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Regulation of Tyrosine Hydroxylase Expression in *tottering* Mouse Purkinje Cells

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Tottering (*tg*) mice inherit a missense mutation in the α_{1A} subunit of P/Q-type calcium channels. This mutation results in an increased density of L-type calcium channels in the cerebellum and abnormal regulation of tyrosine hydroxylase (TH) gene expression in a subset of cerebellar Purkinje cells, a cell type that does not normally express TH. The behavioral phenotype includes attacks of dyskinesia, which can be blocked by L-type calcium channel antagonists. To test the hypothesis that cerebellar TH mRNA expression can be manipulated *in vivo* by L-type calcium channel blockade, control and tottering mice were chronically treated with the L-type calcium channel antagonist nimodipine. Chronic nimodipine treatment significantly reduced the expression of TH mRNA in tottering mouse Purkinje cells. This effect was observed without altering the increased density of L-type calcium channels in tottering mouse cerebella. Chronic nimodipine treatment had no effect on TH mRNA expression in tottering mouse catecholaminergic neurons, including those of the locus coeruleus and substantia nigra. However, a small reduction in TH mRNA expression in the substantia nigra of control mice was observed after drug treatment. These data suggest that the abnormal expression of TH in tottering mouse Purkinje cells is regulated by Purkinje cell excitability.

Keywords: Tottering; Gene expression; Dihydropyridine; Cerebellum; Mouse mutant calcium channel; Nimodipine

INTRODUCTION

The influx of calcium ions through voltage-dependent calcium channels regulates several events in the central nervous system, including neurotransmitter release, activation of intracellular second messengers, and transcription. Six subtypes of voltage-dependent calcium channels regulate calcium influx across electrically charged cell membranes in mammalian systems. These subtypes (L-, N-, P-, Q-, R-, and T-type) have distinct expression patterns, developmental profiles and physiologic responses (for reviews see Catterall, 1995; Perez-Reyes and Schneider, 1995). Additionally, the voltage-dependent calcium channel subtypes subserve unique functions. N-type and P/Q-type calcium channels located at nerve termini appear to be responsible for the calcium influx triggering neurotransmitter release (Sheng *et al.*, 1998). In contrast, L-type calcium channels at the soma (Hell *et al.*, 1993) provide a critical link between neuronal activity and both short and long-term changes in neuronal gene expression (Finkbeiner and Greenberg, 1998).

Mice harboring mutations in neuronal calcium channel subunits provide animal models of calcium misregulation. The tottering missense mutation in the α_{1A} subunit gene (Fletcher *et al.*, 1996), which encodes P- and Q-type calcium channels (Bourinet *et al.*, 1999), results in a behavioral phenotype that includes polyspike discharges, ataxia and paroxysmal dyskinesia. At the cellular level, this mutation leads to a reduction in the current density of P-type calcium channels (Wakamori *et al.*, 1998) and the abnormal expression of tyrosine hydroxylase in tottering mouse Purkinje cells, a cell type that does not normally express this catecholaminergic enzyme in adults (Hess and Wilson, 1991; Austin *et al.*, 1992). The decrease in P-type calcium current density and the increase in TH expression in tottering mouse Purkinje cells is surprising, as

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experiments performed *in vitro* have demonstrated that an increase in calcium promotes the expression of TH mRNA (Vidal *et al.*, 1989; Brosenitsch *et al.*, 1998; Cigola *et al.*, 1998). However, *in vivo* regulation of abnormal TH expression in these mutants is likely influenced by events downstream of the primary mutation and more complex than a direct genotype to phenotype association might predict. First, an increase in L-type calcium channel density, which occurs as a secondary effect of the P/Q-type calcium channel mutation, is observed in the cerebella of tottering mice (Campbell and Hess, 1999). Consistent with *in vitro* studies of TH gene regulation, an increase in calcium influx through L-type calcium channels *in vivo* may promote the expression of TH, regardless of the level of whole-cell calcium current density through other voltage-dependent calcium channel subtypes (Vidal *et al.*, 1989; Brosenitsch *et al.*, 1998; Cigola *et al.*, 1998). Next, the attacks of dyskinesia exhibited by tottering mice cause an increase in cerebellar activation, including activation of Purkinje cells (Campbell and Hess, 1998). This increase in activity may also drive the abnormal expression as depolarization increases TH expression *in vitro* (Kessler, 1985; Katz *et al.*, 1986; Kilbourne *et al.*, 1992; Menezes *et al.*, 1996; Cigola *et al.*, 1998; Brosenitsch and Katz, 2001).

