Indian Journal of Experimental Biology
Vol. 55, May 2017, pp. 271-278

Long term exposure to deltamethrin causes a dual effect on plasma membrane Ca^{2+}-ATPase activity and reduces its mRNA levels in human lymphocytes

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Received 13 March 2015; revised 09 March 2016

Deltamethrin (Delt), a type II pyrethroid used worldwide, is chemically and biologically stable and displays mild toxicity in mammals, but chronic exposure causes toxic effects on the immune system. In order to clarify the damage of deltamethrin to lymphocytes, we studied the effect of deltamethrin on the catalytic activity and expression levels of the plasma membrane Ca^{2+}-ATPase (PMCA) using human lymphocytes as a model. Lymphocytes were incubated with Delt at concentrations ranging from 2 to 133 µM for 36 h, followed by plasma membrane purification and analysis of PMCA activity. A significant increase of 122% in PMCA activity was observed with 2 µM Delt, while concentrations of 40 and 60 µM inhibited activity of PMCA to 58.3% below the control. The viability of lymphocytes was most affected at concentrations higher than 133 µM, the concentration that inhibited PMCA activity to 30.5%. Expression of PMCA was assessed by qPCR and the data showed a decrease in the amount of PMCA transcripts as a result of treatment with deltamethrin. Our results have shown that deltamethrin treatment of human lymphocytes in culture influences PMCA activity as measured in isolated plasma membranes as well as the PMCA mRNA levels sampled at different time points. It indicates disruption of the enzyme activity which would compromise cellular signaling and eventually may lead to cell death.

Keywords: Insecticide toxicity, Neurotoxin, PMCA, Pyrethroid, T-lymphocytes

The pyrethorid deltamethrin (Delt), a potent neurotoxin for both insects and humans, is widely used in crop pest management and also for control of insect-borne diseases[1]. Nonetheless, Delt is a risk for human health; it accumulates in different tissues resulting in histological and biochemical changes, including toxic effects to the immune system[1-3].

In general, pyrethroids bind voltage-gated sodium channels and modify their activity[4], increase phosphorylation levels of synapsin I, and of protein kinase C (PKC) and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$)[5,6]. Therefore, it is important to understand the effects of Delt on Ca^{2+} homeostasis, especially in proteins involved in the regulation of intracellular Ca^{2+}, such as Ca^{2+}-ATPase from sarcoplasmic reticulum (SERCA), which is known to be affected by Delt[7] or the Ca^{2+}-ATPase from plasma membrane (PMCA). Based on this evidence, our hypothesis was that Delt can affect PMCA. There are four PMCA isoforms, PMCA 1-4 encoded by four different genes, which are also processed by alternative splicing[8,9]. However, the predominant isoform in the plasma membrane of lymphocyte and erythrocyte is PMCA4, which has a calmodulin (CaM) binding-site at the C-terminus (CaM-BD)[9,10].

PMCA is an ATP-dependent enzyme that transports Ca^{2+} across the plasma membrane, from the cytosol to the extracellular media. Topologically, PMCA shows 10 transmembrane domains with both cytoplasmic N- and C-terminus. Two conformational states of the enzyme have been described, E1 and E2 stages, where E1 has high-affinity and E2 lower-affinity for Ca^{2+} [10]. The high-affinity Ca^{2+}-binding site in PMCA is located in the cytoplasmic loops suggesting a role in controlling free Ca^{2+} under resting conditions[11]. The binding of PMCA to CaM decreases the K_d for Ca^{2+} from 20 µM to 1 µM[12]. Physiological, transient and high cytosolic Ca^{2+} concentrations are involved in a broad number of cellular pathways, in some cases leading to different pathologies[13]. However, only in few cases, the effect on PMCA and a pathological state had been shown to be correlated[14,15].

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In lymphocytes, PMCA maintains Ca^{2+} homeostasis through the regulation of cytosolic Ca^{2+} levels[16]. The effect of Delt on human PMCA in T-lymphocytes and activated T-lymphocytes remains unexplored, though it is known that thymus is affected by Delt. Therefore, in this study, we investigated if Delt is affecting PMCA activity in human T-lymphocytes.

