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Regulation of Pteridine-Requiring Enzymes by the Cofactor Tetrahydrobiopterin

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Abstract

Tetrahydrobiopterin (BH4) is synthesized from guanosine triphosphate (GTP) by GTP cyclohydrolase I (GCH), 6-pyruvoyltetrahydropterin synthase (PTS), and sepiapterin reductase (SPD). GCH is the rate-limiting enzyme. BH4 is a cofactor for three pteridine-requiring monooxygenases that hydroxylate aromatic L-amino acids, i.e., tyrosine hydroxylase (TH), tryptophan hydroxylase (TPH), and phenylalanine hydroxylase (PAH), as well as for nitric oxide synthase (NOS).

The intracellular concentrations of BH4, which are mainly determined by GCH activity, may regulate the activity of TH (an enzyme-synthesizing catecholamines from tyrosine), TPH (an enzyme-synthesizing serotonin and melatonin from tryptophan), PAH (an enzyme required for complete degradation of phenylalanine to tyrosine, finally to $CO_2 + H_2O$), and also the activity of NOS (an enzyme forming NO from arginine),

Dominantly inherited hereditary progressive dystonia (HPD), also termed DOPA-responsive dystonia (DRD) or Segawa's disease, is a dopamine deficiency in the nigrostriatal dopamine neurons, and is caused by mutations of one allele of the GCH gene. GCH activity and BH4 concentrations in HPD/DRD are estimated to be 2–20% of the normal value. By contrast, recessively inherited GCH deficiency is caused by mutations of both alleles of the GCH gene, and the GCH activity and BH4 concentrations are undetectable. The phenotypes of recessive GCH deficiency are severe and complex, such as hyperphenylalaninemia, muscle hypotonia, epilepsy, and fever episode, and may be caused by deficiencies of various neurotransmitters, including dopamine, norepinephrine, serotonin, and NO. The biosynthesis of dopamine, norepinephrine, epinephrine, serotonin, melatonin, and probably NO by individual pteridine-requiring enzymes may be differentially regulated by the intracellular concentration of BH4, which is mainly determined by GCH activity. Dopamine biosynthesis in different groups of dopamine neurons may be differentially regulated by TH activity, depending on intracellular BH4 concentrations and GCH activity. The nigrostriatal dopamine neurons may be most susceptible to a partial decrease in BH4, causing dopamine deficiency in the striatum and the HPD/DRD phenotype.

Index Entries: Tetrahydrobiopterin; pteridine; GCH; TH.

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Introduction

Tetrahydrobiopterin [(6*R*)-L-*erythro*-5,6,7,8-tetrahydrobiopterin, BH4] is an essential cofactor of several pteridine-requiring enzymes, each of which has an important physiological role for biosynthesis of various neurotransmitters or hormones. BH4 is synthesized *de novo* from guanosine triphosphate (GTP) by three BH4-synthesizing enzymes. Therefore, a deficiency of BH4 attributable to genetic mutations of BH4-synthesizing enzymes may cause various symptoms depending on the species and degree of decrease in bioactive factors.

A partial reduction (2–20% of the normal value) in the enzyme activity of GTP cyclohydrolase I (GCH), the first and rate-limiting enzyme in BH4 biosynthesis, produces the phenotype of autosomal dominant dystonia termed hereditary progressive dystonia with marked diurnal fluctuation (HPD), also termed DOPA-responsive dystonia (DRD) or Segawa's disease, a movement disorder caused by a partial dopamine deficiency in the nigrostriatal dopamine neurons of the brain. HPD/DRD is caused by mutations of one allele of the GCH gene. By contrast, recessively inherited GCH deficiency is caused by mutations of both alleles of the GCH gene producing severe neurological phenotypes, such as hyperphenylalaninemia, muscle hypotonia, epilepsy, and fever episode. These symptoms in GCH deficiency may be caused by deficiencies of various neurotransmitters, including dopamine, norepinephrine, serotonin, and nitric oxide (NO). Various degrees of decreases in GCH activity and in intracellular BH4 concentrations in HPD/DRD and in GCH deficiency may produce different degrees of decrease in the activities of pteridine-requiring enzymes and in the levels of the bioactive substances produced and may cause different physiological or pathological effects. In HPD/DRD, only dopamine deficiency in the nigrostriatal dopamine neurons is manifested, producing dystonia. This finding suggests that the degree of decrease in GCH activity and BH4 level affects TH activity and dopamine level to different degrees in different dopamine neurons. The nigrostriatal dopamine neurons may be most susceptible to a partial decrease in BH4 and dopamine levels.

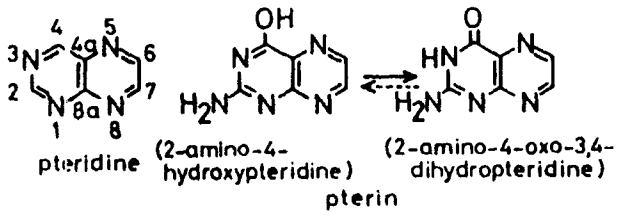

Fig. 1. Structures of pteridine and pterin.

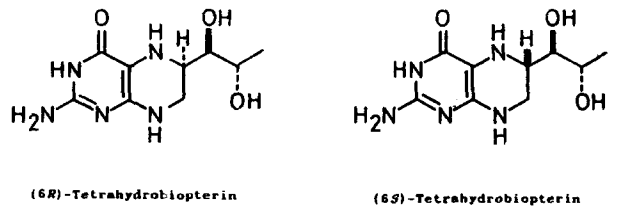

Fig. 2. Structures of natural (6*R*)-[or 6*(L)*]-L-*erythro*-tetrahydrobiopterin and unnatural (6*S*)-L-*erythro*-tetrahydrobiopterin.

Tetrahydrobiopterin (BH4) as a Cofactor of Pteridine-Requiring Enzymes

The term *pterin*, which originally meant a factor in the pigments of butterfly wings, is now used for the natural pteridine compounds that mostly have the structure of 2-amino-4-hydroxypteridine. Pterin in the body fluids has the structure of the oxoform, 2-amino-4-oxo-3, 4-dihydrobiopteridine (Fig. 1). There are two groups of natural pteridine compounds: one group consists of the conjugated pterins, i.e., folic acid derivatives, which have a methylaminobenzoyl glutamic acid side chain at the 6-position; the other group consists of the unconjugated pterins, which contain neither a *p*-aminobenzoate substituent at the 6-position of the ring nor glutamate derivatives (*1*).

An unconjugated pterin, BH4, was discovered to be the natural pteridine cofactor (*2*); its stereochemical structure was determined to be 6*R* (6*R*-BH4) (*3*) (Fig. 2). Biopterin in the tissues

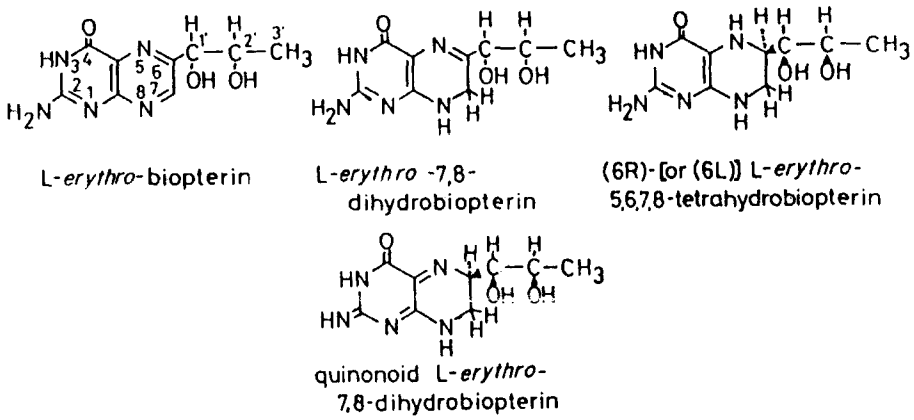

Fig. 3. Structures of four forms of (6*R*)-L-*erythro*-tetrahydrobiopterin.

exists mostly as the tetrahydro form (*R*-BH4), but a small amount of the oxidized form, i.e., quinonoid dihydrobiopterin (qBH2), 7,8-dihydrobiopterin (BH2), and biopterin (B), is also present in vivo (Fig. 3).

BH4 is an essential cofactor of pteridine-requiring enzymes (Table 1). Three of these pteridine-dependent monooxygenases, which hydroxylate aromatic L-amino acids, are phenylalanine 4-monooxygenase (EC 1.14.16.1, phenylalanine hydroxylase; PAH) (*2,4*), tyrosine 3-monooxygenase (EC 1.14.16.2, tyrosine hydroxylase, TH) (*5*), and tryptophan 5-monooxygenase (EC 1.14.16.4, tryptophan hydroxylase, TPH) (*6*). BH4 is also essential for the activity of alkylglycerol monooxygenase (EC 1.14.16.5) (*7,8*), and nitric oxide synthase (NOS) (EC 1.14. 13.39) (*9,10*) (*see* Fig. 4).

