Murine models of inherited monoaminergic and GABAergic neurotransmitter disorders

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Monoamine and amino acid neurotransmitters perform diverse biological functions in mammals, including the regulation of inhibitory/excitatory neurotransmission in the brain and spinal cord, movement and sleep, autonomic function, mood and reward, and numerous other processes. The primary transmitters involved include dopamine, serotonin, epinephrine, norepinephrine and γ-aminobutyric acid (GABA). With the exception of the amino acid transmitter GABA, the cofactor integrating these systems is tetrahydrobiopterin, an oxidizable intermediate found in high concentrations in dopaminergic neurons. With growing awareness of the clinical phenotypes, expanding numbers of patients with monoaminergic and GABAergic neurotransmitter disorders are being identified. For some people, therapeutic intervention demonstrates remarkably positive benefits; conversely, for most other disorders therapy offers limited efficacy. Decoding of the complete mouse genome, coupled with methodology capable of ablating specific genes, has revolutionized how geneticists understand and treat human genetic disease. This is well-exemplified in the disorders covered in this review, which focuses predominantly on monoaminergic (tetrahydrobiopterin-dependent) and GABAergic signaling neurotransmitter disorders.

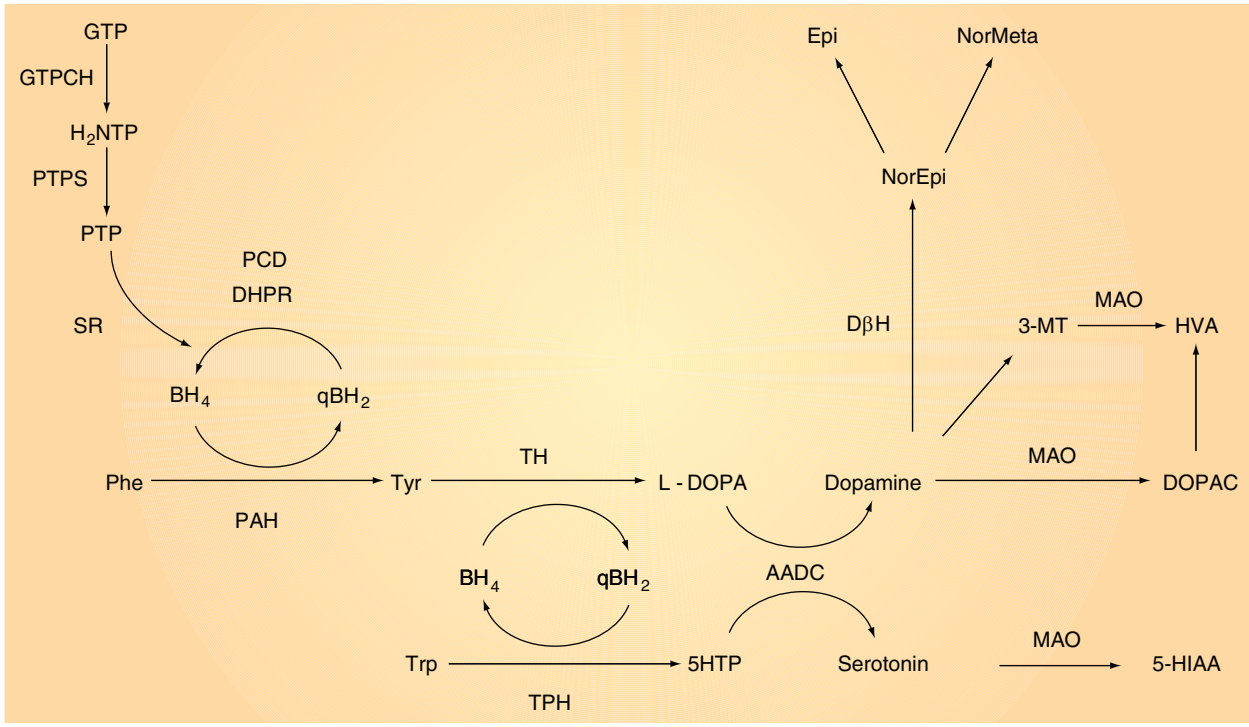
Keywords: dopamine, γ-aminobutyric acid, knockout mice, Mendelian diseases, monoamines, monogenic disorders, receptor mechanisms, serotonin, tetrahydrobiopterin

future medicine

In humans, disorders of monoaminergic and γ-aminobutyric acid (GABA)ergic neurotransmitter synthesis and degradation are rare, with the exception of phenylketonuria (PKU) and Segawa disease (autosomal dominant guanosine triphosphate [GTP] cyclohydrolase deficiency I; GTPCH) [1]. Many of these diseases can be categorized as deficiencies in those enzymes involved in the synthesis of tetrahydrobiopterin (BH_4) from GTP (**Figure 1**). These include both autosomal recessive (with hyperphenylalaninemia) and dominant GTPCH deficiency (Segawa disease; without hyperphenylalaninemia), 6-pyruvoyl tetrahydropterin synthase (PTPS) deficiency (with hyperphenylalaninemia) and sepiapterin reductase (SR) deficiency (without hyperphenylalaninemia). Two additional disorders, pterin carbinolamine dehydratase deficiency (with transient hyperphenylalaninemia) and dihydropteridine reductase (DHPR) deficiency (with hyperphenylalaninemia) are involved in the recycling reactions between BH_4 and quinoid dihydrobiopterin (qBH_2) [1]. Diagnostic approaches to the differentiation of these disorders include an oral loading test with BH_4, the measurement of BH_4 and neurotransmitter metabolites in cerebrospinal fluid and/or urine, determination of enzymatic activities in red blood cells or dermal fibroblasts and genetic analysis [2].

