Toxicology 335 (2015) 1–10

Contents lists available at ScienceDirect

Toxicology

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

Developmental neurotoxic effects of two pesticides: Behavior and neuroprotein studies on endosulfan and cypermethrin

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ARTICLE INFO

Article history:
Received 26 May 2015
Received in revised form 25 June 2015
Accepted 29 June 2015
Available online 2 July 2015

Keywords:
Behaviour
Protein
Developmental Neurotoxicology
Neonatal
Endosulfan
Cypermethrin

ABSTRACT

Developmental neurotoxicity of industrial chemicals and pharmaceuticals have been of growing interest in recent years due to the increasing reports of neuropsychiatric disorders, such as attention deficit hyperactivity disorder (ADHD) and autism. Exposure to these substances during early development may lead to adverse behavior effects manifested at a later phase of life. Pesticides are a wide group of chemicals which are still actively used and residues are found in the environment and in food products.

The present study investigated the potential developmental neurotoxic effects of two different types of pesticides, endosulfan and cypermethrin, after a single neonatal exposure during a critical period of brain development. Ten-day-old male NMRI mice were administrated an oral dose of endosulfan or cypermethrin (0.1 or 0.5 mg/kg body weight, respectively). Levels of proteins were measured in the neonatal and adult brain, and adult behavioral testing was performed. The results indicate that both pesticides may induce altered levels of neuroproteins, important for normal brain development, and neurobehavioral abnormalities manifested as altered adult spontaneous behavior and ability to habituate to a novel home environment. The neurotoxic behavioral effects were also presentseveral months after the initial testing, indicating long-lasting or even persistent irreversible effects. Also, the present study suggests a possible link between the altered levels of neuroprotein and changes in behavior when exposed during a critical period of brain development.

1. Introduction

Pesticides are widely used in, for instance, commercial applications to sustain agricultural yield and commodities, pest control and as household agents. Pesticides may be commonly grouped according to their target pest and/or chemical structure; the more well-known types of insecticides include carbamates, organochlorines (OC), organophosphates (OP) and pyrethroids. The annual usage of pesticides is estimated to be thousands of tons and more than several hundred types of pesticides are reported as contaminants in food products (EFSA, 2013; EPA, 2011a; Eurostat, 2003). The most toxic pesticides have been banned and phased-out in numerous countries,as they are classed as persistent organic pollutants (Stockholm Convention, 2014), however residues and metabolites are still detected in the environment and in humans (Bedi et al., 2013; Dalvie et al., 2014; Mage et al., 2004; Toan et al., 2013; Weber et al., 2010). Numerous insecticides are constructed to target the nervous system of the pest, however due to the similarities of the neurochemical signaling systems these insecticides are also, in varying degree due to dose and potency, neurotoxic to humans.

The OC pesticides are a diverse group of compounds which are classified into different structural classes; however, they share the common characteristics of chemical stability, potential for bioaccumulation and slow degradation rate. Depending on their structural class, these pesticides exert their neurotoxic effect by different mechanisms. In short, they disrupt the signal transduction in the nervous system by affecting the ion transport across the cell membrane (Coats, 1990). Endosulfan is a restricted OC-classed pesticide and is being voluntary phased-out and scheduled for cancellation in 2016 (EPA, 2010). Nevertheless, it is still detected in food products ranging from <0.1–100 ppb and the primary exposure route for the general population are through dietary intake. In general, endosulfan is found in higher concentrations in the kidneys and liver than the brain. The estimated elimination half-lives for endosulfan and it metabolites ranges from 1 to 7 days in adult humans and animals (ATSDR, 2013). It acts as a non-competitive gamma-aminobutyric acid (GABA) antagonist at the

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http://dx.doi.org/10.1016/j.tox.2015.06.010
0300-483X/

Cl-channel within the brain (Silva and Gammon, 2009). Endosulfan's neurotoxic effects are well known, however few studies have investigated the developmental neurotoxic effects of endosulfan, and the investigations that have been made are regarded as inconclusive as the neonatal effects occurred at doses that also caused reduced maternal weight during pregnancy (ATSDR, 2013).

The pyrethroid class of pesticides was derived from the naturally occurring pyrethrins from the *Chrysanthemum* genus of plants. There exist two types of pyrethroid structures, type I and II, and they differ in regards to the absence or presence of an α-cyano-group. The primary neurotoxic effect of pyrethroids is the disruption of sodium channel function. The type I compounds induce a prolonged opening of the channel causing repetitive firing of action potentials, whereas the type II compounds induce an even longer prolongation of the channel compared to type I, leading to a depolarization of the membrane potential and no firing of action potentials (Shafer et al., 2005). Cypermethrin is a type-II pyrethroid and is known to cause CS-syndrome i.e., choreoathetosis (whole body writhing) and salivation after acute exposure. The major route of exposure is via dietary intake, even though dermal and inhalation exposure also occur, and estimated dietary exposure for the general U.S. population is between 0.111–1.85 μg/kg/day (EPA, 2011b). The highest tissue concentrations of cypermethrin are found in body fat, skin, liver, kidneys, adrenals and ovaries, whereas only negligible levels are found in brain. The half-life for cypermethrin ranges from 3.4–18.9 days depending on the isomer form and tissue (INCHEM, 1996). Studies investigating the potential developmental neurotoxic effects of pyrethroids have been conducted and reviewed, however it is still not well understood and gaps in knowledge remain in many aspects, such as age-related sensitivity and linkage between behavioral and biochemical endpoints (Shafer et al., 2005).

