Ca\(^{2+}\) movement and apoptosis induced by deltamethrin in Madin–Darby canine kidney canine renal tubular cells

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Abstract This study explored the effect of deltamethrin, a pesticide, on free Ca\(^{2+}\) concentration [Ca\(^{2+}\)]\(_i\), viability, and apoptosis in Madin–Darby canine kidney (MDCK) canine renal tubular cells. Deltamethrin at concentrations between 10\(\mu\)M and 40\(\mu\)M evoked [Ca\(^{2+}\)]\(_i\), rises in a concentration-dependent manner. The Ca\(^{2+}\) entry was inhibited by nifedipine, econazole, phorbol 12-myristate 13-acetate, and SKF96365. Treatment with the endoplasmic reticulum Ca\(^{2+}\) pump inhibitor 2,5-di-tert-butylhydroquinone (BHQ) in a Ca\(^{2+}\)-free medium abolished deltamethrin-induced [Ca\(^{2+}\)]\(_i\) rise. Treatment with deltamethrin also abolished BHQ-induced [Ca\(^{2+}\)]\(_i\) rise. Inhibition of phospholipase C (PLC) activity with U73122 abolished deltamethrin-evoked [Ca\(^{2+}\)]\(_i\) rise. Deltamethrin killed cells at 30–60\(\mu\)M in a concentration-dependent manner. The cytotoxic effect of deltamethrin was not reversed by prechelating...
Introduction

Pyrethroids are pesticides with high selectivity for insects [1]. Deltamethrin is a photostable pyrethroid providing valuable insecticidal activity against a large number of pests. Because it has potential uses for crop, cattle, and human health protection, extensive research work was carried out to evaluate its safety. Extensive pre- and postharvest use of a single pesticide demands thorough knowledge of residues on each crop and how they are affected by food processing [2].

Deltamethrinic acid is a synthetic compound whose structure is inspired from those present in the flower head of the plant Chrysanthemum cinerariifolium. Its ester deltamethrin exhibits an extremely high insecticidal activity (DDT×35.000) and an extremely low toxicity to mammals [3]. The mechanism underlying deltamethrin-induced toxicity is under extensive research.

At the signal transduction level, deltamethrin was shown to lower cell survival rate and increase intracellular free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) in astrocytes of rat [4], and to increase [Ca\(^{2+}\)]\(_i\) and apoptosis in rat neural cells [5]. Deltamethrin induces mitochondrial membrane permeability and alters expression of cytochrome C in rat brain [6]. Deltamethrin induces apoptosis in cultured cerebral cortical neurons [7] and rat brain [8]. At the molecular level, Wu and Liu [9] reported that deltamethrin induces altered the expression of p53, Bax, and Bcl-2 in rat brain. Furthermore, deltamethrin induces testicular apoptosis in rats [10].

Alteration in [Ca\(^{2+}\)]\(_i\), is a key regulator of many cellular processes involved in apoptosis [11]. To allow a precise regulation of [Ca\(^{2+}\)]\(_i\), and various signaling pathways, cells possess many mechanisms to control [Ca\(^{2+}\)]\(_i\), both globally and at the subcellular level. Among these are many members of the superfamily of G-protein-coupled receptors, which are characterized by the presence of seven transmembrane domains [12]. Typically, these receptors are able to activate phospholipase C (PLC), resulting in the release of Ca\(^{2+}\) from intracellular stores, which subsequently influences Ca\(^{2+}\) entry across the plasma membrane [13].

The effect of deltamethrin on [Ca\(^{2+}\)]\(_i\), and viability in renal cells is unclear. Lesions in the kidney tissues of fish exposed to deltamethrin were characterized by degeneration in the epithelial cells of renal tubules, pycnotic nuclei in the hematopoietic tissue, dilation of glomerular capillaries, degeneration of glomerulus, intracytoplasmic vacuoles in epithelial cells of renal tubules with hypertrophied cells, and narrowing of the tubular lumen [14]. In addition, the relationship between deltamethrin-induced [Ca\(^{2+}\)]\(_i\), rise and possible cell death is unknown. Thus, the aim of this study was to answer these questions by exploring the effect of deltamethrin on [Ca\(^{2+}\)]\(_i\), viability, and apoptosis in Madin–Darby canine kidney (MDCK) cells. This cell line is a useful model for renal cell research. It has been shown that in this cell, [Ca\(^{2+}\)]\(_i\) rises and death can be induced by stimulation with several agents including sulforaphane [15], bisphenol A [16], melittin [17], and safrole [18].

