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Research Report

Enhanced expression of hypothalamic nitric oxide synthase in rats developmentally exposed to organophosphates

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ABSTRACT

Nitric oxide synthase (NOS) is highly expressed in the hypothalamus, and nitric oxide (NO) specifically contributes to the regulation of neuronal activity within distinct hypothalamic regions. We studied the long-lasting effects of developmental exposure to low doses of organophosphate chlorpyrifos (CPF) and diazinon (DZN) on the expression of NOS in the hypothalamic subnuclei that subserve neuroendocrine, autonomic and cognitive functions. A daily dose of 1 mg/kg of either CPF or DZN was administered to developing rats during gestational days 15–18 or postnatal days (PND) 1–4. Brain sections from PND 60 rats were processed using NADPH-diaphorase (NADPH-d) and neuronal NOS (nNOS) immunohistochemistry. The number of labeled neurons and the optical density (OD) were assessed in the supraoptic (SON), paraventricular (PVN), medial septum, vertical limb, and horizontal limb of the diagonal band. Developmental exposure to organophosphates increased the number of labeled neurons and OD in different subnuclei in the hypothalamus without gender selectivity. The effect on OD was more pronounced and was significant for more cases. Prenatal exposure to CPF and DZN significantly increased the OD in all regions studied with the exception of PVN. Neonatal exposure to DZN also consistently increased OD in all studied subnuclei. For rats that treated with CPF during early postnatal period, this effect was statistically significant only for the SON and PVN. These findings suggest that overexpression of NOS in the hypothalamus may contribute to the mechanisms inducing or compensating for endocrine, autonomic and cognitive abnormalities after developmental exposure to organophosphates.

Abbreviations: AVP, arginine vasopressin; CPF, chlorpyrifos; DMSO, dimethylsulfoxide; DZN, diazinon; GD, gestational day; HDB, horizontal limb of diagonal band; MS, medial septum; NADPH-d, nicotinamide adenine dinucleotide phosphate-diaphorase; NO, nitric oxide; NOS, nitric oxide synthase; nNOS-IR, neuronal NOS-immunoreactive; OD, optical density; OP, organophosphate; OT, oxytocin; PND, postnatal day; PVN, paraventricular nucleus; SON, supraoptic nucleus; VDB, horizontal limb of diagonal band

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1. Introduction

The hypothalamus is a critical site of integration for autonomic and neuroendocrine responses and indirectly plays a key role in the modulation of cognitive performance. The diversity of neurotransmitters and neuromodulators in hypothalamic nuclei reflects the complex interplay of chemical signals that balance their functions (Affleck et al., 2012; Li et al., 2001, 2003; Martins-Pinge et al., 2013). Evidence of the functional significance of nitric oxide (NO) and nitric oxide synthase (NOS) expression indicates that the hypothalamus is a locus for the central actions of NO (Liu et al., 1997; Stern, 2004; Ueta et al., 1995a). NOS-expressing neurons are abundant in the supraoptic nucleus (SON) and paraventricular nucleus (PVN) (Huang et al., 2007; Vincent and Kimura, 1992). The PVN and SON contain the most of arginine vasopressin (AVP) and oxytocin (OT) secreting neurons, and interestingly, NOS is co-expressed with these neuropeptides (Villar et al., 1994; Yamada et al., 1996). These nuclei are central to neuroendocrine and autonomic functions, especially by the release of AVP and OT into the pituitary and through PVN projection to the brain stem and spinal cord. This contributes to the physiological control of fluid balance, cardiovascular function, parturition and lactation (Stern, 2004).

A number of projecting cholinergic neurons that have been implicated in the modulation of memory and arousal are located within hypothalamus. These cell groups do not respect traditional nuclear boundaries and represent an intermixture with other non-cholinergic neurons. They are generally classified according to their association with the medial septal nucleus (MS), vertical nucleus of the diagonal band (VDB), and horizontal limb of the diagonal band nucleus (HDB). Many cholinergic neurons localized in the MS and VDB project to the hippocampus and express NOS. Furthermore, scattered NOS positive neurons are present in the HDB, the source of cholinergic projection for the olfactory bulb (Sugaya and McKinney, 1994). These cholinergic projections provide a substrate for cognitive processes, particularly those involved in attention, learning and memory (Ma and Luo, 2012; Craig et al., 2011). It has been shown that NO contributes to the control of the basal and modulated release of neurotransmitters and neurohormones acting in the hypothalamus (Affleck et al., 2012; Martins-Pinge et al., 2013; Prast and Philippu, 1992; Stern, 2004). Expectedly, many physiological and pathological conditions that alter the function of the hypothalamus also change the expression of NOS in particular hypothalamic areas (Gadek-Michalska et al., 2012; Heesch et al., 2009; Ueta et al., 1995a, 1995b; Whitaker and Molina, 2013).

