Neurotoxicology and Teratology 46 (2014) 57–61

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera

Repeated forced swim stress has additive effects in anxiety behavior and in cathecolamine levels of adult rats exposed to deltamethrin

Soraya F. Habr [a], Daclé J. Macrini [a], Helenice de S. Spinosa [c], Jorge C. Florio [c], Maria M. Bernardi [a,b,1]

[a] Graduate Program of Environmental and Experimental Pathology, Paulista University, UNIP, Rua Dr. Bacelar, 1212, São Paulo, SP 04026-002, Brazil
[b] Graduate Program of Dentistry, Paulista University, UNIP, Rua Dr. Bacelar, 1212, São Paulo, SP 04026-002, Brazil
[c] Department of Pathology, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, SP, Brazil

ARTICLE INFO

Article history:
Received 7 April 2014
Received in revised form 18 September 2014
Accepted 7 October 2014
Available online 18 October 2014

Keywords:
Deltamethrin
Pyrethroid
Pesticide
Forced swimming
Anxiety
Stress

ABSTRACT

Deltamethrin (DTM) is a type II pyrethroid insecticide that elicits autonomic and neuroendocrine responses that indicate high levels of stress, presumably caused by the neurotoxic effect of the insecticide. This study investigated the effect of DTM exposure (10 mg/kg, p.o.) and an additional stress induced in the forced swim test (FST) in behavioral tasks related to anxiety, serum corticosterone levels, and striatal neurotransmitter levels. Open field behavior and social interaction were evaluated after DTM administration (10 mg kg^{-1}, p.o). DTM per se reduced rearing frequency in the open field, but no alterations in locomotion frequency or immobility duration were detected. Stress increased immobility duration compared with non-stressed animals. DTM reduced social interaction and increased corticosterone levels, and these effects were enhanced in stressed animals. Mainly stress affected dopaminergic and serotoninergic activity. In anxiety behavior and in both neurotransmitters and metabolites levels it was observed an additive effect of stress in DTM treated rat data. These results indicate that DTM enhanced the anxiogenic responses and stress had an additive effect over the DTM stress. The neurochemical data did not indicate an interaction between stress and DTM exposure. The present results may be important for implementing pyrethroid insecticide safety standards.

1. Introduction

Synthetic pyrethroids can be classified into two categories: first and second generation. First-generation pyrethroids include esters of chrysanthemic acid derivatives and alcohols that have a furan ring and terminal side-chain moieties with high sensitivity to light, air, and temperature. Second-generation pyrethroids, generally 3-phenoxybenzyl alcohol derivatives, have excellent insecticidal activity and sufficient environmental stability (Kaneko, 2011). Pyrethroids have greater potency and environmental stability and are used in a wide array of indoor and outdoor applications, including medicinal, veterinary, and agricultural usages (Kaneko, 2010). Synthetic pyrethroids are known to have high insecticidal activity and low toxicity in mammals and leave little residue in the biosphere (Sanchez-Hernandez, 2006).

Exposure to pyrethroid insecticides can induce neurobehavioral effects in rodents and other species, including humans (Wolansky and Harrill, 2008). Various behaviors can be affected by these insecticides in adult animals, including open-field behavior and catalepsy (Mandhane and Chopde, 1997), conditioned behavior (Moniz et al., 1994), operant behavior (Stein et al., 1987), schedule-controlled behavior (Peele and Crofton, 1987), motor activity (McDaniel and Moser, 1993), and anxiety-related behavior (de Souza Spinosa et al., 2000).

Deltamethrin (DTM), a second-generation, light-stable Type II synthetic, is widely used in agriculture and claimed to be one of the most potent products of this class. Deltamethrin was shown to induce a syndrome associated with pronounced hyperglycemia, a response likely linked to the sympathoadrenal medullary system (de Boer et al., 1988). Elevated plasma norepinephrine concentrations also occur in this syndrome, suggesting the involvement of the adrenal medulla. Additionally, low doses of DTM elicit vigorous autonomic and neuroendocrine responses, indicating high levels of stress that are presumably caused by the neurotoxic properties of the insecticide (de Boer et al., 1988).

Although beneficial for a short period of time, chronically elevated blood levels of glucocorticoids, as seen with prolonged or severe stress, provoke enhanced demands on the body's resources. With regard to neurotoxicity, only a few studies have evaluated the possible role of chronic stress in enhancing the central effects of pesticides (Ehrich et al., 2004; Jortner et al., 2005). No studies of which we are aware have investigated the effects of chronic stress on pyrethroid neurotoxicity. Because pyrethroids per se also induce stress either by stimulation of the sympathoadrenal medullary system (de Boer et al., 1988), increases in corticosterone levels (Righi and Palermo-Neto, 2003) as well as by behavioral methods (De Souza Spinosa et al., 1999; Righi

E-mail address: marthabernardi@gmail.com (M.M. Bernardi).
[1] Paulista University, UNIP, Campus Indianapolis, Rua Dr. Bacelar, 1212, São Paulo, SP 04026-002, Brazil.

http://dx.doi.org/10.1016/j.ntt.2014.10.001
0892-0362/

and Palermo-Neto, 2003), we investigated the possible interaction between chronic stress and pyrethroid-induced neurotoxicity. We employed a repeated daily forced swim stress (FST) protocol in conjunction with acute DTM administration and evaluated behavior in the open field and social interaction tasks. These behavioral models are used to assess emotional- or anxiety-like behavior. Furthermore, the effects of stress and DTM administration on serum corticosterone levels and striatal and hypothalamic neurotransmitter levels were investigated.

