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Bisphenol A (BPA) modulates the expression of endocrine and stress response genes in the freshwater snail *Physa acuta*

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ABSTRACT

Bisphenol A (BPA), a known endocrine disrupting chemical (EDC) that can mimic the action of oestrogens by interacting with hormone receptors, is potentially able to influence reproductive functions in vertebrates and invertebrates. The freshwater pulmonate *Physa acuta* is a sensitive organism to xenobiotics appropriate for aquatic toxicity testing in environmental studies. This study was conducted to explore the effects of BPA on the Gastropoda endocrine system. The effects following a range of exposure times (5–96 h) to BPA in *P. acuta* were evaluated at the molecular level by analysing changes in the transcriptional activity of the endocrine-related genes *oestrogen receptor* (*ER*), *oestrogen-related receptor* (*ERR*), and *retinoid X receptor* (*RXR*), as well as in genes involved in the stress response, such as *hsp70* and *hsp90*. Real-time reverse transcriptase-polymerase chain reaction (qRT-PCR) analysis showed that BPA induced a significant increase in the mRNA levels of *ER*, *ERR*, and *RXR*, suggesting that these receptors could be involved in similar pathways or regulation events in the endocrine disruptor activity of this chemical at the molecular level in Gastropoda. Additionally, the *hsp70* expression was upregulated after 5 and 72 h of BPA exposures, but *hsp90* was only upregulated after 5 h of BPA exposure. Finally, we assessed the glutathione-S-transferase (GST) activity after BPA treatment and found that it was affected after 48 h. In conclusion, these data provide, for the first time, evidences of molecular effects produced by BPA in the endocrine system of Gastropoda, supporting the potential of *ER*, *ERR* and *RXR* as biomarkers to analyse putative EDCs in ecotoxicological studies. Moreover, our results suggest that *P. acuta* is an appropriate sentinel organism to evaluate the effect of EDCs in the freshwater environment.

1. Introduction

Molluscs have received increasing interest in freshwater ecotoxicological studies, but so far the results of how gene expression is affected by anthropogenic pollutants are scarce. The Organisation for Economic Co-operation and Development (OECD) maintained the need to implement standardised test protocols using species of molluscs (OECD, 2010; Bandow and Weltje, 2012) as they are sensitive to endocrine disruption (EDCs) and to other environmental chemicals. However, there is still a relatively poor understanding of molluscan endocrinology. Gastropod snails have been reported to be exceptional test organisms for the determination of endocrine-disrupting effects (Schulte-Oehlmann et al., 2000; Oehlmann et al., 2000; Schmitt et al., 2008). One advantage is their hormone system, unique among invertebrate species and, to some extent, comparable to those of vertebrates (Thornton et al., 2003). On the other hand, snails play important roles in aquatic ecosystems and some gastropod species have been reported to be sensitive to EDC pollution but tolerant to various stressors (Schmitt et al., 2008). For these reasons, the development of new mollusc tests will provide options for the replacement of tests with vertebrate animals. The hermaphroditic freshwater pulmonate snail *Physa acuta* is an invasive species widely distributed in freshwater bodies around the world (Ait Alla et al., 2006; Guo et al., 2009). Moreover, its high sensitivity to different toxicants (Essawy et al., 2009; Laguerre et al., 2009; Sánchez-Argüello et al., 2009; Musee et al., 2010; Hossain and Aditya, 2013; De Castro-Català et al., 2013; Seeland et al., 2013; Martínez-Paz et al., 2017a).

