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Endogenous Control of Hippocampal Epileptogenesis: A Molecular Cascade Involving Brain-Derived Neurotrophic Factor and Neuropeptide Y

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Summary: *Purpose:* Seizures increase the expression of brain-derived neurotrophic factor (BDNF) in the hippocampus. Because this neurotrophin exerts modulatory effects on hippocampal neuronal excitability, it may play an important role in epileptogenesis initiated in this structure. Moreover BDNF is known to regulate the expression of neuropeptide Y (NPY), which displays modulatory properties on seizure activity. This suggests that the effects of BDNF on epileptogenesis may be mediated by NPY.

Methods: Adult male rats received a 7-day chronic intrahippocampal infusion of BDNF, BDNF antisense oligodeoxynucleotides, NPY, or anti-NPY immunoglobulin G during kindling of the hippocampus. The long-term regulation of NPY expression by BDNF was also studied by immunohistochemistry and radioimmunoassay.

Results: BDNF applied during the first week of hippocampal stimulation significantly delayed the progression of kindling, an effect that outlasted the end of the infusion by at least 7 days. Conversely, infusion of BDNF antisense oligodeoxynucleotides to reduce the expression of endogenous BDNF in the hippocampus aggravated the electroencephalographic expression of seizures. Chronic infusion of BDNF increased the expression of NPY in the hippocampus, with a time course similar to that of the protective effect of the neurotrophin on kindling. Finally, chronic infusion of NPY in the hippocampus delayed the progression of hippocampal kindling, whereas anti-NPY antibodies had an aggravating effect.

Conclusions: Our results suggest that the seizure-induced increase in BDNF expression in the hippocampus may constitute an endogenous protective mechanism able to counteract hippocampal epileptogenesis. This protective effect appears to be mediated at least in part through the regulation of NPY expression. **Key Words:** Brain-derived neurotrophic factor—Hippocampus—Epilepsy—Kindling—Neuropeptide Y.

One of the pathophysiological hallmarks of temporal lobe epilepsy is the neuroplasticity observed in the hippocampus (1). These anatomical and functional alterations are seen either as causal mechanisms underlying the development of hyperexcitability or as inhibitory homeostatic processes (2,3). Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, supports neuronal growth, differentiation, and survival in the developing and adult hippocampus (4). The strong up-regulation of BDNF and its high-affinity receptor (TrkB) in the hippocampus in many different models of seizures (5,6) suggests its involvement in epilepsy-related plasticity, in which it could act either as a promoting factor or as an endogenous antiepileptogenic substance (7). Electrophysiological data suggest that BDNF could participate in the establishment and maintenance of hippocampal hyperexcitability. In vitro and in vivo, BDNF potentiates excitatory synaptic transmission in the hippocampus (8–11). On the contrary, as has been shown during development, BDNF could promote activity-dependent inhibitory synaptogenesis (12). In this study, we examined the effects of BDNF on epileptogenesis induced by hippocampal kindling. In this model, the first electrical stimulation of the hippocampus leads to a rapid increase of BDNF expression in the hippocampus, suggesting that BDNF may already modulate the initial phase of epileptogenesis (13). Therefore, the effects on epileptogenesis of recombinant BDNF or BDNF antisense oligodeoxynucleotides infused chronically during the first week of hippocampal kindling were analyzed.

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This article reviews our recent findings suggesting a key role of endogenous BDNF in epileptogenesis through the regulation of neuropeptide Y (NPY) expression.

BDNF DELAYS HIPPOCAMPAL EPILEPTOGENESIS

Effect of a chronic intrahippocampal infusion of BDNF on kindling of the hippocampus

The role of BDNF in epileptogenesis was first examined by studying the effects of chronic exposure to the neurotrophin on hippocampal kindling progression. Bilateral infusion of human recombinant BDNF (12 μg/side/d) in the hippocampus significantly retarded kindling of this structure (Fig. 1, A and B). Seizure scores were significantly reduced for up to 11 days after the end of the infusion period (Fig. 1A). Only 25% of the animals developed generalized convulsive seizures, whereas all control animals displayed such seizures by the end of the experiment. Hippocampal afterdischarge durations in the BDNF-treated group remained significantly shorter throughout the experiment compared with those in animals that received phosphate-buffered saline (PBS) (Fig. 1B). Unilateral infusions of BDNF (6 or 12 μg/d) also

