

# Glucocorticoid-Induced Hypertension and Tetrahydrobiopterin (BH4), a Common Cofactor for the Production of Vasoactive Molecules

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**Abstract:** Excess glucocorticoids, whether produced endogenously or over-prescribed for immunosuppression and anti-inflammation, can lead to hypertension and cardiovascular disease. Humans and animals with glucocorticoid-induced hypertension exhibit reduced nitric oxide (NO) and serotonin, and have increased sensitivity to catecholamines. The common cofactor for the production of these vasoactive molecules is tetrahydrobiopterin (BH4). Recent research has focused on the effects of excess glucocorticoids on BH4 biosynthesis because reduced BH4 cofactor levels can alter the production of NO, serotonin, and catecholamines by NO synthase and the aromatic amino acid hydroxylases. This review will focus on the mechanisms and consequences of excess glucocorticoids on the BH4 biosynthesis pathway and the enzymes that utilize BH4 as a cofactor. Alterations in the production of BH4 contribute to glucocorticoid-induced hypertension and an understanding of the mechanisms may provide therapeutic targets to either develop synthetic glucocorticoids that do not affect BH4 biosynthesis or increase BH4 levels in conditions where glucocorticoids are elevated.

**Keywords:** GTP cyclohydrolase 1, tetrahydrobiopterin, glucocorticoids, endothelial nitric oxide synthase, endothelium.

## INTRODUCTION

Glucocorticoids (GCs) are steroid hormones endogenously produced in the adrenal gland and have many physiological functions including altering glucose metabolism, decreasing inflammation, suppressing the immune system, and increasing protein catabolism. GCs are known to have receptor-mediated genomic effects as well as acute, non-receptor-mediated effects on various cell types [1]. The GC receptor (GR), a 777 amino acid protein located in the cytoplasm, has been identified in endothelial and vascular smooth muscle cells, and contains a zinc finger motif [2-5]. The GR is associated with 2 heat shock protein (HSP) 90 molecules, HSP 56, HSP 70, and a peptide named p 23 in the absence of GCs. Upon binding of GCs to the GR, the chaperone molecules are released and the complex enters the nucleus where it binds a GC response element (GRE) to induce or suppress gene transcription. More specifically, GR side groups of glycine-458 and serine-459 directly bind to the GRE [6].

Synthetic GCs are among the most widely prescribed drugs by physicians for immunosuppression and anti-inflammation, however, high doses of GCs and/or extended use may lead to hypertension in humans and animals [7-9]. Patients with excess GC levels, or Cushing's Syndrome, also suffer from depression, as previous clinical studies have found low levels of serotonin and melatonin in these patients [10]. It has been reported that even after successful treatment of Cushing's Syndrome patients have a 4-fold increase in morbidity and mortality compared to healthy controls, much of which is due to cardiovascular disease [11].

In addition to the low levels of serotonin and melatonin, nitrate/nitrite levels are extremely low in patients with Cushing's Syndrome, suggesting reduced nitric oxide (NO) production. Additionally, clinical studies have shown that plasma catecholamine levels (norepinephrine) are inversely related to urinary GC levels in humans with GC-induced hypertension [10,12]. These observations have lead several laboratories to investigate the effects of GCs on tetrahydrobiopterin (BH4). Why BH4? BH4 is an allosteric and essential cofactor in the production of NO, catecholamines, serotonin, and melatonin (Fig. 1). BH4 is produced by two pathways, by *de novo* synthesis and a regenerating salvage pathway (Fig. 2), see references 13 and 14 for more details on the biosynthesis of BH4. It is through these pathways that various cell types are able to ensure the availability of the potent redox molecule and critical cofactor.

