Evidence that oestrogen exerts an equivalent negative feedback action on LH secretion in male and female ferrets

R. S. Carroll and M. J. Baum

Department of Biology, Boston University, Boston, MA 02215, USA

Summary. Gonadally intact male ferrets in breeding condition, which received an aromatase inhibitor, 1,4,6-androstatriene-3,17-dione (ATD) s.c. in Silastic capsules, had significantly more LH pulses and higher mean LH concentrations in plasma than did control males implanted with empty capsules. Aromatase activity in the hypothalamus + preoptic area and temporal lobe was strongly suppressed by ATD treatment whereas circulating concentrations of testosterone and oestradiol were not affected. These results suggest that oestradiol, formed via neural aromatization of circulating testosterone, contributes to the feedback regulation of LH secretion in breeding male ferrets just as oestradiol of ovarian origin controls LH secretion in females. No sex difference was observed in the rate at which mean plasma LH concentrations rose after the removal from gonadectomized ferrets of s.c. Silastic capsules containing oestradiol. Daily s.c. injections of oestradiol in oil caused an equivalent, dose-dependent inhibition of LH pulse frequency and mean LH concentrations in plasma of male and female ferrets. These findings suggest that the negative feedback control of pulsatile LH secretion by oestrogen is not sexually differentiated in this reflexly ovulating species. The ferret appears to differ from spontaneously ovulating mammalian species in which the female is generally more sensitive than the male to the inhibitory feedback action of oestradiol on LH secretion.

Keywords: ferret; oestrogen; LH; negative feedback

Introduction

Several studies indicate that the negative feedback action of oestradiol on the hypothalamic–pituitary tissues controlling luteinizing hormone (LH) secretion may be sexually differentiated, with females being more sensitive than males to the feedback action of sex steroid. To date, only spontaneously ovulating mammalian species have been used in such experiments. Several investigators (Gay & Midgley, 1969; Blackwell & Amoss, 1971; Tapper *et al.*, 1972; Hiatt *et al.*, 1987) working with rats have approached this question by comparing the rate at which plasma LH concentrations rose in the days after the withdrawal of circulating sex steroids due to gonadectomy. In all of these studies plasma LH values increased dramatically within hours after castration of the male whereas after ovariectomy of the female the rise in plasma LH was delayed for several days. A similar sexually dimorphic pattern of LH secretion occurred in gonadectomized male and female rats after withdrawal of exogenous oestradiol or testosterone treatments, respectively (Gay & Hauger, 1977). It is widely inferred from these results that the female is more sensitive than the male to the negative feedback action of sex steroids on LH secretion. Damassa *et al.* (1983) suggested that this difference in sensitivity to sex steroids results from the prenatal action of testosterone in the male rat brain. A more direct sex comparison of the negative feedback action of oestradiol has

been carried out with the rhesus monkey (Steiner *et al.*, 1976). Normal females were more sensitive than either males or prenatally testosterone-treated females to the long-term feedback inhibition of LH secretion by oestradiol, which was administered in increasing dosages after gonadectomy in adulthood. These results suggest that in the monkey, as in the rat, the sex difference in LH responsiveness to oestradiol results from the prenatal action of testosterone on the developing male neuroendocrine system.

The ferret is a reflexly ovulating carnivore, in which preovulatory LH surges occur in females only after an intromission from a male (Carroll *et al.*, 1985). In ferrets, unlike most other non-primate mammals, the ability of oestradiol to induce receptive coital behaviour is not sexually differentiated (Baum & Gallagher, 1981). We therefore asked whether the same might be true of the ability of oestradiol to inhibit the tonic secretion of LH in this species?

Materials and Methods

Animals and housing

Adult male and female Fitch ferrets (*Mustela furo*) were purchased while in breeding condition from Marshall Research Animals, North Rose, NY, USA. The ferrets were housed individually in rabbit cages with water available *ad libitum*. Purina cat chow was provided in bowls each morning. The lights in the ferret colony were on between 06:00 and 22:00 h.

