Activation of the Hypothalamo-Pituitary-Adrenal Axis by Isolation and Restraint Stress during Lactation in Ewes: Effect of the Presence of the Lamb and Suckling

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We investigated the effect of the presence and absence of lambs and suckling by lambs to attenuate activation of the hypothalamo-pituitary-adrenal (HPA) axis to isolation and restraint stress in lactating sheep. In experiment 1, blood samples were collected every 10 min from nonlactating (n = 5) and lactating (n = 5) ewes for 4 h before and during stress. In experiment 2, ewes (n = 6) were allocated to 1) nonlactating, 2) lactating with lambs absent, 3) lactating with lambs present but unable to suckle, and 4) lactating with lambs present and able to suckle. Blood samples were collected over 8 h with no stress (control day) and for 4 h before and 4 h during stress (stress day). In experiment 1, the mean (±SEM) cortisol concentrations increased significantly (P < 0.05) in nonlactating ewes during stress but did not change in lactating ewes. In experiment 2, cortisol did not vary on the control day or pretreatment of the stress day but increased (P < 0.05) during stress in all groups except lactating ewes with lambs present and able to suckle. The greatest cortisol response occurred in nonlactating ewes followed by lactating ewes with lambs absent and lactating ewes with lambs present but unable to suckle. During stress, the ACTH concentrations increased (P < 0.05) in nonlactating ewes and lactating ewes with lambs absent but not in lactating ewes with lambs present. We conclude that the activity of the HPA axis during isolation and restraint is reduced in lactating ewes and that the presence of lambs increases this level of attenuation. (Endocrinology 147: 3501–3509, 2006)

LACTATING FEMALES of a range of species show attenuated neuroendocrine responses to stress (1–3) and lower levels of anxiety-related behavior (3–5). A large body of research with rats has consistently shown that, during lactation, there is an increase in the basal secretion of glucocorticoids and ACTH, loss of the normal diurnal rhythm of activity of the hypothalamo-pituitary-adrenal (HPA) axis and reduced responsiveness of the HPA axis to stress (for reviews see Refs. 1–6). Consequently, lactating rats generally have elevated basal concentrations of ACTH and corticosterone (6–8), decreased levels of CRH mRNA expression in neurons of the paraventricular nucleus (9–12), and attenuated secretion of ACTH (7, 8, 11, 13, 14) and corticosterone (7–9, 14) in response to various stressors. Similarly, in response to stress, lactating rats have reduced circulating concentrations of catecholamines (15), oxytocin (16–18), and prolactin (15, 19) compared with nonlactating rats. Attenuated stress responses also occur in lactating women. Lactating women were found to have lower basal concentrations of noradrenaline and reduced ACTH, cortisol, and glucose responses to treadmill exercise than nonlactating women (20), and lactating women also had a blunted increase in heart rate and reduced sympathetic nervous activity compared with postpartum lactating women in response to watching video tapes of their infants (21). There was no difference between lactating and nonlactating women in response to social stress (22) or in response to CO₂ inhalation (23), which may suggest that stress responses during lactation will vary with the stressor. In sheep, the basal plasma concentrations of cortisol were higher in lactating than nonlactating ewes, whereas the plasma concentrations of cortisol after introduction of an audiovisual stress (barking dog) were higher in nonlactating than lactating ewes (24).

The mechanisms by which lactation results in attenuated neuroendocrine responses to stress are not understood, but suckling by the infant and/or the presence of the infant may provide important stimuli that induce attenuated responses to stress. In rats, it has been found that removal of the pups generally results in measurements of activity of the HPA axis in lactating females that are similar to nonlactating females. This has been shown for levels of CRH mRNA (6) and ACTH (13) and corticosterone secretion (11, 25). Removal of pups was also found to partially restore the ACTH response to CRH, which has been found to be reduced in lactating females with pups present (26). Nevertheless, none of these studies delineated the importance of the presence of the offspring and suckling per se in attenuating stress responses during lactation. Removal of pups from lactating rats resulted in similar plasma concentrations of corticosterone to nonlactating rats, and reduced increases in corticosterone responses were found in lactating rats when various stressors were applied immediately after suckling (27). The importance of suckling was also highlighted in a study where it was found that ACTH and corticosterone responses to ether stress in thelecomized (removal of nipples) rats exposed continuously to pups were higher than in lactating rats in

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Abbreviations: AVP, Arginine vasopressin; HPA, hypothalamo-pituitary-adrenal.

