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L-Type Calcium Channels Contribute to the Tottering Mouse Dystonic Episodes

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ABSTRACT

Tottering mice inherit a recessive mutation of the calcium channel α_{1A} subunit that causes ataxia, polyspike discharges, and intermittent dystonic episodes. The calcium channel α_{1A} subunit gene encodes the pore-forming protein of P/Q-type voltage-dependent calcium channels and is predominantly expressed in cerebellar granule and Purkinje neurons with moderate expression in hippocampus and inferior colliculus. Because calcium misregulation likely underlies the tottering mouse phenotype, calcium channel blockers were tested for their ability to block the motor episodes. Pharmacologic agents that specifically block L-type voltage-dependent calcium channels, but not P/Q-type calcium channels, prevented the inducible dystonia of tottering mutant mice. Specifically, the dihydropyridines nimodipine, nifedipine, and nitrendipine, the benzothiazepine diltiazem, and the phenylalkylamine verapamil all prevented restraint-induced tottering mouse motor episodes. Conversely, the L-type calcium channel agonist Bay K8644 induced stereotypic tottering mouse dystonic at concentrations significantly below those required to induce seizures in control mice. In situ hybridization demonstrated that L-type calcium channel α_{1C} subunit mRNA expression was up-regulated in the Purkinje cells of tottering mice. Radioligand binding with [^{3}H]nitrendipine also revealed a significant increase in the density of L-type calcium channels in tottering mouse cerebellum. These data suggest that although a P/Q-type calcium channel mutation is the primary defect in tottering mice, L-type calcium channels may contribute to the generation of the intermittent dystonia observed in these mice. The susceptibility of L-type calcium channels to voltage-dependent facilitation may promote this abnormal motor phenotype.

Neurological mutants of the mouse are important tools for studying central nervous system development and provide models of human disease. For many of these mutants, the mutation has been identified but the mechanism by which the mutation causes the abnormal phenotype is unclear. Defining the cellular and neural systems affected by the mutation is necessary for understanding the mutant phenotype in the mouse and for applying the model to human disease states.

The neurological mouse mutation tottering (gene symbol: *tg*) causes spike and wave discharges (Noebels and Sidman, 1979), ataxia, and abnormal motor episodes characteristic of intermittent dystonia (Green and Sidman, 1962). The most extensively studied of these phenotypes is the spike and wave discharge, nonconvulsive bilaterally synchronous epileptic bursts recorded by electroencephalogram. This phenotype is associated with hyperarborization of locus ceruleus axons in tottering mutants (Levitt and Noebels, 1981) as selective lesion of these axons with 6-hydroxydopamine dramatically reduced the spike and wave discharges (Noebels, 1984). By contrast, the intermittent dystonic episodes of tottering mice are neither associated with an abnormal electroencephalographic pattern (Kaplan et al., 1979; Noebels and Sidman, 1979) nor prevented by 6-hydroxydopamine lesion (Noebels, 1984). Although this abnormal motor phenotype of tottering mice is the most behaviorally obvious, the intermittent dystonic episodes have not been well characterized.

Tottering dystonic episodes are highly stereotyped, with the first observable characteristic being extension of the hindlimbs. This initial phase is followed by abduction at the hip and extension at the knee, ankle, and paw with a stiffly arched back that presses the perineum against the cage bottom. The motor dysfunction then spreads to involve the forelimbs and head, with severe flexion of the neck. In the final phase, the mice regain control of the hindlimbs, often rearing, while forepaw and facial muscles continue contractions (Green and Sidman, 1962). The entire episode lasts 30 to 60 min without loss of consciousness.

The molecular basis of the tottering mouse phenotypes was identified recently as a point mutation within the voltage-dependent calcium channel α_{1A} subunit (Fletcher et al., 1996). Simultaneous with the identification of the tottering gene defect, the mutations underlying the human disorders familial hemiplegic migraine and episodic ataxia type-2 were

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ABBREVIATIONS: SSC, standard saline citrate; DTT, dithiothreitol; ANOVA, analysis of variance.

identified as mutations in the human voltage-dependent calcium channel α_{1A} subunit (Ophoff et al., 1996). Voltage-dependent calcium channels consist of four subunits: α_1, α_2, β, and γ. The α_1 subunit is the pore-forming protein, conferring ion selectivity and voltage sensitivity; the other subunits influence the kinetic properties of the channel. Six types of voltage-dependent calcium channels have been distinguished by electrophysiology and pharmacology: L, P, Q, N, R, and T; a multiplicity of genes and alternative splice sites encode the α_1 subunits of the functionally defined channels. All the voltage-dependent calcium channel α_1 subunits have a similar membrane topography with four repetitive domains (I–IV), each consisting of six transmembrane segments (S1–S6) (for reviews see Catterall, 1995; Wheeler et al., 1995). The α_{1A} subunit gene encodes P- and/or Q-type calcium channels, which are distinguished from other types of calcium channels by their sensitivity to blockade by ω-agatoxin-IVA and ω-agatoxin-TK. The tottering missense mutation results in a proline-to-leucine substitution at amino acid 601, within the extracellular loop between S5 and S6 in repetitive domain II of the calcium channel (Fletcher et al., 1996). Although the functional significance of this mutation is not yet known, the region in which the mutation lies is thought to be important in ion selectivity (Catterall, 1995).

Calcium misregulation resulting from the calcium channel mutation is a likely cellular mechanism underlying the abnormal phenotypes of tottering mice. Therefore, calcium channel blockers were tested for their ability to block the intermittent dystonic episodes in tottering mice. The only specific P/Q-type calcium channel antagonists currently available are the spider toxins ω-agatoxin IVA and ω-agatoxin TK, peptides with 71% identity at the amino acid level (Mintz et al., 1992; Teramoto et al., 1993). These peptides have been studied extensively in vitro but are not commonly used in vivo as they do not cross the blood-brain barrier. In contrast, several L-type calcium channel blockers readily cross the blood-brain barrier to exert central effects. Dihydropyridines (e.g., nimodipine, nifedipine, nitrendipine), benzothiazepines (e.g., diltiazem), and phenylalkylamines (e.g., verapamil) have been demonstrated to protect against electrically and chemically induced seizures (Larkin et al., 1992; Palmer et al., 1993; Wurpel and Iyer, 1994). Therefore L-type calcium channel blockers were tested for their ability to prevent dystonia in tottering mutant mice.

Materials and Methods

Mouse Mutants. C57BL/6J-+/*tg* mice were obtained from The Jackson Laboratories and bred at the Pennsylvania State University College of Medicine to produce tottering mutants. Adult tottering mutant mice (2–12 months of age) were identified either by analysis of polymerase chain reaction (PCR) amplification of tightly linked simple-sequence-length polymorphisms in C57BL/6J-+/*tg* x C57BL/6J-+/*tg* cross progeny (Campbell and Hess, 1996, 1997) or by absence of oligosyndactylism in *Os* +/+ *tg* x *Os* +/+ *tg* cross-progeny (Campbell and Hess, 1998). Genotype was confirmed by observation of stereotyped dystonic episodes. Age- and gender-matched C57BL/6J-+/+ mice were used as controls where appropriate. All animals were drug-naive at the beginning of the experiments. All animal procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

In Situ Hybridization. Tottering mutant (*tg*/*tg*), heterozygous (+/*tg*), and normal (+/+) gender-matched littermates (2–3 months of age) were deeply anesthetized with carbon dioxide and sacrificed by decapitation. The brains were rapidly removed, frozen in isopentane at −20 to −40°C, and stored at −70°C. Twenty-micrometer sagittal sections were cut using a cryostat and thaw mounted on Superfrost Plus glass slides (Fisher) with each slide bearing control and mutant sections. After drying, the slide-mounted sections were again stored at −70°C until use in in situ hybridization experiments.

