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Compensation by pulsatile GnRH infusions for incompetence for oestradiol-induced LH surges in long-term ovariectomized gilts and castrated male pigs

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The aim of this study was to investigate incompetence for oestradiol-induced LH surges in long-term ovariectomized gilts and male pigs. Gilts (250 days old; $n = 36$), which had been ovariectomized 30 (OVX 30) or 100 days (OVX 100) before the start of treatment, were challenged i.m. with oestradiol benzoate and were either given no further treatment, fed methallibure to inhibit endogenous GnRH release or fed methallibure and given i.v. pulses of 100 or 200 ng GnRH agonist at 1 h intervals during the LH surge (48–96 h after oestradiol benzoate). The same treatments were applied to long-term orchidectomized male pigs (ORC, $n = 23$). In addition, one ORC group was not injected with oestradiol benzoate but was fed methallibure and given pulses of 200 ng GnRH agonist. Oestradiol benzoate alone induced an LH surge in the OVX 30 group only (5/6 gilts), methallibure suppressed ($P < 0.05$) oestradiol benzoate-induced LH secretion, while pulses of 100 ng GnRH agonist in animals fed methallibure produced LH surges in four of six OVX 30 and four of six OVX 100 gilts. The induced LH surges were similar to those produced by oestradiol benzoate alone in OVX 30 gilts. Pulses of 200 ng GnRH agonist produced LH surges in OVX 30 (6/6) and OVX 100 (6/6) gilts and increased the magnitude of the induced LH surge in OVX 100 gilts ($P < 0.05$ compared with 100 ng GnRH agonist or OVX 30 control). Pulses of 200 ng GnRH agonist also induced LH surge release in ORC male pigs (5/6), but were unable to increase LH concentrations in a surge-like manner in ORC animals that had not been given oestradiol benzoate, indicating that oestradiol increases pituitary responsiveness to GnRH. These results support the hypothesis that oestradiol must inhibit secretion of LH before an LH surge can occur. It is concluded that incompetence for oestradiol-induced LH surges in long-term ovarian secretion-deprived gilts and in male pigs is due to the failure of oestradiol to promote a sufficient increase in the release of GnRH.

Introduction

The ability of gilts to respond to oestrogen stimulation with an LH surge matures gradually as a function of age. This maturation involves a change in both the magnitude and the timing of the LH response to oestradiol benzoate. In contrast to pubertal gilts, immature 60-day-old gilts display low magnitude delayed LH surges after administration of oestradiol benzoate (Elsaesser and Foxcroft, 1978; Dial *et al.*, 1984; Flemming and Dailey, 1985).

The control mechanisms of this maturational process are not fully understood. Central inhibitory opioidergic systems do not seem to be of major importance in preventing mature LH surge responses to oestradiol benzoate at 60 days of age (Küneke *et al.*, 1993). At this age, one cause for the lack of positive feedback responses to oestradiol is the failure of the gonadotrophs to respond to increased GnRH release during the surge period, as is observed in adult animals (Küneke *et al.*, 1993). Foxcroft *et al.* (1984) reported that although maturation of the LH surge mechanism could not be accelerated in immature 60-day-old gilts by pretreatment with oestradiol, later maturation appears to be ovarian and is probably oestradiol-dependent. There was a significant reduction in the size of oestradiol benzoate-induced LH surges on day 160 in gilts ovariectomized on day 60 compared with gilts ovariectomized on day 130. Oestradiol substitution therapy, after ovariectomy on day 60, effectively restored the size of the LH response. According to more recent data (Elsaesser *et al.*, 1998), continuous ovarian secretion is also necessary for final maturation of the LH surge mechanism in late prepubertal gilts and for maintaining the full functionality of the mechanism in sexually mature gilts.

Oestrogen acts on both the hypothalamus and the pituitary to induce an LH surge. General central nervous

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system inhibition using pentobarbitone either blocks or delays the LH surge in most sows anaesthetized during pro-oestrus (Parvizi *et al.*, 1976). The oestrogen-induced LH surge in ovariectomized gilts is blocked after administration of the centrally acting compound methallibure (Kesner *et al.*, 1987), after hypophysial stalk transection (Kesner *et al.*, 1989a), or more specifically after administration of GnRH antiserum (Britt *et al.*, 1991). At the hypothalamus, oestrogen apparently inhibits or greatly reduces the release of GnRH for about 48–54 h, and then causes a punctual release of GnRH which induces an LH surge that is proportional to the amount of GnRH (Britt *et al.*, 1991). At the pituitary, oestrogen acts to suppress the response to GnRH for a period of 12–24 h, thereby increasing the readily releasable pool of LH at the time of the LH surge. Oestrogen does not change the sensitivity of the pituitary to GnRH during the period of positive feedback (Kesner *et al.*, 1987; Britt *et al.*, 1991).

