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Sex dimorphic behaviors as markers of neuroendocrine disruption by environmental chemicals: The case of chlorpyrifos

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ABSTRACT

The complexity of the neuroendocrine level of investigation requires the assessment of behavioral patterns that extend beyond the reproductive functions, which are age- and sex-specific in rodents, described by defined clusters of behavioral items regulated by genetic, hormonal, and epigenetic factors. The study of social behavior in laboratory rodents reveals sex-dimorphic effects of environmental chemicals that may be undetected either by a traditional neurotoxicological approach or referring to the classical definition of endocrine disrupting chemicals. Here we review data on the neurobehavioral effects of developmental exposure to the non-persistent organophosphorus insecticide chlorpyrifos, whose neurotoxic activity at low doses is currently a matter of concern for children's health. In mice exposed to chlorpyrifos in utero and/or in early development social/emotional responses are differently affected in the two sexes in parallel with sex-dependent interference on hypothalamic neuroendocrine pathways regulating social behaviors (vasopressin, oxytocin, and steroid regulated systems). Through the analysis of complex sex-dimorphic behavioral patterns we show that neurotoxic and endocrine disrupting activities of CPF overlap. This widely diffused organophosphorus pesticide might thus be considered as a neuroendocrine disruptor possibly representing a risk factor for sex-biased neurodevelopmental disorders in children.

1. Introduction

In vertebrate species many behaviors, and the neuroendocrine pathways that regulate them, are sexually dimorphic. These sex dimorphisms are the result of sexual selection as they reflect adaptive differences in behavioral strategies of the two sexes, mainly related to their different role in reproduction. Disruptions in these behaviors may reduce social adaptation and impaired the capacity to respond appropriately to eco-ethological niche requirements (Parmigiani et al., 1998). Exposure to environmental substances, which include industrial chemicals, pesticides, fungicides, plasticizers, and even heavy metals and phytoestrogens that interfere with hormonal mechanisms (endocrine disrupting chemicals, EDC) can alter or eliminate behavioral dimorphisms especially when exposure occurs during critical developmental periods. These compounds have in common the ability to derail natural hormonal systems and processes, though different mechanisms of action can be hypothesized. The U.S. Environmental Protection Agency (EPA) defines endocrine disruptors as 'chemicals that either mimic or block the effects of hormones at the target receptor/tissue or by directly stimulating or inhibiting production of hormones by the endocrine system' (EPA, 2007). In this framework many studies have focused on the investigation of effects of xenobiotics acting as xenoestrogens and xenoandrogens on neurobehavioral development (Gore, 2007). However, it has become increasingly clear that the mechanisms of endocrine disruption are complex and include not only direct actions on nuclear hormone receptors, but also membrane steroid receptors, non-steroid receptors (e.g., neurotransmitter receptors), enzymes involved in synthesis/degradation of hormones, and other mechanisms that control levels or activity of hormones (Gore and Patisaul, 2010).

Behavior has a pivotal role in assessing threats to health from exposure to environmental chemicals, as shown in example by the extensive body of data (both experimental and clinical) collected on heavy metals' and polychlorobiphenyls' (PCBs) effects. In animal models, the selection of behavior items is of paramount importance to test or advance hypotheses on the biological basis of outcomes that having been associated to contaminants exposure in epidemiological studies. In a recent provocative review Weiss (2011) underlines that too often sex differences are assigned a secondary role in neurotoxicology research, whereas the ubiquity of sex differences in human behavior and in susceptibility to several pathological conditions and chemical insults would require

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that both sexes are examined in laboratory studies aimed at risk assessment. Furthermore, the effects of sex on neurobehavioral phenotypes go well beyond sexual/reproductive functions and sexual dimorphisms of discrete brain regions. The traditional view of gonadal steroids promoting the formation of male and female circuits in the brain underlying sex differences in behavior has been replaced by a more complex model in which several sex-specific factors, either hormonal, genetic, and epigenetic act in parallel to cause sex differences in brain and behavior possibly with heterogeneous and region-specific mechanisms (McCarthy and Arnold, 2011).

The present review will focus on sexually dimorphic behaviors displayed by laboratory rodents, showing that they may reveal effects of environmental contaminants that may be undetected or underestimated either by a traditional neurotoxicological approach or in the framework of the classical definition of EDC. Many of the behavioral effects of environmental neurotoxicants can be explained by changes in neuroendocrine mechanisms and processes (Frye et al., 2012; Gore and Patisaul, 2010). By adopting a definition of neuroendocrine disruption that encompasses both direct physiological targets and their indirect downstream effects, a more comprehensive picture of the consequences of EDC exposure may emerge. In light of this, in order to define a chemical acting as EDC, it is important reconsidering the full range of integrative processes involved, taking into account the neuroendocrine level of integration which imply several families of inter-system signaling (neuropeptides, neurotransmitters, enzymes, hormones, and receptors) (Waye and Trudeau, 2011). This level of integration underlies animal's reproduction and growth but also its competence in coping with stress, exploring a novel environment or establishing social and affiliative bonds.