Oestrogen receptors (ERs) are ligand activated transcription factors belonging to the nuclear receptor (NR) superfamily that modulate the expression of different genes to induce biological effects. In contrast,

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oestrogen-related receptors (ERRs) are a subfamily of the orphan nuclear receptors with a sequence and structure similar to ERs but not activated by oestrogens. Although the ligand is still unknown, they bind to their own DNA elements and modulate transcription (Horard and Vanacker, 2003). Sequence comparison shows that ERs and ERRs form the same branch of steroid receptors (Giguère, 2002), with a wide distribution in the animal kingdom, including snails (Bannister et al., 2007; Kaur et al., 2015; Martínez-Paz et al., 2017a). While oestrogen receptor has been studied in other snails species (Kajiwara et al., 2006; Castro et al., 2007a; Sternberg et al., 2008a; Stange et al., 2012; Hultin et al., 2014), ERRs are not very well understood in invertebrates. Other member of nuclear hormone receptor family proteins is the Retinoid X Receptor (RXR). It contains two signature domains of nuclear receptor family proteins, *i.e.* DNA-binding domain (DBD) and ligand binding domain (LBD), and is also a ligand-dependent transcription factor. These receptors have been defined in a wide variety of metazoan phyla, including snails (Bouton et al., 2005; Castro et al., 2007b; Janer et al., 2007; Sternberg et al., 2008b; Carter et al., 2010; Martínez-Paz et al., 2017a). Horiguchi (2006) has suggested that RXRs play an important role in the development of imposex in gastropods.

Heat shock proteins (HSPs), a group of highly conserved proteins present in almost all living organisms, function as molecular chaperones in response to a wide range of environmental stressors (Srivastava, 2002). As sensitive stress sensors, *hsp* genes are suitable to evaluate the damaging potential of chemicals, but also to analyse the effects of alterations in physical abiotic parameters, including heat, virus infection, tumorigenesis, chemicals, hypoxia and magnetic field (Parsell and Lindquist, 1993; Bierkens et al., 1998; Feder and Hoffmann, 1999). They are critical to maintain cellular homeostasis in response to changes in the environment and excellent candidates as biomarkers for environmental toxicity tests (Gupta et al., 2010). HSPs constitute a large family of proteins that are often classified based on their molecular weight. HSP70 is one of the most well-known, best-conserved HSPs, and is regulated by several chemical and biological stressors in all organisms, ranging from bacteria to plants and mammals (Beere and Green, 2001; Milani et al., 2002; Srivastava, 2002; Sorensen et al., 2003). In gastropods, the *hsp70* gene has been cloned from several species, such as *Biomphalaria glabrata* (Laursen et al., 1997; Da Silva Cantinha et al., 2017), *Haliotis discus hannai* (Cheng et al., 2007), *Haliotis tuberculata* (Farcy et al., 2007), *Cellana toreuma* (Han et al., 2013), *Pomacea canaliculata* (Xu et al., 2014a), and *P. acuta* (Martínez-Paz et al., 2017a). In addition, the synthesis and overexpression of HSP70 in response to heat shock or environmental stress has been demonstrated in these organisms. In gastropods, HSP90 is less well known than HSP70 but is the major protein in the eukaryotes cytosolic. This environmental approach shows the usefulness of studying *hsp70* and *hsp90* genes as sensitive marker in field of toxicology.

Glutathione S-transferases belong to a family of enzymes that are involved in detoxification of xenobiotic, protection from oxidative damage, endogenous metabolites, intracellular transport of hormones and exogenous chemicals (Hemingway et al., 1991). Insect GST plays important roles in phase II detoxification caused by xenobiotics (Xu et al., 2014b). BPA was able to alter the levels of GST activity in *C. riparius* (Martínez-Paz et al., 2012) but there is still insufficient knowledge of the response of these enzymes in the invertebrates.

