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Effects of exposure to pyrethroid cyfluthrin on serotonin and dopamine levels in brain regions of male rats

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ABSTRACT

The effects of cyfluthrin oral exposure (1, 5, 10 and 20 mg/kg bw, 6 days) on brain region monoamine levels of male rats were examined. Cyfluthrin-treated rats (1, 5 and 10 mg/kg bw, orally 6 days), had no visible injury, i.e., no clinical signs of dysfunction were observed. However, rats treated with cyfluthrin at the highest dose (20 mg/kg bw, orally 6 days) showed skeletal muscle contraction in the hind limbs, slight movement incoordination without any signs of dyskinesia and tremor after 1–2 h of treatment. These signs were reversible at 6 h after dose. After last dose of cyfluthrin, dopamine (DA) and serotonin (5-HT) and its metabolites levels were determined in brain regions hypothalamus, midbrain, hippocampus, striatum and prefrontal cortex by HPLC. Cyfluthrin (1 mg/kg bw, orally 6 days) did not affect the DA, 5-HT and metabolites levels in the brain regions studied. Cyfluthrin (5, 10 and 20 mg/kg bw, orally 6 days) caused a statistically significant decrease in DA and its metabolites DOPAC and HVA levels and in 5-HT and its metabolite 5-HIAA levels in a brain region- and dose-related manner. Moreover, cyfluthrin (20 mg/kg bw, orally 6 days) evoked a statistically significant increase in 5-HT turnover in striatum and midbrain, and in DA turnover in striatum and prefrontal cortex. These findings indicate that serotoninergic and dopaminergic neurotransmission is affected by exposure to cyfluthrin and may contribute to the overall spectrum of neurotoxicity caused by this pyrethroid.

1. Introduction

Pyrethroid insecticides are synthetic analogs of the natural pyrethrins. Pyrethrins are components of extracts from the flowers of *Chrysanthemum* spp. Pyrethroids are more stable in light and air than natural pyrethrins. Today, they are one of the most widely used domestic and agricultural pesticides (Heudorf and Angerer, 2001; Lothrop et al., 2007), they are used to control pests in residential and agricultural settings, to treat head lice and scabies in humans and fleas in pets, for public health vector control, and for disinfection of commercial aircrafts (Anadón et al., 2009, 2013a; Wei et al., 2012; USEPA, 2013). Pyrethroid insecticides account for more than 30% of insecticides used worldwide (Barr et al., 2010). Because of they are generally more toxic to insects than nontarget species (Soderlund et al., 2002), the use of pyrethroids has increased dramatically over the past decade and will likely increase further as they are replacing other pesticides (e.g., organophosphate pesticides) that are considered to have higher mammalian toxicity (Williams et al., 2008).

Substantial information about the toxicity of pyrethroid insecticides exists; their primary mode of action is on the voltage-sensitive channels in neurons. Pyrethroids induce neurotoxicity mainly by slowing the opening and closing of voltage-gated sodium channels (Na_v^+) altering the release of neurotransmitters, however, the effect of pyrethroids on neurotransmitter release may be dual stimulatory or inhibitory or both (Hossain et al., 2004). Moreover, they are generally good "knockdown" agents due to their ability to induce repetitive firing in axons, resulting in restlessness, un-coordination and hyperactivity followed by prostration and paralysis. Currently, it knows that the pyrethroids act on the expressions of the kdr gen (S4,S5 and S6- domain II of Na_v^+), and mutating this gene produces the resistance of insects to pyrethroids (Ranson et al., 2000; Tan et al., 2012). According to the clinical symptoms in animals receiving acute toxic doses, the pyrethroids form two groups. Type I pyrethroids such as permethrin cause hyper-excitation, ataxia, tremors and paralysis. Type II pyrethroids, such as cyfluthrin, have an α-cyanophenoxybenzyl substituent, and cause hypersensitivity, salivation, and choreoathetosis (Ray and Fry, 2006). Although voltage-gated calcium channels and interaction with the GABA receptor–ionophore

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complex, or other mechanisms may also play a neurotoxic role (Lawrence and Casida, 1983; Shafer and Meyer, 2004). The synthetic pyrethroids are subjected for review as probable developmental neurotoxicants (Shafer et al., 2005). Moreover, high occupational and environmental human exposure to pyrethroids could interact with the normal metabolism of drugs and xenobiotics. Induction of cytochrome P450 enzymes by Type I and Type II pyrethroids has been demonstrated (Yang et al., 2009; Anadón et al., 2013b).

