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Stressor Specificity of Sex Differences in Hypothalamo-Pituitary-Adrenal Axis Activity: Cortisol Responses to Exercise, Endotoxin, Wetting, and Isolation/Restraint Stress in Gonadectomized Male and Female Sheep

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Sex differences in the stress-induced activity of the hypothalamo-pituitary-adrenal axis in sheep appear to be dependent on the stressor encountered and occur irrespective of the presence of gonadal steroids. We tested the hypotheses that cortisol responses to exercise, endotoxin, wetting (experiment 1), and isolation/restraint (experiment 2) stress differ between gonadectomized male and female sheep. At weekly intervals (in experiment 1), we subjected gonadectomized rams and ewes (n = 6/group) to control conditions, to exercise stress, to iv injection of endotoxin, and to wetting stress. In a second experiment (experiment 2), we subjected gonadectomized rams and ewes (n = 5/group) to control conditions or to isolation/restraint stress. In both experiments, we measured plasma concentrations of cortisol before, during, and after stress at a frequency of at least 15 min with samples collected (from an indwelling jugular catheter) at a greater frequency around the time of the stressor. Cortisol responses to wetting (experiment 1) and isolation/restraint (experiment 2) stress were significantly higher in females compared with males but in response to exercise (experiment 1) and endotoxin (experiment 1) stress, there were no differences between the sexes. For some stressors, there are sex differences in sheep in the stress-induced activity of the hypothalamo-pituitary-adrenal axis that are independent of the presence of the sex steroids, but the existence of these sex differences and the direction of these sex differences differs, depending on the stressor imposed. (***Endocrinology*** **151: 4324–4331, 2010)**

Sustained or repeated stress can lead to a range of chronic disease states such as obesity, metabolic syndrome, hypertension, autoimmunity, allergy, insomnia, depression, pain syndromes, and fatigue syndromes (1). Stress can be defined as a complex physiological state that embodies a range of integrative physiological and behavioral processes that occur when there is a real or perceived threat to homeostasis (2). A common response to stress is the activation of the hypothalamo-pituitary-adrenal axis, which results in the sequential secretion of CRH and arginine vasopressin from the hypothalamus, ACTH from the anterior pituitary, and glucocorticoids such as cortisol from the adrenal cortex. Together with other pathways, activation of the hypothalamo-pituitary-adrenal axis returns the organism to a state of homeostasis, but pathologies can develop when the activation of these pathways is severe and/or prolonged. Cortisol is thought to be responsible for many of the longer term effects of stress.

There is an increasing amount of evidence to show that different physiological characteristics of an individual can influence cortisol responses to stress (3). For example, cortisol responses to stress can be influenced by the sex of an individual (4–6), the amount of visceral adipose tissue an individual is carrying (7), and whether an individual is lactating (8). There also appear to be individuals who are naturally more or less responsive to stress, possibly due to factors such as genetic predisposition, early life experience, and/or environmental effects (3). In this study, we focused on differences between the sexes.

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It is well established that gonadal steroids can influence the glucocorticoid response to stress. For instance, in female rats, estrogens are known to enhance and in male rats, androgens are known to suppress the glucocorticoid response to stress (9). As a consequence of the ability of the gonadal steroids to influence cortisol responses to stress, stage of the estrous cycle can influence cortisol responses to stress in female rats (10). Nevertheless, it is also clear that sex differences are present in the absence of circulating gonadal steroids because we have found sex differences in cortisol responses to stress in gonadectomized sheep (4, 5) and in 8-wk-old lambs (6). In this study, we considered sex steroid-independent differences between the sexes in gonadectomized males and females and have focused on the role that different types of stressors have in determining sex differences in the magnitude of the cortisol response to stress.

Different stressors are known to activate the stress pathways to varying extents. In rats, there is evidence that different stressors activate central neuroendocrine pathways in a stressor-specific manner (11) and that distinct footprints of neuronal activity have been identified for stressors that were categorized as physical or psychological (12). Our studies to date show that different secretory signatures may also be associated with different types of stressors. For example, the magnitude of the cortisol response to stress in sheep depends on the sex of the animal and the type of stressor encountered. We have shown that females had a greater cortisol response than males to isolation/restraint stress (4), an audiovisual stress that involved exposure to a barking dog (5), and tail docking at 8 wk of age (6). In contrast, males had a greater cortisol response than females to insulin-induced hypoglycemia (4). In other studies (13–15), we found no differences between the sexes in the cortisol response to isolation/restraint stress. These findings suggest that vulnerability to stress may differ between the sexes and that the sex with the greater vulnerability may be specific to the stressor encountered. In the current study, we extend our earlier findings into three new stressors, thus testing the hypothesis in experiment 1 that cortisol responses to exercise, endotoxin, and wetting stress differ between gonadectomized male and female sheep.

