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Effect of permethrin plus antioxidants on locomotor activity and striatum in adolescent rats

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ABSTRACT

Pyrethroids are important insecticides used largely because of their high activity as an insecticide and their low mammalian toxicity. Some studies have demonstrated that these products show neurotoxic effects on the mammalian central nervous system.

The aim of the present study was to investigate the propensity of permethrin to induce oxidative stress in adolescent rats and its possible attenuation by Vitamin E alone or + Coenzyme Q_{10}. Data indicated that adolescent rats exposed to permethrin exhibited alteration in the locomotor activity and plasma membrane fluidity of striatum. Vitamin E + Q_{10} and Vitamin E alone supplementation reversed the negative effect on central nervous system. Permethrin alteration of striatum plasma membrane fluidity was restored by Vitamin E + Q_{10}. Data obtained from red blood cells showed that permethrin did not induce any modification of plasma membrane fluidity in adolescent rats, whereas antioxidants supplementation induced pro-oxidant effect.

In summary some differences between antioxidant treatments were observed at striatum level: Coenzyme Q_{10} + Vitamin E maintains plasma membrane fluidity, while Vitamin E is more effective to preserve GSH level.

1. Introduction

There are serious concerns on the potential risks of exposure to pyrethroid insecticides with increasing production and application (Adelsbach and Tjeerdema, 2003). Pyrethroids are more hydrophobic than other classes of insecticides and this feature indicates that the site of action is the biological membrane. The main target site is neuronal sodium channels and it increases sodium entry into the nerve cells and induces depolarization of the nerve membrane and block of the nerve conduction at high concentrations (Narahashi, 1996). Permethrin (PERM), a member of the family of synthetic pyrethroids, belongs to type II class pyrethroids and is widely used in agricultural and other domestic applications. Workers may be exposed to PERM during occupational exposure; the other people may be exposed through pest control operation, contaminated food and water (Gorell et al., 1998).

The involvement of reactive oxygen species (ROS) have been implicated in the toxicology of pyrethroid inducing oxidative damage not only in the brain but also in others targets such as liver, erythrocytes, lymphocytes, etc. (Song and Narahashi, 1996; Dayal et al., 2003; Kale et al., 1999). Our previous studies indicated that oxidative damage induced by permethrin treatment in adult rats involving DNA damage in lymphocytes, cellular damage in erythrocytes, impaired phagocytosis of monocytes and in the neonatal rats behaviour changes, alterations of striatal monoamine levels, striatal protein oxidation were observed (Cantalamessa, 1993; Gabbianelli et al., 2002; Nasuti et al., 2003, 2007). In order to reduce the extent of oxidative damage, a mixture of antioxidants, Vitamins C and E, were employed in order to contain pyrethoids toxicity (Gabbianelli et al., 2004). Partial positive effects were obtained with these antioxidants: Vitamin E could protect the erythrocyte plasma membrane against the oxidative injury following on the use of the pyrethroids, whereas Vitamin C localized in the hydrophilic region was not effective.

In the light of these results obtained in blood cells of adults rats, we investigate if a mixture of Vitamin E + coenzyme Q_{10} (Q_{10}) could reduce permethrin-induced oxidative stress in adolescent rats. The choice derives from the hydrophobic character of Q_{10} that can

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interact with Vitamin E and its capacity to react with tocopheroxyl radical to regenerate α-tocopherol (Navas et al., 2007). The study is focalized mainly on central nervous system because our previous studies indicated that oxidative damages on striatum of pups rats after short exposure time (from 6 to 15 postnatal days) to permethrin (Nasuti et al., 2007). The aim of this report is to investigate if a supplementation of Vitamin E and CoQ10 could protect the striatum from permethrin-induced oxidative stress in adolescent rats. After administration of Vitamin E + CoQ10 and 1/10 of LD_{50} permethrin daily for 60 days to adolescent rats, we analysed the effects on behavioural activities, plasma membrane fluidity and lipid peroxidation in striatum. In addition, we presented data on erythrocyte plasma membrane fluidity that could be prognostic biomarkers of oxidative stress.