The L-type calcium channel blockers nimodipine, nifedipine, and nitrendipine readily cross the blood-brain barrier to exert central effects. These potent and selective compounds prevent episodes of dyskinesia in tottering mice (Campbell and Hess, 1999; Fureman *et al.*, 2002) and reduce TH expression *in vitro* (Vidal *et al.*, 1989; Brosenitsch *et al.*, 1998; Cigola *et al.*, 1998). To test the hypothesis that cerebellar TH mRNA expression can be manipulated *in vivo* by L-type calcium channel blockade, control and tottering mice were chronically treated with the specific L-type calcium channel blocker nimodipine.

MATERIALS AND METHODS

Animals

C57BL/6J +/*tg* mice were obtained from The Jackson Laboratories and bred at the Pennsylvania State University College of Medicine. Adult tottering male and female mice (16-24 weeks of age) were identified either by analysis of PCR amplified simple sequence length polymorphisms in C57BL/6J +/*tg* x C57BL/6J +/*tg* cross progeny (Campbell and Hess, 1997), or by the absence of oligosyndactylism in *Os* +/+ *tg* x *Os* +/+ *tg* cross progeny. Age- and gender-matched C57BL/6J +/+, +/*tg* or *Os* +/+ *tg* mice were used as controls. Animal procedures were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Drug Administration

Nimodipine (RBI, Natick, MA, USA) was prepared at 2 mg/ml in 14.5% ethanol/ 2.25% Tween80 in 0.9% saline. The mice received a 5 ml/kg volume of nimodipine or vehicle twice daily (20 mg/kg/d, s.c.) at 12 h intervals for a total of 14 days. Because it was impractical to video-monitor all subjects for dyskinetic attacks over the course of the 14 day chronic treatment, a time-course was performed to estimate the protective effects of nimodipine. For the nimodipine time-course, a separate group of tottering mice were injected with 10 mg/kg nimodipine, the same dose used for the chronic treatment. A separate group was used to avoid the confound of excess restraint stress in the test group. At 30 min, 1 h, 2 h, 4 h, 6 h and 8h after injection (n = 12/timepoint; 72 mice total), mice were restrained in a 60 cc syringe for 10 min and released to a novel cage to induce an attack of dyskinesia, as previously described (Campbell and Hess, 1998). Mice were scored for the presence or absence of an attack during the ensuing 40 min. The results were analyzed by logistic regression and compared to the responses of drug-naïve restrained tottering mice.

In Situ Hybridization

Mice were deeply anesthetized with carbon dioxide and killed by decapitation. Brains were rapidly removed and frozen in isopentane at -40°C and stored at -70°C. Sagittal sections (20 µm) were cut using a cryostat and thaw-mounted on Superfrost Plus glass slides (Fisher, Pittsburgh, PA, USA). Because slight variations in hybridization intensity can occur from slide to slide, sagittal sections were taken to promote quantification of TH expression in the substantia nigra, locus coeruleus, olfactory bulb and Purkinje cells within the same section, with sections from each experimental condition mounted on a single slide. After drying, the slide-mounted sections were stored at -70°C.

A 1.7 kb fragment of the mouse TH cDNA in pBluescript KS+ (gift from T. Nagatsu, Fujita Health University, Japan) was the template for sense and antisense transcription reactions. *In vitro* transcription was performed for 2 h at 37°C in a 25 µl volume containing 40 mM Tris, pH 7.9, 6 mM $MgCl_2$, 2 mM dithiothreitol (DTT), 40 U RNase inhibitor (Promega, Madison, WI,

USA), 400 µM each ATP, GTP and UTP, 10 µM [^{35}S]CTP (800 Ci/mmol), 1 µg linearized plasmid and 20 U RNA polymerase (Promega). Following transcription reactions, DNA templates were removed by RNase-free DNase (Promega) digestion for 30 min at 37°C. Transcripts were size-reduced by alkalai treatment with 0.2 M NaOH for 45 min on ice. Probes were extracted with phenol:chloroform:isoamyl alcohol (25:24:1) and separated on a G50 Sephadex Nick column (Pharmacia, Piscataway, NJ, USA) to remove unincorporated nucleotides.

Slide-mounted sections were pretreated by fixation in buffered 4% formaldehyde for 15 min at room temperature followed by a 5 min rinse in 0.1 M phosphate buffered saline (PBS). Slides were treated with 0.25% acetic anhydride in 0.1 M triethanolamine-HCl/0.15 M NaCl (pH 8.0) for 10 min and rinsed in 2X standard sodium citrate (1X SSC; 0.15 M NaCl, 0.015 M sodium citrate). Sections were dehydrated in graded ethanols for 1 min each followed by two 5 min incubations in chloroform. One min incubations in 100% and 95% ethanol were followed by air drying.