In this study, we evaluated the expression of *ER*, *ERR*, *RXR*, *hsp70*, *hsp90* and a parameter related with the detoxification route GST activity in *P. acuta* in response to xenobiotic exposure to bisphenol A (BPA), a compound that frequently contaminates the aquatic environment. 2,2-bis(4-hydroxyphenyl)propane (BPA) is a high production volume chemical, with a total worldwide production rate over 3.8 million pounds per year (Michałowicz, 2014). It is used in polycarbonate plastics and epoxy resin products, as an antioxidant in plasticisers, and as an additive in other plastics (Staples et al., 1998), which are extensively used in the production of medical devices, dental fillings, water bottles sealants, sports equipment, coatings on metal lids, protective linings for canned foods, consumer goods and beverages, and household electronics. Due the large quantities and widespread application BPA, it has been frequently detected in various environmental matrices, such as river, air, sewage, coastal sea waters, lake, soil, drinks, sediments, and biological samples (human urine and blood samples) (Zhu and Zuo, 2013). Human exposure to BPA has also been reported (Geens et al., 2011). Until now, studies that have used *P. acuta* as a biomonitoring organism in response to exposure to chemicals compounds in freshwater ecosystems are very scarce. Understanding the response of *P. acuta* to environmental challenges will provide information about the mechanisms used by this snail to manage stress and allow its sensitivity and specificity to the presence of chemicals with putative toxic effects to be evaluated. Our study provides new data at the molecular level for a mollusc species and could be helpful for covering gaps about physiological processes involved after chemical injury in this group of invertebrates. Furthermore, this study reveals the potential role of *ER, ERR, RXR, hsp70*, and *hsp90* genes as sensitive markers of exposure to BPA in the aquatic snail *P. acuta*.

2. Materials and methods

2.1. Animals and treatments

P. acuta were maintained in a controlled-climate room (20 °C) for several generations prior to the experiment. The culture conditions were defined previously (Sánchez-Argüello et al., 2009; Martínez-Paz et al., 2017a).

For expression analysis, the snails were exposed to 100 and 500 µg/L BPA (Aldrich) diluted in DMSO (Sigma) (0.1%) for 5, 24, 48, 72, and 96 h at 20 ± 1 °C. Non-treated control snails were exposed to the same concentration of DMSO as the corresponding treatment. The test medium (2 mM $CaCl_2$, 0.5 mM $MgSO_4$, 0.77 mM $NaHCO_3$, and 0.08 mM KCl) was supplemented with 12 mg of commercial shrimp food flakes at 48 h. No mortality was observed during the exposures. Ten snails were exposed for concentration and exposure time; five of them were used for RNA analysis and the five other for the enzymatic assay. The treatment consisted in three independent experiments. After shell separation, snails exposed to BPA and untreated snails were stored at −80 °C until RNA and protein extraction were carried out.

BPA is easily biodegradable in natural aquatic environments and in acclimated wastewater treatment plants (Staples et al., 1998). Previous studies reported half-lives in the range of 48–96 h for 3 mg/L of BPA in water samples collected from the effluent of a manufacturing facility and the receiving stream (Klecka et al., 2001). Since biodegradation is expected to be the dominant process for removal of BPA from the aquatic environment the previous study conducted with *Physa acuta* exposed to BPA (Sánchez-Argüello et al., 2012) included chemical determination of BPA. This chemical determination of BPA consisted in a solid-phase extraction-gas chromatography-mass spectrometry as described by Liu et al. (2004) and showed that nominal concentrations of 0.5, 1, 5 and 10 mg/L were maintained constant in the test medium under our laboratory conditions for 96 h. Based on these results it can be concluded that in contrast to ambient water there is no loss of BPA in laboratory test medium which is free of microbial populations, hence BPA renewal was not necessary for the present experiments.

2.2. RNA extraction and quantitative RT-PCR (qRT-PCR)

RNA extraction and reverse transcription were performed following the methods of Martínez-Paz et al. (2017a). PCR conditions and primers for *ER*, *ERR*, *RXR*, *hsp70*, *hsp90*, *rpL13*, and *MT* genes are those used in Martínez-Paz et al. (2017a).

2.3. Glutathione-S-transferase activity

To evaluate the glutathione S-transferase (GST) activity, a pool of

five control snails and five treated snails were collected at 5, 24, 48, 72, and 96 h after the BPA experiments. Protein isolation and GST activity were performed according to the procedures of previous works of our group (Martínez-Paz et al., 2012; Morales et al., 2014).

2.4. Statistical analysis

Statistical analysis were conducted using SPSS 22® (IBM). The results are presented as the mean of at least three separate experiments of at least three replicates for each experiment. Normal distribution and variance homogeneity of data were assessed using the Kolmogorov-Smirnov and Levene tests, respectively. The normalised levels of the specific gene transcripts in treated groups were compared to those of the non-exposed controls using analysis of variance (ANOVA). A $p \leq 0.05$ (*) and $p \leq 0.01$ (**) were considered to indicate significant differences. All results are expressed as the mean ± standard error of the mean (SEM) of three experiments.

