available at www.sciencedirect.com

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Organophosphate exposure during a critical developmental stage reprograms adenylyl cyclase signaling in PC12 cells

*Abayomi A. Adigun, Ian T. Ryde, Frederic J. Seidler, Theodore A. Slotkin**

Department of Pharmacology & Cancer Biology, Duke University Medical Center, Durham, NC 27710, USA

ARTICLE INFO

Article history:
Accepted 8 March 2010
Available online 16 March 2010

Keywords:
Adenylyl cyclase
Chlorpyrifos
Diazinon
Organophosphate insecticide
Parathion

ABSTRACT

Early-life organophosphate (OP) exposures elicit neurobehavioral deficits through mechanisms other than inhibiting cholinesterase. Cell signaling cascades are postulated as critical noncholinesterase targets that mediate both the initial alterations in neurodevelopment as well as subsequent abnormalities of synaptic function. We exposed PC12 cells to chlorpyrifos, diazinon or parathion in the undifferentiated state and during neurodifferentiation; we then assessed the function of the adenylyl cyclase (AC) signaling cascade, measuring basal AC activity as well as responses to stimulants acting at G-proteins or on the AC molecule itself. In undifferentiated cells, a 2 day exposure to the OPs had no significant effect on AC signaling but the same treatment in differentiating cells produced deficits in all AC measures when exposure commenced at the initiation of differentiation. However, when exposure of the differentiating cells was continued for 6 days, AC activities then became supranormal. The same increase was obtained if cells were exposed only for the first two days of differentiation, followed by four subsequent days without the OPs. Furthermore, the OP effects on cell signaling were entirely distinct from those on indices of cell number and neurite outgrowth. These results indicate that OP exposure reprograms the AC pathway during a discrete developmental stage at the commencement of neurodifferentiation, with effects that continue to emerge after OP exposure is discontinued. Importantly, the same sequence is seen with OP exposures in neonatal rats, indicating that direct effects of these agents to reprogram cell signaling provide a major mechanism for functional effects unrelated to cholinesterase inhibition.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

The systemic toxicity of organophosphate pesticides (OPs) reflects their ability to inhibit cholinesterase (Mileson et al., 1998; Pope, 1999), leading to accumulation of acetylcholine and associated signs of excessive cholinergic stimulation. Nevertheless, it is increasingly clear that the developmental neurotoxicity of these agents involves mechanisms unrelated to cholinesterase inhibition (Casida and Quistad, 2004; Colborn, 2006; Gupta, 2004; Perera et al., 2005; Slotkin, 2005). Signal transduction cascades that regulate cell replication, differentiation and function are among the most sensitive targets for noncholinesterase actions of OPs and in particular, these agents affect the synthesis and utilization of the second

* *Corresponding author.* Box 3813 DUMC, Duke University Medical Center, Durham, NC 27710, USA. Fax: +1 919 684 8922.
E-mail address: t.slotkin@duke.edu (T.A. Slotkin).

Abbreviations: AC, adenylyl cyclase; ANOVA, analysis of variance; CPF, chlorpyrifos; DZN, diazinon; PRT, parathion; NGF, nerve growth factor; OP, organophosphate

0006-8993/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2010.03.025

messenger, cyclic AMP (Schuh et al., 2002; Slotkin, 2005; Wardle et al., 2008; Yanai et al., 2002, 2004). We recently showed how early-life exposures to chlorpyrifos (CPF), diazinon (DZN) or parathion (PRT) all evoke lasting effects on components of the adenylyl cyclase (AC) cascade, the pathway that transduces signals from the vast array of G-protein coupled receptors to the generation of cyclic AMP (Adigun et al., 2010a; Meyer et al., 2004a,b; Song et al., 1997). Notably, these effects extend outside the central nervous system; indeed, neonatal OP exposures produce subsequent gain-of-function of hepatic AC signaling that contributes to the emergence of metabolic dysregulation akin to prediabetes (Adigun et al., 2010b; Auman et al., 2000; Lassiter et al., 2010; Meyer et al., 2004b; Slotkin et al., 2005).

