Cardiac Epinephrine Synthesis
Regulation by a Glucocorticoid

Brian Kennedy, PhD, and Michael G. Ziegler, MD

Background. The heart can synthesize epinephrine. Homogenates of rat heart, which contain the enzymes phenylethanolamine N-methyltransferase (PNMT) and nonspecific N-methyltransferase (NMT), methylate norepinephrine to form epinephrine. The cardiac atrium contains primarily PNMT and the cardiac ventricle contains both PNMT and NMT.

Methods and Results. Rats were given the glucocorticoid dexamethasone at doses ranging from 0.2 to 20 mg/kg. Twenty-four hours later, cardiac atria, ventricle, skeletal muscle, and adrenal had increases in PNMT activity to as much as 230% of baseline. NMT activity was unchanged. Longer-term treatment with 1 mg/kg dexamethasone daily for 12 days increased cardiac PNMT activity about fivefold and also increased atrial epinephrine levels. Dexamethasone did not alter ventricular epinephrine levels but increased levels of both PNMT and catechol-O-methyltransferase, the major catabolic enzyme for epinephrine. After dexamethasone treatment, greater volumes of anti-PNMT antiserum were needed to decrease PNMT enzymatic activity, indicating that dexamethasone treatment resulted in greater amounts of PNMT and did not just activate existing PNMT molecules. Denervation of the masseter muscle of rats by unilateral superior cervical ganglionectomy markedly diminished tissue norepinephrine and epinephrine levels but had no effect on masseter PNMT or NMT levels. We have previously shown that chemical sympathectomy with 6-hydroxydopamine increases cardiac PNMT levels. These findings suggest that PNMT is an extraneuronal enzyme in both cardiac and skeletal muscle.

Conclusions. Glucocorticoids have several cardiovascular effects, including increased cardiac output and blood pressure. Enhanced cardiac epinephrine synthesis may mediate some of these glucocorticoid effects. (Circulation 1991;84:891–895)

Epinephrine is a potent agonist at cardiac α- and β-receptors.1 Physiological concentrations of plasma epinephrine increase stroke volume and cardiac output.2 Chronic epinephrine elevations result in cardiac hypertrophy3 and very high epinephrine levels induce cardiomyopathy.4 Epinephrine is concentrated in the adrenal medulla and is often referred to as adrenaline. In the adrenal, phenylethanolamine N-methyltransferase (PNMT) synthesizes epinephrine from norepinephrine.5 Axelrod5 and Torda et al6 presented evidence that PNMT is also present in the heart. We recently showed that rat atria contain mainly PNMT, whereas ventricles contain both PNMT and a nonspecific N-methyltransferase (NMT).7 Both adrenal and cardiac PNMT readily methylate β-hydroxylated amines such as norepinephrine but minimally methylate non-β-hydroxylated amines such as dopamine.5 NMT readily methylates both types of amines.8 Adrenal PNMT is regulated by glucocorticoids.9–11 We reasoned that if cardiac PNMT is a functional enzyme it should be regulated and should synthesize epinephrine in vivo. We present studies of the effects of the glucocorticoid dexamethasone on cardiac PNMT activity and epinephrine levels.

Methods

Acute Dexamethasone

Groups of five or six male Sprague-Dawley (180–250 g) rats were injected with vehicle alone or with 0.2, 1, 5, 10, or 20 mg/kg dexamethasone. Twenty-four hours later, rats were killed by decapitation, and cardiac atria, ventricle, a 300-mg segment of masseter muscle, and adrenal were collected, weighed, and frozen at −70°C. The tissues were then thawed and homogenized in 1 ml of 0.1 M Tris (pH 7) with 0.1% Triton X-100. The homogenates were then centrifuged and assayed for catecholamines and epinephrine-forming enzyme activity.
Chronic Dexamethasone

Superior cervical ganglionectomy was performed on three rats, and sham surgery was performed on 15 other rats. Three days later, the three ganglionectomized rats and nine sham-operated rats were adrenalectomized. The remaining six rats underwent sham adrenalectomy. Adrenalectomized rats were given 0.9% saline instead of drinking water. Three days later, six of the adrenalectomized rats including the three ganglionectomized rats were given the first of 12 daily injections of dexamethasone (1 mg/kg s.c.).

One day after the final dexamethasone injection, all rats were decapitated, and trunk blood was collected. The plasma was later assayed for epinephrine levels to verify complete adrenalectomy. Atria, ventricle, and a 300-mg segment of masseter muscle were removed, weighed, and frozen at −70°C until assay for epinephrine levels and PNMT and NMT activity.

