ACUTE STRESS SUPPRESSES PLASMA ESTRADIOL LEVELS IN FEMALE ALLIGATORS (ALLIGATOR MISSISSIPPIENSIS)

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Abstract—1. Five adult, female alligators (Alligator mississippiensis) were captured at night during the breeding season, and a blood sample taken within 5 min of capture.

2. The alligators were physically restrained (tied to boards) and additional blood samples taken at 4, 8, 12, 16, 22, 28, 38, and 48 hr after capture. After the last blood sample was collected the animals were released.

3. Plasma estradiol-17β and corticosterone were measured by radioimmunoassay. Estradiol declined significantly from initial values by 22 hr post capture, but remained unchanged for 48 hr.

4. Plasma corticosterone rose from a mean of 0.8 ng/ml at capture to 12.6 ng/ml after 4 hr. Corticosterone continued to rise up to 16 hr then declined after 22 hr. From 28 until 48 hr corticosterone again increased significantly.

5. These results demonstrate that acute stress in female alligators causes significant suppression of plasma estradiol and a biphasic pattern of corticosterone secretion.

INTRODUCTION

It is well known that environmental stress, crowding, or social subordination can result in reproductive failure in many animal species including reptiles (see reviews by Stephens, 1980; Moberg, 1985; Greenberg and Wingfield, 1987). Until recently, very little was known about the effects of stress in reptiles, but several recent studies have shown that reptiles respond to various stressors in a similar manner to other vertebrates (Lance, 1990). Crowding stress in juvenile alligators maintained in controlled environmental chambers resulted in chronically elevated plasma corticosterone in those held at the highest densities. Those held at the lowest density grew significantly faster and had significantly lower plasma corticosterone than those at the higher densities (Elsey et al., 1990a). Adult alligators maintained for captive breeding experiments also showed chronically elevated plasma corticosterone and reduced egg production associated with increased stocking density (Elsey et al., 1990b). Similarly, juvenile alligators held under osmotically stressful conditions had chronically elevated plasma corticosterone (Lauren, 1985).

In a previous report we documented the effect of acute stress (restraint) on adult, wild-caught, male alligators (Lance and Elsey, 1986). Plasma corticosterone rose dramatically and plasma testosterone declined to non-detectable levels within 24 hr of capture. Likewise, in the male painted turtle, Chrysemys picta, Licht et al. (1985) showed that plasma testosterone declined precipitously within 24 hr of capture. Curiously, in male snapping turtles, Chelydra serpentina, plasma testosterone rose during the first 24 hr after capture and then declined rapidly (Mahmoud et al., 1989). Plasma androgens in male lizards, Tilqua rugosa, appeared to be unaffected by captivity (Bourne et al., 1986).

Only two studies on the effect of the stress of capture in female reptiles have been published. In the turtle, Chelydra serpentina, both progesterone and estradiol increased dramatically 24 hr after capture in gravid females, but in non-gravid females only progesterone showed a significant increase by 24 hr. Both hormones returned to initial values, but did not decline significantly from baseline during one week of captivity. Plasma corticosterone was not measured (Mahmoud et al., 1989). In the lizard, Lacerta vivipara, Dauphin-Villemant and Xavier (1987) showed that both corticosterone and aldosterone increased significantly after capture. Considering the contradictory results cited above we investigated the effect of acute stress on plasma estradiol and corticosterone in another reptilian species, reproductively active, adult female alligators.

MATERIALS AND METHODS

During May, when plasma estradiol levels are high (Lance, 1989), five adult female alligators were captured at night on the Rockefeller Wildlife Refuge by snare as described in Chabreck (1963). Using a heparinized syringe a 15 ml blood sample was taken within 5–10 min of capture from the supravertebral branch of the internal jugular (Olson et al., 1975). The blood was stored on ice in a portable cooler until all five alligators had been caught. The alligators were then taken back to the field laboratory where they were tied securely to boards. Each animal was measured to the nearest cm (total length) and additional blood samples taken at 4, 8, 12, 16, 22, 28, 38, and 48 hr from the time of capture. The animals were released in good condition.

Plasma estradiol and corticosterone were measured by radioimmunoassay. Estradiol declined significantly from initial values by 22 hr post capture, but remained unchanged for 48 hr. Corticosterone continued to rise up to 16 hr then declined after 22 hr. From 28 until 48 hr corticosterone again increased significantly.
Condition after the last sample was taken. Red blood cells were separated from plasma in a clinical desk-top centrifuge immediately after the sample was drawn. The plasma was rapidly frozen on dry ice, and then stored at -20°C until assayed. Corticosterone and estradiol were measured by radioimmunoassay in duplicate aliquots of plasma using highly specific antisera as previously described (Lance and Callard, 1978; Lance and Lauren, 1984). The data were analysed using a repeated measureANOVA followed by Duncan’s multiple range test.