Materials and Methods

Phytohemagglutinin (PHA), trypan blue, Tween-20, were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Deltametrin (pyrethroid deltamethrin ((S)-a-cyano-3phenoxybenzyl (1R, 3R)-3-(2,2-dibro-movinil)-2,2-dimethyl cyclopropane- carboxylate), 99.7% pure) was purchased from PESTANAL, (Sigma-Aldrich, Mexico). Deltamethrin was diluted in deionized water. Percoll was obtained from General Electric (GE Healthcare), McCoy-5A modified medium and Fetal Serum Bovine (FBS) were obtained from GIBCO (InVitroGen, Mexico, D.F.). Human healthy volunteers ranging 20-25 years age, were recruited among the student population from our institution (Instituto de Ciencias Biomédicas, UACJ). Our procedure was approved by the Institute´s Ethics Committee

Isolation of human lymphocytes

Lymphocytes were purified from human blood as previously described by de la Rosa *et al*.[17]. Peripheral blood was extracted from ten apparently healthy donors, faculty and students volunteers where from the Institute of Biomedical Sciences, Autonomous University of Juarez City (Mexico). The blood was collected in tubes containing PBS-EDTA (pH 7.4) (1:1, v/v), then we added 4 mL of isotonic Percoll (57.5%) and spin at 1000×g for 25 min at room temperature (25°C) in order to form a gradient. Following the manufacturer's directions, in the gradient we were able to separate T-lymphocytes from the rest of white cells; the second fraction (from top to bottom) corresponding to 1.06-1.08 g/mL was collected.

T-lymphocytes were washed twice, and then collected in a selective lymphocyte McCoy-5A modified medium.

Cell culture and treatments

The purified cells collected in McCoy-5A were supplemented with 10 mg/mL phytohemagglutinin (PHA, for stimulation of T cells only), 1% penicillin/streptomycin, and 10% FBS. Cell viability was analyzed with the trypan blue exclusion assay (0.4% (w/v) in 0.85 % (w/v) NaCl)[18]. T-lymphocyte cultures were treated with -133 µM of Delt under the conditions described by Mankame *et al*.[19], with paired cultures in which we added no Delt and these functioned as age-matched culture for every Delt dose applied. Cells were cultured for 72 h and counted every 24 h, before collecting the cells, culture medium was absorbed and the cells washed thrice thoroughly, (adding volume two-fold that of the medium) no Delt was added after this step. The results were represented as viability percentage values.

Isolation of plasma membranes

The cell membranes were prepared by the method described in Grosman and Diel[20]. The isolation of cell membrane was performed after 36 h of exposure to treatment with Delt. Cells were washed 4 times with a buffer of 10 mM Tris-HCl (pH 7.2), containing 131 mM NaCl, 2.4 mM KCl, 1 mM $CaCl_2$ and 0.5 mg/mL bovine serum albumin. Afterwards, cells were exposed to hypotonic shock with 5 mM Tris-HCl (pH 8.1), followed by sedimentation at 14000 ×g. Membranes were washed twice with the same solution. Finally, membranes were resuspended in 0.32 M sucrose, 10 mM imidazole, 1 mM EDTA (pH 7.5) and stored in aliquots at −80°C until they were used in enzymatic assays. Membrane protein was determined by the method of Lowry *et al*.[21].

Determination of Ca^{2+} concentration

Free Ca^{2+} concentration in the media was determined with the aid of $CaCl_2$ and EGTA solutions as described by Fabiato[22]. The software considers the absolute affinity constant for the Ca^{2+}-EGTA complex[23], the EGTA protonation equilibria[24], and the presence of relevant electrolytes in the reaction medium.

Ca^{2+}-ATPase activity assay

The Ca^{2+}-ATPase activity was assessed by a colorimetric method measuring the amount of orthophosphate formed by the hydrolysis of ATP nmol P_i /sample, (Lanzetta *et al*.[25]). The stabilizing agent Sterox was substituted with 0.18% (v/v) Tween-20 as described by Baykov *et al*.[26]. The assessment was carried out in a medium containing 30 mM MOPS (pH 7.2), 130 mM KCl, 3 mM $MgCl_2$, 0.5 mM EGTA, 0.5 mM $CaCl_2$ (10 µM free Ca^{2+}), 0.12 mg membrane protein/mL in the presence of 0.1 mM Ouabain a specific inhibitor of the Na^+-K^+ ATPase. The reaction mix was incubated at 37°C and started

by adding 1 mM ATP. For substrate affinity assays, we used ATP concentrations from 0.1-2 mM (cells were previously exposed to Delt for 36 h, then treatment stopped). In these assay conditions, the ATP hydrolysis can be augmented by 1 µg/mL calmodulin (data not shown), and thus is only attributed to the PMCA activity excluding the activity of Sarco/ Endoplasmic Reticulum Ca^{2+} ATPase. Calmodulin was not included in every experiment and there was no Delt in any of the assays. Thus, effects on enzymatic data are due to the effect of Delt during exposure of the cells in culture.