TH catalyzes the hydroxylation of L-tyrosine to L-3,4-dihydroxyphenylalanine (DOPA), the first step of catecholamine (dopamine, norepinephrine, and epinephrine) biosyhthesis. Catecholamines are synthesized in the following pathway: tyrosine—(1)→DOPA—(2)→dopamine—(3)→norepinephrine—(4)→epinephrine. In the brain, there are dopamine, norepinephrine, and epinephrine neurons. In the periphery, norepinephrine is the neurotransmitter of sympathetic neurons, whereas epinephrine and norepinephrine are the hormones in the adrenal medulla. Four enzymes are involved in the biosynthetic pathway of epinephrine: (1) TH, (2) aromatic L-amino acid decarboxylase, (3) dopamine β-monooxygenase (dopamine β-hydroxylase), and (4) phenylethanolamine *N*-methyltransferase. All genes of these four catecholamine-synthesizing enzymes have been cloned (*11*).

TPH catalyzes the hydroxylation of L-tryptophan to L-5-hydroxytryptophan, which is the first step in the biosynthesis of indoleamines (serotonin and melatonin). Serotonin is a neurotransmitter in the brain and a local hormone in mast cells, whereas melatonin is a hormone in the pineal gland. Indoleamines are synthesized in the following pathway: tryptophan — (1) → 5-hydroxytryptophan — (2) → serotonin (5-hydroxytryptamine) — (3) → *N*-acetylserotonin — (4) → melatonin (*N*-acetyl-5-methoxytryptamine). Thus, four enzymes are required in the biosynthesis of melatonin from tryptophan: (1) TPH, (2) aromatic L-amino acid decarboxylase, which is the same enzyme as that catalyzing catecholamine biosynthesis, (3) serotonin *N*-acetyltransferase, and (4) hydroxyindoleamine *O*-methyltransferase.

PAH catalyzes the obligatory step of phenylalanine degradation, i.e., the hydroxylation of phenylalanine to tyrosine, the latter of which is finally decomposed to $CO_2 + H_2O$. Thus, decreased PAH activity causes an accumulation of phenylalanine in tissues and blood (hyperphenylalaninemia); if not prevented, this accumulation causes mental retardation

Table 1
Human Pteridine-Requiring Enzymes

Enzyme	Gene		Protein	
	Chromosome	Exons	M_r (kDa × Subunit No.)	Amino acid residues
Phenylalanine 4-monooxygenase (Phenylalanine hydroxylase, PAH)	12q24.1	13	50 × 4	452
Tyrosine 3-monooxygenase Tyrosine hydroxylase (TH)	11p15.5	14		
Human TH type 1 (hTH1)			55 × 4	497
Human TH type 2 (hTH2)			56 × 4	501
Human TH type 3 (hTH3)			58 × 4	524
Human TH type 4 (hTH4)			59 × 4	528
Tryptophan 5-monooxygenase Tryptophan hydroxylase (TPH)	11p15.3–14	11	50 × 4	444
Nitric oxide synthase (NOS)				
Neuronal NOS (nNOS, NOS1)	12q24.2–24.31	28	161 × 2	1433
Inducible NOS (iNOS, NOS2)	17cen–11.2	27	131 × 2	1153
Endothelial NOS (eNOS, NOS3)	7q36	26	133 × 2	1203

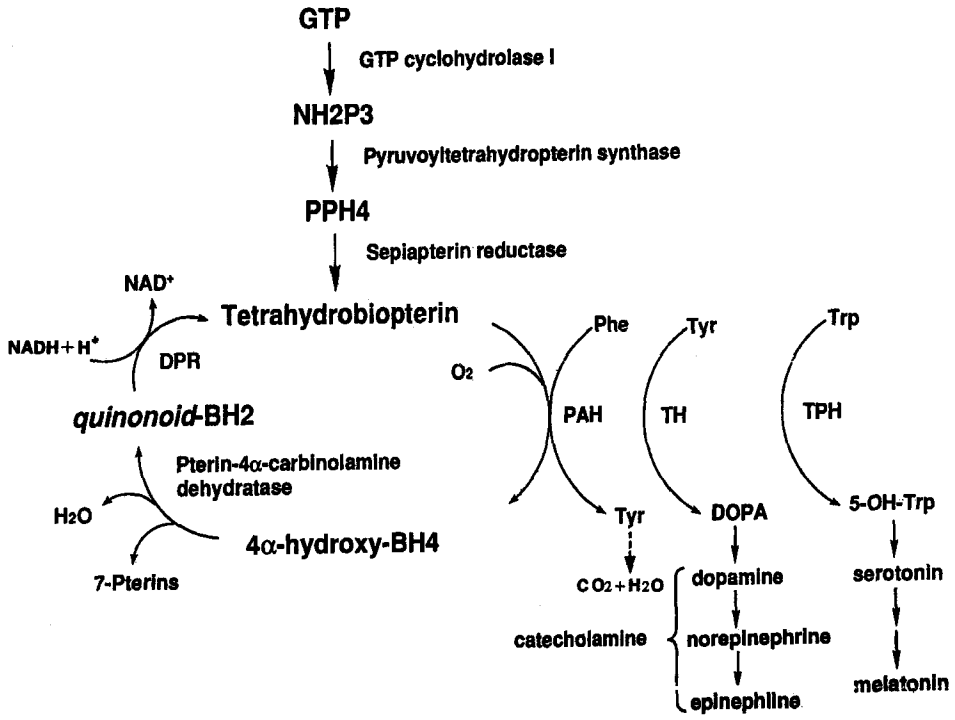

Fig. 4. Relationship between biosynthetic pathway of tetrahydrobiopterin and pteridine-requiring aromatic amino acid hydroxylases. BH4, tetrahydrobiopterin; BH2, dihydrobiopterin; DPR, dihydropteridine reductase; GTP, guanosine triphosphate; NH2P3, 7,8-dihydroneopterin triphosphate; PPH4, 6-pyruvoyltetrahydropterin; PAH, phenylalanine hydroxylase; TH, tyrosine hydroxylase; TPH, tryptophan hydroxylase.

Table 2
Human Tetrahydrobiopterin-Synthesizing Enzymes

Enzyme	Gene		Protein	
	Chromosome	Exons	M_r (kDa × Subunit No.)	Amino acid residues
Guanosine triphosphate (GTP) cyclohydrolase I (GCH)	14q22.1–22.2	6	30 × 10	250
Pyruvoyltetrahydropterin synthase (PTS)	11q22.3–23.3	6	17 × 6	145
Sepiapterin reductase (SPR)	2p14–12	3	25 × 2	261
Pterin-4α-carbinolamine dehydratase (PCD)	10q22	4	11 × 2	104
Dihydropteridine reductase (QDPR)	4q15–31	(–)	25 × 2	244

associated with the disease phenylketonuria (PKU).

Biosynthesis of BH4

BH4 is synthesized from GTP *de novo* in the following pathway (Fig. 5): GTP — (1) → D-*erythro*-6,7-dihydroneopterin triphosphate (NH2P3) — (2) → 6-pyruvoylterahydropterin (PPH4)—(3)→*R*-BH4. Three enzymes are required for the biosythesis of BH4: (1) GCH, (2) pyruvoyltetrahydropterin synthase (PTS), and (3) sepiapterin reductase (SPR) (*12*) (Table 2). GCH is the rate-limiting step.

In the hydroxylation reaction catalyzed by pteridine-requiring monooxygenases, such as hydroxylation of tyrosine to DOPA by TH, BH4 is stoichiometrically oxidized to 4α-carbinolamine tetrahydropterin, which is then converted to qBH2 by pterin-4α-carbinolamine dehydratase. qBH2 is reduced back to BH4 by dihydropteridine reductase (DPR) (Fig. 6). BH4 is also nonenzymatically oxidized to BH2, which is converted to BH4 by dihydrofolate reductase (salvage pathway). As shown for TH in Fig. 6. PAH, TH, and TPH require BH4 as cofactor for complete degradation of phenylalanine, biosynthesis of catecholamines (dopamine, norepinephrine, and epinephrine), and indoleamines (serotonin and melatonin), respectively.

Physiological Roles of BH4 as a Cofactor of Pteridine-Requiring Enzymes

BH4 is an essential cofactor for pteridine-requiring monooxygenases, such as PAH, TH, and TPH. The intracellular concentration of BH4, which is determined mainly by the *de novo* synthesis of BH4 from GTP, is thought to be an important regulatory factor for various pteridine-requiring enzymes. The intracellular concentration of BH4 is also determined by its rate of degradation (*13, 14*). However, little is known regarding the cellular metabolism of BH4. The oxidative elimination of the 6-hydroxypropyl side-chain of BH4 may be the initial step of degradation.

