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Involvement of serotoninergic pathways in the control of luteinizing hormone secretion in red deer hinds

Luis G. Villa-Diaz and Graham K. Barrell[A]

Animal and Food Sciences Division, P. O. Box 84, Lincoln University, Canterbury, New Zealand.
[A]To whom correspondence should be addressed. email: barrell@whio.lincoln.ac.nz

Abstract. Two experiments were conducted to determine whether serotoninergic pathways, which are implicated in the neuroendocrine regulation of luteininzing hormone (LH) secretion in domestic animals, have a similar action in red deer hinds. In the non-breeding season (August), ovariectomized ($n = 5$) and ovariectomized-thyroidectomized ($n = 5$) hinds received a vehicle solution followed 4 h later by either serotonin (66 μg kg^{-1} i.v.) every 10 min for a further 4 h or the serotonin antagonist, cyproheptadine (3 mg kg^{-1} i.v.) as a single injection. This procedure was repeated in the breeding season (June). In the non-breeding season serotonin was without effect, but cyproheptadine reduced LH pulse frequency and amplitude in ovariectomized-thyroidectomized hinds ($P<0.01$). During the breeding season, serotonin reduced LH pulse amplitude in ovariectomized hinds ($P<0.05$) and cyproheptadine reduced LH pulse frequency in both ovariectomized and ovariectomized–thyroidectomized hinds ($P<0.05$ and $P<0.01$, respectively). On each occasion, cyproheptadine increased ($P<0.01$) plasma prolactin concentration, whereas serotonin had no effect. These results indicate a stimulatory role for serotoninergic neurons on the hypothalamic GnRH pulse generator mechanism of red deer hinds during the breeding season. In a second experiment, the LH response to GnRH (5 μg i.v.) was examined in ovariectomized hinds ($n = 5$) following administration of a serotonin infusion (6.6 μg kg^{-1} min^{-1} i.v. for 15 min), cyproheptadine (3 mg kg^{-1} i.v. as a single dose) or vehicle, in the breeding season (July) after induction of halothane anaesthesia and in the non-breeding season (December) without anaesthesia. Halothane anaesthesia eliminated endogenous pulses of LH. In comparison with the vehicle-treated controls, the response of plasma LH to exogenous GnRH was not altered by serotonin or cyproheptadine in either season, which shows that serotonin has no effect on LH release at the pituitary gland level in these animals. It was concluded that in the regulation of LH release in red deer hinds, serotoninergic pathways have a stimulatory role operating at the hypothalamic level.

Extra keywords: cyproheptadine, prolactin, serotonin.

Introduction

Red deer *(Cervus elaphus)* hinds are seasonally breeding animals that display an annual variation in plasma luteinizing hormone (LH) concentration (Meikle and Fisher 1996; Anderson and Barrell 1998*a*), which has characteristics of both steroid-dependent and steroid-independent modes of regulation. In the case of steroid-dependent (e.g. oestradiol) inhibition of LH secretion, any effect at the hypothalamic level must be relayed by neurons other than the GnRH neurons, because the latter do not appear to possess oestradiol receptors, as shown in the ewe (Lehman and Karsch 1993), guinea pig (Watson *et al.* 1992) and rat (Shivers *et al.* 1983). It has been suggested that dopaminergic neurons might be involved in this pathway in sheep (Meyer and Goodman 1986); however, this was not supported by a study of LH secretion in red deer hinds treated with the dopaminergic agonist, bromocriptine, or its antagonist, sulpiride (Anderson and Barrell 1998*b*). Those authors also reported no effect of an opioidergic agonist, morphine, or antagonist, naloxone, on LH secretion in the hinds in the same study, yet opioid peptides appear to be involved in seasonal suppression of LH secretion in sheep (Schillo *et al.* 1985; Yang *et al.* 1988; Schall *et al.* 1991). For the steroid-independent mechanism, serotoninergic neurons have been implicated in the reduction of LH pulse frequency in ovariectomized ewes (Meyer and Goodman 1986; Whisnant and Goodman 1990) during anoestrus. In red deer hinds the steroid-independent inhibition of LH secretion appears to require a specific action of thyroid hormones (Anderson and Barrell 1998*a*). Because the mechanism of action of the thyroid hormones in this phenomenon has not been ascertained, we used ovariectomized hinds, which had also been thyroidectomized, in the present study to investigate putative neurotransmitter effects. Given the lack of action of dopaminergic and opioidergic compounds on LH secretion in red deer hinds (Anderson and Barrell 1998*b*), we decided to examine the possible effects of serotonin and its antagonist, cyproheptadine, on the steroid-independent control of LH secretion in this species. This was achieved by monitoring pulsatile LH concentrations in the plasma of ovariectomized and ovariectomized–thyroidec-

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tomized hinds that had been treated with these compounds. Plasma prolactin concentration was measured in an attempt to verify the effectiveness of the drug treatments, because it has been shown that secretion of this hormone in sheep is influenced by serotoninergic compounds (Thomas *et al.* 1988; Donnelly and Dailey 1991).