Surgical procedures

Animals were anaesthetized using xylazine (Rompun: Haver-Miles Laboratories, Shawnee, KS, USA; 4.0 mg/kg) and ketamine (Ketaset, Bristol Laboratories, Syracuse, NY, USA; 35 mg/kg) administered i.m. Sterile surgical procedures were used at all times. Silastic capsules were implanted s.c. in the nape of the neck. Females were ovariectomized via a single midline incision and males were castrated via a midline scrotal incision. Jugular catheters were constructed and implanted as described previously (Carroll *et al.*, 1987). Catheters were kept patent by flushing them with 1 ml heparinized saline (0.154 M-NaCl + 100 U heparin/ml) every 2nd day.

Assays

LH. A heterologous radioimmunoassay (RIA) was used to measure plasma concentrations of ferret LH (Carroll *et al.*, 1985, 1987). Over 10 standard curves, the minimum detectable limit of the assay was 0.50 ± 0.04 ng/ml plasma, and 50% displacement occurred at 95 ± 2.1 pg NIH-LH-S20 standard/tube. The intra-assay coefficient of variation was 8.6% and the interassay coefficient of variation was 9.8%, based on a pool of ovariectomized ferret plasma which was run on 10 separate assays. Concentrations of LH were calculated using the 4-parameter logistic method (Rodbard & Hutt, 1974). In some experiments plasma LH values were further analysed using the PULSAR algorithm (Merriam & Wachter, 1982) to determine the frequency and amplitude of LH pulses in individual ferrets. The criteria used to identify individual LH pulses were based on both the peak height and the number of sequential LH values which made up the pulse. The G values (cutoff criteria) in standard deviation units used were selected empirically based on data from 7 representative ferrets, as suggested by Merriam & Wachter (1982) and Plant (1982). In the present studies these G values were $G(1) = 10.0$, $G(2) = 4.0$, $G(3), (4), (5) = 3.0$.

Testosterone. Plasma concentrations were estimated by RIA (Erskine & Baum, 1982) following steroid separation using Celite partition chromatography. The sensitivity of this assay was 12 pg/ml plasma. The intra-assay coefficient of variation was 12.0%, and the interassay coefficient of variation was 14.5%. Concentrations of androgen were calculated using the 4-parameter logistic method (Rodbard & Hutt, 1974).

Oestradiol. Plasma concentrations were determined by RIA after Sephadex LH-20 chromatography, as described by Erskine & Baum (1984). The sensitivity of this assay was 5 pg/ml plasma. Inter- and intra-assay coefficients of variation were 18.8% and 11.2% respectively. Concentrations of oestradiol were calculated using the 4-parameter logistic method (Rodbard & Hutt, 1974).

Aromatase activity. Aromatase activity in homogenates of different brain regions was estimated as previously described (Tobet *et al.*, 1985) using [19-³H]hydroxyandrostenedione (sp. act. 5 Ci/nmol; final concentration 0.5 μ M) as androgenic substrate and 2 mM-NADPH as cofactor before the chromatographic isolation of oestrone product. Results are expressed as pmol oestrone/mg protein/h (mean of duplicate estimations for each tissue sample).

Experimental procedures

In female ferrets, as in females of numerous spontaneously ovulating species, oestradiol of ovarian origin is the principal steroid involved in the negative feedback regulation of LH secretion (Donovan & Ter Haar, 1977; Ryan *et al.*, 1985; Baum *et al.*, 1986). In order to determine whether oestradiol, formed either in the brain or in other tissues from circulating testosterone, normally participates in the negative feedback control of LH secretion in male ferrets, we first studied the ability of the aromatase inhibitor, 1,4,6-androstatriene-3,17-dione (ATD), to augment the pulsatile pattern of LH secretion in this sex. A positive outcome of this study justified further comparisons of the negative feedback actions of oestradiol in male and female ferrets. Initially we compared the rise in plasma LH concentrations following the removal of s.c. Silastic capsules containing oestradiol from gonadectomized males and females. When no significant sex difference was found, we proceeded to compare the ability of several dosages of oestradiol, injected s.c. in oil, to inhibit LH pulses in gonadectomized ferrets of each sex.

