Treatment with tetrahydrobiopterin reduces blood pressure in male SHR by reducing testosterone synthesis

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Submitted 26 July 2004; accepted in final form 10 December 2004

Fortepiani, Lourdes A., and Jane F. Reckelhoff. Treatment with tetrahydrobiopterin reduces blood pressure in male SHR by reducing testosterone synthesis. Am J Physiol Regul Integr Comp Physiol 288: R733–R736, 2005. First published December 16, 2004; doi:10.1152/ajpregu.00500.2004.—Treatment with tetrahydrobiopterin (BH₄) reduces blood pressure in spontaneously hypertensive rats (SHR). In the present study, we tested the hypothesis that chronic BH₄ reduces blood pressure in male SHR by reducing testosterone biosynthesis mediated by increasing nitric oxide (NO). Male SHR, aged 17–18 wk, intact or castrated, were treated for 1 wk with BH₄ (20 mg·kg⁻¹·day⁻¹ ip). After 1 wk, mean arterial pressure (MAP), serum testosterone, and nitrate/nitrite excretion (NOₓ) were measured. MAP was significantly higher in intact males than castrated males (179 ± 2 vs. 155 ± 4 mmHg, P < 0.001). In intact males, BH₄ caused a 17% reduction in MAP (148 ± 2% (24.09 ± 2.37 vs. 3.72 ± 0.73 ng/dl; P < 0.001). In castrated males, BH₄ had no effect on MAP (152 ± 5 mmHg) but increased NOₓ by 38%. When castrated males were supplemented with testosterone, MAP increased to the same level as in intact males (180 ± 7 mmHg), and BH₄ had no effect on MAP (182 ± 7 mmHg) or NOₓ. NO has been shown to decrease testosterone biosynthesis. Chronic sodium nitrite (70 mg·kg⁻¹·day⁻¹ × 1 wk) decreased MAP in intact males (150 ± 4 mmHg) but had no effect on serum testosterone (21.46 ± 3.08 ng/dl). The data suggest that BH₄ reduces testosterone synthesis and thereby reduces MAP in male SHR, an androgen-dependent model of hypertension. The mechanism(s) by which BH₄ reduces serum testosterone levels are not clear, but the data do not support a role for NO as a mediator of hypertension; castration; androgens; nitric oxide; oxidative stress.

TETRAHYDROBIOPTERIN (BH₄) is a requisite cofactor for the nitric oxide (NO) synthases (20). In transgenic mice that are deficient in the rate-limiting enzyme necessary to synthesize BH₄, GTP cyclohydrolase I, hypertension occurs (1). In addition BH₄ has been shown to play a role in “uncoupling” of NO synthase because in conditions of increased oxidative stress, BH₄ is oxidized to dihydrobiopterin, and Landmesser and colleagues (12) reported that NO synthase is then capable of producing superoxide rather than NO.

The spontaneously hypertensive rat (SHR) is a model of androgen-dependent hypertension in the male that also exhibits oxidative stress. When male SHR are castrated, a reduction in blood pressure occurs (15), and when ovarioectomized female SHR are supplemented with testosterone, their blood pressures increase in a dose-dependent manner (15, 16). Treatment of male SHR with tempol, a superoxide scavenger, causes a reduction in blood pressure, suggesting that oxidative stress is important in maintaining the hypertension. The SHR is thought to be a model of reduced NO either because of a superoxide-mediated reduction in NO bioavailability or a BH₄ deficiency mediated by oxidative stress, which causes NO synthase to produce superoxide. When BH₄ is given to male SHR, there is a reduction in blood pressure that does not occur in Wistar-Kyoto (WKY) males (8), which tends to support this hypothesis.

However, BH₄ is also a cofactor for the aromatic amino acid hydrolases that are responsible for the synthesis of catecholamines and serotonin (20). NO, the catecholamines, and serotonin have all been shown to reduce steroidogenesis (2, 3, 6, 11, 18, 19). Because the SHR is a model whose hypertension is mediated in part by androgens, we tested the hypothesis that a reduction in androgen biosynthesis may play a role in the BH₄-mediated reduction in blood pressure in male SHR via increase in NO. To test this hypothesis, BH₄ was given to intact males, castrated males, and castrated males with testosterone supplements for 1 wk, and blood pressure, nitrite/nitrate excretion (NOₓ), an index of NO), and serum testosterone were measured.

METHODS

Rats

Male SHR were obtained from Taconic Farms (Germantown, NY). Some of the males were castrated by the vendor at 7–8 wk of age. The rats were maintained on standard rat chow (Teklad, Harlan SD, Indianapolis, IN) and tap water unless otherwise stated, in an environment with a 12:12-h light-dark cycle. The protocols complied with the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health and were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center.