A cDNA specific to the calcium channel α_{1A} subunit gene, but not other calcium channel α_1 subunit genes, was generated by reverse transcription-PCR. A 554-bp DNA segment corresponding to base pairs 5736 to 6289 of the mouse calcium channel α_{1A} subunit cDNA (GenBank U76716) was amplified and inserted into pBluescript II SK(−). A 418-bp *Eco*RI fragment corresponding to base pairs 8596 to 9014 of the α_{1C} subunit 3′ untranslated region was subcloned into pBluescript II SK(−); this sequence is unique to the α_{1C} subunit; the original clone was obtained from the I.M.A.G.E. Consortium (Lawrence Livermore National Laboratory, Livermore, CA) cDNA clone ID#AA462894 (Lennon et al., 1996). The α_{1D} subunit cDNA (residues 2803–3246) subcloned in pGEM was a generous gift from Dr. Hemin Chin (National Institutes of Health). In vitro transcription was performed at 37°C for 2 h in a 25-μl volume containing 40 mM Tris, pH 7.9; 6 mM $MgCl_2$; 2 mM dithiothreitol (DTT); 40 U of RNase inhibitor (Promega, Madison, WI); 400 μM each of ATP, GTP, and UTP; 10 μM [^{35}S]CTP (800 Ci/mmol, Amersham Corp., Arlington Heights, IL); 1 μg of linearized plasmid; and 20 U of RNA polymerase (Promega). After transcription, the DNA template was removed by digestion with RNase-free DNase (Promega Biotec) for 30 min at 37°C, and the transcripts were size reduced to 150 to 250 nucleotides by alkalai treatment with 0.2 N NaOH for 45 min on ice. The probes then were extracted with phenol/chloroform/isoamyl alcohol (25:24:1), and the unincorporated nucleotides were removed by fractionation on a G50 Sephadex Nick column (Pharmacia, Piscataway, NJ).

Pretreatment of the slide-mounted sections included fixation in buffered 4% formaldehyde for 15 min at room temperature followed by a 5-min rinse in 0.1 M phosphate-buffered saline. Slides then 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 2× standard saline citrate (SSC; 0.15 M NaCl, 0.015 M sodium citrate). Sections then were dehydrated in graded ethanols followed by two 5-min incubations in chloroform. One-minute incubations in 100 and 95% ethanol were followed by air drying.

Slides were hybridized with 100 μl of buffer containing 7.5 ng of 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, 5× Denhardt's solution (0.02% bovine serum albumin, 0.02% Ficoll, 0.02% polyvinylpyrrolidone), 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, and hybridized for 16 h at 56°C.

After hybridization, coverslips were removed in 4× SSC plus 300 mM 2-mercaptoethanol at room temperature, incubated in this solution for 15 min, and incubated in 4× SSC without 2-mercaptoethanol for 15 min at room temperature. Slides were treated with 1:1 formamide/buffer (0.6 M NaCl, 40 mM Tris base, 2 μM EDTA, 20 mM HCl) at 60°C for 20 min followed by a 5-min rinse in room temperature 2× SSC. Sections then were treated with 50 μg/ml pancreatic RNase A in 0.5 M NaCl, 50 mM Tris, pH 8.0, and 5 mM EDTA for 30 min at 37°C, washed in graded salt solutions (2×, 1×, and 0.5× SSC each for 5 min at room temperature), with a final wash in 0.1× SSC at 65°C for 30 min. Slides then were cooled to room temperature in 0.1× SSC, dipped in 60% ethanol with 0.33 M ammonium acetate, and air dried. Sections were exposed to X-ray film (DuPont Cronex) for 8 to 96 h and subsequently dipped in Kodak NTB-2 photographic emulsion (diluted 1:2 with dH_2O), exposed at 4°C, and developed in Kodak D-19 developer.

Quantitative analyses were performed using NIH Image software on captured darkfield images of the emulsion-dipped sections. Images of representative areas from each brain were captured at 40×

magnification. Silver grain densities in the Purkinje cell were determined in boxes of 900 μm^2; cerebellar granule cells were assessed in 8100-μm^2 boxes; nucleus of the brachium of the inferior colliculus and thalamic nuclei were assessed in 112,000-μm^2 boxes; all other regions were assessed in 225,000-μm^2 boxes. Data were analyzed by two-way analysis of variance (ANOVA) or by Student's t test where appropriate.

Induction of Dystonia in Tottering Mutant Mice. Intermittent dystonic episodes in tottering mutant mice are inducible. For all experiments assessing the effects of calcium channel blockers on tottering mouse motor episodes, adult mice were transported daily from the vivarium to the laboratory and acclimated for at least 4 h. The extensive acclimation period provided sufficient recovery time for tottering mutants, which routinely express dystonia upon transport. Mice then were injected with vehicle or drug and placed back in the home cage for 30 min. To induce dystonic episodes after drug treatment, mice were restrained in a 60-ml syringe for 10 min, released into a novel plastic cage for 30 min, then returned to the home cage. The mice were scored for the presence or absence of dystonia from the time of injection until 10 min after return to the home cage, a total of 80 min.

Intracerebroventricular Injections of L-Type Calcium Channel Blockers. At least 4 h after transport to the laboratory, tottering mutant mice were anesthetized with methoxyflurane (Pittman-Moore, Inc., Mundelein, IL). After anesthetization (3–3.5 min in methoxyflurane), a small slit was made at the base of the skull and a 27-gauge syringe needle, blunted to a length of 5 mm, was inserted at the midline between the skull and first vertebra. Saline vehicle or drug was injected directly into the fourth ventricle in a volume of 5 μl. Drug masses tested were diltiazem (20 μg) and verapamil (10 μg). All drugs were dissolved in saline. Dihydropyridines were not used i.c.v. because they are relatively insoluble; the vehicle in which dihydropyridines could be solubilized for s.c. administration caused massive intracerebral bleeding, prohibiting the use of these drugs i.c.v. Mice recovered from the anesthesia within 15 min. Thirty minutes after injection, the mice were subjected to the dystonia-induction paradigm described above. All mice were sacrificed imme-

