Environmental Toxicology and Pharmacology 27 (2009) 410–414

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

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

Impact of the UV-B filter 4-(Methylbenzylidene)-camphor (4-MBC) during prenatal development in the neuroendocrine regulation of gonadal axis in male and female adult rats

M.E. Carou[a], M.L. Deguiz[a], R. Reynoso[a], B. Szwarcfarb[c], S. Carbone[c], J.A. Moguilevsky[b,c], P. Scacchi[a,c], O.J. Ponzo[a,*]

[a] Department of Physiology, Medicine School, University of Buenos Aires (UBA), Buenos Aires, Argentina
[b] Medicine School, University Favaloro, Buenos Aires, Argentina
[c] CONICET, Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 5 August 2008
Received in revised form 6 January 2009
Accepted 18 January 2009
Available online 31 January 2009

Keywords:
4-MBC
GnRH
LH
FSH
Glutamate
Aspartate
GABA

ABSTRACT

4-(Methylbenzylidene)-camphor (4-MBC), a UV-B ray filter, is an endocrine disruptors (ED). Our goal was to study the effect of 4-MBC on the neuroendocrine parameters that regulate reproduction in adult female and male rats that received this disrupter during prenatal development. The 4-MBC was administered (sc) to female rats (FO) since pregnancy onset, in doses of 100 mg/kg every other day. The litters (F1) were sacrificed at 70 days to determine gonadotrophin serum levels and also GnRH and the amino acids glutamate, aspartate and GABA release from the hypothalamus. The male litter rats (F1) present at adult age a decrease in serum LH and FSH concentration and so also GnRH, excitatory amino acids and GABA hypothalamic secretion. The female litters (F1) rats present at adult age an increase in serum LH and FSH concentration, whereas hypothalamic GnRH release was not modified. In these animals a significant increase of hypothalamic aspartate release as well as GABA secretion decrease were observed. Glutamate secretion was not modified. All these changes were accompanied by an advance (3 days) on the vaginal opening in 4-MBC rats group. In conclusion, prenatal administration of 4-MBC disrupts the gonadal axis in a sexual dimorphic mode that could be connected with the physiological sexual differences in the development of gonadotrophin secretion hypothalamic control mechanisms.

1. Introduction

4-(Methylbenzylidene)-camphor (4-MBC) is a UV ray filter normally used in cosmetic products such as sunscreens, to protect the skin from ultraviolet rays UV-B. This substance belongs to a group of chemicals called endocrine disrupters (EDs) that have been released into the environment in great quantities in past years and can also be found in natural waters and in fish (Poiger et al., 2004). Many of these substances either synthetic or natural have oestrogenic and/or antiandrogenic activity, and this would lead to serious alterations in the ecosystem and especially in the normal development of the human endocrine axis.

The EDs have been linked to the function of the reproductive and thyroid systems (Schmutzler et al., 2004; Maerkel et al., 2007). They act as agonists or antagonists of hormone receptors and would have some effect on the mRNA expression of steroid hormone receptors and genes regulated by these hormones (Schmutzler et al., 2004; Maerkel et al., 2007; Klann et al., 2005; Durrer et al., 2005).

4-MBC proved to generate alterations in the reproductive axis through a weak oestrogenic effect in rats after oral and subcutaneous administration (Schlumpf et al., 2001, 2004a; Seidlová-Wuttke et al., 2006a,b); for example rats exposed to 4-MBC during the pubertal stage showed an increase in uterus (Schlumpf et al., 2001, 2004b; Seidlová-Wuttke et al., 2006b; Tinwell et al., 2002) and ovarian weight (Schlumpf et al., 2004b). Also chronic administration of ED since the embryonic stage produced a delay of puberty onset and ontogenic differences in the testicular weight in males (Schlumpf et al., 2004b).

Subtypes of oestrogen receptor mRNA expression have different distribution in the rat central nervous system (Shughrue et al., 1997). Oestrogens regulate the gonadal axis acting on peripheral organs, but also change the release of GnRH and gonadotrophins (Herbison, 1998). One of the mediators of these changes is the hypothalamic amino acid system in the regulation of GnRH secretion (Brann and Mahesh, 1992; Jarry et al., 1992). 4-MBC is a partial agonist of alpha and beta oestrogen receptors (ERα and ERβ), but

* Corresponding author at: Laboratorio de Endocrinología, Departamento de Fisiología, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155, Piso 7, CP 1113 Buenos Aires, Argentina.Tel.: +54 11 5950 9500×2146.
E-mail address: oponzo@fmed.uba.ar (O.J. Ponzo).

