

Expression of tyrosine hydroxylase in cerebellar Purkinje cells of ataxic mutant mice : its relation to the onset and/or development of ataxia

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Abstract : This report describes recent studies on tyrosine hydroxylase (TH) expression in Purkinje cells of the cerebellum of ataxic mutant mice. An increased expression of TH in some Purkinje cells has been observed in two allelic groups of mutant mice, *tottering* and *dilute*. TH-positive Purkinje cells appeared preceding the onset of ataxia. Northern blot analysis revealed 2.1 kb of TH mRNA in the mutant cerebella, and the size was identical to that of TH transcripts in other brain regions. However, TH in Purkinje cells did not seem to participate in catecholamine biosynthesis. In vitro studies showed that cultured non-catecholaminergic neurons expressed the TH transcripts following Ca^{2+} influx. Therefore, abnormal TH expression in the mutant Purkinje cells may indicate neuronal dysfunction caused by misregulation of intracellular Ca^{2+} concentrations. *J. Med. Invest.* **48** : 5-10, 2001

Keywords : Purkinje cells ; ataxia ; tyrosine hydroxylase ; calcium channel ; tottering ; rolling mouse Nagoya ; dilute-lethal mouse

INTRODUCTION

Tyrosine hydroxylase (TH), the first step enzyme for catecholamine (CA) synthesis, is mainly expressed in CAergic neurons in the brain. TH was also expressed in some non-CAergic neurons of various brain regions during development (1-5), in ataxic mutant mice (6-9), and in the experimental conditions such as organotypic tissue culture (10,11) and transplantation (12). This report describes recent studies on TH expression in cerebellar Purkinje cells of ataxic mutant mice and its relation to the onset and/or development of the ataxia.

TH EXPRESSION IN CEREBELLA OF ATAXIC MUTANT MICE

Normal mice expressed TH gene (6,7) and immunoreactivity (9,13,14) in Purkinje cells at low levels. TH-positive Purkinje cells transiently increased from the first to second weeks of postnatal life (6,13). Then the number of TH-positive Purkinje cells decreased, maintained a low level, and increased again by 11 months of age (13).

Hess and Wilson first reported increased expressions of both TH mRNA and immunoreactivity in some Purkinje cells of adult *tottering* and *leaner* mice (6). Our recent study also exhibited an enhanced TH immunoreactivity in some Purkinje cells of rolling mouse Nagoya (RMN) on day 14 and thereafter (9). These three mutant mice carry recessive mutant alleles of the *tottering* (*Cch11a4*) locus on chromosome 8 (15-17). They commonly express ataxia, whereas varying degrees of several abnormal neurological phenotypes have been exhibited (Table1). Thus, abnormal TH expression in Purkinje cells appears in all three mutants of

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Table 1. Summary of neurological phenotypes in ataxic mutant mice exhibiting abnormal expression of tyrosine hydroxylase in cerebellar Purkinje cells

Mutant name	Mutant gene	Chromosome	Neurological phenotypes
<i>tottering</i>	<i>tottering (tg)</i> or <i>Cchlla 4</i>	8 ; 38.5 cM	mild ataxia generalized absence-like seizures (petit mal-like epilepsy) sporadic focal myoclonic seizures
<i>leaner</i>	<i>tottering (tg^{la})</i> or <i>Cchlla 4</i>	8 ; 38.5 cM	severe immobilizing ataxia absence seizures
rolling mouse Nagoya	<i>tottering (tg^{rol})</i> or <i>Cchlla 4</i>	8 ; 38.5 cM	ataxia
<i>dilute-lethal</i>	<i>dilute (d^l)</i> or <i>Myo5a</i>	9 ; 42.0 cM	ataxia opisthotonic seizures

the *tottering* allelic group.

We have further reported an increased TH immunoreactivity in some Purkinje cells of *dilute-lethal* mice (DL)(9). DL carry the mutation allele in the *dilute (Myo5a)* locus on chromosome 9 (18), and show the ataxia and opisthotonic seizures (Table1). Therefore, abnormal TH expression in Purkinje cells may not be specific to the allelic group, but rather related to the ataxic symptoms.