Slides were hybridized with 100 µl of hybridization buffer containing 7.5 ng cRNA probe in 50% formamide, 0.75 M NaCl, 20 mM 1,4-piperazine diethane sulfonic acid (pH 6.8), 10 mM EDTA, 10% dextran sulfate, 5X Denhardt's solution (0.02% bovine serum albumin, 0.02% ficoll, 0.02% polyvinylpyrolidone), 50 mM DTT, 0.2% sodium dodecyl sulfate and 100 µg/ml each salmon sperm DNA and yeast tRNA. Slides were coverslipped, sealed with Royalbond Grip contact cement (Columbia Aluminum Products, CA, USA) and hybridized for 16 h at 56°C.

Following overnight hybridization, coverslips were removed in 4X SSC with 300 mM 2-mercaptoethanol at room temperature. Slides were incubated in this solution for 15 min followed by 15 min in 4X SSC alone. The slides were treated with 50 µg/ml pancreatic RNase A in 0.5 M NaCl, 50 mM Tris, pH 8.0, 5 mM EDTA for 30 min at 37°C, washed in graded salt solutions (2X, 1X, and 0.5X SSC each for 5 min at 56°C), and in 0.1X SSC at 65°C for 30 min. Slides were dipped in 60% ethanol with 0.33 M ammonium acetate and air dried. Sections were exposed to x-ray film (DuPont Cronex) and subsequently dipped in Kodak NTB-2 photographic emulsion (diluted 1:2 with dH_2O), exposed at 4°C and developed in Kodak D19 developer.

Analysis of mean grain density was performed on a Macintosh computer using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). For each region, mean grain density was measured as square pixels remaining after the images were normalized by adjusting the threshold in NIH Image. To measure TH mRNA expression in tottering mouse cerebellum, darkfield views of anterior and posterior cerebellum were image-captured (using the primary fissure as the boundary between anterior and posterior), inverted, and the entire anterior or posterior cerebellum quantified; 3 sections/animal were assessed with n=4-5. For single Purkinje cell quantification, darkfield images were captured and inverted. Cells were randomly selected for quantification using random number tables or www.randomizer.org. At least 150 anterior cerebellar Purkinje cells and at least 300 posterior cerebellar Purkinje cells were assessed for each subject. For single cell quantitation of TH mRNA in the substantia nigra and locus coeruleus, grain density over individual cells was counted in inverted darkfield views. For the substantial nigra and locus coeruleus, 16 cells/mouse were assessed with n=4-5. To quantify TH mRNA expression in periglomerular cells of the olfactory bulb, darkfield images were captured, inverted, and counted over periglomerular cells with 9 counts/mouse with n=4-5.

[^{3}H]PN200-110 Saturation Binding Assays

Following the fourteenth day of nimodipine administration, age- and gender-matched tottering and control mice were allowed a 24 h clearance period to eliminate residual nimodipine. The mice were then deeply anesthetized with carbon dioxide and killed by decapitation. Brains were rapidly removed, dissected into cerebellum and forebrain (including all tissue rostral to the inferior colliculus), frozen in isopentane at -40°C and stored at -70°C. In most cases, saturation analysis was performed using tissue from a single brain; in some cases, it was necessary to pool tissue from two brains to obtain enough tissue for analysis. Tissue was homogenized in 100 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.5) with a Tekmar tissue homogenizer (Cincinnati, OH, USA) at setting 70 for 15 sec. The homogenates were centrifuged at 30,000g for 12 min and the supernatant discarded. Pellets were resuspended by homogenization in ice-cold buffer and centrifuged at 30,000g for 12 min. Pellets were resuspended at 10 mg wet weight tissue/ml buffer (forebrain) and 20 mg wet weight tissue/ml buffer (cerebellum). Binding assays were performed in a total volume of 2 ml in 50 mM Tris-HCl buffer for 45 min at 37°C. Each