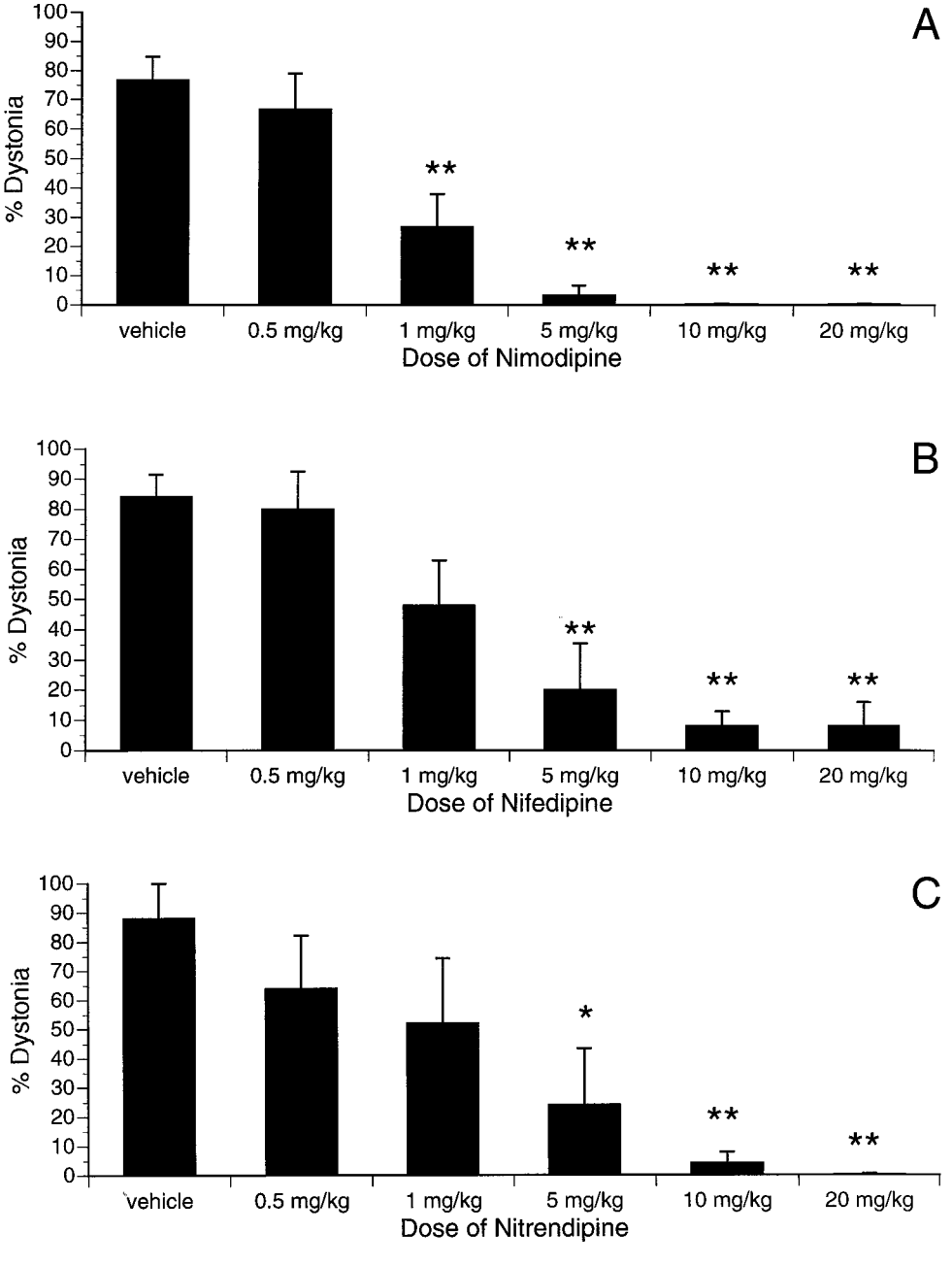

Fig. 1. Effect of s.c. injection of the dihydropyridine class of L-type calcium channel blockers on restraint-induced dystonia in tottering mutant mice. A, nimodipine prevented tottering mouse dystonia in a dose-dependent manner. Doses of 1 mg/kg and above significantly reduced the frequency of dystonic events, completely preventing the events at 10 and 20 mg/kg. B, nifedipine also prevented tottering mouse dystonia in a dose-dependent manner. The effect reached statistical significance at doses of 5 mg/kg and above. C, nitrendipine significantly reduced the occurrence of restraint-induced tottering mouse dystonia at doses of 5, 10, and 20 mg/kg. Nitrendipine was completely protective at 20 mg/kg. Data represent mean ± S.E.M. (n = 5–6). For each drug, values that are significantly less than vehicle as determined by one-way ANOVA followed by Dunnett t test are denoted by ** ($P < .01$) and * ($P < .05$).

diately after the observation period. Data were analyzed by one-way ANOVA followed by Dunnett *t* test.

Subcutaneous Injections of L-Type Calcium Channel Blockers and Activators. The L-type calcium channel blockers nimodipine, nifedipine, nitrendipine, diltiazem, and verapamil were injected s.c. into tottering mutant mice in a volume of 5 ml/kg. Groups of five to six tottering mice were used for each dose-response analysis with five trials at each drug concentration. Mice were injected and challenged in the dystonia induction paradigm (see above) once daily for 5 consecutive days at each dose. The order of drug doses was randomized for each mouse. The mice were allowed 2 days of recovery between drug doses when progressing to a higher dose; 9 days elapsed before treatment with a lower dose of drug. Data were analyzed by one-way factorial ANOVA followed by Dunnett *t* tests comparing the response at each drug dose with vehicle.

To assay the effects of s.c. injection of the L-type calcium channel activator Bay K8644 on tottering mouse dystonia, five tottering mutant females and five age-matched C57BL/6J-+/+ control females (5–7 months of age) were transported to the laboratory daily. After a period of acclimation of at least 4 h, Bay K8644 or vehicle at a volume of 5 ml/kg was injected s.c. The mice were returned directly to the home cage and observed for 60 min with no attempts to induce the motor episodes environmentally. The mice were injected with vehicle on the first day followed by increasing doses of Bay K8644 on subsequent consecutive days. Data were analyzed by one-way factorial ANOVA followed by Dunnett *t* tests comparing the response at each drug concentration with vehicle.

[^{3}H]Nitrendipine-Binding Assays. Tottering (*tg*/*tg*) mutant mice and gender-matched normal (+/+) controls 2 to 3 months of age were deeply anesthetized with carbon dioxide and decapitated. The brains were removed rapidly, dissected into cerebellum and forebrain discarding brainstem and olfactory bulbs, and frozen at −70°C until use. Tissues were homogenized in 100 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4) with a Tekmar tissue homogenizer (Cincinnati, OH) at setting 70. The homogenates were centrifuged at 30,000*g* for 10 min, and the supernatant was discarded. Pellets were resuspended in ice-cold buffer and centrifuged again at 30,000*g* for 10 min. Pellets were resuspended to 25 mg wet weight tissue/ml buffer (forebrain) or 50 mg/ml buffer (cerebellum). Protein concentrations were determined with the Pierce (Rockford, IL) BCA protein assay.

Binding assays were performed in a total volume of 2 ml in 50 mM Tris-HCl buffer for 90 min at 22°C (Marangos et al., 1982). Each reaction contained either 5 mg/ml (cerebellum) or 2.5 mg/ml (forebrain) tissue. Six concentrations of [^{3}H]nitrendipine (78.5 Ci/mmol) ranging from 20 to 1000 pM defined total binding; nonspecific binding was determined in the presence of 1 μM nifedipine. Reactions were terminated by rapid filtration over Whatman GF/C filters, which were washed rapidly three times with 3 ml of ice-cold buffer. Filters were allowed to dry and then were placed in ScintiVerse (Fisher) scintillation fluid. Radioactivity was determined by liquid scintillation spectroscopy at an efficiency of 35 to 40%. Data were analyzed using GraphPad Prism 2.0 software.

Drugs. [^{3}H]nitrendipine was purchased from NEN (Boston, MA). All L-type calcium channel blockers and activators were obtained from Research Biochemicals International (Natick, MA). Verapamil was dissolved in 0.9% saline. Nimodipine and nitrendipine were dissolved in 14.5% ethanol/4.5% Tween 80 (Sigma)/81% 0.9% saline. Nifedipine and Bay K8644 were dissolved in 24% ethanol/4% Tween 80/72% 0.9% saline.

