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Bisphenol A effect on glutathione synthesis and recycling in testicular Sertoli cells

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ABSTRACT. ***Background and objective:*** **Controversial effects of bisphenol A (BPA) have been reported on testicular function. These differences might reflect dissimilar exposure conditions. Dose responses to toxicants may be non-linear, e.g. U-shaped, with effects at low and at high levels of exposure and lower or inexistent effects at intermediate levels. Sertoli cells produce high levels of glutathione (GSH) as a cell defense mechanism. In this study, we addressed the question whether the exposure to different doses of BPA could influence Sertoli cell GSH synthesis and recycling.** ***Materials and methods:*** **Primary Sertoli cell cultures were exposed to various doses of BPA (0.5 nM-100 μM). Cell viability was measured as an outcome of toxic effect. GSH cell content was determined to evaluate cell response to toxicant exposure. Glutamate-cysteine ligase catalytic (GCLC) and modulatory (GCLM) subunit expression were assessed to estimate GSH synthesis, and GSH reductase (GR) expression to estimate GSH recycling.** ***Results:*** **BPA 100 μM, but not lower doses, decreased cell viability. BPA 10 and 50 μM, but not lower doses, induced an increment in Sertoli cell GSH levels, due to a rapid upregulation of GCLC and GR and a slower upregulation of GCLM.** ***Conclusions:*** **High doses of BPA are deleterious for Sertoli cells. Intermediate doses do not affect Sertoli cell viability and increase cell content of GSH owing to increased GSH synthesis and recycling enzyme expression. Lower doses of BPA are not capable of eliciting a cell defense response. These observations may explain a non-linear dose response of Sertoli cells to BPA.**

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INTRODUCTION

Bisphenol A (BPA) [2,2-bis(4-hydroxyphenyl)propane] is a monomer of polycarbonate plastics and a constituent of epoxy and polystyrene resins that are extensively used in the food-packaging industry and also as a component of dental sealant. BPA was first considered a potential endocrine disruptor owing to its experimentally shown estrogenic activity (1-3). Furthermore, detectable concentrations of BPA have been found in human fluids (4, 5), and concern was raised especially by the facts that fetal and neonatal periods of life might be particularly sensitive to endocrine disruptors (6, 7), resulting in adverse effects later in life in diverse organs (3, 8), and that baby feeding bottles may release BPA (9).

Regional decline in sperm count and increase in developmental male reproductive tract disorders, like cryptorchidism, hypospadias, and testicular cancer, have been associated with an increasing exposure to environmental pollutants during fetal life (10-12). Although sperm production begins at puberty, a normal development of testicular cell populations from early embryonic life is essential for the development of a fully competent reproductive capacity. Sertoli cells are the somatic component of the seminiferous tubules and function as primary supporting cells creating the structural and physiological environments necessary for spermatogenesis. In the interstitial tissue, Leydig cells are responsible for the secretion of androgens and insulin-like factor 3. While the latter is involved in testicular descent during fetal life (13), androgens are essential for both testicular descent in fetal life and Sertoli cell maturation and spermatogenic development at puberty (14).

The effect of BPA on testicular function has been controversial. Whereas certain studies have clearly shown that Leydig cell testosterone production (15), Sertoli cell junctional complex formation (16), and sperm output (7, 17) are affected by exposure to the toxicant in fetal and/or early post-natal life, other experimental designs have failed to demonstrate a BPA-dependent deleterious effects in the male gonad (18, 19). Whether these different findings on BPA effects are the result of dissimilar experimental conditions (*in vivo*/*in vitro*, cell type, BPA dose, *etc.*) that might reflect a differential sensitivity of the various cell populations of the testis to BPA remains to be elucidated. On the other hand, to add to the controversy, a non-linear dose response curve has been described for many toxicants. In fact, in some non-monotonic dose responses, the curve may be U-shaped – i.e. high responses at low and at high levels of exposure with lower responses at intermediate levels – or shaped like an inverted U – with the greatest responses at intermediate doses (3).

Glutathione [L-γ-glutamyl-L-cysteinylglycine (GSH)] is a non-protein thiol with an essential role in cell protection against oxidative stress and xenobiotic detoxification (20-23). High levels of GSH have been localized in Sertoli cells (23-27), suggesting that protection of seminiferous epithelium against oxidative stress can be achieved through Sertoli

Key-words: Bisphenol A, sertoli cell, glutamate-cysteine ligase, glutathione, glutathione reductase, glutathione-S-transferase.