With regard to isolation/restraint stress, we have reported varying results. One study (4), which included gonadectomized and gonad intact sheep (luteal phase females), found that females had a greater cortisol response than males, and in three other studies (13–15) in gonadectomized sheep, we found no sex differences. The reasons for these differences between studies are not clear. Our second experiment (experiment 2) used gonadectomized sheep to retest the hypothesis that cortisol responses to isolation/restraint stress differ between gonadectomized males and females.

Materials and Methods

Animals

Adult Romney Marsh sheep were used in two experiments that were conducted at the Monash University Large Animal Research Facility, Werribee, Victoria, Australia (38° South latitude). Experiment 1 was conducted in October and November, which is the nonbreeding season for this breed of sheep (16), and experiment 2 was conducted between mid-February and early June, which is the breeding season for this breed of sheep. We would not expect season to impact stress responsiveness (17–19). All sheep were gonadectomized at least 1 month before experimentation.

In both experiments, 1 d before each experimental day, sheep were penned indoors and indwelling jugular catheters (Dwellcath; Tuta Laboratories, Lane Cove, Australia) were inserted. Our experience is that 1 d is sufficient for animals to adjust from being outdoors on pasture to being penned indoors as in previous studies (4, 7, 13, 15, 20). Animals were weighed before the commencement of the studies. In experiment 1, females were 71.4 ± 1.8 kg (mean ± SEM) and males were 94.1 ± 1.8 kg, and in experiment 2, females were 66.6 ± 3.8 kg and males were 84.8 ± 2.7 kg. During experimentation, sheep were penned indoors with at least one other flock-mate sheep in an adjacent pen. At other times, sheep were kept on pasture. While housed indoors, a maintenance ration of Lucerne chaff was provided and water was available *ad libitum*. To avoid any possible confounding effects of feeding on any of the parameters measures, food was provided to sheep at 1600 h on the day before each experimental day. On experimental days, no food was given to sheep before the conclusion of sample collection. On experimental days in experiment 1, sampling commenced at 0800 h and on experimental days in experiment 2, sampling commenced at 0920 h.

All animal procedures in both experiments were conducted with prior institutional ethical approval under the requirements of the Australian Prevention of Cruelty to Animals Act 1986 and the National Health and Medical Research Council/Commonwealth Scientific and Industrial Research Organization/Australian Research Council Code of practice for the care and use of animals for scientific purposes.

Experimental designs

Experiment 1: plasma concentrations of cortisol in response to exercise, endotoxin, and wetting stress in gonadectomized rams and ewes

Animals in this experiment (n = 6 males and n = 6 females) underwent 4 experimental days at weekly intervals (wk 1: control sampling; wk 2: exercise stress; wk 3: endotoxin stress; and wk 4: wetting stress). After the first week of sampling (control sampling), one of the original males was replaced with another castrated male. The replacement animal continued in the study for the remainder of the experiment.

Control sampling. On the first experimental day, blood samples (5 ml) were collected every 15 min for 8 h. In the fifth hour of sampling (the time equivalent to the first hour after the im-

position of the stressor on days on which stress was imposed), blood samples were collected at 0, 2, 5, 10, 15, 20, 25, 30, 45, and 60 min. No stress treatment was imposed on the control day.

Exercise stress. One week later, on the second experimental day, this blood-sampling schedule was repeated and exercise stress was imposed after 4 h of sampling (time 0). For exercise stress, sheep (unconditioned) were run three times around a 0.6-km circuit (total 1.8 km) at an average rate of 11 km/h (first circuit: 3:38 min, second circuit: 4:07 min, third circuit: 4:51 min). Blood samples were collected after each circuit; hence, on this day, the timing of the blood samples in the first hour after the commencement of the stress was 0, 8, 17, 28, 38, 48, and 60 min. Sheep did not reach exhaustion. This treatment was based on previous studies in which sheep were exercised on treadmills at various rates, for various durations, with or without inclination of the treadmill, with or without prior conditioning of the sheep, and with or without the sheep reaching exhaustion (21–25).