The aim of this study was to understand the endocrine causes of the failure to induce positive oestrogen feedback in long-term ovariectomized gilts, and to distinguish between hypothalamic and pituitary sites of incompetence in the positive feedback action of oestrogen. The approach was to determine the effect of GnRH agonist pulses administered at 1 h intervals during the LH surge (48–96 h after oestradiol benzoate treatment) on the LH surge characteristics in short- and long-term ovariectomized gilts, in which the endogenous GnRH secretion had been blocked by the noradrenaline synthesis inhibitor methallibure (Kesner *et al.*, 1987). In addition, orchidectomized male pigs were used, providing another animal model in which oestradiol fails to induce positive feedback surge release of LH. The incompetence for oestradiol-induced LH surges in male pigs is due to sexual differentiation in brain centres controlling LH secretion by androgens secreted from the testis during a critical period in fetal life (Elsaesser and Parvizi, 1979).

Materials and Methods

German Landrace gilts and barrows from the Institut für Tierzucht und Tierverhalten (FAL) experimental farm, in which first oestrus is observed between 190 and 210 days of age, were used at 250 and 220 days of age, respectively. Gilts ($n = 36$) were ovariectomized under general anaesthesia (5–8 ml Stressnil® and 6–9 ml Hypnodil®) either 30 (OVX 30) or 100 days (OVX 100) before the start of the experiments (oestradiol benzoate treatment). Male pigs ($n = 23$) were orchidectomized at about 3 weeks of age (ORC). After surgery, gilts were housed individually under natural daylight conditions at the experimental farm. From about 10 days before challenge with oestradiol benzoate (Progynon B Oleosum, 5 mg ml^{-1}; Schering AG, Berlin) gilts were housed individually in animal crates in a purpose-built environmentally controlled building under the natural seasonal photoperiod and constant temperature (20–22°C). Animals were fed to appetite at 7:00 h and 13:00 h (approximately 1.8 kg day^{-1}). All animals were surgically fitted with either one or two indwelling jugular catheters (right and left external jugular veins) 3–4 days before the first blood sample was taken, as described by Ellendorff *et al.* (1977). Injections of oestradiol benzoate were administered i.m. to all male castrates at 220 days of age and to all ovariectomized gilts at 250 days of age. Thirteen OVX 30 gilts and 10 male castrates received another treatment after 2 weeks. OVX 100 gilts were not used in the experiments for a second treatment to avoid any possible carry over effect of oestradiol benzoate treatment on the functioning of the LH surge mechanism (induction of competence). Exposure of male pigs orchidectomized neonatally to oestradiol via Silastic implants for various periods (day 60–130 or day 60–160) did not induce competence for positive feedback at 160 days of age (F. Elsaesser and N. Parvizi, unpublished).

Animals were randomly allocated within litters to the various treatments. All animals (OVX 30, OVX 100, ORC), except for a group of male castrates that received sesame oil, were treated with 10 µg oestradiol benzoate kg^{-1} body weight i.m. and either received no further treatment (control), i.v. pulses of 2 ml 0.9% saline solution at 1 h intervals from 48 to 96 h after oestradiol benzoate (sham control), 120 mg methallibure per animal per day after oestradiol benzoate injection, or methallibure as described plus i.v. pulses of either 100 or 200 ng GnRH agonist (des-Gly^{10} [D-Ala^{6}] GnRH ethylamide; Sigma, Deisenhofen) at 1 h intervals from 48 to 96 h after oestradiol benzoate. Methallibure (also named AIMAX, Suisynchron-Premix, Serumwerke Bernburg; 6 g per animal per day) was fed once a day 30 min before the morning feed for 5 days, except for day 1, when it was given immediately after oestradiol benzoate treatment before the 13:00 h feed. Methallibure is thought to act centrally (Malven, 1971) to inhibit LH secretion and blocks LH surges induced by oestradiol benzoate in mature ovariectomized gilts without compromising LH release in response to exogenous GnRH (Kesner *et al.*, 1987). Methallibure is thought to suppress noradrenaline and increase the dopamine content in the hypothalamus in a manner similar to that reported for diethyldithiocarbamate (Chang *et al.*, 1995). The pulsatile GnRH agonist treatments were chosen because identical regimens in ovariectomized gilts, in which the positive oestrogen feedback was blocked by GnRH antiserum or hCG, stimulated an LH surge that did not differ from oestradiol-primed controls (Ziecik *et al.*, 1988; Britt *et al.*, 1991). Infusions were performed through PVC tubings 3–4 m in length. The infusion volume was 2 ml per pulse with a pulse duration of 1 min. Either a Havard (Millis, USA) or Braun (Melsungen) pump was used for infusion.