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cell GSH production. Recently, we demonstrated that GSH content in post-natal Sertoli cells is modulated by FSH and the paracrine regulator basic fibroblast growth factor through stimulation of GSH *de novo* synthesis and recycling (27), suggesting that protection of seminiferous tubule cells against oxidative stress can be achieved through an increase in Sertoli cell GSH content. Here, we hypothesized that post-natal Sertoli cells might respond differently according to the dose of BPA exposure by modifying its GSH content, as a mechanism of cell protection. There are several mechanisms by which cells maintain their GSH content: *de novo* synthesis from its constituent amino acids, GSH redox recycling from oxidized GSH – catalyzed by GSH reductase (GR) – and direct uptake (20). Glutamate-cysteine ligase (GCL) is the initial and rate-limiting enzyme in the *de novo* synthesis of GSH. This enzyme catalyzes the binding of L-glutamate and L-cysteine to form L-γ-glutamyl-L-cysteine. GCL is a heterodimer composed of a 72-kDa catalytic subunit (GCLC) and a 30-kDa modulatory subunit (GCLM) (28, 29). GR catalyzes the reduction of oxidized GSH (GSSG) to reduced GSH using NADPH as the electron donor. The presence of GCL (26) and GR (25, 27, 30) has been demonstrated in rat Sertoli cells. Glutathione-S-Transferases (GST) are a family of detoxification isoenzymes present in different tissues including the testis (25). GST catalyzes the conjugation of many toxic substrates to GSH resulting in the formation of a less toxic product (31). In this study, we addressed the question whether the exposure to different doses of the environmental pollutant BPA could influence post-natal Sertoli cell GSH synthesis, recycling, and loss.

MATERIALS AND METHODS

Materials

Tissue culture media were purchased from Grand Island Biological Co. (Grand Island, NY, USA). Bisphenol A 99% (Aldrich 239658) and other drugs and reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All experimental procedures and animal use were in accordance with the requirements of NIH Guide for the Care and Use of Laboratory Animals.

Sertoli cell isolation and culture

Sertoli cells from early pubertal (18-day-old) Sprague-Dawley rats were isolated as previously described (32). Briefly, decapsulated testes were digested with 0.1% collagenase and 0.006% soybean trypsin inhibitor in Hanks' balanced salt solution for 5 min at room temperature. Seminiferous tubules were saved, cut, and submitted to 1 M glycine-2 mM EDTA (pH 7.4) treatment to remove peritubular cells. The washed tubular pellet was then digested again with collagenase for 10 min at room temperature to remove germ cells. The Sertoli cell suspension, collected by sedimentation, was resuspended in culture medium consisting of a 1:1 mixture of Ham's F-12 and Dulbecco's modified Eagle medium, supplemented with 20 mM Hepes, 1.8 mg/ml sodium bicarbonate, 100 IU/ml penicillin, 2.5 µg/ml amphotericin B, 10 µg/ml transferrin, 5 µg/ml insulin, 5 µg/ml vitamin E, and 4 ng/ml hydrocortisone. Sertoli cells were cultured at 34 C in a mixture of 5% CO2: 95% air.

Purity of Sertoli cells was assessed in monolayers prepared as described above and compared to the purity of monolayers submitted to a brief hypotonic treatment as described by Galdieri et al. (33). After 5 days in culture, germ cells represented 3.89±0.79% and 2.95±0.55%, respectively (ns using a paired t-test). Sox-9 and inhibin-α subunit immunocytochemistry was used to identify Sertoli cells. Myoid and Leydig cell contamination was assessed by immunocytochemistry for alkaline phosphatase and 3β-hydroxysteroid dehydrogenase (3β-HSD) respectively, as described below.

Culture conditions

Sertoli cells were allowed to attach for 48 h in the presence of insulin (5 µg/ml); the medium was then replaced with insulin-free fresh medium. BPA dissolved in Dimethyl Sulfoxide (DMSO)-containing vehicle was added to culture medium at various concentrations (0.5 nM-100 µM). For control conditions, DMSO-containing vehicle without BPA was added to the medium (DMSO final concentration <0.25%). DMSO-containing vehicle or DMSO-dissolved BPA was added 24, 3 or 1 h before the end of the culture period. Cells were harvested on day 5 and disrupted by sonication. Adequate aliquots for DNA determinations were saved and analyzed (34).

