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Different effects by sex on hypothalamic–pituitary axis of prepubertal offspring rats produced by in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP)

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ABSTRACT

This study investigated the effect of pre and perinatal exposure to di-(2-ethylhexyl) phthalate (DEHP) on the neuroendocrine parameters that regulate reproduction in prepubertal male and female rats. DEHP at doses of 3 and 30 mg/kg bw/day was administered orally in the drinking water to dam rats since pregnancy onset until the moment of pups sacrifice at 15 days of age. In these animals gonadotropin serum level and the hypothalamic contents of the amino acids aspartate, glutamate and gamma-aminobutyric acid were determined. No changes in gonadotropin levels and amino acid neurotransmitters were detected at the low dose in both sexes. However, DEHP administered at high dose (30 mg/kg bw/day) to dams produced a significant decrease in the inhibitory neurotransmitter GABA and an increase in the stimulatory neurotransmitter aspartate in prepubertal male offspring rats. These modifications were accompanied by gonadotropin serum levels increase. On the contrary, in treated female rats this chemical increased both, aspartate and GABA, which exert a characteristic stimulatory action on gonadotropin in 15-day-old normal females. This study provides new data about changes produced by DEHP on the hypothalamic amino acid neurotransmitters involved in the neuroendocrine reproductive regulation, in prepubertal male and female rat offspring from dams exposed during gestational and lactational periods. These alterations induced by DEHP exposure could be related to the gonadotropin modifications also described in this work, and with changes in the production of sexual hormones previously reported by other authors.

1. Introduction

Reproductive abnormalities in laboratory and wildlife animals were associated with exposure to industrial chemicals. This fact generated public concern that this chemicals, named "environmental oestrogens" or "xenoestrogens may impair human reproductive health (Akingbemi et al., 2001; Sharpe, 2001). Several high weight phthalates, including the widely used plasticizer di-(2-ethylhexyl) phthalate (DEHP), have been early identified as endocrine disruptors capable of altering male sexual differentiation in animals (Sharpe, 1995; Joblin, 1995; Sharpe, 2001; Foster et al. 2001; Gray et al., 2000; Akingbemi et al., 2001). DEHP is used in a variety of polyvinyl chloride-based consumer products including infant toys, food wraps, cosmetics and many surgical and medical consumables and devices such us tubing, blood bags and dialysis equipment (Heudorf et al., 2007; Latini et al., 2003; NTP-CERHR, 2006). This substance can leach readily from plastic products into foods, beverages or directly into body fluids (Latini, 2000; Lovekamp-Swan and Davis, 2003; Gartner et al., 2009). Consequently the potential for non-occupational exposure to DEHP is high and humans are daily exposed to DEHP through ingestion, inhalation and by dermal contact (NTP-CERHR, 2006; Schettler, 2006). Moreover, occupational and medical exposure increase body burden levels (Calafat et al., 2004; Calafat and McKee, 2006; Doull et al., 1999; Faouzi et al., 1999; Koch et al., 2006; Latini et al., 2003; Pak et al., 2007) reaching much higher concentrations in this population.

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DEHP induces anti-androgenic action and abnormalities of the reproductive system in animals depending of the dose, time and stage of development at exposure (Latini, 2000; Akingbemi and Hardy, 2001). Several mechanisms by which DEHP affects reproductive function in rodents are relevant to humans, e.g., an androgen receptor-independent mechanism of anti-androgenicity (Akingbemi et al., 2004; Bonefeld-Jorgensen et al., 2001; Foster et al., 2001; Gray et al., 2000; Latini et al., 2003; Parks et al., 2000), reduction in the expression of steroidogenesis related factors and activation of peroxisome proliferator-activated receptors (Borch et al., 2006; Howdeshell et al., 2007; Lovekamp-Swan and Davis, 2003; Wilson et al., 2008).

