Endocrine, *vol. 11, no. 1, 75–82, August 1999* 0969–711X/99/8:75–82/$12.00 © 1999 by Humana Press Inc. All rights of any nature whatsoever reserved.

Inhibition of Hypothalamic GnRH Secretion in the Ewe by Antigonadotropic Decapeptide During the Estrous Cycle and Nonbreeding Season

Hsu Lee, Eduardo Esquivel, and Mark E. Wise

Department of Animal Science, University of Arizona, Tucson AZ

Previous experiments from our laboratory and others have shown that the peptide antigonadotropic decapeptide (AGD) has marked inhibitory effects on luteinizing hormone (LH) secretion in rats and ewes. The first objective of this study was to determine whether AGD inhibits LH secretion by regulating hypothalamic release of gonadotropin hormone (GnRH). AGD (200 μg in 200 μL of 0.3% bovine serum albumin [BSA] saline) or vehicle was infused into the lateral ventricle of ovariectomized (OVX) ewes with hypophyseal-portal cannulae, and GnRH secretion was monitored. The frequency of GnRH and LH pulses in AGD-treated ewes was significantly decreased ($p < 0.05$) but did not change in the control ewes. The second objective of this investigation was to evaluate changes in hypothalamic sensitivity to AGD in the ewe during the estrous cycle and nonbreeding season. During the estrous cycle, the effects of AGD on LH secretion were assessed following ovariectomy, during the metestrous, diestrous, and proestrous phases of the estrous cycle. The response to AGD during the estrous cycle was compared to its effect during the anestrous season. LH, cortisol, and prolactin (PRL) concentrations were assayed in peripheral blood samples obtained at 10-min intervals over a 6-h period prior to injection of either vehicle (200 μL of 0.3% BSA in 0.9% saline) or AGD (200 μg in 200 μL of vehicle), and for an additional 10 h following treatment. LH pulse frequency decreased after treatment with AGD ($p < 0.05$) at all times in OVX and intact ewes compared to vehicle-treated controls. During the anestrous season, AGD treatment was more effective in inhibiting LH pulse frequency than during the breeding season ($p < 0.05$). Furthermore, there was a significant increase ($p < 0.05$) in mean cortisol concentrations after AGD infusion in all AGD-treated groups compared to controls independent of season or reproductive status. PRL concentrations were also increased ($p < 0.05$) following treatment with AGD. These results suggest that inhibition of pulsatile LH release induced by AGD is modulated by alterations in frequency of hypothalamic discharges of GnRH. Furthermore, changes in the inhibitory actions of AGD may contribute to the seasonal regulation of hypothalamic GnRH secretion in the ewe.

Key Words: Gonadotropin-releasing hormone; luteinizing hormone; ewe; seasonality.

Introduction

In seasonally breeding animals, such as the ewe, variation in luteinizing hormone (LH) pulse frequency accompanies seasonal changes in reproductive activity *(1–3)*. Decreasing length of day stimulates pulsatile LH secretion and inhibits prolactin (PRL) secretion while increasing length of day has the opposite effect *(2,4–6)*. The effects of photoperiod on both of these hormones are thought to be mediated indirectly by changes in melatonin secretion *(7)*. A diurnal change in melatonin secretion results in an endogenous circannual rhythm of reproductive neuroendocrine activity *(8)*. The effects of melatonin on pulsatile LH secretion are mediated by central nervous system mechanisms that regulate the activity of the gonadotropin-releasing hormone (GnRH) pulse generator system *(9)*. Melatonin induces a decrease in PRL secretion associated with a stimulation of LH secretion and a decrease in dopamine content in median eminence *(6)*.

Seasonal variation in the pattern of LH secretion also appears in ovariectomized (OVX) ewes when treated with exogenous estradiol *(10)*, with lower LH pulse frequency being observed in OVX ewes during the anestrous season when compared to the breeding season *(11)*. Treatment with low levels of estrogen reduces LH pulse frequency markedly in OVX ewes during anestrus but is ineffective during the breeding season *(10,12)*.

Received March 1, 1999; Revised April 29, 1999; Accepted April 29, 1999.

Author to whom all correspondence and reprint requests should be addressed: Mark E. Wise, Department of Animal Sciences, Shantz 228, University of Arizona, Tucson, AZ 85721. E-mail: mwise@ag.arizona.edu

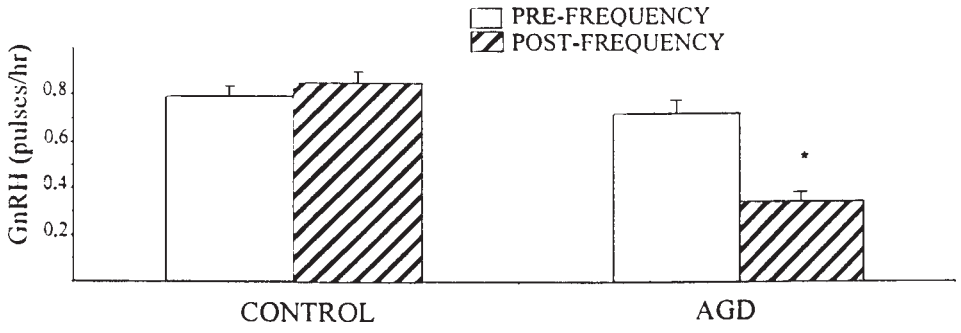

Fig. 1. GnRH pulse frequency in OVX control ewes ($n = 3$) and AGD-treated ewes ($n = 3$; 200 µg AGD). Pretreatment frequencies (□) were established over a 6-h period and posttreatment frequencies (□) were established over an 8-h period following treatment with AGD or vehicle. Statistical significance is indicated by an asterisk (*$p < 0.05$).