Immunocytochemistry

Immunocytochemistry was performed in 18-day-old rat Sertoli cell cultures. Cells were washed with Tris-buffered saline (TBS) and fixed by immersion in methanol at 4 C. Fixed cells were incubated in specific rabbit polyclonal antibodies raised against Sox-9 (Chemicon International Inc., Temecula, CA, USA) or 3β-HSD (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), diluted 1:100, or mouse monoclonal antibodies raised against inhibin-α subunit (35), diluted 1:10 or placental alkaline phosphatase (Dako, Carpinteria, CA, USA), diluted 1:100 in TBS. After incubation with the primary antibodies, cells were washed with TBS and the Dako Cytomation LSAB+System-HRP (Dako, Carpinteria, CA, USA) was used with 3,3'-diaminobenzidine as chromogen. Negative controls were obtained by incubating with nonimmune rabbit or nonimmune mouse serum replacing the primary antibody. Nuclear counterstain was performed with hematoxylin.

Viability assay

Cell viability was determined by an MTS assay using the Cell Titer 96 Aqueous non-radioactive cell proliferation assay (Promega Corp., Madison, WI). This colorimetric method determines the number of viable cells in cytoxicity assays by measuring MTS conversion; this is accomplished by dehydrogenase enzymes found in metabolically active cells and is directly proportional to the number of living cells in culture. The method measures the conversion of a tetrazolium salt compound, MTS to an aqueous soluble formazan product at 490 nm.

GSH assay

Determination of intracellular concentration of total GSH was performed as described by Baker et al. (36) with slight modifications. Briefly, Sertoli cell monolayers in 24-multiwell plates (10 µg DNA/well, corresponding to approximately 1.4×10^6 cells/well at the beginning of the culture) were homogenized in 0.15 M Tris-HCl buffer pH 7.4 and disrupted by sonication. Adequate aliquots were saved for DNA determinations. In microtitration plates, two different aliquots of the homogenate were added to the reaction buffer (0.1 ml) consisting of a mix of 100 mM sodium phosphate, pH 7.5 – 1 mM EDTA (5.75 ml), 1 mM NADPH (5 ml) and 1 mM 5.5'-Dithiobis (2-nitrobenzoic acid) (5 ml). GR from baker's

yeast (20 Units) was added to the mix to initiate the assay. The rate of 5-thio-2-nitrobenzoic acid formation was followed at 405 nm every 2-min intervals for 10 min using a spectrophotometer. Absorbance was proportional to the sum of GSH and GSSG present. It was compared to a standard curve generated with GSH. Results were expressed as pmol GSH/µg DNA.

Western blots

Western blot analyses for GR, GCLC, and GCLM were performed. Sertoli cell monolayers in 6-multiwell plates were disrupted by sonication in PBS (NaCl 137 mM, ClK 2.7 mM, Na_2HPO_4 8 mM, KH_2PO_4 1.5 mM, pH 7.4) containing 1% protease inhibitor cocktail (P-8340, Sigma Chemical Co., St. Louis, MO, USA) and phenylmethanesulfonyl fluoride 2 mM. Protein concentration was determined as described by Bradford et al. (37). Proteins (20-40 µg/lane) were resolved in 12.5% SDS/PAGE and then transferred onto polyvinylide difluoride membranes using a MiniTrans-blot Cell (Bio-Rad, Hercules, CA, USA). Membranes were then blocked by incubation with 5% skim milk in TBS (Tris 20 mM, ClNa 137 mM, pH 7.6) containing 0.1% Tween-20 (TBS-Tween) for 3 h at room temperature. After washing them with TBS-Tween, membranes were incubated overnight with a rabbit anti-GR antibody (1:6000 dilution) (38), generously provided by Dr J. Fujii; a rabbit anti-GCLC antibody (1:10.000 dilution) or a rabbit anti-GCLM antibody (1:12.000 dilution) (39), generously provided by Dr. T. Kavanagh. Membranes were then washed with TBS-Tween and incubated with peroxidase-conjugated goat anti-rabbit IgG (Cell Signal Technology Inc., Beverly, MA, USA) for 1-2 h. The antibody-antigen complexes were detected by chemiluminescence (Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG HRP-linked, Cell Signal Technology Inc, Beverly, MA, USA). Blots were subsequently reprobed with β-actin antiserum (monoclonal anti-actin antibody Clone AC-40, A-4700, Sigma) as a loading control. The intensities of the autoradiographic bands were estimated by densitometric scanning using NIH Image software (Scion Corporation, Frederick, MD, USA)