3. Results

3.1. Normalisation and transcription profiles of rpL13 and metallothionein genes

RpL13 and *MT* were selected as reference genes to normalise the transcript expression of target genes, because these genes presented a coefficient of variation < 0.25 and an *M*-value < 0.5 (Hellemans et al., 2007) (Table 1).

3.2. Effects of BPA exposure on endocrine-related genes

The transcriptional activity of *ER* increased at 24 and 96 h from control levels in snail exposed to 500 µg/L of BPA but decreased at 48 h in the 100 µg/L exposure (Fig. 1). Meanwhile, *RXR* mRNA levels were downregulated with 100 and 500 µg/L of BPA at different times (48 and 24 h, respectively), but this gene was upregulated at 96 h and 500 µg/L. Finally, *ERR* mRNA expression increased at 24 h with 500 µg/L. Thus, BPA differentially affects these hormonal receptor genes and alters the endocrine system of *P. acuta*.

3.3. Effects of BPA exposure on hsp70 and hsp90 transcriptional activity

The *hsp70* and *hsp90* mRNA levels were analysed by qRT-PCR, following normalisation with the reference genes *rpL13* and *MT*. In each case, the gene expression patterns were compared with those observed in control animals exposed to the same concentration of solvent. As shown in Fig. 2, *P. acuta* adults showed a significant increase in the expression of the *hsp70* and *hsp90* after 5 h of treatment with 100 and 500 µg/L BPA. In contrast, the transcriptional activities of *hsp70* and *hsp90* decreased after 24 and 48 h of exposure to BPA. The expression of the *hsp70* and *hsp90* increased again after 72 h of BPA exposure (Fig. 2). Nevertheless, only the differences in *hsp70* expression were significant, and changes in the mRNA levels of *hsp70* and *hsp90* after 96 h of treatment with 100 and 500 µg/L BPA were not significant (Fig. 2).

Table 1
Results of the normalisation procedures using *rpL13* and *metallothionein* as a reference genes.

Treatment	*rpL13*		*Metallothionein*		Mean value	
	CV	*M*-value	CV	*M*-value	CV	*M-value*
BPA-5h	0.1758	0.4818	0.1569	0.4818	0.1665	0.4818
BPA-24h	0.1592	0.4745	0.1689	0.4745	0.1640	0.4745
BPA-48h	0.1905	0.5489	0.1879	0.5489	0.1892	0.5489
BPA-72h	0.0978	0.2808	0.0968	0.2808	0.0973	0.2808
BPA-96h	0.1175	0.3190	0.1041	0.3190	0.1108	0.3190

3.4. GST enzyme activity in response to BPA exposure

GST activity was analysed to assess the effect of BPA. As shown in Fig. 3, the activity of GST was similar all analysed conditions, except for exposure to 500 µg/L BPA for 48 h.

4. Discussion

Mihaich et al. (2009) reported EC50 data for BPA of 2.7 mg/L and LC50's > 4.03 and 2.24 mg/L from acute tests with the midge (*Chironomus tentans*) and the snail (*Marisa cornuarietis*), respectively. These authors compared their findings with other results on acute invertebrate toxicity of BPA in the literature (1.1–16 mg/L) and found good agreement. Similarly, the existing literature on chronic tests showing NOECs values between 0.025 and ≥ 3.2 mg/L were comparable to those from Mihaich's study (0.49 mg/L for *Hyalella* chronic test and 1.8 mg/L for *Brachionus* chronic test). The effect data observed by Mihaich et al. (2009) not only compare well with published effect data on invertebrate but also, more broadly, correspond to the available effect data of aquatic organisms including fish and aquatic plants. More recently Guo et al. (2015) based on 15 acute toxicity values and 18 chronic toxicity values derived water quality criteria for BPA using the species sensitivity distribution (SSD) model. They obtained the criterion maximum concentration (CMC) and the criterion continuous concentration (CCC) of 1.5 mg/L and 2.19 µg/L, respectively.

