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.

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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* (*Cchl1a4*) 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

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
tottering	*tottering (tg)*	8 ; 38.5 cM	mild ataxia
	or *Cchl1a 4*		generalized absence-like seizures
			(petit mal-like epilepsy)
			sporadic focal myoclonic seizures
leaner	*tottering* (tg^{la})	8 ; 38.5 cM	severe immobilizing ataxia
	or *Cchl1a 4*		absence seizures
rolling mouse Nagoya	*tottering* (tg^{rol})	8 ; 38.5 cM	ataxia
	or *Cchl1a 4*		
dilute-lethal	*dilute* (d^{l})	9 ; 42.0 cM	ataxia
	or *Myo5a*		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 decalboxylase (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 Ca^{2+} response element was present in the TH promoter, and non-CAergic neurons expressed the TH transcripts following Ca^{2+} influx (22-24). These results indicate that abnormal TH expression in the mutant Purkinje cells may be caused by misregulation of intracellular Ca^{2+} concentration.

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

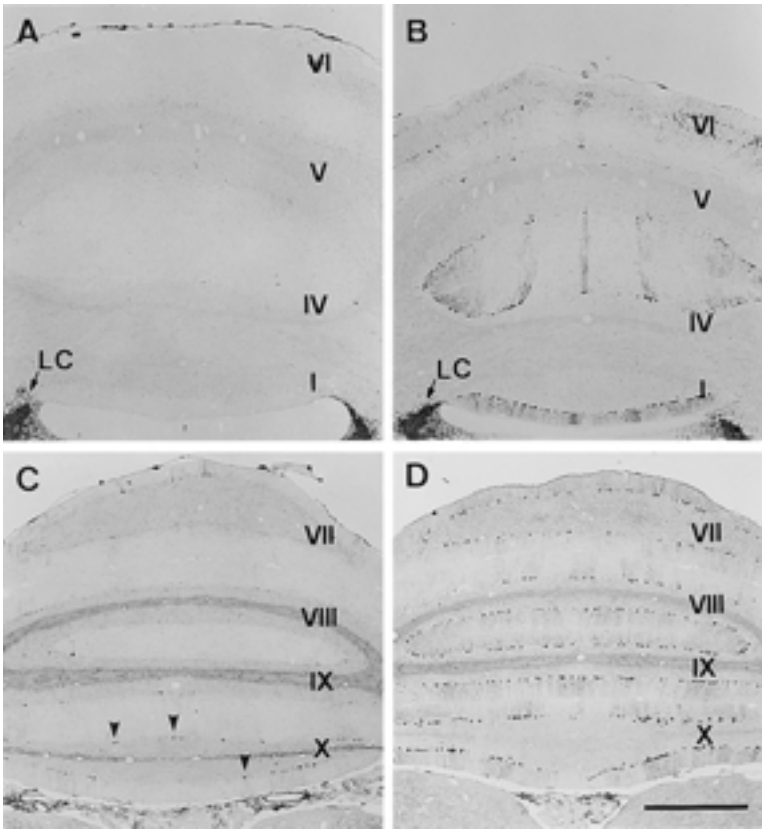

Fig. 1. Immunostaining for tyrosine hydroxylase (TH) in the cerebellum of a control mouse (A, C) and rolling mouse Nagoya (RMN)(B, D). A, B. Anterior vermis, C, D. Posterior vermis. In RMN, definite TH staining was observed in the perikarya and dendrites of some Purkinje cells. TH-positive Purkinje cells 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 μm