Results

Effect of Bay K8644 on Tottering Mouse Dystonic Episodes. Tottering mouse dystonia and the seizures induced by the L-type calcium channel activator Bay K8644 in normal mice are behaviorally distinct. In contrast to the stereotypic spastic contractions and extensions that characterize the tottering mouse phenotype, Bay K8644-induced seizures in normal mice are characterized by a stiff tail, arched back, squeaking, forelimb and hindlimb clonus, flexion and extension of hindlimbs, jumping, catatonia, and loss of righting reflex (Palmer et al., 1993).

Bay K8644 did not induce seizures in control (C57BL/6J-+/+) mice at concentrations below 10 mg/kg (Table 1). At 10 mg/kg the characteristic Bay K8644 seizure was observed in all normal controls. In tottering mutants, although dystonia was not observed after vehicle injection, 40% of the mice exhibited stereotypical tottering mouse dystonic events in response to a 1-mg/kg dose of the L-type calcium channel activator. Bay K8644 induced dystonia in 100% of tottering mutant mice at a dose of 2 mg/kg. The dystonic episodes induced by 2 mg/kg Bay K8644 were behaviorally indistinguishable from restraint-induced tottering mouse dystonia with an average latency to onset of 22 ± 6 (mean ± S.E.M.) min. A dose of 5 mg/kg in tottering mutants induced stereotypical tottering mouse dystonic events accompanied by occasional squeaking, the first characteristic of Bay K8644-induced seizures observed in tottering mice. Other characteristics of Bay K8644 induced seizures, specifically jumping and catatonia, were observed in tottering mutants after a 5 mg/kg dose, but these were not observed until after the tottering mouse dystonic episodes were complete. Tottering mutants treated with 10 mg/kg Bay K8644 expressed stereotypical tottering mouse dystonia with an average latency of 3.7 ± 0.3 min. This highest dose also produced more of the characteristics of Bay K8644 seizures in tottering mutants: the mice vocalized, jumped, and exhibited catatonia. In summary, mutants demonstrated stereotypical dystonic episodes at every dose tested with additional characteristics of Bay K8644 seizures observed at 5 and 10 mg/kg, and

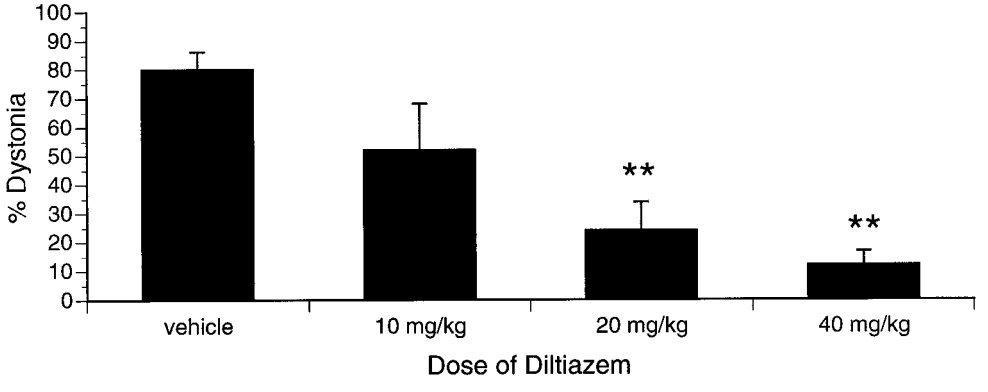

Fig. 2. Effect of s.c. injection of the benzothiazepine diltiazem on restraint-induced tottering mouse dystonia. Diltiazem prevented tottering mouse dystonia, with the effect reaching significance at 20 and 40 mg/kg. The drug specifically blocks L-type calcium channels. Data represent mean ± S.E.M. (n = 5). ** indicates significant difference (P < .01) from vehicle control ANOVA followed by Dunnett *t* test.

the latency to expression of dystonia in tottering mutants decreased with increasing concentration of drug.

Effect of Subcutaneous Injection of L-Type Calcium Channel Blockers on Tottering Mouse Dystonic Episodes. The dihydropyridines nimodipine, nitrendipine, and nifedipine each prevented restraint-induced tottering mouse dystonia in a dose-dependent manner (Fig. 1). Nimodipine significantly reduced the occurrence of these episodes at 1, 5, 10, and 20 mg/kg and was completely protective against tottering mouse dystonia at 10 and 20 mg/kg. Nifedipine and nitrendipine each significantly prevented the expression of the movement disorder at 5, 10, and 20 mg/kg. Nitrendipine was completely protective at 20 mg/kg. Even the highest doses of dihydropyridines tested caused no observable side-effects: the mice were active and the ataxia of tottering mice was not noticeably altered.

The benzothiazepine diltiazem also prevented the tottering mouse motor episodes in a dose-dependent fashion (Fig. 2). The ability of diltiazem to block the dystonia reached significance at 20 and 40 mg/kg. No behavioral side-effects were observed at 20 mg/kg. However, tottering mutant mice injected with 40 mg/kg diltiazem exhibited severe side-effects: the mice were reproducibly inactive, sitting in the corner of the cage for several hours; when provoked to move, these mice exhibited markedly severe ataxia, which subsided several hours after injection.

Injection of the phenylalkylamine verapamil, s.c., had no effect on the tottering mouse phenotype at sublethal doses (Fig. 3). Doses of verapamil of 10 mg/kg or greater were reproducibly lethal in both tottering mutants and normal (C57BL/6J-+/*tg*) controls. Verapamil is a specific L-type calcium channel blocker but does not cross the blood-brain barrier in appreciable quantities (Hamann et al., 1983).

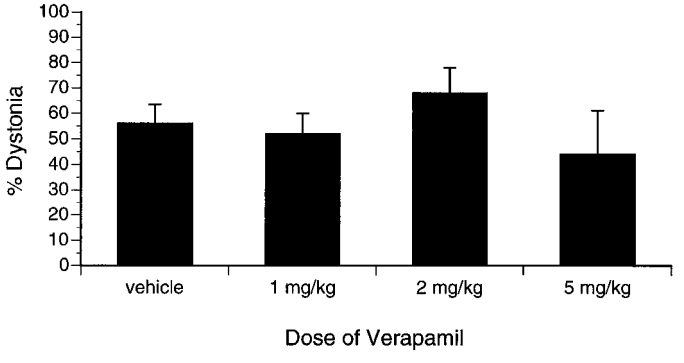

Fig. 3. Effect of s.c. injection of the phenylalkylamine verapamil on tottering mouse dystonia. Verapamil had no effect on the frequency of dystonic events by this route of administration (ANOVA, $P > 0.50$). Doses of 10 mg/kg or greater were invariably lethal in both tottering mutants and C57BL/6J-+/*tg* controls. Although the drug is a potent L-type calcium channel blocker, verapamil does not cross the blood-brain barrier efficiently. Data represent mean ± S.E.M. ($n = 5$).

TABLE 1

Frequency and latency of Bay K8644-induced seizures in control (C57BL/6J-+/+) mice and dystonia in tottering (C57BL/6J-*tg*/*tg*) mice

Bay K8644 induced tottering mouse dystonic events at doses subconvulsive in control mice. Seizure and dystonia data are expressed as % observed; latency data represent mean ± S.E.M. minutes from injection to first observed sign of seizure or dystonia ($n = 5$).

Bay K8644 Dose	Control Mice		Tottering Mutants	
	Seizure Frequency	Latency	Dystonia Frequency	Latency
		min		*min*
Vehicle	0%		0%	
1 mg/kg	0%		40%	
2 mg/kg	0%		100%*	22 ± 6
5 mg/kg	0%		100%*	5 ± 1
10 mg/kg	100%*	29 ± 3	100%*	3.7 ± 0.3

* $P < .01$ from vehicle control by ANOVA followed by Dunnett t test.

