Role of Neurotrophins in Epilepsy – a Progress Report*

Svenja Landweer, PhD student
University of Basel

Summary

At present, epilepsy is among the most prevalent neurological disorders worldwide. Current treatment protocols focus exclusively on the prophylaxis or suppression of seizures and thus provide merely a symptomatic treatment, without influencing the cause of the disease. There is an urgent need for new drugs that may act at different molecular targets than currently available antiepileptic drugs, and for new therapies aiming to inhibit the process of epileptogenesis.

The mechanism underlying the rare coexistence of partial temporal lobe and non-convulsive generalized epilepsies is still poorly understood and a rat model of absence seizures may be a unique opportunity for exploring the pathophysiology of epileptogenesis in animals.

Genetic Absence Epilepsy Rats from Strasbourg (GAERS) serve as a valid model for human absence epilepsy. Goal of this study is to define the potential role of neurotrophins such as BDNF and its receptors in recurrent seizures. Biochemical, developmental, functional as well as immunohistochemical studies were performed in GAERS with regard to novel therapeutic approaches in prevention and treatment of epilepsy.

Epileptologie 2008; 25: 35 – 42

Key words: GAERS, neurotrophins, BDNF, absence seizures, epileptogenesis

Funktion der Neurotrophine in der Epilepsie – Ein Zwischenbericht

Epilepsie gehört zu den weltweit häufigsten neurologischen Erkrankungen. Ihre aktuelle Behandlung ist auf die Prophylaxe oder Unterdrückung von Anfällen fokussiert. Zurzeit gibt es keine Medikation, welche die Ursachen der Erkrankung bekämpft. Deshalb besteht ein dringender Bedarf an Medikamenten, welche die Entstehung von Epilepsien verhindern können.

Temporallappenepilepsie und nicht-konvulsive generalisierte Epilepsien kommen nur sehr selten gekoppelt vor. Der zugrunde liegende Mechanismus ist kaum bekannt. Ein Rattenmodell für Absencen könnte eine einzigartige Möglichkeit darstellen, die Pathophysiologie der Epileptogenese in Tieren aufzuklären.

Genetic Absence Epilepsy Rats from Strasbourg (GAERS) dienen als akzeptiertes Tiermodell für menschliche Absence-Epilepsie. Ziel dieser Studie ist es, eine mögliche Rolle der Neurotrophine wie BDNF und ihrer Rezeptoren in wiederkehrenden Anfällen aufzuklären. Biochemische, immunhistochemische wie auch funktionelle Untersuchungen werden an jungen und erwachsenen GAERS im Hinblick auf die Erforschung von neuen therapeutischen Zugängen zur Prävention und Behandlung von Epilepsien durchgeführt.

Schlüsselwörter: GAERS, Neurotrophine, BDNF, Absence-Epilepsie, Epileptogenese

Rôle des neurotrophines dans l'épilepsie – un rapport intermédiaire

De nos jours, l'épilepsie est l'une des maladies neurologiques les plus fréquentes au monde. Les protocoles de traitement usuels se concentrent exclusivement sur la prophylaxie ou la suppression des attaques fournissant ainsi simplement des traitements symptomatiques sans combattre la cause de la maladie. Il y a un besoin urgent de trouver de nouveaux médicaments qui pourraient agir différemment sur les molécules cibles, ainsi que de nouveaux traitements qui viseraient à inhiber le processus de la genèse de l'épilepsie.

L'épilepsie temporale et l'épilepsie non convulsive sont rarement observées ensemble et les mécanismes sous-jacents sont encore peu compris. Le modèle de rat avec l'épilepsie-absence est probablement l'unique possibilité pour étudier la physiopathologie de la genèse de l'épilepsie chez l'animal.

Genetic Absence Epilepsy Rats de Strasbourg (GAERS) est un modèle valable pour l'épilepsie-absence humaine. Le but de cette étude est de définir le rôle éventuel des neurotrophines tel que le BDNF et de ses récepteurs dans les attaques répétitives. Des études biochimiques, fonctionnelles, immunohistochimiques et de développement ont été pratiquées chez GAERS afin de trouver de nouvelles approches pour la prévention et le traitement de l'épilepsie.

Mots clés: GAERS, neurotrophines, BDNF, épilepsie-absence, genèse de l'épilepsie

***Acknowledgement**
I am deeply indebted to my supervisors Prof. Dr. Uwe Otten, PD Dr. Dieter Kunz and Dr. Raymond Bernasconi from the Institute of Physiology, University of Basel, whose stimulating suggestions and encouragement help me in all the time of my research.
This work is supported by grants from the „Schweizerische Liga gegen Epilepsie" and "Stiftung Emilia Guggenheim-Schnurr der Naturforschenden Gesellschaft in Basel".