The concentration of BH4 is considered to be subsaturating in vivo for TH, TPH, and PAH. The mean intracellular concentration of BH4 in various tissues is estimated to be approxi 10

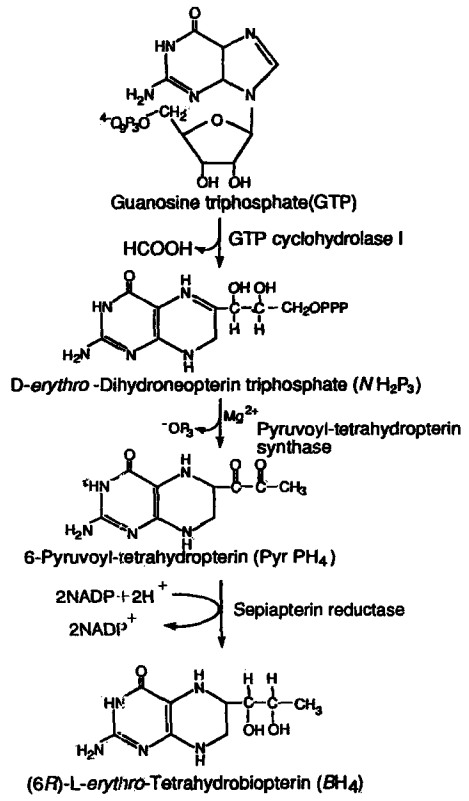

Fig. 5. Biosynthetic pathway of tetrahydrobiopterin.

μ*M*. In some cells, for example, in the nigrostriatal dopamine neurons, the intracellular BH4 concentrations are estimated to be high, approx 100 μM (*15*). The half-life of BH4 in rat dopamine neurons is estimated to be about 4.5 h, which can replenish the entire pool of BH4 every 6.5 h. Thus, the turnover rate of BH4 is sufficiently rapid, and alterations in its synthesis and degradation could acutely modify the rate of dopamine synthesis (*13*).

The approximate K_m values of pteridine-requiring enzymes [TH (*16*), TPH (*17*), PAH(18), alkyl glycerol-ether monooxygenase (*8*), and iNOS (*19*)] in vitro are shown in Table 3. The in vitro K_m values of pteridine-requiring enzymes for (6*R*)-BH4 (Table 3) may only suggest the in vivo K_m values, because phosphorylation of the BH4-requiring aromatic amino acid hydroxylases, such as TH (*20*), under various physiological (e.g., under stress) or pathological conditions (e.g., Parkinson's disease) may lower the K_m value for the predidine cofactor. Phosphorylated, activated TH under stress conditions has a smaller K_m value for BH4. Thus, the K_m values presented in Table 3 indicate rough estimations.

Biosynthesis of catecholamines (dopamine, norepinephrine, and epinephrine) is known to be regulated in vivo by the intracellular concentration of BH4, which regulates TH activity. After acute stimulation of the peripheral sympathetic nerves, the rate of catecholamine formation is rapidly increased as a result of activition of TH (*21*). Intracerebroventricular administration of BH4 increases dopamine biosynthesis through the activation of TH (*22*). BH4, but not tyrosine, stimulates TH activity in tissue slices of the striatum of the rat brain (*23*). Peripherally administered (6*R*)-BH4 increases in vivo TH activity in the striatum measured by microdialysis both in normal mice and in transgenic mice carrying human TH (*24,25*).

(6*R*)-BH4, but not (6*S*)-BH4, stimulates TPH activity in rat raphe slices (*26*). Peripherally administered (6*R*)-BH4, but not (6*S*)-BH4, stimulates TH activity in the adrenal gland in vivo (*27*). Inhibition of GCH activity in vivo in the brain decreases BH4 biosynthesis, resulting in decreases in dopamine and serotonin levels produced by decreases in TH and TPH (*28,29*). Kapatos et al. (*14*) showed that TH activity in cultured rat sympathetic norepinephrine neurons was maintained at 25% of control levels despite a 90–95% decline in BH4 content; these investigators explained this apparent discrepancy by the nonlinear relationship between BH4 concentration and TH activity; if the K_m value of TH for BH4 is 10 μ*M* a 90% decline in BH4 would decrease the TH activity by only 45% (*14*).

Since the glycol- and glycerol-ether monooxygenase is a pteridine-requiring enzyme, it can be expected that patients with decreased BH4 may show abnormally high concentrations of ether lipids (*8*). The function of these lipids is unknown. Further work will be required in order to identify the symptoms

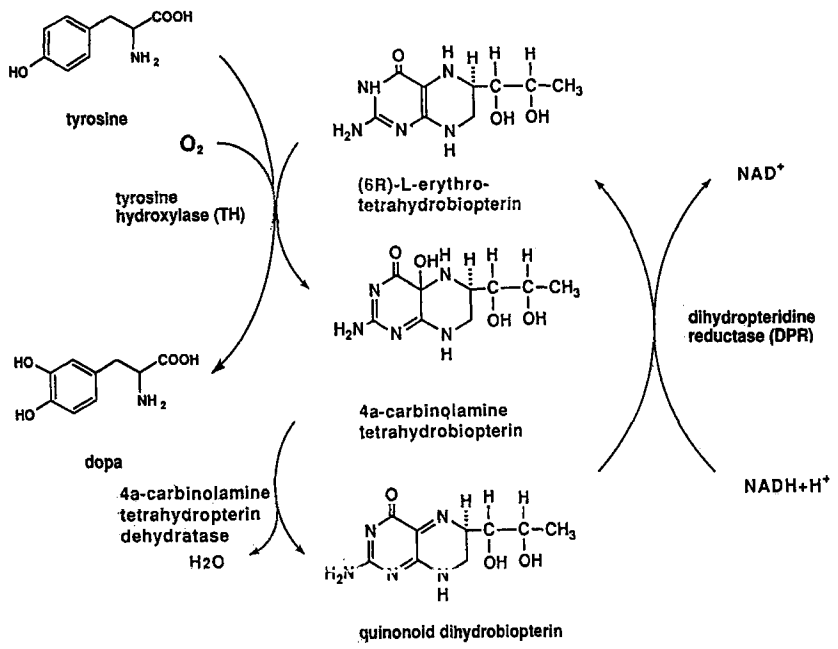

Fig. 6. Tyrosine hydroxylase reaction with tetrahydrobiopterin as cofactor.

Table 3
Estimated K_m Values of Pteridine-Requiring Enzymes for (6*R*)-Tetrahydrobiopterin

Tetrahydrobiopterin (BH4)-dependent enzymes	Species	Tissues	Km (6R-BH4) (μ*M*)	References
Tyrosine 3-monooxygenase (tyrosine hydroxylase, TH)	Bovine	Adrenal medulla	30 (50 μ*M* tyrosine, 50 μ*M* O_2)	*16*
Tryptophan 5-monooxygenase (tyrptophan hydroxylase, TPH)	Mouse	Mastocyte	30 (200 μ*M* tryptophan)	*17*
Phenylalanine 4-monooxygenase (phenylalanine hydroxylase, PAH)	Rat	Recombinant	3	*18*
Alkyl glycerol-ether monooxygenase	Rat	Liver	40	*8*
Nitric oxide synthase (inducible NOS, iNOS)	Mouse	Macrophages	0.02	*9*

that might be expected from a disturbance of ether lipid degradation (*8*).

(6*R*)-BH4 is an essential cofactor for NOS and may regulate NOS activity in the brain. NO-producing neurons constitute a subclass distinct from that of catecholaminergic neurons in the rat (*30,31*). Inducible NOS (iNOS) purified from macrophages requires BH4 for its activity (*32*). iNOS requires the addition of BH4 for activity owing to the low affinity of the enzyme for BH4, whereas endothelial constitutive NOS (eNOS) does not require BH4, because the latter is tightly bound to the enzyme (*33*). BH4, a cofactor for nNOS from

rat cerebellum, does not function as a reactant in the oxygenation of arginine. The role of BH4 is considered to be allosteric or to act in stabilizing NOS in keeping some essential groups in their reduced form (*33–35*). When BH4 synthesis in porcine arterial endothelial cells is blocked by 2,4-diamino-6-hydroxypyrimidine (DAHP), NO formation is also blocked, indicating that NOS requires BH4 for its activity (*36*). The subunit of macrophage iNOS contains FAD, FMN, and calmodulin with NADPH-dependent reductase activity, but without NOS activity. Addition of L-arginine, BH4, and heme produces the dimer with NOS activity. This result suggests that BH4 is essential for the formation of the NOS dimer with NOS activity (*37*). NOS activity in human umbilical vein endothelial cells (HUVEC) is stimulated by various cytokines such as interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), or lipopolysaccharide (LPS), with concomitant increases in GCH activity, BH4 concentration, NO formation, and cyclic guanosine monophosphate (cGMP) concentration (*38*). Activation of microglia by LPS, IFN-γ, and TNF-α induces parallel increases in NO and intracellular BH4 levels. Overproduction of NO caused by increased BH4 levels in glial cells may produce damage in the brain in acute infections (*39*). BH4 biosynthesis and GCH activity are enhanced by LPS stimulation in murine neuroblastoma cell line N1E-115 (*40*). In contrast to that in microglia (*39*), induction of BH4 and NO in mouse neuroblastoma N1E-115 cells may occur in different ways (*41*). Liver cells contain iNOS and PAH. Basal BH4 synthesis appears adequate to support iNOS activity, whereas BH4 synthesis is increased to support PAH activity (*42*). This can be expected from the higher K_m value for PAH than that for NOS.

Proinflammatory cytokines enhance mRNA expression of the rate-limiting enzyme of BH4 biosynthesis. The ability of glial cells and vascular tissues to synthesize BH4 may play an important role in the regulation of NOS under physiological conditions, as well as during inflamation and sepsis (*43*).