The experiments were performed in two replicates of up to 14 animals each. Each replicate included control treatments and three or four of the other treatment combinations.

Blood sampling

Blood samples (2.5 ml) were collected for analysis of LH, starting 4 h before and continuing until 120 h after i.m. injection of oestradiol benzoate (or sesame oil in one ORC group; see Table 1). Sample intervals were 8 h from 4 to 44 h (negative feedback) and 100 to 116 h, and 4 h during all other periods. In the dark phases, red-light forehead torches were

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Table 1. Mean (± SEM) LH surge characteristics (52 to 96 h after treatment with oestradiol benzoate) in ovariectomized and orchidectomized pigs

Treatment	Proportion with mature LH surge	Area under curve of LH surge (ng ml^{-1} plasma per 48 h)	Time to peak (h)	Peak LH amplitude (ng ml^{-1})
OVX 30				
Control	5/6	13.4 ± 2.7^{c}	65.3 ± 1.7^{ab}	3.3 ± 0.6^{c}
Methallibure	0/7	2.0 ± 0.8^{a}	69.7 ± 4.7^{b}	1.0 ± 0.2^{a}
GnRH agonist 100	4/6	11.8 ± 1.7^{c}	63.3 ± 1.6^{ab}	2.4 ± 0.3^{abc}
GnRH agonist 200	6/6	12.4 ± 1.1^{c}	55.3 ± 1.9^{a}	3.7 ± 0.3^{bc}
OVX 100				
Control	0/4	3.3 ± 0.5^{a}	71.0 ± 6.6^{ab}	1.5 ± 0.2^{a}
Methallibure	0/6	2.9 ± 1.3^{a}	86.7 ± 4.7^{b}	1.4 ± 0.3^{a}
GnRH agonist 100	4/6	17.9 ± 2.4^{c}	61.3 ± 3.0^{a}	4.3 ± 0.9^{bcd}
GnRH agonist 200	6/6	24.4 ± 2.2^{d}	53.3 ± 0.8^{a}	7.2 ± 1.0^{d}
ORC				
Control	0/7	8.4 ± 1.9^{b}	85.1 ± 4.9^{b}	2.4 ± 0.4^{b}
Methallibure	0/6	0.1 ± 0.6^{a}	60.7 ± 9.1^{ab}	1.0 ± 0.3^{a}
GnRH agonist 100	3/8	7.0 ± 1.9^{b}	65.5 ± 3.2^{ab}	2.7 ± 0.7^{b}
GnRH agonist 200	5/6	29.7 ± 4.7^{d}	59.3 ± 1.2^{ab}	6.4 ± 1.2^{d}
Placebo + GnRH agonist 200	0/5	3.1 ± 1.1^{a}	67.2 ± 19.3^{ab}	1.3 ± 0.2^{a}

OVX 30: ovariectomized 30 days before treatment with oestradiol benzoate.
OVX 100: ovariectomized 100 days before treatment with oestradiol benzoate.
ORC: orchidectomized neonatally.
Methallibure: 120 mg methallibure per animal per day after treatment with oestradiol benzoate.
GnRH agonist 100 and 200: 120 mg methallibure per animal per day after oestradiol benzoate treatment plus i.v. pulses of 100 or 200 ng GnRH agonist at 1 h intervals for 48–96 h after oestradiol benzoate treatment.
Placebo + GnRH agonist 200: oestradiol benzoate replaced by sesame oil; treatment with methallibure and GnRH agonist as for GnRH agonist 200.
Means within a column with different letters are significantly different ($P < 0.05$).

used during sampling. Heparinized samples were kept on ice, centrifuged for 20–30 min at 1000 *g* within 5 h of collection and plasma was stored at –20°C until analysis.

