PHARMACOLOGICAL REVIEWS
Vol. 24, No. 2

Printed in U.S.A.

Adrenocortical Control of the Biosynthesis of Epinephrine and Proteins in the Adrenal Medulla

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I. Introduction

PROBABLY all vertebrates contain sympathetically innervated chromaffin cells, derived from the neuroectoderm, which stain with chromate salts and which synthesize and secrete catecholamines. Only in mammals, however, do these chromaffin cells form a compact body, the adrenal medulla, which becomes surrounded during embryonic life with cortex of steroid-secreting tissue (11, 32, 45). The cells of this adrenal cortex are strikingly different from the medullary chromaffin cells by almost any criterion that one might choose to apply: Their embryonic anlage is not neuroectoderm but coelomic epithelium; they synthesize not water-soluble amines but lipid-soluble steroids; they secrete their hormones not in response to neuronal inputs but to other circulating signals such as adrenocorticotropic hormone (ACTH) and angiotensin.

The unique evolution of the mammalian adrenal gland as two concentric organs has provoked considerable speculation concerning possible functional interactions between its cortex and medulla. Studies performed during the past few years have provided compelling evidence that this novel juxtaposition of adrenal chromaffin and cortical cells does have at least one major physiological consequence: Glucocorticoid hormones, secreted from the cortex and delivered preferentially to the medulla *via* an intra-adrenal portal vascular system, induce the synthesis of the medullary enzyme phenylethanolamine-N-methyl transferase (PNMT) (40, 41) which catalyzes the terminal step in epinephrine biosynthesis (fig. 1) (1, 21). Were the mammalian adrenal medulla to lack a cortical envelope and depend upon the arterial blood for its supply of cortisol or corticosterone, its capacity to synthesize (39, 40) and secrete (43) epinephrine would be markedly reduced. Moreover, the mass of medullary chromaffin cells would decline (25, 30, 32), and the synthesis of most medullary proteins would decrease (28).

The recognition that a special relationship exists between the availability of glucocorticoid hormones and the functional activity of the adrenal medulla has provided a useful general principle for interpreting the responses of the medulla under a variety of physiological and pathological states (*c.f.* ref. 18). This review will examine the evidence supporting this special relationship and its operation.

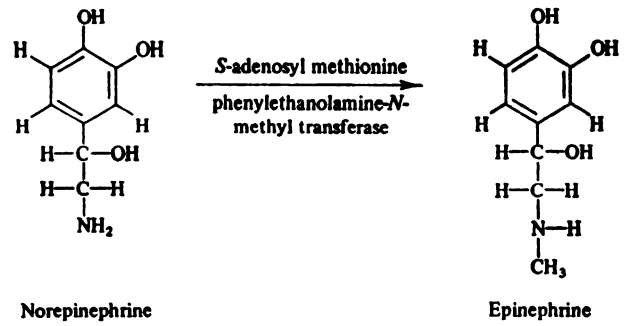

FIG. 1. Phenylethanolamine-N-methyl transferase.

II. Effects of Adrenocortical Hormones on Phenylethanolamine-N-methyl Transferase (PNMT) Activity in the Adrenal Medulla

In 1953, Coupland, an English anatomist, drew attention to the correlation between the morphology of adrenal glands and their contents of epinephrine and norepinephrine (9, 11). He noted that, among species whose adrenal chromaffin tissue was completely surrounded by a cortex (as in man and the rat), epinephrine was the predominant medullary catecholamine; however, among animals whose chromaffin tissue lacked a continuous cortical envelope (as in the rabbit), little or no epinephrine was present in the portion of the medulla not adjacent to cortical cells (10). The chromaffin cells of the dogfish lack any contiguity with the steroid-secreting cells; they also lack epinephrine. On the basis of this correlation Coupland suggested that the adrenal cortex normally secretes a "methylation factor" which stimulates the conversion of norepinephrine to epinephrine. Four years later, Kirshner and Goodall (21) characterized the adrenomedullary enzyme, PNMT, which catalyzes the N-methylation of norepinephrine. Subsequently, Axelrod demonstrated (1) that almost all of the PNMT present in adult mammals is localized within the adrenal gland; hence most or all of the epinephrine released into the circulation is the product of adrenomedullary PNMT.

In 1965 one of us (R. J. W.) participated in the care of several patients suffering from pituitary disease, whose chief symptoms resulted from an unusual sensitivity to insulin. For several hours after receiving insulin or after consuming a carbohydrate meal, these subjects continued to manifest severe hypoglycemia, often leading to convulsions and the loss of consciousness. At that time, it was generally believed that the causes of this insulin sensitivity were related to the absence of growth hormone, or to impaired gluconeogenesis secondary to the lack of ACTH. However these explanations could not easily be reconciled with the long periods of time required for blood glucose concentrations to be elevated by exogenous growth hormone, or by the activation of gluconeogenesis (by exogenous ACTH). It seemed more likely that the secretion of a different, fast-acting hyperglycemic factor was compromised in hypopituitarism. Epinephrine was, *a. priori*, the best candidate for this unknown factor, since it was known to be secreted within minutes of insulin administration, and to accelerate the breakdown of liver and

muscle glycogen within a comparable period. Coupland's correlation of a contiguous adrenal cortex and a high medullary ratio of epinephrine to norepinephrine now suggested a new explanation for the insulin sensitivity of hypopituitary patients: Perhaps an adrenocortical hormone, secreted in response to pituitary ACTH, was required for the synthesis of physiological quantities of epinephrine; pituitary failure would reduce the secretion of this cortical hormone, thereby suppressing epinephrine synthesis and secretion, and rendering the hypoglycemic subject less able to restore blood glucose levels after insulin. The most likely site at which the presumed adrenocortical hormone might act would be at the methylation of norepinephrine. Accordingly, a set of experiments was initiated to determine whether hypophysectomy or various hormone treatments modified the activity of adrenal PNMT, as assayed *in vitro*.