Drug Treatment

BH₄. Rats were given BH₄ (20 mg·kg⁻¹·day⁻¹) by intraperitoneal injection daily for 1 wk. Control rats received the vehicle (saline) by intraperitoneal injection.

Sodium nitrite. To begin to dissect the mechanism by which BH₄ could be affecting androgen biosynthesis, we determined whether an increase in NO could be involved. To address this, the NO donor sodium nitrite (70 mg·kg⁻¹·day⁻¹) was given for 1 wk to intact males in drinking water, and serum testosterone was measured. We have used this NO donor in previous studies (13).

Testosterone. Silastic pellets containing testosterone propionate (10 mg) were implanted subcutaneously in the backs of SHR, as we have previously reported (16). The pellets were changed every 2 wk, and rats were treated for a total of 5 wk. Sham-operated rats received just the empty pellets. In these rats, BH₄ was given during the final week of testosterone supplementation.

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Measurement of Mean Arterial Blood Pressure

At 17–18 wk of age, SHR were anesthetized with the thiobarbiturate Inactin (RBI, Natick, MA) (110 mg/kg) and placed on a temperature-regulated surgery table. Tracheostomy was performed, and femoral arterial catheters were placed for continuous monitoring of blood pressure and for blood sampling. After a 50-min equilibration period, mean arterial pressure (MAP) was measured during two 30-min periods and averaged.

Measurement of Urinary NOx

Nitrate and nitrite in urine were measured by the Griess reagent method, using Escherichia coli to convert nitrate to nitrite, as we have described previously (14). The data are presented as NOx excreted per day per kilogram body weight.

Measurement of Serum Testosterone

Serum testosterone was measured by radioimmunoassay kit (Diagnostic Products), as we have previously described (16).

Statistical Analyses

Statistical differences for all data were determined by ANOVA and Dunnett’s test (5). Data are expressed as means ± SE.

RESULTS

Effect of Chronic BH4 in Intact Male SHR

As shown in Fig. 1, MAP in intact male SHR averaged 179 ± 2 mmHg. Chronic BH4 reduced blood pressure by ∼35 mmHg without significantly affecting NOx excretion (see Fig. 2). BH4-treated rats exhibited an 85% reduction in serum testosterone compared with untreated males (Fig. 1).

Effect of Castration

Blood pressure was significantly lower in chronically castrated rats than in intact males (155 ± 4 mmHg) (Fig. 1). NOx in castrated rats was similar to intact rats, and serum testosterone was very low compared with intact males. BH4 failed to reduce blood pressure in castrated male SHR (Fig. 1) but increased NOx (see Fig. 2).

DISCUSSION

BH4 had been shown previously to reduce blood pressure in male SHR (8). In the present study, we evaluated the role that androgens could be playing in the BH4-mediated reduction in blood pressure. We found that treatment of intact male SHR with BH4 did indeed significantly reduce blood pressure, but it also caused an 85% reduction in testosterone in their serum. There was a tendency for NO metabolites to increase, but this did not reach significance. To determine whether the reduction in testosterone played a role in the depressor response to BH4, castrated male rats were given BH4. Castration prevented the fall in blood pressure with BH4 found in intact males despite a significant increase in NO metabolite excretion. Finally, to further determine whether a reduction in androgens mediates the reduction in blood pressure with BH4, castrated males were treated with testosterone supplements to “clamp” serum testosterone. Again BH4 failed to reduce the blood pressure. These data suggest that the mechanism by which BH4 reduces blood pressure in male SHR is via a reduction in testosterone biosynthesis.

We have previously shown that castration causes a reduction in blood pressure in male SHR, while ovariectomy has no effect in females (15, 16). When ovariectomized rats were treated chronically with testosterone supplements, the females exhibited a dose-dependent increase in blood pressure (15, 16).
We have also found that treatment of intact male SHR with flutamide, the androgen receptor antagonist, reduces blood pressure (17). However, Iliescu and colleagues (10) have recently reported that flutamide may work as a vasodilator in vivo, since testicular feminized (tfm) rats that have no androgen receptors also exhibit reductions in blood pressure with flutamide. In any case, the male SHR is an androgen-dependent model of hypertension. Thus anything that interferes with the production of androgens would also cause a reduction in blood pressure.

