

Serum Corticosterone in Fetal Mice: Sex Differences, Circadian Changes, and Effect of Maternal Stress

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Received 9 August 1990

MONTANO, M. M., M.-H. WANG, M. D. EVEN AND F. S. VOM SAAL. *Serum corticosterone in fetal mice: Sex differences, circadian changes, and effect of maternal stress.* *PHYSIOL BEHAV* 50(2) 323-329, 1991.—The serum concentration of corticosterone was examined in control and stressed pregnant female mice (*Mus domesticus*) as well as male and female fetuses due to our interest in the behavioral effects of maternal stress on offspring in mice. Pregnant females were restrained under flood lights (2 sessions/day, 45 min/session) from Day 13–17 of pregnancy. On Day 17 of pregnancy a significant increase in maternal serum corticosterone was exhibited 1 h after the onset of a stress session, and serum corticosterone did not return to baseline until 16 h later. We also observed a significant increase in serum corticosterone in male fetuses during the first 4 h after maternal stress, while no significant change in serum concentration of corticosterone was observed in female fetuses throughout 24 h after maternal stress. Daily variation in serum concentration of corticosterone was also determined at 4-h intervals in pregnant mice and their fetuses from Day 16–18 of pregnancy. Pregnant females maintained on a 12 L:12 D cycle exhibited peak serum corticosterone concentrations at and just before the onset of the darkness. Daily fluctuations in serum concentrations of corticosterone in male and female fetuses reflected the pattern observed in the mothers. A sex difference in serum corticosterone in fetuses was observed at some, but not all times of the day, with the difference being greatest during the dark phase of the mother's light:dark cycle.

Maternal stress Sex differences Circadian rhythm Corticosterone Fetal development

THE present studies were undertaken due to our interest in the mechanisms underlying the effects of maternal stress on serum adrenal and gonadal steroid concentrations in fetuses and the subsequent reproductive behavior and physiology of prenatally stressed mice (18). The fetal pituitary-adrenal system starts functioning between Days 17 and 18 of pregnancy in rats (6,12), and an important modulating role of corticosterone of maternal origin is well established (20). While studies have been conducted to examine serum concentrations of corticosterone in nonstressed and stressed pregnant female mice, there is no information concerning the effects of maternal stress on serum concentrations of corticosterone in fetuses.

During the second half of pregnancy in mice, maternal serum concentrations of corticosterone increase dramatically. By Day 16 of pregnancy, serum corticosterone concentrations are approximately 60 times higher than in nonpregnant mice (2). There is circadian variation in baseline serum concentrations of corticosterone in virgin female mice (13,14) and the secretion of corticosterone in response to stress, such as being placed under bright lights, can also vary as a function of the time of day during which animals are stressed (5, 7, 10, 17). We thus also examined whether pregnant female mice and their male and female fetuses showed a daily rhythm in serum corticosterone similar to that observed in virgin female mice.

We report here that serum concentrations of corticosterone differed in control male and female fetuses, but only at some times of day. Male and female fetuses also showed a different pattern of change in serum corticosterone in response to maternal stress.

METHOD

Animals and Light Cycle

CF-1 mice were housed in 18×29×13 cm polypropylene cages on Aspen bedding. Mouse breeder chow (Purina) and water were available ad lib. Animal rooms were maintained at 22±1°C on a 12:12 light:dark cycle, with lights on at 1200 h. All times of day will be presented relative to the beginning of the light phase, which will be referred to as Time 0 for each day of pregnancy. All work in the animal rooms during the dark phase of the cycle was conducted under a 25 W red light to which mice do not respond.

Mating

Adult CF-1 female mice were time-mated by being placed with stud males for 4 h beginning at 0900 h. Mating was con-

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firmed by the presence of a copulatory plug (Day 0 of pregnancy). Mated animals were considered to have been fertilized at the onset of the light phase (1200 h), which was labelled as Time 0 of Day 0 of pregnancy. Mated females were housed three per cage until assignment to treatment groups.

Maternal Light and Heat Stress

Randomly selected pregnant females were stressed using procedures similar to those previously described by Ward and Weisz (19). Pregnant females were placed in Plexiglas mouse restraining chambers (9×6.3×5 cm) under a bank of 150 W flood lights (350 fc; 38°C=ambient temperature inside chamber). Females were stressed two times per day at 0400 h and 2000 h beginning at 2000 h on Day 12 of pregnancy. Each stress session lasted 45 min. Control pregnant females were left undisturbed in their cages.