Endotoxin stress. After another week, on the third experimental day, blood samples were collected every 15 min for 4 h before to 5 h after time 0 (time 0 was the time at which endotoxin was injected) and every 30 min from 5 to 8 h after time 0. In the first hour after injection of endotoxin, blood samples were collected at 0, 5, 10, 20, 30, 45, and 60 min. For endotoxin stress, sheep were injected with endotoxin (400 ng lipopolysaccharide per kilogram body weight, iv; *Escherichia coli* 0127; B8, Sigma, St. Louis, MO). Rectal temperatures were recorded hourly from 3 h before to 8 h after time 0. This treatment induced transient fever symptoms such as high temperature in all animals (*vide infra*), and in some animals it induced hunched stance, intermittent coughing, and trembling. This dose of endotoxin was based on studies that have previously used this dose of endotoxin as a stressor in sheep (26, 27).

Wetting stress. On the fourth experimental day, after another week, the blood sampling schedule used in the first week (control day) was repeated (*i.e.* blood samples were collected every 15 min for 4 h before to 4 h after time 0, plus, in the first hour after time 0, blood samples were collected at 0, 2, 5, 10, 15, 20, 25, 30, 45, and 60 min). Wetting stress was imposed at time 0. For wetting stress, sheep were penned indoors in the standard manner (*i.e.* adjacent to other sheep in the experiment) and were sprayed with water from a hand held hose for 30 min (equivalent to rainfall of 1 ml/min). This treatment was based on studies in sheep in which 6 h of artificial wetting using a sprinkler system was used to induce environmental stress (28, 29).

On the final experimental day, after another week, no blood samples were collected and no treatments were imposed. Rectal temperatures were recorded hourly for 11 h for comparison with those collected on the day of endotoxin stress.

Blood samples were centrifuged at 4 C and plasma was harvested and stored at −20 C for subsequent measurement of plasma concentrations of cortisol by RIA.

Experiment 2: plasma concentrations of cortisol in response to isolation/restraint stress in gonadectomized rams and ewes

Animals were allocated to four treatment groups: control males (n = 5), control females (n = 5), stress males (n = 5), and stress females (n = 5). On an experimental day, jugular blood samples were collected every 10 min for 8 h. In control animals, no treatments were imposed during the 8 h. In animals in which stress was imposed, isolation/restraint stress was imposed for 4 h commencing after 4 h of sampling.

Isolation/restraint stress. Isolation/restraint stress is a well-characterized stressor that we have used often in our laboratory (4, 7, 8, 13–15, 20). Isolation/restraint stress involves moving the animal from a pen adjacent to a familiar sheep to an unfamiliar pen that has no sheep housed in adjacent pens, securing the animal to the side of the pen with a harness, and enclosing the pen using opaque materials (13). In this experiment, there was one modification made to the procedure. Sheep were not moved to an unfamiliar pen but remained in their home pen. Any sheep in adjacent pens were removed. As usual, the sheep was subsequently harnessed to the side of the pen and the pen enclosed in opaque materials.

Blood samples were centrifuged at 4 C and plasma was harvested and stored at −20 C for subsequent measurement of plasma concentrations of cortisol by RIA.

Cortisol RIA

Total plasma concentrations of cortisol were measured by a previously described RIA (30) using cortisol (H-4001; Sigma) as standard. For experiment 1, the mean (±SEM) sensitivity of the assay was 0.54 ± 0.08 ng/ml (n = 13). The intraassay coefficient of variation was 16% at 8.4 ± 0.6 ng/ml and 15% at 109.1 ± 6.7 ng/ml, and the interassay coefficient of variation was 11% at 11.1 ± 0.4 ng/ml and 16% at 131.2 ± 6.0 ng/ml. For experiment 2, the mean (±SEM) sensitivity of the assay was 0.43 ± 0.01 ng/ml (n = 14). The intraassay coefficient of variation was 7.0% at 31.0 ± 0.9 ng/ml and the interassay coefficient of variation was 16.7% at 93.1 ± 4.3 ng/ml.

Statistical analyses

Rectal temperatures (Celsius) and plasma concentrations of cortisol (nanograms per milliliter) were analyzed using repeated-measures ANOVA. The within-subjects factor was sampling time and the between subjects factor was sex (male or female). Homogeneity of variance was checked using Levene's test for equality of error variances. Kolmogorov-Smirnov tests were used to test for normality.