Experiment 1. Groups of adult males in breeding condition were anaesthetized and implanted s.c. with Silastic capsules (i.d. 1.47 × o.d. 1.96 mm; Dow Corning Co., Midland, MI, USA) which contained the aromatase inhibitor, ATD (150 mm/kg; N = 5) or no steroid (N = 4). Other groups of males were castrated at the same time and also implanted with capsules containing ATD (N = 5) or no steroid (N = 5). After 7 days all males were implanted with jugular catheters. At 10 days after capsule implantation, blood samples (0.3 ml) were taken every 5 min for 4 h via a polyvinyl extension which was attached to the jugular catheter while the animal remained in its home cage. A blood replacement mixture (0.9 ml) was infused via the jugular catheter after the withdrawal of every fourth blood sample (Sisk & DeJardins, 1986). This regimen of blood replacement maintained the ferrets in an active, healthy condition throughout the blood collection period. Blood samples were immediately placed on ice before being centrifuged (5°C, 1006 g, 30 min). The plasma was removed and stored at -70°C until hormone radioimmunoassays were performed. At the end of the study the males were killed using CO₂ asphyxiation, decapitated, and their brains removed. The hypothalamus + preoptic area (H + POA) and temporal lobes (TL) were dissected out (Vito *et al.*, 1985) onto an ice-cold plate and were stored at -70°C before being assayed for aromatase activity.

Experiment 2. Adult male (N = 5) and female (N = 5) ferrets in breeding condition were gonadectomized upon arrival at the animal care facility. After 1 week they were implanted with s.c. Silastic capsules (1.47 mm i.d. × 1.96 mm o.d.; Dow Corning Co.), of oestradiol diluted with cholesterol (1.9 w/w; 65 mm/kg). This dose of oestradiol has previously been shown to induce behavioural receptivity and full vulval swelling in gonadectomized female ferrets (Baum *et al.*, 1982). These capsules produced plasma oestradiol concentrations of 124 ± 33 pg/ml (N = 5) in the gonadectomized males and 116 ± 44 pg/ml (N = 5) in gonadectomized females ($P > 0.05$), values that were considerably higher than the mean concentrations previously measured in intact, oestrous female ferrets (63 pg/ml; Erskine & Baum, 1984) and in intact, breeding males (8 pg/ml; Exp. 1). Beginning 7 days after capsule implantation, all animals were anaesthetized, fitted with jugular catheters, and the s.c. oestradiol capsules were removed. With the animals still anaesthetized, blood samples were taken every 15 min (5 samples) for 1 h. Subsequently, 2, 4, 6, 8 and 10 days after capsule removal, 5 blood samples were collected via the jugular catheter from each ferret over a 1 h period without the use of anaesthesia. These samples were placed on ice, centrifuged, and the plasma was stored at -70°C before being assayed for LH. Mean LH values for the 5 plasma samples taken over each 1 h bleeding period were computed for each subject, and these values were used to compute group means.

Experiment 3. Groups of adult male and female ferrets in breeding condition were gonadectomized 1–2 days after arriving at the animal care facility. Beginning 1 week later, s.c. 'pulsed' oestradiol injections or oil were given at 08:00 and 16:00 h for 6 days. Four independent groups of ferrets of each sex received the following dosages of oestradiol dissolved in sesame oil: 0 (N = 5 males; 5 females), 0.3 (N = 5; 5), 0.6 (N = 7; 7) or 2.5 (N = 5; 5) µg/kg twice/day. Preliminary data (R. S. Carroll & M. J. Baum, unpublished findings) from 4 intact, oestrous female ferrets which were fitted with jugular catheters and bled at 15 min intervals over a 4 h period showed that plasma concentrations of oestradiol fluctuated considerably in each animal and between animals (individual values ranged from 0.5–100 pg/ml). Therefore, we reasoned that a pulsed regimen of oestradiol treatment would more closely approximate the endogenous pattern of oestradiol secretion than would either a single daily s.c. injection of oestradiol or s.c. implantation of a Silastic capsule containing oestradiol. The doses administered in the present study were chosen on the basis of preliminary experiments with ovariectomized females which showed that the lowest dose (0.3 µg/kg twice/day) of oestradiol partly inhibited pulsatile LH secretion whereas the high dose (2.5 µg/kg twice/day) almost totally suppressed LH secretion. The plasma concentrations of oestradiol resulting from these different doses of injected hormone were not estimated; however, in a previous study (Carroll *et al.*, 1987) daily s.c. injections of oestradiol at a dosage (5 µg/kg twice/day) 2 times higher than the highest dosage used in the present study induced behavioural and vulval oestrus in ovariectomized ferrets and was compatible with the mating-induced secretion of LH. On the 5th day of oestradiol treatment animals were fitted with jugular catheters. Two days later (16 h after the final oestradiol injection) a polyvinyl extension was attached to each animal's jugular catheter, and it was bled in its home cage. Blood samples (0.3 ml) were taken every 5 min for 4 h. Blood replacement mixture (0.9 ml) was infused via the jugular catheter after the withdrawal of every 4th blood sample. Blood samples were immediately placed on ice before being centrifuged. The plasma was removed and stored at -70°C until RIAs were performed. Plasma LH values were further analysed using the PULSAR computer programme as described above. At 1 day after blood sampling all the animals began receiving the highest dose of oestradiol (2.5 µg/kg twice/day) for 7 days. We took this opportunity, using a different regimen of oestrogen treatment from that used in Exp. 2, again to compare the rates at which plasma LH values rise in gonadectomized male and female ferrets after oestradiol withdrawal. Blood samples were taken every 15 min for 1 h