Blood Collection

Pregnant females were housed individually on the day before blood collection. Pregnant females were decapitated and blood was collected in test tubes within 15 s of the cage being touched. All fetuses were removed from the uterine horns, and fetal blood was collected in heparinized micropipettes by aspiration after fetal decapitation within 2 min of maternal decapitation. Fetal sex was determined by examining the length of the anogenital space, which unambiguously distinguishes males from females at the times examined in these studies. Blood from fetuses of each sex within a litter was pooled to control for litter effects. After centrifugation, the sera were frozen at -30°C until assayed.

Extraction of Steroids From Serum

Corticosterone was extracted twice from 5–10 µl serum with 2 ml of an ethylacetate:chloroform (80:20) mixture, dried under nitrogen, and reconstituted in 140 µl buffer. It was necessary to further dilute maternal sera (1:40) in buffer to obtain corticosterone concentrations that fell within the working range of the assay. Recovery was monitored by addition of 2800 dpm (5 pg) of [1,2,6,7-³H]-corticosterone (85.8 Ci/mmol; New England Nuclear, Boston, MA) to quality control samples before extraction. A 20 µl aliquot for recovery and duplicate 50 µl aliquots for steroid radioimmunoassay (RIA) were withdrawn after reconstitution. Recovery was routinely greater than 90%.

Corticosterone RIA

[1,2,6,7-³H]-Corticosterone (85.8 Ci/mmol) was obtained from the New England Nuclear, and unlabelled corticosterone was obtained from Steraloids, Inc. (Wilton, NH). Corticosterone antiserum (rabbit) was obtained from Dr. Gordon Niswender (Ft. Collins, CO). Information regarding cross-reactivity was provided with the antisera. With the exception of 11-deoxy corticosterone, cortisol, and progesterone, cross-reactivity with all other steroids was reported to be less than 0.1%. We determined the percentage of cross-reaction of the corticosterone antiserum to be 0.39% with progesterone and 0.44% with cortisol.

Duplicate aliquots of 50 µl extracted steroid in buffer were incubated for 4 h at 24°C with 18,000 dpm (33 pg) of ³H-corticosterone and antiserum to corticosterone (diluted 1:600). Free and bound steroids were separated by addition of second antibody (diluted 1:11; goat, anti-rabbit; Radioassay Systems Laboratories, Inc., Carson, CA). Tubes were incubated overnight at 4°C and then centrifuged at 1500×g for 1 h. The supernatant

was decanted, and the precipitate (representing the bound portion) was counted in a liquid scintillation counter (Beckman LS 5801, efficiency for tritium: 56%) after addition of 5 ml of Scintiverse II (Fisher).

Charcoal-treated mouse serum was extracted and assayed as described above, and the corticosterone values were indistinguishable from the baseline of our assay. After addition of corticosterone (286 pg) to different volumes (9, 18 and 36 µl) of charcoal-treated serum, we were able to measure more than 90% of the added steroid (mean±S.D. corticosterone concentrations, uncorrected for extraction losses, 277±20, 258±20, 267±35 pg/tube, respectively). This demonstrates that there were no effects of serum volume on the concentration of corticosterone measured. The range of the standard curve for corticosterone was 60–2000 pg/tube. The blank of the assay was indistinguishable from baseline. The inter- and intra-assay coefficients of variability were 11% and 2%, respectively.

Statistical Analysis

Serum concentrations of corticosterone were compared by analysis of variance using the Statistical Analysis System (SAS), General Linear Model. Planned comparisons were made using the LS Means test in SAS.

RESULTS

Serum Concentrations of Corticosterone in Pregnant Mice

The objective of this initial study was to characterize serum concentrations of corticosterone throughout pregnancy in CF-1 mice, prior to examining the effects of stress on serum corticosterone during the last third of pregnancy. While serum corticosterone in pregnant females has been examined in other stocks of mice (2,3), the considerable strain differences which have been reported in circulating gonadal steroid concentrations during pregnancy in mice (1) led to the decision to obtain our own normative data in CF-1 females.

Blood was collected from pregnant female mice immediately after the onset of the light phase of the light:dark cycle (0 h–0300 h) from Day 6 (one day after implantation) through Day 19. In addition, blood was collected after females had delivered one pup (mean time=0300 h, S.D.=±8 h) on Day 19 of pregnancy and 24 h later beginning at 0230 h on Day 20 after mating. At least 5 females were examined at each time point.