Despite the extensive world-wide use of pyrethroids, there are relatively few reports of human poisoning. Occupational, the main route of pyrethroid absorption is through the skin with paresthesia effects. Inhalation is much less important but increase when pyrethroids are used in confined spaces or under conditions of misuse or inadequate user protection. Once absorbed, pyrethroid gives rise within minutes nausea, vomiting, abdominal pain; systemic effects occur from 4 to 48 h after exposure such as dizziness, headache, fatigue, blurred vision, coma, and convulsions (Bradberry et al., 2005; Ray and Fry, 2006). In rodents, it is known that early exposure to pyrethroids can lead to permanent changes in cholinergic and behavioral variables (Eriksson and Fredriksson, 1991), as well as they may specifically target the dopaminergic and serotoninergic systems (Karen et al., 2001; Bloomquist et al., 2002; Martínez-Larrañaga et al., 2003; Gillette and Bloomquist, 2003; Elwan et al., 2006), which may be a contributory factor in the etiology of human neurodegenerative disorders such as of Parkinson's disease, disorder characterized by the loss of nigrostriatal dopamine neurons (Olanow and Tatton, 1999). Several epidemiological studies have identified pesticide exposure as a significant risk factor for neurodegenerative diseases (Gorell et al., 1998; Priyadarshi et al., 2001). The increasing use of pyrethroid insecticides both in agriculture and in the residential setting and their diverse toxicological endpoints require regulatory assessment reviews to safeguard public health. Research should be devoted to enhance our knowledge in understanding the complexity of pyrethroid insecticides exposure patterns. Because major information regarding pyrethroid neurotoxic effects and underlying mechanisms involved after mammalian exposures are needed for risk evaluation, the present study examined in rats the effects of the pyrethroid cyfluthrin on serotonin and dopamine and their metabolites levels, as well as the neurotransmitter rate (turnover), a measure of presynaptic neuronal activity (Dam et al., 1999; Seider and Slotkin, 1990) in five brain regions (hypothalamus, midbrain, hippocampus, striatum and prefrontal cortex), major areas of monoaminergic systems involved in cognitive performance, learning and memory, and motor activity, which could be targets for this pyrethroid. Cyfluthrin is a Type II pyrethroid, it was first registered for use in the United States in 1987 (USEPA, 1987), frequently it is used in veterinary medicine, agriculture against grasshoppers and pests, industrial and residential settings, and public health and, in some countries for the protection of stored products (Ritter and Chappel, 1997; FAO, 1999).

2. Materials and methods

2.1. Chemicals

Cyfluthrin [cyano (4 – fluoro – 3 – phenoxy - phenyl) methyl 3 - (2,2 - dichloroethenyl) - 2, 2 - dimethylcyclopropanecarboxylate] contains three chiral centers and therefore exists in 8 enantiomeric forms, giving four pairs of diastereoisomers. In this study, cyfluthrin was provided by Bayer AG, Wuppertal-Elberfeld, Germany and was a defined mixture of the 4 diastereoisomeric enantiomer pairs [enantiomer pair I (cis) (23.6%), enantiomer pair II (cis) (17.9%), enantiomer pair III (trans) (31.6%) and enantiomer pair IV (trans) (21.1%)], 94% purity.

2.2. Animals and experimental design

All experiments using live animals were undertaken in accordance with the ethics requirements and authorized by the official ethical committee of our university.

