

REVIEW ARTICLE**Avian adrenal medulla: cytomorphology and function**Asok Ghosh, Stephen W. Carmichael^{1*}, Monisha MukherjeeDepartment of Zoology, University of Calcutta, Calcutta, India, ¹Department of Anatomy, Mayo Clinic/Foundation, Rochester, Minnesota, USA

ABSTRACT The purpose of this review is to explore the world literature on the avian adrenal medulla from the last 20 years. Unlike the mammalian adrenal medulla, the adrenal gland in birds has chromaffin cells mixed with cortical cells. Studies have investigated the ultrastructure (both transmission and scanning electron microscopy), biochemistry, and physiology (particularly interactions with other endocrine glands) of the avian adrenal medulla. Although progress has been made, it is apparent that research on the avian adrenal medulla still lags behind work on the mammalian organ.

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The adrenal glands of birds, like those in mammals, are paired yellow- or orange-colored pear- or triangle-shaped glands that are next to the kidneys. The intermingling nature of cortical and medullary components constitutes a major characteristic of avian adrenal medulla (Vestergaard and Willeberg 1978). This is in sharp contrast with its mammalian counterpart, where the medulla always occupies the central portion and remains encircled by the adrenal cortex with its three concentric zones.

Compared to the adrenal cortex, the avian adrenal medulla was a much-neglected subject until the 1950s. Ghosh (This will refer to Asok Ghosh. His son, also an active scientist in the field, will be referred to as Subho Ghosh) and his collaborators pioneered efforts to elucidate various aspects of avian endocrinology, particularly the adrenal gland (Ghosh 1977). This first review article on this subject was published by Ghosh, that covered works up to 1977. Later, a second review by Ghosh in 1980 gave literature coverage from 1978 to 1980. The present article will emphasize the world's literature on the avian adrenal medulla from 1981 through 2000.

Ghosh and his collaborators (see Ghosh 1977, 1980) studied the relative proportion of epinephrine (E)- and norepinephrine (NE)-secreting cells and cellular areas in the adrenal medulla of a number of birds representing several orders and families. The data shows extreme variation in the relative concentration of E and NE cells in avian adrenal medulla that is not seen in other vertebrate groups. Light and ultrastructural studies (Subho Ghosh 1977) revealed that the orders with a more primitive ancestry contain more NE cells, whereas the more recently evolved birds possess more E cells

in the adrenal medulla. This profound variation of medullary E/NE ratio in birds suggests a distinct evolutionary pattern (Ghosh 1977, 1980). The avian phylogeny used in this study was essentially based on palaeontological evidences (Gregory 1957). We feel that our "claim" of hormonal taxonomy is to be re-examined in view of cladistic (Cracraft 1988) and molecular (Mindell 1997) analyses of avian taxa (also Bhattacharyya, B. 1999, personal communication). Later Mahata and Ghosh (1986a, c, 1988) reported that NE and E contents vary not only between birds of different phylogenetic groups, but also with the age of birds of the same species.

I. Morphological considerations**A. Development**

Elegant work from Unsicker's laboratory (Ross et al. 1995) shows that by embryonic day 15 (E₁₅) sympathetic ganglia of chick embryos contain a glucocorticoid-responsive progenitor population that can differentiate into medullary cells. Another important work by Sanchez-Montesinos et al. (1996) examined the development of the chick sympathoadrenal system by identifying antibodies that recognized signal molecules. Three expression patterns were found in the developing adrenal gland defining early permanent markers (chromogranins A and B, GaO, TH, and galanin); others (DbH, somatostatin, enkephalin, secretogranin 11, NPY, and PNMT) follow as development proceeds.

B. Subcellular morphology

During the last three decades, the cytophysiology of the avian adrenal medulla has been thoroughly worked out by Ghosh and his associates by means of histological, histochemical (Ghosh 1980), transmission (TEM; Ghosh et al. 1996a), and scanning (SEM; Guha et al. 1990) techniques.

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Maitra and Ghosh (1980) differentiated the two types of tinctorially different adrenomedullary cells in the plum-headed parakeet (*Psittacula cyanocephala*) by application of a modification of the Wood (1963) technique. The E cell islets become densely granulated and reddish-brown colored, whereas the NE cells are homogeneously yellowish in color, smaller in size, and fewer in number. The E- and NE-secreting cells are also different on the basis of their morphology, size, opacity, and fine structure of the vesicles (see Unsicker 1973a, b, c; Ghosh and Guha 1988).