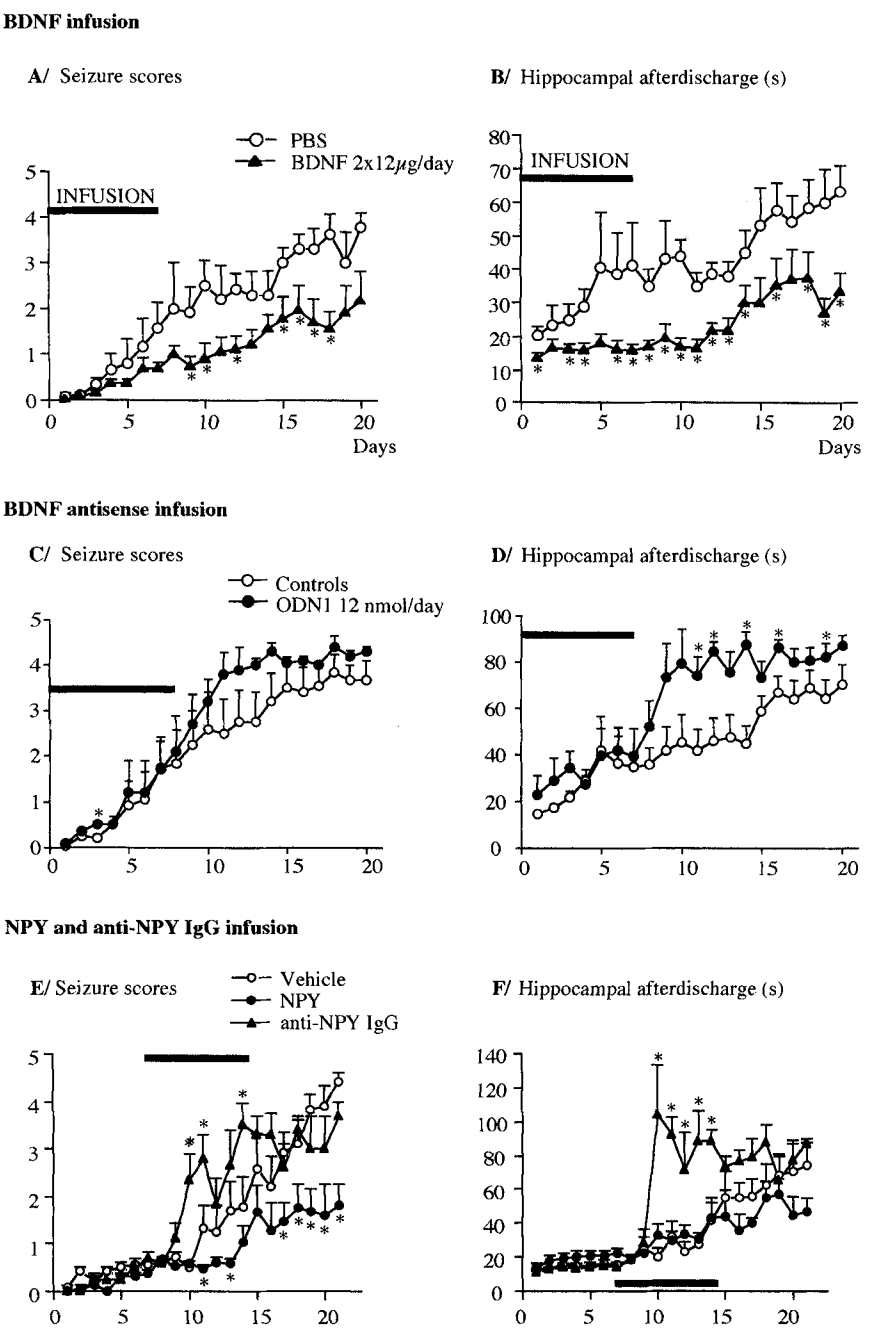

FIG. 1. Effect of a 7-day intrahippocampal infusion of BDNF, BDNF antisense oligodeoxynucleotides, NPY, or anti-NPY IgG on the progression of kindling of the hippocampus. Values represent mean seizure scores ± SEM and mean hippocampal afterdischarge durations ± SEM. **A, B:** Effect of a bilateral infusion of PBS (n = 6) or BDNF (12 μg/side/d; n = 8). **C, D:** Effect of a unilateral infusion of BDNF antisense oligodeoxynucleotides (12 nmol/d; n = 5). Controls were infused with PBS (n = 3) or mismatched oligodeoxynucleotides (n = 3). **E, F:** Effect of a unilateral infusion of vehicle (PBS + 1% bovine serum albumin; n = 7), NPY (30 nmol/d; n = 8), or anti-NPY IgG (n = 6). The solid bars represent the infusion period. *$p < 0.05$ versus controls by Mann-Whitney *U* test.

delayed the progression of kindling, but with less efficacy (data not shown) (14; S. Reibel et al., unpublished data), in agreement with the notion that both hippocampi are involved in kindling development (15).

These data confirm our preliminary report of the suppressive effects of BDNF (120 μg/d) on hippocampal kindling (16). In a more recent study, amygdala and perforant path kindling was also shown to be delayed by intrahippocampal application of BDNF (17). The antiepileptogenic properties of BDNF appear to be specific to this neurotrophic factor; on the contrary, nerve growth factor promotes kindling development and glial cell line–derived neurotrophic factor (GDNF) has no effect (18; S. Reibel et al., unpublished data). Our results are in contrast with the general agreement that BDNF participates in the establishment and maintenance of hippocampal excitability. BDNF enhances glutamatergic synaptic transmission in the normal and epileptic hippocampus (9–11). A very rapid excitatory action of BDNF observed in cultured hippocampal cells has even led to the proposition that it may act as a neuroexcitant per se (8). The down-regulation of TrkB receptors induced by BDNF infusion in vitro and in vivo could protect hippocampal neurons from BDNF excitatory effects (19). However, this receptor desensitization hypothesis does not entirely fit with our observations, because BDNF-induced TrkB down-regulation is limited to the duration of BDNF infusion and is not accompanied by an attenuation of the biological effects of BDNF. In our study in particular, BDNF infusion led to long-lasting overexpression of NPY, a phenomenon that would not be expected after TrkB desensitization (see below). In an attempt to understand the role of the endogenous neurotrophin, several authors have analyzed the susceptibility to seizures of mice that express reduced levels of BDNF. Amygdala