Cypermethrin, deltamethrin and glyphosate affect the activity of the Ca^{2+}-ATPase from human erythrocyte

Cipermetrina, deltametrina y glifosato afectan la actividad enzimática de la Ca^{2+}-ATPasa de eritrocito humano

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Abstract

The extensive use of pesticides can cause many human health problems. However, the effects of pesticides on a biochemical level are still poorly understood. In this study we analyzed the effect of different pesticides on the plasma membrane Ca^{2+}-ATPase (PMCA) and on the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) activities. Different amounts of pesticides were added to the Ca^{2+}-dependent ATPase assays in order to determine if they were affecting the ATPase activity. The results showed that PMCA activity was partially inhibited by deltamethrin to 51.85% ± 3.7 when its concentration in the reaction medium was 0.5 mM, while cypermethrin and glyphosate stimulated the PMCA activity at the lowest concentrations tested. The maximum stimulatory effect of cypermethrin was of 155% ± 9.0 at a concentration of 0.2 mM. In addition, the herbicide glyphosate stimulated the activity 111% ± 2.0 at a concentration of 0.2 mM. In conclusion, our results showed that PMCA activity was partially inhibited by deltamethrin, but cypermethrin and glyphosate stimulated their activity. Our findings suggest that cypermethrin and deltamethrin have different structure-activity relationships. However, SERCA was not sensitive to deltamethrin or glyphosate. These differences may be reflected in disturbs over cellular calcium regulation.

Keywords: PMCA (plasma membrane Ca^{2+}-ATPase), SERCA (sarco/endoplasmic Ca^{2+}-ATPase) enzymatic activity, cypermethrin, deltamethrin, glyphosate.

Resumen

Se ha observado que la exposición de personas a plaguicidas puede causar problemas a la salud. Sin embargo, el estudio del efecto de plaguicidas a un nivel bioquímico ha sido pobremente estudiado. En este estudio, analizamos el efecto de cipermetrina, deltametrina y glifosato sobre la actividad enzimática de las Ca^{2+}-ATPasa de membrana plasmática (PMCA) y de retículo sarcoplásmico (SERCA). Diferentes concentraciones de estos pesticidas fueron añadidas a los experimentos de actividad enzimática como estrategia para determinar si estos compuestos eran capaces de afectar la actividad enzimática de PMCA. Nuestros resultados demuestran que la actividad enzimática de PMCA fue parcialmente inhibida por deltametrina en un 51.85% ± 3.7 cuando su concentración fue de 0.5 mM, mientras que cipermetrina y glifosato estimularon la actividad enzimática de PMCA a menores concentraciones. El máximo efecto estimulatorio de cipermetrina fue de 155% ± 9.0 cuando el compuesto alcanzó una concentración de 0.2 mM. Además, el herbicida glifosato fue capaz de estimular la actividad enzimática de PMCA en un 111% ± 2.0 a concentraciones de 0.1-0.2 mM. En conclusión, nuestros resultados demostraron que la actividad enzimática de PMCA fue también parcialmente inhibida con deltametrina, pero al contrario de cipermetrina y glifosato la estimularon. Nuestros resultados sugieren que las diferencias en las estructuras químicas de cipermetrina y deltametrina se ven reflejadas en el efecto provocado sobre PMCA. Sin embargo, una enzima similar, SERCA, no fue afectada ni por deltametrina ni glifosato. Estas diferencias se pudieran ver reflejadas en disturbios sobre la regulación celular del calcio.

Palabras clave: PMCA (ATPasa de Ca^{2+} de membrana plasmática), SERCA (ATPasa de Ca^{2+} de retículo endo/sarcoplásmico), actividad enzimática, cipermetrina, deltametrina, glifosato.

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Introduction

Pesticides are chemicals used worldwide in agriculture and in household settings, resulting in continuous human exposure from different sources including food, water, and occupational exposure. The effect of pesticides is targeted to specific organisms, however, pesticides are toxic substances widely released into the environment, thus posing a threat to human health (Alavanja *et al*., 2004; Weiss *et al*., 2004). The damage caused by pesticide exposure has been a matter of concern for many years, but the toxic effects induced by most of these chemical compounds remain enigmatic (Alavanja *et al*., 2004).

