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Changes in pituitary secretion during the early postnatal period and anovulatory syndrome induced by neonatal oestrogen or androgen in rats

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The following experiments were performed: (i) concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and prolactin in plasma were measured at 2, 5, 8, 10 and 15 days in female Wistar rats treated on the first day of life with 100 μg oestradiol benzoate or vehicle; (ii) females injected on day 1 with 100 μg of oestradiol benzoate or 1 mg of testosterone propionate and from day 1 to day 10 or 15 with FSH and LH were killed on day 90; (iii) females injected from day 1 to day 10 or 15 with prolactin or vehicle were killed on day 90; (iv) females injected on day 1 with oestradiol benzoate and from day 1 to day 15 with a luteinizing-hormone-releasing hormone (LHRH) agonist were killed on day 90; (v) groups of females injected on days 1, 4, 7, 10, 13 and 16 with an LHRH antagonist were killed on day 90. Onset of puberty, vaginal cycles, organ weights and hormonal plasma concentrations were measured. Females treated on the first day of life with 100 μg oestradiol showed inhibition of gonadotrophin secretion and stimulation of prolactin secretion during the neonatal period. Females injected on the first day of life with oestradiol benzoate or testosterone propionate showed, in adulthood, anovulation, ovarian atrophy, reduced FSH plasma concentrations, increased prolactin plasma concentrations and reduced pituitary prolactin content. These alterations were due neither to blocked gonadotrophin secretion nor to stimulated prolactin secretion observed immediately after steroid injection, since: (i) development of the anovulatory syndrome was not blocked by the administration of exogenous gonadotrophins or LHRH-agonist; and (ii) blockade of gonadotrophin secretion immediately after birth with an LHRH antagonist or neonatal injection of prolactin did not induce the anovulatory syndrome. It is concluded that anovulation induced by administration of neonatal steroid was mediated neither by the early inhibition of gonadotrophin secretion nor by the stimulation of prolactin secretion.

Introduction

The role of the neonatal pituitary in the organization of reproductive function is different in female and male rats. Neonatal administration of luteinizing-hormone-releasing hormone (LHRH) antisera or antagonists induced permanent changes in males (Bercu *et al.*, 1977; Kolho *et al.*, 1988; Kolho and Huhtaniemi, 1989), but not in females (van den Dungen *et al.*, 1989).

Neonatal injection of androgens or oestrogens induced permanent anovulation in female rats (Barraclough, 1961; Gorski, 1963). The reduced effectiveness of steroids when injected simultaneously with neuroactive drugs (Kikuyama, 1961; Raum and Swerdloff, 1981; González-Mariscal *et al.*, 1982; Vidal *et al.*, 1986) and the morphological changes observed in the hypothalamus (Gorski, 1983) suggest that steroids act at the level of the brain, and, during the last decades, it has been assumed that neonatal steroids affect brain structures irreversibly and induce anovulation. It is therefore possible that different mechanisms are involved in the effects of steroids (Gorski, 1990), and a pituitary or ovarian site of action cannot be excluded (Fink, 1990). This possibility is supported by the following experimental findings: (i) the reduced concentrations of gonadotrophins in plasma and the increase in concentrations of prolactin observed in males shortly after neonatal oestrogenization (Aguilar *et al.*, 1987; Pinilla *et al.*, 1989), and the blockade of the effects of neonatal oestrogenization by the simultaneous administration of gonadotrophins (Bellido *et al.*, 1990); (ii) the existence of a delayed anovulatory syndrome induced by low doses of steroids, which appears several weeks after puberty (Swanson and van der Werff Ten Bosch, 1964a, b; Ericsson and Baker, 1966; Arai and Gorski, 1968) and is mediated by ovarian alterations (Napoli and Gerall, 1970); (iii) recent data showing that oestrogens in adulthood induce reversible changes in the neurones of the arcuate nucleus (Naftolin *et al.*, 1990); and (iv) the inhibition of masculinization of neonatal males after suppression of the secretion of gonadotrophins by dihydrotestosterone (van der Schoot *et al.*, 1976).

Thus, the following experiments were designed to establish (i) the pituitary secretion immediately after neonatal oestrogenization; (ii) the capacity of gonadotrophins and LHRH to block

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the effects of neonatal oestrogenization and androgenization; and (iii) the effects of the blockade of neonatal gonadotrophin secretion after administration of an LHRH antagonist and of neonatal prolactin administration on the reproductive function in adulthood.