Figure 1 shows that on Day 6 through Day 10, pregnant females had serum concentrations of corticosterone (mean±SEM: 2.8±0.9, 2.9±1.3, 3.8±0.8, 3.2±1.7, 1.7±0.2 µg/100 ml, Days 6–10, respectively) that were similar to those observed in nonpregnant females at the onset of the light phase (Fig. 2). Between Day 11 (4.9±1.4 µg/100 ml) and Day 14 (152.8 µg/100 ml) serum corticosterone concentrations increased to 53 times higher than the mean (2.9 µg/100 ml) for sera collected on Days 6–10. A drop in serum corticosterone was observed on Day 15 followed by an increase on Day 16. Corticosterone concentrations observed at the beginning of the light phase on Day 19 were lower than those observed on Day 18. However, higher concentrations were observed after delivery of one fetus which, on average, occurred 3 h later. At 24 h after the mean time of the start of delivery, serum corticosterone dropped to 6 times nonpregnant values.

Daily Variation in Serum Corticosterone in Pregnant Females and Fetuses

A recent study has shown that the circadian rhythm in corticosterone in rats may control the duration and possibly the mag-

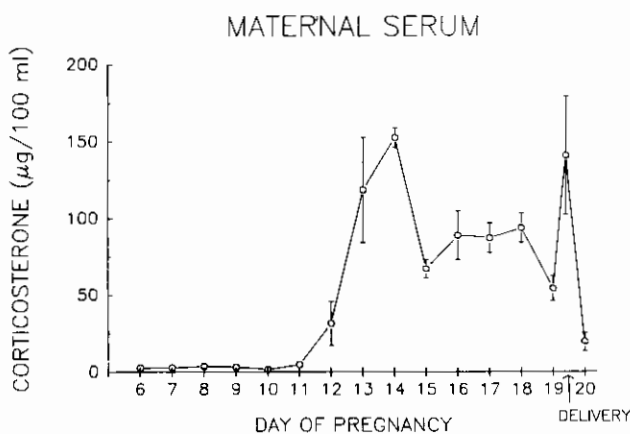


FIG. 1. Serum corticosterone concentrations (mean ± SEM) in pregnant females just after lights on (at 0000 h) from Day 6 through Day 19. Blood was also collected after the delivery of one pup on Day 19 (mean time = 0300 h) and 24 h after the onset of delivery. Group sizes for Day 17 and Day 18 were 12/group (all other days, 5-7/group).

nitude of the ACTH response to stress (9). While there is considerable literature reporting a light:dark rhythm in circulating corticosterone in virgin mice, the existence of changes in serum corticosterone concentrations throughout the light:dark cycle in pregnant female mice and their fetuses has not been examined. Obtaining this information in pregnant mice and fetuses was considered to be important, since baseline changes in serum corticosterone during the light:dark cycle affects the relative magnitude of the corticosterone response to stress in virgin mice (5, 7, 10, 17). For purposes of comparison, we also measured serum corticosterone in virgin female mice at 4-h intervals throughout the light:dark cycle.

A daily rhythm in serum corticosterone was observed in virgin CF-1 female mice (Fig. 2), which is in agreement with prior findings with other stocks of mice (13,14). A 7-fold increase in serum corticosterone concentrations was observed at 12 h (at the beginning of the dark phase) when compared with the end of the dark phase (20 and 24 h) and the first 4 h of the light phase. Values at 8, 12, and 16 h were significantly ($p < 0.01$) elevated

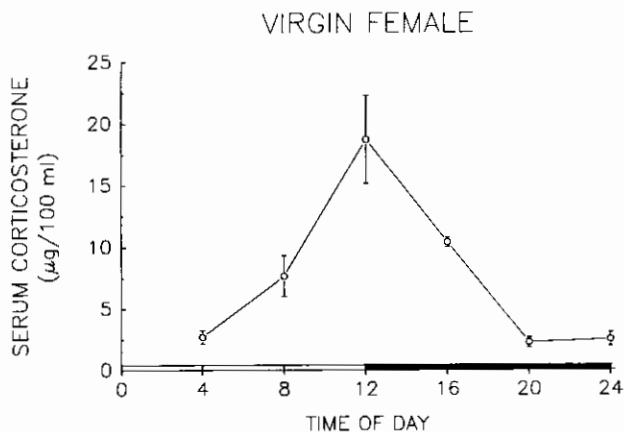


FIG. 2. Serum corticosterone concentrations (mean ± SEM) in virgin females during the light:dark cycle (6 females/time point).