Organophosphate (OP) insecticides are conventional acetylcholinesterase inhibitors but can induce several long lasting neurochemical, endocrine and cardiovascular alterations through mechanisms unrelated to their primary action (ElMazoudy and Attia, 2012; Meyer et al., 2004; Tait et al., 2009). Late-arising behavioral effects induced by developmental exposure to chlorpyrifos (CPF), diazinon (DZN), and other OPs are attributed to the alteration of different neurotransmitter systems (Levin et al., 2001, 2002; Slotkin et al., 2008; Timofeeva et al., 2008). The expression of nNOS can be detected directly by immunohistochemistry using specific antibodies against nNOS or indirectly using the nicotinamide adenosine dinucleotide phosphate-diaphorase (NADPH-d) method, which reliably stains nNOS expressing neurons in many brain structures, including the hypothalamus (Bredt et al., 1990; Valtschanoff et al., 1993). Both methods were previously employed in our published studies on the effects CPF and DZN on NOS-expressing neurons in forebrain regions that subserve memory and cognition (Naseh et al., 2013; Vatanparast et al., 2013). It was noted at the time that developmental exposure to these OPs apparently increases the level of staining in some hypothalamic regions. Aside from the detrimental effects on cognitive function, OP exposure is known to modulate the function of the hypothalamo-neurohypophysial system (ElMazoudy and Attia, 2012; Tait et al., 2009). Hypothalamic neurons are under the control of several neurotransmitters; of these, NO has been a focus of attention because of its relative importance. Because of the lasting effects on neuroendocrine, cardiovascular and cognitive function after developmental exposure to CPF and DZN, the present study tested the hypothesis that NOS expression is affected in the SON, PVN and MS/DB hypothalamic regions that contribute to these functions.

2. Results

Although the reactivity to NADPH-d and nNOS immunostaining was detected within all studied areas of hypothalamus, the distribution of labeled neurons and the labeling intensity of neurons and neuropil showed considerable regional variability (Fig. 1). In the studied regions within the hypothalamus of the control animals, the highest density of NADPH-d$^+$/nNOS-immunoreactive (IR) neurons was detected in the SON that also showed the most prominent staining (Fig. 1C and Fig. 2A). While staining of NADPH-d$^+$/nNOS-IR neurons and the neuropil in the PVN was weaker, it was sufficient for distinguishing this nucleus from the surrounding region (Fig. 1C and Fig. 4D). The NADPH-d$^+$/nNOS-IR neurons in the MS/DB region were more widely scattered and the lowest density was found in the MS. In MS and VDB, the NADPH-d$^+$/nNOS-IR neurons were almost evenly scattered within the region; on the other hand, in the HDB, the labeled neurons were mainly clustered along the ventral border of the nucleus (Fig. 1D, Fig. 2D and Fig. 4G).

For both neuronal count and optical density (OD), the initial comparison conducted by global analysis of variance showed that the main effect of subregions is significant ($p<0.001$). On the other hand, neither treatment × sex interaction nor the main effect of sex was significant. Accordingly, the effect of treatments was analyzed separately for each subregion and data from male and female rats were combined. Prenatal exposure to CPF significantly increased the number of NADPH-d$^+$/NOS-IR neurons in the SON and the VDB of adult rats. In contrast, prenatal exposure to DZN did not significantly affect the number of labeled neurons in any of the studied regions (Fig. 3A). The effect of prenatal exposure to CPF and DZN on the OD was more evident than on the neural count, as both of OPs significantly increased OD in all studied regions with exception to the PVN (Fig. 2 and Fig. 3B). Neonatal exposure to CPF significantly increased the