Neurotoxicology and Teratology 31 (2009) 11–17

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

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

Developmental neurotoxicity of parathion: Progressive effects on serotonergic systems in adolescence and adulthood

Theodore A. Slotkin [a,b,*], Edward D. Levin [a,b], Frederic J. Seidler [a]

[a] Department of Pharmacology & Cancer Biology, Duke University Medical Center, Durham, North Carolina, USA
[b] Department of Psychiatry & Behavioral Sciences, Duke University Medical Center, Durham, North Carolina, USA

ARTICLE INFO

Article history:
Received 23 May 2008
Received in revised form 4 August 2008
Accepted 4 August 2008
Available online 20 August 2008

Keywords:
Organophosphate pesticides
Parathion
Serotonin receptors
Serotonin transporter
Sex-selective neurotoxicity

ABSTRACT

Neonatal exposures to organophosphates that are not acutely symptomatic or that produce little or no cholinesterase inhibition can nevertheless compromise the development and later function of critical neural pathways, including serotonin (5HT) systems that regulate emotional behaviors. We administered parathion to newborn rats on postnatal days (PN) 1–4 at doses spanning the threshold for detectable cholinesterase inhibition (0.1 mg/kg/day) and the first signs of loss of viability (0.2 mg/kg/day). In adolescence (PN30), young adulthood (PN60) and full adulthood (PN100), we measured radioligand binding to $5HT_{1A}$ and $5HT_2$ receptors, and to the 5HT transporter in the brain regions comprising all the major 5HT projections and 5HT cell bodies. Parathion caused a biphasic effect over later development with initial, widespread upregulation of $5HT_{1A}$ receptors that peaked in the frontal/parietal cortex by PN60, followed by a diminution of that effect in most regions and emergence of deficits at PN100. There were smaller, but statistically significant changes in $5HT_2$ receptors and the 5HT transporter. These findings stand in strong contrast to previous results with neonatal exposure to a different organophosphate, chlorpyrifos, which evoked parallel upregulation of all three 5HT synaptic proteins that persisted from adolescence through full adulthood and that targeted males much more than females. Our results support the view that the various organophosphates have disparate effects on 5HT systems, distinct from their shared property as cholinesterase inhibitors, and the targeting of 5HT function points toward the importance of studying the impact of these agents on 5HT-linked behaviors.

1. Introduction

It is increasingly evident that organophosphate pesticides damage the developing brain at exposures below the threshold for overt signs of intoxication and even below that required for cholinesterase inhibition, the biomarker used for risk assessment [12,14,22,23,30,36–38,46–48,63]. Numerous studies have detailed how organophosphates disrupt the basic patterns of neural cell replication and differentiation, alter axonogenesis and synaptogenesis, and discoordinate the development of neural circuits, ultimately producing widespread behavioral deficits [7,9,10,12,18,38–40,46–48,56,65]. Because of the initial focus on cholinergic actions, many reports of the effects of organophosphate exposure have concerned the targeting of acetylcholine systems and cognitive/learning deficits related to these pathways [15,16,19,21,22,26,27,41,46–48,63]. However, recent research indicates that organophosphates target serotonin (5HT) systems to an even greater extent, contributing to adverse outcomes related to emotional and social behaviors [1–5,33,42,43,51,53–55,57,58,62]. Indeed, evidence is now accumulating that relate organophosphate exposures to depression and suicide [8,20,25,28].

