Adrenal Cortical Control of Adrenal Medullary Function (33576)

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The adrenal gland of most mammals is composed of two chemically and anatomically discrete structures; an outer cortex of cells producing steroid hormones which surrounds an inner medulla of cateocholamine-producing chromaffin cells. These two adrenal components originate embryologically from different sources and come into juxtaposition following an unusual process of tissue migration. Until recently, functional relationship between the adrenal cortex and the adrenal medulla had not been studied.

In 1965, Wurtman and Axelrod described an effect of glucocorticoids upon the formation of epinephrine which was through the effect of cortical hormones on the *N*-methylation of norepinephrine. The enzyme phenylethanolamine-*N*-methyl-transferase (PNMT) responsible for this methylation was found to be localized in highest concentrations in the adrenal medulla of mammals. Wurtman and Axelrod noted the depression of PNMT activity following hypophysectomy as well as an increase in activity after the administration of ACTH or dexamethasone. They concluded that the adrenal medulla is a "target organ" of the pituitary-adrenocortical system (1). In such a scheme, glucocorticoid-mediated regulation of enzymatic methylation of norepinphrine may in fact, be rate-limiting in the adrenal medullary secretion of epinephrine (1a).

However, Fuller and Hunt found that in the intact resting rat, the corticoid concentration is such that the PNMT activity appears to be maximal, and further stimulation of adrenocortical output does not increase PNMT activity. Rather, they found that only after a period of reduced corticoid secretion, as seen after hypophysectomy, could one observe an increase in the PNMT activity produced by glucocorticoid or ACTH administration (2).

The purpose of the present research was to study adrenal medullary function under conditions of adrenal-cortical insufficiency, and to examine medullary activity as a time-function of adrenal cortical activation.

Materials and Methods. Sixty male albino rats weighing 150–170 g were divided into 15 groups of four per group.

The first group, used as controls, were decapitated and adrenals were removed immediately. The other groups were hypophysectomized while under ether anesthesia. The completeness of the hypophysectomy was checked at the end of the experiment. Seven groups of rats were hypophysectomized, and adrenals were removed at time intervals of 15-30 min, and in 6 weeks. The remaining groups were hypophysectomized and, following a single dose of ACTH, adrenals and blood samples were taken at time intervals of 3–15 min for 30 min.

In both experiments, abdominal aorta blood was drawn and both adrenals immediately were removed, weighed, and quick-frozen with acetone and dry ice for storage. Paired adrenals were assayed for epinephrine and norepinephrine by the method of Von Euler and Lishajko (3), and for PNMT by the method described by Axelrod involving the use of *S*-adenosylmethionine-^{14}C (4). (One unit of enzyme activity catalyzes the formation of 1 mμmole of product/hr.) Corticosterone was measured in the plasma by Silber's modification of Sweat's fluorometric procedure (5). The ACTH used in this experiment was the USP Third International Reference standard injected intravenously at a dosage of 0.8 milliunits.

Results. The results of the first experiment (Table I), demonstrate no significant change from control values in the epinephrine content or PNMT activity after hypophysectomy during the entire time period through 120 min. There is a significant decrease in norepinephrine content after hypophysectomy. The increase in corticosterone

TABLE I. Effects of Hypophysectomy on Adrenal Cortical and Medullary Response.[a]

Group no.	Remarks	Treatment	Corticosterone[b] (μg/100 ml of plasma)	Epinephrine[b] (μg/g of gland)	Norepinephrine[b] (μg/g of gland)	PNMT[b] (units)
1		Control	11.5 ± 4.9	19.4 ± 0.33	6.6 ± 1.0	3.8 ± 0.05
2	15 min	Hypox	33.5 ± 7.3	22.6 ± 0.68	5.2 ± 0.3	3.8 ± 0.4
3	30 min	Hypox	21.2 ± 2.5	20.4 ± 1.7	3.2 ± 0.4	3.3 ± 0.3
4	45 min	Hypox	2.3 ± 1.9	21.6 ± 0.7	2.6 ± 0.1	3.4 ± 0.07
5	60 min	Hypox	2.5 ± 0.5	19.6 ± 0.5	1.2 ± 0.2	3.4 ± 0.1
6	90 min	Hypox	0.3 ± 0.3	24.2 ± 0.4	1.4 ± 0.3	3.4 ± 0.2
7	120 min	Hypox	0	21.6 ± 0.4	1.8 ± 0.3	3.2 ± 0.16
8	24 hr	Hypox	0	21.4 ± 1.0	0.6 ± 0.08	2.9 ± 0.07
9	6 weeks	Hypox	0	12.0 ± 0.1	3.4 ± 0.3	0.8 ± 0.07

[a] Rats were hypophysectomized and tissue and blood samples were taken at specified time intervals. Each group contained four animals.

