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Biochemical and Biophysical Research Communications 338 (2005) 267–270
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Review

Molecular genetics of tyrosine 3-monooxygenase and inherited diseases

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Received 16 July 2005
Available online 10 August 2005

Abstract

Tyrosine 3-monooxygenase (tyrosine hydroxylase, TH) catalyzes the initial and rate-limiting step in the catecholamine biosynthesis. Alteration in TH activity is involved in the pathogenesis of certain disorders derived from catecholaminergic dysfunction. In the present review, we focus on recent advances in molecular genetic study of TH function and inherited diseases. Knockout mice lacking TH gene show severe catecholamine depletion and perinatal lethality. Mice heterozygous for the TH mutation exhibit defects in some neuropsychological functions. Dopamine-deficient mice impair motor control and operant learning during postnatal development. In addition, some point mutations in the human TH gene underlie the inherited diseases, including the recessive form of L-DOPA-responsive dystonia, parkinsonism in infancy, or progressive encephalopathy. These mutations indeed appear to reduce TH activity or influence expression of TH protein. Advances in molecular genetic studies provide a deeper understanding of the relationship between the alteration in TH activity and the pathology of catecholaminergic systems.

Keywords: Tyrosine 3-monooxygenase; Catecholamine; Dopamine; Noradrenaline; Knockout mouse; Dystonia; Parkinsonism; Encephalopathy

Tyrosine 3-monooxygenase (tyrosine hydroxylase, TH) (EC 1.14.16.2) catalyzes the initial and rate-limiting step in the biosynthetic pathway of catecholamines, including dopamine, noradrenaline, and adrenaline [1,2]. These catecholamines play important roles in a variety of physiological and behavioral functions in the central and peripheral nervous systems as well as the endocrine system. Therefore, the regulation of expression and activity of TH is considered to be important for the neuronal and hormonal functions that are dependent on the activity of catecholaminergic cell types. In addition, alteration in TH activity is involved in the pathogenesis of certain disorders derived from catecholaminergic dysfunction.

There have been several reviews of TH regulation through different mechanisms [3,4]. Short-term regulation of TH activity is dependent on phosphorylation of the enzyme by a variety of protein kinases [3]. TH activity is also regulated through feedback inhibition and through allosteric effectors [4]. Medium- to long-term regulation of TH activity occurs at some phases of gene expression, such as transcription, alternative RNA splicing, RNA stability, and translation [4]. Transcriptional activation of TH gene involves various factors, including cyclic AMP, phorbol esters, glucocorticoids, nerve growth factor, and epidermal growth factor [4]. Protein stability also appears to contribute to the maintenance of TH activity that requires tetrahydrobiopterin [5].

The emphasis in the present review is on recent advances in molecular genetic study of TH function and inherited diseases. We summarize the TH deficiency generated by gene targeting in mice and describe the implication of the mutation in the TH gene in human inherited diseases.

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doi:10.1016/j.bbrc.2005.07.186

TH deficiency in mouse models

Recent advances in gene targeting technique with homologous recombination in mouse embryonic stem cells provide an excellent system for studying the in vivo function of specific genes and for generating animal models for inherited diseases. Some genetically modified mice carrying targeted mutation in the TH gene were generated [6–10] and their phenotypic changes are given in Table 1. Knockout mice lacking the TH gene (homozygous mutation) die at a late stage of embryonic development or soon after birth [6,7]. Lack of TH activity in these mice causes severe depletion of dopamine, noradrenaline, and adrenaline in tissues. These changes do not affect gross development of the cells that normally produce catecholamines. However, surviving newborn mutants display abnormal electrocardiograms characterized by reduced heart rate and prolonged P–Q interval, suggesting alterations in cardiovascular function in the mutant mice. Knockout of TH function causes perinatal lethality probably because of cardiovascular failure.

