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Immunohistochemical Evidence of Seizure-Induced Activation of trkB Receptors in the Mossy Fiber Pathway of Adult Mouse Hippocampus

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Genetic and pharmacological perturbations suggest that tyrosine receptor kinase B (trkB) receptor activation promotes limbic epileptogenesis, but whether or where trkB activation occurs during epileptogenesis is uncertain. Because activation of trk receptors involves phosphorylation of specific tyrosine residues (Segal et al., 1998), the availability of antibodies that selectively recognize the phosphorylated form of trk receptors at the Shc site permits an immunohistochemical assessment of trk receptor activation. We reported previously increased phospho-specific trk (p-trk) immunoreactivity in the mossy fiber pathway of the hippocampus during epileptogenesis in rats (Binder et al., 1999b). Because the p-trk antibody does not distinguish among trkA, trkB, and trkC, the identity of the neurotrophin receptor(s) undergoing phosphorylation was uncertain. The development of mice carrying a point mutation of the Shc binding site (Y515F) in the trkB gene (trkB<sup>shc</sup>) provided an opportunity to test the hypothesis that trkB is the neurotrophin receptor undergoing phosphorylation. Epileptogenesis in wild-type (WT) mice was associated with increased p-trk immunoreactivity in both the mossy fiber pathway and CA3 stratum oriens of hippocampus. In contrast, the epileptogenesis-associated increase of p-trk immunoreactivity was reduced in trkB<sup>shc</sup> mutant mice. The development of epileptogenesis as measured by electrophysiological and behavioral indices did not differ between trkB<sup>shc</sup> mutant and WT mice. These data demonstrate that the neurotrophin receptor trkB undergoes phosphorylation in the mossy fiber pathway and CA3 stratum oriens of the hippocampus during limbic epileptogenesis. In addition, the signaling pathways activated by the Shc site of trkB exert no detectable regulatory effects on limbic epileptogenesis.

Key words: epileptogenesis; kindling; seizures; neurotrophin; trkB receptor; Shc; hippocampus; phosphorylation
hypothesis. That is, substitution of phenylalanine for tyrosine at residue 515 of trkB (Y515F) eliminates the binding site for the phospho-trkB antibody in trkB but not trkA or trkC. This mutation of trkB disrupts the binding of Shc adaptor protein to trkB and abolishes Shc site-mediated downstream signaling events (Minichiello et al., 1998). If our hypothesis is correct, induction of epileptogenesis should produce increased phospho-trkB immunoreactivity in the mossy fiber pathway of wild-type (WT) but not trkB<sup>shc</sup> mutant mice.

**MATERIALS AND METHODS**

*Mice.* trkB<sup>shc</sup> mutant mice were generated as described previously (Minichiello et al., 1998). In brief, PCR-aided mutagenesis was used to introduce a single point mutation (A to T, position 2055) in trkB receptor that resulted in a change of tyrosine 515 into phenylalanine (Y515F). Nonphosphorylatable F515 disrupted the binding of adaptor proteins to the Shc site in trkB and abolished Shc site-mediated down-stream signaling events. The amount of autophosphorylated trkB was reduced in trkB<sup>shc/shc</sup> neurons after BDNF or neurotrophin-4 (NT-4) stimulation. In the present study, the genotype of each animal was assessed twice using PCR of genomic DNA isolated from tail (before experiment) and liver (after being killed). Nine wild-type (+/+) , 14 heterozygous (+/-), and 13 homozygous (-/-) trkB<sup>shc</sup> mice (20-35 gm) were used in this experiment.