[b] Data are expressed as means ± 1 SEM.

seen at 15 min is probably due to the stress of hypophysectomy, which by 30 min starts decreasing and is not detectable at 120 min. After 6 weeks, there is a significant decrease from control values in catecholamine content, enzyme activity, and steroid secretion.

The second experiment was designed to examine the immediate effects of ACTH on the adrenals of rats which had been hypophysectomized only one day prior to the experiment. These results, (Table II), show a significant decrease in PNMT activity, corticosterone concentration, and norepinephrine content 24 hr after hypophysectomy. Epinephrine concentration remains unchanged from control values. Three min after 0.8 milliunits of ACTH is given intravenously, there is an increase in PNMT activity, catecholamine content, and corticosterone secretion. Epinephrine and norepinephrine peak during the 3–12 time range as does the PNMT, then all three of these parameters decline. The corticosterone in arterial blood showed a progressive rise as seen in earlier studies (6).

Discussion. A relationship between adrenal cortical and medullary function was first suggested almost 15 years ago. At this time, Coupland of the University of Leeds, using the recognized anatomic separation of adrenal cortex from medulla in the dogfish, made the observation that pressor activity of adrenal medullary tissue is due exclusively to

TABLE II. Effects of ACTH on Adrenal Medullary and Cortical Activity in Hypophysectomized Rats.[a]

Group no.	Remarks	Treatment + time (min)	Corticosterone[b] (μg/100 ml of plasma)	Epinephrine[b] (μg/g of gland)	Norepinephrine[b] (μg/g of gland)	PNMT[b] (units)
1	Control	None	11.5 ± 4.9	19.4 ± 0.3	6.6 ± 1.0	3.8 ± 0.05
2	Hypox	None	0	21.4 ± 1.0	0.6 ± 0.08	2.9 ± 0.07[c]
3	Hypox	ACTH + 3	1.5 ± 0.0	26.2 ± 0.3	1.2 ± 0.3	3.6 ± 0.17
4	Hypox	ACTH + 6	10.8 ± 1.1	22.0 ± 0.4	1.2 ± 0.1	3.7 ± 0.07
5	Hypox	ACTH + 9	26.7 ± 4.6	26.0 ± 1.2	6.0 ± 0.8	3.5 ± 0.18
6	Hypox	ACTH + 12	17.3 ± 0.9	26.0 ± 1.3	3.8 ± 0.9	3.1 ± 0.06
7	Hypox	ACTH + 15	16.7 ± 2.5	15.0 ± 0.8	1.8 ± 0.3	3.2 ± 0.17
8	Hypox	ACTH + 30	33.0 ±10.1	18.2 ± 0.09	1.4 ± 0.3	3.1 ± 0.08

[a] Rats were hypophysectomized 1 day prior to assay. All animals except for controls were given ACTH, 0.8 milliunits intravenously. Each group contained four animals.

[b] Data are expressed as means ± 1 SEM.

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

norepinephrine. On the other hand, when he studied species, (man and rabbit), where cortex and medulla were anatomically juxtaposed, adrenal medullary (chromaffin) tissue contained both noradrenaline and adrenaline. In these same higher species, study of adjacent extraadrenal chromaffin tissue, which is not surrounded by cortex, revealed norepinephrine exclusively. Coupland proposed that the adrenal cortex exerts an influence upon adrenal medullary function and suggested that the cortex may be necessary for the methylation of noradrenaline to form its methylated product, adrenaline (7). At this time, the methylating enzyme had not been identified.

Shortly after the work of Coupland, an *N*-methylating enzyme was identified and characterized in adrenal medullary tissue (8). This was the final link in elucidation of the biosynthesis of adrenaline.

Upon this basis, Wurtman and Axelrod recently suggested an effect of ACTH on epinephrine synthesis, which they subsequently showed to be due to activation by glucocorticoids of the *N*-methylating enzyme (9).