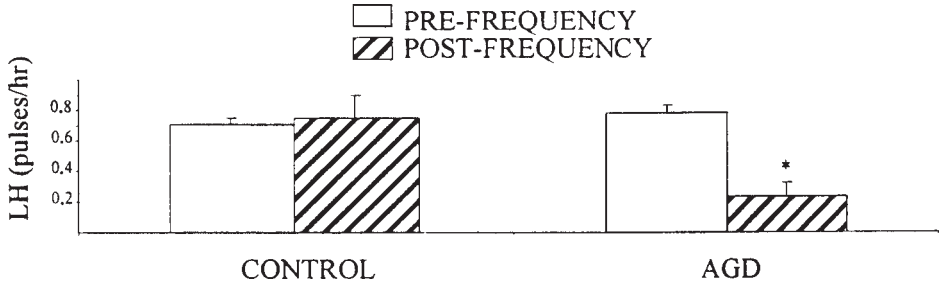

Fig. 2. LH pulse frequency in OVX control ewes ($n = 3$)and AGD-treated ewes ($n = 3$; 200 mg AGD). Pretreatment frequencies (□) were established over a 6-h period and posttreatment frequencies (□) were established over an 8-h period following treatment with AGD or vehicle. Statistical significance is indicated by an asterisk (*$p < 0.05$).

Some studies suggest that the reduction of LH pulse frequency by estrogen during the anestrous season is mediated by a catecholaminergic pathway *(13–15)*. Catecholamines have been implicated in the photoperiodic inhibition of gonadotropin secretion in both male and female sheep *(16)*. Alternatively, other evidence indicates that the regulation of dopaminergic activity in the median eminence (ME) of ewes may be considered a probable step in the photoperiodic regulation of LH secretion *(6)*. The reduction of LH pulse frequency in the absence of estrogen appears to be regulated by a serotonergic pathway *(17,18)*. How steroid-independent regulation of these serotonergic pathways is mediated is unknown but may involve novel regulatory pathways.

Antigonadotropic decapeptide (AGD) was isolated from bovine pineal glands by serial semipreparative liquid chromatography *(19)*. The primary structure of AGD (NH_2-Ser-Phe-Pro-Thr-Thr-Lys-Thr-Tyr-Phe-Pro-COOH) was determined by automated amino acid and microsequence analysis *(20)*. In the rat, intracerebroventricular injection of AGD suppresses LH and PRL secretion *(21,22)*. This action of AGD in the rat is associated with activation of dopamine release from the ME *(19,20,23)*. In OVX ewes, AGD injected into the brain via the lateral ventricle attenuates pulsatile LH secretion but has no effect on anterior pituitary response to exogenous GnRH *(24)*.

Little is known about the mechanisms of action of AGD, but the observation that AGD increases dopamine release from the ME of the rat *(19,23)* suggests that this peptide may interact with dopaminergic neurons in the ME of the ewe in a similar manner to inhibit GnRH secretion. Given the implications of dopaminergic involvement in the regulation of seasonal anestrus and the original isolation of AGD from the pineal gland, we hypothesized that AGD may play a role in regulating pulsatile LH secretion associated with seasonal reproduction in the ewe.

The objectives of this study were first to determine whether AGD acts directly in the brain to inhibit GnRH secretion. Second, experiments were designed to determine whether the responsiveness of the GnRH/LH axis to AGD changes during seasonal anestrus and the estrous cycle in which GnRH secretion is under varying steroid feedback.

Results

GnRH and LH Response to AGD

In both control and AGD-treated groups, GnRH and LH secretory profiles were pulsatile with pulses of GnRH being coincident with LH pulses. There was no difference in GnRH and LH pulse frequencies following treatment with vehicle in the control group (Figs. 1 and 2). However, following treatment with AGD, pulsatile GnRH and LH secretion were decreased significantly ($p < 0.05$) in a con-

