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The Effects of Chronic Norepinephrine Transporter Inactivation on Seizure Susceptibility in Mice

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Epilepsy and depression are comorbid disorders, but the mechanisms underlying their relationship have not been identified. Traditionally, many antidepressants have been thought to increase seizure incidence, although this remains controversial, and it is unclear which medications should be used to treat individuals suffering from both epilepsy and depression. Since the neurotransmitter norepinephrine (NE) has both antidepressant and anticonvulsant properties, we speculated that NE transporter (NET) inhibitor antidepressants might be therapeutic candidates for comorbid individuals. To test this idea, we assessed the effects of chronic administration (via osmotic minipump) of the selective NET inhibitor reboxetine on flurothyl-induced seizures in mice. We found that reboxetine had both proconvulsant and anticonvulsant properties; it lowered both seizure threshold and maximal seizure severity. NET knockout (NET KO) mice essentially phenocopied the effects of reboxetine on flurothyl-induced seizures, and the trends were extended to pentylenetetrazole and maximal electroshock seizures (MES). Furthermore, reboxetine had no further effect in NET KO mice, demonstrating the specificity of reboxetine for the NET. We next tested the chronic and acute effects of other classes of antidepressants (desipramine, imipramine, sertraline, bupropion, and venlafaxine) on seizure susceptibility. Only venlafaxine was devoid of proconvulsant activity, and retained some anticonvulsant activity. These results suggest that chronic antidepressant drug treatment has both proconvulsant and anticonvulsant effects, and that venlafaxine is a good candidate for the treatment of epilepsy and depression comorbidity.

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INTRODUCTION

Epilepsy and depression are comorbid diseases; depressive disorders are the most common type of psychiatric comorbidity in patients with epilepsy, and patients with major depression have a higher frequency of epilepsy than the general population (Kanner and Nieto, 1999; Harden, 2002; Kanner and Balabanov, 2002; Barry, 2003). The mechanisms underlying this relationship, however, are unknown. Although there is some debate about the magnitude of the risk factors for comorbidity because of variance in study design and diagnostic criteria, most estimates place the risk for epileptics developing depression and for depressed patients developing epilepsy at four- to five-fold higher than the general population (Harden, 2002; Kanner and Balabanov, 2002). Treating comorbid individuals can be problematic; some anticonvulsants exacerbate depressive symptoms (Brent *et al*, 1987; Wiegartz *et al*, 1999; Kanner and Balabanov, 2002), and some antidepressants (eg bupropion, clomipramine) are reported to increase seizure susceptibility. Many of the newer antidepressants (eg sertraline, venlafaxine) appear safer but have not been systematically tested (Kanner *et al*, 2000; Kanner and Balabanov, 2002; Lee *et al*, 2003).

The neurotransmitter norepinephrine (NE) has both antidepressant and anticonvulsant properties. Multiple lines of evidence have accumulated over the years to suggest that depression is associated with changes in the noradrenergic system, while pharmacologically increasing NE potently alleviates depression (Ressler and Nemeroff, 1999; Frazer, 2000; Brunello *et al*, 2002). Likewise, endogenous NE is a critical inhibitor of seizure activity; stimulation of NE signaling powerfully inhibits seizures, whereas depletion of NE increases seizure susceptibility and accelerates epileptogenesis in nearly every animal model tested (Weinshenker and Szot, 2002; Giorgi *et al*, 2004). Furthermore, the anticonvulsant effects of multiple therapies for epilepsy

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are attenuated in rodents with NE deficiencies (Szot *et al*, 2001; Weinshenker and Szot, 2002; Schank *et al*, 2005), demonstrating that an intact NE system is important for the anticonvulsant activity of some epilepsy therapies.

Since NE is both antidepressant and anticonvulsant (Jobe *et al*, 1999), we speculated that NET inhibitors, which increase extracellular NE, might be ideal candidates for the treatment of individuals suffering from both epilepsy and depression. Acute administration of NET inhibitors is typically anticonvulsant (McIntyre *et al*, 1982; Clifford *et al*, 1985; Yan *et al*, 1993, 1998), while proconvulsant effects have been observed in a few studies where drug was administered chronically (McIntyre *et al*, 1982; Peterson *et al*, 1985; Escorihuela *et al*, 1989; Arai *et al*, 2003). There are, however, two caveats associated with these studies. First, most of them used desipramine (DMI) as the NET inhibitor. While DMI is a potent NET inhibitor and has good selectivity for NET over other monoamine transporters, it antagonizes other proteins, including receptors for histamine, acetylcholine, and adrenergic transmitters (Frazer, 1997). In particular, DMI blocks α_1-adrenoreceptors, and blockade of these receptors is typically proconvulsant (Weinshenker *et al*, 2001; Weinshenker and Szot, 2002). Second, the studies using chronic administration typically gave once daily bolus injections of drug. While this treatment regimen may mimic human administration (ie one pill per day), it likely produces a transient exposure instead of the chronically high serum levels seen therapeutically in patients because the half-lives of these drugs are much shorter in rodents than they are in humans (eg Lemberger *et al*, 1985; Caccia *et al*, 1990). Furthermore, some of the molecular changes in the brain that are thought to underlie the efficacy of antidepressant drugs only occur in rodents using paradigms that mimic chronic drug serum levels, such as osmotic minipump administration (Benmansour *et al*, 1999; Weinshenker *et al*, 2002).

