

Noradrenergic blockade prevents attacks in a model of episodic dysfunction caused by a channelopathy

Brandy E. Fureman¹ and Ellen J. Hess*

Department of Neurology, Johns Hopkins University, Baltimore, MD 21152, USA

Department of Neuroscience, Johns Hopkins University, Baltimore, MD 21152, USA

Received 19 July 2004; revised 6 December 2004; accepted 4 March 2005

Available online 5 April 2005

Episodic neurological dysfunction often results from ion channel gene mutations. Despite knowledge of the mutations, the factors that precipitate attacks in channelopathies are not clear. In humans, mutations of the calcium channel gene *CACNA1A* are associated with attacks of neurological dysfunction in familial hemiplegic migraine and episodic ataxia type-2. In tottering mice, a mutation in the same gene causes attacks resembling paroxysmal dyskinesia. Stress, a trigger associated with human episodic disorders, reliably elicits attacks in tottering mice. Because noradrenergic neurotransmission is critical to the stress response and because noradrenergic hyperinnervation is observed in tottering mice, the role of norepinephrine in stress-induced attacks was investigated. Drugs that act at α -adrenergic receptors to block noradrenergic transmission prevented attacks. However, agents that facilitate noradrenergic neurotransmission failed to induce attacks. These results suggest that, while noradrenergic neurotransmission may be necessary for attacks, an increase in norepinephrine is not sufficient to induce attacks.

© 2005 Elsevier Inc. All rights reserved.

Keywords: Tottering; Calcium channel; Adrenergic; Familial hemiplegic migraine; Episodic ataxia; Paroxysmal dyskinesia; Dystonia; Mutation; Animal model

Introduction

The group of disorders caused by ion channel mutations exhibit distinct symptoms, including pain (Ophoff et al., 1996), muscle weakness/paralysis (Ptacek et al., 1991, 1992, 1994a,b), seizures (Charlier et al., 1998; Singh et al., 1998; Steinlein et al., 1995), and abnormal movements (Browne et al., 1994, Ophoff et

al., 1996). Despite the disparate symptoms, these channelopathies share both episodic expression and similar factors that trigger attacks of symptoms. Well-known triggers include stress, caffeine or alcohol consumption, hormonal changes, and fatigue (Battistini et al., 1999; Boel and Casaer, 1988; Bressman et al., 1988; Cooper and Jan, 1999; Demirkiran and Jankovic, 1995; Jarman et al., 2000; Jen, 1999; Lance, 1977; Mount and Reback, 1940; Pittock et al., 2000; Ptacek, 1998, 1999; Richards and Barnett, 1968). The mechanisms by which triggers initiate attacks of symptoms in channelopathies are largely unclear. A better understanding of the mechanistic interaction of triggers with altered ion channel systems may help to develop therapeutics designed to prevent or reduce the frequency of attacks in patients with a channelopathy disorder.

Mice harboring mutations in ion channels may offer animal models of episodic neurological dysfunction useful for studying human channelopathies. The tottering mutant mouse results from a missense mutation in the *Cacna1a* gene (Fletcher et al., 1996), which encodes the α_{1A} subunit (Ca_v2.1) of the P/Q-type calcium channel (Bourinet et al., 1999). In humans, mutations of the *CACNA1A* gene are associated with familial hemiplegic migraine, episodic ataxia type-2, or spinocerebellar ataxia type-6 (Ophoff et al., 1996; Zhuchenko et al., 1997). Tottering mice exhibit an episodic motor disorder similar to human paroxysmal dyskinesia (Campbell and Hess, 1999; Campbell et al., 1999; Green and Sidman, 1962), in addition to polyspike discharges resembling human absence epilepsy (Kaplan et al., 1979; Noebels and Sidman, 1979). Motor attacks are reliably elicited in tottering mice by triggers such as restraint stress (Campbell and Hess, 1998), caffeine, and alcohol exposure (Fureman et al., 2002). The genotypic and phenotypic similarities with human channelopathies suggest that the tottering mouse is a good model in which to study the effects of triggers on episodic neural dysfunction (Fureman et al., 2002).

There are as yet few clues into the mechanisms by which a trigger, such as stress, exerts physiological effects that lead to an attack. Both stress hormones and the catecholaminergic neurotransmitter norepinephrine have been implicated in behavioral responses to stress (Koob, 1999). One of the few neurotransmitter

* Corresponding author. Department of Neurology, Meyer, 6-181, Johns Hopkins University School of Medicine, 600 N. Wolfe Street, Baltimore, MD 21287, USA. Fax: +1 410 502 6737.

E-mail address: ehess@jhmi.edu (E.J. Hess).

¹ Present address: Office of Science Policy and Planning, National Institute of Neurological Disorders and Stroke, Bethesda, MD 20892, USA.