1382-6689/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.etap.2009.01.007

shows a preference for ERβ (Klann et al., 2005; Schlumpf et al., 2004a; Seidlová-Wuttke et al., 2006a; Mueller et al., 2003), whereas its metabolite, produced by hepatic first-pass in oral (Volkel et al., 2006), but not dermal (Schauer et al., 2006) administration, shows preference for alpha type receptors. During the prenatal development the hypothalamic and hipophyseal sensitivity could be altered by the EDs, due to the above mentioned estrogenic effects of 4-MBC.

The aim of this work is to study the effect of ED on neuroendocrine parameters, which regulate the reproduction in male and female rats while administered during prenatal.

2. Materials

2.1. Animals and drug used

Wistar rats used were provided by the Department of Physiology, School of Medicine, University of Buenos Aires. Animals were raised under light (lights on from 7 a.m. to 7 p.m.), temperature (22 to 24 °C) and humidity controlled conditions. Rats were fed on balanced diet and water *ad libitum* until time of sacrifice and were weighed daily. In all cases, adequate measures were taken to minimize pain or discomfort, in accordance with the principles and procedures outlined in the European Communities Council Directives (86/609/EEC) and FRAME guidelines (*FRAME Reduction Committee November* 1999).

Each time ED was prepared, 4000 mg of 4-MBC (*BASF*, *Ludwigshafen*, *Germany*, 99.9% *purity*) was dissolved in 1 ml ethyl alcohol at 100% and left at room temperature for the evaporation of the latter. Then the solution was diluted in 40 ml olive oil to get a final concentration of 100 mg/kg (final volume of 0.1 ml per 100 mg body weight was injected).

3. Methods

3.1. Experimental scheme

Female young adult rats with weights from 240 to 250 g (F0) received ED during pregnancy (21 days). For that purpose, each female was put to mate with a male in individual cages for 4 days (one estrous cycle). The animals were daily examined for a mucous plug, taking as first day of pregnancy the day the plug was formed and detected. That first day the female was estranged from the male and began to receive the ED.

4-MBC was subcutaneously (sc) administered in doses of 100 mg/kg, every other day to pregnant female rats (F0), since the mucous plug were detected (gestational day 0) until the litter (F1) was born (gestational day 21). Every animal received 0.1 ml per 100 g body weight of the solution. This dose was well tolerated in adult F0 rats. Control animals (Co) only received the vehicle. In all case, the site of injection was changed to avoid an inflammatory reaction. We chose the subcutaneous pathway because this route is the most similar to that use by humans that apply sunscreens on their skin; the dermal application was not used because in rats the pelage makes the absorption erratic. The doses were chosen according with the bibliography (Tinwell et al., 2002), in this experiment the authors did not observed toxicity at the doses used by us.

Adult offspring (F1) of both sexes, of F0 female rats, were used 10–12 animals per group, with a body weight of about 250 g and 70 days old (adults). All pups were weaned at 21 days old. Adult female offspring (F1) were cycled every day during four oestrous cycles by vaginal smear before sacrifice on day one of diestrous. Also vaginal opening day in female rats was observed.

At 70 days animals of both sexes were weighed and sacrificed by decapitation. Trunk blood (3 ml) was gathered and storage at −70 °C for later gonadotrophin serum determination (LH and FSH). Hypothalamuses were dissected for *in vitro* tissue incubation to study GnRH and excitatory and inhibitory amino acids release. In females, uterus were also dissected and weighed immediately after sacrifice.

3.2. Hypothalamic incubation

Hypothalamus samples with anterior preoptic and basal media areas (APOA–MBH) were dissected with the help of a stereomicroscope. These areas were bordered laterally by the hypothalamic groove, rostrally 3 mm previous to the optic chiasm, caudally by the mamillary body. The thickness of each sample was less than 2 mm. The median eminence was included in this tissue. Tissues were immediately weighed, weighing between 10 and 11 mg each one.

Later, the APOA–MBH were placed in plastic chambers containing 350 μl of Earle's saline medium with glucose (1 mg/ml) and bacitracin (20 mM), pH: 7.4. Each chamber was incubated in a Dubnoff shaker at 37 °C with constant shaking (60 cycles/min) under an atmosphere of 95% O_2, 5% CO_2. After 30 min of preincubation the medium was discarded and 350 μl from the buffer was immediately added to start incubation for 60 min. Then the incubation medium was collected (basal samples) and immediately frozen at −80 °C for GnRH and excitatory and inhibitory amino acid determination. Tissue viability was confirmed after incubation, by adding KCl (56 mM) to the medium and obtaining an increase in the GnRH release (Carou et al., 2008).