Pyrethroids are among the most used pesticides, and unlike first generation pyrethroids, the second generation of pyrethroids are more resistant to the environmental conditions and therefore are more persistent and toxic to living organisms. Two members of the second generation group are cypermethrin and deltamethrin, and they were studied in the present work. The toxic action of pyrethroids mainly focuses on the nervous system by interfering with the ionic channels, which causes membrane depolarization, synaptic alterations and immunotoxicity (Madsen *et al*., 1996; Narahashik, 1996; Soderlund *et al*., 2002; Diel *et al*., 2003). Glyphosate, a frequently used organophosphate herbicide, has been reported to cause toxic effects such as nausea, renal failure, hepatic alterations, and behavioral disturbances and also affect the animal cell cycle (Steinrucken *et al*,. 1980; Marc *et al*., 2002; De Roos *et al*., 2005). Calcium (Ca^{2+}) signaling is a key parameter for cell survival. Calcium acts as a carrier of signals for different cellular processes, including muscle contraction, synaptic transmission and apoptosis. This means that intracellular Ca^{2+} concentration must be strictly regulated (Carafoli, 2002). The plasma membrane Ca^{2+}-ATPase (PMCA) plays a key role in the maintenance of Ca^{2+} homeostasis. PMCA is a membrane-bound enzyme responsible for Ca^{2+} transport from the cytosol to the extracellular space using the energy provided by the ATP hydrolysis. Typically the erythrocyte is the selected cell to obtain PMCA preparations, but the enzyme is present in all eukaryotic plasma membranes. The hydrolytic activity of PMCA can be directly stimulated by calmodulin (CaM), a small cytosolic Ca^{2+}-binding protein (Carafoli, 1992). Another enzyme responsible for the maintenance of low Ca^{2+} concentration in the cytoplasm is the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA). This enzyme translocates Ca^{2+} from the cytosol to the lumen of the sarcoplasmic or endoplasmic reticulum in an ATP-dependent mechanism (Khan *et al*., 2000).

The toxic mechanisms of pesticides should be different for each case. However, since these molecules are generally lipophilic, the phospholipid bilayer of biological membranes could be a suitable toxicity target (Chefurka *et al*., 1987). This could interfere with membrane function and fluidity, thus affecting membrane-associated enzymes such as PMCA and SERCA. For instance, parathion, an organophosphate pesticide, is known to alter membrane organization, and it also has been shown that hexachlorobenzene, a cyclic organochlorine and hydrophobic pesticide, affects the functionality of two membrane-bound enzymes: 5'-nucleotidase and Na^{+}/K^{+}-ATPase (Courtney, 1976; Antunes-Madeira *et al*., 1994; Randi *et al*., 1998). Damage to the erythrocyte and sarcoplasmic reticulum (SR) membranes by some pesticides has been previously reported (Duchnowicz and Koter, 2003; Bhalla and Agrawal, 1998; Sahib and Desaiah, 1987). In addition, the functionality of PMCA and SERCA can be affected by a variety of hydrophobic molecules, including some studied pesticides (Price, 1976; Jones *et al*., 1985; Michelangeli *et al*., 1990; Janik and Wolf, 1992; Salas and Romero, 1996; Plenge-Tellechea *et al*., 1999). The aim of the present study was to test if

cypermethrin, glyphosate and deltamethrin were able to affect the Ca^{2+}-ATPase hydrolytic activity of PMCA; specifically the human PMCA found in membranes from erythrocyte. Findings from this investigation will shed light on the possible toxicological damage induced by these widely used pesticides to this human Ca^{2+}-ATPase.

Material and methods

Biological materials. Plasma membrane-containing PMCA (EC=3.6.3.8) were obtained from packed human erythrocytes of non-outdated human blood. Packed human blood was a donation from the General Hospital in the city of Juárez, México. For the isolation of SR vesicles enriched with SERCA (EC=3.6.3.8), female New Zealand rabbit weighing 2 kg were obtained from the biotherium and sacrificed in a CO_2 chamber. All the biological materials used were obtained in accordance with the moral and ethical principles of the University Autonomous of Juárez City (UACJ).

Chemicals. Adenosine 5' triphosphate (ATP) and bovine serum albumin were purchased from Sigma Inc. (Mexico). $CaCl_2$ was obtained from J. T. Baker (Mexico). Cypermethrin (alpha-methrine) (CAS: 52315-07-8), deltamethrin (CAS: 52918-63-5) and glyphosate (glyphosate-ammonium) (CAS: 1071-83-6) were acquired from Supelco (USA). The Ca^{2+} ionophore A23187 (calcimycin), EGTA (ethylene glycol-bis (β-aminoethyl ether)-N,N,N',N'-tetra acetic acid), Mops were purchased from Sigma-Aldrich Co. (Mexico). Other chemicals used in this investigation were reagent grade. Cypermethrin and deltamethrin were dissolved in methanol. The concentration of methanol added in the experiments never was higher than 1% (v/v). Glyphosate was dissolved in warm water.

Isolation of erythrocyte membranes containing PMCA. Hemoglobin-free erythrocyte membranes depleted of endogenous CaM were obtained from non-outdated packed human erythrocytes according to a previously described method (Niggli *et al*., 1979), except for the use of 10 mM Mops-K^+ (Mops: 4-morpholine-propane-sulfonic acid), pH 7.4 in the last four washes to remove EDTA (ethylenediamine-tetraacetic acid). The erythrocyte membrane preparations were stored in aliquots at -80°C until they were used.

