Zebrin II Expressing Purkinje Cell Phenotype–Related and –Unrelated Cerebellar Abnormalities in Ca\textsubscript{v}2.1 Mutant, Rolling Mouse Nagoya

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Rolling mouse Nagoya is an ataxic mutant mouse that carries a mutation in a gene encoding for the $\alpha_{1A}$ subunit of the voltage-gated P/Q-type Ca\textsuperscript{2+} channel (Ca\textsubscript{v}2.1). This report summarizes our studies and others concerning cerebellar abnormalities in rolling mice based on chemical neuroanatomy. While there are no obvious cerebellar deformations in this mutant mouse, the altered functions of Purkinje cells can be revealed as a reduced expression of type 1 ryanodine receptor (RyR1) in all Purkinje cells uniformly throughout the cerebellum, and as an ectopic expression of tyrosine hydroxylase (TH) in the Purkinje cell subsets with the zebrin II–immunopositive phenotype. As the mutated Ca\textsubscript{v}2.1 channel is expressed at uniform levels in all Purkinje cells, its copresence with RyR1 staining suggests that a Ca\textsubscript{v}2.1 channel dysfunction links with the expression of RyR1 in Purkinje cells of rolling mice. However, an ectopic expression of TH in the Purkinje cells is topologically related to the projection of corticotrophin-releasing factor–immunopositive climbing fibers rather than expression of the mutated Ca\textsubscript{v}2.1 channel. On the other hand, increased levels of serotonin (5-HT) in 5-HTergic fibers were revealed immunohistochemically in Purkinje cells of the vermis of rolling cerebellum. Thus, to determine whether or not cerebellar abnormalities are related to Purkinje cell populations revealed by zebrin II expression is essential for enhancing our understanding of the pathogenesis of hereditary cerebellar ataxic mutants such as rolling mice.

**KEYWORDS:** Ca\textsuperscript{2+} channelopathy, zebrin, tyrosine hydroxylase, ryanodine receptor, CRF, serotonin

**INTRODUCTION**

Rolling mouse Nagoya is an ataxic mutant mouse that carries a mutation in a gene encoding for the $\alpha_{1A}$ subunit of the voltage-gated P/Q-type Ca\textsuperscript{2+} channels (Ca\textsubscript{v}2.1)[1], as do tottering, leaner[2], rocker[3], and
wobbly mice[4]. Specifically, nucleotide position 3784 of the Ca,2.1 gene is changed from C to G, resulting in a charge-neutralizing amino acid change from a highly conserved arginine to glycine at position 1262 in the voltage sensor-forming segment of the Ca,2.1 channel protein[1]. By this mutation, the voltage sensitivity and the activity of P/Q-type Ca,2.1 channel are selectively reduced in cerebellar Purkinje cells[1]. In humans, defects in this gene are responsible for several neurological disorders, such as episodic ataxia type-2 and spinocerebellar ataxia type-6[5]. Motor deficits of rolling mice are characterized by frequent lurching of the mice and abnormal cyclic movements of the hindlimbs when walking, but they do not exhibit epilepsy as seen in tottering and leaner mice[6,7]. However, no obvious cerebellar deformations are seen in rolling mice[8]. As the Ca,2.1 channel gene mutation selectively reduces the voltage sensitivity and activity of this channel[1,2,9], Ca,2.1 channel dysfunctions are considered to be fundamentally attributable to the motor deficits of rolling mice[8]. However, the underlying pathogenesis associated with or resulting from this mutation is not easy to understand because a complex relationship between Ca,2.1 expression and cerebellar abnormalities was already revealed in our previous studies and those of others. The Purkinje cells of the cerebellar cortex form a complex arrangement of parasagittal stripes and transverse zones, which are reflected in the diversity of gene expression patterns, such as zebrin II, phospholipase CB4, and the small heat shock protein HSP25[10,11,12,13,14]. This report summarizes our and other studies regarding cerebellar abnormalities either related or unrelated to specific Purkinje cell populations revealed by zebrin II expression in rolling mice.

**EXPRESSION OF MUTATED CA,2.1 CHANNELS IN ROLLING MOUSE CEREBELLM**

The mutated Ca,2.1 channel is expressed uniformly in all Purkinje cells in rolling[8,15] and other Ca,2.1 mutant mice[2,16]. The Ca,2.1 channel gene mutation selectively reduces the voltage sensitivity and activity of this channel[1,2,9]. Such dysfunctions of the Ca,2.1 channel alter Purkinje cell morphology. Electronmicroscopic observations revealed multiple synapses between the dendritic spines of Purkinje cells at single parallel fiber varicosity in rolling mice[17] and other Ca,2.1 mutants[18]. Furthermore, abnormal swellings (or “torpedoes”), which are known to be a neuropathological sign of axonal transport impairments[19], were seen in Purkinje cell axons of rolling mice (Fig. 1A, 1A‘)[8] as well as in other Ca,2.1 mutant mice[20].