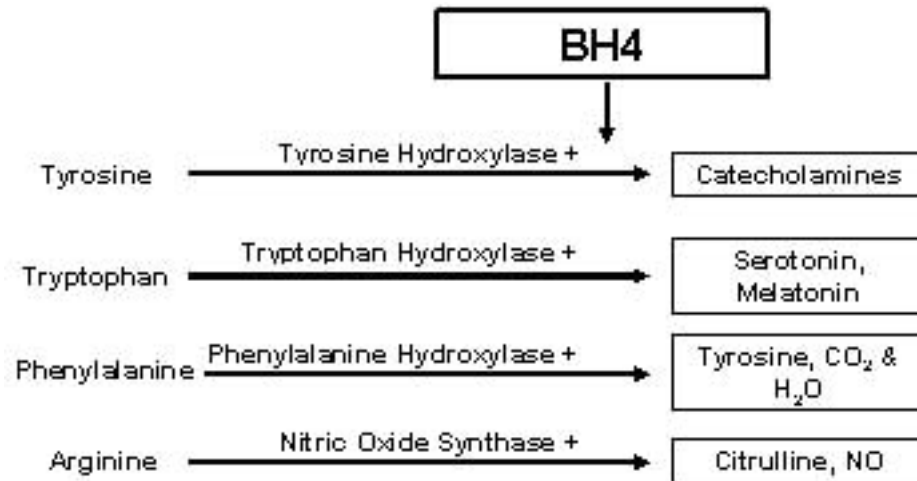
Recent studies have shown that BH4 plays an important role in vascular biology and blood pressure regulation, as decreased BH4 biosynthesis can lead to decreased vasodilation and elevated blood pressure [15,16]. In the presence of low BH4, endothelial NO synthase (eNOS) may become uncoupled, thus generating increased superoxide and decreased NO, leading to decreased vasodilation [17,18]. This review will focus on the effects of GCs on BH4 (See Reference 19 for a review on GC-induced hypertension) and the possible mechanisms by which GCs alter BH4. The effects of GCs on the enzymes in cellular BH4 biosynthesis will be discussed, as well as the effects of GCs on the enzymes that utilize BH4 as a cofactor (eNOS and the aromatic amino acid hydroxylases).

## GC EFFECTS ON BH4 BIOSYNTHESIS

### GTP Cyclohydrolase

GTP cyclohydrolase 1 (GTPCH) is the first and rate-limiting enzyme in the 3-step *de novo* production of BH4.

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**Fig. (1).** Reactions that require the cofactor, tetrahydrobiopterin (BH4). NO = nitric oxide.

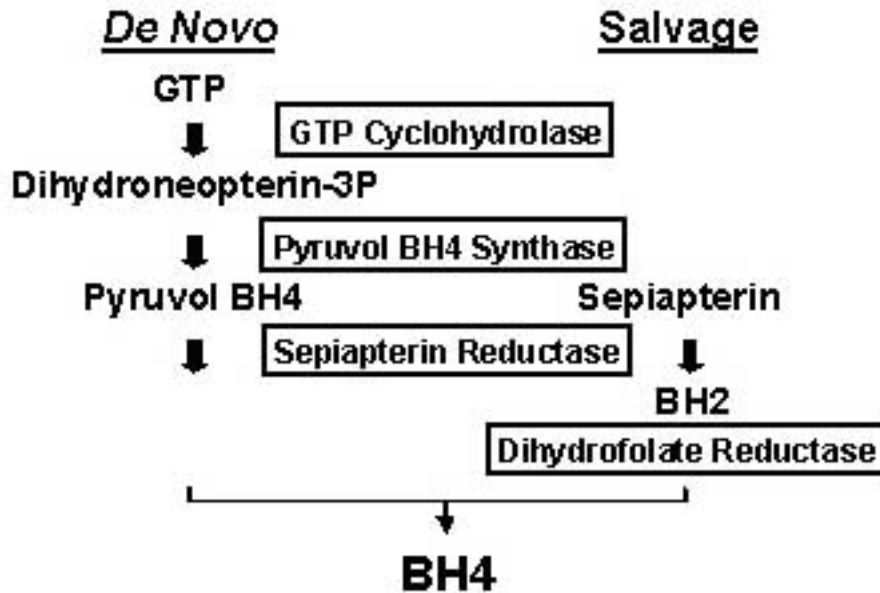
Inhibition of BH4 biosynthesis by a GTPCH inhibitor, di-amino hydroxy-pyrimidine (DAHP), or a genetic deficiency, the *hph-1* mouse mutant which has a 90% decrease in GTPCH activity, leads to uncoupled eNOS, decreased cellular NO production, and hypertension [15,20]. We have previously shown that aortic BH4-dependent vasodilation and GTPCH mRNA are significantly decreased in rats implanted with the synthetic GC dexamethasone (DEX; 0.79 mg/kg/day) after 4 and 15 days [21]. Similarly, aortic rings from normotensive control rats showed a reduced endothelium-dependent dilation and GTPCH mRNA after a 6 hour incubation with DEX [21,22]. Taken together, these findings implicate a possible genomic effect of GCs to reduce GTPCH mRNA and BH4 production. Recently, we hypothesized that GCs have a GR-mediated genomic effect to down-regulate GTPCH leading to decreased endothelium-dependent relaxation. We examined the effects of GCs on aortic GTPCH mRNA using isolated aortic segments taken from control rats and incubated them with DEX and mifepristone, a GR antagonist, for either 2 or 6 hours [23]. Additionally, we co-incubated with cycloheximide, a protein synthesis inhibitor, to determine if new proteins were needed to down-regulate GTPCH mRNA. Our results indicated that GCs act through the GR to decrease GTPCH mRNA, which translated into decreased endothelium-dependent relaxation. The GR antagonist mifepristone completely blocked the GC-induced decrease in GTPCH mRNA and endothelium-dependent dilation, whereas spironolactone, a mineralocorticoid receptor antagonist, had no effect. In addition, cycloheximide had no effect on the down-regulation of GTPCH by GCs, suggesting that GCs may negatively regulate GTPCH by repressing transcription by binding to negative GREs or interfering with other transcription factors without directly contacting the DNA (transrepression). It is unknown if the GTPCH gene contains a GRE and further studies are needed to identify the mechanism of GTPCH down-regulation.

