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

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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 4A receptor binding with [3H]OH-DPAT, 5HT 3 receptor binding with [3H]ketanserin and 5HT transporter binding with [3H]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.

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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 neurotoxins 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.
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–pituatory–adrenal axis function. We measured three 5HT synaptic proteins: the 5HT1A and 5HT3 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 (Christman 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 D50 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
exposure was chosen based on the rate of AChE activity recovery. In rat brain, the synthesis of new enzyme occurs with a mean half-life of approximately 11 days (Giacobini, 2000). The brain regions were dissected by making blunt cuts through the cerebellar peduncles, whereupon the cerebellum (including flocculi) was lifted from the underlying tissue. The cerebral cortex (forebrain with removal of the hippocampus) was separated from the midbrain + brainstem by a cut made rostral to the thalamus. The midbrain was then dissected from the hindbrain by making a cut caudal to the inferior colliculus, so that the midbrain contained the entire dorsal raphe nucleus but no descending serotonergic nuclei (Fumagalli et al., 1996; Trauth et al., 2000). After tissue dissection, cerebral cortex, midbrain and brainstem were frozen in liquid nitrogen, and stored at −85 °C until assayed. For the midbrain and brainstem, the biochemical assays were performed in “pool” tissue from 2 mice. For each treatment group and period, 7–9 animals or “pools” were examined.

As described elsewhere (Lima et al., 2009), most animals (n = 113) used in the present study were submitted to a sequence of behavioral tests. Sacrifice occurred immediately after the behavioral tests. Additional mice (n = 33) were distributed among the treatment groups, submitted to the same procedures, except for the behavioral tests, and sacrificed at the same time points as those that were also submitted to the behavioral tests.

2.2. Tissue preparation and assays

Tissues were thawed and homogenized (Ultra-Turrax T10 basic, IKA, São Paulo, SP) in ice-cold 50 mM Tris (pH 7.4), and homogenates were sedimented at 40,000 × g for 15 min. The pellets were washed by resuspension (Ultra-Turrax) in homogenization buffer followed by resedimentation, and were then dispersed with a homogenizer (smooth glass fitted with Teflon pestle) in the same buffer. An aliquot was assayed for measurement of membrane protein (Smith et al., 1985).

Two radioligands were used to determine 5HT receptor binding: 1 nM [3H]8-hydroxy-2-(di-n-propylamino)tetralin for 5HT1A receptors, and 0.4 nM [3H]ketanserin for 5HT2 receptors. Binding to the presynaptic 5HT transporter was evaluated with 85 pM [3H]paroxetine (Aldridge et al., 2005c). For 5HT1A receptors, incubations lasted for 30 min at 25 °C in a buffer consisting of 50 mM Tris (pH 8), 0.5 mM MgCl2, and 0.5 mM sodium ascorbate; 100 μM 5HT was used to displace specific binding. For 5HT2 receptors, incubations lasted 15 min at 37 °C in 50 mM Tris (pH 7.4) and specific binding was displaced with 10 μM methysergide.

For binding to the presynaptic 5HT transporter, incubations lasted for 120 min at 20 °C in a buffer consisting of 50 mM Tris (pH 7.4), 120 mM NaCl, and 5 mM KCl; 100 μM 5HT was used to displace specific binding. Incubations were stopped by the addition of excess of ice-cold incubation buffer (without radioligand or displacing agent) and the labeled membranes were trapped by rapid vacuum filtration onto glass fiber filters that were presoaked in 0.15% polyethyleneimine. The filters were then washed with incubation buffer and radiolabel was determined (Aldridge et al., 2005c).

The overall strategy was to examine binding at a single ligand concentration in preparations from all regions in every animal/pool, focusing on a concentration above the Kd but below full saturation. We can thus detect changes that originate either in altered Kd or Bmax but cannot distinguish between the two possible mechanisms. In spite of this limitation, the interpretation of results of the present study does not depend upon whether the changes are specific to concentration or affinity. This strategy was needed due to the amount of tissue available for each determination and due to technical limitations caused by the requirement to measure binding in three treatment groups at two different ages in multiple brain regions, with at least six animals. Thus, there were hundreds of separate membrane preparations, each of which had to be evaluated for binding of three different ligands.

