

Mechanisms of Dexamethasone-Induced Hypertension

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Abstract: Hypertension is a well-recognized complication of excess glucocorticoids, both naturally-occurring and synthetic. Dexamethasone is a potent synthetic glucocorticoid, which has widespread clinical applications. As dexamethasone has purely glucocorticoid activity with negligible mineralocorticoid effects, dexamethasone-induced hypertension (DEX-HT) models have been used for studying the mechanisms of glucocorticoid-induced hypertension. This review examines the characteristics and mechanisms of DEX-HT, both in the human and experimental animal models. The roles of hemodynamics, volume, renin-angiotensin-aldosterone system, sympathetic nervous system, vasodilators including nitric oxide, vasoconstrictors and reactive oxygen species in the pathogenesis of DEX-HT are reviewed and differences from hypertension due to naturally occurring steroids discussed.

Key Words: Dexamethasone-induced hypertension, glucocorticoid, nitric oxide, oxidative stress, pathogenesis, reactive oxygen species.

INTRODUCTION

Dexamethasone is the most potent synthetic glucocorticoid which, unlike the naturally-occurring cortisol and corticosterone, has virtually pure glucocorticoid activity. The potent anti-inflammatory and immunosuppressant properties of dexamethasone render it useful in various inflammatory and autoimmune diseases. In addition, dexamethasone is used to prevent vasogenic edema secondary to cerebral tumors, in conjunction with other chemotherapeutic agents in multiple myeloma and as a replacement hormone in adrenal insufficiency.

Hypertension is a common manifestation of chronic dexamethasone use. The exact mechanism is unknown. Perturbations in the various pathophysiological systems affecting blood pressure such as plasma volume, renin-angiotensin-aldosterone system, sympathetic activity, vasopressor and vasodepressor systems have been proposed as contributing to dexamethasone-induced hypertension (DEX-HT). Mechanisms of DEX-HT including the role of these factors and the interactions between them will be explored in this review (Table 1).

MECHANISMS OF DEXAMETHASONE-INDUCED HYPERTENSION

Sodium and Volume

The notion that glucocorticoids induce hypertension through activation of renal mineralocorticoid receptors arises from observations that cortisol produces renal sodium reten-

Table 1. Postulated Mechanisms of Dexamethasone-Induced Hypertension

1.	Sodium retention and volume expansion
2.	Hemodynamic changes
3.	Increased vascular pressor responsiveness
4.	Increased sympathetic nervous system activity
5.	Vasopressor hormone excess <ol style="list-style-type: none"> i. renin-angiotensin system ii. arginine vasopressin iii. endothelin iv. catecholamine v. neuropeptide Y
6.	Vasodilator hormone deficiency <ol style="list-style-type: none"> i. Atrial natriuretic peptide ii. Prostanoids iii. Nitric oxide
7.	Oxidative stress <ol style="list-style-type: none"> i. NADPH oxidase ii. Xanthine oxidase iii. Uncoupling of eNOS iv. Mitochondria v. Cyclooxygenase
8.	Central stimulation of blood pressure

tion and hypertension [1]. However, evidence for a causal relationship between sodium retention and glucocorticoid-induced hypertension (GC-HT) is lacking. It is now clear that sodium retention is not the cause of either synthetic or naturally-occurring GC-HT, in humans or rats [2-5]. Experi-

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mental cortisol-induced hypertension in humans [2, 4] and adrenocorticotrophic hormone-induced hypertension (ACTH-HT) in rats [5] are not prevented by mineralocorticoid receptor blockade with spironolactone. Synthetic glucocorticoids elevate blood pressure in humans without any sodium retention or plasma volume expansion [3]. Grunfeld and colleagues have also demonstrated in rats that the mineralocorticoid receptor antagonist RU28318, at a dose that lowered blood pressure in mineralocorticoid hypertension [6], failed to modify hypertension induced by a glucocorticoid receptor agonist RU26988 [7]. They have also found that the glucocorticoid receptor antagonist RU38486 prevented and improved glucocorticoid receptor agonist RU26988-induced hypertension without altering body weight, urinary water and sodium excretion [7].

Dexamethasone is a potent glucocorticoid that has high affinity for glucocorticoid receptors and low affinity for mineralocorticoid receptors. It effectively induces nuclear translocation of both glucocorticoid and mineralocorticoid receptors but stimulates mineralocorticoid receptor-mediated transactivation at a much lower capacity than aldosterone. 11-Ketodexamethasone, the oxidation product of 11 β hydroxysteroid dehydrogenase 2, also demonstrated a weak binding affinity for mineralocorticoid receptors and requires a high concentration to achieve nuclear translocation without activating mineralocorticoid receptor-mediated transcription of a reporter gene [8]. Even though it can bind to mineralocorticoid receptors, dexamethasone raises blood pressure in man without mineralocorticoid effects as evidenced by the absence of urinary sodium retention and increase in body weight [3]. It produces diuresis in preterm infants [9] and promotes excretion of a water load in adrenal insufficiency in rats [10, 11]. DEX-HT in humans was accompanied by natriuresis without any changes in body weight or plasma volume [3]. In the rat, dexamethasone decreases body weight [12-14] and increases hematocrit by 5% [12].

The increase in blood pressure due to dexamethasone is independent of sodium loading or retention. Okuno *et al.* confirmed that the hypertensive effect of dexamethasone (2.5mg/L drinking fluids or approximately 30-60 μ g/day, given orally) in rats was not influenced by sodium loading with oral 1% NaCl [15].

Hemodynamic Changes

Hemodynamic studies provide insights into the effects of dexamethasone in the different vascular beds. Hypertension due to a large dose of dexamethasone (0.5mg/kg/day, given orally) in dogs was accompanied by a reduction in cardiac output and an increase in calculated total peripheral resistance [16, 17]. In humans, dexamethasone (1mg orally three times daily for 7 days) increased mean arterial pressure from 82 \pm 3 to 91 \pm 3 mmHg (p <0.001) and calculated total peripheral vascular resistance from 21.9 to 24.3 mmHg/L per minute (\pm 0.4, p <0.01) without affecting the cardiac output [18].

Studies of the effect of dexamethasone on the regional hemodynamics are limited. In a study evaluating the effects of dexamethasone (125 μ g/kg/hour, intravenous infusion over

24 hours) on the regional hemodynamic responses to lipopolysaccharide in rats, dexamethasone treatment alone (before administration of lipopolysaccharide) increased the mean arterial pressure, hindquarter blood flow and conductance; and decreased renal and mesenteric blood flow and conductance [19].

Currently available data indicate that DEX-HT, in both humans and dogs, is characterized by increases in calculated total peripheral resistance. It remains unclear whether this is a coexisting feature or a pathogenic mechanism of DEX-HT.