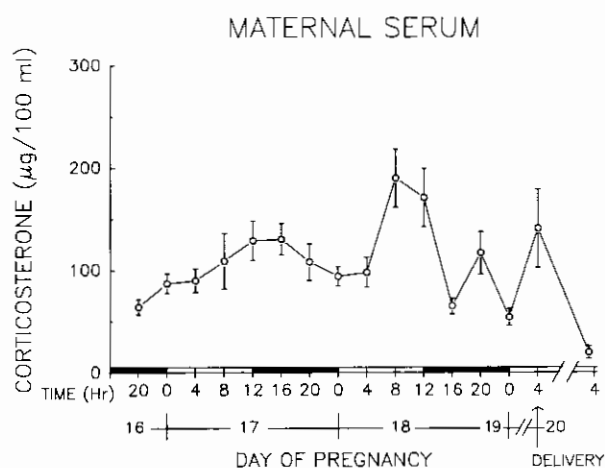


FIG. 3. Serum corticosterone concentrations (mean ± SEM) during the light:dark cycle in pregnant females. Blood was collected at 4-h intervals from Day 16 (2000 h) through the onset of delivery. Blood was also collected 24 h after the onset of delivery. Beginning on Day 16, 2000 h, group sizes for successive time points were: 9, 13, 17, 8, 10, 5, 10, 12, 6, 7, 12, 8, 10, 7, 5, 6.

relative to other time points.

To determine whether there were changes in serum concentrations of corticosterone throughout the light:dark cycle in pregnant female mice and their fetuses, blood was collected at 4-h intervals throughout the light:dark cycle from Day 16 (2000 h) of pregnancy through the onset of delivery (139 litters were used).

Analysis of variance on serum corticosterone concentrations in pregnant females showed a significant effect of time of blood collection, $F(5,133) = 5.3, p < 0.001$. The highest values were recorded just at and 4 h prior to the onset of the dark phase (Fig. 3). Specifically, values at the end of the light phase at both 8 h and 12 h (mean for these 2 time points = 150 µg/100 ml) were, on average, 60% ($p < 0.01$) higher than values from 16 h (during the middle of the dark phase) through 4 h (after the beginning of the light phase; mean for these 4 time points = 93 µg/100 ml). The values at 8 and 12 h did not differ significantly, which was also true for the values at 16, 20, 0 and 4 h.

Analysis of variance on serum corticosterone concentrations in fetuses (Fig. 4) also showed a significant effect of time of blood collection, $F(5,241) = 7.1, p < 0.001$, and exactly the same daily pattern observed in mothers was observed in fetuses. Specifically, serum corticosterone concentrations at both 8 h and 12 h (mean for these two time points = 28.1 µg/100 ml) were, on average, 30% higher ($p < 0.01$) than values from 16 h (during the middle of the dark phase) through 4 h (after the beginning of the light phase; mean for these 4 time points = 21.8 µg/100 ml). As was true for mothers, the values at 8 and 12 h did not differ significantly, which was also true for the values at 16, 20, 0 and 4 h. The 4-hr interval between blood collection in this study was too great to justify testing for the relationship between serum corticosterone concentrations in pregnant females and fetuses, and we cannot conclude from this study that changes in serum concentrations of corticosterone in fetuses reflect changes in pregnant females throughout the light:dark cycle.

There was also a significant effect of fetal sex on serum concentrations of corticosterone, $F(1,241) = 6.5, p < 0.01$. Post hoc

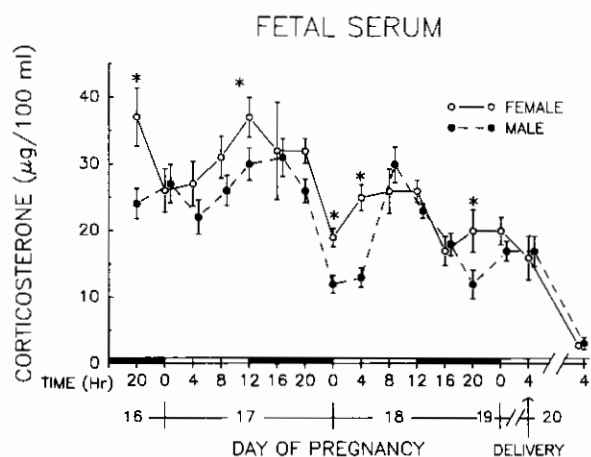


FIG. 4. Serum corticosterone concentrations (mean \pm SEM) in male and female fetuses during the light:dark cycle. Blood was collected at 4-h intervals from Day 16 (2000 h) through the onset of delivery. Blood was also collected from pups 24 h after the onset of delivery. The following number of samples (representing pooled sera from fetuses of the same sex in each litter) were assayed for successive time points: Male fetuses = 7, 8, 8, 5, 11, 6, 11, 9, 8, 8, 12, 11, 12, 7, 6; Female fetuses = 5, 8, 8, 5, 8, 5, 7, 10, 9, 7, 12, 11, 10, 7, 6. *Significant difference between male and female fetuses ($p < 0.05$).

analysis showed that significant sex differences in fetal serum corticosterone occurred only at some times of the day, in particular, toward the end of the dark phase and the beginning of the light phase (Fig. 4). The sex difference appeared to be associated with a greater decrease in serum corticosterone in male fetuses than in female fetuses at these times. The sex by hour interaction was not significant.