2.3. Material

Radioisotopically labeled compounds came from PerkinElmer Life Sciences (Boston, MA): [3H]-8-hydroxy-2-(di-n-propylamino)tetralin (specific activity 170.2 Ci/mmol), [3H]ketanserin (specific activity 67.0 Ci/mmol) and [3H]paroxetine (specific activity, 24.4 Ci/mmol). Sigma Chemical Co. (St. Louis, MO) was the source for bovine albumin, BCA kit, methamidophos, serotonin, methysergide and polyethyleneimine. VETEC Quimica Fina Ltda (Rio de Janeiro, RJ) was the source for all other reagents.

2.4. Statistical analysis

All data were compiled as means and standard errors. Significance was assumed at the level of P < 0.05. Initially, analyses of variance (ANOVA) on each variable (5HT1A receptors, 5HT2 receptors and the 5HT transporter) were carried out. Treatment (high dose, low dose and control) and Period (during and after exposure) were used as between-subjects factors. Whenever applicable, significant Treatment × Period interactions were followed by lower order ANOVAs and by pairwise post hoc analyses by using Fisher’s Protected Least Significant Difference (FPLSD). Main Treatment effects were followed by FPLSD. To facilitate comparisons across the different measurements, the effects of methamidophos were compiled as the percent change from control values, but statistical evaluations involved only the
original data; for reference, control values for all variables are shown in Table 1.

3. Results

Treatment of adult male mice with methamidophos had profound effects on 5HT synaptic markers. The cerebral cortex (Fig. 2), a brain region containing a considerable number of 5HT projections, showed significant reductions in binding sites for 5HT₁A receptors (Treatment: \( F_{2,36} = 3.4, P = 0.04 \)), 5HT₂ receptors (Treatment: \( F_{2,42} = 7.1, P = 0.002 \)) and 5HTT (Treatment: \( F_{2,42} = 7.1, P = 0.002 \); Treatment \( \times \) Period: \( F_{2,42} = 4.3, P = 0.02 \)). All significant results were identified in mice exposed to the higher dose of methamidophos: The HighD group presented reduced 5HT₁A and 5HT₂ receptor binding during exposure, an effect that persisted even eleven days post-treatment. The LowD group did not differ from the CT group, even though there was a trend for reduced binding for 5HT₁A receptors. The effects on 5HTT only reached significance eleven days post-exposure when again the HighD group presented reduced binding when compared to the CT group.

Methamidophos also elicited robust reductions in 5HT binding markers in brain regions containing 5HT cell bodies and dendritic arbor; however, in these regions, both doses elicited significant alterations: In the midbrain (Fig. 3), 5HT₁ (Treatment: \( F_{2,46} = 7.8, P = 0.001 \)) and 5HTT (Treatment: \( F_{2,48} = 10.9, P = 0.001 \); Treatment \( \times \) Period: \( F_{2,48} = 4.4, P = 0.02 \)) binding sites were reduced, while there were no significant reductions for 5HT₂. Both the HighD and the LowD groups presented a reduction in binding for 5HT₂ receptors and this effect was still evident eleven days post-treatment. The reduction in binding sites for 5HTT was evident for both HighD and LowD groups during exposure but failed to persist after the end of treatment. In the brainstem (Fig. 4), all serotonergic markers were affected (5HT₁A – Treatment: \( F_{2,38} = 9.4, P = 0.005 \) and Treatment \( \times \) Period: \( F_{2,38} = 7.4, P = 0.002 \); 5HT₂ – Treatment: \( F_{2,40} = 6.5, P = 0.004 \); 5HTT – Treatment: \( F_{2,41} = 16.2, P < 0.0001 \)). Both HighD and LowD groups showed significant reductions in 5HT₁A receptor binding during methamidophos exposure but no alterations were observed after the end of treatment. For 5HT₂ receptors and the 5HTT, both doses elicited a reduction in binding during and after exposure.