Unsicker (1973a, b, c) reported that E and NE cells could be distinguished from each other on the basis of their structure and granulations. E cells exist in all avians, but in corvids (includes crows, ravens, rooks, magpies, and jackdaws) and in some passers, they possess low electron dense vesicles and lack complete membranes. The average size of NE vesicles in domestic chicken (*Gallus domesticus*) is greater (224 nm) than the E (168 nm) vesicles (Coupland 1971). The E and NE vesicles follow a similar pattern in the rose-ringed parakeet (*Psittacula krameri*; Carmichael et al. 1983) and in duck and geese (Guzsal and Hassan 1975). In the migratory snipe (*Capella gallinago*), only NE types of chromaffin cells are observed (Carmichael et al. 1985). In pigeons (*Columba livia*) and bulbuls (*Pycnonotus cafer*), two distinct cell types are present. Although the vesicles of cells in pigeons do not manifest size difference, in bulbuls the E vesicles are comparatively large (Ghosh and Guha 1988). Cuello (1970) found a wide range of sizes of NE vesicles (80-500 nm) in the gentoo penguin (*Pygoscelis papua*). The TEM studies by Guzsál and Hassan (1975) reveal presence of the so-called "dark cells" and the transitory sympathicoblasts in the adrenal medulla of ducks and geese.

TEM studies of adrenal gland enables differentiation of the E and NE cells by their morphology, size, opacity, and fine structure of the vesicles (*vide supra*). But, in contrast to the mammalian adrenal medulla, it has not been resolved whether E and NE cells originate from the same, or two separate cell types, of the adrenal medulla (Ghosh and Guha 1988). Recently, an electron microscopic study of the chromaffin cells in the adrenal gland of 13 species of birds from the Indian subcontinent was performed by Subho Ghosh et al. (1996a). The study revealed that the cytoarchitecture of the adrenal medulla is similar in many respects in all the birds. However, differences were noted in the density of chromaffin vesicles, subunit organization in certain vesicles, compartmentalization of electron dense vesicles and electron lucent vesicles in a single cell, presence of four cell types (dark, clear, vacuolated, and mixed). There were also some differences among species in the prominence of the interface between the cortical and medullary tissues and in the frequency of exocytotic profiles.

The three-dimensional surface information of the adrenomedullary parenchymal cells is available from studies

on woodpecker, kingfisher, parakeet, and common snipe (Guha et al. 1990). The presence of blebs, cords, globules, granular particulates, filamentous, and coral-like aggregates on the adrenal surfaces also show great inter-specific variations. In the rose-ringed parakeet, SEM observations reveal densely granulated medullary cells, and a cytoplasm containing a single central or eccentric nucleus. Prominent microvillus-like projections are seen that appear to connect nucleus with surrounding cytoplasm. The vesicles are oval, round, or cylindrical and are frequently seen to fuse with one another. Also there occurs conglomeration of bleb-like structures in the blood spaces. The endothelium has rufflings and breakages at points from which blebs appear to be discharged (Carmichael et al. 1983).

C. Immunocytochemistry

By immunocytochemistry, it is suggested by Ohmori et al. (1997) and Ohmori (1998) that serotonin, galanin, cholecystokinin, met-enkephalin, somatostatin, and natriuretic polypeptides exist in chicken chromaffin cells, in addition to E and NE. Different biogenic amines and neuropeptides localized in medullary cells may regulate corticosterone secretion from interrenal cells via a paracrine mode of communication (*cf.* Nussdorfer 1996).

II. Biochemical aspects

A. Biosynthesis

Aspects of biosyntheses have been discussed earlier (Ghosh 1977, 1980). The dopamine antagonist haloperidol stimulates aldosterone and corticosterone secretion in rats (Goebel et al. 1992) and inhibits specific high affinity binding sites in the membrane of adrenal medulla (Rogers et al. 1989). Recent work in rats by Mukherjee et al. (1995) shows significant release of corticosterone following haloperidol treatment. The catecholamine content of adrenal medulla remains non-responsive, probably due to the specific blocking of the D₂-dopamine receptors. This aspect has remained unresolved in avians and needs intense attention.

Studies on rats show that the enzyme phenylethanolamine N-methyltransferase (PNMT) is localized in the E cells only. Using an immunocytochemical technique, our laboratory group (Guha et al. 1992) indicates that PNMT location is similar at least among phylogenetically close avian species. Unlike mammals, no particular cell specificity for this enzyme exists in birds. From their studies, it may be conjectured that PNMT does not act as a marker enzyme in differentiating E and NE cells. Rather, it is presumed that PNMT exists in all adrenomedullary chromaffin cells of avians, and only in some cells it exists in an active form and plays role in conversion of NE to E.

Ascorbic acid acts as co-factor in the α -hydroxylation of dopamine, which is the rate-limiting step in the synthesis of

NE (Subramanian 1980). Experimental administration of vitamin C in the red-vented bulbul (*Pycnonotus cafer*; Ghosh and Chatterjee 1985) shows significant rises of NE and fall in E within the adrenal medulla. This indicates that vitamin C lowers glucocorticoid levels necessary for methylation process (cf. Pohorecky and Wurtman 1971). In experimentally scorbutic bulbuls (Chatterjee and Ghosh 1982), E content of adrenal medulla becomes conspicuously high as compared to its non-methylated counterpart, NE. In avians, vitamin C also plays role in the release of catecholamines (mainly NE) and possibly, like mammals, it is also involved in the catabolism of NE to normetanephrine (Ghosh and Chatterjee 1986).