kindling is delayed in heterozygous mutant mice that carry a deletion of the BDNF gene (20), and transgenic mice that overexpress BDNF appear more sensitive to seizures (21). However, considering the determining role of neurotrophins during brain ontogenesis, it may be hypothesized that reduction or overexpression of BDNF during development leads to abnormal functional properties of the hippocampus, introducing a bias in the sensitivity to seizures of these genetically modified animals. To examine the consequences of a reduction in the expression of endogenous BDNF on kindling development, therefore, we chose an antisense strategy.

Effect of a chronic infusion of BDNF antisense oligodeoxynucleotides on kindling of the hippocampus

The antisense oligodeoxynucleotides used (22) decreased by 56% the levels of BDNF measured by enzyme-linked immunosorbent assay and BDNF immunostaining in the hippocampus of rats subjected to kainic acid–induced status epilepticus on the third day of intrahippocampal antisense oligodeoxynucleotides infusion (12 nmol/d) (S. Reibel et al., unpublished data).

Chronic unilateral infusion of BDNF antisense oligodeoxynucleotides (12 nmol/d) in the hippocampus during the first week of kindling significantly increased hippocampal (Fig. 1D) and cortical (data not shown) afterdischarge durations during the week after the end of the infusion compared with controls. This increase did not reach significance for seizure scores (Fig. 1C). The aggravation consecutive to BDNF antisense oligodeoxynucleotides infusion suggests that seizure-induced overexpression of the endogenous neurotrophin may counterbalance hippocampal epileptogenesis. It also confirms that the effects of exogenously applied BDNF actually relate to biological properties of the neurotrophin, although the quantity of endogenous BDNF released in the hippocampus after a seizure is expected to be lower than the doses infused in our experiments (13). An opposite conclusion on the function of BDNF was drawn from inhibition of kindling development after infusion of TrkB fusion proteins (23). These compounds are purported to sequester the endogenous ligand, thereby preventing receptor activation. Although they act as selective antagonists of BDNF in vitro (24), their efficacy in vivo is not proven. In particular, it has been shown in vivo that they can enhance the distribution of exogenous BDNF without inhibiting its biological activity (25). Moreover, the inhibitory action of TrkB fusion proteins may also reflect scavenging of NT-4/5 or NT-3, which can bind to TrkB. The effects of NT-4/5 on seizure susceptibility are not known, and kindling development is suppressed in NT-3 mutant mice (26).