Additional human disorders of monoamine metabolism convert key amino acids into neurotransmitters with BH_4 or pyridoxine (B_6) as a cofactor. Tyrosine hydroxylase (TH) metabolizes tyrosine to L-dopa, while the two distinct tryptophan hydroxylase (TPH) isoforms, peripheral TPH1 and brain TPH2, convert tryptophan to 5-hydroxytryptophan (5-HTP) [3]; all three enzymes utilize BH_4. Human TH deficiency has been described [4], but a patient with TPH deficiency has not yet been reported, although mutations (or polymorphisms) in the *TPH1* or *TPH2* genes have been implicated in the pathogenesis of psychiatric diseases [5–9]. Further metabolism of L-dopa and 5-HTP is catalyzed by aromatic L-amino acid decarboxylase (AADC), which converts L-dopa and 5-HTP to the active neurotransmitter forms, dopamine and serotonin, respectively. This enzymatic step is also the site of human deficiency [10]. Whilst its substrate is not considered a neurotransmitter, phenylalanine hydroxylase (PAH) catalyzes the formation of tyrosine with BH_4 as the cofactor, and its deficiency is one of the most common single-gene Mendelian disorders in humans, PKU. Thus, when encountered with the patient manifesting hyperphenylalaninemia by newborn screening, an oral BH_4 loading test and BH_4 analysis must be carried out to differentiate if the disorder is a primary defect in PAH or one of the other disorders involved in BH_4 production, as described previously. Glycine is a ligand of the brain *N*-methyl D-amino (NMDA) receptor, and there are glycine receptors (inhibitory, strychnine-sensitive) in the

Figure 1. Integrated overview of catecholamine and monoamine synthesis and metabolic sequences (not all steps are depicted).



Cofactors: BH_4: Tetrahydrobiopterin; qBH_2: Quinoid dihydropterin.
Amino acids: Phe: Phenylalanine; Trp: Tryptophan; Tyr: Tyrosine.
Enzymes: AADC: L-aromatic amino acid decarboxylase; DβH, dopamine-β-hydroxylase; DHPR: Dihydropteridine reductase; GTPCH: Guanosine triphosphate cyclohydrolase; MAO: Monoamine oxidase; PAH: Phenylalanine hydroxylase; PCD: Pterin-4α-carbinolamine dehydratase; PTPS: 6-pyruvoyl tetrahydropterin synthase; SR: Sepiapterin reductase; TH: Tyrosine hydroxylase; TPH: Tryptophan hydroxylase.
Intermediates/metabolites: 3-MT: 3-methoxytyramine; 5-HIAA: 5-hydroxyindoleacetic acid; 5HTP: 5-hydroxytryptophan; DOPAC: 3,4-dihydroxyphenylacetate; Epi: Epinephrine; H_2NTP: Dihydroneopterin; HVA: Homovanillic acid; L-DOPA: 3,4-dihydroxyphenylalanine; NorEpi: Norepinephrine; NorMeta: Normetanephrine; PTP: 6-pyruvoyl-tetrahydropterin.
MAO is also important in the further metabolism of NorEpi, Epi and NorMeta (not shown on diagram).

spinal cord [11]. Thus, patients with nonketotic hyperglycinemia may be considered as having a disorder of neurotransmitter metabolism. Additional disorders in monoamine metabolism in humans, including dopamine β-hydroxylase (DβH) deficiency (associated with a deficiency of norepinephrine and noradrenaline) and monoamine (A and B) oxidase deficiency, are infrequent [1]. However, characterization of knockout mice in these genes has provided novel insights into the role of norepinephrine and noradrenaline in embryonic development and behavior [12,13].

This review will focus primarily on disorders of catecholamine and monoamine metabolism, in which BH_4 is directly involved. Furthermore, the GABA-metabolic knockout mouse with deficiency in the *Aldh5a1* gene (succinate semialdehyde dehydrogenase [SSADH] deficiency) will be described, as this animal reveals new insights into epileptic phenomenology. In addition, a brief discussion of the PAH-deficient mouse will be presented.

Mouse models for primary or secondary deficiencies in neurotransmitters

Knockout mouse models are described for three of the four human defects in primary biogenic amine metabolism, including TH, DβH and monoamine oxidase-A (MAO-A), whereas no such model is available for AADC (**Table 1**). Regarding secondary disorders of neurotransmitter metabolism, mouse models were generated for three defects in BH_4 metabolism (autosomal dominant GTPCH deficiency, PTPS deficiency and SR deficiency). No animal

Table 1. List of inherited disorders of neurotransmitters and available mouse models.