In deer, pituitary responsiveness, in terms of elevation of plasma LH concentration after an injection of GnRH, is lower in the non-breeding season than in the breeding season (Suttie *et al.* 1989; Baker *et al.* 1995; Meikle and Fisher 1996), which indicates that some degree of regulation of the seasonal pattern of LH secretion in deer is exercised at the level of the pituitary gland. In sheep there is evidence for a stimulatory effect of serotonin on GnRH-induced LH release (Deaver and Dailey 1982) and on LH pulse amplitude and mean plasma concentration after pituitary stalk transection (Donnelly and Dailey 1991). These findings give rise to the hypothesis that serotonin has a permissive role on LH secretion at the pituitary gland level, and we have developed an experiment to test this in red deer hinds. We used ovariectomized red deer hinds, which were treated with either serotonin or cyproheptadine, and examined the LH response to exogenous GnRH, in both the breeding and non-breeding seasons. However, a problem arises from using this approach in the breeding season when pulsatile GnRH release in ovariectomized hinds is at a high frequency, making it impossible to differentiate LH responses following exogenous GnRH from those induced by endogenously released GnRH. We attempted to overcome this problem by using halothane anaesthesia to suppress endogenous release of GnRH. Lack of supporting evidence in the scientific literature obliged us to perform a pilot study to determine if LH pulsing in red deer hinds ceases during such anaesthesia. Plasma prolactin concentration provided an independent measure of the ability of halothane to suppress hypothalamic function, because secretion of this hormone is tonically inhibited by the hypothalamus (Clarke and Doughton 1983).

In summary, the work reported here involved two experiments and a pilot study. The first experiment (Experiment 1) investigated the possible involvement of a serotoninergic pathway in the control of LH secretion at the hypothalamic level in red deer hinds, as it was assumed that the effects on LH pulse frequency would specifically implicate an action on the hypothalamic pulse generator mechanism. The pilot study investigated halothane anaesthesia as a means for suppressing GnRH release in red deer. Finally, Experiment 2 was conducted to determine if serotoninergic drugs affected the LH response to GnRH at the pituitary gland level in this species.

Materials and methods

Animals and management

Red deer (*Cervus elaphus*) hinds were maintained outdoors at the Lincoln University Research Farm (lat 43°39′S, long 172°28′E) on pasture with ryegrass silage supplementation during winter and unlimited access to water at all times. The hinds were either ovariectomized–euthyroid or ovariectomized–thyroidectomized and had been prepared surgically (Shi and Barrell 1992; Anderson and Barrell 1998*a*) 3 years previously. Long-term thyroidectomy does not appear to cause any harmful side effects in red deer, one minor consequence being a slight reduction in heart rate. All procedures used in these studies were approved by the Lincoln University Animal Ethics Committee.

Blood sampling

Blood samples (10 mL), collected via indwelling jugular cannula while the hinds were manually restrained, was transferred to glass tubes containing 110 units of sodium heparin and the plasma obtained following centrifugation was stored at –20°C until assayed. The cannulae were inserted 1 or 2 days prior to intensive blood sampling during light sedation with 0.3–0.5 mL i.v. of 5% xylazine hydrochloride (Thiazine 50; RWR Veterinary Products Pty Ltd, NSW, Australia). All hinds were injected subcutaneously with 500 000 IU procaine penicillin, 500 000 IU benzathine penicillin and 1250 mg dihydrostreptomycin base (5 L Penstrep LA, A/S Rosco, Denmark) immediately after cannulation.

Hormone assays

Plasma LH concentrations were measured in duplicate 100 µL aliquots by heterologous radio-immunoassay, as described previously (Anderson and Barrell 1998*a*, 1998*b*). Values are expressed in terms of the ovine standard, NIAMDD-LH S20; iodinated ovine LH (NIDDK-oLH-I-2) was used as tracer, and the primary antiserum was NIDDK-anti-oLH-1 (AFP-192279). Assay sensitivity was 0.34 ng mL^{-1}; the intra-assay coefficient of variation (CV) averaged 16.3% for plasma pools displacing radiolabelled oLH to approximately 72% of the total bound, and the interassay CV was 14.6% for the same plasma pools.

Plasma prolactin concentrations were determined in duplicate 50 µL aliquots of plasma by the ELISA assay of Lewis *et al.* (1992) as described previously (Anderson and Barrell 1998*b*). Values are expressed in terms of the standard, ovine prolactin (NIADDK-oPRL-19), which was provided by NIDDK. Assay sensitivity was 7.6 nmol L^{-1} (6 separate assays involving 12 ELISA microtitre plates), and the intra-assay CV averaged 13.6% for a plasma pool displacing thyroglobulin-conjugated prolactin to approximately 48% of the total bound. Inter-assay CV was 10.6% for a plasma pool displacing thyroglobulin-conjugated prolactin to approximately 25% of the total bound.

Experimental procedures

Experiment 1

At the beginning of the non-breeding season (late August 1997), five ovariectomized and four ovariectomized–thyroidectomized hinds (mean live weight 94.3 ± 2.9 kg) were allocated randomly into two groups and received vehicle intravenously followed 4 h later by either serotonin (66 µg kg^{-1} i.v. as creatine sulphate complex; Sigma Chemical Co., St Louis, MO, USA) every 10 min for 4 h or cyproheptadine (3.0 mg kg^{-1} i.v., Merck Sharp & Dohme Ltd, Auckland, NZ) as a single bolus. Seven days later the hinds received the same treatment, but were injected with the alternative drug, so that all nine animals received both agonist and antagonist treatments. The vehicle used was 5 mL of 0.9 % saline solution for serotonin and 5 mL of 50% ethanol in water for cyproheptadine. For plasma LH analysis blood samples were taken every 10 min, from the time of vehicle injection until 4 h after drug injection, and for plasma prolactin analysis were taken at –240, –120, 0, 10, 20, 40, 80, 160 and 300 min relative to the drug injections. The procedure was repeated in the following breeding season (June 1998).

