

## Adrenocortical Modulation Following ACTH, Corticoid, and Medullary Hormone Administration in the Soft-shelled Turtle *Lissemys punctata punctata* (Bonnoterre) (Family: Trionychidae)

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**Prajna P. Ray, Santasri Chaudhuri-Sengupta and Biswa R. Maiti (2003)** Adrenocortical modulation following ACTH, corticoid, and medullary hormone administration in the soft-shelled turtle *Lissemys punctata punctata* (Bonnoterre)(Family:Trionychidae). *Zoological Studies* 42 (1): 165-172. Our aim was to study the role of ACTH and adrenal hormones in adrenocortical function in the soft-shelled turtle, *Lissemys punctata punctata* (Family: Trionychidae). All experiments were carried out in sexually immature animals. 1) ACTH treatment at all doses (0.5, 1.0, and 2.0 IU/100 g body wt. daily for 10 d) produced adrenocortical hypertrophy with increased nuclear diameters followed by a rise in acid and alkaline phosphatase activities, and depletion of cholesterol and ascorbic acid levels from the adrenal gland. The magnitude of the response was nearly the same for all doses. 2) Dexamethasone treatment only at the moderate or higher dosage (50 or 100 µg/100 g body wt. daily for 10 d) suppressed adrenocortical activity by causing changes counter to those of ACTH, and showed dose-dependent action, with no significant effect at the lower dose (25 µg/100 g body wt. daily for 10 d). But ACTH treatment (0.5 IU/100 g body wt. daily for 10 d after DMS treatment of 100 µg/100 g body wt. daily for 10 d) prevented adrenocortical suppression by inducing the same manifestations as those of ACTH alone in dexamethasone (DMS) recipients. 3) Corticosterone and aldosterone administration only at the moderate or higher dose (50 or 100 µg/100 g body wt. daily for 10 d) had dose-dependent inhibitory effects on adrenocortical function by causing changes that were the same to those of dexamethasone recipients. 4) Norepinephrine and epinephrine treatments, however, caused dose-dependent adrenocortical stimulation only at the moderate or higher dose (50 or 100 µg/100 g body wt. daily for 10 d) by inducing changes that were comparable to those of ACTH. These findings are briefly discussed. <http://www.sinica.edu.tw/zool/zoolstud/42.1/165.pdf>

**Key words:** Corticotropin, Adrenal hormones, Adrenal cortex, *Lissemys*.

Some information is available on the corticotropic control of adrenocortical function in reptiles (Chester Jones and Henderson 1978, Bentley 1998). Hypothalamic lesions suppressed corticosterone level in lizards of *Sceloporus* and *Dipsosaurus dorsalis* (Daugherty and Callard 1972, Callard et al. 1975a b). Dexamethasone implants in the basal hypothalamus of lizards (*Sceloporus cyanogenys*) prevented metyrapone-induced adrenocortical hypertrophy (Callard and Willard 1969). But anterior pituitary extracts stimulated dose-dependent corticosteroid production in dexamethasone-treated *Anolis carolinensis* (Licht

and Bradshaw 1969), *Amphibolurus inermis* (Bradshaw 1975), and *Caiman crocodilus* (Gist and Kaplan 1976). A hypophysectomy caused reductions in adrenal size, weight, and corticosterone levels in snakes and lizards (Licht and Bradshaw 1969, Daugherty and Callard 1972). Whereas mammalian ACTH treatment elevated aldosterone levels in *Naja naja* (Huang et al. 1969) and induced adrenocortical hypertrophy in both hypophysectomized and intact lizards (Callard and Chester Jones 1971), it also caused elevation of corticosterone levels in *Sceloporus cyanogenys* (Daugherty and Callard 1972) and adrenal hyper-

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trophy with elevation of corticosterone and aldosterone levels in the sea snake, *Hydrophis cyanocinctus* (Duggan and Lofts 1979). But ACTH was only moderately effective in stimulating corticosteroid secretion in an alligator (Gist and de Roos 1966), *Natrix*, *Python*, some Lacertans (Leloup Hately 1968), *Caiman sclerops* (Nothstine et al. 1971), and *C. crocodilus* (Gist and Kaplan 1976), or was even ineffective in inducing corticosterone synthesis after infusion of mammalian ACTH in other reptiles (Bentley 1998).