Table 1

	Control	4-MBC 100
Females		
No. of litters	12	10
No. of animals investigated	11	10
Body weight PND 70 (g)	266.88 ± 5.95	300.57 ± 20.42
Males		
Number of litters	13	12
Number of animals investigated	12	12
Body weight PND 70 (g)	344.18 ± 6.78	349.49 ± 6.83

3.3. Determination of LH, FSH, and GnRH

Serum gonadotrophins were determined in duplicate by using a double antibody radioimmunoanalysis (RIA) and precipitated with polyethylene glycol. The material was kindly provided by the NIAMD rat pituitary program. Results were expressed in serum ng/ml, in accordance with the referenced preparation (rat LH RP-1 and rat FSH RP-1). Intra and inter-assay coefficients of variation were 6% and 11%, respectively. All samples were analyzed in the same assay (Carou et al., 2008).

Determination of GnRH release in the samples obtained from the incubation medium was carried out by single antibody RIA and precipitated with ethyl alcohol. Antiserum was kindly handed over by Dr. Wuttke (Goettingen, Germany). Results were expressed in pg/ml/10 mg of fresh tissue. Limit for GnRH detection was 0.2 pg/ml. Intra and inter-assay coefficients of variation were 7% and 14%, respectively.

3.4. Determination of amino acids

The concentrations of glutamate (GLU), aspartate (ASP) and GABA were determined by HPLC after derivatization with phenylisothiocyanate and UV detection at 254 nm, as previously described (Jarry et al., 1992). The drugs used did not interfere in the derivatization process. The mobile phase consisted of 0.57 M sodium acetate buffer (pH 6.5) containing 10% acetonitrile (Sintorgan, Buenos Aires, Argentina). Amino acid standards used were: ASP, GLU and GABA (Sigma Chemical Co St. Louis, MO, USA). Mean inter - and intra-assay coefficients of variation were 4.0, 5.0 and 8.0%, respectively. Results are expressed as pmol/100 μl medium. The detection limit was 10 pmol for GLU, 20 pmol for ASP and 5 pmol for GABA.

3.5. Statistical analysis

Results are expressed as the mean ± S.E.M. Significance was assessed by analysis of variance (ANOVA) and the Bonferroni post-test. Student *t*-test was also used and a $p < 0.05$ was considered significant.

4. Results

The number of pregnancy achieved by control and 4-MBC exposed female rats were not different. So also, there was no difference in litter size between control and treated groups. In males and females 4-MBC administration did not modify body weight. Table 1 indicate number of litters and animals investigated per group and body weight al postnatal day 70 in each sex.

In F1 adult male rats a decrease in serum LH ($p < 0.02$) and FSH ($p < 0.001$) concentrations was observed (Fig. 1A). The GnRH hypothalamic secretion was reduced also with 4-MBC 100 mg/kg ($p < 0.001$) (Fig. 2A). 4 MBC induced a significant reduction in the hypothalamic release of excitatory amino acids aspartate ($p < 0.001$) and glutamate ($p < 0.001$), and inhibitory amino acid GABA ($p < 0.001$) (Fig. 3A).

In F1 adult cycling female rats uterine weight was not modified by 4-MBC (Control: 130.67 ± 5.20, 4-MBC: 120.32 ± 6.68 mg/100 g animal). Vaginal opening was on average 3 days before in 4-MBC rats than in controls (Control: day 35 ± 0.58, 4-MBC: day 32 ± 0.77, $p < 0.001$). On the other hand 4-MBC produced an increment of serum LH ($p < 0.001$) and FSH ($p < 0.01$) concentrations (Fig. 1B). Hypothalamic GnRH secretion was not significantly modified by 4-MBC treatment (Fig. 2B). In these 4-MBC treated