Increased Vascular Pressor Responsiveness

Hypertension is associated with structural, mechanical (compliance and distensibility) and functional abnormalities of blood vessels that result in decreased lumen diameter of small arteries and arterioles [20]. Structural impairment of the blood vessel wall is unlikely to play a major role in experimental dexamethasone-induced hypertension where the hypertensive action of dexamethasone occurs rapidly within 1-2 days [12-14, 16, 18]. However, there are no reports confirming the presence of vascular hypertrophy histologically in dexamethasone-induced hypertensive subjects or animals.

Functional aberrations of the vascular smooth muscle cells, manifesting as increased sensitivity and reactivity to vasoconstrictors (i.e. increased vascular pressor responsiveness) have been described in DEX-HT in humans, rats and dogs [16, 18, 21-23]. Infusions of angiotensin II and norepinephrine in dexamethasone-induced (3mg/day, given orally) hypertensive human subjects resulted in increased forearm vascular resistance with dexamethasone increasing the sensitivity to these vasoconstrictors [18]. Pretreatment with oral dexamethasone (0.3mg/dL drinking water, approximately 0.1mg/day or 0.5mg/kg/day) in rats enhanced blood pressure elevation due to norepinephrine [21]. Similar pressor response to norepinephrine was observed in DEX-HT (0.5mg/kg/day, given orally) in dogs but in contrast to the human study [18], pressor response to angiotensin II was unaltered [16]. *In vitro* perfusion of isolated rat mesenteric vasculatures resulted in a reduced threshold and increased maximal response to norepinephrine in dexamethasone-induced hypertensive rats (2 μ g/rat/day, approximately 7-9.5 μ g/kg/day, given orally for 28 days) but not in controls [22, 23]. However, these effects were not reproducible with AVP and potassium chloride infusions [22, 23]. Ijima and Malik demonstrated that DEX-HT (1.8mg/kg/week or average of approximately 257 μ g/kg/day, for 2 weeks) in rats was associated with increased pressor response to arginine vasopressin but not to norepinephrine or angiotensin II [24].

These studies, which were undertaken in different species under different experimental conditions, showed some discrepancies in the responses (or lack of response) to vasoconstrictors including angiotensin II, norepinephrine and arginine vasopressin [16, 18, 21-23]. Nevertheless, these results suggested a role for dexamethasone in increasing the sensitivity of vascular smooth muscle cells to vasoconstrictors, and vasoconstriction and potentiation of the effects of vasoconstrictors may contribute to DEX-HT.

The mechanism underlying this heightened response to vasoconstrictors induced by dexamethasone has not been fully elucidated. Sato *et al.* demonstrated a dexamethasone-induced decrease in threshold for the production of inositol triphosphate by cultured vascular smooth muscle cells in response to angiotensin II and arginine vasopressin. This effect was thought to be mediated by the glucocorticoid receptor as it was completely blocked by a specific glucocorticoid antagonist RU38486 [25]. This group later identified that dexamethasone (30mg/L drinking water, approximately 0.1mg/rat/day or 0.5mg/kg/day) in rats stimulated vascular angiotensin II type 1 (AT_{1A}) receptor mRNA even prior to the onset of hypertension [26].

Another possible mechanism for dexamethasone-induced increased vascular pressor reactivity is alteration in sodium/potassium pump activity [27, 28]. In an *in vitro* study, direct stimulation of sodium/potassium-pump-mediated cation transport in cultured rat aortic smooth muscle cells was demonstrated after 18-24 hour incubation with 10⁻⁹ M dexamethasone [27]. Sodium/potassium pump activity in the tail artery of dexamethasone-induced hypertensive (initial dexamethasone dose of 12.5mg followed by 6.5mg weekly, subcutaneous injections) uninephrectomized rats was significantly increased [28].

Whilst increased vascular sensitivity or reactivity to vasoconstrictors may be a feature of dexamethasone administration, it remains unclear whether these changes are sufficient to account, if at all, for the hypertension. Despite the evidence presented above, there are reports that have not found any pressor effects either acute or chronic dexamethasone treatments (acute: 20mg intravenously to human subjects [29]; and chronic: 0.5mg/day for 14 days intramuscularly to mongrel dogs [30]) on arterial responses to norepinephrine and angiotensin II.

Other factors contributing to increased pressor responsiveness in DEX-HT include decreased synthesis of vasodilator prostanoids and increased expression of angiotensin 1A and AVP receptors, discussed in sections 6b, 5a and 5b, respectively.

Increased Sympathetic Nervous System Activity

Several studies have evaluated the effect of dexamethasone on sympathetic nerve activity and the role of sympathetic nervous system as a mediator of DEX-HT, but with conflicting results. This is, in part, due to the methods used to measure sympathetic nerve function. There are no published reports evaluating the role of sympathetic nervous system in DEX-HT by means of ganglionic blockade. However, there are several other pieces of evidence from both direct (muscle sympathetic activity) and indirect (expression of adrenergic receptors, catecholamine synthesis, plasma catecholamine and plasma and tissue neuropeptide Y) assessments of sympathetic nerve activity in DEX-HT which are presented below.

Changes in Adrenergic Receptors

Dexamethasone can alter the balance of α 1-adrenergic receptor availability in vascular smooth muscles [31]. In

DDT1 MF2 hamster smooth muscle cell culture, dexamethasone (10⁻⁶M) was shown to result in 2.8±0.7 (mean±SEM) fold increase in the expression of α 1B adrenergic receptor genes and 1.8±0.2-fold increase in the expression of α 1B adrenergic receptors [32]. As discussed below, these alterations may contribute to the increase in vascular reactivity and pressor responsiveness seen in dexamethasone-treated humans and experimental animals [16, 18, 21-23].

Changes in Catecholamine Synthetic Pathway

Tyrosine Hydroxylase

Kumai *et al.* showed that hypertension in rats induced by subcutaneous dexamethasone injections at 1mg/kg/day for 2 days was associated with elevated epinephrine and norepinephrine levels in plasma and adrenal medulla; and increased expression and activity of tyrosine hydroxylase, a rate-limiting enzyme that converts L-tyrosine to dihydrophenylalanine (precursor for dopamine) [33]. They have also demonstrated that inhibition of tyrosine hydroxylase with α -methyl-p-tyrosine reversed DEX-HT [33]. Intravenous dexamethasone (2mg, single dose) in normotensive human subjects resulted in elevated plasma dopamine and epinephrine levels that could be blocked by the tyrosine hydroxylase inhibitor α -methyl-p-tyrosine [34].

Phenylethanolamine N-Methyltransferase

The activity of phenylethanolamine N-methyltransferase (PNMT), an enzyme that converts norepinephrine to epinephrine, in peripheral tissues (atria, ventricle and skeletal muscle) was significantly increased with chronic subcutaneous dexamethasone treatment at 1mg/kg/day for 12-14 days in both intact and adrenalectomized rats [35, 36]. The increase in activity, which was secondary to dexamethasone-induced increase in PNMT level rather than activation of existing enzyme, was associated with restoration of adrenalectomy-induced decrease in epinephrine production in the atria [36]. In another experiment, these authors demonstrated that administration of a highly selective peripheral PNMT inhibitor in adrenalectomized dexamethasone-induced hypertensive rats successfully normalized blood pressure [35].

