MINIREVIEW

Tetrahydrobiopterin and Nitric Oxide: Mechanistic and Pharmacological Aspects

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In previous minireviews in this journal, we discussed work on induction of tetrahydrobiopterin biosynthesis by cytokines and its significance for nitric oxide (NO) production of intact cells as well as functions of H_4-biopterin identified at this time for NO synthases (Proc Soc Exp Biol Med 203: 1–12, 1993; Proc Soc Exp Biol Med 219: 171–182, 1998). Meanwhile, the recognition of the importance of tetrahydrobiopterin for NO formation has led to new insights into complex biological processes and revealed possible novel pharmacological strategies to intervene in certain pathological conditions. Recent work could also establish that tetrahydrobiopterin, in addition to its allosteric effects, is redox-active in the NO synthase reaction. In this review, we summarize the current view of how tetrahydrobiopterin functions in the generation of NO and focus on pharmacological aspects of tetrahydrobiopterin availability with emphasis on endothelial function. Exp Biol Med 228:1291–1302, 2003

Key words: tetrahydrobiopterin; nitric oxide synthase; endothelial dysfunction; reaction mechanism

Aromatic amino acid hydroxylases (i.e., phenylalanine, tyrosine, and tryptophan hydroxylase), all three of crucial importance for neurotransmitter synthesis, as well as glyceryl-ether monoxygenase were long believed to be the only (6*R*)-5,6,7,8-tetrahydro-L-biopterin (H_4-biopterin)-dependent enzymes. However, when nitric oxide synthase (NOS) was characterized some 15 years ago, H_4-biopterin was soon identified as one of its essential cofactors (1, 2). Discovering the multifaceted roles of NO for neurotransmission, endothelial function, and the immune response also fueled the interest in H_4-biopterin and regulation of its biosynthesis. Particularly intriguing was the finding that both H_4-biopterin and NO biosynthesis are under control of cytokines and that intracellular H_4-biopterin levels could modulate not only the activity of inducible NOS (iNOS, NOS2) but also of constitutive NOS, as was shown for the endothelial isoenzyme (eNOS, NOS3).

In previous reviews in this journal, we have focused on the regulatory network of cytokines, H_4-biopterin, and NO in various mammalian cell types as well as aspects of endothelial function and mechanism of the NOS reaction related to H_4-biopterin (3, 4). More recently, we reviewed the current knowledge on H_4-biopterin biosynthesis regulation as well as on pharmacological actions of H_4-biopterin regarding endothelial function, neuronal cell survival, and its role for development and differentiation (5). In addition, we summarized pharmacological aspects of H_4-biopterin analogs (6). Therefore, the present review will focus on new insights into the role H_4-biopterin is playing in the NOS reaction and impact of H_4-biopterin availability for certain NO-related pathological conditions, in particular endothelial dysfunction.

In addition to treatment of endothelial dysfunction, H_4-biopterin treatment of patients with H_4-biopterin responsive

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mutations in the phenylalanine hydroxylase gene has recently received attention (7). Since a surprisingly high proportion (70%) of patients responded to H_4-biopterin treatment (8), and since H_4-biopterin can induce phenylalanine hydroxylase gene expression in the partially H_4-biopterin–deficient hph-1 mouse, it is assumed that in addition to its co-factor action, H_4-biopterin may increase expression of the phenylalanine hydroxylase gene (9). H_4-biopterin treatment allows these patients, who thus far had to consume a special diet low in phenylalanine, to live on a normal diet.

Mechanism of Stimulation of NO Synthases by H_4-Biopterin

Nitric Oxide Synthase Structure and Mechanism. The main synthetic pathway of NO in mammals is the enzymatic transformation of L-arginine into L-citrulline, catalyzed by NOS (EC 1.14.13.39). For overviews on various aspects of NOS enzymology and earlier studies not cited here, the reader is referred to recent reviews (10–13). The active site of NOS is formed by a heme-containing substrate-binding cavity, where L-arginine (Arg) and O_2 are converted to L-citrulline and NO. The electrons required for reductive O_2 activation are transferred from NADPH via the NOS-bound flavins FMN and FAD. All NOS isoforms are only active as homodimers. This dependence of NOS activity on the dimeric structure of the protein may be related to the essential role of "domain swapping", (i.e., reduction of the heme in one subunit by electrons derived from the flavins in the other subunit) (14). The three mammalian isoforms, neuronal, endothelial, and inducible NOS (nNOS, eNOS, and iNOS, also designated as NOS1, NOS3 and NOS2) have distinct physiological functions, are localized to distinct cellular compartments, and are regulated by distinct signaling pathways. However, despite subtle differences in their biochemical properties, the basic mechanism of NO synthesis appears to be the same in all NOS isoforms, including the proteins cloned from lower organisms, such as invertebrates (15) or a myxomycete (16).

Generation of NO occurs in two discrete steps, with intermediate formation of *N*-hydroxy-L-arginine (NHA). NHA formation consumes one molecule of O_2 and two electrons. Conversion of NHA to L-citrulline and NO requires another molecule of O_2 and one more electron. The transformation of Arg to NHA is a cytochrome P450-type monooxygenase reaction. Accordingly, reduction of ferric heme [Fe(III)] followed by binding of O_2 yields the oxyferrous (superoxy ferric) complex [Fe(II) • O_2 <–> Fe(III) • O_2^-]. Further reduction by a second electron is thought to generate the superoxy ferrous/peroxy ferric state [Fe(II) • O_2^- <–> Fe(III) • O_2^{2-}]. In the presence of Arg, uptake of two protons will eventually result in formation of NHA and regeneration of ferric heme via several as yet poorly characterized intermediates.

The conversion of NHA to L-citrulline and NO is also a cytochrome P450-like reaction, but as the -NOH group undergoes atypical three- rather than two-electron oxidation, the second step of NOS catalysis is thought to deviate from the P450 pathway after formation of the oxyferrous complex. Based on the fact that the overall stoichiometry of the second cycle requires only one electron, it is generally assumed that the heme is activated by bound NHA (i.e., that no further reduction by "exogenous" electrons is involved at this stage of the reaction).

Uncoupled Catalysis. One of the most striking differences between NOS and cytochrome P450 is that NOS requires H_4-biopterin as a co-factor. H_4-biopterin binds in the immediate vicinity of the heme at the dimer interface, interacting with residues from both subunits (17, 18). The conversion of Arg to NHA and of NHA to L-citrulline and NO both depend on the presence of H_4-biopterin. In the absence of substrate or pterin, NADPH oxidation by NOS is accompanied by formation of O_2^- and H_2O_2. It is not clear yet whether H_2O_2 is a genuine product of uncoupled NOS catalysis or if H_2O_2 derives exclusively from the disproportionation of O_2^- (19, 20). The extent of uncoupled catalysis varies between different isoforms. For nNOS, the rate of NADPH oxidation is unaffected by the presence of either of the two components that inhibit uncoupling when present together (i.e., substrate or pterin) (21). This is indicative of complete uncoupling in the absence of either of these two components. For iNOS, however, NADPH oxidation is strongly inhibited unless both Arg and H_4-biopterin are present (22).

Most studies point to the heme as the main site of uncoupled catalysis (23–28), despite some dissenting reports (19, 25). Most evidence suggests that uncoupling is only inhibited when H_4-biopterin and substrate are both present, although there are some reports demonstrating the ability of Arg or H_4-biopterin alone to suppress uncoupling (25, 26, 29). In the past few years it has become clear that the dependence of the coupled reaction on H_4-biopterin is not absolute. Some residual Arg oxidation ($\leq$10%) occurs in the absence of pterin (30, 31). The transformation of NHA into L-citrulline in the absence of H_4-biopterin has also been reported (30, 32), although that reaction is most likely mediated by O_2^- (33). Both reactions purportedly yield NO^- instead of NO as a reaction product (30, 32).

Allosteric and Structural Effects of H_4-Biopterin and their Relevance to Catalysis. Since the discovery of H_4-biopterin as a co-factor of NOS, the function of the pterin has been a controversial issue, which was not settled before 1999 (i.e., after the previous update of this Minireview) (4). In aromatic amino acid hydroxylation, H_4-biopterin is involved in binding and activation of O_2. After hydroxylation of the substrate, the hydrated and 2-electron oxidized pterin dissociates and is enzymatically recycled to H_4-biopterin (34, 35). A similar role of H_4-biopterin in NO synthesis was ruled out soon, shifting the attention to structural and allosteric effects of pterin binding.

H_4-biopterin induces a shift of the NOS heme from low- to high-spin, and this effect correlates with enzyme activation (36, 37). H_4-biopterin substantially increases the

substrate affinity of NOS as was demonstrated in equilibrium binding studies (38), and, more recently, in activity assays as well (39, 40).

The best-characterized structural effect of H_4-biopterin is the stabilization of NOS dimers; for many years triggering NOS dimerization has been assumed to reflect the main function of the pterin. This effect is most striking for the inducible isoform, which forms the least stable dimers (41). Under certain conditions iNOS dimerization is strictly dependent on H_4-biopterin. However, dimeric forms of all three isoforms, including iNOS, can be obtained in the absence of H_4-biopterin (42, 43).

There are two other potentially beneficial effects of H_4-biopterin on NOS catalysis. First, H_4-biopterin binding prevents formation of inactive P-420 heme (44), and, secondly, the pterin may scavenge NOS-derived reactive nitrogen and oxygen species (40, 45). Both phenomena could contribute to prevent enzyme inactivation during catalysis. An interesting difference between iNOS and nNOS is that binding of Arg and H_4-biopterin increases the redox potential of the heme in iNOS but not in nNOS (46), an observation that may explain why iNOS shows less uncoupling than the neuronal isoform. Recent work using ultrafast time resolved absorption spectroscopy of eNOS suggested pterin-dependent formation of a new, non-heme NO binding site, which might control NO escape from the enzyme (47).

In summary, H_4-biopterin exhibits a range of allosteric and structural effects, presumably resulting from binding of the pterin in the dimer interface in close proximity to the heme and the substrate-binding site (17, 18). However, modulation of NOS activity through these effects appears to be relatively moderate, pointing to a distinct key function of H_4-biopterin in NO synthesis that is unrelated to allosteric effects.

