Norepinephrine-Deficient Mice Have Increased Susceptibility to Seizure-Inducing Stimuli

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Several lines of evidence suggest that norepinephrine (NE) can modulate seizure activity. However, the experimental methods used in the past cannot exclude the possible role of other neurotransmitters coreleased with NE from noradrenergic terminals. We have assessed the seizure susceptibility of genetically engineered mice that lack NE. Seizure susceptibility was determined in the dopamine β-hydroxylase null mutant (Dbh−/−) mouse using four different convulsant stimuli: 2,2,2-trifluoroethyl ether (flurothyl), pentylenetetrazol (PTZ), kainic acid, and high-decibel sound. Dbh−/− mice demonstrated enhanced susceptibility (i.e., lower threshold) compared with littermate heterozygous (Dbh+/−) controls to flurothyl, PTZ, kainic acid, and audiogenic seizures and enhanced sensitivity (i.e., seizure severity and mortality) to flurothyl, PTZ, and kainic acid. c-Fos mRNA expression in the cortex, hippocampus (CA1 and CA3), and amygdala was increased in Dbh−/− mice in association with flurothyl-induced seizures. Enhanced seizure susceptibility to flurothyl and increased seizure-induced c-fos mRNA expression were reversed by pretreatment with l-threo-3,4-dihydroxyphenylserine, which partially restores the NE content in Dbh−/− mice. These genetically engineered mice confirm unambiguously the potent effects of the noradrenergic system in modulating epileptogenicity and illustrate the unique opportunity offered by Dbh−/− mice for elucidating the pathways through which NE can regulate seizure activity.

Key words: dopamine β-hydroxylase; c-fos mRNA; norepinephrine; flurothyl; epilepsy; seizure; kainic acid

Chen et al. (1954) first suggested that the noradrenergic system modifies seizure activity. Since then, four major observations have supported an anticonvulsant role for norepinephrine (NE): (1) selective lesioning of noradrenergic neurons (with 6-hydroxydopamine or DSP-4) increases seizure susceptibility to a variety of convulsant stimuli (Arnold et al., 1973; Jerlicz et al., 1978; Mason and Corcoran, 1979; Snead, 1985; Trottier et al., 1988; Sullivan and Osorio, 1991; Mishra et al., 1994); (2) direct stimulation of the locus coeruleus (LC, the major concentration of noradrenergic cell bodies in the CNS) and the subsequent release of NE reduce CNS sensitivity to convulsant stimuli (Libet et al., 1977; Turski et al., 1989); (3) genetically epilepsy-prone rats (GEPRs), a widely used animal model of epilepsy, have deficient presynaptic NE content, NE turnover, tyrosine hydroxylase levels, dopamine β-hydroxylase (DBH) levels, and NE uptake (Jobe et al., 1984; Dailey and Jobe, 1986; Browning et al., 1989; Lauterborn and Ribak, 1989; Dailey et al., 1991); and (4) adrenergic agonists acting at the α2 adrenoceptor (α2-AR) have anticonvulsant action (Papanicolaou et al., 1982; Baran et al., 1985; Loscher and Cruzewar, 1987; Fletcher and Forster, 1988; Jackson et al., 1991).