Available online on ScienceDirect (www.sciencedirect.com).

abnormalities to be described in the tottering brain is hyperinnervation of the targets of noradrenergic axons from the locus coeruleus (Noebels, 1984). Therefore, the impact of noradrenergic neurotransmission on stress-induced attacks of dyskinesia was investigated. The hypothesis that noradrenergic transmission is required for the expression of tottering mouse attacks was tested using a pharmacological approach. Agents with broad activity at α - or β -adrenergic receptors were initially tested to determine whether these receptor types were involved in the expression of attacks, followed by administration of drugs with greater selectivity for adrenergic receptor subtypes (Table 1).

Materials and methods

Animals

Tottering mice (*tg*; *Cacna1a*^{tg}) on a C57BL/6J background were bred at the Johns Hopkins University School of Medicine. Adult tottering mice (8–24 weeks of age) were identified by the absence of oligosyndactylism in *Os*^{+/+tg} × *Os*^{+/+tg} cross progeny. All procedures conformed to the NIH *Guidelines for the Care and Use of Animals* and were approved by the Johns Hopkins University Animal Care and Use Committee.

Drug administration

Mice were transferred from the vivarium to the laboratory and allowed to habituate for at least 2 h before the start of the experiment. A group of 36 tottering mice was used. Vehicle plus 5 doses of drug were tested simultaneously with $n = 6$ /dose/day. Each drug was tested over several days with at least 2 test days for each drug. Test days were always followed by at least one recovery day. Mice were pseudo-randomly assigned to a drug dose whereby an individual mouse never received the same drug dose over the course of the experiment. All drugs were injected subcutaneously (with the exception of prazosin, given intraperitoneally). Mice received a 10 ml/kg volume of the drug or

vehicle. Except where noted otherwise, drugs were obtained from Sigma (St. Louis, MO).

Agents expected to inhibit noradrenergic neurotransmission

Clonidine, phentolamine, and propranolol were prepared in 0.9% saline. Phenoxybenzamine and prazosin were prepared in DMSO and diluted to a final concentration of 5% DMSO with water (phenoxybenzamine) or 0.5% DMSO with 0.9% saline (prazosin). Mice were injected with drug, or the appropriate vehicle. Then, 10 min later, mice were restrained in a 60 cm³ 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. Mice injected with propranolol or saline vehicle were subjected to 10 min of restraint-stress 30 min after injection, released to a novel cage, and scored for the next 40 min. If an attack occurred within the first 40 min after release, the total number of 10 min intervals for the entire attack (up to 2 h) was determined as a measure of the severity of the attack.

Agents expected to facilitate noradrenergic neurotransmission

Amphetamine, cirazoline (Tocris; Ellisville, MO), and isoproterenol were prepared in 0.9% saline. Yohimbine (Tocris; Ellisville, MO) was first dissolved in water and then diluted in 0.9% saline. Desipramine was prepared in water. Mice were injected with drug or saline vehicle and 10 min later scored for the presence or absence of an attack. For desipramine, scoring began 20 min after injection. No restraint was used in order to assess the ability of the drug alone to induce an attack. Scoring continued every 10 min during the ensuing 40 min.

Statistical analyses

Because these are nonparametric data, results were analyzed using a logistic regression. Responses for each drug dose were compared to the responses of the vehicle-treated control group using logistic likelihood ratio tests. The duration of attack was analyzed by ANOVA and a post-hoc Dunnett's analysis; drug doses with 4 or fewer attacks were excluded from this analysis due to the low sample size.

Results

Doses for all drugs were selected prior to the start of the experiment based on the response of normal mice and based on dose ranges reported for humans. Generally, drug doses in mice are 10-fold greater than humans due to rapid drug metabolism in mice. Drug doses that caused sedation, seizures, or any unusual behavioral effects were excluded.

Tottering mice were treated with agents expected to inhibit noradrenergic neurotransmission before being exposed to restraint stress, a trigger that normally elicits attacks in 70–80% of tottering mice (Campbell and Hess, 1999; Fureman et al., 2002). To identify broad receptor classes that mediate attacks, mice were first challenged with nonselective α - or β -adrenergic receptor antagonists. The nonselective α -adrenergic receptor antagonists phentolamine and phenoxybenzamine inhibited the expression of attacks in a dose-dependent manner (Figs. 1A and B). However, neither drug significantly reduced the duration of attacks compared to

Table 1
Noradrenergic agents used in this study

Drug	Pharmacology	Site of action
<i>Agents expected to inhibit NE transmission</i>		
Phentolamine	Antagonist (nonselective)	α -adrenergic receptors
Phenoxybenzamine	Antagonist (nonselective)	α -adrenergic receptors
Clonidine	Agonist	α_2 -adrenergic autoreceptors
Prazosin	Antagonist	α_1 -adrenergic receptors
Propranolol	Antagonist (nonselective)	β -adrenergic receptors
<i>Agents expected to facilitate NE transmission</i>		
Amphetamine	Disrupts vesicular stores, reverse transport	Vesicles and transporters
Desipramine	Antagonist	Norepinephrine transporter
Cirazoline	Agonist	α_1 -adrenergic receptors
Yohimbine	Antagonist	α_2 -adrenergic autoreceptors
Isoproterenol	Agonist (nonselective)	β -adrenergic receptors