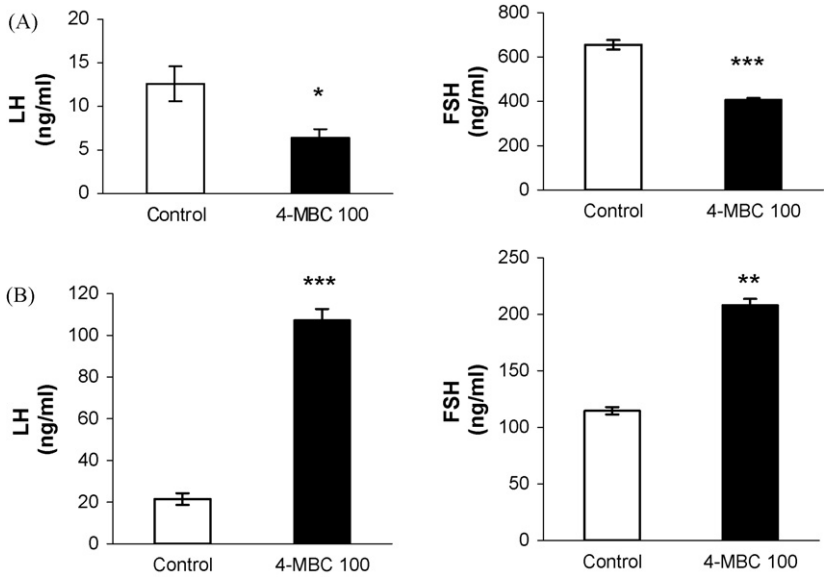

Fig. 1. Serum gonadotrophins concentration in adult male (A) and female (B) rats ($n = 10–12$ per group) exposed during prenatal development to 4-MBC 100 mg/kg (4-MBC 100) ($^{*}p < 0.02$, $^{**}p < 0.01$ and $^{***}p < 0.001$).

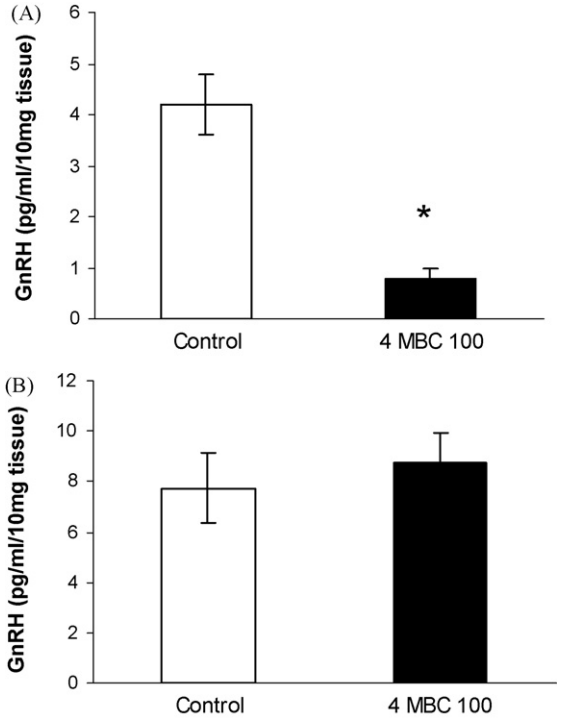

Fig. 2. Hypothalamic GnRH secretion in adult male (A) and female (B) rats ($n = 10–12$ per group) exposed during prenatal development to 4-MBC 100 mg/kg (4-MBC 100) ($^{*}p < 0.001$).

animals, hypothalamic aspartate secretion increased (<0.01), glutamate secretion was not modified and GABA decreased ($p < 0.001$) (Fig. 3B).

5. Discussion

Endocrine disrupters alter the endocrine system. 4-(Methylbenzylidene)-camphor (4-MBC) belongs to this group of substances and has shown to have effects on the reproductive axis either *in vitro* or *in vivo*, prompting physiological mechanisms – in general – controlled by oestrogens. Humans would be in contact with this type of EDs when applying sunscreens on their skin that contain this drug in their formulas (Schauer et al., 2006). For this reason we chose the subcutaneous pathway to administer the drug, a similar path to the topical route used in daily life. In addition, we avoided the hepatic first-pass that the drug endures when administered orally (Volkel et al., 2006).

In the present work we analyzed the impact of 4-MBC on male and female adult rat reproductive axis regulation, exposed during the prenatal development. The results show that intrauterine exposure to 4-MBC modified the neuroendocrine parameters of gonadal axis in both sexes of adult rats. This finding is consistent with a recent publication describing a change in gonadal weight and sexual steroids concentration (Durrer et al., 2005; Schlumpf et al., 2004b). In our model, the male rats receiving 4-MBC, have serum LH and FSH and also in vitro hypothalamic GnRH secretion reduction. This is in concordance with the inhibition of male gonadal axis produce in adult rats, by direct administration of low dose 4-MBC doses (Carou et al., 2008). Nevertheless, in humans it has been shown that topical application of this drug together with other disrupters does not change the gonadotropin levels but causes a decrease in testosterone levels (Janjua et al., 2004).

