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The δ Isomer of Hexachlorocyclohexane Induces Rapid Release of the *myo*-Inositol-1,4,5-trisphosphate-Sensitive Ca^{2+} Store and Blocks Capacitative Ca^{2+} Entry in Rat Basophilic Leukemia Cells

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SUMMARY

Antigenic stimulation of rat basophilic leukemia cells releases Ca^{2+} from internal stores and increases membrane permeability to Ca^{2+}. The δ isomer of hexachlorocyclohexane (δ-HCH) is structurally similar to *myo*-inositol-1,4,5-trisphosphate (IP_3) and is a potent releaser of stored Ca^{2+} from permeabilized cells. This release of Ca^{2+} is not mediated by a competitive interaction with the IP_3 receptor on the Ca^{2+} release channel on the endoplasmic reticulum. In intact cells, δ-HCH and, to a lesser extent, lindane (γ-hexachlorocyclohexane) transiently increase the intracellular Ca^{2+} concentration. The return to basal concentrations is mediated by the plasma membrane Ca^{2+} pumps and not by resequestration of Ca^{2+} into intracellular stores. Treatment of cells with δ-HCH (25–100 μM), but not lindane, leads to a progressive inhibition of the antigen- and thapsigargin-stimulated Ca^{2+} signal. Caffeine, a modulator of the ryanodine receptor Ca^{2+} channel, attenuates the rise in intracellular Ca^{2+} induced by δ-HCH, suggesting that ryanodine receptor-like Ca^{2+} channels may be present in RBL cells. At 25 μM δ-HCH, a concentration that does not inhibit the antigen-stimulated Ca^{2+} signal, the release of [^{3}H]serotonin from antigen-stimulated cells is enhanced as is secretion of [^{3}H]serotonin from cells pretreated with 25–100 μM lindane. The depletion of Ca^{2+} from intracellular stores by δ-HCH should evoke Ca^{2+} entry into the cells by a capacitative mechanism; however, divalent cation permeability across the plasma membrane (Mn^{2+} influx) is not increased but rather is decreased by δ-HCH. An understanding of the mechanism of action of δ-HCH in releasing stored Ca^{2+} and blocking Ca^{2+} influx across the plasma membrane may provide insights into the regulation of capacitative Ca^{2+} entry in nonexcitable cells.

The regulation of $[Ca^{2+}]_i$ is an important function of cells. Interactions between Ca^{2+} permeability pathways in the plasma membrane and Ca^{2+}-sequestering organelles are responsible for the concerted regulation of $[Ca^{2+}]_i$ and, in turn, modulation of Ca^{2+}-dependent reactions. Because the regulation of $[Ca^{2+}]_i$ is related to the health and function of cells, interactions between xenobiotic toxins and key components of the cellular Ca^{2+} signaling cascade may lead to inappropriate regulation of intracellular Ca^{2+} signaling and, as a consequence, altered cellular function.

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The chlorinated alkane pesticide lindane has potent convulsant activity that is attributed to blockade of chloride currents mediated by the transmitter γ-aminobutyric acid at the γ-aminobutyric $acid_a$ receptor complex of inhibitory neurons (1). One isomer of hexachlorocyclohexane, δ-HCH, is structurally similar to IP_3, an important second messenger in the regulation of $[Ca^{2+}]_i$ (2). δ-HCH is a central nervous system depressant (3). Pessah *et al.* (4) demonstrated that δ-HCH is significantly more potent than lindane in modulating the binding of [^{3}H]ryanodine to the Ca^{2+}-induced Ca^{2+} release channels of cardiac SR. The actions of δ-HCH at the cardiac ryanodine receptor are correlated with stimulation of release of Ca^{2+} from cardiac SR vesicles, potent positive inotropy, and contracture in atrial strip preparations. Lindane is approximately 30-fold less potent at increasing atrial contractility and does not produce contracture. Initial experiments indicated that δ-HCH has similar activities toward

ABBREVIATIONS: $[Ca^{2+}]_i$, intracellular concentration of free ionized calcium; HCH, hexachlorocyclohexane; lindane, γ-hexachlorocyclohexane; ER, endoplasmic reticulum; IP_3, D-*myo*-inositol-1,4,5-trisphosphate; PKC, protein kinase C; PMA, phorbol-12-myristate; RBL, rat basophilic leukemia; SERCA, sarcoplasmic and endoplasmic reticulum Ca^{2+}-ATPase; SR, sarcoplasmic reticulum; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; [^{3}H]serotonin, 5-[1,2-^{3}H]hydroxytryptamine binoxalate; fluo-3, Ca^{2+}-sensitive fluorescent dye (K^+ salt).

modulation of the binding of [^{3}H]ryanodine to skeletal and brain receptors but lower potency (4).

The structural similarity of δ-HCH to IP_3 (Fig. 1) raises the important question of whether this chlorinated hydrocarbon influences the phosphoinositide signaling pathway in nonexcitable cells where receptor-activated IP_3 pathways are very important in intracellular Ca^{2+} signaling. In this respect, research has focused on the role of HCH isomers in inhibiting various enzymes in the phosphatidylinositol cascade (5, 6) or in stimulating PKC activity (7). No evidence of a direct action of HCH isomers with intracellular Ca^{2+} stores of nonexcitable cells has been demonstrated. However, lindane has been shown to release Ca^{2+} from intracellular stores in excitable cells, e.g., smooth muscle myocytes (8).

RBL cells, a cell line of transformed mast cells, are an excellent model for studying Ca^{2+} regulation in nonexcitable cells. These cells may also serve as a model for studying how pesticides alter the normal pattern of Ca^{2+} signaling and secretion in immune cells. Stimulation with polyvalent antigen of RBL cells that have been sensitized with IgE bound to high affinity receptors on the plasma membrane results in an increase in the intracellular IP_3 concentration (9) and in the $[Ca^{2+}]_i$ (10, 11). IP_3 has been shown to interact specifically with a receptor localized to the microsomal fraction of RBL cells (12) and to release stored Ca^{2+} from permeabilized RBL cells (13). The identity of the IP_3-sensitive Ca^{2+} store in RBL cells has not been elucidated but is most likely a portion of the ER. Other nonmitochondrial Ca^{2+} stores have not been characterized in RBL cells. Antigen stimulation of RBL cells also leads to an increase in the influx of Ca^{2+} through permeability pathways located in the plasma membrane (14). Antigen-stimulated Ca^{2+} influx is modulated by the membrane potential (11), by the intracellular ATP concentration (15), and by other intracellular signals, such as PKC (16). It also appears that the pathway that allows Ca^{2+} to enter cells during antigen stimulation is the same pathway that allows Ca^{2+} to enter resting nonstimulated cells (17). Ca^{2+} release from ER stores and Ca^{2+} influx across the plasma membrane constitute a Ca^{2+} signal in antigen-stimulated RBL cells that is one of the important components involved in stimulating secretion of allergic and inflammatory mediators.

In RBL cells, a relationship exists between the Ca^{2+} content of intracellular stores and Ca^{2+} influx across the plasma membrane. When luminal Ca^{2+} is passively released from the ER by inhibition of the ER Ca^{2+} pump with thapsigargin, an SERCA inhibitor (18), an unknown signal is produced that stimulates an increase in Ca^{2+} permeability across the plasma membrane (19). Ca^{2+} influx pathways stimulated by both antigen and thapsigargin appear to share many features (19).

The present study demonstrates that the δ isomer of HCH induces a rapid and irreversible release of luminal Ca^{2+} from the Ca^{2+} stores of RBL cells. The depletion of the ER Ca^{2+} store, however, is not associated with increased influx of Ca^{2+} across the plasma membrane. Thus, capacitative Ca^{2+} entry into RBL cells is blocked by δ-HCH. The δ isomer is significantly more active than lindane in releasing Ca^{2+} from intracellular stores and in blocking Ca^{2+} influx, indicating a structural specificity for these actions.

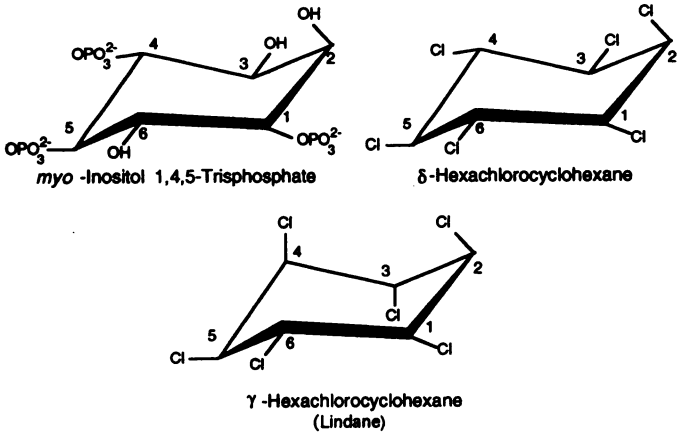

Fig. 1. Structures of IP_3, δ-HCH, and lindane.