The experiments presented here were designed to systematically test the effects of chronic and acute NET inhibitor administration on seizure susceptibility. To address issues related to drug specificity, we used the selective NET inhibitor reboxetine, which does not interact with other transporters or receptors (Wong *et al*, 2000), and NET knockout (NET KO) mice, which have a specific deletion of the gene encoding NET. To mimic human antidepressant administration as closely as possible, we administered reboxetine via osmotic minipump for 3 weeks at a dose that produced therapeutic serum levels of drug. Finally, we systematically tested the chronic and acute effects of antidepressants from five other classes on seizure susceptibility (DMI, tricyclic NET inhibitor; imipramine, tricyclic NET and serotonin transporter (SERT) inhibitor, sertraline, selective SERT inhibitor, venlafaxine, selective NET and SERT inhibitor, and bupropion, selective NET and dopamine transporter (DAT) inhibitor).

MATERIALS AND METHODS

Animals

NET KO and wild-type (WT) control mice, maintained on a pure C57BL6/J background, were generated from NET +/− heterozygote breeders obtained from Mark Caron (Duke University). NET +/− mice were crossed, producing NET +/+ (WT) and NET −/− (KO) mice. WT and KO mice were then bred separately to produce the mice used, and all NET WT mice were age-matched to NET KO mice in the initial reboxetine experiments. For the second set of experiments that compared the effects of different antidepressant drugs, mice of a mixed C57BL/6J and 129SvEv background were used. These mice were heterozygote (*Dbh* +/−) controls from our dopamine β-hydroxylase knockout (*Dbh* −/−) colony. We originally used these mice because we wished to include the analysis of some *Dbh* −/− mice that completely lack NE (Thomas *et al*, 1995, 1998). However, the *Dbh* −/− mice did not tolerate the minipump surgeries well and showed signs of general malaise, and were not included in the final analysis. *Dbh* +/− mice have normal NE levels and were indistinguishable from WT littermates for all previously tested phenotypes, including flurothyl seizure susceptibility (Thomas *et al*, 1995, 1998; Thomas and Palmiter, 1997, Szot *et al*, 1999). The basal and reboxetine-induced seizure phenotypes of *Dbh* +/− mice were also similar to those observed for the NET WT mice. Thus, these mice were phenotypically WT. Adult male and female mice (3–7 months old at time of seizure testing) were used in all experiments, and control and experimental groups were age matched. No sex or age differences were observed and results were combined. Throughout the course of the experiment the colony room was maintained at 22°C with lights on from 0700 to 1900. Food and water were available *ad libitum*, and animals were maintained according to guidelines outlined in the *NIH Guide for Care and Use of Laboratory Animals*. All experiments were approved by the Emory University and Georgetown University Institutional Animal Care and Use Committees.

Drugs

Antidepressant drugs used in this study were: reboxetine (Pfizer, Groton, CT), DMI (Sigma-Aldrich, St Louis, MO), imipramine (Sigma-Aldrich), sertraline (Pfizer), bupropion (Sigma-Aldrich), and venlafaxine (Wyeth, Monmouth Junction, MJ).

Antidepressant Drug Administration

For the chronic studies, drug was administered via Alzet® osmotic minipumps (Model #2004, 0.25 µl/h, 28 d; Durect, Cupertino, CA). Antidepressant drugs were dissolved in either 0.9% NaCl (reboxetine, imipramine, venlafaxine, bupropion) or an aqueous solution containing 50% ethanol and 0.9% NaCl (DMI, sertraline), and loaded into pumps. Minipumps containing 0.9% NaCl or an aqueous solution containing 50% ethanol and 0.9% NaCl were used as vehicle controls. All pumps were placed in a sterile 37°C saline bath for 2 days before implantation. Mice were anesthetized with isoflurane and minipumps were implanted in the intraperitoneal cavity. All mice were given buprenorphine (2.5 mg/kg, s.c.) immediately following surgery. Flurothyl seizure susceptibility was tested 21 days following minipump implantation. For the acute studies, drug was administered i.p. 30 min prior to seizure testing.