2. Materials and methods

2.1. Materials

All reagents were of pure and analytical grade. Collagenase and coenzyme Q_{10} were obtained from Sigma Chemical Co. 1,6-diphenyl-1,3,5-hexatriene (DPH) and 6-lauroyl-2-dimethylaminonaphtalene (LAURDAN) were purchased from Molecular Probes (Eugene, OR, USA). Vitamin E was obtained from Roche S.p.A., Milano. Technical grade (75:25, *trans*:*cis*; 94% purity) 3-phenoxybenzyl-(1*R*,*S*)-*cis*, *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxyl-ate, PERM (NRDC 143) were generously donated by Dr. A. Stefanini of ACTIVA, Milan, Italy.

2.2. Animals

Male Wistar rats from Charles River (Calco, LC, Italy), weighing 120–130 g and about 5 weeks old were used. The animals were housed in plastic (Makrolon) cages (five rats/cage) in a temperature-controlled room (21 ± 5 °C) and maintained on a laboratory diet (pellet 4RF from Mucedula, Settimo Milanese, Italy) with tap water ad libitum. The light/dark cycle was from 7 a.m. to 7 p.m. Animal use in this study complied with the Italian government's guidelines for the care and use of laboratory animals (D.L. n.116 of 27 January 1992).

2.3. Treatment

Permethrin was dissolved in corn oil and administered orally (5 ml/kg) ($n = 30$) for 60 days, at a dose of 150 mg/kg body weight/day (1/10 of LD_{50}) (Cantalamessa, 1993) by intragastric tubing. The animals treated with PERM were divided into three groups: one group was treated only with PERM ($n = 14$), the second group was treated with PERM + Vitamin E ($n = 8$) and the third group was treated with PERM + Vitamin E + CoQ_{10} ($n = 8$). Vitamin E was dissolved in corn-oil and administered orally (200 mg/kg body weight/day). CoQ_{10} was also solubilized in corn-oil and administered intraperitoneally (4 mg/kg body weight) in order to achieve high tissue levels (Ishrat et al., 2006). We did not use oral administration because of variability in absorption of Q_{10} from the gastrointestinal tract due to its high molecular weight and low water solubility (Scalori et al., 1990).

Rats serving as controls ($n = 8$) received 5 ml/kg body weight of corn oil orally for 60 days by intragastric tubing. The substances were administered daily in the rats and the volume administered was based on body weight. The animals were observed daily and weighed at regular intervals; the behaviour of the animals, treated with PERM + Vitamin E, PERM + Vitamin E + Q_{10} and PERM were compared with that of the control group.

After 60 days of treatment at the end of the behavioural tests, the rats were killed by CO_2 asphyxia for biochemical experiments.

2.4. Open field studies

After 60 days of treatment, eight rats from each group (PERM, PERM + Vitamin E, PERM + Vitamin E + Q_{10} and control) were submitted to the open field test. Automated locomotor activity boxes (MedAssociates, VT 05478) were used to quantify behavioural activity. Each animal was placed in the activity box, a square plastic box measuring 43 cm × 43 cm × 30 cm, and spontaneous locomotor activity parameters were monitored during the dark phase. Activity was recorded for 5 min, starting 2 min after placing the animal in the test cage. Movements from each rat were automatically recorded by interruption of two orthogonal light beams (3.5 and 13.0 cm above the activity box floor), which were connected to automatic softwares (Activity Monitor, MedAssociates). All tests were carried out during the same period of time (9:00–11:00 a.m.) and each rat was used only once. The behavioural parameters observed were locomotion (distance travelled), rearings (number of rears), stereotype counts (number of grooming movements) and the number of entries into the central square of the arena. Locomotion counts were recorded when the low row of photocells was interrupted, while rearing counts were recorded by interruptions in the higher row of photocells. The open field is divided into two squares, and the number of entries into the central area (25 cm × 25 cm) which can be considered an unprotected area for rats, were recorded. Entries into the central squares of the arena provided a measure of anxiety-like behaviour (Contò et al., 2005). Time spent and distance travelled in the 25 cm × 25 cm central zone of the field are expressed as percentage of total testing time and distance travelled, respectively. Low percentage of time spent and distance travelled in the center could represent behaviour correlated with anxiety (Ramboz et al., 1998; Levay et al., 2007). Between each test session, the apparatus was cleaned with alcohol (10%) and dried with a cloth.