Results

Experiment 1: plasma concentrations of cortisol in response to exercise, endotoxin, and wetting stress in gonadectomized rams and ewes

Rectal temperatures

On the control day of measuring rectal temperatures (Fig. 1A), there was a significant effect of time ($P < 0.001$) but no effect of sex and no time × sex interaction. It is not obvious why there were changes in rectal temperatures with time. These changes were not related to feeding because no food was provided during the sampling period.

On the day that endotoxin was injected (Fig. 1B), there was a significant effect of time ($P < 0.001$) and a time ×

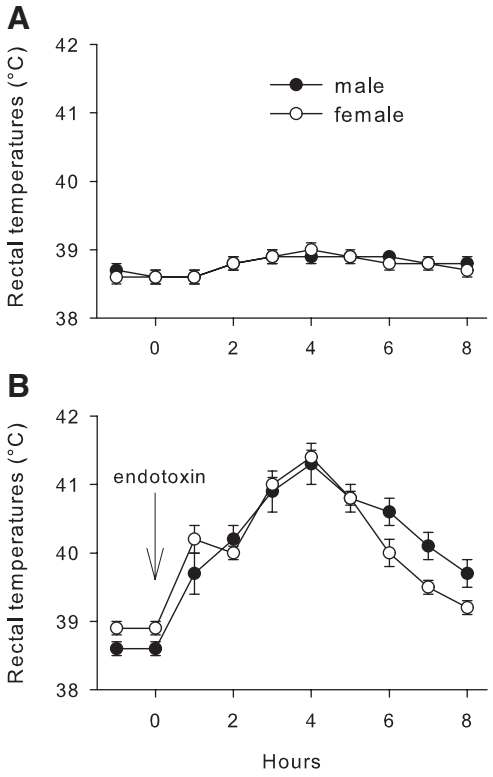

FIG. 1. Mean (±SEM) rectal temperatures (Celsius) in gonadectomized male (n = 6) and female (n = 6) sheep for 9 h on a control day when no endotoxin was administered (A) and from 1 h before to 8 h after injection of endotoxin (indicated by the *arrow*; B) in experiment 1. There was a time × sex interaction ($P < 0.001$) on the day of endotoxin injection but not on the control day.

sex interaction ($P < 0.001$) but no main effect of sex. Mean (±SEM) pretreatment rectal temperatures were higher ($P = 0.056$) in females (38.9 ± 0.1 C) than males (38.6 ± 0.1 C). Conversely, rectal temperatures were higher ($P = 0.052$) in males (40.1 ± 0.2 C) than females (39.6 ± 0.1 C) 6–8 h after the endotoxin injection. Otherwise, there were no significant differences between the sexes after injection of endotoxin.

Cortisol

On the control day of sampling (Fig. 2A), there was a significant effect of time ($P = 0.004$) that was no longer present when the first hour of sampling was removed ($P = 0.093$). There was no time × sex interaction and no effect of sex.

On the day of exercise stress (Fig. 2B), there was a significant effect of time ($P < 0.001$) but no time × sex interaction and no effect of sex. In both sexes, plasma concentrations of cortisol were significantly ($P < 0.001$) higher during the first 90 min after the commencement of exercise stress (38.3 ± 3.8 ng/ml) compared with pretreatment concentrations (6.5 ± 0.7 ng/ml).

On the day of endotoxin stress (Fig. 2C), there was a significant effect of time ($P < 0.001$) but no time × sex