B. Phospholipid profile

Although study of adrenal phospholipid has been fairly well known in mammals, a systematic work in this field was not documented in avians until the early sixties (Ghosh 1962). Recently, the phospholipid content of the adrenal of nine bird species belonging to different phylogeny was worked out by Chakrabarti et al. (1996). Eight different adrenal phospholipid fractions (phosphatidic acid, cardiolipin, phosphatidyl ethanolamine, phosphatidyl glycerol, lecithin, sphingomyelin, lysolecithin, and phosphatidyl inositol) were identified. The study shows that percentage composition of phospholipids, excepting phosphatidyl inositol, does not manifest a difference among species. The major phospholipids of the medulla, phosphatidyl ethanolamine, lecithin, and sphingomyelin, are suggested to play significant role in the secretory process of the chromaffin cells.

C. Release mechanisms

It is well established in mammals that the mode of adrenomedullary secretion is exocytosis (Diner 1967). In birds, however, the process of medullary granular extrusion is not yet understood as clearly (Carmichael et al. 1985, 1987). Unsicker (1973a, b, c) studied the ultrastructure of the adrenal medulla of a number of avian species and found that only the chaffinch (*Fringilla coelebs*) exhibits exocytosis. In a comparative TEM study of the adrenal medulla of thirteen subtropical birds, Subho Ghosh et al. (1996a) observed "exocytotic profiles" only in woodpecker (*Dinopium benghalense*) and kingfisher (*Halcyon smyrnensis*). Very recently, Subho Ghosh et al. (1999a) further found no "exocytotic figures" of the adrenomedullary chromaffin vesicles after treatment of the domestic pigeon (*Columba livia*) with lithium chloride (LiCl). It is, therefore, still not proven that exocytosis is the only mechanism of secretion of chromaffin vesicles in birds, contrary to what had earlier been indicated by Chungsmarnyart and Fujioka (1982). The intricate problem of the release mechanism of avian adrenomedullary chromaffin vesicles has yet to be resolved.

For example, it has been found that G₀ protein is associated with the mammalian chromaffin cell membranes. Activation of this protein causes inhibition of exocytosis. When the G₀ protein is inactivated, exocytosis takes place (Vitale et al. 1994). Studies such as this needs to be done to better appreciate if exocytosis is the mechanism of secretory release in birds.

C. Role of monoamine oxidase isozymes

The recent study of Subho Ghosh et al. (1995) reveals that the adrenal medulla of pigeons possess 60% type A and 40% type B isozymes of monoamine oxidase (MAO). Application of pargyline (a MAO-B inhibitor) or clorgyline (MAO-A inhibitor) together with guanithidine sulfate (an adrenal catecholamine releaser) in pigeons reveal that guanithidine action on adrenal medulla becomes more prominent when the MAO-B isozyme is inhibited, suggesting that MAO-B suppresses catecholamine release.

D. Role of lithium

Lithium (Li) has a well-documented efficacy in psychiatric disorders. The salts of Li act as a mood-stabilizer and thus is used for treatment of manic depression. Although the effects of Li on peripheral endocrine glands have been elucidated in recent years, literature on its role on the adrenal gland is scarce. Recently, Subho Ghosh et al. (1997b) have shown that in pigeons (*Columba livia*) Li reduces tissue stores of catecholamines significantly by activating MAO. Pargyline completely blocks Li-induced depletion of catecholamines from pigeon adrenal medulla, whereas clorgyline fails to exhibit a response. It is thought that in pigeon, the inhibitor of MAO-A and -B isozymes act in a different pattern. Li reduces the catecholamine content of adrenal medulla by utilizing MAO-B.

An integrated approach involving ultrastructural (TEM), histologic, and biochemical probes were employed to study the effect of Li on the pigeon adrenal medulla (Subho Ghosh et al. 1999a). Both E and NE vesicles were found to decrease after treatment with Li. The overall degeneration, i.e. atrophy, of the medulla along with significant depletion of the catecholamines (E and NE) was observed after lithium chloride (LiCl) administration. It was shown that LiCl had a degenerative effect on the pigeon adrenal medulla.

Of the eight phospholipid fractions studied, only two fractions (phosphatidyl glycerol and phosphatidyl ethanolamine) showed significant changes (increase and decrease, respectively) as a result of LiCl treatment. Absence of any detectable change in phosphatidyl inositol content of Li-treated birds suggested different modes of Li action in the adrenal medulla of birds and mammals (Chakrabarti et al. 1999).

The effect of LiCl on the adrenal hormones was studied during experimental hyperthyroidism in the pigeon. It revealed that experimentally altered thyroid function had no effect on the adrenomedullary catecholamines, but significantly increased the corticosterone level. The investigation suggested that lithium has a different effect on the adrenal cortex and medulla during hyperthyroidism (Subho Ghosh et al. 1999b).

