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Stress-Induced Suppression of Testosterone Secretion in Male Alligators

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ABSTRACT In order to test the effect of acute stress on gonadal hormone secretion in reptiles, six mature male alligators were captured, and a blood sample was taken within 5 min of capture. Additional blood samples were taken at timed intervals for up to 41 hr, and plasma testosterone and corticosterone were measured by radioimmunoassay. Plasma testosterone declined to 50% of the initial value by 4 hr and dropped to less than 10% of initial by 24 hr. Plasma corticosterone increased during the first 12 hr, declined at 24 hr, and rose again at 40 hr. Blood samples from male alligators collected in North and South Carolina, south Florida, and in south Louisiana in two consecutive breeding seasons were also assayed for testosterone and corticosterone. In these populations there were significant differences in mean plasma testosterone and corticosterone levels. Elevated corticosterone levels were consistently seen in alligators caught in traps and from which a blood sample was taken several hours later. Plasma testosterone, although consistently lower in trapped alligators, did not show a negative correlation with plasma corticosterone. Farm-reared alligators bled once, released, and bled again at 24 hr also showed a highly significant suppression of testosterone secretion. These results demonstrate that stress has a rapid and dramatic effect on testicular steroid secretion in both farm-reared and wild alligators.

It is well known that environmental stress can cause reproductive failure in domestic animals (see review by Stephens, '80), and stress-induced or pharmacologically induced increases in plasma corticosteroid concentrations result in a rapid decline in plasma testosterone in male rats (Charpenet et al., '82), dogs (Tcholakian and Eik-Nes, '71), boars (Liptrap and Raeside, '78), bulls (Welsh and Johnson, '81), baboons (Goncharov et al., '79), and humans (Cumming et al., '83). Although there is a wealth of data on the effect of stress on reproduction in poultry (Stephens, '80), the effect of stress on reproductive function in other nonmammalian vertebrates has received little attention. Licht et al. ('83) demonstrated that plasma androgen and gonadotropin declined to basal levels in bullfrogs (*Rana catesbeiana*) 35 hr after capture, and Moore and Zoeller ('85) provide evidence that the stress of captivity results in an increase in plasma corticosterone and a corresponding decrease in the secretion of luteinizing hormone-releasing hormone from the hypothalamus of the rough-skinned newt, *Taricha granulosa*. The latter authors also showed that an injection of corticosterone could inhibit testosterone secretion in this animal. It has been suggested that stress affects reptiles in a similar manner (Lance, '84), but data are lacking.

A number of studies on the annual reproductive cycle of male reptiles have presented data on plasma testosterone concentrations without indicating how long after captivity blood samples were taken. Given the rapidity with which the stress of captivity can inhibit testosterone secretion in a wide variety of vertebrates it is unclear what these differences in plasma levels of androgen mean. In lizards, for example, we have data on hormonal cycles that show variations in plasma testosterone from the pg/ml range (Leyton et al., '77) to those with peak levels well over 300 ng/ml (Courty and Dufaure, '79). Whether these extreme differences are simply a result of species differences or are a result of different responses to the stress of captivity is not known (see review by Lance, '84).

During a study on the annual reproductive cycle of the American alligator, *Alligator mississippiensis* (Lance, '85), it was noted that plasma testosterone concentrations in sexually active males ranged from less than 0.5 ng/ml to over 100 ng/ml. These extreme differences in hormone concentrations in animals that were apparently in identical physiological condition were later shown to correlate with the time after captivity a blood sample was taken. We decided to further investigate this effect of immobilization stress on plasma testosterone and corticosterone concentrations in freshly caught male alligators at the height of the breeding season. Testosterone and corticosterone were also measured in blood samples taken from wild male alligators in North Carolina, South Carolina, and south Florida to assess the effect of capture methods on hormone levels.

MATERIALS AND METHODS

Six male alligators were captured at night on the Rockefeller Wildlife Refuge, Louisiana, using a noose snare as described by Chabreck ('69). The experiment was conducted in April when plasma testosterone levels are expected to be high (Lance, '85). A 30-cc blood sample was taken from the supravertebral branch of the internal jugular vein as described by Olson et al. ('75) within 5 min of capture. The blood sample in the syringe was thoroughly mixed with the heparin and stored on ice until all six alligators had been bled. The animals were then taken back to the field laboratory at the refuge where they were held under restraint. The jaws of each alligator were held shut with several large rubber bands, and forelegs, hindlegs, and tail were securely tied with rope. Total length to the nearest cm was recorded, and additional blood samples (10 cc per sample) were taken at 4, 8, 12, 16, 22, 28, and 41 hr from the time of capture. After the last blood sample was taken, the animals were released. Plasma was separated from red cells in a desk top clinical centrifuge and frozen immediately on dry ice, then stored frozen at −20 C until assayed for testosterone and corticosterone.

