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Adrenomedullary and Glycemic Responses to ACTH, Corticosterone, Aldosterone, Epinephrine and Norepinephrine Administrations in the Soft-Shelled Turtle

Prajna P. RAY, S. CHAUDHURI-SENGUPTA and B. R. MAITI

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The aim of the current investigation was to ascertain the role of ACTH and adrenal hormones on adrenomedullary and glycemic functions in soft-shelled turtles, *Lissemys punctata punctata*. All the experiments were carried out on sexually immature animals. Findings revealed that: (1) ACTH administration (0.5 IU/1.0 IU/2.0 IU per 100 g body wt. daily for 10 days) in all doses stimulated adrenomedullary function by increasing medullary cell nuclear diameter with elevations of norepinephrine, epinephrine and blood sugar levels. Only moderate and higher doses (50 μg/100 μg per 100 g body wt. daily for 10 days) of dexamethasone suppressed adrenomedullary activity and blood sugar level by reversing the changes to those of ACTH; the responses were dose-dependent. But these changes were no longer observed after ACTH treatment in dexamethasone (DMS) recipients (DMS: 100 μg / 100 g body wt daily for the first 10 days and ACTH: 0.5 IU / 100 g body wt daily for the next 10 days); (2) Only moderate and higher doses (50 μg/100 μg per 100 g body wt daily for 10 days) of corticosterone increased adrenomedullary activity and blood sugar level and the responses were also dose-dependent. But aldosterone treatment in all doses (same as for corticosterone) had no significant effect on the adrenal medulla or blood sugar level; (3) Only moderate and higher doses of norepinephrine or epinephrine (same as for corticosterone) caused adrenomedullary atrophy with depletions of norepinephrine and epinephrine levels but elevated the glycemic level. The findings are briefly discussed.

Key words: Adrenal medulla, blood sugar, ACTH, corticosterone, aldosterone, epinephrine, norepinephrine, turtle.

Prajna P. RAY, S. CHAUDHURI-SENGUPTA, B. R. MAITI, Histophysiology Laboratory, Department of Zoology, University of Calcutta, 35 Ballygunge Circular Road, Calcutta - 700019, India. E-mail: brmaiti@vsnl.net

Exogenous ACTH is known to stimulate adrenomedullary phenylethanolamine N-methyltransferase (PNMT) activity resulting in a rise of epinephrine synthesis in inact rat (WURTMAN 1967). Hypophysectomy decreased PNMT activity and ACTH restored it in the hypophysectomized rats (WEINSHILLBOUM & AXELROD 1970). POHORECKY & WURTMAN (1971), and PARVEZ & PARVEZ (1972) have reported that in mammals the catecholamine content of the adrenal gland is maintained by high levels of corticosteroids. A positive correlation between the well developed adrenal cortex and increased levels of catecholamines has also been indicated in birds (WASSERMANN & BERNARD 1971). Exogenous corticosterone increased catecholamine content, but metyrapone (a corticosteroid blocker) decreased their levels in birds (ZACHARIASEN & NEWCOMER 1975). Both corticosterone and hydrocortisone also increased the epinephrine content of the adrenal gland of pigeons (SITARAMAN & GHOSH 1977). Catecholamine treatments increased catecholamine storage in mammals (DAIRMAN & UDENFRIEND 1971; DAIRMAN *et al*. 1972).

Adrenocorticotropin is known to induce hyperglycemia in mammals and birds (STURKIE 1976; CHESTER JONES & HENDERSON 1978; BENTLEY 1998). But in reptiles the findings are inconsistent. Hypophysectomy caused a marked hypoglycemia in *Dipsosaurus dorsalis* (FOGLIA *et al.* 1955, CALLARD & CHAN 1972) but leads to only a mild hypoglycemia in *Eumeces obsoletus* (MILLER & WURSTER 1959). While ACTH was ineffective in inducing hyperglycemia in *Alligator mississippiensis* (COULSON & HERNANDEZ 1964) and snake, *Xenodon* (HOUSSAY & PENHOS 1960), but effective when infused slowly in *Emys orbicularis*

