:**1168-1176
32. Agrawaal D, Subramoiam A, Afaq F: **Influence of hexachlorocyclohexane on phosphoinisitides in rat erythrocyte membranes and brain.** *Toxicology* 1995, **95:**135-140
33. Sauviat M-P, Meunier FA, Kreger A, Molgó J: **Effects of trachynilysin, a protein isolated from stonefish (*Synanceia trachynis*) venom, on frog atrial heart muscle.** *Toxicon* 2000, **38:**945-959
34. Sauviat M-P, Benoit AG, Debitus C, Pouny I, Laurent D: **Alterations of transmembrane currents in frog atrial heart muscle induced by photoexcited gymnochrome a purified from the crinoid, *Gymnochrinus richerii*.** *Photochem Photobiol* 2001, **74:**115-119