Adrenal and ovarian sources of progesterone secretion in young female fallow deer, *Dama dama*

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Summary. Six young female fallow deer, including 3 that were ovariectomized at 9 months of age, were blood sampled at frequent intervals after i.v. injections of (1) ACTH analogue (tetracosactrin), (2) GnRH analogue (buserelin) and (3) saline solution on separate occasions at 11, 13, 15 and 18 months of age. Relative to pre-challenge plasma values, ACTH administration resulted in a 4–10-fold increase in mean plasma progesterone concentrations, but only a 10–45% increase in mean plasma cortisol concentrations, within 40 min for entire and ovariectomized does during the prepubertal periods (11, 13 and 15 months) and for ovariectomized does during the post-pubertal period (18 months). Post-pubertal entire does exhibited high mean basal plasma progesterone concentrations (3–4 ng/ml) indicating a luteal source of secretion, with the ACTH-induced progesterone response being additive to the luteal progesterone but of similar magnitude to responses in the ovariectomized does. There was no significant ACTH challenge effect on mean plasma LH concentrations for entire or ovariectomized does at all ages.

GnRH administration had no significant effects on mean plasma concentrations of progesterone and cortisol of entire and ovariectomized does, although there was a small increase in mean plasma progesterone values in post-pubertal does that may have reflected a luteal response to GnRH (via LH). GnRH challenge resulted in marked increases in mean plasma LH concentrations but the response patterns were different for the 2 types of does, being more rapid and of higher magnitude for ovariectomized does.

Saline injections had no significant effects on mean plasma hormone concentrations for entire and ovariectomized does at any age.

It is concluded that the adrenal glands are secondary and major sources of progesterone in fallow deer and that there exists a two-phase response of adrenal glands to stress/ACTH stimulation in terms of cortisol and progesterone secretion.

Keywords: fallow deer, progesterone, cortisol; adrenal gland; corpus luteum

Introduction

The corpus luteum appears to be the principal source of progesterone in female deer, particularly during the oestrous cycle (e.g. fallow deer, *Dama dama*: Asher, 1985; Asher & Smith, 1987; red deer, *Cervus elaphus*: Adam *et al.*, 1985; Pere David's deer, *Elaphurus davidianus*: Curlewis *et al.*, 1988; white-tailed deer, *Odocoileus virginianus*: Harder & Moorhead, 1980) and early pregnancy (e.g. fallow deer: Asher, 1986, 1987; red deer: Kelly *et al.*, 1982; Adam *et al.*, 1985). However, certain anomalies in plasma progesterone concentrations observed occasionally in female red deer and fallow deer cannot be explained in terms of luteal secretion of the hormone. Kelly *et al.* (1985)

recorded erratic fluctuations in plasma progesterone concentrations during the peri-oestrous period of mature red deer hinds. Similarly, a single short-duration (2–3 h), high-amplitude (> 5 ng/ml) pulse of plasma progesterone was observed at the onset of oestrus of a mature fallow doe (Asher *et al.*, 1986). Unusual incidences of progesterone secretion are not confined to adult female deer. Asher (1986) recorded an increase in mean plasma progesterone concentrations, from 0.5 to 2.0 ng/ml, in immature fallow does between 10 and 15 months of age and erratic fluctuations in plasma progesterone concentrations (up to 7 ng/ml) occurred in twice-weekly samples from immature does (Asher *et al.*, 1988b). As significant quantities of active luteal tissue are unlikely to be present before sexual maturity or during the peri-oestrous period, it seems likely that the progesterone observed in these studies was derived from extra-ovarian sources.