Effect of Intracerebroventricular Injection of L-Type Calcium Channel Blockers on Tottering Mouse Dystonic Episodes. Tottering mutant mice injected i.c.v. with saline vehicle exhibited dystonic events in 64% of trials (Table 2). The L-type calcium channel blocker diltiazem eliminated the motor episodes when injected i.c.v., similar to s.c. injection. The phenylalkylamine verapamil, which does not cross the blood-brain barrier and failed to prevent tottering mouse dystonia upon s.c. injection, significantly reduced the frequency of the episodes in tottering mutant mice to 20% when administered i.c.v. (Table 2). Thus, injection of L-type calcium channel blockers directly into the central nervous system effectively prevented the intermittent dystonic episodes exhibited by tottering mutant mice.

L-Type Calcium Channel α_{1C} and α_{1D} Subunit mRNA Expression. Consistent with previous reports (Tanaka et al., 1995), the calcium channel α_{1C} subunit mRNA was expressed throughout the brain including cerebral cortex, thalamus, hippocampus, and cerebellum (Fig. 4). Quantitative analyses of grain density in parasagittal brain sections from

TABLE 2

Ability of L-type calcium channel blockers injected i.c.v. to prevent restraint-induced tottering mouse dystonic episodes

The specific L-type calcium channel blockers verapamil and diltiazem each significantly reduced the occurrence of restraint-induced tottering mouse dystonic episodes. Data are expressed as % dystonic events observed.

Drug Injection, i.c.v.	% Tottering Mice Exhibiting Dystonia
Saline vehicle	64% ($n = 11$)
Verapamil, 10 μg	20%[a] ($n = 10$)
Diltiazem, 20 μg	0%[b] ($n = 6$)

[a] $P < .05$ versus saline vehicle control by ANOVA followed by Dunnett t-test.
[b] $P < .01$ versus saline vehicle control by Student's t test.

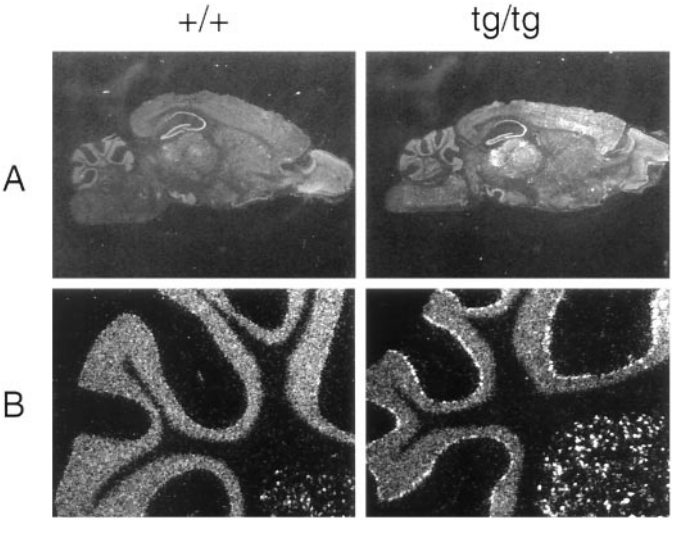

Fig. 4. Darkfield photomicroscopy of in situ hybridization for calcium channel α_{1C} subunit mRNA expression in control and tottering mice. The α_{1C} subunit calcium channel gene was expressed predominantly in thalamus, cortex, and cerebellum. A, photomicrograph (magnification, 0.7×) of sagittal +/+ and *tg*/*tg* brain sections. B, photomicrograph (magnification, 5×) of the cerebellum of the section shown in A.

control (+/+) and tottering (*tg*/*tg*) mice (n = 3 per genotype) revealed a significant increase in the expression of calcium channel α_{1C} subunit mRNA in Purkinje cells of the cerebellum in tottering mice (Table 3). Quantitation was performed on Purkinje cells from both Lobule V and Lobule VIII; in both lobules, expression of the α_{1C} subunit was increased significantly in tottering mice. In fact, α_{1C} subunit mRNA is expressed at extremely low levels in Purkinje cells of control mice, but expressed abundantly in Purkinje cells of tottering mice (Fig. 4). However, α_{1C} subunit mRNA was not increased equally in all Purkinje cells whereby a dramatic increase in expression was observed in some cells but little increase in expression was observed in others (Fig. 4). In contrast, calcium channel α_{1C} subunit mRNA expression was comparable in cerebellar granule cells and deep cerebellar nuclei in control and tottering mice (Table 3), indicating a cell-type-specific regulation of the α_{1C} subunit resulting from the calcium channel α_{1A} subunit mutation. A significant increase in α_{1C} subunit mRNA also was observed in the nucleus of the brachium of the inferior colliculus in tottering mice whereas thalamic nuclei, hippocampus, and olfactory bulb expression did not significantly differ from control mice (Table 3). Although an increase in α_{1C} subunit expression was observed in the cortex, this trend did not reach significance ($P < .1$).

The calcium channel α_{1D} subunit was expressed throughout brain, similar to the α_{1C} subunit, but with abundant Purkinje cell expression observed in both control and tottering mice (data not shown); α_{1D} subunit mRNA distribution was as described in previous reports (Tanaka et al., 1995). The α_{1D} subunit was not expressed in the nucleus of the brachium of the inferior colliculus where a significant increase in α_{1C} subunit mRNA expression was observed. Quantitative analyses of grain density in parasagittal brain sections demonstrated that α_{1D} subunit expression was similar in control (+/+) and tottering (*tg*/*tg*) mice (n = 3 per genotype; Table 4).

P/Q-Type Calcium Channel α_{1A} Subunit mRNA Expression. The calcium channel α_{1A} subunit mRNA was expressed in the cerebellum, inferior colliculus, and hippocampus as described in previous reports (Tanaka et al., 1995; Fletcher et al., 1996; data not shown). Hippocampal expression appeared uniform throughout the dentate gyrus and the cornu ammonus subdivisions. The calcium channel α_{1A} subunit mRNA expression was most abundant in cerebellar cortex with high levels in cerebellar granule cells and Purkinje cells.

Quantitative analyses of grain density in parasagittal brain sections from control (+/+), heterozygous (+/*tg*), and tottering (*tg*/*tg*) mice (n = 3 per genotype) were performed in the cerebellum, where an increase in α_{1C} subunit mRNA expression was observed. Grain densities in four to six representative high-power section images from individual cerebellar lobules (III–X) of each genotype were analyzed. A two-way factorial ANOVA indicated no significant differences among the three genotypes in either the Purkinje cell (ANOVA, $P > 0.24$) or granule cell (ANOVA, $P > 0.86$) layers. Similarly, no significant differences among lobules were observed in Purkinje cells (ANOVA, $P > 0.25$) and no genotype-by-lobule interaction was observed in either the Purkinje cell (ANOVA, $P > 0.95$) or granule cell (ANOVA, $P > 0.62$) layers. Although a significant effect of lobule in the granule cell layer was suggested by the two-way ANOVA ($P < .02$), a posthoc Scheffe F-test revealed no significant differences among the mean grain densities ($P > .05$). Thus, the tottering mutation does not affect expression of the calcium channel α_{1A} subunit mRNA within cerebellar lobules III through X.