2. Materials and methods

2.1. Animals

Adult male Wistar rats, weighing 200–250 g and approximately 90 days of age, were maintained under controlled laboratory conditions. The animals were housed in polypropylene cages (32 × 40 × 18 cm) under controlled temperature (22–25 °C) and a natural light cycle (lights at 6:00 h am and dark 18:30 h pm, experiments performed between January and March), with free access to food and water. The animals used in this study were maintained in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council, USA.

2.2. Insecticide

Deltamethrin (*S*-alpha-cyano-3-phenoxybenzyl-[*R*]-cis-3-[2-,2-dibromovinyl]-2,2-cimethylcyclopropane carboxylate; 10 mg/kg; Quimio-Ind. Química S/A) was dissolved in saline solution and administered orally by gavage in volumes that did not exceed 1 ml/kg body weight. The control solution also is administered by gavage [stainless steel needle BD-12, cannula diameter 1.2 mm with 2.3 mm ball, ray—40 mm, length—54 mm, Insight Equipamentos, ensino e pesquisa Ltda] in volume that did not exceed 1 ml/kg body weight.

2.3. Treatments

Rats were submitted to forced swimming test daily for 4 days. In the last day of training the animals received orally 10 mg/kg of DTM or 1 ml/kg of vehicle 60 min and were submitted to the fourth forced swimming session. After 30 min of this procedure, the animals were evaluated in the open field or in the social interaction tasks and the brains were dissected and employed to neurotransmitters levels evaluation. Different groups of rats were used in each experiment.

2.4. Forced swim test

The rats were individually placed into a vertical glass cylinder (45 cm height, 38 cm diameter) that contained water at a depth of 25 cm and temperature of 25 °C. After 5 min in the cylinder, the animals were removed, dried, and moved to a warm cage. The water in the cylinder was changed after each animal treatment to avoid olfactory cues left by the previous animal.

2.5. Open-field test

The apparatus was similar to the one described by Broadhurst (1960) and consisted of a round arena diameter (96 cm) painted white and wall cardboard with 30 cm high. The floor of the arena is divided into 25 regions with approximately equal areas, bounded by two concentric circles of different radii and straight radial segments marked in black on its surface. The arena sits on a counter located inside the cubicle observation, illuminated with white fluorescent lamps. Hand-operated counters were used to score locomotion frequency (i.e., the number of floor sections entered) and rearing frequency (i.e., the number of times the animal stood on its hind legs). A chronometer was used to measure immobility duration (i.e., the total time in seconds without spontaneous movements). For open-field observations, each rat was individually placed in the center of the arena, and its behavioral parameters were recorded for 5 min. The apparatus was washed with a 5% ethanol solution before each behavioral test. Control and experimental rats were intermixed, and the observations were made between 2:00 PM and 6:00 PM. Two observers blinded to experimental conditions performed this test (r—0.98).

2.6. Social interaction test

This test was performed according to the methods published by File (File and Briley, 1991; File, 1992). The rats were singly housed for 5 days prior to testing. The social interaction test was performed in the open-field apparatus. Fifty rats were divided into four groups. The experimental groups and control groups were subjected to two 7.5-min familiarization sessions in the test arena. The rats were then paired by weight (≥10 g), received the respective treatments, and subjected or not to stress. Thirty minutes after this procedure, the pairs of rats of same treatments were placed in the center of the test arena to evaluate social interaction. The total time in seconds spent by the test rat pairs in active social interaction (e.g., sniffing, following, grooming, kicking, boxing, biting, wrestling, and crawling under or over the partner) was scored for 7.5 min. The apparatus was washed with a 5% ethanol solution before each behavioral test. Control and experimental rats were intermixed, and the observations were made between 2:00 PM and 6:00 PM. Two observers blinded to experimental conditions performed this test (r—0.97).

2.7. Serum corticosterone levels

Serum corticosterone levels were determined using commercial kits (Coat-A-Count, DPC). The limit of corticosterone detection was 16.45 nmol/L, and the intra-assay and inter-assay variation coefficients were 0.88% and 4.04%, respectively.

2.8. Neurotransmitter and metabolite levels

On the last day of the experiment, male rats that were exposed or not to stress and DTM were decapitated 15 min after stress exposure between 5.00 and 5:30 PM, and blood was collected. The brains were dissected on dry ice and prepared as previously described by Felicio et al. (1996). The striatum was dissected within 3 min on an ice-cold plate, weighed, and homogenized (Polytrons) in 0.1 M perchloric acid. A volume of 20 μl/mg tissue (wet weight) of this solution was used for the analysis. To precipitate the proteins completely, the homogenates were left overnight in a refrigerator at 4 °C and then centrifuged at 3000 rotations per minute for 10 min. The supernatants were analyzed by high-performance liquid chromatography (HPLC; Shimadsu, model 6A) with a C-1 column (Shimpak-ODS), electrochemical detector (Shimadsu, model 6A), sample injector (valve for 20 μl), and integrator (Shimadsu, model 6A Chromatopac). The levels of the following monoamines and their metabolites were measured: dopamine (DA), 3,4-dihidroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT), and 5-hydroxyindolacetic acid (5-HIAA).

2.9. Statistical analysis

The treatment effects, additional effects of stress, and interactions between factors were analyzed using two-way analysis of variance (ANOVA) followed by the Bonferroni post hoc test. In all cases, results were considered significant at $p < 0.05$.