4. Discussion

Epidemiologic studies suggest that farm workers are prone to develop depressive disorders. In the present study, we describe that the serotonergic system of adult mice is altered by a subchronic exposure to the OP pesticide methamidophos at doses below the threshold for cholineriger hyperstimulation. Considering that in a previous study we observed an increase in the depressive-like behavior of mice exposed to methamidophos at adulthood, which reinforces the hypothesis that OP exposure is associated with an increased risk of depression, our current findings are consistent with the view that the disruption of the serotonergic function by methamidophos plays a role in the depressive-like symptomatology identified in adult mice.

Even though the classic, toxic effects of OPs are caused by the inhibition of AChE, it is increasingly clear that OPs also act through multiple mechanisms, not necessarily related to AChE inhibition, that culminate in biochemical and/or behavioral deficiencies. Regarding the serotonergic system, there is evidence that the effects are highly dependent on the OP, so that the direction of the alteration and the magnitude of the effect may be different among

Table 1

<table>
<thead>
<tr>
<th></th>
<th>During exposure</th>
<th></th>
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<th>After exposure</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5HT₁A</td>
<td>5HT₂</td>
<td>5HTT</td>
<td>5HT₁A</td>
<td>5HT₂</td>
<td>5HTT</td>
</tr>
<tr>
<td>Cortex</td>
<td>25 ± 1.2</td>
<td>38 ± 3.0</td>
<td>191 ± 9.1</td>
<td>26 ± 1.3</td>
<td>39 ± 2.5</td>
<td>198 ± 7.8</td>
</tr>
<tr>
<td>Midbrain</td>
<td>12.5 ± 1.1</td>
<td>22.9 ± 3.9</td>
<td>411 ± 19.5</td>
<td>10.1 ± 1.1</td>
<td>24.4 ± 2.4</td>
<td>380.5 ± 14.9</td>
</tr>
<tr>
<td>Brainstem</td>
<td>13.1 ± 1.0</td>
<td>13.9 ± 1.1</td>
<td>226 ± 12.4</td>
<td>9.9 ± 0.4</td>
<td>14.3 ± 1.1</td>
<td>219.5 ± 7.1</td>
</tr>
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Values are mean ± SEM in fmol/mg of protein.
distinct OPs (Levin et al., 2010; Slotkin and Seidler, 2008; Slotkin et al., 2009). Besides the OP used, there may be differences among effects according to the stage of development. Here, we found that methamidophos exposure at adulthood elicited a reduction in serotonergic receptors and presynaptic transporter binding. Distinctively, Slotkin et al. (2009) described that neonatal exposure to parathion elicited upregulation of 5HT receptors in adolescent rats and downregulation at adulthood. Neonatal diazinozine exposure evoked downregulation of the 5HT1A receptor in male rats accompanied by upregulation of the 5HT transporter in females (Slotkin et al., 2008) while the effects of chlorpyrifos include suppression of serotonergic biomarkers when exposure occurs early in gestation and increase in 5HT1A and 5HT2 binding due to neonatal exposure (Aldridge et al., 2003).

Here, robust changes in serotonergic biomarkers were identified even when there was little inhibition of AChE. As an example, in our previous study, we showed that the lower dose group presented 15% inhibition of AChE activity during exposure (Lima et al., 2009) and in the present study this group presented a robust reduction in 5HT2 receptor binding relative to the control group (e.g. 42% in the midbrain). Eleven days after exposure, AChE activity was recovered (Lima et al., 2009) while the alteration of the serotonergic receptor was still evident (e.g. 32% in the midbrain). Altogether, these results corroborate previous findings during development that suggest that the alterations in the serotonergic system are not related to the inhibition of AChE.