Long-term regulation of BH4-synthesizing enzymes may be modified under conditions that alter TH expression (*44*). Nicotinic stimulation of chromaffin cells and long-term treatment of PC12 cells with dibutyryl cyclic adenosine monophosphate (cAMP) increase BH4 concentrations in these cells (*45,46*). GCH activity is elevated in the adrenal medulla by agents that increase cAMP, and their effect is blocked by inhibiting protein synthesis (*47*). In cultured dopamine neurons of the hypothalamus or mesencephalon maintained in monolayer culture, the BH4 content is increased by long-term membrane depolarization, and cAMP and GCH mRNA levels are increased (*48*); 24-h activation of murine neuroblastoma cell line N1E-115 with LPS resulted in statistically significant increases in the amounts of the mRNAs of all three BH4-synthesizing enzymes, but most predominantly in the amount of GCH mRNA (*49*).

(6*R*)-BH4 may play other roles besides its role as a cofactor of known pteridine-requiring enzymes, although the molecular mechanisms are unclear. For example, (6*R*)-BH4 releases dopamine directly, independent of its cofactor activity for TH and NOS (*50,51*). Rabphilin-3A is a putative target protein for Rab-3A small GTP-binding protein that is implicated in neurotransmitter release. One of the rabphilin-3A-interacting proteins is GCH. The interaction of rabphilin-3A with GCH may regulate NOS activity, thus influencing the neurotransmitter release. GCH is phosphorylated in intact PC12 cells stimulated with high KCl. It is unknown whether the interaction of rabphilin-3A with GCH is of physiological significance (*52*). BH4 is synthesized by, and regulates, the proliferation of erythroid cells (*53*). BH4 synthesis precedes NO-dependent inhibition of insulin secretion in rat pancreatic β cells (*54*).

BH4 Deficiency and Pathological Phenotypes

Because BH4 has such wide functions in the neuronal, endocrine, and immune systems as a

cofactor of pteridine-requiring enzymes, as decribed above, BH4 deficiency is supposed to produce various pathological phenotypes, i.e., disease symptoms, depending on the degree of BH4 deficiency.

BH4 is a unique enzyme cofactor, in that the level determined mainly by BH4-synthesizing enzymes may regulate the activity of BH4-requiring enzymes, and as a concequence, the levels of important neurotransmitters or hormones, such as dopamine, norepinephrine, epinephrine, serotonin, melatonin, and NO. Disease phenotypes from BH4 deficiency are expected to be produced by decreased concentrations of dopamine, norepinephrine, setotonin, melatonin, and NO: movement disorder (dystonia or parkinsonism) for dopamine deficiency; muscle hypotonia and cerebellar symptoms for norepinephrine deficiency; depression, altered thermogenesis, and insomnia for serotonin deficiency; and various brain disorders such as epilepsy, peripheral nerve disorders, autoimmune diseases, and vascular disturbances for NO deficiency.

HPD/DRD is an autosomal dominant movement disorder caused by a partial dopamine deficiency in the nigrostriatal dopamine neurons. HPD/DRD is also called Segawa's disease, since Segawa et al. (*55*) first described the disease in 1972. We discovered the disruption of one autosomal allele of the GCH gene in HPD/DRD in 1994 (*56*). As a result of this mutation, GCH activity expressed in mononuclear blood cells was decreased to 2–20% of the normal value (*56*). A partial decrease in GCH activity (<20% of the normal values when the activity was measured in stimulated mononuclear blood cells) would be expected to cause partial deficiencies of BH4, TH activity, and finally dopamine in the nigrostriatal dopamine neurons. After the first discovery of GCH mutations in HPD/DRD in 1994 (*56*), 19 families/patients of HPD/DRD have been reported in Japan, England, and Spain (*56–63*) (Fig. 7, Table 4). A partial (2–20% of the normal value) decrease in GCH activity in HPD/DRD suggests that TH in the nigrostriatal dopamine neurons would be most susceptible to a decrease in BH4 levels. The level of phenylalanine in the blood controlled by PAH activity in the liver was found to be normal in HPD/DRD patients. However, a decrease in the BH4 cofactor for PAH in the liver was manifested after phenylanine loading with higher phenylalanine levels and lower tyrosine levels after load (*64*).

HPD/DRD is a rare example of how partial reduction in a metabolic enzyme caused by disruption of one autosomal allele can lead to a disease phenotype (*65*). Disruption of one autosomal allele of the GCH gene leads to a partial BH4 and probably dopamine deficiency only in the nigrostriatal dopamien neurons with a phenotype of dystonia. In contrast to autosomal dominant HPD/DRD, autosomal recessive GCH deficiency, first reported in 1984 by Niederwieser et al. (*66*), results in nearly complete loss of GCH activity and BH4. The phenotype of this recessive condition is complex: hyperphenylalaninemia caused by low PAH activity in the liver, and severe neurological symptoms probably produced by both low TH activity (resulting in catecholamine deficiency), and low TPH activity (resulting in serotonin deficiency) in the brain. Reduction in NO production as a result of decreased NOS activity may also contribute to the severe neurological phenotypes.

In 1995 we in collaboration with Blau et al. (*67,68*) found three different mutations in the GCH gene in recessive GCH deficiency (Table 5). The mutated enzyme proteins expressed in *Escherichia coli* had no enzymatic activity (*68*). GCH activity in mononuclear blood cells and in liver biopsy samples from patients with autosomal recessive GCH deficiency was extremely low (*68,69*).

The possibility that a partial decrease in TH activity is manifested only in the nigrostriatal dopamine neurons, producing a phenotype of DRD is supported by recessively inherited DRD caused by mutations in the TH gene itself (*70–74*) (Fig. 8, Table 6). Interestingly, a partial decrease in TH activity (15% of the normal activity when expressed in *E. coli*) showed a phenotype of Segawa's syndrome (*70*), but a

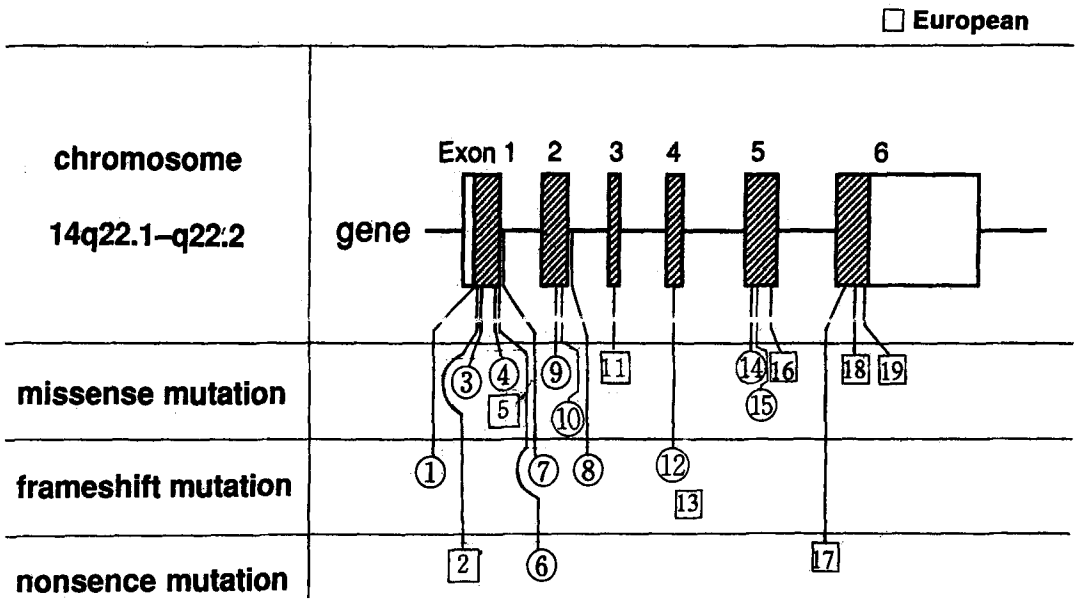

Fig. 7. Mutations of GTP cyclohydrolase I (GCH) gene in hereditary progressive dystonia with marked diurnal fluctuation (HPD)/DOPA-responsive dystonia (DRD). Numbers refer to patients listed in Table 4.

more severe decrease (1.5% of the normal value when expressed in *E. coli*) resulted in more severe, parkinsonism-like symptoms (*71,72*).

Complete loss of TH activity as a result of TH gene mutations in humans would be lethal, since complete disruption of the TH gene in mice by gene targeting was found to produce perinatal death, indicating that TH is essential for survival of the animals during the late gestational development and after birth (*75,76*).

