Published in final edited form as:

Neurosci Lett. 2005 July 1; 382(1-2): 51–55.

Genetic deletion of the norepinephrine transporter decreases vulnerability to seizures

Rafal M. Kaminski^{a,b,*}, Toni S. Shippenberg^a, Jeffrey M. Witkin^{b,1}, and Beatriz A. Rocha^{c,2}

^a Integrative Neuroscience Section, NIDA/IRP, Baltimore, MD, USA

^b Drug Development Group, NIDA/IRP, Baltimore, MD, USA

^c Behavioral Neuroscience Branch, NIDA/IRP, Baltimore, MD, USA

Abstract

Norepinephrine (NE) has been reported to modulate neuronal excitability and act as endogenous anticonvulsant. In the present study we used NE transporter knock-out mice (NET-KO), which are characterized by high levels of extracellular NE, to investigate the role of endogenous NE in seizure susceptibility. Seizure thresholds for cocaine (i.p.), pentylenetetrazol (i.v.) and kainic acid (i.v.) were compared in NET-KO, heterozygous (NET-HT) and wild type (NET-WT) female mice. The dose-response curve for cocaine-induced convulsions was significantly shifted to the right in NET-KO mice, indicating higher seizure thresholds. The threshold doses of pentylenetetrazol that induced clonic and tonic seizures were also significantly higher in NET-KO when compared to NET-WT mice. Similarly, NET-KO mice displayed higher resistance to convulsions engendered by kainic acid. For all drugs tested, the response of NET-HT mice was always intermediate. These data provide further support for a role of endogenous NE in the control of seizure susceptibility.

Keywords

Pentylenetetrazol; Kainic acid; Cocaine; Norepinephrine transporter; Seizures; Knock-out mice

Norepinephrine (NE), released from neurons in the CNS, has been implicated in the modulation of seizure susceptibility. Inhibition of NE neurotransmission was reported to exacerbate seizures [5,21], while enhancement of NE neurotransmission appears to have anticonvulsant effects [18]. More recently, studies in mice lacking NE due to the genetic deletion of the enzyme dopamine beta-hydroxylase showed an increased susceptibility to seizures in the mutants [27] further supporting the hypothesis that NE might act as an endogenous modulator of convulsive seizures (for reviews see [28,7]). However, a fundamental question remains as to whether an elevation of extracellular levels of endogenous NE has an opposite effect. The recent generation of mice lacking the NE transporter (NET-KO), offered the opportunity to explore this question since extracellular NE levels are 100% higher in these animals compared to wild-type litter mates (NET-WT) [32]. Therefore, in the present study we have compared the susceptibility of NET-KO, heterozygous (NET-HT) and NET-WT mice to seizures induced by three distinct convulsant agents, i.e. cocaine, pentylenetetrazol and kainic acid.

*Corresponding author. Present address: Epilepsy Research Section, NINDS/NIH, 35 Lincoln Drive, MSC 3702, Building 35, Room 1C1000, Bethesda, MD 20892-3702, USA. Tel.: +1 301 402 1315; fax: +1 301 480 1978; E-mail address: kaminskr@mail.nih.gov (R.M. Kaminski).

¹Present address: Neuroscience Discovery Research, Lilly Research Labs, Indianapolis, IN, USA.

²Present address: Department of Pharmacology, Merck Research Laboratories, Rahway, NJ, USA.

Female NET-KO, NET-HT and NET-WT mice (25-35 g) were generated on a mixed C57BL/6J $\times$ 129/SvJ background [32] and derived from heterozygous mating (F4 generation). The animals were kept in a vivarium under controlled laboratory conditions (temperature 22-26 °C, humidity 40-50%) with an artificial 12-h light/dark cycle. They were housed five per cage, and maintained with free access to food and water in a facility fully accredited by the American Association for Accreditation of Laboratory Animal Care. The experiments were performed during the light cycle after 30-min acclimation to the experimental room. Experimental groups consisted of five to seven age-matched mice of each genotype. Each mouse was naïve and used only once in the experimental procedures and received only one injection of a given convulsant.