A Novel Role for H_4-Biopterin as a 1-Electron Donor to the Heme. Although a classical function of H_4-biopterin in NO synthesis as a 2-electron donor has been definitely ruled out, studies with H_4-biopterin analogs suggested some kind of redox function of the pterin co-factor (for detailed discussion see References 4, 13). Although the natural 6R-(L-erythro-1′, 2′-dihydroxylpropyl) side chain of H_4-biopterin is required for high affinity to NOS (48), several tetrahydropterins, but no dihydropterins were able to support the reaction when used in high concentrations. 7,8-Dihydrobiopterin, on the other hand, bound fairly well to NOS (38) and was able to provoke allosteric effects identical to those seen with H_4-biopterin, but did not support the reaction (4, 13, 19, 22, 37, 45, 49–53). Since the electrochemical properties of 7,8-dihydropteridines preclude their participation in enzymatic redox cycling (54), these observations strongly suggested a redox function of H_4-biopterin in NOS. More recently we have shown that 5-methyl- H_4-biopterin (55) and other N5-substituted pteridines (50), which can undergo reversible 1- but not 2-electron oxidation (54), support NO synthesis, in agreement with the function of H_4-biopterin as a 1-electron donor suggested by the EPR and light absorbance spectroscopic studies described below.

A major breakthrough in this puzzle was achieved in low-temperature optical studies, designed to detect short-lived intermediates formed in the reaction of reduced nNOS with O_2 (56). An intermediate observed in the absence of Arg or H_4-biopterin was identified as the oxyferrous complex, which decayed to the ferric state within a few minutes. In the presence of both Arg and H_4-biopterin, accumulation of the oxyferrous complex was prevented and stoichiometric amounts of NHA were formed. These results suggested that Arg and H_4-biopterin were essential for continuation of the reaction cycle beyond the oxyferrous complex, whereas in the absence of either substrate or pterin, this complex decayed to the ferric state and O_2^-, giving rise to uncoupled NADPH oxidation (see previous discussion in this review). In this experimental set-up, there was only one electron (on the ferrous heme) available, but two electrons are required for Arg hydroxylation yielding NHA. This apparent inconsistency in reaction stoichiometry led us to suggest that the "missing electron" is provided by H_4-biopterin. Albeit without precedence, such a function of H_4-biopterin as 1-electron donor would explain both the strict pterin dependence of the NOS reaction under single turnover conditions and earlier observations pointing to a redox role of H_4-biopterin distinct from its classical function as 2-electron donor in aromatic amino acid hydroxylation (56). Similar spectroscopic observations were reported by others, although some groups arrived at different conclusions (20, 52, 57–60). The stoichiometric formation of NHA from L-arginine in single-turnover experiments was initially disputed (60, 61) but confirmed in later studies (20, 52, 62, 63).

Of course, the proposed novel function of H_4-biopterin as a 1-electron donor required experimental support. Early support for our hypothesis was provided by crystallographic studies showing that the pterin binding site of eNOS is lined with negatively charged residues that might help to stabilize a trihydropterin ($BH3^{\bullet} \bullet H^+$) intermediate (18), Definitive evidence for the ability of H_4-biopterin to donate an electron to the oxyferrous heme was provided one year later by detection of the trihydropterin radical using electron paramagnetic resonance (EPR) spectroscopy (62). This crucial observation was confirmed for all three NOS isoforms (20, 64, 65). In addition, it was shown that the pterin radical is indeed formed in the N5-protonated cationic state (65), as predicted by the crystal structure of the eNOS oxygenase domain (18), and careful kinetic studies have linked formation of the radical to decay of the oxyferrous state and product formation (20, 66). Intriguingly, the 5-methyl derivative exhibited a 3-fold faster rate of radical formation as compared to unsubstituted H_4-biopterin (66).

The reaction of NOS with the intermediate product NHA exhibits similar H_4-biopterin dependency as the initial hydroxylation of Arg (67). Single-turnover studies showed accumulation of the oxyferrous complex in the absence of NHA and/or H_4-biopterin but completion of the reaction

cycle with stoichiometric formation of L-citrulline when both NHA and H_4-biopterin were present (52, 60–62, 68). Because of these similarities and the lack of an obvious reason to assume that H_4-biopterin should not be able to donate an electron in the second cycle, we proposed that H_4-biopterin has an obligatory role as a transient electron donor in both reaction cycles (64, 68). However, this issue has remained controversial, in particular because the 1-electron stoichiometry of NHA oxidation might implicate that a second, H_4-biopterin-derived electron is not required (10, 60–62). Moreover, we and others failed to detect a pterin radical in the course of NHA oxidation (62, 63, 65).

However, although there is no net requirement for two electrons in the second reaction cycle, the oxyferrous heme must be reductively activated to allow O-O bond scission, and there is evidence that bound NHA is unable to activate the heme, even though it appears to serve as the ultimate electron donor (61, 68, 69). Based on the failure to detect a pterin radical in the course of NHA oxidation, it appears conceivable that the radical is rapidly reduced back to H_4-biopterin concurrently with L-citrulline formation by the "extra" electron available in the second reaction cycle, whereas the H_4-biopterin-derived electron is consumed to produce NHA in the first cycle, rendering the pterin radical sufficiently long-lived to be detectable by EPR (20, 52). In summary, the recent developments in the field have corroborated and provided a mechanistic explanation for the notion, originally suggested a decade ago (70), which indicates the main function of H_4-biopterin is to tightly couple NADPH oxidation to NO synthesis.

The presence of H_4-biopterin is necessary but not sufficient to prevent uncoupling of the NOS reaction. To explain the additional requirement for an amino acid substrate (Arg or NHA), it has been proposed that reduction of oxyferrous heme to Fe(II) • O_2^- by H_4-biopterin would result in uncoupled decay to ferric heme and H_2O_2 in the absence of substrate (20). Although this hypothesis cannot be ruled out, an alternative scenario is also feasible. On the basis of electrochemical studies, H_4-biopterin is expected to be a poor 1-electron donor (54). Thus, albeit kinetically fast because of the close proximity of the pterin to the heme, electron transfer from H_4-biopterin to the oxyferrous complex may be energetically unfavorable. Accordingly, the species $Fe(II)O_2$··· H_4-biopterin and $Fe(II)O_2^-$···BH3• may be in rapid equilibrium with the tetrahydropterin state predominating. In that case uncoupling will still occur, unless substrate is present to pull the reaction forward along the catalytic cycle (13). In addition, this hypothesis explains why H_4-biopterin cannot provide the first electron in the NOS reaction cycle, a redox reaction that would involve reduction of ferric heme against a pronounced potential gradient. Figure 1 summarizes our views on the catalytic mechanism of NO synthesis.

Modulation of Nitric Oxide Synthase Product Distribution by H_4-Biopterin. Since H_4-biopterin prevents uncoupling of NADPH oxidation from NO synthesis, decreasing concentrations of the pterin will result in increased O_2^-/H_2O_2 and concomitantly decreased NO formation. Indeed, there is ample evidence that H_4-biopterin deficiency provokes NOS-derived O_2^-/H_2O_2 production in conditions of oxidative stress (see below). However, alternative ways of how H_4-biopterin might modulate the outcome of the NOS reaction are also feasible. NOS was found to be only half-saturated with H_4-biopterin (39, 61, 63). This phenomenon has been explained by anticooperative binding of H_4-biopterin to the NOS dimer (36). If this hypothesis holds, NOS may catalyze the formation of equal amounts of NO and O_2^- over a wide range of pterin concentrations (13, 68, 71). Consequently, as first noted in 1995 (72) NOS may function as a NO/O_2^- (perxoynitrite) synthase under certain conditions. In standard enzyme incubation mixtures, NO is detected as reaction product only in the presence of the autooxidation resistant N5-substituted pteridines: For this property, these derivatives may constitute a class of potentially useful enzyme activators. However, the low binding affinity of the lead compound, 5-methyl-H_4-biopterin, so far has precluded its use in pharmacological studies (50, 54, 55, 71). More recently, a new twist was added to the plot when it was demonstrated that the simultaneous generation of NO and O_2^- by pterin-subsaturated NOS resulted in the efficient formation of S-nitrosoglutathione (GSNO) in the presence of physiological concentrations of GSH (73). While peroxynitrite formation is expected to exhibit deleterious biological effects, nitrosothiols may serve as relatively long-lived stores and/or transport forms of bioactive NO (10).

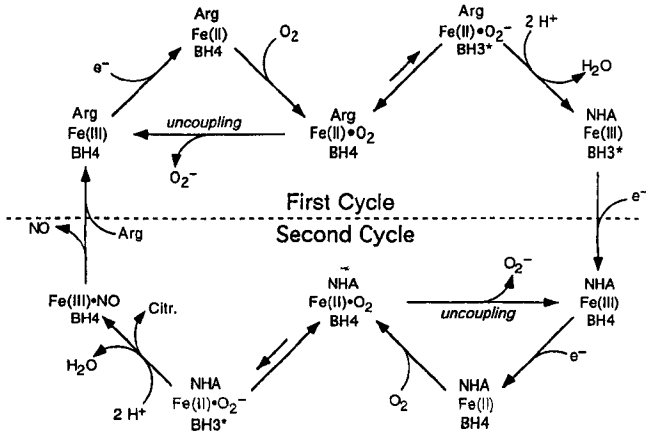

Figure 1. Proposed role of H_4-biopterin in NO synthesis. Reduction of ferric heme is followed by oxygen binding. The oxyferrous complex will yield ferric heme and superoxide in the uncoupled reaction, unless H_4-biopterin and substrate (Arg or NHA in the first and second cycle, respectively) are both present to allow continuation of the cycle. In the reaction with Arg, the BH3• radical is detectable by EPR in single turnover studies, whereas H_4-biopterin may be rapidly regenerated in the reaction with NHA.