Interestingly, despite the fact that occupational contamination by pesticides is more frequent at adulthood (Dharmani and Jaga, 2005), the investigation of biochemical alterations promoted by OPs is scarce at this stage. Our findings indicate that the serotonergic system is sensitive to the methamidophos OP at adulthood. It is well established that 5HT receptors are altered in depressive disorders (for review: Carr and Lucki, 2010; Savitz et al., 2009). In these situations, a depletion of serotonin may lead to a compensatory increase in the density or affinity of target receptors (upregulation) (Arora and Metzer, 1989; Matsubara et al., 1991; Pandey et al., 2002), which is in a clear contrast to our current findings. Interestingly, the upregulation of receptors may not be the only alteration associated with impaired serotonergic transmission. A recent study that evaluated the mRNAs encoding the proteins for serotonin biosynthesis, storage and degradation, as well as for 5HT receptors in undifferentiated and differentiating PC12 cells exposed to chlorpyrifos or diazinon described that both agents increase the expression of genes that encode tryptophan hydroxylase, the rate-limiting enzyme in serotonin biosynthesis, suppress the expression of 5HT transporter genes and that diazinon evokes overall suppression of 5HT receptor subtypes (Slotkin and Seidler, 2008). Furthermore, a decreased expression of the mRNA encoding the 5HT1A receptor (López-Figueroa et al., 2004) and in 5HT2 receptor binding (Mintun et al., 2004) in patients with major depression were demonstrated. One possible explanation for the present results is that the initial event elicited by methamidophos is the reduced 5HTT binding. As a result of the decrease in serotonin uptake, serotonin accumulates in the synaptic cleft, which would lead ultimately to a downregulation of receptors. However, we cannot discard the possibility that our results reflect a direct action of methamidophos on other components of the serotonergic system.

Regarding the action of OPs on neurotransmitter levels, chlorpyrifos was shown to elicit an increase in serotonin content in the cerebral cortex and a decrease in the hippocampus of adult rats (Pung et al., 2006). Late-emergent decreases in serotonin content in the nucleus accumbens and in serotonin turnover (given by the ratio metabolite/neurotransmitter that represents a dynamic index of synaptic activity) in the striatum were also described (Moreno et al., 2008). Aldridge et al. (2005b) detected decreased content and increased serotonin turnover in several brain regions of adult rats exposed to chlorpyrifos during the gestational period. Gestational exposure to chlorpyrifos elicited an increased turnover of 5HT in the cerebral cortex, midbrain, brainstem and hippocampus of adolescent male rats (Slotkin and Seidler, 2007b). In addition, a more recent study found increased turnover of serotonin in the brainstem during the weaning period of rats exposed to chlorpyrifos later during development (PN11 to PN14) (Slotkin and Seidler, 2007a). If similar events occur due to methamidophos exposure, it is possible that our results are a consequence, at least in part, of changes in serotonin levels and/or turnover. Since we identified decreased serotonergic biomarkers, we would expect to find increased neurotransmitter levels. This effect could be associated with decreased turnover, however, it is also possible that a compensatory increase in turnover takes place as a result of long-term exposure at adulthood.

In a previous study using the same experimental design used in the present study (Lima et al., 2009) we have detected increased depressive-like behavior both during and after exposure to methamidophos at adulthood: Mice exposed to the lower dose presented increased depressive-like behavior during exposure while the higher dose elicited increased depressive-like behavior after the end of exposure. As we have shown in the present study, these behavioral effects were paralleled by the decreased binding of receptors and the serotonergic transporter both in the brain region rich in serotonergic projections (cerebral cortex) and in regions rich in serotonergic cell bodies (brainstem and midbrain), which suggests that the behavioral and biochemical alterations are associated. Among 5HT receptors, evidence for a role in the pathogenesis and treatment of depressive disorders is more extensive for 5HT1A and 5HT2 receptors. Besides, serotonin reuptake inhibitors, which act at the 5HTT, are the first-line drugs in the treatment of depression, while agonists at 5HT1A receptors have been reported to exert antidepressive activity in double-blind, placebo-controlled, and comparative trials (Blier and Ward, 2003). In addition, there is evidence of altered 5HT1A and 5HT2 receptor as well as 5HTT binding in brain regions of suicide victims and in patients with major depression (Arango et al., 2001; Malison et al., 1998; Mintun et al., 2004; Yatham et al., 2000). Even though it is not established whether serotonergic alterations precede depressive symptoms or result from depressive episodes, the present results reinforce an association between serotonergic and behavioral alterations. Despite that, some biochemical alterations