Our results indicate that the anovulatory syndrome induced by neonatal steroid treatment is not mediated by changes in gonadotrophin secretion during the early postnatal period. Neonatal prolactin concentrations are unrelated to reproductive function in adult female rats.

Materials and Methods

Animals and drugs

Female Wistar rats were raised in our laboratory under controlled light (12 h light:12 h darkness; lights on at 07:00 h) and temperature (20 ± 2°C). The day when the litters were born was considered as day 1. At that moment, the litter size was adjusted to eight animals, which were weaned on day 21 and housed in groups (four to five per cage). Oestradiol benzoate, testosterone propionate and the LHRH-agonist D-ala^{6}-D-Gly^{10}- LHRH-ethylamide were obtained from Sigma (St Louis, MO). The LHRH antagonist Org.30276 (Ac-D-pClPhe--DpClPhe-D-Trp-Ser-Tyr-D-Arg-Leu-Arg-Pro-D-Ala-NH_2CH_3COOH) was generously supplied by Organon (Oss, Netherlands). FSH (NIADDK oFSH-16), LH (USDA bLH-B-5) and prolactin (NIADDK oPrl-18) were supplied by the NIADDK (Bethesda, MD).

Experiment 1

On day 1, females were injected subcutaneously (0.1 ml) with either 100 μg oestradiol benzoate or olive oil. Control and oestrogenized females were decapitated at the ages of 2, 5, 8, 10 and 15 days. Trunk blood was collected and the serum was separated by centrifugation and stored at −20°C until assayed. The samples from the first days of the study from several animals were pooled; pituitaries were homogenized in 1 ml physiological saline containing urea (2.5 mol l^{-1}) and subjected to ultrasonic treatment (Haggi and Aoki, 1981). Samples were centrifuged at 2800 ***g*** for 10 min and the supernatants frozen at −20°C until analysed for hormone content.

Experiment 2

On day 1, female pups were injected s.c. (0.1 ml) with 100 μg oestradiol benzoate (oestrogenized females), 1 mg of testosterone propionate (androgenized females) or olive oil (control females). The animals were submitted to a combined treatment with FSH (80 μg per 100 g body weight) and LH (40 μg per 100 g body weight), or vehicle (0.5% bovine serum albumin (BSA) in 0.9% NaCl), subcutaneously, once a day from day 1 to day 10. Other groups of oestrogenized animals received higher doses of FSH (100 μg per 100 g body weight) and LH (50 μg per 100 g body weight), twice a day, from day 1 to day 15. The rats were inspected at least once a day for vaginal opening. Thereafter, the vaginal cytology was monitored daily. Animals were decapitated in the first dioestrus after day 90. The weights of animals, ovaries, uteri, adrenals and pituitary glands were recorded.

Experiment 3

Animals were injected subcutaneously with ovine prolactin (200 μg per 100 g body weight from day 1 to day 10 or with 400 μg per 100 g body weight from day 1 to day 15) given as two injections at 08:00 and 22:00 h. Control groups received vehicle (0.15 mol NaCl l^{-1}, 0.03 mol $NaCO_3H$ l^{-1}, 0.1% BSA, pH 9) twice a day. Additionally, female rats were injected from day 21 to vaginal opening with vehicle (n = 4) or the highest dose of prolactin (n = 3). Experimental procedure was similar to that in Expt 2.

Experiment 4

Female rats injected with oestradiol on day 1 were injected from day 1 to day 15 with the LHRH-agonist (in doses of 0.02, 0.2 and 2 μg kg^{-1} day^{-1}, divided into two injections given at 08:00 and 22:00 h). Blood samples were obtained by jugular venepuncture after ether anaesthesia on day 15, 2 h after the last vehicle or LHRH-agonist injection. Thereafter, the animals were studied as in Expt 2.

Experiment 5

Female rats received an s.c. injection of either 500 μg per 100 g body weight of LHRH-antagonist or of saline at days 1, 4, 7, 10, 13 and 16 of age. Groups of rats were killed by decapitation on day 10 or maintained until day 90. At the time rats were killed, trunk blood was collected and pituitaries, ovaries and uteri were dissected, weighed and frozen. In rats maintained until adulthood, the onset of puberty and vaginal cycles were recorded.