Removal of the pituitary from adult rats produced a rapid and significant decline in PNMT activity: Within a week of surgery, enzyme activity fell to 15 to 25% of normal (41) (fig. 2). PNMT activity could be restored by treating the rats with ACTH, but not by administering other pituitary hormones (39). The administration to intact rats of large doses of dexamethasone, a synthetic glucocorticoid, inhibited ACTH secretion and lowered the weight of the adrenal

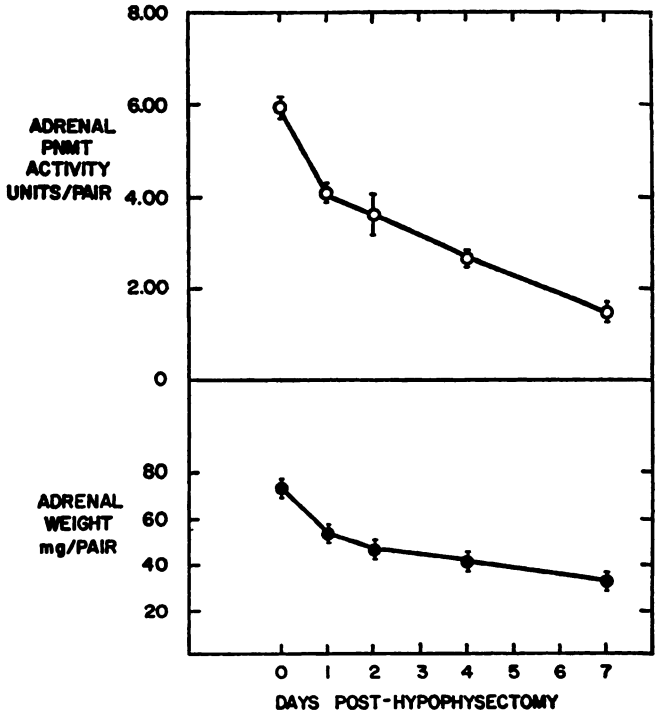

FIG. 2. Decline in adrenal weight and PNMT activity after hypophysectomy in the rat. Each group contained six animals. One unit of PNMT activity catalyzes the formation of 1 nmole of product per hour. Vertical bars indicate standard errors of the mean. [Reproduced from Wurtman, R. J. and Axelrod, J.: J. Biol. Chem., 241: 2301-2305, 1966 (41).]

cortex; however, this treatment did not cause PNMT activity to decline (39, 40). Similarly, small doses of methopyrapone (an inhibitor of glucocorticoid biosynthesis) which increased ACTH secretion and adrenal weight without enhancing corticosterone secretion did not elevate PNMT activity (40). On the basis of these observations, it was concluded that the effect of ACTH on PNMT activity was indirect and was mediated by its actions on adrenocortical secretion. This interpretation was confirmed by the demonstration that the treatment of hypophysectomized rats with large doses of dexamethasone (1 mg per day) fully restored PNMT activity (fig. 3) (40, 41).

The administration of usual "replacement" doses of dexamethasone, corticosterone, or hydrocortisone to rats did not restore PNMT activity following hypophysectomy; as much as 30 mg of hydrocortisone per day was needed to produce a significant increment in the epinephrine-forming enzyme (39, 41). This initially discouraging observation eventually led to the recognition of the paramount importance of the special location of the adrenal medulla within its envelope of adrenal cortex (39). The adrenal medulla normally receives the undiluted venous effluent from the adrenal cortex; the concentration of gluco-

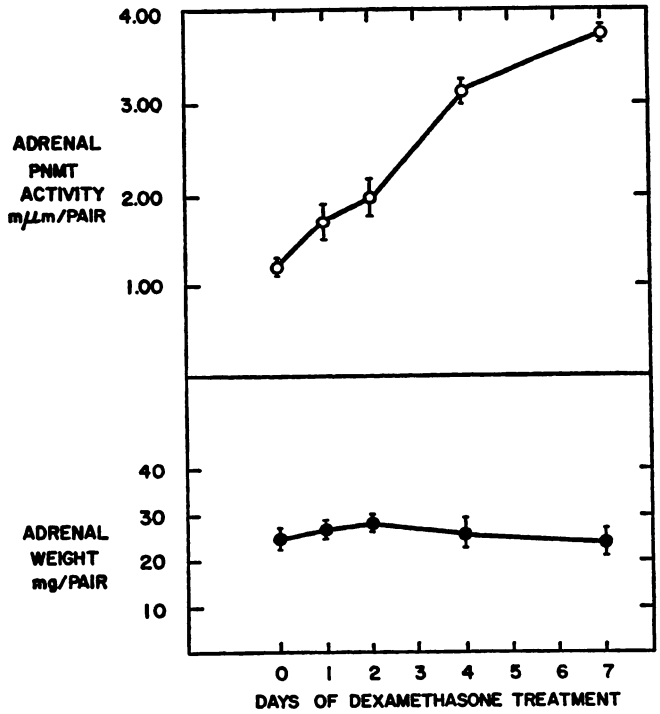

FIG. 3. Effect of treatment with dexamethasone on adrenal weight and PNMT activity in the hypophysectomized rat. Animals were given dexamethasone (1 mg intraperitoneally per day) for various periods, and killed 17 days after hypophysectomy and 1 day after the last dose of dexamethasone. [Reproduced from Wurtman, R. J. and Axelrod, J.: J. Biol. Chem. 241: 2301-2305, 1966 (41).]

