Glucocorticoid Hypertension and Nonadrenal Phenylethanolamine N-Methyltransferase

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Several drugs that block epinephrine synthesis by inhibiting phenylethanolamine N-methyltransferase (PNMT) lower blood pressure in hypertensive rats. We investigated the mechanism by which these drugs lower blood pressure in rats made hypertensive with the glucocorticoid dexamethasone. We performed adrenalectomy or sham operation on several rats and then gave them either dexamethasone chronically or vehicle. The dexamethasone-treated adrenalectomized rats also received either the centrally acting PNMT inhibitor SKF 64139 chronically or an equal dose of the primarily peripherally acting PNMT inhibitor SKF 29661. Both SKF 64139 and SKF 29661 reduced blood pressure by more than 25 mm Hg. SKF 64139 also reduced PNMT activity in hypothalamus, medulla oblongata, skeletal muscle, and cardiac atria and ventricles; SKF 29661 inhibited PNMT in muscle and heart tissue by 40–75%, did not inhibit PNMT in hypothalamus, and inhibited PNMT by only 29% in medulla oblongata. PNMT activity in peripheral tissues was also more highly correlated with blood pressure than was PNMT activity in the brain areas studied. Neither drug reduced tissue epinephrine levels, but SKF 64139 elevated dopamine or norepinephrine levels or both in several tissues. We conclude that the blood pressure–lowering action of PNMT-inhibiting drugs in glucocorticoid hypertensive rats may be due to inhibition of peripheral nonadrenal PNMT. We speculate that elevations in nonadrenal PNMT may mediate glucocorticoid hypertension.

KEY WORDS • hypertension, glucocorticoid • epinephrine • dexamethasone • phenylethanolamine N-methyltransferase

Prolonged administration of glucocorticoids causes hypertension in both humans and experimental animals by an unknown mechanism. Glucocorticoids increase the activity of the epinephrine-forming enzyme phenylethanolamine N-methyltransferase (PNMT) in hypothalamus, medulla oblongata, or both, and this may raise blood pressure. PNMT activity and epinephrine levels are increased in certain brain loci in genetically hypertensive and deoxycorticosterone acetate–salt hypertensive rats, and centrally active PNMT inhibitors lower blood pressure in several animal models of hypertension. However, some data fail to support the view that increases in central nervous system PNMT mediate glucocorticoid hypertension. Central nervous system PNMT elevations have been postulated to raise blood pressure by increasing sympathetic outflow, but most studies indicate that sympathetic activity is reduced or unchanged after glucocorticoid administration. In addition, blood pressure remains elevated in glucocorticoid hypertensive rats after sympathetic function is disrupted by pithing.

We have recently shown that long-term treatment with the glucocorticoid dexamethasone increases heart and skeletal muscle PNMT activity several fold and elevates epinephrine levels in cardiac atria. Epinephrine increases heart rate and contractility, so increased cardiac epinephrine synthesis may mediate the increased cardiac output and blood pressure that follow glucocorticoid treatment. Interestingly, the PNMT inhibitor SKF 64139 markedly reduces heart rate in glucocorticoid hypertensive rats.

To determine whether central or peripheral PNMT elevations are involved in glucocorticoid hypertension, we gave adrenalectomized glucocorticoid hypertensive rats either a centrally and peripherally acting PNMT inhibitor, SKF 64139, or a drug that has been reported to inhibit only peripheral PNMT, SKF 29661. We then compared the effects of these drugs on blood pressure. Recent advances in assays for PNMT and catecholamines also allowed assay of PNMT activity in heart and muscle and tissue epinephrine levels in adrenalectomized animals.

Methods

We performed adrenalectomy on 20 male Sprague-Dawley (Harlan) rats while they were under ether anesthesia (250–270 g) and sham operation on 10 others in accordance with the guidelines of the University of California San Diego Animal Subjects Committee. All of the adrenalectomized rats and five of the sham-operated rats received 1 mg/kg per day dexamethasone for 14 days. Adrenalectomized rats were given normal saline to drink. The remaining rats were injected with vehicle alone and given water to drink. During this period, rats were repeatedly placed in a restrainer for gradually longer

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times to accommodate them to the restraint necessary to measure blood pressure by the tail-cuff method.