[^{3}H]Nitrendipine Saturation Binding. [^{3}H]nitrendipine saturation-binding assays were performed in control and tottering mouse forebrain and cerebellum. Saturation analyses demonstrated specific and saturable binding of [^{3}H]nitrendipine to sites in mouse forebrain and cerebellum homogenates; representative saturation-binding curves are shown in Fig. 5. Scatchard plots of saturation data demonstrated that [^{3}H]nitrendipine binding was increased in the forebrain of *tg*/*tg* mice; however, this trend did not reach significance, likely because the increase observed in α_{1C} subunit mRNA expression occurred in only very limited regions of the forebrain whereas α_{1D} subunit mRNA expression was not altered by the mutation. In contrast, [^{3}H]nitrendipine binding was significantly increased (+75%) in tottering mouse cerebellum compared with control mouse cerebellum ($P < .05$; Table 5); these data are consistent with the increase in α_{1C} subunit mRNA expression in tottering mouse Purkinje cells. The K_D values did not differ significantly among the four tissues (Table 5). Scatchard plots were linear, indicating a single binding site and suggesting that the *tg* mutation had not created a new dihydropyridine-binding site.

Discussion

These data suggest that L-type calcium channels are up-regulated in response to the mutation in the P/Q-type calcium channel in tottering mice. Furthermore, L-type calcium channels appear to influence the expression of the intermittent dystonic episodes in tottering mice. The *tg* mutation directly alters the mouse calcium channel α_{1A} subunit gene,

TABLE 3

Quantitative analysis of calcium channel α_{1C} subunit mRNA expression in control (+/+) and tottering (*tg*/*tg*) mouse brain

Data represent mean ± S.E.M. (n = 3) with number of sections assessed in parentheses. Granule cell and Purkinje cell assessments were also performed in Lobule V of the cerebellum with similar results. An increase in cerebral cortex expression was also observed, but this trend did not reach significance ($P < .1$).

Brain Region	+/+	*tg*/*tg*
Cerebellar Purkinje cell layer (Lobule VIII)	110.6 ± 3.6 (11)	132.6 ± 3.6 (11)**
Cerebellar granule cell layer (Lobule VIII)	107.6 ± 3.7 (11)	110.4 ± 4.3 (11)
Deep cerebellar nuclei	68.5 ± 1.7 (11)	74.3 ± 3.0 (11)
Nucleus of the brachium of the inferior colliculus	80.3 ± 3.5 (7)	98.3 ± 4.4 (6)*
Medical geniculate nucleus	75.8 ± 2.7 (10)	83.3 ± 4.4 (9)
Thalamic ventrobasal complex	73.0 ± 2.5 (11)	78.2 ± 3.1 (10)
Hippocampal dentate gyrus	59.1 ± 2.1 (11)	58.9 ± 2.5 (11)
Cerebral cortex	57.8 ± 3.3 (11)	67.2 ± 3.8 (11)
Olfactory bulb	83.5 ± 4.9 (11)	78.8 ± 4.1 (11)

* $P < .01$ and ** $P < .001$ as determined by two-tailed Student's t test.

which encodes the pore-forming protein of P/Q-type calcium channels. Diffusable P/Q-type calcium channel blockers currently are unavailable, and, as such, the role of the mutant P/Q-type calcium channel in the expression of the motor phenotype is unclear at this time. However, pharmacologic blockade of L-type calcium channels with several distinct agents prevented tottering mouse dystonia. Additionally, L-type calcium channel mRNA expression and binding was increased in tottering mice. The increase in L-type calcium channels coupled with the ability of L-type channel blockers to prevent the intermittent dystonic episodes suggest that L-type calcium channels play a role in the abnormal motor phenotype expressed by tottering mutant mice.

Several agents that specifically alter L-type calcium channel activity affected the behavioral expression of the tottering mouse dystonic episodes. L-type calcium channel antagonists of the dihydropyridine class, nimodipine, nifedipine, and nitrendipine, prevented restraint-induced dystonia in a dose-dependent manner. Diltiazem, an L-type calcium channel blocker of the benzothiazepine class, prevented restraint-induced dystonia when injected either s.c. or i.c.v. The dihydropyridines and diltiazem cross the blood-brain barrier efficiently (Naito et al., 1986; Larkin et al., 1992), and both drug types bind extracellular sites on L-type calcium channels involving the S6 segments of repetitive domains III and IV (Kraus et al., 1996; Peterson et al., 1996). The binding sites appear allosterically linked but distinct because diltiazem enhances the binding of dihydropyridines (Yamamura et al., 1982). The phenylalkylamine verapamil also prevented dystonia when injected i.c.v.; the phenylalkylamine-binding site on L-type calcium channels is clearly distinct from the dihydropyridine and benzothiazepine sites, involving an intracellular site between S6 of repetitive domain IV and the C-terminal tail (Striessnig et al., 1990). Thus, five different L-type calcium channel blockers from three different drug classes all prevented the expression of the dystonic phenotype attesting to the specificity of the effect.

The ability of verapamil to prevent tottering mouse dystonia when injected i.c.v. underscores the role of central nervous system L-type calcium channel blockade in the prevention of this phenotype. The same drug injected peripherally failed to prevent dystonia in tottering mice at sublethal concentrations. Verapamil, a potent and specific L-type calcium channel antagonist, does not cross the blood-brain barrier in appreciable amounts (Hamann et al., 1983). The differential effects of the drug injected via two different routes indicates that blockade of peripheral L-type calcium channels in cardiac and skeletal muscle, where L-type calcium channels are the predominant carriers of calcium current, is insufficient to prevent dystonia. These data suggest that L-type calcium channel blockers prevent the tottering mouse motor abnormalities by acting within the central nervous system rather than on cardiac or skeletal muscle.

Activation of L-type calcium channels with the dihydropyridine Bay K8644 induced stereotypical tottering mouse dys-

TABLE 4

Quantitative analysis of calcium channel α_{1D} subunit mRNA expression in control (+/+) and tottering (*tg/tg*) mouse brain

Data represent mean ± S.E.M. (n = 3) with number of sections assessed in parentheses. Granule cell and Purkinje cell assessments were also performed in Lobule V of the cerebellum with similar results. Significant differences between +/+ and *tg/tg* mRNA expression were not observed as determined by two-tailed Student's t test. No detectable (N.D.) hybridization was observed in the nucleus of the brachium of the inferior colliculus.

Brain Region	+/+	*tg/tg*
Cerebellar Purkinje cells (Lobule VIII)	151.0 ± 7.0 (9)	151.7 ± 7.6 (9)
Cerebellar granule cell layer (Lobule VIII)	152.9 ± 8.9 (9)	146.5 ± 5.9 (9)
Deep cerebellar nuclei	71.5 ± 3.1 (9)	68.0 ± 2.6 (9)
Nucleus of the brachium of the inferior colliculus	N.D.	N.D.
Medical geniculate nucleus	75.2 ± 3.4 (8)	68.8 ± 5.2 (6)
Thalamic ventrobasal complex	69.9 ± 2.2 (9)	71.2 ± 4.0 (8)
Hippocampal dentate gyrus	77.2 ± 2.7 (9)	73.4 ± 3.9 (9)
Cortex	87.0 ± 4.4 (9)	78.1 ± 5.4 (9)
Olfactory bulb	109.0 ± 4.1 (9)	99.4 ± 6.0 (9)

