Control of Enzymatic Synthesis of Adrenaline in the Adrenal Medulla by Adrenal Cortical Steroids

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SUMMARY

The activity of phenylethanolamine N-methyltransferase, the enzyme that catalyzes the N-methylation of noradrenaline to form adrenaline, falls following hypophysectomy. Enzyme activity can be restored by injections of adrenocorticotropic hormone (ACTH) or of dexamethasone, a potent synthetic glucocorticoid. The effect of ACTH on the adrenaline-forming enzyme is not direct, but involves stimulation of the release of endogenous glucocorticoids from the adrenal cortex. These steroids then act to elevate the activity of the transferase, which is principally localized in the adrenal medulla. The stimulation by glucocorticoids of phenylethanolamine N-methyltransferase activity can be blocked by the concurrent administration of puromycin or actinomycin D. Glucocorticoids do not stimulate the activity of other adrenal enzymes involved in catecholamine biosynthesis or metabolism, such as tyrosine hydroxylase, catechol O-methyltransferase, or monoamine oxidase.

The adrenal medulla is unique in mammals by virtue of its high concentration of an enzyme, phenylethanolamine N-methyltransferase, which synthesizes adrenaline from noradrenaline (1). Although very low levels of adrenaline-forming activity have also been shown in the brain and the heart (1), it is likely that almost all of the adrenaline secreted into the circulation is produced by this enzyme within the adrenal medulla (2).

In most mammals, the adrenal gland consists of two anatomically and chemically distinct structures; an inner medulla of catecholamine-producing chromaffin cells, and an outer cortex of cells which synthesize steroids. Although these two adrenal components originate embryologically from different sources, and come into close juxtaposition only through an unusual process of tissue migration, no definite functional relationship has thus far been established between them. The studies described here will show that the activity of phenylethanolamine N-methyltransferase within the adrenal medulla is regulated by glucocorticoids produced in the adrenal cortex.[1]

[1] A preliminary report of these studies has appeared (3).

EXPERIMENTAL PROCEDURE

Preparation of Animals—Hypophysectomized, sham-operated, and unoperated Sprague-Dawley female rats, weighing 180 g at the time of operation, were obtained from the Hormone Assay Laboratories, Chicago. All of the hypophysectomized animals failed to gain weight, and showed atrophy of the adrenals and ovaries. The animals were received 1 day after the operation, and were kept in artificial light from 7 a.m. to 7 p.m. and fed Purina chow, oranges, and milk *ad libitum*. Under these conditions, all of the hypophysectomized rats survived for the duration of the experiment.

Groups of rats were treated with corticosterone, hydrocortisone hemisuccinate, methopyrapone[2] ditartrate, or dexamethasone 21-phosphate dissolved in water, or adrenocorticotropic hormone in 16% gelatin. All drugs were injected subcutaneously, except where noted.

Assay of Phenylethanolamine N-Methyltransferase—Phenylethanolamine N-methyltransferase activity was assayed in the rat adrenal gland by a method described previously (1, 3). This assay depends on the transfer of the methyl group from ^{14}C-S-adenosylmethionine to the nitrogen of normetanephrine, to form ^{14}C-metanephrine. This compound is then separated from unreacted ^{14}C-S-adenosylmethionine by extraction into toluene-isoamyl alcohol, 3:2.

Rats were killed by neck fracture, and both adrenal glands were immediately removed and were dissected free of fat. The glands were weighed and then homogenized in 2.0 ml of ice-cold isotonic KCl solution in an all-glass homogenizer. The homogenate was then centrifuged for 20 min at 50,000 × g in the cold. A 50-μl aliquot of the supernatant fluid was transferred to a 15-ml centrifuge tube containing 37.5 μg of DL-normetanephrine-HCl, 1.0 mμmole of ^{14}C-S-adenosylmethionine (New England Nuclear, 50 μC per μmole), and 100 μmoles of phosphate buffer, pH 7.9, in a total volume of 250 μl. After 1 hour of incubation at 37°, the reaction was stopped by the addition of 0.5 ml of borate buffer, pH 10 (0.5 M), and the ^{14}C-metanephrine formed was extracted into 6 ml of a mixture of toluene and isoamyl alcohol, 3:2. A 4-ml aliquot of the organic phase was transferred to a vial containing 1 ml of ethanol and 10 ml of phosphor, and the radioactivity was measured in a

[2] The following trivial names are used: methopyrapone for 2-methyl-1,2-bis(3-pyridyl)-1-propanone; and dexamethasone for 9α-fluoro-16α-methyl-Δ^1-dehydrocortisol.