Assays

Concentrations of LH, FSH and prolactin in serum and pituitary were determined by a double-radioimmunoassay method using kits supplied by the NIADDK (Bethesda, MD). Rat LH-I-6, rFSH-I-6 and rPrl-I-5 were labelled with ^{125}I by the chloramine T method (Greenwood *et al.*, 1983). The LH, FSH and prolactin concentrations are expressed using rLH-RP-2, rFSH-RP-2 and rPrl-RP-3 as standards. Concentrations of oestradiol in plasma were measured using a kit from Diagnostics Products Corporation (Los Angeles, CA). All samples were measured in duplicate in the same assay, the intra-assay variations being 6, 7, 9 and 8%, respectively. The sensitivities were 7.5, 20, 10 and 1 pg for LH, FSH, prolactin and oestradiol, respectively.

Statistical analysis

Data are expressed as means ± SEM. Statistical analyses were carried out by the Student's t test or by two-way analysis of

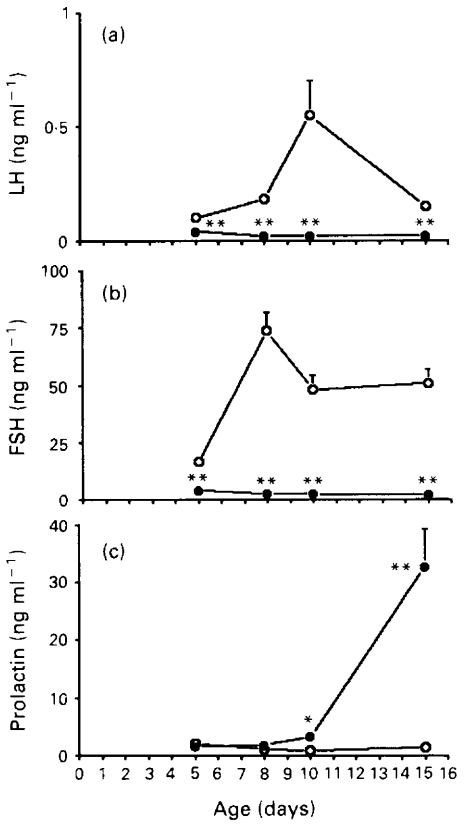

Fig. 1. Concentration of (a) luteinizing hormone (LH), (b) follicle-stimulating hormone (FSH) and (c) prolactin in plasma in female rats injected on day 1 with vehicle (○) or 100 μg of oestradiol benzoate (●). Values are means ± SEM (note the linear scale and that SEM is not given when bar is smaller than the symbols used in the figure). Each group consisted of 10–12 animals; * and ** $P \leqslant 0.05$ and 0.01, respectively, compared with control groups (ANOVA followed by Tukey's test).

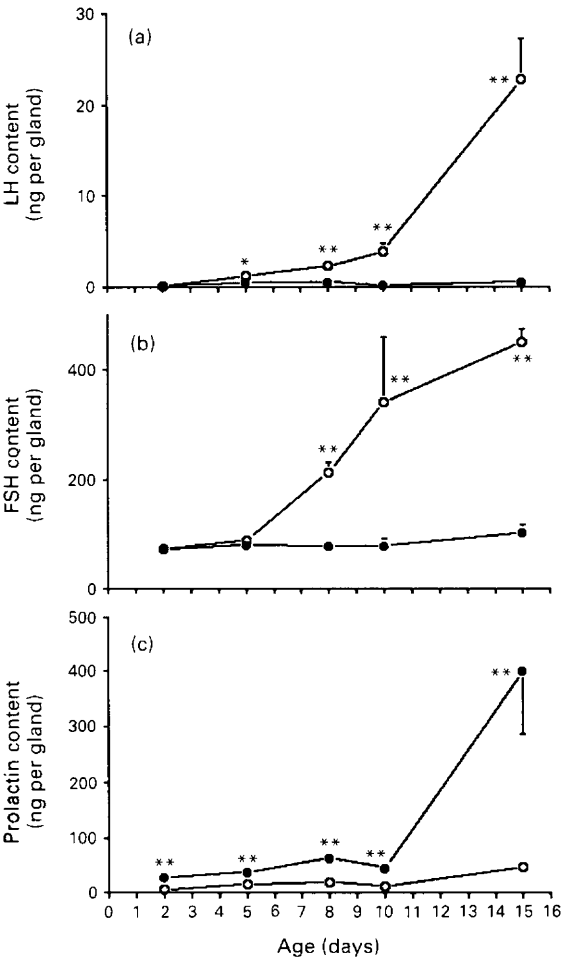

Fig. 2. (a) Pituitary luteinizing hormone (LH), (b) follicle-stimulating hormone (FSH) and (c) prolactin content in female rats injected on day 1 with vehicle (○) or 100 μg of oestradiol benzoate (●). Values are means ± SEM (note the linear scale and that SEM is not given when bar is smaller than the symbols used in the figure). Each group consisted of 10–12 animals; * and ** $P \leqslant 0.05$ and 0.01, respectively, compared with control groups (ANOVA followed by Tukey's test).

variance (ANOVA) and Tukey's multiple-comparison method for comparison among means.