The reproductive toxicity of gestacional and lactational exposure to DEHP is attributable to the action of its primary metabolite, mono-(2-ethylhexyl) phthalate (MEHP), which cross the placental barrier and pass into breast milk (Latini et al., 2003; Main et al., 2006; Stroheker et al., 2005). However, in humans the urine excretion of MEHHP, MEOHP and MECCP, secondary oxidized metabolites of DEHP, reflect better the exposure level than MEHP (Koch et al., 2006). At gonadal level, in uterus and lactational exposure to DEHP produces abnormalities in androgen-dependent processes (Akingbemi et al., 2001; Albro, 1987; Borch et al., 2005; Gray et al., 2000; Parks et al., 2000), alters sexual differentiation (Andrade et al., 2006), and affects the reproductive function and sexual behavior in male rat offspring (Dalsenter et al., 2006). At hypothalamic-pituitary level, prenatal and lactational DEHP exposure at a dose of 30 mg/kg bw/day modifies the content of amino acid neurotransmitters in hypothalamus and FSH secretion in 30-day-old peripubertal male rats (Carbone et al., 2010). The possibility that DEHP exerts adverse effects on androgen synthesis and thereby interferes with reproductive tract development in the human male fetus has been raised in light of evidences indicating that boys born from women exposed to phthalates during pregnancy have an increased incidence of inborn genital malformations, such as shortened anogenital distance (Swan et al., 2005; Swan, 2008), cryptorchidism and hypospadias which are symptoms of one entity named testicular dysgenesis syndrome (TDS) (Skakkebaek et al., 2001). Phthalates induce these adverse effects as a result of abnormal Leydig cells function and the decrease in testosterone synthesis (Hu et al., 2009). Also, prenatal phthalates exposure has been associated with alterations in the neurodevelopment in humans, as neonatal and childhood behaviour, and executive functioning (Engel et al., 2009, 2010).

Little information concerning the reproductive effects of pre and perinatal exposure to DEHP on female rats is presently available, although they have been associated with an increase in the number of ovarian atretic tertiary follicles in adult female offspring rats (Grande et al., 2007). Exposure of adult rats to DEHP results in hypoestrogenic anovulatory cycles and polycystic ovaries (Davis et al., 1994; Lovekamp-Swan and Davis, 2003). MEHP, the active metabolite of DEHP, reduces estradiol production by decreasing aromatase, the rate-limit enzyme that converts testosterone to estradiol, in granulose cell cultures of immature rats (Lovekamp and Davis, 2001). In vitro assays demonstrated that DEHP and the metabolite MEHP may directly inhibit antral follicle growth in adult mice, via a mechanism that includes reduction in levels of estradiol production and a decreased expression of cell cycle regulators (Gupta et al., 2010). On the pituitary–gonadal axis of immature female rats DEHP exerts dual effects stimulating the hormonal function of the pituitary and, at the same time, inhibiting steroidogenesis by the ovarian granulose cells (Svechnikova et al., 2007).

The hypothalamic amino acid system appears to play an important role in the different neuroendocrine processes involved in sexual maturation and in the onset of puberty (Moguilevsky and Wutke, 2001). The excitatory amino acid aspartate (ASP) stimulates GnRH release (Brann and Mahesh, 1994). Previously we have demonstrated that N-methyl-D-aspartate and non-N-methyl-D-aspartate receptors of the excitatory amino acid system stimulate the release of Gn-RH from hypothalamic fragments *in vitro* and also increase serum gonadotropin levels during maturation in male and female rats (Carbone et al., 1996; Moguilevsky et al., 1995). Gamma amino butyric acid (GABA) exerts the inhibitory control of the hypothalamic–pituitary–gonadal (HPG) axis in peripubertal and adult rats. However, this neurotransmitter stimulates gonadotropin secretion in prepubertal (15 days of age) female rats (Feleder et al., 1996; Moguilevsky et al., 1991). It is also demonstrated that the regulatory effects of amino acid neurotransmitters system on GnRH neurons are modulated by sexual hormones acting on different receptor-subtypes of these neurotransmitters in the anterior preoptic and medial basal areas (APOA-MBH) of the hypothalamus (Moguilevsky et al., 1995; Szwarcfarb et al., 1994).

Despite the potential adverse effects on reproductive system associated with DEHP exposure, there is little information about the neuroendocrine mechanisms by which this endocrine disruptor could alter sexual development. The purpose of this work was to investigate whether DEHP modifies gonadotropin levels and the hypothalamic content of the amino acid neurotransmitters ASP, GLU (glutamate) and GABA in 15-day-old prepubertal male and female rat offspring in DEHP-exposed dams during gestational and nursing periods. An oral route in DEHP administration was chosen for this study to mimic the most likely route.