Chronic Dexamethasone in Adrenal-Demedullated Rats

In rats it is possible to remove only the adrenal medulla, which secretes epinephrine, or to remove the entire adrenal. To determine the effect of chronic dexamethasone treatment on rats with no adrenomedullary function but intact adrenocortical function, the adrenal medullae of 12 rats were surgically removed while they were under pentobarbital anesthesia. Both adrenal glands (corpus plus medulla) were removed from six other rats. These six adrenalectomized rats were maintained on saline. Three days after surgery, half of the adrenal-demedullated rats were given the first of 12 daily injections of dexamethasone (1 mg/kg s.c.). The remaining rats received vehicle alone. One day after the final injection, all rats were decapitated, and trunk blood was assayed for epinephrine to verify removal of the adrenal medulla. Atria and ventricles were collected, weighed, and frozen at −70°C. Homogenates of these tissues were later assayed for epinephrine levels and PNMT and NMT activity. The homogenates were also assayed for catechol-O-methyltransferase (COMT) activity according to the method of Zurcher and Da Prada to determine whether dexamethasone changed this catecholamine catabolic enzyme.

Immunotitration

To determine if changes in atrial PNMT activity were due to an increased amount of enzyme or activation of enzyme, we performed immunotitration experiments using a modification of the procedure of Wong et al. Eight 50-μl aliquots of atrial homogenates from each of three adrenalectomized dexamethasone-treated (1 mg/kg/day s.c. for 12 days) and each of three vehicle-treated adrenalectomized rats were prepared. Fifty microliters of buffer containing 0.125, 0.0625, 0.03125, 0.0156, 0.0078, 0.0039, 0.00195, or 0 μl of anti-bovine adrenal PNMT antiserum was added to the eight aliquots from each rat. Samples were vortexed and incubated for 1 hour at 37°C, then for 16 hours at 4°C. Samples were then centrifuged at 11,000g for 6 minutes at 4°C. Aliquots of the supernates were then assayed for PNMT activity in the usual manner.

The antiserum was a generous gift from Dr. Dona Lee Wong and was generated in male New Zealand White rabbits against S-adenosylhomocysteine·AH-Sepharose 4B purified bovine PNMT. One microliter of antiserum failed to inhibit NMT from atria, ventricle, or skeletal muscle homogenates when dopamine was used as substrate. In contrast, very low antiserum concentrations inhibited PNMT in these tissues when norepinephrine was used as substrate.

Assay Techniques

Tissue PNMT and NMT activity were measured in the supernate using the method of Ziegler et al. Briefly, tissue homogenate supernates were incubated for 2 hours at 25°C in the presence of either 10⁻³ M norepinephrine (for PNMT) or 10⁻³ M dopamine plus the PNMT inhibitor SKF29661 at 10⁻⁴ M (for NMT) and ³H-S adenosylmethionine. The ³H-E or ³H-epinephrine formed was extracted by alumina adsorption and quantified by liquid scintillation spectrometry. Tissue catecholamines were determined using the method of Kennedy and Ziegler. This assay involves solvent extraction of catecholamines from tissue homogenate supernates, then incubation in the presence of excess ³H-S adenosylmethionine and rat liver COMT. The resulting ³H-O-methylated catecholamines are separated by thin-layer chromatography and quantified using a liquid scintillation counter.

Results

Twenty-four hours after doses of dexamethasone ranging from 0.2 to 20 mg/kg, the higher doses increased PNMT activity in atria, ventricle, and muscle. The adrenal medulla, which is bathed in corticosteroids produced by the adrenal cortex, increased its PNMT activity only at the highest dose of dexamethasone. In contrast, NMT activity was unaffected by dexamethasone in atria, ventricle, and muscle (see Figure 1).

Both epinephrine and corticosteroids are secreted by the adrenal glands, and epinephrine can be taken up by innervated tissues. Decapitation is a powerful stimulus for adrenal epinephrine release into the bloodstream. After decapitation, plasma epinephrine levels in sham-operated animals were 6,423 pg/ml. However, adrenalectomized rats had mean ± SEM epinephrine levels of 33±2 pg/ml, and adrenalectomized rats given dexamethasone had levels of 22±5 pg/ml. The dramatic reduction in plasma epinephrine levels in both groups of adrenalectomized rats indicates complete removal of the adrenal medulla.

