Mechanisms of altered LH secretion in neonatally oestrogenized male rats

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Abstract

It is well known that the control of LH secretion depends on the steroid milieu during the postnatal period. In this study LH secretion was analysed in adult male rats injected neonatally with 500 μg oestradiol benzoate (1) after orchidectomy, (2) after selective elimination of androgens by destruction of Leydig cells with ethylene dimethane sulphonate (EDS), and (3) after removal in orchidectomized animals of Silastic capsules containing testosterone. In addition, (4) *in vivo* and *in vitro* LH secretion in response to LHRH agonist and antagonists, (5) the hypothalamic LHRH content, (6) the basal and stimulated *in vitro* LHRH release, and (7) the LH responses after administration of naloxone (2 mg/kg), α-methyl-p-tyrosine (α-MPT; 250 mg/kg), N-methyl-D-aspartic acid (NMDA, 15 mg/kg) or kainic acid (KA; 15 mg/kg) were also examined. Our data indicated that (1) the LH response after orchidectomy, after EDS administration and after removal of Silastic capsules containing testosterone was diminished in oestrogenized male rats, (2) the pituitaries from oestrogenized males retained responsiveness to LHRH, (3) hypothalamic LHRH content was reduced in oestrogenized males, but the hypothalamus from oestrogenized males released more LHRH than those of control groups both under basal conditions or after depolarization, (4) α-MPT decreased LH secretion only in oestrogenized males, and (5) NMDA and KA stimulated LH only in oestrogenized males. We conclude that in oestrogenized male rats the loss of sensitivity to the negative feedback action of testosterone on LH secretion was not due to decreased pituitary responsiveness to LHRH stimulation or to the inherent damage of LHRH neurones. In contrast, changes in the mechanisms governing LHRH release seem to be involved. A lack of activation of the excitatory noradrenergic and aminoacidergic systems seems to be part of the neurochemical basis of altered gonadotrophin secretion in neonatally oestrogenized male rats.

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Introduction

It is widely accepted that the feedback mechanisms that regulate luteinizing hormone (LH) secretion are sexually differentiated. In male rats, LH secretion increases significantly a few hours after orchidectomy, whereas in females the increase is observed only a week after ovariectomy (Zanisi & Martini 1975). This different pattern of LH response after gonadectomy is unrelated to the removal of different sex steroids (Gay & Hauger 1977).

Neonatal modification of the steroid milieu profoundly affects the mechanisms governing LH release. Females neonatally injected with testosterone or oestradiol show a lack of positive feedback of LH by oestrogens (Mennin & Gorski 1975, Harlan & Gorski 1977, Aguilar *et al.* 1983) and decreased LH response to ovariectomy (Damassa *et al.* 1983, Aguilar *et al.* 1984).

Males neonatally injected with oestrogens show complex and multiple alterations, such as atrophy of testes and sexual accessory glands (Aguilar *et al.* 1984), impaired maturation of germ (Dhar & Setty 1976), Sertoli and Leydig cells (Gaytan & Aguilar 1986, Gaytan *et al.* 1986), and decreased LH response to castration (Aguilar *et al.* 1984). These alterations are produced by different mechanisms. The testicular damage is due to the decreased gonadotrophin secretion immediately after administration of oestrogens (Aguilar *et al.* 1987) and can be blocked by neonatal administration of gonadotrophins (Bellido *et al.* 1990). The atrophy of sexual accessory glands is due to a permanent alteration in their androgen responsiveness (Naslund & Coffey 1986). In addition, mechanisms governing gonadotrophin secretion are permanently modified by neonatal oestrogenization (Aguilar *et al.* 1984).