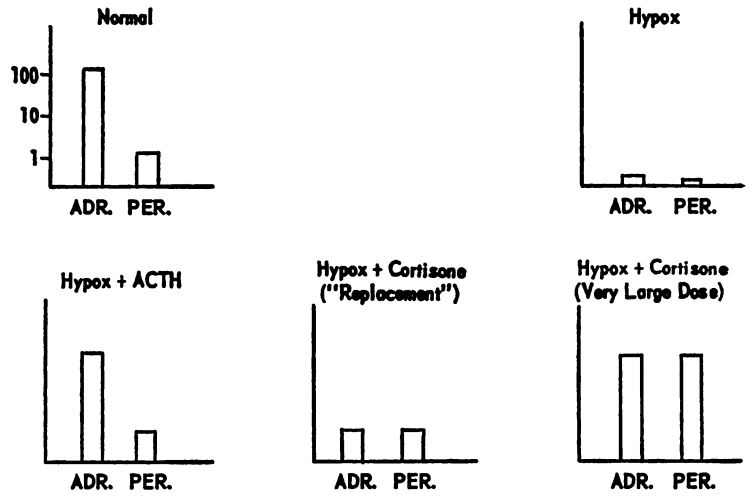

FIG. 4. Schematic diagram showing anticipated effects of hypophysectomy and various hormone treatments on the concentrations of glucocorticoids in adrenal venous blood and peripheral venous blood. Concentrations are given in arbitrary units. [Reproduced from Pohorecky, L. A. and Wurtman, R. J.: Pharmacol. Rev. **23**: 1-35, 1971 (32).]

corticoids in this blood is at least 100-fold greater that that present in systemic arterial blood. After hypophysectomy, the concentrations of glucocorticoids in the adrenomedullary sinusoids and in the general circulation both decline precipitiously. If hypophysectomized rats are treated with exogenous corticoids in concentrations sufficient to restore the steroid levels normally perfusing the liver or brain, these doses will provide the medulla with glucocorticoid concentrations less than $\frac{1}{100}$ of those normally available to it (fig. 4). "Replacement" of the steroid levels normally perfusing the medulla requires doses of glucocorticoids so great that, if continued, they will ultimately kill the animal. However, treatment with ACTH restores the steroid concentrations of both the blood perfusing the adrenal medulla and of the general circulation (fig. 4).

The hypothesis that the medulla requires unusually high concentration of glucocorticoids is supported by the following observations:

1. Hypophysectomized rats were treated with 1 or 10 units of ACTH or 3 or 30 mg of hydrocortisone daily for 5 days. At the end of this period, adrenal PNMT activity was assayed, and the weight of the spleen, a steroid-sensitive organ that receives its glucocorticoids from the general circulation, was measured. Doses of ACTH that were equipotent with hydrocortisone in depressing splenic weight were 100 times *more* effective in restoring PNMT activity (39).

2. *Intact* rats received a particular dose of dexamethasone (0, 1, 10, 100 or 1000 μg) daily for 9 days. At the end of this time, their adrenals were weighed and assayed for PNMT activity and for epinephrine content. The daily administration of 10 μg of dexamethasone (an amount equivalent to the usual "replacement dose" given hypophysectomized animals) caused a marked decline in PNMT activity as well as a significant fall in adrenal epinephrine content (44).

The 100-μg dose of the steroid produced a greater suppression of adrenal weight, but caused a *smaller* decline in PNMT activity or epinephrine content. The largest steroid dose (1000 μg daily) caused a maximal decrease in adrenal weight but *no* decline in either PNMT activity or epinephrine content. This experiment was interpreted as implying that the 10-, 100- and 1000-μg doses all suppressed to varying degrees the delivery of endogenous glucocorticoids to the medulla, but only the largest dose provided the medulla with sufficient exogenous steroid to replace the endogenous corticosterone.

3. Explants of one adrenal medulla, dissected free of cortex, were placed in the anterior chamber of a rat eye; the other adrenal gland was allowed to remain intact and in place. The explanted tissue rapidly took on an extensive vascularization; however its PNMT activity declined precipitously, despite the continued presence in the animal of an intact adrenal gland secreting at least normal quantities of corticosterone (29). The administration of dexamethasone (1000 μg daily for 7 days) caused a 20-fold increase in PNMT activity within the explant, but no change in PNMT activity of the intact gland. This study demonstrated that if the preferential delivery of glucocorticoids to the medulla was interrupted the levels of corticosterone in arterial blood were inadequate to sustain PNMT activity; enzyme activity could be restored, however, by administering very large amounts of glucocorticoid.