The role of corticoids on adrenocortical activity has not been extensively explored in reptiles (Chester Jones and Henderson 1978, Bentley 1998). Cortisone injection caused degenerative changes in the adrenal cortex of some lizards, *Xantusia vigilis* (Miller 1952) and *Uromastix hardwickii* (Ramaswami 1967), and reduced adrenal weight with cellular degeneration in *Natrix natrix* (Wright and Chester Jones 1957). Callard and others (1975a) also observed reductions in plasma corticosterone levels following corticosterone or aldosterone treatment in *D. dorsalis*. But deoxycorticosterone acetate (DCA) had no effect on adrenal weight of *Natrix* sp. in winter (Yip 1974), and neither DCA (Wright and Chester Jones 1957) nor corticosterone (Chan et al. 1970) had any effect on the weight of the regressed adrenals of hypophysectomized *Natrix* and *Dipsosaurus*. Exogenous dexamethasone induced adrenocortical atrophy in *Hydrophis cyanocinctus* (Duggan and Lofts 1979). Corticosterone also caused degenerative changes in the adrenal cortex of several species of turtles, *Testudo elegans*, *Kachuga tectum tenoria*, and *Geoemyda trijuga* (Ramaswami 1967).

Relatively little is known of the action of medullary hormones on adrenocortical function even in homeothermic animals. Exogenous epinephrine induced ACTH release from the anterior pituitary gland (Dallman and Jones 1973) as well as stimulated adrenocortical activity in mammals (Inaba and Kamata 1975, Turner and Bagnara 1976, Greenspan and Strewler 1997). Also in birds, epinephrine activated the pituitary-adrenal axis, increased adrenal weight (Jailer and Boas 1950, Zarrow and Baldwin 1952), and stimulated the hypothalamo-hypophysial-adrenocortical axis and plasma corticosterone levels (Freeman and Manning 1979).

Information relating to the role of corticotropin and corticoids on adrenocortical function is relatively scanty in turtles. To our knowledge there is no information concerning the role of medullary

hormones on the turtle adrenal cortex. Thus, in the current article, these problems are investigated in soft-shelled turtles.

## MATERIALS AND METHODS

Fifty juvenile female turtles (body weight of 250-300 g), *Lissemys punctata punctata* (Bonnoterre), were procured from local natural populations near Calcutta in Jan. 2000. Experiments were conducted on female specimens as they were plentiful compared to males in Jan. Juvenile specimens were selected for the current investigation under the assumption that their endogenous hormonal levels were low (Sen and Maiti 1988) and thus might be suitable for evaluation of the effect of exogenous hormones on adrenocortical function. Animals were maintained in aquaria (5 per aquarium, 150 cm x 90 cm x 90 cm) in the laboratory with controlled light (11L: 13D) and temperature (25°C). Food (tubifex and shrimp) was available ad libitum throughout the experiments. Animals were divided into 10 groups of 5 each. Groups I, II, and III served as controls for different treatment groups (Table 1). Control group I received saline, II oil, and III ethanol-saline. They were kept in the laboratory for 5 d prior to the study. Hormones were injected intramuscularly in alternate hind limbs of animals consecutively for 10 d. Control animals received vehicle without hormone for a similar duration (Table 1). All animals were killed by decapitation 24 h after the last injection (on day 11 of the experiments, except for animals in groups III and VI which were autopsied 20 d after injections) at a particular time (10.00 h) of the day to avoid effects due to diurnal rhythms (Chowdhury et al. 1982). Adrenal glands were quickly dissected out, and the left adrenals were fixed in Bouin's fluid and processed for routine microtomy. Paraffin sections of 5  $\mu$  thickness were prepared and stained by Masson's trichrome technique for histological study. Adrenocortical cell nuclear diameters (mean of short and long axes) ( $\mu$ ) were measured using an ocular micrometer from the subcapsular and central zones of the gland. One hundred nuclei each from the subcapsular and central zones of the adrenal cortex were counted from 10 widely separated random sections of the adrenal gland of each specimen.