(5 samples) at 2, 4, 6, 8 and 10 days after the last injection of oestradiol. Blood samples were immediately placed on ice before being centrifuged. The plasma was removed and stored at -70°C until RIAs were performed. Mean LH values for each of these sets of 5 samples were computed for each subject and were used to compute group means. Initial LH group means for males and females were computed from data derived from the samples collected beginning 16 h after the last injection of oestradiol. The mean LH values of individual animals were based on the 5 plasma samples taken at 15 min intervals over the first of the 4 h of frequent sampling.

Results

Experiment 1: role of endogenous oestrogen in regulating the pulsatile secretion of LH in intact, breeding males

Examples of the pattern of LH secretion exhibited by representative males from each of the 4 groups of males are shown in Fig. 1. Gonadally intact, breeding males which received ATD exhibited more LH pulses than did intact controls. Castrated males exhibited more LH pulses than did intact animals; however, ATD treatment exerted no further effect in castrates. A two-way analysis of variance (ANOVA) performed on grouped data (see Table 1) revealed a significant

Fig. 1. Effect of s.c. administration of Silastic capsules containing the aromatase inhibitor, ATD, on plasma LH concentrations in representative male ferrets which were either gonadally intact and in breeding condition or castrated. Plasma samples were collected every 5 min via an indwelling jugular catheter. Brackets designate LH pulses as identified by the Pulsar program. Open circles (○) designate LH values below the sensitivity of the assay (0.5 ng/ml).

Table 1. Effect of the aromatase inhibitor ATD on parameters of plasma LH in intact-breeding and castrated male ferrets

Groups	No.	LH pulse amplitude (ng/ml)	LH pulse frequency	Mean LH conc. (ng/ml)
Intact-breeding males				
Control	4	0.94 ± 0.19	6.25 ± 0.48	0.86 ± 0.05
ATD	5	1.29 ± 0.18	8.20 ± 0.58*	1.90 ± 0.34*
Castrated males				
Control	5	1.41 ± 0.21	7.80 ± 0.20	2.70 ± 0.25
ATD	5	1.40 ± 0.11	7.80 ± 0.37	2.17 ± 0.25

All values are expressed as mean ± s.e.m.

* $P < 0.05$ compared with respective control group.

Table 2. Effect of the aromatase inhibitor ATD on brain aromatase activity and plasma steroid concentrations in intact-breeding and castrated male ferrets

Groups	No.	Oestrone conc. (fmol/mg protein/h)		Plasma steroids (pg/ml)	
		Hypothalamus + preoptic area	Temporal lobes	Testosterone	Oestradiol
Intact-breeding males					
Control	4	30 ± 9	124 ± 11	7077 ± 2667	8 ± 3
ATD	5	3 ± 1*	4 ± 1*	4777 ± 683	11 ± 5
Castrated males					
Control	5	15 ± 7	49 ± 10	30 ± 10	12 ± 7
ATD	5	5 ± 1*	3 ± 1*	50 ± 23	8 ± 3

Values expressed as mean ± s.e.m.