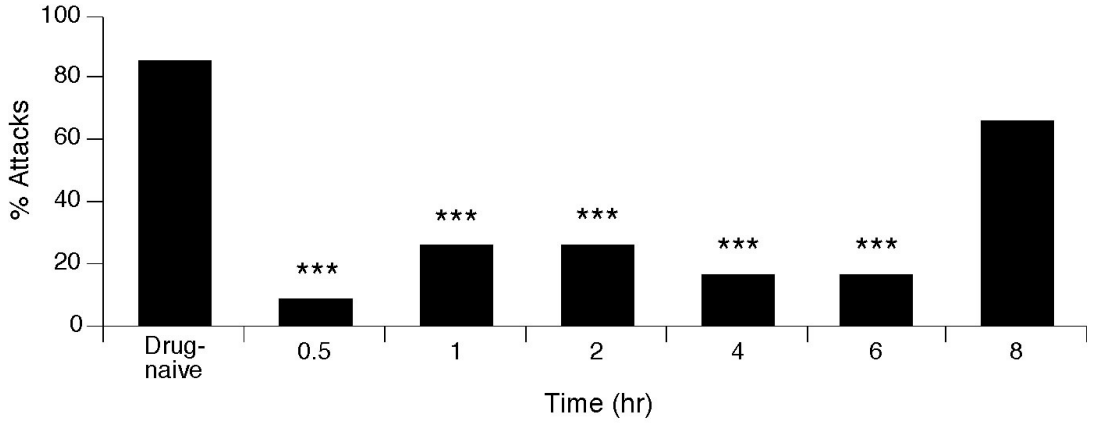

FIGURE 1 Time-course of the effect of a single injection of 10 mg/kg nimodipine on restraint-induced attacks of dyskinesia in tottering mice. Data are expressed as a percent of mice exhibiting attacks at each timepoint. Logistic regression analysis revealed that attacks of dyskinesia were significantly reduced at 30 min (Chi-square = 12.42; p <.001), 1 h (Chi-square = 10.99; p <.001), 2 h (Chi-square = 10.99; p <.001), 4 h (Chi-square = 12.625; p <.001) and 6 h (Chi-square = 12.625; p <.001) after nimodipine injection (n=12/timepoint) compared to drug-naïve tottering mice (n=27) (Fureman et al., 2002). *** indicates significant difference from drug-naive restrained tottering mice.

reaction contained either 1.0 mg/ml (forebrain) or 2.0 mg/ml (cerebellum) tissue. Concentrations of [^{3}H]PN200-110 (82 Ci/mMol, Amersham, Piscataway, NJ, USA) ranging from 0.0125 to 0.4 nM defined total binding; nonspecific binding was determined in the presence of 1 µM nifedipine. Saturation analyses consisted of five or six concentrations of [^{3}H]PN200-110 depending on tissue availability. Reactions were terminated by rapid filtration over Whatman GF/C filters and washed three times with an equal volume of ice-cold buffer. Filters were air-dried, then placed in ScintiVerse scintillation fluid. Radioactivity was measured by liquid scintillation spectroscopy at an efficiency of ~55%. Data were analyzed using Ligand software (Munson and Robard, 1980).

RESULTS

Timecourse of the Inhibition of Dyskinetic Attacks by Nimodipine

To estimate how long chronically-treated mice were protected from attacks of dyskinesia following each injection during the chronic experiment, the timecourse of the protective effect of a single dose of 10 mg/kg nimodipine was investigated. Nimodipine significantly reduced restraint-induced attacks at 30 min, 1 h, 2 h, 4 h, and 6 h after injection. At 8 h post-injection, 66% of tottering mice had a dyskinetic attack after restraint, similar to drug-naive tottering mice (FIG. 1).

Effect of Chronic Nimodipine Treatment on TH mRNA Expression

The cerebellum was divided into anterior and posterior

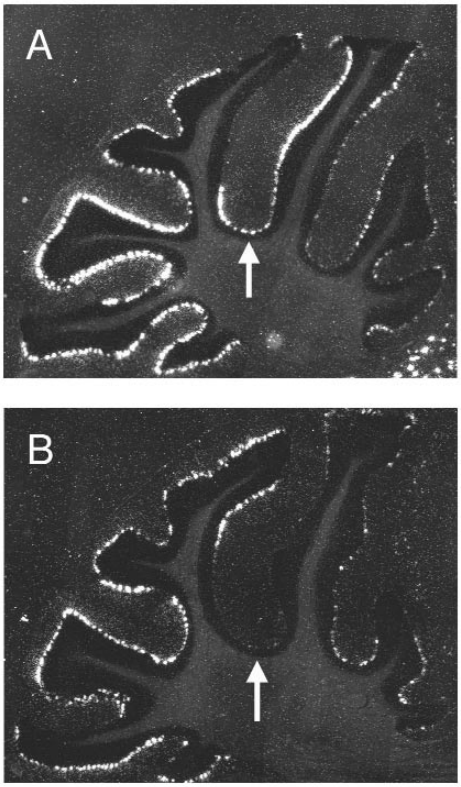

FIGURE 2 Darkfield photomicrosgraph of *in situ* hybridization for TH mRNA in vehicle- (A) and nimodipine- (B) treated tottering mouse cerebella. Arrowheads indicate the primary fissure.