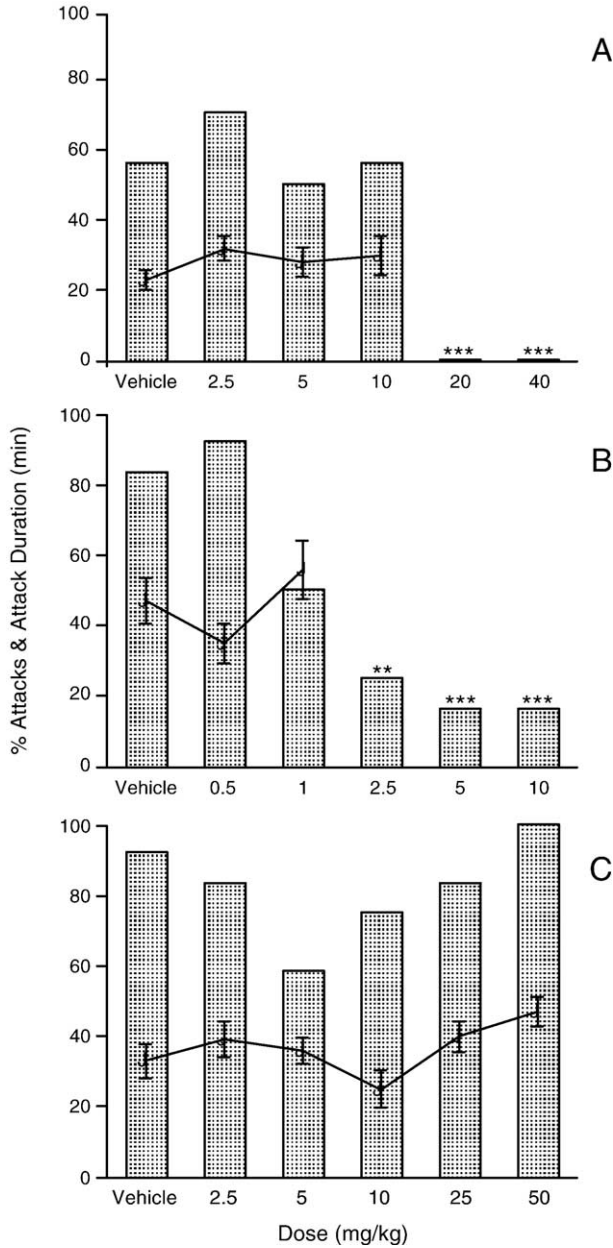


Fig. 1. Effect of nonselective α - or β -adrenergic receptor antagonists on restraint-induced tottering mouse attacks. Tottering mice were injected with phentolamine (A; $n = 10$ – 18 /dose), phenoxybenzamine (B; $n = 12$ /dose), or propranolol (C; $n = 12$ /dose), restrained, and scored for the presence of an attack for 40 min after release. Bars represent percent of mice exhibiting attacks and filled circles represent average duration of attack. Duration of attack was not included for doses where fewer than 5 attacks occurred due to the small sample size. Data for percent attacks were analyzed using a logistic regression and are shown as the percentage of mice exhibiting an attack. Propranolol had no effect on the frequency of restraint-induced attacks. Phentolamine and phenoxybenzamine significantly reduced restraint-induced attacks (** $P < 0.01$; *** $P < 0.001$). The duration of attack was analyzed by ANOVA and a post-hoc Dunnett's analysis; no difference in attack length was observed with any drug, regardless of dose.

vehicle treatment. That is, regardless of dose, phentolamine and phenoxybenzamine did not change the quality of the attack but rather reduced the quantity of attacks. The mixed β -adrenergic receptor antagonist propranolol had no effect on tottering mouse

attacks (Fig. 1C). These results suggest that the reduction in the frequency of attacks observed with the α -adrenergic receptor antagonists was not likely due to potential hypotensive effects as propranolol has similar peripheral effects but was ineffective against attacks.

Because the non-selective β -adrenergic receptor antagonist propranolol did not affect attacks, subtype-selective β -adrenergic receptor antagonists were not tested. However, given the efficacy of the non-selective α -adrenergic receptor antagonists, more selective antagonists were tested to determine the receptor subtype(s) involved. A dose-dependent inhibition of attacks was also observed after the administration of clonidine, an agonist at α_{2A} -adrenergic autoreceptors that blocks the release of norepinephrine (Fig. 2A). Furthermore, prazosin, an antagonist at α_1 -adrenergic receptors, prevented stress-induced attacks in tottering mice (Fig. 2B). Here again, although both clonidine and prazosin reduced the frequency of attacks, the duration of attacks that occurred in the presence of drug was not affected, regardless of