Seizure Testing

Flurothyl. Flurothyl seizure thresholds were determined as described previously (Szot *et al*, 1999). Mice were placed in an air-tight, clear Plexiglas® chamber, and the volatile convulsant flurothyl (2,2,2-trifluroethylether; Sigma-Aldrich) was infused via syringe pump at a rate of 20 μl/min onto filter paper from which it vaporized. The latency in seconds to the first myoclonic jerk (MJ) and clonic–tonic seizure (CT) was measured. MJ, the first behavioral sign of seizure, is evidenced as a brief, large-scale muscle twitch, and is commonly thought of as an index of seizure induction. CT, which appears later as repetitive, full-body convulsions with loss of posture, can be thought of as an index of seizure generalization. Also recorded was the number of mice progressing to tonic extension of the hindlimbs and death. Each mouse was tested individually, removed immediately from the chamber after completion of seizure behavior, and received only one exposure to flurothyl.

Pentylenetetrazole (PTZ). PTZ (Sigma-Aldrich) seizure induction was performed as previously described (Szot *et al*, 1999). PTZ was administered to NET KO and WT mice at a dose of 40 mg/kg, i.p. All mice were placed in a clear Plexiglas® chamber and closely monitored for 10 min. This observation time was chosen because mice that displayed seizure activity did so within the first few minutes after PTZ administration. Latency to the first MJ and CT seizure was recorded.

Maximal electroshock (MES). Shocks, each 0.9 ms in duration, were delivered via ear-clip electrodes at a frequency of 299 pulses/s for 100 ms at 20 mA using a constant-current device (Ugo Basile ECT Unit 7801, Varese, Italy). Seizure severity was determined by measuring the duration of tonic hindlimb extension, flexion, and the extension/flexion (E/F) ratio. Flexion duration was timed from the instant the shock was delivered until the hindlimbs went through an angle of 90° to the plane of the body and extension was timed from that point until the hindlimbs relaxed. As the end of extension was variable, abrupt palpable relaxation of the body was taken as the end of the extension phase.

Analysis of Serum Drug Levels

Trunk blood was collected in Microtainer serum tubes (Fisher Scientific, Pittsburgh, PA) from mice either immediately following seizure (in the cases where the seizure was lethal) or 1–3 h following seizure (in the cases where seizure was not fatal). Tubes were spun for 5 min at 10 K, and serum was placed in a sterile tube and stored at −80°C until analysis.

Serum concentrations of sertraline, DMI, reboxetine, venlafaxine, imipramine, and bupropion were determined using HPLC with UV detection (214 nm). After addition of NaOH to buproprion, DMI, imipramine, sertraline, and reboxetine samples, these drugs were extracted into a mixture of 5% isopropanol/95% hexane, then back-extracted into 12 mM phosphate buffer (pH 2.5). Venlafaxine samples were treated with saturated sodium borate solution, extracted into ethyl ether, and then back-extracted into 10 mM HCl (pH 2.0). Extracted samples of DMI, imipramine, sertraline, and reboxetine were analyzed using a Waters Spherisorb CN column (5 μm, 4.6 mm × 250 mm) and a mobile phase containing 70% acetonitrile, 13% methanol, and 17% of 10 mM phosphate buffer (pH 6.7). Extracted samples of venlafaxine and bupropion were analyzed using an Alltech Alltima C18 column (5 μm, 4.6 mm × 150 mm) and a mobile phase containing 15% acetonitrile and 85% of 100 mM phosphate buffer (pH 2.5). Internal standards were used for all analyses except venlafaxine. All reagents were HPLC grade. Serum concentrations were expressed in ng/ml.

After pilot experiments to determine optimal doses, we achieved serum levels that fell within or very close to human therapeutic range for each drug, with the exception of sertraline (Table 1). Even at the solubility limit of sertraline (a concentration that delivered 40 mg/kg/day), the mean sertraline level was far below human therapeutic levels and was undetectable in some mice.

Table 1 Serum Concentrations of Antidepressants

Drug	Serum level		
	Dose (mg/kg/day)	Steady state (ng/ml)	Therapeutic (ng/ml)
Reboxetine ($n=19$)	20	335±65	100–400
Desipramine ($n=14$)	20	275±49	125–600
Imipramine ($n=9$)	120	312±38	200–300
Sertraline ($n=13$)	40	8±5	30–150
Bupropion ($n=13$)	40	76±17	50–100
Venlafaxine ($n=11$)	20	122±26	100–400
Venlafaxine ($n=8$)	40	500±96	100–400

Serum concentrations (mean±SEM) of antidepressants were after 21 days of treatments by osmotic minipump. As a reference, the human therapeutic drug level guidelines are also listed (Baldessarini, 1989; Kaye *et al*, 1989; Charlier *et al*, 2000).

Statistics

For analyses of seizure threshold and duration, *T*-tests were used to compare two groups, and one-way ANOVA followed by Dunnett's *posthoc* test was used when comparing multiple groups to vehicle. For number of mice progressing to tonic extension and death, treatment groups were compared to controls using Fisher's Exact Test. A *P*-value of <0.05 was considered significant. Graphpad Instat and Prism for Macintosh were used for all statistical analysis.