Our results described here are in agreement with their studies. Additionally, earlier evidence suggesting that hypophysectomy leads to reduction in PNMT activity, and epinephrine content, was confirmed by these studies. Our studies demonstrated that addition of ACTH produces enzyme activation in an hypophysectomized animal through stimulation of glucocorticoid secretion. The critical factor from our studies, however, suggests that the decrease in medullary enzyme activation and epinephrine content following hypophysectomy is not an immediate response as one sees in adrenal cortical functoin following hypophysectomy. Rather, there is a lag period demonstrated by our studies which suggests that the adrenal cortex may retain sufficient glucocorticoids to maintain basal-levels of enzyme in the medulla, or that other factors may exist in the biosynthesis of epinephrine which have not been considered. This may represent an example of the permissive action of glucocorticoids.

The immediate activation of the enzyme PNMT following ACTH administration in the hypophysectomized animal occurs simultaneously with the appearance of corticosterone secretion in peripheral blood. While a cause and effect relationship is suggested by this observation, it is of interest that in the face of a continuing rise in corticosteroid levels in peripheral blood, enzyme levels plateau shortly after the initial rise. These observations lend some support to Fuller and Hunt's suggestion that in the intact animal the concentration of PNMT is near maximal, such that further stimulation of adrenocortical output does not significantly further increase PNMT activity.

The observations described here suggest the concept of a unique relationship between adrenal cortical and adrenal medullary function. This relationship consists of a subtle control by steroids of medullary levels of the enzyme required for the conversion of norepinephrine to epinephrine. Under conditions of *normal* adrenal *cortical* function, medullary levels of this enzyme, and adrenaline, are essentially preserved; and in the normal situation the relationship is one essentially of maintenance. This dependency of enzyme levels of PNMT cannot in fact, be demonstrated either experimentally or under clinical circumstances *except* when adrenal cortical function is diminished.

Our studies in the experimental animal, demonstrate that only under conditions of *diminished* adrenal cortical function can one see parallel and concomitant diminution in medullary levels of epinephrine, norepinephrine, and PNMT. Conversely, the administration of ACTH to animals with normal adrenal cortical function, while leading to increases in glucocorticoids, fails to produce appreciable increases in enzyme content. Because of these observations, we have reexamined the clinical literature for the counterparts of these experimental conditions.

In Addisonian patients, low levels of glucocorticoids were associated with equally low levels of epinephrine excretion (10). In Cushing's syndrome, on the other hand, where adrenal glucocorticoids are normal, or elevated, there were no measurable effects

upon adrenal medullary function reported.

While the mechanism of this control of medullary function by cortical tissue is at present not clearly understood, the findings here are reminiscent of the permissive role of hormones in other systems. Whether or not this type of control constitutes a permissive or a direct effect of adrenal cortical hormones in the regulation of adrenal medullary function is currently under investigation.

Summary. Hypophysectomy in the rat leads to a decrease in adrenal medullary concentrations of phenylethanolamine-*N*-methyltransferase (PNMT), the enzyme which catalyzes the conversion, *in situ*, of norepinephrine to epinephrine. Unlike the immediate decrease of glucocorticoids, this decrement takes several days (weeks) to occur. When ACTH is now given to such animals, not only is steroid synthesis resumed, but there is an immediate activation of PNMT and epinephrine synthesis.

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Received Sept. 4, 1968. P.S.E.B.M., 1969, Vol. 130.

Collagen Synthesis in Cultured Cells: The Influence of Beta-Aminopropionitrile* (33577)

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Experimental lathyrism is a connective tissue disease exhibiting changes in collagen, elastin and ground substance (1–3). The nature of the defect in collagen has been shown to be a failure in cross-linking which in turn is manifested by an increase in neutral salt soluble collagen in lathyritic animals (4–6). There appears, however, to be no direct *in vivo* effect of lathyrogens on the total synthesis of collagen. Similar *in vivo* studies of the effect of lathyrogens on polysaccharide synthesis have been conflicting with reports in the literature of no change, an increase, and decrease in total synthesis (7–10).

* Supported by the National Institutes of Health, United States Public Health Grant DE-AM-02547.

In vitro studies, however, have consistantly indicated an increase in overall polysaccharide production in cell cultures treated with lathyrogens. There are reports of increased ^{35}S uptake (11) and, increased glycogen and uronic acid synthesis (12) in lathyrus-treated strain L fibroblasts, and an increase in protein bound hexose in fibroblasts isolated from human gingiva (13). The *in vitro* effect of lathyrogens on established collagen-producing cell lines has not been investigated. The purpose of this study, therefore, was to investigate the production of collagen by lathyrogen-treated cells in culture.

Materials and Methods. Culture. The 3T6 fibroblasts, known to produce collagen (14–16), were grown in 60 × 15-mm plastic