(VLADESCU 1965), the South American Caiman *Caiman sclerops* (GIST 1972) and *Dipsosaurus dorsalis* (CALLARD *et al.* 1975). Corticoids (especially glucocorticoids) are well known hyperglycemic agents in mammals, birds and reptiles (STURKIE 1976; CHESTER JONES& HENDERSON 1978; BENTLEY 1998). Moderate to intense effects of cortisone, cortisol, hydrocortisone and corticosterone on blood glucose level have also been reported in alligators (COULSON & HERNANDEZ 1964) and lizards (VLADESCU *et al.* 1970; CALLARD & CHAN 1972). But aldosterone has no such effect on blood glucose level in *Dipsosaurus dorsalis* (CALLARD & CHEN 1972), and both aldosterone and corticosterone are ineffective in *Anolis* (GIST 1978).

Infusion of epinephrine and norepinephrine produced marked hyperglycemia in mammals and birds (STURKIE 1976; CHESTER JONES & HENDERSON 1978; BENTLEY 1998). Exogenous epinephrine is also known to be hyperglycemic in ophidians (PRADO 1947), alligators and lizards (AKBAR *et al.* 1978), and also in a turtle species, *Pseudemys d'orbignyi* (LOPES *et al.* 1954).

Interdependence of adrenomedullary activity on the adrenal cortex is fairly well established in mammals and birds unlike poikilotherms, especially in turtles amongst reptiles. Besides, adrenomedullary hormones are directly related to carbohydrate metabolism, vital for their survival. Therefore, in the current article, this problem has been resolved in a species of soft-shelled turtle, *Lissemys punctata punctata*.

Material and Methods

Experiments were conducted in female specimens as they were abundantly available especially in the winter season. Fifty healthy juvenile female turtles (body weight: 250-300 g), *Lissemys punctata punctata* (Bonnoterre), were procured from natural populations near Calcutta in January. Sexually immature specimens were selected for the present experiment since their endogenous hormonal level was low (SEN & MAITI 1988). Consequently adrenomedullary hormonal changes after hormonal administrations could be largely due to exogenous hormones. Animals were maintained in aquaria (5′x 3′x 3′) in small groups (5 per aquarium) in controlled laboratory conditions (temperature 25°C and photoperiod, 11L: 13D). Experiments were conducted in temperature and photoperiod conditions as found in the natural environment at the time of experiments in winter in Calcutta. Food (*Tubifex* and shrimp) was given *ad libitum* throughout the experiments. Turtles were kept in the laboratory for 5 days prior to experiments. Hormones were dissolved in appropriate solvents (ACTH: 0.68% saline, Norepinephrine and Epinephrine: ethanol-saline and corticoids: oil). Each hormone was dissolved 0.1 ml of solvent and injected intramuscularly in alternate hind limbs of animals. Each hormone/drug was administered in three different doses (low: 0.5 IU ACTH / 25 μg for others, moderate: 1.00 IU ACTH / 50 μg for others and high: 2 IU ACTH / 100 μg for others per 100 g of body weight daily to 3 different groups of turtles consecutively for 10 days. Control animals received solvents (0.68% saline / ethanol--saline / oil) without hormones for a similar duration. All animals were killed by decapitation 24 hrs after the last injection (on day 11 of the experiments), except Groups III and IV which were autopsied 20 days (first 10 days for DMS and next 10 days for ACTH) after the treatments, at a particular time of day i.e., 10 a.m. to avoid effects due to diurnal rhythms (CHOUDHURY *et al*. 1982). Adrenal glands were quickly dissected out and left adrenals were fixed in Bouin's fluid and processed for routine microtomy. 5 μm thick paraffin sections were prepared and stained by Masson's trichrome technique for histological study. The nuclear diameter (μm) of adrenomedullary cells was measured by an ocular micrometer from subcapsular and central zones of the gland. One hundred nuclei each for the subcapsular and central zones were considered from 10 widely separated random sections of the adrenal gland of each specimen. Only those nuclei that had a complete nuclear membrane outline were considered (ABERCROMBIE 1946).