### Sepiapterin Reductase

The third enzyme in the *de novo* synthesis of BH4, and first enzyme of the salvage pathway, is sepiapterin reductase. A study by Schoedon and colleagues showed that phenprocoumon, a sepiapterin reductase inhibitor, decreased NO production in murine macrophages by interfering with BH4 biosynthesis [24]. However, no studies to date have examined the effects of GCs on this important enzyme, which aids in the bioavailability of cellular BH4. It is possible that GCs may modulate vascular sepiapterin reductase through either genomic or non-genomic mechanisms leading to altered levels of available BH4. It would be interesting to see if genetic or pharmacological stimulation of sepiapterin reductase could increase BH4 and NO in cells or intact tissues treated with excess GCs.

### Dihydrofolate Reductase

The second enzyme in the salvage pathway of BH4 biosynthesis is dihydrofolate reductase, which reduces dihydrobiopterin (BH2) into the fully reduced pterin, BH4. Recent studies in vascular biology have revealed the importance of the BH4 to BH2 ratio in determining NO production [18,25]. One study found that although total vascular biopterin levels did not change in mineralocorticoid-induced hypertension, BH2 was significantly increased and BH4 was concomitantly decreased [18]. Additionally, Vasquez-Vivar and colleagues have used electronic spin resonance trapping to examine the roles of BH2 and BH4 as cofactors for NO production [17]. They reported that BH2 is able to displace BH4 in the binding site of eNOS leading to uncoupling and decreased NO synthesis. Due to the important balance required between BH2 and BH4, it is possible that GCs may also decrease dihydrofolate reductase mRNA and protein levels leading to increased BH2 levels and preventing full reduction to BH4. Several studies have also reported that exogenous sepiapterin, which has been



**Fig. (2).** Biosynthesis of tetrahydrobiopterin (BH4). BH2 = dihydrobiopterin, GTP = guanosine triphosphate.

shown to improve endothelium-dependent responses under certain conditions [26,27], further decreases vasodilation in certain forms of hypertension [25,28]. Alterations in dihydrofolate reductase, as well as sepiapterin reductase, may play a role in preventing exogenous sepiapterin from being converted into BH4, thus resulting in increased levels of cellular BH2. Whether this mechanism occurs in GC-induced hypertension is unknown.

#### Degradation of BH4

BH4 is susceptible to oxidation in vascular tissue and the subsequent formation of BH2 leads to eNOS uncoupling as mentioned above. The major oxidant that causes this effect appears to be peroxynitrite. Landmesser and colleagues demonstrated that increased peroxynitrite production in mineralocorticoid-induced hypertensive mice leads to a decreased ratio of BH4/BH2, eNOS uncoupling, and impaired endothelium-dependent dilation [18]. GC-induced hypertension is also associated with increased reactive oxygen species. In addition to the genomic effects on BH4 biosynthesis, GC-induced increases of reactive oxygen species in endothelial cells, including peroxynitrite, may exert acute negative effects by degrading BH4 and NO. Evidence to support this comes from an *in vitro* study showing that treatment of human umbilical vein endothelial cells with DEX increased intracellular amounts of peroxynitrite and decreased NO [29].