In the present work were evaluated alterations by BPA in mRNA levels of genes related with the endocrine system and the stress response in *P. acuta*. NRs of *Biomphalaria glabrata* and *Lottia gigantea* are conserved with vertebrate NRs, showing the potential for molluscs as model organisms for endocrine function (Kaur et al., 2015). BPA has been showed to disrupt the endocrine system in several species and in the gastropod *Marisa cornuarietis* acts as an oestrogen receptor (ER) agonist and induces superfeminisation, an alteration that produces the formation of additional female organs, stimulation of egg and egg mass production, gross malformations of the pallial oviduct, and enlarged accessory sex glands (Oehlmann et al., 2000, 2006; Schulte-Oehlmann et al., 2000). Moreover, BPA invoked an upregulation of *ER* gene in the freshwater mudsnail *Potamopyrgus antipodarum* (Stange et al., 2012). ERs are nuclear receptors that modulate gene expression in response to oestrogens, and they can be altered by EDCs in invertebrates (Ciocan et al., 2010; Stange et al., 2012; Zhang et al., 2012). In *P. acuta,* BPA upregulated the transcriptional activity of *ER* at a concentration of 500 µg/L at 24 and 96 h, analogous to results in some vertebrates (Luo et al., 2015; Driessnack et al., 2016). Conversely, BPA downregulated the expression of *ER* at a concentration of 100 µg/L at 48 h, showing similar results to those observed in *P. acuta* exposed to Cd (Martínez-Paz et al., 2017a) and in some vertebrates (Ishitobi et al., 2007; Mehinto et al., 2014; Chen and Chan, 2016). This response may have an explanation that fits the characteristics of the non-monotonic dose-response (NMDR) described for several hormones (Welshons et al., 2003; Vandenberg et al., 2012; Lagarde et al., 2015). The NMDR describes a dose-response characterized by a curve in which slope changes direction within the range of tested doses. This profile, called an inverted-U shape, involves a response at intermediate doses and no response or a diminished response at low and high exposure levels. On the other hand, U-shaped profiles, observed in some studies, show the highest response to low and high concentrations (Lagarde et al., 2015). *ERR* is an orphan receptor with homology to *ER* described in vertebrates and invertebrates. It has been studied as biomarker of EDC exposure in vertebrates (Schlecht et al., 2004; Tohmé et al., 2014) and invertebrates (Park and Kwak, 2010; Bannister et al., 2013; Morales et al., 2013, 2014; Martínez-Paz et al., 2017a, 2017b). BPA upregulated the transcriptional activity of *ERR* in *P. acuta* at 500 ng/L, a similar result observed with cadmium in this organism (Martínez-Paz et al., 2017a). The increase of *ERR* mRNA levels are coincident with ER upregulation at the same time and concentration, suggesting a temporal coordination in the