2. Materials and methods

2.1. Animals and drug

Wistar rats used for this work were provided by the Department of Physiology, School of Medicine, University of Buenos Aires. They were allowed at least 7-day acclimatization-period and observed for signs of illness before starting experimental procedures. Animals were raised under light (lights on from 7 am to 7 pm), temperature (22–24 °C) and humidity controlled conditions. Rats were fed with a pellet diet (ACA Animal Nutrition Division, Complete balanced animal aliment, Argentine Industry) and water *ad libitum* until time of sacrifice. The diet contains soybean meal, but as all animals were exposed to the same levels of phytoestrogen the feed intake was equivalent for control and DEHP treated rats. Moreover, the same lots of diet were provided to animals from both groups at the same time during the course of the study to control across groups for possible variation in the content of the diet. Adequate measures were taken to minimize pain or discomfort in accordance with protocols of the National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals. Approval to conduct the study was granted by the Animal Care and Ethics Committee of the University of Buenos Aires.

DEHP (99% pure, Cat D 20,115-4, Aldrich Chemical Company, Inc. Milwaukee, WI, USA) was used in drinking water. The estimated average dose of exposure to dams was 3 and 30 mg/kg bw/day of DEHP for each experimental group. Taking into account that each dam was individually placed in metallic cages, the estimated dose of exposure were based on the measurements of the difference in the amount of water placed in the water bottle each day and the amount remaining on the following day. It was assumed that all DEHP solution missing in the bottle had been consumed by the animal. Assessments do not account for possible leakage or evaporation of the solution or for potential loss of DEHP activity during the 24-h period. The level of DEHP affecting the fetuses during gestation or that was postnatally ingested by the offspring during the lactation period, was not assessed in this work. The administration pathway and the doses were chosen based on

the previous paper (Carbone et al., 2010) in which we demonstrated that the selected experimental doses of DEHP have shown to effectively elicit response, but not toxicity in both, dams and pups. These doses were less and greater than 3.7 and 14 mg/kg bw/day, respectively, which represent the range of the no-observed-adverse-effect level (NOAEL) determined by the National Toxicology Program Center for the Evaluation of Risks to Human Reproduction for testis/developmental effects of DEHP by oral administration in rodents (NTP-CERHR, 2006).

2.2. Experimental design

Female young adult rats with weights from 240 to 250 g on the day after mating) received DEHP or vehicle during pregnancy and lactation. For that purpose, each female was mated with a male in individual metallic cages for 4 days (one estrous cycle). The animals were daily examined for mucous plug. The presence of copulatory plugs in the cage was taken as evidence of mating and designated as first day of gestation (GD1). At that time, each mated rat was separated from the male. Pregnant rats ($n = 9$) were randomly assigned (3 per group) to one of the three experimental groups (Control; DEHP 3 mg/kg bw/day and DEHP 30 mg/kg bw/day). The control group received water and no DEHP was added. Dams exposed to DEHP with doses of 3 or 30 mg/kg bw/day in drinking water, every day from GD1 to weaning. The solutions were made up fresh daily by sonicating for 30 min, which ensures a permanent and homogenized solution. During the treatment, each dam was weighed daily and DEHP solution consumption was measured to determine the dosage to be administered. Female ($n = 10$) and male ($n = 10$) pups from dams of each different three treatment group rats, were assigned randomly to sacrifice at 15 days of age.

2.3. Tissue collection

When the target age was reached, the animals were weighed and sacrificed by decapitation at 16.00–17.00 h. Blood was collected from the trunks and the samples centrifuged for 10 min at 2500 rpm, the serum separated and stored at −20 °C until gonadotropin determination. Testes, uterus and ovaries were also dissected and weighed immediately after sacrifice. Brains were rapidly removed and hypothalami dissected out with a single razor blade and weighed. Hypothalamic samples containing the anterior preoptic and medial basal areas (APOA-MBH) were dissected with the help of a stereomicroscope. The hypothalamic samples were obtained at a depth of 3–4 mm and were bordered laterally by the hypothalamic sulci; rostrally 3 mm anterior to the optic chiasma and caudally, by the mammillary bodies. The samples were less than 2 mm thick and no significant differences in weight were observed.