Abbreviation	Human deficiency or disorder	McKusick No. [88]/ Physician's guide [89]	Mouse model	Ref.
PAH	Phenylalanine hydroxylase	261600 / 1.1	Available (*Pah-enu2*)	[35]
TH	Tyrosine hydroxylase	191290 / 2.1	Available (*TH*)	[18,19]
AADC	Aromatic L-amino acid decarboxylase	107930 / 2.2	Not available	
DβH	Dopamine β-hydroxylase	223360 / 2.3	Available (*DβH*)	[85]
MAO-A	Monoamine oxidase-A	307850 / 2.4	Available (*MAO-A*)	[13]
GTPCH	GTP cyclohydrolase I, recessive	233910 / 1.2	Not available	
DRD	Dopa-responsive dystonia, GTPCH dominant	600225 / 1.6	Available (*hph-1*)	[77]
PTPS	6-pyruvoyl-tetrahydrobiopterin synthase	261640 / 1.3	Available (*Pts*)	[25,26]
SR	Sepiapterin reductase	182185 / 1.7	Available (*Spr*)	[17]
DHPR	Dihydropteridine reductase	261630 / 1.4	Not available	
SSADH	Succinate semialdehyde dehydrogenase deficiency (γ-hydroxybutyric aciduria)	271980 / 3.2 610045	Available (*Aldh5a1*$^{-/-}$)	[41]

AADC: Aromatic L-amino decarboxylase; DβH: Dopamine-β-hydroxylase; DHPR: Dihydropteridine reductase; DRD: Dopa-responsive dystonia; GTPCH: Guanosine triphosphate cyclohydrolase; MAO: Monoamine oxidase; PAH: Phenylalanine hydroxylase; PTPS: 6-pyruvoyl tetrahydropterin synthase; SR: Sepiapterin reductase; SSADH: Succinic semialdehyde dehydrogenase; TH: Tyrosine hydroxylase.

models are available for the autosomal recessive form of GTPCH deficiency and DHPR defects. TH-, DβH- and PTPS-deficient mice show embryonal or perinatal lethality due to severe monamine deficiency. MAO-A-deficient mice have elevated serotonin concentrations and a distinct behavioral syndrome, including aggression in males. The *hph-1* mouse, which was produced by ethylnitrosourea (ENU) mutagenesis, has an undefined genetic lesion that maps to a congenic interval containing the gene for GTPCH [14], exhibits a transient form of BH_4 deficiency associated with decreased GTPCH activity, and manifests some characteristics of dominant GTPCH deficiency (e.g., dopa-responsive dystonia). SR deficiency, an autosomal recessive disease of BH_4-dependent neurotransmitter deficiency with impaired bodily movements, but without hyperphenylalaninemia [15–17], presents with hyperphenylalaninemia in the mouse with a complete *Spr*-gene knockout, which contrasts distinctly with the human disease.

Tyrosine hydroxylase deficiency in transgenic mice

An interesting series of transgenic mice with primary or secondary deficiencies in dopamine production has been generated. As mentioned previously, a complete deficiency of dopamine and norepinephrine due to TH knockout, or a deficiency in only norepinephrine due to DβH knockout, causes embryonic lethality in mice [18,19,85]. In order to understand the developmental roles for dopamine and norepinephrine/epinephrine, a selective dopamine-deficient mouse was generated by restoring TH function in noradrenergic cells by expressing TH under the noradrenergic-specific DβH promoter. This was achieved by targeting the TH coding sequence to the DβH promoter through homologous recombination in embryonic stem cells [20]. These selective dopamine-deficient mice survive after birth, but dopamine deficiency results in an inability to initiate those activities required to obtain food, perhaps best described as a lack of motivation, especially for rewarding or appetitive stimuli. These mice actually do consume food when it is placed directly in their mouth. Under continuous administration of the precursor L-Dopa, or viral gene delivery to counteract dopamine deficiency, nearly normal growth and survival was achieved [21,22]. With these selective TH-deficient mice, dopamine was shown to be essential for movement and to control feeding behavior via appetite stimulation. Conversely, in the case of norepinephrine and epinephrine-deficient mice, it has been hypothesized that deficits in neuronal development occuring during embryogenesis result in lethality, but no abnormalities in neuronal development have been described, either pre- or post-natally. Most likely, these mice die as fetuses due to an inability of the cardiovascular system to respond to hypoxic stressors such as spontaneous uterine contractions [45].

Effect of BH_4 on TH & the other aromatic amino acid hydroxylases: utility of another lethal knockout model