The differences in the effect of BH4 deficiency on various BH4-requiring enzymes may be partly explained by the K_m values of the enzymes for BH4. As described earlier, the order of K_m values in vitro is estimated to be TH ≥TPH>PAH>NOS (Table 3). Thus, TH might be most sensitive to a partial decrease in BH4 concentration. However, the K_m value of TH for BH4 changes in diseases depending on the phosphorylation and dephosphorylation. As an example, in the striatum from patients with Parkinson's disease, both the TH protein and TH activity (V_{max}) are decreased in parallel, but the homomospecific activity, i.e., activity per enzyme protein, is significantly increased (*77*). The results suggest that surviving TH molecules in the striatum are activated and in a low K_m form. This may also be the case for HPD/DRD. Human TH exists in four isoforms produced by alternative mRNA splicing from a single gene (*78,79*). The mRNA levels of the four TH isoforms in the substantia nigra from patients with Parkinson's disease decrease in parallel, which may result in decreases in their proteins (*80*). The four isoform TH molecules in Parkinson's disease may be activated by increased phosphorylation, resulting in lower K_m values. If the K_m value of TH is low for BH4, the neuron could maintain synthesis of catecholamines despite a precipitous decline in BH4 concentration (*14*). Thus, not only K_m values, but also the amount of GCH, would explain the fact that the nigrostriatal dopamine neurons are the most sensitive to a BH4 decrease among catecholaminergic cells in HPD/DRD. GCH immunostaining was observed in catecholamine neurons, serotonin neurons, adrenal medulla, and liver cells and was particularly strong in serotonin neurons

Table 4
Mutations of GTP Cyclohydrolase I in Hereditary Progressive Dystonia (HPD)/ DOPA-Responsive Dystonia (DRD)

Patient/ Nationality	Exon/Intron	Base-pair change	Amino acid change	Mutation	Reference
1 Sa/Japanese	Exon 1	3insGG ATG GAG →ATG GG GAG	Glu2Ter	Frameshift	Ichinose et al. (*56*)
2 —/English	Exon 1	G193T GAG→TAG	Glu65Ter	Nonsense	Furukawa et al. (*60*)
3 Y/Japanese	Exon 1	T236C CTG→CCG	Leu79Pro	Missense	Ichinose et al. (*57*)
4 K/Japanese	Exon 1	C262T CGG→TGG	Arg88Trp	Missense	Ichinose et al. (*56*)
5 Mo/English	Exon 1	G263C CGG→CCG	Arg88Pro	Missense	Bandmann et al. (*59*)
6 —/Japanese	Exon 1	C341A TCA→TAA	Ser114Ter	Nonsense	Furukawa et al. (*60*)
7 —/Japanese	Intron 1	G(−1)A AG→AA	delExon2	Frameshift	Furukawa et al. (*60*)
8 —/Japanese	Intron 2	G(+1)C GT→CT	delExon2	Frameshift	Hirano et al. (*58*)
9 Su/Japanese	Exon 2	A401T GAC→GTC	Asp134Val	Missense	Ichinose et al. (*56*)
10 —/Japanese	Exon 2	A431C CAC→CCC	His144Pro	Missense	Hirano et al. (*61*)
11 Ro/English	Exon 3	A458C CAT→CCT	His153Pro	Missense	Bandmann et al. (*59*)
12 I/Japanese	Exon 4	511del13bp GATT GTA GAA ATC TAT →GA^{511}————T	Iso171Ter	Frameshift	Ichinose et al. (*57*)
13 —/Spanish	Exon 4	A534C AGA→AGC	Arg178Ser	Missense	Beyer et al. (*62*)
14 N/Japanese	Exon 5	G602A GGA→GAA	Gly201Glu	Missense	Ichinose et al. (*56*)
15 Be/English	Exon 5	G607A GGG→AGG	Gly203Arg	Missense	Bandmann et al. (*59*)
16 Ha/English	Exon 6	C646T CGA→TGA	Arg216Ter	Nonsense	Bandmann et al. (*59*)
17 Hu/English	Exon 6	A671G AAA→AGA	Lys224Arg	Missense	Bandmann et al. (*59*)
18 Sm/English	Exon 6	T701C TTC→TCC	Phe234Ser	Missense	Bandmann et al. (*59*)
19 —/Japanese	Exon 5	C557A ACA→AAA	Thr186Lys	Missense	Hirano et al. (*63*)

Table 5
Mutations of GTP Cyclohydrolase I (GCH) in Recessive GCH Deficiency

Exon	Base-pair change	Amino acid change	Mutation	Reference
1	C328T	Gln110Ter	Nonsense	Ichinose et al. (unpublished observations)
5	G551A	Arg184His	Missense	Ichinose et al. (*68*)
6	G633A	Met211Ile	Missense	Blau et al. (*67*), Ichinose et al. (*68*)

Table 6
Mutations of Human Tyrosine Hydroxylase (hTH1) in Recessive DOPA-Responsive Dystonia

Exon	Activity	Mutation	Symptoms	References
11	15% (*Escherichia coli*)	Q381K	Mild, dystonia	Knappskog et al. (*71*)
5	1.5% (*E. coli*)	L205P	Severe, parkinsonism	Lüdecke et al. (*72*)
5	—	R202H	Severe, parkinsonism	Flatmark et al. (*73*)

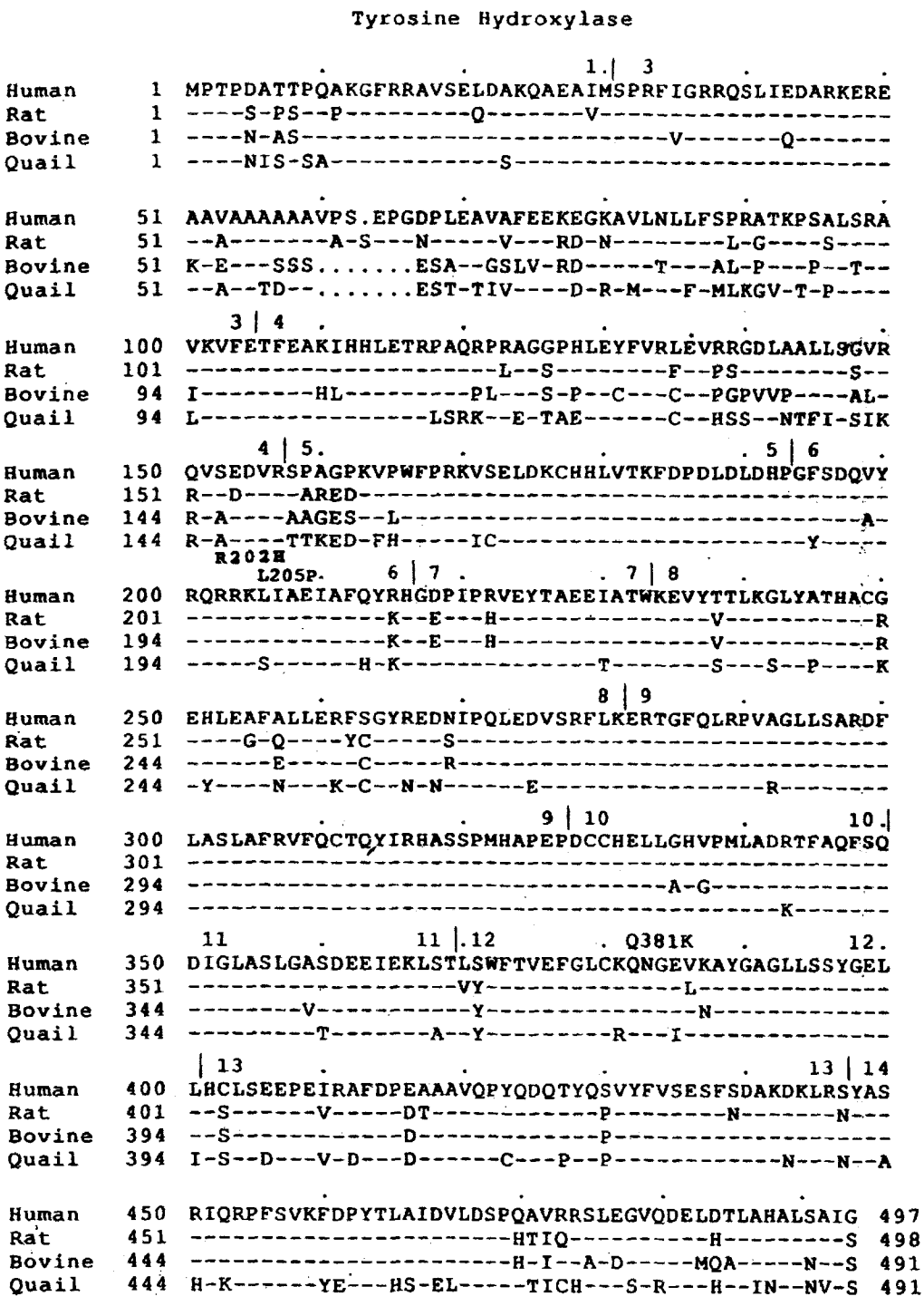

Fig. 8. Comparison of the structures of tyrosine hydroxylase (TH) from human (type 1), rat, bovine, and quail. Amino acids of rat, bovine, and quail TH identical with those of human TH are expressed by hyphens. Vertical bars and the numbers above the human amino acid sequence represent breakpoints of exons and the exon numbers in the human TH gene. From Nagatsu and Ichinose (*74*). Three mutations in recessive DOPA-responsive dystonia (DRD) are shown, Q381K, L208P, and R202H.