Quantitative polymerase chain reaction (qPCR) from T lymphocytes

Human lymphocytes were homogenized in TRIZOL (Promega, 5×10^6 cells in 250 µL TRIZOL). Chloroform was added next to the mixture and spinned at 12000 × g. Total RNA was precipitated from aqueous phase using isopropanol, following by sedimentation at 12000 × g. The pellet was washed twice with 75% ethanol and sedimented at 9000 × g. RNA quality was analyzed on a 0.8% agarose gel and quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific). From each sample 1 µg of total RNA was reverse transcribed with the MMLV Reverse Trascriptase kit (Promega). Quantitative PCR was performed with Sybr Green mastermix (Applied Biosystems) using a MyiQ2 (BioRad) thermal cycler, using 1 µg of cDNA. Identity of expected amplicons was verified by agarose gel electrophoresis. The relative change in PMCA gene expression was calculated by 2-ddCt method[27] corrected to the expression of GAPDH assayed under the experimental and control conditions. Primer sequences are shown in Table 1. Samples were taken from isolated lymphocytes, following by 36 h in culture under control conditions or with Delt treatment at 2 µM and 133 µM. Each condition was done in triplicates and repeated four times.

Statistical analysis

The average and standard error of the mean (SEM) from three independent experiments were noted as results. The data were analyzed by analysis of variance (ANOVA), and *post-hoc* analysis using a significance level of $P < 0.05$ (Sigma Plot 11).

Table 1—Primer sequences of target and housekeeping genes

	Sequence	Amplicon exp. size
PMCA		
	Forward Primer 5'-GTCTGTCTGGGAACCCTGC-3'	
	Reverse Primer 3'-GGGGTAGTTGCGACTTGACC-5'	222 bp
GAPDH		
	Forward Primer 5'-TTGATTTTGGAGGGATCTCG-3'	
	Reverse Primer 3'-CGAGATCCCTCCAAAATCAA-5'	238 bp

Results

Dose-response effect of deltamethrin on T-lymphocytes in culture

Human T-lymphocytes in culture were exposed to deltamethrin (Delt) at different concentrations from 24- 72 h and results of this experiment are shown in Fig. 1A. Without treatment, cell viability decreased over time (50-60% of reduction in cell number, $P < 0.05$ compared to control at time=0). In contrast, Delt treatment between 2-40 µM increased cell viability (76 to 88.3%) compared to control without Delt ($P < 0.05$). On the other hand, high concentrations of Delt 60-133 µM reduced cell viability to 11.2-13% after 24 h of treatment ($P < 0.05$ compared to control without Delt).

Cell viability of human lymphocytes stimulated with mitogen phytohemagglutinin (PHA, from *Phaseolus vulgaris*) is shown in Fig. 1B. Cell cultures with PHA increased in number of cells in a time-dependent manner. After 24 h and 48 h, cells where

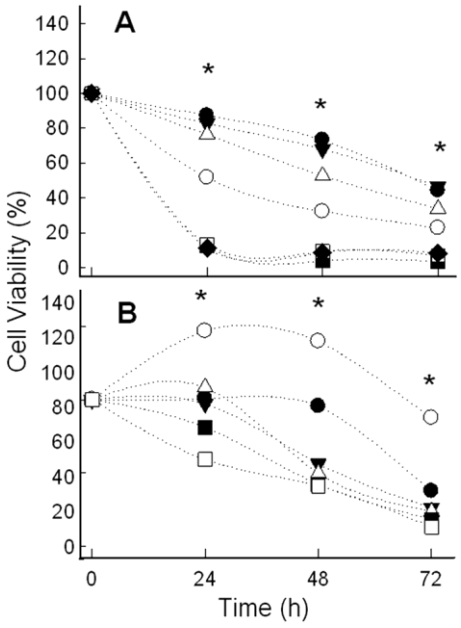

Fig. 1—Delt dose-response curves on cell viability of human lymphocytes. Effect of different doses of (A) Delt in cultures of T-lymphocytes; and (B) Delt on T-lymphocytes stimulated with PHA. [Data are expressed as mean ± SEM (for some points symbols are bigger than error bars) of 4 independent experiments. An asterisk indicates a significant difference compared to control ($P \leq 0.05$). Symbols: empty circles are control cells with no treatment, filled circles represent Delt at 2 µM; filled arrowheads, Delt at 20 µM; empty triangles Delt at 40 µM; filled squares, Delt at 60 µM; empty squares, Delt 99.5 µM, filled diamonds, Delt 133 µM]