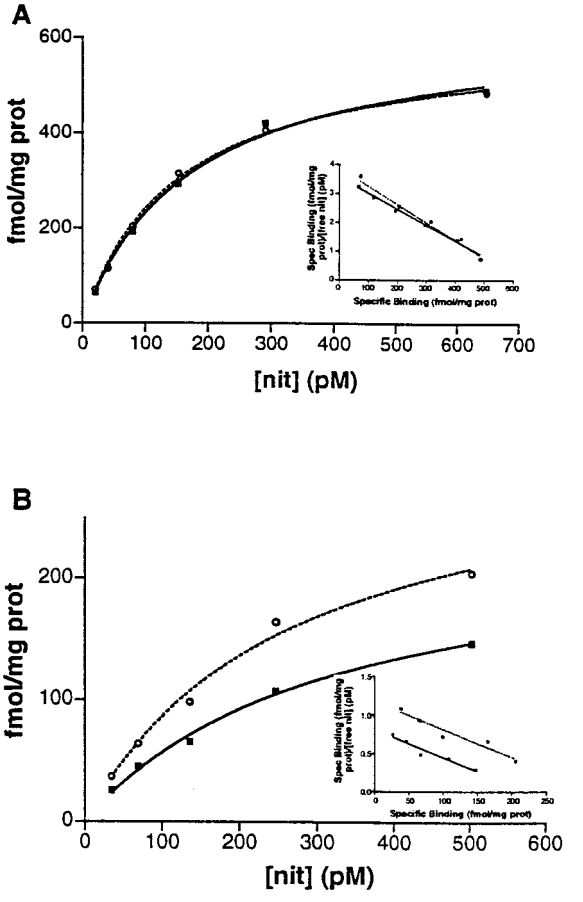

Fig. 5. Representative [^{3}H]nitrendipine saturation binding curve for +/+ (■) and *tg/tg* (○) forebrain (A) and cerebellum (B). Insets, Scatchard plot of the same data.

TABLE 5

Equilibrium dissociation constant, K_D (in pM), and number of binding sites, B_{max} (in fmol/mg protein), for [^{3}H]nitrendipine saturation binding in control (+/+) and tottering (*tg/tg*) mouse brain

The number of [^{3}H]nitrendipine-binding sites was increased in tottering mouse cerebellum. Data are expressed as mean ± S.E.M. of three to five experiments.

	Cerebellum		Forebrain	
	K_D	B_{max}	K_D	B_{max}
	pM	*fmol/mg protein*	*pM*	*fmol/mg protein*
+/+	197 ± 40	145 ± 14	155 ± 36	569 ± 90
tg/tg	203 ± 42	254 ± 41*	145 ± 15	585 ± 45

* $P < .05$ versus control (+/+) B_{max} by two-tailed Student's t test.

tonia at low doses and seizures more typical of this drug at higher doses. The two different disorders induced by Bay K8644 in tottering mice presumably result from regionally distinct calcium channel activation. In a tottering mouse, the dystonia may be induced at low doses through up-regulated L-type calcium channels. Specific increases in calcium channel α_{1C} subunit mRNA were observed in cerebellar Purkinje cells and the nucleus of the brachium of the inferior colliculus. As the dose of Bay K8644 is increased, the "classical" Bay K8644 seizures likely were induced through normosensitive L-type calcium channels located elsewhere in brain. This also would explain why wild-type mice injected with Bay K8644 do not exhibit tottering-like motor abnormalities as the up-regulated expression of α_{1C} subunits is required for expression of dystonia.

P/Q-type calcium channels are expressed throughout brain with the highest expression levels corresponding to regions of high cell density including cerebellar cortex, hippocampus, and olfactory bulb (Stea et al., 1994; Tanaka et al., 1995; Volsen et al., 1995). Thus, any compensatory changes in calcium channel expression may be more evident in cerebellar cortex, but it is not clear why L-type calcium channels are not increased in other brain regions that express high levels of P/Q-type calcium channels. Consistent with the mRNA expression patterns, P/Q-type calcium channel currents predominate in cultured rat cerebellar cortex neurons, carrying 85 to 95% of the total calcium current in Purkinje cells (Mintz et al., 1992) and nearly half the calcium current in granule cells (Randall and Tsien, 1995). In contrast, L-type currents account for a small fraction of the total current (<20%) in both cell types. Thus, an increase in L-type calcium channels could lead to a dramatic change in the conductance of calcium ions in these neurons. The potential for dramatic functional effects is particularly evident in Purkinje cells where L-type channels are restricted to the soma and proximal dendrites (Hell et al., 1993) and thus are positioned to have greater influence on the axon hillock than would be expected by a random distribution.

Although the functional significance of the tottering mutation on P/Q-type calcium channels is as yet unknown, functional differences between L-type and P/Q-type calcium channels are well characterized. In experiments directly comparing α_{1A} (P/Q-type) and α_{1C} (L-type) calcium channels under standard conditions, Sather et al. (1993) found that the single-channel conductance of α_{1C} channels is measurably greater than that of α_{1A} channels; the same is true of native L-type channels compared with native P/Q-type channels. Both L-type and P/Q-type calcium channel currents can be facilitated by a prepulse (Bourinet et al., 1994), with P/Q-type currents facilitated by relief of G-protein-mediated inhibition (Currie and Fox, 1997). The facilitated L-type currents can be very large (more than three times the control currents) and are inducible by either a single strong depolarization or a short train of pulses (Bourinet et al., 1994), whereas the facilitation of P/Q-type currents is more subtle, with only ~18% of the inhibition being voltage-sensitive (Currie and Fox, 1997). This facilitation of L-type calcium currents may explain how L-type calcium channel expression promotes the expression of intermittent dystonia in tottering mice. It is possible that temporal summation of calcium influx through voltage-dependent calcium channels, blunted in normal mice by a paucity of facilitation-susceptible L-type calcium channels, may be augmented by the increased density of L-type channels in tottering mutants.

Mutations of the calcium channel α_{1A} subunit are associated with three distinct human diseases and two distinct mouse neurologic phenotypes. The human spinocerebellar ataxia type 6 (SCA6) and mouse leaner (tg^{la}) mutations both disrupt the intracellular carboxyl tail of the calcium channel (Fletcher et al., 1996; Zhuchenko et al., 1997) and cause a persistent debilitating ataxia associated with severe degeneration of cerebellar neurons (Herrup and Wilczynski, 1982; Zhuchenko et al., 1997). In contrast, the human disorders familial hemiplegic migraine (FHM) and episodic ataxia type-2 (EA-2) mutations as well as the mouse tottering (tg) mutation alter the transmembrane and/or pore regions of the channel (Fletcher et al., 1996; Ophoff et al., 1996) and cause episodic attacks of neurologic dysfunction with little or no obvious changes in cerebellar morphology (Isaacs and Abbott, 1994; von Brederlow et al., 1995; Terwindt et al., 1996). FHM is characterized by brief periods of hemiparesis or weakness accompanied by migraine headaches. EA-2 patients exhibit intermittent attacks of ataxia precipitated by stress or exercise. The genotypic and phenotypic similarities between these human diseases and the intermittent motor phenotype of tottering mutant mice suggest the tottering mouse is a useful model for determining how mutations of the calcium channel α_{1A} subunit can generate episodic neurologic dysfunction in humans. Furthermore, tottering mice are an important tool for determining cell-type-specific regulation of calcium channel subtype expression and function in mammalian brain.

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References

Bourinet E, Charnet P, Tomlinson WJ, Stea A, Snutch TP and Nargeot J (1994) Voltage-dependent facilitation of a neuronal α_{1C} L-type calcium channel. *EMBO J* **13:**5032–5039.