Hormone analysis

Plasma LH was determined in duplicate 100 ml samples by a homologous radioimmunoassay (Pomerantz *et al.*, 1974; Ponzilius *et al.*, 1986). A specific antiserum (UCB porcine-anti-LH; UCB, Brussels) raised in rabbits against pig LH was diluted 1:80 000. Purified pig LH (LER-786-3) had a biological activity of 0.65 NIH-LH-S1 U mg^{-1} and was used for both the standard stock solution and for ^{125}I-labelled hormone tracer. The sensitivity of the LH assay was 0.2 ng ml^{-1} plasma at 90% B:B_0. The intra- and interassay coefficients of variation were 3.5 and 6.0%, respectively. Gilts from different treatments were represented in each assay.

Statistical analysis

The following criteria were used to determine treatment effects on positive feedback characteristics. On the basis of previous findings (Elsaesser and Foxcroft, 1978, Foxcroft *et al.*, 1984), the positive feedback (LH surge) period was defined as the period from 52 to 96 h after oestradiol benzoate treatment. Two-way ANOVA within the surge period using the General Linear Model procedure for repeated measurement revealed abnormal distribution and unequal variances, even after logarithmic transformation of LH concentrations. Therefore, LH surge area under the curve (ng LH ml^{-1} plasma per 48 h) was calculated for each animal by substracting the mean LH concentration during the negative feedback period from the LH concentrations during positive feedback (samples 52 to 96 h after oestradiol benzoate) and summing these values. Treatment effects on the characteristics of the induced LH surges were evaluated (Elsaesser *et al.*, 1998) using the following criteria: A = time to the onset of the LH surge (h), calculated as the first time after the period of LH inhibition that LH concentration increased above 1 ng ml^{-1} plasma; B = time to LH surge peak (h) defined as the time to the highest LH concentrations occurring in the first 20 h after the time of onset defined as A; (this should ensure that high LH concentrations due to recovery from negative feedback are not used); C = peak LH amplitude (ng ml^{-1}) corresponding to LH concentration at time B. Each LH surge was also classified as being immature or mature. LH surges were classified as immature on the basis of the findings of Foxcroft *et al.* (1984) that immature 60-day-old gilts respond to oestradiol benzoate with small LH surges and that in 160-day-old gilts, ovariectomized at 60 days of age, the maximal LH concentration during the surge period does not exceed the maximal LH concentration in the

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period before oestradiol benzoate treatment. In LH surges classified as mature, the maximum LH concentration during the surge period exceeded the LH concentrations in the period before oestradiol benzoate treatment, again indicating that the increase in LH concentrations does not represent a removal of oestradiol negative feedback. Data from animals in which an LH surge could not be induced were included in the statistical analysis. Since there was no apparent carry over effect of pretreatment on LH surge characteristics after the second treatment, results from identical treatments were pooled for subsequent analyses. Data from replicates were pooled, as there was no difference among replicates. Results from controls and sham controls were pooled, since there was no difference in the data from these groups. Data were subjected to least squares one-way ANOVA using SigmaStat™ statistical software (Jandel Scientific, Erkrath). When treatment effects were significant, the Student–Newman–Keuls method was used for pairwise multiple comparisons.

Results

Oestradiol benzoate induced LH surges in OVX 30 control gilts (Fig. 1) and peak concentrations that averaged 3.3 ± 0.6 ng ml^{-1} plasma occurred 65 h after steroid injection (Table 1). Methallibure blocked the oestradiol benzoate-induced LH surge completely and maintained plasma LH concentrations below the values before oestradiol benzoate treatment. Administration of 100 ng GnRH agonist at 1 h intervals during the LH surge period to methallibure-treated OVX 30 gilts restored the LH surge to a magnitude similar to that in the control group (Table 1). Administration of 200 ng GnRH agonist did not change the characteristics of the induced LH surge ($P > 0.05$) compared with the 100 ng GnRH agonist group or the OVX 30 controls.