Norepinephrine and epinephrine concentrations were studied from the remaining (right) adrenal glands. As catecholamines, especially norepinephrine, are also substantially produced by extramedullary sources and are delivered into the general circulation (MAHAPATRA *et al*. 1989; MAHATA & MAHATA 1992; WILSON *et al.* 1998; RAY & MAITI 2001), these hormones were studied in the homogenates of 2 adrenal glands, instead of blood plasma. Catecholamines were extracted, purified (COX & PERHACH 1973) and measured by the method of LAVERTY and TAYLOR (1968) in a HITACHI fluorescence spectrophotometer (Model 650-10M). Blood was drawn from the heart of specimens (unfed for 10 h) by a heparinised syringe prior to autopsy and glucose levels were measured immediately by the method of BERGMEYER and BURNT (1963). The colorimetric samples were measured in duplicate on a PERKIN-ELMER spectrophotometer (550 S, West Germany). All data were analysed by one way analysis of variance (ANOVA) and those were found significant F: 0.01 were further analysed by Students' *t*-test (SNEDECOR & COCHRAN 1971).

Results

Results of adrenomedullary histology, hormonal concentrations, and blood sugar level did not show wide variations among different groups of control animals whether they received saline, ethanol-saline or oil. Therefore, the findings observed in all the control animals were pooled together and served as a single control group for all experiments.

The gross histology of the adrenal gland of *Lissemys* turtles has been described earlier (RAY *et al.* 1987). Adrenomedullary islets were intermingled with cortical cords. The medullary cells showed a granulated cytoplasm. Norepinephrine and epinephrine concentrations of the adrenal gland and blood glucose level of control turtles are presented in Figure 2.

Treatments

(I) ACTH Treatment (0.5 I.U. or 1.0 I.U. or 2.00 I.U. per 100 g body weight daily for 10 days)

Histology. The adrenomedullary cells were hypertrophied and showed cytoplasmic degranulation with increased nuclear diameter. These changes were the same in all the doses of the hormone administered (Fig. 1).

Catecholamines. Both norepinephrine (NE) and epinephrine (E) levels of the adrenal gland increased significantly after ACTH treatment, and the degree of response was nearly the same in all doses (Fig. 2).

Blood glucose. The level of blood glucose increased after ACTH treatment, but the values were the same under all doses of the hormone administered (Fig. 2).

(II) Dexamethasone Treatment (25 μg or 50 μg or 100 μg per 100 g body weight daily for 10 days)

Histology. The medullary cells were loaded with cytoplasmic granules and their nuclear diameter decreased only in moderate (50 μg) or higher (100 μg) doses but not in the lower dose (25 μg) and the degree of response was dose-dependent (Fig. 1).

Catecholamines. Both norepinephrine (NE) and epinephrine (E) contents of the adrenal gland declined after dexamethasone treatment only in moderate and higher doses and the degree of response was dose-dependent (Fig. 2).

Blood glucose. The level decreased significantly only in moderate and higher doses and the degree of response was dose-dependent (Fig. 2).

(III) Dexamethasone (DMS: 100 μg per 100 g body weight daily for first 10 days) plus ACTH (0.5 I.U. per 100 g body weight daily for next 10 days)

Histology. Catecholamines (NE and E) and blood glucose levels were similar to those of ACTH alone (Figs 1 and 2).

(IV) Corticosterone (25 μg or 50 μg or 100 μg per 100 g body weight daily for 10 days)

Histology. The medullary cells were hypertrophied and degranulated, and their nuclear diameter increased only in moderate or higher doses and the degree of response was dose-dependent (Fig. 1).

Catecholamines. Norepinephrine (NE) and epinephrine (E) levels increased only under moderate or higher doses and the degree of response was dose-dependent (Fig. 2).

Blood glucose. The level increased only in moderate and higher doses and the degree of response was dose-dependent (Fig. 2).

(V) Aldosterone (25 μg or 50 μg or 100 μg per 100 g body weight daily for 10 days)

Histology. No conspicuous histological changes were seen in the adrenomedullary cells under any dose of aldosterone treatments (Fig. 1).

Catecholamines. Norepinephrine (NE) and epinephrine (E) concentrations of the adrenal gland were not significantly altered under any dose in the treatments (Fig. 2).

Blood glucose. The level remained unchanged under all the doses of aldosterone treatments (Fig. 2).