Effect of Maternal Stress During the Last Third of Pregnancy on Maternal and Fetal Serum Corticosterone Concentrations

In this study we examined whether there was a sex difference in the serum concentrations of corticosterone in fetuses throughout a 24-h period after a maternal stress session on Day 16. Blood was collected throughout Day 17 rather than throughout Day 18 so that the results would not be confounded by stress associated with parturition, which occurs early on Day 19. Prior to 2000 h on Day 16, very little serum can be obtained from mouse fetuses.

Pregnant females were stressed as described above beginning at 2000 h on Day 12. The concentrations of corticosterone were determined in maternal and fetal serum collected 1, 4, 8, 12, 16 and 24 h after the beginning of the last stress session. The last stress session for all pregnant females began at 1900 h and ended at 1945 h near the end of Day 16. Blood was collected beginning at 2000 h on Day 16 through 2000 h on Day 17.

The control and stressed animals examined in this study were randomly assigned to treatment conditions. Control animals were killed at 4-h intervals between 2000 h on Day 16 (at the same time as the animals killed 1 h after the onset of stress) and 2000 h on Day 17 (at the same time as the animals killed 24 h after the onset of stress).

Other randomly selected control pregnant females from the same mating were also killed throughout Day 18 of pregnancy and after the onset of parturition. Figures 3 and 4 show the changes in serum corticosterone at 4-h intervals throughout the

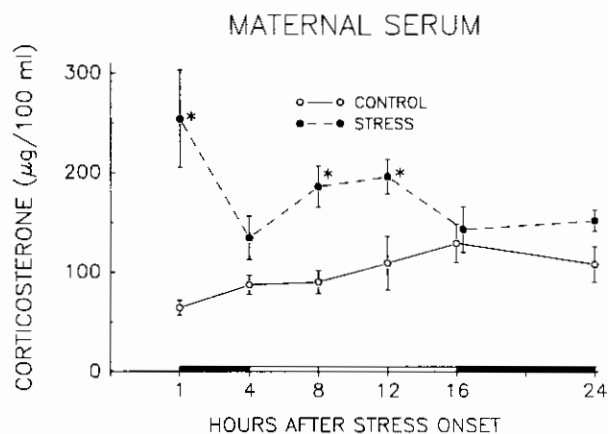


FIG. 5. Serum corticosterone concentrations (mean \pm SEM) in control and stressed mothers on Day 17 of pregnancy. Stress = stressed 2 times per day starting on Day 12. Blood was collected at different times after the onset of the last stress session, which began at 1900 h on Day 16. Control = left undisturbed in their cages until blood collection at corresponding time points. Sample sizes for successive time points: Control = 9, 13, 17, 8, 10, 10; Stress = 7, 13, 11, 8, 8, 7. *Significant difference between control and stressed mothers ($p < 0.01$).

last 2 days of pregnancy in all control pregnant females and fetuses from this experiment. Since the control animals killed on Day 16 through Day 17 in this experiment were also examined at the same time as stressed animals, these same control data are again presented along with data from stressed animals at corresponding times in Fig. 5 and 6. The design of this experiment was intended to reduce the number of animals that had to be killed.

Analysis of variance performed on serum corticosterone concentrations in control vs. stressed pregnant females revealed that the interaction between time and treatment was significant, $F(4,106) = 4.5$, $p < 0.01$. Post hoc analysis revealed that stress resulted in a 4-fold increase in maternal serum corticosterone concentrations 1 h after the onset of stress relative to control values ($p < 0.001$; Fig. 5). Maternal serum concentrations of corticosterone decreased to approximately 1.5 times control values 4 h after the onset of stress ($p = 0.07$). Higher concentrations ($p < 0.01$) of corticosterone relative to control values were observed at 8 h and 12 h, but not at 16 h or 24 h after the onset of the last stress session.

Analysis of variance on serum corticosterone in male and female fetuses showed a significant effect of sex, $F(1,164) = 4.9$, $p < 0.05$, as well as an hour \times treatment interaction, $F(5,164) = 4.3$, $p < 0.001$. Post hoc analysis revealed an increase ($p < 0.01$) in serum corticosterone concentrations in stressed male fetuses relative to control male fetuses at both 1 h and 4 h after the onset of the last stress session (Fig. 6). Stress did not induce a significant change in serum corticosterone concentrations in female fetuses relative to control female fetuses at any time throughout the sampling period.