Results

Hormonal concentrations in neonatal female rats

Control animals showed great variability in the plasma concentration of LH, which increased after day 5 and decreased on day 15. Oestrogenized females showed lower concentrations at 5, 8, 10 and 15 days. Plasma concentrations of FSH increased after day 5 in control animals and were significantly lower in oestrogenized females at 5, 8, 10 and 15 days. Prolactin plasma concentrations were higher in oestrogenized females at 10 and 15 days (Fig. 1). Pituitary FSH and LH content was significantly reduced and pituitary prolactin content increased in oestrogenized females (Fig. 2).

Effects of neonatal administration of gonadotrophins, steroids or both

Neonatal administration of oestrogen induced precocious vaginal opening, which occurred at lower body weights than in controls. In contrast, females injected with testosterone had no vaginal opening. Neonatal injection of gonadotrophins did not modify the onset of puberty in controls or in oestrogenized and androgenized females (Table 1).

Oestrogenized females showed anovulatory cycles characterized by alternately long periods of oestrus and dioestrus. The regular vaginal cycles in control females and the anovulatory cycles in oestrogenized females were unaffected by neonatal treatment with gonadotrophins (Table 1).

Ovaries from androgenized and oestrogenized females treated or not with gonadotrophins were atrophied on day 90 (Fig. 3), and the absence of fresh corpora lutea was revealed by histological examination.

Anovulatory female rats showed reduced FSH and increased prolactin plasma concentrations (Fig. 4). Pituitary concentrations of prolactin were reduced (Fig. 4), whereas those of FSH and LH were unaltered (data not shown).

Table 1. Effects of neonatal injections of oestradiol benzoate, testosterone propionate, gonadotrophins, prolactin and luteinizing-hormone-releasing-hormone (LHRH) agonist and antagonist on the age and body weight at vaginal opening and first oestrus and on the incidence of anovulation in rats

Treatment	Number of rats	Vaginal opening		First oestrus		Incidence of anovulation (%)
		Age (days)	Weight (g)	Age (days)	Weight (g)	
Oil + vehicle	8	39.13 ± 1.24	114 ± 4.7	41.3 ± 1.1	122 ± 4	0
Oestradiol benzoate + vehicle	12	16.75 ± 0.42**	29 ± 0.8**			100
Testosterone propionate + vehicle	6	ND	ND			100
Oil + gonadotrophins[a]	8	40.75 ± 1.23	115 ± 3.5	41.4 ± 1.2	123 ± 5	0
Oestradiol benzoate + gonadotrophins[a]	12	16.00 ± 0.70	25 ± 1.0**			100
Testosterone propionate + gonadotrophins[a]	7	ND	ND			100
Oestradiol benzoate + vehicle	9	18.10 ± 0.30	26 ± 1.1			100
Oestradiol benzoate + gonadotrophins[b]	10	19.00 ± 0.27	33 ± 3.2			100
Oil + vehicle	6	39.80 ± 0.90	116 ± 3.4	41.4 ± 1.2	128 ± 5	0
Oestradiol benzoate + vehicle	10	17.10 ± 0.30	32 ± 0.9			100
Oestradiol benzoate + LHRH-agonist[c]	10	16.45 ± 0.80	34 ± 0.7			100
Oestradiol benzoate + LHRH-agonist[d]	10	17.25 ± 0.90	36 ± 0.6			100
Oestradiol benzoate + LHRH-agonist[e]	9	18.00 ± 1.10	38 ± 0.5			100
Vehicle	13	39.90 ± 1.10	109 ± 1.7	42.9 ± 1.8	121 ± 7.2	0
Prolactin[f]	18	39.50 ± 0.60	114 ± 2.5	42.6 ± 1.0	124 ± 3.3	0
Vehicle	9	35.50 ± 0.80	133 ± 4.2	41.1 ± 2.1	127 ± 6.4	0
Prolactin[g]	7	35.10 ± 0.90	129 ± 9.7	42.4 ± 1.8	129 ± 5.6	0
Vehicle	18	38.83 ± 1.14	123 ± 3.6	40.7 ± 1.5	139 ± 5.1	0
LHRH-antagonist[h]	18	33.44 ± 2.16**	95 ± 7.2**	37.5 ± 0.6**	118 ± 4.4**	0