Cholesterol, ascorbic acid, acid phosphatase, and alkaline phosphatase concentrations were measured from the remaining (right) adrenal glands. Total and free cholesterol concentrations of

the adrenal gland were estimated by the revised method of Schoenheimer-Sperry (Sperry and Webb 1950). The esterified cholesterol level was

determined by subtracting the free cholesterol value from the total value. An acetone-alcohol mixture was used to precipitate protein.

**Table 1.** Experimental schedule

Group <sup>a</sup>	Treatment	Dose and duration
Control (Groups I, II, and III)	Saline (0.68%), Ethanol (100%), and saline (0.68%)/oil (vol. 1: 9)	0.1 ml/100 g body wt. daily for 10 d
Treated	IV. ACTH (Porcine, Sigma, USA) dissolved in saline	a) 0.5 IU (in 0.1 ml saline)/100 g body wt. daily for 10 d b) 1.0 IU (in 0.1 ml saline)/100 g body wt. daily for 10 d c) 2.0 IU (in 0.1 ml saline)/100 g body wt. daily for 10 d
	V. Dexamethasone-21-acetate (Sigma, USA) dissolved in oil	a) 25 µg (in 0.1 ml oil)/100 g body wt. daily for 10 d b) 50 µg (in 0.1 ml oil)/100 g body wt. daily for 10 d c) 100 µg (in 0.1 ml oil)/100 g body wt. daily for 10 d
	VI. Dexamethasone-21-acetate (DMS; Sigma, USA) + ACTH (Porcine, Sigma, USA)	DMS: 100 µg (in 0.05 ml oil)/100 g body wt. daily for the first 10 d + ACTH : 0.5 IU (in 0.05 ml saline)/100 g body wt. daily for the next 10 d
	VII. Corticosterone (Sigma, USA) dissolved in oil	a) 25 µg (in 0.1 ml oil)/100 g body wt. daily for 10 d b) 50 µg (in 0.1 ml oil)/100 g body wt. daily for 10 d c) 100 µg (in 0.1 ml oil)/100 g body wt. daily for 10 d
	VIII. <i>d</i> -Aldosterone (Sigma, USA) dissolved in oil	a) 25 µg (in 0.1 ml oil)/100 g body wt. daily for 10 d b) 50 µg (in 0.1 ml oil)/100 g body wt. daily for 10 d c) 100 µg (in 0.1 ml oil)/100 g body wt. daily for 10 d
	IX. Norepinephrine (arterenol bitartrate; Sigma, USA) dissolved in ethanol (100%) and saline (0.68%) (1: 9)	a) 25 µg (in 0.1 ml ethanol-saline)/100 g body wt. daily for 10 d b) 50 µg (in 0.1 ml ethanol-saline)/100 g body wt. daily for 10 d c) 100 µg (in 0.1 ml ethanol-saline)/100 g body wt. daily for 10 d
	X. L-epinephrine bitartrate (Sigma, USA) dissolved as in group IX	a) 25 µg (in 0.1 ml ethanol-saline)/100 g body wt. daily for 10 d b) 50 µg (in 0.1 ml ethanol-saline)/100 g body wt. daily for 10 d c) 100 µg (in 0.1 ml ethanol-saline)/100 g body wt. daily for 10 d

<sup>a</sup>Number of animals (n):5 for each group.

Cholesterol and cholesterol esters were extracted from the sample. Cholesterol was precipitated with digitonin-ether before saponification (for free cholesterol) or after saponification (for total cholesterol). The digitonin-ether mixtures, containing free or total cholesterol, were purified and subjected to the Liebermann-Burchard color reaction using the acetic anhydride-sulfuric acid reagent, and the absorbance was measured at 625 nm.

Ascorbic acid was estimated by the modified method of Roe and Kuether (1943) (Bessey et al. 1947). Ascorbic acid was oxidized to dehydroascorbic acid, and subsequently to diketogulononic acid, followed by coupling with 2,4-dinitrophenylhydrazine. The osazone formed was dissolved in conc.  $H_2SO_4$  to produce color, and the absorbance was measured at 540 nm.