4. Coupland has measured epinephrine synthesis in cultures of extra-adrenal chromaffin tissue obtained from 2-day-old rabbits. The addition of hydrocortisone to the medium in concentrations of 10 μg/ml causes a significant increase in the formation of epinephrine (12). This concentration is much greater than that usually found in arterial blood.

5. Margolis *et al.* (24) have shown that epinephrine begins to accumulate in the adrenal of the fetal rat 1 or 2 days after the gland first has demonstrable PNMT activity, and that the enzyme first appears soon after the gland begins to synthesize large amounts of glucocorticoids. The sequential appearance of corticosterone, then PNMT, and then epinephrine is compatible with the hypothesis that glucocorticoids normally induce the epinephrine-forming enzyme.

Estradiol or testosterone administration does not increase PNMT activity in hypophysectomized animals (39). Aldosterone treatment does elevate the activity of the epinephrine-forming enzyme, but its potency is less than that of steroids with predominantly glucocorticoid activity (39). In general, the potency of natural C_{21} steroids in restoring PNMT activity varies according to the number of hydroxyl groups present on their —11, —17, and —21 positions; hence hydrocortisone is slightly more potent than corticosterone (31).

The mechanism by which glucocorticoids elevate PNMT activity appears to involve stimulation of its synthesis. The effects of dexamethasone can be blocked in hypophysectomized animals by concurrent administration of actinomycin D or puromycin (fig. 5) (41). Natural or synthetic glucocorticoids do not enhance PNMT activity *in vitro* in adrenal homogenates obtained from control or hypophysectomized animals; steroid concentrations above 10^{-4} M actually inhibit the enzyme (40, 41). As described below (pp. 422–424) there are, after hypophy-

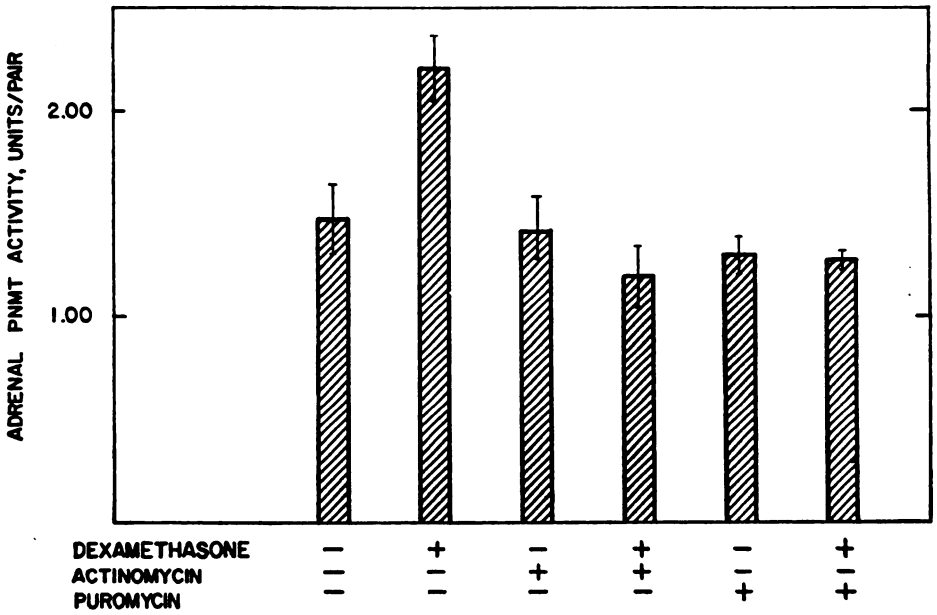

FIG. 5. Effects of actinomycin D and puromycin on the response of adrenal PNMT to dexamethasone in the hypophysectomized rat. Hypophysectomized animals (21 days after surgery) were given a 4-hr intravenous infusion of dexamethasone (1 mg/hr) and killed 5 hr later. Some rats were treated with actinomycin D (1 mg/kg) or puromycin (40 mg/kg) in a single intravenous injection before the infusion. [Reproduced from Wurtman, R. J. and Axelrod, J.: J. Biol. Chem. 241: 2301-2305, 1966 (41).]

sectomy, marked changes in the ratio of adrenal polyribosomes to monosomes. These changes are reversed by the administration of ACTH or dexamethasone. The protein-synthesizing units are largely disaggregated in tissues taken from hypophysectomized dogs or rats; reaggregation can be demonstrated as soon as 1 day after hypophysectomized rats receive dexamethasone. The rate at which adrenal PNMT protein [purified by electrophoresis and assayed immunochemically (26)] becomes labeled with ^{3}H-tyrosine is markedly depressed in hypophysectomized dogs, but is largely restored if the animals are treated with ACTH (table 1) (28).

The changes in PNMT activity that follow hypophysectomy (fig. 2) or treatment with ACTH or glucocorticoids (fig. 3) require hours, and even days, to become manifest. This suggests that glucocorticoid availability is not an important determinant of *minute-to-minute* changes in the rate of epinephrine synthesis, and that the daily rhythm in adrenocortical secretion will not produce a significant parallel rhythm in PNMT activity. In the short run, PNMT activity may be controlled by end-product inhibition. Fuller and Hunt (14) have shown that epinephrine is a non-competitive inhibitor of PNMT activity; it is possible that the secretion of epinephrine from medullary chromaffin cells lessens its inhibition *in vivo* of PNMT, thereby accelerating the N-methylation of norepinephrine without changing the cellular content of PNMT enzyme protein.