**Fig. 4.** Effects of methamidophos subchronic exposure on 5HT1A receptors, 5HT2 receptors and 5HT transporter (5HTT) binding in the brainstem during and after exposure of adult male mice, presented as the percent change from control values. For 5HT1A, a Treatment × Period interaction was detected, so that differences among subgroups (Control during exposure, Control after exposure LowD during exposure, LowD after exposure, HighD during exposure and HighD after exposure) were followed by FPLSD tests. For 5HT2 receptors and 5HTT, only a Treatment effect was detected, accordingly, differences among groups (Control, LowD and HighD) were followed by FPLSD tests on groups collapsed across periods. Values are means ± SEM. **P < 0.01, ***P < 0.001 vs. Control group. LowD during: lower dose after exposure; HighD during: higher dose during exposure; HighD after: higher dose after exposure groups.
were not accompanied by behavioral effects, which might indicate that other serotonergic alterations or even alterations in other neurotransmitter systems play a role. In this regard, in addition to the changes in serotonin levels and turnover described above for OPs other than methamidophos (Aldridge et al., 2005b; SLOTKIN and Seidler, 2007a,b), OP exposure has been associated with dopaminergic and noradrenergic alterations (Ali et al., 1980; Moreno et al., 2008; Pung et al., 2006; SLOTKIN and Seidler, 2007b). Finally, several effects on serotonergic markers that were present during exposure were still detectable after exposure. However, some markers seem to recover post-exposure; particularly the 5HT in the midbrain and the 5HT1A in the brainstem. These results suggest that recovery is more efficient in regions rich in serotonergic cell bodies, where serotonergic proteins are synthesized.

5. Conclusions

Many studies that investigate the impact of OP exposure on the serotonergic system focus on the effects of these substances during the perinatal period (for review: SLOTKIN, 2004; SLOTKIN and Seidler, 2007c). However, occupational contamination by OP pesticides is more frequent at adulthood (Dharmani and Jaga, 2005). Our results indicate that methamidophos is deleterious to brain function and that susceptibility can also occur at adulthood, a period generally considered to be of greater resistance to low dose effects, as opposed to the perinatal period. Considering the association between the serotonergic system and depression, the present results, together with previous findings describing behavioral alterations consistent with depressive-like behavior due to OP exposure (Brocardo et al., 2007; Lima et al., 2009; Ramos et al., 2006) suggest that exposure to pesticides, particularly those in the OP class, should be taken into account in designing health programs that aim to prevent and control mental disorders in rural populations, especially among those that work in the fields. Considering that the ill effects of exposure could take a long time to manifest, such programs should include long-term follow-ups. Furthermore, legislation addressing maximum exposure levels to OP should take into account that even low doses can be harmful to adult humans.

Conflict of interest

The authors declare they have no competing financial interests including grant support, employment, patents, payment for expert witness or testimony, personal financial interests and forms of compensation.

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Organophosphate pesticides that inhibit acetylcholinesterase activity can cause cholinergic symptoms, including muscle weakness, slurred speech, and seizures. These effects are typically reversible with cholinesterase inhibitors such as physostigmine. However, exposure to high levels of organophosphates can lead to more severe neurological effects, such as respiratory failure and coma. In these cases, intensive care management and supportive therapy are critical. The use of organophosphates as neurotoxicants highlights the importance of understanding the mechanisms of action and potential for unintended consequences in the environment.