Materials and Methods

Cells, IgE sensitization, and saline solutions. All experiments were performed with a secreting subline (2H3) of RBL cells (20) maintained in monolayer culture as described previously (21). When indicated, RBL cells in suspension were sensitized with 1 μg/ml IgE [mouse monoclonal IgE directed against the dinitrophenyl hapten (22), a gift from Barbara Baird and David Holowka, Department of Chemistry, Cornell University] for 1 hr at 37° before the start of the experiments. The stimulating antigen (0.1 μg/ml) was bovine γ-globulin, to which an average of 20 DNP groups/molecule had been coupled (11).

Experiments with intact cells were performed in a modified Tyrode's solution (135 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5.6 mM glucose, 0.05% gelatin, and 10 mM HEPES, adjusted to pH 7.4 with NaOH). Saline solutions used in binding and cell permeabilization experiments are described elsewhere in the text.

Experiments were performed at least three times on different days except for the experiment represented by Fig. 4, which was performed twice. Unless otherwise noted, the figures represent single experiments.

Measurement of intracellular free ionized Ca^{2+} and Mn^{2+} influx. Cells (1×10^6/ml) were suspended in a modified Tyrode's solution and then incubated for 1 hr at 37° with 0.5 μM Fura-2/AM. During this step, 0.1% bovine serum albumin was substituted for gelatin (10) to aid in the loading of Fura-2/AM into the cells, and 250 μM sulfinpyrazone was added to minimize leakage of incorporated dye (23). After Fura-2 loading, the cells were washed and suspended in modified Tyrode's solution containing 250 μM sulfinpyrazone. Three milliliters of cell suspension (1×10^6 cells/ml) were added to quartz cuvettes maintained at 37° and constantly stirred. Fluorescence was monitored with a Perkin-Elmer LS-5B fluorescence spectrophotometer (excitation, 340 nm; emission, 510 nm). $[Ca^{2+}]_i$ was determined as previously described by Mohr and Fewtrell (11). Figures were drawn by computer based on calculated values of the Ca^{2+} concentration derived from the fluorescence measurements.

The entry of Mn^{2+} into RBL cells was assessed through measurement of Mn^{2+}-induced quenching of intracellular Fura-2 fluorescence (24). Fura-2 was incorporated into the cells as described. The excitation wavelength was 362 nm (Ca^{2+}-independent wavelength), and emission was 510 nm. The cells were constantly stirred and maintained at 37°. Mn^{2+} (0.5 mM) was added, and the rate of fluorescence decrease was monitored after the addition of vehicle, HCH isomers, or antigen. At the end of the experiment, ionomycin (0.25 μM) was added to the cells to promote Mn^{2+} influx to estimate the degree of dye saturation by Mn^{2+} during the experiment. At the concentrations used and in the presence of cells, neither δ-HCH nor lindane interfered with the fluorescence of Fura-2 or fluo-3 (see later).

Measurement of Ca^{2+} release from permeabilized cells. RBL cells were suspended (2×10^6/ml) in a saline solution (110 mM

K^+-glutamate, 10 mM NaCl, 2 mM K_2HPO_4, and 20 mM HEPES, adjusted to pH 7.2 with KOH). The saline solution was mixed with Chelex-100 resin (Bio-Rad) for 30 min to lower the free Ca^{2+} concentration; $MgCl_2$ (1 mM) was added after treatment. To this saline solution, 1.8 mM Mg^{2+}-ATP and an ATP-regenerating system (5 mM phosphocreatine and 10 units/ml creatine phosphokinase) were also added. All reagents were Chelex treated before being added; Mg^{2+} was added to the treated solution of ATP. The cells were warmed to 37°, and 0.2 units/ml streptolysin-*O* was added. Permeabilization was monitored by trypan blue dye exclusion. Once the cells were permeabilized (10–15 min), 1 μM fluo-3 was added to the cell suspension, and 3 ml of cells (2×10^6/ml) was added to quartz cuvettes that were maintained at 37° and constantly stirred. Fluorescence was excited at 506 nm, and emission was 526 nm. In these experiments, fluo-3 was used to monitor the Ca^{2+} concentration of the bathing saline solution, which was freely exchanging with the cytosolic compartment of the cells. Therefore, an increase in fluorescence indicated that Ca^{2+} was being released from intracellular stores, and a decrease in fluorescence indicated that Ca^{2+} was being sequestered into stores. The free ionized Ca^{2+} concentration of the bathing saline solution was calculated as previously described by Mohr *et al.* (12).

[^{3}H]IP$_3$ binding to permeabilized RBL cells. Equilibrium binding experiments were performed with permeabilized cells according to the method of Hershey *et al.* (13). Cells (4×10^6/ml) were suspended in a solution [140 mM KCl and 30 mM HEPES, pH 7.4 adjusted with KOH (K^+ saline)]. Streptolysin-*O* (0.2 units/ml) was added to the cell suspension and incubated at 37° for 30 min. The cells were centrifuged and resuspended (1×10^6/ml) into K^+ saline solution containing 1 mM EDTA. The calculated free Ca^{2+} and Mg^{2+} concentrations were <1 nM. Aliquots of cells (1 ml total volume) were added to glass tubes and mixed with 10 μl DMSO (vehicle) or δ-HCH and 10 μl [^{3}H]IP$_3$ (17 Ci/mmol) to give a final concentration of 0.5 nM [^{3}H]IP$_3$. The reaction was allowed to proceed for 30 min at 4° with gentle shaking. Each concentration of δ-HCH was tested in duplicate. Nonspecific binding was measured in the presence of a 100-fold excess of IP$_3$. The reaction was terminated by rapid filtration through Whatman GF/C filters presoaked in ice-cold K^+ saline followed by one wash (2.5 ml) with ice-cold K^+ saline. The filters were placed into a scintillation cocktail and counted with a Beckman scintillation counter. In the experiment discussed, 11% of the [^{3}H]IP$_3$ was bound. The specific binding of [^{3}H]IP$_3$ to permeabilized cells in the presence of different concentrations of δ-HCH was expressed as a percentage of the specific binding of [^{3}H]IP$_3$ to vehicle-treated cells.

Measurement of total [^{3}H]inositol phosphates. The level of [^{3}H]inositol phosphates in RBL cells was determined with ion exchange chromatography (25). Monolayer cultures of RBL cells were incubated for 48 hr with [^{3}H]inositol (4 μCi/ml). After sensitization with IgE, the cells (1×10^6 cells/ml) were suspended in modified Tyrode's solution containing 5 mM lithium chloride in glass tubes (0.2 ml final volume). All experiments were performed in triplicate at 37°. Isomers of HCH (100 μM), antigen (0.1 μg/ml), or vehicle (DMSO) was added to cells for 5 min. The reaction was quenched by the addition of 0.75 ml of a mixture of chloroform/methanol/4 N HCL (100:200:4) to each sample. The mixture was vortexed at room temperature, and 0.5 ml of a mixture of chloroform/0.1 N HCL (1:1) was added. The samples were vortexed and allowed to sit at room temperature for 10 min. The tubes were centrifuged ($400 \times g$ for 5 min at room temperature), and 0.6 ml of the aqueous phase was added to plastic tubes and stored frozen at −90° for up to 2 weeks. Thawed samples (0.5 ml) were added to AG 1-X8 anion exchange columns made from disposable Pasteur pipettes (0.25 g resin/column) (26). Each column was washed three times with 5 mM *myo*-inositol (2 ml) to remove free [^{3}H]inositol. [^{3}H]Inositol phosphates were eluted with 1.5 ml of 1 M sodium formate in 0.1 M formic acid. An aliquot (0.5 ml) of the eluate was counted for ^{3}H by liquid scintillation counting (efficiency of 43%). The results were expressed as the mean total [^{3}H]inositol phosphates (in dpm) released into the aqueous phase.

Secretion assays. Secretion was assessed by measurement of the release of incorporated [^{3}H]serotonin with a standard tube assay as described previously (11). Monolayer cultures were loaded overnight with [^{3}H]serotonin (2 μCi/ml) before trypsinization and sensitization with IgE (1 μg/ml). All experiments were performed in duplicate in glass tubes at 37°. Isomers of HCH were added to the cells (1×10^6/ml) for 10 min before the addition of antigen (0.1 μg/ml). After an additional 55 min, secretion was terminated by the addition of ice-cold quenching solution (135 mM NaCl, 5 mM KCl, and 10 mM HEPES, pH 7.4). An aliquot from each supernatant was removed for determination of [^{3}H]serotonin by liquid scintillation counting. [^{3}H]Serotonin release into the supernatant was expressed as a percentage of the total [^{3}H]serotonin in the cells before stimulation.

ATP assay. Total cellular ATP was measured in pellets of cells with the firefly luciferase assay (Sigma Chemical Co., St. Louis, MO). Luminescence was measured with a Turner TD-20E Luminometer. Total ATP associated with cells was expressed as a concentration assuming a volume of 1 μl for 1×10^6 RBL cells (15).