16% more than what it was at 0 h. The increment in cell number was significant compared to 0 h (P = 0.05). Cells treated with 2 and 20 µM Delt were not stimulated with PHA and the number of cells did not change. Clearly, Delt prevented the effect of PHA on T-lymphocytes.

After-effect of deltamethrin on PMCA activity

The plasma membrane Ca^{2+}-ATPase (PMCA) is a key enzyme in the regulation of $Ca^{2+}$and its dysfunction is related to cell viability. PMCA is known to be the major enzyme transporter of Ca^{2+} in several cell types, including immortalized cell lines[9] and T-lymphocytes[16]. Thus, it is important to assess the effect(s) of Delt on the Ca^{2+}-ATPase activity of human T-lymphocytes. With this purpose, plasmatic membranes from cells treated with Delt were isolated and used to measure the enzymatic activity of PMCA. We analyzed the effect of different concentrations of Delt, characterized by calmodulin positive modulation, as determined by the release of P_i from the hydrolysis of ATP under conditions that inhibit Na-K ATPase (see Methods) from the membrane fractions derived from T-lymphocytes exposed to Delt for 36 h (Fig. 2A). Our results clearly showed that

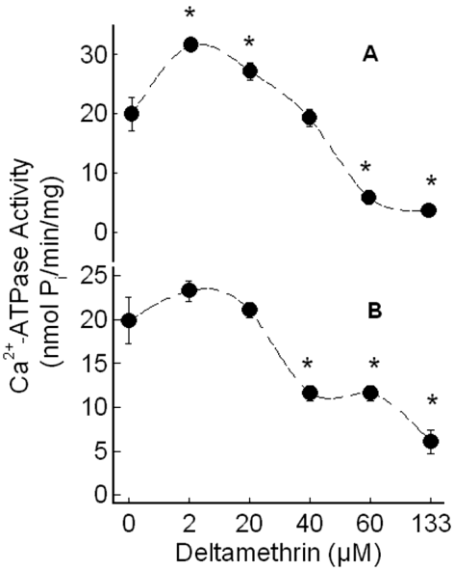

Fig. 2—Effects of Delt on Ca^{2+}-ATPase activity. PMCA activity was determined in plasma membrane isolated from lymphocytes in culture treated with different doses of Delt for 36 h. Shows PMCA activity in plasmatic membranes of (A) T-lymphocyte; and (B) PHA-activated T-lymphocyte and treated with different doses of Delt. [Data are expressed as mean ± SEM of 4 independent experiments. An asterisk indicates a statistical significance difference compared with control cells with no treatment (ANOVA Shapiro-Wilk, F= 21.809, P <0.001, all pairwise multiple comparison, Bonferroni t Test P <0.001)]

Table 2—PMCA activity values[2]

Parameters	Activity (nmol mean ± SEM) T-lymphocytes	Activated T-lympho
Control	19.33 ± 2.9	19.88 ± 2.64
Inhibitor (La^{3+})	3.10 ± 0.63*	3.10 ± 0.63*
Deltamethrin (µM)		
2	31.60 ± 0.95*	23.26 ± 1.20
20	27.12 ± 1.47*	21.06 ± 0.90
40	19.28 ± 1.47	11.59 ± 0.88*
60	5.74 ± 0.63*	11.59 ± 0.88*
133	3.58 ± 0.66*	6.08 ± 1.33*

[ATPase activity was measured by the release of inorganic pho after the hydrolysis of ATP catalyzed by PMCA. Values are me SEM nmoles P_i/mg prot/min, n= 4.]

