Chemosphere 181 (2017) 518–529

Contents lists available at ScienceDirect

Chemosphere

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

Amitraz changes NE, DA and 5-HT biosynthesis and metabolism mediated by alterations in estradiol content in CNS of male rats

Javier del Pino [a, *, 1], Paula Moyano [b, 1], Matilde Ruiz [b], María José Anadón [b], María Jesús Díaz [a], José Manuel García [b], Elena Labajo-González [b], María Teresa Frejo [a]

[a] Department of Toxicology and Pharmacology, Veterinary School, Complutense University of Madrid, 28040 Madrid, Spain
[b] Department of Toxicology and Legal Medicine, Medical School, Complutense University of Madrid, 28041 Madrid, Spain

HIGHLIGHTS

- Amitraz did not inhibit MAO at 20, 50 and 80 mg/kg doses.
- Amitraz altered MAO, COMT, DBH, TH and TRH expression and TH and TRH activity.
- Enzymes' expression alteration was partially mediated by estradiol levels alteration.
- Amitraz alter monoaminergic systems by estradiol levels disruption.

ARTICLE INFO

Article history:
Received 29 January 2017
Received in revised form
20 April 2017
Accepted 23 April 2017
Available online 26 April 2017

Handling Editor: A. Gies

Keywords:
Amitraz
Male rats
Neurotoxicity
Monoaminergic neurotransmitters
Estradiol

ABSTRACT

Amitraz is a formamidine insecticide/acaricide that alters different neurotransmitters levels, among other neurotoxic effects. Oral amitraz exposure (20, 50 and 80 mg/kg bw, 5 days) has been reported to increase serotonin (5-HT), norepinephrine (NE) and dopamine (DA) content and to decrease their metabolites and turnover rates in the male rat brain, particularly in the striatum, prefrontal cortex, and hippocampus. However, the mechanisms by which these alterations are produced are not completely understood. One possibility is that amitraz monoamine oxidase (MAO) inhibition could mediate these effects. Alternatively, it alters serum concentrations of sex steroids that regulate the enzymes responsible for these neurotransmitters synthesis and metabolism. Thus, alterations in sex steroids in the brain could also mediate the observed effects. To test these hypothesis regarding possible mechanisms, we treated male rats with 20, 50 and 80 mg/kg bw for 5 days and then isolated tissue from striatum, prefrontal cortex, and hippocampus. We then measured tissue levels of expression and/or activity of MAO, catechol-*O*-metyltransferase (COMT), dopamine-β-hydroxylase (DBH), tyrosine hydroxylase (TH) and tryptophan hydroxylase (TRH) as well as estradiol levels in these regions. Our results show that amitraz did not

Abbreviations: AD, aldehyde dehydrogenase; AMZ, amitraz; ARO, aromatase; AAADC, aromatic amino acid decarboxylase; CNS, central nervous system; COMT, catechol-*O*-metyltransferase; Ct, cycle threshold; DA, dopamine; DBH, dopamine-β-hydroxylase; DOPA, 3,4-dihydroxyphenylalanine; DOPAC, 3,4-hydroxyphenylacetic acid; DHT, dihydrotestosterone; E2, 17β-estradiol; FC, prefrontal cortex; HC, hippocampus; HPLC, high performance liquid chromatography; HVA, homovanillic acid; 5-HT, serotonin; 5-HTP, 5-hydroxytryptophan; 5-HIAA, 5-hydroxy-3-indolacetic acid; LD_{50}, Lethal Dose 50; LOQ, Quantification limit; MAO, monoamine oxidase; MHPG, 3-metoxy-4-hydroxyphenylethyleneglycol; NE, norepinephrine; NSD-1015, *m*-hydroxymethylhydrazine; SD, standard deviation; ST, striatum; T, testosterone; TH, tyrosine hydroxylase; TMX, tamoxifen; TRH, tryptophan hydroxylase.

* Corresponding author. Department of Toxicology and Pharmacology, Veterinary School, Universidad Complutense de Madrid, Avda. Puerta de Hierro s/n, 28040 Madrid, Spain.
E-mail address: jdelpino@pdi.ucm.es (J. del Pino).
[1] These authors have contributed equally to this work.

http://dx.doi.org/10.1016/j.chemosphere.2017.04.113

inhibit MAO activity at these doses, but altered MAO, COMT, DBH, TH and TRH gene expression, as well as TH and TRH activity and estradiol levels. The alteration of these enzymes was partially mediated by dysregulation of estradiol levels. Our present results provide new understanding of the mechanisms contributing to the harmful effects of amitraz.

1. Introduction

Amitraz (1,5 di-(2,4-dimethylphenyl)-3-methyl-1,3,5-triaza-penta-1,4-diene), a formamidine pesticide, is used worldwide on both animals and crops to control pests as an insecticide and acaricide (Yilmaz and Yildizdas, 2003). The pesticidal action of amitraz in invertebrates is related to activation of octopamine receptors (Nathanson, 1985). A cause of concern for health authorities is the number of amitraz human poisoning cases that have been attributed and are still being reported (Demirel et al., 2006; Veale et al., 2011).