H_4-Biopterin and Endothelial Dysfunction

Effects of H_4-Biopterin Treatment. The potential involvement of H_4-biopterin in endothelial dysfunction has recently received much attention. Since lack of H_4-biopterin causes an alteration of the ratio of superoxide to nitric oxide

by the purified enzyme, and since endothelial dysfunction was found to be correlated with decreased NO and increased superoxide production, partial H_4-biopterin deficiency is an attractive hypothesis for the cause of impaired vasodilatatory responsiveness in certain clinical settings such as the insulin-resistant state, diabetes, or atherosclerosis. In addition to the concentration of H_4-biopterin, the ratio of 7,8-H_2-biopterin to H_4-biopterin might be important, since this ratio has been shown to control superoxide release from endothelial NO synthase (53). Indeed, depletion of H_4-biopterin in canine (74) and rat (75) arteries as well as in rats *in vivo* (76) led to endothelial dysfunction within a few hours. Apart from these investigations, the hypothesis of H_4-biopterin deficiency as cause for impaired vasodilatation was tested primarily by the effects of H_4-biopterin or sepiapterin treatment on the responsiveness of blood vessels to stimulation by agents acting via NO (e.g., acetylcholine). NOS has a more than 10-fold lower K_M for H_4-biopterin compared with aromatic amino acid hydroxylases, and may therefore be the primary target for administered H_4-biopterin. It cannot be excluded, however, that biosynthesis of catecholamines, which have vasoconstrictive properties, might be potentially increased by H_4-biopterin. This issue, however, has not yet been addressed experimentally in attempts to manage vascular dysfunction. Influence of H_4-biopterin on catecholamine biosynthesis might be of minor importance in these settings, since H_4-biopterin consistently improves endothelial function in a number of vascular diseases. Table I summarizes these attempts of H_4-biopterin treatment regimens. With one exception (77), H_4-biopterin

Table I. Effect of Application of Agents Modulating H_4-Biopterin Concentrations in Various Experimental and Clinical Settings

Species/study design	Effect of H_4-biopterin (related) treatment	Reference
Dog, artery	H_4-biopterin depletion leads to endothelial dysfunction	74
Human, hypercholesterolemia	H_4-biopterin plus L-arginine restore impaired vasodilatation	130
Human, hypercholesterolemia	H_4-biopterin improves vasodilatation	131
Human, pig, atherosclerosis	Sepiapterin improves vasodilatation	132
Human, saphenous vein	H_4-biopterin improves vasodilatation	133
Human, coronary heart disease	H_4-biopterin attenuates endothelial dysfunction	134
Human, normo-or hypertensive	H_4-biopterin augments vasodilatation in both groups	77
Human, vasospastic angina	H_4-biopterin improves coronary function, but not spasm	135
Human, chronic heart failure	H_4-biopterin improves vasodilatation	136
Human, long-term smokers	H_4-biopterin improves vasodilatation	137
Human, chronic smokers	H_4-biopterin, but not H_4-neopterin, attenuates endothelial dysfunction	80
Human, EAhy926 cells	GTP-cyclohydrolase I gene transfer augments eNOS	82
Mouse, Apo-E deficient	Sepiapterin improves vasodilatation	138
Mouse, Apo-E deficient, + eNOS	H_4-biopterin counteracts damage of eNOS overexpression	81
Mouse, Apo-E deficient	Ascorbate lowers H_2-biopterin and improves vasodilatation	90
Mouse, partially H_4-biopterin deficient	H_4-biopterin counteracts catalase sensitivity of vasorelaxation	113
Rat, spontaneously hypertensive	H_4-biopterin lowers O_2^-/H_2O_2 and restores NOS activity	109
Rat, glucocorticoid-treated	Sepiapterin restores vasorelaxation	106
Rat, mouse, deoxycorticosterone-treated	H_4-biopterin blunts deoxycorticosterone-induced hypertension	107
Rat, cerebral arterioles	Nicotine impairs vasodilatation; H_4-biopterin counteracts	139
Human, diabetes mellitus	H_4-biopterin improves vasodilatation	140
Human, diabetes mellitus	H_4-biopterin corrects increased NADPH oxidation	104
Mouse, spontaneously diabetic	H_4-biopterin and sepiapterin improve vasodilatation	141
Mouse, mesangial cells	H_4-biopterin attenuates inhibition of iNOS induction by glucose	142
Rat, insulin-treated, fructose-fed	Insulin-resistant state leads to lower H_4-biopterin, higher H_2-biopterin	99
Rat, fructose-fed	Oral H_4-biopterin prevents endothelial dysfunction	100
Rat, fructose-fed	H_4-biopterin improves vasodilatation	143
Rat, diabetic, aortic rings	6-methyl-H_4-pterin augments relaxation	144
Rat, diabetic, arterioles	Sepiapterin improves vasodilatation	75
Rat, PC-12 cells	H_4-biopterin protects from NO-mediated glucose toxicity	145
Pig, coronary arteries	Sepiapterin restores vasodilatation after reperfusion	146
Mouse, rat, primary neurons	Sepiapterin protects against vulnerability due to GSH depletion	147
Rat, primary neurons	Sepiapterin protects against toxicity due to GSH depletion	148
Rat, neuronal cultures	H_4-biopterin depletion causes vulnerability to hypoxia	149
Rat, human cells; ischemia	H_4-biopterin protects against ischemia-reperfusion damage	150
Rat, isolated heart	H_4-biopterin protects against ischemia-reperfusion damage	91
Rat, renal allografts	H_4-biopterin increases NO; diminishes tyrosine nitration	151
Human, endothelial cells	Sepiapterin blunts ascorbate-mediated increase of NO production	85
Human, endothelial cells	Ascorbate stabilizes H_4-biopterin and increases eNOS activity	87
Human, endothelial cells	Ascorbate increases H_4-biopterin and increases eNOS activity	88
Human, placenta	Ascorbate stabilizes H_4-biopterin and thus activates eNOS	93
Pig, endothelial cells	Ascorbate increases H_4-biopterin and increases eNOS activity	86

shows effects only in the pathological state, but not in healthy controls. In the conditions shown in Table I, the mechanism of vasodilatation downstream of NO production, however, appears to be intact and not affected by H_4-biopterin treatment as judged by the responses of the vessels or cells to, for example, glyceryl trinitrate. H_4-biopterin appears to be an efficient drug in restoring endothelial dysfunction in a surprisingly wide range of experimental settings (Table I). See also previous reviews by Katusic (78), and Tiefenbacher (79). Abrogation of the effects of tetrahydrobiopterin by NOS inhibitors confirms that the effects observed are mediated by NOS. Further confirmation that H_4-biopterin exerts its action via NOS comes from the observed stereospecificity of the effect. In contrast with H_4-biopterin, H_4-neopterin had no effect on forearm bloodflow in chronic smokers (80). Since H_4-neopterin has chemical properties similar to H_4-biopterin, but does not bind to NOS due to its different side chain at C6 of the pterin ring (48), this finding strongly suggests that H_4-biopterin exerts its action on vasodilatation primarily by acting as co-factor of NOS rather than by chemical reactions. A striking example for the importance of H_4-biopterin for endothelial function is the attempt to correct vascular dysfunction in ApoE-deficient mice by overexpression of eNOS (81). Overexpression of eNOS in these animals had a detrimental effect that could be corrected by administration of H_4-biopterin. On the other hand, overexpression of GTP-cyclohydrolase I, the key enzyme of H_4-biopterin biosynthesis, in an endothelial type cell line by an adenoviral system dramatically increased intracellular H_4-biopterin levels and NO production by these cells (82).

Vascular Effects of Ascorbate—often Mediated by H_4-Biopterin Stabilization. In addition to H_4-biopterin, ascorbate has also been shown to restore impaired vasodilatation in a variety of clinical settings (83, 84). Experiments with endothelial cells in culture demonstrated that ascorbate leads to increased NO production, an effect that could be blinded by sepiapterin treatment, suggesting that the ascorbate effect was mediated by H_4-biopterin (85). Subsequently, increased H_4-biopterin was detected in cells treated with ascorbate (86–88). Detailed investigation of H_4-biopterin biosynthetic activities and content of the various oxidation states of biopterin in cells and culture media demonstrated that ascorbate increased intracellular H_4-biopterin and thereby NO synthesis via a chemical stabilization of H_4-biopterin (87). In agreement with the above-mentioned *in vitro* findings, pretreatment with ascorbate abolished the effect of H_4-biopterin on forearm bloodflow in chronic smokers (80). In addition, ascorbate, like H_4-biopterin in diabetic subjects, restores endothelium-dependent vasodilatation impaired by acute hyperglycemia in healthy humans (89). Ascorbate treatment of ApoE-deficient mice restored impaired vascular eNOS activity and lowered vascular 7,8-H_2-biopterin levels (90). On the other hand, in ischemia-reperfusion injury, H_4-biopterin, but not ascorbic acid or superoxide dismutase, protects from organ damage (91).