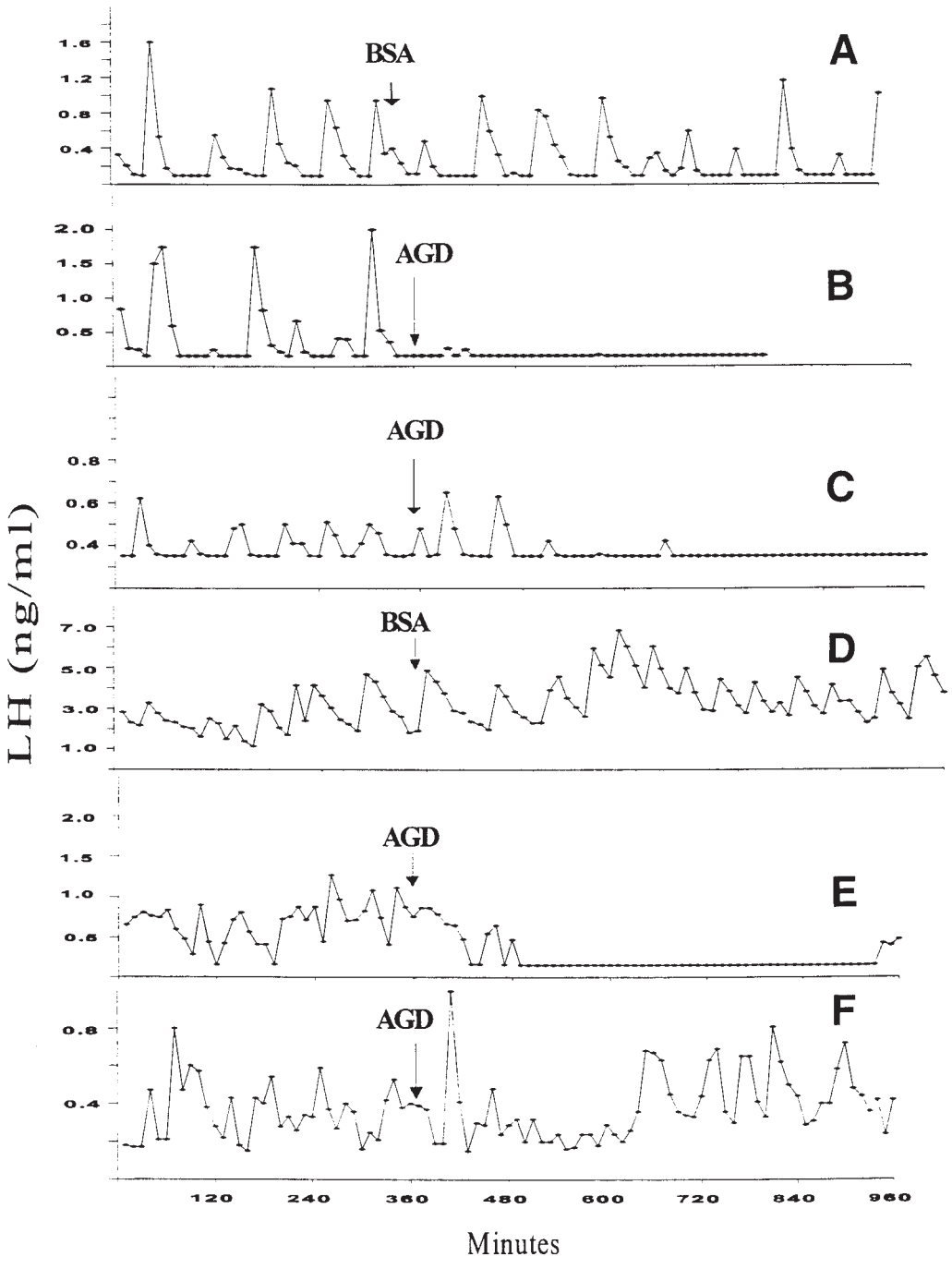

Fig. 3. Representative profiles of LH secretion in ewes following treatment. **(A)** OVX ewe in the nonbreeding season treated with BSA-saline vehicle; **(B)** OVX ewe in the nonbreeding season treated with AGD (200 μg); **(C)** ovary intact ewe in the nonbreeding season treated with AGD; **(D)** an OVX ewe in the breeding season treated with BSA-saline vehicle; **(E)** OVX ewe in the breeding season treated with AGD; and **(F)** ovary intact ewe on d 10 of the estrous cycle treated with AGD. Arrows indicates time of treatment relative to sampling.

comitant manner. AGD treatment did not influence amplitude (peak minus nadir) of GnRH (pre-, 25.3 ± 2.2 vs post-, 25.4 ± 0.8 pg/mL) or LH pulses (pre-, 0.3 ± 0.05 vs post-, 0.28 ± 0.02 ng/mL).

Seasonal LH Pulse Frequency and Amplitude Responses to AGD

Representative patterns of pulsatile LH release of ewes with different ovarian status and during the breeding and nonbreeding seasons are illustrated in Fig. 3A–F. In control animals, there was no change in LH pulse frequency following infusion of vehicle. By contrast, AGD decreased LH pulse frequency by 20–70% in all treatment groups. Mean LH pulse frequency for all treatment groups during the pre- and post-AGD treatments were 1.001 ± 0.078 and 0.463 ± 0.122 pulses/h, respectively. When comparing pre-AGD to post-AGD periods in all treatment groups, LH pulse frequency was decreased significantly ($p < 0.05$, Fig. 4)

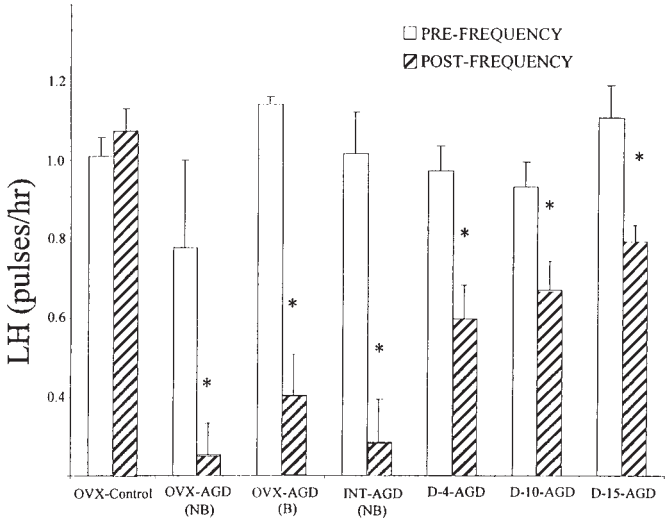

Fig. 4. Mean (+SEM) LH pulse frequency prior to (□) and following treatment (□) in (INT) intact and OVX ewes during the breeding (B) and nonbreeding seasons (NB). Data are expressed as pre- vs posttreatment with AGD or vehicle (6 h vs 10 h, respectively). Control animals (OVX-control) were treated with vehicle (BSA saline). Intact (INT) ewes received AGD (200 μg) during the breeding season on d 4 (D-4), d 10 (D-10), or d 15 (D-15) of the estrous cycle (d 0 being the first day of estrus). Statistically significant changes in LH pulse frequency from the pre-compared to the posttreatment period is indicated by an asterisk (*$p < 0.05$).

following AGD administration. During the nonbreeding seasons in OVX animals, AGD inhibited LH pulse frequency sooner (Table 1) to a greater extent ($p < 0.05$; Table 2, Fig. 4) compared to OVX animals in the breeding season. AGD was more effective in decreasing LH pulse frequency during the nonbreeding season in intact animals than in intact animals at any time during the estrous cycle ($p < 0.05$; Fig. 4). During different phases of the estrous cycle (d 4, d 10, and d 15), AGD inhibited LH pulse frequency in a similar manner (Fig. 4). AGD had no effect on LH pulse amplitude in any treatment group during the breeding or nonbreeding season.