TABLE 1

Effects of hypophysectomy and ACTH on PNMT synthesis in the adrenal medulla

Treatment	PNMT Activity	^{3}H-Tyrosine Specific Activity		^{3}H-PNMT	
		3 hr	17 hr	3 hr	17 hr
	units/gland	*CPM/μg*		*CPM/mg protein*	
Control	166	15.3	11.7	450	410
Hypophysectomy	41	14.0	11.3	180	171
Hypophysectomy and ACTH	101	16.1	12.0	300	265

Dogs were hypophysectomized or sham-operated 3 months prior to sacrifice and where indicated, received ACTH (40 units/day) daily. ^{3}H-tyrosine (1 mC, intravenously) was administered 3 or 17 hr prior to sacrifice.

III. Effects of Adrenocortical Hormones on Epinephrine Secretion

The observation that hypophysectomy caused PNMT activity to fall suggested that impaired adrenocortical function might also interfere with epinephrine synthesis; it did not, however, prove this relationship. Theoretically, it was possible that adrenal chromaffin cells contained a superabundance of PNMT, such that even a 90% decrease in PNMT activity would still have left adequate amounts to catalyze epinephrine biosynthesis. To study the possible effects of hypophysectomy on epinephrine synthesis, groups of operated and control animals were killed at intervals of 1 to 7 weeks after surgery, and the epinephrine and norepinephrine in their adrenals were assayed. Adrenal epinephrine levels were found to decline postoperatively, with a half-life of about 30 days; 7 weeks after hypophysectomy, adrenal epinephrine levels were only 25 to 30% of normal (39). Studies utilizing tracer techniques have shown that the half-life of adrenal epinephrine in intact animals is on the order of 7 to 14 days (36). The slower rate of epinephrine disappearance observed in the hypophysectomized rats suggests that these animals are still able to synthesize some epinephrine in the absence of ACTH; moreover, the release of the catecholamine from the gland might also be impaired after hypophysectomy. Norepinephrine levels in the adrenals of hypophysectomized animals show a tendency to rise; however, this increase is not stoichiometric with the decrease in epinephrine (39). This observation suggests that adrenal norepinephrine is present in at least two distinct metabolic pools, *i.e.*, one that serves as the precursor for epinephrine and another that does not normally undergo N-methylation. This hypothesis is consistent with the evidence that the adrenal medulla contains two histologically distinct populations of cells, one of which stores epinephrine, and the other, norepinephrine (30). Presumably the catecholamine in the latter cell population constitutes the metabolic pool that is not precursor to epinephrine. After hypophysectomy, the ratio of "norepinephrine cells" to "epinephrine cells" in the rat adrenal medulla increases; this change is especially evident near the periphery of the medulla (30, 32). The normal pattern of "epinephrine cells" and "norepinephrine cells" can be restored by treating the hypophysectomized animals with ACTH. These ob-

servations suggest that the mammalian adrenal medulla contains only a single clone of chromaffin cells, whose members do or do not produce PNMT (and epinephrine) depending upon the extent to which they are stimulated by glucocorticoids.

The effects of hypophysectomy on epinephrine *secretion* were studied in dogs whose pituitaries had been removed 3 to 5 months previously (43). Animals were anesthesized and one adrenal vein was cannulated; blood was collected at intervals, and the plasma concentrations of glucose, epinephrine, and norepinephrine were measured. After a control period of 30 to 60 min, each animal received insulin (0.1 unit/kg body weight) intravenously; some control animals received as much as 0.3 unit/kg in an effort to produce comparable decreases in plasma glucose concentrations. As expected, hypophysectomy caused the PNMT activity and epinephrine content of the dog adrenal to decline markedly. The basal secretion of epinephrine in hypophysectomized dogs was significantly less than that in intact animals, and its secretion in response to insulin hypoglycemia was less than half that of control animals (43). The adrenals of hypophysectomized dogs secreted considerably more norepinephrine, both basally and in response to insulin hypoglycemia, than those of intact animals. Since norepinephrine has only a minute fraction of the glycogenolytic potency of epinephrine, the secretion of the unmethylated amine in response to hypoglycemia must be regarded as physiologically wasteful. Treatment of hypophysectomized animals with ACTH (40 units per day) for 4 weeks fully restored adrenal PNMT activity, epinephrine content, and epinephrine secretion (43). These observations on dogs tended to confirm our hypothesis concerning the mechanism of the insulin sensitivity found in hypopituitary human subjects (45).

IV. Phenylethanolamine-N-methyl Transferase (PNMT) Activity in the Brain

PNMT activity has now been identified in several regions of the brains of rats, hens, cats, turtles and man (1, 8, 22, 33, 45). In mammals, enzyme activity is greatest in the olfactory bulb and tubercle and in the hypothalamus (33). Small amounts of epinephrine (5–15% of the total catecholamine content) have been identified in the mammalian brain with bioassay methods (13, 38); fluorescence techniques, however, have not consistently yielded conclusive evidence for its presence (6). The existence of epinephrine in mammalian olfactory structures has apparently not yet been examined. It has been demonstrated, however, that both the olfactory bulb and tubercle are able to convert isotopically labeled norepinephrine (taken up from the lateral cerebral ventricles) to epinephrine, and then to store the methylated catecholamine (33). Nerve terminals in the frog brain store epinephrine, and sympathetic neurons in the frog heart have been observed to liberate the catecholamine when stimulated electrically (3, 23). On the basis of this evidence it seems likely that epinephrine can function as a neurotransmitter in amphibians. No similar evidence exists that epinephrine serves as a neurotransmitter in mammals.