TH activity and thus catecholamine neurotransmitter synthesis, is dependent on the availability of its cofactor, BH_4. Other BH_4-dependent enzyme systems are PAH, TPH, all types of nitric oxide synthases (NOS), and glyceryl-ether mono-oxygenase [23]. When the first mouse unable to synthesize BH_4 was generated by completely knocking out the 6-pyruvoyl-BH_4 synthase gene (*Pts*), it was somewhat surprising to find that homozygous *Pts* newborns died immediately after birth, as human patients do not [25,26]. The monoamine neurotransmitters are required during embryogenesis, but mice only die after birth as the BH_4 cofactor is supplied by the mother during pregnancy. Repeated administration of BH_4 in combination with the neurotransmitter precursors L-dopa and 5-HTP rescued the perinatal lethality with 100% survival of *Pts* knockout mice, but resulted in dwarfism [26]. A detailed analysis of these dwarf mice revealed that BH_4 levels normalized in the brain and other tissues, and that hepatic PAH and neuronal TPH and NOS displayed normal activity. However, brain TH immunoreactivity, enzyme activity, and dopamine levels remained very low despite normal TH gene expression [25]. Furthermore, the pituitary-derived growth hormone and the TSH-dependent thyroxin (T_4) were both normal, indicating that the pituitary gland has developed normally, whereas insulin-like growth factor (IGF)-1 was only 15% of wild-type control. From these results, in combination with observations above, it was hypothesized that the dwarfism was due to low appetite and thus chronic under-nutrition, with subsequently low IGF-1 owing to the selectively low TH and dopamine levels [26]. Furthermore, catecholamine and serotonin synthesis are regulated differentially by BH_4, as lack of BH_4 leads to brain TH but not TPH protein depletion [25]. In parallel, newborn patients with BH_4 deficiency due to *PTPS* gene alterations show a reduction of plasma IGF-1.

The potential direct effect of BH_4 on the aromatic amino acid hydroxylases prompted us to study the hepatic PAH in more detail in *Pts* mice with BH_4 deficiency. From various *in vitro* studies with PAH, including a coupled transcription–translation assay system, it was known that a 1:1 ratio of BH_4 and PAH stabilized the PAH–BH_4 complex and that the BH_4 cofactor had a direct stabilizing effect on PAH protein expression [27,28]. Whether BH_4 also has such a chaperon-like effect on the TH and the TPH protein in the brain remains to be shown. In the liver of newborn *Pts* knockout heterozygous and wild-type mice, a correlation was observed between the amount of BH_4, PAH protein expression and PAH-enzyme activity, whereas gene expression and PAH-mRNA stability was unchanged in all three genotypes. Moreover, under-reduced BH_4 concentrations in heterozygous mice (57% of normal), or near absence of BH_4 in knockout animals (2% of normal), degradation products of the PAH protein in liver were observed, indicating that the cofactor also stabilizes or protects PAH *in vivo* [29]. Interestingly, a similar observation had been made almost 30-years ago, when Kaufman and colleagues discovered the first patient with DHPR deficiency: they found that the level of hepatic PAH was low and speculated that the lack of BH_4 could lead to an accelerated rate of PAH degradation [29].

The PAH (enu2) (PKU) mouse: a model of human PKU

Although phenylalanine is not a neurotransmitter, the product of its immediate metabolism (tyrosine) produces dopamine and other downstream neurotransmitters (**Figure 1**). In addition, many of the disorders described in this review (e.g., Segawa disease, PTPS deficiency) manifest with frank hyperphenylalaninemia. Thus, it is worthwhile to briefly describe the murine model.

The most common cause of human PKU is absence of the liver-specific enzyme PAH due to mutations in the *PAH* gene [30]. PAH catalyzes the irreversible hydroxylation of phenylalanine to tyrosine (**Figure 1**). PAH is a homotetramer that is found exclusively in the liver of humans [31] but is also expressed in the kidneys and pancreas of the rodent [32]. BH_4 is required as a cofactor for *in vivo* PAH activity. During phenylalanine hydroxylation, BH_4 is oxidized to qBH_2. BH_4 is subsequently regenerated via a reaction catalyzed by DHPR in the presence of reduced nicotinamide adenine dinucleotide. The presence of DHPR and sufficient BH_4 are fundamental for maximal PAH activity in hepatic tissue.

The *Pah* [enu2] (hitherto referred to as PKU) mouse was generated via random chemical mutagenesis (ENU) [33]. The *Pah* [enu2] mouse harbors a T to C transition at nucleotide 835

(amino acid 263) of exon 7 of the mouse PAH cDNA. The missense allele substitutes serine (Ser [S]) for conserved phenylalanine (Phe [F]), thus resulting in complete inactivation of the PAH protein. While PAH polyA$^+$ RNA and PAH protein in the liver are detectable via northern and western blotting, respectively, PAH activity is undetectable by enzyme activity determination in organ extracts of animals harboring two copies of the F263S allele. Mutant mice manifest hyperphenylalaninemia, hypopigmentation (tyrosine is a precursor of melanin), mild growth retardation and neurological dysfunction in comparison to their wild-type littermates [34,35]. Comparable to what is observed in human maternal PKU, offspring of mutant dams often manifest structural defects, especially in the heart [36].

The utility of this mouse for long-term correction of PKU has recently been demonstrated. Elegant studies from Hamman and colleagues demonstrated that, under selective growth conditions, transplantation of 5% or less of wild-type hepatocytes in PKU mice appears sufficient to improve blood phenylalanine concentrations significantly [37], suggesting that hepatocyte repopulation may have efficacy in human PKU. In 2006, two independent groups demonstrated that the administration of recombinant adeno-associated virus serotype 2/8 to PAH$^{-/-}$ mice resulted in normalization of hyperphenylalaninemia [38,39], while a third group utilized a bacteriophage integrase system for introduction of the PAH cDNA into the liver of PAH$^{-/-}$ mice [40]. The latter study also demonstrated normalization of blood phenylalanine levels in gene-ablated mice. These elegant cell- and gene-therapy experiments suggest that long-term amelioration of PKU should be translatable from the bench (experimental animal models) to the clinic with human patients.

