

# Stress and the Adrenocortical Control of Epinephrine Synthesis

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Psychologic states produced by environmental or physiologic stresses are usually associated with hypersecretion of adrenal hormones, particularly epinephrine and the glucocorticoids (hydrocortisone in humans or corticosterone in rats). A common mechanism links the secretion of these hormones, even though the adrenal medulla and cortex have different embryologic origins and biochemical properties and very different mechanisms controlling their secretory activities, ie, a cholinergic nervous input stimulates medullary secretion while a hormone, corticotropin (ACTH), activates secretion from the cortex. This mechanism is made possible by an intra-adrenal portal vascular system, which provides the medulla with uniquely high concentrations of glucocorticoids. These high concentrations are needed to induce the medullary enzyme, phenylethanolamine-N-methyltransferase (PNMT), which controls the synthesis of epinephrine from norepinephrine. By suppressing glucocorticoid secretion, pituitary failure compromises epinephrine synthesis and decreases the rate at which epinephrine is secreted; in contrast, prolonged chronic stress can enhance epinephrine synthesis and secretion within the adrenal, the brain, or both organs. This control mechanism could be involved in the long-term consequences of stress.

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**T**HE CLASSIC PERIPHERAL manifestations of severe environmental or physiological stress—increased plasma levels of epinephrine and hydrocortisone (in humans) or corticosterone (in rats)—result from hypersecretion of these hormones from the adrenal medulla and cortex, respectively. A common mechanism links these processes, even though the medulla and cortex have different embryologic origins and biochemical properties, and very different chemical signals (the neurotransmitter acetylcholine and a pituitary hormone, corticotropin [ACTH], respectively) control their secretory activities. For several decades, on the basis of experiments performed on rats, it had been thought that circulating epinephrine acted directly on the pituitary to control the release of ACTH. In this formulation the first step in the adrenal “stress response” was thought to be the liberation of epinephrine; this in turn released ACTH, leading to hypersecretion by the adrenal cortex. However, subsequent experiments on humans failed to demonstrate any consistent effect of circulating epinephrine on ACTH secretion. Hence, it is no longer believed that epinephrine controls glucocorticoid secretion. Rather, the converse is true: glucocorticoids control epinephrine synthesis, and thereby affect its secretion.<sup>1,2</sup>

This perception had its origins in a patient that I saw as a student in 1965. She had become unusually sensitive to endogenous insulin after suffering a pituitary infarction during a difficult labor. Several hours after eating a carbohydrate-rich meal she would develop persistent hypoglycemia, sometimes even leading to seizures.

The enhanced insulin sensitivity seen in patients with pituitary insufficiency had generally been attributed to the absence of a hyperglycemic factor normally secreted from the pituitary (eg, growth hormone) or to the impairment in gluconeogenesis that might be expected to result from the ACTH deficiency and resulting decrease in glucocorticoid secretion.<sup>3,4</sup> However, this explanation seemed suspect, given that growth hormone or ACTH need many hours to raise plasma glucose levels. More likely, hypopituitary patients also lacked the substance that normally acts most rapidly to restore plasma glucose levels after induction of hypoglycemia. Since epinephrine seemed the best candidate for this hypothetical fast-acting hyperglycemic agent, we designed studies, in collaboration with Julius Axel-

rod, to determine whether pituitary failure could be shown to affect the synthesis or secretion of this catecholamine. These studies led to the discovery that the very high concentrations of glucocorticoids that the adrenal cortex normally delivers to the adrenal medulla (via the adrenal’s portal vascular system) induce the enzyme, PNMT, that is rate-limiting in epinephrine synthesis. By suppressing glucocorticoid secretion, pituitary failure not only compromised epinephrine synthesis, but also decreased the levels of epinephrine in the adrenal medulla and the rate at which epinephrine is secreted in response to hypoglycemia.<sup>5</sup>

A severe prolonged stress, which chronically elevates glucocorticoid secretion, can have opposite effects.