In the present study we tried to analyse the mechanisms involved in the altered control of LH secretion in oestrogenized males. Thus, we specifically studied (1) LH secretion after orchidectomy, after selective elimination of androgens by destruction of Leydig cells with ethylene dimethane sulphonate (EDS) (Jackson & Morris 1977, Morris *et al.* 1986, Tena-Sempere *et al.* 1993, 1994), and after removal of Silastic capsules containing testosterone, (2) the *in vivo* and *in vitro* LH secretion in response to

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LH-releasing hormone (LHRH) agonist and antagonists, (3) basal and stimulated LHRH release, and (4) the effects of pharmacological manipulations of noradrenergic, opiatergic and aminoacidergic systems, given the major role of these systems in the regulation of LHRH release (Kalra & Kalra 1983, Leadem *et al.* 1985, Brann & Mahesh 1992, Cowell 1993).

Materials and Methods

Animals

Wistar male rats bred in our laboratory were used. The day on which animals were born was considered to be day 1 of age. At this time the litter size was adjusted to eight pups per dam, and the animals were injected with oestradiol benzoate (500 μg dissolved in 0·1 ml olive oil) or vehicle. Animals were weaned at 21 days of age and housed in groups of four to five per cage. They were maintained under controlled conditions of temperature (20 °C) and light (12 h light:12 h darkness) with free access to pelleted food (Panlab, Barcelona, Spain) and tap water. Experiments were performed in 60-day-old controls and oestrogenized males. Efficiency of neonatal oestrogenization was confirmed by the reduced weight of testes, ventral prostate and seminal vesicles.

Drugs

Oestradiol benzoate, testosterone, α-methyl-p-tyrosine (α-MPT), naloxone, N-methyl-D-aspartic acid (NMDA) and kainic acid (KA) were purchased from Sigma (St Louis, MO, USA). LHRH was from Peninsula Laboratories Inc. (St Helens, Merseyside, UK). Ac-D-pClPhe-DpClPhe-D-Trp-Ser-Tyr-D-Arg-Leu-Arg-Pro-D-Ala-NH_2CH_3COOH (Org.31276) was kindly supplied by Organon (Oss, The Netherlands). EDS was synthesized by us as previously described (Gaytan *et al.* 1990). The dosages of the various drugs were chosen on the basis of previous studies (Ojeda & McCann 1973, Aguilar *et al.* 1983, Tena-Sempere *et al.* 1993, Pinilla *et al.* 1994). All drugs were dissolved in physiological saline, except EDS which was dissolved in DMSO:water (1:3)

Experiment 1

Control and oestrogenized males were orchidectomized after light ether anaesthesia. Blood samples were obtained by jugular venipuncture after ether anaesthesia before and at different times after orchidectomy. Other animals were decapitated before or 1, 2 or 3 weeks after orchidectomy.

Experiment 2

To analyse whether the differences in LH responses to orchidectomy in control and oestrogenized males properly reflected specific changes in the sensitivity to androgen inhibition, control and oestrogenized males were injected i.p. with 80 mg EDS/kg, a specific toxin for Leydig cells (Jackson & Morris 1977, Morris *et al.* 1986, Tena-Sempere *et al.* 1993, 1994) or vehicle. Blood samples were obtained by jugular venipuncture after light ether anaesthesia before and 1 or 2 weeks after injection of EDS or vehicle (DMSO:water).

Experiment 3

Since serum testosterone concentrations were lower in oestrogenized than in control males (Pinilla *et al.* 1989, Tena-Sempere *et al.* 1993) and to analyse the LH responses after removal of similar amounts of testosterone, control and oestrogenized males were orchidectomized and implanted with Silastic capsules (Dow Corning, Midland, MI, USA; length 3·0 cm; internal diameter 0·062 mm; outside diameter 0·125 mm) containing testosterone. Other groups of orchidectomized males were implanted with empty capsules. Seven days later these animals were anaesthetized with ether, blood samples were obtained by jugular venipuncture, and the Silastic capsules were removed. Blood samples were obtained 7 days after removal of the Silastic capsules.

Experiment 4

To analyse the pituitary response to LHRH, control and oestrogenized males were orchidectomized and a week later injected i.p. with vehicle, or 100 or 1000 ng LHRH. Blood samples were obtained by jugular venipuncture at 0, 30 and 60 min after light ether anaesthesia.