The present study's protocol was approved by the Animal Care and Use Committee of the National Institute on Drug Abuse, according to the Guide for Care and Use of Laboratory Animals (National Research Council, 1996, National Academy Press, Washington, DC).

Mice received an i.p. injection of cocaine (0-110 mg/kg) (NIDA Drug Supply Program, Rockville, MD) and were immediately placed in individual Plexiglas boxes (25 cm $\times$ 15 cm $\times$ 36 cm) for observation. The endpoint for convulsions was the occurrence of clonic movements of all four limbs accompanied by loss of the righting response for at least 5 s. Although seizures typically occurred within 10 min, the presence or absence of convulsions was recorded for 30 min after injection. The percentage of animals exhibiting clonic seizures was determined for each dose of cocaine tested, and the convulsive threshold dose was expressed as the CD₅₀ value, which defines the dose predicted to induce convulsions in 50% of animals. The CD₅₀ values and their 95% confidence intervals were calculated and statistically compared with the use of computer software implementing probit analysis method [19].

Mice were briefly restrained in a Rotating Tail Injector (Braintree Scientific Inc., Braintree, MA), and a 30-gauge needle attached to a 0.3-m long polyethylene tubing (PE-10) was inserted into the lateral tail vein. The needle was gently secured to the tail with plastic tape. The tubing was connected with a 10-ml syringe mounted on the CMA 100 microinfusion pump (Carnegie Medicine AB, Stockholm, Sweden). Following catheterization, mice were placed in a Plexiglas cage (25 cm $\times$ 20 cm $\times$ 15 cm) for behavioral observation. Pentylentetrazol (10 mg/ml) (Sigma) or kainic acid (Sigma) (7.5 mg/ml) were infused at the rate of 0.15 ml/min. The behavioral endpoints of seizure activity were as follows: (i) the first myoclonic twitch or behavioral arrest (in case of kainic acid), (ii) clonic convulsions for at least 5 s, and (iii) tonic hind limb extension. The time from the start of the infusion to the onset of each behavioral endpoint was recorded. The threshold doses were calculated according to the following formula: threshold dose [mg/kg] = (drug concentration [mg/ml] $\times$ infusion rate [ml/s] $\times$ infusion duration [s] $\times$ 1000)/weight of mouse [g]. One-way analysis of variance (ANOVA) on genotype, followed by the Student-Newman-Keuls test when appropriate, was used to compare the threshold doses at each seizure endpoint.

Cocaine induced convulsions in all genotypes and the incidence of seizures increased in a dose-dependent manner. The dose-response curve of cocaine-induced convulsions was significantly ($p < 0.05$) shifted to the right in NET-KO mice when compared to NET-WT control; an intermediate shift that did not reach statistical significance was observed in NET-HT mice (Fig. 1A and B). Thus, the CD₅₀ of cocaine was approximately 30% and 13% higher in NET-KO and NET-HT than in NET-WT mice, respectively (Fig. 1B). Nonetheless, when convulsions did occur, their behavioral phenotype did not differ across genotypes.

The i.v. infusion of pentylentetrazol produced an array of behaviors that rapidly progressed from myoclonic twitching to full-blown clonic seizures followed by tonic hindlimb extension in all genotypes. However, the doses of pentylentetrazol necessary to induce each stage were 40-50% higher in NET-KO as compared to NET-WT mice whereas the response of NET-HT

mice was intermediate (Fig. 2A). ANOVA revealed a significant effect of genotype upon clonic ($F = 20.8$, d.f. = 2, 14; $p < 0.001$) and tonic convulsions ($F = 4.3$, d.f. = 2, 14; $p < 0.05$). Although the minimum dose of pentylenetetrazol producing the first myoclonic twitch was higher in NET-KO mice, this effect did not reach statistical significance ($F = 3.0$, d.f. = 2, 14; $p = 0.09$).