## ADRENAL MORPHOLOGY AND EPINEPHRINE SYNTHESIS

On the basis of the adrenal’s unique property of containing 2 distinct organs, of which one (the cortex) surrounds the other (the medulla), it had been suspected for a while that one of these organs played some special role in the physiology of the other. In 1953, Coupland, an English anatomist, drew attention to the correlation between the morphology of the mammalian adrenal gland in any particular species and its contents of epinephrine or norepinephrine.<sup>6</sup> He noted that in species in which the adrenal cortex and medulla are juxtaposed (ie, in rats and humans), epinephrine is the major medullary catecholamine, while in those which the medullary chromaffin tissue lacks a cortical envelope, it contains little or no epinephrine. In the rabbit, part of the medulla is surrounded by cortex, and this part contains essentially all of the medullary epinephrine. On the basis of these findings, Coupland suggested that the adrenal cortex secreted a “methylation factor” that influenced the N-methylation of norepinephrine to form epinephrine.

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**Table 1. Effect of Hypophysectomy, ACTH, and Dexamethasone on PNMT**

Treatment*	Adrenal Weight (mg/pair)	PNMT ( $\mu$ mol/pair)
Control	68.9 $\pm$ 3.4†	6.16 $\pm$ 0.66†
Hypophysectomy	26.5 $\pm$ 2.1	1.46 $\pm$ 0.11
Hypophysectomy plus ACTH	46.7 $\pm$ 1.5†	4.76 $\pm$ 0.26†
Hypophysectomy plus dexamethasone	26.0 $\pm$ 2.3	7.80 $\pm$ 0.44†

\*Animals were sham-operated or hypophysectomized and killed 17 to 21 days later. Some of the hypophysectomized rats were given ACTH or dexamethasone for 6 days prior to autopsy.

† $P < .001$  v hypophysectomy.

In 1959, Kirshner identified the adrenomedullary enzyme, PNMT, responsible for the synthesis of epinephrine from norepinephrine.<sup>7</sup> Subsequently, Axelrod demonstrated that most of the epinephrine-forming ability found in adult mammals was localized within the adrenal gland<sup>8</sup>; hence, most of the epinephrine released into the circulation had very likely been produced by PNMT within the adrenal medulla. An isotopic assay was subsequently developed for PNMT, which simplified measuring the effects of various physiologic states and drugs on epinephrine biosynthesis.

#### PITUITARY ACTH AND EPINEPHRINE SYNTHESIS

Based on the pathologically prolonged hypoglycemia of our hypopituitary patient, we initiated studies to see whether the glucocorticoid hormones were the unknown "methylation factor." Hypophysectomy was found to be associated with a marked reduction in epinephrine-forming ability (Table 1).<sup>1</sup> This reduction was of even greater magnitude than the fall in adrenal weight (which was due largely to a reduction in the mass of the adrenal cortex). When hypophysectomized rats were treated with ACTH, PNMT activity was largely restored with 6 days (Table 1). No other pituitary hormone restored adrenal PNMT levels after hypophysectomy.

Most of the fall in adrenal PNMT activity occurred within 1 week of hypophysectomy; by this time, epinephrine-forming ability was reduced in the majority of rats to 15% to 20% of normal. Subsequently, PNMT activity decreased much more slowly, reaching 10% of control levels 10 weeks after hypophysectomy. The content of epinephrine in the adrenals declined significantly within 1 week of hypophysectomy; it continued to fall for as long as animals could be maintained, reaching levels of about 25% to 35% of normal after 10 weeks. This slow fall in adrenal epinephrine levels was thought to be consistent with the epinephrine's known low rate of turnover in adrenals (ie, 7 to 14 days). Adrenal norepinephrine rose somewhat following hypophysectomy, as might be anticipated. The adrenals of chronically hypophysectomized dogs were subsequently shown not only to contain subnormal epinephrine levels and PNMT activities, but also to secrete subnormal quantities of epinephrine basally or when made hypoglycemic by injecting insulin.<sup>9</sup> Normal epinephrine secretion was restored in this species by physiologic doses of ACTH, but not by physiologic doses of glucocorticoids (which, as discussed below, perfuse the me-

dulla in much lower concentrations than those available after ACTH administration).