Mice heterozygous for the TH mutation show a reduced TH activity in the tissues (approximately 40% of the wild type), although they are apparently normal in development and gross behavior [8]. In these mice, noradrenaline accumulation in the brain regions is moderately reduced to 73–80% of the wild type levels, and noradrenaline release in the frontal cortex in response to depolarization is also reduced relative to the wild type level. These mice display impairments in the water-finding task associated with latent learning performance. They also exhibit impairments in long-term memory formation of associative learning. These include active avoidance, cued fear conditioning, and taste aversion, which are known to require mainly the amygdala function. In contrast, the hippocampus-dependent spatial learning and long-term potentiation appear to be normal in the mutants. Interestingly, these behavioral deficits are rescued by treatment of a noradrenaline reuptake inhibitor, which stimulates noradrenergic function. The reduction in TH activity in the heterozygous mutants causes defects in some neuropsychological functions at least associated with noradrenaline transmission in the brain.

Expression of TH gene in noradrenergic and adrenergic cell types by using the dopamine β-hydroxylase gene promoter was introduced into the TH knockout mice [9,10]. This causes deletion of TH expression in the cell types that normally produce dopamine, resulting in a marked reduction of dopamine level in the brain regions. Dopamine-deficient mice display growth retardation beginning from postnatal 2 weeks and then die until postnatal 4 weeks. These mice show a reduction in spontaneous locomotion, cataleptic behavior, and blockade of dopamine receptor agonist-induced motor activation.

Table 1
Phenotypes of genetically modified mice carrying TH mutation

Genotype	Enzyme activity	Phenotype	References
Knockout (homozygous mutation)	Complete loss	Abnormal electrocardiography, perinatal lethality	[6,7]
Heterozygous mutation	Reduction to 45% of control	Defect in latent learning, defect in conditioned learning (cued fear conditioning, taste aversion), defect in operant conditioning	[8]
Conditional knockout in dopaminergic neurons	Loss in dopaminergic neurons	Akinesia, cataleptic behavior, loss of drug response, defect in operant conditioning, adipsia, aphagia, postnatal lethality, growth retracdation	[9,10]

They also show defect in the acquisition of operant conditioning including the active avoidance task. Knockout of TH function in dopaminergic neurons impairs motor control and operant learning during postnatal development.

TH mutations in human inherited diseases

Mutations in the human TH gene have been reported in some inherited neurological diseases as given in Table 2. A missense point mutation (C to A at the nucleotide 1234, which is numbered on the basis of human TH type 4 mRNA sequence in [11]) was first found in the recessive inherited form of L-DOPA-responsive dystonia or Segawa's disease [12]. L-DOPA-responsive dystonia is characterized by progressive symptoms with marked diurnal fluctuation and these symptoms are recovered by a treatment of low dose of L-DOPA. This mutation causes a substitution of the amino acid at the residue 412 from Gln to Lys. The recombinant protein carrying the mutation, when expressed in *Escherichia coli*, shows a reduced affinity for L-tyrosine and the activity of the mutant enzyme is approximately 15% of the wild type activity [13]. The altered enzyme property supports the clinical phenotype of the patients homozygous for the missense point mutation. Another missense mutation (A to G at the nucleotide 698) was found in the recessive L-DOPA-responsive dystonia [14,15]. This mutation substitutes the amino acid at the residue 233 from Arg to His. In addition, a deletion of a single nucleotide C at the nucleotide 291, that generates a truncated form of TH protein, was identified in the patient who is compound heterozygous for this mutation and the mutation A to G at the nucleotide 698 [15,16].

The missense point mutation (T to C at the nucleotide 707) in the TH gene was also reported in a patient showing parkinsonism in early infancy, and the symptoms were accompanied by sympathetic dysfunction (ptosis) and by being responsive to L-DOPA therapy [17]. This mutation causes the amino acid substitution (Leu to Pro) at the residue 236. The recombinant mutant protein possesses a lower TH activity relative to the wild type protein, and the activity ranges from 1.5% to 16% depending on the expression systems of the recombinant enzyme [17]. In addition, other point mutations were found in progressive encephalopathy in infancy with L-DOPA-nonresponsive dystonia [18]. One mutant allele is a missense point mutation, by which G at the nucleotide 1076 is converted to T, causing the amino acid substitution (Cys to Phe) at the residue 359. Another mutant allele is a point mutation around the splice acceptor site in intron 11, resulting in alternative splicing to generate an aberrant mRNA.

The aforementioned point mutations appear to remove partially TH activity in the patients carrying the homozygous or compound heterozygous mutations. On the other hand, there has been debate whether mutations in the human TH gene are associated with the pathogenesis of certain neuropsychiatric diseases. A recent research suggests a link between the TH locus and bipolar affective disorders [19]. Another study also suggests an association of the DNA polymorphism in the TH locus with disturbances in the catecholaminergic system in schizophrenia [20].