Activity of GST

Total enzymatic activity of GST was determined by the method of Habig and Jakoby (40). Activity was measured at room temperature by determining the rate of conjugate formation between GSH and 1-chloro-2,4-nitrobenzene in a spectrophotometer at 340 nm. Results are expressed as nmol of product/µg DNA/min. The change in absorbance is a linear function of enzyme concentration.

Statistical analyses

Data presented as means±SEM. Analysis of variance (ANOVA) was used to compare more than two experimental conditions, followed by adequate *post-hoc* tests (Dunnet's test for comparisons of all conditions against control, Tukey's test for comparisons of all conditions against each other). A one-sample t-test was used to compare results of an experimental condition to an expected theoretical value. Results were considered significantly different if $p<0.05$; all tests were two-tailed. Statistical analyses were performed using GraphPad version 4.00 (GraphPad Software, San Diego, CA, USA).

RESULTS

Identity of cell monolayers

Cells in primary culture were obtained following a validated method for isolation of Sertoli cells (41-43). We verified the identity of cell monolayers by immunocytochemistry. The vast majority of cells were positive for Sox-9 (Fig. 1A) and inhibin-α subunit (Fig. 1B); this combined expression is specific of Sertoli cells. Very few germ cells were identified by their morphology and negative reaction for the above-mentioned markers. No cell was positive to 3β-HSD, thus ruling out Leydig cell contamination (Fig. 1C). Extremely scarce myoid cells were identified by a positive reaction to alkaline phosphatase (Fig. 1D).

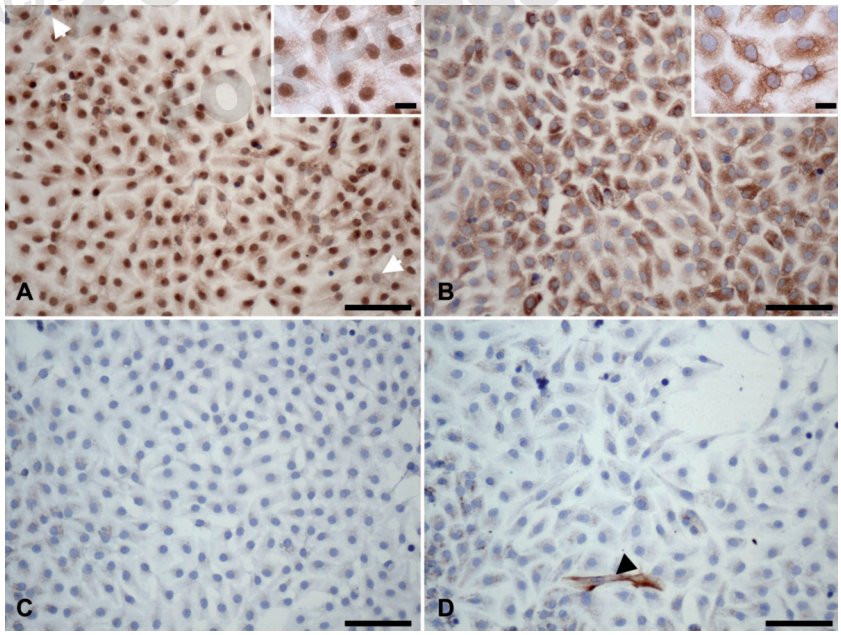

Fig. 1 - *Immunocytochemistry for Sox-9 (A), inhibin-α subunit (B), 3β-hydroxysteroid dehydrogenase (C), and placental alkaline phosphatase (D) in cell monolayers. Inserts are higher magnifications of figures A and B. Bars represent 50 µm in all Figures except for inserts to figures A and B, where they represent 10 µm. Arrows indicate germ cells in Figure A and peritubular cells in D.*