Whilst these studies suggest a role for tyrosine hydroxylase and non-adrenal PNMT in the pathogenesis of DEX-HT, it is not possible to draw any definitive conclusions about the role of increased sympathetic activity in DEX-HT as both tyrosine hydroxylase and PNMT activities are very indirect measures of sympathetic function.

Changes in Neuropeptide Y

Neuropeptide Y (NPY) is a 36 amino acid peptide that is found in abundance within neurons both in the central [37, 38] and peripheral [39, 40] nervous system. In the periphery, it coexists with norepinephrine in sympathetic neurons that innervate the blood pressure-controlling structures: blood vessels, heart and kidneys [41]. It is released upon sympathetic stimulation and thus, is used as an index of sympathetic activity. NPY can modulate vascular tone by inhibiting the release of norepinephrine and NPY itself at the presynap-

tic NPY receptors [42, 43]. At the postsynaptic receptor, NPY causes direct vasoconstriction (especially coronary, cerebral, mesenteric and renal vascular beds) [44-46] and enhances the vasoconstricting effect of vasopressors including norepinephrine and histamine [46].

A role for NPY in GC-HT was proposed following observations that dexamethasone can influence NPY gene expression and tissue content in neuroendocrine tissue and cell lines [47-50]. Dexamethasone-induced NPY expression in islet cells (*in vivo* dexamethasone dose 2mg/kg/day, intraperitoneal injections, 12 days) [48] and insulin-producing cell line RINm5F (*in vitro* dexamethasone concentration 100nM, 5 days) [49] has been demonstrated. Increases in rat hypothalamic NPY mRNA [50] and content [47, 50] have also been shown following dexamethasone treatment (0.4mg/kg/day, subcutaneously [50] - 0.5mg/kg/day, intraperitoneally [47]). The only evaluation of plasma NPY levels in glucocorticoid hypertension was conducted by Tabarin *et al.* who found that plasma NPY was not elevated in either hypertensive (n=15) or normotensive patients (n=11) with Cushing's syndrome [51]. There is insufficient evidence to conclude that changes in plasma or tissue NPY content play a role in DEX-HT as direct measurement of NPY tissue content and plasma level, consequent on alterations in its formation, release and clearance, in DEX-HT has not been reported.

Changes in Plasma Norepinephrine and Epinephrine Levels

Dexamethasone effects on plasma norepinephrine and epinephrine concentrations are inconsistent in the literature (Table 2). Acute dexamethasone (2.5, 25 and 250µg or approximately 0.01, 0.1 and 1mg/kg, respectively, given subcutaneously) did not alter plasma epinephrine and norepinephrine levels in normal rats [52]. On the other hand, rats made hypertensive with 2-day dexamethasone injections (1mg/kg/day, given subcutaneously) had significantly raised plasma epinephrine and norepinephrine [33]. In another study in rats, 0.1mg/kg subcutaneous dexamethasone raised plasma epinephrine but did not alter plasma norepinephrine concentration [53].

Similar variations in plasma epinephrine and norepinephrine concentrations were documented in normotensive human subjects following dexamethasone treatment [34, 54] (Table 2).

Whilst plasma epinephrine and norepinephrine concentrations have been used as an index of sympathetic activity, they have limitations. Static measurements are influenced by changes in renal function and hence, plasma catecholamine clearance. Discrepancies probably also reflect differences in species studied, dose and duration of dexamethasone treatments.

Muscle Sympathetic Activity

Direct assessment of sympathetic vasomotor drive to skeletal muscle has been evaluated in six healthy male subjects, treated with oral dexamethasone (3mg/day) for five days. Dexamethasone significantly increased systolic blood pressure from a pre-dexamethasone pressure of 115±1 to 125±2mmHg (mean±SEM, $p<0.01$) but completely suppressed resting spontaneous sympathetic activity in five subjects and markedly reduced it in the other [55]. Stimulated sympathetic activity induced by cold pressor stimulus, end-inspiratory and end-expiratory apnoea were also attenuated by dexamethasone [55].

Summary

Although indirect methods have provided some useful information about the effects of dexamethasone on sympathetic nerve activity, direct recording of sympathetic nerve traffic remains a more reliable technique [56]. Based on the data of Macefield *et al.* using the latter technique, it is evident that DEX-HT, at least in humans, is not due to increased sympathetic drive [55].

Excess Vasopressor Hormone

Angiotensin II

Dexamethasone (4mg/kg, intraperitoneally) given to rats 16 and 24 hours before sacrifice significantly increased plasma and liver renin substrate (angiotensinogen) without significant alteration in plasma renin activity [57]. In DEX-HT in rats (2.5mg dexamethasone/L drinking water, approximately 30-60µg/day or 0.17-0.27mg/kg/day), plasma renin substrate was increased [15] but plasma renin activity was within normal range [15, 58]. The absence of raised plasma renin activity in these studies suggests that glucocorticoid-induced increase in plasma renin substrate does not necessarily indicate activation of the RAS.

Table 2. Responses of Plasma Norepinephrine and Epinephrine Concentrations Induced by Dexamethasone Treatment in Normotensive Humans and Rats. EPI: Epinephrine, NE: Norepinephrine

Species	Dexamethasone Dose	Plasma NE	Plasma EPI	Reference
Human	2mg, i.v. <i>stat.</i>	↑	↓	[34]
Human	1mg, p.o. <i>stat.</i>	↔	↔	[54]
Rat	0.01, 0.1, 1mg/kg, s.c. <i>stat.</i>	↓	↓	[52]
Rat	1mg/kg, s.c. 2 days	↑	↑	[33]
Rat	0.1mg/kg, s.c. <i>stat.</i>	↔	↑	[53]

Another way of evaluating the role of angiotensin II in the development of DEX-HT is by utilizing AII receptor blockers or angiotensin converting enzyme inhibitors in *in vivo* models. Suzuki *et al.* found that dexamethasone (2.5mg/L drinking water, approximately 30-60 μ g/day or 0.17-0.27mg/kg/day) raised blood pressure, daily urine volume, urinary sodium excretion and fluid intake without altering plasma renin activity [58]. There was partial prevention of DEX-HT with the angiotensin antagonist saralasin and partial reversal with both saralasin and angiotensin converting enzyme inhibitor SQ14225 without significant differences in volume status, as indicated by body weight, daily urine volume and fluid intake, between the groups. The blood pressure lowering effects of saralasin and SQ14225 were abolished by bilateral nephrectomy [58]. These results highlight a partial contribution of vasoconstrictor angiotensin II in the development of DEX-HT but do not differentiate whether this is due to angiotensin II excess or glucocorticoid-induced increase in angiotensin II sensitivity.