Murine succinic semialdehyde dehydrogenase (*Aldh5a1*) deficiency (SSADH$^{-/-}$ mice)

Patients with inherited SSADH deficiency manifest a nonspecific neurological picture of psychomotor retardation, speech delay, ataxia, hypotonia and seizures. In order to obtain insight into the diffuse pathophysiology of the human disorder, and to develop novel preclinical treatment paradigms, the *SSADH* gene was ablated in the mouse [41]. Heterozygous mice show no clear biochemical or clinical abnormalities. Mutant mice are born at the expected Mendelian frequencies, fail to thrive and manifest neurological abnormalities, and succumb in lethal status epilepticus by the age of 26 days [42]. Some of the more intriguing findings thus far have involved:

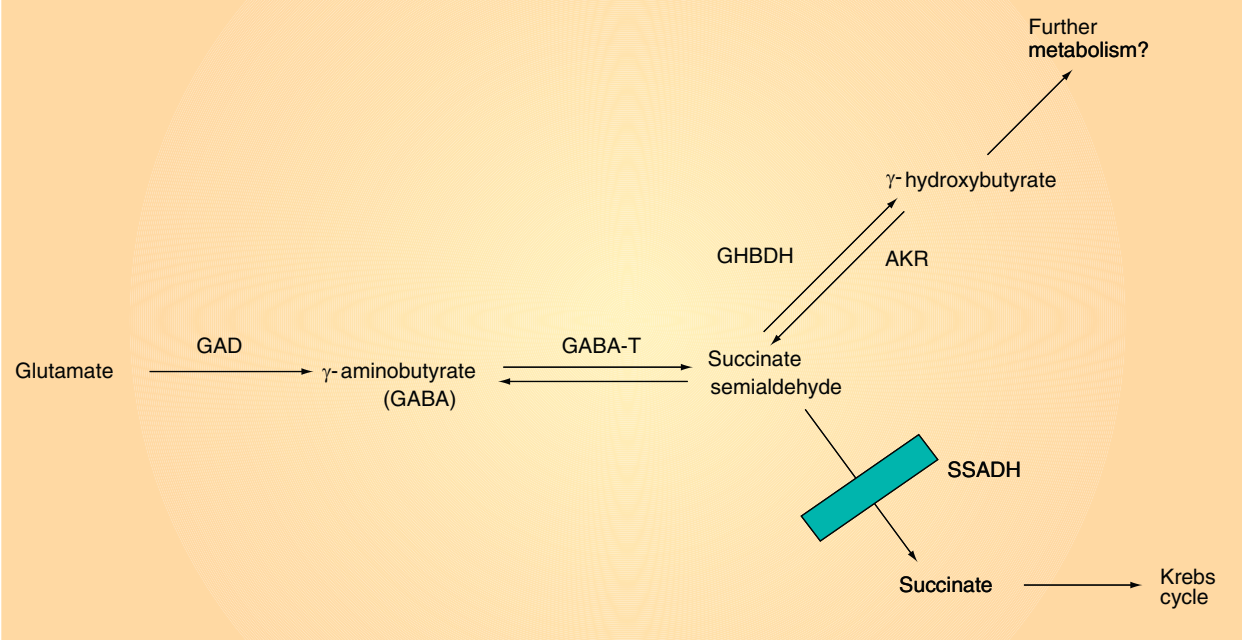
- Definition of the seizure transition in mutant mice;
- Rescue of mutant mice from early lethality with a variety of pharmacological agents;
- Rescue of the lethal phenotype and improvement in brain concentrations of γ-hydroxybutyric (GHB) utilizing gene therapy targeting the liver.

In vivo electrocorticograms of unrestrained SSADH$^{-/-}$ mice revealed a transition from absence seizures at postnatal (PN) day 14 to generalized tonic–clonic convulsions at PN 20, with eventual evolution to lethal status epilepticus, as noted above [41]. Absence seizures were blocked by the standard anti-absence intervention, ethosuximide. That these seizures had a prominent $GABA_B$ receptor component was shown further by a blockade utilizing the $GABA_B$ receptor antagonist CGP 35348 [43]. The rationale for use of a $GABA_B$ receptor antagonist centered on the observations that both GHB and GABA levels are high in the CNS of these animals, potentially leading to receptor downregulation with chronic exposure; therefore, the blockade of this effect by an antagonist might be expected to have therapeutic efficacy. A cloned GHB receptor has recently been presented, and there is molecular evidence that the GHB receptor and $GABA_B$ receptor are distinct entities [51,86]. Among its various pharmacological roles in the CNS, GHB has been shown to be a weak $GABA_B$ receptor agonist, so that high GHB and GABA in the CNS of SSADH$^{-/-}$ mice could lead to compounded effects on the $GABA_B$ receptor. In support of these observations, recent results have revealed abnormalities in the quantity of $GABA_B$ receptor isoforms [47]. In addition, Wu and colleagues have reported altered $GABA_A$ receptor function in these mice [46]. Taken together, the majority of data indicates decreased GABAergic activity (inhibitory) in the face of unchanged glutamatergic (excitatory) function, which may underlie the genesis of the epileptic phenotype. These findings have clear treatment ramifications for patients with heritable SSADH deficiency, in relation to agonism/antagonism of GABAergic systems.