In males, oestrogens take part in the negative feedback mechanisms at hypothalamic level (Mooradian et al., 1987; Rochira et al., 2006); moreover oestradiol causes the repression of GnRH promoter (Roy et al., 1999). Since it has been stated that 4-MBC has an oestrogenic action, a probable mechanism of this disrupter would be the facilitation of the negative feedback mechanism produced by oestrogen action in the hypothalamus. Moreover, another probable mechanism that might be participating in these alterations, would be changes in the expression of oestrogen receptors in the hypothalamus, induced by the 4-MBC. The GnRH neuron of the preoptic area, in male rats, expresses oestrogen receptor beta mRNA and its protein (Hrabovszky et al., 2001), probably responsible for the oestrogen negative feedback. Micromolar concentrations of 4-MBC generate a surge in the amount of ER mRNA similar to the one induced by higher concentrations than 1 nmol/L of 17-beta oestradiol (Klann et al., 2005). All the above mentioned would support the hypothesis of a possible exacerbation of the negative hypothalamic feedback with the subsequent decrease of GnRH and gonadotrophic hormone release as described in our animals exposed to 4-MBC.

On the contrary, in adult female rats we observed a rise in serum LH and FSH concentration with 4-MBC. However the “in vitro”

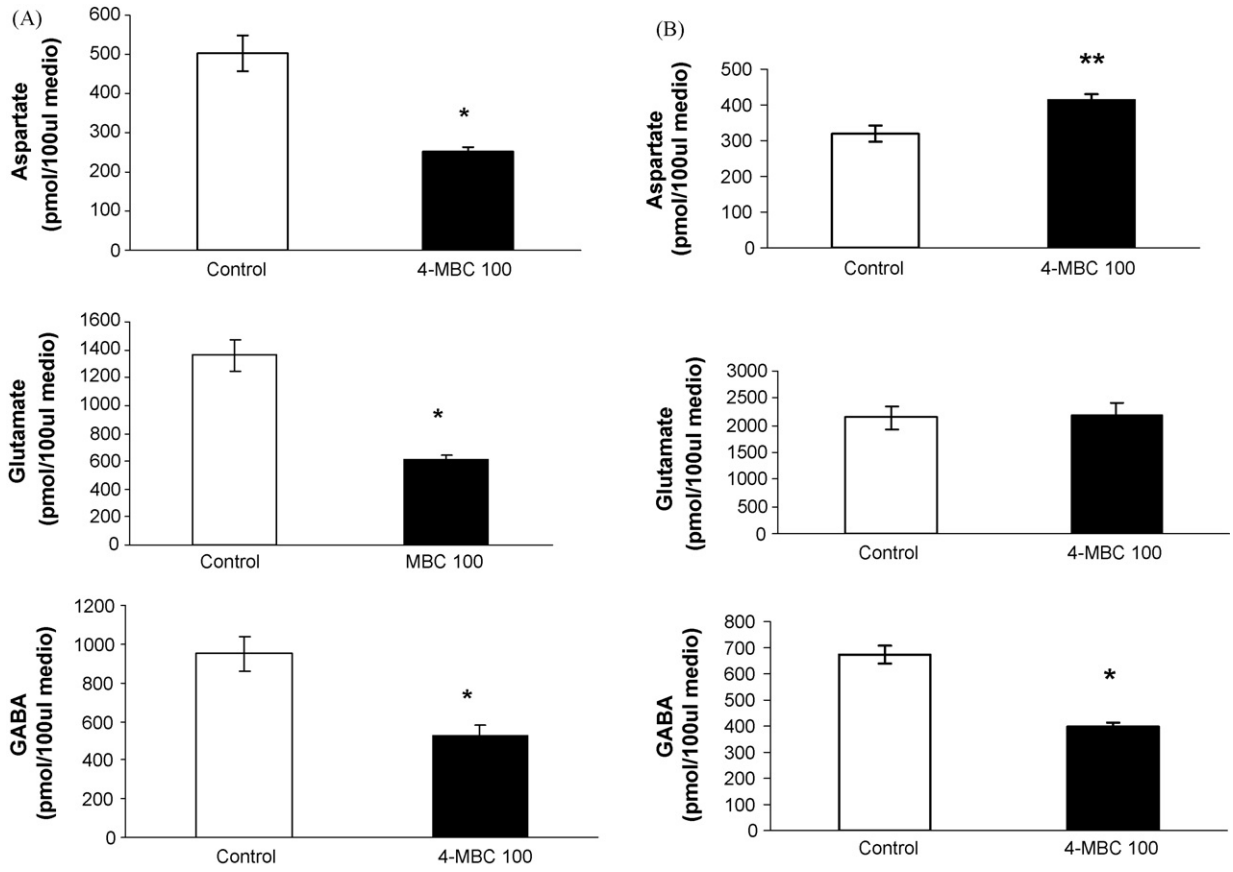

Fig. 3. Hypothalamic excitatory and inhibitory amino acids secretion in male (A) and female (B) rats exposed during prenatal development to 4-MBC 100 mg/kg (4-MBC 100) ($n = 10–12$ per group) ($^{*}p < 0.001$ and $^{**}p < 0.01$).

