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"Permethrin chronic exposure alters motor coordination in rats: Effect of calcium supplementation and amlodipine"

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ARTICLE INFO

Article history:
Received 25 October 2013
Received in revised form
21 February 2014
Accepted 24 February 2014
Available online 2 March 2014

Keywords:
Permethrin
Motor coordination
Calcium
Behavior
Amlodipine
Rat

ABSTRACT

Recently was observed that pyrethroids decrease motor coordination and that calcium channels can be important targets for this effect. To contribute with this observation, this work studied the motor coordination and exploration (using hole-board apparatus), and locomotion (using open-field apparatus) of rats exposed to following treatments: permethrin (PM), PM plus calcium gluconate (CG) and PM plus amlodipine (AML). The results obtained show that CG or AML alone not changed the motor coordination while PM decreases it. CG kept the effect of permethrin; AML, however, decreased the values of permethrin to the control. Locomotor activity and exploration, which could confound results of motor coordination, were not modified by treatments. The concentration of PM in brain tissue was increased by the CG and AML. The neurosomatic index (weight brain/body weight) was increased by the PM and PM + CG. In conclusion, the combined results here obtained indicates that the calcium ion and the channels in which it is involved can be important targets for the toxic effect of pyrethroid insecticide permethrin on motor nerve activity of rats.

1. Introduction

The toxicity of pesticides is not restricted to the target-organisms and the intentional and indiscriminate use has revealed that these compounds may affect the environment as well as put in risk the survival of non-target species, such as humans (Colosio and Moretto, 2008). Among the most widely used pesticides, include the pyrethroids.

Pyrethroids are derivatives of pyrethrins, natural insecticides extracted from plants like *Chrysanthemun cinerariaefolium*. These compounds have been modified to become more photostable and lipophilic, improving its action spectrum. Present lower acute toxicity to non-target species such as mammals, when compared to other classes of insecticides, such as organochlorines, organophosphates and carbamates (Pine et al., 2008).

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http://dx.doi.org/10.1016/j.etap.2014.02.017
1382-6689/

Pyrethroids are usually divided into two classes: type I, which does not contain the alpha-cyano group in the carboxyl group and type II, which feature this alpha-cyano group (Verschoyle and Aldridge, 1980). Permethrin is known for being one of the most potent pyrethroids insecticides of type I (Cox, 1998).

The widespread use of pyrethroids in agricultural and domestic environments has encouraged research on these compounds and evidence suggests that despite being relatively safe for humans (Naumann, 1990), epidemiological data, clinical reports and laboratory studies indicate that pyrethroids can cause a series of neurotoxic and immunotoxic effects in humans and animals; in addition, exposure to pyrethroids has been linked to acute reproductive effects and developmental deficiency suggesting a character of endocrine disrupter (Landrigan et al., 2000). On the other hand, children and adolescents are risk groups that has worried the toxicologists due to the fact of being a target more sensitive to the action of agents such as insecticides, presenting increased risk of reproductive problems, cancer and behavior (Garry, 2004). Therefore, it is relevant the development of studies that explore the mechanisms of toxicity of insecticides substances, in individuals at different ages, such as puberty and old age.

The mechanism of action of pyrethroids seems be analogous to the organochlorine insecticide DDT (dichlorodiphenyl-trichloroethane), which prolongs the opening of sodium channels of the cell membrane retarding the repolarization (Nahasashi et al., 1992), being its toxic action primarily on the nervous system, causing nervous paralysis due to repetitive discharges of fibers and nerve endings, causing hyperexcitation; moreover, pryrethroids have a weak action as liver enzyme inducer (Soderlund et al., 2002; Ferrer, 2003).

Recently it was evidenced that insecticides pyrethroids cause decreased motor coordination in experimental animals (Wolansky and Harrill, 2008) and calcium ion might be involved in this process (Yan et al., 2011). As changes in homeostasis of the cytosolic Ca^{2+} concentration can affect the regulation of many neural functions (Alshuaib and Byerly, 1996; Putney, 1999) emerged the hypothesis that the voltage-dependent calcium channels can be also important targets for these pyrethroids compounds, however this hypothesis requires experimental evidence *in vitro* and *in vivo* primarily.

Neuronal excitation causes a transient rise in the Ca^{2+} intracellular which in turn mediates a neuronal response. The increase of intracellular Ca^{2+} depends on voltage-dependent channels and also of its stockpile release of intracellular storage. The Ca^{2+} intracellular is quickly restored to basal level using extrusion neuronal through Ca^{2+} ATPases (exchange Na/Ca^{2+}), binding of Ca^{2+} to proteins and Ca^{2+} sequestration to the endoplasmic reticulum and mitochondria (Siesjö, 1994; Alshuaib et al., 2003).

It is well known that coordination of the movement and maintenance of body posture and equilibrium are key functions of the body regulated at cerebellum level (Ito, 1984). The cerebellum consists of two anatomically distinct structures: the cortex and deep nuclei. The circuits of the cerebellar cortex receive and integrate a large amount of sensory and cortical information. These informations are relayed to further processing to neurons of the cerebellar deep nuclei (DCN) and this whole process occurs through mechanisms modulators that itself develop using processes via cell calcium (Putney, 1999).

Calcium is a mineral provided by diet, involved in numerous biochemical processes in the organism, many of which are important for the development and maintenance of normal neurobiological functions. Disturbance of Calcium Homeostasis is a primary mechanism by which cells become injured (Catterall, 2000). For this reason the level of the intracellular calcium must always be perfectly adjusted and processes such as calcium influx through channels dependent of ions ligands (Frandsen and Schousboe, 1993) or voltage-dependent channels (Choi, 1988), associated with calcium release from internal locals as nonlipid reticulum and mitochondria (Parsons et al., 1997; Paschen et al., 1999), relate to each other to maintain cellular calcium homeostasis, since prolonged elevation of intracellular calcium levels could lead to excessive activation of several calcium-dependent mechanisms.

Hamilton and Smith (1992) studying the calcium currents in motor nerve terminals of rats observed that such currents seem to involve intracellular calcium accumulation as a prerequisite for subsequent release of synaptic transmitter.

Despite the amount of accumulated knowledge about the mechanisms of toxic action suggested for the pyrethroids, exists lack of work that bring scientific evidence regarding the latest discoveries on calcium's involvement in the process, especially in experiments performed with mammals.

Because of the fact that humans are exposed gradually to pyrethroids insecticides due to anthropogenic action, we aimed to study experimentally the importance of calcium for the effect of insecticide pyrethroid permethrin in the motor nervous coordination of rats. Here were used animals exposed to amlodipine, a calcium channel blocker, and diet augmented in calcium, for contribute to the elucidation of the mechanisms of neurotoxicity action of these compounds.

2. Material and methods

The product used for the experiments was the technique formulation Pounce®, produced by FMC, containing 38.4% permethrin [3-Phenoxybenzyl (1RS.3RS.1RS.3SR)-3-(2,2-dichloro-vinyl)-2,2-dimethyl-cyclopropanecarboxylate].

2.1. *Animals*

Experiments utilized male Wistar rats obtained from the colony housed at the Sao Paulo State University and kept under a constant 12 h light/dark cycle and controlled temperature ($23 \pm 2\,^{\circ}C$). Standard pellet chow (BioBase®, Santa Catarina/SC, Brazil) and tap water were available *ad libitum*. The Committee of Ethics in Experimentation Animals (CEEA) of the Institute of Biosciences at Sao Paulo State University approved the experimental protocols. All procedures were performed in accordance with institutional guidelines for animal use and care.