These findings raise the possibility that, during a critical developmental period, OP exposures directly reprogram the functioning of the AC signaling pathway, a hypothesis that would be difficult to evaluate in vivo, given the myriad systemic changes elicited when these agents are given to animals. In the current study, we used an in vitro model to examine the effects of different OPs on AC signaling under conditions spanning different developmental stages from cell replication through early and later stages of differentiation. We had three specific objectives: first, to determine if OPs affect AC signaling during a discrete stage of cell development; second, to establish whether the effects persist so long as OP exposure continues or rather whether effects emerge beyond the exposure period; and third, to evaluate whether the effects on AC signaling are separable from effects on general aspects of cell growth. We conducted our evaluations in PC12 cells, a well-characterized neurodevelopmental model (Teng and Greene, 1994) that reproduces many of the key mechanisms and features of the adverse effects of OPs in vivo (Bagchi et al., 1995, 1996; Crumpton et al., 2000a,b; Das and Barone, 1999; Flaskos et al., 1994; Jameson et al., 2006a; Li and Casida, 1998; Nagata et al., 1997; Qiao et al., 2001, 2005; Slotkin, 1999, 2004, 2005; Song et al., 1998; Tuler et al., 1989; Yanai et al., 2002). When nerve growth factor (NGF) is introduced, PC12 cells exit the mitotic cycle and undergo neurodifferentiation (Fujita et al., 1989; Song et al., 1998; Teng and Greene, 1994). Here, we used these features to examine the effects of CPF, DZN and PRT on AC signaling in the undifferentiated state, at the initiation of differentiation, and after a more prolonged period of differentiation. Our AC assessments focused on measures that evaluate pathway function at sequential steps: basal enzymatic activity, the response to global stimulation of G-proteins by fluoride, and the responses to two direct AC stimulants, forskolin and Mn^{2+}. Because the two stimulants act at different epitopes on the AC molecule, the preferential effects for one versus the other define shifts in the expression and catalytic activities of different AC isoforms (Auman et al., 2000; Zeiders et al., 1997, 1999a). We then compared the effects on AC signaling to those on cell growth, focusing on measures of cell number and neurite formation. Each neural cell contains a single nucleus, so that measuring the DNA content evaluates the number of cells (Winick and Noble, 1965), whereas the expansion of the membrane surface area that accompanies the formation of neurites during neurodifferentiation leads to an increase in the membrane protein/DNA ratio (Abreu-Villaça et al., 2005; Jameson et al., 2006b; Slotkin et al., 2007b; Song et al., 1998).

2. Results

In control cells, NGF treatment elicited the expected switch from cell replication to neurodifferentiation, as evidenced by significantly lower numbers of cells (Fig. 1A) and greater membrane surface area (Fig. 1B) as compared to undifferentiated cells cultured for the same amount of time. NGF also elicited a significant overall reduction in AC activities relative to membrane protein ($p<0.0001$ for the main effect of NGF), with selective effects on the responses to the various AC stimulants ($p<0.0001$ for the interaction of NGF × stimulant measure). For basal activity (Fig. 1C), the net reduction was completed within 2 days of NGF treatment and showed no further reduction by the 6 day time point. In contrast, the response to fluoride showed a progressive loss over time (Fig. 1D). The decline in responses to forskolin (Fig. 1E) and Mn^{2+} (Fig. 1F) resembled that of basal activity, with a complete effect evident after 2 days of NGF; furthermore, with the onset of differentiation, there was a small but significant decline in the Mn^{2+}/forskolin response ratio (Fig. 1G). The patterns for basal AC and the forskolin and Mn^{2+}-mediated responses were thus entirely distinct from the changes in cell number and membrane protein/DNA ratio, which showed distinct progression between 2 and 6 days of NGF exposure. Differentiation also altered the relative response to each of the stimulants. For fluoride, the increase over basal activity was 8-fold in undifferentiated cells, rising to 13-fold after NGF treatment; for forskolin, the increase was from 25-fold to nearly 40-fold, and for Mn^{2+} the values were 12-fold and 18-fold, respectively.

In undifferentiated cells, a 2 day exposure to CPF or PRT had little or no effect on DNA content but DZN produced a significant, albeit small, decrement (Fig. 2A). In differentiating cells, the 2 day OP treatment had no discernible effect on DNA but extending the exposure to 6 days produced a significant decline with all three agents. In contrast, when OP exposure was limited to the first 2 days of differentiation, followed by a 4 day recovery period, there were no DNA deficits. For the membrane protein/DNA ratio, undifferentiated cells showed no significant effects after a 2 day OP exposure (Fig. 2B). Differentiating cells showed a trend toward increases at 2 days that became statistically significant for CPF and PRT after 6 days. Again, limiting the exposure of differentiating cells to the first 2 days, followed by a 4 day recovery period, completely obtunded the increases and instead, there was a slight but significant decline seen for DZN.

Exposure of undifferentiated cells to the three OPs for a period of 2 days did not have any statistically significant overall effects on AC signaling parameters (Fig. 3A) but in differentiating cells there was a robust suppression of activity regardless of stimulant condition, an effect that was statistically significant overall as well as individually for CPF, DZN and PRT (Fig. 3B). With continued exposure of differentiating cells for 6 days, there was a complete reversal of the inhibitory effect of the OPs on AC signaling parameters (Fig. 3C). Indeed, the response to forskolin became significantly elevated for all three agents, and similar but nonsignificant trends were present for fluoride and Mn^{2+}; the nonsignificant increases for the latter two were statistically indistinguishable from the significant increase in the forskolin response. Importantly, the

Download English Version:

https://daneshyari.com/en/article/4327094

Download Persian Version:

https://daneshyari.com/article/4327094

Daneshyari.com