2.4. Hypothalamic homogenization and amino acid measurement

Each APOA-MBH was weighed and homogenized in $HClO_4$ acid 0.6 N, glass Potter homogenizer refrigerated with ice. The homogenate was centrifuged at 13,000 rpm for 2 min, and the supernatant obtained frozen at −80 °C until amino acids were determined. ASP and GABA concentrations were determined by high performance liquid chromatography after derivatization with phenylisothiocyanate and UV detection at 254 nm (Jarry et al., 1992). The drugs used did not interfere in the derivatization process. Mean inter and intra assay coefficients of variability were 5% (ASP) and 8% (GABA). The detection limit was 20 pmol for ASP and 5 pmol for GABA. The mobile phase consisted of 0.57 M sodium acetate buffer (pH 6.5) containing 10% acetonitrile (Sintorgan, Buenos Aires, Argentina). Amino acid standards were: ASP and GABA (Sigma Chemical Co., St. Louis, MO, USA). The results are expressed as pmol/mg of tissue.

2.5. LH and FSH determination

Serum LH and FSH was determined in duplicate by using the double-antibody radioimmunoassay technique (Niswender et al., 1968). The material for this assay was provided by the National Hormone and Peptide Program of the National Institutes of Diabetes and Digestive and Kidney Diseases (NIDDK) (Harbor-UCLA Medical Center, Torrance, CA, USA). Results were expressed in serum ng/ml, in accordance with the referenced preparation (rat LH RP-1, rat FSH RP-1). All samples were analyzed in the same assay and intra-assay coefficient of variation was 6%.

2.6. Statistical analysis

Data were analyzed on individual means basis. Results were expressed as the mean ± S.E. Statistical analysis was conducted using GraphPad InStat (version 3.00 for Windows 95, GraphPad Software, San Diego CA, USA). Analysis was performed non-parametrically with Kruskal–Wallis test followed by Dunn's multiple comparisons test. Differences were considered to be statistically significant at a probability level of 5% ($p < 0.05$). In must be noted that while there is the potential for litter specific effects, our statistical analysis was based on individual offspring numbers given the absence of any overt toxicity in the dams.

3. Results

Litter size was not altered by maternal DEHP treatment (8–10 pups per litter in DEHP and control groups). Also, exposure to DEHP did not produced changes on pup weights at birth (control: 6.14 ± 0.26; DEHP 3 mg/kg bw/day: 6.58 ± 0.38 and 30 mg/kg bw/day: 5.94 ± 0.25) and viability (number of pups at sacrifice relative to number of pups at birth).

3.1. Prepubertal male rats

Table 1 shows the effect of DEHP exposure in 15-day-old male rats. No modifications in body weight, absolute testicular weight, plasmatic gonadotropin levels and in the hypothalamic ASP and GABA concentrations were detected in prepubertal male rats exposed to DEHP at dose of 3 mg/kg bw/day. However, the exposure to DEHP 30 mg/kg bw/day significantly decreased the absolute testicular weight without changes in body weight. On the other hand, there was a significant increased in serum gonadotropin levels and in the hypothalamic ASP concentration. Contrary, decreases in GABA content were found in these prepubertal rats exposed to a high dose of DEHP during pregnancy and lactation. No changes were detected in GLU content.

3.2. Prepubertal female rats

As it can be seen in Table 2, no changes in body weight, absolute weight of gonads, serum gonadotropin levels and in hypothalamic ASP and GABA concentrations were found in 15-day-old female rat offspring to dams exposed to DEHP at dose of 3 mg/kg bw/day. A very important increase in serum LH level and in the hypothalamic concentration of the neurotransmitters ASP and GABA was observed in the prepubertal rats exposed to DEHP 30 mg/kg bw/day. There was not modification in the hypothalamic content of GLU at the same dose.

Table 1
Prepubertal male rat offspring from dams exposed to low and high doses of DEHP.