Because organophosphates cause developmental neurotoxicity through mechanisms beyond their shared property as cholinesterase inhibitors, the various members of this pesticide class could differ in their impact on 5HT systems. In recent studies, we showed that exposures of neonatal rats on postnatal days (PN) 1–4 to three different organophosphates, chlorpyrifos, diazinon and parathion, at doses spanning the threshold for detectable but nonsymptomatic cholinesterase inhibition, produced dissimilar initial effects on 5HT systems, as monitored on PN5 [4,52,58]. Notably, parathion was entirely distinct, eliciting deficits in $5HT_{1A}$ receptor expression, whereas the other two organophosphates produced increases. In subsequent work, we showed some basic similarities in the long-term effects of chlorpyrifos and diazinon on 5HT systems, but also some significant disparities that emerged between adolescence and adulthood [3,5,51,55], contributing to divergent effects on emotional behaviors [1,45]. Accordingly, in the present study, we evaluated the long-term effects of neonatal parathion exposure, again conducting studies from adolescent through adult stages. We gave parathion on PN1–4, an exposure window identified in our earlier work with

Abbreviations: 5HT, 5-hydroxytryptamine, serotonin; 5HTT, 5HT transporter; ANOVA, analysis of variance; PN, postnatal day.

* Corresponding author. Department of Pharmacology & Cancer Biology, Box 3813 DUMC, Duke University Medical Center, Durham, NC 27710-3813, USA. Tel.: +1 919 681 8015; fax: +1 919 684 8197.
E-mail address: t.slotkin@duke.edu (T.A. Slotkin).

0892-0362/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ntt.2008.08.004

chlorpyrifos as a peak of sensitivity for disrupting 5HT systems [2,4,5,54]. We focused on two parathion treatments spanning the maximum tolerated dose, 0.1 mg/kg/day, which produces 10% cholinesterase inhibition [58], well below the 70% inhibition required for the symptoms of cholinergic hyperstimulation [13], and 0.2 mg/kg/day, just past the threshold for the first signs of systemic toxicity in neonates [50]. Our measurements focused on three 5HT synaptic proteins known to be highly affected by developmental exposure to chlorpyrifos [3–5,55] or diazinon [51,58], 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 [6,17,66,67], and the transporter, which regulates the synaptic concentration of 5HT, is the primary target for antidepressant drugs [29,34,35]. We evaluated effects in all the brain regions comprising the major 5HT projections (frontal/parietal cortex, temporal/occipital cortex, hippocampus, striatum) as well as those containing 5HT cell bodies (midbrain, brainstem). The study design and assays were all identical to those in our previous work on chlorpyrifos and diazinon [1,5,51,55,58], so as to foster comparison of the outcomes of exposure to the three different organophosphates.

2. Methods

2.1. Animal treatments

All experiments were carried out humanely and with regard for alleviation of suffering, with protocols approved by the Duke University Institutional Animal Care and Use Committee and in accordance with all federal and state guidelines. Timed-pregnant Sprague–Dawley rats were housed in breeding cages, with a 12 h light–dark cycle and free access to food and water. On the day after birth, all pups were randomized and redistributed to the dams with a litter size of 10 (5 males, 5 females) to maintain a standard nutritional status. Because of its poor water solubility, parathion was dissolved in dimethylsulfoxide to provide consistent absorption [50,58,64] and was injected subcutaneously in a volume of 1 ml/kg once daily on postnatal days (PN) 1–4; control animals received equivalent injections of the dimethylsulfoxide vehicle, which does not itself produce developmental neurotoxicity [64]. Doses of 0.1 and 0.2 mg/kg/day were chosen because they straddle the threshold for detectable cholinesterase inhibition and the first signs of impaired viability [50,58]: the low dose produces 5–10% inhibition without mortality, whereas the higher dose elicits 5–10% mortality. The PN1–4 regimen was chosen because it represents a peak period for sensitivity of 5HT systems to the developmental neurotoxicity of chlorpyrifos [46–48] and because the systemic toxicity and cholinesterase inhibition in response to parathion have already been characterized for this treatment window [50,58]. Accordingly, both the toxicodynamic effects and treatment window parallel those used in our prior studies with chlorpyrifos and diazinon [1,5,51,55,58]. Randomization of pup litter assignments within treatment groups was repeated at intervals of several days up until weaning, coordinated with weighing of the animals and changes of cage bedding, and in addition, dams were rotated among litters to distribute any maternal caretaking differences randomly across litters and treatment groups. Each treatment group comprised 12 litters and all pups within a reconstituted litter belonged to the same treatment group to ensure that dams did not discriminate between control and treated pups in the maternal caretaking or nursing behaviors. Offspring were weaned on PN21.