Progesterone is known to be secreted in significant amounts by the adrenal glands of rats (Resko, 1969; Fajer *et al.*, 1971), ewes (Green & Moor, 1977) and cows (Gwazdauskas *et al.*, 1972; Wagner *et al.*, 1972). Furthermore, Wesson *et al.* (1979) observed a significant rise in plasma progesterone concentrations in female white-tailed deer 30 min after immobilization with the muscle relaxant, succinyl-choline chloride, and concluded that increased stress while under non-sedated chemical restraint resulted in increased adrenal secretion of progesterone. From a series of experiments involving the use of anaesthetic drugs and ACTH stimulation/dexamethasone depression of adrenal activity, Plotka *et al.* (1983) provided further evidence of adrenal progesterone secretion in female and male white-tailed deer.

These studies lend support to the hypothesis that the adrenal glands may be a secondary source of progesterone in fallow deer (Asher, 1986). The present study aimed to test this hypothesis for entire and ovariectomized fallow does between 10 and 18 months of age by measuring the hormonal responses to ACTH-analogue challenge of adrenal tissue and GnRH-analogue challenge (and hence LH challenge) of luteal tissue.

Materials and Methods

Animals and management. Six immature fallow does, born in December 1985, were selected in July 1986 (8 months of age) at a uniform liveweight (25–27 kg). Three of the does were ovariectomized on 13 August (9 months) using anaesthetic and surgical procedures similar to those described by Asher *et al.* (1988a) for hysterectomy surgery on adult does.

The deer were contained in high-fenced paddocks with 18 other does of the same age and were grazed on ryegrass-clover pastures.

Blood sampling. During the months of October (11 months), January (13 months), March (15 months) and June (18 months, after sexual maturation) the 6 does were held indoors from 08:00 to 17:00 h (9 h) on 3 separate occasions and subjected to an intensive blood sampling regimen following physiological challenges with ACTH analogue, GnRH analogue or saline solution (i.e. control).

On 7 October, 14 January, 31 March and 8 June each doe received an i.v. injection of 125 µg tetracosactrin (0.5 ml Synacthen; Ciba Geigy Ltd, Basle, Switzerland) at 09:00 h. They were blood sampled at –60, –40, –20, 0, 10, 20, 40, 60, 90, 120, 150, 180, 240, 300, 360 and 480 min from the challenge. The entire procedure was repeated from an i.v. challenge of 4 µg buserelin (1.0 ml Receptal; Hoechst Veterinar GmbH, Munchen, FRG) on 29 October, 7 January, 25 March and 15 June, and for an i.v. challenge of 1.0 ml saline solution (9 g NaCl/l) on 16 October, 21 January, 3 April and 19 June.

The does were blood sampled (5 ml) by jugular venepuncture (21-gauge needles) into heparinized Vacutainers while individually restrained in a cradle device. Their necks had been shaven previously to facilitate venepuncture from the right external jugular vein. Intravenous injections were administered into the left external jugular vein. Blood samples were centrifuged immediately after collection and the plasma was stored at -10°C until assayed.

Hormone assays. Plasma samples ($n = 1152$) were assayed for progesterone, cortisol and luteinizing hormone (LH) by radioimmunoassay techniques.

Plasma progesterone concentrations were measured by a procedure similar to that described by Fairclough *et al.* (1975a) and validated previously for fallow deer serum (Asher, 1985). The antiserum was raised in a rabbit against progesterone-11-BSA conjugate and used at a final dilution of 1:3000. The only significant cross-reaction of a wide range of steroids, including cortisol, tested in the assay was cholesterol (1.5%). Sensitivity of the standard curve was 0.03 ng per tube (0.15 ng/ml plasma) and the intra- and inter-assay coefficients of variation were 6.2% and 15.8% respectively.