Figure 2 summarizes the current knowledge of how enzymatic activities and ascorbate are thought to influence interconversion between different oxidation states of H_4-biopterin. In addition to the NOS reaction, which generates a H_3-biopterin radical cation (65) (for detailed discussion see previous section), a neutral H_3-biopterin radical is formed upon reaction of H_4-biopterin with various radicals (92), which can be recycled to H_4-biopterin by ascorbate. Alternatively, the H_3-biopterin radical can disproportionate to the quinonoid 6,7-[8H]-H_2-biopterin, which is also reduced by ascorbate to H_4-biopterin (93). In addition, the quinonoid 6,7-[8H]-H_2-biopterin is the product of H_4-biopterin catalyzed aromatic amino acid hydroxylation followed by cleavage of the 4a-hydroxy intermediate by carbinolamine dehydratase (34, 94). In contrast with the NOS reaction, theoretical considerations of aromatic amino acid hydroxylation do not require the formation of a pterin radical (35). The quinonoid 6,7-[8H]-H_2-biopterin can be enzymatically reduced to H_4-biopterin by dihydropteridine reductase (DHPR). Once the quinonoid 6,7-[8H]-H_2-biopterin

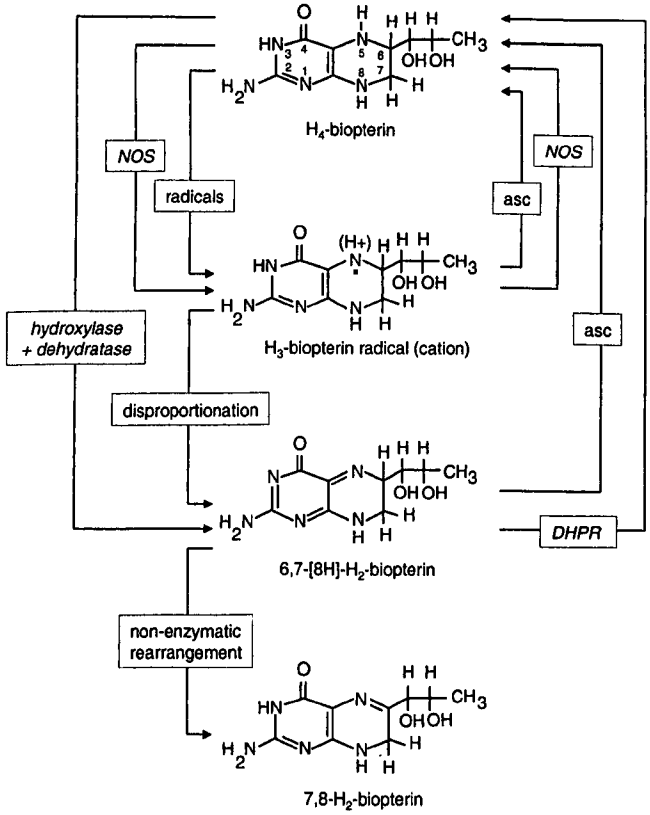

Figure 2. Pathways for the interconversion between different oxidation states of H_4-biopterin. While the H_3-biopterin radical formed in NOS has spectra best fitted by a cation (65), chemical oxidation leads to a neutral species at neutral pH (92). 7,8-H_2-biopterin can be converted to H_4-biopterin by dihydrofolate reductase. This pathway, however, is omitted from the figure since it appears not to be relevant physiologically due to the K_M in the 50 μM range, unless high concentrations of 7,8-H_2-biopterin are reached in cells (e.g., upon sepiapterin treatment). Asc, ascorbic acid; DHPR denotes dihydropteridine reductase. See text for details.

has undergone non-enzymatic rearrangement to 7,8-H_2-biopterin, it appears to be lost as co-factor and is excreted by cells to the culture medium (87). Although it is known that 7,8-H_2-biopterin can serve as substrate of dihydrofolate reductase yielding H_4-biopterin, this reaction appears to be of little importance unless high concentrations of 7,8-H_2-biopterin occur (e.g., by treatment with sepiapterin), which is efficiently converted to H_4-biopterin by cells due to the sequential action of sepiapterin reductase and dihydrofolate reductase. A reason for this behavior might be the high K_M value of dihydrofolate reductase (DHFR) for 7,8-H_2-biopterin (43–88 μM) (95, 96). In contrast, K_M values for the physiologically relevant reductions of 7,8-dihydrofolate by dihydrofolate reductase and 6,7-[8H]-H_2-biopterin by dihydropteridine reductase are 0.1 μM (95) and 0.1 μM to 0.3 μM (97), respectively.

Mechanisms Leading to Partial H_4-Biopterin Deficiency in Diabetes. The possible mechanisms leading to the suspected H_4-biopterin deficiency have received less attention than the above-mentioned effects of H_4-biopterin administration. In insulin-resistant patients, impaired vasodilatation was found paralleled by a decreased H_4-biopterin to H_2-biopterin ratio, as well as decreased dihydropteridine reductase activities (98). Similar observations have been made in diabetic rats (99, 100). The mechanisms of how hyperglycemia leads to H_4-biopterin deficiency and endothelial dysfunction are only poorly understood. It has been known for a long time that insulin can augment GTP-cyclohydrolase I activities (101), and a more recent study confirmed that insulin-induced vasodilatation depends on H_4-biopterin biosynthesis (102). These mechanisms may be impaired in the insulin-resistant state. Remarkably, high glucose leads to endothelial dysfunction already 6 hrs after increase in concentration, and this effect can be blocked by inhibition of protein kinase C (103, 104).

Other Conditions Leading to Impaired Vasodilation Paralleled by Partial H_4-Biopterin Deficiency. In addition to diabetes, partial H_4-biopterin deficiency associated with endothelial dysfunction has been observed in other conditions such as 17β-estradiol depletion (105), glucocorticoid (106) and deoxycorticosterone (107) treatment, hypercholesterolemia (108), and in the hyperphenylalaninemic mouse (hph-1) mutant (109). Ovariectomized rats show impaired vasodilatation, which can be restored by H_4-biopterin (105). Similarly, glucocorticoid-induced hypertension was found to be associated with decreased NO-dependent vasodilatation in aortic rings, which could be corrected by sepiapterin. In contrast with diabetes, where dihydropteridine reductase appears to be affected, GTP-cyclohydrolase I expression was found to be lowered by dexamethasone in these animals (106). Deoxycorticosterone treatment, on the other hand, leads to oxidation of tetrahydrobiopterin, uncoupling of eNOS, and hypertension (107). When rabbits were fed with hyperlipidemic chow, this resulted in highly increased cholesterol and dramatically lowered H_4-biopterin levels. Remarkably, sepiapterin could not reverse impaired vasodilatation in aortae cultured from these animals (108). The anticipated effect might have been achieved, however, by coincubation with superoxide dismutase, since in cultured dog arteries, sepiapterin could augment cGMP levels only when administered together with superoxide dismutase (110). See also comment in (111). Hph-1 mice are mutants with a partially reduced GTP cyclohydrolase I activity, leading to lowered H_4-biopterin levels that can be corrected by administration of H_4-biopterin (112). These animals show altered responsiveness of vasorelaxation to catalase and superoxide dismutase when compared with normal control animals, a difference that vanishes after H_4-biopterin treatment (113).

In addition to partial H_4-biopterin deficiency, another attractive mechanism of how peroxynitrite, high glucose, or hypercholesterolemia leads to dysfunction of endothelial NOS is the removal of $Zn(2^+)$ from eNOS, which resulted in decreased NO and increased superoxide production (114). Although this has not yet been addressed experimentally, it seems possible that H_4-biopterin, which stabilizes particularly eNOS dimers (41), might counteract the mechanisms removing the zinc and thereby confer its protective action. Increased vascular oxidant stress seems partially to be due to an increased expression of NADPH oxidase, as has been shown in experimental left ventricular hypertrophy (115) and in diabetes (104). Moreover, electron spin resonance measurements revealed increased xanthine and NADPH oxidase activities in coronary arteries from patients with coronary artery disease (116). Increased production of superoxide by NADPH oxidase might lead to oxidation of tetrahydrobiopterin and NOS uncoupling, an effect that was overcome by tetrahydrobiopterin supplementation (107) Taken together, the diverse indications for successful H_4-biopterin administration in various conditions remain intriguing (Table I). Lack of effect of H_4-biopterin has been reported only in exceptional cases, such as impaired endothelial regulation of ventricular relaxation in cardiac hypertrophy in the guinea pig (115).

Catabolism of H_4-Biopterin. Catabolism of H_4-biopterin (117–119) as well as its renal excretion (120) has been studied in some detail in rats more than two decades ago by Heinz Rembold *et al.* Using ^{14}C-labeled H_4-biopterin, these authors found that only a small portion (10%–15%) of the administered H_4-biopterin reappeared as fully oxidized biopterin. Most of the administered H_4-biopterin lost its side chain at C6 to yield 7,8-dihydroxanthopterin and xanthopterin (11%–19%), and was further deaminated at C2 to yield 6-hydroxylumazine (27%–28%). Other catabolic products of tetrahydrobiopterin identified include isoxanthopterin and leucopterin. While these degradation reactions can occur to some extent by chemical reactions in neutral solution, they have been shown to be greatly assisted by enzyme activities present in tissue homogenates. One of these activities contributing to degradation has been identified as xanthine oxidase. To our knowledge no comparable studies have been performed in humans thus far. While the

catabolic reactions might be largely similar in humans and rats, the comparatively lower concentrations of lumazines in human urine (121) may indicate that enzymatic activities yielding deamination of C2 of the pterin ring might be less active in humans than in rodents.

Pharmacological Effects of Inhibitory H_4-Biopterin Analogues

One of the best-studied NOS inhibitory pteridines is 4-amino-H_4-biopterin (6, 13), a compound in which the keto function at C4 is replaced by an amino group. This structural analogue of the well-known inhibitor of tetrahydrofolate reductase, methotrexate, proved to be a very potent H_4-biopterin-competitive inhibitor of NO synthesis, even though this compound is virtually identical to H_4-biopterin with respect to its electrochemical properties and allosteric effects (e.g., low-to-high-spin transition of the heme and stabilization of NOS dimers) (4, 13). In addition, the orientation of this analogue in crystals of iNOS oxygenase domain exactly resembles that of the natural co-factor H_4-biopterin (122). Thus, the mechanism of inhibition of NOS by 4-amino-H_4-biopterin differs from the mechanism of inhibition of dihydrofolate reductase by methotrexate, which binds in an altered orientation to the active center of dihydrofolate reductase (123). An attractive explanation of the mechanism of inhibition of NOS by 4-amino-H_4-biopterin is the altered electrostatic potential at N3 (124), which is the position of the molecule interacting with the heme (17). While 4-amino-H_4-biopterin inhibits all three purified isoforms of NOS in the micromolar range, in cultured cells as well as in aortic strips a selectivity for inhibition of the inducible isoform of NOS is observed (125, 126). Phenylalanine hydroxylase, in contrast, is not inhibited by 4-amino-H_4-biopterin in concentrations up to 1 mM. In a rat model of septic shock, a single dose of 10 mg/kg of 4-amino-H_4-biopterin was able to save the animals from the lethal effects of endotoxin (127). In a murine model of cardiac allograft rejection, the 4-amino H_4-biopterin was as efficient as high-dose cyclosporin A treatment in suppressing allograft rejection (128). In addition to 4-amino-H_4-biopterin, a series of other pterin-based compounds has also been investigated for inhibition of NOS (129).