2.5. Erythrocyte membrane preparation

After 60 days of pesticide treatment plus or less antioxidants, blood was drawn by cardiac puncture from all rats, collected in vials containing heparin (250 I.U.) and washed three times with physiological solution before the experiments. Plasma membranes were prepared by hypotonic hemolysis from 10 mM Tris pH 7.4 to 2.5 mM Tris pH 7.4 according to Bramley et al. (1971).

Protein concentration was evaluated using Lowry's method (Lowry et al., 1951).

2.6. Fluorescence measurements

The samples of striatum were homogenized in PBS containing 1.25 mg/ml collagenase at 4 °C and used for fluorescence and GSH measurements. A Hitachi 4500 spectrofluorometer was used for fluorescence measurements on striatum and erythrocytes. Steady-state fluorescence anisotropy (r) of DPH was calculated using an excitation and emission wavelengths of 360 and 430 nm, respectively, according to the Shinitzky and Barenholz (1978) equation:

$$r = \frac{(I_{II} - I_{\perp})\, g}{(I_{II} + 2I_{\perp})g}$$

where g is an instrumental correction factor, and I_{II} and $I_{\perp}$ are the intensities measured with the polarization plane parallel and perpendicular to that of the exciting beam.

Generalized polarization of Laurdan (GP_{340}) was calculated according to Parasassi equation (Parasassi et al., 1990):

$$GP_{340} = \frac{I_B - I_R}{I_B + I_R}$$

where I_B and I_R are the intensities at the blue (440 nm) and red (490 nm) edges of the emission spectrum and correspond to the fluorescence emission maximum in the gel and liquid-crystalline phases of the bilayer, respectively (Parasassi et al., 1991).

The final protein concentration in the assay was 0.4 mg/ml while the probe concentration was 10^{-6} M. The buffer used for the assay was PBS.

2.7. GSH content in striatum

The method of Lowry et al. (1951) was used to normalize the sample while the method of Butler et al. (1994) was used to determine the GSH content in the cells from striatum.

2.8. Lipid peroxidation in striatum

Lipids were extracted from the striatum homogenate using a chloroform/methanol mixture (2:1) according to the method of Folch et al. (1957). The absorbances at 233 and 215 nm were measured on a Cary 1 Varian spectrophotometer at 25 °C in order to evaluate the oxidation index (A233/A215) used as a relative measurement for conjugated dienes of lipids (Konings, 1984).

2.9. Statistical analysis

Statistical analysis was carried out using one-way ANOVA followed by the Newman-Keuls test. A P-value lower than 0.05 was considered statistically significant.

3. Results

3.1. General findings

Rats treated daily during 2 months with PERM, PERM + Vitamin E, PERM + Vitamin E + Q_{10}, by intragastric tubing showed no signs of poisoning or gross behavioural abnormalities throughout the experimental period.

Data on body weight in rats treated with PERM, PERM + Vitamin E, PERM + Vitamin E + Q_{10}, and control are reported in Fig. 1. As shown, body weight values increased as a function of age and no

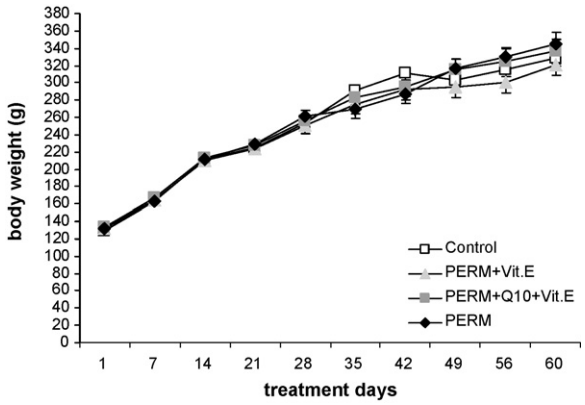

Fig. 1. Body weight in PERM-treated ($n = 14$), PERM + Vitamin E ($n = 8$) and PERM + Vitamin E + Q10 ($n = 8$) and control ($n = 8$) rats during 60 days of PERM exposure (150 mg/kg). Data are presented as mean ± S.D.

significant differences were observed in the body weight of treated versus control rats.