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which pups could suckle (28), but no comparisons were made with nonlactating females. In humans, a protective role of breast-feeding against postpartum stress has been suggested (29). Mothers who breast-fed their infants were calmer and less anxious (21) and had less perceived stress (30, 31) and reduced negative mood (31) compared with mothers who bottle-fed. Reduced blood pressure and cortisol levels (32) have also been associated with breast-feeding. It has also been suggested that suckling is important in causing the suppression of the HPA axis in response to stress in women because the salivary and plasma concentrations of cortisol in response to mental stress were blunted in women who were breast-feeding compared with those who were only holding their infants (33), but no comparisons were made with nonlactating women. In sheep, the presence of the offspring (without suckling) appears to be sufficient to attenuate stress responses during lactation because plasma concentrations of cortisol after audiovisual stress were reduced in lactating ewes that had their lambs restrained alongside, allowing body contact but no suckling compared with nonlactating ewes (24). Nonetheless, it is unclear whether suckling would have further reduced the cortisol response to stress because comparisons were not made with ewes that were suckled by their lambs.

Although it appears that suckling can modify the activity of the HPA axis during lactation, there have been no experiments to systematically determine the relative role of suckling and the presence of offspring in modifying the activity of the HPA axis in response to stress in lactating females. Therefore, we used sheep to test the hypothesis that attenuation of the HPA axis during stress in lactating ewes does not require suckling by the lamb, but suckling causes greater attenuation of stress responses.

Materials and Methods

Animals

Thirty-four adult Romney Marsh ewes were used in two experiments that were conducted during October, which is nonbreeding season for this breed of sheep (34), at the Prince Henry’s Institute of Medical Research Biological Resource Centre at Werribee, Victoria, Australia (38° 5'). In experiment 1, five nonlactating ewes and five lactating ewes were used, whereas in experiment 2, six nonlactating ewes and 18 lactating ewes were used. All ewes were selected randomly from a flock of 200 ewes. In experiment 1, five nonlactating ewes and five lactating ewes with lambs present were collected from each of five nonlactating ewes and five lactating ewes with lambs present every 10 min for 8 h. During the first 4 h of sampling, all ewes were housed in individual pens (1.4 m long × 0.4 m wide × 0.9 m high) that were adjacent to pens containing other sheep. The lambs of the lactating ewes were present in the same pens as their mothers and were able to move freely and to suckle. After 4 h of sampling, isolation and restraint stress was imposed on all ewes for the remaining 4 h as previously described (33). Briefly, each ewe was moved to a novel pen of the same size that contained no sheep in adjacent pens and was completely enclosed on all sides and top by hessian (RN & G Lowin, Fitzroy, Australia) to prevent visual contact with other sheep. Each ewe was fitted with a harness that was used to restrain it to the side of the pen. This restricted the movement of the animals so that only the head could move freely. The ewes had access to water during this time. The lambs of lactating ewes were not restrained. The plasma concentrations of cortisol were measured in all samples.

Experiment 2: importance of the presence and absence of the lamb and suckling by the lamb on the plasma concentrations of ACTH and cortisol during isolation and restraint stress in lactating and nonlactating ewes. The effectiveness of isolation and restraint stress to induce an increase in the plasma concentrations of ACTH and cortisol was determined in the following four experimental groups (n = 6 ewes per group): 1) nonlactating ewes, 2) lactating ewes with lambs absent, 3) lactating ewes with lambs present but unable to suckle, and 4) lactating ewes with lambs present and able to suckle. In group 2 (lactating ewes with lambs absent), the lambs were removed from their mothers 16 h before the commencement of the sampling and were kept in a separate building for the duration of the sampling. In group 3 (lactating ewes with lambs present but unable to suckle), the lambs were present in the pens with their mothers, but a partition was placed in the pen that prevented the lambs from having access to the udder of the ewe and, therefore, from being able to suckle. Auditory, olfactory, and limited tactile stimulation was possible between mother and lamb. In group 4, as for the lactating groups in experiment 1, the lambs were kept in the same pens as their mothers and could move freely and suckle without restriction. A small video camera (Micromark, CCTV Camera System; Dick Smith, Melbourne, Australia) was placed in the pens of ewes in groups 3 and 4 to establish whether or not lambs suckled.