The most striking feature of the protective effects of BDNF was the prolonged kindling suppression, which outlasted the end of the infusion by 2 weeks (Fig. 1, A and B). The long-lasting presence of the neurotrophin is probably not the explanation for this enduring effect, because weight loss and appetite suppression secondary to BDNF application are limited to the infusion period (16). Rather, BDNF may trigger in the hippocampus long-lasting genomic regulations, which in turn are responsible for the enduring delay in kindling progression. These prolonged inhibitory properties could overcome the acute excitatory neurotransmitter-like effects that have been described for BDNF in the hippocampus and that may not be seen in our experimental paradigm. Such an indirect mechanism could also explain the delayed aggravation observed after BDNF antisense oligodeoxynucleotides infusion.

ARE THE PROTECTIVE EFFECTS OF BDNF MEDIATED BY NPY?

Several lines of evidence point to NPY as a particularly attractive candidate for mediating the protective ef-

fects of BDNF on hippocampal epileptogenesis. BDNF is known to up-regulate NPY, which is expressed mainly in inhibitory interneurons of the hippocampus (27). Moreover, this neuropeptide displays antiepileptic properties (28).

Effect of a chronic BDNF infusion on the expression of NPY in the dorsal hippocampus

Because BDNF induces a prolonged delay in kindling progression, we first examined the long-term effects of BDNF infusion on NPY expression in the dorsal hippocampus by immunohistochemistry and radioimmunoassay (29). In PBS-treated animals, NPY-immunopositive cell bodies were mostly located in CA1 strata oriens and radiatum and in the hilus of the dentate gyrus (Fig. 2A). Immediately at the end of a 7-day infusion with BDNF (day 7), a strong increase in the intensity of NPY immunoreactivity was seen in the dorsal hippocampus ipsilateral to treatment compared with PBS-treated rats (Fig. 2B). A greater number of NPY-immunostained somata were observed in CA1 and counted in the dentate hilus of BDNF-treated animals (Fig. 2D). Moreover, strongly immunopositive somata were observed in the granule cell layer, from which NPY immunolabeling is normally absent (Fig. 2B). One week after the end of the BDNF infusion (day 14), NPY immunoreactivity remained significantly increased in CA1 and the dentate hilus (Fig. 2, C and D). Quantitative evaluation of the total amount of NPY in the hippocampus ipsilateral to infusion was also obtained by radioimmunoassay. As shown in Figure 2E,

