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DISRUPTION OF THE ENZYME AROMATASE

PESTICIDES AND THE DISRUPTION OF THE ENZYME AROMATASE

J Thomas Sanderson, Associate Professor, Institut National de la Recherche Scientifique-Institute Armand-Frappier, Université du Québec, Montréal, QC, H9R 1G6, Canada tells of the importance of the aromatase enzyme, pesticide interactions with the enzyme and the possibility of endocrine disruption effects

Keywords

Society is increasingly concerned that many chemicals produced by industry for commercial use are contributing to the increased incidence of endocrine-related pathologies (such as breast, prostate and testicular cancer) and decreased male fertility observed in the Western world. In particular, concern has been focused on synthetic pesticides for the obvious reason that they are deliberately applied to crops intended for human consumption. Agricultural industry is large-scale and highly intensive by necessity to provide sufficient supply to an ever increasing population and to satisfy the demand for diversity and continuous availability by the affluent. Pesticides form an integral part of this agricultural process and their area of use has increased dramatically since the 1940s. Pesticides have also evolved over the years due to efforts of pesticide toxicologists in the agrochemical industry and pressure from society to find ever more selective, and less toxic and persistent chemicals.

Attention of the general public was first drawn to the adverse effects of pesticides by Rachel Carson’s 1962 book Silent Spring in which she described a world without birds to convey her concern about the egg shell-thinning effects of the highly persistent pesticide DDT. Interestingly, the mode of action of DDT-induced eggshell thinning is still not entirely understood, but does not appear to involve the mechanisms of endocrine disruption which are currently under such intense investigation. Renewed awareness of potential harmful effects of synthetic chemicals, including pesticides, was triggered by Theo Colburn’s 1996 book Our Stolen Future, which postulates that numerous man-made chemicals introduced into the environment are causing endocrine disruption, thus resulting in decreased fertility, learning-ability and increasing the risk of developing cancers. However, scientific evidence to support the claims made in Our Stolen Future was spurious at the time and scientific opinion remains greatly divided today, 10 years later. Although Our Stolen Future never approached the impact on society that Silent Spring had, the book did give momentum to scientific research efforts in this area of concern over the last decade.

The current definition of an endocrine disruptor is ‘an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism or its progeny or (sub)populations’. Inherent in this definition is the extremely wide-scope of potential adverse effects and underlying mechanisms of actions of endocrine disrupting chemicals that need to be considered. The daunting goal of toxicologists, pharmacologists, epidemiologists and other life science researchers is to determine whether current environmental exposures to various chemicals, including pesticides, cause endocrine disruption, and if so, how, and do they pose any form of health risk to either humans or wildlife?

Where does one start? Initially, research has focused mainly on interactions of chemicals with thyroid hormone and sex hormone receptors, particularly the estrogen receptor. Numerous pesticides have been shown to be agonists (or antagonists) for the estrogen receptor, although usually with very low affinities relative to the endogenous hormone 17-beta-estradiol. Many of these chemicals, except for certain halogenated compounds such as DDT and methoxychlor, and metabolites, do not bioaccumulate or biomagnify in the environment. The resultant biological potencies of these chemicals are invariably low and, in most situations, it appears unlikely that environmental concentrations are sufficiently high to compete effectively with estradiol and other endogenous estrogens for the receptor. It should be remembered that specific local situations should never be ruled out, particularly in agricultural settings where run-off of pesticides can result in relatively high concentrations in the immediate aquatic environment. However, other mechanisms of interference with endocrine functions are increasingly being considered, including effects on enzymes involved in steroid hormone synthesis and metabolism (Sanderson et al., 2000). Particularly the cytochrome P450 (CYP) enzymes responsible for the highly specific reactions in the steroid biosynthetic pathway are of interest, given their key role in the formation of highly potent endogenous steroid hormones. It is possible for certain chemicals to cause or contribute to hormonal disruption and subsequent reproductive and developmental toxicities by interfering with the function of key enzymes involved in steroid synthesis and breakdown. Aromatase (CYP19) is one of these key enzymes and is responsible for the conversion of androgens to estrogens in various endocrine and non-endocrine tissues. Its activity is especially high in the female ovary during the
reproductive years, but it plays an important role in bone tissue, sperm production, and is critical in brain development and sexual behaviour in males and females. In fact, neither aromatase nor estrogens should be considered strictly female-specific attributes; the enzyme is highly conserved among species and plays crucial roles in both sexes.

The catalytic activity of aromatase in vitro or ex vivo can be determined by exposing cells or tissue fractions to 14C-estrone or 14C-androstenedione. During the highly stereospecific aromatization process estrone is formed, while the tritiated label is incorporated into water, which is readily extracted and measured in a liquid scintillation counter. This tritiated water-release assay is useful for the identification of aromatase inhibitors and inducers. To obtain more mechanistic information about inducers, alterations in aromatase expression can be assessed either at the protein or mRNA levels. Protein levels can be determined by immunoblotting with highly selective antibodies raised against the protein segment of the aromatase enzyme. mRNA levels are typically determined by reverse-transcriptase-polymerase chain reaction (RT-PCR) using specific primers that uniquely recognize the coding sequence of the aromatase gene. It should be kept in mind, however, that neither protein nor mRNA levels provide information on the catalytic activity of the enzyme. Thus, whereas catalytic activity is affected by inhibitors and inducers, inhibitors may have little to no effect on mRNA or protein expression, emphasizing the importance of using reliable methodologies for catalytic assays.

