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Review

Esterification of vertebrate like steroids in molluscs: A target of endocrine disruptors?

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ABSTRACT

Alterations of the reproductive organs of gastropod molluscs exposed to pollutants have been reported in natural populations for more than 40 years. In some cases, these impacts have been linked to exposure to endocrine-disrupting chemicals (EDCs), which are known to induce adverse impacts on vertebrates, mainly by direct binding to steroid receptors or by altering hormone synthesis. Investigations on the mechanisms of action of endocrine disruptors in molluscs show that EDCs induce modifications of endogenous titres of androgens (e.g., testosterone, androstenedione) and oestrogens (e.g., 17ß-oestradiol). Alterations of the activity of enzymes related to steroid metabolism (i.e., cytochrome P-450 aromatase, acyltransferases) are also often observed. In bivalves and gastropods, fatty acid esterification of steroids might constitute the major regulation of androgen and oestrogen homeostasis. The present review indicates that metabolism of steroid hormones to fatty acid esters might be a target of synthetic EDCs. Alterations of this process would impact the concentrations of free, potentially bioactive, form of steroids.

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1. Introduction

Alterations of the reproductive organs of gastropod molluscs exposed to pollutants have been reported in natural populations for more than 40 years (Blaber, 1970). The development of male sex organs in females has now been described in more than 200 mesogastropods and neogastropods species (Horiguchi et al., 2012). This phenomenon called *imposex* can lead, in the most adverse cases, to the sterilisation of females, which may thus affect population survival (Bauer et al., 1997). The occurrence of male characteristics in females has been linked to exposure to tributyltin (TBT), the main constitutive compound of boat antifouling paints, which has been found in harbours and marinas at high concentrations; i.e., in the μg/L range (IPCS, I.P.o.C.S., 1990). Other xenobiotics

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(e.g., pharmaceuticals, organochlorines) have also been shown to alter reproduction (e.g., superfemales, delayed gametogenesis, alteration of spawning) in many gastropod species, (Oehlmann et al., 2000; Tillmann et al., 2001; Oehlmann et al., 2006; Gust et al., 2009). Sex alterations such as masculinisation and feminisation of bivalve species (e.g., occurrence of spermatids in female gonads, presence of female follicles in male gonads) have also been observed in estuaries and rivers contaminated by anthropogenic wastes (Gagné et al., 2002; Ortiz-Zarragoitia and Cajaraville, 2010; Gagné et al., 2011; Tankoua et al., 2012). Some xenobiotics (e.g., organotin, pharmaceuticals, organochlorinated molecules) are called endocrine-disrupting chemicals (EDCs) because these contaminants are known to induce adverse impacts on the reproduction of vertebrates, mainly by binding directly to steroid receptors or altering hormone metabolism; e.g., by interfering with enzymes involved in steroidogenesis (IPCS, I.P.o.C.S., 2002).

Over the last decade, the knowledge on mollusc endocrinology has increased and evidence of synthesis and biological activity of vertebrate-like steroid hormones has been observed in at least 3 mollusc classes: Bivalvia, Gastropoda and Cephalopoda (Gottfried and Dorfman, 1970; Naokuni, 1979; Santos et al., 2002; Porte et al., 2006; Köhler et al., 2007; Lafont and Mathieu, 2007; Sternberg et al., 2010). In vertebrates, steroid hormone concentrations are controlled through glucuronidation and sulfonation pathways. The formation of glucuronic and sulphate steroid conjugates, catalysed by UDP-glucuronosyl transferase (UGT) and sulfotransferase (SULT) enzymes, respectively, reduces the biological activity of the hormones and facilitates their excretion from cells as these metabolites are more polar (James, 2011). In molluscs, the regulation of endogenous concentrations of free forms of steroids (i.e., testosterone and 17ß-oestradiol) through sulfonation has been shown to be low, whereas steroid esterification to fatty acid esters has been observed to be the major steroid metabolic pathway (Gooding and LeBlanc, 2001; Janer et al., 2005b). Apolar steroid fatty acid esters are stored in lipid rich tissues. This fatty acid esterification of steroids may constitute the major regulation of homeostasis of androgen and oestrogen in molluscs (Gooding and LeBlanc, 2001; Janer et al., 2004, 2006; Sternberg and LeBlanc, 2006; Labadie et al., 2007).