The i.v. infusion of kainic acid produced a seizure pattern similar to that of pentylenetetrazol, but in this case the animals initially exhibited a behavioral arrest. This behavior progressed to clonic seizures and was followed by a rapid transition into tonic limb extension. As with pentylenetetrazol the pattern of seizures did not differ between genotypes, but the minimum doses of kainic acid that produced behavioral arrest ($F = 8.8$, d.f. = 2, 18; $p < 0.005$) and tonic convulsions ($F = 4.7$, d.f. = 2, 18; $p < 0.05$) were significantly higher in NET-KO mice. As shown in Fig. 2B the minimum dose of kainic acid producing behavioral arrest and tonic convulsions was 92% and 48% higher in NET-KO mice than in NET-WT mice, respectively. A similar difference between genotypes in vulnerability to kainic acid induced clonic convulsions was apparent, however, this effect did not reach statistical significance ($F = 3.1$, d.f. = 2, 18; $p = 0.07$). As in the case of pentylenetetrazol, NET-HT mice always showed an intermediate response to kainic acid induced seizures (Fig. 2B).

The present study demonstrates that female NET-KO mice are more resistant than their NET-WT littermates to seizures induced by i.p. cocaine, i.v. pentylenetetrazol or i.v. kainic acid. While the behavioral pattern of the seizures induced by each of these drugs did not change among genotypes, the threshold doses were significantly higher in the mutants. These observations suggest that the absence of NET conferred a general protection against convulsions, which was apparently independent of the agent's route of administration or specific mechanism of action. Given that the potencies of the convulsants were dampened in an orderly manner by genotype suggests that it was the difference in circulating NE that accounted for the anticonvulsant effects of NET deletion.

It is widely accepted that NE plays a major role in regulation of the cardiovascular system. Thus, the present results may have been affected by pharmacokinetic factors. In fact, a lower heart rate and arterial pressure in resting conditions, but increased tachycardia in response to mechanic stimulation (i.e. tactile startle) that were recently described in NET-KO mice [15, 14] suggest that different hemodynamic conditions following experimental manipulation might have induced a higher rate of brain distribution in the mutants. However, if this was the case, increased instead of decreased sensitivity to seizures would have been observed. NE has been suggested to modify permeability of the blood brain barrier [10], which may have also altered the penetration of convulsants to the brain. Regardless of whether hemodynamic factors altered brain concentrations of the convulsants, NET deletion was sufficient to reduce seizure vulnerability of the mutants. The fact that the anticonvulsant effect was observed after administration of convulsants via different routes, i.e. i.p. and i.v., may also suggest that pharmacokinetic factors were less likely to contribute to these results. Nonetheless, careful evaluation of brain levels of the convulsants in NET-KO and NET-WT female mice is necessary to fully understand the contribution of pharmacokinetic factors to the present data.

Depending on the pharmacological agent used, diverse neurochemical pathways could be responsible for generation of seizure activity. Cocaine, pentylenetetrazol and kainic acid affect mainly monoaminergic, GABA-ergic or glutamatergic neurotransmission, respectively, but the specific mechanism of seizure-induction caused by these drugs awaits elucidation.

The two main actions of cocaine that seem to be responsible for its convulsant effect are inhibition of voltage-dependent sodium channels and blockade of monoamine transporters [17], but other neurotransmitter systems, such as GABA and glutamate, have also been implicated [6,31]. The protective effect of NET deletion against cocaine-induced seizures

seems to be difficult to interpret since, as it was mentioned before, cocaine itself has been shown to block NE uptake. Thus, the effects of NET deletion in NET-KO mice would be partially overlapped with NET inhibition by cocaine in NET-WT. Consequently, the relative influence of NET-KO on cocaine-induced convulsions could be smaller than that observed in case of pentylenetetrazole or kainate, which do not have inhibitory effects on monoamine transporters. In fact, NET deletion produced approx. 30% increase of the seizure threshold for cocaine-induced seizures, while it yielded 40-50% increase for pentylenetetrazole and kainate.

Pentylenetetrazol is believed to induce seizures primarily due to blockade of the GABA_A receptor [11], and kainic acid due to the activation of kainate receptors, particularly those containing the GluR6 subunit [3]. Increased seizure threshold for cocaine, pentylenetetrazol and kainic acid in NET-KO mice suggests a common NE-dependent mechanism, which occurs independently of the agent's primary pharmacological action.