Halothane anaesthesia: pilot study

In the breeding season (May 1998), five ovariectomized hinds (mean live weight 112 ± 8.7 kg) were removed from pasture approximately 24 h prior to experimentation and kept in yards without access to food or water. Anaesthesia was induced with a single (i.v.) dose of 25 mg kg^{-1} 10% sodium thiopentone (Pentothal, Techvet Laboratories Ltd, Auckland, NZ) to permit endotracheal intubation. Anaesthesia was usually maintained in the deep surgical plane (Booth 1977) with a gas mixture of 2.5% v v^{-1} halothane (Fluothane, Imperial Chemical Industries, New Zealand Ltd) in oxygen using a closed circuit system for 4 h and was monitored by testing digital and palpebral reflexes. In some cases, noted later, animals did not remain in the fully anaesthetized state during halothane administration. Blood samples were taken every 20 min for plasma LH analysis from 4 h prior to induction of anaesthesia and during the 4 h period of administration of anaesthetics. Plasma prolactin concentration was measured in samples obtained at –240, –120, 0, 20, 40, 80, 160 and 300 min relative to the injection of thiopentone. Of the five animals, one (no. 2) did not reach the desired plane of anaesthesia and after 2 h another (no. 5) started to regain consciousness; however, in both cases blood samples were collected and processed as for the other hinds.

Experiment 2

Non-breeding season In December 1997, five ovariectomized hinds (as used in the pilot study) were treated intravenously with a single injection of cyproheptadine (3 mg kg^{-1} in 5 mL of 50% ethanol in water), or vehicle (5 mL of 50% ethanol in water), followed by 5 μg of synthetic GnRH (L-7134, LH-RH, acetate salt, human synthetic; Sigma Chemical Co) given intravenously in 1 mL of 0.9% saline solution 1 h later. In late October 1998 the hinds received two intravenous injections of either serotonin (each one 66 μg kg^{-1} in 5 mL of 0.9% saline solution) or vehicle (5 mL of 0.9% saline solution), which were given at 10 min and 1 min before a single injection of 5 μg of GnRH (as described earlier). In all cases the treatments were applied in a balanced manner on two occasions so that all five hinds received both drug and vehicle treatments. Plasma LH concentration was measured in samples collected at –60, –30, 0 and 10 min relative to the GnRH injection.

Breeding season In July 1998 the five hinds were anaesthetized as described before, then 30 min later were treated (i.v.) either with serotonin infused at 6.6 μg kg^{-1} min^{-1} in 20 mL of 0.9% saline solution for 10 min, or a single injection of 3.0 mg kg^{-1} cyproheptadine in 5 ml of 50% ethanol in water, or a single injection of 5 mL 0.9% saline solution, followed 10 min later by GnRH (5 μg as before). The treatments were allocated in a balanced manner over a few days so that all five hinds received all three treatments. Plasma LH concentration was measured in samples collected at –10, 0 and 10 min relative to GnRH injection.

Statistical analysis

Hormone concentrations below assay sensitivity were assigned a value equal to the sensitivity for statistical analysis.

In Experiment 1, LH pulse parameters, defined as described by Goodman and Karsch (1980), were obtained by using the pulse detection 'Cluster' algorithm of Veldhuis and Johnson (1986). LH pulse frequency was the number of identified pulses in 4 h. Means for the drug treatments and seasonal effects were examined using the paired Student's *t*-test. Plasma prolactin concentrations were pooled from ovariectomized and ovariectomized–thyroidectomized hinds in each season and transformed to their logarithms prior to analysis.

For the halothane pilot study, mean plasma LH concentration and LH pulse frequency values during halothane anaesthesia were compared with mean pre-induction values using paired Student's *t*-test. Plasma prolactin concentrations were transformed to logarithms and the means analysed using the paired Student's *t*-test. Data from animal no. 2 and the last 2 h samples of animal no. 5 were removed from the analysis because the animals were not at the required surgical plane of anaesthesia at these times.

In Experiment 2, pituitary responsiveness to exogenous GnRH was calculated as the plasma LH concentration measured at 10 min after injection of GnRH minus the plasma LH concentration immediately prior to injection (Suttie *et al.* 1989; Meikle and Fisher 1996) and treatment means were analysed using Student's *t*-test.

Values are presented as mean ± SEM.

Results

In some hinds it was noted that serotonin injections increased urination and defaecation and appeared, with jaw and tongue movements, to cause a brief taste sensation. Cyproheptadine caused some haemolysis (not seen in vehicle-only treated animals), occasional vocalization and disorientation, plus agitation when handled, in all hinds. In two cases these effects of cyproheptadine were particularly severe and lasted for 2 h.

Experiment 1

During the breeding season, cyproheptadine reduced mean LH pulse frequency in both ovariectomized and ovariectomized–thyroidectomized hinds ($P<0.05$ and $P<0.01$, respectively), but did not affect plasma LH concentration or pulse amplitude (Table 1, Fig. 1). Serotonin treatment caused a reduction ($P<0.05$) in LH pulse amplitude in ovariectomized hinds, otherwise it was without effect in both groups of hinds in either season (Table 1, Fig. 2).

Mean plasma LH concentration, pulse frequency and pulse amplitude during the period prior to drug injections were lower ($P<0.01$) in the non-breeding season than in the breeding season in ovariectomized hinds (Table 2), but not in the case of ovariectomized–thyroidectomized hinds. All three parameters were lower ($P<0.01$) in ovariectomized hinds than in ovariectomized–thyroidectomized hinds across both the breeding and non-breeding seasons (Table 1).