Dexamethasone treatment (1 mg/kg per day, for 7 days) produces an elevation

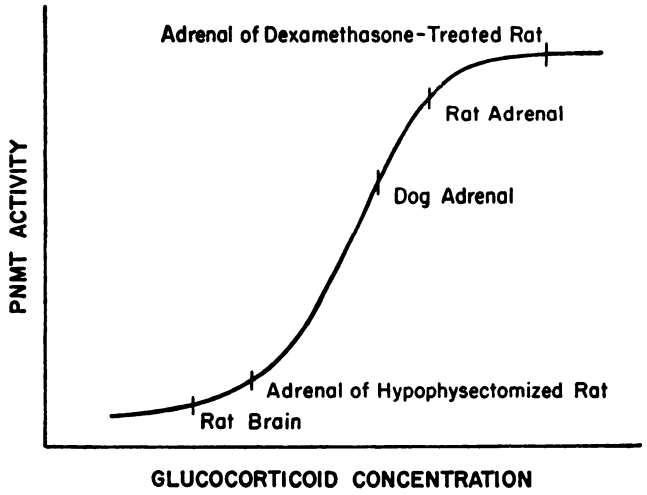

FIG. 6. Theoretical curve describing the relationship between the glucocorticoid concentration perfusing a PNMT-containing tissue and its PNMT activity. [Reproduced from Pohorecky, L. A. and Wurtman, R. J.: Pharmacol. Rev. 23: 1-35, 1971 (32).]

in PNMT activity in the olfactory tubercles of intact rats (33); however hypophysectomy does not cause brain PNMT activity to decline. The responses of brain tissues to changes in the availability of glucocorticoids are thus distinct from those of adrenomedullary chromaffin cells: In the latter, a decrease in glucocorticoid perfusion causes a marked fall in PNMT activity, while an increase in adrenocortical secretion produces only minor elevations in reactivity of PNMT (37). This difference can be resolved by locating the brain and adrenal medulla on a theoretical curve that relates PNMT activity to the availability of glucocorticoids (fig. 6): At relatively low glucocorticoid levels, an increase in their availability will increase PNMT activity, but a decrease will have little or no effect on the enzyme. This situation would be expected to exist in the brain, an organ that depends on the arterial blood for its supply of glucocorticoids. At high levels of glucocorticoid perfusion within the adrenal medulla, an increase in glucocorticoid input will have little or no effect on PNMT activity, but a decrease should (and does) cause a marked decline in the enzyme.

V. Heterogeneity of Phenylethanolamine-N-methyl Transferase (PNMT) Enzymes

In the frog, both epinephrine (3, 23) and PNMT activity (42) are widely distributed: PNMT activity has been identified within the brain, heart, and spleen (42) as well as in the adrenal. Since none of these organs is normally perfused with very high concentrations of glucocorticoids (*i.e.*, the frog adrenal lacks concentric zones and a portal vascular apparatus), studies were performed to characterize the physiocochemical properties and steroid dependence of frog PNMT. After hypophysectomy, PNMT activity remained unchanged in all of the frog organs, including the adrenal, even though the concentration of cor-

ticosterone within the adrenal gland declined significantly (42). The physiochemical properties of frog PNMT differed from those in the rat in several ways: the frog enzyme was thermolabile at 47°C; its catalytic activity was the same when incubated at 30°C as at 38°C; and its electrophoretic mobility on starch block at pH 8.6 was considerably less than that of rat PNMT (2, 42). The substrate specificity of frog PNMT was similar to that in the rat, and both enzymes were largely unassociated with subcellular particles (2, 42).

The PNMT activity present in mammalian adrenal was subsequently shown to be associated with as many as five proteins with differing immunochemical properties, molecular weights and electrophoretic mobilities (2, 19, 27). After hypophysectomy, the activity of one of the two isozymes found in dog adrenal became undetectable (27); however, the other PNMT protein persisted, suggesting that it was related to the uninducible "frog-type" protein. The physicochemical properties of PNMT enzymes isolated from several human pheochromocytomas have been examined (17, 19); some of these proteins resemble the principal mammalian enzyme, while others exhibit the thermolability and immunological characteristics of the frog enzyme. The presence of an uninducible form of PNMT in some metastatic pheochromocytomas may explain the ability of these tumors to synthesize epinephrine even though they lie at some distance from an adrenal cortex.

VI. Effects of Adrenocortical Hormones on Other Enzymes in the Adrenal Medulla

Hypophysectomy causes small but significant changes in a variety of adrenomedullary enzymes involved in catecholamine metabolism besides PNMT: tyrosine hydroxylase (34, 41), dopamine-β-oxidase (15), catechol-O-methyl transferase (41), and monoamine oxidase (41) activities all fall. All of these enzyme activities are partially or totally restored if the operated animals are treated with ACTH; however, except for dopamine-β-oxidase, none appears to be increased after administration of dexamethasone. It is possible that in the inductions of tyrosine hydroxylase, catechol-O-methyl transferase, and monoamine oxidase, the medullary chromaffin cells respond directly to circulating ACTH, without the mediation of glucocorticoids. However, the apparent restorations of these enzymes when hypophysectomized animals are treated with ACTH could be artifactual, perhaps reflecting the formation of other enzymes within adrenal cortex that non-specifically catalyze a broad range of reactions. (In general, these enzymes have been assayed with homogenates of whole adrenal.) Adrenal dopamine-β-oxidase activity *is* restored when hypophysectomized animals are treated with either ACTH or dexamethasone (15).