On the seventh day of dexamethasone administration, we injected seven of the adrenalectomized rats intraperitoneally with 50 mg/kg SKF 29661 twice daily for 8 days, six of the adrenalectomized rats with SKF 64139 (gifts from Dr. J. Paul Hieble) at the same dose, and the remaining rats with vehicle alone. On the seventh day of injection with PNMT inhibitor or vehicle, all rats had their systolic blood pressure measured by an automated tail cuff (model PE 300, NARCO Bio-Systems, Houston, Tex.). The order of blood pressure measurement was randomized and performed within 30 minutes after rats received their first daily injection of drug or vehicle. One day later, rats had approximately 1 mL blood removed by cardiac puncture under pentobarbital anesthesia for determination of plasma epinephrine levels to verify adrenalectomy. Rats were then killed by decapitation, and medulla oblongata, hypothalamus, a 300-mg segment of masseter muscle and cardiac atria, and ventricles were removed, weighed, frozen in liquid nitrogen, and stored at −70°C until assay. Just before assay, tissues were thawed and diluted with 0.1 M Tris, pH 7, with 0.1% Triton X-100. They were homogenized with a polyclot and then centrifuged at 4°C for 10 minutes at 6,000g. The resulting supernatants were assayed for PNMT activity by a modification of the radioenzymatic method of Ziegler et al.18 in which a 10-fold lower concentration of norepinephrine substrate was used to minimize displacement of inhibitor by norepinephrine and to more accurately reflect norepinephrine concentrations in vivo. Samples were assayed for catecholamines by the catechol-O-methyltransferase-based radioenzymatic method of Kennedy and Ziegler.19 The overall statistical significance of intergroup differences in blood pressure, PNMT activity, or catecholamines was determined by one-way analysis of variance using the PC-INFO program (Retriever Data Systems, Seattle, Wash.). The significance of differences between any two specific groups was assessed by the Newman-Keuls test. The degree of correlation between the blood pressure of all 30 rats and PNMT activity in the five tissues studied was assessed using the PC-INFO linear regression program.

Results

Thirteen days of dexamethasone increased systolic blood pressure by more than 50 mm Hg in rats with intact adrenals. Adrenalectomy nonsignificantly reduced the magnitude of this elevation by 17 mm Hg. Seven adrenalectomized dexamethasone-treated rats received the centrally and peripherally acting PNMT inhibitor SKF 64139 for 7 days, and six rats received SKF 29661, which penetrates the brain poorly. Both PNMT inhibitors lowered blood pressure in glucocorticoid hypertensive rats by 25 to 30 mm Hg (Figure 1). Dexamethasone markedly increased PNMT activity in peripheral tissues but not in brain (Figure 2) regardless of adrenalectomy. The centrally acting PNMT inhibitor SKF 64139 reduced PNMT activity in both brain and peripheral tissues (Figure 2). In contrast, SKF 29661 reduced PNMT activity in peripheral tissues by 40 to 75%, in medulla oblongata by 29%, and did not change PNMT activity in the hypothalamus. Blood pressures from the 30 rats in the study were highly correlated with PNMT activity in atria, ventricle, and medulla oblongata, hypothalamus, and medulla oblongata, respectively. The correlation between the blood pressure of all 30 rats and PNMT activity in the five tissues studied was assessed using the PC-INFO linear regression program.

Discussion

These experiments suggest that PNMT located outside both the adrenal and central nervous system mediates glucocorticoid hypertension. Dexamethasone increased heart and muscle PNMT dramatically but had only minor effects on brain PNMT in this and a previous study.4 Also, both PNMT inhibitors brought blood pressure back to normal levels, but only one of them markedly inhibited brain PNMT. This suggests that inhibition of peripheral PNMT mediates the antihypertensive action of these drugs.

PNMT makes epinephrine, and infused epinephrine can raise blood pressure in humans and animals.21 The hypertensive action of epinephrine is probably mediated, as epinephrine penetrates the brain poorly. In several peripheral tissues, epinephrine enhances release of the vasoconstrictor norepinephrine from sympathetic nerve endings, and this may mediate the hypertensive effect of epinephrine.22 The heart may be a site at which both epinephrine and glucocorticoids act to raise blood pressure, as both hormones increase cardiac output.
the PNMT inhibitor SKF 64139 reduces heart rate in glucocorticoid hypertensive rats is compatible with this view.10

We were surprised to find that SKF 29661 inhibited PNMT activity in medulla oblongata, because a previous study found no radioactivity in the brains of rats that were given radiolabeled SKF 29661 acutely at 10 mg/kg.17 Furthermore, no in vitro inhibition of medulla oblongata PNMT was found when SKF 29661 was given to rats at doses as high as 100 mg/kg three times within 24 hours.17 These authors measured PNMT activity in vitro using substrate at a concentration of approximately 1.6 mM. This concentration may have been high enough to completely displace SKF 29661 from PNMT, as SKF 29661 competes with substrate for binding sites. We may have been able to detect medulla oblongata PNMT inhibition by SKF 29661, because we used a 16-fold lower substrate concentration to assay PNMT activity. Alternatively, it is possible that central nervous system penetration occurred in our study because we gave a higher cumulative dose of PNMT inhibitor.