TABLE I

Effect of hypophysectomy, ACTH, and dexamethasone on adrenal phenylethanolamine N-methyltransferase activity

Animals were sham-operated or hypophysectomized, and killed 17 to 21 days later. Some of the hypophysectomized rats were given ACTH (4 units subcutaneously per day) or dexamethasone (1 mg intraperitoneally per day) for 6 days prior to autopsy. Each group contained 8 to 12 animals. Data are expressed as mean ± standard error of the mean.

Treatment	Adrenal weight	Phenylethanolamine N-methyltransferase	Adrenaline
	mg/pair	*units/pair*	*µg/pair*
Control	68.9 ± 3.4[a]	6.16 ± 0.66[a]	31.8 ± 1.5[b]
Hypophysectomy	26.5 ± 2.1	1.46 ± 0.11	24.3 ± 1.7
Hypophysectomy and ACTH	46.7 ± 1.5[a]	4.76 ± 0.26[a]	25.3 ± 1.6
Hypophysectomy and dexamethasone	26.0 ± 2.3	7.08 ± 0.44[a]	29.0 ± 2.6

[a] $p < 0.001$ differs from hypophysectomy.
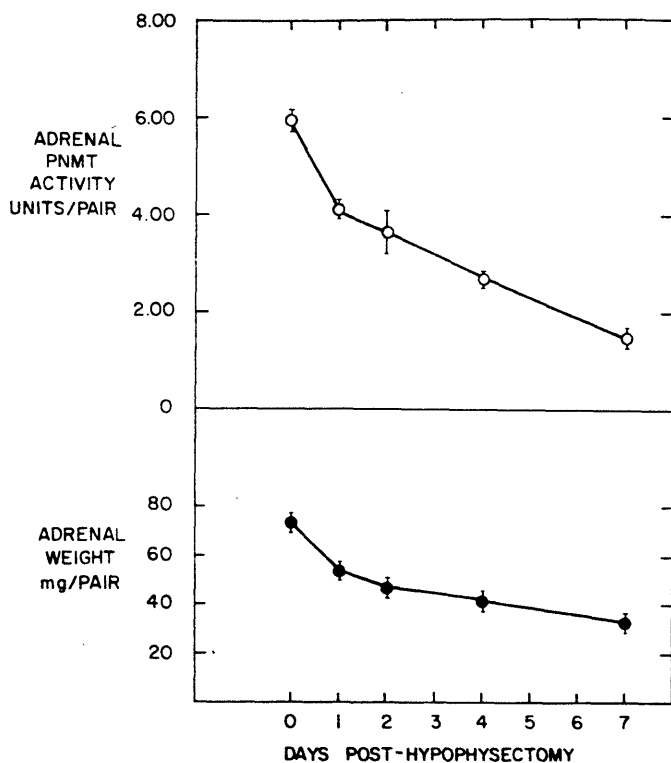[b] $p < 0.05$ differs from hypophysectomy.

FIG. 1. Changes in adrenal weight and phenylethanolamine N-methyltransferase activity (*PNMT*) following hypophysectomy in the rat. Each group contained six rats.

liquid scintillation spectrophotometer. Corrections for the small amount of ^{14}C-S-adenosylmethionine that is extracted into the organic solvent were made by omitting the normetanephrine from the incubation medium.