Characterization of AGD Suppression of LH Pulses

The more pronounced effect of AGD on LH pulse frequency in the anestrous season was the result of rapid inhibition of pulsatile LH release. There was no time delay between infusion of AGD and suppression of LH pulse frequency in OVX ewes during the anestrous season (Fig. 3B, Table 1). However, in nonbreeding season intact ewes, the time between AGD infusion and suppression of LH pulses was 116 ± 21 minutes (Table 1). The mean length of time LH pulses were suppressed following AGD treatment was different for OVX and intact ewes ($p < 0.05$; Table 2). Pulsatile LH secretion in OVX ewes was inhibited sooner by AGD in the nonbreeding season compared to the breeding season ($p < 0.05$; Table 1) although the length of suppression was similar (Table 2). Time to inhibition of

Table 1
Length of Time Following Treatment with AGD to Suppression of Pulsatile LH Release During Different Seasons in Each Treatment Group[a]

Treatment	Time to inhibition (min)
OVX-AGD (NB)	0 ± 0[b]
INT-AGD (NB)	116 ± 21.9[c-e]
OVX-AGD (B)	133 ± 54.6[c-e]
D-4-AGD	48.0 ± 20.3[c,d]
D-10-AGD	198 ± 58.7[c-e]
D-15-AGD	290 ± 41.1[d,e]

[a]OVX, ovariectomized. NB; nonbreeding season; B, breeding season; D-4, d 4 of the estrous cycle (metestrus). D-10, d 10 of the estrous cycle (diestrus). D-15, d 15 of the estrous cycle (proestrus).
[b–e]Column values with different superscripts are different; $p < 0.05$.

Table 2
Period of Time Pulsatile LH Secretion Was Suppressed by AGD During Different Seasons in Each Treatment Group[a]

Treatment	Length of suppression (min)
OVX-AGD(NB)	600 ± 0.00[b]
INT-AGD(NB)	483 ± 21.9[c]
OVX-AGD (B)	376 ± 61.2[c]
D-4-AGD	136 ± 31.7[d]
D-10-AGD	208 ± 43.3[d]
D-15-AGD	164 ± 26.2[d]

[a]OVX, ovariectomized; NB, nonbreeding season. B, breeding season; D-4, d 4 of the estrous cycle (metestrus); D-10, d 10 of the estrous cycle (diestrus). D-15, d 15 of the estrous cycle (proestrus).
[b–d]Column values with different superscripts are different; $p < 0.05$.

pulsatile LH release in intact ewes was similar in the breeding and nonbreeding seasons (Table 1). However, length of suppression of pulsatile LH release following AGD in intact ewes was greater ($p < 0.05$) during the nonbreeding than breeding season (Table 2).

During the breeding season, AGD was more effective in suppressing LH secretion in OVX ewes than in intact ewes during the estrous cycle ($p < 0.05$; Table 2). During the estrous cycle (d 4, d 10 and d 15), there was a difference ($p < 0.05$; Table 1) in time to suppression of LH pulses post-AGD treatment between d 4 and d 15. There was no difference in mean time of total suppression among ewes at different days of the estrous cycle (Table 2). AGD suppressed pulsatile LH release in OVX animals to a greater extent than in intact animals in both the breeding and nonbreeding seasons ($p < 0.05$; Table 2).