Cell viability. Release of the cytoplasmic enzyme lactate dehydrogenase was assayed spectrophotometrically (21). Trypan blue exclusion was also used to monitor cell viability.

Reagents. Isomers of HCH were a generous gift of Dr. Robert Joy, Department of Molecular Biosciences, University of California, Davis. Fura-2/AM and fluo-3 were purchased from Molecular Probes (Eugene, OR). D-[1-^{3}H]Inositol-1,4,5-trisphosphate, *myo*-[2-^{3}H]inositol, and [^{3}H]serotonin were purchased from DuPont-NEN (Wilmington, DE). IP$_3$ and thapsigargin were purchased from LC Services Corp. (Woburn, MA). Ionomycin was purchased from Calbiochem (San Diego, CA). Reduced streptolysin-*O* was purchased from Wellcome Reagents (Greenville, NC). All other chemicals were purchased from Sigma Chemical Co. Stock solutions of inositol-1,4,5-trisphosphate and fluo-3 were prepared in distilled water, Fura-2/AM and isomers of HCH were prepared in DMSO, and thapsigargin was dissolved in ethanol. Cells were never exposed to >0.5% ethanol or DMSO.

Results

δ-HCH induced rapid release of Ca^{2+} from permeabilized cells. RBL cells were permeabilized with the bacterial pore-forming toxin streptolysin-*O* (0.2 units/ml), and changes in the free ionized Ca^{2+} concentration in the bathing saline solution were measured with fluo-3. The permeabilized cells were permitted to buffer the ambient Ca^{2+} concentration until a steady state concentration of Ca^{2+} was reached before the addition of agonists. Either 100 μM δ-HCH (Fig. 2A) or 1 μM IP$_3$ (Fig. 2B) induced a rapid release of stored Ca^{2+} from permeabilized RBL cells. The amount of Ca^{2+} released from permeabilized cells was dependent on the δ-HCH concentration (Fig. 2A, inset). The Ca^{2+} concentration of the saline solution bathing the cells stimulated with δ-HCH remained elevated throughout the experiment (Fig. 2A), whereas the Ca^{2+} concentration of the saline solution bathing the cells stimulated with IP$_3$ slowly returned to prestimulatory concentrations (Fig. 2B). In marked contrast to δ-HCH, lindane (100 μM) was significantly less effective at mobilizing Ca^{2+} from permeabilized RBL cells (Fig. 2C). The release of Ca^{2+} from permeabilized cells in response to thapsigargin (100 nM) was slower than the release stimulated by IP$_3$ or δ-HCH and was sustained over the course of the experiment (Fig. 2D). Ionomycin (0.5 μM) was added at the end of the experiment to define the total amount of releasable Ca^{2+} from intracellular stores.

The rapid rates of increase in ambient Ca^{2+} concentration in response to either δ-HCH or IP$_3$ suggested that these

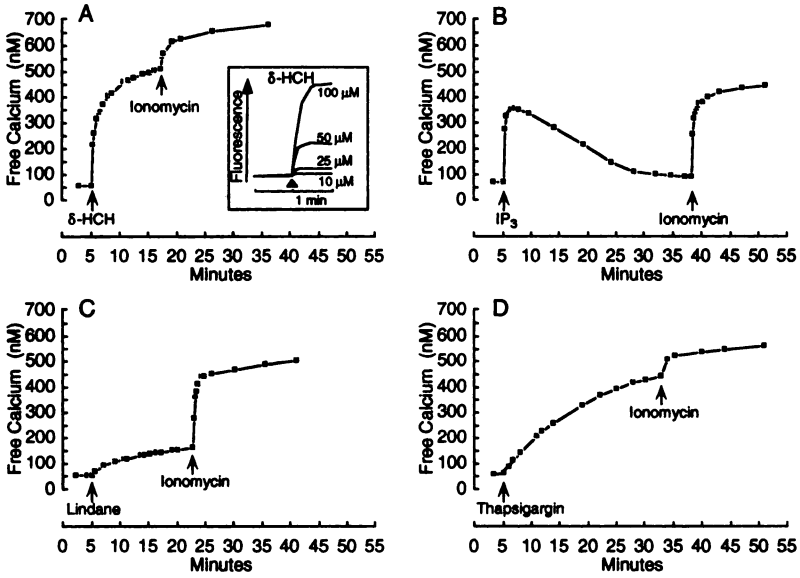

Fig. 2. Isomers of HCH induce the release of Ca^{2+} from permeabilized RBL cells. A, Permeabilized cells incubated with fluo-3 are stimulated with 100 μM δ-HCH. *Inset*, dose-response relationship between δ-HCH (added at *arrowhead*) and Ca^{2+} release (symbols are removed for clarity). B, Permeabilized cells incubated with fluo-3 are stimulated with 1 μM IP_3. C, Permeabilized cells incubated with fluo-3 are stimulated with 100 μM lindane. D, Permeabilized cells incubated with fluo-3 are stimulated with 100 nM thapsigargin. Changes in the Ca^{2+} concentration of the bathing saline solution were monitored over time. Ionomycin (0.5 μM) was added at the end of the experiment to release all Ca^{2+} from intracellular stores.

agents may stimulate Ca^{2+} release by similar mechanisms. One test of this hypothesis is to block δ-HCH-mediated Ca^{2+} release with heparin, an antagonist of IP_3 binding to its receptor on RBL cell ER (12) and of IP_3-mediated Ca^{2+} release (27). Ca^{2+} stored in IP_3-sensitive stores is released by IP_3 (1 μM) in permeabilized cells (Fig. 3A). A second addition of IP_3 (1 μM), after the ambient Ca^{2+} concentration returned to basal levels, again released Ca^{2+} from stores; however, the amount released was approximately 25–50% less than the amount released by the first addition of IP_3 (not shown). In permeabilized cells stimulated with IP_3 (1 μM), the addition of heparin (100 μg/ml), after the ambient Ca^{2+} concentration returned to baseline, inhibited the release of Ca^{2+} stimulated by a second addition of 1 μM IP_3 (Fig. 3A). In contrast, heparin (100 μg/ml) did not block δ-HCH-mediated Ca^{2+} release from permeabilized RBL cells (Fig. 3B).

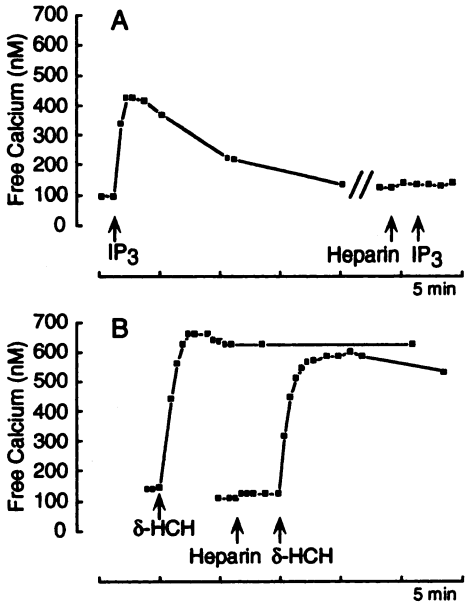

Fig. 3. Heparin does not block δ-HCH-induced release of Ca^{2+} from permeabilized RBL cells. A, Permeabilized RBL cells are treated with 1 μM IP_3 and 100 μg/ml heparin. B, Permeabilized RBL cells are treated with 100 μM δ-HCH and 100 μg/ml heparin. Changes in the Ca^{2+} concentration of the bathing solution are monitored over time.

δ-HCH did not compete with [^{3}H]IP_3 in binding to permeabilized RBL cells. Another test for determining whether there is an interaction between δ-HCH and the IP_3 receptor located on ER Ca^{2+} stores of RBL cells is to determine whether δ-HCH can inhibit the binding of [^{3}H]IP_3 to its receptor. When measured in permeabilized RBL cells, δ-HCH, at concentrations as high as 500 μM, did not significantly reduce the specific binding of [^{3}H]IP_3 to its receptor compared with control cells treated with DMSO (Fig. 4). This finding and the observation that heparin did not inhibit Ca^{2+} release mediated by δ-HCH suggest that δ-HCH is not stimulating Ca^{2+} release from the ER of RBL cells by an interaction with a site that overlaps with the IP_3-binding site on the microsomal receptor.