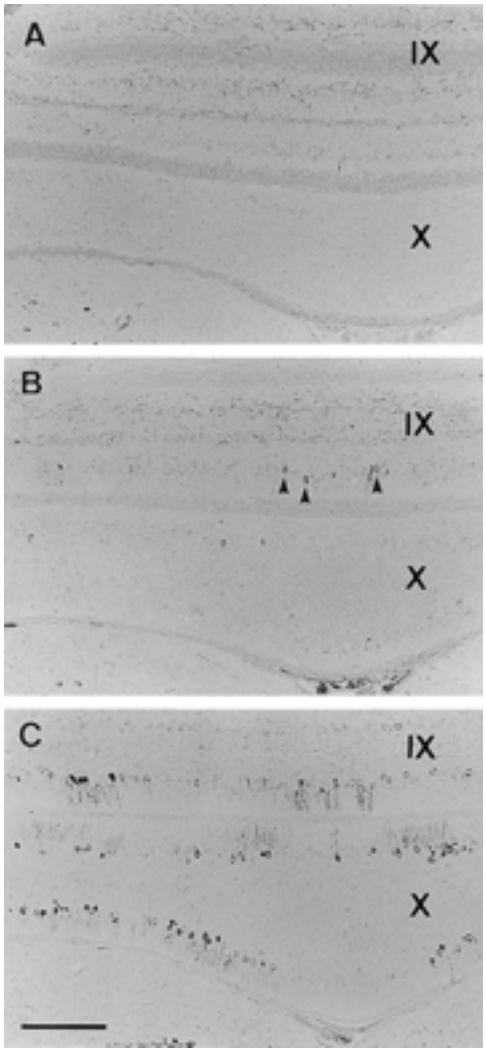

Fig.2. Immunostaining for tyrosine hydroxylase (TH) in lobules IX and X of the posterior vermis of *dilute-lethal* mice (DL). (A) Day 8. Few TH-positive Purkinje cells were found, similarly to wild-type (+/+) or heterozygous (d^{l}/+) mice. DL walked normally on this age. (B) Day 9. TH-positive Purkinje 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 μm

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* (*Cchlla 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*

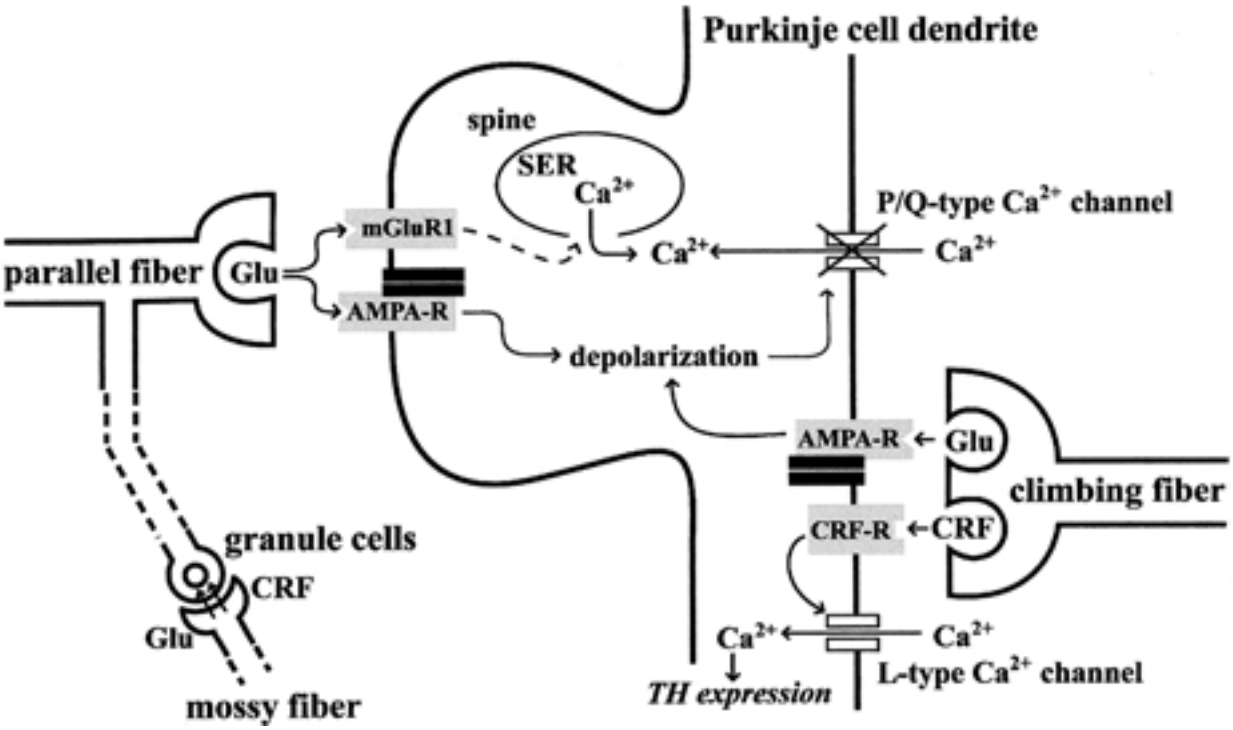

Fig. 3. Presumptive mechanism by tyrosine hydroxylase (TH) expression in Purkinje cells of ataxic mutant mice belonging to the *tottering* allelic group. In *tottering*, *leaner* and rolling mouse Nagoya, the α_{1A} subunit of the P/Q-type Ca^{2+} channel was mutated, and expression of the α_{1c} subunit of the L-type Ca^{2+} channel increased in compensation for altered function of the P/Q-type Ca^{2+} channel. An increased level of corticotropin-releasing factor (CRF) in climbing fibers in the mutants might facilitate Ca^{2+} influx through the L-type Ca^{2+} 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 Ca^{2+} currents through the L-type Ca^{2+} channel (36). Therefore, an increased level of CRF in climbing fibers may facilitate Ca^{2+} influx through the α_{1c} subunit of the L-type Ca^{2+} 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 Ca^{2+}, TH expression in Purkinje cells of the mutant mice may indicate neural dysfunction by alteration of the intracellular Ca^{2+} concentrations.