**TYPE 1 RYANODINE RECEPTOR EXPRESSION**

Our recent study revealed that type 1 ryanodine receptor (RyR1) expression in Purkinje cells was uniformly reduced through all cerebellar lobules in rolling mice[21]. Such an expressional change was not regionally specific or related to any Purkinje cell phenotypes. Coexpression of RyR1 and the Ca,2.1 channel in all Purkinje cells of rolling mice indicates that expressional changes in RyR1 are linked with the Ca,2.1 channel dysfunction[21]. RyR1 expression also overlapped with the L-type Ca\(^{2+}\) channel \(\alpha_{1C}\) subunit (Ca,1.3) in the cerebellum of rolling mice[21]. The Ca,1.3 channel is known as the dihydropyridine receptor (DHPR) and has a direct conformational linkage with RyR1 in the neuronal plasma membrane to induce a Ca\(^{2+}\) release from the ER through RyR1 (DHPR-induced Ca\(^{2+}\) release)[22]. Therefore, DHPR-RyR1 complexes in Purkinje cells of rolling mice may be reduced by a diminished RyR1 expression.

**TYROSYNE HYDROXYLASE EXPRESSION**

Tyrosine hydroxylase (TH), the first-step enzyme for catecholamine synthesis, is mainly expressed in catecholaminergic neurons in the brain, but has also been expressed in some noncatecholaminergic neurons
FIGURE 1. Cerebellar abnormalities of rolling mouse Nagoya. Calbindin D-28k immunostaining in the cerebellum of rolling mouse at low (A) and high (A’) magnifications. Arrowheads indicate abnormal swellings (torpedoes) of Purkinje cell axons. Immunostaining for corticotropin-releasing factor (CRF) in the cerebellum of rolling (B) and control mice (C). Arrowheads in (B) indicate the borders of CRF-immunopositive climbing fiber stripe. The staining intensity is enhanced in the rolling cerebellum, whereas the characteristic stripe pattern of CRF is sustained between rolling and control mice. Serotonin (5-HT) immunostaining in the cerebellar cortex of the vermis (D, E) and the origins of cerebellar 5-HTergic fibers (F, G). 5-HT-immunopositive fibers were densely present in the vermis of a rolling mouse (D), but not a control mouse (E). Arrowheads in (D) and (E) indicate 5-HT-immunopositive fibers. 5-HT immunostaining in the lateral paragigantocellular reticular nucleus of medulla oblongata was also more intense in a rolling mouse (F) than in a control mouse (G). LPGi, lateral paragigantocellular reticular nucleus; mol, molecular layer; P, Purkinje cell layer; ROb, raphe obscurus. Bar = 50 μm in (A) [applied to (B), (C), (D), and (E)], Bar = 20 μm in (A’), Bar = 100 μm in (F) [applied to (G)].

of various brain regions during development and in experimental conditions, such as organotypic tissue culture and transplantation[23]. We have reported that TH was ectopically expressed in particular subsets of Purkinje cells in the rolling mouse cerebellum[23,24]. Ectopic expression of TH in the Purkinje cells is not specific to the Ca2.1 mutant[23,24], but is rather thought to reflect an increase of intracellular Ca2+...
concentrations[23]. Furthermore, the enzymatically active, phosphorylated form of TH has not been identified immunohistochemically in the Purkinje cells of rolling mice[25], suggesting the absence of any aberrant catecholamine synthesis. Interestingly, the expression pattern of TH resembles that of zebrin II[24], which is known as the most extensively studied marker of Purkinje cell phenotypes[26,27]. However, neither of those two distributions completely overlapped; TH-immunopositive Purkinje stripes were also present in the central zone (lobules VI to VII) and nodular zone (ventral IX and lobule X), where zebrin II is uniformly expressed[24]. TH-immunopositive Purkinje cell stripes in these regions formed an alternating array to HSP25-immunopositive Purkinje cell stripes[28]. As described in the next section, such ectopic TH expression in the rolling Purkinje cells is considered to be associated with corticotropin-releasing factor (CRF)–immunopositive climbing fiber innervation and not with expression of the mutated Ca, 2.1 channel.