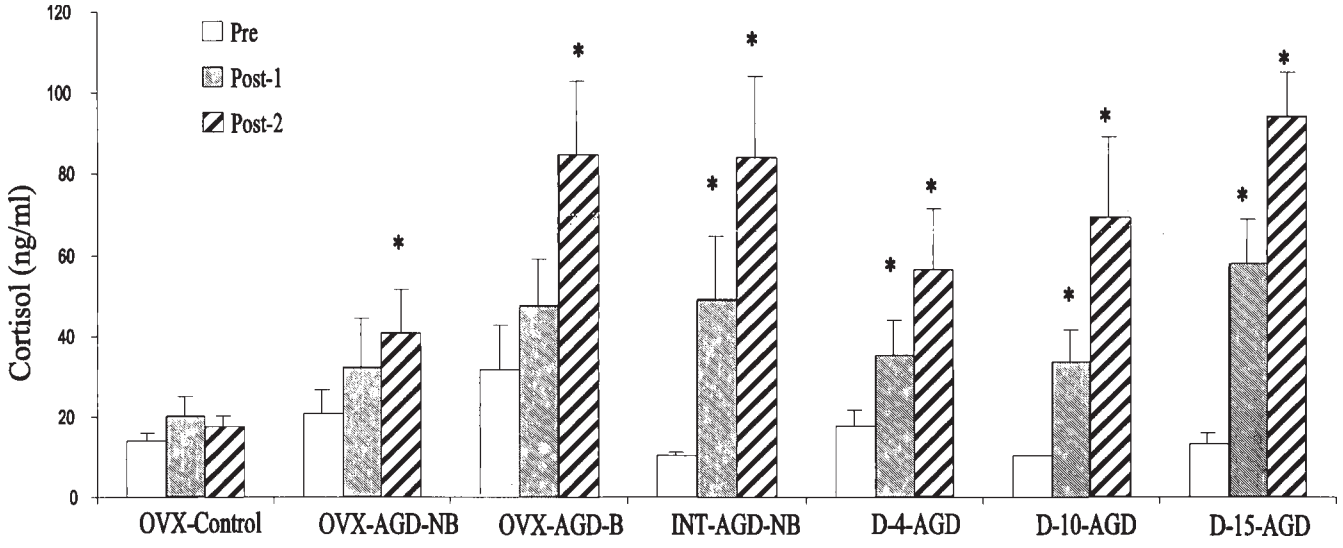

Fig. 5. Mean concentrations of cortisol following treatment in intact (INT) and OVX ewes during the breeding (B) and nonbreeding seasons (NB). Data are expressed as pre-(mean + SEM of pooled samples from the first 6 h of sampling, Pre) vs posttreatment concentrations (mean ± SEM of pooled samples from the first 5 h [Post-1] and second 5 h [Post-2]) following treatment with vehicle or AGD. Control animals (OVX-control) were treated with vehicle (BSA saline). Intact ewes received AGD (200 μg) during the breeding season on day 4 (D-4), d 10 (D-10), or d 15 (D-15) of the estrous cycle (d0 being the first day of estrus). Statistically significant changes in cortisol concentrations from the pre- compared to the posttreatment periods is indicated by an asterisk (*$p < 0.05$).

Cortisol Secretion in Response to AGD

Cortisol concentration increased significantly in all treatment groups during the first 5 h following AGD treatment compared to pre-AGD treatment concentrations (Fig. 5). A further increase in cortisol concentration occurred during the second 5 h sampling period compared to the pre-AGD treatment period and the first 5 h post-AGD (Fig. 5). In control animals, cortisol concentrations were not different after infusion of vehicle over any time during the sampling periods compared to the prevehicle sampling period (Fig. 5). The effects of AGD on cortisol concentration were not influenced by season, ovariectomy, or stage of estrous cycle (Fig. 5).

PRL Secretion in Response to AGD

Serum PRL responded in a similar fashion to cortisol, increasing over the 10-h period after treatment with AGD. In the breeding season ($n = 7$), following AGD infusion, PRL concentrations tended to increase in the first 5-h (23.2 ± 3.3 ng/mL) and second 5-h period (23.8 ± 2.6 ng/mL) compared to pre-AGD concentrations (18.5 ± 2.2 ng/mL; $p < 0.1$ and $p < 0.05$, respectively).

Discussion

This study demonstrates that lateral ventricle administration of AGD is effective during the breeding and nonbreeding seasons in inhibiting LH pulse frequency in both OVX and intact ewes. These data are in agreement with previous findings indicating that the inhibitory actions of AGD are centered in the brain rather than at the level of the anterior pituitary gland *(24)*. The actions of AGD on hypothalamic GnRH release appear to be independent of ovarian status because GnRH/LH secretion were suppressed in both OVX and intact ewes. Furthermore, AGD was equally effective in inhibiting LH secretion during periods of the estrous cycle in which estrogen or progesterone were the dominant ovarian steroids present. Other ovarian factors may contribute to the inhibition of GnRH release by AGD because inhibitory effects were more pronounced in OVX than intact animals.

Our data demonstrate that LH release in response to AGD varies with season in the ewe. The primary reason AGD was more effective in inhibiting LH pulse frequency during the nonbreeding compared to the breeding season was that inhibition of pulsatile LH release was almost immediate following AGD infusion and was maintained over a longer period of time in the nonbreeding season. This may indicate greater hypothalamic sensitivity to AGD during the anestrous season in the ewe.

Changing day length may trigger mechanisms other than those signaled by melatonin *(9,25,26)*. As the peak of the breeding season wanes, ewes become refractory to short-day stimulation *(27)*. The pause of the estrous activity may occur in response to an inhibitory cascade, activated in the photorefractory period, which requires estrogen and activation of dopaminergic receptors *(27)*. Previous studies in the rat suggested that AGD increases dopamine release from the ME and decreases circulating PRL levels *(20,21,23)*. We originally speculated that AGD may also interact with dopaminergic neurons in the ME to inhibit GnRH secretion in the ewe. However, data from the current experiment suggest that in ewes, AGD may have the oppo-

site effect on dopamine release from the ME because an increase in circulating PRL concentrations occurred after AGD infusion during the breeding season. Concentrations of AGD and mRNA levels for AGD are high in the hypothalamus compared to other regions of the brain (unpublished observations), suggesting that AGD may have a functional role in this region of the brain. The effect of AGD on the dopaminergic pathway implicated in seasonal anestrus, however, has yet to be fully examined.

Although the mechanism of action of AGD is unknown, the current data suggest several pathways by which suppression of hypothalamic GnRH release could occur. The ovarian- independent action of AGD indicates that AGD may inhibit the GnRH pulse generator via a serotonergic neural system that mediates inhibition of LH pulse frequency in a steroid-independent manner *(17,28)*. We observed a significant increase in circulating cortisol concentrations after AGD infusion that was independent of season, ovariectomy, or stage of the estrous cycle. The most likely explanation of the increase in cortisol is that AGD stimulates release of corticotropin-releasing hormone (CRH) secretion from the hypothalamus of the ewe. In the hypothalamus, serotonin stimulates CRH-ACTH secretion in the rat *(29–32)*. Previous reports have demonstrated that a serotonergic pathway mediates the steroid-independent inhibition of LH pulse frequency in anestrous ewes *(28)*. Additionally, serotonin plays a role in photoperiodic inhibition of LH pulsatile release in OVX ewes during short days *(18)*. Interestingly, our data also demonstrate that AGD inhibits pulsatile LH secretion seasonally in an ovarian-independent manner. Furthermore, the increase in PRL secretion stimulated by AGD suggests that AGD may stimulate the release of PRL-releasing factor in the ewe. Serotonin stimulation of the hypothalamus has been reported to enhance PRL secretion in the ewe *(33)*. Taken together, these data support the notion that AGD may activate serotonergic neurons that could both inhibit pulsatile GnRH secretion and stimulate CRH release and enhance PRL secretion.