Acid and alkaline phosphatases were assayed by the hydrolysis of *p*-nitrophenyl phosphate in acid (pH 4.8) and alkaline (pH 10.5) media. *p*-Nitrophenyl phosphate was used as a substrate for the determination of acid and alkaline phosphatase activities. After 30 min of incubation, phosphatase reactions were completely inhibited by NaOH, and *p*-nitrophenol liberated by the phosphatases produced a yellow anion. Acid and alkaline phosphatase activities were directly proportional to the amount of *p*-nitrophenol liberated per unit time. Absorbance of the yellow *p*-nitrophenol was measured at 405 nm (Linhardt and Walter 1963). All colorimetric samples were measured on a Perkin-Elmer (550S, Germany) spectrophotometer. All data were analyzed statistically by ANOVA followed by Students' *t*-test (Snedecor and Cochran 1971).

## RESULTS

### Control groups

Findings of the adrenal cortex did not show wide variations among different groups of control animals, whether they received saline, ethanol-saline, or oil. So, findings of all control groups were pooled together and served as a single control group for all experiments.

Findings of histology (Fig. 1B), cortical nuclear diameter, cholesterol and ascorbic acid concentrations, and acid phosphatase and alkaline phosphatase activities of the adrenal glands are presented in figure 1F.

### Treated groups

#### A) Corticotropin

I) ACTH treatment (0.5, 1.0 and 2.0 IU/100 g body wt. daily for 10 d)

##### Histology:

ACTH at all doses caused adrenocortical hypertrophy with conspicuous cell outlines, vesicular nuclear appearance, enlarged cell, and nuclear sizes, and increased nuclear diameters (Fig. 1C,F), without showing much difference between 2 doses.

##### Biochemical changes:

a) Cholesterol: Cholesterol levels were significantly depleted from the adrenal gland for all doses administered (Fig. 1F).

b) Ascorbic acid: Adrenal ascorbic acid concentrations were significantly decreased in treated turtles (Fig. 1F).

c) Acid and alkaline phosphatases: Activities of both phosphatases were significantly increased for all doses (Fig. 1F).

II) Dexamethasone (25, 50, and 100  $\mu$ g/100 g body wt. daily for 10 d)

##### Histology:

Dexamethasone at the low dose had no effect on the adrenal cortex, but at the moderate and higher doses caused cortical atrophy. The cortical cell outline became inconspicuous, the cell size became smaller and the nuclei lost their vesicular appearance. Nuclear diameters decreased, and some of the nuclei became pycnotic. These manifestations were maximum at the higher dose (100  $\mu$ g daily) as compared to those of the moderate dose (50  $\mu$ g daily) (Fig. 1D,F).

##### Biochemical changes:

a) Cholesterol: Levels were not significantly altered at the low dose, but increased at the moderate and high doses of dexamethasone. The effect was maximum with the higher dose as compared to that of the moderate dose (Fig. 1F).

b) Ascorbic acid: Results were similar to those for cholesterol (Fig. 1F).

c) Acid and alkaline phosphatases: Acid and alkaline phosphatase activities of the adrenal gland were not altered at the low dose of dexamethasone, but they significantly declined at the other doses, being most affected at the higher dose than at the moderate dose (Fig. 1F).

III) Dexamethasone (DMS) (100  $\mu$ g/100 g body wt. daily for first 10 d) and ACTH (0.5 IU/100 g body wt. daily for next

10 d)

**Histology:**

ACTH treatment in dexamethasone recipients prevented cortical regression and caused histological stimulation of the adrenal cortex but to a lesser extent than that of ACTH alone at the parallel dose (0.5 IU daily/100 g body wt.). Cell outlines of cortical tissue were conspicuous. Nuclei were partially vesicular. Cell and nuclear sizes were hypertrophied, and nuclear diameters were increased. These manifestations were less intense than those with ACTH alone (Fig. 1F).

**Biochemical changes:**

- a) Cholesterol: Levels significantly declined after the combined treatment, but the magnitude of the response was less than that with ACTH alone (Fig. 1F).
- b) Ascorbic acid: Results were similar to those for cholesterol (Fig. 1F).
- c) Acid and alkaline phosphatases: Acid and alkaline phosphatase activities of the adrenal gland increased, but to a lesser extent than with ACTH alone (Fig. 1F).

**B) Corticoids**

- IV) Corticosterone (25, 50, and 100 µg/100 g body wt. daily for 10 d)

**Histology:**

Corticosterone at the low dose (25 µg daily) was ineffective, but caused degenerative changes at the other doses. Cortical cell outlines were not conspicuous; nuclei were not vesicular, and became smaller and pyknotic as their diameters decreased. These manifestations were more intense with the higher dose (100 µg daily) as compared to those of the moderate dose (50 µg daily) (Fig. 1E,F).