**Antibody.** A peptide affinity-purified polyclonal trkB antibody (pY490) (New England Biolabs, Beverly, MA) directed against a synthetic phospho-tyr 490 peptide corresponding to residues 485–493 (1ENPOY<sup>*</sup>FSD) of human trkB was used for immunohistochemistry as we described previously (Binder et al., 1999b). The sequence of this peptide is highly conserved among trkA, trkB, and trkC receptors and also among human, rat, and mouse. Antibody pY490 is phospho-specific, recognizing the phosphorylated but not the nonphosphorylated form of peptide 490. Additionally, pY490 is phosphorylation site specific, recognizing phosphorylated 490 but not a trk peptide phosphorylated at residues 674/675 (Binder et al., 1999b). Antibody pY490 was used to detect the phosphorylation events. The amount of autophosphorylated trkB was reduced in trkB<sup>shc/shc</sup> neurons after BDNF or neurotrophin-4 (NT-4) stimulation. In the present study, the genotype of each animal was assessed twice using PCR of genomic DNA isolated from tail (before experiment) and liver (after being killed). Nine wild-type (+/+) , 14 heterozygous (+/-), and 13 homozygous (-/-) trkB<sup>shc</sup> mice (20-35 gm) were used in this experiment.

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**Surgery and kindling.** Under pentobarbital anesthesia (60 mg/kg, i.p.), a stimulation–recording bipolar electrode was stereotactically implanted in the right amygdala using the following coordinates, with bregma as the reference: 1.0 mm posterior, 2.9 mm lateral, and 4.6 mm below dura. A wire secured to the skull overlying the left frontal cortex was used as the ground electrode. After a postoperative recovery period of at least 1 week, the electrographic seizure threshold (EST) was determined by application of 1 sec of a 130 Hz biphasic rectangular pulses at 60 Hz beginning at 60 μA; additional stimulations increasing by 20 μA were administered at 1 min intervals until an electrographic seizure lasting at least 5 sec was detected on the EEG recorded from the amygdala. Subsequently, experimental animals were stimulated twice each day at a stimulus intensity 100 μA above the EST, with an interstimulus interval of at least 60 min. For consecutive seizures of class 4 or greater with limb clonus and/or tonic clonus lasting at least 12 sec were evoked. The behavioral manifestations of seizures were classified according to a modification of Racine’s classification (Racine, 1972): 1, facial clonus; 2, head nodding; 3, unilateral forelimb clonus; 4, rearing with bilateral forelimb clonus; 5, rearing and falling (loss of postural control); 6, running or bouncing seizures; and 7, tonic kindling extension. The surgery and kindling procedures were performed by an individual blinded to genotype of the animals. Control animals included unimplanted as well as implanted but unstimulated mice.

**Perfusion and histology.** After completion of kindling and a subsequent stimulation-free period of 2 weeks, the persistence of the hyperexcitability was determined by administration of an additional stimulation of the same intensity used in kindling procedure. Twenty-four hours after stimulation, the experimental and control animals were perfused transectionally under pentobarbital anesthesia with ice-cold 4% paraformaldehyde in 1× PBS containing 1 m sodium orthovanadate (PBSV) for 5 min at a flow rate 7.5 ml/min. The brains were removed, postfixed in the same solution overnight at 4°C, and cryoprotected in 20% sucrose in 1× PBSV until they sank. Coronal sections of 40 μm were cut in a cryostat at −20°C, and two sections per slide were mounted in PBSV on slides, air dried, frozen, and stored at −70°C until use. The sections were stained with methyl green pyronine-Y for determination of electrode placement. Only animals with correct electrode placement in the amygdaloid complex were included in the statistical analysis.
erately high level but more diffuse pattern of p-trk IR was apparent throughout stratum oriens of CA3. In contrast, no overt changes of p-trk IR were evident in CA1, in stratum radiatum of CA3, or in the molecular layer of the dentate gyrus. Quantitative analyses of these data are presented below. Importantly, omission of the primary antibody eliminated immunoreactivity in these WT animals (data not shown).

To verify the specificity of p-trk IR in kindled mice, peptide competition experiments were performed. Preincubation of pY490 antibody with its phosphopeptide immunogen (300 nM) eliminated p-trk IR in sections from kindled mice (Fig. 2, compare A, C) and unstimulated controls (data not shown). Importantly, preincubation with the unphosphorylated form of peptide 490 had no effect on the immunoreactivity (Fig. 2, compare A, B). Moreover, preincubation of pY490 antibody with either phospho- or unphospho-peptides from other sites of the trk receptor, 674/675 and 785, did not reduce p-trk IR (data not shown). Together, the results from these competition experiments support the assertion that the p-trk IR detected by antibody pY490 in wild-type mice reflects the phosphorylated form of a trk receptor at a tyrosine residue within a sequence similar to that of pY490 of human trkA.