δ-HCH altered Ca^{2+} signaling in resting and antigen-stimulated cells. Both δ-HCH and lindane are hydrophobic compounds; therefore, these compounds would be expected to enter readily into intact cells. The addition of δ-HCH (25–100 μM) to Fura-2-loaded RBL cells induced a progressive increase in $[Ca^{2+}]_i$, whereas the addition of DMSO vehicle had no effect (Fig. 5A). The rise in $[Ca^{2+}]_i$ mediated by δ-HCH

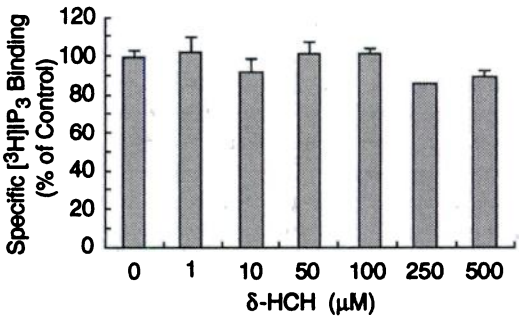

Fig. 4. δ-HCH did not reduce specific binding of [^{3}H]IP_3 in permeabilized cells. Aliquots of permeabilized RBL cells were mixed with increasing concentrations of δ-HCH or DMSO (*0*) and 0.5 nM [^{3}H]IP_3 for 30 min at 4°. Average specific binding of [^{3}H]IP_3 to control cells treated with DMSO vehicle was 1367 ± 68 dpm; average nonspecific binding was 75 ± 13 dpm. *Bars*, upper range of values for each concentration.

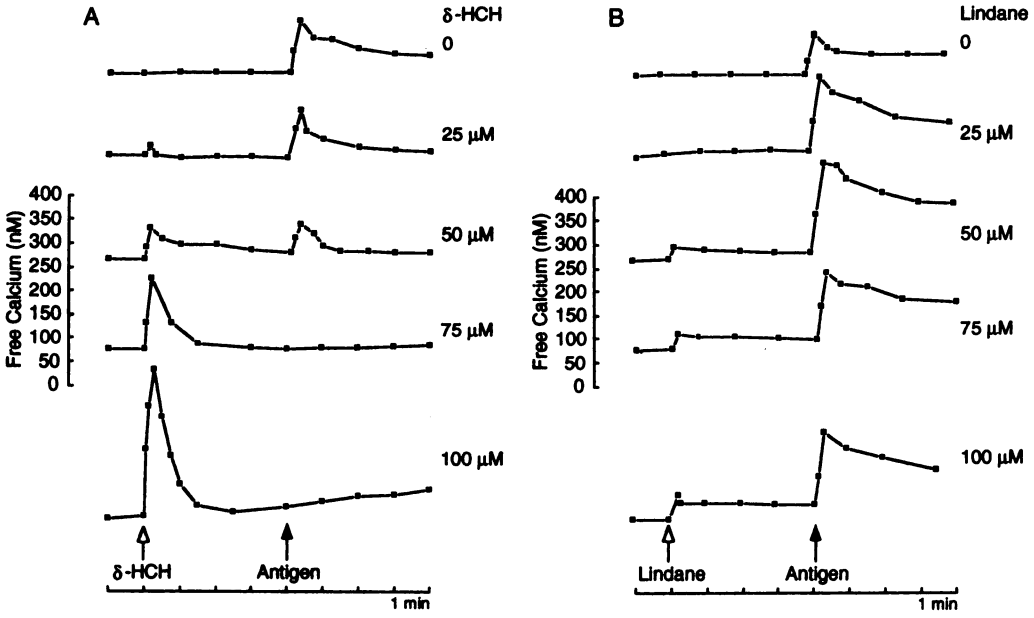

Fig. 5. Effect of δ-HCH on the resting and antigen-stimulated Ca^{2+} signal. Different concentrations of δ-HCH (A) or lindane (B) were added to Fura-2-loaded cells, and changes in the Ca^{2+} signal were followed over time. Four minutes after the addition of δ-HCH or lindane, antigen (0.1 μg/ml) was added to the cells. Traces are offset.

was transient, and after the peak increase, the Ca^{2+} concentration rapidly returned toward baseline. The response of the cells to the subsequent addition of antigen depended on the concentration of δ-HCH to which the cells were pre-exposed. The addition of antigen (0.1 μg/ml) to cells exposed to DMSO vehicle or to 25 μM δ-HCH resulted in a rapid increase in $[Ca^{2+}]_i$ (Fig. 5A). The $[Ca^{2+}]_i$ then decreased before stabilizing to a level above that originally found in the cells before antigen stimulation. Cells exposed to 50 μM δ-HCH transiently responded to antigen, whereas exposure to higher concentrations of δ-HCH (75 and 100 μM) resulted in the complete loss of the antigen response (Fig. 5A).

$[Ca^{2+}]_i$ slowly increased over time in cells treated with 100 μM δ-HCH (Fig. 5A, bottom trace). This slow increase in $[Ca^{2+}]_i$ also occurred in cells not stimulated with antigen. It was correlated with an increase in the release of lactate dehydrogenase from the cells and with an increase in the number of cells that stain positively with trypan blue (nonviable cells). Cells treated with 50 or 75 μM δ-HCH also exhibited a slow increase in $[Ca^{2+}]_i$ over time; however, the onset of the increase was slower and fewer cells were nonviable after 60 min compared with cells exposed to 100 μM δ-HCH. The delayed cytotoxicity did not appear to be responsible for the attenuation of the antigen-mediated Ca^{2+} signal in cells treated with 50–100 μM δ-HCH because at the time of the addition of antigen to the cells, ≥95% of the cells excluded trypan blue and lactate dehydrogenase release was <10%. These values are comparable to those observed in the control (DMSO-treated) cells.

Lindane also stimulated an increase in $[Ca^{2+}]_i$; however, this increase was consistently smaller than increases induced by similar concentrations of δ-HCH (Fig. 5B). In contrast to δ-HCH-treated cells, cells exposed to lindane responded to antigen (0.1 μg/ml) with the expected prolonged increase in $[Ca^{2+}]_i$. In addition, exposure of cells for 60 min to concentrations of lindane of ≤100 μM did not result in significant toxicity to RBL cells.

The inhibition of the antigen-stimulated Ca^{2+} signal by δ-HCH was not due to cellular ATP depletion. Intracellular ATP depletion is known to inhibit antigen-stimulated increases in $[Ca^{2+}]_i$ in RBL cells (15); this could account for the lack of responsiveness seen with δ-HCH. However, 6 min after exposure to 0, 50, or 100 μM δ-HCH, a time at which antigen would be added, there was little change in the intracellular concentration of ATP of the cells (1.00, 0.91, and 0.97 mM ATP, respectively). Changes in cellular ATP could not account for the inhibition of the antigen-stimulated Ca^{2+} signals in δ-HCH-treated cells.

The inhibition of the antigen-stimulated Ca^{2+} signal in RBL cells by δ-HCH was not overcome by increasing the extracellular Ca^{2+} concentration. Antigen-stimulated increases in $[Ca^{2+}]_i$ in RBL cells were inhibited by membrane depolarization (11). This inhibition could be partially overcome by suspending the depolarized cells in a saline solution containing a high concentration of Ca^{2+}, e.g., 10 mM. Possibly, δ-HCH depolarized the plasma membrane of RBL cells, and this was responsible for inhibition of the antigen-stimulated Ca^{2+} signal. Thus, it may be possible to overcome these inhibitory effects by stimulating δ-HCH-treated RBL cells with antigen in the presence of 10 mM Ca^{2+}. However, in three separate experiments, 1 min after the addition of antigen (0.1 μg/ml), $[Ca^{2+}]_i$ remained similar to the Ca^{2+} concentration at 1 min before the addition of antigen to cells previously exposed to 100 μM δ-HCH for 4 min (before antigen: 144, 111, and 104 nM; after antigen: 146, 109, and 102 nM, respectively.

Antigen-stimulated elevation of inositol phosphates is not affected by δ-HCH or lindane. One of the earliest steps in the generation of the IgE receptor-mediated Ca^{2+} signal is the production of inositol phosphates by the hydrolysis of membrane-associated phosphatidylinositol-4,5-bisphosphate with the enzyme phospholipase C. Both lindane and δ-HCH have been reported to affect phosphoinositide turnover in cells (5, 6). Interference by δ-HCH of the phosphoinositide signaling system could lead to the inhibition of the antigen-stimulated Ca^{2+} signal in RBL cells. However, treatment of cells with 100 μM δ-HCH or lindane for 5 min did not alter the levels of [^{3}H]inositol

phosphates produced compared with control cells treated with vehicle alone (Fig. 6). In addition, cells treated with 100 μM δ-HCH or lindane and antigen (0.1 μg/ml) did not have levels of [^{3}H]inositol phosphates that differed from those of control cells treated with vehicle and antigen (Fig. 6).

δ-HCH and thapsigargin released Ca^{2+} from the same intracellular store. In addition to antigen stimulation, a prolonged rise in $[Ca^{2+}]_i$ in RBL cells was induced by thapsigargin. The increase in $[Ca^{2+}]_i$ stimulated by thapsigargin, like antigen, depended on the concentration of δ-HCH to which the cells were previously exposed. Treatment with increasing concentrations of δ-HCH led to progressively larger (and transient) increases in $[Ca^{2+}]_i$ (Fig. 7A), as previously shown for antigen (see Fig. 5A). The addition of thapsigargin (100 nM) to vehicle-treated cells resulted in a prolonged increase in $[Ca^{2+}]_i$, and this response was attenuated in cells that were first treated with 50 μM δ-HCH and was inhibited in cells that were first treated with 75 and 100 μM δ-HCH (Fig. 7A). In contrast, lindane evoked a smaller increase in $[Ca^{2+}]_i$, and the subsequent addition of thapsigargin (100 nM) led to a rapid and prolonged increase in $[Ca^{2+}]_i$ (Fig. 7B).