Depending on their effects on the redox potential of the heme (22), some substrate-based NOS inhibitors, like the N^G-methyl derivative of Arg, attenuate NO synthesis but do not block NADPH-dependent O_2 activation. This type of compound is expected to switch the outcome of the NOS reaction from NO to O_2^-, casting doubt on their clinical benefits. Therefore, it appears essential to clarify how pterin-site inhibitors affect NOS uncoupling before considering this class of compounds for clinical use. Current evidence suggests that some pterin derivatives do indeed support the uncoupled reaction (46, 53), but definitive conclusions await detailed comparative studies evaluating the effects of a wide variety of compounds on the catalytic functions of the three NOS isoforms.

Abbreviations: For a proposal of nomenclature of pteridine compounds compare Reference 152. H_4-biopterin, BH4: 5,6,7,8-tetrahydro-6R-(L-erythro) biopterin, i.e., 5,6,7,8-tetrahydro-6R-(L-erythro-1,2-dihydroxypropyl)-pterin; 6,7-[8H]-H_2-biopterin: 6,7-dihydro-[8H]-6R-(L-erythro)-biopterin, i.e., 6,7-[8H]-dihydro-6R-(L-erythro-1′,2′-dihydroxypropyl)-pterin; H_2-biopterin, 7,8-H_2-biopterin: 7,8-dihydro-6-(L-erythro)-biopterin, i.e., 7,8-dihydro-6-(L-erythro-1,2-dihydroxypropyl)-pterin; 4-amino-H_4-biopterin: 5,6,7,8-tetrahydro-2,4-diamino-6(R,S)-6-(L-erythro-1, 2-dihydroxypropyl)-pteridine; H_4-neopterin: 5,6,7,8-tetrahydro-6(R,S)-D-erythro-neopterin, i.e., 5,6,7,8-tetrahydro-6(R,S)-6-(D-erythro-1,2,3-trihydroxypropyl)-pterin; 6-methyl-H_4-pterin: 5,6,7,8-tetrahydro-6(R,S)-6-methyl-pterin; sepiapterin: 7,8-dihydro-6-lactoyl-pterin, i.e., 7,8-dihydro-6-(1-oxo-2-hydroxypropyl)-pterin. Arg: L-arginine; Asc: ascorbate; DHPR: dihydropteridine reductase; NHA: *N*-hydroxy-L-arginine; NOS: nitric oxide synthase.

1. Tayeh MA, Marletta M. Macrophage oxidation of L-arginine to nitric oxide, nitrite, and nitrate. J Biol Chem **264:**19654–19658, 1989.
2. Kwon NS, Nathan CF, Stuehr DJ. Reduced biopterin as a cofactor in the generation of nitrogen oxides by murine macrophages. J Biol Chem **264:**20496–20501, 1989.
3. Werner ER, Werner-Felmayer G, Wachter H. Tetrahydrobiopterin and cytokines. Proc Soc Exp Biol Med **203:**1–12, 1993.
4. Werner ER, Werner-Felmayer G, Mayer B. Tetrahydrobiopterin, cytokines, and nitric oxide synthesis. Proc Soc Exp Biol Med **219:**171–182, 1998.
5. Werner-Felmayer G, Golderer G, Werner ER. Tetrahydrobiopterin biosynthesis, utilization and pharmacological effects. Curr Drug Metab **3:**159–173, 2002.
6. Werner ER, Werner-Felmayer G. Biopterin analogues:novel nitric oxide synthase inhibitors with immunosuppressive action. Curr Drug Metab **3:**119–121, 2002.
7. Muntau AC, Roschinger W, Habich M, Demmelmair H, Hoffmann B, Sommerhoff CP, Roscher AA. Tetrahydrobiopterin as an alternative treatment for mild phenylketonuria. N Engl J Med **347:**2122–2132, 2002.
8. Bernegger C, Blau N. High frequency of tetrahydrobiopterin-responsiveness among hyperphenylalaninemias: a study of 1,919 patients observed from 1988 to 2002. Mol Genet Metab **77:**304–313, 2002.
9. Blau N, Trefz FK. Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency: possible regulation of gene expression in a patient with the homozygous L48S mutation. Mol Genet Metab **75:**186–187, 2002.
10. Pfeiffer S, Mayer B, Hemmens B. Nitric oxide – Chemical puzzles posed by a biological messenger. Angew Chem Int Ed **38:**1714-1731, 1999.
11. Stuehr DJ. Mammalian nitric oxide synthases. Biochim Biophys Acta **1411:**217–230, 1999.
12. Alderton WK, Cooper CE, Knowles RG. Nitric oxide synthases: structure, function and inhibition. Biochem J **357:**593–615, 2001.
13. Gorren ACF, Mayer B. Tetrahydrobiopterin in nitric oxide synthesis: A novel biological role for pteridines. Curr Drug Metab **3:**133–157, 2002.
14. Siddhanta U, Presta A, Fan B, Wolan D, Rousseau DL, Stuehr DJ. Domain swapping in inducible nitric-oxide synthase. J Biol Chem **273:**18950–18958, 1998.
15. Colasanti M, Venturini G. Nitric oxide in invertebrates. Mol Neurobiol **17:**157–174, 1998.

16. Golderer G, Werner ER, Leitner S, Gröbner P, Werner-Felmayer G. Nitric oxide synthase is induced in sporulation of Physarum polycephalum. Genes Dev **15:**1299–1309, 2001.
17. Crane BR, Arvai AS, Ghosh DK, Wu C, Getzoff ED, Stuehr DJ, Tainer JA. Structure of nitric oxide synthase oxygenase dimer with pterin and substrate. Science **279:**2121–2126, 1998.
18. Raman CS, Li H, Martásek P, Král V, Masters BSS, Poulos TL. Crystal structure of constitutive endothelial nitric oxide synthase: a paradigm for pterin function involving a novel metal center. Cell **95:**939–950, 1998.
19. Vásquez-Vivar J, Hogg N, Martásek P, Karoui H, Pritchard KA Jr, Kalyanaraman B. Tetrahydrobiopterin-dependent inhibition of superoxide generation from neuronal nitric oxide synthase. J Biol Chem **274:**26736–26742, 1999.
20. Wei CC, Wang ZQ, Wang Q, Meade AL, Hemann C, Hille R, Stuehr DJ. Rapid kinetic studies link tetrahydrobiopterin radical formation to heme-dioxy reduction and arginine hydroxylation in inducible nitric oxide synthase. J Biol Chem **276:**315–319, 2001.
21. List BM, Klosch B, Volker C, Gorren AC, Sessa WC, Werner ER, Kukovetz WR, Schmidt K, Mayer B. Characterization of bovine endothelial nitric oxide synthase as a homodimer with down-regulated uncoupled NADPH oxidase activity:tetrahydrobiopterin binding kinetics and role of haem in dimerization. Biochem J **323:**159–165, 1997.
22. Presta A, Siddhanta U, Wu C, Sennequier N, Huang L, Abu-Soud HM, Erzurum S, Stuehr DJ. Comparative functioning of dihydro- and tetrahydropterins in supporting electron transfer, catalysis, and subunit dimerization in inducible nitric oxide synthase. Biochemistry **37:** 298–310, 1998.
23. Stroes E, Hijmering M, van Zandvoort M, Wever R, Rabelink TJ, van Faassen EE. Origin of superoxide production by endothelial nitric oxide synthase. FEBS Lett **438:**161–164, 1998.
24. Vásquez-Vivar J, Kalyanaraman B, Martásek P, Hogg N, Masters BSS, Karoui H, Tordo P, Pritchard KA Jr. Superoxide generation by endothelial nitric oxide synthase:the influence of cofactors. Proc Natl Acad Sci USA **95:**9220–9225, 1998.
25. Xia Y, Roman LJ, Masters BSS, Zweier JL. Inducible nitric-oxide synthase generates superoxide from the reductase domain. J Biol Chem **273:**22635–22639, 1998.
26. Pou S, Keaton L, Surichamorn W, Rosen GM. Mechanism of superoxide generation by neuronal nitric-oxide synthase. J Biol Chem **274:**9573–9580, 1999.
27. Stuehr D, Pou S, Rosen GM. Oxygen reduction by nitric-oxide synthases. J Biol Chem **276:**14533–14536, 2001.
28. Yoneyama H, Yamamoto A, Kosaka H. Neuronal nitric oxide synthase generates superoxide from the oxygenase domain. Biochem J **360:**247–253, 2001.
29. Xia Y, Tsai A-L, Berka V, Zweier JL. Superoxide generation from endothelial nitric-oxide synthase. J Biol Chem **273:**25804–25808, 1998.
30. Adak S, Wang Q, Stuehr DJ. Arginine conversion to nitroxide by tetrahydrobiopterin-free neuronal nitric-oxide synthase. J Biol Chem **275:**33554–33561, 2000.
31. Adhikari S, Ray S, Gachhui R. Catalase activity of oxygenase domain of rat neuronal nitric oxide synthase. Evidence for product formation from L-arginine. FEBS Lett **475:**35–38, 2000.
32. Rusche KM, Spiering MM, Marletta MA. Reactions catalyzed by tetrahydrobiopterin-free nitric oxide synthase. Biochemistry **37:** 15503–15512, 1998.
33. Moali C, Boucher J-L, Renodon-Corniere A, Stuehr DJ, Mansuy D. Oxidations of N^{ω}-hydroxyarginine analogues and various N-hydroxyguanidines by NO synthase II:key role of tetrahydrobiopterin in the reaction mechanism and substrate selectivity. Chem Res Toxicol **14:**202–210, 2001.
34. Fitzpatrick PF. Tetrahydropterin-dependent amino acid hydroxylases. Annu Rev Biochem **68:**355–381, 1999.
35. Bassan A, Blomberg MR, Siegbahn PE. Mechanism of dioxygen cleavage in tetrahydrobiopterin-dependent amino acid hydroxylases. Chemistry **9:**106–115, 2003.
36. Gorren ACF, List BM, Schrammel A, Pitters E, Hemmens B, Werner ER, Schmidt K, Mayer B. Tetrahydrobiopterin-free neuronal nitric oxide synthase: evidence for two identical highly anticooperative pteridine binding sites. Biochemistry **35:**16735–16745, 1996.
37. Rusche KM, Marletta MA. Reconstitution of pterin-free induciible nitric-oxide synthase. J Biol Chem **276:**421–427, 2001.
38. Klatt P, Schmid M, Leopold E, Schmidt K, Werner ER, Mayer B. The pteridine binding site of brain nitric oxide synthase. Tetrahydrobiopterin binding kinetics, specificity, and allosteric interaction with the substrate domain. J Biol Chem **13:**13861–13866, 1994.
39. Gorren ACF, Schrammel A, Schmidt K, Mayer B. Effects of pH on the structure and function of neuronal nitric oxide synthase. Biochem J **331:**801–807, 1998.
40. Kotsonis P, Fröhlich LG, Shutenko ZV, Horejsi R, Pfleiderer W, Schmidt HHHW. Allosteric regulation of neuronal nitric oxide synthase by tetrahydrobiopterin and suppression of auto-damaging superoxide. Biochem J **346:**767–776, 2000.
41. Panda K, Rosenfeld RJ, Ghosh S, Meade AL, Getzoff ED, Stuehr DJ. Distinct dimer interaction and regulation in nitric-oxide synthase types I, II, and III. J Biol Chem **277:**31020–31030, 2002.
42. Ghosh DK, Wu C, Pitters E, Moloney M, Werner ER, Mayer B, Stuehr DJ. Characterization of the inducible nitric oxide synthase oxygenase domain identifies a 49 amino acid segment required for subunit dimerization and tetrahydrobiopterin interaction. Biochemistry **36:**10609–10619, 1997.
43. Abu-Soud HM, Wu C, Ghosh DK, Stuehr DJ. Stopped-flow analysis of CO and NO binding to inducible nitric oxide synthase. Biochemistry **37:**3777–3786, 1998.
44. Huang L, Abu-Soud HM, Hille R, Stuehr DJ. Nitric oxide-generated P420 nitric oxide synthase: Characterization and roles for tetrahydrobiopterin and substrate in protecting against or reversing the P420 conversion. Biochemistry **38:**1912–1920, 1999.
45. Reif A, Fröhlich LG, Kotsonis P, Frey A, Bömmel HM, Wink DA, Pfleiderer W, Schmidt HHHW. Tetrahydrobiopterin inhibits monomerization and is consumed during catalysis in neuronal NO synthase. J Biol Chem **274:**24921–24929, 1999.
46. Presta A, Weber-Main AM, Stankovich MT, Stuehr DJ. Comparative effects of substrates and pterin cofactor on the heme midpoint potential in inducible and neuronal nitric oxide synthases. J Am Chem Soc **120:**9460–9465, 1998.
47. Slama-Schwok A, Negrerie M, Berka V, Lambry JC, Tsai AL, Vos MH, Martin JL. Nitric oxide (NO) traffic in endothelial NO synthase. Evidence for a new NO binding site dependent on tetrahydrobiopterin? J Biol Chem **277:**7581–7586, 2002.
48. Werner ER, Pitters E, Schmidt K, Wachter H, Werner-Felmayer G, Mayer B. Identification of the 4-amino analogue of tetrahydrobiopterin as a dihydropteridine reductase inhibitor and a potent pteridine antagonist of rat neuronal nitric oxide synthase. Biochem J **320:**193–196, 1996.
49. Bömmel HM, Reif A, Fröhlich LG, Frey A, Hofmann H, Marecak DM, Groehn V, Kotsonis P, La M, Köster S, Meinecke M, Bernhardt M, Weeger M, Ghisla S, Prestwich GD, Pfleiderer W, Schmidt HHHW. Anti-pterins as tools to characterize the function of tetrahydrobiopterin in NO synthase. J Biol Chem **273:**33142–33149, 1998.
50. Werner ER, Habisch H-J, Gorren ACF, Schmidt K, Canevari L, Werner-Felmayer G, Mayer B. Contrasting effects of N5-substituted tetrahydrobiopterin derivatives on phenylalanine hydroxylase, dihydropteridine reductase and nitric oxide synthase. Biochem J **348:**579–583, 2000.
51. Kotsonis P, Fröhlich LG, Raman CS, Li H, Berg M, Gerwig R, Groehn V, Kang Y, Al-Masoudi N, Taghavi-Moghadam S, Mohr D, Münch U, Schnabel J, Martásek P, Masters BSS, Strobel H, Poulos T, Matter H, Pfleiderer W, Schmidt HHHW. Structural basis for pterin antagonism in nitric-oxide synthase. J Biol Chem **276:**49133–49141, 2001.