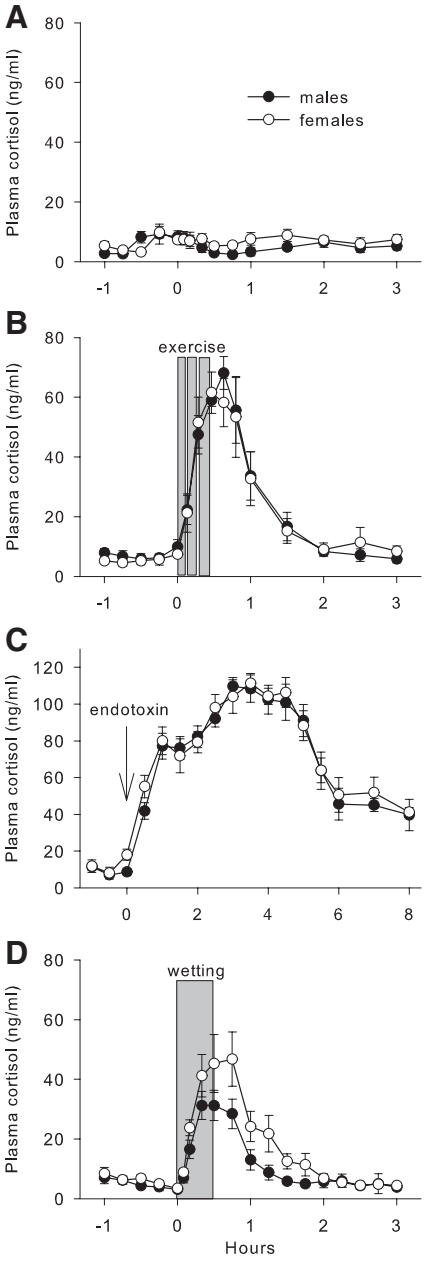

FIG. 2. Mean (±SEM) plasma concentrations of cortisol in gonadectomized male (n = 6) and female (n = 6) sheep for 4 h on a control day of sampling on which no stress was imposed (A); for 1 h before to 3 h after the commencement of exercise stress, which consisted of running 3 × 0.6 km (*shaded regions*; B); for 1 h before to 8 h after injection of endotoxin (indicated by the *arrow*; C); and for 1 h before to 3 h after the commencement of 30 min of wetting stress (*shaded region*; D) in experiment 1. There was a time × sex interaction ($P = 0.027$) on the day of wetting stress but not on any of the other days.

interaction and no effect of sex. Plasma concentrations of cortisol during the 8 h of sampling after endotoxin injection (73.5 ± 3.3 ng/ml) were significantly ($P < 0.001$) higher than those during the pretreatment period (10.6 ± 1.4 ng/ml).

On the day of wetting stress (Fig. 2D), there was a significant effect of time ($P < 0.001$) and a significant

time × sex interaction ($P = 0.027$) but no overall effect of sex. The significant interaction was due to plasma concentrations of cortisol being significantly ($P = 0.027$) higher from the commencement of stress until 105 min in females (23.9 ± 4.0 ng/ml) than males (15.0 ± 2.0 ng/ml). In the period before stress and during the last hour of stress, plasma concentrations of cortisol did not change over time and were not different between sexes.

Experiment 2: plasma concentrations of cortisol in response to isolation/restraint stress in gonadectomized rams and ewes

Cortisol

In control animals, there were no significant variations in mean (±SEM) plasma concentrations of cortisol over time and no significant differences between sexes (Fig. 3A).

In animals subjected to isolation/restraint stress (Fig. 3B), there was a significant effect of time ($P < 0.001$) and a significant time × sex interaction ($P = 0.012$) but no overall effect of sex. During the pretreatment period, there was a significant effect of time ($P = 0.022$) that could not be accounted for by any obvious external factors such as feeding. Also during the pretreatment period, mean (±SEM) plasma concentrations of cortisol were significantly ($P = 0.001$) higher in females (9.7 ± 0.4 ng/ml) than males (6.4 ± 0.6 ng/ml). During isolation/restraint stress, there was a significant effect of time ($P < 0.001$) and a significant time × sex interaction ($P = 0.016$). Mean (±SEM) plasma concentrations of cortisol were significantly ($P = 0.04$) higher in females (33.2 ± 4.4 ng/ml) than males (19.5 ± 3.4 ng/ml) during the first 90 min of isolation/restraint and were significantly higher during the first 90 min of stress compared with pretreatment in both males ($P = 0.012$) and females ($P = 0.005$).