Fura-2 was used as a fluorescent Ca\(^{2+}\)-sensitive dye to measure [Ca\(^{2+}\)]\(_i\), changes in this study. Deltamethrin-induced [Ca\(^{2+}\)]\(_i\), rises in MDCK cells were characterized, the concentration–response plots were established, and the pathways underlying the [Ca\(^{2+}\)]\(_i\), rise were investigated. The effect of deltamethrin on cell viability and apoptosis was also examined.

Materials and methods

Chemicals

The reagents for cell culture were purchased from Gibco (Gaithersburg, MD, USA). acetoxyethyl esters of Fura-2 (Fura-2/AM) and 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA/AM) were purchased from Molecular Probes (Eugene, OR, USA). Deltamethrin and all other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise indicated.

Cell culture

MDCK renal tubular cells obtained from Bioresource Collection and Research Center (Taiwan) were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin.

Solutions used in [Ca\(^{2+}\)]\(_i\) measurements

The Ca\(^{2+}\)-containing medium (pH 7.4) had 140mM NaCl, 5mM KCl, 1mM MgCl\(_2\), 2mM CaCl\(_2\), 10mM HEPES, and 5mM glucose. The Ca\(^{2+}\)-free medium contained chemicals similar to that of the Ca\(^{2+}\)-containing medium except that CaCl\(_2\) was replaced with 0.3mM ethylene glycol-bis(2-
a aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA) and 2mM MgCl2. Deltamethrin was dissolved in dimethyl sulfoxide as a 0.1M stock solution. The other chemicals were dissolved in water, ethanol, or dimethyl sulfoxide. The concentration of organic solvents in the experimental solutions did not exceed 0.1%, and did not affect viability, apoptosis, or basal \( [Ca^{2+}] \).

\[ \text{[Ca}^{2+}\text{], measurements} \]

Confluent cells grown on 6-cm dishes were trypsinized and made into a suspension in culture medium at a density of \( 10^5/mL \). Cell viability was determined by trypan blue exclusion test (adding 0.2% trypan blue to 0.1-mL cell suspension). The viability was usually \( > 95% \) after the treatment. Cells were subsequently loaded with 2\( \mu \)M fura-2/AM for 30 minutes at 25\( ^\circ \)C in the same medium. After loading, cells were washed twice with the \( Ca^{2+}\)-containing medium and were made into a suspension in the \( Ca^{2+}\)-containing medium at a density of \( 10^5/mL \). Fura-2 fluorescence measurements were performed in a water-jacketed cuvette (25\( ^\circ \)C) with continuous stirring; the cuvette contained 1 mL of the medium and 0.5 million cells. Fluorescence was monitored with a Shimadzu RF-5301PC spectrofluorophotometer immediately after the addition of 0.1-mL cell suspension to 0.9 mL \( Ca^{2+}\)-containing or \( Ca^{2+}\)-free medium, by recording excitation signals at 340 and 380 nm and emission signal at 510 nm at 1-second intervals. During the recording, reagents were added to the cuvette by pausing the recording for 2 seconds to open and close the cuvette-containing chamber. For calibration of \( [Ca^{2+}] \), after completion of the experiments, the detergent Triton X-100 (0.1%) and \( CaCl_2 \) (5mM) were added to the cuvette to obtain the maximal fura-2 fluorescence. The \( Ca^{2+}\) chelator EGTA (10mM) was then added to chelate \( Ca^{2+}\) in the cuvette to obtain the minimal fura-2 fluorescence. Control experiments showed that cells bathed in a cuvette had 95% viability after 20 minutes of fluorescence measurements. \( [Ca^{2+}] \) was calculated as previously described [19].

Cell viability assays

The measurement of cell viability was based on the ability of cells to cleave tetrazolium salts by dehydrogenases. An increase in the intensity of color correlated with the number of live cells. Assays were performed according to manufacturer’s instructions (Roche Molecular Biochemical, Indianapolis, IN, USA). Cells were seeded in 96-well plates at a density of 10,000 cells/well in culture medium for 24 hours in the presence of 0–60\( \mu \)M deltamethrin. The cell-viability-detecting tetrazolium reagent 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio-1,3-benzene disulfonate] (10\( \mu \)M pure solution) was added to samples after deltamethrin treatment, and the cells were incubated for 30 minutes in a humidified atmosphere. In experiments using BAPTA/AM to chelate cytosolic \( Ca^{2+}\), cells were treated with 5\( \mu \)M BAPTA/AM for 1 hour prior to incubation with deltamethrin. The cells were washed once with the \( Ca^{2+}\)-containing medium and incubated with or without deltamethrin for 24 hours. The absorbance of samples (\( A_{450} \)) was determined using enzyme-linked immunosorbert assay reader. Absolute optical density was normalized to the absorbance of unstimulated cells in each plate and expressed as a percentage of the control value.