Blood samples (5 ml) were collected every 10 min for 8 h on a day with no stress was imposed (control day) and a day when isolation and restraint stress was imposed for 4 h after 4 h of sampling (stress day). The experiment was conducted in two replicates of 12 ewes so that there were four sampling days in total. The isolation and restraint stress was imposed immediately to experiment 1. All blood samples on each sampling day were assayed for cortisol. ACTH was assayed in all samples collected for 1 h before isolation and restraint stress and in all samples collected during the first 3 h of the isolation and restraint stress on the stress day.

RIAs

ACTH. Plasma concentrations of ACTH were determined using a 125I RIA kit (DiaSorin, Stillwater, MN; catalog no. 24065, 24130) that had a sensitivity of 15 pg/ml. Four assays were conducted, and the intra-assay coefficient of variation was 14.6% at 46.1 pg/ml and 13.9% at 84.4 pg/ml. The interassay coefficients of variation were 15.3% at 36.4 pg/ml and 11.9% at 79.4 pg/ml.

Cortisol. In both experiments, the concentrations of cortisol were measured in unextracted plasma by RIA that was developed and described by Broadbear et al. (36). A total of 16 assays were conducted and the mean (±SEM) sensitivity was 0.7 ± 0.1 ng/ml. The intraassay coefficients of variation were 8.3% at 7.5 ng/ml and 10.3% at 53.0 ng/ml. The interassay coefficients of variation were 16.7% at 9.9 ng/ml and 5.9% at 67.1 ng/ml.

Statistical analyses

All data in both experiments were statistically analyzed using repeated-measures ANOVA. For experiment 1, the within-subjects factor was time and the between-subjects factor was group of ewe (nonlactating or lactating), whereas for experiment 2, the within-subjects factors were time and day (control or stress) and the between subjects factor was...
Results

Experiment 1: plasma concentrations of cortisol during isolation and restraint stress in lactating and nonlactating ewes

Comparison of plasma concentrations of cortisol within groups. The mean (±SEM) plasma concentrations of cortisol were significantly (P < 0.05) higher in nonlactating ewes during isolation and restraint stress than during the pretreatment period from 10–160 min after the commencement of the stressor and 220 and 230 min after the commencement of the stressor (Fig. 1). In contrast, there were no significant differences in plasma concentrations of cortisol between the pretreatment period and isolation and restraint stress in lactating ewes (Fig. 1).

Comparison of plasma concentrations of cortisol between groups. During the pretreatment period, the mean (±SEM) plasma concentrations of cortisol in the nonlactating ewes and lactating ewes differed on seven of the 18 time points. At 240, 230, 180, 170, 160, 50, and 40 min before imposition of the stressor, the mean (±SEM) plasma concentrations of cortisol were significantly (P < 0.05) higher in the nonlactating ewes than in the lactating ewes (Fig. 1). During the period of isolation and restraint stress, the mean plasma concentrations of cortisol were significantly (P < 0.05) higher in the nonlactating ewes than in the lactating ewes from 10–150 min and from 220–240 min after commencement of the stressor (Fig. 1).

Experiment 2: importance of the presence and absence of the lamb and sucking by the lamb on the plasma concentrations of ACTH and cortisol during isolation and restraint stress in lactating and nonlactating ewes

Sucking by the lambs. Video footage showed that lambs in group 3 (lactating ewes with lambs present but unable to suckle) were unable to suckle their mothers, although auditory, olfactory, limited visual, and limited tactile stimulation was possible. In contrast, the lambs in group 4 (lactating ewes with lambs present and able to suckle) showed frequent bouts of suckling throughout the sampling and treatment periods.

Comparison of plasma concentrations of cortisol within groups. On the control day, there were no significant changes in the mean (±SEM) plasma concentrations of cortisol (ng/ml) for all groups of ewes (Fig. 2).

The mean (±SEM) plasma concentrations of cortisol (ng/ml) in the nonlactating ewes on the stress day were significantly (P < 0.05) higher from 10–150 min of isolation and restraint stress than during the pretreatment period. The mean (±SEM) plasma concentrations of cortisol in the nonlactating ewes were also significantly (P < 0.05) higher during isolation and restraint stress than at any time on the control day (Fig. 2).