3.2. Open field behaviour

To evaluate the behavioural activity of different treated groups, the spontaneous locomotor activity of rats was studied. As can be observed in Fig. 2, PERM treatment (1254 ± 95.31) led to a decrease in locomotion compared with both control (1649 ± 72.31) and PERM + Vitamin E (1586 ± 120.41) ($P < 0.05$). No change between PERM and PERM + Vitamin E + Q_{10} (1439 ± 89.44) was measured. All treated groups showed a decrease in grooming compared with the control one (Fig. 3). In Figs. 4 and 5, all treated groups showed a significant ($P < 0.05$) decrease in % of distance traveled (PERM: 1.30 ± 0.32; PERM + Vitamin E: 1.97 ± 0.56; PERM + Vitamin E + Q_{10}: 1.71 ± 0.57) and in % time spent (PERM: 1.84 ± 0.23; PERM + Vitamin E: 2.93 ± 0.44; PERM + Vitamin E + Q_{10}: 2.72 ± 0.29) compared with the control group (% distance traveled: 3.96 ± 0.62; % time spent: 4.05 ± 0.27). Treatment with PERM induced a significant ($P < 0.05$) decrease in % time spent compared with PERM + Vitamin E.

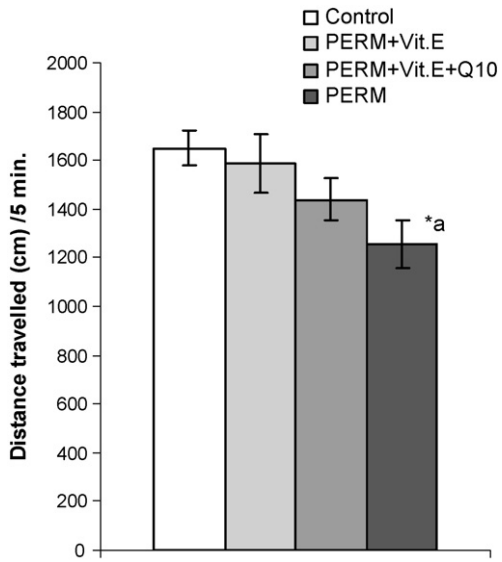

Fig. 2. Locomotion (expressed as distance travelled) in PERM-treated ($n = 8$), PERM + Vitamin E-treated ($n = 8$), PERM + Vitamin E + Q10-treated ($n = 8$) and control ($n = 8$) rats during 60 days of PERM exposure (150 mg/kg). Data are presented as mean ± S.D. *$P < 0.05$ vs. control group; [a]$P < 0.05$ vs. PERM + Vitamin E group.

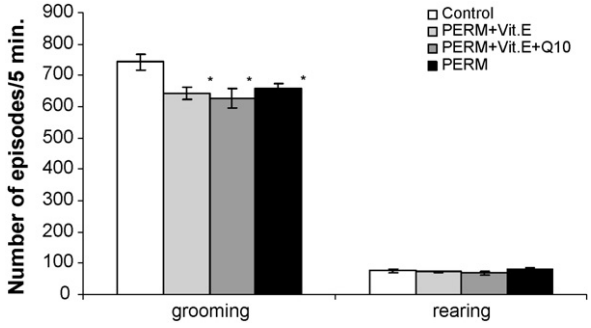

Fig. 3. Grooming and rearing in PERM-treated ($n = 8$), PERM + Vitamin E-treated ($n = 8$), PERM + Vitamin E + Q10-treated ($n = 8$) and control ($n = 8$) rats during 60 days of PERM exposure (150 mg/kg). Data are presented as mean ± S.D. *$P < 0.05$ vs. control group.