Assay of Catechol O-Methyltransferase—Catechol O-methyltransferase was assayed by a modification of the method described previously (4). A 200-µl aliquot of the soluble supernatant fraction of the adrenal medulla (prepared as described above) was transferred to a 15-ml centrifuge tube containing 0.5 µmole of *dl*-epinephrine, 1.0 mµmole of ^{14}C-S-adenosylmethionine, 20 µmoles of MgCl$_2$, 50 µmoles of phosphate buffer, pH 7.9, and water to make a final volume of 500 µl. After 1 hour of incubation at 37°, the reaction was stopped by the addition of 0.5 ml of borate buffer, pH 10 (0.5 M), and the ^{14}C-metanephrine formed was extracted into a toluene-isoamyl alcohol mixture, as described above.

Monoamine oxidase was assayed in whole adrenal homogenates by the method of Wurtman and Axelrod (5). Tyrosine hydroxylase was kindly assayed by Drs. Morton Levitt, Morris Lipton, and James Gibb of the National Heart Institute (6). For all enzyme assays, 1 unit of activity catalyzes the formation of 1 mµmole of product per hour.

A portion of the adrenal supernatant fluid was acidified with an equal volume of 2 N acetic acid, and a 25-µl aliquot was assayed for noradrenaline and adrenaline by the method of von Euler and Lishajko (7).

Actinomycin D and puromycin were generously supplied by Drs. Arnold Osterberg and Alfred Stanley of the National Cancer Institute.

RESULTS

Effect of Hypophysectomy on Adrenal Phenylethanolamine N-Methyltransferase Activity and Catecholamine Content—Groups of 12 rats were hypophysectomized or sham-operated and killed 17 to 21 days later. The adrenals were assayed for phenylethanolamine N-methyltransferase activity, and for their content of adrenaline and noradrenaline (Table I). Removal of the pituitary gland was associated with a decline in adrenal weight, and an even greater fall in phenylethanolamine N-methyltransferase activity. The adrenals of hypophysectomized rats contained significantly less adrenaline than those of control animals, and a smaller fraction of their total content of catecholamine was methylated.

To determine the rate at which phenylethanolamine N-methyltransferase falls after removal of the pituitary gland, the enzyme was measured in other animals killed 0, 1, 2, 4, and 7 days after hypophysectomy. Within 24 hours of surgery, both adrenal weight and adrenaline-forming activity had fallen significantly (Fig. 1). Subsequently, enzyme activity declined more rapidly than adrenal weight, so that by 7 days after hypophysectomy, the activity per g of whole adrenal was less than half that of the control animals. Other animals assayed 5 weeks after hypophysectomy had less than 10% of the normal levels of phenylethanolamine N-methyltransferase activity.

Effect of ACTH[3] on Adrenal Phenylethanolamine N-Methyltransferase—The mammalian pituitary gland secretes at least six hormones. To determine whether the lack of pituitary ACTH was responsible for the decline in adrenal phenylethanolamine N-methyltransferase activity, animals which had been hypophysectomized 11 days earlier were treated for 6 days with daily injections of 4 units of ACTH. This caused adrenal weight and adrenaline-forming ability to return almost to normal (Table I).

Site of Action of ACTH in Increasing Adrenal Phenylethanolamine N-Methyltransferase Activity—There were at least three possible routes by which ACTH could have enhanced the adrenaline-forming activity of the rat adrenal: (*a*) the adrenal cortex could have contained an enzyme capable of N-methylating

[3] The abbreviation used is: ACTH, adrenocorticotropic hormone.

noradrenaline. As the amount of adrenocortical tissue increased (following treatment with ACTH), enzyme activity would have increased proportionately. (b) ACTH could have exerted a direct effect upon the methylating enzyme in the adrenal medulla. (c) ACTH could have stimulated the synthesis of glucocorticoids in the adrenal cortex, and their secretion into the adrenal venous blood. These steroids could have then acted to increase the formation or the activity of phenylethanolamine N-methyltransferase.

To test the first possibility, phenylethanolamine N-methyltransferase activity was assayed in separated adrenocortical and adrenomedullary tissues from 10 rats. It was found that the adrenaline-forming ability of the adrenal cortex was less than one-tenth that of the medulla (Table II). Since there is no capsule separating the adrenal cortex from the medulla in the rat, it is possible that the small amount of enzyme activity found in the cortex was due to contamination with adrenomedullary tissue.