RESULTS

Chronic Reboxetine Administration has Both Proconvulsant and Anticonvulsant Effects

Most previous studies have examined the effects of acute antidepressant administration on seizure susceptibility, and the few that have used chronic administration employed drugs that have targets other than the NET (eg DMI) and

paradigms that did not support therapeutic serum levels (eg daily i.p. injection). To circumvent these caveats, we used a NET inhibitor that has no other known targets (reboxetine) and a paradigm that mimics therapeutic serum levels (chronic infusion via osmotic minipump; Table 1). Chronic administration of reboxetine to WT mice significantly lowered seizure threshold (shorter latency to first MJ and generalized CT seizure; Figure 1a), but also tended to reduce maximal seizure severity, although the effect was not quite significant (4/9 vehicle-treated mice died, while 0/9 reboxetine-treated mice died; $P = 0.08$ by Fisher's Exact Test; Table 2).

We speculated that if the effects of reboxetine on seizures were exclusively mediated by chronic NET blockade, then the seizure phenotype of mice completely lacking NET (NET KO mice) would be similar to reboxetine-treated WT mice. Furthermore, reboxetine should have no further effect on NET KO mice. We found that, similar to WT mice treated chronically with reboxetine, NET KO mice had increased seizure susceptibility (significantly shorter latency to MJ and CT; Figure 1b) and decreased maximal seizure severity (fewer NET KO mice progressed to tonic extension and death; Table 2). In contrast to its effects on WT mice, reboxetine did not alter seizures in NET KO mice (Figure 1c; Table 2). These results suggest that the effects of reboxetine on flurothyl-induced seizures are mediated solely by NET blockade.

We next addressed whether the effects of chronic NET inactivation on seizure phenotypes extended to other methods of seizure induction. We used PTZ seizure susceptibility as an alternate measure of seizure threshold and MES as an alternate measure of maximal seizure severity. Both the PTZ seizure threshold phenotype and the MES maximal seizure severity phenotypes of NET KO mice were similar to that observed with flurothyl; NET KO mice had a shorter latency to MJ and CT for PTZ seizures (Figure 2) and had a shorter duration of tonic extension for MES seizures (Figure 3). These results confirm that chronic

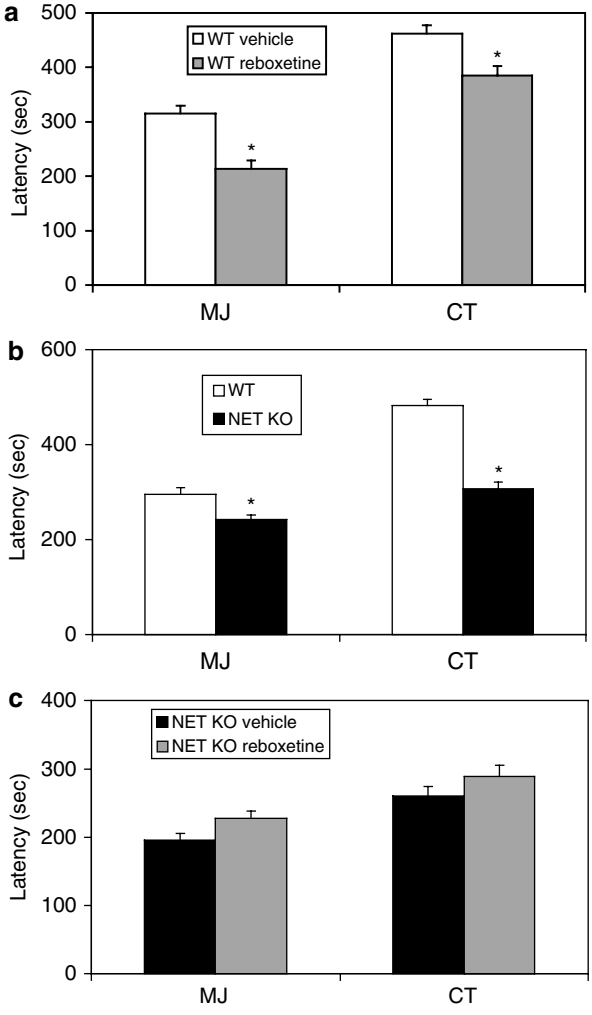

Figure 1 Effects of chronic reboxetine administration and NET genotype on flurothyl-induced seizure susceptibility. Shown is mean ± SEM latency to first myoclonic jerk (MJ) and clonic–tonic seizure (CT) after flurothyl administration in (a) wild-type (WT) mice administered vehicle (0.9% NaCl) or reboxetine (20 mg/kg/d) via osmotic minipump for 21 d ($n = 9$ per group; $^*P < 0.01$ compared to WT vehicle), (b) untreated WT and NET knockout (NET KO) mice ($n = 16$ per group; $^*P < 0.01$ compared to WT), and (c) NET KO mice administered vehicle (0.9% NaCl) or reboxetine (20 mg/kg/day) via osmotic minipump for 21 days ($n = 5$–6 per group).