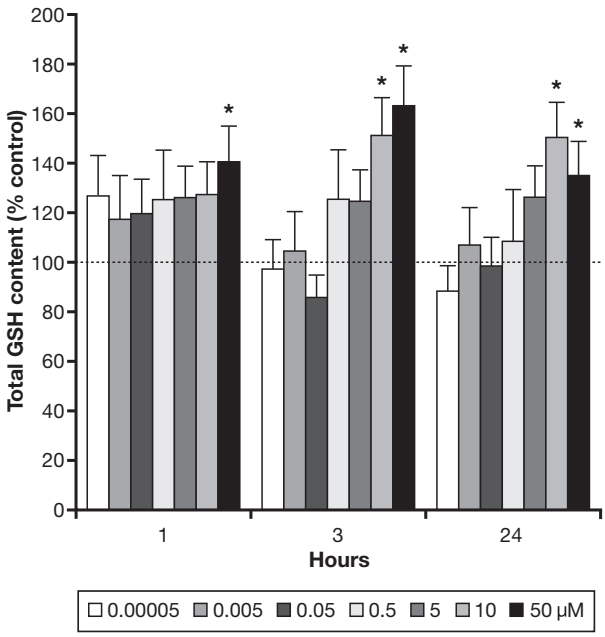

Fig. 2 - *Total glutathione (GSH) levels in Sertoli cell cultures treated for 1, 3, and 24 h with bisphenol A. To normalize for interassay variability, control value of GSH content was considered as 100% in each assay (dotted line). Results are the mean±SEM of total GSH content in treated conditions expressed as percentage of the untreated controls in 3 different experiments (one-sample t-test against the control value considered as 100%; *p<0.05).*

High doses of BPA affect Sertoli cell viability

Sertoli cells in primary culture were incubated for 24 h in medium containing various concentrations of BPA. BPA induced a significant diminution of cell viability when incubated at 100 µM (82.4±5.3% viable cells as compared to control, $p<0.05$), but not at 50 µM (95.2±6.1%, ns) or 10 µM (95.1±4.6%, ns). The 100-µM dose also affected Sertoli cell viability at 1 h (67.6±8.0%, $p<0.05$) and 3 h (80.9±11.8%, $p<0.05$). In all cases, results are the mean±SEM of 3 experiments with 6 replicates each, and were compared by ANOVA followed by Dunnett's test. These results indicate that high doses (100 µM) of BPA are severely toxic for Sertoli cells, whereas lower doses could be managed by Sertoli cells.

Intermediate BPA doses induce an increment in Sertoli cell GSH levels

Subsequently, we investigated the effect of intermediate and low doses of BPA on total GSH levels in primary Sertoli cell culture. Results in Figure 2 show a significant increment in intracellular GSH levels at 3 and 24 h with 10-µM BPA and at 1, 3, and 24 h with 50 µM. No changes in GSH content was observed with lower BPA concentrations. From these results we concluded that intermediate, but not low, doses of BPA are capable of inducing an increase in the GSH content in Sertoli cells. To investigate the mechanisms underlying the observed changes in GSH content, were performed further experiments using BPA at 10 and 50 µM.

Intermediate BPA doses induce an increase in GCL expression

To assess whether BPA-dependent increase in Sertoli cell GSH content was mediated by an upregulation of GCL, the rate-limiting enzyme involved in the *de novo* synthesis of GSH, we studied the effect of BPA on the expression of GCLC and GCLM protein subunits by Western blot. After incubation with BPA, both 10 µM and 50 µM, a rapid increase in GCLC was seen at 1 h, with a decrease to basal levels at 3 and 24 h (Fig. 3). Conversely, GCLM expression was significantly increased only at 24 h (Fig. 4). These results indicate that GCL expression is upregulated in Sertoli cells by intermediate doses of BPA, which might be explained by a rapid increase in GCLC and a later increase in GCLM.

Intermediate BPA doses increase GSH recycling

We also assessed whether BPA effect on GSH content in Sertoli cells was mediated by an increment in GR expression, which reduces GSSG to GSH. Incubation of Sertoli cells with both 10 µM did not affect GR expression, whereas 50 µM of BPA resulted in an increased GR expression at 1 h, but not at 3 h or 24 h (Fig. 5). These results indicate that the increase in Sertoli cell GSH content after incubation with 50 µM of BPA may also result from GSH recycling from GSSG.