The mean (±SEM) plasma concentrations of cortisol (ng/ml) in lactating ewes with lambs absent on the stress day were significantly (P < 0.05) higher from 10–50 min and at 110 min of isolation and restraint than during the pretreatment period and at any time on the control day (Fig. 2).

In ewes with lambs present but unable to suckle, the mean (±SEM) plasma concentrations of cortisol (ng/ml) on the stress day were significantly (P < 0.05) higher from 20–60 min and 110–140 min of isolation and restraint than during the pretreatment. Cortisol concentrations from 30–60 min and 120–140 min of isolation and restraint were also significantly (P < 0.05) higher than at any time on the control day (Fig. 2).

In lactating ewes with lambs present and able to suckle, there was no significant change in the mean (±SEM) plasma concentrations of cortisol during the isolation and restraint stress compared with the pretreatment period, and there were no significant differences in the cortisol concentrations between the stress day and the control day (Fig. 2).

Comparison of plasma concentrations of cortisol between groups. There were no consistent significant differences between groups in the mean (±SEM) plasma concentrations of cortisol (ng/ml) on the control day and the pretreatment period on the stress day (Fig. 2). On the stress day, the mean (±SEM) plasma concentrations of cortisol (ng/ml) during isolation and restraint stress were significantly (P < 0.05) higher in nonlactating ewes than in all other groups (Fig. 2). This was
from 10–100, 120–170, and 210–240 min of stress compared with lactating ewes with lambs absent; 10–100 and 150–170 min of stress compared with lactating ewes with lambs present but unable to suckle; and 10–150, 170, and 210–220 min of stress compared with lactating ewes with lambs present and able to suckle. The mean (±SEM) plasma concentrations of cortisol (ng/ml) during isolation and restraint in lactating ewes with lambs absent were significantly (P < 0.05) higher than in lactating ewes with lambs present but unable to suckle from 10–30, 60, 120–140, and at 220 min of stress. These concentrations were also significantly (P < 0.05) higher than in lactating ewes with lambs present and able to suckle from 3–40, 60, 120–140, and 210 min of stress (Fig. 2).

Comparison of plasma concentrations of ACTH within groups. The mean (±SEM) plasma concentrations (pg/ml) of ACTH in nonlactating ewes on the stress day were significantly (P < 0.05) greater than the pretreatment concentrations from 10–70 min of isolation and restraint (Fig. 3). In the lactating ewes with lambs absent, the mean (±SEM) plasma concentrations (pg/ml) of ACTH were significantly (P < 0.05) greater at 10 and 20 min after the commencement of isolation and restraint than the pretreatment concentrations. In contrast, there were no significant differences in the mean (±SEM) plasma concentrations (pg/ml) of ACTH over time in the lactating ewes with lambs present but unable to suckle and lactating ewes with lambs present and able to suckle (Fig. 3).

Comparison of plasma concentrations of ACTH between groups. There were no significant differences between groups in the mean (±SEM) plasma concentrations (pg/ml) of ACTH during the pretreatment period on the stress day. During isolation and restraint, these concentrations in nonlactating ewes were significantly (P < 0.05) higher than in all groups of lactating ewes from 30–70 min after commencement of the stressor (Fig. 3). The mean (±SEM) plasma concentrations (pg/ml) of ACTH in lactating ewes with lambs absent were significantly (P < 0.05) higher than in lactating ewes with.
lambs present but unable to suckle and in lactating ewes with lambs present and able to suckle at 10 and 20 min after the commencement of isolation and restraint (Fig. 3).