In RMN, TH-positive Purkinje cells were distributed in all lobules of the cerebellum and were arranged into parasagittal bands running through the vermis and hemispheres (9). Five narrow bands were observed in the anterior vermis, distributed symmetrically on each side of the midline band (Fig.1 B). The bands widened in the posterior vermis (Fig.1 D). Consistent results have been obtained in *tottering* and *leaner* mice (7,8). The topology of TH-positive Purkinje cells in the mutant cerebella corresponded to Zebrin-positive Purkinje cells (8). DL also showed similar banding pattern of TH-positive Purkinje cells in lobules IX and X of the vermis (9).

The relationship between TH expression in Purkinje cells and the onset of ataxic symptoms has been reported clearly in DL (19). DL walked normally by day 8. The ataxic symptom was exhibited by about 20% of DL on day 9 and by all DL by day 10. TH-positive Purkinje cells began to appear in lobules IX and X of the vermis of either ataxic or non-ataxic DL on day 9 (Fig.2 B), and they drastically increased between days 9 and 10 (Fig.2 C). We concluded that ataxia in DL may appear immediately following abnormal TH expression in the Purkinje cells.

ROLE OF TH

Northern blot analysis revealed 2.1 kb of TH mRNA in the cerebellum of *tottering* and *leaner* mice (6,7). The size was identical to TH transcripts in other brain regions (7). However, the role of TH in the development of ataxia is unclear. In RMN, GABA immunoreactivity appeared in Purkinje cells, similarly to their littermate controls (20). TH-positive Purkinje cells in *tottering* and *leaner* mice co-expressed mRNA for glutamic acid decarboxylase (GAD), the synthetic enzyme for GABA (6). Aromatic amino acid decarboxylase, the next enzyme of the catecholamine synthesis, could not be detected immunohistochemically in any Purkinje cells in *tottering* and *leaner* mice (6). By biochemical study, noradrenaline content in the RMN cerebellum was not different from that in their controls, although TH activity was higher in the RMN cerebellum (21). Thus, TH-positive Purkinje cells in the mutant mice do not seem to participate in catecholamine biosynthesis, and a phenotypic switch from GABAergic to CAergic does not occur.

MECHANISM OF TH EXPRESSION

In vitro studies showed that the Ca²⁺ response element was present in the TH promoter, and non-CAergic neurons expressed the TH transcripts following Ca²⁺ influx (22-24). These results indicate that abnormal TH expression in the mutant Purkinje cells may be caused by misregulation of intracellular Ca²⁺ concentration.

In DL, the smooth endoplasmic reticulum (SER), which played a crucial role for synaptic regulation

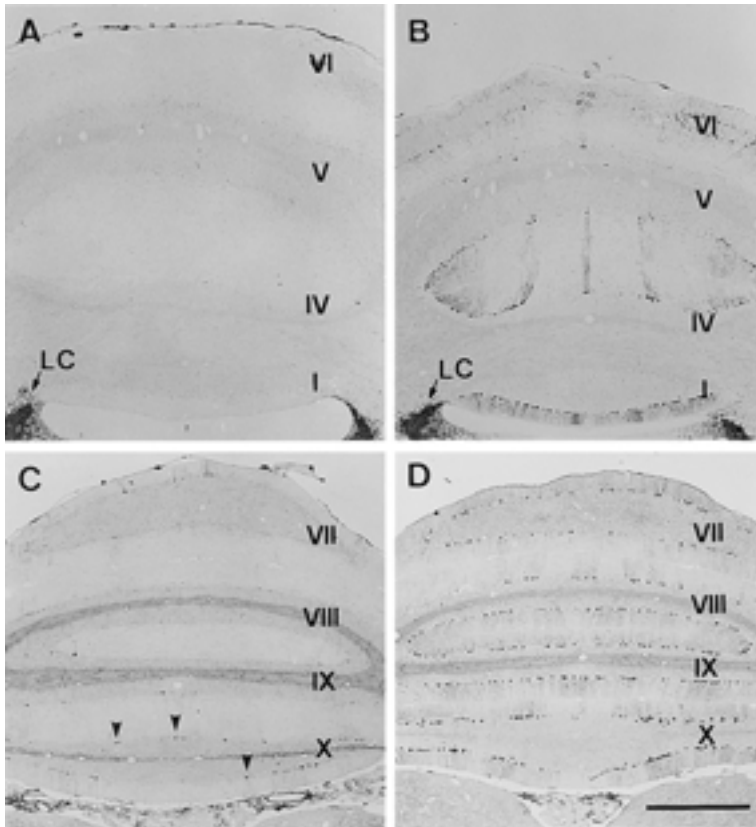


Fig. 1. Immunostaining for tyrosine hydroxylase (TH) in the cerebellum of a control mouse (A, C) and rolling mouse Nagoya (RMN)(B, D). A, B. Anterior vermis, C, D. Posterior vermis. In RMN, definite TH staining was observed in the perikarya and dendrites of some Purkinje cells. TH-positive Purkinje cells were organized into parasagittal bands. Arrowheads indicate a weak TH staining in the Purkinje cell perikarya in the posterior vermis of the control mouse. LC : locus ceruleus ; Bar=500 μ m