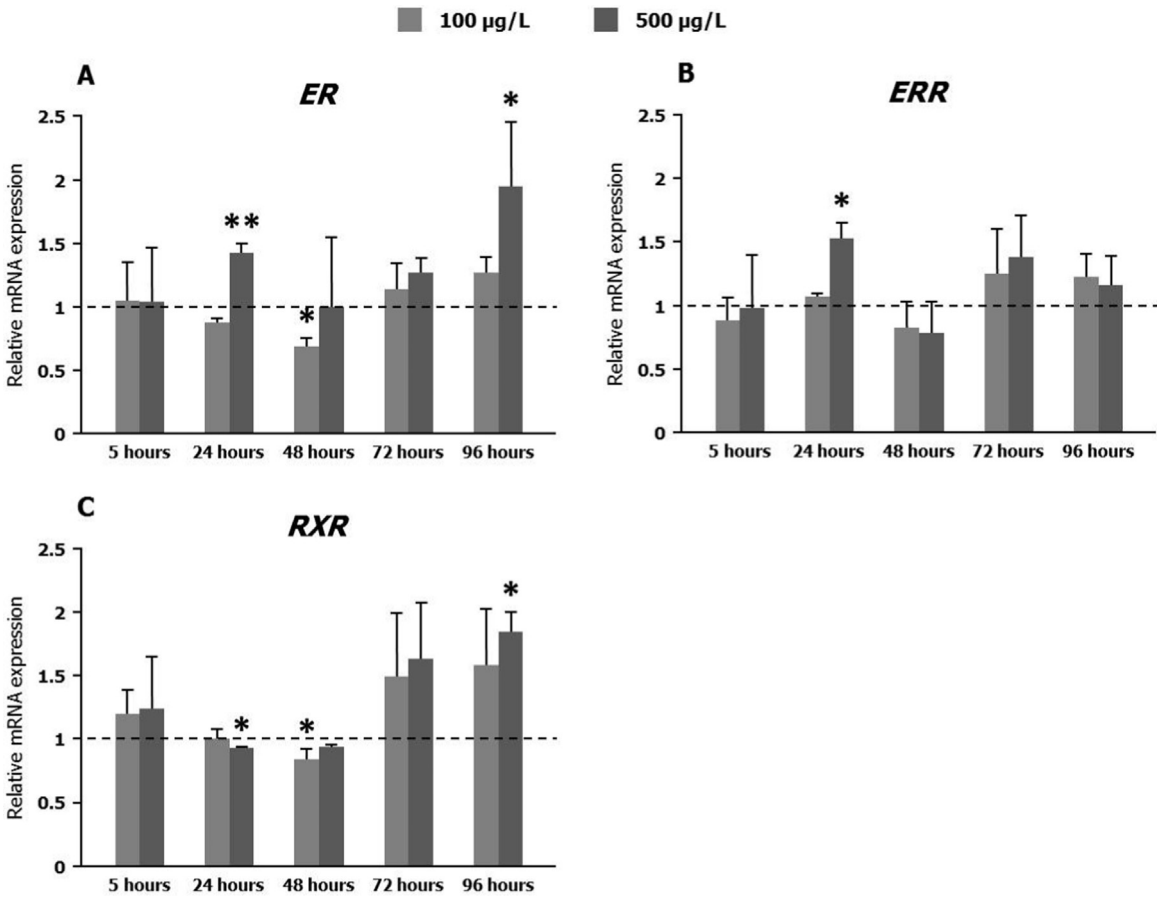

Fig. 1. Effects of BPA on the mRNA levels of *ER* (A), *ERR* (B), and *RXR* (C) in control and treated snails as measured by qRT-PCR with primers and reference genes, as indicated in Materials and Methods. The means ± SEMs of three independent experiments are shown and each sample consisted of three replicates. Values were normalised to the reference genes *rpL13* and *MT* and are presented in relation to the values of the control snails, which were set to 1 (dashed line). Statistical differences: * $p \leq 0.05$; ** $p \leq 0.01$.

expression of these genes. A similar behaviour has been recently described for *ERR* and *RXR*, another NR, in *P. acuta* exposed to Cd (Martínez-Paz et al., 2017a). It should be noted that the changes observed to the lower doses analysed in both studies invokes transcriptional changes at environmental concentrations of BPA and Cd. It could be possible that the behaviour depends on the xenobiotic. ER orthologues in molluscs may be targets for endocrine disruptors, although mechanistic evidence is not available. On the other hand, *RXR* showed decreased mRNA levels in the first 48 h while increased its levels in the two last times analysed. This suggests that BPA affects the expression of this gene using a different and slower pathway than that for *ER* and *ERR*. Previous studies have reported that RXR plays an important role in the development of gastropod imposex (Nishikawa et al., 2004; Sternberg et al., 2008b; Horiguchi et al., 2010; Abidli et al., 2013). BPA appears to act as an agonist of the RXR; thus, it might be possible that it could induce imposex in *P. acuta*, as observed in *Hexaplex trunculus* (Abidli et al., 2013). Further research is needed to confirm this hypothesis since there are no studies on this phenomenon for *P. acuta*.