Table 2 Effects of NET Genotype and Reboxetine on Maximal Seizure Severity

Genotype	Treatment	Tonic extension (over total)	Death (over total)
WT	None	14/16	4/16
NET KO	None	3/16*	0/16
WT	Vehicle	7/9	4/9
WT	Reboxetine	7/9	0/9
NET KO	Vehicle	0/5#	0/5
NET KO	Reboxetine	0/6#	0/6

NET KO and WT control mice were either untreated for administered vehicle or reboxetine (20 mg/kg/day) via osmotic minipump for 21 days prior to seizure induction with flurothyl. Shown is the number of mice that displayed tonic extension and death following generalized seizure over the total number of mice tested for each genotype and treatment group. Data were analyzed by Fisher's Exact Test. $^*P < 0.01$ compared to WT. $^{\#}P < 0.05$ compared to WT vehicle.

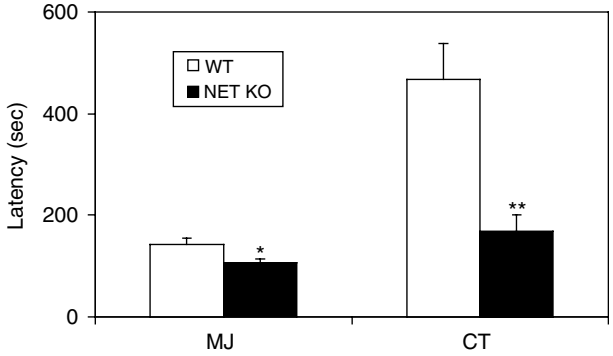

Figure 2 Effects of NET genotype on pentylenetetrazole-induced seizure susceptibility. Shown is mean ± SEM latency to first myoclonic jerk (MJ) and clonic–tonic seizure (CT) in wild-type (WT) and NET knockout (NET KO) mice after administration of pentylenetetrazole (PTZ; 40 mg/kg, i.p.; $n = 10$ per group; $^*P < 0.05$, $^{**}P < 0.01$ compared to WT).

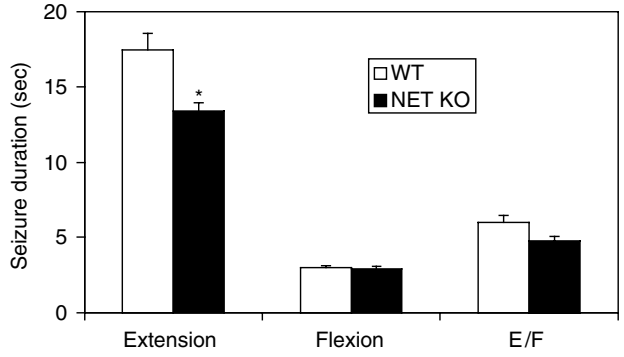

Figure 3 Effects of NET genotype on maximal electroshock seizures. Shown is mean ± SEM duration of tonic extension, flexion, and the tonic extension/flexion ratio in wild-type (WT) and NET knockout (NET KO) mice after seizure induction with maximal electroshock (MES; $n = 13–18$ per group; $^*P < 0.01$ compared to WT).

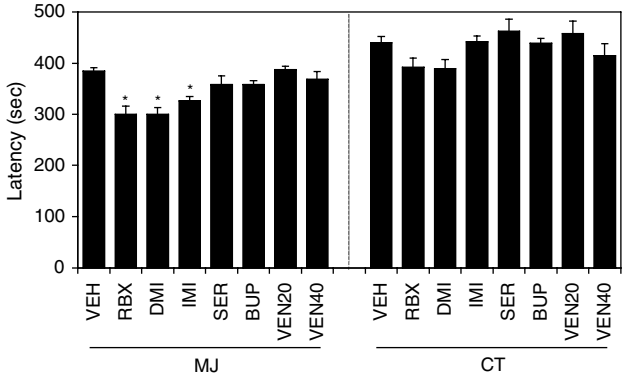

Figure 4 Effects of chronic antidepressant drug treatment on flurothyl-induced seizure susceptibility. Shown is mean ± SEM latency to first myoclonic jerk (MJ) and clonic–tonic seizure (CT) after flurothyl administration in mice administered vehicle (0.9% NaCl or 50% EtOH in 0.9% NaCl), reboxetine (RBX; 20 mg/kg/day), desipramine (DMI; 20 mg/kg/day), imipramine (IMI; 120 mg/kg/day), sertraline (SER; 40 mg/kg/day), bupropion (BUP; 40 mg/kg/day), or venlafaxine (VEN20; 20 mg/kg/day, VEN40; 40 mg/kg/day) via osmotic minipump for 21 d ($n = 7–15$ per group; $^*P < 0.01$ compared to vehicle).