Although there is significant evidence that the NE system is anticonvulsant, there are several considerations that temper one’s confidence in the hypothesis that NE, itself, reduces seizure sensitivity. For example, although the lesioning studies (i.e., chemical destruction of noradrenergic terminals) reduce the amount of NE release, this manipulation also reduces the release of other transmitters coreleased with NE. The neuropeptides galanin and neuropeptide Y (NPY) and the neurotransmitter adenosine (i.e., ATP) are released at noradrenergic terminals and have been shown to exert anticonvulsant effects against several convulsant stimuli (Murray et al., 1985; Mazarrati et al., 1992, 1998; Dichter, 1994; Erickson et al., 1996; Baraban et al., 1997). A similar argument can be made for the anticonvulsant effect of direct LC stimulation, which results in the release not only of NE but also of these cotransmitters. The enhanced seizure sensitivity of the GEPRs may not be caused solely by their abnormal noradrenergic system, because these animals also have abnormalities in their central serotonergic, GABAergic, and excitatory amino acid systems (Faingold et al., 1986; Dailey et al., 1992; Meyerhoff et al., 1992); moreover, other animal models of epilepsy have a higher than normal central NE content (Noebels, 1986; Hara et al., 1993). Finally, the α2-AR pharmacological studies are difficult to interpret because the effect of clonidine (α2-AR agonist) on seizure-induced activity can be biphasic, nonexistent, or even proconvulsant (King and Burnham, 1982; Tacke and Kolonen, 1984; Lapin and Ryzor, 1990). Such multiple responses to α2-AR agonists may be caused by the localization of the affected α2-AR. Activation of presynaptic α2-AR autoreceptors would reduce transmitter released at NE terminals (L’Heureux et al., 1986), whereas activation of postsynaptic α2-ARs would mimic the effect
of released NE. Because it has not been determined whether the anticonvulsant effect of a2-AR agonists is mediated via pre- or postsynaptic receptors, it remains unclear whether increased NE release is anti- or proconvulsant.

Taken together, these studies suggest that changes in noradrenergic functions (terminal NE content or release) can modulate seizure activity, but they do not resolve the issue of whether NE is, itself, anticonvulsant. It is this issue that we have addressed with the DBH null mutant (Dbh−/−) mice. These animals selectively lack NE and epinephrine (dopamine content tends to be elevated) because DBH is required for the conversion of dopamine to NE (Thomas et al., 1998).

MATERIALS AND METHODS

Animals. Mice were derived from a hybrid line (129/Sv/Ev and C57BL/6J), Dbh−/− and heterozygote (Dbh+/−) mice were bred as described previously (Thomas et al., 1995). Mice were maintained on a 12 hr light/dark cycle in a specific pathogen-free facility at the University of Washington (Seattle, WA). Food and water were available ad libitum, and animals were maintained according to the guidelines outlined in the NIH Guide for Care and Use of Laboratory Animals. All animal procedures were approved by the University of Washington Animal Care Committee. Genotype was deduced from phenotype (Dbh−/− mice exhibit delayed growth during adolescence and ptosis), and a subset of mice was confirmed by PCR (Thomas et al., 1995). Dbh−/− mice are indistinguishable from wild-type (+/+) mice as to NE and epinephrine levels (Thomas et al., 1998). Preliminary studies showed no significant difference in seizure susceptibility [2,2,2-trifluoroethanol (flurothyl)-induced seizures] between wild-type (+/+) and heterozygote Dbh (+/−) mice; therefore, Dbh−/− mice were used as controls in all experiments. Adult (3–6 months) male and female littermates of each genotype were evenly distributed to experimental and control groups for each convulsant stimulus. A subset of animals will receive a single intraperitoneal injection of l-threo-3,4-dihydroxyphenylserine (DOPS; 1 mg/gm). DOPS is converted to NE by aromatic L-amino acid decarboxylase, which is expressed. Fluorothyl susceptibility. Fluorothyl seizure thresholds were determined for Dbh−/+ and Dbh−/− mice, with and without previous administration of DOPS. Mice were placed in an air-tight Plexiglas chamber, and the volatile anesthetic ether (2% v/v) was infused (20 μL/min) onto filter paper from which it vaporized (Prichard et al., 1969). The latencies (seconds) to the first myoclonic jerk (focal seizure) and to generalized (clonic/tonic) seizure served as the measurements of seizure susceptibility. Each mouse was tested individually, removed immediately from the chamber after seizure onset, and recovered before the next testing session. Each animal was immediately killed and the brain was removed. The brain was fixed in a 4% formaldehyde, and 30 μm coronal sections were processed, hybridized, and washed in the same experimental conditions. To determine basal c-fos mRNA expression, sections from the cerebellum were hybridized with a 32P-labeled c-fos probe (New England Nuclear). The optical density measurements were made from films using the Microcomputer Imaging Device (Imaging Research, Ontario, Canada).