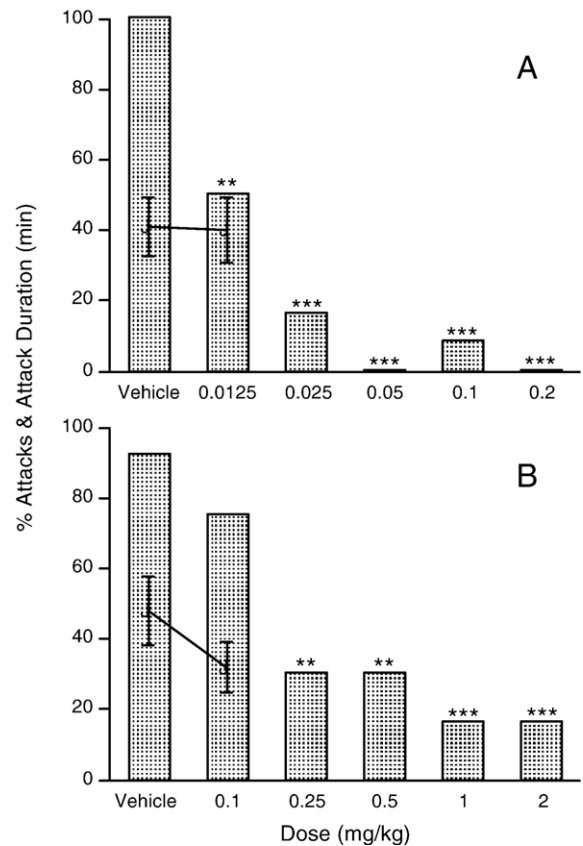


Fig. 2. Inhibition of noradrenergic transmission through subtype selective α -adrenergic receptor compounds reduces restraint-induced tottering mouse attacks. Tottering mice ($n = 12$ /dose) were injected with clonidine (A) or prazosin (B) 10 min prior to restraint and scored for the presence of an attack for 40 min after release. Bars represent percent of mice exhibiting attacks and filled circles represent average duration of attack. Duration of attack was not included for doses where fewer than 5 attacks occurred due to the small sample size. Data were analyzed using a logistic regression and are shown as the percentage of mice exhibiting an attack. Both clonidine and prazosin significantly reduced restraint-induced attacks (** $P < 0.01$; *** $P < 0.001$). The duration of attack was analyzed by ANOVA and a post-hoc Dunnett's analysis; no difference in attack length was observed with any drug, regardless of dose.

dose. In fact, no difference in the duration of attacks was observed even at the highest doses where attacks were observed but the sample size was too small for statistical analyses.

To determine if drugs that facilitate noradrenergic neurotransmission might induce attacks, both indirect and direct agonists were tested. Amphetamine, which increases both norepinephrine and dopamine in the synaptic cleft by promoting reverse transport, did not increase the attack frequency (Fig. 3). Likewise, no attacks were observed at any dose of desipramine, which increases the concentration of norepinephrine in the synaptic cleft by blocking norepinephrine reuptake transporters (1.25–20 mg/kg; $n = 12$ /dose; data not shown). The α_1 -adrenergic agonist cirazoline did not induce attacks in tottering mice in the absence of another trigger, and in fact, cirazoline suppressed attacks at the highest dose (Fig. 4A). However, the biological significance of this effect is not clear, given the very small reduction in absolute numbers of attacks relative to vehicle-induced attacks. Yohimbine, an α_2 -adrenergic autoreceptor antagonist, did not initiate tottering mouse attacks (Fig. 4B). Because the mixed β -adrenergic receptor agonist isoproterenol failed to induce any attacks at any dose tested (0.1–2.0 mg/kg; $n = 12$ /dose; data not shown), more selective β -adrenergic receptor agonists were not tested.

Discussion

The connections between chronically deleterious mutations in ion channel genes, episodically expressed symptoms, and the agents that provoke attacks are not well defined. However, common triggers have been noted throughout the clinical literature for many years. Regardless of the type of ion channel affected or the neurological dysfunction, the most common precipitants of attacks in episodic disorders include psychological or emotional stress, caffeine or alcohol consumption, hormonal changes, and fatigue (Battistini et al., 1999; Boel and Casaer, 1988; Bressman et al., 1988; Cooper and Jan, 1999; Demirkiran and Jankovic, 1995; Jarman et al., 2000; Jen, 1999; Lance, 1977; Mount and Reback, 1940; Ptacek, 1998, 1999; Pittcock et al., 2000; Richards and Barnett, 1968). The fact that these diverse agents are all potent

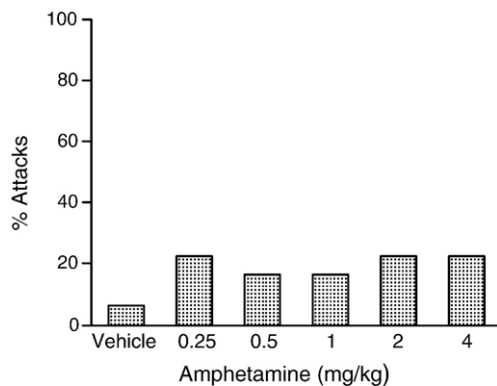


Fig. 3. Effect of amphetamine on the frequency of spontaneous tottering mouse attacks. Tottering mice ($n = 18$ /dose) were injected with amphetamine and scored for the presence of an attack for 40 min. Data were analyzed using a logistic regression and are shown as the percentage of mice exhibiting an attack. Amphetamine had no effect on the frequency of attacks.