The changes in the various adrenomedullary enzymes that follow hypophysectomy can best be explained by assuming that the lack of glucocorticoids thus produced causes three kinds of alterations in chromaffin cells: 1) The *number* of cells decreases (25, 30, 32), probably because glucocorticoids in high concentrations are "tropic hormones" for mammalian chromaffin cells; 2) The *rate of protein synthesis in general* declines (see. pp. 422–424 and figs. 7–9); and, 3) The *synthesis*

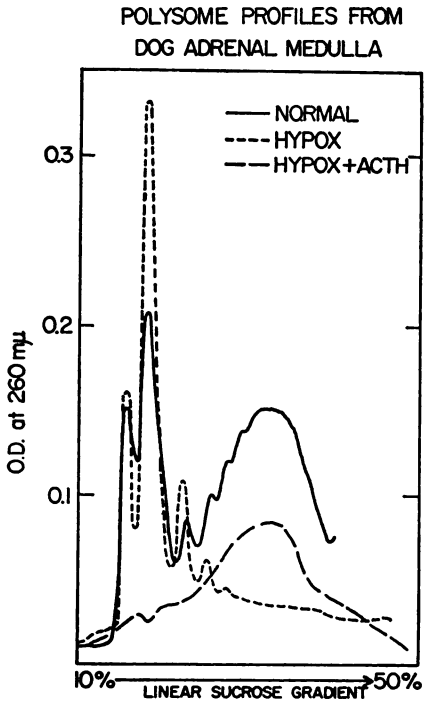

FIG. 7. Absorbance profiles of adrenal medullary polysomes obtained from normal dogs hypophysectomized animals, and hypophysectomized dogs treated with ACTH (40 units/day). Hypophysectomy was performed 3 months prior to sacrifice.

of PNMT is specifically suppressed. The formation of new PNMT protein (figs. 3 and 9; table 1) and the reaggregation of available ribosomes (fig. 8) are restored fairly rapidly by treatment with dexamethasone. However, the complete restoration of enzymes other than PNMT appears to require longer periods of hormonal therapy, *i.e.*, time intervals consistent with those probably needed for the mass of chromaffin cells to return to normal.

It appears that neural inputs to the adrenal chromaffin cells are of much greater importance than glucocorticoids in controlling the activities of tyrosine hydroxylase (34) and dopamine-β-oxidase (15). Chronic increases in the preganglionic neuronal input to medullary chromaffin cells can elevate PNMT activity by a transsynaptic mechansim that is independent of glucocorticoid secretion (35).

VII. Effects of Adrenocortical Hormones on Protein Synthesis in the Adrenal Medulla

The observations that glucocorticoids had no effect *in vitro* on PNMT activity, that actinomycin D or puromycin blocked the increase *in vivo* in PNMT activity caused by dexamethasone, and that the dexamethasone effect took hours or even days to reach a plateau, all suggested that glucocorticoids increased PNMT by inducing the synthesis of the enzyme. This hypothesis was confirmed

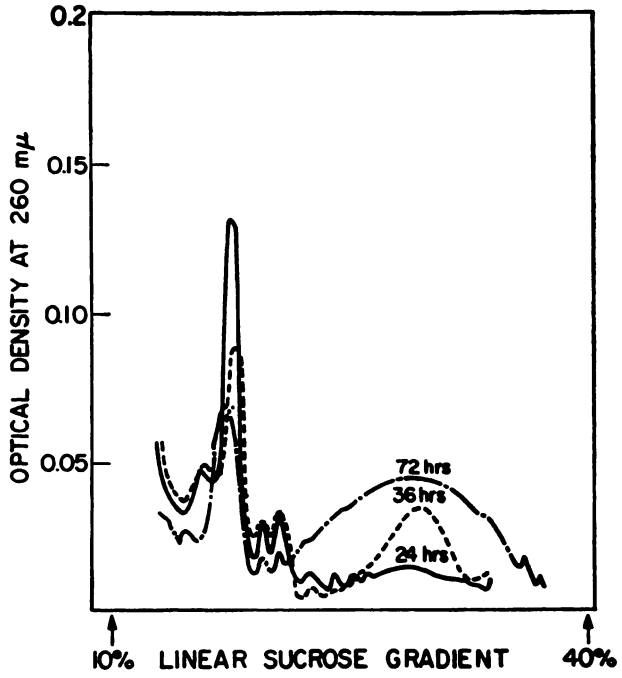

FIG. 8. Absorbance profiles of adrenal polysomes obtained from hypophysectomized rats killed at various times after receiving a single dose (1.6 mg/rat, intraperitoneally) of dexamethasone.