Alexa Fluor 488 annexin V/prodipidum iodide staining for apoptosis

Annexin V/prodipidum iodide (PI) staining assay was used to further detect cells in the early apoptotic and late apoptotic/necrotic stages. Cells were exposed to deltamethrin at concentrations of 0–50\( \mu \)M for 24 hours. Cells were harvested after incubation and washed in cold phosphate-buffered saline. Cells were resuspended in 400 \( \mu \)L reaction solution with 10mM of HEPES, 140mM of NaCl, 2.5mM of CaCl2 (pH 7.4). Alexa Fluor 488 annexin V/PI staining solution (Probes Invitrogen, Eugene, OR, USA) was added in the dark. After incubating for 15 minutes, the cells were collected and analyzed in a FACScan flow cytometry analyzer. Excitation wavelength was at 488 nm and the emitted green fluorescence of annexin V (FL1) and red fluorescence of PI (FL2) were collected using 530- and 575-nm band pass filters, respectively. A total of 20,000 cells were analyzed for each sample. Light scatter was measured on a linear scale of 1024 channels and fluorescence intensity was measured on a logarithmic scale. The percentage of early apoptosis and the percentage of late apoptosis/necrosis were determined, respectively, as the percentage of annexin V+/PI− or the percentage of annexin V+/PI+ cells. Data were later analyzed using the flow cytometry analysis software WinMDI 2.8 (by Joe Trotter, freely distributed software). The X and Y coordinates refer to the intensity of fluorescence of annexin and PI, respectively.

Statistical analysis

Data are reported as mean \( \pm \) standard error of the mean of three separate experiments and were analyzed by one-way analysis of variances using the SAS (SAS Institute Inc., Cary, NC, USA). Multiple comparisons between group means were performed by post hoc analysis using Tukey’s honest significance test. A \( p \) value \( < 0.05 \) was considered significant.

Results

Effect of deltamethrin on \( [Ca^{2+}] \)

The effect of deltamethrin on basal \( [Ca^{2+}] \) was examined. Fig. 1A shows that the basal \( [Ca^{2+}] \) was 50 \( \pm \) 2nM. At concentrations between 10\( \mu \)M and 40\( \mu \)M, deltamethrin induced a \( [Ca^{2+}] \) rise in a concentration-dependent manner in the \( Ca^{2+}\)-containing medium. At a concentration of 40\( \mu \)M, deltamethrin evoked a \( [Ca^{2+}] \) rise that attained a net increase of 120 \( \pm \) 2nM (n = 3) followed by a slow decay. The \( Ca^{2+}\) response saturated at 40\( \mu \)M deltamethrin because at a concentration of 60\( \mu \)M, deltamethrin evoked a similar response as that induced at 40\( \mu \)M. Fig. 1B shows that in the \( Ca^{2+}\)-free medium, 10–40\( \mu \)M deltamethrin induced a concentration-dependent rise in \( [Ca^{2+}] \). Fig. 1C shows the concentration—response plots of deltamethrin-induced \( [Ca^{2+}] \) rises. The half maximal effective concentration
was 20/C6
m in the Ca2
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-containing or Ca2
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-free medium, which was obtained by fitting to a Hill equation.

Pathways of deltamethrin-induced Ca2
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entry

Experiments were conducted to explore the Ca2
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entry pathway of the deltamethrin-induced [Ca2
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]i rise. Nifedipine and the store-operated Ca2
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entry inhibitors, namely, econazole (0.5μM) and SK&F96365 (5μM); phorbol 12-myristate 13-acetate [1nM; a protein kinase C (PKC) activator]; and GF109203X (2μM; a PKC inhibitor) were applied 1 minute prior to treatment with 20μM deltamethrin. Except for GF109203X, these agents significantly inhibited deltamethrin-induced [Ca2
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]i rise (Fig. 2).