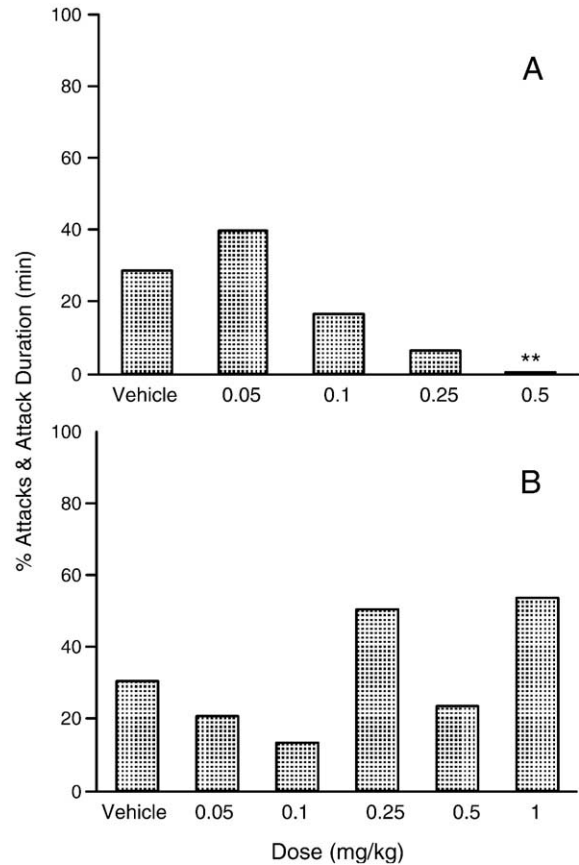


Fig. 4. Facilitation of noradrenergic transmission through subtype selective α -adrenergic receptor compounds does not affect restraint-induced tottering mouse attacks. Tottering mice were injected with cirazoline (A; $n = 18$ /dose) or yohimbine (B; $n = 30$ /dose) and scored for the presence of an attack for 40 min. Data were analyzed using a logistic regression and are shown as the percentage of mice exhibiting an attack. Cirazoline significantly reduced the frequency of attacks at only the highest dose (** $P < 0.01$). Yohimbine had no effect on the frequency of attacks.

triggers in many episodic disorders suggests that they might converge on a final common pathway to initiate abnormal symptoms. However, the mechanisms by which these triggers contribute to the onset of symptoms in a patient who is otherwise normal are unknown.

Emotional and/or psychological stress is a common trigger in episodic neurological disorders. The cascade of events leading to the physiological stress response appears to be under the control of noradrenergic neurotransmission, particularly those signals originating from the brainstem nucleus locus coeruleus (Koob, 1999). The firing rate of noradrenergic neurons in the locus coeruleus dramatically increases when awake, behaving rats are exposed to various environmental stimuli (Aston-Jones and Bloom, 1981; Foote et al., 1980), suggesting that this brainstem nucleus plays a role in arousal and stress responses. Projections from the locus coeruleus to the hypothalamus may signal the release of the central stress hormone, corticotropin-releasing factor (CRF) (Alonso et al., 1986). Conversely, the release of CRF may activate noradrenergic neurons of the locus coeruleus (Koob, 1999). These reciprocal pathways provide connections between autonomic and neuroendocrine systems and establish the hypothalamus as an integration center for neuroendocrine responses to visceral stimuli (Saw-

chenko and Swanson, 1981). Thus, the interactions between the noradrenergic system and the hypothalamus comprise a system that is sensitive to changes in both external and internal environments and capable of responding with physiologic mechanisms to regain homeostasis in response to stress.

One of the few neurotransmitter abnormalities to be described in the tottering brain is that noradrenergic axons originating from the locus coeruleus hyperinnervate their target regions (Levitt and Noebels, 1981). Norepinephrine content in target areas such as the cerebellum, hippocampus, neostriatum, and dorsal lateral geniculate nucleus is increased approximately 2-fold or more. In the anterior hypothalamus, NE content is 3-fold greater than in controls. Hyperinnervation results from an increase in the number of noradrenergic axon terminals, as the number of cells within the locus coeruleus is unchanged. This abnormality is specific to the locus coeruleus, since the terminal number and catecholamine content in targets innervated by neurons from another noradrenergic nucleus, the lateral tegmentum, are unperturbed (Levitt and Noebels, 1981). Although we and others have demonstrated that modest reductions in the noradrenergic hyperinnervation to hippocampus and cerebellum in tottering mice are not sufficient to eliminate attacks (Campbell et al., 1999; Noebels, 1984), prior to this report, potent and direct challenges to noradrenergic signaling had not been examined in tottering mice.