In addition, control and oestrogenized males were orchidectomized on day 60 and injected on days 60, 63 and 66 of age with the LHRH antagonist (5 mg/kg, i.p.) or vehicle. Blood samples were obtained by decapitation 2 h after administration of the last injection.

Experiment 5

Control and oestrogenized males were orchidectomized and killed a week later. Pituitaries were removed and the posterior lobe was dissected out and discarded. Anterior pituitary glands were halved and incubated in a Dubnoff shaking incubator under an atmosphere of 95% O_2 and 5% CO_2 at 38 °C in 2 ml Krebs–Ringer–bicarbonate glucose buffer (KRB), pH 7·4. After 1 h of preincubation, the medium was changed by adding fresh medium with or without LHRH (10^{-9} to 10^{-6} M) and after 2 h of incubation the medium was collected and immediately frozen at −25 °C until use. The median eminence and a block of neural tissue encompassing the medial basal hypothalamus (MBH), delimited by the hypothalamic fissures, the optic chiasm and the rostral portion of the mammillary bodies were dissected under a stereomicroscope by a horizontal cut of about 2 mm in depth using fine iris scissors and halved. Fragments of MBH were incubated (one fragment per vial) with 500 µl KRB. Thirty minutes after preincubation, the medium was removed and changed twice. In the first incubation period (0–30 min), the hypothalamic fragments were incubated with KRB and in the second period (30–60 min) with KRB or KRB with 56 mM K^+. The osmolarity of this medium was maintained by reducing the NaCl concentration proportionally to the amount of KCl added.

Experiment 6

Control and oestrogenized males were orchidectomized and a week later injected i.p. with vehicle or α-MPT (250 mg/kg), an inhibitor of the rate-limiting step in catecholamine synthesis. Blood samples were obtained by jugular venipuncture before and 4 h after vehicle or α-MPT injection.

Experiment 7

Control and oestrogenized males, either intact or orchidectomized, were injected i.p. with vehicle, naloxone (2 mg/kg), an opiatergic antagonist, NMDA (15 mg/kg), an agonist of NMDA receptors or KA (15 mg/kg), an agonist of non-NMDA receptors. Blood samples were obtained by decapitation 15 min later.

RIA of gonadotrophins

After centrifugation, serum was collected, frozen and stored at − 20 °C until use. The concentration of LH was determined by a double RIA using kits supplied by the NIDDK (Bethesda, MD, USA). Rat-LH I-6 was labelled with ^{125}I by the chloramine T method (Greenwood *et al.* 1963). LH concentrations are expressed using rat-LH-RP-3 as a standard. Intra- and interassay coefficients of variation were 6% and 8% respectively. The sensitivity of the assay was 150 pg/ml.

RIA of LHRH

MBH fragments were homogenized (ultrasound) in 100 µl 0·1 M acetic acid and, after centrifugation, samples were stored frozen until assayed for LHRH. Samples from hypothalamic tissue or incubation medium were measured in duplicate by RIA using the LHRH antibody HU-60 (generously provided by Dr H F Urbanski, Neuroscience Division, Oregon Regional Primate Research Center, Beaverton, OR, USA). Synthetic LHRH (Peninsula Laboratories, Belmont, CA, USA) was used as the reference standard and for labelling with ^{125}I. Samples and standards were incubated at 4 °C for 24 h with antiserum (HU-60) at an initial dilution of 1:25 000. The tracer (10 000 c.p.m./tube) was added on the second day and also incubated at 4 °C for 24 h. On the third day of incubation, bound LHRH was precipitated with 1 ml cold ethanol and pelleted by centrifugation (30 min at 2000 *g*). The assay buffer to dilute samples, antibody and tracer consisted of 0·01 M sodium phosphate, 0·145 M NaCl, 0·032 M EDTA, 0·0001 M sodium azide and 0·25% human serum albumin (pH 7·4). All samples were run in the same assay. Intra-assay variability was 9% and the sensitivity of the assay was 10 pg/ml.