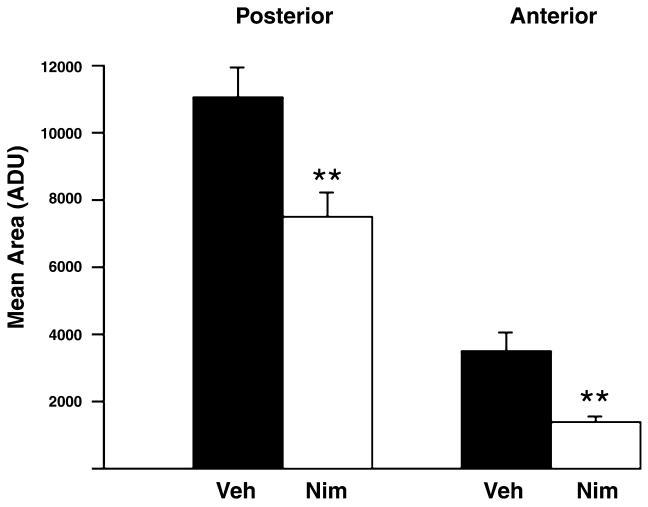

FIGURE 3 Effect of chronic nimodipine treatment on TH mRNA expression in tottering mouse Purkinje cells. Data are mean ± S.E.M. from measurements of the entire anterior or posterior cerebellum in vehicle- (filled bar) and nimodipine - (open bar) treated tottering mice. TH mRNA expression was significantly greater in the posterior cerebellum (n=4-5, $p < 0.0001$, two-way repeated measures ANOVA) and nimodipine treatment significantly reduced TH mRNA expression ($p < 0.01$). ** indicates significant difference from vehicle-treated tissue.

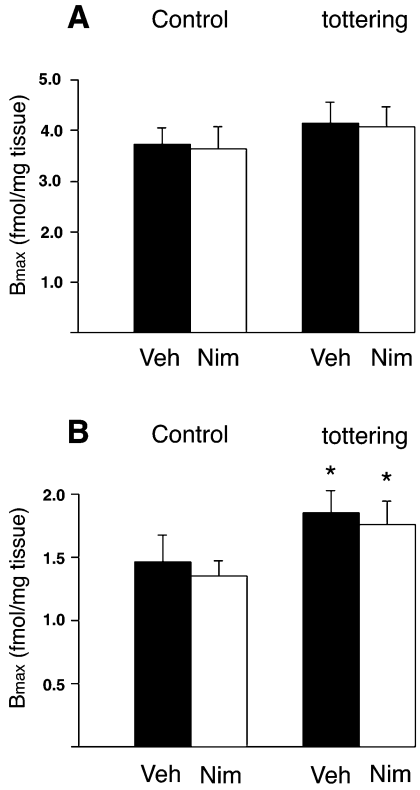

FIGURE 4 Density of [^{3}H]PN200-110 binding sites after chronic drug treatment in control and tottering mice. Data represent mean ± S.E.M. (n= 8) in forebrain (**A**) or cerebellum (**B**) of vehicle- (filled bar) and nimodipine - (open bar) treated tottering mice. The density of L-type calcium channels was significantly greater in tottering mouse cerebellum, regardless of drug treatment ($p < 0.05$, two-way repeated measures ANOVA). Equilibrium dissociation constants (KD) were comparable in all groups. * indicates significant difference from wild type tissue.

Table I Tyrosine hydroxylase mRNA expression after chronic nimodipine treatment

	Substantia nigra	Locus coeruleus	Olfactory bulb
Vehicle-treated control mice	837 ± 32	868 ± 123	3409 ± 417
Nimodipine-treated control mice	704 ± 29	679 ± 96	3470 ± 594
Vehicle-treated tottering mice	828 ± 54	975 ± 138	4416 ± 439
Nimodipine-treated tottering mice	853 ± 21	979 ± 72	3809 ± 353

Data are expressed in arbitrary densitometric units and represent means ± SEMs.

regions with the primary fissure defining the border between regions. TH mRNA was abundantly expressed in tottering mouse Purkinje cells (FIG. 2). TH mRNA was differentially expressed in tottering mouse cerebella with significantly greater expression in the posterior cerebellum than anterior cerebellum ($p < 0.0001$). After chronic treatment with nimodipine, a significant reduction in the expression of TH mRNA was observed in tottering mouse Purkinje cells (FIG. 3). In both anterior and posterior cerebellum, TH mRNA expression was significantly reduced while the rostral-caudal gradient of the TH mRNA expression was still present after chronic treatment (FIG. 3). Quantification of cerebellar TH mRNA expression by either single cell assessments or measurements of the entire region yielded similar results. The effect of chronic nimodipine treatment in normal mouse cerebellum was not assessed because the population of TH-positive Purkinje cells was too small to provide an accurate measurement of the drug effect.

Dopaminergic neurons in the substantia nigra expressed abundant TH mRNA in both tottering and normal mice. Although tottering mice appeared to express more TH mRNA in this region, the trend was not significant compared to control mice ($p < 0.1$). However, there was a small but significant genotype by treatment interaction effect ($p < 0.05$) whereby nimodipine treatment decreased the expression of TH mRNA in the substantia nigra of control mice by ~15%, but did not alter TH mRNA expressed in dopaminergic cells in tottering mice (Table I).