* $P < 0.05$ compared with respective control group.

effect of castration on LH pulse frequency ($F(1,15) = 5.10$, $P < 0.05$) and a significant interaction between the effects of castration and ATD treatment ($F(1,15) = 5.10$, $P < 0.05$). Post-hoc analysis (Duncan's multiple range tests) indicated a significant difference ($P < 0.05$) between groups of intact males given ATD versus no steroid. Castrated males bearing either ATD or empty capsules exhibited significantly more ($P < 0.05$) LH pulses than did intact males bearing empty capsules. There was also a significant effect of castration on mean LH values ($F(1,15) = 15.93$, $P < 0.01$) and a significant interaction between the effects of castration and ATD treatment ($F(1,15) = 8.49$, $P < 0.01$). Post-hoc analysis indicated that there was a significant difference ($P < 0.05$) between groups of intact males which received ATD versus no steroid. Castrated males given empty capsules had significantly higher mean LH levels than did intact males with empty capsules ($P < 0.05$). Mean LH concentrations tended to be higher in castrated control males than in intact males given ATD (2.70 ± 0.25 vs 1.90 ± 0.34 ng/ml), but this difference was not significant. Mean LH levels were similar in intact and castrated males treated with ATD. There were no significant group differences in the amplitude of LH pulses. Aromatase activity in different brain regions was significantly lower in intact and castrated males which received ATD capsules (Table 2). A two-way ANOVA performed on the temporal lobe data showed significant effects of gonadal status ($F(1,15) = 30.19$, $P < 0.01$), ATD treatment ($F(1,15) = 143.18$, $P < 0.01$) and a significant gonadal status × ATD treatment interaction ($F(1,15) = 29.76$, $P < 0.01$). For the H + POA, there was a significant effect of ATD treatment ($F(1,15) = 22.98$, $P < 0.01$) and a significant gonadal status × ATD treatment interaction ($F(1,15) = 4.71$, $P < 0.05$). Post-hoc analyses revealed that in the absence of ATD treatment castration of males caused significant reductions ($P < 0.05$) in

Fig. 2. Plasma LH in gonadectomized male and female ferrets before (Day 0) and after the removal of s.c. Silastic capsules containing oestradiol (E_2). Data are expressed as mean $\pm$ s.e.m.; the absence of an s.e.m. bar indicates that all LH values were below the sensitivity limit of the assay (0.5 ng/ml), and these mean values are designated as 0.5 ng/ml.

aromatase activity in both the temporal lobes and H + POA, confirming a previous report from this laboratory (Tobet *et al.*, 1985). Plasma oestradiol concentrations were very low and not significantly different among these groups of males (Table 2). As expected, plasma testosterone concentrations were significantly higher in gonadally intact males than in castrated subjects; ATD treatment had no effect on plasma testosterone either in intact or gonadectomized males (Table 2).

Experiment 2: plasma LH rise after removal of oestrogen feedback inhibition in gonadectomized male and female ferrets

Silastic capsules of oestradiol suppressed plasma LH values in both sexes. After removal of the capsules, there was a non-significant trend for mean plasma LH levels to increase more rapidly in females than in males (Fig. 2). A two-way ANOVA with repeated measures revealed that LH concentrations increased significantly across time ($F(5,40) = 13.4$, $P < 0.01$); however, there were no significant sex ($F(1,40) = 3.25$, $P > 0.05$) or sex $\times$ time interaction ($F(5,40) = 1.48$, $P > 0.05$) effects.

Experiment 3: negative feedback effect of oestradiol on pulsatile LH secretion in gonadectomized male and female ferrets

Representative examples of the pattern of LH secretion exhibited by gonadectomized male and female ferrets in response to increasing dosages of oestradiol, given in a 'pulsed' fashion, are shown in Fig. 3. Oestradiol treatment decreased the pulsatile secretion of LH equally in gonadectomized male and female ferrets in a dose-dependent manner. Statistical analyses of grouped data (see Fig. 4) showed that mean LH concentrations decreased significantly as the dose of oestradiol increased ($F(3,36) = 10.3$, $P < 0.01$); however, there was no significant difference between males and females ($F(1,36) = 1.33$, $P > 0.05$). LH pulse frequency decreased significantly as the dose of oestradiol increased ($F(3,36) = 9.53$, $P < 0.01$). Again, however, this effect was equivalent in the two sexes

Fig. 3. Effect of daily s.c. injections of different dosages (/kg twice/day) of oestradiol (E_2) in sesame oil on plasma LH concentrations in representative male and female ferrets which had previously been gonadectomized. Plasma samples were collected every 5 min via an indwelling jugular catheter. Brackets designate LH pulses as identified by the Pulsar program. Open circles (○) designate LH values below the sensitivity of the assay (0.5 ng/ml).