Statistics

Data are expressed as means ± S.E.M. Statistical differences between groups were determined by Student's *t*-test or ANOVA followed by Tukey's test.

Results

Effect of orchidectomy on LH secretion

Serum LH concentrations were lower in oestrogenized (0·82 ± 0·21 ng/ml) than in control males (1·47 ± 0·25 ng/ml). In control males, serum concentrations of LH increased significantly 12 h after orchidectomy and further increases were observed later. The LH response to orchidectomy was diminished in oestrogenized males and only the levels measured 3 and 7 days after orchidectomy were significantly higher than the basal ones. After orchidectomy, serum concentrations of LH were always higher in control than in oestrogenized males. Reduced LH secretion in oestrogenized males was also observed in males decapitated 7, 14 and 21 days after orchidectomy (Table 1).

Effect of EDS treatment on LH secretion

Serum LH concentrations remained unchanged after injection of DMSO:water (data not shown) and increased a week after EDS administration in both control and oestrogenized males. However, the response was significantly blunted in oestrogenized males. Two weeks after EDS treatment, LH concentration returned to basal levels in oestrogenized males (Table 1), perhaps because of the restoration of testosterone secretion by repopulation of the Leydig cells, which occurs faster than in control males (Tena-Sempere *et al.* 1993).

Serum LH concentrations after implantation and removal of Silastic capsules containing testosterone

In control orchidectomized males implanted with empty Silastic capsules, LH secretion increased significantly 7 and 14 days after orchidectomy. In contrast, in oestrogenized

TABLE 1. Serum LH concentration (ng/ml) before (0), 7, 14 and 21 days after orchidectomy or EDS administration in adult males treated on day 1 of age with vehicle (controls) or 500 μg oestradiol benzoate (oestogenized). Values are expressed as means ± S.E.M. (n=8–12)

	LH concentration (ng/ml)			
	0 days	7 days	14 days	21 days
Treatment				
Orchidectomized				
Controls	0·54 ± 0·1	8·76 ± 0·9	12·26 ± 0·8	13·57 ± 1·0
Oestrogenized	0·58 ± 0·2	3·74 ± 1·0**	3·74 ± 1·5**	5·21 ± 1·0**
EDS treated				
Controls	0·52 ± 0·2	7·06 ± 1·0	9·53 ± 0·8	0·96 ± 0·2
Oestrogenized	0·36 ± 0·1	4·90 ± 1·0**	1·35 ± 0·2**	1·20 ± 0·2

**$P \leq 0·01$ vs controls (ANOVA followed by Tukey's test).

males LH only increased 14 days after orchidectomy, with levels lower than in control orchidectomized males (data not shown).

Implantation of Silastic capsules containing testosterone blocked the LH increase after orchidectomy in both control and oestrogenized males, and the LH levels were similar in control and oestrogenized males implanted with testosterone (Fig. 1). LH increased significantly in control and oestrogenized males a week after removal of Silastic capsules. However, the LH levels remained lower in the oestrogenized group that in controls (Fig. 1).