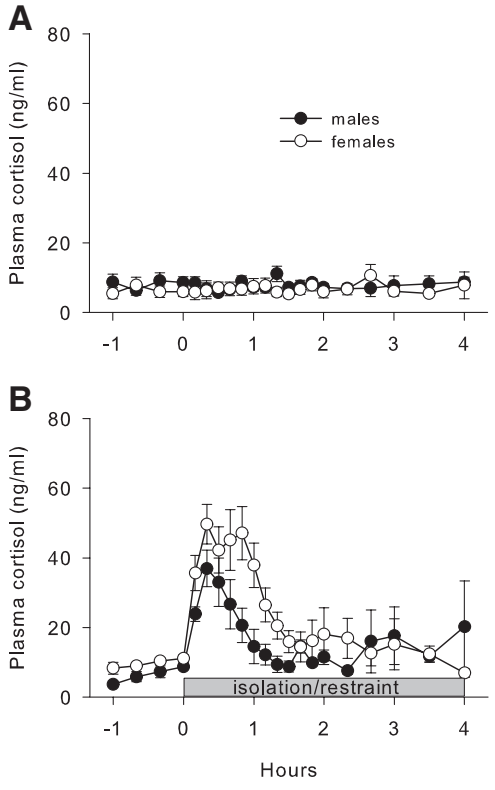

FIG. 3. Mean (±SEM) plasma concentrations of cortisol in gonadectomized male (n = 5) and female (n = 5) sheep for 5 h on a control day of sampling on which no stress was imposed (A) and for 1 h before to 4 h after the commencement of 4 h of isolation/restraint stress (indicated by the *shaded bar*; B) in experiment 2. There was a time × sex interaction ($P = 0.012$) in animals subjected to isolation/restraint stress but not in those subjected to control conditions.

Discussion

In experiment 1 of this study, we have shown that there are differences between the sexes in the cortisol response to wetting stress but not to exercise or endotoxin stress. We can therefore accept our hypothesis pertaining to sex differences in cortisol responses for wetting stress but not for exercise stress or endotoxin stress. These data extend our previous findings around different stressors eliciting different cortisol responses in males and females and add further weight to the notion that an individual's vulnerability to the detrimental effects of stress may be stressor specific. Moreover, these data further strengthen the argument that studies in stress should either use different types of stressors within a study or alternatively, that studies in stress using only one stressor should not make generalizations about stress in general but be limited to conclusions about that stressor in particular.

In rodent studies, a distinction has been made between reactive glucocorticoid responses to stress and anticipatory glucocorticoid responses to stress (31). Reactive glucocorticoid responses to stress are those induced by a genuine challenge to physiological homeostasis that is recognized by sensory pathways. Such challenges may include a change in cardiovascular tone, respiratory distress, pain, or circulating cytokines. In such cases, there is a direct neuronal pathway to CRH neurons in the paraventricular nucleus via the brainstem to activate the hypothalamo-pituitary-adrenal axis. In contrast, anticipatory glucocorticoid responses to stress are not mounted in response to an actual disruption to physiological homeostasis but to the anticipation of such a disruption. These responses require some higher cortical processing involving limbic pathways (31).

We have used three stressors to date that could be considered as imposing no actual disruption to physiological homeostasis but that required higher processing to anticipate a disruption to homeostasis: wetting stress (current study), isolation/restraint stress (current study and Refs. 4, 13–15), and audio/visual stress that involved a barking

dog (5). In each case, females had a significantly greater cortisol response compared with males, although in some of the studies using isolation/restraint stress (13–15), there were no differences between the sexes. Although it is tempting to make generalizations about sex differences in reactive and anticipatory responses to stress, it is not that straightforward because females also had a significantly higher cortisol response to tail docking (6), a stimulus that involves pain that was categorized by Herman *et al.* (31) as reactive rather than anticipatory. Nevertheless, there may be some anticipatory components to tail docking. Also, wetting stress may not be purely anticipatory but may have some reactive components if body temperature is affected. Either way, trying to generalize about stressors is likely impossible because each stressor is likely to activate unique pathways (11).

Exercise and endotoxin used in this experiment are both likely to have induced reactive cortisol response through the elevation of cardiovascular tone and through the elevation of circulating cytokines, respectively. In both cases, there were no significant differences between the sexes. Although our experience is that the design of experiment 1 left adequate time between imposition of the stressors to avoid any carryover habituatory effects (13), we acknowledge that this has been explored only for isolation/restraint and not for exercise, endotoxin, and wetting. It is possible that sex differences in responsiveness to stress may be due, in part at least, to sex differences in habituation to particular stressors. This hypothesis has hitherto not been tested. In sheep, we have found *in vitro* differences between the sexes at the adrenal, pituitary (32), and hypothalamic (32, 33) levels of the hypothalamo-pituitary-adrenal axis so the etiology of the difference we find in cortisol responses to different stressors may arise at any level or at multiple levels.