Apart from its vasoconstrictive capacity, angiotensin II stimulates angiogenesis, vascular smooth muscle hypertrophy and hyperplasia, myocardial hypertrophy and vascular extracellular matrix synthesis [59]. It is also a powerful stimulus of superoxide production by the mitochondria, via the activation of mitochondrial ATP-sensitive potassium channel openers, and NADPH oxidase pathway [60, 61] (Fig. 1). The role of oxidative stress including NADPH oxidase-mediated superoxide in DEX-HT is discussed below.

Arginine Vasopressin

Administration of arginine vasopressin (AVP) to normotensive rats resulted in dose dependent increases in mean arterial pressure [24]. This increase was accentuated by dex-

amethasone administration (1.8mg.kg/week, given as weekly subcutaneous injections for 2 weeks). Administration of d(CH₂)₅Tyr(Me)AVP, an AVP V₁ receptor antagonist, reduced mean arterial pressure in dexamethasone-treated rats but not in control rats [24]. The contribution of AVP to the pathogenesis of DEX-HT appears to be mediated by increased expression of the V_{1a} AVP receptor due to increased mRNA stability [62] and not increased plasma AVP concentration [16].

Endothelin

Dexamethasone has been shown to induce endothelin release from cultured human umbilical vein endothelial cells [63] and cultured rat and rabbit vascular smooth muscle cells [64, 65]. Plasma endothelin level was reported to be increased in rats receiving dexamethasone (2mg/kg/day for 12 days) [66]. A 50% increase in circulating endothelin concentration was also reported in human subjects treated with another synthetic glucocorticoid, prednisolone (50mg on day 1, 25mg on days 2 and 3, and 10mg on days 4 and 5) [67]. It is unclear whether this association represents the pathological process responsible for DEX-HT. Further *in vivo* studies using an endothelin antagonist in DEX-HT models is necessary to confirm this hypothesis.

Other Vasoconstrictors

The influences of other vasoconstrictors such as catecholamines and neuropeptide Y have been discussed earlier.

Deficiency in Vasodilator Hormone

Of the naturally-occurring vasodilators, atrial natriuretic peptide, prostanoids and nitric oxide have been examined in DEX-HT models. There are no data on the role of other

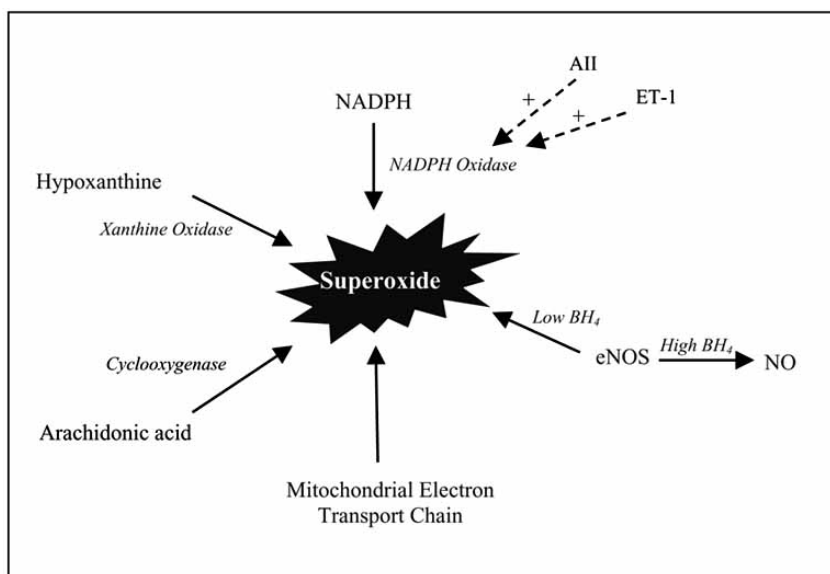


Fig. (1). Major sources of superoxide in the vasculature. AII: angiotensin II, BH₄: tetrahydrobiopterin, ET-1: Endothelin-1, NADPH: reduced nicotinamide adenine dinucleotide phosphate, NO: nitric oxide.

vasodilators (eg endothelium-derived hyperpolarizing factor) in the pathogenesis of DEX-HT.

Atrial Natriuretic Peptide

The role of atrial natriuretic peptide (ANP) in DEX-HT is controversial. Currently available evidence from both *in vivo* and *in vitro* studies reports conflicting results on the effect of dexamethasone on tissue and plasma ANP concentrations. On one hand, plasma atrial natriuretic peptide (ANP) concentrations were significantly lower in rats made hypertensive with subcutaneous dexamethasone infusions at low dosages (1, 2, 5 and 10 µg/day) [68]. In these rats, the ANP values were negatively correlated with blood pressure [68]. On the other hand, dexamethasone treatment (1mg/day or 4mg/kg/day, subcutaneously for 2 days) increased expression of atrial, ventricular and pulmonary ANP gene, and plasma ANP levels in both intact and adrenalectomized rats [69]. The dexamethasone-induced increases in plasma ANP and atrial ANP mRNA levels were independent of volume status as they were not suppressed by water deprivation for 96 hours prior to sacrifice and sample collection [69]. Garcia *et al.* demonstrated that the synthesis and release of ANP by the atria of adrenalectomized rats are regulated by dexamethasone (0.1mg/kg/day *via* miniosmotic pump subcutaneous infusion for 5 days) and can only occur when co-administered with a mineralocorticoid which exerts a permissive effect on the regulatory role of dexamethasone on ANP production [70]. In another study, ANP levels from atrial slices and extract obtained from dexamethasone (0.1mg/kg/day, intraperitoneal injection, 4 days)-treated rats were significantly higher compared to control rats but were not reproducible by *in vitro* incubation of atrial slices with varying concentrations of dexamethasone (10^{-7} - 10^{-4} M, for 1 and 4 hours) [71]. The latter result was likely due to inadequate incubation time.

It is unclear whether the contrasting effects of dexamethasone on tissue and plasma ANP concentrations are related to the dose and administration of dexamethasone and/or variations in experimental conditions, rat strain or model. Nevertheless, the role of ANP availability and activity in DEX-HT merits further investigation.

Prostanoids

The notion that a reduction in vasodilator prostanoids plays a role in the development of DEX-HT arose from observations in *in vitro* studies that dexamethasone can inhibit the biosynthesis of prostaglandins *via* the inhibition of phospholipase A₂ activity, mediated by a transferable phospholipase A₂ inhibitory protein [72-74], and subsequent reduction in arachidonic acid.