NET inactivation has both proconvulsant (lowering of seizure threshold) and anticonvulsant (reduction of maximal seizure severity) properties.

Effects of Chronic Antidepressant Drug Treatment on Seizure Susceptibility

Our results suggest that, contrary to our hypothesis, reboxetine is not a good candidate to treat comorbid depression and epilepsy due to its proconvulsant effects. To determine whether a different type of antidepressant would be a better therapeutic candidate, we tested the effects of chronic treatment with a tricyclic NET inhibitor (DMI), a tricyclic NET and SERT inhibitor (imipramine), a selective SERT inhibitor (sertraline), a selective NET and DAT inhibitor (bupropion), and a selective NET and SERT inhibitor (venlafaxine) on flurothyl seizure susceptibility. We achieved therapeutic serum levels with all antidepressants tested except sertraline (Table 1). We found that, like reboxetine, DMI and imipramine lowered seizure threshold (significantly reduced latency to MJ, strong trend towards reducing latency to CT), while sertraline, bupropion, and venlafaxine had no effect (Figure 4). As we observed previously, reboxetine had an anticonvulsant effect in terms of seizure severity (reduced number of mice progressing to death; Table 3). DMI also tended to reduce seizure severity, but the results did not quite reach significance ($P = 0.07$ for both tonic extension and death by Fisher's Exact Test; Table 3).

Since the NET and SERT inhibitor imipramine had proconvulsant properties, we were intrigued by the lack of a proconvulsant effect for the NET and SERT inhibitor venlafaxine. Although venlafaxine serum levels were in therapeutic range, they were on the low end (Table 1). To determine whether a higher dose of venlafaxine would be proconvulsant, we doubled the venlafaxine dose from 20 to 40 mg/kg/day, which elevated serum drug levels from 122 to 500 ng/ml, and retested seizure susceptibility. The higher dose of venlafaxine still did not significantly lower seizure thresholds (Figure 4), and in fact tended to decrease maximal seizure severity (fewer mice died; $P = 0.09$ by Fisher's Exact Test; Table 3). These results suggest that the effects of venlafaxine in the brain are fundamentally different in some way from imipramine.

Table 3 Effects of Chronic Antidepressant Treatment on Maximal Seizure Severity

Drug	Dose (mg/kg/day)	Tonic extension (over total)	Death (over total)
Vehicle	NA	15/16	12/16
Reboxetine	20	10/10	0/10*
Desipramine	20	4/7	2/7
Imipramine	120	5/7	4/7
Sertraline	40	4/7	4/7
Bupropion	40	9/12	7/12
Venlafaxine	20	7/9	5/9
Venlafaxine	40	6/8	3/8

Antidepressants were administered via osmotic minipump for 21 days prior to seizure induction with flurothyl. Shown are the number of mice that displayed tonic extension and death following generalized seizure over the total number of mice tested for each drug. Data were analyzed by Fisher's Exact Test.
$^*P < 0.05$ compared to vehicle control.

Effects of Acute Antidepressant Drug Treatment on Seizure Susceptibility

In order to compare the effects of chronic and acute antidepressant treatment on seizure susceptibility, we administered each antidepressant (a single i.p. bolus of the chronic therapeutic daily dose) to seizure-naïve mice 30 min prior to induction of seizures with flurothyl. In contrast to the effects of chronic treatment, most of the antidepressants did not significantly affect seizure thresholds (Figure 5). The one exception was sertraline, which was profoundly proconvulsant. Reboxetine and the lower dose of venlafaxine reduced the number of mice that died, while

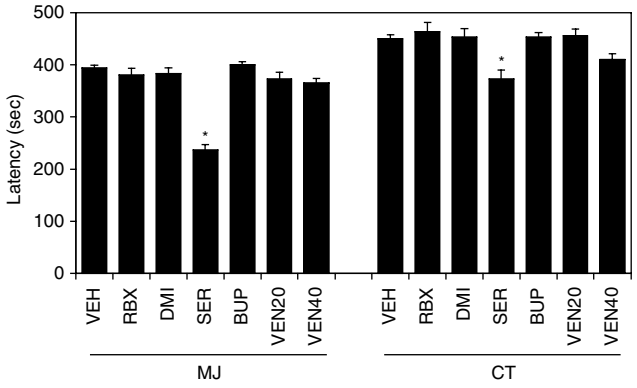

Figure 5 Effects of acute antidepressant drug treatment on flurothyl-induced seizure susceptibility. Shown is mean ± SEM latency to first myoclonic jerk (MJ) and clonic–tonic seizure (CT) in mice administered vehicle (0.9% NaCl), reboxetine (RBX; 20 mg/kg), desipramine (DMI; 20 mg/kg), sertraline (SER; 40 mg/kg), bupropion (BUP; 40 mg/kg), or venlafaxine (VEN20; 20 mg/kg, VEN40; 40 mg/kg) via i.p. injection 30 min prior to seizure induction with flurothyl ($n = 6$–11 per group; $^*P < 0.05$ compared to vehicle).