The SSADH mutant mouse may further represent a novel model in which to evaluate the mechanisms of seizure transition. Many patients who develop absence seizures (with and without febrile convulsions) in preadolescence demonstrate remittance in adolescence or adulthood, while others transition to generalized tonic–clonic convulsions [48]. The mechanism of the transition process remains undefined, but could be evaluated more thoroughly in the SSADH-deficient mouse. In essence, this animal model represents a compressed system (10–15-day period) in which absence seizures transition to generalized tonic–clonic seizures. Interestingly, the β3 $GABA_A$ receptor knockout mouse manifests a similar pattern of seizure transition [44]. Thus, detailed characterization of the receptor, pharmacological and electrophysiological processes during this transition window in the SSADH mutant model may provide new insights into a variety of human epilepsies.

Early lethality in $SSADH^{-/-}$ mice provided a unique opportunity in which to attempt novel preclinical treatment paradigms. Antagonists of the GHB receptor (NCS-382), $GABA_B$ receptor (CGP 35348), and a suicide inhibitor of GABA-transaminase (vigabatrin; **Figure 2**) were all successful in extending the survival of mice [50], with NCS-382 providing optimal survival (~60%). The rationale in these studies (employing NCS-382 and CGP 35348) was that CNS tissues would be exposed chronically to high-level GHB and GABA, and the hypothesis was that a corresponding alteration of GHBergic/GABAergic receptor function would likely ensue in these animals. As noted previously, we have shown altered $GABA_B$ receptor subunit composition in these mice [47]. Conversely, detailed studies demonstrated no significant alterations in GHB receptor number or binding [87]. The use of vigabatrin in these mice was empirical, since the blockade of GABA-transaminase activity would be expected to decrease the concentration of GHB. The nonprotein amino acid taurine also significantly improved the lifespan of mutant mice, although its mechanism of action is still not understood. Taurine intervention was attempted since mutant mice transitioned to generalized tonic–clonic convulsions at (or near) the weaning period, and taurine has a high concentration in mother's milk. The working hypothesis for the use of taurine was that breast milk from the dam protected the weanlings from tonic–clonic seizures due to its high taurine concentration [50].

Figure 2. Formation and degradation of aminobutyrate.



Not all steps for GABA synthesis are shown. The site of the block in patients (and mice) with SSADH (*Aldh5a1*) deficiency is depicted by the cross-hatched box. Due to the metabolic block, accumulated succinate semialdehyde (normally converted to succinate) is metabolized to γ-hydroxybutyrate, a biochemical hallmark of the human and murine disorder.
AKR: Aldo–keto reductase (succinate semialdehyde reductase); GABA: γ-aminobutyric acid GABA-T: GABA-transaminase; GAD: Glutamate decarboxylase; GHBDH: γ-hydroxybutyrate dehydrogenase; SSADH: Succinate semialdehyde dehydrogenase.

Early lethality in this mutant model was exploited further by attempting hepatic gene therapy as a rescue mechanism. In those studies, the working rationale was the high concentration of SSADH protein in the liver [52] and the fact that GHB may readily traverse the blood–brain barrier. The hypothesis tested centered upon the potential to correct liver SSADH activity and thereby decrease the brain concentration of GHB. Using a second generation adenoviral-SSADH construct and intraperitoneal/periorbital administration, a maximum of approximately 20% wild-type SSADH activity in the liver at 72 h post-administration was obtained, with no expression in the brain [53]. GHB concentrations in the periphery (liver, kidney) decreased by up to 80%, and brain GHB decreased by approximately 50%. These data provide proof-of-principle that liver-directed gene therapy may have efficacy in SSADH deficiency, which is primarily considered a neurometabolic disorder.

Transgenic mouse models for neurotransmitter deficiencies, for which corresponding human diseases remain undetected

Additional knockout animals have been generated with alterations in release, clearance, transport or receptor signaling in the monoamine neurotransmitter system, including TPH deficiency, but to our knowledge corresponding human disorders have not yet been assigned (**Table 2**). These include dopamine defects for genes encoding vesicular monoamine transporter-2, the DA transporter, catechol-*O*-methyltransferase and the dopamine receptor subtypes D1 to D5; and for the serotonin system, the genes encoding TPH1 and the serotonin receptor genes *5-HT$_{1A}$* and *5-HT$_{1B}$* [3,54–60]. The spectrum of murine phenotypes spans from neonatal lethality and/or major biochemical and behavioral abnormalities to no visible phenotype due to potential redundancy.