52. Hurshman AR, Marletta MA. Reactions catalyzed by the heme domain of inducible nitric oxide synthase: evidence for the involvement of tetrahydrobiopterin in electron transfer. Biochemistry **41:**.3439–3456, 2002.
53. Vásquez-Vivar J, Martásek P, Whitsett J, Joseph J, Kalyanaraman B. The ratio between tetrahydrobiopterin and oxidized tetrahydrobiopterin analogues controls superoxide release from endothelial nitric oxide synthase:an EPR spin trapping study. Biochem J **362:**733–739, 2002.
54. Gorren ACF, Kungl AJ, Schmidt K, Werner ER, Mayer B. Electrochemistry of pterin cofactors and inhibitors of nitric oxide synthase. Nitric Oxide **5:**176–186, 2001.
55. Riethmüller C, Gorren ACF, Pitters E, Hemmens B, Habisch H-J, Heales SJR, Schmidt K, Werner ER, Mayer B. Activation of neuronal nitric-oxide synthase by the 5-methyl analog of tetrahydrobiopterin. J Biol Chem **274:**16047–16051, 1999.
56. Bec N, Gorren ACF, Voelker C, Mayer B, Lange R. Reaction of neuronal nitric-oxide synthase with oxygen at low temperature. J Biol Chem **273:**13502–13508, 1998.
57. Abu-Soud HM, Gachhui R, Raushel FM, Stuehr DJ. The ferrous-dioxy complex of neuronal nitric oxide synthase. J Biol Chem **272:** 17349–17353, 1997.
58. Sato H, Sagami I, Daff S, Shimizu T. Autooxidation rates of neuronal nitric oxide synthase: Effects of the substrates, inhibitors, and modulators. Biochem Biophys Res Commun **253:**845–849, 1998.
59. Ledbetter AP, McMillan K, Roman LJ, Masters BSS, Dawson JH, Sono M. Low-temperature stabilization and spectroscopic characterization of the dioxygen complex of the ferrous neuronal nitric oxide synthase oxygenase domain. Biochemistry **38:**8014–8021, 1999.
60. Boggs S, Huang L, Stuehr DJ. Formation and reactions of the heme-dioxygen intermediate in the first and second steps of nitric oxide synthesis as studied by stopped-flow spectroscopy under single-turnover conditions. Biochemistry **39:**2332–2339, 2000.
61. Abu-Soud HM, Presta A, Mayer B, Stuehr DJ. Analysis of neuronal NO synthase under single-turnover conditions:conversion of Nw-hydroxyarginine to nitric oxide and citrulline. Biochemistry **36:** 10811–10816, 1997.
62. Hurshman AR, Krebs C, Edmondson DE, Huynh BH, Marletta MA. Formation of a pterin radical in the reaction of the heme domain of inducible nitric oxide synthase with oxygen. Biochemistry **38:**15689–15696, 1999.
63. Witteveen CFB, Giovanelli J, Kaufman S. Reactivity of tetrahydrobiopterin bound to nitric-oxide synthase. J Biol Chem **274:**29755–29762, 1999.
64. Bec N, Gorren ACF, Mayer B, Schmidt PP, Andersson KK, Lange R. The role of tetrahydrobiopterin in the activation of oxygen by nitric-oxide synthase. J Inorg Biochem **81:**207–211, 2000.
65. Schmidt PP, Lange R, Gorren ACF, Werner ER, Mayer B, Andersson KK. Formation of a protonated trihydrobiopterin radical cation in the first reaction cycle of neuronal and endothelial nitric oxide synthase detected by electron paramagnetic resonance spectroscopy. J Biol Inorg Chem **6:**151–158, 2001.
66. Wei CC, Wang ZQ, Arvai AS, Hemann C, Hille R, Getzoff ED, Stuehr DJ. Structure of tetrahydrobiopterin tunes its electron transfer to the heme-dioxy intermediate in nitric oxide synthase. Biochemistry **42:**1969–1977, 2003.
67. Stuehr DJ, Kwon NS, Nathan CF, Griffith OW, Feldman PL, Wiseman J. N omega-hydroxy-L-arginine is an intermediate in the biosynthesis of nitric oxide from L-arginine. J Biol Chem **266:**6259–6263, 1991.
68. Gorren ACF, Bec N, Schrammel A, Werner ER, Lange R, Mayer B. Low-temperature optical absorption spectra suggest a redox role for tetrahydrobiopterin in both steps of nitric oxide synthase catalysis. Biochemistry **39:**11763–11770, 2000.
69. Huang H, Hah JM, Silverman RB. Mechanism of nitric oxide synthase. Evidence that direct hydrogen atom abstraction from the O-H bond of NG-hydroxyarginine is not relevant to the mechanism. J Am Chem Soc **123:**2674–2676, 2001.
70. Heinzel B, John M, Klatt P, Böhme E, Mayer B. Ca2+/calmodulin dependent formation of hydrogen peroxide by brain nitric oxide synthase. Biochem J **281:**627–630, 1992.
71. Gorren ACF, Schrammel A, Riethmüller C, Schmidt K, Koesling D, Werner ER, Mayer B. Nitric oxide-induced autoinhibition of neuronal nitric oxide synthase in the presence of the autoxidation-resistant pteridine 5-methyl-tetrahydrobiopterin. Biochem J **347:**475–484, 2000.
72. Mayer B, Werner ER. In search of a function for tetrahydrobiopterin in the biosynthesis of nitric oxide. Naunyn-Schmiedebergs Arch Pharmacol **351:**453–463, 1995.
73. Mayer B, Pfeiffer S, Schrammel A, Koesling D, Schmidt K, Brunner F. A new pathway of nitric oxide/cyclic GMP signaling involving S-nitrosoglutathione. J Biol Chem **273:**3264–3270, 1998.
74. Cosentino F, Katusic ZS. Tetrahydrobiopterin and dysfunction of endothelial nitric oxide synthase in coronary arteries. Circulation **91:** 139–144, 1995.
75. Bagi Z, Koller A. Lack of nitric oxide mediation of flow-dependent arteriolar dilation in type I diabetes is restored by sepiapterin. J Vasc Res **40:**47–57, 2003.
76. Yamashiro S, Kuniyoshi Y, Arakaki K, Miyagi K, Koja K. The effect of insufficiency of tetrahydrobiopterin on endothelial function and vasoactivity. Jpn J Thorac Cardiovasc Surg **50:**472–477, 2002.
77. Higashi Y, Sasaki S, Nakagawa K, Fukuda Y, Matsuura H, Oshima T, Chayama K. Tetrahydrobiopterin enhances forearm vascular response to acetylcholine in both normotensive and hypertensive individuals. Am J Hypertens **15:**326–332, 2002.
78. Katusic ZS. Vascular endothelial dysfunction:does tetrahydrobiopterin play a role? Am J Physiol Heart Circ Physiol **281:**H981–H986, 2001.
79. Tiefenbacher CP. Tetrahydrobiopterin:a critical cofactor for eNOS and a strategy in the treatment of endothelial dysfunction? Am J Physiol Heart Circ Physiol **280:**H2484–H2488, 2001.
80. Heitzer T, Brockhoff C, Mayer B, Warnholtz A, Mollnau H, Henne S, Meinertz T, Munzel T. Tetrahydrobiopterin improves endothelium-dependent vasodilation in chronic smokers: evidence for a dysfunctional nitric oxide synthase. Circ Res **86:**E36–E41, 2000.
81. Ozaki M, Kawashima S, Yamashita T, Hirase T, Namiki M, Inoue N, Hirata K, Yasui H, Sakurai H, Yoshida Y, Masada M, Yokoyama M. Overexpression of endothelial nitric oxide synthase accelerates atherosclerotic lesion formation in apoE-deficient mice. J Clin Invest **110:**331–340, 2002.
82. Cai S, Alp NJ, McDonald D, Smith I, Kay J, Canevari L, Heales S, Channon KM. GTP cyclohydrolase I gene transfer augments intracellular tetrahydrobiopterin in human endothelial cells:effects on nitric oxide synthase activity, protein levels and dimerisation. Cardiovasc Res **55:**838–849, 2002.
83. Carr AC, Zhu BZ, Frei B. Potential antiatherogenic mechanisms of ascorbate (vitamin C) and alpha- tocopherol (vitamin E). Circ Res **87:** 349–354, 2000.
84. May JM. How does ascorbic acid prevent endothelial dysfunction? Free Radic Biol Med **28:**1421–1429, 2000.
85. Heller R, Munscher-Paulig F, Grabner R, Till U. L-Ascorbic acid potentiates nitric oxide synthesis in endothelial cells. J Biol Chem **274:**8254–8260, 1999.
86. Huang A, Vita JA, Venema RC, Keaney JF Jr. Ascorbic acid enhances endothelial nitric-oxide synthase activity by increasing intracellular tetrahydrobiopterin. J Biol Chem **275:**17399–17406, 2000.
87. Heller R, Unbehaun A, Schellenberg B, Mayer B, Werner-Felmayer G, Werner ER. L-ascorbic acid potentiates endothelial nitric oxide synthesis via a chemical stabilization of tetrahydrobiopterin. J Biol Chem **276:**40–47, 2001.
88. Baker TA, Milstien S, Katusic ZS. Effect of vitamin C on the availability of tetrahydrobiopterin in human endothelial cells. J Cardiovasc Pharmacol **37:**333–338, 2001.