A second explanation for the divergent effects of AGD on GnRH and cortisol secretion is via novel gaseous neurotransmitters. Recent studies suggest that nitric oxide (NO) and carbon monoxide (CO) may act as neurotransmitters that can inhibit the secretion of CRH in the rat *(34,35)* These novel neurotransmitters are involved in both the induction of the preovulatory LH surge and stimulation of GnRH release from the hypothalamus of estrogen-primed rats *(36)*. Both gases modulate guanylate cyclase activity and increase the cyclic guanosine S^1-monophosphate (cGMP) level for activation of a phosphorylation cascade (44,45). Activation of glutamate receptors (*N*-methyl-D-asparate) in the medial preoptic area results in an NO-dependent increase in cGMP efflux, which, in turn, activates the GnRH-LH axis *(39)*. Hematin, a product of heme molecule cleavage by heme oxygenase to yield CO, dose dependently stimulates GnRH release by regulation of cGMP *(40)*. The inhibition of GnRH secretion and stimulation of CRH observed in the current experiment could be owing to inhibition of the release of NO from neurons in the hypothalamus. That is, removal of NO stimulation from the hypothalamus would be inhibitory to GnRH release and stimulatory to CRH release. Differences in the seasonal response to AGD could be attributed to AGD blocking NO neurotransmission, which is required for NMDA-receptor activation of GnRH release *(39)*. In the ram *(41)*, hamster *(42)*, and ewe *(26)*, NMDA administration results in increased LH secretion regardless of the physiological state of the animal, but the stimulation is larger in photoinhibited than in photostimulated animals. Thus, seasonal differences in LH release in response to AGD could be attributed to AGD blocking the NMDA-NO cascade, which may be more active in the ewe during the anestrous season.

In summary, AGD may function as a regulator of neurosecretory activity of GnRH neurons in the ewe. AGD inhibits pulsatile GnRH/LH in an ovarian-independent manner, possibly via a serotonergic pathway, which is more effective during nonbreeding than breeding seasons. Alternatively, AGD may act as an inhibitor of gaseous neurotransmitters, NO and/or CO, which have divergent effects on GnRH secretion and CRH release. Further investigation will be needed to confirm these hypotheses.

Materials and Methods

Surgical Procedure

All studies with animals were approved by the Institutional Animal Care and Use Committee at the University of Arizona. Lateral ventricle cannulation or hypophyseal-portal cannulation was performed 5 d prior to infusion of AGD or vehicle. Each ewe was anesthetized with sodium pentobarbital (14 mg/kg of body weight), intubated, and maintained under general anesthesia with halothane gas (2–3%). A single steel cannula was inserted into the skull at a position 1 cm lateral and 1 cm rostral to the intersection of the coronal and sagittal cranial suture *(43)*. A 14 gauge steel cannula, which opened internally into the lateral ventricle of the brain, was fixed in place with dental acrylic and bone screws. Correct placement of the cannula was confirmed during surgery by withdrawal of cerebral-spinal fluid via an 18 gauge needle inserted approx 15–25 mm into the cannula. The length from the top of the cannula to the lateral ventricle was measured and recorded for each animal. The cannula opened externally just above the skin and was sealed with a sterile rubber cap. Following collection of blood samples, ewes were anesthetized with an overdose of sodium pentobarbital and exsanguinated. Placement of the lateral ventricle cannula was confirmed by postmortem inspection following injection of 0.5 mL of Coomassie blue dye into the lateral ventricle cannula at the depth of AGD or vehicle infusion *(44)*.

For hypophyseal-portal cannulation, modification of the procedure described by Clarke and Cummins *(46)* was used. A transnasal, transphenoidal route was taken to expose the anterior face of the pituitary gland, and two specially designed cannulae were fixed in place with dental acrylic *(46)*. Body temperature, hay and water consumption, and general behavior of the ewes were monitored postsurgery. Cannulae were flushed daily with sterile saline.

Sexually mature western range ewes were utilized in this study during the anestrous season (April–July) and the breeding season (September–December). Ewes were randomly assigned to OVX or intact groups. Ovariectomies were performed at least 3 wk prior to lateral ventricle cannulation. During the anestrous and breeding seasons, ewes were randomly assigned to be OVX or to remain intact and receive either AGD or vehicle treatment.

Hypophysial-Portal Sampling

Six ewes were randomly assigned to either AGD treatment ($n = 3$) or control groups ($n = 3$). On the day of sampling, 20,000 IU of heparin (Sigma, St. Louis, MO) in saline was infused intravenously followed by 5000 IU of heparin every hour as needed to maintain hypophyseal-portal blood collection. Ewes received heparin and a hypodermic trochar was used to cut the hypophyseal-portal vessels through the upper cannula. Continuous aspiration was applied to the lower cannula via peristaltic pump, and hypophyseal-portal blood was collected into tubes that were maintained on ice and changed at 10-min intervals. Plasma from samples was harvested within 20 min of collection by centrifugation (600*g*) and immediately frozen at –20°C. At the beginning of portal blood sampling, venous blood samples were also collected at 10 min intervals for the duration of the experimental period via an indwelling jugular catheter (16 g). Samples were collected 6 h prior to treatment with either AGD (200 μg in 200 μL of 0.3% BSA saline) or vehicle (0.3% BSA saline) through the lateral ventricle cannula. Blood samples were collected for an additional 8 h following treatments.