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REFERENCES

1. Jaeger CB, Joh TH : Transient expression of tyrosine hydroxylase in some neurons of the developing inferior colliculus of the rat. Dev Brain Res 11 : 128-132, 1983
2. Berger B, Verney C, Gaspar P, Febvret A: Transient expression of tyrosine hydroxylase immunoreactivity in some neurons of the rat neocortex during postnatal development. Dev Brain Res 23 : 141-144, 1985
3. Verney C, Gaspar P, Febvret A, Berger B : Tran-

sient tyrosine hydroxylase-like immunoreactive neurons contain somatostatin and substance P in the developing amygdala and bed nucleus of the stria terminalis of the rat. Dev Brain Res 42 : 45-58, 1988.
4. Satoh J, Suzuki K:Tyrosine hydroxylase-immunoreactive neurons in the mouse cerebral cortex during the postnatal period. Dev Brain Res 53 : 1-5, 1990
5. Nagatsu I, Takeuchi T, Sakai M, Karasawa N, Yamawaki Y, Arai R, Nagatsu T : Transient appearance of tyrosine hydroxylase-immunoreactive non-catecholaminergic neurons in the medial geniculate nucleus of postnatal mice. Neurosci Lett 211 : 183-186, 1996
6. Hess EJ, Wilson MC : Tottering and leaner mutations perturb transient developmental expression of tyrosine hydroxylase in embryologically distinct Purkinje cells. Neuron 6 : 123-132, 1991
7. Austin MC, Schultzberg M, Abbott LC, Montpied P, Evers JR, Paul SM, Crawley JN : Expression of tyrosine hydroxylase in cerebellar Purkinje neurons of the mutant tottering and leaner mouse. Mol Brain Res 15 : 227-240, 1992
8. Abbott LC, Isaacs KR, Heckroth JA : Co-localization of tyrosine hydroxylase and zebrin II immunoreactivities in Purkinje cells of the mutant mice, tottering and tottering/leaner. Neuroscience 71 : 461-475, 1996
9. Sawada K, Komatsu S, Haga H, Sun X-Z, Hisano S, Fukui Y:Abnormal expression of tyrosine hydroxylase immunoreactivity in cerebellar cortex of ataxic mutant mice. Brain Res 829 : 107-112, 1999
10. Østergaard K, Schou JP, Gähwiler BH, Zimmer J : Tyrosine hydroxylase immunoreactive neurons in organotypic slice cultures of the rat striatum and neocortex. Exp Brain Res 83 : 357-365, 1991
11. Seil FJ, Johnson ML, Nishi R, Nilaver G: Tyrosine hydroxylase expression in non-catecholaminergic cells in cerebellar cultures. Brain Res 569 : 164-168, 1992
12. Park JK, Joh TH, Ebner FF : Tyrosine hydroxylase is expressed by neocortical neurons after transplantation. Proc Natl Acad Sci USA 83 : 7495-7498, 1986
13. Fujii T, Sakai M, Nagatsu I : Immunohistochemical demonstration of expression of tyrosine hydroxylase in cerebellar Purkinje cells of the human and mouse. Neurosci Lett 165 : 161-163, 1994
14. Nelson TE, King, JS, Bishop GA : Distribution of tyrosine hydroxylase-immunoreactive afferents to the cerebellum differs between species. J Comp Neurol 379 : 443-454, 1997
15. Green MC, Sidman RL : Tottering-a neuromuscular mutation in the mouse. J Hered 53 : 233-237, 1962
16. Tsuji S, Meier H : Evidence for allelism of leaner and tottering in the mouse. Genet Res Camb 17 : 83-88, 1971
17. Oda S : A new allele of the tottering locus, rolling mouse Nagoya, on chromosome no.8 in the mouse. Jpn J Genet 56 : 295-299, 1981
18. Strobel MC, Seperack PK, Moore KJ, Copeland NG, Jenkins NA : The dilute coat-color locus of mouse chromosome 9. Prog Clin Biol Res 256 : 297-305, 1988
19. Sawada K, Komatsu S, Haga H, Oda S, Fukui Y : Abnormal expression of tyrosine hydroxylase immunoreactivity in Purkinje cells precedes the onset of ataxia in *dilute-lethal* mice. Brain Res 844 : 188-191, 1999
20. Sawada K, Haga H, Fukui Y : Ataxic mutant mice with defects in Ca^{2+} channel α_{1A} subunit gene : morphological and functional abnormalities in cerebellar cortical neurons. Cong Anom 40 : 99-107, 2000
21. Muramoto O, Ando K, Kanazawa I : Central noradrenaline metabolism in cerebellar ataxic mice. Brain Res 237 : 387-395, 1982
22. Kilbourne EJ, Nankova BB, Lewis EJ, McMahon A, Osaka H, Sabban, DB, Sabban EL : Regulated expression of the tyrosine hydroxylase gene by membrane depolarization. J Biol Chem 267 : 7563-7569, 1992
23. Nankova B, Hiremagalur B, Menezes A, Zeman R, Sabban E. Promoter elements and second messenger pathways involved in transcriptional activation of tyrosine hydroxylase by ionomycin. Mol Brain Res 35 : 164-172, 1996
24. Nagamoto-Combs K, Piech KM, Best JA, Sun B, Tank AW : Tyrosine hydroxylase gene promoter activity is regulated by both cyclic AMP-responsive element and AP1 sites following calcium influx. J Biol Chem 272 : 6051-6058, 1997
25. Takagishi Y, Oda S, Hayasaka S, Dekker-Ohno K, Shikata T, Inouye M, Yamamura H : The *dilute-lethal* (*d^l*) gene attacks a Ca^{2+} store in the dendritic spine of Purkinje cells in mice. Neurosci Lett 215 : 169-172, 1996