Plasma cortisol concentrations were measured by a procedure similar to that described for progesterone (Fairclough *et al.*, 1975b). The antiserum was raised in an ovariectomized ewe against cortisol-3-CMO-BSA and used at a final dilution of 1:3000. Cross-reactivity with other steroids occurred to the following extent: deoxy-17-hydroxycorticosterone 5.7%, cortisone 1.2%, corticosterone 0.8%, dexamethasone 0.02%, deoxycorticosterone 0.005%, progesterone and all other steroids tested <0.005%. The mean recovery ($\pm$ s.d.) of cortisol added to charcoal-stripped plasma was $94.8 \pm 10.1\%$ at 50 ng/ml ($n = 50$ determinations), $100.7 \pm 13.3\%$ at 100 ng/ml ($n = 48$), $100.2 \pm 14.4\%$ at 200 ng/ml ($n = 47$) and $90.0 \pm 12.8\%$ at 500 ng/ml ($n = 32$). Extraction efficiency of radioactive cortisol in plasma was $94.2 \pm 2.7\%$ ($n = 10$ determinations). All samples from an individual challenge were included in a single assay. The inter-assay coefficients of variation were 10.6%, 13.2%, 14.4% and 14.4% for 50, 100, 200 and 500 ng/ml control samples respectively. The intra-assay coefficient of variation, calculated from multiple determinations of the 100 ng/ml control sample was 8.8% and the sensitivity of the standard curve was 0.10 ng per tube (10 ng/ml plasma).

Luteinizing hormone (LH) concentrations were determined using a heterologous radioimmunoassay procedure described for sheep plasma by Scaramuzzi *et al.* (1970). The entire assay has been validated previously for fallow deer serum and plasma (Asher *et al.*, 1986). Sheep pituitary LH preparations used for standards and iodinated tracer were NIH-LH-S11. The LH antibody, which had been raised in a rabbit also using NIH-LH-S11 as the antigen, was used in the assay at a final dilution of 1:200 000. Cross-reactivity with other proteins has been described previously (Kelly *et al.*, 1982; Asher *et al.*, 1986). Serial dilutions of pooled serum or plasma from oestrous does with high concentrations of immunoreactive LH gave inhibition curves parallel to those generated for NIH-LH-S11 in buffer (Asher *et al.*, 1986). The inter-assay coefficients of variation, calculated from determinations of sheep (mean concentration = 4.8 ng/ml) and deer (mean = 3.2 ng/ml) control samples in each assay ($n = 4$ assays) were 12.7% and 14.2% respectively. The intra-assay coefficients of variation for multiple determinations of the same control samples were 5.2% and 7.0% respectively. The sensitivity of the standard curve was 0.03 ng NIH-LH-S11 per tube (0.30 ng/ml).

Statistical analysis. The data were log-transformed and subjected to analysis of variance in which challenge type (ACTH, GnRH and saline), age (11, 13, 15 and 18 months) and their interaction, plus interactions of these with doe type (entire and ovariectomized), were tested against corresponding variation between does. The data are presented graphically as non-transformed means (± 1 s.e.m.).

Results

Progesterone responses

During the 3 prepubertal age periods (11, 13 and 15 months) there was a significant effect of challenge type (ACTH vs GnRH and saline; $P < 0.001$), but no apparent effect of doe type ($P > 0.1$), on plasma progesterone concentrations (Fig. 1). For entire and ovariectomized does, ACTH injection resulted in a 4–10-fold increase in mean plasma progesterone concentrations to attain peak values (4–8 ng/ml) within 40 min after challenge. The concentrations declined progressively from 40 to 180 min after challenge. GnRH challenge and saline injection did not result in increases in mean plasma progesterone concentrations during the prepubertal period. As with ACTH challenge, mean values towards the end of each sampling session were generally lower than corresponding pre-challenge values.

During the post-pubertal period (18 months) there was a significant difference between entire and ovariectomized does in mean progesterone concentrations before and after challenge with GnRH ($P < 0.001$), being 3–4 ng/ml for entire does and 0.3–1.0 ng/ml for ovariectomized does (Fig. 1). However, the response to ACTH challenge was of similar magnitude for both types of does (~ 6 ng/ml above respective basal values) and of similar magnitude to ACTH-induced changes observed in the prepubertal period. The entire does exhibited a small, non-significant ($P > 0.1$) rise in mean plasma progesterone concentrations immediately after GnRH challenge (i.e. $\sim 25\%$ increase) that was not apparent for the ovariectomized does or for both types of does following saline injection.