Introduction

Absence epilepsy is an idiopathic, generalized and non-convulsive form of epilepsy with an unknown polygenetic background. It may be related to an excess of GABAergic inhibition within the thalamus. Absence seizures are brief episodes of staring during which awareness and responsiveness are impaired. They are usually not realized by the patient. There is no signal before a seizure, and the person is completely alert immediately afterwards. Bilateral synchronous 3Hz discharges, the so-called spike-and-wave discharges (SWDs), can be detected on the electroencephalogram (EEG). Seizures occur as frequently as several hundred times a day, mainly during quite wakefulness, inattention and the transition between sleep and awakening.

GAERS is one of the best validated animal models of absence epilepsy; it is an inbred Wistar line, which exhibits spontaneous absence-like seizures. The EEG demonstrates 5-8Hz generalized SWDs similar to human absence epilepsy. The animals manifest no other neurological deficits. During seizures, the animals have an arrest in activity, often associated with some mild clonic activity of neck muscles and whisker twitching. Data in GAERS demonstrate that transmission of SWDs is inherited. Inter-individual variability for age of appearance and duration of SWDs is extremely high, suggesting that the inheritance is probably not due to a single gene locus [1].

Neurotrophins (NTs) are a family of related proteins consisting of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5). All of them are synthesized as pre-pro-proteins of approximately 30kDa and cleaved to the mature form of about 13kDa. All mature NTs share common elements in structure and sequence. The precursor forms proNGF and proBDNF may themselves have independent biological activity.

The NT receptor system consists of the low-affinity transmembrane receptor $p75^{NTR}$ which binds all mature NTs as well as proNGF and proBDNF. In addition, NTs bind with high affinity to one of the Tyrosine kinase receptor (Trk) family: NGF to TrkA, BDNF and NT-4/5 to TrkB, NT-3 to TrkC. The receptors act as dimers with or without $p75^{NTR}$. Activation of Trk blocks apoptosis and promotes cell survival and differentiation. However, if $p75^{NTR}$ is expressed in the absence of Trk or if the ratio of $p75^{NTR}$/Trk is high, NTs can activate the $p75^{NTR}$ pathway and promote apoptosis [2].

It was reported that GAERS cannot be kindled. Although maximal stimulations were applied, GAERS remained at stage 2 of the Racine scale and no motor seizures were observed [3]. In this rat model, the resistance to kindling is related to the total duration of spike-and-wave discharges. Thus, the mechanisms underlying generalized absence seizures may be responsible for the resistance to the development of kindling and provide evidence for specific thalamo-limbic interactions.

The observation that the expression of genes encoding NTs and their receptors in the central nervous system is regulated prominently by seizure activity suggests a central role of NTs in epileptogenesis. However, the function of endogenous NTs, particularly of BDNF, is complex. Xu and coworkers [4] compared the effects of continuous BDNF infusion versus bolus injections of BDNF on kindling induction. They found that multiple bolus microinjections of BDNF accelerated kindling development whereas, in contrast, continuous infusion of BDNF inhibited the development of seizures.

Acute BDNF treatment induces a clear increase of neuronal excitability suggesting a pro-convulsive role for BDNF. This view is supported by the fact, that BDNF can also act as neurotransmitter and cause an inward flow of sodium ions leading to membrane depolarization within milliseconds. This model does not require a contribution from soluble second messengers and thus explains the speed of the response. Binding of BDNF to TrkB induces a conformational change of the receptor that immediately affects the gating of the specific sodium channel such that it briefly becomes permeable to sodium ions [5].

In contrast, chronic intrahippocampal infusion of BDNF delays kindling development in rats.

A rationale for these contradictory results might be that on a longer time scale BDNF overexpression results in TrkB receptor downregulation. In spite of this downregulation, remaining TrkB receptor phosphorylation and signal transduction are sufficient to trigger transcription of genes involved in neuronal plasticity including the inhibitory neuropeptide Y (NPY) [6]. NPY expression is increased following acute seizures induced by kainic acid injection as well by electrically- and chemically-induced kindling, and has a protective action against different seizures [7].

Recent studies demonstrated that limbic motor seizures can be suppressed by electrical stimulation of specific thalamic nuclei [8] suggesting that neuronal excitatory pathways between thalamic reuniens nucleus and hippocampal CA1 regulate BDNF levels and thereby inhibit epileptogenesis.