III. Physiological considerations

A. Neural regulation of catecholamine release

(a) Role of the splanchnic nerve

The chromaffin cells of adrenal gland in homeothermic vertebrates are mainly innervated by preganglionic sympathetic nerve fibers that travel in a splanchnic nerve (Coupland 1971). Mahata and Ghosh (1986b) presented the first evidence of neural regulation of catecholamine release in birds (pigeon, *Columba livia*). They showed that splanchnic denervation of the adrenal medulla has no significant effect on adrenal weight, medullary histology, NE fluorescence, and catecholamine content in a species of bird (Mahata and Ghosh 1986b). However, splanchnic nerve modulates adrenomedullary functions under various experimental conditions. Splanchnic denervation in the pigeon (*Columba livia*) and duck (*Anas querquedula*) fails to affect reserpine-induced depletion of E, whereas it prevents reserpine-induced depletion of NE only at a low dose of reserpine (Mahata and Ghosh 1989). Unilaterally denervated pigeons treated with reserpine and protein hormones show that the splanchnic nerve modulates synthesis, release, and resynthesis of catecholamines in the avian adrenal medulla (Mahata and Ghosh 1991a). The presence of adrenergic nerve fibers in the avian adrenal medulla has been demonstrated from exhaustive work on fifteen avian species. All of these studies indicate that post-ganglionic adrenergic nerve fibers are exclusively associated with NE cells, and thus the splanchnic nerve plays a vital role in the synthesis of NE, but not E (Mahata and Ghosh 1988).

To determine neural regulation of adrenomedullary functions in birds, Mahata and Ghosh (1989) applied reserpine at three doses (0.05, 0.2, and 0.8 mg/100 g body weight) to seven unilaterally splanchnic denervated birds. Results show that reserpine at high doses cause 66-92% depletion of total catecholamines from both innervated and denervated gland in all species investigated. A low dose of reserpine depleted 40-84% of catecholamines from the adrenal gland in passerine birds (common myna and bulbul) whether their nerve supply was intact or not. However, in non-passerine birds (duck and pigeon), a low dose of reserpine depleted 66-71% of NE from innervated gland as compared to only a 2-13% reduction from denervated glands. In these birds, E

depletion was 60-85% that was independent of neural regulation. The findings suggest that at high dose of reserpine, depletion of catecholamines is controlled by some non-neurogenic mechanism. At a low dose of reserpine, the splanchnic nerve probably modulates depletion of catecholamines from the adrenal medulla of non-passerine birds, whereas the same is governed by some non-neurogenic mechanisms in the passerine birds. Role and Perlman (1983), Wakade and Wakade (1983), Marley et al. (1985), and Khalil et al. (1986a,b, 1987) also reported that in mammals both neural and non-neural mechanisms are involved in release of catecholamines from the adrenal medulla.

A study by Mahata and Ghosh (1991a) has demonstrated that administration of steroid hormones (corticosterone, dexamethasone, deoxycorticosterone, progesterone, testosterone, and estradiol) on unilaterally splanchnic denervated pigeons causes significant changes in catecholamine content. Specifically, the E content between the innervated and denervated glands is markedly different. From this finding it becomes evident that the splanchnic nerve regulates steroid-induced alterations of E content in the pigeon. The results further reveal that the glucocorticoid hormones augment reserpine-induced resynthesis of catecholamines in the innervated glands. These results confirm that the splanchnic nerve is essential for the synergistic action of glucocorticoid- and reserpine-accelerated resynthesis of catecholamines.

(b) Role of the vagus nerve

Pilo and his collaborators (1984) have demonstrated an influence of the vagus nerve on the adrenal functions in homeothermic vertebrates. Histomorphologic studies carried out on adrenal glands of bilaterally vagotomized pigeons show that the adrenal gland, to some extent, is dependent on the vagus for its structural and functional integrity. Vagotomy results in an increase of adrenal weight. Both acetylcholinesterase and ascorbic acid levels become significantly lower in the adrenal gland of vagotomized birds. It also leads to a reduction in the proportion of medullary cells. It can be surmised that both in birds and mammals vagotomy causes a hypertrophy of cortical tissue and an atrophy of chromaffin cells.

B. Role of adrenal medulla in carbohydrate metabolism

The participation of the adrenal medulla in carbohydrate metabolism has been well elucidated in mammals. On the contrary, only a few references exist on the role of E and NE from the adrenal medulla in the avian glycemic response. Chattopadhyay (1986) performed investigations on the role of adrenomedullary hormones in the regulation of glucose homeostasis in birds. Starvation experiments on birds of different food habits as the rose-ringed parakeet (*Psittacula krameri*, which is granivorous), the house crow (*Corvus*

splendens, which is omnivorous), and the spotted owl (*Athene brama*, which is carnivorous) show simultaneous depletion and release of catecholamines and glycogen. Similarly, glucose loading in pigeons (*Columba livia*) increases glycogen level and store of catecholamines in the adrenal medulla. Administration of various autonomic effector agents that modify adrenal catecholamine levels (E, NE, isoproterenol and acetylcholine), causes simultaneous change in the glycemic response of the pigeon. The study further shows that α -receptors mediate the effect of catecholamines on carbohydrate metabolism in pigeons.