Effect of a Single Maternal Stress Session on Maternal and Fetal Serum Corticosterone Concentrations

Pregnant females were stressed at one time only on Day 17 beginning at 1900 h to determine whether the effects on serum corticosterone were different than in the previous study due to either sensitization (leading to an increase in the magnitude of

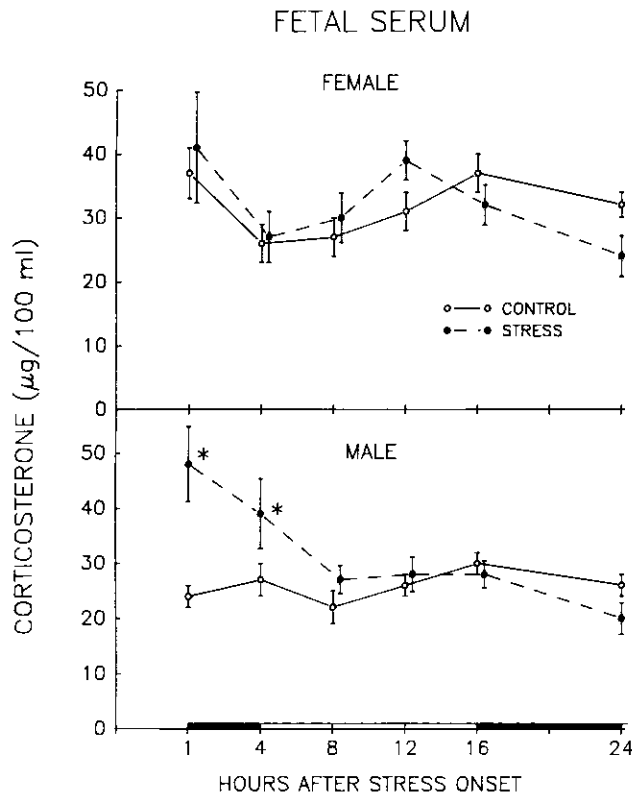


FIG. 6. Serum corticosterone concentrations (mean \pm SEM) in male and female fetuses delivered from control and stressed mothers on Day 17 of pregnancy (Fig. 5). Mothers were stressed 2 times per day starting on Day 12. Blood was collected at different hours after the onset of the last stress session, which began at 1900 h on Day 16. The following number of samples (representing pooled sera from stressed fetuses of the same sex from each litter) were assayed for successive time points: Male fetuses = 5, 6, 11, 8, 9, 11; Female fetuses = 6, 8, 9, 8, 8, 7. *Significant difference between control and stressed fetuses ($p < 0.01$).

response to stress) or habituation (leading to a decrease in the magnitude of response to stress) as a result of repeated stress. Blood was collected from these mothers and their fetuses at either 1 h or 4 h after the onset of the single stress session (at 2000 h and 2300 h on Day 17). A total of 51 pregnant females was used in this experiment. Corticosterone values obtained for stressed pregnant females and their fetuses were then compared with those obtained at corresponding time periods from different control pregnant females than those used in previous experiments. All control and stressed females used in this experiment were randomly assigned to treatment conditions and were killed at the same time.

When pregnant females that were stressed at one time only for 45 min beginning at 1900 h on Day 17 were compared to control females killed at corresponding time periods, the interaction between time of blood collection and treatment was significant, $F(1,30) = 15.3$, $p < 0.001$. Serum corticosterone concentrations in stressed females ($260 \pm 4.0 \mu\text{g}/100 \text{ ml}$) were 2.4 times higher ($p < 0.001$) than those in control females ($110 \pm 20 \mu\text{g}/100 \text{ ml}$) 1 h after the onset of the stress session. By 4 h after the onset of the stress session, values for stressed females ($91 \pm 10 \mu\text{g}/100 \text{ ml}$) and control females ($94 \pm 10 \mu\text{g}/100 \text{ ml}$) did not differ significantly.