The roles of prostaglandins and prostacyclins in the development of DEX-HT have been examined, but with conflicting results. The discrepancies may be ascribed to variability in species and experimental protocols. There have been reports indicating that hypertension in rats due to dexamethasone (3mg/L, drinking water, approximately 146-160 µg/day, 7 days) was associated with decreased urinary excretion of PGI-M, a marker of prostacyclin (PGI₂) biosyn-

thesis [75]. Handa *et al.* concluded that DEX-HT in the rat (dexamethasone dose: 0.1mg/day or 0.5mg/kg/day, orally) was associated with sustained inhibition of prostaglandin synthesis based on their finding of reduced urinary PGE₂ excretion prior to the onset of hypertension [21]. There are no *in vivo* studies in humans evaluating the role of prostanoid in DEX-HT but studies using cultured human umbilical vein endothelial cells showed that thrombin- and histamine-stimulated PGI₂ [63, 76] and histamine-stimulated PGE₂ [76] release from this human cell line were inhibited by dexamethasone (10^{-7} M or approximately 0.04 µg/mL [76] and 1 µg/mL [63]).

On the other hand, Nasjletti *et al.* demonstrated that DEX-HT in rats (dexamethasone dose: 2.5mg/kg/week, given subcutaneously) was associated with an increase in circulating and urinary excretion of PGE₂ in the setting of reduced renomedullary production [77]. This was attributed to decreased degradation of this vasodilator prostaglandin in the kidney and other extrapulmonary tissues [77]. This conclusion was further supported by another study which found increased renal and urinary PGE₂ and 6-oxo-PGF_{1α}, a stable derivative of PGI₂, in dexamethasone-induced hypertensive rats (dexamethasone dose: 2.5mg/L drinking water or approximately 0.3mg/kg/day) [78]. Furthermore, they have shown that cod liver oil diet prevented DEX-HT despite causing a fall in vasodilator prostaglandins [78]. In a recent study from our own laboratory, low dose dexamethasone (1 µg/rat/day, subcutaneous injection) resulted in hypertension in rats without altering urinary PGI₂ [79].

Whilst there are data in keeping with the hypothesis that DEX-HT is linked to a reduction in vasodilator prostanoids, it remains unclear if this is a significant cause of DEX-HT.

Nitric Oxide

Nitric oxide (NO) deficiency due to administration of nitric oxide synthase inhibitors [80, 81] or endothelial nitric oxide synthase (eNOS) gene inactivation [82] is associated with hypertension. Although there are no published data on the role of NO based on acute NO synthase blockade or sequential blockade of vasoactive systems in DEX-HT, there is accumulating evidence suggesting a role for NO deficiency in the pathogenesis of DEX-HT. Plasma nitrate/nitrite, a marker of total body NO synthesis, are reduced in rats [83] and mice [84, 85] made hypertensive by dexamethasone treatment. The reduced availability of NO might result from a range of influences on the NO biosynthetic pathways: i) alteration in the activity and expression of NOS ii) decreased availability of tetrahydrobiopterin (BH₄), a NOS cofactor, iii) decreased NO precursor L-arginine and iv) increased NO removal *via* its interaction with superoxide to form peroxynitrite (Fig. 2).

Altered Nitric Oxide Synthase Expression

Dexamethasone substantially suppressed the expression of eNOS mRNA and protein in cultured human and bovine endothelial cells, as well as in aorta, kidney and liver of dexamethasone-induced hypertensive rats [84]. Furthermore, the hypertensive effect of dexamethasone seen in wild type mice

was not evident in their eNOS knockout counterparts [85]. This is compatible with the proposal that DEX-HT involves downregulation of eNOS gene expression and resultant decrease in vasodilator NO.

Decreased Tetrahydrobiopterin Availability

Tetrahydrobiopterin (BH₄) is an essential cofactor for all the nitric oxide synthase isoforms [86] (Fig. 2). Simmons *et al.* demonstrated that dexamethasone-induced decrease in intracellular BH₄ content in cultured rat cardiac microvascular endothelial cells, mediated by suppression of guanosine triphosphate (GTP) cyclohydrolase gene expression, was associated with suppressed nitrogen oxides production [87]. In another study, *ex vivo* analysis of aortic segments of dexamethasone (5mg/kg, subcutaneous implant)-induced hypertensive rats revealed decreased GTP cyclohydrolase gene expression, dampened eNOS activity, increased vasoconstrictor response to phenylephrine and absent vascular contraction to NOS inhibitor N^ω-nitro-L-arginine [88]. Based on these results, they proposed that BH₄ deficiency consequent on downregulation of GTP cyclohydrolase results in eNOS-dependent regulation of vascular contractility and may play a role in the development of GC-HT.

In contrast, BH₄ administration *in vivo* in dexamethasone-hypertensive rats did not alter systolic blood pressure [89].

Decreased L-Arginine Availability

Nitric oxide is synthesized from L-arginine *via* the catalytic action of NOS with co-production of L-citrulline (Fig. 2). Induced depletion of L-arginine in rats resulted in hypertension and decreased NO synthesis, as evidenced by reduced urinary nitrate and cyclic GMP [90], illustrating the importance of L-arginine in the maintenance of nitric oxide homeostasis. However, L-arginine supplementation did not prevent or reverse DEX-HT [12, 91]. This could mean that either L-arginine deficiency is not a predominant feature of DEX-HT or there is a defective L-arginine delivery system that results in a state of relative intracellular L-arginine deficiency. Dexamethasone-induced abnormalities in L-arginine transport have been demonstrated in cardiac microvascular endothelial cells [87]. The state of L-arginine transporter in the dexamethasone-induced hypertensive model has not been examined.

Increased Nitric Oxide Inactivation

Nitric oxide has a very short half life. Under normal circumstances, it diffuses rapidly across cell membrane into red blood cells where it is removed by its interaction with hemoglobin [92]. It can also be inactivated within the vessel wall by its interaction with superoxide to form a powerful oxidant, peroxynitrite [93]. This reaction occurs faster than the