Table 1. LH pulsatility in ovariectomized ($n = 5$) and ovariectomized–thyroidectomized hinds ($n = 4$) treated with serotonin and cyproheptadine during the breeding season

Data are means ± SEM.

	Ovariectomized		Ovariectomized–thyroidectomized	
Pulse parameter	Serotonin	Cyproheptadine	Serotonin	Cyproheptadine
Concentration (ng mL^{-1})				
Before	1.7 ± 0.4	4.3 ± 1.2	11 ± 3.8	10.4 ± 3.0
After	1.1 ± 0.2	2.6 ± 0.8	8.9 ± 1.4	8.0 ± 3.0
Number per 4 h				
Before	2.2 ± 0.6	2.8 ± 0.3[a]	4.0 ± 0.4	4.2 ± 0.8[a]
After	1.0 ± 0.4	1.2 ± 0.6[b]	3.7 ± 0.4	1.7 ± 0.2[b]
Amplitude (ng mL^{-1})				
Before	4.0 ± 1.3[a]	4.9 ± 2.1	7.6 ± 1.6	8.8 ± 1.9
After	1.5 ± 0.4[b]	1.5 ± 0.1	6.8 ± 0.8	5.6 ± 2.3

Means assigned with a different superscript letter within a pulse parameter and column are significantly different ($P<0.05$ and $P<0.01$).

In the non-breeding season, cyproheptadine caused a reduction ($P<0.01$) in both LH pulse frequency and amplitude in ovariectomized–thyroidectomized hinds, but did not affect plasma LH in ovariectomized hinds (Table 3, Fig. 3).

Table 2. LH pulsatility in ovariectomized ($n = 5$) and ovariectomized–thyroidectomized hinds ($n = 4$) treated with serotonin and cyproheptadine during the breeding season

Data are means ± SEM.

Pulse parameter	Ovariectomized	Ovariectomized-thyroidectomized
Concentration (ng mL^{-1})		
Breeding	3.0 ± 0.7^a	10.7 ± 2.2^b
Non-breeding	0.9 ± 0.1^c	5.3 ± 0.9^d
Number per 4 h		
Breeding	2.5 ± 0.3^a	4.1 ± 0.3^b
Non-breeding	1.5 ± 0.3^c	5.0 ± 0.5^b
Amplitude (ng mL^{-1})		
Breeding	4.4 ± 1.1^a	8.2 ± 1.2^b
Non-breeding	1.8 ± 1.0^c	6.0 ± 1.1^b

Data pooled from the two intensive sampling dates in each season before treatment. Means assigned with a different superscript letter within a pulse parameter are significantly different ($P<0.01$).

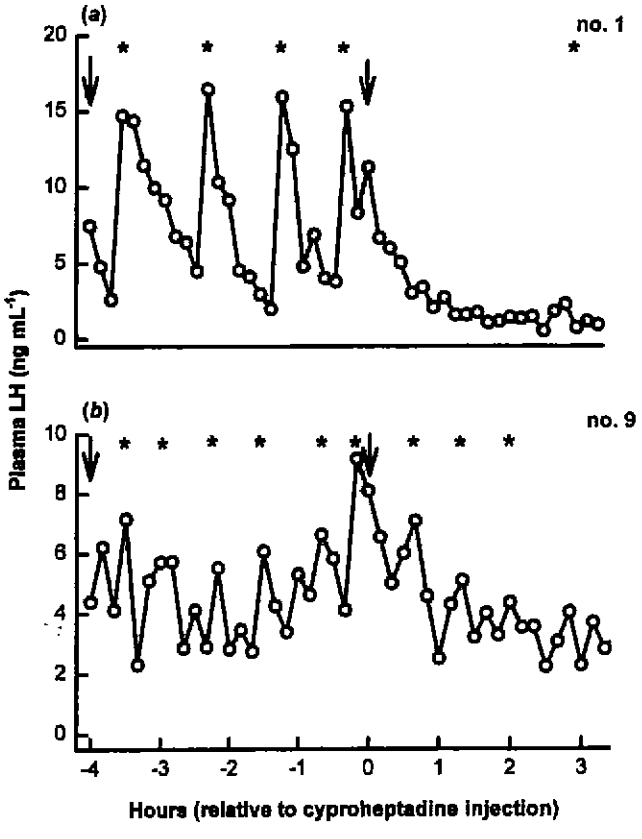

Fig. 1. Plasma LH profiles of two representative individual red deer hinds treated with cyproheptadine during the breeding season: *(a)* ovariectomized hind, *(b)* ovariectomized-thyroidectomized hind. Vertical arrows (left, vehicle; right, drug) indicate time of injection. Note reduction in pulse frequency ($P<0.05$ and $P<0.01$, respectively) in both animals. Note differing y-axis scales on graphs. Asterisks denote significant pulses.

Prior to drug injections, mean plasma prolactin concentrations during the breeding season and non-breeding season were similar (67.5 ± 19.7 ng mL^{-1} and 59.67 ± 14.4 ng mL^{-1} respectively; $P>0.05$). Cyproheptadine caused an increase ($P<0.01$) in mean plasma prolactin concentration whereas serotonin was without effect (Fig. 4).

Halothane pilot study

Anaesthesia effectively abolished LH pulsing from a mean of 2.6 ± 0.2 pulses per 4 h prior to induction to 2 pulses from one animal and no pulses at all from three animals during the period of anaesthesia (Fig. 5). The animal with two pulses (no. 1) had one pulse at the onset of the induction and then a period of 3 h in which no pulses occurred (Fig. 5). Consequently, mean plasma LH concentration was reduced ($P<0.05$) from 2.8 ± 0.7 ng mL^{-1} prior to induction to 1.1 ± 0.1 ng mL^{-1} during the anaesthesia period. Mean plasma prolactin concentration was increased ($P<0.05$) by anaesthesia (116 ± 27.5 and 301.8 ± 70.6 ng mL^{-1}, pre and during anaesthesia respectively; Fig. 6).