Source of deltamethrin-induced Ca2
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release

In most cell types including MDCK cells, the endoplasmic reticulum has been shown to be the main Ca2
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store [20]. Thus, the role of endoplasmic reticulum in deltamethrin-evoked Ca2
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release in MDCK cells was explored. The experiments were conducted in the Ca2
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-free medium to exclude the involvement of Ca2
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 influx. Fig. 3A shows that addition of 50μM 2,5-di-tert-butylhydroquinone (BHQ) [21], an endoplasmic reticulum Ca2
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pump inhibitor, induced a [Ca2
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]i rise of 80 ± 2nM. Addition of 40μM deltamethrin afterward failed to induce further Ca2
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 release. Conversely, Fig. 3B shows that after 40μM deltamethrin-induced [Ca2
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]i rise, addition of 50μM BHQ also failed to induce a [Ca2
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]i rise.

Role of PLC in deltamethrin-induced [Ca2
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]i rise

PLC is one of the crucial enzymes regulating the release of Ca2
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 from Ca2
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 stores. Because deltamethrin released Ca2
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was 20 ± 1μM in the Ca2
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-containing or Ca2
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-free medium, which was obtained by fitting to a Hill equation.

Pathways of deltamethrin-induced Ca2
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entry

Experiments were conducted to explore the Ca2
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 entry pathway of the deltamethrin-induced [Ca2
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]i rise.

Figure 1. Effect of deltamethrin on [Ca2
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]i in fura-2-loaded cells. (A) Deltamethrin-induced [Ca2
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]i rise in fura-2-loaded cells. Deltamethrin was added at 30 seconds in the Ca2
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-containing medium. The concentration of deltamethrin is indicated. (B) Effect of deltamethrin on [Ca2
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]i in the Ca2
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-free medium. Deltamethrin was added at 30 seconds in the Ca2
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-free medium. (C) Concentration–response plot of deltamethrin-induced [Ca2
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]i rise. Y axis is the percentage of the net (baseline subtracted) area under the curve (25–200 seconds) of the [Ca2
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]i; rise induced by 20μM deltamethrin in the Ca2
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-containing medium. Data are mean ± standard error of the mean of three experiments. * p < 0.05 compared with open circles.

Figure 2. Effect of Ca2
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channel modulators on deltamethrin-induced [Ca2
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]i rise. In the blocker- or modulator-treated groups, the reagent was added 1 minute prior to the addition of deltamethrin (20μM). The concentration was 10nM for phorbol 12-myristate 13-acetate (PMA), 2μM for GF109203X, 1μM for nifedipine, 0.5μM for econazole, and 5μM for SK&F96365. Data are expressed as the percentage of control (1st column) that is the area under the curve (25–200 seconds) of 20μM deltamethrin-induced [Ca2
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]i rise and are mean ± standard error of the mean of three experiments. p < 0.05 compared with the 1st column.
from the endoplasmic reticulum, the role of PLC in this process was examined. The PLC inhibitor U73122 [22] was applied to examine whether the activation of this enzyme was required for deltamethrin-evoked Ca\textsuperscript{2+} release. Cellular activation by many agonists results in the stimulation of PLC and the subsequent hydrolysis of phosphatidylinositol 4,5-bisphosphate to inositol 1,4,5-triphosphate (IP\textsubscript{3}) and diacylglycerol (DAG) [20]. Each of these two molecules exerts a specific effect on the cell. The increased DAG concentration leads to the activation of PKC while IP\textsubscript{3} binds to the IP\textsubscript{3} receptor, an intracellular Ca\textsuperscript{2+}-release channel located in the endoplasmic reticulum, thereby inducing Ca\textsuperscript{2+} release from internal stores [20].

First, adenosine triphosphate (ATP) was used to test the activity of U73122. Fig. 4A shows that ATP (10 \mu M) induced a [Ca\textsuperscript{2+}]i rise of 75 \pm 2 nM. ATP is a PLC-dependent agonist of [Ca\textsuperscript{2+}]i rise, in most cell types [23], and therefore, was chosen as a tool to examine whether U73122 effectively inhibited the activity of PLC. Fig. 4B shows that incubation with 2 \mu M U73122 did not change basal [Ca\textsuperscript{2+}]i, but abolished ATP-induced [Ca\textsuperscript{2+}]i rise. This suggests that U73122 effectively suppressed PLC activity. The data also show that incubation with 2 \mu M U73122 did not alter basal [Ca\textsuperscript{2+}]i, but abolished 40 \mu M deltamethrin-induced [Ca\textsuperscript{2+}]i rise. However, U73343 (2 \mu M), a U73122 analog, did not have the inhibitory effect (data not shown).

