NeuroToxicology 58 (2017) 161–170

Contents lists available at ScienceDirect

NeuroToxicology

Full Length Article

Behavioral effects and neuroanatomical targets of acute atrazine exposure in the male Sprague-Dawley rat

V.M. Rodriguez, M.S. Mendoza-Trejo, I. Hernandez-Plata, M. Giordano*

Departamento de Neurobiología Conductual y Cognitiva, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Boulevard Juriquilla 3001, Juriquilla, Qro, 76230, Mexico

ARTICLE INFO

Article history:
Received 16 June 2016
Received in revised form 17 December 2016
Accepted 19 December 2016
Available online 21 December 2016

Keywords:
Dopamine
c-Fos
GABA
Glutamate
Locomotor activity
Herbicides

ABSTRACT

Atrazine (ATR) is an herbicide broadly used in the world to control weeds in corn and sorghum fields, and it is potentially toxic for the dopaminergic system. Alterations in dopaminergic markers after ATR administration in rats and C57BL/6 mice have been reported. Behaviorally, it has been observed that ATR exposure causes hypoactivity shortly after its administration. To understand how acute ATR administration induces hypoactivity, we set out to map the brain areas responsive to ATR using c-Fos as a marker of neuronal activity, and tyrosine hydroxylase (TH) as a marker of dopaminergic neurons. The levels of glutamate and gamma-aminobutyric acid (GABA) were measured using high performance liquid chromatography, and spontaneous locomotor activity was evaluated as well. Male Sprague-Dawley rats received a systemic injection of 1% methyl cellulose (vehicle) or 100 mg ATR/kg body weight to evaluate locomotor activity immediately after injection, c-Fos and TH immunohistochemistry in forebrain, midbrain and hindbrain, or glutamate and GABA content in various brain areas 90 min after injection. To assess the possible involvement of the GABAergic system on ATR effects we tested the effects of a GABA-B antagonist. We found statistically significant decreases in locomotor activity, which were partially reversed by the GABA-B antagonist, and increases in the number of c-Fos-positive cells in thalamus, central amygdala, subthalamic nucleus, superior colliculus, and substantia nigra, TH positive cells were not selectively activated by ATR. The acute administration of ATR did not affect GABA or glutamate tissue levels but significantly decreased locomotor activity. These results corroborate the hypoactivity-inducing effect of ATR, and show that non-dopaminergic cells respond to the acute administration of ATR. The activation of cell populations in the basal ganglia and their target nuclei may contribute to the acute behavioral effects of ATR.

1. Introduction

Atrazine (6-chloro-N2-ethyl-N4-isopropyl-1,3,5-triazine-2,4-diamine; ATR) belongs to the group of triazinic organochloride herbicides, it is persistent, and its solubility is 35 mg/L in water at 25 °C (Hansen et al., 2013), exposure can be dietary or occupational (Gammon et al., 2005; Catenacci et al., 1993; Vonberg et al., 2014). It has been among the most heavily used pesticides in the world, and was finally banned in the European Union in 2004 (Jablonowski et al., 2010), and in Mexico it has been estimated that the herbicide is applied on 59% of the irrigated districts of the country at 0.1 to 4 kg/hectare/year, mainly on corn, sugarcane and sorghum crops (Villada-Canela, 2006). The evidence of its activity as an endocrine disruptor at environmentally relevant concentrations is cause for serious concern, since it can persist in water and soil for decades (Vonberg et al., 2014). Currently, the maximum allowable level for ATR in drinking water is 0.1 μg/L (European Union) (Jablonowski et al., 2010). The precautionary value without toxicological effects for humans is 2 μg/L according to the World Health Organization (World Health Organization, 2003) or 3 μg/L (United States Environmental Protection Agency, USEPA) (Jablonowski et al., 2010). In an Italian study of human exposure to ATR during its industrial production, exposure was estimated to vary between 28 and 356 μmol per work shift (Catenacci et al., 1993), and dietary exposure has been estimated to be between 0.046 and 0.857 μg/kg/day, depending on type of exposure (Gammon et al., 2005).

* Corresponding author.
E-mail address: giordano@unam.mx (M. Giordano).

http://dx.doi.org/10.1016/j.neuro.2016.12.006
0161-813X/

In addition to its potential as a neuroendocrine disruptor, previous studies from our group and others using in vivo and in vitro models have shown that ATR has significant effects on the dopaminergic system (Rodriguez et al., 2005, 2007; Bardullas et al., 2013). Acute effects of ATR include decreases in dopamine (DA) release in the striatum that can be reversed by haloperidol (Rodriguez et al., 2005), decreased levels of DA and its metabolites in the striatum of Sprague-Dawley rats after repeated exposure to 100 mg/kg ATR (Rodríguez et al., 2013), and loss of dopaminergic cells in C57BL/6 juvenile male mice (Coban and Filipov, 2007). Fraites et al. (Fraites et al., 2009) suggested that the effects of ATR on the hypothalamic pituitary adrenal (HPA) axis could be mediated by alterations in central catecholaminergic systems, and Foradori et al. (Foradori et al., 2013) suggested that the inhibition of gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) release may be mediated by endogenous opioid neurons in the arcuate nucleus. Alternatively, alteration of gamma-aminobutyric acid (GABA) A receptor activity has been suggested to contribute to the disruption of hypothalamic release of GnRH in female rats (Shafer et al., 1999). These authors found that ATR, at concentrations of 30 μM or greater, decreases binding to rat cortical membranes of Ro15-4513, a imidazobenzodiazepinone derivative that has the activity profile of a partial inverse (low efficacy) agonist at the benzodiazepine receptor (Wallner et al., 2014). ATR may also affect more generalized targets such as phosphodiesterases (PDEs) and cyclic adenosine monophosphate (cAMP) (Roberge et al., 2004).