Pharmacological and/or anatomical manipulations of NE transmission have been inversely correlated with seizure vulnerability in several species, and more recently such correlation was confirmed with a genetic approach in mice [27]. The ablation of the gene encoding the enzyme dopamine beta-hydroxylase, and the resultant disruption of NE biosynthesis, was associated with increased vulnerability to convulsant stimuli [27]. Conversely, the genetic ablation of the NET, and the resultant high extracellular levels of NE [32], was associated with decreased sensitivity to seizures as described here. The present data add support to the hypothesis that NE has an overall anticonvulsant role (for reviews see [28,7]), and further illustrate the bi-directional utility of the genetic approach to help test such hypotheses, albeit with the limitations imposed by compensations that occur in response to constitutive gene deletion.

The exact mechanism by which increased levels of NE protect against seizures is still unknown. Studies addressing this issue have been hampered by the existence of multiple adrenergic receptor subtypes, their pre-synaptic versus post-synaptic localizations and the degree of endogenous noradrenergic tone controlling a given seizure state. In addition, different effects of adrenoceptors ligands have been reported depending on their selectivity and the seizure model used [28]. For instance, β -adrenergic antagonists were shown to have both convulsant and anticonvulsant effects (for review see [28]). On the other hand, activation of α_1 - and/or α_2 receptor sub-types has been consistently implicated in the anticonvulsant effect of endogenous NE [28,29]. In this regard it is interesting to note that NET deletion is associated with a down-regulation of α_1 receptors in the hippocampus [32]. Consequently, such down-regulation of α_1 receptors may have triggered some other compensatory changes that resulted in increased seizure vulnerability of NET-KO mice. Interestingly, overexpression of α_{1B} receptors was associated with spontaneous seizures in mutant animals [16], despite the fact that α_1 receptors generally have anticonvulsant properties [28,29]. α_2 receptor agonists also exert anticonvulsant effects, particularly against pentylenetetrazol [24,26,20], kainic acid [2,8] and cocaine [30]. However, exogenously injected α_2 receptor agonists can act on neurons other than noradrenergic, such as dopaminergic or serotonergic neurons. In fact, dexmedetomidine, a highly selective α_2 receptor agonist, inhibited cocaine seizures, but this effect was closely associated with the decreased extracellular dopamine level in the nucleus accumbens [30]. Actually, it had been suggested that anticonvulsant effects of α_2 receptor agonists could be a result of decreased monoamine release [24,23]. Additional studies, however, are required to delineate the contribution of α_1 versus α_2 receptors in mediating the decreased seizure vulnerability of NET-KO mice.

Dysregulation of the NE system has been implicated in the pathophysiology of various psychiatric and neurological syndromes, such as depression, pain and epilepsy, for which comorbidity has been increasingly reported [12,22,13]. Antiepileptic drugs show efficacy against these syndromes [9,1] further suggesting a common mechanism linking these pathological

states [12,13,25]. The increased resistance of NET-KO mice to convulsant insults (present study) together with the increased nociceptive threshold [4], and the antidepressant-like phenotype [32] described in these mutants are consistent with this hypothesis, and indicate that the NET may be an effective target for the treatment of such complex disorders.

Acknowledgments

We are grateful to Dr. Marc G. Caron for the gift of NETHT mice used as breeding pairs, and to Dr. David Weinshenker for helpful comments and discussion during the preparation of the manuscript.