#### GC EFFECTS ON ENZYMES THAT USE BH4 AS A COFACTOR

##### Endothelial Nitric Oxide Synthase

It is well known that NO plays an important role in vascular homeostasis and blood pressure regulation. Evidence shows that the NO system is altered in GC-induced hypertension and eNOS knockout mice do not display a further increase in blood pressure when treated with high

doses of GCs [30,31]. In addition to the effects on GTPCH, GCs are known to have genomic effects on eNOS mRNA and protein expression [32,33]. The GR has been shown to mediate down-regulation of eNOS mRNA and protein, and decrease nitrate/nitrite/NO release in aortic tissue and endothelial cells [32,33]. A previous study showed a 40% decrease in eNOS mRNA in aortas from rats treated with DEX in the drinking water after 3 days [32]. Wallerath and colleagues also reported that although the eNOS promoter lacks a GRE, GCs reduce the binding of the transcription factor GATA to the eNOS gene. Previous studies have demonstrated the importance of GATA in eNOS transcription and data to support this comes from experiments in which a mutation in the GATA binding site on the eNOS promoter reduces eNOS promoter activity by about 30% [34,35]. Rogers *et al.* demonstrated that cortisol dose-dependently reduced nitrate/nitrite levels and eNOS protein in cultured bovine coronary artery endothelial cells through activation of GRs [33]. Our laboratory has also shown a time-dependent down-regulation of aortic eNOS mRNA in rats treated with high doses of GCs [21]. However, studies in isolated rat aortas have shown that a 6-hour incubation with GCs had no effect on eNOS mRNA levels, whereas GTPCH was down-regulated at this time point [23]. These findings suggest that down-regulation of GTPCH by GCs, leading to decreased BH4 biosynthesis and uncoupled eNOS, may precede the down-regulation of eNOS mRNA.

In contrast to GR-mediated down-regulation of eNOS, recent studies have shown an increase in eNOS as a result of activation by an acute administration of GCs [36,37]. These studies examined the neuroprotective effects of GCs during cerebral ischemia and determined that GCs, after binding to the GR, activated phosphatidylinositol 3-kinase and protein kinase Akt, leading to eNOS activation most likely through serine 1179 phosphorylation, increased NO-dependent vasodilation and increased blood flow to the brain. Although

these studies used high doses of GCs acutely, the long-term effects were not measured. GCs may show promise in the acute treatment of cerebral ischemia (i.e., within 2 hours of ischemia), however long-term administration of GCs may lead to reduced eNOS expression and activity.

### **Tyrosine Hydroxylase**

Tyrosine hydroxylase is the first and rate-limiting enzyme in the production of catecholamines. Previous studies have found elevated tyrosine hydroxylase mRNA and activity in models of chronic stress, which is associated with high levels of endogenous GC release [38]. Recently, a GC-responsive GRE was discovered in the 5' flanking region of the mouse tyrosine hydroxylase gene promoter in PC12 cells [39]. Additionally, this GRE is sufficient for increased tyrosine hydroxylase gene transcription following GR activation [40]. Contrary to the effects of GCs on GTPCH and eNOS, GCs seem to induce tyrosine hydroxylase gene transcription through a genomic effect. These findings support the idea of an increased vasoconstriction and reduced vasodilation in GC-induced hypertension. However, clinical studies have shown that plasma catecholamine levels (norepinephrine) are inversely related to urinary cortisol levels in humans with GC-induced hypertension [10,12]. These patients do not experience a nocturnal fall in blood pressure, therefore catecholamine biosynthesis may decrease as a compensatory mechanism or the production of catecholamines becomes reduced due to the decreased BH4 from high concentrations of GCs. The latter mechanism seems more likely because BH4 can regulate catecholamine biosynthesis by activating tyrosine hydroxylase [41]. This decrease in circulating catecholamines may lead to receptor upregulation, manifesting as an increased sensitivity to norepinephrine shown in isolated vessels of animals treated with excess GCs [7,9].

### **Tryptophan Hydroxylase**

Tryptophan hydroxylase aids in the conversion of tryptophan into 5-hydroxytryptamine (5-HT or serotonin) and melatonin. Studies on the effects of GCs on serotonin have been contradictory. However, a study performed 6 years ago showed that GCs have a cell-specific action to alter tryptophan hydroxylase mRNA [42]. Although no studies to date have examined the vascular effects, GC-treatment of adrenalectomized rats leads to a significant increase of tryptophan hydroxylase in the melatonin-producing pineal gland and a significant decrease in the serotonin-producing raphe nuclei in the brain [42]. Furthermore, these authors found that GCs significantly reduced tryptophan hydroxylase mRNA and serotonin production in a neuronal-like cell line (CA 77 C cells).