We set out to describe c-Fos expression in the male rat brain, including in tyrosine hydroxylase (TH)-positive cells, after one administration of ATR [100 mg/kg body weight (BW), i.p]. We also recorded changes in locomotor activity and the effect of a GABA-B antagonist on the hypoactivity caused by ATR, and measured glutamate and GABA levels in the areas with the greatest c-Fos expression. c-Fos immunohistochemistry has been used previously in studies of how the brain is affected by pesticides, including the organophosphates chlorpyrifos, (Carvajal, 2005), paraoxon (Charoenying et al., 2011), methyl parathion (Betancourt et al., 2007), and deltamethrin, a synthetic pyrethroid (Wu and Liu, 2003). c-Fos is a transcription factor encoded by *c-fos,* one of the so-called cellular immediate-early genes (IEG), and is induced by both cAMP and Ca2+ signaling (Okuno, 2011). IEG expression shows a strong correlation with recent neuronal activity in the brain, thus, it provides information regarding where and when neurons were activated (Okuno, 2011) and can be used to map potential targets of acute ATR exposure.

Our previous results showed that ATR induces sustained hypoactivity (Rodríguez et al., 2013). In this study we stopped recording at 90 min, during that time animals treated with ATR never returned to control levels of activity. This result can be due to the continued presence of ATR in brain. Indeed, in C57BL/6 mice an acute administration (oral gavage) of 5, 25, or 125 mg ATR/kg reached ~0.4 M ATR brain levels at 4 h, while 250 mg ATR/kg peaked at 1.5 M ATR brain levels 1 h after herbicide administration, ATR brain levels fell to <0.1 μM 12 h after dosing in all groups (Ross et al., 2009).

In addition, we found several brain areas that responded to ATR, but the response cannot be described as widespread. On the contrary, from eleven areas selected, only five showed significant increases in c-Fos expression after ATR administration. We selected both, dopaminergic as well as all other areas that, at first glance, showed increased expression. Double immunostaining using c-Fos and TH indicated that TH+ cells were not selectively activated by ATR administration. In particular, the substantia nigra showed increased c-Fos expression when all cell types were considered, but when only TH+ cells were considered there was no increase in c-Fos expression. These results suggest that ATR may be affecting a variety of cell populations. To evaluate the possibility that the effects of ATR could involve GABAergic receptors, we administered saclofen, a GABA-B receptor antagonist, and observed a partial reversal of ATR effects.

2. Materials and methods

2.1. Animals

One-hundred eighteen male Sprague-Dawley rats weighing between 250 and 400 g were obtained from the vivarium of the Instituto de Neurobiologia-UNAM. Rats were housed two per polycarbonate cage and maintained in a controlled environment at 23 ± 1 °C and a 12-h/12-h inverted dark/light cycle (lights on at 21:00 h) with water and food *ad libitum.* We chose to use only males to replicate previous results with ATR, and explore possible mechanisms of action. The animals were cared for and treated according to the Norma Oficial Mexicana (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 the protocol was approved by the local Committee on Research Ethics.

2.2. Chemicals

ATR was obtained from Chem Service (West Chester, PA, USA). Methyl cellulose (MC) and all other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated.

2.3. Effects of acute ATR administration on locomotor activity

Locomotor activity was evaluated using an automatic recording system in a group of eleven animals (Digiscan Animal Activity Monitors, Accuscan Inc., Columbus, OH, USA). Each animal was placed in a locomotor activity chamber for 15 min before and for 90 min immediately after the injection of vehicle (1% MC, n = 6) or ATR (100 mg ATR/kg of BW, n = 5), in a volume of 2 mL/kg. This dose was chosen based on the information available from previous microdialysis and behavioral studies. ATR was diluted in 1% MC because it is only slightly soluble in water (35 mg/L at 25 °C (Hansen et al., 2013)). Total distance (defined as the distance traveled by the rat in a given period), vertical activity (defined as total number of beam interruptions of the vertical sensor during a given period) and stereotypy counts (defined as the number of beam breaks due to repetitive movements that occurred during a determined period) were used to evaluate the effects of ATR on locomotor behavior.

2.4. Effects of acute ATR administration on c-Fos activation in the brain

Male Sprague-Dawley rats under deep anesthesia (70 mg/kg ketamine and 6 mg/kg xylazine) received an i.p. injection of 1% MC (control, n = 9) or 100 mg ATR/kg BW (n = 11), 90 min later the rats were injected with a lethal dose of pentobarbital and perfused through the left ventricle of the heart with 0.1 M phosphate-buffered saline (PBS, pH 7.4) and then with 4% paraformaldehyde (PFA). Deep anesthesia was used to avoid the effect of other stimuli on c-Fos expression. The brain was removed from the skull and post-fixed in 4% PFA overnight, immersed in a post-fixative solution (30% sucrose in 0.1 M PBS) for 7 days and then cut into 50-μm sections using a freezing microtome (Leica SM2000R, Nussloch, Germany) and placed in a cryoprotectant solution (0.025 mM polyvinylpyrrolidone-40, 30% sucrose, 30% ethylene glycol in 0.05 M Tris buffered saline, pH 7.6).