In spite of continued administration of halothane, anaesthesia as monitored by testing digital and palpebral reflexes,

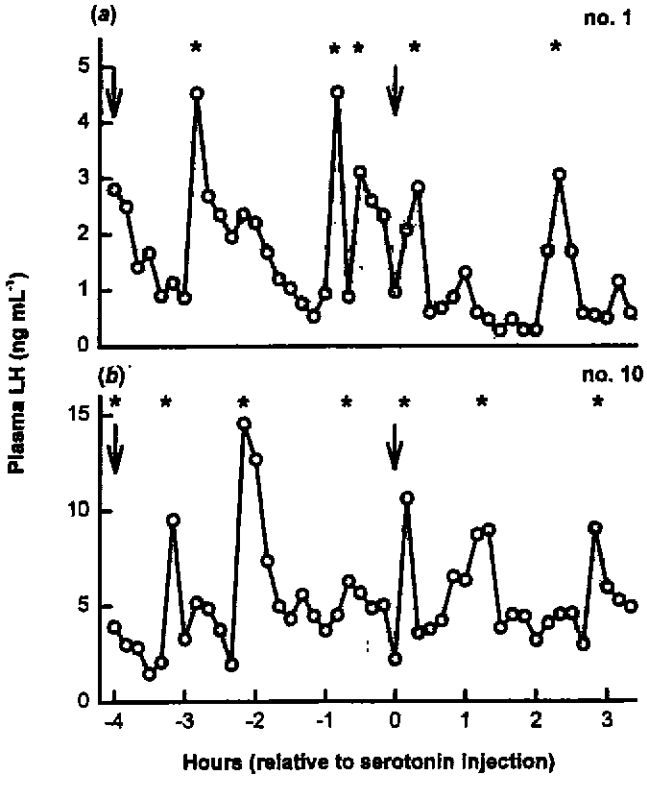

Fig. 2. Plasma LH profiles of two representative individual red deer hinds treated with serotonin during the breeding season: *(a)* ovariectomized hind, *(b)* ovariectomized-thyroidectomized hind. Vertical arrows (left, vehicle; right, drug) indicate time of injection Note differing y-axis scales on graphs. Asterisks denote significant pulses.

indicated that one hind (no. 5) had recovered consciousness in the last 2 h of this period. During that time there was an increase in plasma LH concentration in this hind (Fig. 5). In another case (no. 2) anaesthesia was not achieved by the halothane administration and the plasma LH data show no evidence of suppression of secretion (Fig. 5).

Experiment 2

In the breeding season, mean plasma LH concentration during the anaesthesia period and before GnRH injection was 1.1 ± 0.2 ng mL^{-1} and the mean plasma LH concentration at 10 min after the exogenous GnRH challenge (i.e. pituitary responsiveness) was 36.7 ± 2.8 ng mL^{-1}. During the non-breeding season, without anaesthesia, the corresponding values were 0.4 ± 0.3 ng mL^{-1} (equivalent to assay sensitivity) and 3.3 ± 1.1 ng mL^{-1}, respectively. Pituitary LH responsiveness to exogenous GnRH was not different ($P>0.05$) between serotonin and its vehicle either in the breeding season (35 ± 6.3 *v.* 41.1 ± 10.1 ng mL^{-1}, respectively) or the non-breeding season (5.1 ± 1.1 *v.* 5.8 ± 0.5 ng mL^{-1}, respectively) (Fig 7*a*,*b*). Also there was no difference ($P<0.05$) in pituitary LH responsiveness between cyproheptadine treatment and its vehicle during the breeding season (34.1 ± 9.6 *v.* 41.1 ± 10.1 ng mL^{-1}, respectively) or in the non-breeding season (2.3 ± 0.7 *v.* 4.3 ± 2.1 ng mL^{-1}, respectively) (Fig. 7*a*,*c*).

Table 3. LH pulsatility in ovariectomized ($n = 5$) and ovariectomized–thyroidectomized hinds ($n = 4$) treated with serotonin and cyproheptadine during the non-breeding season

	Ovariectomized		Ovariectomized–thyroidectomized	
Pulse parameter	Serotonin	Cyproheptadine	Serotonin	Cyproheptadine
Concentration (ng mL^{-1})				
Before	0.9 ± 0.4	0.8 ± 0.1	4.6 ± 1.0	6.1 ± 1.6
After	0.5 ± 0.1	0.7 ± 0.1	4.1 ± 1.1	4.6 ± 1.4
Number per 4 h				
Before	1.4 ± 0.6	1.6 ± 0.4	4.6 ± 0.3	5.5 ± 0.9^a
After	1.0 ± 0.5	1.5 ± 0.3	3.0 ± 0.8	2.0 ± 0.7^b
Amplitude (ng mL^{-1})				
Before	2.7 ± 2.5	1.2 ± 0.9	4.6 ± 0.9	7.9 ± 2.0^a
After	0.3 ± 0.1	1.5 ± 0.6	3.2 ± 0.6	2.1 ± 1.0^b

Means not assigned with a common superscript letter within a pulse parameter and column are significantly different ($P<0.01$).