Table 4 Effects of Acute Antidepressant Treatment on Maximal Seizure Severity

Drug	Dose (mg/kg/day)	Tonic extension (over total)	Death (over total)
Vehicle	NA	11/11	10/11
Reboxetine	20	8/8	3/8*
Desipramine	20	7/8	7/8
Imipramine	120	NA	NA
Sertraline	40	1/6*,[a]	1/6*,[a]
Bupropion	40	1/8*,[a]	0/8*,[a]
Venlafaxine	20	8/8	3/8*
Venlafaxine	40	2/8*	1/8*

Antidepressants were administered via i.p. injection 30 min prior to seizures induction with flurothyl. Shown are the number of mice that displayed tonic extension and death following generalized seizure over the total number of mice tested for each drug. Mice given imipramine were not tested for seizure susceptibility because they displayed ataxia and sedation following drug administration. Data were analyzed by Fisher's Exact Test. $^*P < 0.01$ compared to vehicle control.
[a]Denotes drugs that induced status epilepticus.

the higher dose of venlafaxine suppressed both tonic extension and death (Table 4). Sertraline and bupropion also appeared to reduce maximal seizure severity, but that result is somewhat deceiving. Mice treated with vehicle or other antidepressants that did not progress to tonic extension typically had one short (~10 s) CT seizure, and then entered a prolonged postictal period with no obvious seizure activity. For this reason, mice are removed from the flurothyl chamber after 10 min (Szot *et al*, 1999) and thus the numbers listed in Table 4 are for the first 10 min after flurothyl administration. In contrast, after the first CT seizure, sertraline- or bupropion-treated mice entered a state of status epilepticus; they had repeated CT seizure activity that continued for up to ~20 min, at which time most of them went into tonic extension and died (data not shown).

DISCUSSION

Chronic NET Inactivation has Both Proconvulsant and Anticonvulsant Effects

Since NE is anticonvulsant in nearly all known seizure models (Weinshenker and Szot, 2002), we speculated that NET inhibitors, which increase extracellular NE, would suppress seizures. In support of this hypothesis, we found that the selective NET inhibitor reboxetine reduced maximal flurothyl seizure severity when administered both chronically and acutely. Flurothyl and MES seizures in NET KO mice were also less severe, and reboxetine had no further effect in NET KO mice. Thus, as predicted, selective genetic or pharmacological inactivation of NET had anticonvulsant properties.

Paradoxically, chronic NET inactivation was also proconvulsant, as both NET KO and reboxetine-treated WT mice had a reduction in seizure threshold. How can we reconcile the anticonvulsant effect of NE and the proconvulsant effect of NET blockade, which increases extracellular NE? One clue is that while both chronic and acute NET blockade elevate extracellular NE, acute blockade of NET lacked the proconvulsant effect on seizure threshold but retained the ability to suppress seizure severity. This result indicates that increasing NE is not proconvulsant *per se*, but rather suggests that chronic NET blockade activates compensatory mechanisms that are proconvulsant. It is well established that chronic but not acute NET inactivation results in many changes in the noradrenergic system, including a downregulation of tyrosine hydroxylase (the rate-limiting enzyme in NE synthesis; Nestler *et al*, 1990), burst firing of the locus coeruleus (LC, the major brain noradrenergic cell group; Grant and Weiss, 2001), the NET (Benmansour *et al*, 1999; Weinshenker *et al*, 2002), and adrenergic receptors (Bergstrom and Kellar, 1979; Xu *et al*, 2000; Invernizzi and Garattini, 2004). A decrease in LC activity after chronic NET blockade was associated with an increase in the activity of hippocampal neurons that received noradrenergic innervation (Huang *et al*, 1980), and hippocampal hyperexcitability often contributes to seizures. Finally, an increase in extracellular NE could enhance the activation of inhibitory α_2-adrenergic autoreceptors. Thus, although basal extracellular NE levels are elevated, chronic NET blockade may result in an overall decrease in NE signaling under some conditions, which could produce a proconvulsant effect. Another intriguing possibility is the interaction between the noradrenergic and GABAergic systems. NE can enhance hippocampal GABA function, and some studies have shown that chronic antidepressant treatment downregulates not only adrenergic receptors but also $GABA_A$ receptors (Suzdak and Gianutsos, 1985; Dennis *et al*, 1994; Sanacora *et al*, 2000). A decrease in GABA function could contribute to the proconvulsant effects of antidepressants.