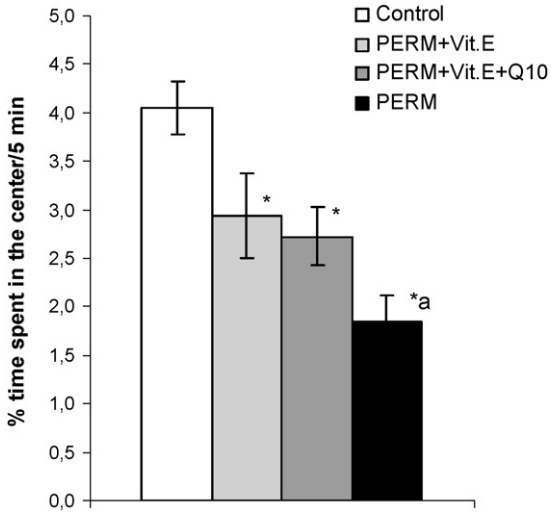

Fig. 4. % Time spent in PERM-treated ($n = 8$), PERM + Vitamin E-treated ($n = 8$) and PERM + Vitamin E + Q10-treated ($n = 8$) and control ($n = 8$) rats during 60 days of PERM exposure (150 mg/kg of PERM). Time spent in the 25 cm × 25 cm central zone of the open field are expressed as percentage of total testing time. Data are presented as mean ± S.D. *$P < 0.05$ vs. control group; [a]$P < 0.05$ vs. PERM + Vitamin E group.

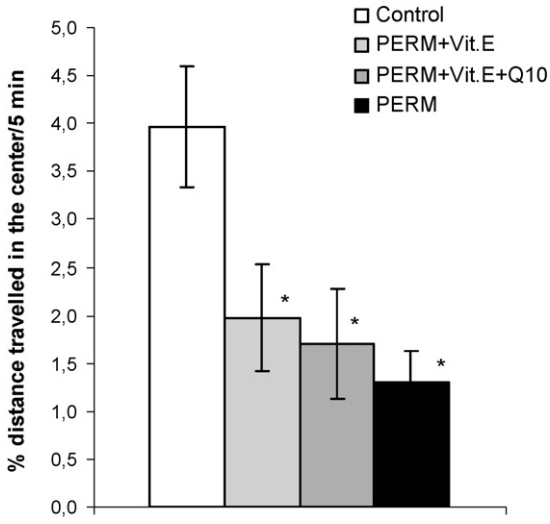

Fig. 5. % Distance travelled in PERM-treated ($n = 8$), PERM + Vitamin E-treated ($n = 8$) and PERM + Vitamin E + Q10-treated ($n = 8$) and control ($n = 8$) rats during 60 days of PERM exposure (150 mg/kg of PERM). Distance travelled in the 25 cm × 25 cm central zone of the open field are expressed as percentage of total distance travelled. Data are presented as mean ± S.D. *$P < 0.05$ vs. control group.

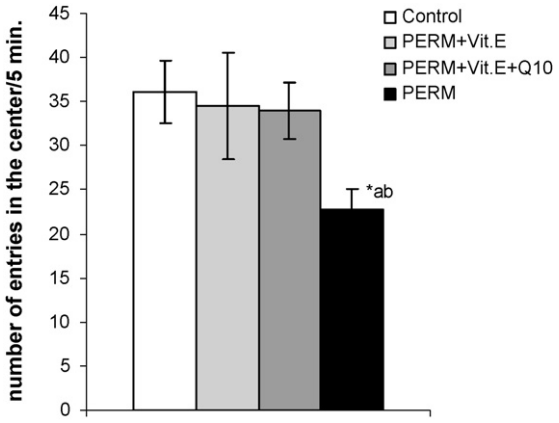

Fig. 6. Number of entries in the center in PERM-treated ($n=8$), PERM + Vitamin E-treated ($n=8$) and PERM + Vitamin E + Q10-treated ($n=8$) and control ($n=8$) rats during 60 days of PERM exposure (150 mg/kg of PERM). Data are presented as mean ± S.D. $^{*}P<0.05$ vs. control group; $^{a}P<0.05$ vs. PERM + Vitamin E group; $^{b}P<0.05$ vs. PERM + Vitamin E + Q_{10}.