(VI) Noradrenaline (25 μg or 50 μg or 100 μg per 100 g body weight daily for 10 days)

Histology. Adrenomedullary cells were densely granulated and atrophied, and their nuclear diameters decreased only under moderate or higher doses. These manifestations were clearly visible under the higher dose of the hormone (Fig. 1).

Catecholamines. Norepinephrine (NE) and epinephrine (E) levels of the adrenal gland declined only under moderate and higher doses and the degree of response was dose-dependent (Fig. 2).

Blood glucose. The level increased significantly only under moderate and higher doses and the degree of response was dose-dependent (Fig. 2).

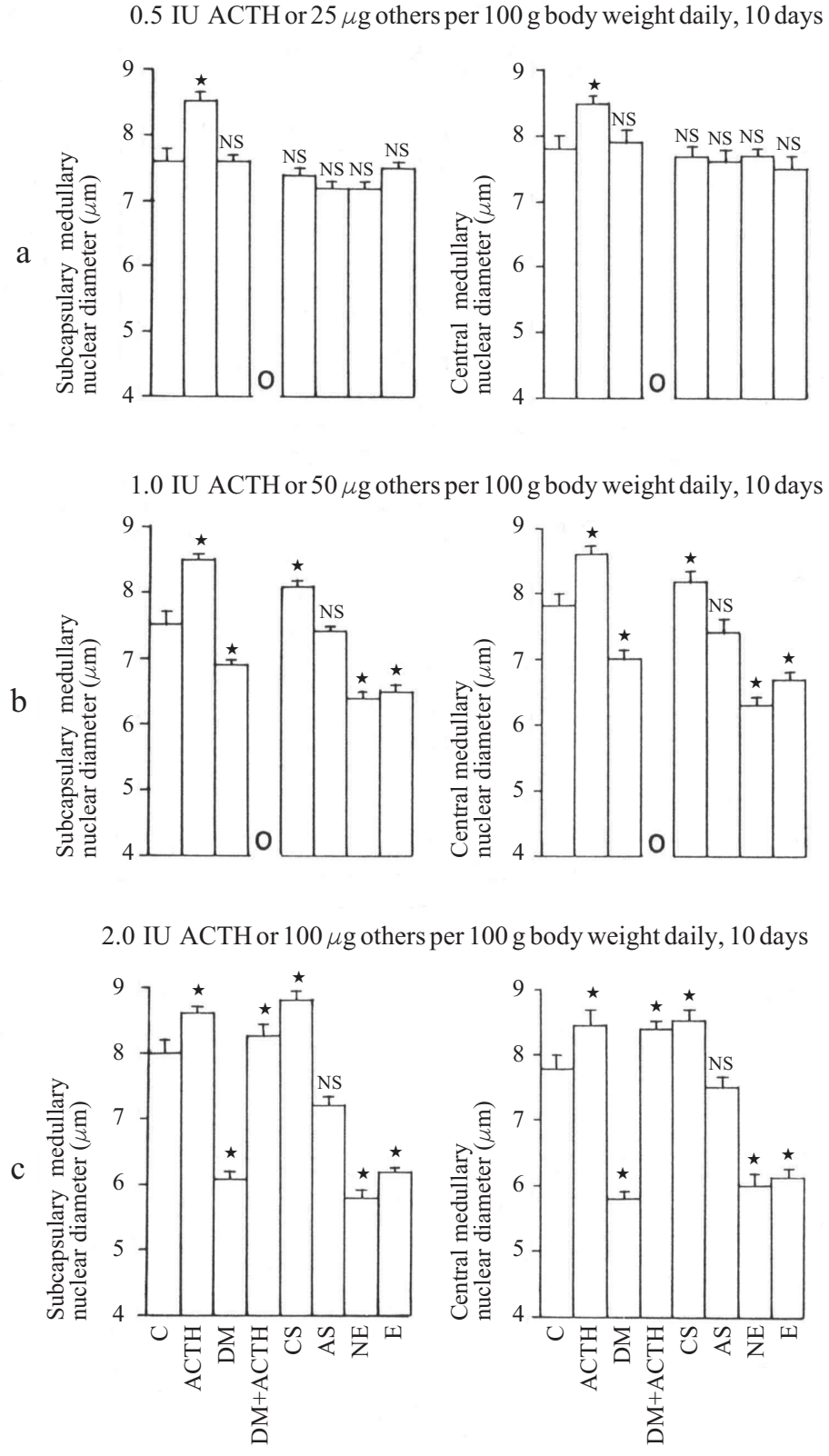

Fig. 1. Histograms show changes in nuclear diameters of the subcapsular and central zones of the adrenal gland following treatments of hormones (ACTH, dexamethasone, dexamethasone plus ACTH, corticosterone, aldosterone, norepinephrine and epinephrine in three different doses (a – low, b – moderate and c – high) in turtles. Histograms represent mean values. Unidirectional vertical bars represent SE. O – Dexamethasone and ACTH were not administered simultaneously in low or moderate doses, but only in higher doses in order to achieve maximum effects on adrenal medulla and glycemia in turtles. (C – control, ACTH – Adrenocorticotropic hormone, DM – Dexamethasone, CS – Corticosterone, AS – Aldosterone, NE – Norepinephrine, E – Epinephrine). Statistical analysis by ANOVA : * $P<0.025$, ** $P<0.01$, *** $P<0.05$, **** $P<0.005$, ***** $P<0.001$. NS – Not significant.