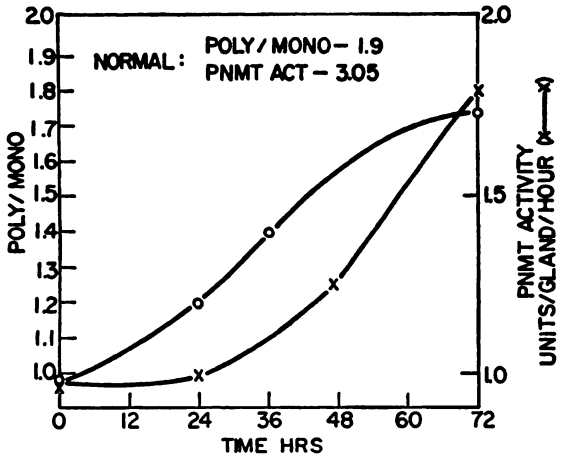

FIG. 9. Increases in polysome/monosome rats and PNMT activity in adrenals from hypophysectomized rats treated with a single dose (1.6 mg/rat) of dexamethasone. The polysome and monosome regions were measured planimetrically.

by measuring the incorporation of ^{3}H-tyrosine, administered 3 or 17 hr prior to sacrifice, into the PNMT protein present in dog adrenal medullas (table 1). (The isotopically labeled PNMT was purified by column chromatography and gel electrophoresis, and precipitated with a specific antibody (26, 28).) Adrenals of

hypophysectomized dogs accumulated less ^{3}H-PNMT at both times after ^{3}H-tyrosine administration than medullas from control animals or hypophysectomized dogs that had been treated with ACTH (table 1) (28). These changes were not the consequence of differences in the specific activities of the ^{3}H-amino acid precursor within the adrenals.

Since the synthesis of at least one adrenomedullary protein, PNMT, has thus been shown to depend upon the availability of glucocorticoid hormones, we have recently initiated studies to examine the hormone-dependence of adrenomedullary protein synthesis in general. The subcellular units on which peptide synthesis is thought to occur are the polyribosomes. These structures, each composed of a single strand of messenger ribonucleic acid (RNA) plus multiple pairs of ribosomes, are denser than either messenger RNA or free ribosomes; hence they can be separated from these other RNA species by ultracentrifugation over linear sucrose gradients. Treatments that cause the polyribosomes, or polysomes, in a tissue to disaggregate are, in general, associated with a profound decrease in the overall rate of protein synthesis (5). Polysome profiles prepared from postmitochondrial supernatant fractions of whole adrenals or adrenomedullary homogenates are similar to those obtained with other tissues; they contain small monosome and disome peaks, and a large mass of polysomes (figs. 7 and 8) (4, 28). Hypophysectomy is associated with a near-total disappearance of polysomes; the polysomes are restored in dog adrenal medullas (fig. 7) by treatment with ACTH (28) or in rat whole adrenals by treatment with dexamethasone (fig. 8) (4). A small polysome peak is discernible in the adrenals of hypophysectomized rats within 24 hr of dexamethasone treatment; this peak continues to rise for 72 hr. Since glucocorticoids are known to suppress protein synthesis in the adrenal *cortex* (7), the reappearance after dexamethasone administration of polysome peaks in homogenates of whole adrenals (as shown in fig. 8) almost certainly represents the reaggregation of messenger and ribosomal RNA within the chromaffin cells. The ratio of polysomal to monosomal RNA increases for several days after dexamethasone administration; this increase precedes the restoration of PNMT activity (fig. 9).

VIII. Summary

The activity of phenylethanolamine-N-methyl transferase (PNMT) in the adrenal medulla declines markedly after hypophysectomy. Enzyme activity is restored if animals are treated with ACTH or very large doses of glucocorticoids, but not by "replacement" doses of glucocorticoids or by other pituitary or adrenal hormones. These observations indicate that basal levels of PNMT activity require that the medulla receive the very high concentrations of hydrocortisone or corticosterone that are available to it by virtue of its unique location within an envelope of adrenal cortex.

The resotration by dexamethasone treatment of the PNMT activity in medullas of hypophysectomized animals is blocked by concurrent administration of actinomycin D or puromycin. Moreover, hypophysectomy is associated with decreases in the quantity of immunochemically assayable PNMT protein in the

medulla and in the rate at which PNMT is synthesized from isotopically labeled amino acid precursors. These changes are also reversed by dexamethasone. Adrenomedullary polysomes are markedly disaggregated after hypophysectomy; however, polysome patterns revert to normal if animals are treated with ACTH or dexamethasone. These observations all suggest that glucocorticoids stimulate the synthesis not only of PNMT, but also of a wide variety of medullary proteins.

The decrease in adrenal PNMT activity caused by hypophysectomy is associated with corresponding decreases in the mass of chromaffin cells and in the amounts of epinephrine stored in the adrenal medulla and secreted in response to physiological stimuli. After the induction of insulin hypoglycemia, adrenals of hypophysectomized dogs release considerably less epinephrine, and more norepinephrine, than those of control animals. Inasmuch as norepinephrine is far less potent than epinephrine in accelerating the breakdown of glycogen, the impairment in epinephrine synthesis caused by pituitary insufficiency may be related to the insulin sensitivity that often characterizes this disease.

Acknowledgments. These studies were supported in part by grant no. AM-11237 from the U.S. Public Health Service. The authors acknowledge with thanks the assistance of Dr. Julius Axelrod, in whose laboratory these studies were initiated, and of Mr. Orest Hurko.

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