Here we will summarize data on the effects produced by an environmental chemical, namely the organophosphorus (OP) insecticide chlorpyrifos (CPF) on different sex dimorphic behavioral responses characteristic of laboratory rodents. The developmental neurotoxicity of CPF has been traditionally associated with its well-known interference, common to the other OPs, with brain acetylcholinesterase (AChE) activity. However, going beyond AChE inhibition and considering the whole spectrum of effects induced by this OP on neurobehavioral development of exposed offspring, neurotoxic and endocrine disrupting activities of CPF have been discovered.

2. Chlorpyrifos

2.1. The case of CPF developmental exposure

OPs make up approximately 50% of all insecticides used in the world (Colborn, 2006; http://epp.eurostat.ec.europa.eu), and are the subject of intensive investigation for their suspected developmental neurotoxicity. Starting from 2004, several epidemiological studies involving agricultural and urban communities have indicated that developmental OP exposure may affect children's neuropsychological maturation (Rosas and Eskenazi, 2008). These compounds, largely used in agriculture, as well as in the home and garden, for pest control, exert their acute neurotoxic effects through AChE inhibition and consequent cholinergic hyper stimulation. However, increasing evidences indicate that, as other environmental chemicals, OPs exert developmental toxicity at low doses with mechanisms different from those observed at higher doses (Eaton et al., 2008; Slotkin and Seidler, 2012).

Since the late nineties, most of experimental and clinical research has focused on CPF, the most widely applied compound in the OP class in the USA and Europe (Bjorling-Poulsen et al., 2008). In 2001 the US Environmental Protection Agency (EPA) imposed a ban on its sale for residential use due to safety concerns on aggregate children exposure. In the European Union no regulatory decision has been reached so far, but there is intense debate on this topic among scientists and regulators (Saunders et al., 2012). In a recent US study (Rauh et al., 2011) cognitive and motor development of a inner-city children cohort was analyzed as a function of CPF levels in umbilical cord plasma, and demonstrated a delayed development in children with the higher CPF exposure. Further, Landrigan (2010) in discussing the environmental contribution to autism etiology included CPF among the environmental chemicals (thalidomide, valproate, and misoprostol) whose developmental exposure in vulnerable individuals might contribute to this group of neurodevelopmental disorders.

Most environmental exposure to CPF is well below the level of systemic toxicity caused by cholinesterase inhibition and thus goes undetected. Nevertheless, experimental studies demonstrated that, at these lower levels, CPF interferes with brain development through a variety of cellular and molecular mechanisms, that are largely independent from inhibition of AChE. Gestational and neonatal exposure to CPF impairs DNA synthesis, neuronal differentiation, synaptogenesis, and gene expression in rats (Crumpton et al., 2000; Dam et al., 1998), and affects neural systems beyond the cholinergic one, such as serotoninergic and dopaminergic transmission, in a sex-dimorphic fashion (Slotkin and Seidler, 2007). Developmental CPF also causes long-term alterations in behaviors related to these neural effects (Aldridge et al., 2005a,b; Icenogle et al., 2004; Levin et al., 2001, 2002). Studies with rats revealed that early neonatal exposure to CPF induces long term changes in cognitive performance in the 16-arm radial maze that selectively impaired males (Levin et al., 2001). In addition to cognition, CPF effects include changes in emotional functions: increased risk taking behavior was seen in male rats treated in early postnatal periods with a low dose of CPF as indicated by significantly increased time spent in the open arms of the elevated plus maze (Aldridge et al., 2005a). Notably, persisting neurobehavioral effects of developmental CPF exposure have been evidenced in a fish model too, the zebrafish, where individuals show increase startle response, decrease predatory avoidance, increase hyper-activity, and impairment in learning upon exposure to low-dose CPF for the 5 days following eggs' fertilization (Levin et al., 2003; Sledge et al., 2011).

In the past years, our laboratory has carried out a detailed characterization of the behavioral effects of CPF, at doses devoid of systemic toxicity and not inhibiting brain AChE, given either during prenatal and postnatal development in the CD1 outbred mouse strain, modeling the doses and exposure schedules applied by Slotkin et al. in the rat species. Our studies confirmed the large window of susceptibility of central nervous system (CNS) development to CPF, with both exposure in late gestational and neonatal phases [those in which brain sexual differentiation takes place (Aldridge et al., 2005b)] yielding long-term behavioral changes in both male and female mice. We basically evaluated two different treatment windows, the late gestational phase [gestational day (GD) 15–18] and the late neonatal stage [postnatal day (PND) 11–14], characterized by different CNS maturational events and representing critical phases of susceptibility to CPF action in rodents (Garcia et al., 2003), using CPF doses within the range (1–6 mg/kg) of suspected fetal and childhood exposure (Gurunathan et al., 1998; Ostrea et al., 2002). We chose to focus on sexually dimorphic behaviors in adult mice. These are complex behaviors with a typical pattern described by defined clusters of activities that are regulated by genetic, neural, hormonal, and experience factors and their interactions, which can be analyzed by means of reliable and standardized ethological methods. They include behavior patterns directly linked to reproductive functions – such as maternal behavior and aggression related to nest defence in lactating females, and the agonistic interactions between adult