	Control	DEHP (3 mg/kg bw/day)	DEHP (30 mg/kg bw/day)
Body weight (g)	29.3 ± 2.4	31.6 ± 1.8	32.1 ± 2.3
Testes weight (mg)	132.92 ± 11.04	141.78 ± 9.82	102.67 ± 4.76*
LH (ng/ml)	17.16 ± 3.18	15.25 ± 4.64	39.66 ± 13.10*
FSH (ng/ml)	36.00 ± 12.16	40.50 ± 8.55	171.66 ± 46.22**
Aspartate (pmol/mg tissue)	1413.75 ± 221.28	1250.10 ± 127.28	2123.28 ± 290.88*
Glutamate (pmol/mg tissue)	6631.50 ± 791.94	6836.26 ± 743.42	7703.32 ± 448.42
GABA (pmol/mg tissue)	488.20 ± 84.82	490.40 ± 63.35	256.75 ± 65.80*

Parameters were measured at 15 days of age. Each value represents the mean ± SEM of 8–10 animals. No statistically significant differences between control and DEHP (3 mg/kg bw/day) groups were present.
* $p < 0.05$ DEHP (30 mg/kg bw/day) vs. control.
** $p < 0.001$ DEHP (30 mg/kg bw/day) vs. control.

Table 2
Prepubertal female rat offspring from dams exposed to low and high doses of DEHP.

	Control	DEHP (3 mg/kg/bw day)	DEHP (30 mg/kg bw/day)
Body weight (g)	24.7 ± 1.9	23.8 ± 2.2	26.3 ± 2.7
Uterus weight (mg)	27.04 ± 2.84	31.39 ± 3.62	33.59 ± 3.22
Ovary weight (mg)	14.58 ± 2.15	13.73 ± 2.54	14.41 ± 1.87
LH (ng/ml)	22.75 ± 8.15	21.87± 6.77	102.85 ± 34.86**
FSH (ng/ml)	1272.22 ± 167.91	1015.55 ± 357.11	1286.36 ± 279.37
Aspartate (pmol/mg tissue)	673 ± 44.72	889.22 ± 149.8	1056.22 ± 189.67**
Glutamate (pmol/mg tissue)	648.83 ± 97.78	664.83 ± 111.44	657.70 ± 113.08
GABA (pmol/mg tissue)	77.56 ± 46.81	95.88 ± 55.73	459.27 ± 27.16**

Parameters were measured at 15 days of age. Each value represents the mean ± SEM of 8–10 animals. No statistically significant differences between control and DEHP (3 mg/kg bw/day) groups were present.
** $p < 0.001$ DEHP (30 mg/kg bw/day) vs. control.

4. Discussion

The processes of sexual maturation and adult reproduction function are under the control of numerous hormone-mediated feed back mechanisms that are sensitive to the regulation of many endogenous and exogenous factors, including the environmental endocrine disruptors. Since several of these mechanisms begin to operate during fetal life, the pre and perinatal exposure to chemicals that alter the hormonal balance become very significant in these stages.

Previous studies of phthalates were conducted with high doses and short exposure periods. Typically, DEHP at 500 mg/kg and above were associated with decreased testosterone production and lower sperm counts (Sjöberg et al., 1985; Parks et al., 2000; Foster et al., 2001) and also shorted anogenital distance and reduced testis weights (Gray et al., 2000). Taking into account this, we explored the possibility that DEHP, at low doses, administered to female rats during pregnancy and lactation could affect the neuroendocrine regulation of the reproductive axis in prepubertal male and female pups.

The results show that exposure to DEHP at low dose (3 mg/kg bw/day) was unable to produce significant changes in the reproductive axis of 15-day-old male and female rat offspring, not only in the absolute weight of gonads but also in the gonadotropin serum levels and in the hypothalamic contents of amino acid neurotransmitters.