89. Beckman JA, Goldfine AB, Gordon MB, Creager MA. Ascorbate restores endothelium-dependent vasodilation impaired by acute hyperglycemia in humans. Circulation **103**:1618–1623, 2001.
90. d'Uscio LV, Milstien S, Richardson D, Smith L, Katusic ZS. Long-term vitamin C treatment increases vascular tetrahydrobiopterin levels and nitric oxide synthase activity. Circ Res **92**:88–95, 2003.
91. Yamashiro S, Noguchi K, Matsuzaki T, Miyagi K, Nakasone J, Sakanashi M, Koja K, Sakanashi M. Beneficial effect of tetrahydrobiopterin on ischemia-reperfusion injury in isolated perfused rat hearts. J Thorac Cardiovasc Surg **124**:775–784, 2002.
92. Patel KB, Stratford MR, Wardman P, Everett SA. Oxidation of tetrahydrobiopterin by biological radicals and scavenging of the trihydrobiopterin radical by ascorbate. Free Radic Biol Med **32**:203–211, 2002.
93. Toth M, Kukor Z, Valent S. Chemical stabilization of tetrahydrobiopterin by L-ascorbic acid:contribution to placental endothelial nitric oxide synthase activity. Mol Hum Reprod. **8**:271–280, 2002.
94. Thony B, Auerbach G, Blau N. Tetrahydrobiopterin biosynthesis, regeneration and functions. Biochem J **347**:1–16, 2000.
95. Stone KJ. The role of tetrahydrofolate dehydrogenase in the hepatic supply of tetrahydrobiopterin in rats. Biochem J **157**:105–109, 1976.
96. Reinhard JF Jr, Chao JY, Smith GK, Duch DS, Nichol CA. A sensitive high-performance liquid chromatographic-fluorometric assay for dihydrofolate reductase in adult rat brain, using 7,8- dihydrobiopterin as substrate. Anal Biochem **140**:548–552, 1984.
97. Whiteley JM, Varughese KI, Nguyen NH, Matthews DA, Grimshaw CE. Dihydropteridine reductase. Pteridines **4**:159–173, 1993.
98. Shinozaki K, Hirayama A, Nishio Y, Yoshida Y, Ohtani T, Okamura T, Masada M, Kikkawa R, Kodama K, Kashiwagi A. Coronary endothelial dysfunction in the insulin-resistant state is linked to abnormal pteridine metabolism and vascular oxidative stress. J Am Coll Cardiol **38**:1821–1828, 2001.
99. Shinozaki K, Kashiwagi A, Nishio Y, Okamura T, Yoshida Y, Masada M, Toda N, Kikkawa R. Abnormal biopterin metabolism is a major cause of impaired endothelium- dependent relaxation through nitric oxide/O2- imbalance in insulin- resistant rat aorta. Diabetes **48**:2437–2445, 1999.
100. Shinozaki K, Nishio Y, Okamura T, Yoshida Y, Maegawa H, Kojima H, Masada M, Toda N, Kikkawa R, Kashiwagi A. Oral administration of tetrahydrobiopterin prevents endothelial dysfunction and vascular oxidative stress in the aortas of insulin- resistant rats. Circ Res **87**:566–573, 2000.
101. Viveros OH, Lee CL, Abou-Donia MM, Nixon JC, Nichol CA. Biopterin cofactor biosynthesis: independent regulation of GTP cyclohydrolase in adrenal medulla and cortex. Science **213**:349–350, 1981.
102. Verma S, Arikawa E, Yao L, Laher I, McNeill JH. Insulin-induced vasodilation is dependent on tetrahydrobiopterin synthesis. Metabolism **47**:1037–1039, 1998.
103. Beckman JA, Goldfine AB, Gordon MB, Garrett LA, Creager MA. Inhibition of protein kinase Cbeta prevents impaired endothelium-dependent vasodilation caused by hyperglycemia in humans. Circ Res **90**:107–111, 2002.
104. Guzik TJ, Mussa S, Gastaldi D, Sadowski J, Ratnatunga C, Pillai R, Channon KM. Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxide synthase. Circulation **105**:1656–1662, 2002.
105. Lam KK, Ho ST, Yen MH. Tetrahydrobiopterin improves vascular endothelial function in ovariectomized rats. J Biomed Sci **9**:119–125, 2002.
106. Johns DG, Dorrance AM, Tramontini NL, Webb RC. Glucocorticoids inhibit tetrahydrobiopterin-dependent endothelial function. Exp Biol Med **226**:27–31, 2001.
107. Landmesser U, Dikalov S, Price SR, McCann L, Fukai T, Holland SM, Mitch WE, Harrison DG. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. J Clin Invest **111**:1201–1209, 2003.
108. Vasquez-Vivar J, Duquaine D, Whitsett J, Kalyanaraman B, Rajagopalan S. Altered tetrahydrobiopterin metabolism in atherosclerosis: implications for use of oxidized tetrahydrobiopterin analogues and thiol antioxidants. Arterioscler Thromb Vasc Biol **22**:1655–1661, 2002.
109. Cosentino F, Patton S, d'Uscio LV, Werner ER, Werner-Felmayer G, Moreau P, Malinski T, Luscher TF. Tetrahydrobiopterin alters superoxide and nitric oxide release in prehypertensive rats. J Clin Invest **101**:1530–1537, 1998.
110. Tsutsui M, Milstien S, Katusic ZS. Effect of tetrahydrobiopterin on endothelial function in canine middle cerebral arteries. Circ Res **79**: 336–342, 1996.
111. Tarpey MM. Sepiapterin treatment in atherosclerosis. Arterioscler Thromb Vasc Biol **22**:1519–1521, 2002.
112. Canevari L, Land JM, Clark JB, Heales SJ. Stimulation of the brain NO/cyclic GMP pathway by peripheral administration of tetrahydrobiopterin in the hph-1 mouse. J Neurochem **73**:2563–2568, 1999.
113. Cosentino F, Barker JE, Brand, Heales SJ, Werner ER, Tippins JR, West N, Channon KM, Volpe M, Luscher TF. Reactive oxygen species mediate endothelium-dependent relaxations in tetrahydrobiopterin-deficient mice. Arterioscler Thromb Vasc Biol **21**:496–502, 2001.
114. Zou MH, Shi C, Cohen RA. Oxidation of the zinc-thiolate complex and uncoupling of endothelial nitric oxide synthase by peroxynitrite. J Clin Invest **109**:817–826, 2002.
115. MacCarthy PA, Grieve DJ, Li JM, Dunster C, Kelly FJ, Shah AM. Impaired endothelial regulation of ventricular relaxation in cardiac hypertrophy:role of reactive oxygen species and NADPH oxidase. Circulation **104**:2967–2974, 2001.
116. Spiekermann S, Landmesser U, Dikalov S, Bredt M, Gamez G, Tatge H, Reepschlager N, Hornig B, Drexler H, Harrison DG. Electron spin resonance characterization of vascular xanthine and NAD(P)H oxidase activity in patients with coronary artery disease:relation to endothelium-dependent vasodilation. Circulation **107**:1383–1389, 2003.
117. Rembold H, Metzger H, Sudershan P, Guttensohn W. Catabolism of pteridine cofactors. I. Properties and metabolism in rat liver homogenates of tetrahydrobiopterin and tetrahydroneopterin. Biochim Biophys Acta **184**:386–396, 1969.
118. Rembold H, Simmersbach F. Catabolism of pteridine cofactors. II. A specific pterin deaminase in rat liver. Biochim Biophys Acta **184**: 589–596, 1969.
119. Rembold H, Chandrashekar V, Sudershan P. Catabolism of pteridine cofactors. IV. In vivo catabolism of reduced pterins in rats. Biochim Biophys Acta **237**:365–368, 1971.
120. Haberle DA, Schiffl H, Mayer G, Hennings G, Rembold H. Renal balance of pterin cofactors in the rat. A clearance and micropuncture study. Pflugers Arch **375**:9–16, 1978.
121. Wachter H, Hausen A, Grassmayr K. Increased urinary excretion of neopterin in patients with malignant tumors and with virus diseases. Hoppe Seylers Z Physiol Chem **360**:1957–1960, 1969.
122. Crane BR, Arvai AS, Ghosh S, Getzoff ED, Stuehr DJ, Tainer JA. Structures of the N(omega)-hydroxy-L-arginine complex of inducible nitric oxide synthase oxygenase dimer with active and inactive pterins. Biochemistry **39**:4608–4621, 2000.
123. Bolin JT, Filman DJ, Matthews DA, Hamlin RC, Kraut J. Crystal structures of Escherichia coli and Lactobacillus casei dihydrofolate reductase refined at 1.7 A resolution. I. General features and binding of methotrexate. J Biol Chem **257**:13650–13662, 1982.
124. Reibnegger G, Pauschenwein J, Werner ER. Electronic structure of tetrahydropteridine derivatives. Pteridines **10**:91–94, 1999.
125. Schmidt K, Werner-Felmayer G, Mayer B, Werner ER. Preferential inhibition of inducible nitric oxide synthase in intact cells by the 4-amino analogue of tetrahydrobiopterin. Eur J Biochem **259**:25–31, 1999.
126. Gibraeil HD, Dittrich P, Saleh S, Mayer B. Inhibition of endotoxin-induced vascular hyporeactivity by 4-amino- tetrahydrobiopterin. Br J Pharmacol **131**:1757–1765, 2000.