Preparation of SR vesicles enriched with SERCA. Microsomal membranes were obtained by differential centrifugation after homogenization of low density SR of skeletal fast-twitch muscles. They were preserved in 10 mM Mops pH 7.0 and 30% (w/v) sucrose as previously described (Eletr and Inesi, 1972), and stored at -80°C until they were used.

Protein concentration. The total membrane protein concentration was determined by the colorimetric method described by Lowry *et al*. (1951), using bovine serum albumin as the standard protein.

Free Ca^{2+} concentration in the reaction medium. The free Ca^{2+} concentration used in the experiments was determined using a computer program (Fabiato and Fabiato, 1979). The computer program *Calcium* takes into account the absolute stability of the constant value for the Ca^{2+}-EGTA complex, the EGTA protonation equilibria (Blinks *et al*., 1982), the presence of Ca^{2+} ligands, and the pH in the medium.

PMCA activity. The PMCA activity was performed at 37°C in a typical reaction medium containing 30 mM Mops buffer pH 7.0, 130 mM KCl, 3 mM $MgCl_2$, 0.5 mM EGTA, 0.5 mM $CaCl_2$ (10 μM free Ca^{2+}) and 0.12 mg protein/ml. The reaction was started with the addition of 1 mM of ATP. The PMCA activity was determined using the colorimetric method described by Lanzetta *et al*. (1979), following the appearance of P_i (inorganic phosphate) during the ATP hydrolysis reaction. The stabilizing agent Sterox was substituted by 0.18% (v/v) of Tween-20 as described by Baykov *et al*. (1988). Different concentrations of the pesticides cypermethrin, deltamethrin, and glyphosate were also present in the mixture as indicated in the corresponding figure. The enzymatic activity was measured in the absence of CaM.

SERCA activity. The hydrolytic reaction was measured at 25°C by determining the appearance of P_i using a previously described colorimetric method by Lin and Morales (1977). The reaction mixture contained 20 mM Mops buffer pH 7.0, 80 mM KCl, 5 mM $MgCl_2$, 1 mM EGTA, 0.967 mM $CaCl_2$, (10 µM free Ca^{2+}), and 0.75 µM A23187. The enzymatic reaction started by the addition of 1 mM ATP. Different concentrations of the pesticides deltamethrin and glyphosate were also present in the mixture as indicated in the corresponding figure.

Statistical analysis and other specifications. Statistical analysis such as Pearson product moment correlation, linear regression and standard error were calculated with the computer software Sigma Plot Graph System. Pesticides were always included in the incubation/reaction medium after erythrocyte membranes or SR vesicles had been added. The mixtures were always pre-incubated for at least 2 minutes before the reactions were started. The experimental data corresponds at least to three independent measurements performed in duplicates and using more than one membrane preparation.