The results presented here suggest that β -adrenergic receptors are not involved in the expression of attacks in tottering mice. In contrast, a reduction in noradrenergic neurotransmission by drugs that act via α -adrenergic receptors was extremely effective in blocking attacks. Although these compounds reduced the frequency of attacks, the duration of attack was not affected suggesting that these drugs may increase the threshold for the initiation of attacks without modifying the quality of attack, once initiated. In contrast, increasing noradrenergic transmission by blocking re-uptake or by antagonizing presynaptic autoreceptors did not initiate attacks in the absence of another trigger. Therefore, it appears that noradrenergic transmission is necessary for the expression of an attack triggered by stress, but an increase in norepinephrine alone is not sufficient to trigger an attack.

Although the noradrenergic abnormalities in tottering mouse brain made these studies a logical place to initiate our investigation of triggering mechanisms, it appears that noradrenergic neurotransmission is not the final common step in the pathway to initiating an attack of dyskinesia in tottering mice. Nonetheless, the results suggest that α -adrenergic antagonists may reduce the frequency of attacks for patients where stress is a clear trigger and may provide a new treatment strategy. α_1 -adrenoceptors are coupled to phosphoinositide hydrolysis; agonist binding at this receptor results in the release of calcium from intracellular stores and activation of calcium-dependent currents (Kirischuk et al., 1996; Okuda et al., 2001; Paladini and Williams, 2004). We have previously demonstrated that inhibition of L-type calcium channels is extremely effective in blocking attacks. As calcium buffering may be inadequate in these mice (Cicale et al., 2002), both L-type calcium channel inhibitors and α -adrenergic antagonists may prevent gross fluctuations in intracellular calcium and stabilize normal signaling.

Effective therapies for disorders caused by calcium channel mutations, such as episodic ataxia type-2, are currently limited to carbonic anhydrase inhibitors, including acetazolamide (Griggs et al., 1978; Harno et al., 2004; Jen, 2000). For the paroxysmal dyskinesias, acetazolamide, carbamazepine, and valproate are

effective in some patients, but there is no satisfactory treatment in many cases (Bhatia, 2001; Zorzi et al., 2003). Clearly, more treatment options are necessary. Here, we demonstrate that the tottering mouse mutant is a valuable tool for screening drugs that may prove effective in human episodic disorders.

Acknowledgments

We thank Bryan Lee and Catherine Weisz for technical assistance. Supported by PHS NIH NS 33592.

References

- Alonso, G., Szafarczyk, A., Balmefrezol, M., Assenmacher, I., 1986. Immunocytochemical evidence for stimulatory control by the ventral noradrenergic bundle of parvocellular neurons of the paraventricular nucleus secreting corticotropin releasing hormone and vasopressin in rats. *Brain Res.* 397, 297–307.
- Aston-Jones, G., Bloom, F.E., 1981. Nonrepinephrine-containing locus coeruleus neurons in behaving rats exhibit pronounced responses to non-noxious environmental stimuli. *J. Neurosci.* 1, 887–900.
- Battistini, S., Stenirri, S., Piatti, M., Gelfi, C., Righetti, P.G., Rocchi, R., Giannini, F., Battistini, N., Guazzi, G.C., Ferrari, M., Carrera, P., 1999. A new CACNA1A gene mutation in acetazolamide-responsive familial hemiplegic migraine and ataxia. *Neurology* 53, 38–43.
- Bhatia, K.P., 2001. Familial (idiopathic) paroxysmal dyskinesias: an update. *Semin. Neurol.* 21, 69–74.
- Boel, M., Casaer, P., 1988. Familial periodic ataxia responsive to flunarizine. *Neuropediatrics* 19, 218–220.
- Bourinet, E., Soong, T.W., Sutton, K., Slaymaker, S., Mathews, E., Monteil, A., Zamponi, G.W., Nargeot, J., Snutch, T.P., 1999. Splicing of α_{1A} subunit gene generates phenotypic variants of P- and Q-type calcium channels. *Nat. Neurosci.* 2, 407–415.
- Bressman, S.B., Fahn, S., Burke, R.E., 1988. Paroxysmal non-kinesigenic dystonia. *Adv. Neurol.* 50, 403–413.
- Browne, D.L., Gancher, S.T., Nutt, J.G., Brunt, E.R., Smith, E.A., Kramer, P., Litt, M., 1994. Episodic ataxia/myokymia syndrome is associated with point mutations in the human potassium channel gene, KCNA1. *Nat. Genet.* 8, 136–140.
- Campbell, D.B., Hess, E.J., 1998. Cerebellar circuitry is activated during convulsive episodes in the tottering (tg/tg) mutant mouse. *Neuroscience* 85, 773–783.
- Campbell, D.B., Hess, E.J., 1999. L-type calcium channels contribute to the tottering mouse dystonic episodes. *Mol. Pharmacol.* 55, 23–31.
- Campbell, D.B., North, J.B., Hess, E.J., 1999. Tottling mouse motor dysfunction is abolished on the Purkinje cell degeneration (pcd) mutant background. *Exp. Neurol.* 160, 268–278.
- Charlier, C., Singh, N.A., Ryan, S.G., Lewis, T.B., Reus, B.E., Leach, R.J., Leppert, M., 1998. A pore mutation in a novel KQT-like potassium channel gene in an idiopathic epilepsy family. *Nat. Genet.* 18, 53–55.
- Cicale, M., Ambesi-Impombato, A., Cimini, V., Fiore, G., Muscettola, G., Abbott, L.C., de Bartolomeis, A., 2002. Decreased gene expression of calretinin and ryanodine receptor type 1 in tottering mice. *Brain Res. Bull.* 59, 53–58.
- Cooper, E.C., Jan, L.Y., 1999. Ion channel genes and human neurological disease: recent progress, prospects, and challenges. *Proc. Natl. Acad. Sci. U. S. A.* 96, 4759–4766.
- Demirkiran, M., Jankovic, J., 1995. Paroxysmal dyskinesias: clinical features and classification. *Ann. Neurol.* 38, 571–579.
- Fletcher, C.F., Lutz, C.M., O'Sullivan, T.N., Shaughnessy, J.D., Hawkes, R., Frankel, W.N., Copeland, N.G., Jenkins, N.A., 1996. Absence epilepsy in tottering mutant mice is associated with calcium channel defects. *Cell* 87, 607–617.