**INNERVATION OF CRF-IMMUNOPOSITIVE CLIMBING FIBERS**

CRF immunostaining is found in climbing fiber subsets in the cerebellum, and a striking parasagittal pattern of CRF-immunopositive climbing fibers has been reported in several mammalian species[29,30,31,32,33,34]. In normal mice, the array of CRF-immunopositive climbing fiber stripes resembles the zebrin II–immunopositive Purkinje cell stripes, whereas these two distributions did not completely overlap[34]. CRF immunostaining in particular subsets of climbing fibers is enhanced in the rolling cerebellum (Fig. 1B,C)[35]. Our previous studies revealed that the overall distribution of CRF-immunopositive climbing fibers was not altered[35]. Furthermore, the expression of CRF mRNA increased in the inferior olivary origin of the CRF-immunopositive climbing fibers in rolling mice[36]. It is interesting to note that TH is ectopically expressed in the rolling mouse cerebellum corresponding to the terminal fields of CRF-immunopositive climbing fibers[35]. This suggested that CRF from a particular component of olivocerebellar tracts is involved in ectopic TH expression in the Purkinje cells of rolling mice. While the Ca, 2.1 channel is strongly expressed in the inferior olivary nucleus[37], the relationship is unclear between the Ca, 2.1 channel function and CRF expression in this nucleus.

On the other hand, CRF serves as a neuromodulator in the cerebellar circuit to enhance the glutamate sensitivity and reduce the GABA sensitivity of Purkinje cells[38,39] by suppressing the afterhyperpolarization[40]. Furthermore, CRF from climbing fibers plays a critical role in the induction of long-term depression (LTD) at the parallel fiber synapses of Purkinje cells[41]. Therefore, increased levels of CRF in climbing fibers of the rolling mouse cerebellum may alter the excitability and/or LTD induction of zebrin II–like, TH-defined Purkinje cell populations.

**SEROTONERGIC INNERVATION**

The enhancement of the serotonin (5-HT) metabolism and an increased 5-HTergic innervation have been revealed in the cerebellum of other ataxic mutant mice[42,43]. 5-HTergic fibers are distributed throughout the cerebellar cortex[44,45], and reduce Purkinje cell activity either directly or via Lugaro cells[46,47]. The distribution of 5-HT–immunopositive fibers in the vermis of the cerebellar cortex was much denser in rolling mice than in wild-type mice (Fig. 1D, E). However, 5-HT–immunopositive fibers were few in the hemisphere in both rolling and control mice. In addition, the distribution of 5-HT–immunopositive fibers in the rolling cerebellum did not involve either zebrin II– or TH-defined Purkinje cell compartments.

5-HT immunostaining of the origins of cerebellar 5-HTergic fibers (the lateral paragigantocellular reticular nucleus of medulla oblongata) was also enhanced in rolling mice (Fig. 1F, G). Since an enhancement of 5-HT concentration in both the cerebellum and brainstem has been reported in rolling mice[48], much denser 5-HT–immunopositive fibers in the rolling mouse than in the wild-type cerebellum may reflect increased levels of 5-HT in the cerebellar 5-HTergic fibers in the former. This
suggests that such increased 5-HTergic innervations of the cerebellar cortex may alter the regulation of Purkinje cell excitability and/or of the LTD in rolling mice.

The Ca_{2.1} channel is expressed in the medullary raphe nuclei[37] and blockade of this channel is known to enhance the firing rate of medullary raphe neurons by reducing afterhyperpolarization[49]. Therefore, the increased 5-HTergic innervations of the rolling cerebellum may be involved in the enhanced excitability of medullary raphe neurons by dysfunction of the mutated Ca_{2.1} channel.

CONCLUSIONS

Although motor deficits of rolling mice are characterized by frequent lurching of the mice and abnormal cyclic movements of the hindlimbs when walking[6,7], no obvious cerebellar deformations have been reported in this mutant. Our studies of the rolling mouse cerebellum have revealed the cerebellar abnormalities based on chemical neuroanatomy. The reduced RyR1 expressions in Purkinje cells and the increased 5-HTergic innervations of the cerebellar cortex in rolling mice may be linked with Ca_{2.1} channel expression[21]. Ectopic TH expression appeared within a specific Purkinje cell population revealed by zebrin II expression[23,24] and was related to the innervations of CRF-immunopositive climbing fibers[35], whereas the relationship between the Ca_{2.1} channel function and CRF expression of the inferior olivary nucleus was unclear. Thus, to determine whether or not cerebellar abnormalities are related to Purkinje cell populations revealed by zebrin II expression is essential for enhancing our understanding of the pathogenesis of hereditary cerebellar ataxic mutants such as rolling mice.

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