An antihypertensive action of SKF 29661 has been found previously. Black et al9 gave SKF 29661 to rats twice during a 1-hour period at an intravenous dose of approximately 20 mg/kg and measured blood pressure at 30-minute intervals for 3 hours. They found that blood pressure was unchanged in normotensive rats but was reduced by 7–10 mm Hg in both two-kidney, one clip and deoxycorticosterone acetate–salt hypertensive rats for a period beginning 30 minutes after the final drug injection until the end of the experiment. The rapid onset of this antihypertensive effect suggests that it was not due to depletion of adrenal epinephrine, because the half-life of adrenal epinephrine in rats is approximately 5 days.24

Nevertheless, adrenal epinephrine or aldosterone may play a role in glucocorticoid hypertension, as adrenalectomized rats had a smaller dexamethasone-induced increase in blood pressure than rats with intact adrenals, although this reduction did not reach statistical significance. Glucocorticoids do not elevate circulating epinephrine,10–13 so it seems likely that glucocorticoids may potentiate the hypertensive effects of epinephrine by upregulating or sensitizing β-receptors. Interestingly, isolated cardiac atria from stressed rats have enhanced chronotropic responsiveness to β-agonists,25,26 and this effect appears to be mediated by glucocorticoids.25

Surprisingly, although both PNMT inhibitors lowered blood pressure and significantly reduced PNMT activity in peripheral tissues, they did not lower epinephrine levels in any tissue. Epinephrine levels are not always indicative of the rate of epinephrine synthesis, because they are also influenced by the rate of epinephrine breakdown. In the case of SKF 64139, the lack of epinephrine reduction we observed may be due to inhibition of monoamine oxidase, an enzyme that catabolizes a substantial fraction of the catecholamines present in tissues.27 Mefford et al28 have reported that SKF 64139 has an ED50 for inhibition of brain stem monoamine oxidase of 3–4 mg/kg. Our data support this finding in
that both norepinephrine and dopamine were markedly elevated in several tissues of SKF 64139–treated rats. SKF 64139 may have failed to reduce epinephrine because of the opposing effects of simultaneous inhibition of PNMT and monoamine oxidase leading to inhibition of both epinephrine synthesis and epinephrine destruction. Catecholamines accumulate extraneuronally when monoamine oxidase is inhibited, and it is likely that synaptic catecholamine levels are also elevated. The unchanged epinephrine levels in hypothalamus and medulla oblongata after long-term SKF 64139 may therefore be indicative of unaltered intrasynaptic epinephrine levels. This suggests that SKF 64139 may lower blood pressure by a mechanism other than reducing the activity of central nervous system epinephrine neurons, which, in turn, leads to decreased sympathetic outflow. This assertion is further supported by the finding that, although SKF 64139 reduces blood pressure in glucocorticoid hypertensive rats, it tends to increase plasma norepinephrine and epinephrine levels.

In addition to inhibiting PNMT and monoamine oxidase, SKF 64139 has appreciable α2-receptor-blocking activity. This action is unlikely to cause the anti-hypertensive action we observed, because other α2-blocking drugs, such as yohimbine, tend to raise blood pressure.

It is not readily apparent why SKF 29661 did not reduce tissue epinephrine levels. We have previously shown that muscle PNMT is not neuronal, so neuronal storage sites are probably not available for epinephrine synthesized by muscle PNMT. We did not measure epinephrine turnover and thus cannot determine if SKF 29661 reduced epinephrine breakdown, thereby countering the effect of reduced epinephrine synthesis on epinephrine levels. However, SKF 29661 does not inhibit monoamine oxidase. The lack of monoamine oxidase inhibition by SKF 29661 may explain why the minimum dose of this drug required to reduce adrenal epinephrine levels is eightfold lower than SKF 64139.
despite the fact that it is a sixfold less potent inhibitor of PNMT in vitro than SKF 64139. 

In vitro, SKF 29661 has no effect on α- or β-adrenergic receptor binding or catechol-O-methyltransferase activity at concentrations as high as 30 μM, which is 300 times the Kᵢ for its inhibition of adrenal PNMT. It is therefore unlikely that any of these actions can account for the antihypertensive effect of SKF 29661.

In summary, the marked elevations in peripheral PNMT induced by dexamethasone and the pronounced antihypertensive actions in adrenalectomized dexamethasone-treated rats of a highly selective peripherally acting PNMT inhibitor suggest that elevations in non-adrenal peripheral PNMT play a role in the pathogenesis of glucocorticoid hypertension.

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