Contrary to what was described in the case of low dose, we detected significant alterations in the hypothalamic–pituitary–gonadal axis of prepubertal rats exposed to DEHP at high dose (30 mg/kg bw/day). In males, we observed a reduction in absolute testes weight, an increase in LH and changes in the hypothalamic content of amino acid neurotransmitters. It is well known that the excitatory amino acid system exerts a stimulatory effect on GnRH and gonadotropins release and GABA has an inhibitory one in normal prepubertal male rats. In this work we found an increase in the excitatory amino acid ASP and a decrease in the inhibitory tone of GABA in 15-day-old male rats offspring from dams exposed to DEHP at dose of 30 mg/kg bw/day, in comparison with the controls. These changes were associated with the LH enhancement observed in the same animals (see Fig. 1). LH increase produced by DEHP could be related with a possible reduction in the negative feedback mechanism of testosterone on gonadotropins, probably induced by the recognized antiandrogenic action of this phthalate or its metabolites. The normal development is accompanied by a progressive increase in the capacity for testosterone secretion, which produces the negative feedback mechanism. Therefore, a decrease in serum testosterone concentration produces a reduction in the negative feedback mechanism in male rats, inducing an increase in LH output from pituitary. High serum LH levels stimulate androgen biosynthesis in Leydig cells which acting via the same pathway restore pituitary LH secretion to normal levels in the short term. However, LH overstimulation may cause premature deterioration of steroidogenic capacity if prolonged and induce Leydig cell proliferation with adverse consequences for male fertility (Akingbemi et al., 2004; Shinji et al., 2005). On the other hand, sexual steroids can modulate different receptor-subtypes of excitatory or inhibitory amino acid neurotransmitters regulating Gn-RH release from the APOA-MBH areas of the hypothalamus (Moguilevsky et al., 1995; Szwarcfarb et al., 1994). In consequence, male serum LH level is the result of neuroendocrine mechanisms that involved the modulatory actions of testosterone and the amino acid neurotransmitter system. In this way, the effects induced by DEHP high dose on hypothalamic-pituitary axis of prepubertal male offspring rats could be related with its antiandrogenic activity (Andrade et al., 2006; Parks et al., 2000) and also with the ability of this chemical to impair Leydig cell function (Akingbemi et al., 2004, 2001).

The effect of DEHP 30 mg/bw/day on hypothalamic amino acid neurotransmitters content in 15-day-old male rats (aspartate increase and GABA decrease) that was observed in this work, are different to those previously published in 30-day-old peripubertal male rat offspring from dams submitted to similar treatment. At

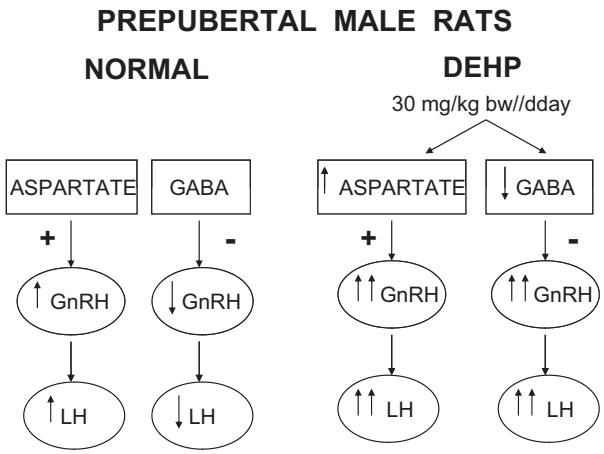

Fig. 1. A schema summarizing DEHP (30 mg/kg bw/day) effects on hypothalamic–pituitary axis in prepubertal male rats offspring from dams exposed to the phthalate during pregnancy and lactation. DEHP increased the stimulatory effect of ASP and decreased the inhibitory action of GABA, producing to LH enhancement in 15-day-old male rats.

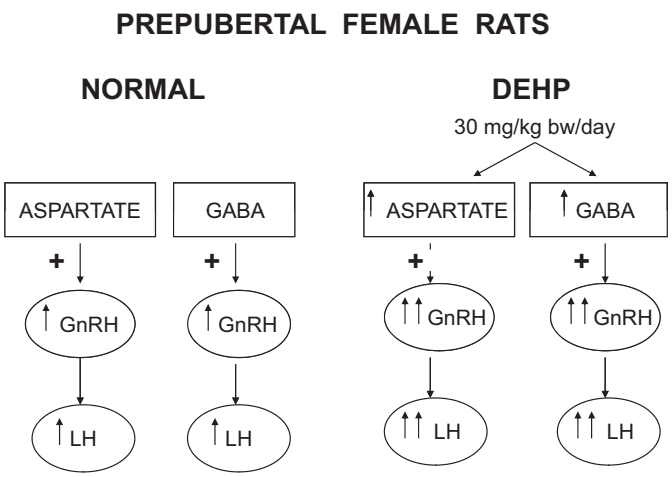

Fig. 2. A schema summarizing DEHP (30 mg/kg bw/day) effects on hypothalamic–pituitary axis in prepubertal female rat offspring from dams exposed to the phthalate during pregnancy and lactation. DEHP increases the stimulatory effects of ASP and GABA, producing LH enhancement in 15-day-old male rats.