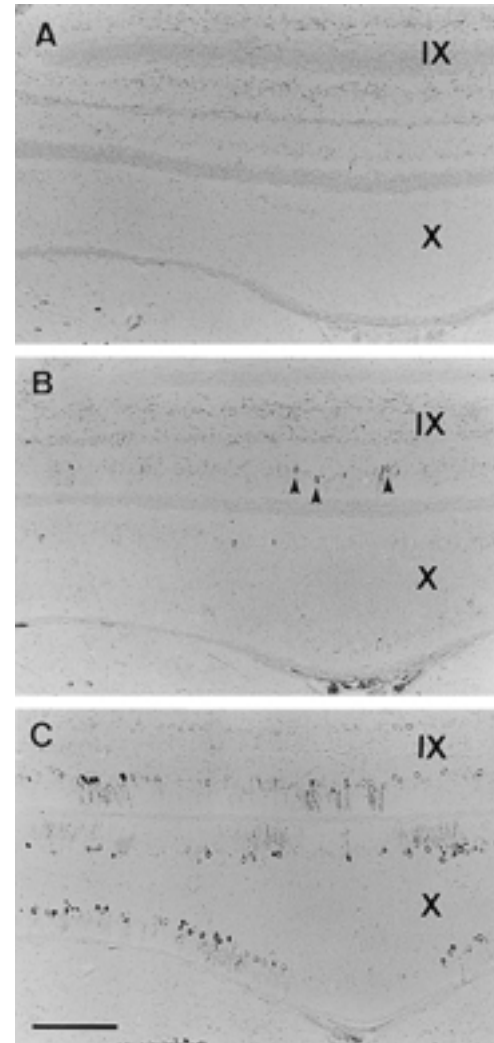


Fig.2. Immunostaining for tyrosine hydroxylase (TH) in lobules IX and X of the posterior vermis of *dilute-lethal* mice (DL). (A) Day 8. Few TH-positive Purkinje cells were found, similarly to wild-type (+/+) or heterozygous (*d¹/+*) mice. DL walked normally on this age. (B) Day 9. TH-positive Purkinje cells appeared in lobules IX and X of the vermis (arrowheads). About 20% of DL showed falling over when walking. (C) Day 10. TH-positive Purkinje cells drastically increased in the lobules IX and X. All DL showed falling over. Bar=200 μ m

as an intracellular Ca^{2+} store, was missing in the dendritic spine of Purkinje cells (25). Depletion of intracellular Ca^{2+} stores by inositol 1,4,5-triphosphate, ionomycin and an excess of EDTA induced a sustained Ca^{2+} entry through the plasma membrane of mast cells (26). Therefore, Ca^{2+} influx followed by a failure of Ca^{2+} mobilization from SER may cause abnormal TH expression in Purkinje cells of DL.

The presumptive mechanism by TH expression in Purkinje cells of the mutant mice belonging to the *tottering* allelic group is summarized in Figure 3. In *tottering*, *leaner* and RMN, mutation alleles

are located on the *tottering* (*Cch11a 4*) locus (15-17), which encodes the α_{1A} subunit of the P/Q-type Ca^{2+} channel (27,28). The mutated Ca^{2+} channel α_{1A} subunit was prominently expressed throughout cerebellar Purkinje cells (29), and the P-type Ca^{2+} channel currents of those neurons were reduced (28, 30-32). In compensation for altered function of the α_{1A} subunit, expression of the α_{1C} subunit of the L-type Ca^{2+} channel increased in Purkinje cells of *tottering* mice (33). Chronic injections of the L-type Ca^{2+} channel blockers decreased TH mRNA expression in the cerebellum of *tottering*