A group of mature male alligators hatched from artificially incubated eggs and raised on a commercial farm in Florida was also sampled. For a detailed description of the facilities, see Lance et al. ('85). Blood samples were taken within 5 min of capture, and the animals were released. The following day (approximately 24 hr later) they were recaptured and a second blood sample taken. Testosterone, but not corticosterone, was assayed in these samples.

In addition to the above animals. a large number of blood samples from wild male alligators captured during population studies in North Carolina, South Carolina, and the Florida Everglades were compared with samples taken from wild male alligators at the Rockfeller Wildlife Refuge, Louisiana. The alligators sampled in North and South Carolina were caught by means of a noose snare (Murphy and Fendley, '73), in which the animal often struggles for hours before the wildlife workers are able to collect a sample, whereas in Louisiana during the first year of study alligators were either caught on baited hooks and a blood sample taken some hours later or were noosed and held overnight before being bled. In subsequent sampling in Louisiana, alligators were caught by noose at night and bled within 5 min of capture. In Florida the alligators were caught by noose from airboats during the day and a blood sample taken immediately. All of these blood samples were assayed for corticosterone and testosterone.

Corticosterone and testosterone were measured in duplicate plasma samples by radioimmunoassay (RIA) as described previously (Lance and Lauren, '84; Lance et al., '77). The data from the acute stress experiment were subjected to a single factor repeated measure ANOVA followed by the Duncan's multiple range test using a Human Systems Dynamics software package. A correlation coeffecient for plasma testosterone and corticosterone was calculated using the individual values from each animal in each population.

RESULTS

The male alligators used in the acute stress experiment ranged from 180 to 253 cm total length. Weight range was estimated as 35–75 kg from the growth curve of Chabreck and Joanen ('79). Only four of the six had elevated plasma testosterone levels. The two with testosterone less than 0.1 ng/ml were the two smallest animals and may have been sexually immature (Table 1). Sexual maturity is attained at a total body length of about 183 cm (Joanen and McNease, '75). Testosterone was measured in all six alligators throughout the experiment, but only the results from the four with initial concentrations greater than 5 ng/ml were used to

TABLE 1. Body lengths and initial plasma hormone levels in alligators used in the stress experiment

Body length in cm	Testosterone (ng/ml)	Corticosterone (ng/ml)
253	14.99	1.40
230	5.88	0.88
271	51.09	1.99
244	13.50	1.46
180	0.09	0.47
188	0.10	0.27

construct the graph of the testosterone data. Testosterone concentrations declined rapidly during the 41 hr of restraint in these four sexually mature alligators. As testosterone levels at the beginning of the experiment were so variable, the results are presented as percent change from the initial values. The mean percent decline in testosterone concentration is shown in Figure 1, lower panel. Plasma testosterone was significantly lower than at time 0 ($P < .01$) at all time periods after the initial bleed. Plasma corticosterone concentration in the samples collected at the time of capture are given in Table 1. The plasma corticosterone results from all six of the male alligators were used to construct the graph. Mean corticosterone concentrations during the experiment are shown in Figure 1, upper panel. Corticosterone levels at 12 and 41 hr were significantly higher ($P < .01$) than at time 0, 4, 22, and 28 hr. Levels at 8 hr were significantly higher ($P < .05$) than at time 0, and those at 12 and 41 hr were significantly higher ($P < .05$) than at 16 hr.

Range of body lengths and range of plasma testosterone concentrations in alligators from the different populations sampled are given in Table 2. Mean testosterone levels and mean corticosterone levels in the plasma of alligators collected during April and May in two consecutive years in Louisiana are shown in Figure 2. Mean plasma testosterone and corticosterone levels in alligators captured in May in North Carolina, in April and May in South Carolina, and in April and May in south Florida are also shown in Figure 2. Mean testosterone was significantly higher, and mean corticosterone was significantly lower ($P < .01$) in Louisiana in the second