**Biochemical changes:**

- a) Cholesterol: Cholesterol levels were not altered at the low dose of corticosterone, but increased significantly with other doses. Changes were more intense with the higher dose (100 µg daily) than with the moderate dose (50 µg daily) (Fig. 1F).
- b) Ascorbic acid: The results were similar to those for cholesterol (Fig. 1F).
- c) Acid and alkaline phosphatases: Acid phosphatase activity of the adrenal gland was not altered after treatments at all doses. Adrenocortical alkaline phosphatase activity, however, significantly

decreased at both the moderate and higher doses, with a much higher response at the higher dose (100 µg daily) than at the moderate dose (50 µg daily), with no clear change at the low dose (25 µg daily) (Fig. 1F).

- V) Aldosterone (25, 50, and 100 µg/100 g body wt. daily for 10 d)

Both the histological and biochemical findings were the same as those for corticosterone (Fig. 1F).

- VI) Norepinephrine (25, 50 and 100 µg/100 g body wt. daily for 10 d)

**Histology:**

Norepinephrine at the low dose had no effect on adrenocortical histology, but caused changes at other doses. Cell outlines were conspicuous, and nuclei appeared vesicular. Cells and nuclei were hypertrophied. Nuclear diameters increased. These manifestations were more intense at the higher dose (100 µg daily) than at the moderate dose (50 µg daily) (Figs. 1E,F).

**Biochemical changes:**

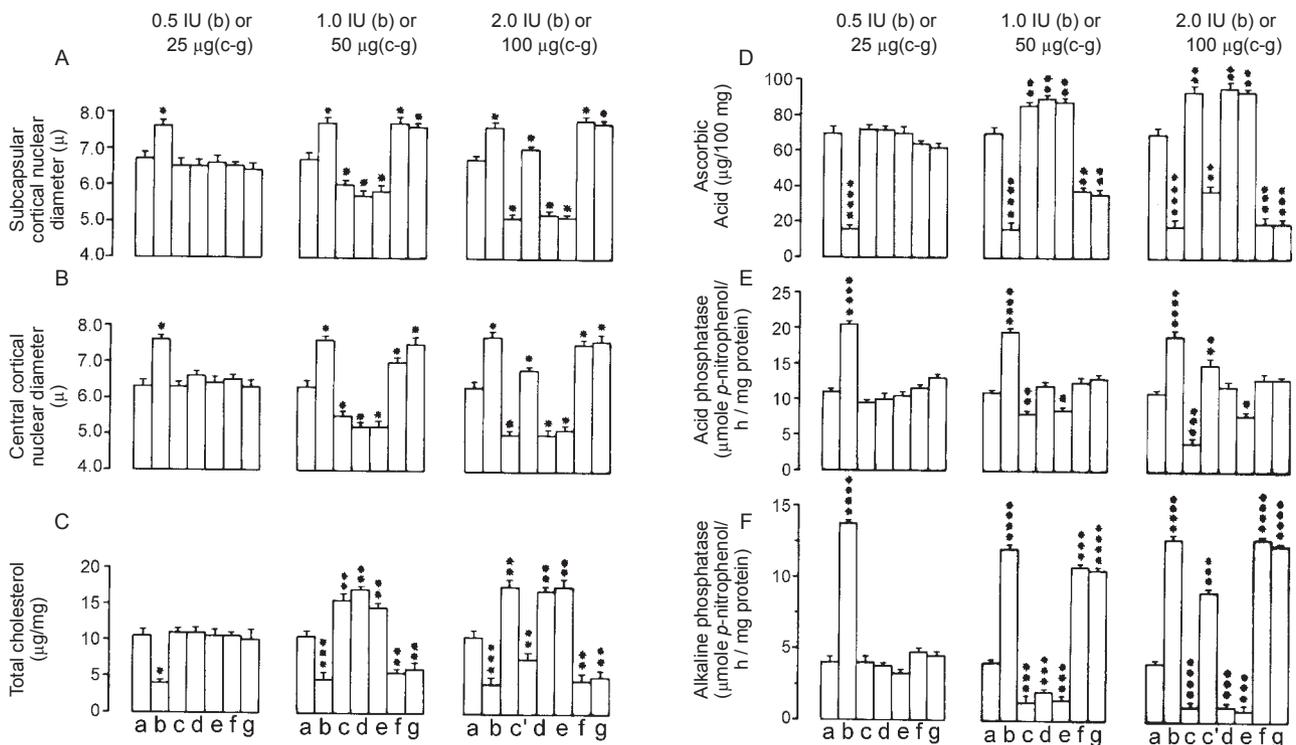
- a) Cholesterol: Cholesterol levels remained unaltered at the low dose, but decreased significantly at the other doses, with a much higher response at the higher dose (100 µg daily) than at the moderate dose (50 µg daily) (Fig. 1F).
  - b) Ascorbic acid: Results showed similar trends to those for cholesterol (Fig. 1F).
  - c) Acid and alkaline phosphatases: Acid phosphatase activity of the adrenal gland was not altered at any dose of the hormone administered. Alkaline phosphatase activity of the gland, however, significantly increased at both the moderate and higher doses, with a maximum response at the higher dose (100 µg daily) compared to that at the moderate dose (50 µg daily), with no clear change at the low dose (25 µg daily) (Fig. 1F).
- VII) Epinephrine (25, 50 and 100 µg/100 g body wt. daily for 10 d)
- Changes were the same as those for norepinephrine (Fig. 1F).