TH mRNA is also expressed in the dopaminergic periglomerular cells of the olfactory bulb and the noradrenergic neurons of the locus coeruleus. There was no significant difference in TH mRNA expression between tottering and control mouse periglomerular cells or locus coeruleus neurons, nor did chronic nimodipine administration affect TH mRNA expression in either region (Table I).

Effect of Chronic Nimodipine Treatment on the Regulation of L-type Calcium Channels

The density of L-type calcium channels was assessed in the forebrain and cerebellum of normal and tottering mice by radioligand binding using the L-type specific calcium channel compound [^{3}H]PN200-110 (Weiland and Oswald, 1985). The density of L-type channels in the cerebellum of tottering mice was significantly greater than in control mice ($p < 0.05$, FIG. 4B), consistent with previous reports (Campbell and Hess, 1999). In contrast, L-type calcium channel density was comparable in tottering and control mouse forebrain (FIG. 4A). Chronic nimodipine treatment did not affect L-type calcium channel density in either tottering or control mice. The tottering mutation did not affect the channel binding affinity for the [^{3}H]PN200-110 ligand. Likewise, chronic nimodipine treatment did not alter channel affinity for the ligand, suggesting that little residual nimodipine was left in the tissue following the 24 h drug clearance period.

DISCUSSION

Expression of TH mRNA in tottering mouse Purkinje cells was significantly reduced following L-type calcium channel blockade. Several mechanisms of action may account for this result. The most parsimonious explanation for the nimodipine-induced reduction in TH mRNA expression is blockade of L-type calcium channels directly on Purkinje cells, thereby decreasing the calcium signals regulating TH gene expression. However, L-type calcium channels are also expressed by cerebellar granule cells (Tanaka *et al.*, 1995) and cells in the inferior olivary nucleus (Hillman *et al.*, 1991), which both provide excitatory input to Purkinje cells. Blockade of L-type calcium channels on cerebellar granule cells and olivary climbing fibers would decrease excitatory input to Purkinje cells. Attacks of dyskinesia are also prevented by L-type calcium channel blockade, and although the relationship between attacks of dyskinesia and abnormal cerebellar TH gene expression is not clear, it is possible that intense cerebellar activation during an attack (Campbell and Hess, 1998) influences TH gene expression in these animals, particularly as calcium buffering may be impaired in these mutants (Cicale *et al.*, 2002). It is likely that twice-daily injections of nimodipine greatly diminished the number of attacks the animals experienced during the course of the two-week long experiment, as nimodipine was protective up to six hours after a single administration. Regardless of the site of action, the net effect of nimodipine administration is a decrease in Purkinje cell excitability and consequently a decrease in calcium influx.

Although the current density of P-type calcium channels is reduced in tottering Purkinje cells (Wakamori *et al.*, 1998), cerebellar TH mRNA expression *in vivo* appears to result from excessive Purkinje cell activation as blockade of L-type calcium channels reduced the expression of this cellular abnormality. The apparent paradox between tottering mouse Purkinje cell physiology and TH mRNA expression may be

explained by the differences in the function of L-type and P/Q-type calcium channels. The calcium channels directly affected by the tottering mutation are P/Q-type channels (Fletcher *et al.*, 1996), the predominant carriers of calcium current in Purkinje cells (Llinas *et al.*, 1989; Usowicz *et al.*, 1992). P/Q-type calcium channels have been associated with neurotransmitter release and are localized mainly on neuronal dendrites and to a lesser extent on the cell soma (Hillman *et al.*, 1991). L-type calcium channels are largely localized on the soma (Hell *et al.*, 1993) where they influence excitability and regulate gene expression (Finkbeiner and Greenberg, 1998). The increase in L-type calcium channels may be responsible for increased calcium signaling to the nucleus and subsequent alterations in transcriptional regulation. The decrease in TH mRNA expression in tottering mouse Purkinje cells following chronic nimodipine administration supports this hypothesis.

It is important to note that a reduction in whole cell calcium current was observed in immature tottering mouse Purkinje cells (Wakamori *et al.*, 1998). However, calcium currents in developing tottering mouse Purkinje cells may not reflect adult states as L-type calcium channel expression is differentially regulated during cerebellar development. Postnatal expression of L-type calcium channels is low in the first week and peaks between the second and third weeks of development. This sharp increase in L-type calcium channels is followed by a steady decline in channel density to reach intermediate levels in normal adult rodents (Erdman *et al.*, 1983; Litzinger *et al.*, 1993; Tanaka *et al.*, 1995). In contrast, L-type calcium channel expression remains high in adult tottering mouse cerebellum, which may contribute to the persistence of TH mRNA expression in mature tottering mouse Purkinje cells.