Analyses of brain regions outside the hippocampal formation in unstimulated controls disclosed p-trk immunoreactivity in multiple areas of forebrain, including neocortex, lateral habenula, and some hypothalamic nuclei (data not shown). In contrast to the hippocampal formation, no marked increases were noticed in any of these regions after seizures evoked in WT mice. Thus, subsequent analyses were confined to the hippocampal formation in these studies.

Reduction of seizure-induced increased phospho-trk immunoreactivity in trkB	extsuperscript{shc} mutant mice

Because the pY490 antibody can detect the phosphorylated tyrosine residue in trkA, trkB, or trkC (Segal et al., 1996), the precise trk receptor undergoing phosphorylation in the hip-
hippocampus during epileptogenesis is uncertain. To determine whether phosphorylation of trkB in particular accounts for the increased immunoreactivity in the hippocampus during epileptogenesis, p-trkB immunohistochemistry was performed in WT and trkBshc mice, which were either not stimulated or were killed 1 d after a stimulation-evoked seizure. As described in the preceding section, a marked increase of p-trkB IR was evident in stratum lucidum and dentate hilus with a moderate increase in stratum oriens of CA3 after a seizure evoked in WT mice (Fig. 1, compare A, B). The trkBshc mutation reduced the seizure-evoked increase of immunoreactivity. That is, seizures evoked in trkBshc null mice triggered no significant increase of immunoreactivity in stratum lucidum, dentate hilus, or stratum oriens of CA3 (Fig. 1, compare B, F). Results with heterozygous trkBshc were intermediate between the WT and trkBshc nulls (Fig. 1, compare B, D, F, in lucidum, dentate hilus, and stratum oriens of CA3).

The immunoreactivity within hippocampus was quantified in distinct subregions (Fig. 3) and confirmed the findings evident by visual inspection. First, analyses of WT mice confirmed the spatially selective, twofold to threefold increase of phospho-trkB immunoreactivity in stratum lucidum, the dentate hilus, and hilus border after a seizure compared with unstimulated controls (Fig. 4A, B). Significant (p < 0.05; ANOVA) increases of a smaller magnitude were also evident in stratum oriens of CA3 (Fig. 4A). In contrast, comparison of stimulated with unstimulated trkBshc null mutants disclosed no significant seizure-induced increases of immunoreactivity in stratum lucidum, the dentate hilus or hilus border, or CA3 stratum oriens (Fig. 4A, B). In addition to the above comparisons of unstimulated versus stimulated mice of a given genotype, comparisons of seizure-treated mice of distinct genotypes revealed significant increases of immunoreactivity in the mossy fiber pathway and stratum oriens of CA3 in WT compared with trkBshc null mutants (Fig. 4A, B). Similar analyses of trkBshc heterozygotes disclosed results intermediate between wild-type and null mutants, but no significant increases of immunoreactivity were detected in the mossy fiber pathway and stratum oriens of CA3 after a kindled seizure compared with unstimulated controls of the same genotype (Fig. 4A, B). Interestingly, the increases of p-trkB IR after seizures exhibited striking heterogeneity among the trkBshc heterozygotes (data described but not shown). Robust staining was seen in three of nine heterozygous mice after a seizure with intensity and distribution similar to WT mice after a seizure. However, no detectable increase was evident in four heterozygous mice and a slight increase in the remaining two mice. Together, these quantitative analyses demonstrate that substitution of phenylalanine for tyrosine at residue 515 of trkB reduces the increased p-trkB IR after limbic seizures.

Figure 3. Locations at which densitometric measures of p-trkB immunoreactivity were performed: 1, strata radiatum; 2, lucidum; 3, oriens; 4, deep hilus; 5, hilus-GCL border; 6, IML; 7, MML; 8, OML.