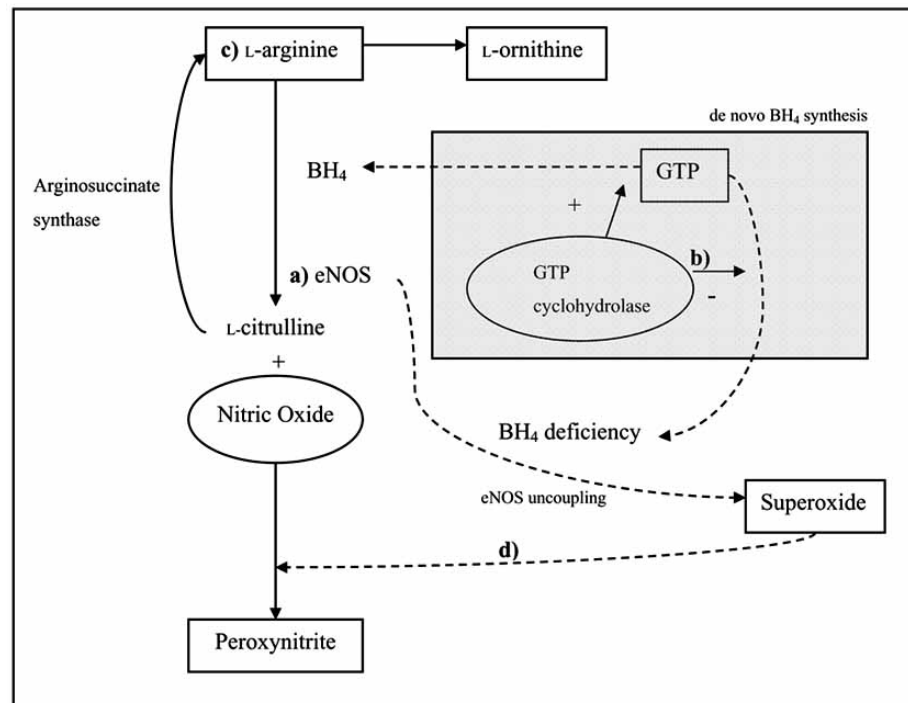


Fig. (2). Biochemical pathways controlling nitric oxide bioavailability. Dexamethasone treatment can decrease nitric oxide by altering four main pathways marked in this diagram. a) Downregulation of eNOS or decrease in eNOS activity by dexamethasone can decrease nitric oxide synthesis; b) Downregulation of GTP cyclohydrolase by dexamethasone results in decreased de novo BH₄ biosynthesis and BH₄ deficiency; c) Decrease in L-arginine availability due to alterations to its transporter by dexamethasone; d) dexamethasone-induced oxidative stress increases nitric oxide inactivation through peroxynitration, a reaction that is significantly faster than dismutation of superoxide by superoxide dismutase. BH₄: tetrahydrobiopterin, eNOS: endothelial nitric oxide synthase, GTP: guanosine triphosphate.

removal of superoxide by superoxide dismutase. In the presence of vascular oxidative stress, endothelial NO can be rapidly quenched by excess superoxide resulting in a state of NO deficit. The role of oxidative stress in DEX-HT will be reviewed below.

Oxidative Stress

Reactive oxygen species (ROS), which include oxygen radicals and highly reactive non-radicals, are continually produced as by products of normal cellular metabolism (Table 3). Superoxide, the first ROS generated *via* one electron reduction of molecular oxygen, serves as a precursor for other ROS through a cascade of catalytic processes. In the vasculature, superoxide is predominantly generated by NAD(P)H oxidase, xanthine oxidase, uncoupled eNOS, mitochondria and cyclooxygenase (Fig. 1).

Table 3. Reactive Oxygen Species

Oxygen Radicals	Highly Reactive Non Radicals
Superoxide	Hydrogen peroxide
Hydroxyl	Hypochlorous acid
Carbonate	Fatty acid hydroperoxides
Peroxyl	Reactive aldehydes
Alkoxy	Singlet oxygen

There is increasing evidence implicating vascular oxidative stress in the pathogenesis of hypertension, mediated by decreased NO bioavailability [94, 95]. Oxidative stress has also been implicated in DEX-HT (10µg/rat/day or approximately 40-50µg/kg/day, subcutaneously) as the antioxidants tempol, apocynin, N-acetylcysteine, folic acid and aspirin prevent and/ or reverse DEX-HT in rats [12, 14, 89, 96, 97]. Dexamethasone (10⁻⁷M) treatment of cultured human umbilical vein endothelial cells has been shown to enhance ROS production [98].

The association of DEX-HT with oxidative stress has led to studies to delineate the specific pathways of vascular superoxide generation that are likely to contribute to the pathogenesis of DEX-HT. The specific roles of NAD(P)H oxidase, xanthine oxidase, uncoupled eNOS and mitochondria were evaluated by inhibiting these pathways in dexamethasone-hypertensive rats (Fig. 1).

NADPH Oxidase

NADPH oxidase is one of the major producers of vascular superoxide, expressed in all layers of the blood vessel wall [99-102]. It is a multimeric enzyme composed of a membrane-bound catalytic domain (gp91phox/Nox2, Nox1 or Nox4), regulatory cytosolic subunits (p22phox, p47phox, p67phox, p40phox) and low molecular weight G-protein (Rac 1 or Rac 2) [103, 104]. Upon activation, this enzyme undergoes conformational change in the cytosolic complex followed by migration and assembly at the membrane [105]. Apocynin, an antioxidant and a known inhibitor of the phagocytic NADPH oxidase enzyme, prevented and reversed

DEX-HT in rats [12]. Whether this reflects a non-specific antioxidant effect of apocynin or specific vascular NADPH oxidase inhibition is unclear [106].

Further examination of the role of NADPH oxidase enzyme using other strategies such as NADPH oxidase subunit knockout mice, will be necessary to confirm its role in DEX-HT.

Xanthine Oxidase

Xanthine oxidase catalyses the oxidation of hypoxanthine and xanthine to uric acid, with superoxide formed as by-products. Whilst this superoxide-generating pathway is implicated in the development of hypertension in spontaneously hypertensive [107, 108] and Dahl salt-sensitive rats [109], it has been shown not to be the major source of superoxide in DEX-HT in the rat as the xanthine oxidase inhibitor allopurinol failed to prevent and reverse DEX-HT despite lowering plasma uric acid [13].

Uncoupling of eNOS

Fully-reduced tetrahydrobiopterin (BH₄) serves as an important cofactor for endothelial nitric oxide synthase (eNOS) which catalyses the conversion of L-arginine to L-citrulline and NO (Fig. 2). Decreased BH₄ availability, which promotes eNOS uncoupling, leads to the formation of superoxide instead of NO [110]. In a BH₄-free environment, L-arginine did not inhibit superoxide production but rather, augmented superoxide generation that could only be reversed by the addition of reduced BH₄ [111].

Dexamethasone-induced hypertension (5mg subcutaneous dexamethasone pellet delivering approximately 0.79 mg/kg/day) in the rat is associated with downregulation of vascular GTP cyclohydrolase I, the rate-limiting enzyme for *de novo* biosynthesis of BH₄ [112]. Abnormal endothelium-dependent vasorelaxation in the aortic rings of the dexamethasone-induced hypertensive rats was restored by sepiapterin, a BH₄ donor [112]. However, *in vivo* administration of BH₄ failed to prevent DEX-HT (10µg/rat/day, given subcutaneously) in the rat despite appropriate increases in plasma biopterin levels [89]. Furthermore, L-arginine supplementation did not prevent, reverse or exacerbate DEX-HT (10µg/rat/day, given subcutaneously) in the rat [91]. As eNOS is downregulated by dexamethasone treatment [84, 113], its involvement in superoxide production *via* its uncoupled state is unlikely to be significant. At this stage, there is insufficient evidence to draw any conclusion about the role of eNOS uncoupling in DEX-HT. Minimizing eNOS activity using NOS inhibitors and increasing BH₄ production *via* a salvage pathway involving the conversion of sepiapterin to dihydrobiopterin and then, to BH₄ could in theory minimize the extent and effects of eNOS uncoupling.