In recent years,studies have indicated that environmental factors may be a cause for impaired neurodevelopment due to neurotoxic effects in the developing brain; however, none of these studies show conclusive evidence. This inference is based upon the vulnerability of the developing brain and its higher susceptibility to toxic insults compared the adult brain (Bjorling-Poulsen et al., 2008; Burns et al., 2013; Grandjean and Landrigan, 2006; Shelton et al., 2014). Essentially, the mammalian central nervous system (CNS) goes through several development stages before reaching full maturation. A critical phase in the brain development is the "brain growth spurt (BGS)" and is characterized by rapid increase in size and biochemical changes of the brain (Dobbing and Sands, 1979; Kolb and Whishaw, 1989). The onset and duration of the BGS varies among species. In humans, this phase starts around the third trimester of pregnancy; peaks around birth and continues up to the first two years of life, whereas in mice the BGS is neonatal, beginning from birth and extending up to the first four weeks of life, with a peak around postnatal day (PND) 10. Among the many biochemical processes taking place during the BGS are, for example, the establishment of neuronal connections, synaptogenesis, neuronal pruning and myelination (Davison and Dobbing, 1968). These processes involve the expression and regulation of neuroproteins such as calcium/calmodulin-dependent kinases II (CaMKII), growth-associated protein 43 (GAP-43), glutamate receptor 1(GluR1), postsynaptic density protein-95 (PSD95), synaptophysin and tau (Benowitz and Routtenberg, 1997; Ehrlich and Malinow, 2004; Navone et al., 1986; Rongo and Kaplan, 1999; Traynelis et al., 2010; Wang and Liu, 2008; Wiedenmann and Franke, 1985). These proteins have also been shown to express specific ontogenetic patterns during the postnatal period in the mouse and rat nervous system development (Brown et al., 2002; Gottschall et al., 2010; Viberg, 2009; Viberg et al., 2008a).

The BGS have shown to be sensitive to toxic insults by xenobiotics such as polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), perfluorinated compounds (PFCs), pharmaceuticals, nicotine and bisphenol A (Eriksson, 1998; Eriksson et al., 2000; Johansson et al., 2008, 2009; Lee and Viberg, 2013; Viberg et al., 2006, 2008b, 2011, 2013, 2014; Viberg and Lee, 2012); and we have previously seen that certain of these xenobiotics affect the level of neuroprotein expression when exposed during this critical phase. Also, previous studies on pyrethroids and organochlorines have shown to cause developmental neurotoxicity in mice, manifested as deranged spontaneous behavior and changes in muscarinic acetylcholine receptor density in the brain, when neonatally exposed (Ahlbom et al., 1994; Eriksson et al., 1992, 1993; Talts et al., 1998).

In regard to the increasing incidences of observed neurobehavioral and cognitive disorders, and to our previous studies, the objective of this study was to investigate if neonatal exposure, during the peak of the BGS, to a single dose of either endosulfan or cypermethrin may cause neurochemical changes in the protein levels of CaMKII, GAP-43, GluR1, PSD95, synaptophysin and tau in the mouse brain, and adult neurobehavioral aberrations.

2. Material and methods

2.1. Animals and chemicals

Pregnant NMRI mice were purchased from Scanbur, Sollentuna, Sweden and housed individually in plastic cages in a room with an ambient temperature of 22 °C and 12/12 h cycle of light and dark. The animals had free access to standardized food pellets (Lactamin, Stockholm, Sweden) and tap water ad libitum. The day of birth was assigned PND 0; the litters were culled to 10–12 pups within 48 h after birth. At the age of 3–4 weeks male and female mice were separated, the female mice were euthanized and the male mice were kept in their respective treatment groups, together with their siblings. Each litter contained 4–7 animals. Experiments were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC), after approval from the local ethical committee (Uppsala University and Agricultural Research Council) and by the Swedish Committee for Ethical Experiments on Laboratory Animals, approval number C195/12.

Endosulfan (purity >99%, CAS number 115-29-7 and linear formula $C_9H_6Cl_6O_3S$) and cypermethrin(purity >99%, CAS number 67375-30-8 and linear formula $C_{22}H_{19}Cl_2NO_3$) were purchased from Sigma–Aldrich, Stockholm, Sweden. The endosulfan and cypermethrin were dissolved in an egg lecithin (Merck, Darmstadt, Germany) and peanut oil (*Oleum arachidis*) mixture (1:10) and sonicated with water to yield a 20% (w:w) fat emulsion vehicle containing: 0.01, 0.05 mg endosulfan/ml; 0.01 or 0.05 cypermethrin/ml, respectively. This was done to emulate the fat content of mouse milk (∼14%) for a physiologically appropriate absorption and hence distribution (Keller and Yeary, 1980; Palin et al., 1982).

2.2. Treatment

On PND10 the mice were given:0.1, 0.5 mg endosulfan/kg body weight (0.25 or 1.25 μmolendosulfan/kg body weight); 0.1 or 0.5 mg cypermethrin/kg body weight (0.24 or 1.20 μmol cypermethrin/kg body weight), respectively, as a single oral dose via a metal gastric tube. These doses were chosen in accordance to our previous studies with environmental pollutants, for comparison (Ahlbom, 1995; Talts, 1996). Control mice received 10 ml of the 20% fat emulsion vehicle/kg body weight. The animals were euthanized 24 h or 5 months after endosulfan or cypermethrin exposure and the cerebral cortex and hippocampus brain regions were dissected