Figure 4. Quantitative analyses of p-trkB immunoreactivity in hippocampal subregions of stimulated and unstimulated WT, heterozygous, and null (trkBshc) mutant mice. The data are the mean ± SEM of the percentage reduction in gray value in the given subregions compared with corpus callosum (see Materials and Methods); high values reflect more intense immunoreactivity. *p < 0.05 compared with unstimulated WT mice; φp < 0.05 compared with kindled and stimulated WT mice (one-way ANOVA with post hoc Bonferroni’s test).

Development and persistence of kindling in trkBshc mutant mice

No significant differences in the development of kindling were detected among WT (+/+), heterozygous, and null trkBshc mutants, as evident in the following observations. First, the current required to evoke an afterdischarge (i.e., EST) was similar in the three groups (180.0 ± 40.1 µA for +/+; n = 4; 151.1 ± 22.6 µA for +/−, n = 9; 180 ± 24.8 µA for −/−, n = 8; mean ± SEM; p > 0.05; one-way ANOVA). Second, no significant differences were detected in the numbers of stimulations required to evoke the initial clonic motor seizures (class 4 or greater) in WT (5.5 ± 0.9 stimulations) compared with heterozygous (6.4 ± 0.5) or null (6.8 ± 0.7) mutants. Third, no significant differences were found in the numbers of stimulations required to evoke three consecutive class 4 (or greater) seizures, the numbers of stimulations being 9.8 ± 1.5, 10.3 ± 0.5, and 10.8 ± 0.9 in WT, heterozygous, and null mutants, respectively (Fig. 5A). Finally, no significant
Figure 5. Behavioral (A) and electrographic seizure (B) measures of kindling development in WT and trkBshc mutant mice. Kindling development is presented as behavioral seizure class (A) and electrographic seizure duration as a function of the number of stimulations that evoked electrographic seizures (y-axis). Wild type (+/+; n = 4), heterozygous (+/−; n = 9), and homozygous (−/−; n = 8) mutant mice. ADD# (x-axis) refers to the number of stimulations that evoked an afterdischarge with duration of at least 5 sec. Data are mean ± SEM. Note that neither seizure class nor electrographic seizure duration was significantly different among the three groups (one-way ANOVA).

Differences were noted in the duration of electrographic seizures during kindling development among the three experimental groups (Fig. 5B). In summary, kindling development appears to proceed normally in trkBshc mutant mice.

To determine whether the Shc binding site of the trkB receptor influenced the persistence of the hyperexcitability after completion of kindling, WT and trkBshc mutants were maintained for a stimulation-free period of 2 weeks after kindling and then subjected to a single stimulation. The stimulation evoked a generalized seizure in all animals from each of the three groups (+/+; +/−, and −/−). There was no significant difference among the groups in duration of electrographic seizure (41.5 ± 8.1, 41.4 ± 4.0, and 35.4 ± 2.1 sec, respectively) or seizure class (5.5 ± 0.5, 5.8 ± 0.2, and 5.3 ± 0.2, respectively; p > 0.05; one-way ANOVA). Thus, the persistence of kindling was unaffected in the trkBshc mutant mice.

DISCUSSION

Our previous ex vivo analyses of limbic epileptogenesis in rats demonstrated increased pY490 immunoreactivity in the hippocampal mossy fiber pathway, providing immunohistochemical evidence for activation of trk receptor tyrosine kinase. Which trk receptor(s) in particular accounted for the immunoreactivity was unclear. To test the hypothesis that phosphorylation of trkB contributed to the increased immunoreactivity, we studied mutant mice (trkBshc) in which phenylalanine was substituted for tyrosine at residue 515 of trkB, a site homologous to residue 490 of human trkA. Three principal findings emerge. First, the previous results in rats were confirmed and extended by the studies of mice. A single, brief seizure induced by stimulation of the right amygdala in a kindled mouse in a WT background induced increased pY490 immunoreactivity bilaterally in the hippocampal mossy fiber pathway. In addition, increased pY490 immunoreactivity was evident in stratum oriens of CA3 bilaterally. Second, in contrast to WT mice, seizures evoked in null mutant (trkBshc) mice induced no significant increase of pY490 immunoreactivity in either the mossy fiber pathway or CA3 stratum oriens. Third, despite the reduction of the seizure-induced increase of pY490 immunoreactivity, the development and persistence of kindling as measured by electrophysiological and behavioral indices did not differ between trkBshc mutant and WT mice. These data demonstrate that the neurotrophin receptor trkB undergoes phosphorylation in the mossy fiber pathway and CA3 stratum oriens of the hippocampus during limbic epileptogenesis. In addition, the signaling pathways activated by the Shc site of trkB exert no detectable regulatory effects on limbic epileptogenesis.

