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ROY O. GREEP

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PREFACE

The 1974 Laurentian Hormone Conference, held at Mount Tremblant, Quebec, Canada, August 25–30, 1974, opened on a high plane with the annual Gregory Pincus Memorial Lecture given by our esteemed Nobel Laureate Dr. Julius Axelrod. He described new techniques originated by himself and his co-workers that have made possible the local quantitation of brain biogenic amines and opened inroads to the significance of their presence. There followed a series of five stirring papers on the interaction of hormones with their target cell-binding or receptor sites, the isolation and nature of receptors and the further intracellular translocation and function of the hormone–receptor complex. A paper on endocrine neurons described an exquisite electrophysiological means of identifying and studying single neurons concerned with the secretion and release of neurohypophysial hormones. This nicely set the stage for a paper based on years of study of the Brattleboro rat that "leaks like a sieve" due to the hereditary absence of vasopressin and of a mouse that does the same due to an inherited inability of the kidney to respond to vasopressin. What's missing in the mouse?—most likely that starter fuel of all cell chemistry, adenylate cyclase.

Each year one of the highlights of the conference is a half-day symposium on some rapidly moving and unsettled forefront. This one was on those wondrous and sometimes mystifying hypothalamic emanations that exercise such complete stop and go control over the secretory output of pituitary hormones in health and disease and how such CNS control is modified by the sleep–wake cycle of daily life.

Exophthalmos of the most severe variety was revisited. This time not as a pituitary- but a thyroid-induced symptom. Evidence of dramatic corrective treatment by orbital irradiation was presented but the etiological mechanisms remained elusive. Next the gut hormones, too long in the wings, moved center stage in brilliant display. GIP, the gastric inhibitory polypeptide, comprised of 43 amino acids in straight-chain sequence and distinct from secretin or glucagon joined with them to form a trio of insulinotropins. The GI hormones are here to stay.

The concluding session traversed in historical perspective the circuitous and often baffling maze that led to the formulation of new concepts of the gonad–pituitary feedback system. The surprise that was pulled from underneath wraps was *pleomorphism* of FSH—i.e., FSH that may be His or Hers or Neuter.

Personally, and on behalf of the Program Committee, I want to thank

Drs. Lutz Birnbaumer, Abraham White, Claude A. Villee, Samuel M. McCann, W. P. Vander Laan, H. Maurice Goodman, Monte A. Greer, and M. B. Nikitovitch-Winer who chaired the several sessions with warmth, command, and fairness. It is again our pleasure to acknowledge the efficient and skillful work of Miss Joanne Sanford and her teammates, Mrs. Mina Rano and Mrs. Lucy Felicissimo in transcribing tapes of the discussions. A special note of grateful appreciation goes to Miss Sanford for her many years of devoted service as Executive Secretary to the Committee on Arrangements of the Laurentian Hormone Conference. The headquarters of the Conference have been transferred to 45 Shattuck Street, Boston, Massachusetts, and the new Executive Secretary is Miss Martha P. Wright.

ROY O. GREEP

CONTENTS

Relationship between Catecholamines and Other Hormones[1]

JULIUS AXELROD

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I. Introduction

During the past decade there has been a rapid increase in our understanding about the biochemistry, anatomy, and pharmacology of catecholamines and their regulation of sympathetic nerves and in the adrenal medulla. These advances were made possible by the availability of radioactive catecholamines of high specific activity, the development of sensitive methods for measuring these biogenic amines, the use of drugs affecting the sympathetic nervous system and of histofluorescent techniques for visualizing adrenergic nerve tracts. In the course of studies on the storage of catecholamines and the regulation of their biosynthetic enzymes, it became apparent that pituitary, adrenal, and thyroid hormones affect the disposition of catecholamines and in turn catecholamines regulate the formation of pineal hormones.