**Discussion**

It is clear from this study that the presence of the offspring and suckling by the offspring modify the stress-induced activity of the HPA axis in lactating ewes. In both experiments, activation of the HPA axis during isolation and restraint stress was substantially reduced in lactating ewes, and in experiment 2, the greatest attenuation in cortisol response occurred in lactating ewes with lambs present and able to suckle. Both the maximal plasma concentrations of ACTH and cortisol and the periods of significant elevation in plasma concentrations of these hormones were reduced in the lactating ewes. Our data support other reports that lactating females have attenuated stress responses (see introductory section), but importantly, we have extended our understanding of the stress-induced responsiveness of the HPA axis in lactating females by investigating the effects of the presence and absence of the offspring and suckling by the offspring to attenuate stress responsiveness. Although the activation of the HPA axis during isolation and restraint was attenuated in all groups of lactating ewes in experiment 2, there was a progression in responses, with greater increases in plasma concentrations of ACTH and cortisol occurring in the lactating ewes with lambs absent than in lactating ewes with lambs present but unable to suckle. In both of these groups, the stress-induced rise in cortisol was greater than in lactating ewes with lambs present and able to suckle. There were no differences, however, in the stress-induced elevation in plasma ACTH concentrations between lactating ewes with lambs present and unable to suckle and in lactating ewes with lambs present and able to suckle. Our finding that the activity of the HPA axis was attenuated in lactating ewes with lambs absent differs from studies in rats where removal of pups has been found to restore the activity of the HPA axis to a level similar to that in nonlactating females (see introductory section). This may represent a difference between species or in experimental approach. Previous studies have not systematically addressed the role of the presence and absence of the offspring and suckling by the offspring in influencing the stress-induced activity of the HPA axis as we have done here. Our data illustrate that there are mechanisms...
independent of the effects of the presence of the lambs and suckling by the lambs that attenuate the stress-induced activity of the HPA axis in lactating ewes. Nevertheless, the presence of the lambs clearly provides additional stimuli to reduce the activity of the HPA axis during stress.

Our results suggest that the mechanisms for reduced stress-induced activity of the HPA axis during lactation involve modification at the level of the hypothalamus and/or anterior pituitary gland because the increase in plasma concentrations of ACTH during isolation and restraint stress were substantially reduced in lactating ewes compared with nonlactating ewes. This could be the result of reduced secretion of CRH and/or arginine vasopressin (AVP) from the hypothalamus into the hypophysial portal system and/or reduced responsiveness of the anterior pituitary to the actions of CRH and/or AVP. Indeed, there is evidence that reductions in the synthesis of CRH and AVP and alterations in the responsiveness of the pituitary to CRH and AVP contribute to attenuated stress responsiveness in lactating females. For example, decreases in the stress-induced levels of mRNA for CRH (9, 37–39) and c-fos (12, 40–42) in neurons in the paraventricular nucleus have been found in lactating compared with nonlactating rats. Nonlactating rats also had a greater increase in primary transcripts for AVP after immune stress than did lactating rats (12). Nonetheless, it is unknown how these changes translate into the secretion of CRH and AVP because there have not been direct measurements of these neuropeptides in the hypophysial portal blood of conscious lactating females of any species. It is unknown whether pituitary responsiveness to CRH and AVP is altered during lactation in sheep, although this has been demonstrated in lactating rats. Treatment with CRH resulted in greater increases in ACTH in nonlactating than in lactating rats (26). In contrast, increased pituitary responsiveness to AVP has been found during lactation in rats (2, 26), suggesting that the pituitary actions of CRH and AVP may be altered differently during lactation. It has also been suggested that attenuated responsiveness of the HPA axis during lactation results from altered negative feedback by glucocorticoids and a reduction in stimulatory noradrenergic inputs from the brain stem to the paraventricular nucleus (1, 3, 43). Because the pattern of secretion of ACTH was similar to that of cortisol in experiment 2, it appears that altered adrenal responsiveness to ACTH is unlikely to be a major mechanism for reduced HPA activity during stress in lactating ewes, although additional investigations would be necessary to confirm this. Additional research is also necessary to establish the extent to which attenuated stress responsiveness of the HPA axis in lactating females is a result of mechanisms operating at the level of the brain and the anterior pituitary gland.