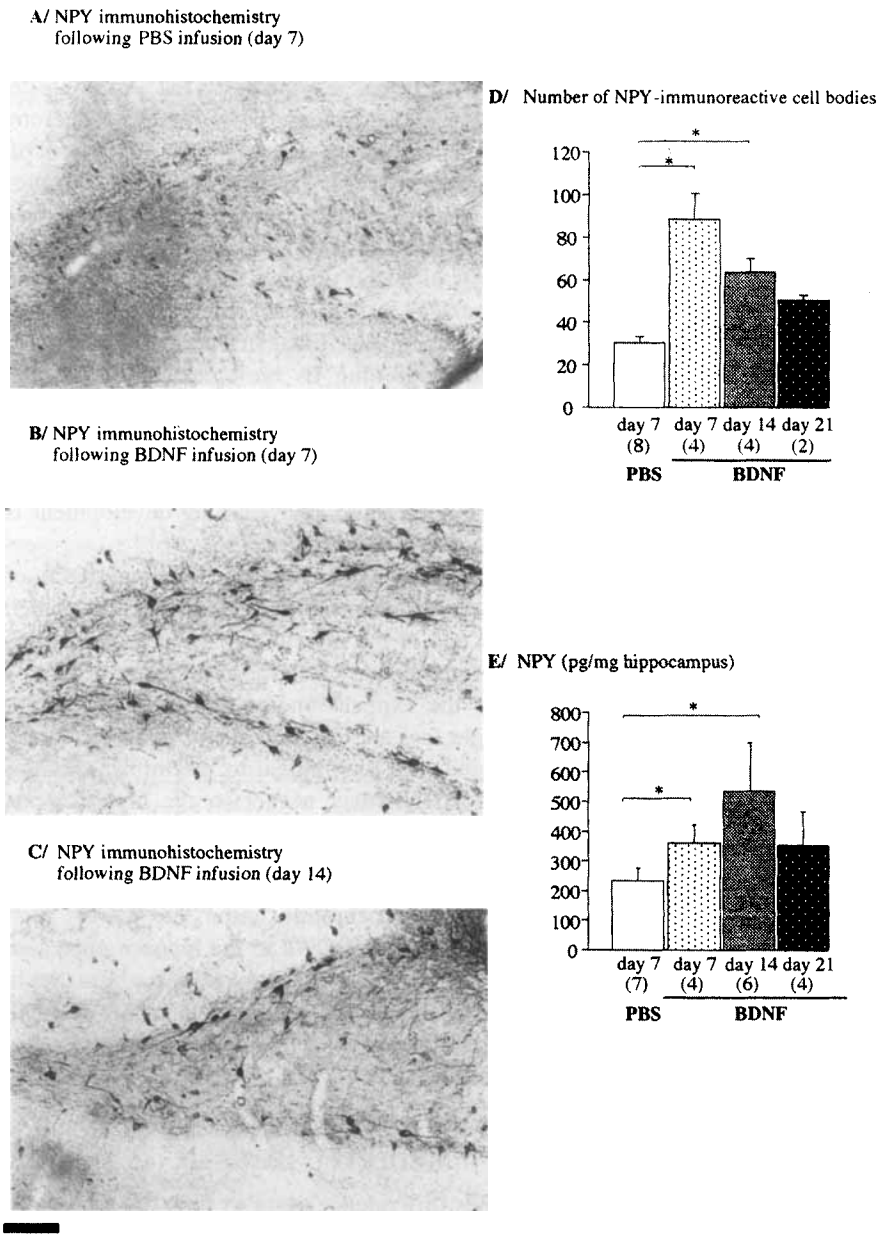

FIG. 2. Effect of BDNF infusion on NPY expression in the hippocampus. **A, B, C:** Micrographs of representative sections showing NPY immunoreactivity in the dentate hilus of the dorsal hippocampus of PBS-treated (**A**) and BDNF-treated (12 μg/d) (**B, C**) rats on the last day of a 7-day infusion (**A, B**) and 1 week after the end of the infusion (**C**). Scale bar, 100 μm. **D:** Number of NPY-immunoreactive cell bodies in the polymorphous layer of the dentate hilus ipsilateral to the infusion site. Cell counts were carried out on the last day of a 7-day infusion (day 7) with PBS or BDNF (12 μg/d), 1 week (day 14) after the end of the BDNF infusion, and 2 weeks (day 21) after the end of the BDNF infusion. Values represent mean cell counts ± SEM. **E:** NPY-like immunoreactivity in the hippocampus measured by radioimmunoassay after infusion of PBS or BDNF (12 μg/d). Values represent mean concentrations of NPY (pg/mg hippocampus) ± SEM. *$p < 0.05$ versus PBS-treated rats by Mann-Whitney *U* test. Figures in parentheses indicate number of animals in each experimental group.

NPY-like immunoreactivity was significantly increased in the dorsal hippocampus of BDNF-treated animals at days 7 and 14.

