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Exposure to methamidophos at adulthood adversely affects serotonergic biomarkers in the mouse brain

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ABSTRACT

Epidemiologic studies describe a potential risk of depression and suicide in farm workers exposed to organophosphates (OPs). In a previous study we observed an increase in depressive-like behavior in adult mice exposed to the OP pesticide methamidophos. Considering the association between depression and the serotonergic (5HT) system, in the present study we investigated whether a subchronic exposure to methamidophos affects the serotonergic system of adult mice. From postnatal day 60 to 89 (PN60 to PN89), one of two concentrations of methamidophos (higher dose: 5.25 μg/ml; lower dose: 1.31 μg/ml) or vehicle was administered in the drinking water of male Swiss mice. We evaluated three serotonergic biomarkers during (PN89) and after (PN100) the exposure period: $5HT_{1A}$ receptor binding with [^{3}H]OH-DPAT, $5HT_2$ receptor binding with [^{3}H]ketanserin and 5HT transporter binding with [^{3}H]paroxetine. Methamidophos elicited robust decreases in binding for all 5HT markers. These decreases were evident in brain regions containing 5HT cell bodies and dendritic arbors (midbrain, brainstem) as well as in the cerebral cortex, which contains 5HT projections. In the cerebral cortex, effects were identified in mice exposed to the higher dose of methamidophos while in the midbrain and brainstem, both doses elicited significant effects. Overall, effects were present both during and after exposure, even though there were some regional disparities regarding the persistence of effects. Our results indicate that exposure to methamidophos affects synaptic transmission promoting decreases of specific serotonergic biomarkers. These data suggest a mechanism of action of this pesticide that might explain the increased depressive-like behavior in adult mice.

1. Introduction

Organophosphate pesticides (OPs) are known to elicit neurotoxic actions with neurobehavioral consequences (Aldridge et al., 2003, 2005a; Raines et al., 2001; Slotkin et al., 2006). In fact, the effects of OP exposure during development have been widely studied and the consequences include alterations in the motor system, in sleep (Timofeeva and Gordon, 2002), in anxiety levels (Braquenier et al., 2010) and in depressive-like behavior (Aldridge et al., 2005a). It is increasingly clear that some of these findings are not restricted and may not even be related to the effects of the inhibition of acetylcholinesterase (AChE), the enzyme responsible for the breakdown of the neurotransmitter acetylcholine in the central and peripheral nervous system (Sultatos, 1994), which is the classical action of OP pesticides and which triggers the cholinergic hyperstimulation typical of acute poisoning by OPs. These agents also act as developmental neurotoxicants through a family of mechanisms, some of which operate at exposures below the threshold for cholinesterase inhibition (Slotkin, 2004, 2005). It has been demonstrated that exposure to OPs such as diazinon, parathion, chlorpyrifos and dichlorvos during prenatal or early postnatal periods elicit widespread abnormalities in indices of cholinergic, dopaminergic and serotonergic (5HT) synaptic function (Aldridge et al., 2005b; Levin et al., 2010; Slotkin and Seidler, 2008; Slotkin et al., 2008).

In spite of the fact that OPs have undergone several restrictions regarding their use in several countries such as United States (U.S.

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EPA, 2002) and Brazil (ANVISA Agência Nacional de Vigilância Sanitária, 2004), these substances are still used worldwide and account for about 25% of the insecticide world market value (Casida et al., 2008), besides the existence of important OP herbicides and fungicides (Casida et al., 2008). In this regard, agricultural workers are at particular risk of exposure and, in fact, epidemiologic studies suggest that the annual incidence of pesticide poisonings among agricultural workers in developing countries varies from 3 to 10%, so that a conservative estimative suggests that 25 million pesticide poisonings occur among agricultural workers each year in developing countries alone (Koh and Jeyaratnam, 1996). Accordingly, besides the effects during development, several important changes in glutamatergic, noradrenergic, dopaminergic and serotonergic systems occur as a result of exposure at adulthood. Among these changes are reductions of norepinephrine levels in rats exposed to dichlorvos (Ali et al., 1980), increases in glutamate and GABA uptake in rats exposed to methamidophos (Gubert et al., 2011) and changes in dopamine metabolism and serotonin content and turnover after exposure to chlorpyrifos (Moreno et al., 2008; Pung et al., 2006). These findings suggest that the susceptibility to OPs extends beyond the perinatal period, so that the central nervous system of adults is still vulnerable to non-cholinesterase effects of OPs.