II. Catecholamines: Biochemistry, Disposition, and Regulation

The three important catecholamines are norepinephrine (noradrenaline), epinephrine (adrenaline), and dopamine. Norepinephrine is primarily localized in the sympathetic nerves of the peripheral organs (von Euler, 1946) and in the nerve tracts of the brain (Vogt, 1954). Noradrenergic pathways in the brain originate mainly in the cell bodies of the locus ceruleus of the brain stem (Ungerstedt, 1971). These neurons then pass through the medial forebrain bundle and septum and give off branches to the hypothalamus, cerebral cortex, hippocampus, and cerebellum. The main function of dopamine was previously thought to serve as a precursor of norepinephrine, but it has been shown to have a role of its own (Carlsson, 1959). It is localized mainly in the nerve tracts in the brain. Dopamine cell bodies originate in the substantia nigra in the midbrain (Ungerstedt, 1971), and the axons course through the lateral hypothalamus and terminate in the caudate nucleus and putamen of the corpus striatum. A few dopamine cell bodies are located in the arcuate

[1] The Gregory Pincus Memorial Lecture.

nucleus of the hypothalamus and give rise to short axons which descend into the median eminence.

Epinephrine is found mainly in the adrenal medulla. Recent work, however, has uncovered the presence of adrenergic tracts where cell bodies originate in the midbrain and send axons into parts of the hypothalamus (Hokfelt *et al.*, 1973; Saavedra *et al.*, 1974).

Histofluorescent studies have shown that catecholamine-containing nerves consist of a cell body, a long axon, and highly branched nerve terminals. These nerve terminals arising from a single cell body contain thousands of swellings or varicosities. The catecholamine neurotransmitters are stored in dense core vesicles present in the nerve terminals (Wolfe *et al.*, 1962). In the adrenal medulla, catecholamines are stored in the chromaffin granules (Blaschko and Welch, 1953).

The synthesis of catecholamines proceeds by the following steps: tyrosine → dopa → dopamine → norepinephrine → epinephrine. The first step is catalyzed by the enzyme tyrosine hydroxylase (Nagatsu *et al.*, 1964), the second by aromatic amino acid decarboxylase (Holtz *et al.*, 1938), the third by dopamine β-hydroxylase (DBH) (Levin *et al.*, 1960), and the final step by phenylethanolamine *N*-methyltransferase (Axelrod, 1962). Tyrosine hydroxylase and amino acid decarboxylase are present in all catecholamine-containing nerves, and dopamine β-hydroxylase is found in noradrenergic nerves. Phenylethanolamine *N*-methyltransferase (PNMT) is highly localized in the adrenal medulla together with the other catecholamine biosynthetic enzymes. Tyrosine hydroxylase, aromatic amino acid decarboxylase, and PNMT are localized in the cytosol, while DBH is found in vesicles of nerves and chromaffin granules of the adrenal medulla. Catecholamines in nerves act as neurotransmitters on discrete postjunctional cell populations. Norepinephrine and epinephrine, when discharged from the adrenal medulla into the blood stream, act on distant target organs.

The biosynthetic enzymes are made in the cell body of the sympathetic neurons. They are then transported down the axon to nerve terminal varicosities where they synthesize the catecholamine neurotransmitters (Dahlström, 1965). The catecholamine neurotransmitters are discharged from the nerve terminals by a process of exocytosis (Geffen *et al.*, 1969; Weinshilboum *et al.*, 1971). When the nerve is depolarized the storage vesicle fuses with the inner membrane of the nerve terminal; this is followed by the formation of an opening large enough to extrude the catecholamine neurotransmitters as well as soluble DBH. The exocytotic release of catecholamines and DBH requires the presence of Ca^{2+}, intact microtubes and microfilaments (Thoa *et al.*, 1972). This finding suggests that a contractile mechanism activated by Ca^{2+} might be involved in neu-

rotransmitter release (Axelrod, 1973; Berl *et al.*, 1973). Once released, the catecholamines interact with a specific receptor on a postjunctional cell to produce the characteristic biological response of that cell.

The actions of the catecholamine neurotransmitters are terminated by several mechanisms: O-methylation by catechol-*O*-methyltransferase, deamination by monoamine oxidase, physical removal by the blood stream, and ultimate metabolism in the liver and kidney and reuptake by nerve terminals (Hertting and Axelrod, 1961). In most instances, reuptake is the predominant means of inactivation of the neurotransmitters.