RESULTS

Dbh−/− mice have increased susceptibility to epileptic stimuli

Flurothyl

Dbh−/− mice without DOPS had significantly reduced latencies to the first myoclonic jerk (MJ) and clonic/tonic (C/T) seizure compared with Dbh−/+ controls (Fig. 1A, B, without DOPS). The latency to the first MJ was affected to a greater degree (46% reduction) than the latency to C/T convulsion (29% reduction). The percent of Dbh−/− and Dbh−/+ mice progressing to tonic extension after a C/T seizure was identical (45%); however, 100% of the Dbh−/− mice died after C/T seizure, whereas only 60% of the Dbh−/+ mice died after tonic extension (Table 1).

The higher mortality rate of Dbh−/− mice was not a function of the duration of flurothyl exposure, because the average duration of exposure was shorter for the Dbh−/− than for the Dbh−/+ animals.

NE levels are partially restored in the CNS of Dbh−/− mice by the administration of DOPS (Thomas et al., 1998). Administration of DOPS to Dbh−/− mice significantly lengthened the latency to the first MJ and C/T convulsion (Fig. 1A, B with DOPS); latencies to MJ and C/T convulsions in Dbh−/− mice
A significantly shorter latencies to MJ (induced seizures. Latencies (seconds) to first MJ (A) and C/T seizures (B) recorded in Dbh +/− and Dbh −/− mice, with and without the administration of DOPS. Dbh −/− mice without DOPS had significantly shorter latencies to MJ (A) and C/T seizures (B) compared with Dbh +/− mice without DOPS (mean ± SEM; single asterisks denote p < 0.05). Administration of DOPS (1 mg/gm) 6 hr before flurothyl significantly increased flurothyl latencies in the Dbh −/− mice compared with the Dbh +/− mice without DOPS for both MJ (A) and C/T seizures (B) (mean ± SEM; double asterisks denote p < 0.05). Latencies to both MJ and C/T convulsions in Dbh −/− mice with DOPS were not significantly different compared with those in Dbh +/− mice with DOPS.

with DOPS were not statistically different from latencies in Dbh +/− mice with DOPS. Administration of DOPS to Dbh −/− mice did not significantly alter the latency to the first MJ but significantly increased the latency time to C/T seizures (Fig. 1). Administration of DOPS to Dbh −/− and Dbh +/− mice did not affect the number of animals progressing to tonic extension but did reduce the number of animals dying after tonic extension in both groups (Table 1).

Pentylenetetrazol
Dbh −/− and Dbh +/− mice were challenged with PTZ at 30 and 40 mg/kg, and the latencies (seconds) to the first MJ, forelimb clonus (FC), and C/T were measured (Fig. 2). PTZ (40 mg/kg) induced generalized seizures in all Dbh −/− mice (eight of eight) but in only four of seven Dbh +/− mice. Latencies to MJ, FC, and C/T seizures in Dbh −/− mice were significantly shorter than those in Dbh +/− mice (Fig. 2A). The percent of animals exhibiting tonic extension was greater in Dbh −/− mice (100%) than in Dbh +/− mice (29%); however, for both genotypes, all animals exhibiting tonic extension died (Fig. 2A).

PTZ (30 mg/kg) induced C/T seizures in 8 of 10 Dbh −/− mice and in 2 of 9 Dbh +/− mice. Of these animals exhibiting seizures, the Dbh −/− mice had significantly shorter latencies to the first MJ, FC, and C/T seizures than did Dbh +/− mice (Fig. 2B). Again 100% of the Dbh −/− mice that exhibited seizure activity progressed to tonic extension and death; however, only 11% of the Dbh +/− mice that exhibited seizure activity had tonic extension, and of those, only 44% died.