C. Role of the adrenal medulla in lipid metabolism

Sturkie (1965) reported that the adrenal medulla is less important in the mobilization of free fatty acids (FFA) in birds than in mammals. It appears that glycogen may be the prime lipolytic factor in birds.

A study by Sen and Bhattacharya (1984) on two avian species with distinct physiologic and metabolic differences, show that 18 hour fasting in the graminivorous rose-ringed parakeet (*Psittacula krameri*) causes a significant drop in plasma glucose and tissue glycogen and a simultaneous increase in plasma glucose, tissue glycogen, and plasma FFA levels. It also indicates an involvement of the adrenal glands in restoring metabolic homeostasis. Similar changes in glucose, glycogen, and FFA levels are found in the carnivorous collared scops owl (*Otus bakkamoena*), also. However, when the fasting birds are subjected to a single dose of E, NE, insulin, or glucagon (that are directly involved in carbohydrate and lipid metabolism), the pathway of response becomes altered. This apparently denotes some physiological and metabolic differences amongst various species of birds.

D. Response to stress

A stress-induced osmotic alteration leads to changes in the adrenal gland functions in birds. However, the response of the adrenal medulla to osmotic stress is highly variable. The findings of Ghosh and Sitarman (1975), and later by De and Ghosh (1993), reveal that E possibly is the hormone that mediates stress in the pigeon (*Columba livia*), as also reported earlier by Kobayashi et al. (1980) in the Japanese quail (*Coturnix coturnix japonica*). In the house sparrow (*Passer domesticus*), both E and NE are required for counteracting the stress situation (De and Ghosh 1993).

Water deprivation induced stress in three species of munias adapted to different habitats (i.e. swampy, *Lonchura malacca*; grassland, *Lonchura malabarica*; and arid, *Lonchura punctulata*) yields differential responses (Subho Ghosh et al. 1993). Those adapted to swampy and grassland areas show significant depletion of catecholamines, whereas those inhabiting arid lands exhibit depletion of NE only. Differ-

ential secretion of E and NE in munias inhabiting swampy and arid habitats are related with the degree of osmotic and/or hypovolemic cues as determinant factor(s) for triggering specific sites within the diencephalon (Banerjee et al. 1994). The details of stress adrenal interactions in birds will be discussed below.

E. Stress recovery action of vitamin A

In mammals, vitamin A plays a role in ameliorating the effects of stress (Olson 1984). However, no such information was available until 1992, when Ghosh et al. (1992) first explored this in birds. Chronic vitamin A therapy causes hyperglycemia and rise in glandular E in pigeons (*Columba livia*), but birds exposed to psychophysical stress (cold-wet immobilization) become normoglycemic with a rise in NE. It is presumed that vitamin A modulates the synthesis and release of catecholamines under conditions of stress in pigeons.

IV. Endocrine interactions

A. Adrenal corticomedullary relationship

The relationship between adrenal medulla and cortex is well established. Mammalian glucocorticoids regulate the activities of tyrosine hydroxylase (TH; Lucas and Thoenen 1977) and PNMT (Wurtman and Axelrod 1965; Ciaranello 1978). Aldosterone treatment also elevates PNMT activity, but its potency is less than that of the glucocorticoids (Wurtman 1966). Glucocorticoid administration increases NE content in the chick (Wasserman and Bernard 1971) and E content in pigeon adrenal glands (Chaudhuri et al. 1966; Sitarain and Ghosh 1977). Recently Mahata and Ghosh (1991a) have proved the effect of cortical steroids (corticosterone, dexamethasone, and deoxycorticosterone) on the neural regulation of adrenomedullary catecholamines. The study indicates that splanchnic nerve is essential for the synergistic action of glucocorticoids and reserpine in augmenting resynthesis of CA in pigeons.

B. Relationship with the endocrine pancreas

Avian adrenal medulla and pancreatic hormones hold a close relationship. Mahata and Ghosh (1986b) have demonstrated that the splanchnic nerve modulates insulin-induced depletion of NE in pigeons (*Columba livia*). Administration of insulin (4 IU/100 g body weight) caused depletion of NE from both intact and denervated adrenal glands in both newly hatched and adult pigeons. This treatment failed to modulate E content of the innervated and denervated glands. This indicates that the splanchnic nerve and age have definite influences on the effect of insulin on adrenal medulla. The study by Mahata et al. (1990a) on the effect of insulin on adrenomedullary catecholamine contents in a number of avian species (chicken, pigeon, parakeet, common myna, red-

vented bulbul, and babbler) points out that, with the exception of the babbler and parakeet, the action of insulin on avian adrenal medulla follows the mammalian pattern closely.