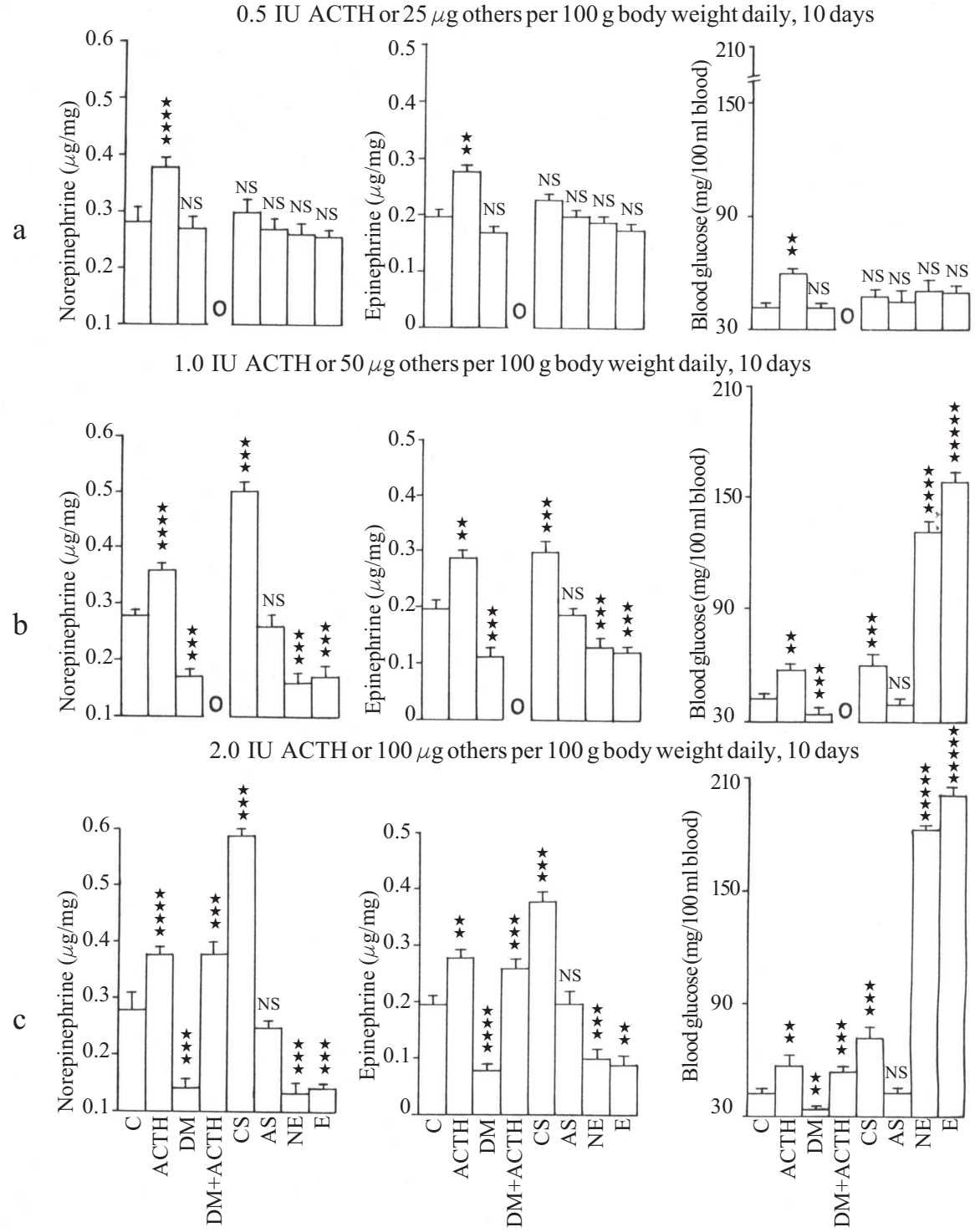

Fig. 2. Changes in the concentrations of epinephrine, norepinephrine and blood glucose following treatments of hormones in 3 different doses (a – low, b – moderate and c – high) in turtles. For other legends see Fig. 1.

(VII) Epinephrine (25 μg or 50 μg or 100 μg 100 g body wt daily for 10 days).

The results of histological examination, catecholamine and blood glucose levels were the same as those of norepinephrine (Fig. 1).

Discussion

Adrenal medulla

Exogenous corticotropin, corticoids (corticosterone and aldosterone) and adrenomedullary

hormones (norepinephrine and epinephrine) modulated adrenomedullary activity in soft-shelled turtles. Exogenous ACTH caused adrenomedullary stimulation by inducing adrenomedullary cell hypertrophy with increased nuclear diameter followed by a rise in both norepinephrine and epinephrine titres. In reptiles (YIP 1974), birds (BHATTACHARYYA & GHOSH 1965) and mammals (MILLER 1952), cellular activity is expressed by nuclear size. The increased nuclear diameter of adrenomedullary cells may be indicative of cellular hypertrophy in the experimental turtles. But the treatment with dexamethasone suppressed adrenomedullary activity by causing reverse changes to those of ACTH. Activity was restored by ACTH treatment in the dexamethasone recipients. It is known that L-dopamine hydroxylase helps in the conversion of L-dopamine to norepinephrine which in turn is converted to epinephrine by the PNMT enzyme (Phenylethanolamine-N-methyltransferase) (AXELROD 1975). Exogenous ACTH is known to stimulate PNMT activity (WURTMAN 1966) which is decreased following hypophysectomy and is restored by