δ-HCH reduced the Ca^{2+} signal in antigen- and thapsigargin-stimulated cells. The previous experiments demonstrated two prominent effects of δ-HCH on $[Ca^{2+}]_i$ regulation in RBL cells: mobilization of Ca^{2+} from ER stores and inhibition of both antigen- and thapsigargin-stimulated Ca^{2+} permeability (i.e., Ca^{2+} entry) pathways in the plasma membrane. To determine whether δ-HCH could inactivate Ca^{2+} entry pathways once they are activated by antigen stimulation, RBL cells were first stimulated with antigen (0.1 μg/ml) and then, at the plateau phase of the Ca^{2+} signal, exposed to different concentrations of δ-HCH or lindane. Fig. 8A shows that the addition of δ-HCH (25 or 50 μM) at the plateau phase resulted in an immediate reduction in $[Ca^{2+}]_i$. The addition of 75 or 100 μM δ-HCH first resulted in an immediate increase in $[Ca^{2+}]_i$ that was most likely due to additional release of luminal Ca^{2+} from the ER Ca^{2+} stores. This increase in Ca^{2+} was then followed by a decrease in $[Ca^{2+}]_i$. When antigen-stimulated cells were treated with 50–100 μM lindane (Fig. 8B) during the Ca^{2+} plateau, a small decrease in the $[Ca^{2+}]_i$ was observed. However, the magnitude of this reduction in $[Ca^{2+}]_i$ was negligible compared with that observed with similar concentrations of δ-HCH.

Similar findings were observed if the cells were first stimulated with thapsigargin (100 nM) and then exposed to δ-HCH at the plateau phase of the Ca^{2+} signal (Fig. 9A). Lindane (50–100 μM) caused a comparatively small reduction in the Ca^{2+} signal when added at the plateau of the thapsigargin-mediated Ca^{2+} signal (Fig. 9B).

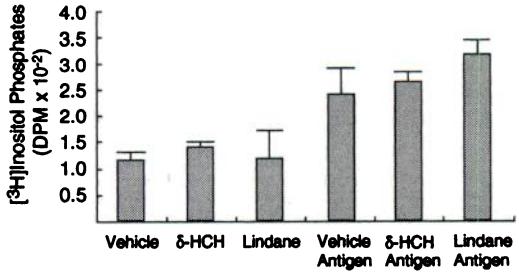

Fig. 6. Effects of δ-HCH and lindane on resting and antigen-stimulated production of [^{3}H]inositol phosphates. DMSO, 100 μM δ-HCH, or 100 μM lindane was added with or without 0.1 μg/ml antigen to RBL cells for 5 min before incubation was stopped. Results are expressed as total [^{3}H]inositol phosphates released into the aqueous phase. *Columns with standard deviation bars*, mean value of three separate experiments.

δ-HCH reduced Mn^{2+} influx across the plasma membrane. The rapid increase in $[Ca^{2+}]_i$ that was observed when intact RBL cells were treated with δ-HCH (Figs. 5A and 7A) may have been due not only to the release of Ca^{2+} from ER stores (Figs. 2 and 3) but also to Ca^{2+} influx across the plasma membrane. In intact cells, when monitoring changes in $[Ca^{2+}]_i$ with Fura-2 fluorescence it is difficult to discriminate between the contributions from the release of Ca^{2+} from stores and from Ca^{2+} influx across the plasma membrane. However, it is possible to measure changes in the permeability of divalent cations across the plasma membrane by monitoring the rate of quenching of intracellular Fura-2 fluorescence by Mn^{2+}. Extracellular Mn^{2+} enters into cells via the same permeability pathway(s) used by Ca^{2+} (24); therefore, it serves as a useful substitute for Ca^{2+} in measuring divalent cation permeability across the plasma membrane. When Mn^{2+} (0.5 mM) was added to Fura-2-loaded RBL cells, there was an immediate decrease in Fura-2 fluorescence that was due to the quenching of extracellular Fura-2. Then, a gradual decline of Fura-2 fluorescence was observed, which was attributed to the slow influx of Mn^{2+} into resting RBL cells, presumably through the same permeability pathway that is activated during antigen stimulation (17). The addition of DMSO vehicle had little effect on the rate of fluorescence quenching (Fig. 10, A and B, top traces). However, when δ-HCH (25–100 μM) was added to the cells, there was a slowing of the rate of fluorescence quenching (Fig. 10A). This correlated with a decrease in Mn^{2+} influx into the cells, and it was most pronounced when the cells were treated with 50–100 μM δ-HCH. Also, when 50–100 μM δ-HCH was added, there was an initial rapid but small decrease in Fura-2 fluorescence before the rate of fluorescence quenching slowed. This was attributed to a small increase in Mn^{2+} influx stimulated by δ-HCH before it limited Mn^{2+} influx and not to fluorescence artifact. The rate of Mn^{2+}-induced Fura-2 fluorescence quenching in cells was not significantly slowed by the addition of lindane (Fig. 10B).

The addition of antigen (0.1 μg/ml) to control RBL cells stimulated an increase in the rate of Mn^{2+}-induced Fura-2 fluorescence quenching (Fig. 10, A and B, top traces). When antigen was added to cells that had been exposed to 25 μM δ-HCH, there also was an increase in Mn^{2+} influx into the cells (Fig. 10A). In cells that had been exposed to 50–100 μM δ-HCH, there was an inhibition of antigen-stimulated Mn^{2+} influx. Mn^{2+} influx was increased by the antigen in cells exposed to all concentrations of lindane (Fig. 10B).

Moser and Smart (7) have shown that at concentrations higher than those used in the present study, lindane is able to stimulate PKC activity. It is known that stimulation of PKC with phorbol ester inhibits antigen-stimulated increases in $[Ca^{2+}]_i$ in RBL cells (16). If δ-HCH (50–100 μM) is inhibiting Mn^{2+} influx in resting and antigen-stimulated RBL cells by activating PKC, then the addition of the phorbol ester PMA to RBL cells should also induce an inhibition of Mn^{2+} influx into these cells. However, in contrast to cells treated with 50–100 μM δ-HCH, PMA (50 nM) has no effect on reduction of the rate of Mn^{2+}-induced fluorescence quench-