Values are means ± SEM. **$P \leqslant 0.01$ versus respective oil control groups (ANOVA followed by Tukey's test).
[a]80 μg per 100 g body weight of follicle-stimulating hormone and 40 μg per 100 g body weight of luteinizing hormone were injected daily from day 1 to day 10.
[b]100 μg per 100 g body weight follicle-stimulating hormone and 50 μg per 100 g body weight of luteinizing hormone were injected each 12 h from day 1 to day 15.
[c]0.02 μg kg^{-1} day^{-1} of LHRH-agonist were injected daily from day 1 to day 10.
[d]0.2 μg kg^{-1} day^{-1} of LHRH-agonist were injected daily from day 1 to day 10.
[e]2 μg kg^{-1} day^{-1} of LHRH-agonist were injected daily from day 1 to day 10.
[f]200 μg per 100 g body weight of prolactin were injected daily from day 1 to day 10.
[g]400 μg kg per 100 g body weight of prolactin were injected daily from day 1 to day 15.
[h]500 μg per 100 g body weight of LHRH-antagonist were injected each 72 h from day 1 to day 16.
ND: vaginal opening was not observed.

Effect of neonatal administration of prolactin

The age and body weight at which vaginal opening and first oestrus occurred were unaffected by neonatal administration of prolactin (Table 1) and advanced by 3.6 days in females injected from day 21 (data not shown). Females treated with prolactin showed regular vaginal cycles. Pituitary concentrations of LH and prolactin and concentrations of FSH in plasma in adulthood were greater in the females treated with low doses of prolactin during the neonatal period (Table 2). Similar findings were observed with the higher doses of prolactin (data not shown).

Effects of neonatal administration of LHRH-agonist to oestrogenized females

Reduced FSH and LH plasma concentrations and increased prolactin concentrations in oestrogenized females were also observed on day 15 at the end of treatments with different doses of LHRH-agonist (Fig. 5). Precocious vaginal opening and constant vaginal oestrus were observed in oestrogenized animals injected with different doses of LHRH-agonist (Table 1). Ovarian and uterine atrophy were observed on day 90 (Fig. 3). Absence of corpora lutea was confirmed by histological examination.

Effects of neonatal administration of LHRH-antagonist

Females injected with LHRH-antagonist showed, on day 10, reduced FSH, LH and oestradiol plasma concentrations, pituitary LH and FSH content and weight of uteri (Table 3). Vaginal opening and first oestrus were advanced (Table 1) and regular cycles were observed. Normal ovarian and uterine weights and FSH and LH plasma concentrations were observed on days 90 and 150 (data not shown).

Discussion

Dependence of ovarian function on pituitary secretion during the neonatal period begins in the first week of life. Specific