($F(1,36) = 0.02$, $P > 0.05$). There were no significant effects of oestradiol treatment or sex on the amplitude of LH pulses ($F(3,22) = 2.9$, $P > 0.05$). After cessation of daily injections of the highest dosage of oestradiol, mean LH levels increased significantly ($F(4,52) = 40.0$, $P < 0.01$) (Fig. 5). Although mean LH values tended to increase faster in males than females, there were no significant sex ($F(1,52) = 3.75$, $P > 0.05$) or sex $\times$ time interaction ($F(4,28) = 1.18$, $P > 0.05$) effects. The reverse trend had occurred in Exp. 2 after the removal of oestradiol capsules, further affirming that no reliable sex difference exists in the rise in LH secretion after oestrogen withdrawal.

Discussion

The results of Exp. 1 suggest that oestrogen, formed in the brain via aromatization of circulating testosterone, plays a critical role in the negative feedback control of LH secretion in male ferrets. Treatment of gonadally intact, breeding males with the aromatase inhibitor, ATD, augmented the pulsatile secretion of LH nearly as effectively as castration. Brain aromatase activity was strongly suppressed by ATD treatment whereas plasma concentrations of oestradiol were uniformly low in males, regardless of whether or not they had gonads or received ATD. These findings emphasize the importance of neural as opposed to peripheral aromatization of testosterone in controlling LH secretion in male ferrets. The importance of oestradiol in controlling LH secretion is further supported by the observation (Tang & Sisk, 1988) that oestradiol more effectively suppressed

Fig. 4. Effect of daily s.c. injections of different dosages of oestradiol in sesame oil on LH pulse amplitude, pulse frequency and mean concentration in male and female ferrets which had previously been gonadectomized. Data are expressed as mean $\pm$ s.e.m. The number of ferrets from each independent group which contribute to each mean value is given above each bar.

plasma LH in castrated male ferrets than either testosterone or 5 α -dihydrotestosterone. Our observations (Exp. 1) that pulsatile LH secretion was slightly greater in castrated than in ATD-treated, gonadally intact ferrets raises the possibility that an androgen (either testosterone itself or 5 α -dihydrotestosterone, which like oestradiol is formed in the male ferret brain; Tobet *et al.*, 1985) may synergize with oestradiol to restrain LH secretion. More work is needed to explore this possibility.

Much evidence suggests that oestradiol, formed via aromatization of circulating testosterone, contributes to the regulation of LH secretion in males of several other species including the sheep (Schanbacher, 1984), dog (Worgul *et al.*, 1981), and rhesus monkey (Ellinwood *et al.*, 1984), but not the rat (Krey *et al.*, 1982). Evidence from the ferret and rhesus monkey suggests that oestradiol produced by neural aromatization in the male may ultimately inhibit LH secretion as a result of its action on GnRH neurones in the hypothalamus. This possibility is raised by observations in male ferrets that oestrogen receptors (Vito *et al.*, 1985), aromatase activity (Tobet *et al.*, 1985), and cell bodies of GnRH neurones (Sisk *et al.*, 1988) are all present in the mediobasal hypothalamus. A similar overlap of aromatase activity and immunopositive GnRH neurones occurs in the male

Fig. 5. Plasma LH in gonadectomized male and female ferrets after the termination of 'pulsed' oestradiol injections (2.5 µg/kg twice/day). Data are expressed as mean $\pm$ s.e.m.: the absence of an s.e.m. bar indicates that all LH values were below the sensitivity limit of the assay (0.5 ng/ml), and these mean values were designated as 0.5 ng/ml. The number of ferrets contributing to each mean is given.

rhesus monkey (Roselli *et al.*, 1987). Additional research is needed using ferrets and primates to determine whether oestradiol is directly formed in and bound by GnRH neurones, or whether oestradiol acts indirectly via inter-neurones to inhibit the output of GnRH.