Aromatase has been the subject of intensive research by pharmaceutical companies for the development of highly selective inhibitors for the treatment of estrogen-responsive breast cancer such as fadrozole and letrozole. These drugs evolved from structurally similar fungicides commonly used on fruit and vegetable crops. These so-called azole fungicides were developed to target a specific cytochrome P450 enzyme (CYP51) in yeast and fungi which is responsible for the synthesis of ergosterol, an important component of their cell membranes. However, several azole fungicides have been found to be more or less potent inhibitors of aromatase (CYP19) activity in humans, experimental animals and wildlife. Prochloraz and imazalil are particularly potent in this respect and have been shown to cause endocrine disrupting effects in laboratory rodents and fish. Prochloraz has been shown to cause demasculinization and feminization of male offspring of perinatally exposed dams. In female fish, prochloraz is known to lower estradiol levels resulting in inhibited vitellogenin production, an essential process for formation of the egg yolk. Effects were found to be similar to those of the medicinal aromatase inhibitor fadrozole. Another group of pesticides suggested to be aromatase inhibitors are the organotin compounds, which are used as anti-fouling/biocidal agents in various industrial and commercial applications. The endocrine disrupting effects of organotin compounds such as tributyl tin are well documented, particularly their ability to cause imposex (penis development in females) in molluscs at very low concentrations. However, the frequent suggestion that aromatase inhibition is the specific mechanism linking organotin exposure to imposex has so far not been supported by scientific evidence. There is an early indication that the pesticide glyphosate may inhibit aromatase activity in placental cells, although the only existing study appears to indicate that it is the formulation and not the active ingredient causing aromatase inhibition concurrent with decreased cell viability. Consequences of aromatase inhibition are numerous and include impairment of sperm maturation and demasculinization in males, and induction of menopausal symptoms, such as osteoporosis and arrest of ovulatory cycle in females.

Inducers of aromatase have also been identified although these observations have been made mostly in vitro. For example, the 2-chloro-1,3,5-triazine herbicides, which are used in large quantities on corn crops in North America, have been shown to induce aromatase activity in certain human cell lines (Sanderson et al., 2000). The triazine herbicides, atrazine, simazine and propazine, have been implicated in various estrogenic effects in humans and wildlife, and exposures have been associated with increased incidences of ovarian and breast cancer in experimental animals and in epidemiological studies in humans. However, there is only limited evidence for an interaction with the estrogen receptor. In vivo evidence of the ability of atrazine and related herbicides to induce aromatase in endocrine tissues of, for example, alligators and frogs has so far not been strong. More experimental evidence is necessary to support the hypothesis that aromatase induction may play a role in vivo to explain the estrogenic effects of certain endocrine disrupting chemicals. Nevertheless, induction of aromatase activity as a potential mode of action is of importance, as it would have far reaching consequences for the exposed organism. Potential adverse affects in wildlife would be disruption of androgen-to-estrogen balance and feminization and/or demasculinization of males particularly during the sensitive period of embryonic and perinatal development. Furthermore, over-expression of aromatase in humans is strongly associated with the occurrence of estrogen-dependent breast tumors.
Aromatase inhibition by, for example, azole fungicides has been observed both in vitro and in vivo in various species including rodents, turtles, humans and various species of bird and fish, and also in various tissues such as gonads, placenta, adrenal and brain. Apart from species differences in kinetics such as tissue distribution and rate of metabolism, the inhibition of aromatase activity by such pesticides is considered to be a general effect that can be expected to occur across species. Aromatase induction on the other hand is a more complex response, due to the highly tissue-specific, but also species-specific differences in regulation of the enzyme. Aromatase expression is tightly controlled in all tissues by multiple, different regulatory pathways. In contrast to mammals, fish have two different aromatase genes and resultant protein products, one for gonads and one for brain. It is not known whether the occasionally observed induction of aromatase in vitro also occurs in the tightly regulated in vivo situation (with the possible exception of tumor tissues where control is often lost) The in vitro-to-in vivo extrapolation of any observed aromatase induction is not possible without a more detailed understanding of the way in which aromatase expression is regulated in various species under normal physiological and under perturbed circumstances.

Two important, interrelated questions arise from these scientific findings. Firstly, are the current environmental exposures sufficiently high to cause the effects found in toxicological studies performed in the laboratory? In general, analyses of pesticide residues on consumable products indicate exposure levels that are lower than those applied in laboratory studies. However, relative persistence under various environmental and biological conditions, and potential for bioaccumulation and biomagnification should also be considered. A large number of pesticides have been identified in human liver, breast milk and urine or feces, sometimes for extended periods of time after exposure. Naturally, pesticide workers are at a particular occupational hazard as they may be exposed frequently and to potentially high levels. A second question is, if environmental levels are not sufficiently high to cause the overt toxicities found in laboratory studies, what then are the consequences of chronic low-dose exposures to inhibitors or inducers of aromatase, particularly during critical periods of development, such as embryonic or pubertal, or in females with a predisposition for the development of estrogen-dependent breast cancer? This is simply not known at present and a meaningful answer will require more detailed dose-response studies using lower doses and longer exposure times.


Thomas Sanderson obtained his BSc (1989) from the Free University of Amsterdam, The Netherlands, followed by a PhD (1994) from the University of British Columbia in Vancouver, Canada. After a postdoctoral research position at Michigan State University (1994-1996), and an assistant professorship at Utrecht University (1996-2005), The Netherlands, Thomas Sanderson is currently an associate professor in toxicology at the INRS-Institute Armand-Frappier, Montréal, Canada. His research interests concern the interactions of chemicals with the expression and function of enzymes involved in steroid biosynthesis, and their relation to the development of hormone-dependent cancers and other forms of endocrine disruption. Current research activities aim to elucidate the mechanisms by which a wide variety of chemicals, including environmental contaminants, pesticides, drugs and compounds of natural origin interfere with androgen and estrogen biosynthesis in humans, laboratory animals and wildlife.