Several studies have reported an association between OP exposure and psychiatric disorders (Amr et al., 1997; Fiedler et al., 1997; London et al., 2005; Stephens et al., 1995). Particularly, studies describe a potential risk of depression and suicide in farm workers exposed to OPs (Jaga and Dharmani, 2007; London et al., 2005; van Wijngaarden, 2003). Despite that, to our knowledge, a scant number of studies have investigated the nature of the relation between psychiatric disorders and OP exposure in animal models of OP exposure at adulthood. In a previous study, our research group has identified depressive-like behavior in adult mice subchronically exposed to low doses of methamidophos (Lima et al., 2009), a highly toxic (class I) OP which, in recent years, has been increasingly used as a consequence of the prohibition of other pesticides. These results reinforce evidence that there is an association between pesticide exposure and psychiatric disorders, particularly depression (Beseler et al., 2008; London et al., 2005; Ross et al., 2010). Considering the behavioral changes we have observed and the fact that the serotonergic system is a key system, deeply associated with mood disorders, in the present study we investigated whether the serotonergic system of adult mice is altered by a subchronic exposure to the OP pesticide methamidophos at doses below the threshold for cholinergic hyperstimulation. The analyses of the serotonergic system were performed by the end of the period of exposure and after a period of recovery in order to verify whether the effects persist or emerge post-exposure. We evaluated indices of 5HT synaptic function in the cerebral cortex, midbrain and brainstem. The cerebral cortex contains major 5HT projections and the midbrain and brainstem, in addition to 5HT dendritic arbors, contain the majority of the serotonergic cell bodies of pathways that ascend into the cerebral cortex, hippocampus and other regions involved in affective disorders and serotonergic regulation of hypothalamus–pituitary–adrenal axis function. We measured three 5HT synaptic proteins: the $5HT_{1A}$ and $5HT_2$ receptors, and the presynaptic 5HT transporter (5HTT). The two receptors play major roles in 5HT-related mental disorders, particularly depression (Arango et al., 2001; Fujita et al., 2000; Mintun et al., 2004; Ohno, 2010; Yatham et al., 1999, 2000), and the transporter, which regulates the synaptic concentration of 5HT, is the primary target for antidepressant drugs (Bab and Yirmiya, 2010; ICSI, 2011; Maes and Meltzer, 1995; Nemeroff, 1998; Nutt, 2002; Ohno, 2010).

2. Materials and methods

All experiments were carried out under institutional approval of the Universidade do Estado do Rio de Janeiro in accordance with the declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

2.1. Animals and treatment (Fig. 1)

All mice were bred and maintained in a temperature controlled environment, on a 12:12 h light/dark cycle (lights on at 2:00 a.m.). Access to food and water was *ad lib*. On the first postnatal day (PN1), litters were culled to a maximum of eight mice to ensure standard nutrition. At weaning (PN21) animals were separated by sex and allowed free access to food and water. On PN60, males were housed in groups of 2–4 animals to begin treatment. Previous reports have demonstrated that OP exposure may elicit disparate effects between males and females (Aldridge et al., 2004; Dam et al., 2000; Levin et al., 2001; Meyer et al., 2004). Since agricultural activities are essentially performed by men, who indeed receive the most intense occupational exposures, while women participation is usually secondary, intermittent, and discontinuous (Chrisman et al., 2009; ILO – International Labour Organization, 1999), we opted to focus the study on male subjects.

One hundred forty-six male Swiss mice from 36 litters were distributed into three treatment groups: higher dose (HighD), lower dose (LowD) and control (CT). From PN60 to PN89, one of two concentrations of methamidophos (Sigma, St. Louis, MO) dissolved in methanol (HighD: 5.25 μg/ml; LowD: 1.31 μg/ml) was administered in the drinking water (the sole source of fluid). The doses used are the same as those used in a previous study which evaluated depressive-like behavior in male mice (Lima et al., 2009). The doses were calculated based on the estimated fluid consumption of adult male Swiss mice so that the higher dose delivered 7% and the lower dose delivered 1.8% of the DL50 value of 21 mg/kg (EXTOXNET, 1995). The CT group received methanol dissolved in water at a concentration equivalent to that received by the HighD group. Methanol consumption averaged 8.7 mg/day, well below levels that produce neurotoxic effects (Burbacher, 1993; Shelby et al., 2004; Youssef and Santi, 1997). The present experimental design does not allow us to discard the possibility that methanol exposure affects the results. Despite that, there are no studies describing an association between depression or depressive-like symptomatology and methanol exposure, and monoamine neurotransmission alterations in response to methanol are described at doses at least ten times higher than the highest dose used in our study (Jeganathan and Namasivayam, 1987, 1989). These findings suggest that it is unlikely that methanol exposure, at the doses used here, has affected the results.

Bottles were cleaned and refilled every other day. Body weights and fluid consumption during the period of exposure were also measured every other day. As described elsewhere (Lima et al., 2009), fluid intake data were obtained by dividing the values of fluid intake of each cage by the combined body weight of the animals of the cage. There were no differences in body weight and fluid consumption among groups; as a result, the higher concentration of methamidophos in the drinking solution of the HighD group resulted in a proportionally higher dose of the methamidophos exposure when compared to the LowD group (Lima et al., 2009).

Mice were decapitated at two time points, one toward the end of treatment (PN89) and another eleven days after the end of exposure (PN100). The time points used are the same as those used in a previous study which evaluated depressive-like behavior in male mice (Lima et al., 2009). The time point after the end of