ACTH treatment in rats (WEINSHILLBOUM & AXELROD 1970). However, dexamethasone is known to inhibit ACTH release in reptiles (GIST & KAPLAN 1976). Thus, in the current investigation, exogenous ACTH increased adrenomedullary hormonal level presumably by stimulating L-dopamine -hydroxylase (DBH) and PNMT activities in normal animals. In turtles, the decrease in adrenomedullary hormonal level could be due to a suppression of DBH and PNMT activities via dexamethasone – induced inhibition of ACTH release.

Exogenous corticosterone also stimulated adrenomedullary activity by inducing changes that were similar to those of ACTH in soft-shelled turtles. In contrast, aldosterone treatment had no significant effect on the adrenomedullary activity of turtles. There is evidence that catecholamine levels (norepinephrine and epinephrine) of the adrenal gland depend largely upon the maintenance of high levels of corticosteroids in mammals (POHORECKY & WURTMAN 1971; PARVEZ & PARVEZ 1972). Exogeneous corticosterone has also been shown to increase adrenal catecholamine levels, but metyrapone treatment (a corticoid blocker) reduced the adrenomedullary hormonal level in birds (WASSERMANN & BERNARD 1971; ZACHARIASEN & NEWCOMER 1975). Therefore, the current findings in turtles corroborate those of earlier works in homeothermic animals. As corticosterone is known to stimulate adrenomedullary PNMT activity in mammals (POHORECKY & WURTMAN 1971), a similar mechanism of action of the corticoid might explain the experimental results in turtles. The failure of aldosterone to influence adrenomedullary activity could be due to its inability to involve ACTH or corticosterone in turtles.