Conclusion

In the present review, we summarized the phenotype of some genetically modified mice with TH mutation and described the implication of the TH mutation in human inherited diseases. Phenotypic study of the mouse models represents the physiological and behavioral role of TH function and the pathological state derived from altered TH activity by the mutation. Genetic study of human inherited diseases determines the mutations that cause TH deficiency characterized mainly by recessive L-DOPA-responsive dystonia, parkinsonism in infancy, or progressive encephalopathy. Future genetic study will address the question as to whether mutations in the TH gene are involved in neuropsychiatric diseases. The progress of these studies provides a deeper understanding of

Table 2
TH mutations in human inherited diseases

Symptom	Mutation[a]	Amino acid sequence	Population	References
L-DOPA-responsive dystonia	Missense C → A (1234) in exon 11	Gln → Lys (412)	Germany	[12,13]
	Missense G → A (698) in exon 6	Arg → His (233)	Netherlands	[14,15]
	Deletion ΔC (291) in exon 3[b]	Frame shift	Netherlands	[15,16]
Parkinsonism in infancy	Missense T → C (707) in exon 6	Leu → Pro (236)	Greek	[17]
Progressive infantile encephalopathy with L-DOPA-nonresponsive dystonia	Missense G → T (1076) in exon 10	Cys → Phe (359)	Germany	[18]
	Splice T → A (2 bp upstream of splice accepter site in intron 11)[b]	Skipping exon 12	Germany	[18]

[a] Numbered from the first nucleotide of the initiation Met codon of human TH type 4 mRNA [11].
[b] Compound heterozygous mutants with another mutation G → A (698) in exon 6.

the relationship between the alteration in TH activity and the pathology of catecholaminergic systems.

References

[1] T. Nagatsu, M. Levitt, S. Udenfriend, Tyrosine hydroxylase: the initial step in norepinephrine biosynthesis, J. Biol. Chem. 239 (1964) 2910–2917.

[2] P.F. Fitzpatrick, Tetrahydropterin-dependent amino acid hydrolases, Annu. Rev. Biochem. 68 (1999) 355–381.

[3] P.R. Dunkley, L. Bobrovskaya, M.E. Graham, E.I. von Nagy-Felsobuki, P.W. Dickson, Tyrosine hydroxylase phosphorylation: regulation and consequences, J. Neurochem. 91 (2004) 1025–1043.

[4] S.C. Kumer, K.E. Vrana, Intricate regulation of tyrosine hydroxylase activity and gene expression, J. Neurochem. 67 (1996) 443–462.

[5] C. Sumi-Ichinose, F. Urano, R. Kuroda, T. Ohye, M. Kojima, M. Tazawa, H. Shiraishi, Y. Hagino, T. Nagatsu, T. Nomura, H. Ichinose, Catecholamines and serotonin are differently regulated by tetrahydrobiopterin, J. Biol. Chem. 276 (2001) 41150–41160.

[6] K. Kobayashi, S. Morita, H. Sawada, T. Mizuguchi, K. Yamada, I. Nagatsu, T. Hata, Y. Watanabe, K. Fujita, T. Nagatsu, Targeted disruption of the tyrosine hydroxylase locus results in severe catecholamine depletion and perinatal lethality in mice, J. Biol. Chem. 270 (1995) 27235–27243.

[7] Q.-Y. Zhou, C.J. Quaife, R.D. Palmiter, Targeted disruption of the tyrosine hydroxylase gene reveals that catecholamines are required for mouse fetal development, Nature 374 (1995) 640–643.

[8] K. Kobayashi, Y. Noda, N. Matsushita, K. Nishii, H. Sawada, T. Nagatsu, D. Nakahara, R. Fukabori, Y. Yasoshima, T. Yamamoto, M. Miura, M. Kano, T. Mamiya, Y. Miyamoto, T. Nabeshima, Modest neuropsychological deficits caused by reduced noradrenaline metabolism in mice heterozygous for a mutated tyrosine hydroxylase gene, J. Neurosci. 20 (2000) 2418–2426.