The mechanism by which the presence of the lamb increased the attenuation of the stress-induced activity of the HPA in lactating ewes is not known. For lactating ewes with lambs present but unable to suckle, auditory, olfactory, visual, and limited tactile stimulation was possible between the mother and lamb. Although these cues play varying roles in the establishment of the maternal young bond (44, 45), it is unknown whether they also influence the activity of the HPA axis. Research in the squirrel monkey has shown that social separation causes increases in the plasma concentrations of cortisol in both mothers and infants, whereas companionship has been shown to result in social buffering (for review see Ref. 46). It is unknown whether social buffering of this type is a mechanism for the reduced HPA axis responses to stress in lactating ewes with lambs present but unable to suckle compared with lactating ewes with lambs absent in experiment 2. Nevertheless, the lack of differences in the plasma concentrations of ACTH and cortisol between groups of ewes on the control day and the pretreatment period of the stress day in experiment 2 suggest that separation of the ewes from their lambs for 16 h did not stimulate the HPA axis of the dams. The mechanisms by which suckling by the lambs induced the greatest attenuation of the cortisol response to stress are also unknown, but it is feasible that suckling stimulates specific neuronal pathways that project to the key regulatory areas of the HPA axis, such as the paraventricular nucleus of the hypothalamus and the brain stem. Additional work is necessary to identify such pathways. Furthermore, it is also necessary to determine whether the suckling stimulus induces changes in pituitary responsiveness to CRH and/or AVP in lactating ewes.

Suckling may modify the activity of the HPA axis by influencing the synthesis, secretion, and actions of the neuropeptides oxytocin and prolactin, although this hypothesis has not been systematically tested. Oxytocin and prolactin are essential for normal lactation (47), and both hormones may potentially be involved in attenuation of the HPA axis in response to stress. It has been suggested that central oxytocinergic pathways, which are stimulated during lactation, may mediate some of the attenuated HPA axis responses to stress in lactating females (48). The intracerebroventricular infusion of ovariectomized estradiol-treated rats with oxytocin reduced the response of the HPA axis to noise (49) and restraint (50) stress, illustrating that oxytocin can act centrally to attenuate the stress-induced activity of the HPA axis. Administration of an oxytocin receptor antagonist into the paraventricular nucleus of rats increased both the basal and stress-induced activity of the HPA axis (51, 52), and female mice that had undergone oxytocin gene deletion displayed increased anxiety-related behavior and corticosterone secretion in response to stress compared with wild-type mice (53).

In sheep, infusion of oxytocin into the paraventricular nucleus also suppressed the cortisol response to an audiovisual stressor in nonlactating ewes and, to a lesser extent, in lactating ewes (24), and we have identified oxytocin neurons in the paraventricular nucleus of sheep in close proximity to CRH and AVP neurons (Rivalland, E. T. A., J. Iqbal, I. J. Clarke, A. I. Turner, and A. J. Tilbrook, unpublished data), making them well placed to influence CRH and AVP neurons. It is also possible that oxytocin secreted from the posterior pituitary may contribute to the attenuation of the HPA axis during lactation. Because oxytocin does not readily cross the blood-brain barrier (54), peripheral oxytocin may act directly on the anterior pituitary gland to affect responsiveness to CRH and/or AVP. In support of this, infusion of oxytocin into humans inhibited the plasma ACTH response to CRH (55). Although these studies suggest a role for oxytocin in altering the activity of the HPA axis during lactation, they do not provide direct evidence that suckling reduces...
stress responsiveness by inducing the release of oxytocin, and this requires investigation. Nevertheless, in humans, increased levels of oxytocin as a result of suckling and breast stimulation were associated with decreased levels of ACTH, suggesting that suckling-induced release of oxytocin inhibited the HPA axis (53, 56). Suckling increases the secretion of prolactin (57), and prolactin has also been implicated as a possible mediator of attenuated stress-induced activity of the HPA axis (13, 16, 58). The role of prolactin in attenuation of anxiety and stress responses has been extensively reviewed (59). It is apparent that prolactin secreted into the peripheral circulation can gain access to the brain through receptor-mediated active transport via the choroid plexus cells (60), and central prolactin pathways exist in regions relevant to the regulation of the HPA axis (61, 62). The intracerebroventricular infusion of antisense nucleotides against the long form of the brain prolactin receptor into lactating rats resulted in an increase in stress-induced ACTH (63), providing evidence for an inhibitory role for prolactin on HPA axis during lactation. Nonetheless, the relative contributions of oxytocin and prolactin in causing the attenuation of the stress-induced activity of the HPA axis in lactating females as a result of suckling have not been established.