Except in pigeons (*Columba livia*) and red-vented bulbuls (*Pycnonotus cafer*), insulin-produced hypoglycemia alters, the catecholamine content of the avian adrenal medulla (Carmichael et al. 1987). Therefore, like mammals, adrenomedullary response depends on hypoglycemia also in birds (Mahata et al. 1990a). Administration of insulin in unilaterally splanchnic denervated pigeons at various time intervals show that whereas the splanchnic nerve stimulates the resynthesis of NE, synthesis of E is increased by insulin. Studies further show that insulin acts synergistically with reserpine to accelerate resynthesis of catecholamines in birds (Mahata et al. 1990b).

Glucagon administration in intact and splanchnic denervated pigeons also indicates that the splanchnic nerve regulates the release and/or resynthesis of catecholamines that are induced by glucagon (Mahata and Ghosh 1991).

C. Relationship with the pineal gland

Ultrastructural observations support the concept that adrenomedullary function, including mitotic activity, is controlled, at least temporarily, by the pineal gland (Kachi et al. 1988). In birds, the release of melatonin from the pineal is low during daytime (Takahashi et al. 1980). However, administration of melatonin during this period modulates the catecholamine content of avian adrenal medulla (Mahata et al. 1988). Later, Mahata and De (1991) found that the action of melatonin varies with age, species, dosage, and time of the day and that it modulates both NE and E contents of the adrenal medulla.

D. Relationship with the thyroid gland

The relationship between the adrenal medulla and thyroid gland in birds so far has remained comparatively less studied than in mammals. As mentioned earlier (Ghosh 1980) the work of Bhattacharyya (1971) on pigeon has proved that experimental hypo- and hyper- thyroidal drugs failed to alter adrenomedullary physiology and exogenous catecholamine administration had no effect on the thyroid gland. However under stressful situations, thyroid gland and adrenal medulla show striking synergism to counteract the effect of stress.

Bobek et al. (1996) analyzed the role of reverse triiodothyronine (rT_3) in heat stressed immature chickens. When injected subcutaneously, rT_3 (14 mg/100 g body weight) aggravates heat stress symptoms and increases plasma levels of corticosterone, catecholamines, and free fatty acids in chickens.

E. Relationship with the gonads

Application of gonadal steroids on the unilaterally splanchnic

denervated pigeons results in an increase of E content of the adrenal gland (Mahata and Ghosh 1991). This finding indicates that 1) steroid hormones significantly alter adrenomedullary catecholamine content, and 2) changes of E content induced by steroid hormones are modulated by the splanchnic nerve.

Earlier studies by Pal and Chatterjee (1985) show that in the pigeon, testosterone application along with the corticoid inhibitor metapirone, has no effect on adrenomedullary catecholamines. On the contrary, testosterone treatment in metapirone pre-treated intact pigeons causes an increase of E. Perhaps testosterone influences methylation of NE, particularly in glucocorticoid-deficient pigeons.

The catecholamine-containing nerves of the testes of male birds exert an influence in regulating the functional state of the organ (Ljunggren 1969). Research from this laboratory by Mukherjee (1985, 1988) show that chronic applications of catecholamines and 6-hydroxydopamine (which causes "chemical sympathectomy"; Malmfors and Thoenen 1971) to the male weaver bird (*Ploceus philippinus*) produce extreme testicular degeneration. The unaffected hormonal content of the adrenal medulla under all these experimental conditions proves non-participation of the gland in controlling gonadal status, at least in this avian species.

In pigeons (*Columba livia*), however, a direct role of adrenomedullary catecholamines on testicular physiology was observed (Subho Ghosh et al. 1997a). Guanithidine treatment (which causes "chemical medullectomy") led to testicular degeneration with a concurrent fall in adrenal E and NE contents. Thus, differential responses exist in birds regarding relationship between gonad and adrenal medulla. In the blossom-headed parakeet (*Psittacula cyanocephala*), a phase-dependent relationship exists between adrenomedullary catecholamines and testicular cycle (Maitra and Ghosh 1982).

Studies on annual gonadal activity cycle of some common birds have given distinct indications of a positive correlation with catecholamines in the adrenal medulla. This was found in the Swedish wood pigeon (*Columba palumbus*), rose-ringed parakeet (*Psittacula krameri*), and blossom-headed parakeet (*Psittacula cyanocephala*; Maitra and Dey 1992). Interestingly, the gonadal phase and adrenal catecholamines are out of phase in the domestic pigeon (*Columba livia*) and lal munia (*Estrilda amandava*; Chakravorty et al. 1985).

In fowl, the weaver bird, and the tree pie (*Dendrocitta vagabunda*), adrenomedullary catecholamines exerted a negative influence on gonadal physiology. Mallick and Sarkar (1985) noticed parallelism between the testicular and adrenomedullary functions in the common myna (*Acridotheres tristis*).

