NeuroToxicology 43 (2014) 82–89

Contents lists available at ScienceDirect

NeuroToxicology

Low level chlorpyrifos exposure increases anandamide accumulation in juvenile rat brain in the absence of brain cholinesterase inhibition

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ARTICLE INFO

Article history:
Received 1 November 2013
Accepted 19 December 2013
Available online 25 December 2013

Keywords:
Chlorpyrifos
Developmental neurotoxicity
Organophosphorus
Endocannabinoid

ABSTRACT

The prevailing dogma is that chlorpyrifos (CPF) mediates its toxicity through inhibition of cholinesterase (ChE). However, in recent years, the toxicological effects of developmental CPF exposure have been attributed to an unknown non-cholinergic mechanism of action. We hypothesize that the endocannabinoid system may be an important target because of its vital role in nervous system development. We have previously reported that repeated exposure to CPF results in greater inhibition of fatty acid amide hydrolase (FAAH), the enzyme that metabolizes the endocannabinoid anandamide (AEA), than inhibition of either forebrain ChE or monoacylglycerol lipase (MAGL), the enzyme that metabolizes the endocannabinoid 2-arachidonylglycerol (2-AG). This exposure resulted in the accumulation of 2-AG and AEA in the forebrain of juvenile rats; however, even at the lowest dosage level used (1.0 mg/kg), forebrain ChE inhibition was still present. Thus, it is not clear if FAAH activity would be inhibited at dosage levels that do not inhibit ChE. To determine this, 10 day old rat pups were exposed daily for 7 days to either corn oil or 0.5 mg/kg CPF by oral gavage. At 4 and 12 h post-exposure on the last day of administration, the activities of serum ChE and carboxylesterase (CES) and forebrain ChE, MAGL, and FAAH were determined as well as the forebrain AEA and 2-AG levels. Significant inhibition of serum ChE and CES was present at both 4 and 12 h. There was no significant inhibition of the activities of forebrain ChE or MAGL and no significant change in the amount of 2-AG at either time point. On the other hand, while no statistically significant effects were observed at 4 h, FAAH activity was significantly inhibited at 12 h resulting in a significant accumulation of AEA. Although it is not clear if this level of accumulation impacts brain maturation, this study demonstrates that developmental CPF exposure at a level that does not inhibit brain ChE can alter components of endocannabinoid signaling.

1. Introduction

The organophosphorus (OP) insecticides are the most frequently used class of insecticides in the US accounting for over ~35% of total insecticides used (Grube et al., 2011). The most heavily used OP insecticide is chlorpyrifos (CPF) in spite of the fact that CPF is restricted to agriculture use. Its household use was eliminated in 2000 due to concerns that exposure to OP insecticides exert greater neurotoxic effects in children as compared to adults (U.S. EPA, 2000). However, in agricultural communities, the potential for childhood exposure to CPF, as well as other OP insecticides, still exists (Koch et al., 2002; Arcury et al., 2007). Developmental exposure to OP insecticides has been suggested to have lasting negative impacts including decreased cognitive abilities and motor skills (Ruckart et al., 2004; Marks et al., 2010; Engel et al., 2011; Bouchard et al., 2011). Specifically, children exposed to CPF exhibit increased manifestation of attention deficit disorder and attention-deficit/hyperactivity disorder (ADHD) (Rauh et al., 2006), decreased working memory and IQ (Rauh et al., 2011), and altered brain morphology (Rauh et al., 2012).

The inhibition of brain cholinesterase (ChE), a serine esterase that degrades the widely distributed neurotransmitter acetylcholine (ACh), is considered the canonical target for OP insecticides. At higher levels of OP exposure, significant inhibition of brain ChE activity leads to accumulation of ACh and hyperactivity of the cholinergic system resulting in the disruption of normal physiological functioning. Therefore, it is logical to assume that if such an exposure occurred during critical periods of nervous system development, the resulting cholinergic hyperactivity could alter the process of maturation and establishment of the normal

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0161-813X/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neuro.2013.12.009

pathways and synapses that occurs during this period. However, the environmental levels of CPF to which children would typically be exposed would be unlikely to induce significant inhibition of ChE in the brain, and thus would not induce hyperactivity of the cholinergic system. Nonetheless, this does not mean that low level exposure to OPs such as CPF is safe. In fact, laboratory studies have reported adverse neurochemical and behavioral effects following exposure to CPF and other OP insecticides at levels that induce only minimal amounts of brain ChE inhibition and little hyperactivity in the cholinergic system (Levin et al., 2002; Slotkin et al., 2006, 2007; Timofeeva et al., 2008a,b). This has led to the hypothesis that the developmental toxicological effects of OP insecticides involves a currently unknown "non-cholinergic mechanism of action".