Campbell DB and Hess EJ (1996) Chromosomal localization of the neurological mouse mutations tottering (*tg*), Purkinje cell degeneration (*pcd*), and nervous (*nr*). *Mol Brain Res* **37:**79–84.

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

Campbell DB and Hess EJ (1998) Cerebellar circuitry is activated during convulsive episodes in the tottering (*tg*/*tg*) mutant mouse. *Neuroscience* **85:**773–783.

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

Currie KPM and Fox AP (1997) Comparison of N- and P/Q-type voltage-gated calcium channel current inhibition. *J Neurosci* **17:**4570–4579.

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

Green MC and Sidman RL (1962) Tottering: A neuromuscular mutation in the mouse and its linkage with oligosyndactylism. *J Hered* **53:**233–237.

Hamann SR, Todd GD and McAllister RG (1983) The pharmacology of verapamil: V. Tissue distribution of verapamil and norverapamil in rat and dog. *Pharmacology* **27:**1–8.

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

Herrup K and Wilczynski SL (1982) Cerebellar cell degeneration in the leaner mutant mouse. *Neuroscience* **7:**2185–2196.

Isaacs KR and Abbott LC (1994) Cerebellar volume decreases in the tottering mouse are specific to the molecular layer. *Brain Res Bull* **36:**309–314.

Kaplan BJ, Seyfried TN and Glaser GH (1979) Spontaneous polyspike discharges in an epileptic mutant mouse (tottering). *Exp Neurol* **66:**577–586.

Kraus R, Reichl B, Kimball SD, Grabner M, Murphy BJ, Catterall WA and Striessnig J (1996) Identification of benz(othi)azepine-binding regions within L-type calcium channel α1 subunits. *J Biol Chem* **271:**20113–20118.

Larkin JG, Thompson GG, Scobie G, Forrest G, Drennan JE and Brodie MJ (1992)

Dihydropyridine calcium channel antagonists in mice: Blood and brain pharmacokinetics and efficacy against pentylenetetrazol seizures. *Epilepsia* **33:**760–769.
Lennon G, Auffray C, Polymeropoulos M and Soares MB (1996) The I. M. A. G. E. Consortium: An integrated molecular analysis of genomes and their expression. *Genomic* **33:**151–152.
Levitt P and Noebels JL (1981) Mutant mouse tottering: Selective increase of locus ceruleus axons in a defined single-locus mutation. *Proc Natl Acad Sci USA* **78:** 4630–4634.
Marangos PJ, Patel J, Miller C and Martino AM (1982) Specific calcium antagonist binding sites in brain. *Life Sci* **31:**1575–1585.
Mintz IM, Venema VJ, Swiderek KM, Lee TD, Bean BP and Adams ME (1992) P-type calcium channels blocked by the spider toxin ω-Aga-IVA. *Nature (London)* **355:** 827–829.
Naito K, Nagao T, Otsuka M, Harigaya S and Nakajima H (1986) Penetration into and elimination from the cerebrospinal fluid of diltiazem, a calcium antagonist, in anesthetized rabbits. *Arzneim-Forsch* **36:**25–28.
Noebels JL (1984) A single gene error of noradrenergic axon growth synchronizes central neurones. *Nature (London)* **310:**409–411.
Noebels JL and Sidman RL (1979) Inherited epilepsy: Spike-wave and focal motor seizures in the mutant mouse tottering. *Science (Wash DC)* **204:**1334–1336.
Ophoff RA, Terwindt GS, Vergouwe MN, van Eijk R, Oefner PJ, Hoffman SMG, Lamerdin JE, Mohrenweiser HW, Bulamn DE, Ferrari M, Haan J, Lindhout D, van Ommen G-JB, Hofker MH, Ferrari MD and Frants RR (1996) Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations of the Ca^{2+} channel gene CACNL1A4. *Cell* **87:**543–552.
Palmer GC, Stagnitto ML, Ray RK, Knowles MA, Harvey R and Garske GE (1993) Anticonvulsant properties of calcium channel blockers in mice: *N*-methyl-D-, L-aspartate- and Bay K8644-induced convulsions are potently blocked by the dihydropyridines. *Epilepsia* **34:**372–380.
Peterson BZ, Tanada TN and Catterall WA (1996) Molecular determinants of high affinity dihydropyridine binding in L-type calcium channels. *J Biol Chem* **271:** 5293–5296.
Randall A and Tsien RW (1995) Pharmacological dissection of multiple types of Ca^{2+} channel currents in rat cerebellar granule neurons. *J Neurosci* **15:**2995–3012.
Sather WA, Tanabe T, Zhang JF, Mori Y, Adams ME and Tsien RW (1993) Distinctive biophysical and pharmacological properties of class A (BI) calcium channel α1 subunits. *Neuron* **11:**291–303.
Stea A, Tomlinson WJ, Soong TW, Bourinet E, Dubel SJ, Vincent SR and Snutch TP (1994) Localization and functional properties of rat brain α_{1A} calcium channel reflect similarities to neuronal Q- and P-type channels. *Proc Natl Acad Sci USA* **91:**10576–10580.
Striessnig J, Glossman H and Catterall WA (1990) Identification of a phenylalkylamine binding region within the α1 subunit of skeletal muscle Ca^{2+} channels. *Proc Natl Acad Sci USA* **87:**9108–9112.
Tanaka O, Sakagami H and Kondo H (1995) Localization of mRNAs of voltage-dependent Ca^{2+}-channels: Four subtypes of α1- and β-subunits in developing and mature rat brain. *Mol Brain Res* **30:**1–16.
Teramoto T, Kuwada M, Niidome T, Sawada K, Nishizawa Y and Katayama K (1993) A novel peptide from funnel web spider venom, ω-Aga-TK, selectively blocks P-type calcium channels. *Biochem Biophys Res Commun* **196:**134–140.
Terwindt GM, Ophoff RA, Haan J, Frants RR and Ferrari MD (1996) Familial hemiplegic migraine: A clinical comparison of families linked and unlinked to chromosome 19. *Cephalgia* **16:**153–155.
Volsen SG, Day NC, McCormack AL, Smith W, Craig PJ, Beattie R, Ince PG, Shaw PJ, Ellis SB, Gillespie A, Harpold MM and Lodge D (1995) The expression of neuronal voltage-dependent calcium channels in human cerebellum. *Mol Brain Res* **34:**271–282.
von Brederlow B, Hahn AF, Koopman WJ, Ebers GC and Bulman DE (1995) Mapping the gene for acetazolamide responsive hereditary paroxysmal cerebellar ataxia to chromosome 19p. *Hum Mol Genet* **4:**279–284.
Wheeler DB, Randall A, Sather WA and Tsien RW (1995) Neuronal calcium channels encoded by the α_{1A} subunit and their contribution to excitatory synaptic transmission in the CNS. *Prog Brain Res* **105:**65–78.
Wurpel JND and Iyer SN (1994) Calcium channel blockers verapamil and nimodipine inhibit kindling in adult and immature rats. *Epilepsia* **35:**443–449.
Yamamura HI, Schoemaker H, Boles RG and Roeske WR (1982) Diltiazem enhancement of [^{3}H]nitrendipine binding to calcium channel associated drug receptor sites in rat brain synaptosomes. *Biochem Biophys Res Commun* **108:**640–646.
Zhuchenko O, Bailey J, Bonnen P, Ashizawa T, Stockton DW, Amos C, Dobyns WB, Subramony SH, Zoghbi HY and Lee CC (1997) Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the α_{1A}-voltage-dependent calcium channel. *Nat Genet* **15:**62–69.

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