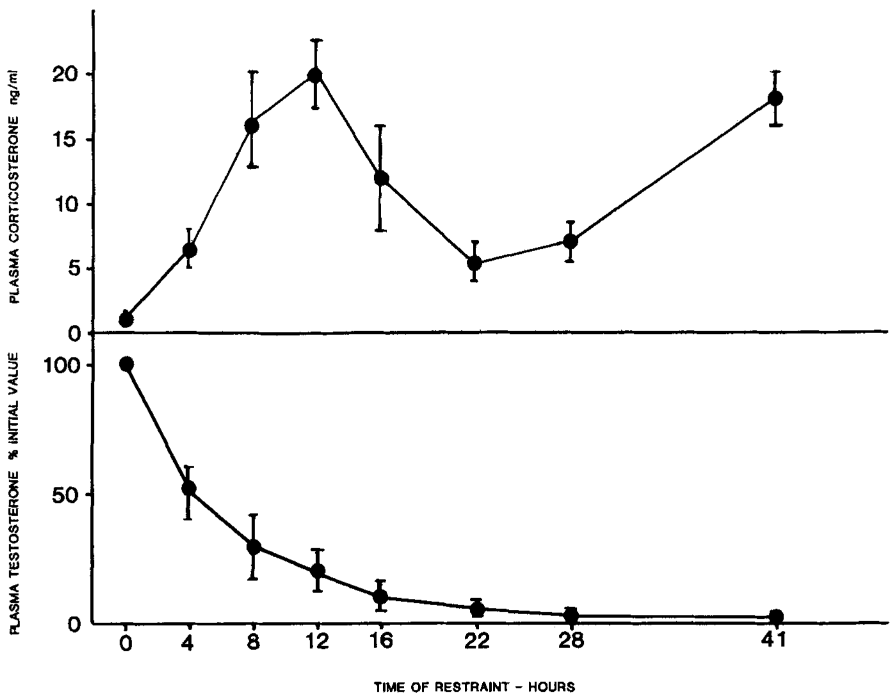

Fig. 1. Changes in plasma corticosterone and testosterone in immmobilized male alligators. Points on each graph represent the means and the bars about the means the standard errors of the mean. Plasma testosterone (lower panel) is presented as percent change of initial value.

TABLE 2. Ranges of body weights and plasma testosterone in alligators from different populations

Group	n	Body length range (cm)	Plasma testosterone range (ng/ml)
LA 1	15	193–310	0.38–19.2
LA 2	21	180–296	8.09–73.7
NC	38	140–287	0.14–33.3
SC	71	153–380	0.05–65.2
FLA	14	183–282	0.16–27.0

year of the study than in the first year of the study. Means of plasma testosterone in the alligators captured in North Carolina, South Carolina and south Florida were significantly lower ($P < .01$) than the mean plasma testosterone from alligators captured in Louisiana in the second year of the study. Mean plasma corticosterone in the alligators captured in North and South Carolina was significantly higher ($P < .01$) than in the Louisiana alligators (LA 2), but mean plasma corticosterone in the Florida sample was not significantly different from mean corticosterone in group LA 2. The correlation coefficient for corticosterone and testosterone for the S. Carolina population, r = 0.158, n = 71, was not significant.

In all of the farm-reared alligators injected with saline, sampled, released, and recaptured 24 hr later, plasma testosterone declined by as much as 90% of the initial value (Fig. 3).

DISCUSSION

The results of these experiments clearly show that alligators respond in a similar manner to mammals to the stress of immobilization, in that adrenocortical secretion increases and testicular testosterone secretion rapidly declines. The kinetics of the response are, however, markedly slower. In rats (Charpenet et al., '82) and baboons (Sapolsky, '85), testosterone concentrations decline to a nadir within 6 hr of restraint, whereas in alligators lowest testosterone levels do not occur until 20–24 hr after the onset immobilization. This difference may be due to the fact that metabolic processes in alligators are considerably slower than those of mammals, largely as a result of a slower circulation time (Coulson and Hernandez, '83). It is still not clear how actue stress inhibits testicular steroid secretion. In rats (Welsh et al., '82) and humans (Cumming et al., '83) there is evidence to suggest that in addition to a hypothalamic site of action, corticosteroids have a direct inhibitory effect on testicular steroidogenesis. In bulls, however, a decline in pituitary LH secretion in response to elevated corticosteroids may be more important (Welsh and Johnson, '81). In the absence of data on levels of pituitary hormones in the circulation in the alligator, it is not clear how acute stress inhibits testicular steroid secretion.