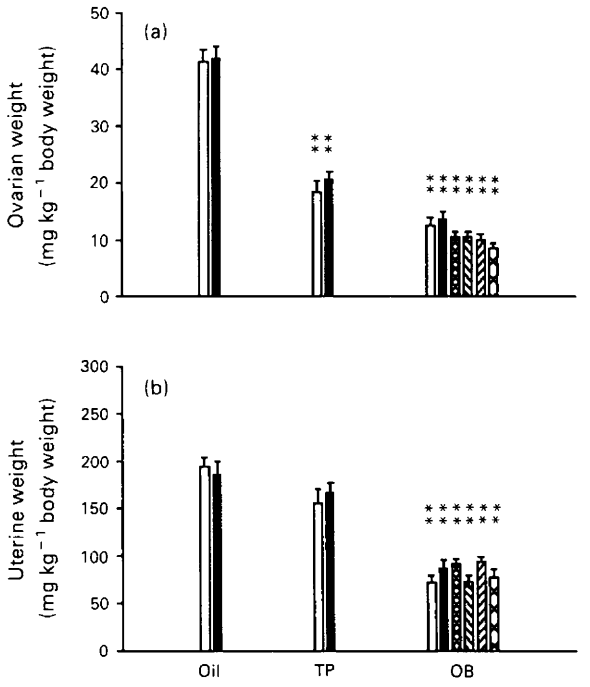

Fig. 3. (a) Ovarian and (b) uterine weights in adult female rats injected on day 1 with 0.1 ml of oil, 100 μg of oestradiol benzoate (OB) or 1 mg of testosterone propionate (TP) and with vehicle (□), follicle-stimulating hormone and luteinizing hormone from day 1 to day 10 (■), or from day 1 to day 15 (▣) or luteinizing-hormone-releasing-hormone agonist (▨: 0.02 μg kg^{-1} day^{-1}; ▨: 0.2 μg kg^{-1} day^{-1}; ⊠: 2 μg kg^{-1} day^{-1}). Values are means ± SEM. Each group consisted of 10–12 animals; ** $P \leqslant 0.01$ compared with the corresponding oil-injected groups (ANOVA followed by Tukey's test).

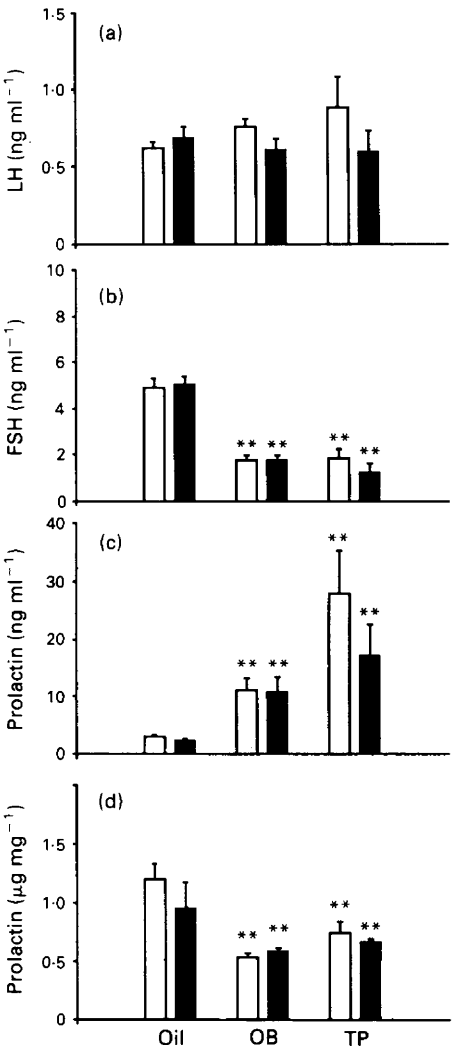

Fig. 4. Concentrations of (a) luteinizing hormone (LH), (b) follicle-stimulating hormone (FSH) and (c) prolactin in plasma and (d) prolactin in pituitary in adult female rats injected on day 1 with 0.1 ml of oil (Oil), 100 μg of oestradiol benzoate (OB) or 1 mg of testosterone propionate (TP) and from day 1 to day 10 with vehicle (□) or FSH and LH (■). Values are means ± SEM. Each group consisted of 10–12 animals; ** $P \leqslant 0.01$ compared with respective oil-injected groups (ANOVA followed by Tukey's test).

binding of LH and FSH has been detected on days 4 and 5 (Peluso *et al.*, 1976; Smith-White and Ojeda, 1981b) and FSH increases ovarian cAMP production (Sokka and Huhtaniemi, 1990) and testosterone aromatization (Funkenstein *et al.*, 1980) in 4-day-old rats. Prolactin receptors appear in the ovary at about 1 week of life (Huhtaniemi and Catt, 1981).

Assuming that neonatal gonadotrophin secretion is essential for development of ovarian FSH receptors (Smith and Ojeda, 1986) and that neonatal oestrogenization reduces gonadotrophin secretion and increases prolactin secretion at least until day 15 of life, when ovarian receptors for gonadotrophins and prolactin are present, the possibility that the anovulatory syndrome induced by neonatal oestrogenization or androgenization is mediated, at least in part, by changes initially induced in pituitary secretion was investigated. Doses of gonadotrophins used were higher than those needed to prevent testicular atrophy induced in male rats after neonatal injection of oestradiol benzoate (Bellido *et al.*, 1990) or LHRH-antagonist (L. Pinilla, P. Garnelo and E. Aguilar, unpublished observations). Our results suggest that the anovulatory syndrome induced in female rats by neonatal oestrogen or androgen administration was mediated neither by the decrease in pituitary secretion of gonadotrophins, nor by the increase in prolactin secretion. The following findings supported this assumption: (i) development of the anovulatory syndrome was not blocked by the administration of either exogenous gonadotrophins or LHRH-agonist; (ii) blockade of gonadotrophin secretion immediately after birth with an LHRH-antagonist or neonatal injection of prolactin did not induce the anovulatory syndrome. Doses of steroids injected were large and traditionally used to ensure complete sterilization. The possibility that gonadotrophins prevent the 'delayed anovulatory syndrome' induced by administration of lower doses of steroids (Swanson and van der Werff Ten Bosch, 1964a, b; Ericsson and Baker, 1966) needs further study.