## DISCUSSION

Exogenous ACTH, corticoid, and medullary hormones can certainly modulate adrenocortical function in turtles since administration of ACTH

caused cortical stimulation as well as prevented adrenocortical atrophy induced by dexamethasone treatment. Conversely, corticosterone and aldosterone administration suppressed cortical activity, whereas both norepinephrine and epinephrine stimulated adrenocortical function. These findings were determined from cortical hypertrophy with increased nuclear diameters, a rise in phosphatase (acid and alkaline) activities as well as depletion of cholesterol and ascorbic acid levels during stimulation, while reverse changes were observed during adrenocortical suppression, because nuclear size (Miller 1952), cholesterol and ascorbic acid concentrations (Shimizu 1970, Maiti and Chatterjee 1980), and phosphatases activities

(Naik and George 1964) are indices of adrenocortical activity including cortical steroidogenesis. It is interesting to note that exogenous ACTH has no dose-dependent action unlike dexamethasone, corticoids, and medullary hormones, which do have dose-dependent actions on adrenocortical functions in turtles. Such a differential action between ACTH and adrenal hormones on adrenocortical function cannot be explained from the present results. Since the adrenal cortex is the target organ of ACTH, it is likely that the latter hormone directly stimulates adrenocortical function in turtles just like it does in other vertebrates (Chester Jones and Henderson 1978). Dexamethasone caused adrenocortical suppression in the turtle *Hydromys*



**Fig. 1.** A: Section of an adrenal gland of an untreated control juvenile turtle showing intermingled cortical and chromaffin tissues. Note the orientation of the cortical cells in the narrow adrenocortical cords with the nuclei facing towards the basement membrane. Cell outlines are inconspicuous. (Masson's trichrome stain, X500). B: ACTH-treated juvenile turtle adrenal tissue gland showing expanded adrenocortical cords with hypertrophied cells and nuclei. Cell outlines are conspicuous. The nuclei are located away from the basement membrane. (Masson's trichrome stain, X500). C: Dexamethasone-treated juvenile turtle adrenal tissue gland showing narrow cortical cords consisting of irregularly oriented atrophied cells and nuclei. Cell outlines are inconspicuous. (Masson's trichrome stain, X500). D: Corticosterone-treated juvenile turtle adrenal tissue gland showing narrow cortical cords with the atrophied cells and nuclei located towards the basement membrane. (Masson's trichrome stain, X 500). E: Norepinephrine-treated juvenile turtle shows wider cortical cords with regular orientation of cells and conspicuous cell outlines. Note the hypertrophied cells with thin nuclei located away from the basement membrane. (Masson's trichrome stain, X500). F: Histograms showing changes in nuclear diameter of adrenocortical cells, cholesterol and ascorbic acid concentrations, and acid phosphatase and alkaline phosphatase activities following ACTH, dexamethasone, dexamethasone plus ACTH, cortical, and medullary hormonal administration in juvenile turtles. Histograms represent the mean  $\pm$  SE (shown by unidirectional error bars) values (number of animals  $n = 5$  for each group). a=Control, b=ACTH, c=Dexamethasone(DS), c'=DS+ACTH, d=Corticosterone, e=Aldosterone, f=Norepinephrine, g=Epinephrine. \* $p < 0.025$ ; \*\* $p < 0.05$ ; \*\*\* $p < 0.005$ ; \*\*\*\* $p < 0.001$