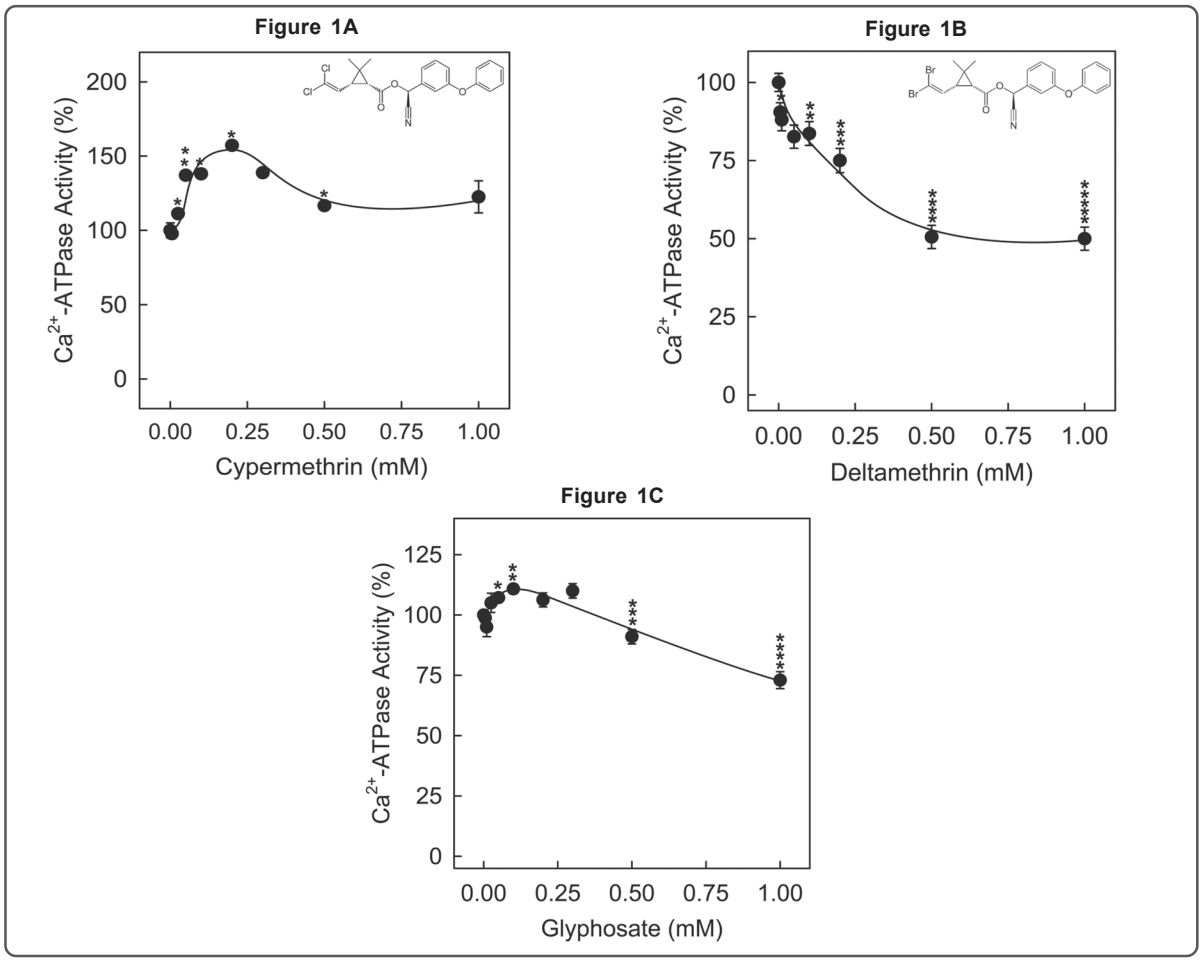
Results

Cypermethrin, deltamethrin and glyphosate affect PMCA activity in different manners. The present study shows the effect of cypermethrin, deltamethrin and glyphosate on the specific ATP hydrolysis mediated by PMCA. This Ca^{2+}-dependent pump utilizes ATP as a source of energy to pump Ca^{2+} across the plasmatic membrane. Our study consisted of different independent treatments in which distinct concentrations of pesticides where added to the PMCA reaction medium. Randomly, different concentrations of cypermethrin, deltamethrin and glyphosate were tested in order to determine which ones affected the Ca^{2+}-ATPase activity (PMCA). Therefore, only the concentrations that produced an effect are shown in this study. This biological system is measured by following the appearance of phosphate during the ATP hydrolysis and it was followed using a spectrophotometric procedure (Lanzetta *et al*., 1979; Baykov *et al*., 1988). The pesticides tested on PMCA activity were cypermethrin, glyphosate and deltamethrin using the appropriate concentrations of K^+, Mg^{2+}, ATP, and Ca^{2+}. This study was conducted in the absence of CaM. The second generation pyrethroids cypermethrin and deltamethrin altered the PMCA activity as shown in Fig. 1. Cypermethrin significantly stimulated the ATP hydrolysis at concentrations from 0.005-0.5 mM without inhibiting the Ca^{2+}-ATPase activity (Fig. 1A).

The maximal stimulation was 55% ± 9.0 over the activity control, at a concentration of 0.2 mM. However, deltamethrin did not stimulate PMCA activity. Interestingly, deltamethrin significantly inhibited the hydrolytic activity of the enzyme to 51.85% ± 3.7 at 0.5 mM (Fig. 1B). In addition, we studied the effect of glyphosate and unexpectedly this pesticide affected PMCA activity in a biphasic manner. As well as cypermethrin, glyphosate significantly stimulated the hydrolytic activity of the enzyme over 11% ± 2.0 with respect the control when its concentration was between 0.05-0.10 mM (Fig. 1C). However, higher concentrations of glyphosate (0.5-1.0 mM) significantly inhibited PMCA activity to 73% ± 3.5 when compared to the control. Our findings prove that cypermethrin and deltamethrin, two second generation pyrethroids, affected PMCA activity, as well as glyphosate.

