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Repeated exposure to the herbicide atrazine alters locomotor activity and the nigrostriatal dopaminergic system of the albino rat

Verónica M. Rodríguez [a,*], Jorge H. Limón-Pacheco [a], Maria Soledad Mendoza-Trejo [a], Adriana González-Gallardo [b], Isela Hernández-Plata [a], Magda Giordano [a]

[a] Departamento de Neurobiología Conductual y Cognitiva, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Boulevard Juriquilla 3001, Querétaro, Querétaro 76230, Mexico
[b] Unidad de Proteogenómica, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Boulevard Juriquilla 3001, Querétaro, Querétaro 76230, Mexico

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ABSTRACT

Atrazine (ATR) is used as a pre- and post-emergent herbicide; although banned in several countries of the European Community, it is still used extensively around the world. A recent study in rats has shown that chronic, daily exposure to 10 mg ATR/kg BW causes hyperactivity, disrupts motor coordination and learning of behavioral tasks, and decreases dopamine levels in the brain. In order to evaluate the short-term effect of ATR exposure on locomotor activity, monoamine markers, and antioxidants, adult male Sprague-Dawley rats received six IP injections of 100 mg ATR/kg BW or vehicle over two weeks. After every ATR injection we found hypoactivity that lasted up to five days, and it was accompanied by reductions in levels of striatal DA, DOPAC, and HVA without any alteration in the striatal expression of the mRNAs for *Mn-SOD*, *Trx-1*, *DAR-D$_1$*, or *DAR-D$_2$*. In contrast, in the nucleus accumbens no changes in monoamine markers were observed, and a down-regulation of *Trx-1* expression was detected shortly after the ATR treatment. Moreover, in the ventral midbrain, we found that ATR induced a down-regulation of mRNA for *Th* and *DAT*, but it increased *VMAT2* mRNA expression. Decreases of monoamine levels and of locomotor activity disappeared three months after ATR treatment; however, an amphetamine challenge (1 mg/kg) given two months after the ATR treatment resulted in a significant stimulation in the exposed group, revealing hidden effects of ATR on dopaminergic systems. These results indicate that ATR exposure differentially modifies the dopaminergic systems, and these modifications may underlie the behavioral changes observed.

1. Introduction

Atrazine (ATR, 2-chloro-4-(ethylamine)-6-(isopropylamine)-*s*-triazine) belongs to the chlorotriazine herbicide group. ATR is heavily used, mainly to control broad-leaf and grassy weeds in farmland, industrial sites, and recreational areas, and it is consistently found in surface waters (rivers and lakes) (Konstantinou et al., 2006). In addition ATR can be found in soil even decades after its application, as recently reported (Jablonowski et al., 2011).

This herbicide is a well-known endocrine disruptor; there are studies showing that ATR alters reproductive systems in amphibians (Fan et al., 2007; Hayes, 2009; Hayes et al., 2010) and rodents (Abarikwu et al., 2010; Juliani et al., 2008), and these alterations have been associated with disruptions of the neuroendocrine axis (Fraites et al., 2009). These characteristics suggest that ATR can be considered a risk factor for the health of humans and wildlife.

Human ATR exposure has been reported by several authors based mainly on the presence of ATR metabolites in urine (Adgate et al., 2001; Bakke et al., 2009; Clayton et al., 2003; Curwin et al., 2007); such herbicide exposure has not been evaluated in detail, and few studies have reported dietary or occupational exposure to ATR. In this regard, Gammon et al. (2005) estimated 0.234–0.857 μg/kg/day and 0.046–0.286 μg/kg/day for acute and chronic ATR dietary exposure, respectively, when considering all commodities with United States Environmental Protection Agency tolerances in drinking water. Catenacci et al. (1993) reported that ATR manufacturing workers were exposed to a total of 10–700 μmol (~2.157–151.004 mg) per work shift, while Gammon et al. (2005) calculated that cornfield workers (mixer-loader-tender applicators) could be exposed to as much as 2.8 mg ATR/day of work with an absorbed dose of 1.8–6.1 μg/kg/day based on a dermal absorption rate of 5.6%. Because of the paucity of information about ATR exposure in human populations, it remains