Chronic dexamethasone significantly increased PNMT activity in atria, ventricle, muscle, hypothalamus, and brain stem but adrenalectomy failed to reduce PNMT activity in any of these tissues (see Figure 2). Chronic dexamethasone treatment of adrenalectomized rats increased epinephrine levels
Chronic dexamethasone treatment of adrenal-demulated rats significantly increased atrial and ventricular PNMT activity and ventricular NMT relative to vehicle-treated adrenal-demulated rats. None of the animals in this experiment had an adrenal source for circulating epinephrine. Nevertheless, dexamethasone again increased atrial epinephrine levels and had no effect on ventricular epinephrine levels (see Figure 3). A number of studies indicate that cardiac epinephrine levels also depend on the rate of destruction of epinephrine by COMT.\textsuperscript{16,17} The major catabolic enzyme for epinephrine. Dexamethasone increased COMT activity in the ventricle but not in the atria (see Figure 3).

We incubated atrial homogenates from dexamethasone-treated and vehicle-treated adrenalectomized rats with increasing amounts of anti-PNMT antiserum before assay to determine whether the increased PNMT activity in atria after dexamethasone was due to increased enzyme levels or to activation of existing enzyme. When the results were expressed as a percent of activity in the absence of antiserum, we found that more antiserum was required to inhibit PNMT activity of dexamethasone-treated than vehicle-treated adrenalectomized rats (see Figure 4). The antiserum did not reduce NMT activity in atria, ventricle, or skeletal muscle.

Both norepinephrine and epinephrine can be taken up and stored in sympathetic nerve terminals.\textsuperscript{18} We unilaterally sympathectomized three adrenalectomized rats and used the contralateral side as control. The rats received dexamethasone for 12 days after denervation. Denervation diminished muscle norepinephrine levels by 96\% and diminished muscle epinephrine to undetectable levels. Despite the destruction of muscle sympathetic nerves, PNMT and NMT activity remained unchanged (see Figure 5).

**Discussion**

PNMT synthesizes epinephrine in the adrenal medulla; it is relatively specific for phenylethanolamines such as norepinephrine and methylates non-\textbeta-\textsubscript{3} hydroxylated phenylethylamines such as dopamine poorly. NMT is present in many nonadrenal tissues and methylates both norepinephrine and dopamine. PNMT in the heart is similar to adrenal PNMT in at least three respects: substrate specificity, inactivation by anti-PNMT antiserum, and regulation by glucocorticoids. NMT differs from PNMT in all three respects. Cardiac atrium has a much higher level of PNMT than NMT, whereas skeletal muscle and cardiac ventricle contain fairly similar levels of both enzymes.

PNMT activity is relatively high in the atria, and dexamethasone causes a further striking increase. Atrial PNMT activity in dexamethasone-treated adrenalectomized rats required more anti-PNMT antiserum to neutralize enzymatic activity than in adrenalectomized rats. This suggests that dexamethasone increased PNMT activity by increasing the amount of enzyme rather than by activating existing PNMT.

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**Figure 1.** Bar graphs showing phenylethanolamine N-methyltransferase (PNMT) and nonspecific N-methyltransferase (NMT) activity in homogenates of adrenal gland, cardiac atria, ventricles, and masseter muscle in groups of five or six rats treated with increasing doses of dexamethasone. Values are mean±SEM. Overall intergroup differences in PNMT activity are significant (p<0.05) by analysis of variance for all tissues. *p<0.05 vs. control by Tukey’s test.

**Figure 2.** Bar graphs showing phenylethanolamine N-methyltransferase (PNMT) activity, nonspecific N-methyltransferase (NMT) activity, and epinephrine (E) levels in homogenates of five tissues of vehicle-treated sham-operated (CON), vehicle-treated adrenalectomized (ADX), and dexamethasone-treated adrenalectomized rats (DEX, 1 mg/kg/day s.c. for 12 days). Values are mean±SEM of determinations made from six rats. Intergroup differences in PNMT activity of all tissues are significant (p<0.05) by analysis of variance. Intergroup differences in atrial epinephrine levels are significant by analysis of variance (p<0.05). *p<0.05 by Tukey’s test vs. ADX. †, p<0.05 vs. CON by Tukey’s test.
Increased levels of PNMT mRNA have been reported in the adrenal after glucocorticoid treatment. Dexamethasone increased atrial epinephrine when compared with basal levels in adrenalectomized (Figure 2) or adrenal-medullectomized (Figure 3) animals. Despite elevated atrial PNMT activity, atrial epinephrine levels in dexamethasone-treated adrenalectomized rats were not elevated relative to sham-operated control rats. This may be because adrenal-ectomy lowers atrial epinephrine levels by about 50%. Epinephrine has about a 70-fold higher affinity for β-receptors than its precursor norepinephrine. Stimulation of β-receptors in cardiac atria increases both the rate and force of contraction in the heart. Thus, augmented atrial epinephrine synthesis after glucocorticoid treatment may act to enhance cardiac output.