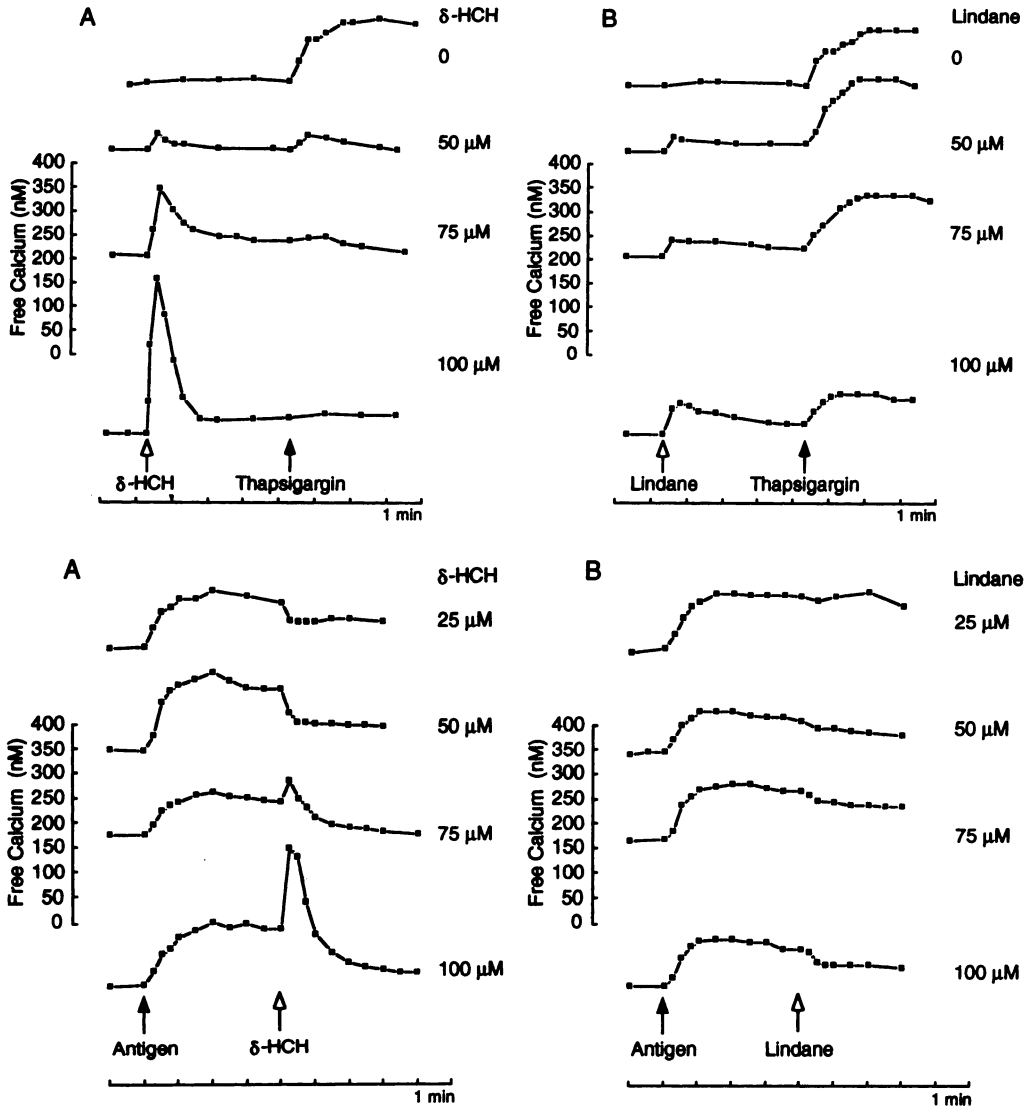

Fig. 7. Effect of δ-HCH on thapsigargin-mediated increases in $[Ca^{2+}]_i$. RBL cells were treated with δ-HCH (A) or lindane (B) for 4 min before addition of thapsigargin (100 nM). Traces are offset.

Fig. 8. δ-HCH terminated antigen-activated Ca^{2+} permeability pathways in RBL cells. RBL cells were stimulated with antigen (0.1 μg/ml), and, at elevated $[Ca^{2+}]_i$, either 100 μM δ-HCH (A) or lindane (B) was added.

ing in resting cells, whereas it does inhibit antigen-stimulated Mn^{2+} influx (Fig. 10, A and B, lower traces).

To assess the extent of Fura-2 fluorescence quenching by Mn^{2+}, 2 min after the addition of antigen to all cells, we added ionomycin (0.25 nM) to stimulate Mn^{2+} influx (not shown). For cells treated with 0, 25, 50, 75, or 100 μM δ-HCH, the addition of ionomycin induced an increase in Fura-2 fluorescence quenching of 18%, 31%, 67%, 75%, and 90%, respectively, above the amount of Mn^{2+}-induced quenching observed in the period between the initial addition of Mn^{2+} (minus the quenching of extracellular Fura-2 fluorescence by Mn^{2+}) and the addition of ionomycin. This finding indicates that dye saturation by Mn^{2+} was not the reason for the slowing of fluorescence quenching by δ-HCH. Furthermore, the correlation of the increase in the percentage of fluorescence quenching by ionomycin in cells with the increasing concentrations of δ-HCH lends support to the interpretation that high concentrations of δ-HCH are inhibiting Mn^{2+} influx into the cells. When ionomycin was added to cells pretreated with 0, 25, 50, 75, or 100 μM lindane, the percentage of quenching of Fura-2 fluorescence was increased by 18%, 25%, 22%, 37%, and 46%, respectively. The increase in ionomycin-induced quenching of Fura-2 fluorescence by Mn^{2+} in cells treated with 75 and 100 μM lindane was not attributed to an inhibition of Mn^{2+} influx into the cells, as was observed with δ-HCH-treated cells. Instead, in this experiment, it was due to a decrease in antigen-stimulated Mn^{2+} influx.

δ-HCH and lindane altered antigen-stimulated secretion. The rate of secretion from RBL cells in response to antigen/IgE receptor interactions is slow and the time to reach completion is variable, but it usually is complete within 1 hr (28). For this reason, secretion in the presence of high concentrations of δ-HCH (≥50 μM) cannot be accurately evaluated due to the delayed cytotoxicity observed at these concentrations. RBL cells treated with 25 μM δ-HCH for 65 min released similar amounts of [^{3}H]serotonin as cells treated with DMSO (Fig. 11A). The addition of antigen (0.1 μg/ml) to cells pretreated with vehicle or δ-HCH stimulated [^{3}H]serotonin release. However, antigen-stimulated cells pretreated with 25 μM δ-HCH release more [^{3}H]serotonin than the corresponding control cells, which are pretreated with DMSO.

Lindane-treated cells showed no cytotoxicity after 65 min of exposure, nor was the release of [^{3}H]serotonin from resting cells altered compared with control cells (Fig. 11B). Like δ-HCH, receptor-mediated release of [^{3}H]serotonin by the

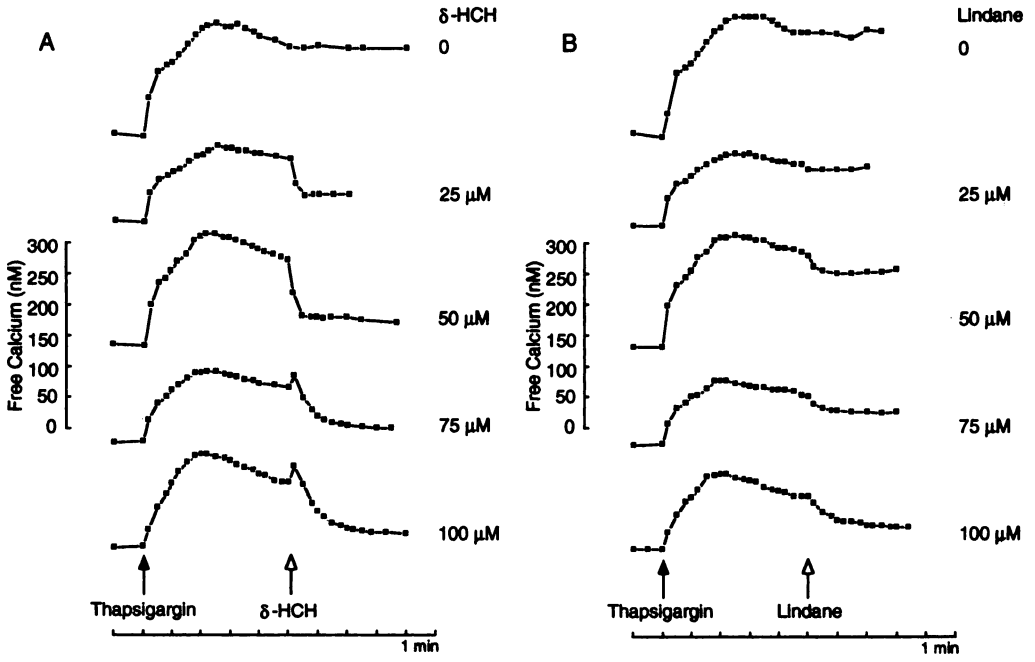

Fig. 9. δ-HCH terminated thapsigargin-activated Ca^{2+} permeability pathways in RBL cells. RBL cells were stimulated with thapsigargin (100 nM) and, at elevated $[Ca^{2+}]_i$, either 100 μM δ-HCH (A) or lindane (B) was added.

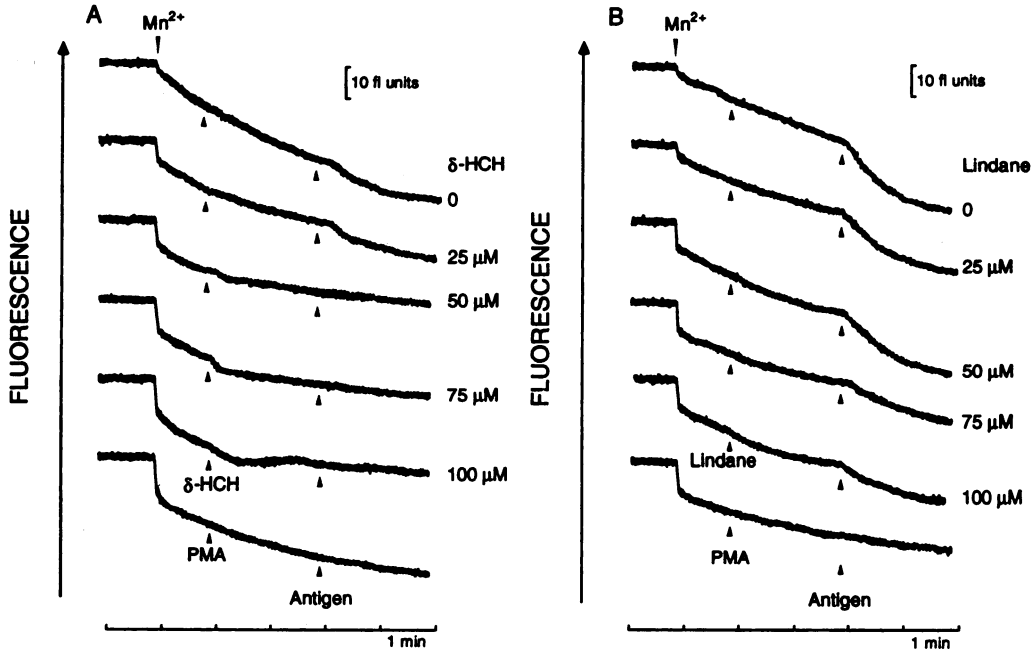

Fig. 10. Effect of δ-HCH on Mn^{2+} influx. Mn^{2+} (0.5 mM) was added to Fura-2-loaded cells, and the rate of Fura-2 fluorescence quenching was measured in cells exposed to δ-HCH (A), lindane (B), or 50 nM PMA (A and B, *bottom traces*). In each trace, antigen 0.1 μg/ml was added after 2 min. Traces are offset.

addition of antigen (0.1 μg/ml) was enhanced in cells treated with 25–100 μM lindane compared with antigen-stimulated release from control cells.