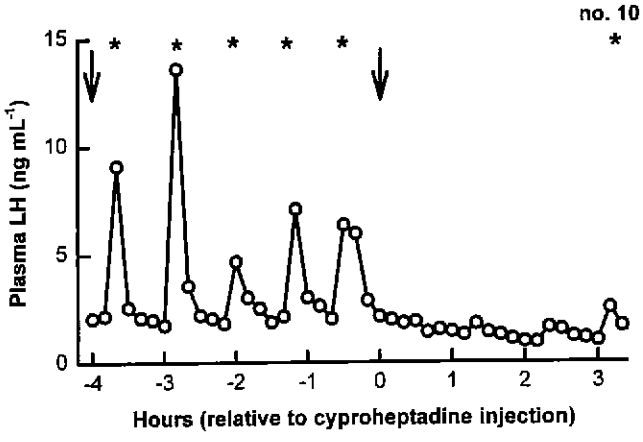

Fig. 3. Plasma LH profile of a representative ovariectomized–thyroidectomized hind treated with cyproheptadine during the non-breeding season. Note reduction in pulse frequency and amplitude. Asterisks denote significant pulses.

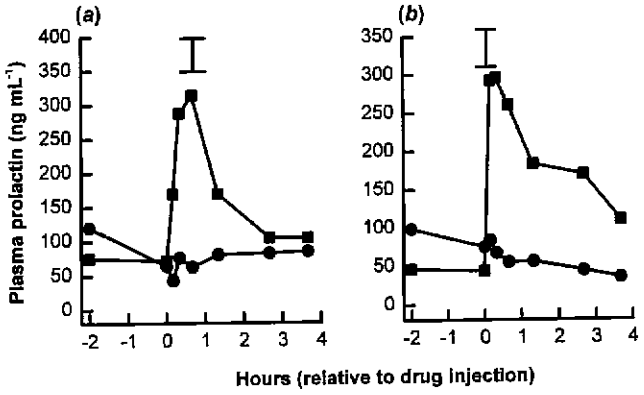

Fig. 4. Mean plasma prolactin concentration following injection of serotonin (●) and cyproheptadine (■) during, *(a)* the breeding season and *(b)* the non-breeding season. Data ($n = 9$) are pooled from five ovariectomized and four ovariectomized–thyroidectomized hinds. Vertical lines indicate pooled 2 × SEM.

Discussion

The inhibitory action of cyproheptadine on LH secretion in ovariectomized and ovariectomized–thyroidectomized animals recorded here indicates a stimulatory role for serotoninergic pathways on the pulsatile secretion of LH in red deer hinds. The finding that serotonin decreased LH pulse amplitude in ovariectomized hinds during the breeding season conflicts with this conclusion. However, the inhibitory effects of cyproheptadine tended to be consistent across treatments and seasons, whereas the effect of serotonin was isolated to the single incident. Also, during the non-breeding season when there was no effect of cyproheptadine in the ovariectomized hinds, inhibition of LH secretion would not have been detectable anyway, as the LH parameters were already at low values in these animals. In contrast with the present findings, an increase in LH pulse frequency following treatment with cyproheptadine has been reported in sheep (Meyer and Goodman 1986; Le Corre and Chemineau 1993; Le Corre *et al.* 1993) indicating an inhibitory role of serotonin on LH secretion in that species. It is thus possible that, in red deer, the serotoninergic pathways are stimulatory and operate throughout both seasons, whereas in sheep (Meyer and Goodman 1986; Le Corre and Chemineau 1993; Le Corre *et al.* 1993), these pathways appear to have an inhibitory action on LH secretion via the