In OVX 100 control gilts, there was no surge in LH secretion after oestradiol benzoate treatment (Fig. 1). Methallibure treatment did not decrease plasma LH concentrations further (Table 1), while pulsatile treatment with 100 ng GnRH agonist at 1 h intervals produced LH surge characteristics in four of six gilts that were similar to those in OVX 30, short-term ovariectomized control gilts. The higher dose of GnRH agonist (200 ng) produced LH surges in six of six gilts and significantly changed the induced LH secretion (ng ml^{-1} per 48 h).

In orchidectomized pigs, oestradiol benzoate alone did not induce an LH surge (Fig. 1). Methallibure suppressed plasma LH concentrations, while pulses of 100 ng GnRH agonist at 1 h intervals induced LH surges in three of eight male castrates (Table 1). The higher dose of GnRH agonist produced LH surges in five of six animals. This treatment differed from all other ORC treatments with respect to surge release of LH (ng ml^{-1} per 48 h) and peak LH concentrations (Table 1). None of the animals challenged with oil instead of oestradiol benzoate responded to pulses of 200 ng GnRH agonist with an LH surge.

In general, with the exception of the ORC group treated with methallibure, the time to the LH surge peak was delayed in treatment groups in which the proportion of animals with mature LH surge responses was low.

Discussion

In accordance with previous work in prepubertal, late pubertal and sexually mature gilts (Foxcroft *et al.*, 1984; Elsaesser *et al.*, 1998), gilts that had been ovariectomized 100 days before the start of the experiments did not respond to oestradiol benzoate treatment with an LH surge. The incompetence for oestradiol-induced LH surges in these gilts is due to lack of ovarian secretions, possibly oestrogens, since in prepubertal gilts, oestrogen replacement therapy after ovariectomy effectively restored the magnitude of the LH response. In addition, positive feedback responses could not be induced in castrated male pigs, which is also in agreement with previous work (Elsaesser and Parvizi, 1979). However, non-functioning of the LH surge mechanism in male pigs is thought to be due to masculinization of control centres in the central nervous system by fetal androgens (Elsaesser and Parvizi, 1979) and not to lack of postnatal oestrogen. This is because in male pigs orchidectomized neonatally, exposure to oestradiol via Silastic implants for various periods (day 60–130 or day 60–160) did not induce competence for positive oestrogen feedback at 160 days of age (F. Elsaesser and N. Parvizi, unpublished).

The present work indicates that the lack of positive feedback response in long-term ovariectomized gilts and in male ORC pigs is not the result of inadequate releasable pools of pituitary LH. This is because a mature LH surge response was induced by the regimen involving administration of 200 ng GnRH agonist in OVX 100 gilts (6/6) and in ORC pigs (5/6). It appears that the incompetence for oestradiol-induced LH surges in long-term ovariectomized gilts and in male pigs is due to lack of a sufficient increase in hypothalamic GnRH release. However, non-functioning of positive oestrogen feedback under different reproductive conditions appears to be due to an endocrine lesion at the pituitary. Sows in mid-lactation, which respond to oestradiol benzoate with fewer LH surges than sows at late lactation, lack adequate releasable LH pools in the pituitary (Sesti and Britt, 1993). Similarly, Küneke *et al.* (1993) suggested that oestradiol fails to generate mature LH surges in 60-day-old gilts because the gonadotrophs in immature gilts cannot respond to increased GnRH release during the surge period, as is observed in adult animals. Studies in diabetic gilts also indicate that the pituitary is the site of the depressed LH response to oestradiol (Angell *et al.*, 1996).

It is possible that oestrogens are involved in the mechanism underlying the incompetence for positive feedback on LH in long-term ovariectomized gilts deprived of ovarian secretions (Foxcroft *et al.*, 1984). In rats, oestrogens accelerate the maturation of synaptic morphology and increase the number of axodentric synapses in the arcuate nucleus, a key hypothalamic nucleus in the control of LH secretion (Arai and Matsumoto, 1978). In addition, antisera against oestrogen attenuate development of neurites by hypothalamic explants from newborn rats (Toran-Allerand, 1976).