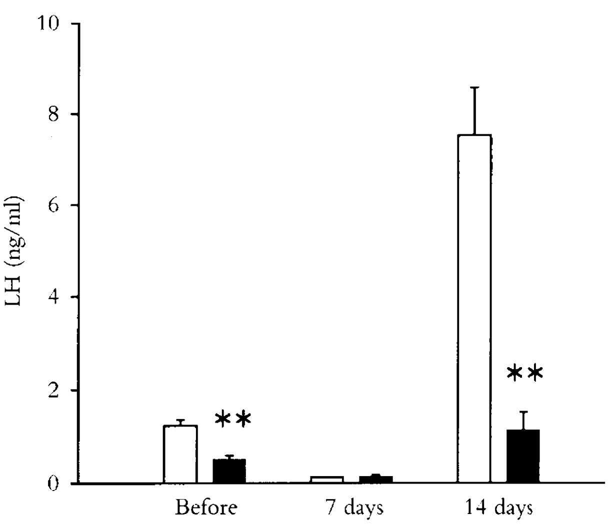

FIGURE 1. Serum LH concentration before, 7 and 14 days after orchidectomy, in control (open bars) and oestrogenized males (solid bars). Animals were implanted with Silastic capsules containing testosterone at the time of orchidectomy and capsules were removed on day 7. Values are the mean ± S.E.M. (n=10 animals per group). **$P \leq 0·01$ vs levels at the same time in the control group (ANOVA followed by Tukey's test).

Serum LH concentration after administration of LHRH or LHRH antagonist

Serum LH concentration remained unchanged 30 and 60 min after vehicle injection (data not shown). LHRH induced a significant dose-dependent increase in LH levels in both control and oestrogenized orchidectomized male rats. However, LH levels remained lower in the oestrogenized group than in controls (Fig. 2).

Since LH preinjection levels were higher in control than in oestrogenized males, we expressed the response as net increments over basal levels. In this way, we found that LH responses were similar in control and oestrogenized males (data not shown).

LHRH antagonist induced a significant decrease in serum LH concentrations in both orchidectomized control and oestrogenized male rats, with similar levels in control (0·35 ± 0·07 ng/ml) and oestrogenized males (0·11 ± 0·01 ng/ml) after treatment with LHRH antagonist.

In vitro *LH responses after LHRH*

The amount of LH secreted in the absence of LHRH by hemipituitaries from control and oestrogenized males was similar. In control and oestrogenized males, all doses of LHRH induced a significant increase in LH release although no clear dose–response was observed in control males. LH secretion after stimulation with the higher doses (10^{-6} and 10^{-7} M) of LHRH was significantly higher in oestrogenized males (Fig. 3).

Serum LH concentrations after α-MPT administration

In orchidectomized control rats, LH levels were significantly reduced 4 h after administration of α-MPT (4·87 ± 0·90 vs 9·72 ± 1·3 ng/ml in the vehicle-injected group). In contrast, no changes were observed in the

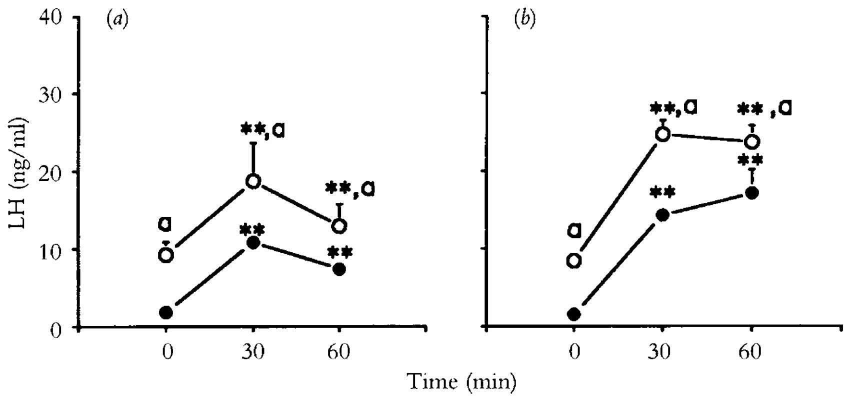

FIGURE 2. Serum LH concentration at 0, 30 and 60 min after injection of (*a*) 100 or (*b*) 1000 ng LHRH in orchidectomized control (○) and oestrogenized (●) males. Values are the mean ± S.E.M. (n=10 animals per group). **$P \leq 0{\cdot}01$ vs levels at 0 min; [a]$P \leq 0{\cdot}05$ vs corresponding levels in oestrogenized groups (two-way ANOVA followed by Tukey's test).