(*81*). Kapatos and coworkers (*82,83*) reported that mRNA and protein expressions of GCH across populations of dopamine, norepinephrine, epinephrine, and serotonin neurons in the brain are different and that nigrostriatal dopamine neurons express low levels of GCH protein. Because dopamine and epinephrine neurons express essentially equal amounts of GCH mRNA, posttranscriptional events may serve to maintain low levels of GCH protein within nigrostriatal dopamine neurons (*83*). Diurnal fluctuation is one of the main characteristics in HPD/DRD. A relatively short half-life of BH4 would explain this phenomenon. Kapatos (*13*) reported that the half-life of BH4 in neuronal cultures of embryonic rat brain was about 4.5 h and did not differ between the mesencephalon containing the nigrostriatal dopamine neurons and the hypothalamus. The average fractional rate constants of BH4 loss for the mesencephalon culture and the hypothalamus culture were equivalent. However, the calculated rate of BH4 synthesis were significantly greater for the hypothalamus than for the mesencephalon, owing to the greater steady-state concentration of BH4 in the hypothalamus than in the mesencephalon. These data indicate that BH4 metabolism may be different between populations of dopamine neurons and that the BH4 synthesis rate in the nigrostriatal dopamine neurons may be lower. Because a low level of GCH activity remains in HPD/DRD patients, they might continue to synthesize BH4 at a low rate. This rate would not be high enough to supplement the consumption of BH4 during the day but would supplement the cofactor during sleep. This may be the reason for marked diurnal fluctuation, in that they become aggravated toward the evening and are partially alleviated in the morning after sleep.

BH4 deficiency is caused by deficiency not only of GCH but of other BH4-synthesizing enzymes, as well, i.e., PTS, DPR, and pterin-4α-carbinolamine dehydratase (PCD) (*84*). Genetic mutations that block BH4 biosynthesis differentially affect the levels of dopamine, norepinephrine, and serotonin in the brain, and the BH4 supplementation therapy is effective (*85*).

Mutations in PTS cause deficiency of BH4, resulting in hyperphenylalaninemia and monoamine neurotransmitter insufficiency (*84,86*). PTS deficiency is recessive and appears in three different phenotypes, a central, a peripheral, and a transient form, which may disappear during infant development. Patients with the central type of PTS deficiency exhibit a general lack of BH4 in all organs and monoamine neurotransmitter shortage in the central nervous system (CNS), whereas patients with the peripheral form do not synthesize BH4 in peripheral organs but have normal BH4 and neurotransmitter levels in the CNS (*87*). PTS deficiency is therefore a very heterogeneous disorder. This may be attributable to phosphorylation and additional, but not yet identified, posttranslational modifications, for its *de novo* function (*87*). Dystonia symptoms such as HPD/DRD were also observed in a patient with PTS deficiency with generalized dystonia and diurnal fluctuation of symptoms (*88*).

All results from molecular changes to the phenotypes of patients with BH4 abnormalites suggest that BH4 has complex regulatory roles in the function of BH4-requiring enzymes and that the elucidation of the mechanism of regulation of BH4 levels in various cell types would be important in establishing therapy against diseases of the central and peripheral nervous systems and immune systems caused by BH4 deficiency.

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References

1. Nixon, J. C. (1985) Naturally occurring pterins, in *Folates and Pterins,* vol. 2 (Blakley, R. L. and Benkovic, S. J., eds.), John Wiley & Sons, New York, pp. 1–42.
2. Kaufman, S. (1963) The structure of the phenylalanine hydroxylase cofactor. *Proc. Natl. Acad. Sci. USA* **50,** 1085–1093.
3. Matsuura, S., Sugimoto, T., Murata, S., Sugawara, Y., and Iwasaki, H. (1985) Stereochemistry of biopterin cofactor and facile methods for the determination of the stereochemistry of a biologically active 5,6,7,8-tetrahydrobiopterin. *J. Biochem.* **98,** 1341–1348.
4. Kaufman, S. (1959) Studies on the mechanism of the enzymatic conversion of phenylalanine to tyrosine. *J. Biol. Chem.* **234,** 2677–2688.
5. Nagatsu, T., Levitt M., and Udenfriend, S. (1964) Tyrosine hydroxylase. The initial step in norepinephrine biosynthesis. *J. Biol. Chem.* **139,** 2910–2917.
6. Lovenberg, W., Jequir, E., and Sjoerdsma, A. (1967) Tryptophan hydroxylase: Measurement in pineal gland, brain stem, and carcinoid tumor. *Science* **155,** 217–219.
7. Tiets, A., Lindberg, M., and Kennedy, E. (1964) A new pteridine-requiring enzyme system for the oxidation of glyceryl ethers. *J. Biol. Chem.* **239,** 4081–4090.
8. Kaufman, S., Pollock, R. J., Summer, G. K., Das, A. K., and Hajira, A. K. (1990) Dependence of an alkyl glycol-ether monooxygenase activity upon tetrahydropterins. *Biochim. Biophys. Acta* **1040,** 19–27.
9. Tayeh, M. A., and Marletta, M. A. (1989) Macrophage oxidation of L-arginine to nitric oxide, nitrite, and nitrate. Tetrahydrobiopterin is required as a cofactor. *J. Biol. Chem.* **264,** 19654–19658.
10. Kwon, N. S., Nathan, C. F., and Stuehr, D. J. (1989) Reduced biopterin as a cofactor in the generation of nitrogen oxides by murine macrophages. *J. Biol. Chem.* **264,** 20,496–20,501.
11. Nagatsu, T. (1991) Genes for human catecholamine synthesizing enzymes. *Neurosci. Res.* **12,** 315–345.
12. Nichol, C. A., Smith, G. K., and Duch, D. S. (1985) Biosynthesis and metabolism of tetrahydrobiopterin and molybdopterin. *Annu. Rev. Biochem.* **54,** 729–762.
13. Kapatos, G. (1990) Tetrahydrobiopterin synthesis rate and turnover time in neuronal cultures from embryonic rat mesencephalon and hypothalamus. *J. Neurochem.* **55,** 129–136.
14. Kapatos, G., Hirayama, K., and Hasegawa, H. (1992) Tetrahydrobiopterin turnover in cultured rat sympathetic neurons: Developmental profile, pharmacological sensitivity, and relationship to norepinephrine synthesis. *J. Neurochem.* **59,** 2048–2055.
15. Levine, R. A., Miller, L. P., and Lovenberg, W. (1981) Tetrahydrobiopterin in the striatum: Localization in dopamine nerve terminals and role in catecholamine synthesis. *Science* **214,** 919–921.
16. Oka, K., Ashiba, G., Sugimoto, T., Matsuura, S., and Nagatsu, T. (1982) Kinetic properties of tyrosine hydroxylase purified from bovine adrenal medulla and bovine candate nucleus. *Biochim. Biophys. Acta* **706,** 188–196.
17. Hasegawa, H., and Ichiyama, A. (1987) Tryptophan 5-monooxygenase from mouse mastocytoma clone P815. *Methods Enzymol.* **142,** 88–92.
18. Citron, B. A., Davis, M. D., and Kaufman, S. (1992) Purification and biochemical characterization of recombinant rat liver phenylalanine hydroxylase produced in *Escherichia coli. Protein Expr. Purif.* **3,** 93–100.
19. Yui, Y., Hattori, R., Kosuga, K., Eizawa, H., Hiki, K., and Kawai, C. (1991) Purification of nitric oxide synthase from rat macrophages. *J. Biol. Chem.* **266,** 12,544–12,547.
20. Zigmond, R. E., Schwarzchild, M. A., and Rittenhouse, A. R. (1989) Acute regulation of tyrosine hydroxylase by nerve activity and by neurotransmitters via phosphorylation. *Annu. Rev. Neurosci.* **12,** 415–461.
21. Spector, S., Gordon, R., Sjoerdsma, A., and Udenfriend, S. (1967) End-product inhibition of tyrosine hydroxylase as a possible mechanism of a regulation of norepinephrine biosynthesis. *Mol. Pharmacol.* **3,** 549–555.
22. Kettler, R., Bartholini, G., and Pletscher, A. (1974) In vivo enhancement of tyrosine hydroxylation in rat striatum by tetrahydrobiopterin. *Nature* **249,** 476–478.
23. Hirata, Y., Togari, A., and Nagatsu, T. (1983) Studies on tyrosine hydroxylase system in rat brain slices using high-performace liquid chromatography with electrochemical detection. *J. Neurochem.* **40,** 1585–1589.
24. Kaneda, N., Sasaoka, T., Kobayashi, K., Kiuchi, K., Nagatsu, T., Kurosawa, Y., Fujita, K., Yokoyama, M., Nomura, T., Katsuki, M., and Nagatsu, T. (1991) Tissue-specific and high-

