Half-life of FSH and LH in the ferret

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Abstract. Anoestrous and oestrous ferrets were injected with luteinizing hormone-releasing hormone (LRH) or a long-acting analogue and subsequently hypophysectomized. Spayed ferrets were hypophysectomized without prior treatment with gonadotrophin releasing factor, and serial blood samples collected from all animals in order to follow the rate of decline in plasma gonadotrophin concentration. The half-life of LH in the spayed female (around 2 h) was much longer than that of the hormone released from the hypophysis of anoestrous females by LRH (25 min) or by the analogue (19 min). The half-life of FSH released by LRH or analogue in anoestrous females was approximately 65 min, while that discharged by the analogue in oestrous females was about 4 h. The fall in plasma FSH concentration in spayed females after hypophysectomy was too slow to allow calculation of a half-life.

The ferret is a carnivore with a well-defined breeding season, during which the female remains on heat until ovulation is triggered by the neural stimuli associated with mating. Thus, distinct phases of reproductive activity (anoestrus, oestrus, pregnancy, or pseudopregnancy) can be studied without the complications introduced by the rhythmic hormonal changes associated with an oestrous cycle. Extension of the hours of illumination during the winter hastens the development of oestrus in sexually quiescent females, but the nature of the change in pituitary gland function brought about by this means is not understood (Donovan 1967; Donovan & Gledhill 1981).

Natural mating is a lengthy process, with coupling being sustained for an hour or so. Ovulation occurs 30–36 h later, and the female becomes pregnant or pseudopregnant (Donovan 1963; Harris & Campbell 1966). Experimentally, ovulation can be induced by electrical stimulation of the hypothalamus, with gonadotrophin secretion being increased only during the period of excitation (Donovan & ter Haar 1977a). In anoestrous females, the surges of gonadotrophin secretion produced by hypothalamic stimulation are much larger, perhaps because the pituitary gland is more sensitive to hypothalamic gonadotrophin-releasing hormone at this time (Donovan & ter Haar 1977b).

The results of several studies on the control of reproduction in the seasonally breeding female ferret indicate that the oestrous condition is not associated with an increase in the plasma level of gonadotrophins. Paradoxically, the plasma levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), as measured by radioimmunoassay, appear to be higher during anoestrus than during oestrus (Donovan & ter Haar 1977a; Donovan & Gledhill 1981). One possible explanation is that the gonadotrophic activity measured immunologically does not reflect biological potency and that a change in the nature of the hormone secreted, significant for oestrus, might pass undetected by the radioimmunoassay. However, such a change in molecular structure might be expected to affect the half-lives of FSH and LH under various reproductive conditions and this possibility has been tested, using radioimmunoassay. Luteinizing hormone-releasing hormone (LRH), or a long-acting analogue (HOE 766), was used to release gonadotrophins from the hypophysis of anoestrous and of oestrous females before hypophysectomy, while the half-lives of FSH and LH in spayed females were also examined. Marked differences in half-life emerged.
Materials and Methods

Anoestrous, oestrous and spayed female ferrets (640–1150 g body weight) were housed under controlled lighting conditions (long days: 16 h light, 8 h dark/day; short days: 8 h light, 16 h dark/day) and fed a tinned ‘dog convalescent diet’ (Petfoods Ltd.) once a day with

Dog Diet A (Cooper Nutritional Products) and water being available ad libitum. Oestrous females were identified by the presence of vulval swelling of 10 mm diameter, or more, and the condition was subsequently confirmed by histological examination of the ovaries and uterus.