Serotonin, released as a neurotransmitter and stored in circulating platelets, has been shown to play a role in hypertension and cardiovascular disease [43]. It has been suggested that agonists of the serotonin receptor may decrease blood pressure in hypertension [7,43]. It is possible that a lack of serotonin receptor activation by decreased levels of serotonin may contribute to GC-induced hypertension, but how do GCs decrease serotonin? In addition to decreasing tryptophan hydroxylase and BH4 cofactor levels, excess GCs may also decrease serotonin

through end-product feedback. Neurons containing serotonin directly innervate corticotropin-releasing hormone-containing cells and upon serotonin-induced activation of the hypothalamic-pituitary-adrenal axis, GCs are released from the adrenal gland [44]. Excess GCs may suppress the release of serotonin from neurons, as clinical studies have reported low amounts of serotonin in patients with Cushing's Syndrome [10]. Whether or not tryptophan hydroxylase and serotonin can be restored with a GR antagonist in GC-induced hypertension remains to be determined.

### **Phenylalanine Hydroxylase**

Phenylalanine hydroxylase, a liver-associated enzyme, utilizes BH4 as a cofactor to convert phenylalanine to tyrosine with carbon dioxide and water produced as byproducts. Previous studies have shown that GCs markedly increase phenylalanine hydroxylase in several cell lines [45]. This finding would be consistent with the early GC-induced increase in catecholamine production. Twenty years ago Haggerty and colleagues showed that adrenalectomy in rats resulted in reduced phenylalanine hydroxylase, whereas GC-treatment increased this enzyme [45]. More recent studies have further examined the mechanism of this GC-induced increase in phenylalanine hydroxylase. Faust and colleagues showed a GC-responsive enhancer at kb -3.5 that contained a GR binding site [46]. More recently, hormone responsiveness of the phenylalanine hydroxylase gene was conferred by a tissue-specific HSIII enhancer and a GR protein bound to GRE half sites [47]. They also showed a synergistic effect of the GR and a transcription factor, hepatocyte nuclear factor I, in augmenting phenylalanine hydroxylase expression in response to GCs.

However, similar to tyrosine hydroxylase, it is possible that long-term exposure to high concentrations of GCs results in decreased tyrosine production by down-regulation of phenylalanine hydroxylase. Alternatively, BH4 is known to increase phenylalanine hydroxylase levels and activity and is administered to reduce the elevated plasma phenylalanine levels in patients with certain mutations leading to phenylketonuria [48,49]. Reduced BH4 biosynthesis by excess GCs may also contribute to the decreased catecholamine production in GC-induced hypertension by reducing the activity of phenylalanine hydroxylase.

### **CONCLUSION**

In summary, excess GCs exert many detrimental effects that may lead to hypertension. One of these effects is a reduction in vascular BH4 biosynthesis. GCs down-regulate GTPCH, the rate-limiting enzyme in the *de novo* synthesis of BH4, through the GR leading to reduced cofactor levels for eNOS and the aromatic amino acid hydroxylases. Enzyme dysfunction due to reduced BH4 results in decreased production of important vasoactive molecules including NO, catecholamines, and serotonin.

Further studies examining the mechanisms of down-regulation of the BH4 biosynthetic enzymes are needed, and include the possible identification of GREs on the various genes. Additionally, elucidation of the mechanisms and characterization of the genomic effects of the GC/GR complex may lead to therapeutic targets that would prevent decreased BH4 biosynthesis in conditions of excess GCs.

The prevention of GTPCH and eNOS down-regulation and/or inhibition of the GR in humans and animals with excess GCs may maintain endothelial function and suppress the development of hypertension and cardiovascular disease.

#### ACKNOWLEDGEMENTS

This work was supported by grant HL 74167 from the National Institutes of Health.

#### LIST OF ABBREVIATIONS

BH2	=	Dihydrobiopterin
BH4	=	Tetrahydrobiopterin
DAHP	=	Di-amino-hydroxy-pyrimidine
DEX	=	Dexamethasone
ENOS	=	Endothelial nitric oxide synthase (NOS 3)
GC	=	Glucocorticoid
GR	=	Glucocorticoid receptor
GRE	=	Glucocorticoid response element
GTPCH	=	Guanosine triphosphate cyclohydrolase
HSP	=	Heat shock protein
NO	=	Nitric oxide

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