Our results agree with other in vitro and in vivo data. Application of BDNF increases the expression of NPY messenger RNA and peptide in hippocampal cell cultures and slices (30). Intracerebral administration of BDNF increases the expression of NPY in the hippocampus of adult rats, with a pattern of labeling very close to the distribution observed in our study (31). The regulation of NPY by BDNF appears to be specific, because immunoreactivity for two other peptides also contained in hippocampal interneurons, somatostatin and glutamic acid decarboxylase, the rate-limiting enzyme for γ-aminobutyric acid synthesis, was not modified after BDNF infusion (29). It has been hypothesized that the seizure-induced overexpression of NPY in the hippocampus is mediated by BDNF. The onset of BDNF increase after seizures precedes that of NPY in overlapping areas (32). Furthermore, our data show that BDNF alone, without any epileptic activity, can change NPY levels in a manner similar to seizures (33). Finally, our results show for the first time that BDNF induces a long-lasting overexpression of NPY in the hippocampus, outlasting the infusion period by at least 1 week (29). This time course resembles that of the effects of BDNF on kindling progression.

Effect of chronic NPY and anti-NPY immunoglobulin G infusion on hippocampal kindling

To further investigate the possible involvement of NPY as a mediator of the protective action of BDNF on epileptogenesis, we examined whether NPY, like BDNF, could modulate hippocampal kindling. NPY was infused unilaterally in the dorsal hippocampus during the second week of kindling, when modulation of NPY expression, release, and binding sites can be seen (33,34). Seizure scores of NPY-treated rats (30 nmol/d) remained significantly below control values from the 11th day of kindling and during the week after the end of the infusion (Fig. 1E). Whereas all control animals displayed generalized convulsive seizures before the end of the stimulation period, only 27% of NPY-treated rats developed such seizures. Hippocampal (Fig. 1F) and cortical (data not shown) afterdischarge durations were not statistically different in control and NPY-treated rats. Conversely, infusion of anti-NPY immunoglobulin G (IgG) in the hippocampus during the same period significantly accelerated hippocampal kindling, as indicated by higher seizure scores and hippocampal and cortical afterdischarge durations (Fig. 1, E and F).

Our observations agree with other antiepileptic properties described for the peptide in vitro and in vivo. In hippocampal slices, NPY reduces neuronal excitability (35). Transgenic mice that do not express NPY are more susceptible to generalized convulsive seizures, and intracerebroventricular injection of NPY diminishes seizure activity in the rat (36–38; S. Reibel et al., unpublished data). We show here that infusion of NPY in the hippocampus delays the behavioral development of seizures without affecting electroencephalographic activity. This finding suggests that NPY prevents diffusion of hippocampal ictal activity to other brain structures responsible for motor expression of seizures. An opposite effect was observed after infusion of anti-NPY IgG, which aggravated both the behavioral and electroencephalographic variables. This finding supports the idea that seizure-induced up-regulation of NPY constitutes an endogenous protection against epileptogenesis.

DO BDNF AND NPY PARTICIPATE IN AN ENDOGENOUS MECHANISM AIMED AT PREVENTING EPILEPTOGENESIS?

Our results suggest that the enduring protective effects on kindling development conferred by intrahippocampal infusion of BDNF are mediated by the inhibitory properties of NPY, which undergoes a long-lasting up-regulation after chronic application of the neurotrophin. Obviously, other morphological and/or neurochemical modifications of BDNF-sensitive hippocampal neurons cannot be ruled out. We have reported previously that BDNF does not appear to suppress kindling through modification of mossy fiber sprouting (16), a morphological reorganization of the hippocampus associated with kindling that could modulate hippocampal excitability. BDNF may also protect from kindling-induced neuronal loss (39), as suggested by its neuroprotective effects against kainic acid–induced neuronal damage in the developing rat (40). Finally, BDNF could regulate the expression of other proteins involved in the kindling process, such as calbindin-D28k (30,41), somatostatin (42,43), or Ca^{2+}/calmodulin-dependent protein kinase II (44,45).

The aggravating effects of BDNF antisense oligodeoxynucleotides and anti-NPY IgG support the concept that endogenous BDNF and NPY reduce hippocampal excitability during the initial phase of kindling. Seizure-induced successive overexpression of BDNF and NPY may thus constitute the first stage of an endogenous mechanism that controls hippocampal epileptogenesis. Such a cascade of molecular events may exist in the human brain, because increased expression of BDNF and NPY has been described in the hippocampus of patients suffering from temporal lobe epilepsy (46,47). However, the loss of NPY-immunoreactive hilar interneurons also observed in these patients may explain why, at some stage, the epileptic process outruns the endogenous protection, allowing seizures to develop.

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