#### GLUCOCORTICOIDS AND EPINEPHRINE SYNTHESIS

There were at least 3 possible mechanisms by which ACTH treatment could have restored PNMT activity in the hypophysectomized rat:

1. The adrenal cortex might have contained an unrecognized epinephrine-forming enzyme, in which case the decline in cortical mass after hypophysectomy, and its restoration with ACTH treatment, would be expected to cause parallel changes in PNMT activity.
2. ACTH could have acted directly on the adrenal medulla to enhance PNMT activity.
3. ACTH could have stimulated the secretion of glucocorticoids by the adrenal cortex; these compounds, delivered to the medulla by the intra-adrenal portal vascular system, could then have increased PNMT activity.

To examine the first possibility, we separated the cortex and medulla from adrenals of hypophysectomized and normal animals, and assayed them individually for PNMT. We found that the cortex normally contains less than 10% of the epinephrine-forming activity of the medulla. (Since no capsule separates the rat cortex from the medulla, it is possible that even this 10% represented contamination of the cortical sample with medullary tissue.)

To rule out the possibility that ACTH acted directly on the adrenal medulla, it was necessary to show that when the level of glucocorticoids available to the medulla was held constant, changes in the rate of ACTH secretion had no effect on PNMT activity.<sup>10</sup> This was done in 2 ways. First, normal rats were treated with large doses of dexamethasone, a highly potent synthetic glucocorticoid. This dose depressed pituitary secretion of ACTH (as demonstrated by a fall in adrenal weight, Table 2), but provided the tissues with more than adequate amounts of glucocorticoid. PNMT activity did not fall. Next, other rats were treated in a like manner with small doses of metyrapone, a compound that interferes with the synthesis of adrenal glucocorticoids by inhibiting 11- $\beta$  oxidation. Animals so treated developed adrenal hypertrophy (Table 2), indicating that ACTH secretion from the pituitary had indeed been elevated. However, epinephrine-forming ability did not show a parallel rise.

On the basis of the above studies, it could be anticipated that glucocorticoids would share with ACTH the ability to elevate PNMT activity in the hypophysectomized animal, if provided

**Table 2. Effects of Dexamethasone and Metyrapone on PNMT Activity in Intact Rats**

Treatment*	Adrenal Weight (mg/pair)	PNMT ( $\mu$ mol/pair)
Control	54 $\pm$ 1.2	5.12 $\pm$ 0.47
Dexamethasone	35 $\pm$ 1.3†	6.02 $\pm$ 0.44
Metyrapone	60 $\pm$ 2.2†	5.12 $\pm$ 0.47

\*Rats were given 1 mg of the drug intraperitoneally for 6 days and killed on the 7th day.

† $P < .001$  v control.

in adequate doses. And when hypophysectomized rats were given the highly potent drug dexamethasone, it, like ACTH, fully restored epinephrine-forming activity (Table 1).

These data were thought to indicate that: (1) adrenal PNMT activity is largely confined to the medulla; and (2) epinephrine synthesis is controlled by the availability of ACTH, but indirectly, via a route that involves the secretion of glucocorticoids from the adrenal cortex. Natural and synthetic steroids that lacked potency as glucocorticoids were found to have no effect on PNMT.<sup>10</sup>

Glucocorticoids added to adrenal homogenates from hypophysectomized rats did not stimulate PNMT activity, and large doses actually inhibited this activity. This suggested that these hormones were affecting PNMT activity by controlling the rate of formation (or destruction) of the enzyme protein. To test this hypothesis, the ability of dexamethasone to restore PNMT activity in hypophysectomized rats was measured in animals pretreated with actinomycin D or puromycin. Neither agent had a direct effect on PNMT activity, but both blocked the anticipated rise in epinephrine-forming ability induced by dexamethasone. This suggested that glucocorticoids secreted from the adrenal cortex act physiologically to induce the synthesis of new PNMT protein within the adrenal medulla, a hypothesis subsequently confirmed by the finding that the hormones also increase PNMT mRNA levels.<sup>11,12</sup>

#### EFFECT OF "REPLACEMENT" DOSES OF GLUCOCORTICOIDS ON EPINEPHRINE-FORMING ABILITY

Although PNMT activity was fully restored by low doses of ACTH (ie, the doses needed to maintain adrenal weight), the amounts of glucocorticoid needed to maintain epinephrine synthesis seemed unusually high. The usual "replacement" dose of glucocorticoid in the rat is thought to be about 0.1 to 0.3 mg (of corticosterone) per day. Doses higher by tenfold had essentially no effect on PNMT. It was necessary to treat rats with 100 to 300 times the replacement dose (or its equivalent in dexamethasone) to produce a significant restoration of PNMT.<sup>10</sup>