RESULTS

The five female alligators ranged from 187 to 222 cm total length. Female alligators are sexually mature at a length of approximately 180 cm (Joanen and McNease, 1989). Estimated weights from published growth curves (Chabreck and Joanen, 1979) were 35 to 40 kg. One of the large females (216 cm) had very low estradiol levels and was probably quiescent. Only about 50% of adult females reproduce each year in Louisiana (Joanen and McNease, 1989). The other four animals had initial estradiol levels ranging from 240 to 735 pg/ml. The results are presented in the upper panel of Fig. 1. Two of the animals showed an initial rise in estradiol followed by a gradual decline, and two of the animals showed a steady decrease from the initial values. The mean values at 22, 28, 38, and 48 hr were significantly lower (P < 0.05) than the initial value. The mean plasma estradiol value after 48 hr of restraint was 334 pg/ml. Plasma corticosterone at the first sampling ranged from 0.07 to 1.86 ng/ml. The results are presented in the lower panel of Fig. 1. Corticosterone rose dramatically in all of the alligators by 4 hr (P < 0.001) and remained elevated for the first 16 hr. At 22 hr, however, mean corticosterone levels dropped markedly, and were not significantly different from the initial value. From 28 hr until the end of the experiment mean plasma corticosterone again increased. At each of these last three points the means were significantly different from the initial sample (P < 0.05).

DISCUSSION

Our results demonstrate that in female alligators acute stress causes a significant decline in plasma estradiol. Two of the animals, however, showed an initial increase in plasma estradiol followed by a decline. These results are somewhat similar to those seen in the turtle, Chelydra serpentina, where plasma estradiol rose 24 hr after capture and then fell back to initial levels (Mahmoud et al., 1989). In the gravid turtle, however, plasma estradiol rose by an order of magnitude (ca 30 pg/ml to more than 300 pg/ml). The source of this transient surge in estradiol secretion in response to stress is not known. Embryonic turtle adrenal tissue has been shown to synthesize and secrete estradiol (White and Thomas, 1990), but it is not known if adult turtle adrenal is capable of producing estradiol. The mean rise in estradiol in the alligators was not significant, and by 22 hr all animals had levels significantly lower than the initial levels. While the drop in plasma estradiol was significant in the female alligators, it was far less dramatic than the drop in plasma testosterone seen in male alligators subjected to the stress of restraint. In males, plasma testosterone was undetectable in the plasma by 28 hr of restraint (Lance and Elsey, 1986), whereas in females plasma estradiol remained over 300 pg/ml after 48 hr of restraint. During the reproductive season plasma testosterone in male alligators ranges from 10 to over 100 ng/ml, whereas plasma estradiol in female alligators only ranges from 300 to 1000 pg/ml (Lance, 1989). The reason for this sustained estrogen titer may be due to the presence of the sex steroid binding protein (SSBP) in alligator blood (Ho et al., 1987). Concentrations of SSBP (30–140 nM) always exceed those of estradiol (0–20 nM), whereas concentrations of testosterone in breeding males greatly exceed those of SSBP. Furthermore, the binding affinity of alligator SSBP for estradiol is about five times that of testosterone (Ho et al., 1987). This suggests that even though the stress of restraint effectively shuts down sex steroid secretion from the ovary, there is sufficient binding capacity in the blood to maintain circulating estradiol concentrations at physiological levels during the 48 hr of the experiment.

The biphasic pattern of corticosterone secretion in female alligators under restraint is remarkable in that it is identical to that seen in the male. In male alligators plasma corticosterone rose dramatically from 4 to 12 hr post capture, dropped at 22 hr, then continued to rise up to 41 hr (Lance and Elsey, 1986). This unusual pattern is difficult to explain. There are no comparable data for other species, and there are no data available on circadian rhythms of corticosterone secretion in adult wild alligators. The normal circadian pattern of corticosterone secretion in young captive alligators (Lance and Lauren, 1984) does not fit the pattern seen in this experiment.
The results of this experiment showing the effects of acute stress on corticosterone and estradiol levels, and the data of Elsey et al. (1990b) documenting an association of reproductive failure with chronically elevated plasma corticosterone levels in alligators, indicate that both acute and prolonged stress can have deleterious effects on reproduction in this species.

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**REFERENCES**