Cortisol responses

Throughout the 4 age periods there was no significant effect of doe type on the pattern of plasma cortisol changes ($P > 0.1$) but there was a significant challenge effect ($P < 0.001$) (Fig. 2). Mean pre-challenge plasma cortisol concentrations of entire and ovariectomized does varied

Fig. 1. Profiles of mean ($\pm$ s.e.m.) plasma concentrations of progesterone before and after an i.v. injection of (1) ACTH analogue, (2) GnRH analogue and (3) saline; to 3 entire (●) and 3 ovariectomized (○) follow does at (a) 11 months, (b) 13 months, (c) 15 months and (d) 18 months. Arrows indicate the time of challenge.

between 60 and 160 ng/ml. ACTH challenge increased basal concentrations by 10–45% within 20 min after challenge. Thereafter, concentrations declined progressively to reach mean values 50–70% lower than pre-challenge values. GnRH challenge and saline injection did not result in any significant increase in mean plasma cortisol concentration ($P > 0.1$) but there was a similar reduction in values towards the end of each sampling session as occurred during ACTH challenge sessions (Fig. 2).

LH responses

There were significant challenge type and doe type effects on mean plasma LH concentrations ($P < 0.001$) (Fig. 3). Throughout the 4 age periods mean plasma LH concentrations before challenge were consistently higher for ovariectomized does (3–4 ng/ml) than for entire does (0.5–1.0 ng/ml). The principal challenge effect was observed following GnRH injection, although the response pattern was markedly different between entire and ovariectomized does. Mean plasma LH concentrations for entire does increased progressively after GnRH administration to attain maximal values 8–24-fold higher (i.e. 12–18 ng/ml) than pre-challenge values within about 150 min. In contrast, maximal values for ovariectomized does (i.e. 20–28 ng/ml) were reached within 40 min. Both types of does subsequently exhibited a progressive decline in mean plasma LH values to reach pre-challenge concentrations by 480 min (Fig. 3).

Neither ACTH challenge nor saline injection had any significant effects on mean plasma LH concentrations ($P > 0.1$).

Discussion

The rapid elevation of plasma progesterone concentrations in ovariectomized and entire fallow does after ACTH administration clearly implicates the adrenal glands as a major source of the hormone in this species. It seems likely, therefore, that the occasional anomalous progesterone profiles observed in fallow deer, and other cervid species, also represent adrenal progesterone secretion in response to various stressors.

Plasma progesterone concentrations following ACTH challenge were similar to peripheral plasma concentrations of apparent luteal origin occurring during the oestrous cycle (Asher, 1985) and pregnancy (Asher, 1987). Likewise, erratic fluctuations in plasma progesterone concentrations during the perioestrous period of some red deer hinds (Kelly *et al.*, 1985) were similar to concentrations presumably derived from luteal sources (Adam *et al.*, 1985). Furthermore, adrenal glands of white-tailed deer does contribute more than 3 ng progesterone/ml to the peripheral circulatory system (Plotka *et al.*, 1983). Thus progesterone of adrenal origin may occasionally confound interpretation of plasma progesterone profiles for a number of deer species. For example, high mean basal concentrations of plasma progesterone (3–4 ng/ml) in the entire fallow does at 18 months of age (Fig. 1d) undoubtedly reflect active corpora lutea after sexual maturation. Progesterone of adrenal origin, as a consequence of ACTH administration, was clearly additive to that of ovarian origin, and in the absence of pre-challenge data (or indeed the effect of stress stimuli) it would be impossible to differentiate the relative contributions of the two sources of secretion.