Breeding Season

During the breeding season, estrus was synchronized with a prostaglandin $F_{2\alpha}$ analog (Lutalyse, Upjohn, Kalamazoo, MI; 7.5 mg/injection; two injections 4 h apart) given 10 d apart. Ewes were housed with a vasectomized ram to detect the onset of estrus (d 0). All animals were fitted with a lateral ventricle cannula 5 d prior to blood sampling. Ewes were assigned to receive AGD (200 μg in 200 μL of 0.3% BSA in 0.9% saline) on one of three days of the estrous cycle (d 4, d 10, or d 15; $n = 4$–6/treatment). Additionally, two groups of ewes ($n = 3$/group) were OVX 3 wk prior to the initiation of the experiment and assigned to receive AGD (200 μg in 200 μL of vehicle) or vehicle (200 μL of 0.3% BSA in 0.9% saline) via lateral ventricle infusion. Peripheral blood samples were collected at 10-min intervals via an indwelling jugular catheter (16 gage) for 6 h prior to treatments. Blood samples were collected for an additional 10 h at 10 min-intervals following treatments. Samples were allowed to clot over a 24-h period at 4°C. Serum was harvested and stored at –20°C until assayed for hormone concentrations.

Anestrous Season

During the nonbreeding season, ewes were exposed to a vasectomized ram over a 3-wk period to confirm that the ewes were anestrus. Both OVX and intact ewes were divided into AGD-treated ($n = 3$) and control ($n = 3$) groups. All animals were fitted with a lateral ventricle cannula 5 d prior to treatment (either 200 μL of 0.3% BSA in 0.9% saline or 200 μg of AGD in 200 μL of vehicle). AGD or vehicle was administered, and blood samples were collected as described previously.

Assays

Hypophyseal-portal GnRH concentrations were determined by radioimmunoassay (RIA) *(47)* using R1245 antibody. All samples were run in a single assay. Sensitivity of the assay was 0.6 pg/tube, and the intraassay coefficient of variation (CV) was 10.2%. LH was measured in duplicate 200-μL aliquots of serum by RIA *(46,48)* using ^{125}I-labeled ovine LH (NIADDK-I-3) as tracer, NIADDK-oLH-25 as standard, and CSU-204 as first antibody. The sensitivity of the assay was 0.15 ng/mL, and intra- and interassay CVs were 10 and 8.9%, respectively.

Pooled serum samples for the cortisol and PRL were created by combining 200 μL of serum from each sample during the 6-h period before treatment (pre-) and each 5-h period posttreatment (post-1 and post-2, respectively). Cortisol was assayed in duplicate 25-μL aliquots of pooled plasma samples by a Coat-A-Count cortisol RIA kit (Diagnostic, Los Angeles, CA). Sensitivity of this assay was 2 ng/tube. PRL was assayed in duplicate 200 μL aliquots of pooled serum samples by the Coat-A-Count (Diagnostic) PRL RIA kit, with a sensitivity of 1.4 ng/tube.

Statistical Analysis

Pulses of GnRH and LH were identified by using the Cluster Analysis Program *(49)*. The size of nadir and peak were set at 2 and 1 points, respectively. The T-score for detecting significant increases and decreases in LH was set at 2 for both the upstroke and downstroke using a linear variance model. The values of LH below the assay sensitivity were assigned a value equal to the detection limit of the assay.

To characterize further the effect of AGD infusion on pulsatile LH secretion, LH pulse frequency and amplitude data were analyzed in the posttreatment period. The length of time from either AGD or vehicle administration to the last regularly occurring LH pulse was expressed as "time to inhibition." The total time that LH pulse frequency was

disrupted was determined by measuring the length of time when no pulses of LH occurred following treatment and was called "length of suppression."

Statistical analysis was performed by SPSS for Windows, Release 7.5 (SPSS, Chicago, IL). Data posttreatment in each group were compared by using paired *t*-test, and the effects of treatment on changes in LH pulse frequency and amplitude, cortisol, and PRL were analyzed using multiple analysis of variance (ANOVA) over time by general linear measurement and one-way ANOVA. Data were expressed as the mean ± SEM.

Acknowledgments

We thank Dr. Bryan Benson for supplying AGD used in these experiments. We are also grateful to Dr. Gordon Niswender and Dr. Terry Nett (Colorado State University, Fort Collins, CO) for providing LH and GnRH antibody and the NIH Pituitary Hormone Distribution Program (Baltimore, MD) for RIA reagents. This work was supported by USDA 96-35203-3313 (M.E.W.).