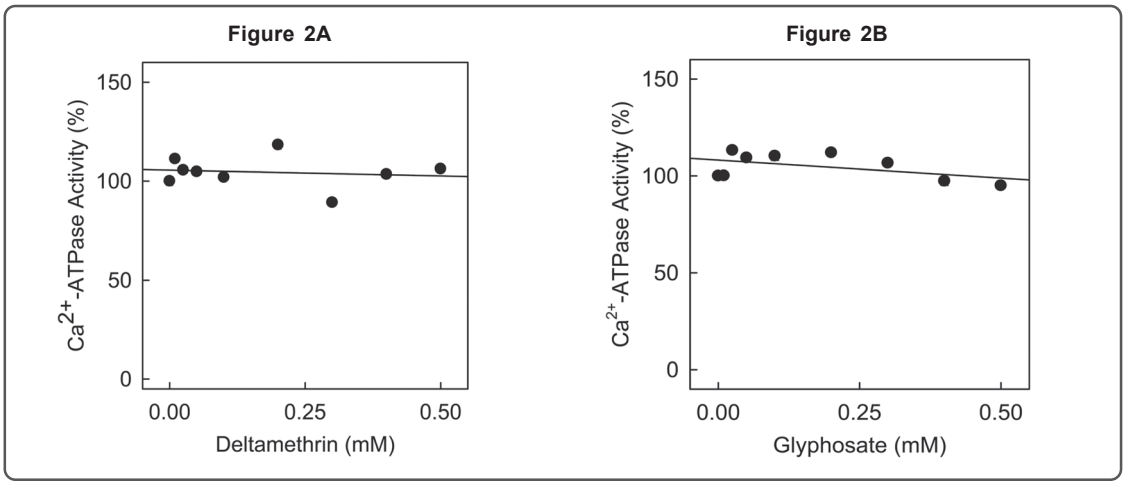
The Ca^{2+}-ATPase SERCA was unaffected by deltamethrin and glyphosate. In order to investigate if our previous findings were general effects for other Ca^{2+}-ATPases, we studied the SERCA activity as a function of the pesticide concentration (deltamethrin and glyphosate). We choose only one compound from the second generation of pyrethroids, and the organophosphorate glyphosate to test our hypothesis. The concentrations of deltamethrin and glyphosate used in this study were in the same ranges as previously used for PMCA. The Ca^{2+}-ATPase activity was measured at 25°C, in a buffered medium containing 10 µM free Ca^{2+} and 1 mM ATP. This is a reliable and well established procedure of measuring SERCA activity.

Fig 1. The effect of cypermethrin, deltamethrin and glyphosate on the hydrolysis of ATP mediated by the Ca^{2+}-ATPase from human plasma membrane. The reactions were performed as described in *Methods* and they were started after the addition of 1 mM ATP. The ATPase activity was measured in the steady state of the enzyme. Different concentrations of pesticides were added into reaction media as indicated at the *x* axes. Closed circles show the average of 3 ± SE independent experiments. For figure A) *, p=<0.001, **, p=0.002; for figure B) *, p=0.020, **, p=0.003, ***, p=0.010, ****, p=<0.001, *****, p=0.002; for figure C) *, p<0.001, **, p=0.006, ***, p=0.012, ****, p=0.005 compared to the control that correspond to the 100% of the Ca^{2+}-ATPase activity. The average (n=3) of ATPase activity control was 18nmol P_i/min/mg of protein and it was normalized to 100%. The chemical structures of cypermethrin and deltamethrin are shown and they differ in substitution of two bromines in deltamethrin for two chlorines in cypermethrin (**PubChem Substance ID:** 24868901 and 40585 respectively).



This data was obtained in the presence of a membrane protein concentration of 0.01 mg SR protein/ml. Deltamethrin (Fig. 2A) was not found to significantly affect SERCA activity, as demonstrated by a Pearson product-moment correlation coefficient -0.135 whit a p=0.7283 (n=9), indicating the lack of a statistically significant relationship between enzyme activity and deltamethrin concentration. Similar results were obtained when we assayed glyphosate (Fig. 2B); since the compound was not found to significantly affect SERCA activity. This was confirmed with a correlation test, which provided a correlation coefficient of -0.502 with a p=0.168 (n=9), once again indicating that there was not a statistically significant relationship between SERCA activity and glyphosate concentration. This conclusion was determined based on the high p-values ($p \geq 0.05$) obtained for each corresponding analysis. Therefore, the pesticides tested on the SERCA activity (glyphosate and deltamethrin) had no significant effect on the ATP hydrolytic activity mediated by this SERCA at the tested concentrations. In conclusion, our results show that SERCA is not sensitive to glyphosate and deltamethrin.

Fig 2. The effect of deltamethrin and glyphosate on the hydrolysis of ATP mediated by the Ca^{2+}-ATPase of sarcoplasmic reticulum. Ca^{2+}-ATPase activity was measured as described in *Methods* and 1 mM ATP was used to start the reaction in the steady state of the enzyme. The pesticides were titrated as indicated at the *x* axes. Each data point is the average of 3 ± SE independent experiments. Lines correspond to a computed linear regressing showing no significant effect ($p \geq 0.05$) of cypermethrin and deltamethrin on the SERCA activity. The ATPase activity was 2.713 ± 0.008 µmol P_i/min/mg of SR and it was normalized to 100%.