Catecholamines are in a constant state of flux. In the nerves they are continuously synthesized, released, and metabolized, yet they maintain a steady level. This is due to a variety of regulatory mechanisms. Some of these are very rapid, and others slower. The rate-limiting enzyme in catecholamine metabolism, tyrosine hydroxylase, is inhibited by catecholamines. When nerves are rapidly firing the level of intraneural catecholamines fall and the activity of tyrosine hydroxylase increases (Weiner and Rabadjija, 1968). This permits a larger fraction of tyrosine to be converted to dopa, and ultimately new dopamine and norepinephrine molecules are formed. In the case of reduced nerve activity intraneural catecholamines are elevated and tyrosine hydroxylase is inhibited.

Another rapid regulatory mechanism of sympathetic nerve activity involves an inhibitory α-adrenergic receptor located presynaptically (Langer, 1974). With neuronal nerve firing, the concentration of catecholamines is elevated in the synaptic cleft. The increased level of the neurotransmitter then stimulates the presynaptic α-adrenergic receptor, which in turn inhibits the release of catecholamine from nerves. When the α-adrenergic receptor is inhibited with phenoxybenzamine, there is an increased discharge of neurotransmitters.

A slower control on catecholamines involves the biosynthetic enzymes tyrosine hydroxylase and DBH (Thoenen *et al.*, 1969; Molinoff *et al.*, 1970). Certain drugs and stress can increase firing of sympathetic nerves and cause an elevation of these two biosynthetic enzymes in nerves and the adrenal gland. This increase in enzyme activity is a transsynaptic event in which receptors on sympathetic ganglia or adrenal medullary cells are stimulated. This results in an increased formation of new enzyme molecules. The elevation of the enzymes is slow and is manifest only after many hours.

III. Control of Epinephrine Synthesis by Adrenal Hormones

The adrenal gland consists of two anatomically and functionally different structures—the outer cortex that synthesizes steroids and the inner

medulla that contains chromaffin cells (Coupland, 1953). The latter cells are involved in the synthesis of catecholamines. It was long suspected that the adrenal cortex, which is in juxtaposition with the medulla, might influence the formation of epinephrine. Sheppard and West (1951) noted that those species which have a larger proportion of cortex surrounding the medulla contain a larger proportion of the methylated catecholamine epinephrine. It was suggested that a factor associated with methylation of catecholamine is present in the adrenal cortex. The isolation and characterization of the enzyme that methylates norepinephrine (PNMT) (Axelrod, 1962) made it possible to examine the role of the adrenal cortex in the formation of epinephrine in the medulla. This enzyme requires *S*-adenosylmethionine as the methyl donor and can methylate not only norepinephrine, but also other β-hydroxylated phenylethanolamines. The development of a sensitive assay for this enzyme (Axelrod, 1962) made it possible to measure changes in this enzyme after various endocrine manipulations.

The experimental approach to examine how the adrenal gland affects epinephrine formation was to reduce the adrenocorticoid content of the adrenal cortex by removal of the pituitary of the rat. The activity of PNMT and the content of epinephrine in the medulla was measured 17 days after hypophysectomy (Wurtman and Axelrod, 1966). A profound fall in PNMT activity and a reduction in the epinephrine content of the adrenal medulla occurred after hypophysectomy. The activity of the epinephrine-forming enzyme could be restored by the injection of large doses of a glucocorticoid, dexamethasone, or ACTH (Fig. 1). The amount of ACTH necessary to restore PNMT in the rat adrenal was similar to that necessary to maintain adrenal weight. The concentration of glucocorticoid in the portal blood from cortex to the medulla is very high and presumably is sufficient to maintain the levels of PNMT. All these results indicated that the conversion of norepinephrine to epinephrine is controlled by glucocorticoids from the cortex prefusing and adrenal medulla. The elevation in PNMT in hypophysectomized rats after dexamethasone treatment can be blocked by protein synthesis inhibitors, thus indicating that glucocorticoids stimulate the synthesis of new enzyme molecules. Under normal conditions PNMT cannot be increased in the intact rat. The enzyme can be induced after implantation of an ACTH-secreting tumor (Vernikos-Danellis *et al.*, 1968), unilateral adrenalectomy (Ciaranello *et al.*, 1969), or repeated stress (Kvetnansky *et al.*, 1970). PNMT in adrenal glands can be elevated in response to endogenous or exogenous glucocorticoids in certain strains of mice whose pituitaries have not been removed (Ciaranello *et al.*, 1972). ACTH and corticosterone have also been shown to be involved in the rapid development of PNMT in newborn