The findings of this study indicate that the incompetence of male pigs for LH surge secretion is due to the failure of oestrogen to activate the hypothalamic pulse generator for

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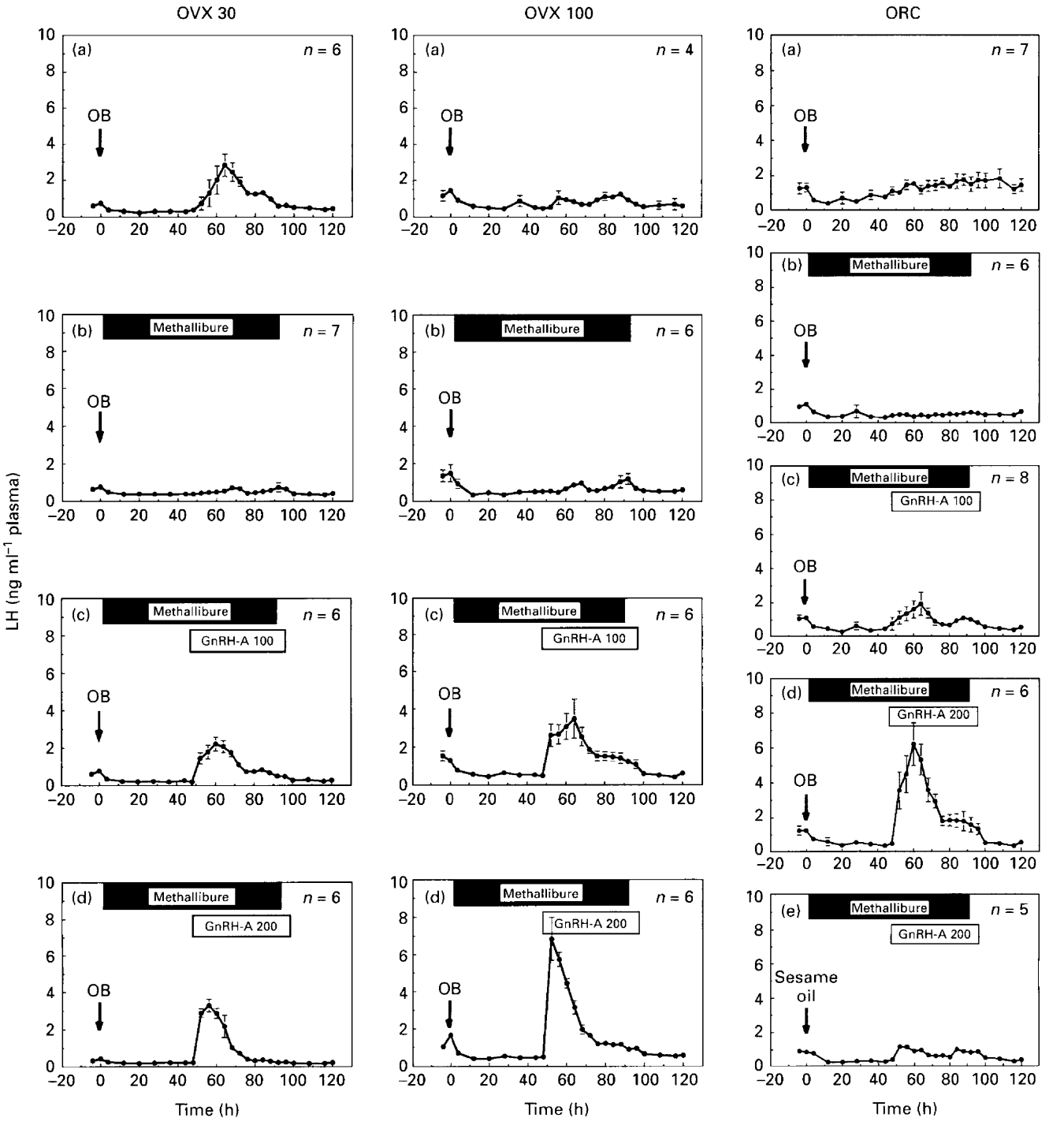

Fig. 1. Mean ± SEM plasma concentration of LH in 250-day-old gilts ovariectomized 30 (OVX 30) or 100 days (OVX 100) before treatment, and in male pigs orchidectomized neonatally (ORC). The following treatments were applied: (a) control, 10 μg oestradiol benzoate (OB) kg^{-1} body weight; (b) oestradiol benzoate and methallibure from 0 to 92 h to suppress LH secretion; (c) oestradiol benzoate, methallibure and i.v. pulses of 100 ng GnRH agonist at 1 h intervals (GnRH-A 100); (d) oestradiol benzoate, methallibure and i.v. pulses of 200 ng GnRH agonist at 1 h intervals (GnRH-A 200); (e) sesame oil, methallibure and i.v. pulses of 200 ng GnRH agonist at 1 h intervals (only in ORC).