Differential gonadal responses of parakeet and weaver bird to a particular photoperiod (22 light:2 dark) during

different phases of the annual gonadal cycle suggest the existence of an endogenous circennial periodicity in these birds. This finding suggests that in these sub-tropical birds, the duration of daily photoperiod possibly acts in the role of an entraining agent (also referred to as Zeitgeber). In the parakeet, entrainment of the photoperiod does not involve the adrenal glands in the mechanism that controls annual testicular cycle. The finding by Subho Ghosh et al. (1996b) shows that in the common myna (*Acridotheres tristis*), both day length and humidity control the testicular cycle. This sub-tropical avian species maintains a parallel type of adrenocortical-gonadal relationship. However, adrenomedullary hormones are also involved in the regulation of reproduction.

F. Relationship with neurohypophyseal hormone

The physiologic relationship between vasopressin and adrenal medullary catecholamines has been explored relatively recently. Nussey et al. (1987) first demonstrated that although arginine vasopressin (AVP) has no effect on basal catecholamine release from bovine chromaffin cells, it inhibited acetylcholine and nicotine-stimulated NE and E release in dose-related manner. Porter et al. (1988) have reported that AVP stimulated secretion of catecholamines from the rat adrenal gland perfused in situ.

Mahata and Ghosh (1991) first demonstrated the role of vasopressin in the avian adrenal medulla. Intraperitoneal injections of lysine vasopressin into unilaterally splanchnic denervated pigeons showed that in innervated gland, vasopressin caused 59-74% decrease of NE when compared to the denervated gland. This supports a role for the splanchnic nerves in preventing vasopressin-induced NE release. On the other hand, the splanchnic nerve has no effect on vasopressin-induced release of E. The resynthesis of both NE and E took place 144 hrs and 216 hrs after the injections, respectively. This points out that like mammals and birds, vasopressin possibly augments the release of ACTH that results in an accelerated resynthesis of both E and NE (Mahata and Ghosh 1991).

G. Role of prostaglandins in catecholamine action

Though extensive work has been done on the effect of prostaglandins (PG) on the adrenal medulla of mammals, findings relating to the regulatory role of PG on the hormonal release in birds are very limited (Das Adhikari and Guha 1991). Sengupta et al. (1994) and Sarkar et al. (1996) from our laboratory have demonstrated that chemically and structurally different PG inhibitors (indomethacin and ibuprofen) differ considerably in their mode of action on the adrenal hormonal profile during stress conditions in the same avian species. Treatment with indomethacin (a methylated

indole derived PG blocker) exhibited no perceptible effect on either E or NE during acute formalin stress in the pigeon (*Columba livia*; Sengupta et al. 1994). On the other hand, Sarkar et al. (1996) observed that administration of ibuprofen (a propionic acid derived PG inhibitor) under acute stress condition significantly reduced the adrenal NE but had no influence on E. More recently, Sarkar et al. (1999) studied the effect of other PG inhibitors with different chemical natures such as aspirin (acetyl salicylic acid), mefenamic acid (fenamates), diclofenac (phenylacetic acid derivative), and piroxicam (an oxycam derivative) on the avian adrenal hormonal physiology during stress (see Table 1).

From the results of these studies it appears that aspirin, piroxicam, and diclofenac fail to alter the effects of formalin stress on the adrenal hormonal profile, whereas mefenamic acid accentuates the effects of the stress. Our studies reveal that chemically and structurally different PG inhibitors show diverse action in the same avian species under similar stress conditions. In this context, it seems worthwhile to explore which specific series of PG are inhibited by the prostaglandin blockers used.

H. Involvement of prolactin

The involvement of prolactin in maintaining electrolyte balance in avians is well documented by Mukherjee et al. (1993). The treatment in parakeet (*Psittacula krameri*) shows perceptible stimulation of the adrenal cortex and chromophobe cells of the adenohypophysis, but fails to modulate adrenomedullary functions at both cytologic and hormonal levels (Mukherjee et al. 1990). In the rat, a dopamine antagonist (haloperidol) induces adrenocortical hypertrophy but fails to generate an adrenomedullary response (Mukherjee et al. 1995).

I. Adrenal medulla — thyroid relationship

It becomes quite evident from the findings of Subho Ghosh et al. (1996b) that a parallel relationship exists between reproductive phases of the common myna (*Acridotheres tristis*) and levels of the hormones, viz. medullary E and NE, and T₃ and T₄.

J. Role of the bursa of Fabricius

The role of the bursa of Fabricius (*bursa fabricii*) during stress-induced modulation of adrenomedullary catecholamines has been studied by Mahata et al. (1990c). Studies on bursa-intact and bursectomized chicks exposed to cold-wet immobilization (CWI) stress demonstrate for the first time that the bursa modulates stress-induced changes of catecholamines in the adrenal medulla. In bursa-intact chicks, CWI stress decreased E and NE within 5 minutes. In bursectomized chicks, CWI stress decreased adrenal NE within 15 minutes and E in 30 minutes. Further, it is found that E

specifically causes size reduction of bursa follicles in the chicken (De and Ghosh 1999).