The rapid rise in corticosterone that accompanies the decline in testosterone secretion

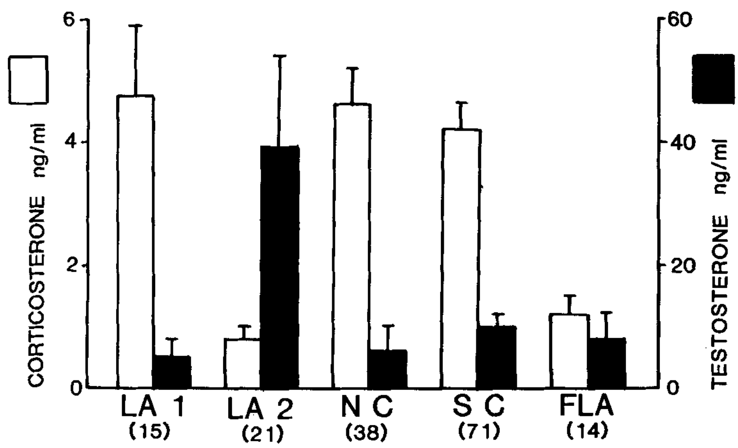

Fig. 2. Plasma corticosterone and plasma testosterone in male alligators from Louisiana (LA 1 and LA 2), North Carolina (NC), South Carolina (SC), and south Florida (FLA). The solid bars represent the means ± SEM of testosterone concentrations in each of the groups, and the open bars represent the corticosterone concentrations. Numbers in parentheses under each group indicates sample size.

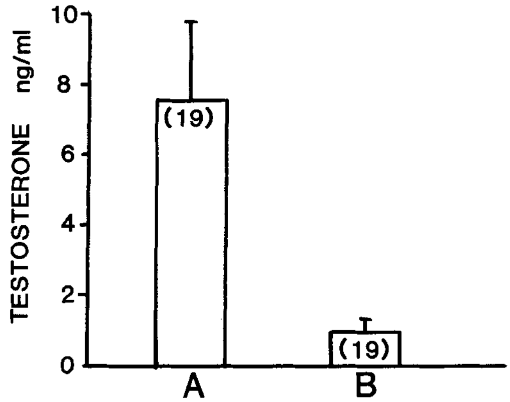

Fig. 3 Plasma testosterone in samples taken from farm-reared male alligators within 5 min of initial capture (group A), and in a second sample taken at 24 hr (group B). The bars represent the means ± SEM, and the numbers in parentheses represent the sample size.

strongly suggests a cause and effect relationship. However, the biphasic corticosterone-response is at present impossible to explain. An identical biphasic response in corticosterone secretion was seen in female alligators held under similar conditions (Lance and Elsey, unpublished).

Although it appears that there is a relationship between method of capture of wild alligators and hormone levels, expecially in the populations in Louisiana and South Carolina, a negative correlation between testosterone and corticosterone was not apparent. The reason for lack of a negative correlation could be due to two factors: (1) there is a strong positive correlation between size of an alligator and plasma testosterone concentration (Lance, in preparation), and (2) the plasma corticosterone levels do not continue to rise after capture, but show a biphasic curve (see Fig. 1). Thus some alligators with a relatively low plasma corticosterone concentration may have been held under restraint for hours, and small alligators with relatively low plasma testosterone and fromwhich a blood sample was taken shortly after capture would have low plasma corticosterone levels. A similar lack of correlation between corticosterone and plasma androgen was noted by Licht et al. ('83) in their study on the bullfrog, despite the fact that capture consistently resulted in elevated corticosterone levels. In comparing alligators collected in Louisiana in years 1 and 2, the correlation is very clear: animals sampled immediately after capture have low plasma corticosterone and high plasma testosterone, and animals bled many hours after capture have high plasma corticosterone and low plasma testosterone. In the other populations it is clear that capture method has an effect on corticosterone level, but it is less clear how testosterone levels were affected.

The sensitivity of alligators to stress is clearly seen in the response of the farm-reared animals to a single period of restraint. These animals, which had been hatched from artificially incubated eggs and reared entirely under environmentally controlled conditions, had been fed, cleaned, and handled frequently by humans over the previous 4 years, yet a single period of not more than 20 min of restraint was enough to suppress testosterone levels for at least the following 24 hr.

These data on the effects of the stress of captivity on plasma testosterone levels in alligators may not, however, apply to all reptilian species. There are clearly interspecific differences in the levels of circulating testosterone and interspecific differences in how an animal responds to the stress of captivity. Many lizards and snakes for example, breed readily in captivity, yet others, such as the Western Diamondback rattle snack, *Crotalus atrox*, usually die in captivity as a result of stress, even under the most carefully controlled conditions (Lhotka, '80). However, unless the time between capture and blood sampling of a wild reptile is known, data on circulating hormone levels from such animals are of little value.

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