Delt stimulated the enzymatic activity of when cells were treated with low concentrations of Delt (2 µM and 20 µM) and enzyme activity was 27.12 and ~31.60 nmol P_i/mg/min, respectively compared to the control (without Delt) that was 19.88 nmol P_i/mg /min (Table 2, ANOVA Shapiro-Wilk, F= 21.809, P <0.001). The enzymatic activity of plasma membranes treated with 40 µM Delt showed no significant effect compared to the control (19.28 ± 1.47 vs 19.88 nmol P_i/mg /min, p=0.706). Higher concentrations of Delt (60 to 133 µM), there was a reduction in the activity of PMCA to 5.74 and 3.58 nmol P_i/mg/min, respectively.

We also analyzed the enzymatic activity of PMCA in membranes from activated T-lymphocytes (Fig. 2B). There was also a significant difference among treatments (ANOVA Shapiro-Wilk, F= 52.974, P <0.001). In membranes from these cells without Delt treatment, PMCA activity was 19.88 nmol P_i/mg/min. Low concentrations of Delt, 2 and 20 µM, stimulated PMCA activity 23.26 and 21.06 nmol P_i/mg/min, respectively. This difference was found statistically insignificant compared to the control (all pairwise multiple comparison, Bonferroni t Test p=.103). However, 40 and 60 µM of Delt, activity of PMCA decreased by ~50% with a significant difference (all pairwise multiple comparison, Bonferroni t test P <0.001). The highest Delt concentration tested, 133 µM, reduced the activity even more until 6 nmol P_i/mg/min. We included a negative control with La^{3+}, a known inhibitor to PMCA activity, showing the specific effects of Delt on this enzyme (Table 2).

Effect of deltamethrin on substrate affinity

In order to analyze the biphasic effect on PMCA (stimulation and inhibition) from cells treated with Delt, it was necessary to evaluate the effect of Delt in the substrate affinity of PMCA (ATP). Therefore, we

analyzed the effects of Delt at 2 and 133 µM on the activity of PMCA at different ATP concentrations ranging 0-2 mM. The PMCA activity from T-lymphocyte membranes treated with 2 µM Delt was stimulated (Fig. 3A) showing a V_{max} of 30 nmol P_i/mg/min compared to the control 21 nmol P_i/mg prot/min. This stimulation was significant (P=0.030). However, there was no significant differences between ATP concentrations between 0.1 to 0.5 mM (p= 0.667, 0.057, respectively). There was a significant inhibition of enzymatic activity of PMCA in lymphocyte membranes with Delt at 133 µM. The enzymatic activity of PMCA from activated T-lymphocyte is shown in Fig. 3B. Concentration of 2 µM Delt produced a V_{max} 22 nmol P_i/mg/min which was not different to control V_{max} 19.5 nmol P_i/mg/min (P =0.227). Delt at 133 µM produced an inhibitory effect on the enzymatic activity of PMCA at all ATP concentrations of Delt tested (P= 0.001).

The affinity of PMCA for its substrate (K_m value) was determined from experiments showed in Fig. 3 and the values are reported in Table 3. PMCA have a low affinity for ATP with K_m of 0.295 ± 0.02 mM. However, 2 and 133 µM Delt did not affect the affinity of PMCA for ATP compared to the control K_m. In activated T-lymphocytes, Delt 2 µM increased K_m value (0.468 ± 0.02), indicating a reduction in the affinity of PMCA for ATP. In contrast, Delt 133 µM reduced K_m value (0.165 ± 0.01), increasing the affinity of PMCA for ATP (p=0.002).

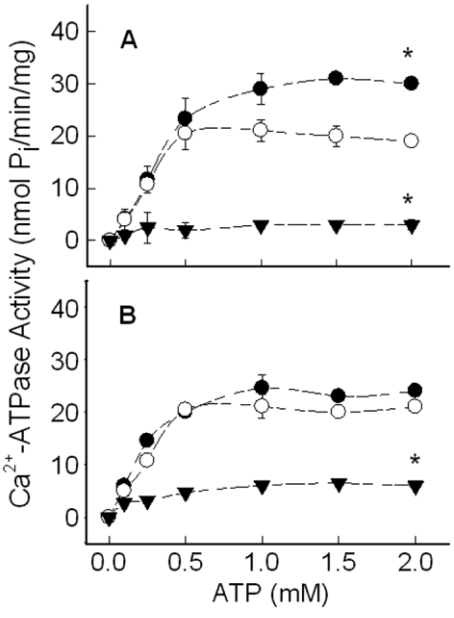

Fig. 3—Effect of Delt on PMCA activity as a function of ATP concentration. Control cells with no treatment (○), with Delt 2 µM (●), and Delt 133 µM (▼). (A) Depicts the PMCA activity in lymphocytes membranes; and (B) in activated lymphocytes membranes (* P <0.05).