**Effect of deltamethrin on cell viability**

Given that acute incubation with deltamethrin induced a substantial and lasting [Ca\textsuperscript{2+}]i rise, and that an unregulated [Ca\textsuperscript{2+}]i rise often alters cell viability [24], experiments were performed to examine the effect of deltamethrin on viability of MDCK cells. Cells were treated with 0–60 \mu M deltamethrin for 24 hours, and the tetrazolium assay was performed. In the presence of 30–60 \mu M deltamethrin, cell viability decreased in a concentration-dependent manner (Fig. 5).

**Lack of effect of BAPTA/AM on reversing deltamethrin-induced cell death**

The next question was whether the deltamethrin-induced cell death was caused by a preceding [Ca\textsuperscript{2+}]i rise. The intracellular Ca\textsuperscript{2+} chelator BAPTA/AM [25] was used to prevent a [Ca\textsuperscript{2+}]i rise during deltamethrin treatment. After treatment with 5 \mu M BAPTA/AM, 40 \mu M deltamethrin failed to evoke a [Ca\textsuperscript{2+}]i rise (data not shown). This suggests that BAPTA/AM effectively chelated cytosolic Ca\textsuperscript{2+}. Fig. 5 also shows that 5 \mu M BAPTA/AM loading did not alter the control value of cell viability. In the presence of 30–60 \mu M deltamethrin, BAPTA/AM loading did not reverse deltamethrin-induced cell death (n = 3; p > 0.05).

**Role of apoptosis in deltamethrin-induced cell death**

Annexin V/PI staining was applied to detect apoptotic cells after deltamethrin treatment. Fig. 6 shows that 30 \mu M and 40 \mu M deltamethrin induced apoptosis in a concentration-dependent manner. At 30–50 \mu M, deltamethrin also induced necrosis.

**Discussion**

Our study is the first to show that deltamethrin induced a [Ca\textsuperscript{2+}]i rise in a noncancer cell. It has been shown that deltamethrin induced a concentration-dependent [Ca\textsuperscript{2+}]i rise at concentrations between 10 \mu M and 40 \mu M. Deltamethrin induced a [Ca\textsuperscript{2+}]i rise by depleting intracellular Ca\textsuperscript{2+} stores and causing Ca\textsuperscript{2+} influx from extracellular milieu because removing extracellular Ca\textsuperscript{2+} reduced 40 \mu M deltamethrin-induced [Ca\textsuperscript{2+}]i rises by 40%. Removal of extracellular Ca\textsuperscript{2+} decreased the deltamethrin-induced response throughout the measurement period, which suggests that Ca\textsuperscript{2+} influx occurred during the whole stimulation period.
period. The data show that deltamethrin induced a concentration-dependent $[\text{Ca}^{2+}]_i$ rise. Deltamethrin appears to cause $\text{Ca}^{2+}$ entry by stimulating store-operated $\text{Ca}^{2+}$ entry, which is induced by depletion of intracellular $\text{Ca}^{2+}$ stores [26], based on the inhibition of deltamethrin-induced $[\text{Ca}^{2+}]_i$ rise by nifedipine, econazole, and SK&F96365. These three compounds have often been used as blockers of store-operated $\text{Ca}^{2+}$ entry in different cells [27–30], although none of them exerts selective inhibition.

Because activation of PLC produces IP$_3$ and DAG, which activates PKC, the effect of modulation of PKC activity on deltamethrin-induced $[\text{Ca}^{2+}]_i$ rise was examined. Activation of PKC inhibited deltamethrin-induced $[\text{Ca}^{2+}]_i$ rise. By contrast, inhibition of the kinase had no effect. The association between PKC activity and $\text{Ca}^{2+}$ signaling is well-known. For example, $\text{Ca}^{2+}$ signaling was shown to play a key role in regulation of chondrogenesis by PKC [31]. Mepivacaine-induced contraction was found to involve increased $\text{Ca}^{2+}$ sensitization mediated by Rho kinase and PKC in endothelium-denuded rat aorta [32].

Regarding the $\text{Ca}^{2+}$ stores involved in deltamethrin-evoked $\text{Ca}^{2+}$ release, the BHQ-sensitive endoplasmic reticulum stores seemed to be the dominant one. The data further show that the $\text{Ca}^{2+}$ release was by a PLC-dependent mechanism, given that the release was blocked when PLC activity was inhibited.