Administrations of norepinephrine and epinephrine suppressed adrenomedullary activity in soft-shelled turtles, since treatments of both catecholamines induced adrenomedullary atrophy manifested by decreased nuclear diameter with depletions of norepinephrine and epinephrine levels from the adrenal gland. However, in mammals, catecholamine treatment has been reported to increase the accumulation of these hormones (DAIRMAN *et al.* 1972), possibly by blocking their release into circulation. Since the sympathetic nervous system largely controls adrenomedullary function (GREENSPAN & STREWLER 1997), their involvement in suppressing adrenomedullary hormonal synthesis following epinephrine or norepinephrine treatment cannot be ruled out in the experimental turtles. But the release of E and NE was not inhibited because their levels were not increased in the adrenal gland after the treatments of NE and E in turtles.

All the hormones and dexamethasone, except ACTH, had dose-dependent action on adrenomedullary function in turtles, since the medullary response increased with an increase in the dose of the hormone/dexamethasone administered, unlike ACTH which had a similar magnitude of response even with increased dose of ACTH.

Blood glucose

The glycemic level in soft-shelled turtles also appears to be modified by the interplay of several hormones. Administration of ACTH caused hyperglycemia, but dexamethasone treatment decreased the glycemic level, while ACTH therapy restored it in the dexamethasone recipients. Corticosterone, like ACTH, also had an hyperglycemic effect, aldosterone did not take part in sugar metabolism. Both norepinephrine and epinephrine also caused hyperglycemia despite their depletions from the adrenal medulla after norepinephrine (NE) and epinephrine (E) treatments in turtles. The present result of hyperglycemia after treatments of catecholamines is quite plausible, since exogenous hormones (epinephrine and norepinephrine) themselves are highly hyperglycemic (BENTLEY 1998). However, all the above hormones, except ACTH, had dose-dependent actions on glycemia in turtles, because the glycemic response was increased with an increase in the dose of the hormones administered, whereas exogenous ACTH in all doses had the same magnitude of response on glycemia in turtles. Such differential response in respect of the dose of hormones between

ACTH and other hormones cannot be explained from the present experiments. Hypophysectomy has been reported to cause a marked decrease in the blood glucose level in turtles (FOGLIA *et al.* 1955) and other reptiles, *Dipsosaurus dorsalis* (CALLARD & CHAN 1972), but ACTH treatment caused hyperglycemia in many reptiles including turtles (CALLARD *et al.* 1975). Additionally, ACTH, corticosterone, epinephrine and norepinephrine are all known to be strong hyperglycemic hormones in homeothermic animals (BENTLEY 1998), and thus earlier findings corroborate the current findings in turtles. Since both ACTH and corticosterone treatments increased norepinephrine and epinephrine levels, these hormones (ACTH and corticosterone) might have exterted their actions directly and/or by involving catecholamines in inducing hyperglycemia in turtles. The glycogenolytic property of epinephrine has also been confirmed in domestic fowl by DICKSON *et al.* (1978). Additionally, glucagon is known to be a strong hyperglycemic agent in vertebrates (BENTLEY 1998), and catecholamines are known to induce the release of glucagon from the pancreas, which in turn stimulates glycogenolysis and consequently induces hyperglycemia in the rat (LECLERQ-MEYER *et al.* 1971) and the duck (TYLER *et al*. 1972). Therefore, glucagon mediated action of adrenomedullary hormones in inducing hyperglycemia cannot be ruled out in the present study. The current findings also indicate that norepinephine and epinephrine are more potent hyperglycemic hormones than all other hormones examined, since catecholamines produced maximum hyperglycemic effects as compared to those of other hormones, thereby indicating that norepinephrine and epinephrine are the principal hormones that largely regulate blood glucose level in turtles, as in birds (GHOSH 1977).

In essence, both ACTH and corticosterone can independently stimulate adrenomedullary function possibly through stimulation of medullary enzymes (DBH and PNMT) resulting in a rise of norepinephrine and epinephrine levels with a concomitant rise in the level of blood glucose, thereby indicating their role in carbohydrate metabolism, whereas aldosterone and medullary norepinephrine and epinephrine are not normally involved in adrenomedullary function in turtles. Furthermore, interdependence of adrenomedullary activity upon adrenal cortex (glucocorticoids) is confirmed in turtles, as in homeothermic animals.

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