Young adult male Wistar rats, at 60 days old each weighting 200–210 g (Charles River Inc., Margate, Kent, UK) were used. The animals were individually housed in polycarbonate cages with sawdust bedding and maintained in environmentally controlled rooms (22 ± 2 ºC and $50 \pm 10\%$ relative humidity) with a 12 h light/dark cycle (light from 08.00 to 20.00 h). Food (A04 rodent diet, Panlab SL) and potable water were available *ad libitum*. Twenty five male rats were assigned randomly to five groups of 5 animals each, a control group and four cyfluthrin treated groups. Five animals per group were selected on the basis of a preliminary study which showed results with no significant deviation (data not shown). Animal treated groups received cyfluthrin orally at the dose of 1, 5, 10 and 20 mg/kg bw [equivalent to 1/250, 1/50, 1/25 and 1/12.5 of the LD_{50} value (mean LD_{50} was previously calculated, data not shown)] for 6 consecutive days. The lower dose was chosen to reflect a possible no-observed-adverse-effect level (NOAEL). The cyfluthrin treated and control group rats were deprived of food for 6 h before the oral administration of cyfluthrin, but were allowed water *ad libitum*. The animals received the treatment at the same time each day, specifically between 10.0 and 11.0 h. Cyfluthrin was dissolved in corn oil and administered orally by gavage in a volume of 1 ml/rat. Control animals received the vehicle (corn oil) on the same schedule.

The animal body weights were measured during the study and food and water consumption of each animal was also assessed. Twenty-four hours after the last dose, the animals were sacrificed by decapitation. The brain was removed quickly and the hypothalamus, midbrain, hippocampus, striatum and prefrontal cortex tissues were rapidly dissected out at 4 ºC (Glowinski and Iversen, 1966). Tissues were rapidly weighed and stored at −80 ºC until analysis.

2.3. Determination of monoamine levels

The five brain regions analyzed in the present study were hypothalamus, midbrain, hippocampus, striatum and prefrontal cortex. Following sample collections, 300–800 μl of 0.4 M $HClO_4$ containing 0.1% (w/v) $Na_2S_2O_5$ was added to the tissues, and the mixture was homogenized (1 min) by sonication (Labsonic U-Braun) before neurochemical evaluation was performed. The homogenates were centrifuged (RC5C, Sorvall Instruments) at 12,000*g* for 20 min at 4 ºC and aliquots of supernatants were taken for analysis of dopamine (DA) and its metabolites [3,4-hydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)] and serotonin (5-HT) and its metabolite [5-hydroxy-3-indolacetic acid (5-HIAA)] using a high performance liquid chromatography (HPLC) technique with electrochemical detection (Colado et al., 1993) with modifications (Martínez-Larrañaga et al., 2003).

For the analysis of DA and its metabolites DOPAC and HVA, the mobile phase consisted of 0.1 M $Na_2HPO_4 \cdot 2H_2O$, 0.1 M citric acid (pH 3.5), 1.6 mM octane sulfonic acid, 0.9 mM EDTA and 10% (v/v) methanol. For the analysis of 5-HT and its metabolite 5-HIAA, the mobile phase consisted of 0.1 M $Na_2HPO_4 \cdot 2H_2O$, 0.1 M citric acid (pH 3.5) and 10% (v/v) methanol. Elution was performed at a flow rate of 1 ml/min and the working electrode potential was set at 0.85 V for DA and metabolites and 0.63V for 5-HT and metabolite. The HPLC system consisted of a Shimadzu liquid chromatograph, model LC-9A equipped with a stainless steel reversed-phase column (Nucleosil 120 C_{18}, 5 μm, 125 mm × 4 mm i.d.) proceeded by a C_{18} precolumn, an electrochemical detector (Shimadzu, model L-ECD-6A), a sample injector (20 μl valve) and a integrator (Shimadzu, model C-R6A Chromatopac).