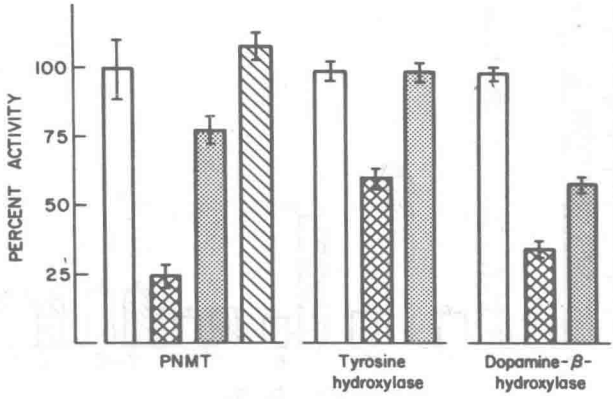

FIG. 1. Regulation of catecholamine biosynthetic enzymes in rat adrenal by glucocorticoids and ACTH. Rats were killed 17 days after hypophysectomy, and phenylethanolamine *N*-methyltransferase (PNMT), tyrosine hydroxylase, and dopamine β-hydroxylase activity were measured in the adrenal gland. Either ACTH (4 units) or dexamethasone (1 mg) was given for 7 days, 10 days after hypophysectomy. Normal, □, hypophysectomy ▩, Hypophysectomy and ACTH ▩, hypophysectomy and dexamethasone ▨.

rat adrenals (Margolis *et al.*, 1966) and in the methylation of norepinephrine in extraadrenal chromaffin tissues (Ciaranello *et al.*, 1973).

PNMT does not decline in hypophysectomized frog adrenals (Wurtman *et al.*, 1968b). This enzyme was found to be different in frogs as compared to mammals with respect to pH and temperature optima and electrophoretic mobility.

Small amounts of PNMT are present in the superior cervical ganglia (Ciaranello *et al.*, 1973) and paraaortic chromaffin tissue (organ of Zuckerhandl) of the newborn rat. This enzyme rapidly disappears 1 week after birth. With repeated injections of glucocorticoids, the activity of the epinephrine-forming enzyme in ganglia and organ of Zuckerkandl can be increased manyfold and maintained for 3 weeks after birth (Fig. 2). The enzyme rapidly disappears within 1 month. This suggests that glucocorticoids may play a role during differentiation of catecholamine-containing cells. Recently PNMT has been found in certain areas of the midbrain and hypothalamus (Saavedra *et al.*, 1974). Whether PNMT in neural tissues is under control of glucocorticoid remains to be established.

The rate-limiting enzyme in catecholamine formation, tyrosine hydroxylase (Nagatsu *et al.*, 1964), is also affected by hypophysectomy. Removal of the pituitary of the rat results in a gradual fall of tyrosine hydroxylase in the adrenal medulla (Mueller *et al.*, 1970). The half-life of decline is about 10 days. ACTH will restore tyrosine hydroxylase ac-

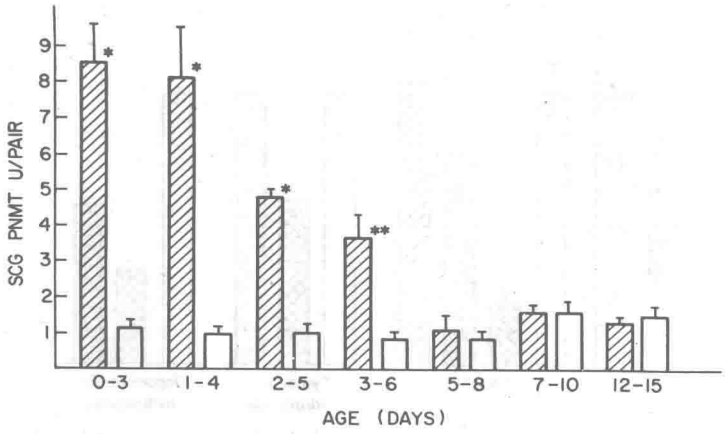

FIG. 2. Induction of the epinephrine-forming enzyme phenylethanolamine *N*-methyltransferase (PNMT) in rat superior cervical ganglia of newborn rats. Dexamethasone (0.1 mg/gm sc) was given in three daily injections. The animals were killed 1 day after the last injection, and the ganglia were assayed for PNMT. Dexamethasone-treated (▨) and saline-treated (□) groups consisted of 5–8 rats. $*P < 0.001$ as compared to untreated rats. $**P < 0.01$ as compared to untreated rats. From Ciaranello *et al.* (1973), with permission of Pergamon Press.

tivity after the rats have been hypophysectomized (cf. Fig. 1). ACTH will not increase tyrosine hydroxylase in the intact animal above normal levels.