Discussion

Our research was conducted to study the effect of cypermethrin, deltamethrin and glyphosate on the hydrolytic activity of the human PMCA, which is found in membranes from erythrocytes.

Pyrethroids, like cypermethrin and deltamethrin, are widely used in combination with organophosphates instead of chlorinated-hydrocarbons such as DDT. In addition, it was established that pyrethroids were designed to target sodium channels (Cremer, 1983; Narahashi, 1996). Later on, pyrethroids were improved into more environmental resistant compounds and they were called pyrethroids of second generation and good examples of these are cypermethrin and deltamethrin. The second generation of pyrethroids, cypermethrin and deltamethrin were both dissolved in methanol; however, the amount of methanol added together with the pyrethroids into the reaction media never exceeded 1% as a final concentration. The main reason for maintaining a low concentration (lower than 1%) of methanol in our assays was to avoid significant effects on PMCA or SERCA activities. Alcohols such as methanol are known to stimulate Ca^{2+}-ATPase activity and this was carefully evaluated in our lab before titrating pyrethroids into our assays (Benaim *et al*., 1994). The studies in the effect of cypermethrin, deltamethrin and glyphosate on Ca^{2+}-ATPases have been described few times in the literature, in different animal and plant models, but not in particular on the human PMCA isoform from membranes of erythrocyte, therefore, the importance of our study (Clark *et al*., 1987; Sahib *et al*., 1987; Kodavanti *et al*., 1993; Souza da Silva *et al*., 2003). To the best of our knowledge, the effect of these compounds have never been reported before for human PMCA from membranes of erythrocyte. Similar studies were done, such as the study of esbiol and cyfluothrin effect on Ca^{2+}-ATPase using rat's leukocyte and synaptosome membranes (Grosmal and Diel, 2005). According to our data, the pyrethroid cypermethrin produced a marked increase on the PMCA enzymatic activity, followed by a partial inhibition on the PMCA activity (Fig. 1A). While the hydrolytic activity was inhibited when deltamethrin was titrated into the PMCA reaction

(Fig. 1B), on the other hand, glyphosate exhibited the same biphasic effect that we observed with cypermethrin, but the stimulation and inhibition was lower (Fig. 3C). The adverse effect produced by these pesticides is related to the chemical structure of each compound (Soderlund *et al*., 2002). Even though the pyrethroid molecules under study have similar structures, they do not affect different enzymes in the same way, hence the importance of studying each individual compound on specific enzymatic systems. Many structure-activity relationship studies have been reported and it has been shown that minor changes in the chemical structure of a compound can dramatically modify the effect of the molecule on different protein systems (Plenge-Tellechea *et al*., 1999). Structurally, both pyrethroids share a similar structure with a minor difference. Cypermethrin is a chlorinated compound, with two chlorides in their structure, in contrast to deltamethrin that contains two bromines instead of chlorides (Inserts in Fig. 1A and 1B respectively). These small modifications in the chemical structure of both cypermethrin and deltamethrin produced a different structure-activity relationship. While the chlorines in cypermethrin are making it a stimulator of PMCA activity at the lower concentrations tested and then an inhibitor at higher concentrations, the bromide-bearing deltamethrin acts as a PMCA inhibitor only.

Different authors mentioned that pyrethroids are highly hydrophobic pesticides with a high-tendency to target biological membranes from which they can disrupt membrane organization, thus affecting the activity of membrane proteins. In addition to our findings, pyrethroids are known to inhibit the ATPase activity derived from rat, squid, toad, and cockroach brain membrane preparations (Michelangeli *et al*., 1990; Berlin *et al*., 1984; Clark and Matsumura, 1987; Sahib *et al*., 1987; Moya-Quiles *et al*., 1996). Additionally, the pyrethroids cypermethrin and permethrin are known to inhibit the activity of the synaptosomal ATPase from rat brain, while permethrin, esbiol, and cyfluthrin affect the activity of the Ca^{2+}-ATPase from leukocyte membranes (Kakko *et al*., 2003; Grosman and Diel, 2005). This is an important issue, because even though ATPases can be similar or the same in different cell types, the lipidic composition of a membrane may change the effect of a compound in a given ATPase. Membrane lipid composition has been shown to be determinant in the effect of compounds like pyrethroids. Comparative studies using Ca^{2+}-ATPase in different rat membrane had been shown to differ in sensibility to pyrethroids (Grosman and Diel, 2005). Therefore, it is very interesting to see how the effect of the same compound varies in ATPases from different derived preparations or cell source.