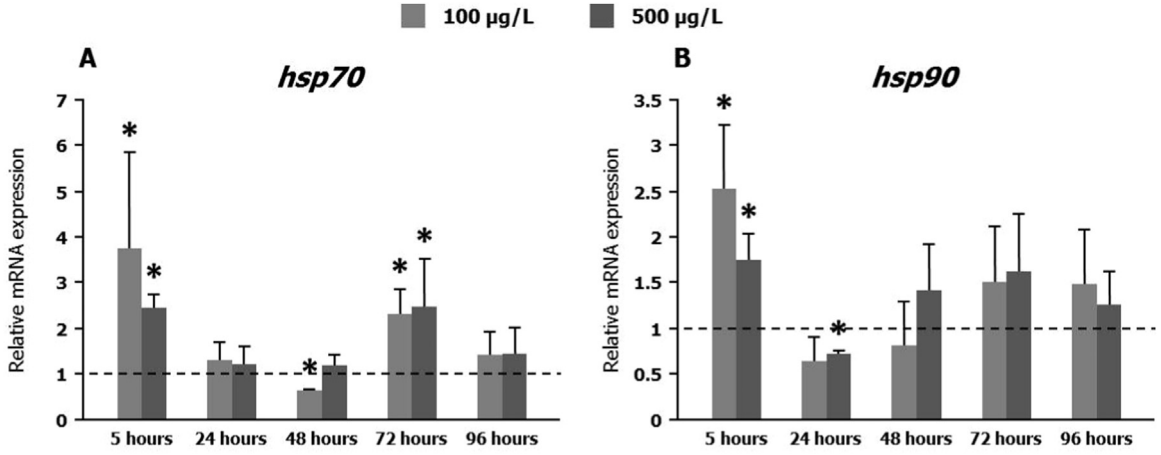

Fig. 2. Relative mRNA levels of *hsp70* (A) and *hsp90* (B) in *P. acuta* at different time points after 100 and 500 µg/L BPA treatments. Values were normalised to reference genes and are presented in relation to the values of the control solvent-exposed snails, which were set to 1 (dashed line). The means ± SEMs from three independent experiments are shown (n = 15), and each sample consisted of three replicates. Differences between BPA-treated and the corresponding solvent control samples were considered significant at * $p \leq 0.05$.

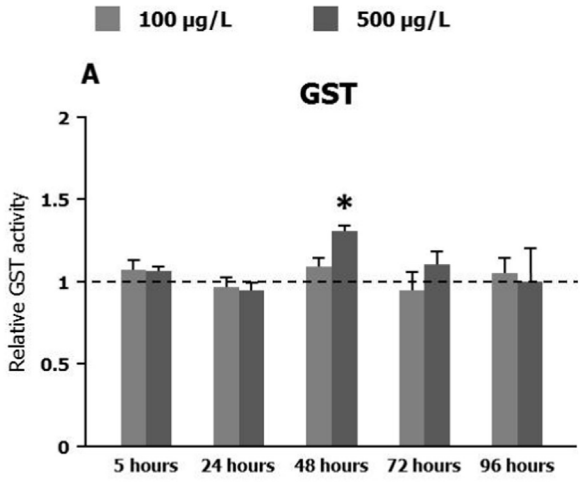

Fig. 3. GST activity after 5, 24, 48, 72, and 96 h of BPA exposure. The means ± SEMs of three independent experiments are shown and each sample consisted of three replicates. The activity level of the untreated control snails was set to 1 (dashed line). The asterisks indicate significant differences between BPA-treated samples and the solvent control: * $p \leq 0.05$.