this age we detected decrease in aspartate and increase in GABA contents (Carbone et al., 2010). The different neuroendocrine effects observed in prepubertal and peripubertal male rats could relate to ontogenic changes in regulation and activation of different receptors-subtypes of these neurotransmitter systems produced by testosterone that increases significantly in normal 30 days old in comparison with 14 days old rats (Hu et al., 2009). Specifically, Szwarcfarb et al. (1994) reported a significant increase in GABA-A receptors in normal male rats of 30 days of age with respect to prepubertal rats, as well as a very important decrease in these receptors when the animals was castrated at birth. In addition to this, Adams et al. (1999), demonstrated that hypothalamic NMDA receptor subunits (NR1, NR2a, and NR2b) levels change significantly and differentially during postnatal development in the preoptic area anterior hypothalamus. These data are in accordance with our findings, which demonstrated that DEHP can produce different effects on the hypothalamic amino acid neurotransmitters, at different stages of sexual development. Also, they were in agreement with our hypothesis that the antiandrogenic action of DEHP could be involved in these effects. On the other hand, it is known that the toxicity of phthalates in males is age dependent, since it is more sensitive in fetal and prepubertal animals than in adult males (Ge et al., 2007). This would be caused by a higher gastrointestinal absorption of the metabolite MEHP DEHP-metabolite in young animals. Therefore, it is suggested that the age-related difference observed in testicular response after oral administration of DEHP may be due to pharmacokinetic rather than tissue sensitivity differences (Sjöbergt et al., 1986) Therefore, it cannot be ruled out that the difference seen between 15 vs. 30-day-old rats, are consequence of different secondary metabolites MEHHP, MEOHP, MECPP concentrations.

In prepubertal female rats exposed to the higher dose of DEHP treatment (30 mg/kg/day) we found significant increase in serum LH and in the hypothalamic contents of ASP and GABA in comparison with the controls (see Fig. 2). No changes in the weights of gonads, FSH level and GLU content were observed. Taking into account that DEHP 30 mg/kg bw/day provoked a significant reduction in the GABA content and in the testicular weight of 15-day-old male rats, our findings indicated sexual differences in the effect of DEHP in prepubertal rats. It is interesting to note that ovarian steroids exert a negative feedback effect on gonadotropin secretion in normal female rats younger than 20–22 days of age and a stimulatory action after this age (Andrews et al., 1981; Caligaris et al., 1972; Scacchi and Moguilevsky, 1973). The maturation of the positive feedback effect of sex hormones is a very important event that characterizes the onset of puberty and the sexual cycles in females and it is also regulated by excitatory and inhibitory hypothalamic neurotransmitters (Carbone et al., 1996; Moguilevsky et al., 1991). It could be possible that exposure to DEHP at a dose of 30 mg/kg bw/day during pre and perinatal stages alters the hormonal balance modifying the neuroendocrine mechanisms involved in sexual maturation. The LH enhancement induced by DEHP was consistent with previous findings in DEHP (500 mg/kg bw/day by oral gavage) treated immature female rats (Svechnikova et al., 2007). In this way, a reduction in progesterone and estradiol plasma levels together with an inhibition of the steroidogenesis by granulose cells in DEHP exposed immature female rats (Svechnikova et al., 2007) could be a probable mechanism to explain the changes in LH and amino acid neurotransmitters described in this work.

Therefore, our study provides new findings regarding sexual differences of effect of DEHP (30 mg/kg bw/day) on the hypothalamic content of the amino acid neurotransmitters, which are involved in the neuroendocrine regulation of reproductive axis, in prepubertal male and female rats exposed to this chemical during gestational and lactational periods. This could be related with the gonadotropin modifications also described in this work.

Finally, we think that DEHP impact at hypothalamic level could have very important consequences on the onset of puberty and on the reproductive function in adult life. Thus, it would be necessary to study the cumulative effect of the exposure to this substance or the simultaneous exposures to other chemicals with similar hormonal action.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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DISCLAIMER

The findings and conclusions in this report are those of the authors.

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