* Corresponding author at: Departamento de Neurobiología Conductual y Cognitiva, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Boulevard Juriquilla 3001, Querétaro, Querétaro 76230, Mexico.
Tel.: +52 442 238 1061; fax: +52 442 238 1046.
E-mail addresses: vermire@yahoo.com, vmrodriguez@comunidad.unam.mx (V.M. Rodríguez).

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to be determined if results obtained from animal models of ATR toxicity are relevant for evaluating the impact on human health of occupational exposure to environmental ATR.

Although several studies have tested the endocrine disruptive effects of ATR exposure, few have examined its neurotoxic effects. ATR crosses the blood brain barrier and enters the brain through still unknown mechanisms, as confirmed by a study in the lactating dam and suckling neonate following an acute exposure to either 2 or 4 mg/kg ^{14}C-atrazine (^{14}C-ATR) by gavage. ^{14}C-ATR residues levels were measured in the brain of the perfused dams and rat pups, and both concentrations resulted in comparable levels of ^{14}C-ATR residues in the anterior and posterior hypothalamus and striatum of the dams, and lower levels in the pups (Stoker and Cooper, 2007). A study using mice showed that 4 h after dosed with 5–250 mg ATR/kg, ATR levels in the brain reached 0.06–1.5 μM. Levels dropped below 0.1 μM after 12 h in all groups (Ross et al., 2009). The importance of evaluating the effects of ATR on the nervous system became evident after recent studies in rodents treated with ATR showed changes in locomotor activity (Bardullas et al., 2011; Belloni et al., 2011; Ugazio et al., 1991), learning (Bardullas et al., 2011; Belloni et al., 2011), and in dopaminergic systems (Bardullas et al., 2011, Coban and Filipov, 2007).

Regarding ATR effects on locomotor activity, weanling male Sprague-Dawley rats exposed to 10 mg ATR/kg BW for one year (Bardullas et al., 2011) showed hyperactivity in the horizontal plane. Interestingly, these authors (Bardullas et al., 2011) found an association between horizontal hyperactivity and decreases in striatal dopamine after ATR exposure. In another study, when a high dose (1000 mg ATR/kg BW) was given for four to eleven days, rats showed hypoactivity in the vertical plane (rearing) when evaluated in the open field (Ugazio et al., 1991). Recently, it was reported that female and male mice delivered from CD1 dams and exposed daily to 1 μg ATR/kg BW from gestational day 14 (GD 14) to postnatal day 21 (PND 21) exhibited increased exploratory behavior at PND 16 (Belloni et al., 2011).

The dopaminergic nigrostriatal system of rodents has been shown to be a target of chronic or repeated ATR exposure. In this regard, reductions in striatal DA and in tyrosine hydroxylase-positive (TH+) cells in the substantia nigra pars compacta (SNpc) and the ventral tegmental area (VTA) were found in mice orally exposed to doses ranging from 5 to 250 mg ATR/kg/day over 14 days (Coban and Filipov, 2007). A more recent study also found decreases in striatal DA content after one year of exposure to 10 mg ATR/kg BW (Bardullas et al., 2011). PC12 cells (a neuronal cell line derived from pheochromocytoma that synthesizes DA and norepinephrine (NE) *in vitro*) showed a significant, concentration-dependent decrease in intracellular DA after incubation with 0, 12.5, 25, 50, 100, and 200 μM ATR (Das et al., 2000). Similarly, dose-dependent reductions in total DA and increases in DA turnover have been observed in striatal tissue slices incubated with up to 500 μM ATR; a reduction in the levels of DA released in response to a high potassium stimulus was also found, suggesting that ATR may interfere with vesicular storage and cellular uptake of DA (Filipov et al., 2007). Others have shown that incubation with 1–250 μM ATR caused dose-dependent inhibition of DA uptake into striatal synaptic vesicles due to a significant drop in the Vmax of the vesicular transporter, but with no alterations of its Km. These alterations led to an increase in the synaptosomal/vesicular dopamine transporter/vesicular monoamine transporter 2 (DAT/VMAT-2) uptake ratio (Hossain and Filipov, 2008). Based on these results, the authors proposed that ATR decreases striatal DA levels, in part by increasing cytosolic DA in the presynaptic terminal.