We showed that BPA upregulates both *hsp70* and *hsp90* in *P. acuta* but at lower levels than in the heat shock response of *P. acuta* to Cd (Martínez-Paz et al., 2017a). BPA increased the transcriptional level of *hsp70* between 2- and 3-fold, whereas *hsp90* expression increased between 1.5- and 2.5-fold. The activation was most apparent after 5 h of exposure, although upregulation could be detected at later time points, especially for *hsp70* at 72 h. Balakrishnan and De Maio (2006) demonstrated that the accumulation of HSP70 protein promotes the degradation of *hsp70* mRNA. The decrease observed at 48 h and the later increase at 72 h of *hsp70* mRNA may reflect these dynamics in *hsp70* gene turnover. On the other hand, the results suggest that BPA activates *hsps* in a different way to that observed in heat shock response, showing a slower increase in comparison with heat stress but relatively fast taking in account that chemical stressors usually induce slower responses. Further research is needed to elucidate the differences in the activation processes. Additionally, it is important to note that the activation of *hsp70* and *hsp90* could be related to the role that these proteins play in the maturation of steroid receptors. HSP70 and HSP90 proteins are needed for the maturation of steroid receptors, proteins that interact with steroid hormones and relay the information from these signalling molecules to the cell through the transcriptional regulation of gene expression (Kimmins and MacRae, 2000; Echeverria and Picard, 2010). The modulation of specific genes by BPA has not yet been reported in *P. acuta*. This report constitutes the first evidence of the ability of BPA to alter the activity of *hsp70* and *hsp90* in snails. Previous studies have reported that Cd increased the amount of HSP70 protein in *Biomphalaria glabrata* (Da Silva Cantinha et al., 2017) and hsp70 mRNA levels in *Physa acuta* (Martínez-Paz et al., 2017a). HSPs have become important tools in environmental studies, and *hsp70* and *hsp90* have been evaluated as putative biomarkers of exposure to different xenobiotics in other invertebrate species (Morales et al., 2011; Qian et al., 2012; Bouétard et al., 2013; Chen et al., 2014; Martínez-Paz et al., 2017a, 2017b). In molluscs, *hsp90* responds to various heavy metals in a dose- and time-dependent manner, *e.g.* in *Chlamys farreri* and *Crassostrea gigas* (Gao et al., 2007; Choi et al., 2008), and dietary Zn can modulate the mRNA expression of *hsp70* and *hsp90* in *Haliotis discus hannai* (Wu et al., 2011). Furthermore, exposure to xenobiotics such as hydrocarbons (Boutet et al., 2004), oils (Snyder et al., 2001), diquat (Bouétard et al., 2013), benzo[a]pyrene, and chrysene (Guo et al., 2017), as well as heavy metals such as Zn (Wu et al., 2011) and Cd (Choi et al., 2008) increases the expression of *hsp70* and *hsp90*. Our data suggest that BPA, a widely distributed industrial and environmental toxicant, induces the expression of these genes. Therefore, *hsp70* and *hsp90* might be useful as biomarkers to assess the toxicity of chemical compounds.

In addition, the enzymatic activity of GST, a major detoxification enzyme, was also evaluated. Interestingly, BPA did not significantly alter the activity of GST after 5, 24, 72, and 96 h of exposure at almost all analysed concentrations of BPA. Specifically, GST activity only increased after 48 h of exposure at the highest concentration of BPA. Similar results were found after BPA exposure in other invertebrates, such as *Mytillus galloprovincialis* (Canesi et al., 2007), *Daphnia magna* (Jemec et al., 2012), and *Chironomus riparius* (Martínez-Paz et al., 2012). On the other hand, GST activity increased in the snail *Lymnaea stagnalis* after exposure to the herbicide diquat (Bouétard et al., 2013). This study demonstrates that BPA does not alter GST enzyme activity, suggesting that in *P. acuta* either BPA does not activate the detoxification process at these concentrations or that enzymes other than GST participate in the process.

In summary, changes in several genes related to the endocrine system and the stress response were observed in the gastropod *P. acuta* after BPA treatment. The data showed that BPA alters the expression of genes involved in endocrine functions, but that it can have contradictory effects depending on the pathway affected. There are different responses for *ER* and for *ERR* and *RXR* genes. The biomarkers of exposure to EDCs in aquatic snails are still scarce, and this study provides additional clues. Moreover, our data indicate that *hsp70* and *hsp90* in *P. acuta* are sensitive to cellular stresses caused by BPA treatment. The description of new molecular biomarkers in *P. acuta* would increase the utility of this snail in toxicology studies and constitute new possibilities for the development of genomic tests to analyse environmental pollution.

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