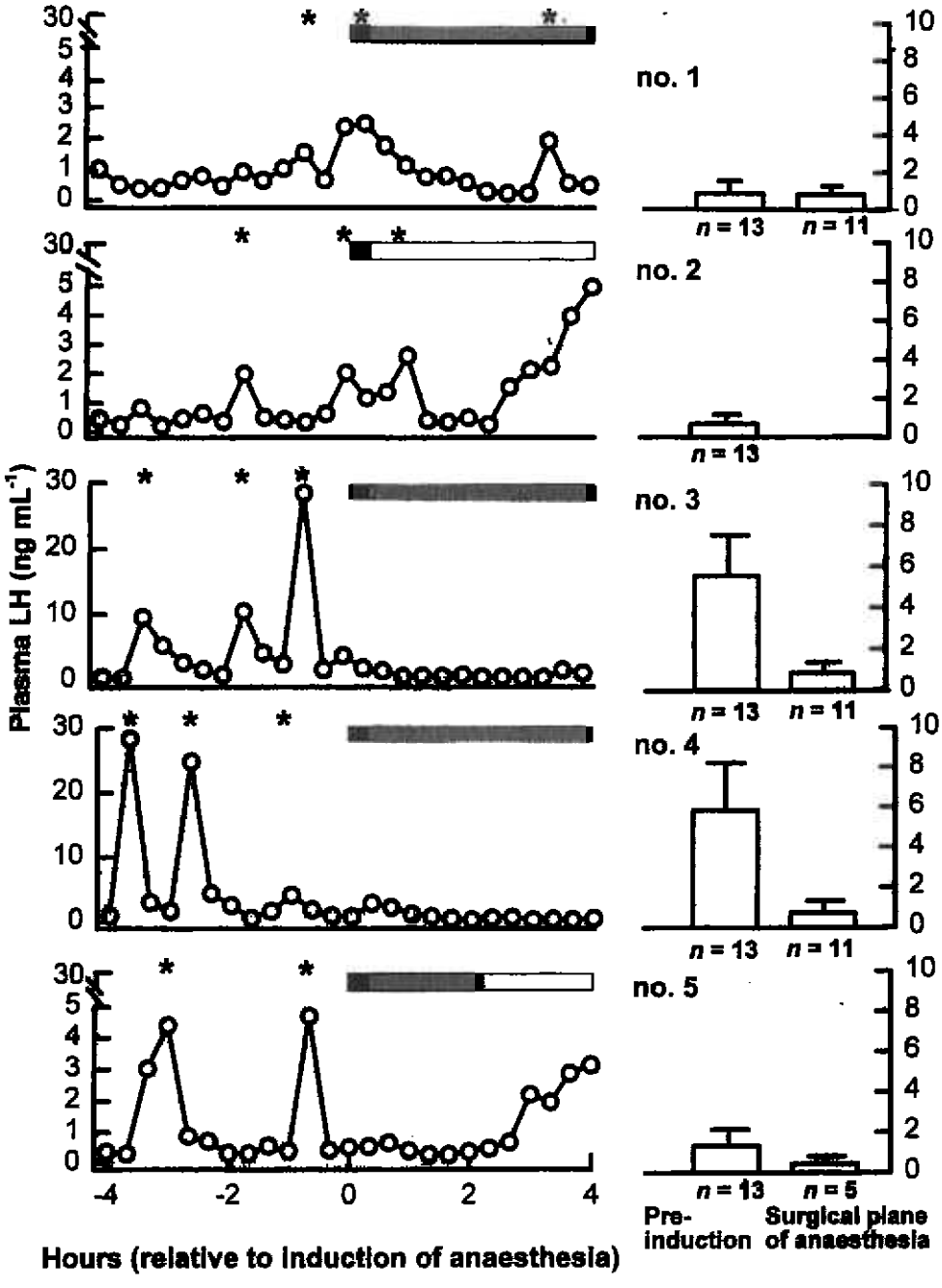

Fig. 5. Individual plasma LH profiles of ovariectomized red deer hinds before and during the period of anaesthesia which is divided into, barbiturate induction period (■) and halothane administration period, with (■) and without (□) surgical plane of anaesthesia. Note differing y-axis scales on graphs. Asterisks denote significant pulses. Histograms show mean plasma LH concentration (ng mL^{-1}, right hand axis). Vertical lines denote SEM. n = number of observations.

steroid-independent mechanism, which is active only during the non-breeding season. The behavioural disturbances seen in hinds treated with cyproheptadine raise the possibility that the effects on LH secretion arose from side effects of the drug, which may include raised concentrations of corticosteroids. However, this is a concern that must apply to all studies involving peripheral administration of drugs, indicating that such studies serve mainly as first-order screens for determination of neurotransmitter pathways in biological processes.

The hypothesis that serotonin has a permissive role on LH secretion at the pituitary gland level to modify LH respon-

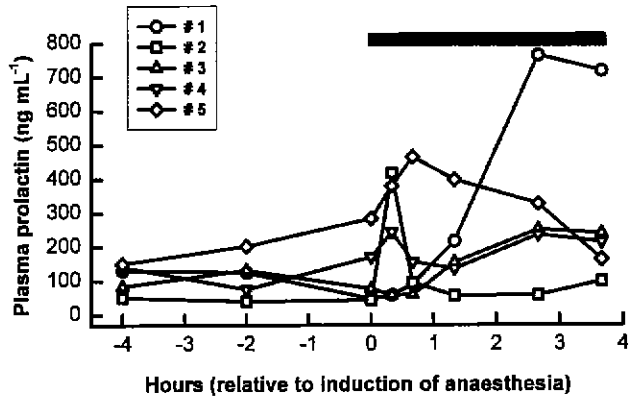

Fig. 6. Individual plasma prolactin profiles before and during anaesthesia in ovariectomized red deer hinds. Barbiturate induction period (■), halothane administration period (■).

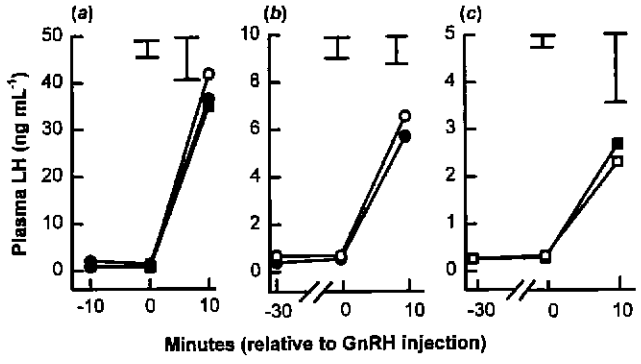

Fig. 7. Mean plasma LH concentration in ovariectomized red deer hinds ($n = 5$) treated i.v. with 5 μg GnRH followed by either serotonin (●) or its vehicle (○), or cyproheptadine (■) or its vehicle (□), during *(a)* the breeding season (July) and in the non-breeding season, *(b)* serotonin (October), *(c)* cyproheptadine (December). Vertical lines indicate pooled 2 × SEM.

siveness to GnRH in red deer hinds is rejected by the results of Experiment 2. A similar finding has been reported in ovariectomized cows treated with serotonin followed by intravenous GnRH (Mondragon *et al.* 1986). In rats, serotonin perfused directly into the anterior pituitary gland also did not affect LH release (Kamberi *et al.* 1970). Likewise, an *in vitro* study showed that serotonin failed to alter LH release from rat pituitaries incubated alone or in the presence of stalk-median eminence tissue (Schneider and McCann 1969). Nevertheless, studies in sheep have shown stimulatory effects of peripherally administered serotonin on LH responsiveness to GnRH challenge (Deaver and Dailey 1982; Donnelly and Dailey 1991) so it is not possible to rule out the lack of effect in deer arising from an inadequate dose level of serotonin.