TH mRNA expression in the olfactory periglomerular region and locus coeruleus was unperturbed by either the tottering mutation or by chronic nimodipine administration, while TH mRNA expression was slightly reduced in the substantia nigra of control mice following nimodipine treatment. TH mRNA expression is tightly regulated in catecholaminergic cells (Cottingham *et al.*, 1990; Buckland *et al.*, 1992) and may be refractory to short-lived drugs such as nimodipine, as alterations in transcription are observed only after extended chronic drug treatment or lesion (Berod *et al.*, 1987; Rodriguez-Gomez *et al.*, 1997). Alterations in transcription may be more likely to occur in non-catecholaminergic Purkinje cells, where such regulatory mechanisms may not exist.

These data suggest that TH expression in the Purkinje cells of tottering mice is a secondary effect of the loss-of-function mutation. Such effects are emerging as significant factors in the generation of abnormal phenotypes in calcium channel mutant mice (Zhang *et al.*, 2002). Investigations of calcium regulation using these *in vivo* models may prove valuable in understanding the etiology, progression and treatment of disease caused by calcium channel mutations in humans, as well as in understanding basic mechanisms controlling calcium homeostasis in the central nervous system.

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References

Austin M, M Schultzberg, LC Abbott, P Montpied, J Evers, S Paul and J Crawley (1992) Expression of tyrosine hydroxylase in cerebellar Purkinje neurons of the mutant *tottering* and *leaner* mouse. *Mol. Brain Res.* **15**, 227-240.

Berod A, N Biguet, S Dumas, B Bloch and J Mallet (1987) Modulation of tyrosine hydroxylase gene expression in the central nervous system visualized by *in situ* hybridization. *Proc. Natl. Acad. Sci. USA* **84**, 1699-1703.

Bourinet E, TW Soong, K Sutton, S Slaymaker, E Mathews, A Monteil, GW Zamponi, J Nargeot and TP Snutch (1999) Splicing of α_{1A} subunit gene generates phenotypic variants of P- and Q-type calcium channels. *Nat. Neurosci.* **2**, 407-415.

Brosenitsch TA and DM Katz (2001) Physiological patterns of electrical stimulation can induce neuronal gene expression by activating N-type calcium channels. *J. Neurosci.* **21**, 2571-2579.

Brosenitsch TA, D Salgado-Commissariat, DL Kunze and DM Katz (1998) A role for L-type calcium channels in developmental regulation of transmitter phenotype in primary sensory neurons. *J. Neurosci.* **18**, 1047-1055.

Buckland PR, MC O'Donovan and P McGuffin (1992) Changes in dopa decarboxylase mRNA but not tyrosine hydroxylase mRNA levels in rat brain following antipsychotic treatment. *Psychopharmacology (Berl.)* **108**, 98-102.

Campbell DB and EJ Hess (1997) Rapid genotyping of mutant mice using dried blood spots for polymerase chain reaction (PCR) analysis. *Brain Res. Brain Res. Prot.* **1**, 117-123.

Campbell DB and EJ Hess (1998) Cerebellar circuitry is activated during convulsive episodes in the tottering (tg/tg) mutant mouse. *Neuroscience* **85**, 773-83.

Campbell DB and EJ Hess (1999) L-type calcium channels contribute to the *tottering* mouse dystonic episodes. *Mol. Pharmacol.* **55**, 23-31.

Catteral WA (1995) Structure and function of voltage-gated ion channels. *Annu. Rev. Biochem.* **64**, 493-531.

Cicale M, A Ambesi-Impiombato, V Cimini, G Fiore, G

Muscettola, LC Abbott and A de Bartolomeis (2002) Decreased gene expression of calretinin and ryanodine receptor type 1 in tottering mice. *Brain Res. Bull.* **59**, 53-58.

Cigola E, BT Volpe, JW Lee, L Franzen and H Baker (1998) Tyrosine hydroxylase expression in primary cultures of olfactory bulb: role of L-type calcium channels. *J. Neurosci.* **18**, 7638-7649.

Cottingham SL, D Pickar, TK Shimotake, P Montpied, SM Paul and JN Crawley (1990) Tyrosine hydroxylase and cholecystokinin mRNA levels in the substantia nigra, ventral tegmental area, and locus ceruleus are unaffected by acute and chronic haloperidol administration. *Cell Mol. Neurobiol.* **10**, 41-50.

Erdman R, H Yamamura and W Roeske (1983) The ontogeny of specific binding sites for the calcium channel antagonist, nitrendipine, in mouse heart and brain. *Brain Res.* **278**, 327-331.

Finkbeiner S and ME Greenberg (1998) Ca^{2+} channel-regulated neuronal gene expression. *J. Neurobiol.* **37**, 171-189.