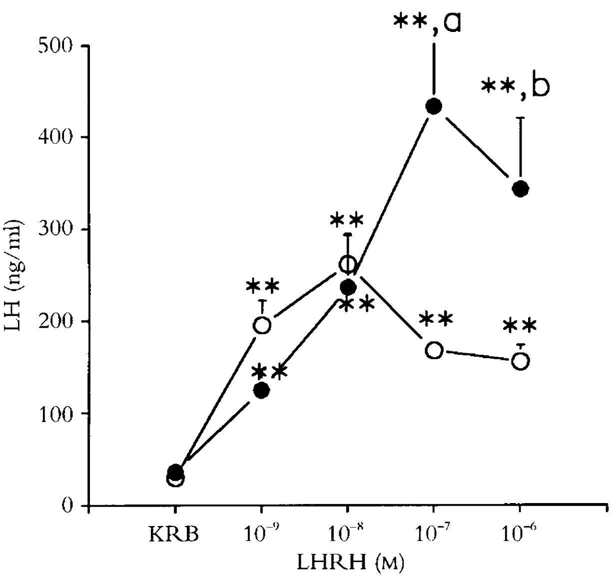

FIGURE 3. Secretion of LH by hemipituitaries from control (○) and oestrogenized (●) males after incubation for 2 h in media without or with LHRH (10^{-9} to 10^{-6} M). Values are the mean ± S.E.M. (n=10 hemipituitaries per group). **$P \leq 0{\cdot}01$ vs KRB; [a]$P \leq 0{\cdot}01$, [b]$P \leq 0{\cdot}01$ vs control males (two-way ANOVA followed by Tukey's test).

oestrogenized group (0·29 ± 0·02 vs 0·41 ± 0·07 ng/ml in the vehicle-injected group).

Serum LH concentrations after naloxone administration

Serum LH concentrations increased 15 min after naloxone administration in orchidectomized controls (10·55 ± 1·42 vs 5·37 ± 0·98 ng/ml in vehicle-injected animals) and oestrogenized males (1·03 ± 0·42 vs 0·18 ± 0·04 ng/ml in vehicle-injected animals). Therefore, the serum LH concentration after naloxone administration remained significantly higher in orchidectomized control males (10·55 ± 1·42 vs 1·03 ± 0·42 ng/ml) than in oestrogenized animals.

Serum LH concentration after NMDA or KA administration

The serum LH concentration in control males (intact or orchidectomized) remained unchanged 15 min after NMDA administration and a slight decrease was observed in intact males after KA administration. In contrast, a significant increase was observed in oestrogenized males (intact or orchidectomized) after NMDA or KA administration (Fig. 4).

Hypothalamic content and secretion of LHRH

The MBH LHRH content in the controls (14·10 ± 1·9 ng/mg tissue) increased significantly compared with the 8·26 ± 1·8 ng/mg tissue measured in oestrogenized males. LHRH release *in vitro* by hypothalamic fragments incubated with KRB or K+KRB increased in oestrogenized males (Table 2).

Discussion

Our results demonstrate that in oestrogenized males the negative feedback between testosterone and LH secretion is deeply affected, since (1) orchidectomy only induced weak increases in LH release between 12 h and 21 days after orchidectomy, (2) LH increase after selective destruction of Leydig cells with EDS was also lower and (3) LH levels after removal of Silastic capsules containing testosterone were significantly lower in oestrogenized than in