Besides, as shown in Fig. 6, PERM-treated group (22.76 ± 2.25) showed a decrease in entries in the center compared with control (36.12 ± 3.5), PERM + Vitamin E (34.57 ± 6.04) and PERM + Vitamin E + Q_{10} (34 ± 3.26) ($P<0.05$).

3.3. Plasma membrane fluidity and lipid peroxidation in striatum

In order to evaluate PERM toxicity at the striatum plasma membrane level, fluorescence probes (DPH and laurdan) were used to analyse the structure and dynamics of biological membranes following pyrethroid treatment. These probes localizing in different regions of the bilayer can give information on the physical state of phospholipids. Steady-state fluorescence anisotropy of DPH shows that PERM treatment induces a significant decrease ($r=0.276\pm0.0151$) in hydrophobic membrane fluidity compared to control ($r=0.230\pm0.0194$) (Fig. 7A). Only supplementation of Vitamin E + Q_{10} ($r=0.238\pm0.0287$) maintained fluidity as control values (Fig. 7A). No changes in hydrophilic-hydrophobic region of the bilayer was observed using Laurdan (Fig. 7B). Fig. 8 shows that conjugated diene levels in lipid extracts from striatum cells did not change following permethrin treatment.

3.4. GSH level in striatum

Our previous study showed that neonatal exposure to permethrin reduces GSH levels in striatum (Nasuti et al., 2007). A similar behaviour was observed in adolescent rats: a significant reduction in GSH levels was measured in the PERM group (6.156 ± 2.467 μg/mg Pr) and PERM + Vitamin E + Q_{10} group (6.584 ± 1.49 μg/mg Pr) compared with the control group (12.005 ± 4.751 μg/mg Pr) (Table 1). No change in GSH levels, with respect to the control, was measured in the group treated with PERM + Vitamin E (10.47 ± 7.926 μg/mg Pr) (Table 1).

Table 1
GSH level in striatum cells of rats from control and PERM-treated groups

	GSH (μg/mg Pr)	S.D.
Control	12.005[a]	4.751
PERM	6.156	2.467
PERM + Vitamin E	10.47	7.926
PERM + Vitamin E + Q_{10}	6.584	1.49

Data represent the means ± S.D.
[a] $P<0.05$, vs. PERM and PERM + Vitamin E + Q_{10}.

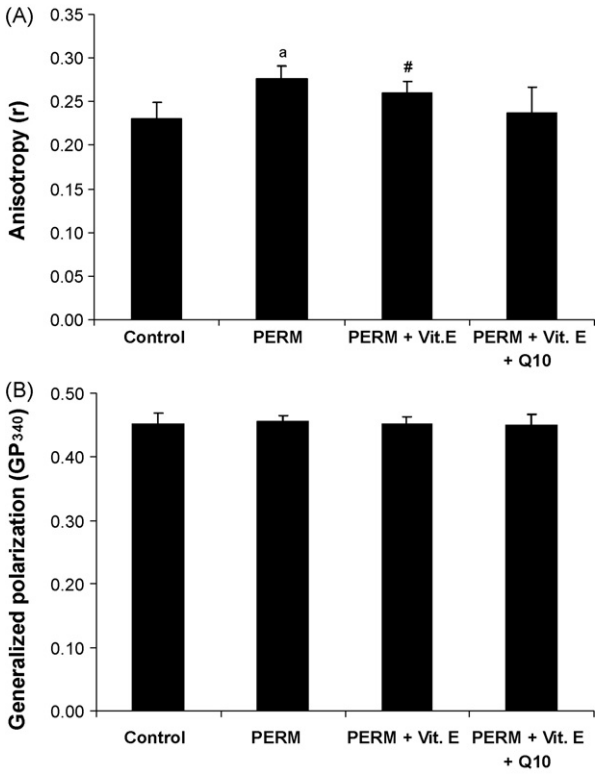

Fig. 7. Steady-state fluorescence anisotropy (r) of DPH (A) and generalized polarization (GP_{340}) of laurdan in striatum cells (B) from control, PERM, PERM + Vitamin E, PERM + Vitamin E and Q_{10} rat groups. Data represent the means ± SD; $^{a}P<0.05$ vs. control, PERM + Vitamin E and PERM + Vitamin E + Q_{10}; $^{\#}P<0.05$ vs. control and PERM + Vitamin E + Q_{10}.