Kainic acid
Kainic acid (KA; 20 mg/kg) induced some seizure behavior (i.e., staring, head nodding, and forelimb clonus) in most animals in both groups; however KA induced generalized C/T convulsions in 100% of the Dbh −/− mice (eight of eight) but in only 38% of the Dbh +/− mice (three of eight). Of the animals showing C/T convulsions, Dbh −/− mice had a significantly shorter latency to generalized seizure (1587 ± 188 sec) than did Dbh +/− mice (2243 ± 120 sec). The Dbh −/− mice also exhibited enhanced sensitivity to KA compared with Dbh +/− mice; 50% of the Dbh −/− mice died after the KA-induced seizure, whereas none of the Dbh +/− mice died.

Audiogenic seizures
The Dbh −/− mice were more sensitive to the acoustic stimuli than were Dbh +/− mice, in that 50% (5 of 10) of the Dbh −/− mice exhibited a generalized seizure during the sound stimulus, whereas only 11% (1 of 9) of the Dbh +/− exhibited a generalized convulsion. Seizures were initiated shortly after onset of the sound (latencies between 3 and 12 sec) and manifested initially as jumping behavior that progressed quickly to explosive running-bouncing activity and finally to tonic extension and death. Sensi-

Figure 2. Responsiveness of Dbh +/− and Dbh −/− mice to PTZ injections at 40 mg/kg (A) and 30 mg/kg (B). Left, Graphs show seizure latencies (seconds) to the first myoclonic jerk, forelimb clonus, and clonic/tonic seizure in Dbh −/− and Dbh +/− mice. At both PTZ concentrations Dbh −/− mice had significantly shorter latencies compared with those in Dbh +/− mice (mean ± SEM; single asterisk denotes p < 0.01; double asterisks denote p < 0.001). Right, Graphs show the percentage of animals progressing to tonic extension and the percentage of animals that died after tonic extension (mortality).
tivity to sound-induced seizure was identical between the groups of animals; all animals (i.e., in both Dbh +/− and Dbh +/+ groups) that exhibited a sound-induced generalized seizure died.

Dbh −/− mice have increased c-fos mRNA associated with flurothyl-induced seizures

The animals that survived flurothyl-induced seizures [Dbh +/− (n = 8) and Dbh −/− (n = 6) without DOPS; Dbh +/− (n = 8) and Dbh −/− (n = 9) with DOPS] were killed 1 hr after C/T seizures to measure c-fos mRNA expression. Seizure-induced c-fos mRNA expression was quantitated in the neocortex, amygdala, and hippocampus [CA1, CA3, and dentate gyrus (DG)] [see Figs. 3 (for representative autoradiograms), 4 (for quantitative comparisons)].

In the neocortex, Dbh −/− mice had significantly greater seizure-induced c-fos mRNA expression than did the Dbh +/− mice, even though the two different genotypes had similar seizure-induced behavior (generalized seizures). Administration of DOPS to Dbh −/− mice reduced seizure-associated c-fos mRNA expression to a level comparable with that seen in the neocortex of Dbh +/− mice, without or with DOPS (Fig. 4). Administration of DOPS to Dbh +/− mice did not alter seizure-associated c-fos mRNA expression in the neocortex.

Similar results were obtained in the hippocampal CA1 and CA3 regions and the amygdala. Flurothyl seizure-associated c-fos mRNA expression in Dbh −/− mice was significantly higher than that in Dbh +/− mice in CA1 and CA3 regions and the amygdala. Administration of DOPS to Dbh −/− mice significantly reduced the flurothyl seizure-associated c-fos mRNA expression (to the level observed in Dbh +/− mice with DOPS). Flurothyl seizure-associated c-fos mRNA expression in Dbh +/− mice was not significantly changed by DOPS pretreatment. The only region where c-fos mRNA expression was not significantly different between Dbh −/− and Dbh +/− mice was in the DG. Administration of DOPS to both genotypes also had no effect on flurothyl-induced c-fos mRNA expression in the DG. Because the Dbh −/− mice had elevated seizure-associated c-fos mRNA expression in the neocortex, hippocampal CA1 and CA3, and amygdala, basal c-fos mRNA was measured in Dbh +/− and Dbh −/− mice. Basal c-fos mRNA expression in Dbh −/− mice was not significantly different from that in Dbh +/− mice (data not shown).