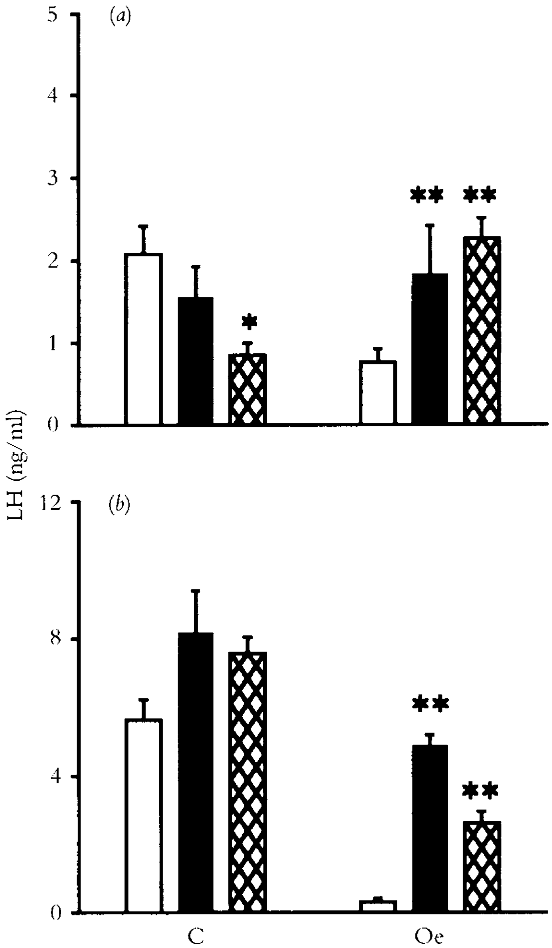

FIGURE 4. Serum LH concentration in (*a*) intact and (*b*) orchidectomized control (C) and oestrogenized (Oe) males 15 min after administration of vehicle (open bars), 15 mg NMDA/kg (solid bars) or 15 mg KA/kg (cross-hatched bars). Values are the mean ± S.E.M. (n=10 animals/per group). *$P \leq 0 \cdot 05$, **$P \leq 0 \cdot 01$ vs vehicle-injected group (ANOVA followed by Tukey's test).

control rats. This abolition of the LH response to orchidectomy cannot be explained by the withdrawal of lower amounts of androgens characteristic of oestrogenized males (Pinilla *et al.* 1989, Tena-Sempere *et al.* 1993), since the diminished response was also observed after removal of Silastic capsules containing testosterone (Fig. 1).

In principle, the loss of negative feedback may be due either to a decrease in pituitary responsiveness to LHRH stimulation or to a decreased secretion of LHRH. Our results show that pituitary responsiveness to LHRH was maintained both *in vivo* and *in vitro*. The *in vitro* response was higher in oestrogenized males after stimulation with the highest doses of LHRH. We do not have a clear explanation for the *in vitro* absence of a dose–response in the LHRH-stimulated LH release (also observed in

TABLE 2. LHRH released (pg/fragment) *in vitro* by hypothalamic fragments from orchidectomized control and oestrogenized male rats. Values are given as means ± S.E.M. of 12–17 determinations

	LHRH (pg/fragment)		
	0–30 min incubation	30–60 min incubation	
	KRB	KRB	K^+ (56 mM)
Treatment			
Controls	8·9 ± 1·2	8·5 ± 1·9	18·6 ± 2·7
Oestrogenized	15·6 ± 1·2**	14·1 ± 1·7**	25·9 ± 2·2**

**$P \leq 0 \cdot 01$ vs controls (ANOVA followed by Tukey's test).

LHRH/follicle-stimulating hormone-stimulated release, data not shown), although it is possible that exposure to high concentrations of LHRH induced a 'down-regulation' at the pituitary level in control males (Ban *et al.* 1990).

Interestingly, the serum concentration of LH after treatment with LHRH antagonist was similar in control and oestrogenized males. These results indicate that the pituitaries of control and oestrogenized males have a similar capacity to release LH in the absence of LHRH. This was confirmed by the similar amounts of LH secreted *in vitro* by pituitaries from control or oestrogenized males in the absence of LHRH. In all, the results obtained after administration of LHRH agonists and antagonists suggest that the functionality of pituitary gonadotrophs was maintained in oestrogenized males.