Our results contrast with those showing that the sterilizing effect of testosterone injected into neonatal rats was mediated by a decrease in gonadotrophin secretion and that the changes induced by puberty, ovarian cycle and sexual behaviour could be blocked by neonatal gonadotrophin administration (Sheridan *et al.*, 1973) despite the administration of higher doses of

Table 2. Effects of daily s.c. injections of prolactin (2 mg kg^{-1} from day 1 to day 10) on concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and prolactin in pituitary and plasma in adult female rats

	Treatment	
	Vehicle (n = 12)	Prolactin (n = 18)
Concentration in pituitary (ng mg^{-1})		
FSH	42.4 ± 1.9	44.8 ± 2.7
LH	106 ± 12	180 ± 17**
Prolactin	610 ± 50	850 ± 40**
Concentration in plasma (ng ml^{-1})		
FSH	4.17 ± 0.2	5.68 ± 0.23**
LH	0.57 ± 0.05	0.59 ± 0.04
Prolactin	2.30 ± 0.34	2.25 ± 0.40

Values are means ± SEM. **Significantly different from control, $P \leqslant 0.01$ (Student's t test).
FSH: follicle stimulating hormone; LH: luteinizing hormone.

gonadotrophins during a longer period. However, our results are consistent with the normal reproductive function described in adult females, in which neonatal secretion of gonadotrophins is blocked with LHRH-agonist (Aguilar *et al.*, 1990) or antagonist (van den Dungen *et al.*, 1989). Suppression of high concentrations of gonadotrophins in the first weeks of life retards follicle development prepubertally, but this is restored at the start of cyclical activity (Meijs-Roelofs *et al.*, 1990).

It was demonstrated by Meijs-Roelofs *et al.* (1987) that LHRH-antagonist given to prepubertal females delayed onset of puberty only if the antagonist was given immediately before the preovulatory LH surge. The precocious puberty observed in our females treated with LHRH-antagonist from day 1 to day 15 seems paradoxical, but agrees with previous data (van den Dungen *et al.*, 1989) and may be related to ovarian action, since LHRH-binding sites are present at 10 days of age (Dalkin *et al.*, 1981), LHRH inhibits granulosa cell differentiation (Hsueh *et al.*, 1984) and the ovarian steroidogenic responsiveness to gonadotrophins increases when the inhibitory local action of LHRH decreased (Smith-White and Ojeda, 1981a).

The only mechanism that is probably involved in the anovulatory syndrome induced by neonatal steroid injection is a permanent alteration in the central nervous system. The volume of the hypothalamic sexually dimorphic nucleus in adulthood is permanently modified in female rats injected shortly after birth with testosterone propionate or the antioestrogen tamoxifen (Gorski, 1983), and sexual differences in postsynaptic membranes of the hypothalamic arcuate nucleus are abolished by oestrogens (Naftolin *et al.*, 1990).

Clear sex differences in reproductive alterations after neonatal steroid injection can be observed. Male rats treated with oestradiol on the first day of life show low gonadotrophin concentrations, either in serum or the hypophysis, in the early postnatal period (Aguilar *et al.*, 1987). The administration of FSH and LH from