Analysis of variance conducted on the data for male and fe-

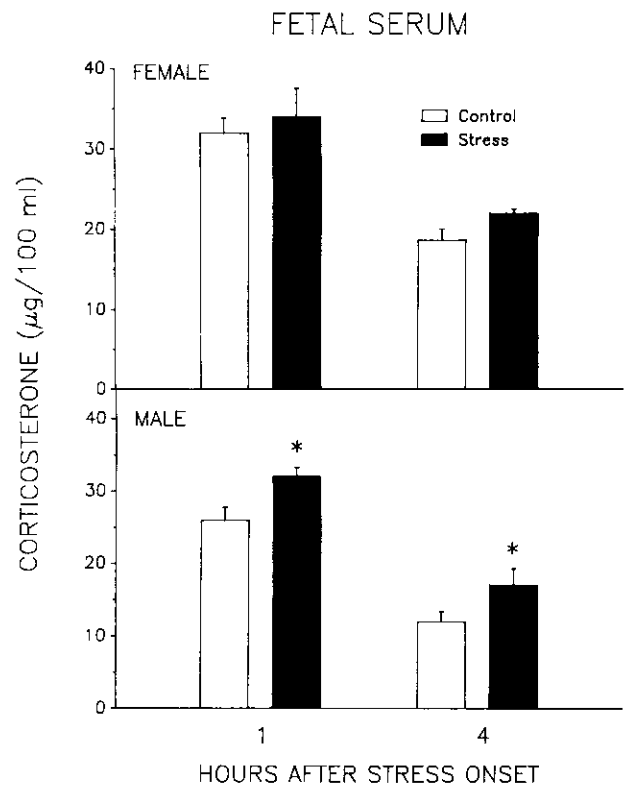


FIG. 7. Serum corticosterone concentrations (mean \pm SEM) in male and female fetuses obtained from pregnant females that were stressed at one time only on Day 17 at 1900 h. Blood was collected 1 h (at 2000 h on Day 17) and 4 h (at 23 h on Day 17) after the onset of the stress session. Sera from control fetuses carried by nonstressed mothers were collected at the same time points. The following number of samples (representing pooled sera from fetuses of the same sex in each litter) were assayed for successive time points: Male fetuses: Control, 1 h, $n = 11$; 4 h, $n = 9$; Stress, 1 h, $n = 7$; 4 h, $n = 6$; Female fetuses: Control, 1 h, $n = 7$; 4 h, $n = 10$; Stress, 1 h, $n = 6$; 4 h, $n = 6$. *Significant difference between control and stressed fetuses ($p < 0.05$).

male fetuses showed significant effects of sex, $F(1,54) = 12.2$, $p < 0.001$, time of blood collection, $F(1,54) = 102.6$, $p < 0.001$, and treatment, $F(1,54) = 10.0$, $p < 0.01$. There were no significant interactions. Post hoc analysis revealed an increase ($p < 0.05$) relative to control values in male fetuses at 1 h and 4 h after the onset of the single stress session (Fig. 7), while no significant change ($p > 0.1$) was observed between control and stressed female fetuses at either 1 h or 4 h after the onset of maternal stress.

We previously found that female fetuses had higher concentrations of corticosterone than male fetuses at both 20 h ($p = 0.09$) and 0 h ($p < 0.01$) at the end of Day 17 of pregnancy (Fig. 4). In addition, both male and female fetuses showed a marked ($p < 0.01$) decrease in corticosterone concentrations at 0 h relative to 20 h on Day 17. In the present study we also found that at both 20 h and 23 h at the end of Day 17, control females had significantly higher ($p < 0.05$) concentrations of corticosterone than control males. We again observed that both males and females showed a decrease ($p < 0.05$) in corticosterone at 23 h relative to 20 h on Day 17.

DISCUSSION

We found that on Days 16-18 of pregnancy, maternal serum corticosterone concentrations were lowest toward the end of the

dark phase and increased gradually during the light phase to peak values toward the beginning of the dark phase. This pattern is very similar to what we observed in virgin female mice, despite the much higher concentrations of serum corticosterone in pregnant females. A contributing factor to the high concentrations of serum corticosterone during pregnancy are the high concentrations of plasma corticosteroid binding globulin (CBG) in pregnant mice, which have been demonstrated to be approximately 20 times those of the nonpregnant adult (16). Since the daily pattern of plasma corticosterone parallels changes in corticosteroid binding capacity (16), our findings suggest changes in serum binding capacity as a function of the time of day. Recent studies have indicated that changes in serum binding activity can be attributed to changes in binding protein concentrations (8).

The pattern of fetal serum corticosterone during the light:dark cycle was similar to that observed in mothers. However, the 4-h interval between collection of samples was too great to assess whether the differences in serum corticosterone throughout the light:dark cycle in fetuses were correlated with the pattern seen in their mothers. In mammals, the suprachiasmatic nuclei of the anterior hypothalamus act as a biological clock generating hormonal rhythms. In the rat, the suprachiasmatic nuclei appear to be functional before birth and are entrained by the mother (15). However, the expression of overt rhythms, such as the daily rhythm in corticosterone secretion, has not been demonstrated until postnatal life in the rat (4). Furthermore, there is no information available as to the nature of the maternal signal which entrains the fetus.

In prior experiments examining changes in circulating corticosterone as a result of stress in rats, corticosterone was only examined within 3.5 h after the onset of maternal stress; a significant increase in maternal serum corticosterone 2–3.5 h after the onset of stress was observed on Day 17 and 18 of pregnancy (19). We observed that on Day 17 of pregnancy in mice, a significant increase in maternal serum corticosterone was exhibited 1 h after the onset of a stress session, and serum concentrations did not return to baseline until 16 h later.