To rule out the possibility that the adrenocorticotropic hormone regulated phenylethanolamine N-methyltransferase activity by a direct action on the adrenal medulla, experiments were designed to determine whether the level of endogenous ACTH in the circulation could be increased or decreased without producing a parallel alteration in enzyme activity. First, groups of six normal rats were treated for 6 days with daily intraperitoneal injections of 1 mg of methopyrapone bitartarate. (This agent interferes with the biosynthesis of glucocorticoids, by inhibiting the steroid 11-hydroxylating enzyme. Since the concentrations of ACTH and the glucocorticoids in the blood generally bear an inverse relation to each other, such treatment would be expected to raise blood ACTH levels (8).) The adrenal weights of animals treated with methopyrapone increased (Table III), suggesting that the secretion of ACTH by the pituitary had indeed been elevated. However, the total enzyme activity per pair of adrenals was not changed. Other normal animals were given daily injections of 1 mg of dexamethasone, a highly potent synthetic glucocorticoid. This steroid produced a fall in the weight of the adrenal glands, suggesting that the release of ACTH from the pituitary had been reduced (Table III). However, phenylethanolamine N-methyltransferase activity rose slightly. These experiments indicated that experimental conditions which are associated with a change in the level of circulating ACTH do not necessarily produce corresponding alterations in phenylethanolamine N-methyltransferase activity. This indicated that the effect of ACTH observed in the hypophysectomized animal was not due to a direct action on the adrenal medulla.

TABLE II

Localization of phenylethanolamine N-methyltransferase activity in adrenal gland

Adrenals from 10 rats were separated into medulla and cortex. Tissues from two rats were pooled, weighed, homogenized in isotonic KCl, and centrifuged, and aliquots representing equal amounts of tissue were assayed for enzyme activity.

Tissue	Phenylethanolamine N-methyltransferase
	unit/mg
Adrenal medulla	0.125 ± 0.007
Adrenal cortex	0.012 ± 0.002

TABLE III

Effects of dexamethasone and methopyrapone on phenylethanolamine N-methyltransferase activity in intact rats

Groups of six Sprague-Dawley female rats were given 1 mg of the drug intraperitoneally for 6 days, and were killed on the 7th day.

Treatment	Adrenal weight	Phenylethanolamine N-methyltransferase
	mg/pair	*units/pair*
Control	54 ± 1.2	5.12 ± 0.47
Dexamethasone	35 ± 1.3[a]	6.02 ± 0.44
Methopyrapone	66 ± 2.2[a]	5.12 ± 0.47

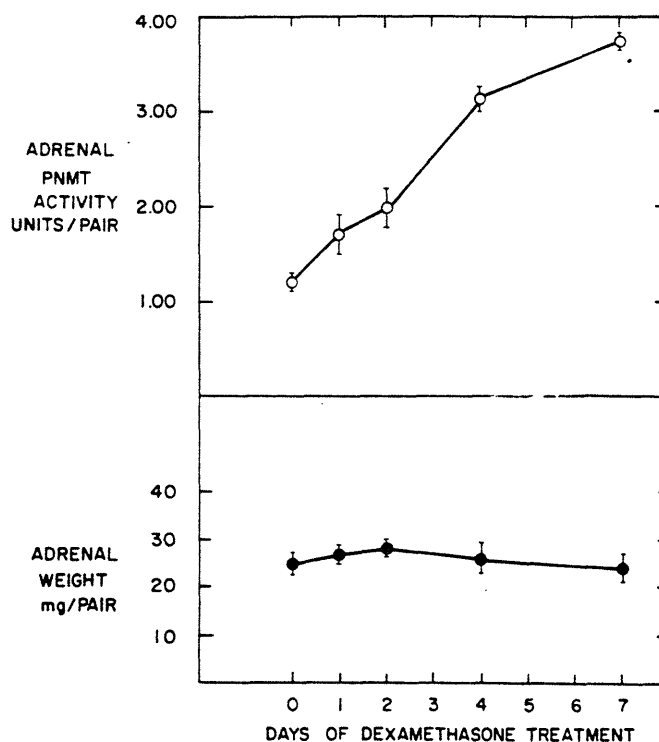[a] $p < 0.001$ differs from control.