3.5. Effect on erythrocyte plasma membrane fluidity

Steady-state fluorescence anisotropy of DPH and generalized polarization of laurdan were employed to evaluate the fluidity changes in erythrocyte plasma membranes from rats treated with pyrethroids ± antioxidant. DPH fluorescence anisotropy shows a decrease of membrane fluidity in both groups treated with antioxidants compared to control and PERM groups (Fig. 9A). No modification in erythrocyte plasma membrane fluidity was measured in the region tested by laurdan (Fig. 9B).

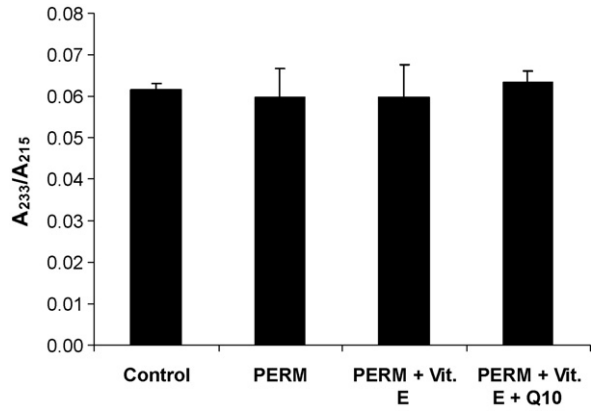

Fig. 8. Oxidation index obtained by the absorbance ratio A_{233}/A_{215} measured on lipids extracted from striatum cells of rats from control, PERM, PERM + Vitamin E, PERM + Vitamin E and Q_{10} rat groups. Data represent the means ± S.D.

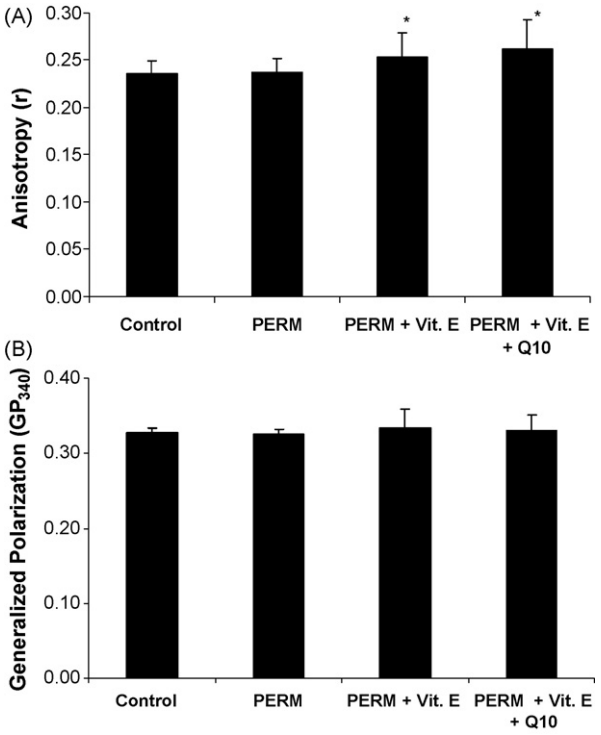

Fig. 9. Steady-state fluorescence anisotropy (r) of DPH (A) and generalized polarization (GP_{340}) of laurdan (B) in erythrocytes from control, PERM, PERM + Vitamin E, PERM + Vitamin E and Q_{10} rat groups. Data represent the means ± S.D.; $^*P < 0.05$ vs. control and PERM.

4. Discussion

Recent evidences implicate the involvement of oxidative stress mechanisms under conditions of pyrethroid-induced toxic effect, hence supplementation with antioxidants could be important for protecting against oxidative injury following the use of these pesticides (Giray et al., 2001; Gupta et al., 1999).

Our previous study shows that Vitamins E (α-tocopherol) and C could only partially protect against PERM damage (Gabbianelli et al., 2004), so in this study we employed a supplementation of Vitamin E and Q_{10}. The choice derives from the hydrophobic character of Q_{10} that could synergically act with Vitamin E in a concerted manner to scavenge radicals during autoxidation (Sohal and Forster, 2007).