Since oestradiol is normally involved in the negative feedback regulation of LH secretion in both male (Exp. 1) and female ferrets (see 'Methods'), we asked whether the responsiveness to this steroid of the hypothalamic-pituitary mechanism controlling LH secretion is sexually differentiated in the ferret? Evidence from Exps 2 and 3 suggests that it is not. First, using gonadectomized ferrets no overall sex difference was seen in the rate at which plasma LH values rose after the removal of Silastic capsules containing oestradiol (Exp. 2) or after the cessation of oestradiol injections (Exp. 3). Second, gonadectomized male and female ferrets showed equivalent reductions in mean plasma LH concentrations and LH pulse frequency in response to increasing dosages of oestradiol (Exp. 3). Our observations corroborate the results of separate studies using male (Sisk & Desjardins, 1986) and female (Ryan *et al.*, 1985) ferrets. In both sexes LH pulses were detected within 24 h after gonadectomy, and their frequency and amplitude increased at similar rates, reaching equivalent peak levels 15–20 days after gonadectomy. Previous research (Carroll *et al.*, 1987) showed that i.v. injections of GnRH into gonadectomized ferrets, treated chronically with oestradiol or no steroid, caused equivalent, dose-dependent increments in plasma LH levels in males and females. These findings, together with those of Exps 2 and 3, imply that the effects of oestrogen on both pituitary responsiveness to GnRH and on activity of the neural GnRH pulse generator are similar in male and female ferrets.

In contrast to the ferret, a reflex ovulator, most data collected to date suggest that in spontaneously ovulating species the female is more sensitive than the male to the negative feedback action of oestradiol. Thus (see 'Introduction'), plasma LH concentrations increased in male rats within hours after castration whereas in females this rise was delayed for several days after gonadectomy. Also, in rhesus monkeys (Steiner *et al.*, 1976) increasing dosages of oestradiol more readily suppressed mean plasma LH levels in ovariectomized females than in gonadectomized males or in females exposed prenatally to testosterone. A possible exception to this pattern of results for spontaneously ovulating species is the marmoset, in which mean plasma LH concentrations rose at equivalent rates in males and females after gonadectomy (Hodges, 1978). A more detailed

assessment of the negative feedback potential of oestradiol, including a comparison of the ability of increasing doses of oestradiol to inhibit LH secretion in male and female marmosets, was not reported in that paper. It would be useful to have such information for the marmoset and for reflex ovulating species in addition to the ferret, such as the vole and rabbit. Although the case has yet to be established conclusively, the available evidence suggests that the responsiveness of the hypothalamic-pituitary axis to the negative feedback action of oestradiol is sexually differentiated in spontaneously ovulating species but not in reflex ovulators.

There are no obvious differences in the neural distribution of oestradiol receptors or in the location of GnRH-containing neurones which would help to explain these apparent species differences in the sexual differentiation of oestradiol-dependent negative feedback control of LH secretion. Autoradiographic studies show that the distribution of oestrogen receptors in the brains of reflex ovulating species such as the ferret (Baum *et al.*, 1986), mink (Morrell *et al.*, 1977), cat (Rees & Michael, 1984) and rabbit (Anderson, 1975) resembles that of several spontaneously ovulating species, including the rat (Pfaff & Keiner, 1973), hamster (Krieger *et al.*, 1976), guinea-pig (Sar & Stumpf, 1975) and monkey (Pfaff *et al.*, 1976). The distribution of GnRH neurones in the ferret (King & Anthony, 1984; Sisk *et al.*, 1988), with numerous immunopositive cell bodies located in the mediobasal hypothalamus and pituitary stalk, more closely resembles that of the human (Barry, 1976) and monkey (Barry & Carette, 1975) than that of the rat (King *et al.*, 1982), in which most GnRH cell bodies are located more rostrally in the hypothalamus and preoptic area. Yet the negative feedback control of LH secretion is sexually differentiated in the rat and monkey, but not in the ferret. More detailed information concerning the relationship between oestrogen-concentrating neurones and the neurones which comprise the GnRH pulse generator in males and females representing both reflex and spontaneously ovulating species might help to explain the species difference in the degree to which the feedback control of LH secretion by oestradiol is sexually differentiated.

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