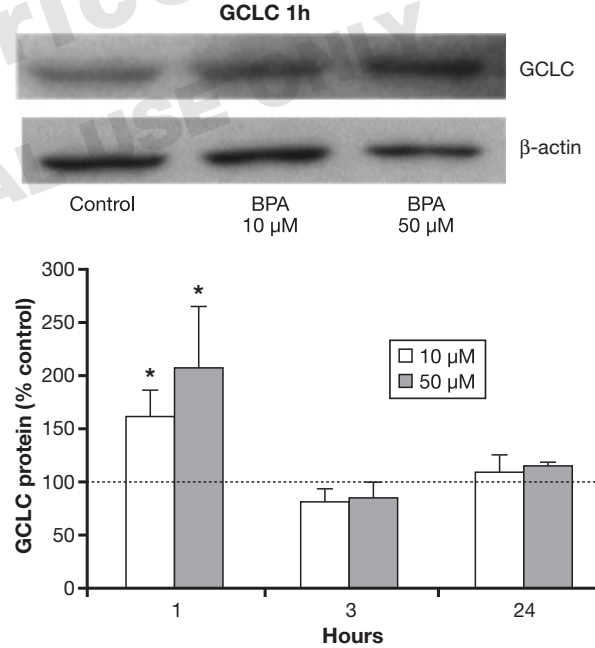

Fig. 3 - *Western blot analysis of glutamate-cysteine ligase catalytic subunit (GCLC) expression in Sertoli cells under bisphenol A (BPA) treatment (10 and 50 µM). Top: Representative Western blot. Bottom: Quantitative analysis: to normalize for interassay variability, control value of GCLC protein level, normalized to β-actin protein level, was considered as 100% in each assay (dotted line). Results are the mean±SEM of GCLC protein levels in treated conditions expressed as percentage of the untreated controls in 3 different experiments (one-sample t-test against the control value considered as 100%; *p<0.05).*

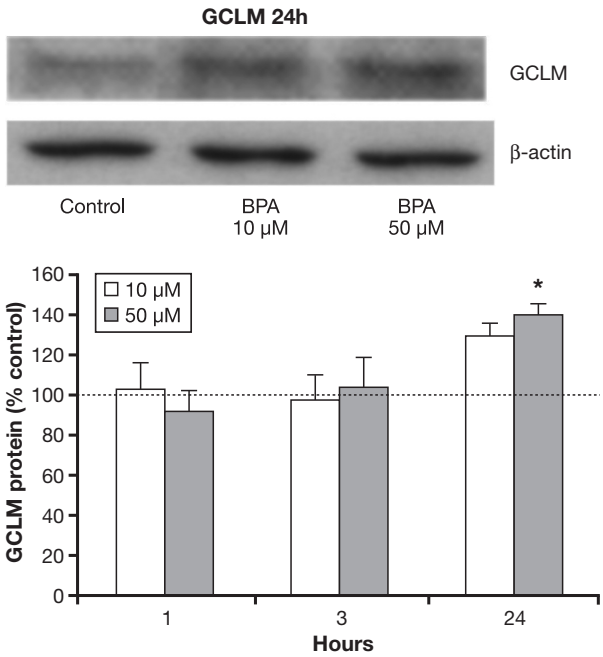

Fig. 4 - *Western blot analysis of glutamate-cysteine ligase modulatory subunit (GCLM) expression in Sertoli cells under bisphenol A (BPA) treatment (10 and 50 µM). Top: Representative Western blot. Bottom: Quantitative analysis: to normalize for interassay variability, control value of GCLM protein level, normalized to β-actin protein level, was considered as 100% in each assay (dotted line). Results are the mean±SEM of GCLM protein levels in treated conditions expressed as percentage of the untreated controls in 3 different experiments (one-sample t-test against the control value considered as 100%; *p<0.05).*

Intermediate BPA doses induce the activity of GST

GST conjugate pollutants to GSH thus reducing their toxic effect. We assessed the effect of BPA 50 µM on total GST enzymatic activity in Sertoli cells. BPA had a moderate but significant stimulatory effect on the activity of GST at 24 h (1 h: 106.4±8.0% as compared to control, ns; 3 h: 109.9±4.4 %, ns; 24 h: 116.1±3.7%, $p<0.05$). In all cases, results expressed as the mean±SEM of 3 experiments with 4 replicates each were compared to the expected theoretical value of 100% corresponding to the control condition by a one-sample t-test. These results suggest that BPA conjugation to GSH could also be envisaged to underlie Sertoli cell detoxification.