26. **Hoth M, Penner R : Depletion of intracellular calcium stores activates a calcium current in mast cells. Nature 335 : 353-356, 1992**
27. **Fletcher CF, Lutz CM, O'Sullivan TN, Shaughnessy JD Jr, Hawkes, R, Frankel WN, Copeland NG, Jenkins NA : Absence epilepsy in tottering mutant mice is associated with calcium channel defects. Cell 87 : 607-617, 1996**
28. **Mori Y, Wakamori M, Oda S, Fletcher CF., Sekiguchi N, Mori E, Copeland NG, Jenkins NA, Matsushita K, Matsuyama Z, Imoto K : Reduced voltage sensitivity of activation of P/Q-type Ca^{2+} channels is associated with the ataxic mouse mutation rolling Nagoya (tg^{rol}). J Neurosci 20 : 5654-5662, 2000**
29. **Lau FC, Abbott LC, Rhyu IJ, Kim DS, Chin H : Expression of calcium channel α_{1A} mRNA and protein in the leaner mouse (tg^{la}/tg^{la}) cerebellum. Mol Brain Res 59 : 93-99, 1998**
30. **Dove LS, Abbott LC, Griffith WH : Whole-cell and single-channel analysis of P-type calcium currents in cerebellar Purkinje cells of leaner mutant mice. J Neurosci 18 : 7687-7699, 1998**
31. **Lorenzon NM, Lutz CM, Frankel WN, Beam KG : Altered calcium channel currents in Purkinje cells of the neurological mutant mouse *leaner*. J Neurosci 18 : 4482-4489, 1998**
32. **Wakamori M, Yamazaki K, Matsunodaira H, Teramoto T, Tanaka I, Niidome T, Sawada K, Nishizawa Y, Sekiguchi N, Mori E, Mori Y, Imoto K : Single tottering mutations responsible for the neuropathic phaenotype of the P-type calcium channel. J Biol Chem 273:34857-34867, 1998**
33. **Campbell DB, Hess EJ : L-type calcium channels contribute to the tottering mouse dystonic episodes. Mol Pharmacol 55 : 23-31, 1999**
34. **Fureman BE, Campbell DB, Hess EJ : L-type calcium channel regulation of abnormal tyrosine hydroxylase expression in cerebella of tottering mice. Ann NY Acad Sci 868 : 217-219, 1999**
35. **Sawada K, Sakata-Haga H, Hisano S, Fukui Y : Topological relationship between corticotropin-releasing factor-immunoreactive cerebellar afferents and tyrosine hydroxylase-immunoreactive Purkinje cells in a hereditary ataxic mutant, rolling mouse Nagoya. Neuroscience *in press***
36. **Kanno T, Suga S, Nakano K, Kamimura N, Wakui M : Corticotropin-releasing factor modulation of Ca^{2+} influx in rat pancreatic β-cells. Diabetes 48 : 1741-1746, 1999**