Another enzyme involved in the biosynthesis of catecholamines affected by removal of the pituitary is DBH. Hypophysectomy results in about 50% fall in enzyme activity in about 21 days (Weinshilboum and Axelrod, 1970) (Fig. 1). ACTH, but not large doses of glucocorticoid, increased DBH activity in hypophysectomized rats. After these experiments were done it was found that glucocorticoids can increase DBH and presumably tyrosine hydroxylase after the rats were hypophysectomized after a short period of time but not after a longer period (G. F. Wooten, R. D. Ciaranello, and J. Axelrod, unpublished, 1974).

IV. Hypophysectomy and Turnover of Dopamine β-Hydroxylase

The steady-state levels of an enzyme reflect a balance between its synthesis and degradation (Schimke and Doyle, 1970). Thus, glucocorticoid hormones regulate the level of catecholamine biosynthetic enzymes by interfering with its synthesis and/or degradation. Decrease in enzyme activity after hypophysectomy could be a consequence of diminished synthesis or increased degradation of catecholamine enzymes. To distinguish between these two possibilities, experiments using the incorporation of

^{3}H- and ^{14}C-labeled amino acids into the enzyme protein were carried out (R. D. Ciaranello, G. F. Wooten, and J. Axelrod, unpublished, 1974). The enzyme containing radioactive amino acids was then isolated by immunoprecipitation with an antibody obtained from the purified enzyme. In examining the effect of hypophysectomy on enzyme synthesis and degradation, DBH was used because we had an antibody for this enzyme. Glutamic acid-^{14}C was injected into a rat 7 days after hypophysectomy, Glutamic acid-^{3}H was given 24 hours later, and the animal was killed 2 hours thereafter. The DBH in the adrenal gland was precipitated with the antibody, and the ^{14}C- and ^{3}H-labeled glutamic acid of the enzyme was measured. The ^{14}C remaining provides an estimate of rate of enzyme degradation, and the short time exposure to ^{3}H-labeled amino acid gives a measure of the rate of DBH synthesis. The ratio of ^{3}H to ^{14}C incorporation into DBH to that of soluble adrenal protein represents the turnover index. Enzymes undergoing rapid turnover have high turnover indexes relative to a slower system.

There was no change in the rate of incorporation of glutamic acid-^{3}H into DBH (Table I) in hypophysectomized rats as compared to intact animals. On the other hand, there was a considerable decrease in the amount of ^{14}C-labeled enzyme remaining in a rat whose pituitary was removed. These results indicate that hypophysectomy accelerates the rate of degradation in DBH with a resultant decrease in the number of enzyme molecules. Hypophysectomy did not appreciably affect synthesis and degradation of soluble adrenal protein. The increased disappearance of DBH was partially reversed by ACTH.

V. Plasma DBH and Vasopressin

A fraction of the DBH released from sympathetic nerves finds its way into the blood stream (Weinshilboum and Axelrod, 1971a). Removal of the adrenal gland does not reduce the plasma levels of DBH in rat blood, but destruction of sympathetic nerves with 6-hydroxydopamine does (Weinshilboum and Axelrod, 1971b). This suggests that plasma DBH comes mainly from sympathetic nerve terminals; thus, plasma DBH can be a measure of sympathetic nerve activity.

The effects of hypophysectomy on plasma DBH were examined in the rat (Lamprecht and Wooten, 1973). After removal of the pituitary, there was a gradual rise in plasma DBH with a doubling of the enzyme level in 5 weeks (Fig. 3). Repeated administration of vasopressin resulted in a decrease of plasma DBH to normal levels. Three days after vasopressin injections were stopped, plasma DBH activity rose to pretreatment levels. Vasopressin also reverses the high DBH levels in rats with hereditary diabetes insipidus. There was also an increase in plasma DBH after re-