surge release of GnRH. Male characteristics are imposed on the developing brain by the organizational action of testicular testosterone during a critical period. In rats, the neonatal period is critical, and in pigs, it is the second third of fetal life (Elsaesser and Parvizi, 1979). In fact, intraneuronal production of oestradiol by the aromatization of testicular testosterone appears to be the hormonal molecule that is responsible for the masculine differentiation of the brain

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centres controlling LH secretion. In rats, marked structural correlates of sexual differentiation have been reported, such as the sexually dimorphic nucleus of the preoptic area (see Gorski, 1985). In addition, gonadal steroids exert organizational effects on arcuate nucleus synaptic connectivity, and a lack of gonadotrophin positive feedback in male rats is associated with lack of oestrogen-induced synaptic plasticity in the arcuate nucleus (for review see Fernandez-Galaz *et al.*, 1997; Horvath *et al.*, 1997). Such information is not available in pigs.

In addition to acting on the hypothalamus, oestrogens exert strong direct or indirect effects on the pituitary. While pulsatile GnRH infusion during the period of anticipated positive feedback induced LH surges in all oestradiol benzoate-treated groups, this was not apparent in the absence of oestrogen, as was evidenced in the ORC group treated with sesame oil. Oestrogen appears to act on the pituitary by increasing pituitary responsiveness to GnRH agonist, presumably because the pituitary is first sensitized by oestradiol benzoate. This effect is apparently oestrogen-specific and is not a result of suppression of LH and GnRH release during the preceding inhibitory phase, since LH suppression by methallibure alone does not enhance responsiveness to GnRH agonist. These findings support a previous study in ovariectomized gilts, in which the responsiveness of the pituitary to GnRH agonist during the period of expected positive feedback differed when GnRH antiserum was used instead of oestradiol benzoate to suppress the secretion of LH. Gilts that received no treatment before receiving GnRH agonist pulses at 1 h intervals between 54 and 96 h did not show LH surges, indicating the importance of oestradiol benzoate-induced negative feedback in the induction of the LH surge (Britt *et al.*, 1991). When inhibition of LH during the period of negative feedback was prevented by pulses of GnRH in ovariectomized oestradiol benzoate-treated gilts (Kesner *et al.*, 1989b), the subsequent LH surge was blocked. Similarly, no LH surge occurred in oestradiol benzoate-treated ovariectomized gilts that were passively immunized against GnRH and then given pulses of GnRH agonist during the negative and positive feedback phases, rather than during the period of expected positive feedback. These observations indicate that negative oestrogen feedback permits accumulation of a readily releasable pool of LH (Britt *et al.*, 1991). Indeed, pituitary LH content increases immediately before the oestradiol-induced LH surge in ovariectomized gilts (Cox and Britt, 1982). The present study indicates that these effects of oestrogen on the pituitary also occur in male pigs, and that the incompetence for oestradiol-induced LH surges in male pigs is due to the failure of oestradiol to increase GnRH release sufficiently.

In agreement with previous findings (Britt *et al.*, 1991), the amplitude but not the duration of the LH surge was positively related to the dose of GnRH agonist, and the readily releasable pool of LH was greater than that typically released in response to oestradiol benzoate alone (treatment OVX 30 control). The ascending limb of the LH surge was steeper in gilts administered GnRH agonist than in the OVX 30 control group, indicating that the natural increase in GnRH release that induces the LH surge is more gradual. The descending limbs of the LH surges were similar among treatment groups. Furthermore, the duration of the LH surge was not increased by treatment with GnRH agonist, indicating that the gonadotrophs became refractory to further stimulation irrespective of the amount of LH that had been released. Therefore, the LH surge induced by oestradiol is terminated for reasons other than a lack of GnRH. A reduction in the number of pituitary GnRH receptors was observed at the end of the LH surge in ewes (Crowder and Nett, 1984), and continuous infusion of GnRH to ewes can decrease the number of receptors (Nett *et al.*, 1981). Therefore, the pulsatile infusion of GnRH agonist may have desensitized the gonadotrophs by downregulating the GnRH receptor, thereby terminating the LH surge.

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