*cyanocinatus* (Duggan and Lofts 1979) and also in the metyrapone recipient *Sceloporus cyanogenys* (whose adrenal cortex was hypertrophic following metyrapone treatment) (Callard and Willard 1969). Furthermore, dexamethasone is known to suppress adrenocortical function by blocking ACTH release as reported in many reptiles (Gist and Kaplan 1976). Thus, in the current study, dexamethasone might have caused adrenocortical suppression possibly by blocking ACTH release in turtles.

Exogenous corticoids appear to have suppressive action on the adrenocortical function of soft-shelled turtles, since both glucocorticoid (corticosterone) and mineralocorticoid (aldosterone) suppressed adrenocortical activity (including corticoidogenesis) as evidenced from changes that were the same as those of dexamethasone recipients (Ray 1986). It is also apparent that both corticoids have dose-dependent actions on the turtle adrenal cortex, since corticoids at higher doses caused higher degrees of adrenocortical suppression. Similar reports are also available on the suppressive actions of both corticosterone and aldosterone on the cortical activity of many reptiles including turtles (Callard 1975). As adrenocortical activity is known to depend largely upon ACTH (Chester Jones and Henderson 1978), it is likely that exogenous corticoids suppressed adrenocortical activity possibly by ACTH suppression through a negative feedback mechanism.

In contrast to the suppressive action of corticoids, adrenomedullary hormones, like ACTH, can stimulate adrenocortical activity in soft-shelled turtles, since both norepinephrine and epinephrine are reported to cause adrenocortical stimulation in a turtle (Ray 1986).

A dose-dependent action of catecholamines on adrenocortical activity has also been recorded in turtles, since these hormones had dose-dependent actions on the adrenal cortex of turtles. There is evidence that epinephrine can activate the adrenal cortex of mammals (Dallman and Jones 1973, Inaba and Kamata 1975), and the hormone under certain conditions promotes ACTH release from the anterior pituitary (Hughes 1971) with a consequent rise in certain adrenocortical steroids in mammals (Greenspan and Strewler 1997, Bentley 1998). It even stimulates the hypothalamo-hypophysial-adrenocortical axis resulting in an increased plasma corticosterone level in birds (Freeman and Manning 1979). Such a mechanism of action of epinephrine and norepinephrine might explain the present results of

adrenocortical stimulation in turtles.

It is pertinent to mention the mechanism of action of exogenous epinephrine (E) and norepinephrine (NE) on adrenocortical function in turtles. Since epinephrine and norepinephrine stimulate adrenocortical activity, it is quite likely that these adrenomedullary hormones either exert their action via ACTH or directly on adrenocortical cells or both. In this context, the receptor concept of hormone action on targets needs to be discussed. Thus E and NE ( $\beta$ -adrenergic) receptors might be present in corticotroph and/or adrenocortical cells which in turn activated corticotroph and/or adrenocortical cells and eventually stimulate adrenocortical function in turtles. Such a stimulatory action of adrenomedullary hormones is substantiated by the works of Kawamura et al. (1984) who clearly showed that epinephrine and norepinephrine can stimulate steroidogenesis in primary cultures of bovine adrenocortical cells in vitro through the  $\beta$ -adrenergic receptor. These observations support the hypothesis that E and NE might have exerted their actions directly on adrenocortical cells in the present experimental turtles. Nevertheless, such a hypothesis of direct action of E and NE on adrenocortical cells needs to be confirmed by examining turtle adrenal tissue glands.

In summary, ACTH, norepinephrine, and epinephrine can influence adrenocortical function, unlike corticoids which inhibit cortical activity possibly by a negative feedback mechanism on pituitary ACTH in turtles like other reptiles, birds, and mammals.

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