The mechanism by which BH$_4$ decreases testosterone levels in male SHR is not apparent from our studies. We had hypothesized that BH$_4$ would stimulate production of NO, and NO has been shown to inhibit steroidogenesis in vitro. For example, DelPunta and colleagues (3) reported that NO donor treatment of Leydig cells reduces steroidogenesis. Kostic and colleagues (11) found that the mechanism involves NO inhibition of 3β-hydroxysteroid dehydrogenase, which converts androstenediol to testosterone and dehydroepiandrosterone to androstenedione for further conversion to testosterone. It is thought that NO binds the heme groups of this cytochrome P-450 enzyme. In the present study, there was a tendency for NO metabolite excretion to increase with BH$_4$ in intact males, although it did not reach significance, which made us conclude that NO did not play a role in the reduction in serum testosterone in males. However, this method of assessing NO has been frequently criticized for its lack of sensitivity. We therefore gave an NO donor, sodium nitrite, for 1 wk, and although sodium nitrite reduced the blood pressure and increased NO metabolite excretion in the intact male SHR, serum testosterone levels were not affected. Our data then do not support a role for NO as the mechanism by which BH$_4$ reduces testosterone in male SHR.

Another possible mechanism by which BH$_4$ could reduce blood pressure in male SHR is by causing a reduction in oxidative stress. The SHR is a model of oxidative stress, and tempol, the superoxide scavenger, has been shown to reduce their blood pressure. We have recently found that testosterone stimulates superoxide production by the kidney in SHR, and castration reduces superoxide production (R. Iliescu and J. F. Reckelhoff, unpublished results). Therefore, a reduction in oxidative stress with BH$_4$ likely did occur, but this is due to the effect of BH$_4$ to inhibit testosterone biosynthesis, rather than other mechanisms.

In addition to NO synthases, BH$_4$ is a cofactor for the enzymes responsible for the synthesis of the catecholamines dopamine, norepinephrine, and epinephrine and for phenylalanine metabolism (20). Because BH$_4$ is a negative effector for phenylalanine hydroxylase (PAH), an increase in BH$_4$ should cause a reduction in PAH activity, leading to an increase in phenylalanine. It is not clear how this would affect testosterone biosynthesis. However, the catecholamines have also been shown to inhibit androgen biosynthesis. For example, Hagag and colleagues (7) reported that the dopamine agonist bromocriptine reduced total testosterone in humans by 40 – 85%. It is possible that BH$_4$ in the SHR could have increased dopamine synthesis, which led to the reduction in serum testosterone. NO has been shown to stimulate dopamine release in the brain (4); thus, in the present studies, the NO donor sodium nitrite reduced blood pressure and should also have increased dopamine. However, sodium nitrite did not reduce serum testosterone, and thus it is unlikely that increased dopamine is the mechanism by which BH$_4$ reduces serum testosterone in SHR.

An inverse relationship has been shown to exist between plasma epinephrine and androgens. Elman and colleagues (6) reported that in healthy men blockade of glucose metabolism, which caused metabolic stress, was associated with a reduction in serum testosterone and concomitant increases in plasma epinephrine and norepinephrine within 60 min (6). Therefore, a BH$_4$-mediated increase in norepinephrine and/or epinephrine could have resulted in the inhibition of androgen synthesis in the SHR. One might expect that if BH$_4$ caused an increase in catecholamines that an increase in blood pressure should also occur in male SHR. However, if serum testosterone was decreasing while catecholamines were increasing, the drop in testosterone would offset an increase in blood pressure due to catecholamines.

Another possible mechanism by which BH$_4$ could have reduced steroidogenesis is via serotonin. It is well known that serotonin reuptake inhibitors that increase serotonin levels also reduce serum testosterone levels (18). Therefore, future studies are needed that will focus on the roles that serotonin, norepinephrine, and epinephrine may play in the BH$_4$-mediated reduction in serum testosterone in SHR.

In summary, our data suggest that BH$_4$ reduces serum testosterone in SHR and causes a concomitant reduction in blood pressure because androgens play an important role in

![Fig. 3. BH$_4$ had no effect on MAP when castrated males were treated with testosterone supplements. *P < 0.05 compared with MAP in untreated castrated male; ‡P < 0.05 compared with serum testosterone in untreated castrated male.](image-url)
mediating the hypertension in male SHR. However, the BH4-mediated inhibition in androgen biosynthesis is not mediated by NO. Our data also suggest that in models of hypertension in which a definite link has been established between androgens and the hypertension, care should be taken when interpreting the effects of BH4 treatment without monitoring the effects on androgen steroidogenesis that could impact blood pressure and oxidative stress.

ACKNOWLEDGMENTS

We thank H. Zhang for excellent technical support.

GRANTS

This work was supported by National Institutes of Health Grant HL-66072.

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