Amitraz is a potent neurotoxic compound that induces signs such as loss of righting reflex, motor incoordination, appetite alteration, hyperreactivity to external stimuli, aggressiveness, among other effects. These effects are mediated in part by disruption of neurotransmitter systems (Del Pino et al., 2015). Amitraz (20, 50 and 80 mg/kg bw) increases serotonin (5-HT), norepinephrine (NE) and dopamine (DA) levels and decreases their metabolites levels and turnover rates in the central nervous system (CNS) of male rats with the striatum, prefrontal cortex, and hippocampus being the most affected (Del Pino et al., 2013). Although the mechanisms responsible for these alterations are not completely understood, amitraz inhibits monoamine oxidase (MAO), the main enzyme that metabolizes monoamine neurotransmitters (Aziz and Knowles, 1973). Therefore, amitraz could mediate the effects observed on monoaminergic neurotransmitters by blocking MAO. In this regard, Florio et al. (1993), showed that amitraz at dose of 100 mg/kg bw increased NE and DA levels in hypothalamus and striatum, respectively, and decreased the homovanillic acid (HVA) levels in striatum and attributed these effects to MAO inhibition. Moser and MacPhail (1989) reported that amitraz inhibits MAO only at doses of 100 mg/kg bw, so this mechanism could not explain the effect seen at lower doses. However, motor incoordination appears from doses lower than 100 mg/kg bw (from 6.25 to 25 mg/kg bw/day) in rats (Moser et al., 1987), and it has been reported that MAO inhibition mediates motor incoordination (Florio et al., 1993), suggesting this enzyme could mediate the monoamine neurotransmitters alteration observed at doses lower than 100 mg/kg bw.

Another explanation for amitraz-induced alterations in the NE, DA and 5-HT and its metabolites levels observed in male rats is that the pesticide may alter sex steroid hormones that regulate the activity and expression of enzymes important for neurotransmitter synthesis and metabolism. Such enzymes include aldehyde dehydrogenase (AD), catechol-*O*-metyltransferase (COMT), dopamine-β-hydroxylase (DBH), MAO, tyrosine hydroxylase (TH), and tryptophan hydroxylase (TRH), all important for neurotransmitters synthesis and metabolism (Babu and Vijayan, 1984; Chaube and Joy, 2011; Donner and Handa, 2009; De Souza Silva et al., 2009; Handa et al., 1997; Lubbers et al., 2010; Luine and Rhodes, 1983; Purves-Tyson et al., 2012; Rahman and Thomas, 2013; Scardapane and Cardinali, 1977; Schendzielorz et al., 2011; Thiblin et al., 1999). This hypothesis is reasonable considering that amitraz alters hepatic metabolism of 17β-estradiol (E2) and testosterone (T) in rats, and increases serum T in male rats at doses of 25 and 50 mg/kg bw (Chou et al., 2008).

Aromatase (ARO) or reductase enzymes can metabolize T in CNS to E2 or dihydrotestosterone (DHT) in a region-specific manner (Castelli et al., 2013; Zhao et al., 2007, 2008). Therefore, the amitraz effect on monoaminergic neurotransmitter in male rats could be mediated by changes in T or its E2 or DHT metabolites. This idea is supported by evidence that E2 regulates the expression of enzymes which form and metabolize 5-HT, DA and NE (Donner and Handa, 2009; Luine and Rhodes, 1983; Luine et al., 1973; Scardapane and Cardinali, 1977; Serova et al., 2002). In this regard, E2 has been reported to induce TRH-2 mRNA expression (Donner and Handa, 2009) and to increase levels of mRNA encoding TH, the major rate limiting enzyme in DA and NE biosynthesis (Serova et al., 2002). In addition, synthesis and metabolism of monoaminergic neurotransmitters are modulated by T and DHT hormones (Thiblin et al., 1999). These hormones increase COMT, MAO-A and MAO-B mRNAs levels and TH protein enzymes (Purves-Tyson et al., 2012). Also, DHT reduces 5-HT, DA and NE turnover rates of gonadectomized animals (Handa et al., 1997). Taking all the above into consideration, we hypothesized that amitraz effects on serotoninergic, noradrenergic and dopaminergic systems may be mediated through a combination of MAO inhibition, and/or by altering sex steroid-dependent expression and activity of the enzymes that metabolize and synthesize these neurotransmitters.

This study sets out to analyze the presented hypothesis on the importance of monoaminergic neurotransmitter dysregulation for explaining amitraz neurotoxic effects. We evaluated this hypothesis by testing dose-dependent effects of amitrz (20, 50 and 80 mg/kg bw for 5 days) in striatum, prefrontal cortex, and hippocampus from male rats. Specifically, we tested whether amitraz inhibits MAO and/or alters the expression and activity enzymes that metabolize or synthesize the monoaminergic neurotransmitters and does so by altering sex hormones in these regions.

2. Materials and methods

2.1. Chemicals

Amitraz (98%), dihydroxybenzoic acid, *S*-adenosyl-L-methionine, *m*-hydroxymethylhydrazine (NSD-1015) were obtained from Sigma (Madrid, Spain). All other chemicals were reagent grade of the highest laboratory purity available.

2.2. Animals and experimental design

European Union guidelines (2003/65/EC) and Spanish regulations (BOE 67/8509-12, 1988) regarding the use of laboratory animals were followed when performing all experiments. We used male Wistar rats, at 60 days old, each weighting 200–210 g (Charles River, Barcelona, Spain), which were individually housed and maintained in a temperature- and light-controlled room (14:10 light:dark cycle; lights on at 0500 h) with food and water available *ad libitum*. We randomly assigned 6 animals to each of the control and treatment groups. We used independent groups of controls and treatments for all different analyses performed. Animals were