[9] Q.-Y. Zhou, R.D. Palmiter, Dopamine-deficient mice are severely hypoactive, adipsic, and aphagic, Cell 83 (1995) 1197–1209.

[10] K. Nishii, N. Matsushita, H. Sawada, H. Sano, Y. Noda, T. Mamiya, T. Nabeshima, I. Nagatsu, T. Hata, K. Kiuchi, H. Yoshizato, K. Nakashima, T. Nagatsu, K. Kobayashi, Motor and learning dysfunctions during postnatal development in mice defective in dopamine neuronal transmission, J. Neurosci. Res. 54 (1998) 450–464.

[11] K. Kobayashi, N. Kaneda, H. Ichinose, F. Kishi, A. Nakazawa, Y. Kurosawa, K. Fujita, T. Nagatsu, Structure of the human tyrosine hydroxylase gene: alternative splicing from a single gene accounts for generation of four mRNA types, J. Biochem. 103 (1988) 907–912.

[12] B. Lüdecke, B. Dworniczak, K. Bartholomé, A point mutation in the tyrosine hydroxylase gene associated with Segawa's syndrome, Hum. Genet. 93 (1994) 123–125.

[13] P.M. Knappskog, T. Flatmark, J. Mallet, B. Lüdecke, K. Batholomé, Recessively inherited L-DOPA-responsive dystonia caused by a point mutation (Q381K) in the tyrosine hydroxylase gene, Hum. Mol. Genet. 4 (1995) 1209–1212.

[14] L.P. van den Heuvel, B. Luiten, J.A. Smeitink, J.F. de Rijk-van Andel, K. Hyland, G.C. Steenbergen-Spanjers, R.J. Janssen, R.A. Wevers, A common point mutation in the tyrosine hydroxylase gene in autosomal recessive L-DOPA-responsive dystonia in the Dutch population, Hum. Genet. 102 (1998) 644–646.

[15] C. Bräutigam, R.A. Wevers, R.J.T. Janssen, J.A.M. Smeitink, J.F. de Rijk-van Andel, F.J.M. Gabreëls, G.F. Hoffmann, Biochemical hallmarks of tyrosine hydroxylase deficiency, Clin. Chem. 44 (1998) 1897–1904.

[16] R.A. Wevers, J.F. de Rijk-van Andel, C. Brautigam, B. Geurtz, L.P. van den Heuvel, G.C. Steenbergen-Spanjers, J.A. Smeitink, G.F. Hoffmann, F.J. Gabreels, A review of biochemical and molecular genetic aspects of tyrosine hydroxylase deficiency including a novel mutation (291delC), J. Inherit. Metab. Dis. 22 (1999) 364–373.

[17] B. Lüdecke, P.M. Knappskog, P.T. Clayton, R.A.H. Surtees, J.D. Clelland, S.J.R. Heales, M.P. Brand, K. Bartholomé, T. Flatmark, Recessively inherited L-DOPA-responsive parkinsonism in infancy caused by a point mutation (L205P) in the tyrosine hydroxylase gene, Hum. Mol. Genet. 5 (1996) 1023–1028.

[18] G.F. Hoffmann, B. Assmann, C. Bräutigam, C. Dionisi-Vici, M. Häussler, J.B.C. de Klert, M. Naumann, G.C.H. Steenbergen-Spanjers, H.-M. Strassburg, R.A. Wevers, Tyrosine hydroxylase deficiency causes progressive encephalopathy and dopa-nonresponsive dystonia, Ann. Neurol. 54 (2003) S56–S65.

[19] C. Smyth, G. Kalsi, D. Curtis, J. Brynjolfsson, J. O'Neill, L. Rifkin, E. Molony, P. Murphy, H. Petursson, H. Gurling, Two-locus admixture linkage analysis of bipolar and unipolar affective disorder supports the presence of susceptibility loci on chromosome 11p15 and 21q22, Genomics 39 (1997) 271–278.

[20] F. Thibaut, J.M. Ribeyre, N. Dourmap, R. Meloni, C. Laurent, D. Campion, J.F. Menard, S. Dollfus, J. Mallet, M. Petit, Association of DNA polymorphism in the first intron of the tyrosine hydroxylase gene with disturbances of the catecholaminergic system in schizophrenia, Schizophr. Res. 23 (1997) 259–264.