hypothalamic GnRH secretion did not show a statistically significant increase. A possible explanation for this is that 4-MBC might cause a change in the pulse frequency of the GnRH neuron, which is not under study in this work. This situation could not be reflected in the total amount of hormone in the culture media, due to the fact that alterations in the pulsatility of GnRH or LH are not always reflected in changes in hormone concentrations (Dong et al., 1994; Kaji et al., 1998).

Probably, the excitatory 4-MBC effect in females could also be produced by a direct action of ED on the gonadotrophin adenohypophyseal secretion. ER expression and its mRNA in the pituitary gland, differs between male and female rodents (Chen and Tu, 1992). ER expression is modified during different stages of life like the intrauterine period (Vaillant et al., 2002). Perhaps, the intrauterine exposure to 4-MBC could alter the ER expression in adenohypophisis in female rats, providing a positive feedback on gonadotrophin secretion.

On the other hand, the different response to 4-MBC among male and female rats might be a result of differences in intrinsic regulation of both sexes. It is well known that amino acids system regulate the adult hypothalamic GnRH secretion, being glutamate and aspartate excitatory and GABA inhibitory (Moguilevsky and Wuttke, 2001; Gore and Roberts, 1994; Brann and Mahesh, 1994; Donoso et al., 1990). In fact, we found that 4-MBC produce dimorphic changes on the amino acid system in rats of both sexes, producing a decrease in the levels of the excitatory amino acids glutamate and aspartate in male rats and an increase of aspartate, together with a decrease in GABA amino acid, in female rats. This fact could be an additional evidence for this point of view.

A few published works have reported the use of the subcutaneous pathway to administer the drug, and it was not administered during prenatal stage (Tinwell et al., 2002). The effect produced by the oral administration of 4-MBC *in vivo* involves its metabolite [3-(4-carboxybenzylidene)-6-hydoxycamphor]. The same would have more affinity for ERα than 4-MBC. This metabolite is produced by the hepatic metabolism, mainly in the hepatic first-pass endured by the drug when orally administered (Volkel et al., 2006). This metabolic pass does not occur with dermal application in humans, and the plasma levels of 4-MBC are higher than with oral administration (Schauer et al., 2006). Therefore, in our work, where the drug was administered subcutaneously, this metabolite may have had a lesser role. Maybe this is the reason why we did not find the uterotrophic effects seen in other works (Maerkel et al., 2007), since this effect is related to ER alfa (Harris et al., 2002) and the 4-MBC has a preference for ER beta (Klann et al., 2005; Seidlová-Wuttke et al., 2006a; Schlumpf et al., 2004b; Mueller et al., 2003), in spite of it must be note that, the uterotrophic effects were observed on immature 4-MBC exposed rats.

These results, obtained in animals exposed to the drug during the prenatal development that reached the adult stage, shown an alteration of the neuroendocrine-gonadal axis regulation in male and female rats. It remains to be studied if the alteration on neuroendocrine-gonadal axis regulation might have, in the long term, any effect on the reproductive capacity.

Conflict of interest

The authors declare that there are no conflicts of interest. The authors declare that have not any financial and personal relationships with other people or organisations that could inappropriately influence their work.

Acknowledgements

This work has been supported by grants from Agencia de Promoción Científica y Técnica (BID 1728. PICT 2003, 05/13509) and University of Buenos Aires (UBACYT M816 and UBACYT M006). We thank Angela Ciocca for the manuscript revision.

References

Brann, D.W., Mahesh, V.B., 1992. Excitatory amino acid regulation of gonadotrophin secretion: modulation by steroid hormone. J. Steroid Biochem. Mol. Biol. 41, 847–850.

Brann, D.W., Mahesh, V.B., 1994. Excitatory amino acids: function and significance in reproduction and neuroendocrine regulation. Front. Neuroendocrinol. 15, 3–49.