While plasma progesterone data clearly indicate the presence of active luteal tissue in 18-month-old entire does there is little indication of a major effect of GnRH challenge (and hence, LH stimulation) on luteal progesterone secretion. This type of challenge may not be a useful indicator of the presence of active luteal tissue in mature fallow deer, as had been postulated at the start of the trial. The complete failure of exogenous GnRH to stimulate progesterone secretion in sexually immature does (i.e. 11–15 months) most probably reflects the absence of luteal tissue for such tissue is only found after sexual maturation at 16 months of age (Armstrong *et al.*, 1969; Chapman & Chapman, 1969, 1975; Baker, 1973). However, plasma LH concentrations of ovariectomized fallow

Fig. 2. Profiles of mean ($\pm$ s.e.m.) plasma concentrations of cortisol before and after an i.v. injection of (1) ACTH analogue, (2) GnRH analogue and (3) saline; to 3 entire (●) and 3 ovariectomized (○) fallow does at (a) 11 months, (b) 13 months, (c) 15 months and (d) 18 months. Arrows indicate the time of challenge.

Fig. 3. Profiles of mean ($\pm$ s.e.m.) plasma concentrations of LH before and after an i.v. injection of (1) ACTH analogue, (2) GnRH analogue and (3) saline; to 3 entire (●) and 3 ovariectomized (○) fallow does at (a) 11 months, (b) 13 months, (c) 15 months and (d) 18 months. Arrows indicate the time of challenge.

does were notably higher than for entire does, indicating that the negative gonadal feedback on pituitary function was effective even in immature animals. Differential responses by ovariectomized and entire does to GnRH challenge indicate possible gonadal modulation of pituitary response to GnRH that was manifest by extremely rapid, high-amplitude LH secretion following GnRH administration in ovariectomized does compared with a more gradual, lower-amplitude response in entire does.

Plasma cortisol concentrations observed in this study were very high at the start of each blood sampling session and declined progressively to attain considerably lower final concentrations. Cortisol concentrations did not increase markedly in response to ACTH challenge, whereas those of progesterone did. Perhaps the high levels of stress at the start of each experiment caused maximal adrenal secretion of cortisol, such that additional stimulation with exogenous ACTH was without a marked response. The present results suggest that large quantities of cortisol and modest quantities of progesterone are secreted by the adrenal cortex of fallow deer in response to most stress stimuli. However, under extreme stress, progesterone, rather than additional quantities of cortisol, is secreted in large amounts.

There has been considerable speculation on how the adrenal gland might influence reproductive function in mammals (reviews: Parkes & Deanesly, 1966; Moberg, 1985). Adrenal progesterone secretion may affect various components of the reproductive process. For example, Plotka *et al.* (1983) postulated that adrenal progesterone in white-tailed deer may help to maintain pregnancy during periods of stress by neutralizing the pregnancy-terminating action of cortisol. Alternatively, high blood progesterone concentrations after adrenal stimulation may inhibit oestrus and ovulation during the breeding season, thus preventing pregnancy under highly stressful conditions. Our blood sampling regimen lacked sufficient precision and duration to detect any possible changes in LH pulse frequency in response to adrenal progesterone secretion in fallow does.

Evidence that progesterone secretion, possibly of adrenal origin, may affect LH secretion during the breeding season of fallow deer was provided by Asher *et al.* (1986) who reported a mature doe with a very high plasma concentration (>5 ng/ml) of progesterone, at the onset of oestrus, which appeared to depress the preovulatory LH surge. However, this situation, and that from the present study, only related to transitory stimulation of the adrenal glands, resulting in transient increases in progesterone secretion. Sustained adrenal stimulation associated with environmental or social stressors could have more long-term effects on reproduction in fallow deer.

We thank M. Langridge, O. C. Chitty and H. H. Humble for assistance with animal handling and blood sampling; R. Shanley, S. Parratt and L. ter Veer for hormone analyses; Dr A. J. Pearson for useful comments on earlier draft manuscripts; and Ciba Geigy Ltd for the kind donation of Synacthen.

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Received 2 August 1988