Mobilization of ER Ca^{2+} by δ-HCH was attenuated by caffeine. The lack of a demonstrable interaction between δ-HCH and the $[^3H]IP_3$-binding site (Fig. 4) or of an antagonism with heparin (Fig. 3) and the unique actions on depletion-activated Ca^{2+} entry (Figs. 5 and 7–10) raise the possibility that the chlorinated alkane mediates its actions by a novel mechanism. Caffeine is known to interact with the ryanodine-sensitive Ca^{2+} channels in a number of excitable and nonexcitable cells (29–31). The ability of caffeine to alter the response of intact RBL cells to δ-HCH is shown in Fig. 12. Aliquots of RBL cells were resuspended in modified Tyrode's solutions containing caffeine (10–40 mM) and subsequently challenged with 50 μM δ-HCH. Caffeine dose-dependently attenuated the increase in $[Ca^{2+}]_i$ induced by δ-HCH (Fig. 12).

Discussion

In addition to the well-documented convulsant activity of lindane, isomers of HCH appear to have other, more subtle, biological activities. Lindane has been shown to alter cellular functions in nonexcitable cells, including neutrophils (32) and macrophages (33). Lindane suppresses lymphocyte (34) and macrophage (35) proliferation and signaling. The mechanism by which lindane alters immune cell function and its relationship with altered intracellular Ca^{2+} regulation remain unclear. With another class of immune cell, the mast

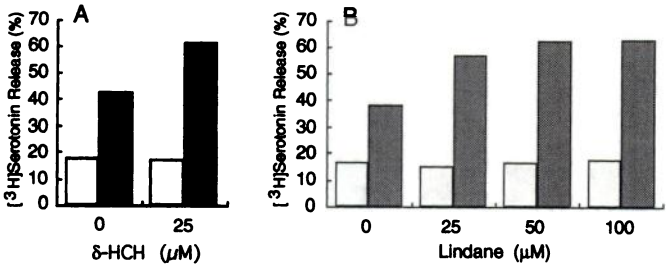

Fig. 11. Effects of δ-HCH and lindane on secretion from RBL cells. Cells were incubated with δ-HCH (A) or lindane (B) for 10 min before the addition of saline solution vehicle (□) or 0.1 μg/ml antigen (■) for an additional 55 min. Range between the duplicate samples of each concentration never varied by >2%.

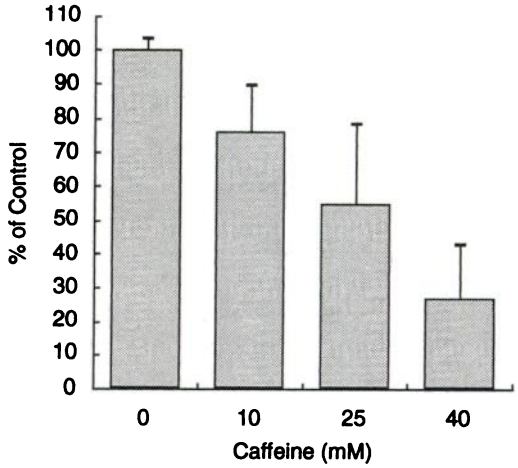

Fig. 12. Caffeine inhibited δ-HCH-induced increases in $[Ca^{2+}]_i$. Fura-2-loaded cells were suspended in modified Tyrode's solution containing 0–40 mM caffeine for at least 10 min before the addition of 50 μM δ-HCH. Net changes in $[Ca^{2+}]_i$ (from resting to peak increase in free Ca^{2+}) are expressed as a percentage of the net changes in $[Ca^{2+}]_i$ in control cells that were stimulated with δ-HCH in the absence of caffeine. *Bars*, standard deviations. Values represent the mean of three separate experiments. Mean ± standard deviation of the net changes in $[Ca^{2+}]_i$ of the control cells was 90 ± 10 nM.

cell, we showed that concentrations of δ-HCH ranging from 10–100 μM induced the release of Ca^{2+} from internal Ca^{2+} stores of RBL cells (Fig. 2A). The release of stored Ca^{2+} by δ-HCH is not reversible; this may be the result of continual stimulation of Ca^{2+} release pathways because δ-HCH is not known to be readily metabolized. Lindane also releases Ca^{2+} from intracellular stores but to a much lesser extent (Fig. 2C). Both isomers alter normal patterns of Ca^{2+} signaling in the RBL cell, but they do so in distinctly different manners (Figs. 5 and 7–9). The actions of δ-HCH and lindane on RBL cells result in enhancement of antigen-stimulated secretion of allergic mediators (Fig. 11). Because polychlorinated hydrocarbons are lipophilic, the question arises as to whether changes in $[Ca^{2+}]_i$ and secretory function are simply the result of compromised lipid membrane barriers. However, the markedly higher potency of δ-HCH over lindane and the observation that δ-HCH increases ER Ca^{2+} permeability while shutting off Ca^{2+} influx through the plasma membrane strongly suggest a more specific mechanism.

Luminal Ca^{2+} can also be released from ER stores by blocking the organelle's Ca^{2+}-ATPase and thereby unmasking a passive leak of stored Ca^{2+} into the cytoplasm. If δ-HCH induces Ca^{2+} release from stores by blocking the SERCA pump, the rates of Ca^{2+} release would be expected to be similar to those evoked by thapsigargin. However, the rate of Ca^{2+} release from permeabilized cells induced by δ-HCH (Fig. 2A) is significantly faster than that observed with thapsigargin (Fig. 2D). Pessah *et al.* observed that 30 μM δ-HCH blocks Ca^{2+}-ATPase activity in rat cardiac SR membrane vesicles by 20%; however, the membrane vesicles are still able to accumulate Ca^{2+} in the presence of ruthenium red, suggesting that Ca^{2+} release induced by δ-HCH in the heart is not mediated by inhibition of the SERCA pumps (4).

In intact RBL cells, the addition of δ-HCH resulted in an immediate increase in $[Ca^{2+}]_i$ (Figs. 5A and 7A). This increase was attributed to the release of luminal Ca^{2+} from ER stores with little contribution from Ca^{2+} influx across the plasma membrane because during the period of time that $[Ca^{2+}]_i$ was increased due to the addition of δ-HCH, the rate of Mn^{2+}-induced quenching of intracellular Fura-2 fluorescence was not increased (Fig. 10A). δ-HCH (25–100 μM) decreased the rate of Mn^{2+} influx to less than that observed in control cells. Ca^{2+} regulation in RBL cells was less sensitive to lindane, which induced a small but rapid increase in $[Ca^{2+}]_i$ (Figs. 5B and 7B). Joy and Burns (36) found that lindane (10–400 μM) significantly increases the $[Ca^{2+}]_i$ in NCB-20 neurohybridoma cells, and, similar to the present findings in RBL cells, $[Ca^{2+}]_i$ returned toward basal levels, although in neurohybridoma cells the decline to basal concentrations occurs over a much longer period of time.

The rapid decline in $[Ca^{2+}]_i$ immediately after the δ-HCH-induced peak was attributed to the pumping of Ca^{2+} out of the cell and not back into ER stores. If Ca^{2+} is resequestered into a store, it should be released either by IP_3-mediated mechanisms during antigen stimulation or by direct inhibition of the SERCA pumps on the ER membrane. However, in cells pretreated with 75 or 100 μM δ-HCH, neither the addition of antigen nor the addition of thapsigargin after the $[Ca^{2+}]_i$ returned to basal levels resulted in further increase in $[Ca^{2+}]_i$ (Figs. 5A and 7A). In resting healthy cells, the mitochondria do not store significant amounts of ionized Ca^{2+} (37); therefore, it is unlikely that δ-HCH is inducing an increase in $[Ca^{2+}]_i$ by affecting mitochondrial Ca^{2+} transport. In RBL cells, the mitochondria may accumulate Ca^{2+} during antigen stimulation (15) but not during periods when $[Ca^{2+}]_i$ approximates the Ca^{2+} concentration in resting, nonstimulated cells. This suggests that the mitochondria are not responsible for returning $[Ca^{2+}]_i$ to basal levels after the peak increase induced by δ-HCH (Figs. 5A and 7A).