Carou, M.E., Ponzo, O.J., Cardozo Gutierrez, R.P., Szwarcfarb, B., Deguiz, M.L., Reynoso, R., Carbone, S., Moguilevsky, J.A., Scacchi, P., 2008. Low dose 4-MBC effect on neuroendocrine regulation of reproductive axis in adult male rats. Environ. Toxicol. Pharmacol. 26 (2008), 222–224.

Chen, T.J., Tu, W.W., 1992. Sex differences in estrogen and androgen receptors in hamster brain. Life Sci. 50, 1639–1647.

Dong, Q., Bergendahl, M., Huhtaniemi, I., Handelsman, J., 1994. Effect of undernutrition on pulsatile luteinizing hormone (LH) secretion in castrate and intact male rats using an ultrasensitive immunofluorometric LH assay. Endocrinology 135, 745–750.

Donoso, A.O., López, F.J., Negro-Vilar, A., 1990. Glutamate receptors of the non-N-methyl-D-aspartic acid type mediate the increase in luteinizing hormone-releasing hormone release by excitatory amino acids in vitro. Endocrinology 126, 414–420.

Durrer, S., Maerkel, K., Schlumpf, M., Lichtensteiger, W., 2005. Estrogen target gene regulation and coactivator expression in rat uterus after developmental exposure to the Ultraviolet filter 4-methylbenzylidene camphor. Endocrinology 146, 2130–2139.

Gore, A.C., Roberts, J.L., 1994. Regulation of gonadotropin-releasing hormone gene expression by the excitatory amino acids kainic acid and N-methyl-D L-aspartate in the male rat. Endocrinology 134, 2026–2031.

Harris, J.A., Katzenellenbogen, J.A., Katzenellenbogen, B.S., 2002. Characterization of the biological roles of the estrogen receptors, ER alpha and ER beta, in estrogen target tissues *in vivo* through the use of an ER alpha-selective ligand. Endocrinology 143, 4172–4177.

Herbison, A.E., 1998. Multimodal influence of estrogen upon gonadotropin-releasing hormone neurons. Endocr. Rev. 19, 302–330.

Hrabovszky, E., Steinhauser, A., Barabás, K., Shughrue, P.J., Petersen, S.L., Merchenthaler, I., Liposits, Z., 2001. Estrogen receptor beta-immunoreactivity in luteinizing hormone-releasing hormone neurons of the rat brain. Endocrinology 142, 3261–3264.

Janjua, N.R., Mogensen, B., Andersson, A.M., Petersen, J.H., Henriksen, M., Skakkebaek, N.E., Wulf, H.C., 2004. Systemic absorption of the sunscreens Benzophenone-3, Octyl-methoxycinnamate, and 3-(4-Methyl-Benzylidene) Camphor after whole-body topical application and reproductive hormone levels in humans. J. Invest. Dermatol. 123, 57–61.

Jarry, H., Hirsch, B., Leonhardt, S., Wuttke, W., 1992. Amino acid neurotransmitter release in the preoptic area of rats during the positive feedback actions of estradiol on LH release. Neuroendocrinology 56, 133–140.

Kaji, H., Saito, S., Shitsukawa, K., Irahara, M., Aono, T., 1998. The endogenous feeding suppressant, 2-buten-4-olide, impairs the pulsatile secretion of luteinizing hormone through endogenous opioid peptides. Eur. J. Endocrinol. 138, 198–205.

Klann, A., Levy, G., Lutz, I., Muller, C., Kloas, W., Hildebrandt, J.P., 2005. Estrogen-like effects of ultraviolet screen 3-(4-methylbenzylidene)Camphor (Eusolex 6300) on cell proliferation and gene induction in mammalian and amphibian cells. Environ. Res. 97, 274–281.

Maerkel, K., Durrer, S., Henseler, M., Schlumpf, M., Lichtensteiger, W., 2007. Sexually dimorphic gene regulation in brain as a target for endocrine disrupters: Developmental exposure of rats to 4-Methylbenzylidene camphor. Toxicol. Appl. Pharmacol. 218, 152–165.

Moguilevsky, J.A., Wuttke, W., 2001. Changes in the control of gonadotropin secretion by neurotransmitters during sexual development in rats. Exp. Clin. Endocrinol. Diab. 109, 188–195.

Mooradian, A.D., Morley, J.E., Korenman, S.G., 1987. Biological actions of Androgens. Endocr. Rev. 8, 1–28.