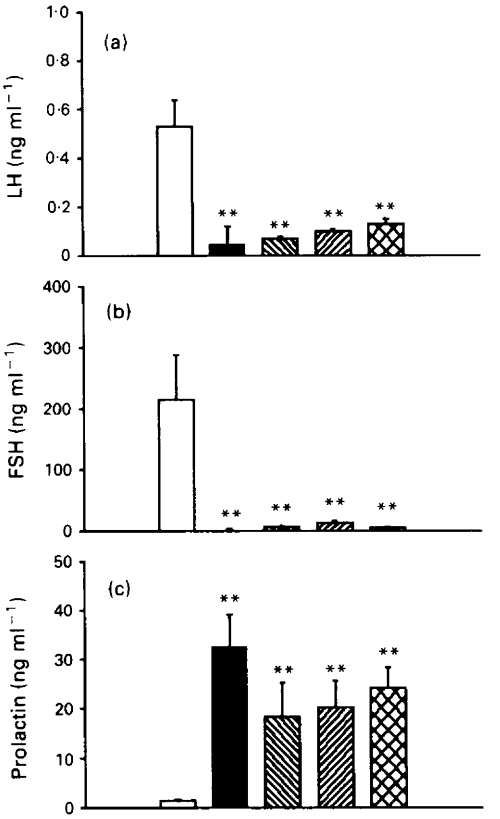

Fig. 5. (a) Luteinizing hormone (LH), (b) follicle-stimulating hormone (FSH) and (c) prolactin concentrations in plasma of 16-day-old female rats injected on day 1 with olive oil (□), 100 μg of oestradiol benzoate (■) or oestradiol benzoate plus LH-releasing-hormone agonist (▧: 0.02 μg kg^{-1} day^{-1}; ▨: 0.2 μg kg^{-1} day^{-1}; ⊠: 2 μg kg^{-1} day^{-1}). Values are means ± SEM. Each group consisted of 10–12 animals; ** $P \leqslant 0.01$ compared with oil-injected group (ANOVA followed by Tukey's test).

Table 3. Hormone concentrations and organ weights on day 10 in female rats injected with LHRH-antagonist

	Treatment	
	Vehicle (n = 8)	LHRH-antagonist (n = 10)
Pituitary FSH (ng)	629 ± 90	43 ± 7.6**
Pituitary LH (ng)	248 ± 22	93 ± 9.2**
Plasma FSH (ng ml^{-1})	58.6 ± 7.4	4.5 ± 0.8**
Plasma LH (ng ml^{-1})	1.73 ± 0.4	0.17 ± 0.02**
Plasma oestradiol (pg ml^{-1})	232 ± 119	1.6 ± 0.9**
Ovarian weight (mg kg^{-1} body weight)	9.55 ± 1.7	9.77 ± 1.4
Uterine weight (mg kg^{-1} body weight)	32.11 ± 1.2	25.47 ± 1.7**

Values are means ± SEM. **$P \leqslant 0.01$ versus vehicle-injected group.
FSH: follicle-stimulating hormone; LH: luteinizing hormone.

day 1 to day 10 abolished the effects of oestrogen (Bellido *et al.*, 1990). Female rats treated with oestradiol or testosterone also showed a decrease in gonadotrophin secretion during the early postnatal period, but, in contrast, simultaneous injection of gonadotrophin did not abolish the effects of steroids.

It has been reported that neonatal prolactin deficiency induced in female pups by treatment of lactating mothers with bromocriptine induced oestrous acyclicity, hyperprolactinaemia and reduced turnover rates of dopamine in the median eminence (Shyr *et al.*, 1986; Shah *et al.*, 1988; Crowley *et al.*, 1990) and that the administration of a prolactin antiserum to neonatal males reduced the pituitary prolactin content in adulthood and the diurnal prolactin surges (Mills *et al.*, 1982).

Implantation of additional pituitaries or exogenous administration of prolactin has been used to induce an excess of prolactin during the neonatal period. The first approach seems to be inconclusive, since neonatal female rats grafted on day 5 with one additional pituitary exhibited increased prolactin plasma concentrations only after day 17 (Tresguerres *et al.*, 1983). In the present work, we demonstrated that prolactin injected during the neonatal period at doses higher than those previously reported as effective (Jones *et al.*, 1983; Chase and Payne, 1985) did not significantly alter the reproductive function in accordance with similar results obtained in males (Bellido *et al.*, 1990). The biological activity of prolactin was confirmed by precocious vaginal opening induced after its injection from day 21, a finding reported by Clemens *et al.* in 1969). The increased LH and prolactin pituitary content and the FSH values in plasma observed in adult females injected shortly after birth with prolactin suggest that, in spite of the regular reproductive cycles of these rats, subtle changes in the regulation of pituitary secretion occur.

The results demonstrate that in females, unlike males, sterility induced after neonatal steroid treatment was not mediated by the initial decrease in gonadotrophin secretion and that an excess of neonatal prolactin did not affect ovarian activity in adulthood, in clear contrast to data obtained by others after induction of neonatal prolactin deficiency (Shyr *et al.*, 1986; Shah *et al.*, 1988; Crowley *et al.*, 1990).

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