FIG 2. Effect of dexamethasone treatment on adrenal weight and phenylethanolamine N-methyltransferase activity (PNMT) in the hypophysectomized rat. Animals were given dexamethasone (1 mg intraperitoneally per day) for various periods, and killed 17 days after hypophysectomy. Each group contained six rats.

Effect of Glucocorticoids on Adrenal Phenylethanolamine N-Methyltransferase—To determine whether the influence of ACTH on adrenal phenylethanolamine N-methyltransferase activity operated indirectly, via changes in the synthesis and secretion of glucocorticoids, animals which had been hypophysectomized 15 days earlier were treated with dexamethasone (1 mg per day intraperitoneally) for 6 days. Treatment with this synthetic glucocorticoid produced a marked rise in phenylethanolamine N-methyltransferase activity, without elevating adrenal weight (Table I).

Other hypophysectomized rats were treated with daily injections of dexamethasone for 0, 1, 2, 4, or 7 days; they were all killed 17 days after hypophysectomy. A rise in enzyme activity could be shown after 1 day of treatment. Phenylethanolamine N-methyltransferase activity continued to rise for the duration of the experiment (Fig. 2). Adrenal weight did not change
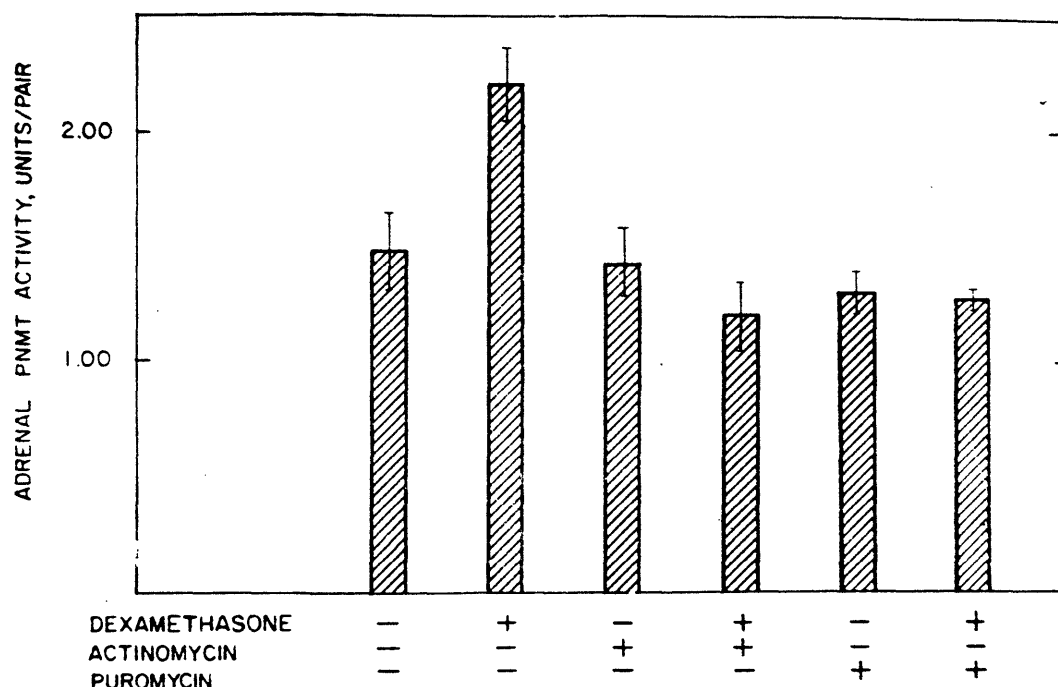
FIG. 3. Effects of actinomycin D and puromycin on the response of adrenal phenylethanolamine N-methyltransferase ($PNMT$) to dexamethasone in the hypophysectomized rat. Hypophysectomized animals (21 days after surgery) were given a 4-hour in-travenous infusion of dexamethasone (1 mg per hour), and killed 5 hours later. Some rats were treated with actinomycin D (1 mg per kg) or puromycin (40 mg per kg), in a single intravenous injection before the infusion. Each group contained six rats.