Each ferret was anaesthetized with sodium pentobarbitone (Sagatal, May and Baker, 36 mg/kg), and a ‘Silastic’ polymer catheter (Dow-Corning) inserted into one external jugular vein to end close to the heart. Blood samples (1 ml) were collected into heparinized syringes and replaced with an equivalent volume of 0.9% sodium chloride solution injected down the catheter. LRH, or LRH analogue, was injected immediately after venous catheterization and the pituitary gland removed by the parapharyngeal route when elevated levels of plasma FSH and LH were expected (Donovan & ter Haar 1977b; Gledhill & Donovan 1981). Blood samples were collected approximately 30, 15 and 2 min before hypophysectomy to monitor control levels and then at 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 120, 150, 180, 210, 240 and 270 min after the pituitary gland was removed. Care was taken to keep the animals warm throughout the experiment. Each ferret was killed with an overdose of anaesthetic and the head and genital tract removed and fixed in formal saline for histological examination. The sella turcica was serially sectioned to check the completeness of hypophysectomy, and only the results from completely hypophysectomized animals are presented here. Plasma concentrations of FSH and LH were measured by heterologous radioimmunoassays validated for the ferret (Donovan & ter Haar 1977a), with all of the samples from a ferret being measured in the same assay. The FSH assays used a rabbit antiserum against ovine FSH (JU 619/II) and a highly purified rat FSH as tracer (NIAMDD-RFSH-1-1), with values being expressed in terms of ng NIAMDD-FSH-RP1/ml. LH was measured using a rabbit antiserum raised against ovine LH (CDN 15) and highly purified ovine LH (LER-1056-C2) as tracer, and the results expressed in terms of ng NIH-LH-S18/ml.

The sensitivities of the assays were 30 ng FSH/ml and 0.05 ng LH/ml and inter- and intra-assay co-efficients of variation were 9.55 (n = 17) and 2.6%, respectively for FSH and 18.6 (n = 12) and 5.8% for LH.

Synthetic LRH (4 µg per ferret; Hoechst) or D-Ser (Bu)9-Des Gly10 LRH ethylamide (1 or 4 µg per ferret; HOE 766 Hoechst) was injected iv through the catheter and flushed in with a 0.5 ml 0.9% sodium chloride solution.

Statistical analysis was by Student’s t-test, applied sequentially between groups at various time intervals after hypophysectomy.

Results

LH

Anoestrous females. Five anoestrous ferrets were given 4 µg LRH before hypophysectomy and the subsequent fall in plasma LH concentration followed. The peptide increased the plasma concentration of LH from 0.11–0.42 to 2.95–5.2 ng/ml at the time of hypophysectomy. For comparative purposes the value just before hypophysectomy for each animal was taken as 100, and the subsequent percentage falls in plasma LH concentration calculated. There was a sharp exponential decline in blood gonadotrophin concentration, with basal levels being reached some 1.5 h after hypophysectomy (Fig. 1). When the values were plotted on semi-logarithmic paper a straight line emerged and an exponential correlation co-efficient of −0.95 calculated. The half-life of the LH released from the hypophysis under these circumstances was 25 min.

Five anoestrous ferrets were similarly treated with 1 µg HOE 766, producing plasma LH values at hypophysectomy ranging between 2.1–5.6 ng/ml and the fall in plasma LH concentration after hypophysectomy followed. Again the decline was exponential in character with a calculated half-life of LH of 19 min, and a correlation coefficient of −0.95. When plotted semilogarithmically the curves for the two groups of anoestrous ferrets were divergent, with respective slopes of −0.029 and −0.038.

Oestrous females. The hypophysis of the ferret is much less sensitive to LRH during oestrus than during anoestrus (Donovan & ter Haar 1977b) so that only a longer acting analogue was administered. However, the secretion of LH in oestrous females was increased little by this peptide. In 5 ferrets given 1 µg HOE 766 the plasma levels of LH immediately after removal of the pituitary gland were 0.15, 0.15, 0.3, 1.26 and 1.30 ng/ml, and the corresponding values in 4 ferrets given 4 µg HOE 766 were 1.05, 1.6, 2.0 and 3.2 ng/ml. Such low plasma gonadotrophin concentrations, and a marked dispersion in the curves for half-life plotted after hypophysectomy, precluded further analysis.

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Fig. 1.
The fall in plasma concentrations of LH (mean ± SEM) after the hypophysectomy (at time zero) of 6 spayed ferrets, or of 5 anoestrous animals given 4µg LRH (anoe: LH-RH), or 5 anoestrous females given 1µg HOE 766 (anoe: HOE 766) in order to enhance gonadotrophin secretion.

Fig. 2.
The fall in plasma concentrations of FSH after hypophysectomy (at time zero) of 4 spayed ferrets, of 9 oestrous ferrets earlier given 1 or 4 µg HOE 766 (oe: HOE 766) to enhance gonadotrophin secretion, and of 5 anoestrous ferrets given 4µg LRH (anoe: LH-RH), and 5 given 1 µg HOE 766 (anoe: HOE 766).
Spayed females. The prevailing plasma concentrations of LH in the spayed ferrets were sufficiently high to allow observations on the decline in plasma LH concentrations after hypophysectomy without the need for prior treatment with gonadotrophin releasing factor. Six animals (spayed 10–34 days earlier) were studied and the findings (plotted in Fig. 1) make it quite clear that the half-life of LH in the circulation (around 2 h) is much longer than in the intact female. The correlation co-efficient for a linear regression curve was −0.66, and for a logarithmic curve −0.27.