References

- [1]. Backonja MM. Use of anticonvulsants for treatment of neuropathic pain. *Neurology* 2002;59:S14–S17. [PubMed: 12221151]
- [2]. Baran H, Sperk G, Hortnagl H, Sapetschnig G, Hornykiewicz O. Alpha 2-adrenoceptors modulate kainic acid-induced limbic seizures. *Eur. J. Pharmacol* 1985;113:263–269. [PubMed: 2995067]
- [3]. Ari, Y. Ben; Cossart, R. Kainate, a double agent that generates seizures: two decades of progress. *Trends Neurosci* 2000;23:580–587. [PubMed: 11074268]
- [4]. Bohn LM, Xu F, Gainetdinov RR, Caron MG. Potentiated opioid analgesia in norepinephrine transporter knock-out mice. *J. Neurosci* 2000;20:9040–9045. [PubMed: 11124980]
- [5]. Corcoran ME, Fibiger HC, McCaughan JA Jr. Wada JA. Potentiation of amygdaloid kindling and metrazol-induced seizures by 6-hydroxydopamine in rats. *Exp. Neurol* 1974;45:118–133. [PubMed: 4153438]
- [6]. Gasior, M.; Kaminski, R.; Witkin, JM. Pharmacological modulation of GABA(B) receptors affects cocaine-induced seizures in mice, *Psychopharmacology (Berl.)*. 2004.
- [7]. Giorgi FS, Pizzanelli C, Biagioni F, Murri L, Fornai F. The role of norepinephrine in epilepsy: from the bench to the bedside. *Neurosci. Biobehav. Rev* 2004;28:507–524. [PubMed: 15465138]
- [8]. Halonen T, Kotti T, Tuunanen J, Toppinen A, Miettinen R, Riekkinen PJ. Alpha 2-adrenoceptor agonist, dexmedetomidine, protects against kainic acid-induced convulsions and neuronal damage. *Brain Res* 1995;693:217–224. [PubMed: 8653412]
- [9]. Harden CL. The co-morbidity of depression and epilepsy: epidemiology, etiology, and treatment. *Neurology* 2002;59:S48–S55. [PubMed: 12270969]
- [10]. Harik SI. Blood-brain barrier sodium/potassium pump: modulation by central noradrenergic innervation. *Proc. Natl. Acad. Sci. U.S.A* 1986;83:4067–4070. [PubMed: 3012548]
- [11]. Huang RQ, Bell-Horner CL, Dibas MI, Covey DF, Drewe JA, Dillon GH. Pentylentetrazole-induced inhibition of recombinant gamma-aminobutyric acid type A (GABA(A)) receptors: mechanism and site of action. *J. Pharmacol. Exp. Ther* 2001;298:986–995. [PubMed: 11504794]
- [12]. Jobe PC, Dailey JW, Wernicke JF. A noradrenergic and serotonergic hypothesis of the linkage between epilepsy and affective disorders. *Crit. Rev. Neurobiol* 1999;13:317–356. [PubMed: 11028680]
- [13]. Kanner AM, Balabanov A. Depression and epilepsy: how closely related are they? *Neurology* 2002;58:S27–S39. [PubMed: 11971130]
- [14]. Keller, NR.; Diedrich, A.; Appalsamy, M. Excessive Stress-Induced Tachycardia in the Norepinephrine Transporter (NET) Knockout Mouse, American Heart Association's Scientific Sessions. 2003.
- [15]. Keller, NR.; Diedrich, A.; Tuntrakool, S.; Caron, MG.; Robertson, D. Excessive Stress-Induced Tachycardia in the Norepinephrine Transporter (NET) Knockout Mouse, American Autonomic Society; 14th International Symposium on the Autonomic Nervous System; 2003;
- [16]. Kunieda T, Zuscik MJ, Boongird A, Perez DM, Luders HO, Najm IM. Systemic over expression of the alpha 1B-adrenergic receptor in mice: an animal model of epilepsy. *Epilepsia* 2002;43:1324–1329. [PubMed: 12423381]
- [17]. Lason W. Neurochemical and pharmacological aspects of cocaine-induced seizures. *Pol. J. Pharmacol* 2001;53:57–60. [PubMed: 11785913]