Glyphosate is a pesticide that belongs to the group of widely used organophosphates. They produce alterations on ATPases and good examples of them are anilofos and paraoxon (Blasiak, 1995; Hazarika *et al*., 2001). In addition, there is evidence suggesting that malathion inhibits ATP hydrolysis in brain membranes preparations from zebrafish (Senger *et al*., 2005). In agreement with our results, where glyphosate inhibited PMCA hydrolytic activity (Fig. 1C), it has been shown that glyphosate inhibits the ATPase of slug nervous system (Souza da Silva *et al*., 2003). It was interesting to find out, that glyphosate affected PMCA activity as well as the herbicide paraquat, which is known to inhibit PMCA activity in membranes from erythrocyte (Janik and Wolf, 1992). However in neurons, paraquat induced the production of ROS and this seems to inactivate neuronal PMCA (Zaidi *et al*., 2009).

Unlike PMCA, SERCA was more resistant to the adverse effects of the selected pesticides in our experiments. None of the pesticides tested (glyphosate and deltamethrin) had a significant effect on the hydrolytic activity of the SERCA, based on the high *p*-values obtained ($p \geq 0.05$) (Fig. 2A and 2B). We performed these experiments in SERCA in order to validate our findings in PMCA, and surprisingly they were

very different. This difference may be due to the closed-membranes (spherical shape) of SR vesicles and to the great quantity of Ca^{2+} pumps per total protein, which represents 0.2 µM of Ca^{2+}-ATPase per 0.05 mg/ml of total membrane protein (Deamer and Baskin, 1969; Lax *et al*., 2002). In contracts with SERCA, PMCA is an open-membranes system. Plasmatic membrane from erythrocytes also contained less Ca^{2+}-ATPase in compared to SR vesicles, as the quantity of functional membrane protein represents only 0.01-0.1% of PMCA from the total erythrocyte membrane protein, the use of this system is highly relevant, given the fact that it is directly derived human tissue (Knauf *et al*., 1974).

A second reason could be the lipid composition of membranes from erythrocytes versus RS membranes from rabbit's muscle. The phospholipids/cholesterol ratio was determined to be 0.96 for membrane of erythrocyte, which means that the amount of both lipids is equilibrated in this membrane type (Gottfriedg, 1967). However, in SR this ratio is totally different and it was determined to be 0.05 (Waku and Nakazawa, 1964). ATPases in general, are lipid-dependent proteins and function of the ATPase is highly dependent of the chemical composition and physical phase of the lipids surrounding the protein (Lee, 1998). This may be the case of the compounds studied here, were they may modify in some degree the lipid environment that surround the ATPase, thus affecting ATPase function.

It has been shown that cypermethrin, deltamethrin and glyphosate induce the production of reactive oxygen species (ROS) in different model organisms; however we did not evaluate the production of ROS in our *in vitro* model and in presence of cypermethrin, deltamethrin or glyphosate (Giray *et al*., 2001; Li *et al*., 2007; Ahsan *et al*., 2008). Even though there is a remote possibility of having ROS production in pure and washed membranes, which will lead to lipid peroxidation of the lipids in our membrane model, we concluded that we did not have an electron source (such as the ones found in a living model) that is required for ROS production. In addition, there is no way for these compounds under study to enter into a redox cycle or be biotransformated in a redox cycler that may lead to ROS production without the cellular components required for ROS production in our test tube. Moreover, several investigations had shown that herbicides like paraquat targets neurons, where it mediated the generation of ROS (Corasaniti *et al*., 1992). Generation of ROS mediated by these compounds demonstrated how toxic these compounds really are when they enter a living cell. However, our findings contributed to show additional toxic effects produced by these compounds. In addition to ROS production cited in the literature, these compounds also affect Ca^{2+} homeostasis, by affecting Ca^{2+}-carriers such as Ca^{2+}-ATPases.

Finally, SERCA and PMCA are different proteins in terms of their pharmacological sensitivity, as it has been demonstrated by the use of several inhibitors. The effects caused by pesticides on the biochemical level are still poorly understood. However, it is clear that some pesticides could exert their toxic effects by interfering with membrane-bound enzymes such as PMCA and SERCA, thus disrupting Ca^{2+} homeostasis. Calcium is critical for cell function, since cells are not able to survive in the absence of Ca^{2+}, but an excess of the ion can also be lethal, since it can lead to apoptosis (Lam *et al*., 1994).

Conclusion

Our results do not necessarily reflect the concentrations that may produce poisoning, but they are helpful in understanding the effects of cypermethrin, deltamethrin and glyphosate on the biological systems studied in this investigation. The present study concludes that PMCA activity was significantly affected by the pesticides studied and future investigations should focus on the molecular mechanism behind this effect. SERCA was unaffected by deltamethrin and glyphosate. This may be due the lipidic nature of this membrane and the closed-membrane (SR

vesicle) system employed in this study. The effect of pyrethroids and organophosphorates in PMCA should affect Ca^{2+} homeostasis and their consequences still need to be elucidated. An inhibition on SERCA and PMCA activities should produce an increase in intracellular Ca^{2+} concentration with may kill a cell.