Deficiency of the GABA-transporter in the mouse leads to tremor, ataxia and nervousness with altered GABA-induced conductances [61,62]. Both isoforms of glutamic acid decarboxylase (GAD_{65}, GAD_{67}) (**Figure 2**) have been ablated in the mouse, individually and synergistically [63,64]. Loss of GAD_{67} results in cleft palate in the mouse and early perinatal lethality; GAD_{65} deletion results in abnormalities in ventilation [65,66]. GABA-receptor subunits have been targeted extensively in a variety of mouse models. The $GABA_A$ receptor is a heteromeric pentamer (Cl^-channel pore), of which the pharmacology and electrophysiological properties vary depending on the subunit composition. Currently, 19 $GABA_A$-receptor subunits have been

Table 2. List of currently available mouse models for which there is no human disease yet identified.

Mouse model	System*	Phenotype	Ref.
Monoamine transporter VMAT-2 (three different knockout mice)‡	Monoamine transporter	Severe growth retardation Neonatal death	[55–57]
Dopamine transporter (knockout/knockdown)	Dopamine clearance	Mild phenotype with hyperactivity and impaired pulse control	[57]
Catechol-*O*-methyltransferase	Catecholamine clearance	Mild to normal phenotype	[56]
Monoamine oxidase B	Catabolism of catecholamines and serotonin	Aggressive behavior	[56]
Dopamine receptor D1-D5	Dopamine receptor signaling	Variable: impaired feeding, growth delay, hyperactivity, no phenotype	[24,49,59]
Norepinehprine	Catecholamine	Reduced locomotor activity and body temperature	[56]
Tryptophan hydroxylase 1	Serotonin	Altered 5-HT synthesis, turnover	[78]
serotonin receptor 5-HT_{1A} and 5-HT_{1B}	Serotonin	Altered behavior	[58,60]
GABA transporter	GABA	Ataxia, tremor	[62,63]
Glu decarboxylase GAD_{65}, GAD_{67}	GABA	Perinatal lethality, ventilation abnormalities	[66,67]
$GABA_B$ receptor 1, 2	GABA	Anxiety, depression	[74,75]
$GABA_A$ receptor (primarily α, β and γ)	GABA	Altered conductances, response to anesthetics	[69–73]

Space considerations preclude inclusion of all known knockout mice without a corresponding human disorder. In particular, no discussion is presented concerning mice with targeted deletion of the genes in other serotonin receptors (beyond 1A/1B) or the nine adrenergic receptors. The reader is directed to the primary literature for further information.

GABA: γ-aminobutyric acid; GAD: Glutamic acid decarboxylase; VMAT: Vesicular monoamine transporter.

**Neurotransmitter system*

‡Double and triple-knockout mice are available.

Executive summary

Introduction

- Monoamine and amino acid neurotransmitters regulate movement, sleep, autonomic function, mood, reward and inhibitory/excitatory neurotransmission, among other processes. Patients with defects in the metabolism of these neurotransmitters have limited treatment options.

Mouse models for primary & secondary deficiencies in neurotransmitters

- Available knockout mice for defects in primary biogenic amine metabolism include tyrosine hydroxylase (TH), dopamine β-hydroxylase (DβH), and monoamine oxidase-A.
- Tetrahydrobiopterin is the main cofactor involved in the metabolism of the monoamine neurotransmitters. Defects in its formation may be considered to result in secondary neurotransmitter disorders.
- Knockout mouse models in tetrahydrobiopterin formation include Segawa disease (autosomal dominant GTP cyclohydrolase deficiency), 6-pyruvoyl-tetrahydropterin synthase (PTPS), and sepiapterin reductase (SR) deficiency.
- TH-, DβH-, and PTPS-deficient mice display severe catecholamine deficiency with embryonal or perinatal lethality.
- SR knockout mice manifest hyperphenylalaninemia, in direct contrast to human patients who have normal blood phenylalanine levels.

Mouse models without an identified corresponding human disease

- Knockout mice encompassing genes for release, metabolism, transport or receptor signaling include the vesicular monoamine transporter-2, dopamine (DA) transporter, catechol-*O*-methyltransferase, DA-receptor subtypes D_1–D_5, tryptophan hydroxylase (TPH)1 and the serotonin receptor genes, *5-HT$_{1A}$* and *5-HT$_{1B}$*, as well as the serotonin transporter.
- Knockout mice have also been generated for additional serotonin receptor genes, the norepinephrine transporter and the nine adrenergic receptors.
- Mice have been generated with deletion of the γ-aminobutyric acid (GABA)-transporter and both isoforms of the GABA-synthesizing enzyme glutamic acid decarboxylase (GAD_{65}/GAD_{67}).
- A total of 19 subunits have been identified that comprise mammalian $GABA_A$ receptors. The predominant form of the heteropentameric $GABA_A$ receptor contains α, β and γ subunits in the mammalian brain; accordingly, most murine knockouts have been produced via ablation of these subunits.
- Some forms of juvenile epilepsy have been associated with inherited mutations in specific $GABA_A$ receptor subunits, primarily α and γ subunits
- Knockout mice have been produced with a specific deletion of the $GABA_B$ receptor subunit 1 and 2.
- In all disorders, the phenotype spans the presentations of neonatal lethality, major biochemical and behavioral abnormalities, to no visible phenotype.