The data presented in this study indicate that the antioxidant supplementation of Vitamin E alone or plus Q_{10} can positively influence the spontaneous locomotor activity of rats treated 60 days with PERM. The decreased locomotion, expressed as distance travelled and numbers of entries in the center, in PERM-treated group is reversed by antioxidant treatments. The alteration of locomotor activity is consistent with previous studies performed with animals treated with PERM for short time (from postnatal days 6 to 15). Since alteration of locomotion was concomitant with increased dopamine turnover in the striatum (Nasuti et al., 2007), in the present study we decided to investigate the oxidative state of the striatum. A decrease of plasma membrane fluidity in the hydrophobic region of the striatal cells was observed after treatment with PERM. The state of striatum plasma membrane could be correlated with locomotor alterations since changes in plasma membrane fluidity can alter signal transmission to cells. Changes in transmission at the striatum level affect the striato-nigral and striato-pallidal pathway thereby influencing motor behaviour (Santini et al., 2008; Fisone et al., 2007). On the contrary, Vitamin E + CoQ_{10} supplementation was effective in maintaining the plasma membrane fluidity at control level. The lack of locomotor alterations could be explained by antioxidant capacity of Vitamin E + CoQ_{10} supplementation to protect the physico-chemical state of striatum plasma membrane. Supplementation with Vitamin E only, even if it could increase plasma membrane fluidity compared to PERM-treated group, it could not restore the anisotropy value to control level (Fig. 7A). Instead of preventing changes in plasma membrane fluidity, Vitamin E supplementation was effective in protecting against neuronal damage by maintaining GSH level in striatum cells at control value. GSH is among one of the most abundant soluble antioxidant molecules in the brain where it prevents damage from free radicals. Depletion of GSH could significantly influence neuron functions. A reduction in GSH following PERM treatment was observed previously in pup rats (Nasuti et al., 2007) and now confirmed also in adolescent rats after long-term exposure. Moreover, the supplementation with Vitamin E + Q_{10} could not prevent the reduction in GSH induced by PERM. This effect could be related to the dual activity of Q_{10} that can act as antioxidant but also as pro-oxidant. The pro-oxidant effect can also be observed from data obtained on blood cells. We analysed the effect of PERM and antioxidant supplementation on erythrocyte plasma membrane fluidity. PERM treatment alone did not induce any change in erythrocyte plasma membrane fluidity compared to the control, in contrast to the same treatment in adult rats observed in previous studies (Nasuti et al., 2003). We suggest that in adolescent rats, the antioxidant capacity is higher than in adult rats and consequently, oxidation induced by PERM treatment can be neutralized by endogenous antioxidants without any modification in erythrocyte plasma membrane fluidity. A decrease of plasma membrane fluidity after supplementation with antioxidants was observed. In this condition, elevated serum levels of antioxidants produced a shift in the equilibrium towards a pro-oxidant status (reduced fluidity in groups treated with antioxidants, Fig. 9A). This condition could also be proposed to justify the observed GSH level reduction in presence of Vitamin E + Q_{10}.

In summary, the above data indicate that adolescent exposure to permethrin has negative effects on central nervous system but do not alter erythrocyte membrane fluidity as observed in adult rats (Nasuti et al., 2003), then pyrethroid toxicity on various target cells is a function of age. Besides, Vitamin E alone or plus CoQ_{10} supplementation shows controversial effects. It is effective to protect striatal cells preserving spontaneous locomotor activity from permethrin treatment, whereas it causes pro-oxidant effect in blood cells. However, some differences between antioxidant treatments were observed at striatum level: Coenzyme Q_{10} + Vitamin E maintains plasma membrane fluidity, while Vitamin E is more effective to preserve GSH level.

Further studies are in progress to better understand the mechanism of antioxidant and pro-oxidant effect of Vitamin E + CoQ_{10} supplementation in permethrin-induced oxidative stress.

Conflict of interest

The authors declare that there are no conflicts of interest.

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