In our second experiment, we found that cortisol responses to isolation/restraint stress were significantly higher in females compared with males. This was consistent with our hypothesis that there would be sex differences in cortisol responses after this stressor. This finding is consistent with one of our earlier studies (4) but contrasts with three of our other earlier studies in which we found no such sex difference (13–15). The reason for these differences in different studies is still not clear. After our first study using this stressor found no sex differences in cortisol response in gonadectomized males and females treated with and without sex steroids (13), we concluded that in contrast to rodents, there are no sex differences in sheep in the stress-induced secretion of cortisol. After our second study found that females had a greater cortisol response than males to isolation/restraint stress in gonadectomized and gonad intact sheep (luteal phase females), we concluded that gonadal factors other than sex steroids may be involved (4). Our subsequent two studies (14, 15), in which no sex differences were found in cortisol response to isolation/restraint stress, used gonadectomized sheep so these shed no light on whether gonadal factors other than sex steroids are responsible for sex differences. One of these studies (14) also suggested that time since gonadectomy might be important because time since gonadectomy was more than 1 yr during which sex differences were found (4) but shorter in studies in which no sex differences were found (13, 14). In another report (15), the lack of sex difference in cortisol response to isolation/restraint stress was concordant with a lack of sex differences in CRH and arginine vasopressin activation in the paraventricular nucleus, and the authors suggested that sex differences found *in vitro* (32) may not be present *in vivo*.

The current study challenges the hypothesis that gonadal factors other than sex steroids are necessary because there were no gonad intact animals in the current study. Time out of gonadectomy may be important. These studies were conducted at least 1 month after gonadectomy but not as long as a year after gonadectomy as was the case in one study (4). As to whether there are sex differences in cortisol responses to isolation/restraint stress in sheep, it seems that there may be other yet-to-be-determined factors at work. Perhaps genetic predisposition, early life experience and/or environmental effects (3) play important roles. For example, conditions in fetal life may be important for determining physiological responses to stress in adulthood (34). We have previously shown that a sex difference in the cortisol response to tail docking and ACTH injection developed between 1 and 8 wk of age in sheep (6).

All four stressors used in this study represent robust stressors in sheep that could readily be used in future stress research in this species. The exercise and wetting stressors used in this study have not been used before but were derived from previous studies in sheep. In previous exercise studies, sheep were exercised on treadmills using a wide variety of conditions for various reasons (21–25), and in previous studies of wetting in sheep, sheep were exposed to 6 h of artificial wetting using a sprinkler system (28, 29). In the form used in this study, these new stressors were efficient (because all 12 sheep were stressed simultaneously) and effective (because both induced a substantial activation of the hypothalamo-pituitary-adrenal axis). The magnitude and duration of the cortisol response to endotoxin were similar to those observed previously using this stressor in sheep (26, 27) as were those for isolation/restraint stress (4, 13–15). Together with other stressors that we have used earlier [audiovisual stress involving exposure to a barking dog (5), insulin-induced hypoglycemia (4), and tail docking (6)], these stressors represent a variety

of physical and/or psychological stressors likely to produce reactive and/or anticipatory responses of the hypothalamo-pituitary-adrenal axis in sheep.

Stress-related pathologies (1) are most likely to develop when stress is prolonged or frequently repeated rather than when imposed for a short period. Nonetheless, we have shown that imposition of stress for periods of as little as 4 h in sheep can have an impact on sexual behavior that can lead to a decrease in the likelihood of a female mating, thereby impacting reproductive success (35). This study extends our understanding of sex differences by including extra stressors that require different processing. Understanding these differences is important to allow a comprehensive appreciation of the mechanisms by which stress will impact physiological systems.

In conclusion, sex is an important factor in determining the stress-induced activity of the hypothalamo-pituitary-adrenal axis in sheep, and it is clear that there are sex differences that are independent of the presence of the sex steroids. Different stressors also play an important role. Nevertheless, caution is needed when trying to generalize about directions of sex differences in sheep and about the effects of different types of stressors. Each stressor needs to be considered individually, and it would seem that there are advantages to using multiple types of stressors in stress research. Nonetheless, it appears that females may have a greater response when higher processing is involved, but further research is necessary to confirm this. It seems that other factors such as genetic predisposition, early life experience, and/or environmental effects may be important in determining stress-induced hypothalamo-pituitary-adrenal axis activity in sheep.

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