Toxicology Letters 230 (2014) 408–412

Contents lists available at ScienceDirect

Toxicology Letters

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

Effect of atrazine and fenitrothion at no-observed-effect-levels (NOEL) on amphibian and mammalian corticosterone-binding-globulin (CBG)

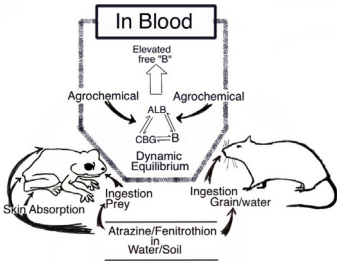
Sandra E. Hernández *, Conrad Sernia, Adrian J. Bradley

School of Biomedical Sciences, The University of Queensland, Brisbane, 4072 Queensland, Australia

HIGHLIGHTS

- The study determines NOEL atrazine/fenitrothion effect on amphibian/mammal CBG.
- Atrazine/fenitrothion compete with B for binding CBG in cane toad and rat plasma.
- Atrazine NOEL would interfere with normal B-CBG interaction in amphibian.
- Displacement of B by the agrochemicals would affect total:free B in plasma.
- Increase of free B will lead to an indirect disruption to stress response.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 17 July 2014
Received in revised form 13 August 2014
Accepted 14 August 2014
Available online 17 August 2014

Keywords:
NOEL
Atrazine
Fenitrothion
CBG
Amphibian
Rat

ABSTRACT

This study determines the effect of atrazine and fenitrothion no-observed-effect-levels (NOEL) on the binding of corticosterone (B) to corticosterone-binding-globulin (CBG) in an amphibian and a mammal. Plasma from five cane toads and five Wistar rats was exposed to atrazine and fenitrothion at the NOEL approved for Australian fresh water residues and by the World Health Organization (WHO). The concentration required to displace 50% (IC_{50}) of B binding to CBG was determined by a competitive microdialysis protein assay. Competition studies showed that both atrazine and fenitrothion at NOEL are able to compete with B for CBG binding sites in toad and rat plasma. The IC_{50} levels for atrazine in toads and rats were 0.004 nmol/l and 0.09 nmol/l respectively. In the case of fenitrothion the IC_{50} level found in toads was 0.007 nmol/l, and 0.025 nmol/l in rats. Plasma dilution curves showed parallelism with the curve of B, demonstrating that these agro-chemicals are competitively inhibiting binding to CBG. The displacement of B by atrazine and fenitrothion would affect the total:free ratio of B and consequently disrupt the normal stress response. This is the first time that the potential disruptive effect of atrazine and fenitrothion on B-CBG interaction at the NOELs has been demonstrated in amphibian and mammalian models.

1. Introduction

Organisms can be affected by environmental contaminants in inconspicuous ways that can subsequently impact on populations and ecosystems. Developmental and metabolic systems are known

* Corresponding author. Tel.: +61 7 33655178; fax: +61 7 33651299.
E-mail addresses: s.hernandez@uq.edu.au, sahm30@hotmail.com (S.E. Hernández).

http://dx.doi.org/10.1016/j.toxlet.2014.08.015
0378-4274/

to be adversely affected by both organic and inorganic contaminants. While toxicological studies of agrochemicals have been directed mostly toward their disruptive effect upon reproductive function, other physiological responses like the stress response have been overlooked. The stress response has an important role in maintaining homeostasis during times of emergency (Wingfield and Kitaysky, 2002). The organism may react to a wide variety of challenges or stressors by activating a complex of physiological regulatory networks to enable adaptation and permit survival. Among all these regulatory networks, the activation of the hypothalamic-pituitary-adrenal (HPA) axis has attracted most attention. The mediators operating on the HPA axis have been linked to important biological functions and, in particular, the glucocorticoids (GCs) cortisol (F), and corticosterone (B), are known to regulate metabolic, immunologic, and developmental functions in vertebrates (Sapolsky et al., 2000). Consequently any disruption of the HPA axis would be expected to adversely affect the biological responses to GCs (McEwen and Wingfield, 2003). In fact, there is already evidence that environmental pollutants are able to increase GCs levels in fish (Miller et al., 2009) and amphibia (Hopkins et al., 1997, 1999; Ward et al., 2007).

There are several critical points in the HPA axis where environmental contaminants can act as disruptors. Studies in fish (Gravel et al., 2005; Hontela et al., 1992; Leblond et al., 2001), rats (Yamamoto et al., 1982) and amphibia (Goulet and Hontela, 2003) have demonstrated that heavy metals and pesticides are able to disrupt the production of GCs in steroidogenic cells. Pollutants have also been reported to act as stressors via a direct effect on the HPA axis. For example, degradation products of the pesticide fenitrothion increase concentrations of adreocorticotrophic hormone (ACTH) in rats (Li et al., 2007).

Another critical point where pollutants may disrupt the HPA axis is by competition with GCs for binding to plasma globulins. Globulins work as carriers and reservoirs of hormones in plasma (Anderson, 1974; Rosner, 1991; Westphal, 1983). They are crucial in maintaining proper levels of free hormone in plasma as well as providing localized tissue delivery (Breuner and Orchinik, 2002). Several studies of sex steroid-binding globulins (SBG) have reported that environmental pollutants can compete for SBG in fish (Gale et al., 2004), green turtles (Ikonomopoulou et al., 2009), and humans (Dchaud et al., 1999). However, to our knowledge no study has reported the same effect for Corticosterone Binding Globulin (CBG).