Despite the attenuation of the stress-induced activity of the HPA in lactating ewes, we found no clear evidence that the basal activity of the axis was influenced by the presence or absence of the offspring or suckling by the offspring. In experiment 1, there were no consistent differences between lactating and nonlactating ewes in plasma concentrations of cortisol during the pretreatment period, and in experiment 2, there were no differences between groups in plasma concentrations of cortisol on the control day and the pretreatment period of the stress day. We also found that the basal plasma concentrations of ACTH did not differ between nonlactating ewes and lactating ewes, irrespective of whether the lambs were absent or present or able to suckle. These findings contrast to some extent with a study where lactating ewes were found to have slightly higher basal concentrations of cortisol than nonlactating ewes (24), although the differences between studies are small and inconsistent. In rats, it appears that the basal as well as the stress-induced activity of the HPA axis is altered during lactation. Nonstressed rats typically have elevated concentrations of ACTH and corticosterone (6, 8, 10, 11, 28). This appears to be caused predominantly by a flattening of the diurnal rhythm of secretion with a rise in nadir concentrations of corticosterone and a decrease in the peak evening concentrations (64). There is also evidence that suckling may stimulate the basal activity of the HPA axis in rats. In one study, greater increases in plasma concentrations of corticosterone occurred in dams when pups suckled compared with when pups did not suckle (65), and in another, elevated plasma concentrations of ACTH and corticosterone were found in lactating rats with their pups present compared with when the pups were absent (8). These findings contrast with the current study where suckling did not alter the plasma concentrations of ACTH and cortisol during the control day and pretreatment period of the stress day. The reason for the differences between studies is not known but may represent a species difference. Although there may be species differences in the extent to which basal activity of the HPA axis is altered during lactation, more extensive studies are required to establish whether the diurnal rhythm of the activity of the HPA axis changes during lactation in sheep. It has been demonstrated that nonlactating ewes show diurnal variation in the secretion of cortisol (66), but this has not been studied in lactating ewes.

Although the data from our experiments clearly show that the activity of the HPA axis in response to the psychogenic and physical stressor of isolation and restraint stress was attenuated in lactating ewes compared with nonlactating ewes, we are unaware of the extent to which this occurs with other classes of stressors in sheep. Reduced responses of the HPA axis to the auditory stress of a barking dog was found in lactating ewes compared with nonlactating ewes (24), but other stressors have not been investigated. There is evidence that different pathways and systems are involved in the relay of critical neural information from different stressors. For example, different stressors are known to activate different areas of the brain in rodents (reviewed in Ref. 67), prepubertal pigs (68), and castrated rams (69, 70). It is also apparent from studies in rodents that distinct afferent pathways mediate stress-specific stress responses (for reviews see Refs. 71 and 72). Nonetheless, reduced stress responsiveness of the HPA axis in lactating rats has been demonstrated for a wide range of stressors, including psychogenic, physical, and immunological stressors (1), which suggests that the influence of lactation on the activity of the HPA axis can be generalized across different types of stressors for this species. In contrast, the type of stressor appears to be important in humans because attenuated activity of the HPA axis was found in lactating compared with nonlactating women in response to treadmill exercise (20), whereas there were no differences between lactating and nonlactating women in response to social stress (22) or in response to CO₂ inhalation (23). It should be noted, however, that the roles of suckling per se and the presence and absence of the offspring on the stress-induced activity of the HPA axis have not been investigated using stressors other than isolation and restraint in sheep.

In conclusion, it appears that the stress-induced activity of the HPA axis is attenuated in lactating ewes by a multifaceted mechanism that ultimately involves decreased secretion of ACTH from the anterior pituitary gland. The extent to which this is a result of changes in the secretion of CRH and AVP from the hypothalamus and in altered pituitary responsiveness to these neuropeptides has not been determined. It is apparent that at least some of the mechanisms for attenuated stress responses in lactating ewes do not involve the presence of the lambs or suckling by the lambs. The lambs clearly provide some form of stimulus that increases the level of attenuation, and maximal inhibition of the stress-induced secretion of cortisol is induced by suckling. In contrast to the stress-induced activity of the HPA axis, we did not find any consistent evidence that the basal activity of the HPA axis varies between lactating and nonlactating ewes, and the influence of lactation on the diurnal rhythm of the HPA axis in sheep has not been determined. There may be differences between species, particularly between sheep and rats, in the key mechanisms by which stress responses are attenuated during lactation. Additional work is necessary to identify the
critical cues provided by offspring that alter the activity of the HPA axis and to determine the neuroendocrine mechanisms by which suckling inhibits responsiveness of the HPA axis to stress.

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