Mueller, S.O., Kling, M., Arifin Firzani, P., Mecky, A., Duranti, E., Shields-Botella, J., Delansorne, R., et al., 2003. Activation of estrogen receptor alpha and ERbeta by 4- methylbenzylidene-camphor in human and rat cells: comparison with phyto- and xenoestrogens. Toxicol. Lett. 142, 89–101.

Poiger, T., Buser, H.R., Balmer, M.E., Bergqvist, P.A., Muller, M.D., 2004. Occurrence of UV filter compounds from sunscreens in surface waters: regional mass balance in two Swiss lakes. Chemosphere 55, 951–963.

Rochira, V., Zirilli, L., Genazzani, A.D., Balestrieri, A., Aranda, C., Fabre, B., Antunez, P., et al., 2006. Hypothalamic-pituitary-gonadal axis in two men with aromatase deficiency: evidence that circulating estrogens are required at the hypothalamic level for the integrity of gonadotropin negative feedback. Eur. J. Endocrinol. 155, 513–522.

Roy, D., Angelini, N.L., Belsham, D.D., 1999. Estrogen directly respresses gonadotropin-releasing hormone (GnRH) gene expression in estrogen receptor-alpha (ER-alpha)- and ER beta-expressing GT1-7 GnRH neurons. Endocrinology 140, 5045–5053.

Schauer, U.M.D., Volkel, W., Heusener, A., Colnot, T., Broschard, T.H., von Landerberg, F., Dekant, W., 2006. Kinetics of 3-(4-methylbenzylidene) camphor in rats and humans after dermal application. Toxicol. Appl. Pharmacol. 216, 339–346.

Schlumpf, M., Cotton, B., Conscience, M., Haller, V., Steinmann, B., Lichtensteiger, W., 2001. In vitro and in vivo estrogenicity of UV screens. Environ. Health Perspect. 109, 239–244.

Schlumpf, M., Jarry, H., Wuttke, W., Ma, R., Lichtensteiger, W., 2004a. Estrogenic activity and estrogen receptor B binding of the UV filter 3-benzylidene camphor Comparison with 4-methylbenzylidene camphor. Toxicology 199, 109–120.

Schlumpf, M., Schmid, P., Durrer, S., Conscience, M., Maerkel, K., Henseler, M., Gruetter, M., et al., 2004b. Endocrine activity and developmental toxicity of cosmetic UV filtres- and update. Toxicology 205, 113–122.

Schmutzler, C., Hamann, I., Hofmann, P.J., Kovacs, G., Stemmler, L., Mentrup, B., Schomburg, L., et al., 2004. Endocrine active compounds affect thyrotropin and thyroid hormone levels in serum as well as endpoints of thyroid hormone action in liver, heart and kidney. Toxicology 205, 95–102.

Seidlová-Wuttke, D., Christoffel, J., Rimoldi, G., Jarry, H., Wuttke, W., 2006a. Comparison of effects of estradiol with those of octylmethoxycinnamate (OMC) and 4-Methylbenzylidene Camphor (4-MBC) on fat tissue, lipids and pituitary hormones. Toxicol. Appl. Pharmacol. 214, 1–7.

Seidlová-Wuttke, D., Jarry, H., Christoffel, J., Rimoldi, G., Wuttke, W., 2006b. Comparison of effects of estradiol (E2) with those of octylmethoxycinnamate (OMC) and 4-Methylbenzylidene Camphor (4-MBC) - 2 filters of UV light - on several uterine, vaginal and bone parameters. Toxicol. Appl. Pharmacol. 210, 246–254.

Shughrue, P.J., Lane, M.V., Merchenthaler, I., 1997. Comparative distribution of estrogen receptor-alpha and -beta mRNA in the rat central nervous system. J. Comp. Neurol. 388, 507–525.

Tinwell, H., Lefevre, P.A., Moffat, G.J., Burns, A., Odum, J., Spurway, T.D., Orphanides, G., et al., 2002. Confirmation of uterotrophic activity of 3(4-methylbenzylidene) camphor in the immature rat. Environ. Health Perspect. 110, 533–536.

Vaillant, C., Chesnel, F., Schausi, D., Tiffoche, C., Thieulant, M.L., 2002. Expression of estrogen receptor subtypes in rat pituitary gland during pregnancy and lactation. Endocrinology 143, 4249–4258.

Volkel, W., Colnot, T., Schauer, U.M., Broschard, T.H., Dekant, W., 2006. Toxicokinetics and biotransformation of 3-(4-methylbenzylidene)camphor in rats after oral administration. Toxicol. Appl. Pharmacol. 216, 331–338.