level expression of the human tyrosine hydroxylase gene in transgenic mice. *Neuron* **6,** 583–594.
25. Nagatsu, T., Nakahara, D., Kobayashi, K., Morita, S., Sawada, H., Mizuguchi, T., and Kiuchi, K. (1994) Peripherally administered (6*R*)-tetrahydrobiopterin increases in vivo tyrosine hydroxylase activity in the striatum measured by microdialysis both in normal mice and in transgenic mice carrying human tyrosin hydroxylase. *Neurosci. Lett.* **182,** 44–46.
26. Sawada, M., Sugimoto, T., Matsuura, S., and Nagatsu, T. (1986) (6*R*)-Tetrahydrobiopterin increases the activity of tryptophan hydroxylase in rat raphe slices. *J. Neurochem.* **47,** 1544–1547.
27. Matsuura, S., Murata, S., Sugimoto, T., Sawada, M., and Nagatsu, T. (1986) Preparation and cofactor activity of (6*S*)-tetrahydrobiopterin. *Chem. Expr.* **1,** 403–406.
28. Niwa, S., Watanabe, Y., and Hayaishi, O. (1985) 6*R*-L-Erythro-5,6,7,8-tetrahydrobiopterin as a regulator of dopamine and serotonin biosynthesis in the rat striatum. *Arch. Biochem. Biophys.* **239,** 234–241.
29. Suzuki, S., Watanabe, Y., Tsubokura, S., Kagamiyama, H., and Hayaishi, O. (1988) Decrease in tetrahydrobiopterin content and neurotransmitter amine biosynthesis in rat brain by an inhibitor of guanosine triphosphate cyclohydrolase. *Brain Res.* **446,** 1–10.
30. Ohta, A., Takagi, H., Matsui, T., Hamai, Y., Iida, S., and Esumi, H. (1993) Localization of nitric-oxide synthase-immunoreactive neurons in the solitary nucleus and ventrolateral medulla oblongata of the rat: Their relation to catecholaminergic neurons. *Neurosci. Lett.* **158,** 33–35.
31. Mayer, B., and Werner, E. R. (1995) in search of a function for tetrahydrobiopterin in the biosynthesis of nitric oxide. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **351,** 453–463.
32. Schoedon, G., Schneemann, M., Hofer, S., Guerrero, L., Blau, N., and Schaffner, A. (1993) Regulation of the arginine-dependent and tetrahydrobiopterin-dependent biosynthesis of nitric oxide in murine macrophages. *Eur. J. Biochem.* **213,** 833–839.
33. Nathan, C., and Xie, Q.-W. (1994) Nitric oxide syntheses: Roles, tolls, and controls. *Cell* **78,** 915–918.
34. Giovanelli, J., Campos, K. L., and Kaufman, S. (1991) Tetrahydrobiopterin, a cofactor for rat cerebellar nitric oxide synthase does not function as a reactant in the oxygenation of arginine. *Proc. Natl. Acad. Sci. USA* **88,** 7091–7095.
35. Marletta, M. A. (1993) Nitric oxide synthase: Structure and mechanism. *J. Biol. Chem.* **268,** 12,231–12,234.
36. Schmidt, K., Werner, E. R., Mayer, B., Wachter, H., and Kukovets, W. R. (1992) Tetrahydrobiopterin-dependent formation of endothelium-derived relaxing factor (nitric oxide) in aortic endothelial cells. *Biochem. J.* **281,** 297–300.
37. Baek, K. J., Thiel, B. A., Lacas, S., and Stuehr, D. J. (1993) Macrophage nitric oxide synthase subunits. Purification, characterization and role of prosthetic groups and substrate in regulating their association into a dimeric groups and substrate in regulating their association into a dimeric enzyme. *J. Biol. Chem.* **268,** 21,120–21,129.
38. Werner-Felmayar, G., Werner, E. R., Fuchs, D., Hausen, A., Reibnegger, G., Schmidt, K., Weiss, G., and Wachter, H. (1993) Pteridine biosynthesis in human endothelial cells. Impact on nitric oxide-mediated formation of cyclic GMP. *J. Biol. Chem.* **268,** 1842–1846.
39. Sakai, N., Kaufman, S., and Milstien, S. (1995) Parallel induction of nitric oxide and tetrahydrobiopterin synthesis by cytokines in rat glial cells. *J. Neurochem.* **65,** 895–902.
40. Ota, A., Yoshida, S., Nomura, T., Matsui, S., Hagino, Y., Umezawa, K., Katoh, S., and Nagatsu, T. (1996) Tetrahydrobiopterin biosynthesis enhanced by lipopolysaccharide stimulation in murine neuroblastoma cell line N1E-115. *J. Neurochem.* **67,** 2540–2548.
41. Yoshida, S., Ota, A., Umezawa, K., and Nagatsu, T. (1996) Dissociated release of lipopolysaccharide from mouse neuroblastoma cells. *Neurosci. Lett.* **212,** 135–138.
42. Pastor, C. M., Williams, D., Yoneyama, T., Hatakeyama, K., Singleton, S., Naylor, E., and Billiar, T. R. (1996) Competition for tetrahydrobiopterin between phenylalanine hydroxylase and nitric oxide synthase in rat liver. *J. Biol. Chem.* **271,** 24,534–24,538.
43. Kinoshita, H., Tsutsui, M., Milstien, S., and Katusic, Z. (1997) Tetrahydrobiopterin, nitric oxide and regulation of cerebral arterial tone. *Prog. Neurobiol.* **52,** 295–302.
44. Sabban, E. L. (1996) Synthesis of dopamine and its regulation, in *CNS Neurotransmitters and Neuromodulators Dopamine* (Stone, T. W., ed.), CRC, Boca Raton, Fl, pp. 1–20.

45. Waymire, J. C., Ayling, J. E., and Graviso, G. L. (1993) Nicotinic cholinergic regulation of tetrahydrobiopterin levels in bovine adrenal chromaffin cells. *Adv. Exp. Med. Biol.* **338,** 235–238.
46. Nakanishi, N., Onozawa, S., Isono, A., Hara, M., Hasegawa, H., and Yamada, S. (1993) Long-term treatment of PC12 pheochromocytoma with dibutyryl cyclic AMP increases biopterin content in the cells but decreases in the medium. *Adv. Exp. Biol. Med.* **338,** 231–234.
47. Abou-Doinia, M., Wilson, S. P., Zimmerman, T. P., Nichol, C. A., and Viveros, O. H. (1986) Regulation of guanosine triphosphate cyclohydrolase and tetrahydrobiopterin levels and the role of the cofactor in tyrosine hydroxylation in primary cultures of adrenomedullary chromaffin cells. *J. Neurochem.* **46,** 1190–1199.
48. Zhu, M., Hirayama, K., and Kapatos, G. (1994) Regulation of tetrahydrobiopterin biosynthesis in cultured dopamine neurons by depolarization and cAMP. *J. Biol. Chem.* **269,** 11,825–11,829.
49. Mori, A., Nakashima, A., Nagatsu, T., and Ota, A. (1997) Effect of lipopolysaccharide on the gene expression of the enzymes involved in tetrahydrobiopterin de novo synthesis in murine neuroblastoma cell line N1E-115. *Neurosci. Lett.* **237,** 1–4.
50. Koshimura, K., Miwa, S., Lee, K., Fujiwara, M., and Watanabe, Y. (1990) Enhancement of dopamine release in vivo from the rat striatum by dialytic perfusion of 6*R*-L-erythro-5,6,7,8-tetrahydrobiopterin. *J. Neurochem.* **54,** 1391–1397.
51. Koshimura, K., Takagi, Y., Miwa, S., Kido, T., Watanabe, Y., Murakami, Y., Kato, Y., and Masaki, T. (1995) Characterization of a dopamine-releasing action of 6*R*-L-*erythro*-tetrahydrobiopterin: Comparison with a 6*S*-form. *J. Neurochem.* **65,** 827–830.
52. Imaizumi, K., Sasaki, T., Takahashi, K., and Takai, Y. (1994) Identification of a rabphilin-3A-interacting protein to GTP cyclohydrolase I in PC12 cells. *Biochem. Biophys. Res. Commun.* **205,** 1409–1416.
53. Tanaka, K., Kaufman, S., and Milstein, S. (1989) Tetrahydrobiopterin, the cofactor for aromatic amino acid hydroxylases, is synthesized by and regulates proliferation of erythroid cells. *Proc. Natl. Acad. Sci. USA* **86,** 5864–5867.
54. Laffranchi, R., Schoedon, G., Blau, N., and Spinas, G. A. (1997) Tetrahydrobiopterin synthesis precedes nitric oxide-dependent inhibition of insulin secretin in ISN-1 rat pancreatic β-cells. *Biochem. Biophys. Res. Commun.* **233,** 66–70.
55. Segawa, M., Hosaka, A., Miyagawa, F., Nomura, Y., and Imai, H. (1976) Hereditary progressive dystonia with marked diurnal fluctuation. *Adv. Neurol.* **14,** 215–233.
56. Ichinose, H., Ohye, T., Takahashi, E., Seki, N., Hori, T., Segawa, M., Nomura, Y., Endo, K., Tanaka, H., Tsuji, S., Fujita, K., and Nagatsu, T. (1994) Hereditary progressive dystonia with marked diurnal fluctuation caused by mutations in the GTP cyclohydrolase I gene. *Nature Genet.* **8,** 236–242.
57. Ichinose, H., Ohye, T., Segawa, M., Nomura, Y., Endo, K., Tanaka, H., Tsuji, S., Fujita, K., and Nagatsu, T. (1995) GTP cyclohydrolase I gene in hereditary progressive dystonia with marked diurnal fluctuation. *Neurosci. Lett.* **196,** 5–8.
58. Hirano, M., Tamaru, Y., Nagai, Y., Ito, H., Imai, T., and Ueno, S. (1995) Exon skipping caused by a base substitution at a splice site in the GTP cyclohydrolase I gene in a Japanese family with hereditary progressive dystonia/dopa responsive dystonia. *Biochem. Biophys. Res. Commun.* **213,** 645–651.
59. Brandmann, O., Nygaard, T. G., Surtees, R., Marsden, C. D., Wood, N. W., and Harding, A. E. (1996) Dopa-responsive dystonia in British patients: New mutations of the GTP cyclohydrolase I gene and evidence for genetic heterogeneity. *Hum. Mol. Genet.* **5,** 403–406.
60. Furukawa, Y., Shimadzu, M., Rajput, A. H., Shimizu, Y., Tagawa, T., Mori, H., Yokochi, M., Narabayashi, H., Hornykiewicz, O., Mizuno, Y., and Kish, S. J. (1996) GTP-cyclohydrolase I gene mutations in hereditary progressive and dopa-responsive dystonia. *Ann. Neurol.* **39,** 609–617.
61. Hirano, M., Tamaru, Y., Ito, H., Matsumoto, S., Imai, T., and Ueno, S. (1996) Mutant GTP cyclohydrolase I mRNA levels contribute to dopa-responsive dystonia. *Ann. Neurol.* **40,** 796–798.
62. Beyer, K., Ladoniga J.-V., Becino-Bilbao B., Cacabelos R., and Fuente-Fernández R. de la. (1997) A novel point mutation in the GTP cyclohydrolase I gene in a Spanish family with hereditary progressive and dopa responsive dystonia. *Lancet* **349,** 420–421.
63. Hirano, M., Imaiso, Y., and Ueno, S. (1997) Differential splicing of the GTP cyclohydrolase I RNA in dopa-responsive dystonia. *Biochem. Biophys. Res. Commun.* **234,** 316–319.
64. Hyland, K., Fryburg, J. S., Wilson, W. G., Bebin, E. M., Arnold, L. A., Guanasekara, R. S., Jacob-