Since the effect of ACTH on PNMT had been shown to be indirect, this great difference between ACTH and glucocorticoid doses suggested that PNMT activity depends not on the levels of glucocorticoid in the general circulation, but on levels within the adrenal gland itself. Ordinarily, adrenal venous blood (which bathes the adrenal medulla after it has perfused the cortex)<sup>6</sup> contains about 100 times as much glucocorticoid as peripheral venous blood. When rats are hypophysectomized, both intra-adrenal and peripheral glucocorticoid levels decline. Treatment of hypophysectomized rats with "replacement" doses of glucocorticoids restores normal hormone levels in the general circulation but does not restore intra-adrenal levels. However, when such animals are given ACTH, intra-adrenal corticoid levels do return to normal. Thus it was concluded that the restoration of PNMT by customary doses of ACTH results from the selective increases they produce in intra-adrenal glucocorticoid levels. In order to test this hypothesis, hypophysectomized rats were treated with several different doses of ACTH or of hydrocortisone, and the effects of these hormones were measured on splenic weight (which depends on corticoid levels

in the general circulation) and on adrenal PNMT activity (which, we hypothesized, depends on intra-adrenal blood levels). It was observed that a dose of ACTH that was equipotent with a certain amount of hydrocortisone in depressing splenic weight was fully 100 times *more* potent in inducing PNMT. Thus, maintaining the "normal" rate of epinephrine synthesis requires that the adrenal medulla be surrounded by, and receive portal venous blood from, a normally functioning adrenal cortex. Hence, the location of the mammalian medulla within the cortex is an important factor in controlling epinephrine synthesis.

#### CONTROL OF EPINEPHRINE SYNTHESIS WITHIN ADRENAL AND BRAIN AMONG STRESSED ANIMALS

To the best of the author's knowledge, only fragmentary data exist on the effects of chronic stress on epinephrine synthesis and secretion in normal human subjects (although it is well known, of course, that acute stress causes the liberation of large amounts of epinephrine). This is surprising, and should be rectified, perhaps initially by comparing adrenomedullary PNMT and epinephrine levels of such stressed people with those in adrenals from people who died without having suffered a chronic stress.

Immobilization stress for 2.5-hour periods has been shown to increase adrenal PNMT activity in rats.<sup>12</sup> This effect is best observed after 7 days of treatment, and does not occur in hypophysectomized animals. Various regions of rat brain contain low levels of PNMT activity, PNMT mRNA, and epinephrine,<sup>13</sup> and the release of intracerebral epinephrine is enhanced by experimental stress.<sup>14</sup> The neonatal administration of dexamethasone apparently increases brain stem PNMT activity permanently, and amplifies the increase in PNMT caused by stress.<sup>15</sup>

If chronic stress increases the synthesis of epinephrine in, and its secretion from, the human's adrenal medulla, and, as a consequence, if it also reduces norepinephrine levels in, and secretion from, the medulla, what might be the functional consequences of these changes? Epinephrine can markedly increase blood glucose levels (by stimulating hepatic glycogenolysis and decreasing peripheral glucose uptake). This would increase the availability of glucose to the brain, but might also exacerbate diabetes. Epinephrine is also more potent than norepinephrine in raising body temperature and in increasing heart rate and cardiac output. The expected decrease in adrenomedullary norepinephrine secretion would probably be compensated by the stress-induced increase in the release of norepinephrine from sympathetic nerve terminals. Plasma levels probably do not fall but may actually rise, increasing peripheral resistance and raising blood pressure. If the pituitary was unable to secrete additional ACTH in response to the stress, this might, by impairing the rise in epinephrine synthesis and secretion, compromise the ability of the brain and heart, and perhaps other organs, to respond appropriately.

A stress-induced increase in brain epinephrine synthesis might be expected to affect the central regulation of cardiovascular function. The exact consequences of this putative increase await characterization.

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