127. Bahrami S, Fitzal F, Peichl G, Gasser H, Fuerst W, Banerjee A, Strohmaier W, Redl H, Werner-Felmayer G, Werner ER. Protection against endotoxemia in rats by a novel tetrahydrobiopterin analogue. Shock **13:**386–391, 2000.
128. Brandacher G, Zou Y, Obrist P, Steurer W, Werner-Felmayer G, Margreiter R, Werner ER. The 4-amino analogue of tetrahydrobiopterin efficiently prolongs murine cardiac-allograft survival. J Heart Lung Transpl **20:**747–749, 2001.
129. Bommel HM, Reif A, Frohlich LG, Frey A, Hofmann H, Marecak DM, Groehn V, Kotsonis P, La M, Koster S, Meinecke M, Bernhardt M, Weeger M, Ghisla S, Prestwich GD, Pfleiderer W, Schmidt HH. Anti-pterins as tools to characterize the function of tetrahydrobiopterin in NO synthase. J Biol Chem **273:**33142–33149, 1998.
130. Stroes E, Kastelein J, Cosentino F, Erkelens W, Wever R, Koomans H, Luscher T, Rabelink T. Tetrahydrobiopterin restores endothelial function in hypercholesterolemia. J Clin Invest **99:**41–46, 1997.
131. Fukuda Y, Teragawa H, Matsuda K, Yamagata T, Matsuura H, Chayama K. Tetrahydrobiopterin restores endothelial function of coronary arteries in patients with hypercholesterolaemia. Heart **87:** 264–269, 2002.
132. Tiefenbacher CP, Bleeke T, Vahl C, Amann K, Vogt A, Kubler W. Endothelial dysfunction of coronary resistance arteries is improved by tetrahydrobiopterin in atherosclerosis. Circulation **102:**2172–2179, 2000.
133. Verma S, Lovren F, Dumont AS, Mather KJ, Maitland A, Kieser TM, Triggle CR, Anderson TJ. Tetrahydrobiopterin improves endothelial function in human saphenous veins. J Thorac Cardiovasc Surg **120:** 668–671, 2000.
134. Maier W, Cosentino F, Lutolf RB, Fleisch M, Seiler C, Hess OM, Meier B, Luscher TF. Tetrahydrobiopterin improves endothelial function in patients with coronary artery disease. J Cardiovasc Pharmacol **35:**173–178, 2000.
135. Fukuda Y, Teragawa H, Matsuda K, Yamagata T, Matsuura H, Chayama K. Tetrahydrobiopterin improves coronary endothelial function, but does not prevent coronary spasm in patients with vasospastic angina. Circ J **66:**58–62, 2002.
136. Setoguchi S, Hirooka Y, Eshima K, Shimokawa H, Takeshita A. Tetrahydrobiopterin improves impaired endothelium-dependent forearm vasodilation in patients with heart failure. J Cardiovasc Pharmacol **39:**363–368, 2002.
137. Ueda S, Matsuoka H, Miyazaki H, Usui M, Okuda S, Imaizumi T. Tetrahydrobiopterin restores endothelial function in long-term smokers. J Am Coll Cardiol **35:**71–75, 2000.
138. Laursen JB, Somers M, Kurz S, McCann L, Warnholtz A, Freeman BA, Tarpey M, Fukai T, Harrison DG. Endothelial regulation of vasomotion in apoE-deficient mice: implications for interactions between peroxynitrite and tetrahydrobiopterin. Circulation **103:**1282–1288, 2001.
139. Fang Q, Sun H, Mayhan WG. Impairment of nitric oxide synthase-dependent dilatation of cerebral arterioles during infusion of nicotine. Am J Physiol Heart Circ Physiol **284:**H528–H534, 2003.
140. Heitzer T, Krohn K, Albers S, Meinertz T. Tetrahydrobiopterin improves endothelium-dependent vasodilation by increasing nitric oxide activity in patients with Type II diabetes mellitus. Diabetologia **43:**1435–1438, 2000.
141. Pannirselvam M, Verma S, Anderson TJ, Triggle CR. Cellular basis of endothelial dysfunction in small mesenteric arteries from spontaneously diabetic (db/db–/–) mice:role of decreased tetrahydrobiopterin bioavailability. Br J Pharmacol **136:**255–263, 2002.
142. Prabhakar SS. Tetrahydrobiopterin reverses the inhibition of nitric oxide by high glucose in cultured murine mesangial cells. Am J Physiol Renal Physiol **281:**F179–F188, 2001.
143. Hyndman ME, Verma S, Rosenfeld RJ, Anderson TJ, Parsons HG. Interaction of 5-methyltetrahydrofolate and tetrahydrobiopterin on endothelial function. Am J Physiol Heart Circ Physiol **282:**H2167–H2172, 2002.
144. Pieper GM. Acute amelioration of diabetic endothelial dysfunction with a derivative of the nitric oxide synthase cofactor, tetrahydrobiopterin. J Cardiovasc Pharmacol **29:**8–15, 1997.
145. Koshimura K, Tanaka J, Murakami Y, Kato Y. Involvement of nitric oxide in glucose toxicity on differentiated PC12 cells:prevention of glucose toxicity by tetrahydrobiopterin, a cofactor for nitric oxide synthase. Neurosci Res **43:**31–88, 2002.
146. Tiefenbacher CP, Chilian WM, Mitchell M, DeFily DV. Restoration of endothelium-dependent vasodilation after reperfusion injury by tetrahydrobiopterin. Circulation **94:**1423–1429, 1996.
147. Nakamura K, Wright DA, Wiatr T, Kowlessur D, Milstien S, Lei XG, Kang UJ. Preferential resistance of dopaminergic neurons to the toxicity of glutathione depletion is independent of cellular glutathione peroxidase and is mediated by tetrahydrobiopterin. J Neurochem **74:** 2305–2314, 2000.
148. Gramsbergen JB, Sandberg M, Moller DA, Kornblit B, Zimmer J. Glutathione depletion in nigrostriatal slice cultures: GABA loss, dopamine resistance and protection by the tetrahydrobiopterin precursor sepiapterin. Brain Res **935:**47–58, 2002.
149. Delgado-Esteban M, Almeida A, Medina JM. Tetrahydrobiopterin deficiency increases neuronal vulnerability to hypoxia. J Neurochem **82:**1148–1159, 2002.
150. Verma S, Maitland A, Weisel RD, Fedak PW, Pomroy NC, Li SH, Mickle DA, Li RK, Rao V. Novel cardioprotective effects of tetrahydrobiopterin after anoxia and reoxygenation: Identifying cellular targets for pharmacologic manipulation. J Thorac Cardiovasc Surg **123:**1074–1083, 2002.
151. Huisman A, Vos I, van Faassen EE, Joles JA, Grone HJ, Martasek P, van Zonneveld AJ, Vanin AF, Rabelink TJ. Anti-inflammatory effects of tetrahydrobiopterin on early rejection in renal allografts: modulation of inducible nitric oxide synthase. FASEB J **16:**1135–1137, 2002.
152. Ferre J, Jacobson KB, Pfleiderer W. Proposal towards a normalization of pteridine nomenclature. Pteridines **2:**129–132, 1990.