son, R. D., Rost-Ruffner, E., and Trugman, J. M. (1997) Oral phenylalanine loading in dopa-responsive dystonia: A possible diagnostic test. *Neurology* **48,** 1290–1296.
65. Ozelius, L. J., and Breakfield, X. O. (1994) Co-factor insufficiency in dystonia-parkinsonian syndrome. *Nature Genet.* **8,** 207–209.
66. Niederwieser, A., Blau, N., Wang, M., Joller, P., Atarés, M., and Cardia-Garcia, J. (1984) GTP cyclohydrolase I deficiency, a new enzyme defect causing hyperphenylalaninemia with neopterin, biopterin, dopamine and serotonin deficiencies and muscular hypotonia. *Eur. J. Pediatr.* **141,** 208–214.
67. Blau, N., Ichinose, H., Nagatsu, T., Heizmann, C. W., Zacchello, F., and Burlina, A. B. (1995) A missense mutation in a patient with guanosine triphosphate cyclohydrolase I deficiency missed in the newborn screening program. *J. Pediatr.* **126,** 401–405.
68. Ichinose, H., Ohye, T., Matsuda, Y., Hori, T., Balu, N., Burliner, A., Rouse, B., Matalon, R., Fujita, K., and Nagatsu, T. (1995) Characterization of mouse and human GTP cyclohydrolase I gene. Mutation in patients with GTP cyclohydrolase I deficiency. *J. Biol. Chem.* **270,** 10,062–10,071.
69. Blau, N., Joller, P., Atarés, M., Cardesa-Garcia, J., and Niederwieser, A. (1985) Increase of GTP cyclohydrolase I activity in mononuclear blood cells by stimulation: Detection of heterozygotes of GTP cyclohydrolase I deficiency. *Clin. Chim. Acta* **148,** 47–52.
70. Lüdecke, B., Dworniczak, B., and Bartholomé, K. (1995) A point mutation in the tyrosine hydroxylase gene associated with Segawa's syndrome. *Hum. Genet.* **95,** 123–125.
71. Knappskog, P. M., Flatmark, T., Mallet, J., Lüdecke, B., and Bartholomé, K. (1995) Recessively inherited L-dopa-responsive dystonia caused by a point mutation (Q381K) in the tyrosine hydroxylase gene. *Hum. Mol. Genet.* **4,** 1209–1212.
72. Lüdecke, B., Knappskog, P. M., Clayton, P. T., Surtees, S. J. R., Brand, M. P., Bartholomé, K., and Flatmark, T. (1996) Recessively inherited L-DOPA-responsive parkinsonism in infancy caused by a point mutation (L205P) in the tyrosine hydroxylase gene. *Hum. Mol. Genet.* **5,** 1023–1028.
73. Flatmark, T., Knappskog, P. M., Bjørgo, E., and Martinez, A. (1997) Molecular characterization of disease related mutant forms of human phenylalanine hydroxylase and tyrosine hydroxylase. *Pteridines* **8,** 58.
74. Nagatsu, T., and Ichinose, H. (1991) Comparative studies on the structure of human tyrosine hydroxylase with those of the enzyme of various mammals. *Comp. Biochem. Physiol.* **98C,** 203–210.
75. Zhou, Q.-Y., Quaife, C. J., and Palmiter, R. D. (1995) Targeted disruption of the tyrosine hydroxylase gene reveals that catecholamines are required for mouse fetal development. *Nature* **374,** 640–643.
76. Kobayashi, K., Morita, S., Sawada, H., Mizuguchi, T., Yamada, K., Nagatsu, I., Hata, T., Watanabe, Y., Fujita, K., and Nagatsu, T. (1995) Targeted disruption of the tyrosine hydroxylase locus results in severe catecholamine depletion and perinatal lethality in mice. *J. Biol. Chem.* **270,** 27,235–27,243.
77. Mogi, M., Harada, M., Kiuchi, K., Kondo, T., Narabayashi, H., Rausch, D., Riederer, P., Jellinger, K., and Nagatsu, T. (1988) Homospecific activity (activity per enzyme protein) of tyrosine hydroxylase increases in Parkinsonian brain. *J. Neural Transm.* **72,** 77–81.
78. Grima, B., Lamouroux, A., Boni, C., Julien, J.-F., Jovoy-Agid, F., and Mallet, J. (1997) A single human gene encoding multiple tyrosine hydroxylases with different predicted functional characteristics. *Nature* **326,** 707–711.
79. Kaneda, N., Kobayashi, K., Ichinose, H., Kishi, F., Nakazawa, A., Kurosawa, Y., Fujita, K., and Nagatsu, T. (1987) Isolation of a novel cDNA clone for human tyrosine hydroxylase: Alternative RNA splicing produces four kinds of mRNA from a single gene. *Biochem. Biophys. Res. Commun.* **146,** 971–975.
80. Ichinose, H., Ohye, T., Fujita, K., Pantucek, F., Lange, K., Riederer, P., and Nagatsu, T. (1994) Quantification of mRNA of tyrosine hydroxylase and aromatic L-amino acid decarboxylase in the substantia nigra in Parkinson's disease and schizophrenia. *J. Neural Transm.* **8,** 149–158.
81. Nagatsu, I., Ichinose, H., Sakai, M., Titani, K., Suzuki, M., and Nagatsu, T. (1995) Immunocytochemical localization of GTP cyclohydrolase I in the brain, adrenal gland, and liver of mice. *J. Neural. Transm.* **102,** 175–188.
82. Lentz, S. I., and Kapatos, G. (1996) Tetrahydrobiopterin biosynthesis in the rat brain: Heterogeneity of GTP cyclohydrolase I mRNA expression in monoamine containing neurons. *Neurochem. Int.* **28,** 569–582.

83. Hirayama, K., and Kapatos, G. (1998) Nigrostriatal dopamine neurons express low levels of GTP cyclohydrolase I protein. *J. Neurochem.* **70,** 164–170.
84. Blau, N., Barnes, I., and Dhondt, J. L. (1996) International detabase of tetrahydrobiopterin deficiencies. *J. Inher. Metab. Dis.* **19,** 8–14.
85. Kaufman, S., Kapatos, G., Rizzo, W., Schulman, J. D., Tamarkin, L., and Van Loon, G. L. (1983) Tetrahydropterin therapy for hyperphenylalaninemia caused by defective synthesis of tetrahydrobiopterin. *Ann. Neurol.* **14,** 308–315.
86. Thöny, B., and Blau, N. (1997) Mutations in the GTP cyclohydrolase I and 6-pyruvayl-tetrahydropterin synthase genes. *Hum. Mutat.* **10,** 11–20.
87. Oppliger, T., Thöny, B., Nar, H., Bürgisser, D., Huber, R., Heizmann, C. W., and Blau, N. (1995) Structural and functional consequences of mutations in 6-pyruvoyltetrahydropterin synthase causing hyperphenylalaninemia in humans. Phosphorylation is a requirement for in vivo activity. *J. Biol. Chem.* **270,** 29,498–29,506.
88. Hanihara, T., Inoue, K., Kawanishi, C., Sugiyama, N., Miyakawa, T., Onishi, H., Yamada, Y., Osaka, H., Kosaka, K., Iwabuchi, K., and Owada, M. (1997) 6-Pyruvoyl-tetrahydropterin synthase deficiency with generalized dystonia and diurnal fluctuation of symptoms: A clinical and molecular study. *Movement Disord.* **12,** 408–411.