References

1. Karsch, F. J., Dahl, G. E., Evans, N. P., Manning, J. M., Mayfield, K. P., Moenter, S. M., and Foster, D. L. (1993). *Biol. Reprod.* **49,** 1377–1383.
2. Karsch, F. J., Bittman, E. L., Foster, D. L., Goodman, R. L., Legan, S. J., and Robinson, J. E. (1984). *Recent Prog. Horm. Res.* **40,** 185–225.
3. Goodman, R. L., Legan, S. J., Ryan, K. D., Foster, D. L., and Karsch, F. J. (1981). *J. Endocrinol.* **889,** 229–240.
4. Kennaway, D. J., Dunstan, E. A., Gilmore, T. A., and Seamark, R. F. (1984). *J. Endocrinol.* **102,** 199–207.
5. Bittman, E. L., Kaynard, A. H., Olster, D. H., Robinson, J. E., Yellon, S. M, , and Karsch, F. J. (1985). Neuroendocrinology. **40,** 409–418.
6. Viguie, C., Thibault, J., Thiery, J. C., Tillet, Y., and Malpaux, B. (1997). *Endocrinology* **138,** 499–506.
7. Arendt, J. (1986). *Oxford Rev. Reprod. Biol.* **8,** 266–320.
8. Woodfill, C. J. I., Wayne, N. L., Moenter, S. M., and Karsch, F. J. (1994). *Biol. Reprod.* **50,** 965–976.
9. Barrel, B. A., Moenter, S. M., Caraty, A., and Karsch, F. J. (1992). *Biol. Reprod.* **46,** 1130–1135.
10. Martin, G. B., Scaramuzzi, R. T., and Henstridge, J. D. (1983). *J. Endocrinol.* **96,** 181–193.
11. Goodman, R. L., Bittman, E. L., Foster, D. L., and Karsch, F. J. . (1982). *Biol. Reprod.* **27,** 580–589.
12. Martin, G. B. (1984). *Biol. Rev.* **59,** 1–87.
13. Meyer, S. L. and Goodman, R. L. (1985). *Endocrinology* **116,** 2054–2061.
14. Havern, R. L., Whisnant, C. S., and Goodman, R. L. (1994). *Endocrinology* **134,** 1905–1914.
15. Goodman, R. L., Havern, R. L., and Whisnant, C. S. (1996). *Biol. Reprod.* **54,** 380–386.
16. Tortonese, D. J. and Lincoln, G. A. (1994). *J. Endocrinol.* **143,** 25–32.
17. Meyer, S. L. and Goodman, R. L. (1986). *Biol. Reprod.* **35,** 562–571.
18. Corre, S. L. and Chemineau, P. (1993). *J. Reprod. Fert.* **97,** 367–373.
19. Benson, B., Ebels, I., and Hruby, V. J. (1990). In: *Advances in pineal research,* vol. 4. Reiter, R. J., and Lukaszyk, A. (eds.). John Libbey and Co.: London.
20. Benson, B. and Ebels, I. (1994). *Life Sci.* **54,** 437–443.
21. Benson, B. and Machen, N. (1994). *Life Sci.* **55,** 263–268.
22. Benson, B., Machen, N., Dunn, A. M., and Wise, M. E. (1996). *Life Sci.* **58,** 1083–1090.
23. Benson, B. and Chawla, M. (1992). In: *Program of the 9th international congress of endocrinology.* Nice, France.
24. Dunn, A. M., Benson, B., Esquivel, E., and Wise, M. E. (1993). *Biol. Reprod.* **48,** A132.
25. Viguie, C., Caraty, A., Locatelli, A., and Malpaux, B. (1995). *Biol. Reprod.* **52,** 1114–1120.
26. Viguie, C., Caraty, A., Locatelli, A., and Malpaux, B. (1995). *Biol. Reprod.* **52,** 1156–1161.
27. Kao, C., Schaeffer, D. J., and Jackson, G. K. (1992). *Biol. Reprod.* **46,** 425–434.
28. Whisnant, C. S., and Goodman, R. L. (1990). *Biol. Reprod.* **42,** 656–661.
29. Calogero, A. E., Bernardini, R., Margioris., A. N., Bagdy, G., Gallucci , W. T., Munson, P. J., Tamarkin, L., Tomai, T. P., Brady, L., Gold , P. W., and Chrousos, G. P. (1989). *Peptides* **10,** 189–200.
30. Feldman, S. and Weidenfeld, J. (1995). *Neurosci. Lett.* **198,** 143–145.
31. Weiner, R. J. and Ganong, W. F. (1978). *Physiol. Rev.* **58,** 905–973.
32. Martire, M., Pistritto, G., and Preziosi, P. (1989). *J. Neural. Trans.* **78,** 109–120.
33. Thomas, G. B., Cummins, J. T., and Clark, I. J. (1988). *Neurosci. Lett.* **85,** 143–146.
34. Costa, A., Trainer, P., and Grossman, A. (1993). *Brain Res.* **605,** 187–192.
35. Pozzoli, G., Mancuso, C., Mirtella, A., Preziosi, P., Grossman, A. B. and Navarra, P. (1994). *Endocrinology* **135,** 2314–2317.
36. Bonavera, J. J., Kalra, P. S., and Kalra, S. P. (1996). *Endocrinology* **137,** 956–1062.
37. Snyder, S. H. (1992). *Science* **257,** 494–496.
38. Verma, A., Hirsh, D. J., Glatt, C. E., Ronnett, G. V., Snyder, S. H. . (1993). *Science* **259,** 381–384.
39. Pu, S., Xu, B., Kalra, S. P., and Kalra, P. S. (1996). *Endocrinology* **137,** 1949–1955.
40. Lamar, C. A., Mahesh, V. B., and Brann, D. W. (1996). *Endocrinology* **137,** 790–793.
41. Lincoln, G. A. and Wu, F. C. W. (1991). *Neuroendocrinology* **3,** 309–317.
42. Hui, Y., Hastings, M. H., Maywood, E. S., and Ebling, F. J. P. (1992). *J. Reprod. Fertil.* **95,** 935–945.
43. Mori, Y., Takeuchi, Y., Shimada, M., Hayashi, S., and Hoshino, K. (1990). *Jpn. J. Vet. Sci.* **52,** 339–349.
44. Baldwin, B. A., Heavens, R. P., Kendrick, K. M., Parrott, R. F., Sharman, D. F., and Thornton, S. N. (1985). *Proc. Physiol. Soc.*, Abstract 9P.
45. Clarke, I. J. and Cummins, J. T. (1982). *Endocrinology* **111,** 1737–1739.
46. Wise, M. E. (1990). *Biol. Reprod.* **43,** 719–725.
47. Nett, T. M. and Adams, T. E. (1977). *Endocrinology* **101,** 1135–1144.
48 Niswender, G. D., Reichert, G. E. Jr., Midgley, A. R. Jr., and Nalbandov, A. V. (1969). *Endocrinology* **84,** 1166–1173.
49. Veldhuis, J. D., and Johnson, M. L. (1986). *Am. J. Physiol.* **250,** E486–E493.