On PN30, 60 and 100, one male and one female were selected from each litter of origin and were decapitated. The cerebellum (including flocculi) was removed and the midbrain/brainstem was separated from the forebrain by a cut rostral to the thalamus. The striatum and hippocampus were then dissected from these larger divisions and the midbrain and brainstem were divided from each other. The cerebral cortex was divided down the midline and then further sectioned into anterior and posterior regions (frontal/parietal cortex and temporal/occipital cortex, respectively). The cerebellum, which is sparse in 5HT projections, was reserved for future studies; also, the midbrain, hippocampus and striatum were not evaluated on PN30 because these regions were utilized in another study of acetylcholine biomarkers [49]. Tissues were frozen with liquid nitrogen and stored at −45 ° C.

2.2. Assays

All of the assay methodologies used in this study have appeared in previous papers [5,50,53,55], so only brief descriptions will be provided here. Tissues were thawed and homogenized (Polytron, Brinkmann Instruments, Westbury, NY) in ice-cold 50 mM Tris (pH 7.4), and the homogenates were sedimented at 40,000 ×*g* for 15 min. The pellets were washed by resuspension (Polytron) 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 [59].

Two radioligands were used to determine 5HT receptor binding: 1 nM [^{3}H]8-hydroxy-2-(di-*n*-propylamino)tetralin for $5HT_{1A}$ receptors, and 0.4 nM [^{3}H]ketanserin for $5HT_2$ receptors. Binding to the presynaptic 5HT transporter was evaluated with 85 pM [^{3}H]paroxetine. For $5HT_{1A}$ receptors and the 5HT transporter, specific binding was displaced by addition of 100 μM 5HT; for $5HT_2$ receptors, we used 10 μM methylsergide for displacement. The overall strategy was to examine binding at a single ligand concentration in preparations from all regions in every animal, focusing on a concentration above the K_d but below full saturation. We can thus detect changes that originate either in altered K_d or B_{max} but cannot distinguish between the two possible mechanisms, albeit that a change in K_d would seem highly unlikely. This strategy was necessitated by the amount of tissue available for each determination and technical limitations engendered by the requirement to measure binding in three treatment groups at three different ages in multiple brain regions, with at least six animals for each sex. Thus, there were hundreds of separate membrane preparations, each of which had to be evaluated for binding of three different ligands.

2.3. Data analysis

Data were compiled as means and standard errors. Because we evaluated binding parameters for multiple proteins all related to 5HT synapses, the initial comparisons were conducted by a global ANOVA (data log-transformed because of heterogeneous variance among ages, regions and the different protein measures) incorporating all the variables and measurements so as to avoid an increased probability of type 1 errors that might otherwise result from multiple tests of the same data set. Where we identified interactions of treatment with the other variables, data were then subdivided for lower-order ANOVAs to evaluate treatments that differed from the corresponding control. Where permitted by the interaction terms, individual groups that differed from control in a given region at a given age were identified with Fisher's Protected Least Significant Difference Test; however, where only main treatment effects were present (without interactions), we present the main effect without subsequent lower-order tests of individual values. Significance was assumed at the level of $p<0.05$. For interactions at $p<0.1$, we also examined whether lower-order main effects were detectable after subdivision of the interactive variables [60]. The $p<0.1$ criterion for interaction terms was not used to assign significance to the effects but rather to identify interactive variables requiring subdivision for lower-order tests of the main effects of parathion, the variable of chief interest [60].

For convenience, the results are presented as the percent change from control values but statistical comparisons were conducted only on the original data. Although not shown here, the control values for