Another possibility is that the dose of GnRH used in the present study resulted in maximal release of LH from the pituitaries of the red deer hinds. A similar magnitude of LH response to exogenous GnRH was recorded here and previously by Anderson and Barrell (1998*a*) in deer and by Donnnelly and Dailey (1991) in sheep, but with the deer given one-fifth of the dose of GnRH (5 *v.* 25 μg), albeit using a different route of administration (i.v. for deer and i.m. for sheep). Furthermore, in wapiti cows challenged intravenously with different doses of GnRH (0.3, 1, 3, 10 and 30 μg) during the breeding season, the maximal LH response was induced with the 3-μg dose and only non-significant changes in LH were observed at higher doses (Baker *et al.* 1995). Thus, if the dose of GnRH used in the present study resulted in maximal release of LH from the pituitary gland it would not be possible for serotonin to increase the size of the LH peak, and so a stimulatory role of serotonin on LH secretion at pituitary gland level can not necessarily be ruled out. However, the lack of inhibition of pituitary responsiveness by cyproheptadine does not support this argument.

A perplexing result in Experiment 1 was the reduction of LH pulse amplitude by both drugs, serotonin and its antagonist, in ovariectomized hinds during the breeding season and in ovariectomized–thyroidectomized hinds during the non-breeding season. One possibility is that the secretion of LH is regulated differentially by the different classes of serotoninergic receptors (Lacau-Mendigo *et al.* 1996). Also, it is feasible that the reduction of LH pulse amplitude caused by the serotonin treatment resulted from a vasoconstrictor effect on blood supply in the portal vessels of the pituitary gland (Douglas 1985), which may mask any direct action of serotonin on LH secretion at the cellular level in the hypothalamus or pituitary gland.

The inhibitory action of cyproheptadine on LH pulse frequency recorded in both the breeding and non-breeding seasons in ovariectomized–thyroidectomized hinds suggests that the action of the thyroid hormones in allowing the transition between the breeding season and anoestrus may be executed by serotoninergic pathways. Neither dopaminergic nor opioidergic pathways appear to be involved in this process in red deer hinds (Anderson and Barrell 1998*b*). Also, it is noteworthy that the plasma concentration, pulse frequency and pulse amplitude of LH in ovariectomized–thyroidectomized hinds were all higher than in ovariectomized hinds in both seasons prior to drug or vehicle treatment. A trend of higher plasma LH concentration during the breeding season in thyroidectomized hinds compared with euthyroid hinds can also be observed in data published by Anderson and Barrell (1998*a*). However, the occurrence of an inhibitory action of thyroid hormones on circulating LH concentrations in both seasons has not been reported in ewes (Moenter *et al.* 1991) or rams (Parkinson and Follett 1994).

In the deer in the present study, halothane anaesthesia achieved a block in the secretion of LH; the reduction in mean plasma LH concentration being a reflection of the reduction in number of LH pulses. This observation is rein-

forced by the finding that for the two hinds in which recovery of consciousness appeared to occur, there was a concomitant increase in plasma LH concentration. Almost certainly the effect of halothane is due to suppression of GnRH pulses.

Like other general anaesthetics, halothane is capable of depressing central nervous system (CNS) function at most higher brain levels and at some lower levels (Booth 1977; Marshall and Wollman 1985). The latter include the hypothalamus, which is the site of the GnRH pulse generator. Suppression of CNS activity at this level by halothane is indicated by the reduction of LH pulsatility achieved in deer hinds (present study) and ewes (Clarke and Doughton 1983). Further evidence for hypothalamic depression is provided by the increase in plasma prolactin secretion recorded in the hinds during the period of anaesthesia. Halothane anaesthesia thus provides a potentially useful animal model for neuroendocrine studies in deer, somewhat akin to the hypothalamus–pituitary disconnected sheep example (Clarke *et al.* 1983), but without the requirement for surgery. A similar case has been made for the use of barbiturate anaesthesia in sheep (Evans *et al.* 1991).

While the deer were anaesthetized, LH was released in response to exogenous GnRH, as has been demonstrated in sheep (Radford and Wallace 1974; Webb *et al.* 1981; Wright and Clarke 1988; Evans *et al.* 1991). However, in anaesthetized ewes, the LH response to GnRH in one case was lower than in their conscious counterparts (Wright and Clarke 1988). Those authors argued that this could have arisen from inadequate priming of the pituitary gland with endogenous GnRH before the GnRH treatment. There was no evidence of this in deer, as the increase in plasma LH concentration of the anaesthetized hinds following exogenous GnRH seen in this study did not differ in magnitude from that of conscious ovariectomized red deer hinds reported by Anderson and Barrell (1998*a*).

Although it was not tested formally in the present study, the LH response to exogenous GnRH varied between seasons, being highest in the breeding season as reported previously in red deer (Suttie *et al.* 1989; Meikle and Fisher 1996; Anderson and Barrell 1998*a*) and wapiti (Baker *et al.* 1995). This indicates a seasonal change in pituitary responsiveness to exogenous GnRH, possibly due to the pituitary gland being more active during the breeding season as a result of priming by the more frequent release of endogenous GnRH.

In summary, these results provide evidence for a stimulatory role of serotoninergic pathways at the hypothalamic level of regulation of LH secretion in red deer, but rule out the possibility of a permissive role at the pituitary gland level.

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