Effect of deltamethrin on PMCA expression in T-lymphocytes

We studied the effect of Delt on PMCA expression in human T-lymphocytes using quantitative PCR (n=4 per time point and dose). Delt produced a dose dependent effect in PMCA expression: Initially, Delt increased the expression of PMCA to 1.2-fold, but at 133 µM Delt, the expression of PMCA was reduced to 0.12-fold compared to the control (Fig. 4).

Table 3—Affinity values of PMCA for ATP in the presence of Delt[3]

	Affinity values (K_m)	
	Means ± SEM	P-values
Activated T-lymphocytes		
Control	0.295 ± 0.0218	
Delt 2 µM	0.271 ± 0.0160	0.403
Delt 133 µM	0.288 ± 0.0832	0.343
T-Lymphocytes		
Control	0.297 ± 0.0218	
Delt 2 µM	0.468 ± 0.0231	0.002
Delt 133 µM	0.165 ± 0.0125	0.002

['t'-test indicating the P-values between control with Delt treatment, where lymphocytes no treatment were *ns* and treatment were significant to 2 µM and 133 µM]

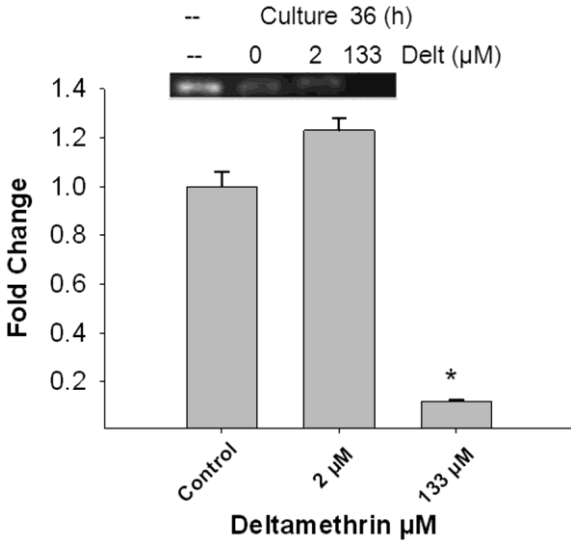

Fig. 4—Expression levels of PMCA in response to Delt. [Expression levels compared to those of GAPDH determined by qPCR, comparison was made using the ddCt method[27]. PMCA expression (fold change) is reduced as a result of culture conditions in the presence of Delt at 133 µM. Inset: representative gel of PCR PMCA amplification from lymphocytes cultured during 36 hours with Delt as indicated. First line, amplification product from purified lymphocytes. One-Way ANOVA (P <0.001). Pairwise comparison procedures Control *vs*. PMCA 133 µM (P <0.05, *)]

Statistical analysis of these data rendered a significant difference among the groups (One-Way ANOVA, P <0.001; Pairwise comparison procedures Control *versus* PMCA 133 µM, P <0.05*).

Discussion

This study was conducted to determine the effects of Delt on human T-lymphocytes and activated T-lymphocytes in culture and the effect on the enzymatic activity of the plasma membrane Ca^{2+}-ATPase (PMCA). Previous studies showed the effect of pesticides, including pyrethroids on enzyme activity of PMCA isolated from eritrocytes[28]. In the present study, we evaluated the effect of pesticides after prolonged treatment, and thereby understand the acute effect as previously shown by others[28], from the chronic (long lasting) which can be produced after a prolonged exposure to the pesticide. Accordingly, we designed a method where human cells were treated with Delt and later their plasma membranes were isolated and used to measure PMCA activity in the absence of Delt. Thus, we considered the effects seen as an effect of the prolonged treatment of the pesticide and in this way, as a long lasting modification on the PMCA itself or the lipid environment.

Our results demonstrated that cell survival of T-lymphocytes was dependent on the Delt concentrations tested (Fig. 1). We observed that Delt increased cell viability at concentrations between 2-40 µM, but it had a negative effect at higher concentrations in both cell viability and PMCA activity.