DISCUSSION

The toxic effects of BPA on testicular cell populations, and especially those of the seminiferous tubules, have been controversial. The mechanisms underlying deleterious effects claimed to exist with low dose exposure and no effects at higher doses have been difficult to explain. In this work, we have assessed the effect of a large range of BPA doses on post-natal Sertoli cells using a previously validated *in vitro* model (44). From our results, we conclude that high doses of BPA result in a severely deleterious effect on Sertoli cells, provoking a decrease in cell viability. Intermediate doses do not affect Sertoli cell viability and increase cell content of GSH, which has a well-known role in cell protection against oxidative stress and xenobiotic detoxification (20-23, 45). Interestingly, lower BPA doses were unable to trigger GSH production in our Sertoli cell model. Altogether, these results might give some insight into the potential mechanisms underlying the disparate effects observed in the reproductive axis after exposure to different concentrations of BPA in the environment or in different experimental conditions, based on the U-shaped curve of non-monotonic dose responses – i.e. toxic effects more evident at low and at high levels of exposure than at intermediate levels. We show that post-natal Sertoli cells are particularly sensitive to high doses of BPA that reduce their viability. They might be less sensitive to intermediate BPA doses because an increase in GSH cell content is triggered. Conversely, low doses do not elicit a response in GSH production; BPA is claimed to have deleterious, estrogen receptor-dependent effects at low concentrations; the absence of an increase in the GSH-dependent cell detoxification mechanisms at these low doses might render Sertoli cells more sensitive to low than to intermediate BPA concentrations. Conclusions from our results are applicable only to BPA exposure during the pubertal period, since we isolated Sertoli cells from 18-day-old rats. *In vi-*

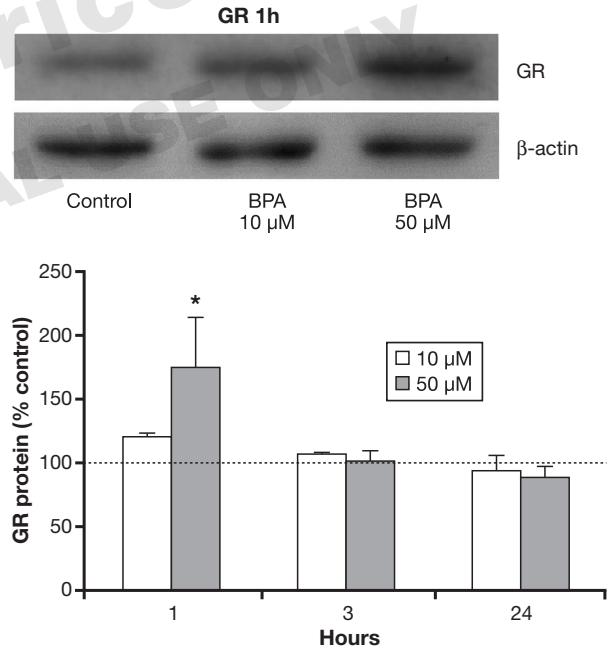

Fig. 5 - *Western blot analysis of glutathione reductase (GR) expression in Sertoli cells under bisphenol A (BPA) treatment (10 and 50 µM). Top: Representative Western blot. Bottom: Quantitative analysis: to normalize for interassay variability, control value of GR protein level, normalized to β-actin protein level, was considered as 100% in each assay (dotted line). Results are the mean±SEM of GR protein levels in treated conditions expressed as percentage of the untreated controls in 3 different experiments (one-sample t-test against the control value considered as 100%; *p<0.05).*