TH deficiency in transgenic mice & the utility of tetrahydrobiopterin-deficient mice

- Embryonic lethality in mice is associated with complete deficiency in DA and norepinephrine due to TH knockout, whereas a DβH knockout leads to isolated deficiency in only norepinephrine.
- To dissect the roles for DA and norepinephrine/epinephrine, a selective DA-deficient mouse was generated by restoring TH function in noradrenergic cells via expression of TH under the noradrenergic-specific DβH promoter.
- General hypoactivity resulted in early lethality associated with feeding difficulties. Rescue of these animals was achieved with L-dopa (normal growth/lifespan), suggesting that DA was integral to feeding and movement, while epinephrine/norepinephrine appeared critical for cardiovascular system development to enable responses to hypoxic stress during embryo development.
- Similarly, PTPS-knockout mice are unable to synthesize tetrahydrobiopterin in association with perinatal lethality. Rescue is afforded by supplementation with transmitter precursors, L-dopa and 5-hydroxytryptophan, but mice remain dwarfed.

A mouse model in the GABA acid degradative pathway

- Two enzymes control GABA degradation, GABA-transaminase and succinate semialdehyde dehydrogenase (SSADH). The former has been documented in only a single family; the latter is more common, and manifests with neurological sequelae and γ-hydroxybutyric (GHB) aciduria.
- SSADH-knockout mice die within 30 days due to status epilepticus (SE), associated with high CNS levels of GABA and GHB; null mice transition from absence to generalized tonic–clonic convulsions prior to SE.
- These mice may be rescued from premature lethality by hepatic gene therapy, as well as pharmacotherapeutic intervention targeting the $GABA_B$ and GHB receptor systems.
- Abnormalities with GABAergic inhibitory transmission associated with unaltered glutamatergic transmission may underlie this seizure transition.

Conclusions

- Murine knockout models of monoamine and amino acid neurotransmitters continue to represent important systems in which to pattern associated Mendelian disorders, develop treatment concepts and investigate important questions of normal systems biology.

identified in mammalian tissue [67]. Most $GABA_A$-receptor knockout mice have targeted the α, β and γ subunits since variations of these three subunits comprise approximately 50% of mammalian $GABA_A$ receptors. Of interest, an expanding number of patients with various epileptic phenotypes (juvenile myoclonic epilepsy, absence seizures, temporal lobe epilepsy and generalized epilepsy with febrile seizures [GEFS+]) have been shown to have specific missense mutations associated with the epileptic phenotype, primarily in α and γ subunits [68]. Both α1 and γ2 receptors have been ablated in mice [69–72]. These animals do not manifest an epileptic phenotype, but do have altered GABAergic conductances and abnormal responses to anesthetics. In addition, the β3 subunit of the $GABA_A$ receptor has been implicated in both epilepsy and Angelman syndrome [44].

The $GABA_B$ receptor is a G-protein coupled dimer composed of nonidentical subunits $GABA_B$ receptor 1a/1b (alternative splice events) and $GABA_B$ receptor 2; both subunits must be expressed for functional receptor activity. Each subunit has been individually ablated in the mouse, with $GABA_B$ receptor 2 deficient mice showing anxiety and depression-related behavior [73,74].

Although at first glance these mice do not appear useful for the identification of human disorders, they may be valuable for analysis of the genetic, biochemical and environmental interrelationships of the complex neurotransmitter systems involved. As an example, the murine TPH1 knockout led to the identification of a second TPH gene in mammals encoding a unique central TPH2 isoform. TPH1 mutants also lack peripheral serotonin but retain neuronal serotonin action and are defective for liver regeneration (i.e., induction of hepatocyte proliferation after loss of hepatic tissue [75,76]). These knockout mice might become an important tool in establishing a link with the hundreds of still unknown monogenic disorders yet to be defined [79], and will certainly be an invaluable aid in understanding various neurochemical phenomena associated with monoamines and amino acid neurotransmitters.

Conclusions

Mouse models are clearly valuable for linking genetic diseases to distinct alleles and investigating pathogenesis and treatment, particularly in light of the hundreds of unknown monogenic diseases including neurological disorders [79], but also for complex genetic maladies such as diabetes. In the context of a genome wide knockout mouse project, it was reported in 2004 that knockouts exist for only approximately 10% of mouse genes [80,81]. However, as pointed out by others, caution must be used regarding the phenotypic reproduction of human diseases, as many signs and symptoms of human abnormalities cannot be reproduced easily in mice [82]. Other hurdles remain, including differences in phenotype assessment and in the phenotypic spectra between mice and man. A 'historical' example for the latter are the hypoxanthine guanine phosphoribosyl transferase mutations responsible for Lesch–Nyhan syndrome and the diverse phenotype of the mouse knockout [83,84]. Murine knockout models of monoamine and amino acid neurotransmitters continue to represent important systems in which to pattern associated Mendelian disorders, develop treatment concepts, and investigate important questions regarding normal systems biology.

Future perspective

Future work in this area is likely to focus on epileptic processes and the role of monoamines in embryology. A particular emphasis on knock-in and regional/conditional gene knockdown systems will be useful in these studies. Systems redundancy is expected to be explored extensively, employing transgenic models, especially for receptor targets that are composed of multiple subunits (e.g., the $GABA_A$ receptor).

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