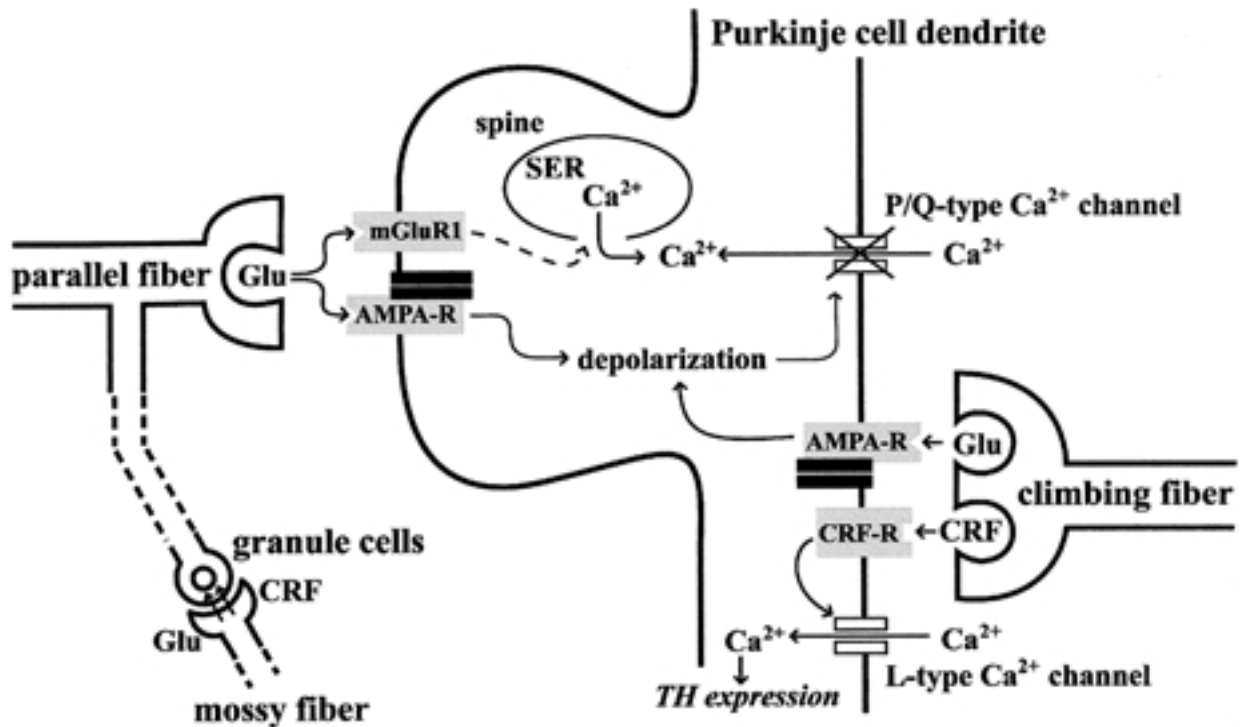


Fig. 3. Presumptive mechanism by tyrosine hydroxylase (TH) expression in Purkinje cells of ataxic mutant mice belonging to the *tottering* allelic group. In *tottering*, *leaner* and rolling mouse Nagoya, the α_{1A} subunit of the P/Q-type Ca²⁺ channel was mutated, and expression of the α_{1c} subunit of the L-type Ca²⁺ channel increased in compensation for altered function of the P/Q-type Ca²⁺ channel. An increased level of corticotropin-releasing factor (CRF) in climbing fibers in the mutants might facilitate Ca²⁺ influx through the L-type Ca²⁺ channel in their target Purkinje cells, resulting in abnormal TH expression in those neurons. Glu: glutamate; SER: smooth endoplasmic reticulum

mice (34). Double immunohistochemistry for corticotropin-releasing factor (CRF) and TH in the RMN cerebellum revealed that distribution of TH-positive Purkinje cells corresponded to terminal fields of CRF-positive climbing fibers (35). CRF potentiated Ca²⁺ currents through the L-type Ca²⁺ channel (36). Therefore, an increased level of CRF in climbing fibers may facilitate Ca²⁺ influx through the α_{1c} subunit of the L-type Ca²⁺ channel in their target Purkinje cells, and may induce abnormal TH expression in those neurons of the mutant mice belonging to the *tottering* allelic group.

CONCLUSIONS

As summarized in Table 1, abnormal TH expression in Purkinje cells has been observed in two allelic groups of mutant mice, *tottering* and *dilute*, and appeared in connection with the onset and/or development of the ataxic symptoms. Since the transcription of the TH gene was regulated by Ca²⁺, TH expression in Purkinje cells of the mutant mice may indicate neural dysfunction by alteration of the intracellular Ca²⁺ concentrations.

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