Mitochondria

Approximately 0.2-2% of oxygen used by the mitochondria is reduced to superoxide in this electron transport chain [114]. As a charged radical, superoxide produced within the mitochondria does not diffuse across the mitochondrial membrane into the cytosol readily [115]. It exits the organ-

elle through voltage dependent anion channels and more commonly, as membrane-permeable hydrogen peroxide following its dismutation by intermembrane Cu, Zn-superoxide dismutase [115].

Apart from being an important source of superoxide, the mitochondrion is also a target of oxidative injury [114]. Oxidative damage to the mitochondria is known to damage mitochondrial DNA, compromise oxidative phosphorylation potentials, decrease energy production and lead to additional production of mitochondrial reactive oxygen species [114].

Mitochondrial DNA damage is increasingly recognised as an important etiological factor in cardiovascular disease including hypertension [116, 117].

Mitochondrial superoxide overproduction has also been implicated in the pathogenesis of hypertension in some models. El Midaoui *et al.* demonstrated that hypertension in rats treated chronically with glucose is associated with increased mitochondrial superoxide production [118]. In these rats, oral supplementation of alpha-lipoic acid (500mg/kg rat chow) blunted the increase in cardiac mitochondrial superoxide and prevented hypertension. Despite this observation, the sequential relationship between these factors remains unclear. It was also uncertain whether the blood pressure lowering effect was due to the antioxidant properties of alpha-lipoic acid or the direct result of a lowered mitochondrial superoxide level. We found that oral alpha-lipoic acid at 10mg/rat/day prevented DEX-HT in the rat without altering mitochondrial superoxide production [119].

Cyclooxygenase

Endothelial cyclooxygenase (COX) is involved in regulation of vascular tone, endothelial function and pressor reactivity to agonists. Arachidonic acid metabolism by endothelial COX is a source of superoxide anions as demonstrated in studies on cerebral arteries in cats and dogs [120, 121].

The role of COX-related superoxide overproduction in DEX-HT has not been fully evaluated. Aspirin, a non-selective COX inhibitor with antioxidant properties, tended to prevent DEX-HT ($P=0.07$) in rats but failed to reverse it [97]. This modest effect could be due to its non-specific antioxidant properties or aspirin-induced stimulation of NO production *via* eNOS acetylation [122] and increased cyclic GMP [123] rather than inhibition of COX-related oxidative stress. Furthermore, indomethacin treatment potentiated the hypertensive effect of low dose dexamethasone (0.1mg/kg/day, given orally) in dogs [17]. However, the extent of oxidative stress was not evaluated in this study. The hypertensive response could be a consequence of relative imbalance of vasodilator and vasoconstrictor prostaglandins from the dexamethasone and indomethacin doses used.

Whether oxidative stress due to COX pathway has a role in DEX-HT remains to be determined.

Summary

There is a body of evidence implicating oxidative stress in the pathogenesis of DEX-HT. Dexamethasone-induced

hypertension is associated with raised plasma F₂-isoprostane and lucigenin-enhanced chemiluminescence. In rats, DEX-HT is prevented and reversed by antioxidants tempol, apocynin, folic acid, N-acetylcysteine and aspirin. Oxidative stress in DEX-HT has been shown to involve the NADPH oxidase pathway but not the xanthine oxidase pathway, uncoupled eNOS and mitochondria. The role of COX-induced superoxide production in DEX-HT is unclear.

Central Stimulation of Blood Pressure

The role of dexamethasone in blood pressure regulation at peripheral sites is better documented than its role in the central nervous system. The delivery of systemically-administered dexamethasone to the brain and cerebrospinal fluid is well-established [124, 125]. This was confirmed in a study on rats where subcutaneous tritium-labeled dexamethasone administration resulted in localization of radioisotope in the thalamus (lateral nucleus), hypothalamus (arcuate, ventromedial, periventricular and paraventricular nuclei) and cell bodies of locus ceruleus, area postrema and nucleus tractus solitarius [126]. Several investigators have studied the role of the central nervous system in the pathogenesis of DEX-HT but with conflicting results [127-129]. Direct microinjections of dexamethasone into bilateral rat nucleus tractus solitarius resulted in a transient hypertensive response acutely at lower doses (12.5 and 25pmol) and a longer hypertensive response at higher doses (50 and 100pmol) [129]. The increase in blood pressure was found to be mediated by glucocorticoid receptor-independent interaction with GABA_A and GABA_B receptors and glucocorticoid-receptor dependent non-transcriptional activation of phosphatidylinositol 3-kinase/protein kinase Akt pathway. However, the possibility of systemic absorption and dexamethasone interactions at peripheral sites was not accounted for in this study. In contrast, intracerebroventricular dexamethasone (1 and 10µg/kg/day for 7 days in dogs [127], and approximately 1µg/kg/day for 24 days in rats [128]) lowered blood pressure. In the former study, co-administration of intracerebroventricular glucocorticoid receptor antagonist, RU38486, abolished the anti-hypertensive effects [127]. Differences in dosages, treatment duration and central sites of dexamethasone administration may account for these discrepancies. Nevertheless, the role of the central nervous system in the pathogenesis of DEX-HT remains unclear.

DIFFERENCES BETWEEN NATURALLY-OCCURRING AND SYNTHETIC GLUCOCORTICOID-INDUCED HYPERTENSION

Dexamethasone, the most potent synthetic glucocorticoid, is commonly used for study of the generic effects of glucocorticoid in different disease states and in various biological pathways. Dexamethasone however exhibits some features that are different from those of naturally-occurring glucocorticoids. The similarities and differences between dexamethasone- and adrenocorticotrophic hormone-induced hypertension (ACTH-HT) are summarized below. ACTH-HT is explicable in terms of ACTH-stimulated cortisol [130, 131] and corticosterone [132] secretions in humans and rats, respectively.

Similarities

Rapid Onset

Development of GC-HT, due to both naturally-occurring and synthetic glucocorticoid hormones, is rapid. Hypertension due to oral cortisol at supraphysiological doses (80 and 200mg/day) is evident within 24 hours with the peak blood pressure occurring at day 4 or 5 of treatment in humans [130, 133]. Adrenocorticotrophic hormone administration produces rapid onset of hypertension in sheep [134], rats [135] and humans [130, 136]. Similarly, dexamethasone raises blood pressure in rats (10µg/rat/day, subcutaneously [12-14]), dogs (0.5mg/kg/day, orally [16, 17]) and humans (3mg/day, orally [18]) within 1-2 days.