The agrochemicals used in this study were chosen based on previous reports of their disruptive effects on the endocrine system in amphibians and mammals. For example atrazine has been reported to induce hermaphrodism and reduce testosterone in male *Xenopus laevis* (Hayes et al., 2002) but on the other hand fenitrothion can interact with androgen receptors in vitro while no conclusive effect was found in vivo (Sohoni et al., 2001). The agrochemicals were also chosen on the likelihood of their presence in the environment at concentrations that could be detrimental to these species.

Atrazine is a 2-chloro-*s*-triazine pesticide that inhibits photosynthesis in plants. It is one of the most widely used herbicides in the world, and one of the most common agrochemical contaminants found in ground and surface water bodies (Graymore et al., 2001; Murphy et al., 2006). Given its potential disruptive effect on reproductive function (Hayes et al., 2002; Stoker et al., 2000) and carcinogenic effects (McElroy et al., 2007), its use has been banned by the European Union (Sass and Colangelo, 2006). However its use is still allowed in the United States of America (U.S. Environmental Protection Agency, 2012) as well as in Australia (Agriculture and Resource Management Council of Australia and New Zealand, 2000; Australian Pesticides and Veterinary Medicines Authority, 2010).

Fenitrothion is a dialkyl-aryl phosphorothioate insecticide that has a cholinergic action and is widely used for plant and forest protection (Miyamoto, 1969; Osicka-Koprowska et al., 1987). Doses higher than 10 ppm have been associated with deformities in the tadpole neural axis (Elliott-Feeley and Armstrong, 1981; Pawar and Katdare, 1983; Pawar and Katdare, 1984), and doses higher than 500 mg/kg (LD_{50}) have been reported to be lethal in rats, mice and guinea pigs (Farmoz Pty Ltd, 2008). Its use in Australia is approved in horticultural and forage crops (Australian Pesticides and Veterinary Medicines Authority, 2012).

The objective of this study was to determine the ability of atrazine and fenitrothion to bind to CBG in plasma of cane toads and rats, and to assess the significance of this binding. When the displacement of B from CBG by agrochemicals occurs in vitro, it is reasonable to hypothesise that such disruption might also occur in vivo. The displacement of B from CBG would affect the total:free ratio of B in plasma and consequently disrupt indirectly the normal stress response in both the cane toad and rat. Finally elevation of free B in plasma will also affect other physiological responses, including reproduction and immune function (Sapolsky et al., 2000).

2. Material and methods

A total of five adult male cane toads (*Rhinella marina*) captured from The University of Queensland Lakes, St Lucia, SE Queensland and five adult male Wistar rats (*Rattus norvegicus*) sourced from The University of Queensland Biological Resources (UQBR) were selected to perform the tests.

All animals were sampled in the laboratory on the day of capture or collection from the animal house. The animals selected for blood sampling were weighed and anesthetised before blood was drawn. Blood (600 μl) was collected via cardiac puncture with heparin treated syringes and placed into Eppendorf tubes and centrifuged at 1300 × *g* for 5 min to separate plasma, which was stored at −20 °C for later analysis. All procedures were approved by The University of Queensland Animal Ethics Committee (SBMS/437/09/URG/GOVTMEX/HSF/CFOC).

2.1. Microdialysis method and data analysis

The range of concentrations for fenitrothion and atrazine used in this study was based upon the no-observed-effect level (NOEL) approved for Australian fresh water residues (Agriculture and Resource Management Council of Australia and New Zealand, 2000).

Microdialysis was used to determine the competition of atrazine and fenitrothion for CBG in cane toad and rat plasma. The technique has been described previously by Ikonomopoulou et al. (2009) in the green turtle, and is carried out by exposing the plasma to radioactive and non-radioactive ligand under equilibrium conditions and determining the percentage bound by plasma globulin.

After running trial dilutions to find the optimum dilution range, cane toad plasma ($n = 5$) and rat plasma ($n = 5$) were diluted 1:30 and 1:10 respectively in a solution of 0.2% dextran coated charcoal in PBS (0.05 M, pH 7.4). The dilute plasma was incubated for 30 min at 24 °C followed by centrifugation at 1500 × *g* for 5 min to strip endogenous hormones from the diluted plasma. Non-radioactive agrochemicals (5–0.5 ng/50 μl) and 30,000 dpm of tritiated corticosterone (Corticosterone [1,2,6,7-^{3}H] 2.59 TBq/mmol, PerkinElmer, Australia) were added to the diluted plasma (100 μl) and allowed to equilibrate in a microdialysis chamber. Samples dialysed only with non-radioactive corticosterone (B) were used as controls. All agrochemicals (analytical grade) were donated by the Queensland Health Scientific Sciences (QHSS). The stock concentrations for both agrochemicals were 1 mg/l. Equilibrium was established after incubation of dialysis cells for 24 h at 4 °C, and radioactivity was measured by removing 50 μl from the saline and plasma chambers of each microdialysis set of chambers. Radioactivity was counted in a liquid scintillation spectrometer (Beckman LS 6000 TA®).