Table 1. Number of animals used for flurothyl-induced seizures with and without the administration of DOPS

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<th>Without DOPS</th>
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<td>Flurothyl-induced seizures</td>
<td>n = 11</td>
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<tr>
<td>% tonic extension</td>
<td>45 (5/11)</td>
<td>45 (5/11)</td>
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<td>% mortality</td>
<td>60 (3/5)</td>
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Percentage of animals exhibiting tonic extension and the percent of animals that died (mortality) after tonic extension for Dbh +/− and Dbh −/− mice after flurothyl-induced seizure.

Figure 3. Representative autoradiograms of c-fos mRNA expression after flurothyl-induced seizures. A, C. Flurothyl-induced c-fos mRNA expression in Dbh +/− mice without (A) and with (C) DOPS (1 mg/gm). B, D. Flurothyl-induced c-fos mRNA expression in Dbh −/− mice without (B) and with (D) DOPS (1 mg/gm). Note the higher c-fos mRNA expression in Dbh −/− animals without DOPS (B). DOPS (1 mg/gm) administration not only reduces c-fos mRNA expression of Dbh −/− mice (compare B with D) but also normalizes c-fos mRNA expression in Dbh −/− mice relative to that in Dbh +/− mice (compare C with D). Scale bar, 2 mm. N, Neocortex.

DISCUSSION

These studies provide evidence that endogenous NE exerts a profound inhibitory effect on seizure induction. The enhanced susceptibility of Dbh −/− mice to such a diverse set of seizure-inducing stimuli (convulsant stimuli potentially acting at excitatory or inhibitory receptors, sodium channels, and brainstem activation) (Olney et al., 1974; Schwob et al., 1980; Woodbury, 1980; Browning, 1985; Snead, 1992) suggests a “global” suppressive action of NE. The loss of NE’s inhibitory action in Dbh −/− mice is also associated with increased c-fos mRNA expression after flurothyl-induced seizures. LC axons have a high degree of collateralization, and a single neuron can innervate several distant regions (Fallon and Loughlin, 1982; Loughlin et al., 1982). This diffuse noradrenergic innervation pattern would allow NE release from LC terminals to suppress neuronal activity throughout the brain, including regions such as the cortex and hippocampus that are important in regulating seizures. Our studies support...
Flurothyl seizure-associated c-fos mRNA expression was significantly higher in Dbh -/- mice without DOPS than in Dbh +/- mice without DOPS in all regions but the dentate gyrus (mean ± SEM: asterisks denote p < 0.01). DOPS (1 mg/gm) administration to Dbh -/- mice significantly reduced c-fos mRNA expression in all regions but the dentate gyrus (mean ± SEM: asterisks denote p < 0.05); c-fos mRNA expression in Dbh -/- mice and Dbh +/- mice with DOPS was not significantly different. Basal c-fos mRNA expression in Dbh -/- mice is not significantly different from basal c-fos mRNA expression in Dbh +/- mice (data not shown).
considered a marker of neuronal activity (Dragunow and Robertson, 1987; Morgan et al., 1987; Sonnenberg et al., 1989; Morgan and Curran, 1991). A correlation of seizure severity and c-fos expression has been observed with different convulsant stimuli (White and Price, 1993; Szot et al., 1997; Robbins et al., 1998). The enhanced seizure-induced c-fos mRNA expression in Dbh+/− mice is not a function of an elevated basal c-fos state, because basal c-fos mRNA expression in Dbh+/+ mice is not different from that in Dbh−/− mice. This enhanced c-fos mRNA expression in the CNS of Dbh−/− mice was measured in animals with a consistent behavioral seizure phenotype, suggesting a relationship between c-fos mRNA expression and seizure threshold. Acute DOPS administration normalized the seizure-associated c-fos mRNA expression in Dbh−/− mice. We conclude that the ability of the noradrenergic system to regulate seizure activity is a direct result of NE-mediated suppression of CNS excitability in such regions as the neocortex, hippocampus, and amygdala.