FSH

Anoestrous females. The decay in plasma FSH content after injection of 4 µg LRH and subsequent hypophysectomy was followed in 5 females (Fig. 2). Plasma FSH concentration was approximately doubled, from 330–550 to 690–920 ng/ml, but the decline from the latter value was slow. The correlation co-efficient for a linear regression curve (−0.84) was closer to unity than that for an exponential curve (−0.80). After the treatment of 5 anoestrous ferrets with 1 µg HOE 766 before hypophysectomy, the mean curve produced was not significantly different from that derived from LRH injection (Fig. 2). The calculated half-life of FSH was approximately 65 min.

Oestrous females. Five oestrous ferrets were injected with 1 µg and 4 with 4 µg HOE 766 before hypophysectomy. Since the plasma concentrations of FSH of both groups overlapped, being 190, 220, 230, 240 and 300 ng FSH/ml after 1 µg HOE 766 and 240, 320, 370 and 480 ng/ml after 4 µg HOE 766, the results were combined and one curve plotted (Fig. 2). This was significantly different from that for the anoestrous females after the 15 min sample (P < 0.05), with the level of significance being P < 0.01 at and after 20 min. At 240 min after hypophysectomy the mean FSH concentration was 52 ± 5% of the initial value so that the half-life of FSH in oestrous ferrets approximates to 4 h.

Spayed females. The plasma level of FSH in 4 spayed females showed no decline over the first 2 h after hypophysectomy (Fig. 2) and at 285 min 60 ± 6% of the initial concentration remained. In absolute terms the plasma FSH concentrations at hypophysectomy ranged between 1930 and 2750 ng/ml and at 285 min lay between 980 and 1800 ng/ml.

Discussion

Gonadotrophin releasing factor was given in order to raise the plasma concentration of gonadotrophin to a sufficiently high level in the intact animals to allow decay curves to be plotted after hypophysectomy. Distinct differences in the half-lives of FSH in anoestrous, oestrous and spayed females, and in the half-life of LH in anoestrous and spayed females were seen, but the half-life of LH could not be determined in oestrous females because the secretion of LH was much less affected by treatment with HOE 766, in accord with previous observations (Donovan & ter Haar 1977b; Gledhill & Donovan 1981). It may be argued that the hormone discharged by releasing factor was not typical of that in circulation during oestrus or anoestrus, but the fact remains that the half-lives differed.

Ovariectomized ferrets were not given LRH or HOE 766 because it was considered that study of the half-life of hormone acutely discharged from a pituitary gland sustaining a high level of secretion would be less informative than study of the untreated spayed female. It emerged that the half-lives of the FSH and LH secreted by gonadectomized females are distinctly different from the hormones released from the glands of anoestrous or oestrous animals.

Although the radioimmunoassays used in the present work have failed to detect any rise in plasma gonadotrophin concentration associated with oestrus, they reliably plot the changes occurring after gonadectomy, steroid administration and LRH treatment (Donovan & ter Haar 1977b), as well as after hypophysectomy. Further, our observations indicate that the plasma levels of gonadotrophins are higher during anoestrus than during oestrus, perhaps as a result of the negative feedback action of gonadal hormones during oestrus (Donovan & Gledhill 1981). The nature of the hormonal change causing oestrus remains unknown, but the present findings are of considerable interest, in that they accord with the possibility that structurally different gonadotrophins could be secreted during anoestrus and during oestrus (Bogdanove et al. 1975). On this basis, the concentration of hormone in the plasma needs to change little, but the biological action of the differing forms could vary considerably. Alternatively, the metabolism of the hormones might vary under differing reproductive conditions, and here study
of the half-lives of gonadotrophins contained in extracts of pituitary tissue from oestrous and from anoestrous ferrets could be of value. However, the hormone stored in the gland may differ markedly from that secreted, so that the results would not necessarily be comparable. It is clear that sensitive bioassays will need to be applied to the resolution of this problem.

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References


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