We found an increase on cell survival of T-lymphocytes exposed to low concentrations of Delt (2-40 µM, Fig. 1A); although it was clear that the viability of activated T-lymphocytes was reduced in the presence of higher concentrations of 40-133 µM, which can be attributed to Delt cytotoxicity. We have also shown that Delt prevented the increasing viability induced by phytohemaglutinin (PHA) on activated T-lymphocytes (Fig. 1B). Reports showed that some pyrethroids like allethrin, cypermethrin and permethrin cause humoral damage, although they were also able to inhibit the mitogenic response of lymphocytes to concanavalin A[29]. In addition, Delt affects other cell types and tissues such as fibroblasts[30], germinal cells, sperm[31], liver and muscle of freshwater fish *Anabas testudineus*[32]. It is reported that lectin PHA acts on lymphocytes increasing their metabolic and the cell division rates, mediated through PKC activation[33,34]. Probably Delt prevented the increase in cell number induced by PHA because Delt also interacts with PKC[5,6].

Long term treatment of T-lymphocytes with Delt showed a dual effect on PMCA activity assayed on isolated membranes. In plasma membrane fractions isolated from T-lymphocytes cultured with lower concentrations of Delt (2-20 µM), PMCA activity was higher compared to membranes isolated from T-lymphocytes cultured in the absence of Delt (Fig. 2A), whereas in fractions isolated from cells cultured at Delt 40-133 µM, we noticed inhibition of PMCA activity refered to membranes isolated from cells with no treatment. Therefore, the effects of Delt treatment on the PMCA activity are related to the cell viability of T-lymphocytes. In contrast to plasma membranes isolated from T-lymphocytes (Fig. 1A), membranes from activated lymphocytes (Fig. 1B) did not have a significant increase in PMCA activity with Delt at 2-20 µM. But in a very similar manner, plasmatic membranes from activated T-lymphocytes treated with 20-133 µM Delt have shown a reduction in PMCA activity (Fig. 2B). Only activated T-lymphocytes had decreased affinity of PMCA for ATP with Delt 2 µM (K_m 0.468 mM) from control (K_m 0.297 mM), whereas after Delt 133 µM treatment increased the affinity of PMCA for ATP (K_m =0.129, Table 3). These changes reflect a decreased enzyme affinity produced by long term exposure at lower Delt concentrations, but a marked increase affinity of PMCA for ATP in membranes isolated from activated T-lymphocytes cultured at higher Delt concentrations, which would explain in part the reduced P_i production due to the fast saturation of the enzyme by substrate. These same effects have been demonstrated with other P-type enzymes like SERCA-type ATPase[7].

It has been suggested that alterations in enzyme activity induced by insecticides are due to the interaction between an insecticide and the plasma membrane in the lipid bilayer, and this interaction reduces the affinity of the enzyme for the substrate[28]. The biphasic effect of Delt on PMCA activity could be attributed to the interaction of plasma membrane with Delt.

To determine if Delt was affecting the expression of PMCA gene, we analyzed mRNA levels of PMCA by qPCR and we used GAPDH as a housekeeping gene. The relative changes induced by Delt treatment at different concentrations was normalized to GAPDH gene expression under each treatment and control

condition by the 2ddCt method[35], and the data are shown in Fig. 4. In lymphocytes treated with 2 µM Delt there was a 1.2 fold increase in PMCA gene expression, whereas at 133 µM Delt decreased PMCA gene expression to 0.12-fold below control. Delt altering gene expression has been reported before[36], mRNA levels of GTP-binding nuclear protein (*Ran)* were increase by treating Kc cells with several concentrations of Delt[37] (10-50 ppm). Our data as well as others' reveal that Delt can induce changes in gene expression.

Conclusion

In summary, Delt long term exposure produces a dual effect on PMCA activity in isolated plasma membranes. Low concentrations of Delt (2-20 µM) increase PMCA activity and higher concentrations of Delt render an enzyme with reduced activity. Delt treatment also decreased mRNA levels of PMCA. This is the first report of Delt affecting gene expression of PMCA in isolated human cells in culture, a question deserving further scrutiny to explain the effect of this insecticide *in vivo* and pursuant of a future research.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgement

The authors acknowledge the technical assistance from Alejandra Vargas-Caraveo (UACJ), Leny Judith Alvarez-Araujo (UACJ), and Dr. Jorge Anibal Sierra-Fonseca (University of Texas at El Paso). This project was supported by CONACyT CHI CO1-57 268 (to FPT) and partially by CONACyT 2008-01, 390-106298 (to JAPL), and by grant institution PIFI 2909 5001-004-09.

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