The present study revealed that the increased p-trkB immunoreactivity evident in the hippocampus during limbic epileptogenesis was reduced in trkBshc mutant mice, implying that phosphorylation of trkB underlies the increased p-trkB immunoreactivity. Whether the increased p-trkB immunoreactivity is attributable to a post-translational modification of a fixed number of constitutively expressed trkB molecules, to post-translational modification of increased numbers of trkB molecules, or some combination of the two is uncertain. The presence of the increased p-trkB immunoreactivity in WT mice adds to previous findings in rats and demonstrates that trkB activation occurs during limbic epileptogenesis in multiple species. Moreover, the induction of the immunoreactivity by a single, brief seizure evoked in a kindled mouse extends the previous studies using chemoselectively induced status epilepticus or massed electrical stimulations and demonstrates that activation of trkB is common to limbic epileptogenesis induced by diverse methods. In light of evidence implicating a causal role for trkB activation in limbic epileptogenesis (Kokaia et al., 1995; Binder et al., 1999a; Croll et al., 1999; Lähteenen et al., 2002), knowing where and when trkB activation occurs will facilitate elucidating its structural and functional consequences and, in turn, provide insight into mechanisms of limbic epileptogenesis.

The light microscopic distribution provides a clue to the cellular localization of the increased p-trkB immunoreactivity. Its presence in the dentate hilus and stratum lucidum of CA3 corresponds to the mossy fiber axons of the dentate granule cells, providing the most parsimonious explanation for its cellular localization. Whereas our studies of rats disclosed increased p-trkB immunoreactivity confined to the mossy fiber pathway (Binder et al., 1999b), the present study revealed increased p-trkB immunoreactivity not only in the mossy fiber pathway but also throughout.
stratum oriens of CA3 extending to the junction with CA2 (Fig. 1B). Whether this p-trkB immunoreactivity is localized to an infrapyramidal projection of mossy fibers, axons of CA3 pyramidal cells, and/or postsynaptic targets such as interneurons or basal dendrites of CA3 pyramidal cells is uncertain. Ultrastructural studies will be required to establish the cellular locale of the p-trkB immunoreactivity in both the mossy fiber pathway and stratum oriens of CA3.

Circumstantial evidence implicates increased release of BDNF as the proximate cause for the increased p-trkB immunoreactivity during limbic epileptogenesis. The binding of neurotrophin to the trk receptor induces dimerization and trans-autophosphorylation of a subset of tyrosine residues (Schlessinger and Ullrich, 1992; Guiton et al., 1994), thereby suggesting that the binding of a neurotrophin to trkB underlies its increased phosphorylation. The occurrence of the increased p-trkB immunoreactivity after seizures suggests the possibility that seizures induced increased expression of a neurotrophin that is translated, transported, released, and thereby activates trkB. Analysis of the temporal and spatial patterns of seizure-mediated regulation of neurotrophins supports the candidacy of BDNF. The seizure-induced increase of BDNF protein peaks at ~24 hr, the time point corresponding to increased p-trkB immunoreactivity (Nawa et al., 1995; Elmér et al., 1998; Rudge et al., 1998; Binder et al., 1999b). Concordance of the anatomic distribution of seizure-induced increases of BDNF and the p-trkB immunoreactivity further implicates BDNF; that is, immunohistochemical studies disclosed increased BDNF in the granule cell soma within 2 hr after seizures and in the mossy fiber pathway 1 to 2 d thereafter (Yan et al., 1997; Vezzani et al., 1999). We favor the idea that BDNF is anterogradely transported to the mossy fiber axons (Conner et al., 1997; Smith et al., 1997), from which it is released and activates trkB on the mossy fibers by a paracrine or autocrine mechanism. Concordance of the anatomic distribution of seizure-induced BDNF (Yan et al., 1997, their Fig. 7D) and p-trkB immunoreactivity in stratum oriens of CA3 implicates BDNF in this site as well. Together with persistence of seizure-induced p-trkB immunoreactivity in NT-4 null mutants (He et al., 1999), this circumstantial evidence favors BDNF as the neurotrophin activating p-trkB in hippocampus.