The ability of NE to have an inhibitory effect on seizures seems inconsistent with its general role on the arousal state of an animal. Noradrenergic neurons are active in awake animals but quiescent during sleep (Jouvet, 1969; Hobson et al., 1975; Aston-Jones and Bloom, 1981; Robbins, 1984). Basal c-fos mRNA expression in the cortex corresponds to the arousal state of the rat (Cirelli et al., 1996). When noradrenergic neurons in the LC were destroyed with 6-hydroxydopamine, the amount of basal c-fos mRNA expression in the cortex of the awake animal was reduced to levels comparable with that in an animal during sleep (Cirelli et al., 1996). Although these studies suggest a relationship between basal c-fos mRNA expression and NE, our study failed to find a change in basal c-fos mRNA expression in Dbh−/− mice relative to Dbh+/− mice. This difference emphasizes the gross effects of lesioning noradrenergic neurons, which results not only in the loss of NE but also affects the level of all neurotransmitters coreleased with NE. These cotransmitters released with NE may contribute to the basal excitability of the neurons.

The dual action of NE as an inhibitory and excitatory neurotransmitter can be attributed to the large diversity of noradrenergic receptors. Iontophoretic application of NE to neocortex or hippocampus results in both excitatory and inhibitory responses (Szabadi, 1979; Langmoen et al., 1981; Nishi et al., 1981; Segal, 1981; Madison and Nicoll, 1986; Waterhouse, 1986; Stanton, 1992). The excitatory response of NE appears to be mediated via the β-receptors and/or α1-ARs, whereas the inhibitory response is mediated via the α2-ARs (Curet and deMontigny, 1988; Parfitt et al., 1988; Licata et al., 1993). This dual action of NE on neuronal activity is apparent when synaptic NE content is elevated with NE reuptake blockers; these agents do not alter the animal’s susceptibility to convulsant stimuli (Kleinrok et al., 1991; Yacobi and Burnham, 1991). We postulate that the anticonvulsant action of NE is mediated via α2-ARs. Indeed, agonists selective for the α2-ARs have been shown to exert anticonvulsant effects against audiogenic seizures in mice, as well as against PTZ-, kainic acid-, and bicuculline-induced seizures; α2-AR antagonists have the reverse effect (Papanicolau et al., 1982; Baran et al., 1985; Loscher and Czuczwar, 1987; Fletcher and Forster, 1988; Jackson et al., 1991). However, it has not been determined whether the anticonvulsant effect of α2-AR agonists is mediated via the pre- or postsynaptic receptors. A recently developed transgenic mouse with nonfunctional α2A-ARs (MacMillan et al., 1996) responded to a kindling paradigm (a process of repetitively applied stimuli resulting in generalized seizures) similarly to wild-type mice treated with an α2-AR antagonist (Janumpalli et al., 1998). Although the Dbh−/− mice are not the same as the α2A-AR mutant, the combined results provide compelling evidence that NE acting at least partially via inhibitory postsynaptic α2-ARs dampens seizure excitability. The lack of spontaneous seizure activity in Dbh−/− mice suggests that NE release may only become important under conditions of high activity (e.g., seizures) when the LC is sufficiently activated; i.e., NE serves as a potent modulator of excitability.

In conclusion, the data presented here show unambiguously that NE is capable of modulating seizure activity induced by different convulsant stimuli. The pervasive inhibitory action of NE on excitability is reflected in the increased seizure-associated c-fos mRNA expression in the Dbh−/− mice. Because galanin and NPY are also inhibitory neuromodulators that are coreleased from the same terminals as NE, it seems that the noradrenergic projection system may use multiple neurotransmitters to dampen excitability. Because of this complexity, the Dbh−/− mice provide an especially useful and new system to examine the pathways through which NE regulates seizure activity.

REFERENCES