Epinephrine synthesis and turnover may be elevated even if epinephrine levels are unchanged when breakdown of epinephrine is enhanced. COMT is the primary catabolic enzyme for extraneuronal catecholamines. Dexamethasone increased ventricular COMT activity but did not alter epinephrine (see Figure 3), suggesting that ventricular epinephrine turnover may be increased after dexamethasone treatment.

PNMT is found in the adrenal medulla and in neurons of the central nervous system, so we suspected that cardiac PNMT might be localized in sympathetic nerves. However, Torda et al. destroyed sympathetic nerves with 6-hydroxydopamine treatment, yet found an increase in cardiac PNMT. In a recent study, we carried this procedure even further using 6-hydroxydopamine to destroy sympathetic nerves, reserpinize to deplete sympathetic nerve vesicles, and adrenal demedullation to deplete circulating epinephrine, and again found increased cardiac PNMT levels. The heart is difficult to denervate surgically; however, it is rela-

**Figure 3.** Bar graphs showing phenylethanolamine N-methyltransferase (PNMT), nonspecific N-methyltransferase (NMT), catechol-O-methyltransferase (COMT) activity, epinephrine (E), and levels in homogenates of atria and ventricle of vehicle-treated adrenalectomized (ADX), vehicle-treated adrenal-demedullated (AMX), and adrenal-demedullated rats treated with dexamethasone (DEX, 1 mg/kg/day for 12 days). Values are mean ± SEM of determinations from six rats. Intergroup differences are significant by analysis of variance for atrial PNMT (p < 0.001), epinephrine (p < 0.001), ventricular PNMT (p < 0.001), NMT (p < 0.004), and COMT (p < 0.001). *p < 0.05 vs. AMX by Tukey’s test.

**Figure 4.** Graph showing phenylethanolamine N-methyltransferase (PNMT) activity of atrial homogenates from three adrenalectomized (ADX) and three dexamethasone-treated (DEX, 1 mg/kg/day for 12 days) adrenalectomized rats in the presence of 0–0.125 μl anti-PNMT antiserum. Values are mean ± SEM and are expressed as a percent of activity in the absence of antiserum. *p < 0.05 vs. ADX by Student’s t test with Bonferroni correction.

**Figure 5.** Bar graphs showing norepinephrine (NE) and epinephrine (E) levels, phenylethanolamine N-methyltransferase (PNMT) and nonspecific N-methyltransferase (NMT) activity in masseter muscle of three rats that underwent adrenalectomy, dexamethasone treatment (1 mg/kg/day for 12 days) and unilateral superior cervical ganglionectomy (SCGX) on one side and sham-ganglionectomy (SHAM) on the other side 12 days before killing by decapitation. Values are mean ± SEM. ***p < 0.001 vs. sham-operated side by Student’s t test.
tively simple to denervate sympathetic input to skeletal muscle by superior cervical ganglionectomy. More than 2 weeks after performing ganglionectomy, we found a dramatic reduction in muscle norepinephrine and epinephrine but no effect on PNMT. This indicates that muscle PNMT is not contained in sympathetic nerves but that locally synthesized epinephrine is stored intraneuronally. Sympathetic nerves have an avid catecholamine uptake mechanism\(^ {18}\) and can store catecholamines made outside the nerves.

Adrenalectomy did not affect cardiac PNMT or NMT levels, suggesting that cardiac epinephrine-forming activity is not affected by the levels of circulating glucocorticoids normally present in rats. Although adrenalectomy did lower some tissue epinephrine levels, this would appear to be a consequence of the loss of circulating epinephrine from the adrenal medulla.

Pharmacological doses of dexamethasone gave a dose- and time-dependent increase in cardiac PNMT. In humans, such doses of glucocorticoids often induce hypertension and can ameliorate septic shock. The antagonism of septic shock is largely the consequence of protection from leukocyte-, platelet-, and endotoxin-mediated damage. However, elevation of cardiac epinephrine synthesis may play a role in glucocorticoid hypertension. Both glucocorticoids and epinephrine increase cardiac output\(^ {2,22}\) and blood pressure.\(^ {23-27}\) Also, the PNMT inhibitor SKF64139 reduces blood pressure in glucocorticoid-hypertensive rats but not in normotensive rats.\(^ {28}\) This suggests that inhibition of epinephrine synthesis or blockade of the \(\alpha\)- and \(\beta\)-adrenergic stimulation caused by epinephrine may be helpful in treating glucocorticoid hypertension.

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B Kennedy and M G Ziegler

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