What consequence(s) of trkB activation, presumably by BDNF, might contribute to the increased excitability evident as limbic epileptogenesis? The fact that at least part of the increased p-trkB immunoreactivity may reside in dentate granule cells refines the question: what functional and structural consequences of enhanced activation of trkB in granule cells by BDNF might promote increased hippocampal excitability? The fact that activation of trkB by BDNF can enhance excitatory synaptic transmission (Lohof et al., 1993; Kang and Schuman, 1995; Levine et al., 1995, Stoop and Poo, 1996) and reduce inhibitory synaptic transmission (Tanaka et al., 1997) in hippocampus raises the interesting possibility that enhanced synaptic activation of CA3 pyramidal cells by mossy fibers, either directly or indirectly by disinhibition of the mossy fiber–interneuron–CA3 pyramidal cell circuit is one consequence of trkB activation. The increased excitability of CA3 pyramidal cells in kindled animals as detected by increased epileptiform bursting induced by elevated K+ or lowered Mg2+, in isolated hippocampal slices (King et al., 1985; Behr et al., 1998) is consistent with this idea. Another possibility relates to the increased recurrent excitatory synapses that have been identified among dentate granule cells in the epileptic brain, synapses that would be expected to promote repetitive firing of the granule cells in response to an isolated excitatory synaptic input (Wuarin and Dudek, 2001). Two distinct morphological plasticities of the granule cells have been identified in the epileptic brain, either of which could underlie increased recurrent excitatory synapses: sprouting of the mossy fiber axons and formation of basal dendrites (Nadler et al., 1980; Spigelman et al., 1998). We found that particle-mediated gene transfer of BDNF, but not NGF, into the dentate granule cells in hippocampal explant cultures is sufficient to induce increases in both axonal branching and basal dendrite number (Danzer et al., 2001). Whether BDNF-mediated activation of trkB during limbic epileptogenesis in vivo mediates these functional and/or structural effects remains to be elucidated.

Despite the evidence that trkB activation promotes limbic epileptogenesis, the signaling pathways mediating the epileptogenic consequences of trkB activation are obscure. After binding a ligand, trk receptors autophosphorylate on cytoplasmic tyrosine residues, which themselves become docking sites for intracellular signaling proteins. Shc adaptor proteins associate directly with a specific site in trk receptors and activate a signaling pathway involving Ras, Raf, MAPK1 [for mitogen-activated protein (MAP) kinase 1], MAPK2, Mek 1 (for MAP kinase kinase 1), and Mek 2, one consequence of which is activation of transcription factors and regulation of gene expression. The present studies reveal that introduction of the Y515F mutation into the germine of the trkBshc/shc mice did not modify either electrophysiological or behavioral indices of epileptogenesis, despite previous evidence that BDNF- and NT-4-mediated activation of ERKs (for extracellular signal-regulated kinase) were attenuated in neurons isolated from Shc mutant mice (Minichiello et al., 1998). These results imply that trkB-mediated activation of the Ras/ERK pathway does not mediate epileptogenesis. The absence of detectable differences in epileptogenesis between WT and trkBshc/shc mice is reminiscent of the absence of detectable differences in long-term potentiation between WT and trkBshc/shc mice (Korte et al., 2000), despite defects in long-term potentiation in BDNF and trkB null mutant mice (Korte et al., 1995; Minichiello et al., 1999; Xu et al., 2000). The trkB signaling pathways mediating epileptogenesis remain elusive, and solving this question will require the generation of additional signaling alleles of the trkB gene.

REFERENCES
Goddard GV, McIntyre DC, Leech CK (1969) A permanent change in


He et al. • Seizures Activate Hippocampal trkB