Enhancement of gonadotrophin secretion after gonadectomy is primarily dependent upon increased pulsatile LHRH secretion from the hypothalamus (Clayton *et al.* 1982, Plant & Dubey 1984, Levine & Duffy 1988), which induced, several days later, a decrease in LHRH content (Badger *et al.* 1978) and LHRH release *in vitro* (Kalra *et al.* 1987). LHRH release is stimulated by noradrenaline (Negro-Vilar *et al.* 1979, Ojeda *et al.* 1982), excitatory amino acids (Donoso *et al.* 1990), galanin (López & Negro-Vilar 1990), neuropeptide Y (Kalra & Crowley 1992) and endothelins (Moretto *et al.* 1993) and is inhibited by opioids and gamma aminobutyric acid (Masotto *et al.* 1989). The decreased LHRH release in oestrogenized males may be due either to inherent damage of LHRH neurones or to an imbalance between inhibitors and stimulators of LHRH release. If one considers that, in the rat, the neurones that concentrate oestradiol and those that contain LHRH do not overlap (Shivers *et al.* 1983), the effect of oestradiol given neonatally is probably exerted on neurones governing the LHRH system.

Our results show that the hypothalamic content of LHRH decreased in oestrogenized males, while the *in vitro* release increased. The reduced hypothalamic content of LHRH and LH secretion are evidence that mechanisms governing the synthesis and secretion of LHRH were damaged in oestrogenized males. In contrast, the *in vitro* LHRH release, increased both in basal conditions and after depolarization with K^+, rules out damage of LHRH neurones by neonatal oestrogenization.

It is well known that opioids inhibit LH secretion (Kalra & Kalra 1983, Leadem *et al.* 1985). The differences in LH secretion remained in control and oestrogenized males after the blocking of the endogenous opiatergic tone with naloxone, which suggests that an increase in the inhibitory effects of opioids is not the major factor responsible for the decreased LH secretion in the oestrogenized males.

Hypothalamic LHRH neurones receive dense inervation by the noradrenergic fibres ascending in the ventral noradrenergic tract. The noradrenergic system is involved in both basal and stimulated LH secretion. Orchidectomy increased the turnover of noradrenaline in the hypothalamus (Donoso *et al.* 1969) and the blocking of noradrenaline synthesis with α-MPT (Spector *et al.* 1965) inhibited LH secretion in male rats (Ojeda & McCann 1973). Our data show that α-MPT attenuated the LH response after orchidectomy in control rats, but not in oestrogenized ones. This different response indicates that the noradrenergic system was not activated after orchidectomy or that, alternatively, the synaptic connection between noradrenergic and LHRH neurones was impaired in oestrogenized males.

It has been reported in recent years that systemic administration of excitatory amino acids stimulates LH release (Price *et al.* 1978, Cicero *et al.* 1988, Strobl *et al.* 1993) and that NMDA and non-NMDA receptors (activated by KA) are involved in the control of LHRH secretion (Bourguignon *et al.* 1990, Donoso *et al.* 1990). The NMDA action on LH release is mediated by the increase of LHRH secretion and blocked by prior LHRH antagonist administration (Strobl *et al.* 1993). Our results show that NMDA and KA stimulated LH secretion in adult oestrogenized males in contrast to data obtained in control males. The greater effectiveness of NMDA in oestrogenized males may be explained by a change in the number or affinity of NMDA receptors, by the existence of a greater releasable pool of LHRH in oestrogenized males or by low testosterone secretion. The similar LH secretion in control and oestrogenized males after activation of NMDA and non-NMDA receptors strongly suggests that a reduced release of NMDA may be involved in the diminished LH response after orchidectomy in oestrogenized males.

In conclusion, the present experiments indicate that, in oestrogenized male rats, the loss of sensitivity to the negative feedback action of testosterone on LH secretion was not due to the removal of lower amounts of testosterone, to decreased pituitary responsiveness to LHRH stimulation or to inherent damage of LHRH neurones. On the contrary, changes in the mechanisms governing LHRH release seem to be implicated. A lack of activation of the excitatory noradrenergic and aminoacidergic system seems to be part of the neurochemical basis of altered gonadotrophin secretion in neonatally oestrogenized male rats.

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