The complete inhibition of antigen- and thapsigargin-mediated increases in $[Ca^{2+}]_i$ in cells pretreated with 75 and 100 μM δ-HCH (Figs. 5A and 7A) indicated that Ca^{2+} influx across the plasma membrane was also inhibited. Lindane, on the other hand, did not inhibit antigen- or thapsigargin-stimulated increases in $[Ca^{2+}]_i$ (Figs. 5B and 7B). The small rise in $[Ca^{2+}]_i$ observed during antigen stimulation in cells pretreated with 50 μM δ-HCH most likely represented the release of residual ER luminal Ca^{2+} that was not released by the initial treatment with a submaximal concentration of δ-HCH. This is correlated with the finding that antigen-stimulated increases in Mn^{2+} influx are fully inhibited in cells exposed to ≥50 μM δ-HCH (Fig. 10A).

Antigen-stimulated Ca^{2+} influx may be inhibited in δ-HCH-treated RBL cells by a number of different mecha-

nisms. Depletion of cellular ATP is one method of inhibiting antigen-stimulated Ca^{2+} influx in RBL cells (15). However, treatment of RBL cells with δ-HCH does not significantly deplete the total cellular ATP concentration (see Results) to a level that would affect Ca^{2+} homeostasis.

Inhibition of the antigen-stimulated Ca^{2+} signal may be explained by the finding that δ-HCH has been reported to inhibit phosphatidylinositol metabolism (6), but precipitating concentrations (≥500 μM) of δ-HCH are required to demonstrate this effect. We found that δ-HCH and lindane did not affect the formation of inositol phosphates in resting or antigen-stimulated RBL cells (Fig. 6). Thus, it is unlikely that the lower concentrations of δ-HCH used in the present study affected the phosphatidylinositol cascade.

Another intracellular signal that is activated by agonist stimulation is PKC. In RBL cells, activation of PKC by phorbol esters inhibits antigen-stimulated increases in $[Ca^{2+}]_i$ (16). Lindane has been shown to stimulate an increase in the activity of this kinase in brain tissue; however, phorbol ester at a 250-fold lower concentration is 2.5 times more effective than lindane in stimulating PKC activity (7). Whether δ-HCH activates PKC is not known. However, the phorbol ester PMA did not alter the rate of Mn^{2+}-induced Fura-2 fluorescence quenching (Fig. 10, A and B, bottom traces), whereas δ-HCH did. These findings suggest that δ-HCH is not inhibiting antigen-mediated increases in $[Ca^{2+}]_i$ by a mechanism involving activation of PKC.

The immediate reduction of Mn^{2+} influx into resting RBL cells on addition of δ-HCH suggests a direct interaction with the plasma membrane that inhibits Ca^{2+} influx. One membrane event known to reduce Ca^{2+} influx in resting (17) and antigen-stimulated (11) RBL cells is plasma membrane depolarization. HCH has been found to interfere with potential-sensitive fluorescent dyes, precluding measurements to determine the effect of the δ isomer on membrane potential. The inhibition of ^{45}Ca influx into antigen-stimulated RBL cells that are depolarized by suspension in a saline solution containing a high concentration of K^+ (140 mM) can be partially overcome by increasing the extracellular Ca^{2+} concentration (11). However, the inhibition of the antigen-stimulated increase in $[Ca^{2+}]_i$ in cells that have been pretreated with 100 μM δ-HCH was not affected by an increase in the extracellular Ca^{2+} concentration to 10 mM (see Results). Although we cannot fully discount the involvement of membrane potential in the actions of δ-HCH on RBL cells, other mechanisms must be involved in mediating the effects of δ-HCH.

A capacitative mechanism that links the depletion of Ca^{2+} from intracellular stores to the influx of Ca^{2+} across the plasma membrane has been proposed as a major mechanism controlling Ca^{2+} influx into nonexcitable cells (38). The nature of the signal that mediates this response has not been elucidated. It is possible that δ-HCH inhibits Ca^{2+} regulation in RBL cells by uncoupling the link between Ca^{2+} stores and influx pathways in the plasma membrane because the release of large concentrations of Ca^{2+} from ER stores is not correlated with increased Ca^{2+} influx across the plasma membrane. The signal that increases Ca^{2+} permeability across the plasma membrane must also maintain the pathway in the open state because the addition of δ-HCH to cells in which Ca^{2+} influx has been previously increased due to antigen- or thapsigargin- stimulation results in a decrease in Ca^{2+} permeability across the plasma membrane (Fig. 8–9A). The finding that δ-HCH reduces basal and antigen-stimulated Mn^{2+} influx (Fig. 10A) suggests that the molecular lesion induced by δ-HCH may be modulating the Ca^{2+} permeability pathway. Lindane also releases Ca^{2+} from intracellular stores in RBL cells (Fig. 2C) and in cultured uterine smooth muscle cells (8). However, in RBL cells, lindane does not affect Ca^{2+} entry through the plasma membrane as noted for δ-HCH, i.e., inhibition of basal Mn^{2+} influx (Fig. 10B). Furthermore, lindane does not alter depletion-activated Ca^{2+} entry stimulated by antigen or thapsigargin, suggesting that (a) the concentration of Ca^{2+} released from ER stores by lindane is below the threshold needed to evoke the signal required to mediate Ca^{2+} entry across the plasma membrane or (b) the actions of δ-HCH and lindane are fundamentally different and isoform specific.

The effects of δ-HCH and lindane on secretion demonstrate that these agents can enhance antigen-mediated release of [^{3}H]serotonin (Fig. 11). At 25 μM, δ-HCH enhances antigen-mediated [^{3}H]serotonin release over that of cells pretreated with DMSO alone. At this concentration of δ-HCH, the antigen-stimulated Ca^{2+} signal, although diminished in magnitude, is still prolonged (Fig. 5A). It is tempting to propose that the additional release of Ca^{2+} induced by 25 μM δ-HCH may be responsible for releasing more [^{3}H]serotonin when subsequently exposed to antigen, although other Ca^{2+}-independent mechanisms may also have an important role. Lindane is not as potent as δ-HCH in inducing delayed toxicity in the RBL cells; therefore, the effects of lindane on antigen-stimulated secretion can be observed over a wider concentration range. At all concentrations of lindane tested, there was significant enhancement of antigen-stimulated [^{3}H]serotonin release from cells compared with cells pretreated with DMSO. The same mechanisms responsible for enhancing antigen-stimulated [^{3}H]serotonin release in cells pretreated with 25 μM δ-HCH may also be responsible for increasing antigen-stimulated secretion in lindane-treated cells. This enhancement is most likely not due to stimulation of PKC by lindane because activation of PKC by phorbol esters has only marginal effects in enhancing antigen-stimulated secretion in RBL cells (16).

Agonist-stimulated mast cells have been shown to be capable of releasing vasoactive amines, enzymes, and cytokines, including interleukins, tumor necrosis factor, and colony-stimulating factors (39). Enhanced release of these mediators from mast cells could therefore have significant effects on the function of cells that are targets for these inflammatory mediators and cytokines. The finding that low concentrations of δ-HCH, lindane, and, perhaps, other chlorinated hydrocarbons can significantly enhance antigen-stimulated release of mediators suggests that by modulating the release of bioactive inflammatory mediators from immune cells such as mast cells, chlorinated hydrocarbon pesticides may indirectly promote functional changes in a wide variety of neighboring cells.

The molecular mechanism(s) by which δ-HCH induces the release of Ca^{2+} from permeabilized RBL cells is not known. δ-HCH does not compete with [^{3}H]IP_3 in binding to the IP_3 receptor (Fig. 4), and δ-HCH-induced Ca^{2+} release from permeabilized cells is not blocked by the IP_3 receptor antagonist heparin (Fig. 3B). Thus, δ-HCH does not appear to induce Ca^{2+} release by a simple mechanism of binding to the IP_3

binding site on the microsomal IP_3 receptor. However, a more complex interaction between δ-HCH and the IP_3 receptor-Ca^{2+} release channel complex cannot be ruled out at the present time. These findings suggest that it is not the structural similarity between δ-HCH and IP_3 that is responsible for the effects of δ-HCH on $[Ca^{2+}]_i$ regulation in RBL cells. The attenuation of the δ-HCH-induced rise in $[Ca^{2+}]_i$ by caffeine suggests the intriguing possibility that ryanodine receptor-like Ca^{2+} channels are present in RBL cells. A direct interaction of δ-HCH with the ryanodine receptor of cardiac SR membranes has been demonstrated (4). It is possible that the molecular target for the interaction of δ-HCH in both cardiac cells and RBL cells is similar. Recent work with a number of different cell types suggests that the ryanodine-sensitive Ca^{2+} channel may be widely expressed in nonexcitable cells (29–31); however, there is no direct evidence that these channels are found in RBL cells. The effects of caffeine on attenuating the δ-HCH response in RBL cells suggest that these cells may contain ryanodine receptor-like Ca^{2+} channels; however, caffeine has also been shown to block IP_3-sensitive Ca^{2+} channels reconstituted in bilayer lipid membranes (40). Further work with RBL cell microsomal vesicles is required to understand the nature of the molecular mechanism by which δ-HCH evokes Ca^{2+} release from ER stores.

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