Deltamethrin has been shown to induce apoptosis in several cell types. This study shows that deltamethrin was cytotoxic to MDCK cells by evoking apoptosis and necrosis. $\text{Ca}^{2+}$ overloading is known to initiate processes leading to alteration in cell viability [20]. In many cell types, $\text{Ca}^{2+}$ mobilization may cause $\text{Ca}^{2+}$ influx across the plasma.

Figure 4. Effect of U73122 on deltamethrin-induced $\text{Ca}^{2+}$ release. Experiments were performed in the $\text{Ca}^{2+}$-free medium. (A) Adenosine triphosphate (ATP; 10$\mu$M) was added as indicated. (B) U73122 (2$\mu$M), ATP, dimethyl sulfoxide (DMSO; 0.1%), and deltamethrin (40$\mu$M) were added as indicated. Data are mean ± standard error of the mean of three separate experiments. *$p < 0.05$ compared with the first bar (control). Control is the area under the curve of 40$\mu$M deltamethrin-induced $[\text{Ca}^{2+}]_i$ rise (25–220 seconds).

Figure 5. Effect of deltamethrin on cell viability. Cells were treated with 0–60$\mu$M deltamethrin for 24 hours, and the cell viability assay was performed. Data are mean ± standard error of the mean of three experiments. Each treatment had six replicates (wells). Data are expressed as percentage of control that is the increase in cell numbers in the deltamethrin-free groups. Control had 10,985 ± 712 cells/well prior to the experiments, and had 13,658 ± 799 cells/well after incubating for 24 hours. *$p < 0.05$ compared with control. In each group, the $\text{Ca}^{2+}$ chelator BAPTA/AM (5$\mu$M) was added to cells followed by treatment with deltamethrin in the $\text{Ca}^{2+}$-containing medium. Cell viability assay was subsequently performed. *$p < 0.05$ compared with control. BAPTA/AM = Acetoxymethyl ester of 1,2-bis(2-aminophenoxy)ethane-$\text{N},\text{N},\text{N},\text{N}$-tetraacetic acid.
membrane by the process of store-operated Ca\textsuperscript{2+} entry \cite{11,20}. Prolonged elevations in [Ca\textsuperscript{2+}] or abnormal regulations of [Ca\textsuperscript{2+}] are known to lead to cell injury and apoptosis \cite{11,20}. In previous studies, deltamethrin increased [Ca\textsuperscript{2+}], and subsequently induced apoptosis in rat neural cells \cite{5}. However, our data show that deltamethrin-induced cell death is independent of a [Ca\textsuperscript{2+}] rise. Nevertheless, the elevated [Ca\textsuperscript{2+}], could alter various Ca\textsuperscript{2+}-dependent downstream responses, thereby affecting cell physiology. Therefore, it appears that the effect of deltamethrin on [Ca\textsuperscript{2+}], and cell viability varied among different cell types.

Figure 6. Apoptosis induced by deltamethrin measured by annexin V/propidium iodide (PI) staining. (A) Cells were treated with 0\, \mu M, 30\, \mu M, 40\, \mu M, or 50\, \mu M deltamethrin, respectively, for 24 hours. Cells were then processed for annexin V/PI staining and analyzed by flow cytometry. (B) The percentage of apoptotic and necrotic cells. *\textit{p} < 0.05 compared with control. Data are mean ± standard error of the mean of three experiments. FITC = fluorescein isothiocyanate.
Because the data show that treatment with 30–50μM deltamethrin induced an increase in the percentage of apoptotic cells, it is suggested that deltamethrin-induced cytotoxicity involved apoptosis. Although 50μM deltamethrin caused cell death by 80% in viability experiments, the same concentration of deltamethrin only induced apoptosis in 15% of cells. It is possible that the significant loss of cell viability is through other pathways such as necrosis or autophagy.

Together, the results show that deltamethrin induced Ca\(^{2+}\) entry by a PKC-dependent, store-operated Ca\(^{2+}\) entry in MDCK cells. Deltamethrin also released Ca\(^{2+}\) from the endoplasmic reticulum in a PLC-dependent fashion. Deltamethrin killed cells by Ca\(^{2+}\) influx and apoptosis in Madin-Darby canine renal tubular cells. Chin J Physiol 2010;53:215–22.

References