Investigations on impacts of putative endocrine disruptors in molluscs have shown that modifications of endogenous titres of androgens (e.g., testosterone, androstenedione) and oestrogens (e.g., 17ß-oestradiol, oestrone) occur in molluscs exposed to EDCs (Gooding et al., 2003; Gust et al., 2010; Abidli et al., 2012). Moreover, these chemicals have been shown to alter the activity of enzymes related to steroid metabolism; e.g., cytochrome P-450 aromatase, acyltransferases (Spooner et al., 1991; Ronis and Mason, 1996; Gooding et al., 2003; Abidli et al., 2012). Exposure of molluscs to natural steroids and to androgenic and oestrogenic xenobiotics has been reported to disturb normal hormonal balance of free versus esterified forms of both androgen and oestrogen hormones (Janer et al., 2004, 2006; Gust et al., 2009). Alteration of the esterification of steroids to fatty acid esters through enzymatic interference is proposed amongst hypotheses on the mechanism of imposex induction of TBT (Gooding et al., 2003; Janer et al., 2005c). Indeed, studies have shown that testosterone can induce imposex (Bettin et al., 1996). Free testosterone levels are higher in tissues of molluscs exposed to TBT and in females affected by imposex (Bettin et al., 1996; Gooding et al., 2003; Abidli et al., 2012). These observations lead to the hypothesis that free testosterone is bioactive in molluscs and can induce imposex. Therefore, an alteration of the regulation of testosterone concentrations in mollusc tissues through esterification would affect the concentrations of the free form of this steroid, which finally induce imposex (Bettin et al., 1996; LeBlanc et al., 2005).

In this review, we synthesise and discuss the impacts of natural and anthropogenic compounds on endogenous concentrations of vertebrate-like steroids in their free and esterified forms in molluscs. We also consider their impacts on the metabolic processes by which vertebrate-like steroids are esterified and the possibility that alteration of this process is a specific target of endocrine disruptors in molluscs.

2. Steroid metabolism in molluscs and seasonal variation of endogenous levels

As shown in *in vivo* studies on cholesterol biosynthesis, oestrogens (e.g., 17ß-oestradiol, oestrone) and androgens (e.g., androstenedione, androsterone) are synthesised *de novo* in molluscs (Gottfried and Dorfman, 1970; De Longcamp et al., 1974). *In vitro* studies also provide evidence of endogenous synthesis of vertebrate-like sex steroids in molluscs (Gottfried and Dorfman, 1970; De Longcamp et al., 1974; Le Curieux-Belfond et al., 2001; Lyssimachou et al., 2009b). Steroidogenesis in gastropod molluscs has been investigated through the injection of radiolabelled steroid precursors involved in vertebrate steroidogenesis (Fig. 1). Metabolites produced have been analysed and the rate of biotransformation of each precursor has been assessed (Gottfried and Dorfman, 1970; De Longcamp et al., 1974). Additionally, cytochrome P-450 aromatase (responsible for the conversion of androgens to oestrogens), 5α-reductase (responsible for the conversion of androgens), 3α/ß- and 17ß-hydroxydehydrogenases (which catalyse the reduction of ketosteroids or the oxidation of hydroxysteroids), sulfotransferases (involved in sulphate conjugation of steroids to polar metabolites) and fatty acid acyl coenzyme A acyltransferases (which convert steroids to apolar fatty acid ester steroids) have been found in molluscs; reviewed by Fernandes et al. (2011). However, Scott (2012) recently argued that molluscs only pick up steroids from their environment and therefore questioned the capacity of molluscs to biosynthesise vertebrate-like steroids *in vivo*. Hormone concentrations in molluscs vary between species, sex and tissues. For example, in natural population of molluscs, endogenous levels of androgens (e.g., androstenedione, testosterone), oestrogens (e.g., 17ß-estradiol) and other steroid hormones (e.g., progesterone) have been shown to vary according to the reproductive status of the animals. In the clam *Ruditapes decussatus* tissues, the highest concentrations of testosterone and progesterone were observed after gametogenesis whereas 17ß-oestradiol levels were found to increase at the beginning of vitellogenesis (Ketata et al., 2007). In tissues of the soft-shell clam *Mya arenaria*, 17ß-oestradiol, progesterone and testosterone concentrations were the highest at previtellogenic and spawning stages (Siah et al., 2002; Gauthier-Clerc et al., 2006). Testosterone and 17ß-oestradiol concentrations in gonads of the cockle *Fulvia mutica* were highest at the onset of gametogenesis and during spawning (Liu et al., 2008). These field studies showed that concentration peaks are observed at different times of the year in males and females according to their reproductive cycle (Gauthier-Clerc et al., 2006; Ketata et al., 2007). In gastropod species, clear seasonal patterns were also observed in sex steroid concentrations. In *Ilyanassa obsoleta*, the highest testosterone concentrations in tissues were observed during the reproductive season in both sexes with no notable differences between genders. 17ß-Oestradiol levels were not modulated during the reproductive cycle in males while higher concentrations were found during the dormant stages in females (Gooding and LeBlanc, 2004; Barroso et al., 2005; Sternberg et al., 2008a). In *Potamopyrgus antipodarum*, females exhibited the highest 17ß-oestradiol levels during the reproductive period while testosterone concentrations were lower during this period (Gust et al., 2011). In the cephalopod *Octopus vulgaris*, concentrations of 17ß-estradiol and progesterone increased significantly just before ovarian maturation in females (Di Cosmo et al., 2001).

Laboratory experiments have provided evidence that exposure of bivalve and gastropod species to sex steroid hormones leads to modifications of sex characteristics such as in superfemales and imposex females in gastropods and skewed sex ratios or alteration of oocytes in bivalves (Bettin et al., 1996; Schulte-Oehlmann et al., 2004; Wang and Croll, 2004) or induces impacts on spawning, gametogenesis and