In the preceding two paragraphs, we have presented evidence that chronic NET blockade could have anticonvulsant and proconvulsant effects, but how can we

explain both effects occurring essentially simultaneously? The proconvulsant effect of chronic NET inactivation is most evident for moderate clonic seizures (MJ), while the anticonvulsant effect is restricted to very severe seizures (tonic extension, death). One possibility is that different brain regions are involved. It is generally accepted that forelimb clonic seizures (eg MJ, rearing, and falling seizures) and hindlimb tonic seizures have partially overlapping but distinct anatomical substrates; clonic seizures predominantly activate forebrain regions, while tonic seizures also recruit brainstem structures (Browning, 1994; Eells *et al*, 2004). The effects of chronic NET blockade on NE signaling may differ between forebrain and brainstem regions. In support of this hypothesis, the decrease in β_1-adrenergic receptors observed after chronic DMI treatment was much more evident in the cortex compared to subcortical regions (Bergstrom and Kellar, 1979), and α_2-adrenergic receptor desensitization after chronic reboxetine was observed in the hippocampus but not the LC (Parini *et al*, 2005). Our results suggest that the compensatory downregulation of NE signaling may preferentially occur in the forebrain. Another possibility is the involvement of noradrenergic cotransmitters. LC neurons coexpress anticonvulsant neuropeptides such as NPY and galanin. Since neuropeptides are typically released under conditions of very high neuronal excitability, NPY and galanin may be preferentially released from LC neurons to prevent severe brainstem seizures under our experimental conditions. In support of this idea, the activation of LC neurons is much greater during brainstem tonic seizures than during forebrain clonic seizures (Eells *et al*, 2004).

Therapeutic Implications

Although depression is the most common comorbid psychiatric disorder in epilepsy, it is still severely underdiagnosed and undertreated in the epileptic population (Wiegartz *et al*, 1999; Harden, 2002; Kanner and Balabanov, 2002). Since depression appears to have a much greater impact on the quality of life of epileptic individuals than seizure frequency or severity (Johnson *et al*, 2004; Boylan *et al*, 2004), finding safe and effective treatment is critical. Historically, this has been problematic because some antidepressants exacerbate seizures, especially when drug doses are high (Pisani *et al*, 1999). SSRIs are typically recommended for alleviating depressive symptoms in epileptics, and appear to be relatively safe (Kanner and Nieto, 1999; Kanner *et al*, 2000; Pisani *et al*, 2002; Isbister *et al*, 2004), although some preclinical studies have suggested otherwise (Raju *et al*, 1999; Zienowicz *et al*, 2005). We were unable to test the effects of chronic sertraline administration due to rapid metabolism and/or excretion that precluded the maintenance of human therapeutic serum levels in the mice, but a high dose administered acutely was proconvulsant.

We speculated that selective NE reuptake inhibitors might be good candidates for treating comorbid individuals because NE has both antidepressant and anticonvulsant properties. However, we found that the selective NET blocker reboxetine possessed proconvulsant properties when administered chronically, as did the tricyclic DMI, probably due to compensatory mechanisms activated by chronic administration. Therefore, NET inhibitor antidepressants may not be safe for use in seizure-prone individuals.

There have been few systematic, preclinical comparisons of different classes of antidepressant drugs on seizure susceptibility, and none to our knowledge has utilized chronic administration at therapeutic serum drug levels. We found that chronic administration reboxetine, DMI, and imipramine had proconvulsant effects. In addition, acute administration of bupropion or sertraline was proconvulsant, and a seizure risk has been documented clinically for bupropion (Richmond and Zwar, 2003). The only antidepressant in our study that was devoid of significant proconvulsant effects was the selective NET/SERT inhibitor venlafaxine, even when administered at a relatively high dose. Venlafaxine is of considerable interest because, unlike other NET inhibitors like reboxetine, DMI, and imipramine, it does not cause a downregulation in NET density in rats after chronic administration, and the lack of effect on the NET cannot be attributed to its dual reuptake-inhibiting properties (Gould *et al*, 2004). Venlafaxine may interact with the NET and the noradrenergic system in a novel way, and it is possible that venlafaxine's lack of proconvulsant activity is related to this difference. Like many antidepressants, venlafaxine overdose has occasionally been reported to cause seizures, but it appears to be safe at therapeutic doses (White *et al*, 1997; Pisani *et al*, 2002). Our results suggest that venlafaxine warrants further investigation as a treatment for depression in the epileptic population.

In general, most antidepressants appear to be proconvulsant under some conditions. Therefore, it may be wise to explore alternative treatments for depression in comorbid individuals (Barry, 2003). Vagus nerve stimulation and the ketogenic diet were originally developed to treat epilepsy, and both treatments also appear to have antidepressant properties (El-Mallakh and Paskitti, 2001; Krahl *et al*, 2004; Murphy *et al*, 2004; Schachter, 2004). Interestingly, an intact noradrenergic system is critical for the anticonvulsant effect of vagus nerve stimulation and the ketogenic diet (Krahl *et al*, 1998; Szot *et al*, 2001). Thus, despite the proconvulsant properties of chronic NET inhibition, increasing NE signaling may still represent an effective therapy for epilepsy and depression comorbidity.

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