- Foote, S.L., Aston-Jones, G., Bloom, F.E., 1980. Impulse activity of locus coeruleus neurons in awake rats and monkeys is a function of sensory stimulation and arousal. *Proc. Natl. Acad. Sci. U. S. A.* 77, 3033–3037.
- Fureman, B.E., Jinnah, H.A., Hess, E.J., 2002. Triggers of paroxysmal dyskinesia in the calcium channel mouse mutant tottering. *Pharmacol. Biochem. Behav.* 73, 631–637.
- Green, M.C., Sidman, R.L., 1962. Tottering—A neuromuscular mutation in the mouse. *J. Hered.* 53, 233–237.
- Griggs, R.C., Moxley III, R.T., Lafrance, R.A., McQuillen, J., 1978. Hereditary paroxysmal ataxia: response to acetazolamide. *Neurology* 28, 1259–1264.
- Harno, H., Hirvonen, T., Kaunisto, M.A., Aalto, H., Levo, H., Isotalo, E., Somer, H., Kallela, M., Palotie, A., Wessman, M., Farkkila, M., 2004. Acetazolamide improves neurological abnormalities in a family with episodic ataxia type 2 (EA-2). *J. Neurol.* 251, 232–234.
- Jarman, P.R., Bhatia, K.P., Davie, C., Heales, S.J., Turjanski, N., Taylor-Robinson, S.D., Marsden, C.D., Wood, N.W., 2000. Paroxysmal dystonic choreoathetosis: clinical features and investigation of pathophysiology in a large family. *Mov. Disord.* 15, 648–657.
- Jen, J., 1999. Calcium channelopathies in the central nervous system. *Curr. Opin. Neurobiol.* 9, 274–280.
- Jen, J., 2000. Familial episodic ataxias and related ion channel disorders. *Curr. Treat. Options Neurol.* 2, 429–431.
- Kaplan, B.J., Seyfried, T.N., Glaser, G.H., 1979. Spontaneous polyspike discharges in an epileptic mutant mouse (tottering). *Exp. Neurol.* 66, 577–586.
- Kirischuk, S., Matiash, V., Kulik, A., Voitenko, N., Kostyuk, P., Verkhratsky, A., 1996. Activation of P₂-purino-, α_1 -adreno and H₁-histamine receptors triggers cytoplasmic calcium signalling in cerebellar Purkinje neurons. *Neuroscience* 73, 643–647.
- Koob, G.F., 1999. Corticotropin-releasing factor, norepinephrine, and stress. *Biol. Psychiatry* 46, 1167–1180.
- Lance, J.W., 1977. Familial paroxysmal dystonic choreoathetosis and its differentiation from related syndromes. *Ann. Neurol.* 2, 285–293.
- Levitt, P., Noebels, J.L., 1981. Mutant mouse tottering: selective increase of locus coeruleus axons in a defined single-locus mutation. *Proc. Natl. Acad. Sci. U. S. A.* 78, 4630–4634.
- Mount, L.A., Reback, S., 1940. Familial paroxysmal choreoathetosis: preliminary report on a hitherto undescribed clinical syndrome. *Arch. Neurol.* 44, 841–847.
- Noebels, J.L., 1984. A single gene error of noradrenergic axon growth synchronizes central neurones. *Nature* 310, 409–411.
- Noebels, J.L., Sidman, R.L., 1979. Inherited epilepsy: spike-wave and focal motor seizures in the mutant mouse tottering. *Science* 204, 1334–1336.
- Okuda, T., Tokutomi, N., Tokutomi, Y., Murai, Y., Negi, A., Nishi, K., 2001. Noradrenaline receptor-mediated potentiation of caffeine-induced Ca(2+)-activated K(+) currents in bovine ciliary muscle cells. *Curr. Eye Res.* 23, 455–462.