One possible hypothesis for the above finding is that exposure to bright lights during the dark phase could lead to a phase shift in the daily rhythm in serum corticosterone concentrations. This phase shift could lead to a significant increase in corticosterone concentrations in stressed animals relative to controls if corticosterone were measured only during the times that values were low in control animals. However, our finding of higher serum corticosterone concentrations across a 12-h period in stressed female mice relative to values observed at any time during Day 17 in control females argues against this as an explanation for our findings.

We found a similar increase in serum corticosterone concentrations 1 h after the onset of stress in pregnant females whether the females were administered two stress sessions per day from Day 12–16 or only administered a single stress session on Day 17. However, the pregnant females stressed two times per day between Day 12–16 showed elevated serum corticosterone concentrations for 12 h after the onset of the last stress session, while females stressed only one time had control levels of corticosterone by 4 h after the onset of stress.

In contrast to the findings in pregnant females, the increase in serum concentrations of corticosterone in response to stress was much greater in male fetuses whose mothers were repeatedly stressed relative to male fetuses carried by mothers stressed only one time. This finding suggests that although the mothers showed a similar corticosterone response to stress regardless of the number of prior stress sessions, the transport of corticoste-

rone across the placenta into male fetuses might have been altered. However, the same qualitative pattern of change in corticosterone was observed in male and female fetuses regardless of the number of stress sessions administered to the mother.

Maternal stress resulted in an increase in serum corticosterone for a 4-h period after maternal stress relative to control levels in male fetuses. Female fetuses, which show significantly higher baseline serum corticosterone concentrations at certain times of the day relative to male fetuses under control conditions, did not show any significant change in serum corticosterone concentrations over a 24-h period after maternal stress.

Dalle et al. (3) did not observe a sex difference in serum corticosterone in fetuses from nonstressed mice when measured between 0900 and 1100 h on Days 18–19 of pregnancy. In rats, Ward and Weisz (19) reported that although serum corticosterone concentrations were slightly higher in control female fetuses relative to males, the changes in serum corticosterone in male and female fetuses due to maternal stress were similar. We observed a sex difference in the effect of maternal stress on serum corticosterone concentrations in fetuses, such that the sex difference in serum corticosterone concentrations in control fetuses was eliminated throughout a 24-h period after a stress session. Studies with adult rats have also indicated that the rise in plasma corticosterone after stress is dependent on sex (11).

In other studies which will be described elsewhere, we did not find a difference in adrenal corticosterone content in control male vs. female fetuses at a time when we observed a sex difference in corticosterone concentration in serum collected from these same fetuses. In addition, the sex difference in serum corticosterone in fetuses carried by nonstressed mothers was eliminated by maternal adrenalectomy. Specifically, as a result of maternal adrenalectomy, the serum concentration of corticosterone in male fetuses increased to that of control female fetuses; there was also no sex difference in adrenal content of corticosterone in these same fetuses, although adrenal corticosterone content was significantly elevated relative to levels in fetuses carried by control mothers with intact adrenals (M. Montano, unpublished observation). These findings lead to the hypothesis that the fetal sex difference in serum corticosterone in control fetuses may be due to differential transport of corticosterone from the mother to female vs. male fetuses rather than a difference in the functioning of the brain-pituitary-adrenal axis of female fetuses as compared to male fetuses.

In the present experiments we observed an increase in maternal serum corticosterone concentrations at the beginning of parturition. The increase in maternal serum corticosterone during parturition, however, was not reflected in the fetal serum, where we observed a continuous decrease after Day 18 of pregnancy. This finding suggests that toward the end of pregnancy, mechanisms may exist which protect the fetus from increases in maternal serum corticosterone, such as a decrease in the passage of corticosterone across the placentae into fetuses.

We also observed no change in serum corticosterone in either male or female fetuses following maternal stress if the stressed pregnant females had previously been adrenalectomized (M. Montano, unpublished observation). This finding showed that the changes in serum corticosterone in fetuses in response to maternal stress were mediated by secretions from the maternal adrenals. This finding also suggests that the difference in the pattern of serum corticosterone after maternal stress observed between male and female fetuses is mediated by differential transport of corticosterone secreted by the maternal adrenals across the placentae to male vs. female fetuses.

ACKNOWLEDGEMENTS

These experiments were approved by the Animal Care and Use Committee of the University of Missouri-Columbia. Support was provided by grants to F.vS. from NSF (DCB 8518094) and the University of Missouri Food for the 21st Century Animal Reproductive Biology Program.

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