TABLE IV

Effect of hypophysectomy and dexamethasone on activity of enzymes in adrenal gland

Animals were sham-operated or hypophysectomized, and killed 30 days later. Some of the hypophysectomized rats were treated with dexamethasone (1 mg daily, intraperitoneally) for 3 days prior to assay. Each group contained six animals.

Treatment	Adrenal weight	Phenyl-ethanol-amine N-methyl-transferase	Mono-amine oxidase	Catechol O-meth-yltrans-ferase	Tyrosine hydrox-ylase
	mg	*units/pair*	*units/pair*	*units/pair*	*units/pair*
Control............	65.0 ± 1.6[a]	4.32 ± 0.32[a]	88.8 ± 0.8[a]	1.06 ± 0.01[a]	41.8 ± 6.6
Hypophysectomy....	22.0 ± 1.4	0.72 ± 0.08	27.6 ± 0.8	0.75 ± 0.01	30.1 ± 1.4
Hypophysectomy and dexamethasone.....	17.2 ± 0.8[b]	1.44 ± 0.04[a]	20.8 ± 0.4[a]	0.50 ± 0.01	29.2 ± 0.5

[a] $p < 0.001$ differs from hypophysectomy.

[b] $p < 0.05$ differs from hypophysectomy.

significantly. In other experiments, it has been observed that when hypophysectomized rats are treated with dexamethasone for 2 weeks, the activity of the adrenaline-forming enzyme rises to levels which are higher by 30 to 50% than those found in normal rats.

Mechanism of Dexamethasone Action on Phenylethanolamine N-Methyltransferase Activity—Dexamethasone could elevate phenylethanolamine N-methyltransferase activity by directly stimulating enzyme activity, or by affecting the synthesis or breakdown of the enzyme protein. To examine the possibility of a direct effect, adrenal homogenates obtained from hypophysectomized and sham-operated animals were assayed for adrenaline-forming activity with and without the addition *in vitro* of dexamethasone or corticosterone (the major natural glucocorticoid in the rat (9)). Both steroids had no effect on phenylethanolamine N-methyltransferase activity in concentrations below 10^{-5} M. Larger amounts of corticosterone actually inhibited enzyme activity. This suggested that the stimulating effect of the steroids *in vivo* was not due to activation of pre-existing enzyme.

To determine whether the corticoid-induced rise in enzyme activity resulted from increased synthesis of the enzyme protein, the effect of dexamethasone was examined in animals in whom RNA-dependent protein synthesis had been inhibited pharmacologically by actinomycin D or puromycin. Hypophysectomized rats were given a 4-hour intravenous infusion of 1 mg of dexamethasone per hour, and killed 5 hours later. Treatment for this short interval produced a significant rise in phenylethanolamine N-methyltransferase activity (Fig. 3). Other rats were treated with actinomycin D (1 mg per kg intravenously) or puromycin (40 mg per kg intravenously), followed in some by a similar infusion of dexamethasone. Neither actinomycin D nor puromycin altered the activity of the adrenaline-forming enzyme in the adrenal medulla (Fig. 3). However both agents blocked the rise in enzyme activity caused by dexamethasone. These results suggest that glucocorticoids elevated phenylethanolamine N-methyltransferase activity by increasing the rate of synthesis of the enzyme protein.