We have recently suggested that the endocannabinoid system could possibly be a non-cholinergic target that contributes to the developmental toxicity of OP insecticides. The endocannabinoid system consists of a group of neuromodulatory lipids that are involved in a variety of physiological processes including appetite, motor learning, synaptic plasticity, and pain sensation. They also play a pivotal role in normal brain development (for reviews see Harkany et al., 2008; Anavi-Goffer and Mulder, 2009). The two main endocannabinoids, arachidonoylethanolamide (AEA or anandamide) and 2-arachidonoylglycerol (2-AG) (Fig. 1), are not stored in vesicles due to their lipophilic nature but are synthesized and released on demand in response to elevations in intracellular calcium (Devane et al., 1992; Di Marzo et al., 1994; Mechoulam et al., 1995; Sugiura et al., 1995; Stella et al., 1997). Endocannbinoid production can also be upregulated by group I metabotropic glutamate, nicotinic, and dopamine D2 receptor activation (Giuffrida et al., 1999; Stella and Piomelli, 2001; Ohno-Shosaku et al., 2002; Kim et al., 2002). In the brain, the endocannabinoids bind primarily to cannabinoid receptor 1 (CB1) resulting in their physiological actions, which can differ based on the brain region, second messenger machinery utilized, and type of synapse involved (for review see Kano et al., 2009). After activating the CB1 receptor, 2-AG and AEA are degraded primarily by the action of monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH), respectively.

It was demonstrated that acute exposure of adult mice to high dosages of OP compounds resulted in the inhibition of 2-AG and AEA hydrolysis in the brain (Quistad et al., 2001, 2002, 2006). Additional studies demonstrated that acute in vivo exposure of adult mice to CPF resulted in increased levels of 2-AG and AEA in the brain (Nomura et al., 2008; Nomura and Casida, 2011). Given the importance of the endocannabinoids in brain development, it is possible that developmental OP exposure could alter the normal endocannabinoid levels and result in deleterious effects on brain maturation. Therefore, we previously initiated investigations on the effects of repeated exposure to CPF in the brain of developing rats (Carr et al., 2011, 2013). Following 7 days of daily CPF exposure, 2-AG and AEA hydrolysis activities in the brain were inhibited in a dose-related manner at 4 h post-exposure, and the extent of inhibition from highest to lowest level was AEA hydrolysis > acetylcholine hydrolysis > 2-AG hydrolysis (Carr et al., 2011). Using the same exposure paradigm, we determined the peak time of inhibition for MAGL (4 h post-exposure) and brain ChE and FAAH (12 h post-exposure) and that levels of 2-AG and AEA were significantly elevated (Carr et al., 2013). However, significant brain ChE inhibition was still observed at all dosages administered and it was not clear whether CPF exposure can induce significant effects on endocannbinoid metabolism in the absence of brain ChE inhibition. Therefore, the goal of the current study was to continue this line of investigation by determining the extent of inhibition of endocannabinoid hydrolysis and accumulation of the endocannabinoids in brains of developing rats following repeated exposure to a dosage of CPF that does not inhibit brain ChE.

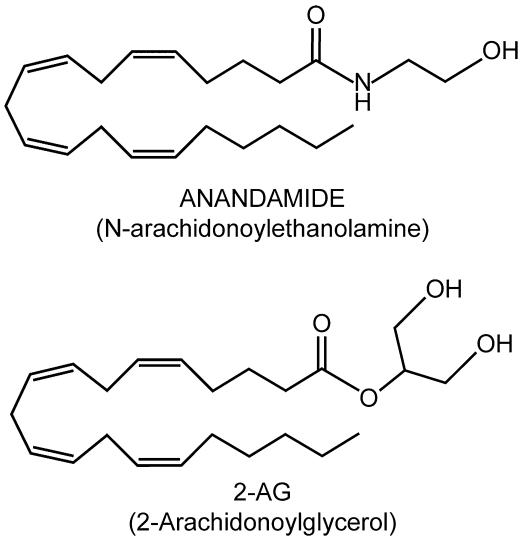

Fig. 1. Chemical structures of the two major endocannabinoids anandamide and 2-arachidonoylglycerol.

2. Materials and methods

2.1. Chemicals

Chlorpyrifos (>99% purity) was a generous gift from DowElanco Chemical Company (Indianapolis, IN). DowElanco did not contribute to or have any control over the data presented in this publication. All other chemicals were purchased from Cayman Chemicals (Ann Arbor, MI) or Sigma Chemical Co. (St. Louis, MO).

2.2. Animal treatment

Adult male and female Sprague Dawley rats [SD] were obtained from Harlan Laboratories (Prattville, AL) and used for breeding. All animals were housed in a temperature controlled environment (22 ± 2 °C) with a 12-h dark-light cycle with lights on between 0700 and 1900 in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited facility. LabDiet rodent chow and tap water were freely available during the experimentation. All procedures were approved by the Mississippi State University Institutional Animal Care and Use Committee. Following parturition, male and female rat pups within the same litter were assigned to different treatment groups. There were always representative control animals of the same sex present in each litter to match the CPF treated animals. For this project, rats from 6 litters were used. The day of birth was considered as postnatal day 0 (PND 0).

Chlorpyrifos was dissolved in corn oil and administered at a volume of 1 ml/kg body weight by oral gavage (*per os*) every day from PND10 through PND16. This period corresponds to the period following birth in humans. This age range is beyond the period of the growth spurt (PND7 and below) but encompasses a time of significant brain maturation (Andersen, 2003; Counotte et al., 2011; Tau and Peterson, 2010). The dosage selected was 0.5 mg/kg. Oral gavage was performed using a 50-μl tuberculin syringe equipped with a 1-inch 24-gauge straight intubation needle (Popper and Sons, Inc., New Hyde Park, NY) to deliver the solution to the back of the throat.

The dosage used in this study was designed to be below the level required to induce inhibition of brain ChE activity. It is below the oral repeated No Observed Effect Level (NOEL) for inhibition of brain ChE activity (0.75 mg/kg) and below the oral repeated NOEL