- Ophoff, R.A., Terwindt, G.M., Vergouwe, M.N., van Eijk, R., Oefner, P.J., Hoffman, S.M., Lamerdin, J.E., Mohrenweiser, H.W., Bulman, D.E., Ferrari, M., Haan, J., Lindhout, D., van Ommen, G.J., Hofker, M.H., Ferrari, M.D., Frants, R.R., 1996. Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca²⁺ channel gene CACNL1A4. *Cell* 87, 543–652.
- Paladini, C.A., Williams, J.T., 2004. Noradrenergic inhibition of midbrain dopamine neurons. *J. Neurosci.* 24, 4568–4575.
- Pittock, S.J., Joyce, C., O'Keane, V., Hugle, B., Hardiman, M.O., Brett, F., Green, A.J., Barton, D.E., King, M.D., Webb, D.W., 2000. Rapid-onset dystonia-parkinsonism: a clinical and genetic analysis of a new kindred. *Neurology* 55, 991–995.
- Ptacek, L.J., 1998. The place of migraine as a channelopathy. *Curr. Opin. Neurol.* 11, 217–226.
- Ptacek, L.J., 1999. Ion channel diseases: episodic disorders of the nervous system. *Semin. Neurol.* 19, 363–369.
- Ptacek, L.J., George, A.L.J., Griggs, R.C., Tawil, R., Kallen, R.G., Barchi, R.L., Robertson, M., Leppert, M.F., 1991. Identification of a mutation in the gene causing hyperkalemic periodic paralysis. *Cell* 67, 1021–1027.
- Ptacek, L.J., George, A.L.J., Barchi, R.L., Griggs, R.C., Riggs, J.E., Robertson, M., Leppert, M.F., 1992. Mutations in an S4 segment of the adult skeletal muscle sodium channel cause paramyotonia congenita. *Neuron* 8, 891–897.
- Ptacek, L.J., Tawil, R., Griggs, R.C., Engel, A.G., Layzer, R.B., Kwiecinski, H., McManis, P.G., Santiago, L., Moore, M., Fouad, G., et al., 1994a. Dihydropyridine receptor mutations cause hypokalemic periodic paralysis. *Cell* 77, 863–868.
- Ptacek, L.J., Tawil, R., Griggs, R.C., Meola, G., McManis, P., Barohn, R.J., Mendell, J.R., Harris, C., Spitzer, R., Santiago, F., et al., 1994b. Sodium channel mutations in acetazolamide-responsive myotonia congenita, paramyotonia congenita, and hyperkalemic periodic paralysis. *Neurology* 44, 1500–1503.
- Richards, R.N., Barnett, H.J., 1968. Paroxysmal dystonic choreoathetosis. A family study and review of the literature. *Neurology* 18, 461–469.
- Sawchenko, P.E., Swanson, L.W., 1981. Central noradrenergic pathways for the integration of hypothalamic neuroendocrine and autonomic responses. *Science* 214, 685–687.
- Singh, N.A., Charlier, C., Stauffer, D., DuPont, B.R., Leach, R.J., Melis, R., Ronen, G.M., Bjerre, I., Quattlebaum, T., Murphy, J.V., McHarg, M.L., Gagnon, D., Rosales, T.O., Peiffer, A., Anderson, V.E., Leppert, M., 1998. A novel potassium channel gene, KCNQ2, is mutated in an inherited epilepsy of newborns. *Nat. Genet.* 18, 25–29.
- Steinlein, O.K., Mulley, J.C., Propping, P., Wallace, R.H., Phillips, H.A., Sutherland, G.R., Scheffer, I.E., Berkovic, S.F., 1995. A missense mutation in the neuronal nicotinic acetylcholine receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. *Nat. Genet.* 11, 201–203.
- Zhuchenko, O., Bailey, J., Bonnen, P., Ashizawa, T., Stockton, D.W., Amos, C., Dobyns, W.B., Subramony, S.H., Zoghbi, H.Y., Lee, C.C., 1997. Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the α_{1A} -voltage-dependent calcium channel. *Nat. Genet.* 15, 62–69.
- Zorzi, G., Conti, C., Erba, A., Granata, T., Angelini, L., Nardocci, N., 2003. Paroxysmal dyskinesias in childhood. *Pediatr. Neurol.* 28, 168–172.