Specificity of Effect of Dexamethasone on Adrenomedullary Phenylethanolamine N-Methyltransferase—Groups of six rats were

hypophysectomized or sham-operated and killed 30 days later. For 3 days prior to autopsy, some of the animals were given daily injections of dexamethasone (1 mg). Four enzymes involved in catecholamine formation or metabolism were then assayed in the whole adrenal gland. These were phenylethanolamine *N*-methyltransferase, tyrosine hydroxylase (which may control the rate of synthesis of noradrenaline (10), the substrate of the adrenaline-forming enzyme), catechol *O*-methyltransferase, and monoamine oxidase. Hypophysectomy was associated with a marked fall in adrenal weight, and an even greater decrease in adrenaline-forming activity (Table IV). Monoamine oxidase activity was depressed to the same extent as adrenal weight, while catechol *O*-methyltransferase and tyrosine hydroxylase activities fell by about 35% (Table IV). The administration of dexamethasone to hypophysectomized rats doubled the activity of phenylethanolamine *N*-methyltransferase per pair of adrenals. On the other hand, dexamethasone treatment produced a slight decrease in the activities of catechol *O*-methyltransferase and monoamine oxidase, and no change in that of tyrosine hydroxylase (Table IV).

DISCUSSION

These studies show that glucocorticoids markedly influence the enzymatic synthesis of adrenaline in the rat adrenal medulla. Removal of the pituitary gland causes a decline in both the phenylethanolamine *N*-methyltransferase activity and the adrenaline content of the adrenals. Enzyme activity can be restored by the administration of ACTH, or by a potent synthetic glucocorticoid, dexamethasone. The restoration of adrenaline-forming activity by ACTH is mediated by an increase in the availability of endogenous glucocorticoids and is not due to a direct action of the pituitary hormone on the adrenal medulla. It thus appears that the chromaffin cells of the adrenal medulla constitute a "target organ" for the glucocorticoids elaborated by the adrenal cortex. It is likely that factors which alter the synthesis and secretion of these corticoids (*e.g.* pituitary insufficiency) may produce some of their biochemical effects as a result of changes in the availability of adrenaline.

Although no direct functional relationship has heretofore been established between the two major components of the adrenal gland, other investigators have noted a correlation between the juxtaposition of the cortex and medulla in the adrenal and its catecholamine content, and have suggested that the adrenal cortex influences the methylation of noradrenaline (11). Thus, in the dogfish the chromaffin tissue is entirely outside the adrenal gland and appears to contain no measurable adrenaline (11). In the rabbit, part of the chromaffin tissue is contiguous with adrenal cortex; this part contains chiefly adrenaline, while the portion of the adrenal medulla which is not in contact with cortex contains only noradrenaline (11). In the human and the rat, the adrenal cortex completely surrounds the adrenal chromaffin

cells, and most of the catecholamine in the gland is adrenaline (12).

Because of its position within an envelope of adrenal cortex, it is likely that the mammalian adrenal medulla is exposed to considerably higher levels of glucocorticoids than any other organ in the body. Preliminary experiments suggest that correspondingly higher levels of cortical hormones are, in fact, needed to maintain the activity of phenylethanolamine *N*-methyltransferase. Low doses of glucocorticoids, which are sufficient to affect such extra-adrenal target organs as splenic weight, do not raise the activity of this enzyme in the hypophysectomized animal.

The stimulatory effect of glucocorticoids on medullary phenylethanolamine *N*-methyltransferase appears to be highly specific; dexamethasone does not enhance the activity of tyrosine hydroxylase, the enzyme that appears to control the rate of synthesis of noradrenaline (10). (Hence its effect on the adrenaline-forming enzyme is probably not the consequence of alterations in the availability of substrate.) It also does not increase adrenal monoamine oxidase or catechol *O*-methyltransferase activities, even though the latter enzyme is also a methylating enzyme, with subcellular localization and cofactor requirements similar to those of phenylethanolamine *N*-methyltransferase (1, 13).

The ability of puromycin to block the rise in phenylethanolamine *N*-methyltransferase activity produced by dexamethasone suggests that the mechanism of this rise involves synthesis of new enzyme protein. Since actinomycin D also prevents this rise, it is probable that the glucocorticoid exerts its effect at the level of RNA transcription from DNA. There is a growing body of evidence that glucocorticoids induce a number of other enzymes by a similar mechanism (14).

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