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Resúmenes curriculares de autor y coautores

Javier Vargas Medrano. Originario de Ciudad Juárez, ingresó al programa de Biología de la Universidad Autónoma de Ciudad Juárez en 1999, como parte de la primera generación del naciente programa. Ahí realizó su función de técnico de investigación y su tesis de licenciatura en el área de bioquímica, bajo la dirección del Dr. Plenge Tellechea, analizando el efecto de diferentes estructuras químicas de diclorobenzenos (precursores de pesticidas) sobre proteínas transportadoras de calcio. Después de obtener su licenciatura, ingresó a la Universidad de Texas en El Paso, donde comenzó su tesis doctoral estudiando como un proceso postraduccional, la fosforilación del transportador de glicina del cerebro era capaz de regular los flujos de glicina, lo cual en la clínica puede ser un prometedor tratamiento a esquizofrenia. Durante sus estudio doctoral fue distinguido dos veces como asistente de investigación con fondos del National Institute of Health and National Institute of Mental Health de los Estados Unidos. Finalmente, obtuvo el doctorado en el 2010. En el 2011, fue distinguido con el Hispanic Training Fellowship para ocupar la posición de Postdoctoral-Research Associate en el Texas Tech Health Science Center en el Paso Texas. Actualmente, el doctor se encuentra inmerso en varias investigaciones sobre una de las proteínas (CCR5) responsables de la entrada del virus del SIDA a las células.

Jorge Aníbal Sierra Fonseca. Se graduó del programa de Licenciatura en Biología de la Universidad Autónoma de Ciudad Juárez (UACJ) en el año 2007. Posteriormente inició sus estudios de posgrado en la Universidad de Texas en El Paso (Texas, EUA), donde fue admitido en el programa de doctorado en Ciencias Biológicas/Patobiología. Actualmente cursa su tercer año en el programa de doctorado, donde además de desarrollar su proyecto de investigación, también funge como instructor asistente en diversos cursos de licenciatura. Ha asistido a diversos congresos locales, regionales, nacionales e internacionales, donde ha presentado los resultados de diversos proyectos de investigación. Actualmente se encuentra estudiando el papel de las proteínas G heterotrimericas en la organización del citoesqueleto durante la diferenciación neuronal y neurodegeneración, utilizando diversos modelos celulares. Sus intereses incluyen biología celular, neurociencias, señalización celular y cáncer.

Manuel David Arellano Carrillo. En el año 2009 obtuvo el grado de Licenciado en Biología en la Universidad Autónoma de Ciudad Juárez con el tema de tesis: Interacción de plaguicidas sobre la actividad enzimática de la ATPasa de Ca2+ de eritrocito humano (PMCA). Además, el biólogo ha participado en diversos congresos nacionales e internacionales entre los que destacan varios congresos nacionales de bioquímica y uno organizado por la American Chemical Society. Los trabajos de investigación donde ha colaborado se han publicado en diversas revisas tales como en la revista Ciencia en la Frontera. Actualmente, el biólogo realiza sus estudios en la Maestría con Orientación Genómica en la UACJ donde evalúa el efecto de deltametrina sobre la expresión de diversos genes en Linfocitos T humanos.

Luis Fernando Plenge Tellechea. Desde 1990 ingresó como becario interno del laboratorio de reproducción en la Facultad de Ciencias de la Universidad Autónoma de Baja California. En 1992 culminó sus estudios de Biología en la misma dependencia. Posteriormente realizó sus estudios de Doctorado en Ciencias Biológicas por la Universidad de Murcia, culminando en 1998. El Dr. Plenge se ha caracterizado por sus estudios bioquímicos en la rama de proteínas asociadas en membranas. Actualmente labora como profesor investigador de tiempo completo en la Universidad Autónoma de Ciudad Juárez. Cuenta con múltiples publicaciones y dirección de tesis de pregrado y de grado. Es actual director en jefe de la revista de ciencias, Ciencia en la Frontera, e imparte la cátedra de Bioquímica en el programa de Biología y de Estructura y función proteínas en la Maestría en Ciencias con orientación en genómica (PNP). Actualmente se encuentra de reingreso a la UACJ posterior a una estancia Posdoctoral en el Border Biomedical Research Center de la Universidad de Texas at El Paso desde 2010 a Julio del presente, donde estudió los mecanismos bioquímicos de neurotransportadores de dopamina y glicina así como escritura de trabajos científicos.