No Requirement for Salt Loading or Volume Expansion

In both forms of GC-HT, salt loading and volume expansion are not prerequisites for their hypertensinogenic effects. Excess sodium is not required for the development of glucocorticoid-induced hypertension. Dexamethasone raises blood pressure independent of sodium and intravascular volume expansion in man [3]. Likewise, ACTH-HT is not entirely dependent on this mechanism even though sodium excess can magnify its hypertensive response in both man and sheep [137, 138]. Furthermore, dietary sodium restriction (15mmol/day) did not prevent ACTH-HT (1mg/day, given intramuscularly) in man despite limiting the mineralocorticoid effects [139]. Cortisol-induced hypertension developed in sheep following sodium depletion (urinary sodium loss of 603±49 mmol) although the extent of the increase was less than that in control sheep [140].

Cortisol, at maximal physiological doses, exhibits mineralocorticoid activity. However, spironolactone blocked the mineralocorticoid effects of cortisol without preventing the blood pressure rise [4]. In addition, the administration of intravenous deoxycorticosterone (1mg/day or 40µg/hour) in man for 5 days reproduced the antinatriuretic properties of cortisol without the hypertensive effects [131].

Nitric Oxide-Redox Imbalance

As discussed in the earlier sections, both dexamethasone and ACTH-HT are associated with increased superoxide production and decreased NO bioavailability.

Plasma F₂-isoprostanes, reliable markers of lipid peroxidation and systemic oxidative stress, were elevated in both ACTH- [89, 141, 142] and DEX-HT in rats [14, 89]. Similarly, plasma reactive nitrogen intermediates (nitrate/nitrite), a marker of NO availability, are reduced in cortisol- [133], corticosterone- [132], ACTH- [143, 144] and DEX-HT [83, 85, 89, 145].

Responses to Antioxidants and BH₄ Supplementation

Both ACTH- and DEX-HT were prevented and reversed by antioxidants: folic acid [89], N-acetylcysteine [96, 146] and superoxide scavenger tempol [14, 141]. Hypertension due to both ACTH and dexamethasone was prevented and reversed by apocynin [12, 142] implicating a role for super-

oxide-generating NADPH oxidase in this condition. Oxidative stress in these models of hypertension was not due to uncoupling of eNOS or the xanthine oxidase pathway as hypertension was not prevented by administration of BH₄ [89, 147] or the xanthine oxidase inhibitor allopurinol [12, 13].

DIFFERENCES

Despite the evidence implicating oxidative stress and nitric oxide-superoxide imbalance, blood pressure responses to treatments known to modify the synthesis of NO and superoxide were variable.

Response to L-Arginine

As discussed earlier, L-arginine deficiency is not a characteristic of DEX-HT. L-Arginine treatment (500mg/kg/day) increased plasma nitrate/nitrite concentrations but failed to prevent hypertension in dexamethasone-treated rats [12, 91]. In contrast, ACTH-HT is associated with decreased plasma L-arginine, L-citrulline and nitrate/nitrite concentrations [143]. Established ACTH-HT was prevented and partially reversed by L-arginine, but not D-arginine supplementation [143, 148].

The blood pressure lowering effect of L-arginine in corticosterone- and ACTH-HT is largely due to restoration of NO by increased availability of NOS substrate as increases in blood pressure with both corticosterone- and ACTH treatments were prevented by L-arginine but not D-arginine [132, 148]; and co-administration of L-arginine and the competitive NOS inhibitor, N-nitro L-arginine negated the blood pressure lowering effect of L-arginine in ACTH-HT [81].

Urinary 20-HETE

Adrenocorticotrophic hormone-induced hypertension, but not DEX-HT, is associated with increased urinary 20-hydroxyeicosatetraenoic acid excretion [79].

20-Hydroxyeicosatetraenoic acid (20-HETE) is a cytochrome P450-derived arachidonic acid metabolite that has potent vasoconstrictive properties. Synthesis of 20-HETE can be inhibited by nitric oxide [149]. Overproduction of 20-HETE in ACTH-HT might be due to NO deficiency although this does not explain the findings in DEX-HT.

Response to Glucocorticoid Receptor Antagonism

Dihydroepiandrosterone (DHEA), an endogenous steroid with anti-glucocorticoid activity, prevented DEX-HT [150] but not ACTH-HT [151].

Dexamethasone-induced hypertension was prevented by the glucocorticoid antagonist RU486 at a dose (50mg subcutaneous pellet) that did not reverse weight loss [152]. With ACTH-HT, a dose of RU486 (70mg/kg every third day) that reversed weight loss did not modify blood pressure [5]. Partial prevention of the blood pressure rise was seen with high dose RU486 (70mg/kg/day) [5].

These differences suggest glucocorticoid receptor activation is more predominant in DEX-HT.

Response to Vasopressin Antagonism

Vasopressin antagonism with the AVP V₁ receptor antagonist [l-(β -mercapto- β , β -cyclopentamethylene propionic acid) 2-(O-methyl)-tyrosine]-AVP significantly decreased mean arterial pressure in dexamethasone-induced hypertensive rats but not in saline-treated control rats [24]. Conversely, ACTH-HT was not modified by either acute or chronic inhibition of the AVP V_{1a} receptor using OPC 21268 [153] at a dose proven to be effective in blocking V₁ receptors [154].

Response to Aspirin

Aspirin, a non-selective cyclooxygenase inhibitor and an antioxidant, prevented and partially reversed ACTH-HT but not DEX-HT in rats [97]. The postulated antihypertensive mechanisms of aspirin in the ACTH-HT model include inhibition of cyclooxygenase- and NADPH oxidase-mediated superoxide production, decrease in superoxide-mediated NO inactivation, increase in NO production and increase in cyclic GMP availability. The reason for the differences between DEX- and ACTH-HT is unclear.

Response to Neomycin

Neomycin attenuates the blood pressure rise in rats following ACTH, corticosterone or prednisolone but not dexamethasone [155]. It remains unclear whether the blood pressure changes were due to altered patterns of enterohepatic handling and metabolism or the intrinsic properties of neomycin.

Summary

The observed differences between naturally-occurring GC-HT and DEX-HT suggest that hypertension due to these glucocorticoids involves different pathophysiological perturbations of nitric oxide-redox imbalance.

CONCLUSION

There is convincing evidence indicating that sodium retention with a resultant volume expansion is not a mechanism of dexamethasone-induced hypertension. Whilst the major cause for DEX-HT remains unclear, there is a body of evidence implicating nitric oxide-redox imbalance, in part, due to increased vascular superoxide production. The likely source of superoxide is the NADPH oxidase pathway. Dexamethasone-induced hypertension is also associated with increased total peripheral resistance and heightened pressor response to vasoconstrictors but not raised sympathetic activity. The roles of endogenous vasoconstrictors and vasodilators in the pathogenesis of DEX-HT are variable, with possible links to nitric oxide, prostanoids, angiotensin II, arginine vasopressin, endothelins, catecholamines, neuropeptide Y and atrial natriuretic peptide.

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