Fletcher CF, CM Lutz, TN O'Sullivan, JD Shaughnessy, R Hawkes, WN Frankel, NG Copeland and NA Jenkins (1996) Absence epilepsy in tottering mutant mice is associated with calcium channel defects. *Cell* **87**, 607-617.

Fureman BE, HA Jinnah and EJ Hess (2002) Triggers of paroxysmal dyskinesia in the calcium channel mouse mutant tottering. *Pharmacol. Biochem. Behav.* **73**, 631-637.

Hell JW, RE Westenbroek, C Warner, MK Ahlijanian, W Prystay, MM Gilbert, TP Snutch and WA Catterall (1993) Identification and differential subcellular localization of the neuronal class C and class D L-type calcium channel α1 subunits. *J. Cell. Biol.* **123**, 949-962.

Hess EJ and MC Wilson (1991) Tottering and leaner mutations perturb transient developmental expression of tyrosine hydroxylase in embryologically distinct Purkinje cells. *Neuron* **6**, 123-132.

Hillman D, S Chen, TT Aung, B Cherksey, M Sugimori and RR Llinas (1991) Localization of P-type calcium channels in the central nervous system. *Proc. Natl. Acad. Sci. USA* **88**, 7076-7080.

Katz DM, JE Adler and IB Black (1986) Expression and regulation of tyrosine hydroxylase in adult sensory neurons in culture: effects of elevated potassium and nerve growth factor. *Brain Res.* **385**, 68-73.

Kessler JA (1985) Differential regulation of peptide and catecholamine characters in cultured sympathetic neurons. *Neuroscience* **15**, 827-839.

Kilbourne EJ, EJ Nankova, EJ Lewis, A McMahon, H Osaka, DB Sabban and EL Sabban (1992) Regulated expression of the tyrosine hydroxylase gene by membrane depolarization. *J. Biol. Chem.* **267**, 7563-7569.

Litzinger M, B Grover, S Saderup and J Abbott (1993) Voltage sensitive calcium channels mark a critical period in mouse neurodevelopment. *Int. J. Dev. Neurosci.* **11**, 17-24.

Llinas R, M Sugimori, JW Lin and B Cherksey (1989) Blocking and isolation of a calcium channel from neurons in mammals and cephalopods utilizing a toxin fraction (FTX) from funnel-web spider poison. *Proc. Natl. Acad. Sci. USA* **86**, 1689-1693.

Menezes A, R Zeman and E Sabban (1996) Involvement of intracellular or extracellular calcium in activation of tyrosine hydroxylase gene expression in PC12 cells. *J. Neurochem.* **67**, 2316-2324.

Munson PJ and D Robard (1980) LIGAND: A versatile computerized approach for characterization of ligand binding systems. *Anal. Biochem.* **107**, 220-239.

Perez-Reyes E and T Schneider (1995) Molecular biology of calcium channels. *Kidney Intl.* **48**, 1111-1124.

Rodriguez-Gomez JA, M Romero-Ramos, ML Vizuete, JL Venero, J Cano and A Machado (1997) Increased activity and expression of tyrosine hydroxylase in the rat substantia nigra after chronic treatment with nomifensine. *Mol. Pharmacol.* **52**, 641-647.

Sheng ZH, RE Westenbroek and WA Catterall (1998) Physical link and functional coupling of presynaptic calcium channels and the synaptic vesicle docking/fusion machinery. *J. Bioenerg. Biomembr.* **30**, 335-345.

Tanaka O, J Sakagami and H Kondo (1995) Localization of mRNAs of voltage-dependent Ca^{2+}-channels: four subtypes of α1 and ß-subunits in developing and mature rat brain. *Brain Res. Mol. Brain Res.* **30**, 1-16.

Usowicz MM, M Sugimori, B Cherksey and R Llinas (1992) P-type calcium channels in the somata and dendrites of adult cerebellar Purkinje cells. *Neuron* **9**, 1185-1199.

Vidal S, B Raynaud and MJ Weber (1989) The role of Ca^{2+} channels of the L-type in neurotransmitter plasticity of cultured sympathetic neurons. *Brain Res. Mol. Brain Res.* **6**, 187-96.

Wakamori M, K Yamazaki and H Matsunodaira (1998) Single tottering mutations responsible for the neuropathic phenotype of the P-type calcium channel. *J. Biol. Chem.* **52**, 34857-34867.

Weiland GA and RE Oswald (1985) The mechanism of binding of dihydropyridine calcium channel blockers to rat brain membranes. *J. Biol. Chem.* **260**, 8456-8464.

Zhang Y, M Mori, DL Burgess and JL Noebels (2002) Mutations in high-voltage-activated calcium channel genes stimulate low-voltage-activated currents in mouse thalamic relay neurons. *J. Neurosci.* **22**, 6362-6371.