Concluding remarks

During the last two decades, research from this laboratory has enlightened different aspects of avian adrenal medulla.

Several histologic and histochemical methods have been employed to explore the cytomorphology of the chromaffin cells. Studies with electron microscopes, both TEM and SEM, have also been carried out to understand the ultrastructure of the E- and NE-containing cells of a number of avian species. Investigation points out that in the normal chromaffin cells of most birds there exists specific E- and NE-storing cells. Furthermore, E/NE ratio in birds seems to bear a distinct relation to the suggested avian phylogeny as described by Gregory (1957). The latter is based on palaeontological evidences of avian phylogeny. As per recent cladistic and molecular analyses, however, the hormonal taxa of avians needs to be re-examined.

Biochemical analyses have established that along with the soluble proteins, phosphatidyl ethanolamine, lecithin, and sphingomyelin exist in the chromaffin vesicles and they play a role in the secretory process of chromaffin cells. However, it has been shown that avian adrenal phospholipid content is not dependent on the relative concentration of E and NE.

Investigation has also enlightened the release mechanism of catechol hormones from the avian adrenal medulla. Experimental application of lithium with MAO-A and MAO-B isozyme inhibitors in pigeons demonstrates a significant rise in medullary catecholamine level after blockade of type B activity. This shows a difference in the mechanism of action of MAO type A and type B in the release of catecholamines. Recent findings in pigeons leads to the inference that exocytosis may not be the only mechanism of depletion of secretory vesicles in birds. The intricate phenomenon of avian catecholamine release requires serious attention.

The neural regulation of catecholamine release from the adrenal gland has been established in this laboratory through splanchnic denervation and other experimental manipulations. Studies point out that the splanchnic nerve modulates the synthesis, release, and resynthesis of catecholamines from chromaffin cells. Moreover, the splanchnic nerve works in synergy with glucocorticoids to regulate the catecholamine content of the adrenal medulla and plays a role in controlling blood sugar levels in insulin-induced hypoglycemic birds.

Research from this laboratory has given information on the influence of catecholamines on carbohydrate and fat metabolism in birds. These studies have stressed the involvement of endocrine glands with adrenomedullary functions in birds. The influence of melatonin in modulating E and NE contents of the adrenal gland deserves a special mention. A comparative study on the effect of insulin on adrenomedullary catecholamine contents shows, that excepting the babbler and parakeet, its action on the avian adrenal medulla follows the mammalian pattern. Avian vasopressin also affects the avian medulla in a similar manner as seen in mammals.

The involvement of the avian adrenal medulla to counteract the various stress effects has been emphasized in this article. The role of vitamin A in ameliorating the stress effect in pigeons is also indicated.

In spite of an extensive research on the avian adrenal medulla, a major gap remains on the understanding of the storage and release mechanisms of catecholamines from chromaffin cells. Exploration of this field will definitely open new frontiers in avian endocrinology.

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Table 1. Effect of PG inhibitors on adrenomedullary responses in formalin stressed domestic pigeon (*Columba livia*).

Treatment	N	Epinephrine (µg/mg tissue)	Norepinephrine (µg/mg tissue)
None	(6)	0.352±0.049*	0.121±0.024
Formalin	(6)	0.227±0.029 P<0.05	0.091±0.004 NS
Apririn	(7)	0.317±0.045 NS	0.089±0.005 NS
Apririn + Formalin	(7)	0.218±0.028 P<0.05 NS**	0.071±0.004 NS NS**
Mefenamic acid	(6)	0.181±0.042 P<0.025	0.079±0.012 NS
Mefenamic acid + Formalin	(6)	0.079±0.019 P<0.001 P<0.005**	0.049±0.008 P<0.001 P<0.001**
Diclofenac	(6)	0.461±0.040 NS	0.140±0.026 NS
Diclofenac + Formalin	(6)	0.237±0.21 P<0.050 NS**	0.084±0.006 NS NS**
Piroxicam	(6)	0.273±0.050 NS	0.124±0.009 NS
Piroxicam + Formalin	(6)	0.210±0.025 P<0.025 NS**	0.104±0.005 NS NS**

* Mean ± SEM

** Formalin vs. PG Inhibitor + Formalin

Figures in parenthesis represent the number of specimens.

NS= non significant

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Glossary of abbreviations

TH	tyrosine hydroxylase (EC 1.14.16.2)
CWI	cold-wet immobilization stress
DbH	dopamine -hydroxylase (EC 1.14.17.1)
E	epinephrine
FFA	free fatty acids
LiCl	lithium chloride
NE	norepinephrine
NPY	neuropeptide Y
PNMT	phenylethanolamine N-methyltransferase (EC 2.1.1.28)
TEM	transmission electron microscopy
SEM	scanning electron microscopy