Oxidative stress (OS) has been implicated in ATR toxicity through the evaluation of specific biomarkers (*i.e.*, antioxidant content, expression and activity of antioxidant-synthesizing enzymes, and lipid peroxidation) in tissues such as liver, erythrocytes, testis, and epididymis in the rat (Abarikwu et al., 2010; Adesiyan et al., 2011; Singh et al., 2011). In the nervous system, ATR forms adducts with proteins involved in metabolism, the cytoskeleton, and apoptosis, and chaperones, antioxidant proteins such as Cu-Zn superoxide dismutase, and the peroxiredoxins in the cortex, preoptic area, and medial basal hypothalamus (Dooley et al., 2010), leaving these brain structures vulnerable to oxidative stress damage.

Studies of ATR effects on gene expression in the brain are still scarce, and additional biomarkers are needed to understand the ATR effects on neurotransmitter systems at different organizational levels of the central nervous system.

In the present study, we examined the changes of the rat nigrostriatal and mesolimbic dopaminergic systems after repeated exposure to 100 mg ATR/kg BW administered over two weeks by evaluating locomotor activity, monoaminergic neurochemistry, and expression of mRNA for the antioxidants mitochondrial superoxide dismutase (*Mn-SOD*) and cytosolic thioredoxin (*Trx-1*). In order to understand the possible targets of ATR toxicity in the dopaminergic systems, we also evaluated TH protein levels and mRNA expression for the dopamine receptor (*DAR*) family members D_1 and D_2 in the striatum and nucleus accumbens, and mRNA expression of *Th*, *DAT*, and *VMAT2* in the ventral midbrain.

2. Materials and methods

2.1. Animals

Forty-four, two-month-old male Sprague-Dawley rats were acquired from the vivarium of the Instituto de Neurobiologia-UNAM and kept under a 12-h inverted dark/light cycle (lights on at 20:00) at constant temperature (23 ± 3 °C). Experiments were carried out according to the Norma Oficial Mexicana de la Secretaría de Agricultura (SAGARPA NOM-062-ZOO-1999), which complies with the guidelines in the Institutional Animal Care and Use Committee Guidebook (NIH Publication 80-23, Bethesda, MD, USA, 1996) and were approved by the local Committee on Bioethics.

2.2. Chemicals

ATR was obtained from Chem Service (West Chester, PA, USA). Methylcellulose and reagents for high performance liquid chromatography with electrochemical detection (HPLC-ED) were acquired from Sigma-Aldrich (St. Louis, MO, USA), and reagents for Western blot were obtained from BioRad (Hercules, CA, USA) unless otherwise stated.

2.3. Experimental design

Once the animals reached 300 g of body weight, or after a one-week habituation period, whichever occurred first, they were divided into four groups. Two groups (n = 8 each) received six IP injections of 1% methylcellulose (VEH), whereas the other two groups (n = 8 each) received six IP injections of 100 mg ATR/kg BW (ATR) over a two-week period (three injections per week, at 48-h intervals); 48 h after the last ATR or VEH administration, rats received an IP saline injection. Administration of atrazine was performed in accordance with "Health Effects Test Guidelines OPPTS 870.6200 Neurotoxicity Screening", available at the website (http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0156-0041). Locomotor activity was evaluated 15 min before and for 2 h immediately after each injection of vehicle, ATR, or saline. Once treatments ended, groups were used for two separate experiments as shown in Fig. 1.