- [18]. Lindvall O, Barry DI, Kikvadze I, Brundin P, Bolwig TG, Bjorklund A. Intracerebral grafting of fetal noradrenergic locus coeruleus neurons: evidence for seizure suppression in the kindling model of epilepsy. *Prog. Brain Res* 1988;78:79–86. [PubMed: 3073428]
- [19]. Litchfield JT, Wilcoxon F. A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Ther* 1949;96:99–113. [PubMed: 18152921]
- [20]. Loscher W, Czuczwar SJ. Comparison of drugs with different selectivity for central alpha 1-and alpha 2-adrenoceptors in animal models of epilepsy. *Epilepsy Res* 1987;1:165–172. [PubMed: 2848694]
- [21]. McIntyre DC. Amygdala kindling in rats: facilitation after local amygdala norepinephrine depletion with 6-hydroxydopamine. *Exp. Neurol* 1980;69:395–407. [PubMed: 7409053]
- [22]. Ottman R, Lipton RB. Comorbidity of migraine and epilepsy. *Neurology* 1994;44:2105–2110. [PubMed: 7969967]
- [23]. Papanicolaou J, Summers RJ, Vajda FJ, Louis WJ. Anti-convulsant effects of clonidine mediated through central alpha2-adrenoceptors. *Eur. J. Pharmacol* 1982;77:163–166. [PubMed: 6277661]
- [24]. Papanicolaou J, Summers RJ, Vajda FJ, Louis WJ. The relationship between alpha 2-adrenoceptor selectivity and anticonvulsant effect in a series of clonidine-like drugs. *Brain Res* 1982;241:393–397. [PubMed: 6286046]
- [25]. Post RM. Do the epilepsies, pain syndromes, and affective disorders share common kindling-like mechanisms? *Epilepsy Res* 2002;50:203–219. [PubMed: 12151130]
- [26]. Scotti DC, Passarelli F, Pezzola A. Study on the anticonvulsant activity of clonidine against pentylenetetrazol-induced seizures in rats: pharmacological evidence of alpha 2-adrenoceptors mediation. *Arch. Int. Pharmacodyn. Ther* 1986;282:209–218. [PubMed: 2876688]
- [27]. Szot P, Weinshenker D, White SS, Robbins CA, Rust NC, Schwartzkroin PA, Palmiter RD. Norepinephrine-deficient mice have increased susceptibility to seizure-inducing stimuli. *J. Neurosci* 1999;19:10985–10992. [PubMed: 10594079]
- [28]. Weinshenker D, Szot P. The role of catecholamines in seizure susceptibility: new results using genetically engineered mice. *Pharmacol. Ther* 2002;94:213–233. [PubMed: 12113799]
- [29]. Weinshenker D, Szot P, Miller NS, Palmiter RD. Alpha(1) and beta(2) adrenoreceptor agonists inhibit pentylenetetrazole-induced seizures in mice lacking norepinephrine. *J. Pharmacol. Exp. Ther* 2001;298:1042–1048. [PubMed: 11504801]
- [30]. Whittington RA, Virag L, Vullemoz Y, Cooper TB, Morishima HO. Dexmedetomidine increases the cocaine seizure threshold in rats. *Anesthesiology* 2002;97:693–700. [PubMed: 12218537]
- [31]. Witkin JM, Gasior M, Heifets B, Tortella FC. Anticonvulsant efficacy of *N*-methyl-D-aspartate antagonists against convulsions induced by cocaine. *J. Pharmacol. Exp. Ther* 1999;289:703–711. [PubMed: 10215643]
- [32]. Xu F, Gainetdinov RR, Wetsel WC, Jones SR, Bohn LM, Miller GW, Wang YM, Caron MG. Mice lacking the norepinephrine transporter are supersensitive to psychostimulants. *Nat. Neurosci* 2000;3:465–471. [PubMed: 10769386]

Fig. 1.

Dose—response curves for the convulsant effects of cocaine and threshold doses of cocaine for the induction of seizures in NET-WT, NET-HT and NET-KO mice. The dose—response curves for convulsant effects of cocaine (**A**: left panel) were expressed as the percentage of animals ($N= 5-7$ mice per data point) displaying seizures in response to cocaine injection. The threshold dose with its 95% confidence intervals (**B**: right panel) for the convulsant effects of cocaine was expressed as a CD_{50} value, which is a dose predicted to induce convulsions in 50% of animals [Litchfield and Wilcoxon (1949)]. $*p < 0.05$ vs. NET-WT mice.

Fig. 2.

Threshold doses of pentylenetetrazol (PTZ) and kainic acid (KA) for the induction of seizures in NET-WT, NET-HT and NET-KO mice. The doses were determined following timed infusion of PTZ (10 mg/ml) or KA (7.5 mg/ml) into the lateral tail vein at the rate of 0.15 (ml/min), $N=5-6$ mice per group. $*p < 0.05$ vs. NET WT mice (Student—Newman—Keuls test).