vo exposure to low doses of BPA in adult rats has been shown to affect spermatogenesis transiently (46, 47).
Normal Sertoli cell development and function is essential for the attainment of adequate sperm production in adult life. Deleterious effects on germ cells have been shown in animals exposed to high doses of BPA (48-50), whereas GSH has been reported to have significant protective functions in reproductive processes and spermatogenesis (26, 45, 51, 52). However, the mechanisms underlying spermatogenic cell protection by GSH is unknown. In this work, we provide evidence that Sertoli cells, while severely affected by high BPA doses, are capable of responding to the insult of moderate doses of BPA by activating their GSH-dependent defense mechanisms. Although our experimental model was not designed to test the final outcome on germ cell survival, a protective effect on germ cells by Sertoli cell-produced GSH could be foreseen. Further work applying the adequate experimental model is necessary to confirm this hypothesis.
Our results are in line with those reported by others demonstrating effects in primary or immortalized Sertoli cell cultures exposed to BPA at micromolar concentrations (48, 49, 53-56), equivalent to those released from plastic dental sealants in saliva, i.e. approximately 10-140 μM (57). BPA toxic effects at these concentrations are due to disruption of the blood-testis barrier, essentially formed by Sertoli cell tight junctions, when administered to post-natal rodents (16, 58), and to increased germ cell apoptosis (17). Interestingly, no such effect was observed in the latter study when somewhat lower concentrations of BPA were used (17).
BPA levels of 0.1 to 10 nM have been measured in human serum, ovarian follicular fluid and amniotic fluid and raised concern on their potential pathophysiological significance (59). Since it was beyond our scope, we did not test whether low BPA concentrations could be deleterious for germ cell development *in vitro*. However, we show that those levels did not elicit a protective GSH increase in our Sertoli cell model. An unsolved issue remains in that low BPA concentration may have adverse effects on Sertoli cells and spermatogenesis, which cannot be neutralized by Sertoli cell protective mechanisms. An alternative explanation is that although BPA may have negligible or no effects at low doses in primary cell culture, *in vivo* exposure is continuous and BPA can bioaccumulate, thus representing a possible threat to human reproductive health especially for occupational workers (60). Unfortunately, primary cell culture is not an adequate model for testing bioaccumulative effects since they cannot be maintained for longer than a week.
When exposed to intermediate doses of BPA, we show that Sertoli cells respond by activating their oxidative defense mechanisms. Our results are in line with those observed *in vivo*, where intratesticular GSH content increased after BPA administration for 5 days (61). Here we provide evidence that the increase in Sertoli cell GSH content is the consequence of stimulated GSH synthesis and recycling mediated by the induction of GCL and GR, respectively, and also by an increase in GST activity. A rapid increase in Sertoli cell GSH content is due, at least in part, to GSH synthesis and recycling. In fact, as early as 1 h after treatment, BPA induced an almost 2-fold increment in GCLC, the catalytic subunit of the rate-limiting enzyme involved in GSH synthesis, and in GR, involved in GSH recycling from GSSG. The increased GSH levels observed at 3 h, despite no increased levels of GCL or GR, may reflect the GSH previously synthesized and not yet consumed. This observation is in line with a lack of an early increase in the conjugation activity of GST. Another possibility, that we did not test in this work, is that GSH peroxidase (GPX) is activated. The activity of GPX is important for maintaining normal sperm activity in men (62). At 24-h treatment, the increased levels of GSH seem to be mainly explained by an upregulation of the modulatory subunit of GCL, GCLM. Differential regulation of the two GCL subunits has already been reported in other tissues (63, 64).
It is known that BPA induces the formation of GSH conjugates. Two hypotheses could explain the formation of GSH conjugates (65): the first one involves the formation of GSH conjugates by an oxidation of BPA into an arene epoxide and its subsequent conjugation to GSH, catalyzed by a GST. The second one, where GST is not involved, is a first-step CYP-mediated oxidation of BPA into 5-hydroxybisphenol A converted lately into bisphenol-o-quinone. This is an electrophile-reactive species that can covalently bind to GSH, a nucleophile compound, producing GSH-BPA conjugates. We tested the first hypothesis analyzing GST enzymatic activity in Sertoli cell culture; finding a moderate but significant increase in GST activity at 24 h. These results indicate that GST also is probably involved in Sertoli cell mechanisms of detoxification when exposed to BPA. However, a GST-independent mechanism cannot be ruled out. An increased GST activity is expected to result in a decrease of GSH intracellular content (20). In our experiments, BPA upregulated both GSH cell content and GST activity after 24-h incubation. GSH cell content results from the balance between its synthesis and its consumption. It therefore seems that the increased GSH synthesis resulting from GCL upregulation at 24 h prevails over GSH consumption resulting from GST conjugating activity.
In conclusion, high concentrations of BPA affect Sertoli cell viability and could underlie the deleterious effects already described on spermatogenesis. Intermediate doses of BPA induce an increment in GSH level in Sertoli cells, which suggests that Sertoli cells – and secondarily spermatogenesis – may be protected. Low doses of BPA do not elicit a GSH protective response; whether this represents a potential hazard to the male reproductive function remains to be elucidated.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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