With regard to developmental differences, we compared juvenile with adult animals. Early alterations in NT expression might have important consequences on developmental changes and therefore contribute to epileptogenesis.

Using the GAERS strain as experimental model the potential role of NTs, in particular BDNF, and their receptors TrkB and $p75^{NTR}$ as well as related molecules for epileptogenesis were investigated. Juvenile GAERS which are younger than 30 days fail to show SWDs [9]. With increasing age the number of GAERS with SWDs gradually increases and reaches 100 % at the age of 3 months. We hypothesized that alterations in the NT system during brain development may significantly contribute to epileptogenesis. In particular, we inten-

ded to investigate whether the protective effects of BDNF might be mediated through an impaired signal transmission either caused by TrkB-downregulation and/or elevated levels of inhibitory neurotransmitters such as NPY. Thus, we performed our studies on juvenile animals at age of 30 days as compared to adult animals at age of 3 months. In order to exclude possible differences due to hormone cycles, only male adult animals were used in the experiments.

Materials and Methods

Animals

GAERS and non-epileptic control rats (NEC) were housed with up to five animals of the same age in one cage. Animals had exposure to food and water ad libitum and were maintained on a 12h-light/12h-dark cycle. They were killed and different brain regions including parietal cortex, hippocampus and thalamus were dissected, immediately frozen in liquid nitrogen and stored at -80°C. Animal tests were performed in accordance with guidelines of Kantonales Veterinäramt, Kanton Basel-Stadt.

Western blot analyses

Brain sections were homogenized by sonification in 500µl of RIPA buffer (NaCl 150mM, Nonidet P-40 1 %, Deoxycholate 0.5 %, SDS 0.1 %, Tris-HCl 50mM, Complete EDTA-free Protease inhibitor cocktail tablet, $Na_4P_2O_7$ 20mM, Na_3VO_4 2mM, NaF 1mM). The homogenate was purified by centrifugation for 1min at 16'000xg; thereafter, the supernatant was further centrifuged for 20min at 50'000xg; the final supernatant was used for the studies. Protein concentrations were determined using a Coomassie dye-based assay (BioRad). For Western blot analyses of BDNF protein, 25µg of total protein were loaded per lane. The homogenates were resolved on 12 % bis-tris gels (Invitrogen) by electrophoresis at 90V for 2h. Proteins were transferred to nitrocellulose membranes for 1h at 30V. For immunostaining, membranes were blocked for 30min at room temperature in 5 % skim milk powder (Fluka) in PBS with 0.1 % Tween-20. Incubation with primary antibodies was performed overnight at 4°C at dilutions of 1:2'000 for BDNF (N-20, Santa Cruz) in 5 % skim milk powder solution. After three 5min washes, blots were incubated for 1h at room temperature with goat anti-rabbit IgG-HRP-conjugated secondary antibodies in 5 % skim milk powder solution at a dilution of 1:2'000. A chemiluminescence system (Roche Diagnostics) was used for the signal detection. Following BDNF analysis, blots were stripped, again blocked in 5 % skim milk powder solution and reprobed with anti-glyceraldehyd-3-phosphate dehydrogenase (GAPDH (6C5, Santa Cruz)) antibody diluted 1:10'000 overnight at 4°C, followed by incubation with goat anti-mouse IgG-HRP-conjugated antibody diluted 1:10'000. Chemiluminescence images were captured using an Alpha Innotech imaging station and quantified using FluorChem software. BDNF pixel values were normalized to GAPDH values for each sample.

Real-time polymerase chain reaction (RT-PCR)

Total RNA was prepared from rat parietal cortex, hippocampus and thalamus using Trizol® Reagent (Invitrogen) and purified by DNase treatment (Promega) according to the manufacturer's instructions. First-strand cDNA synthesis was performed using 2µg of total RNA, random hexamers, dNTPs and Superscript™ II reverse transcriptase (Invitrogen). The reaction mixtures were subjected to PCR amplification using primers specific for BDNF, TrkB, NPY and β-actin (QuantiTect® Primer Assays, Qiagen) and SYBR® Green (Applied Biosystems) as fluorescent dye. PCR amplifications were performed in quadruplicate wells in the ABI Prism 7000 Sequence Detection System (Applied Biosystems) using the following conditions: 2min at 50°C for polymerase activation, 10min at 95°C for denaturation, followed by 40 cycles consisting of 15s at 95°C for denaturation and 1min at 60°C for annealing and elongation. A melting curve analysis of PCR products was performed after each experiment to detect non-specific amplifications. Negative controls were transcribed both by omitting reverse transcriptase (-RT control) or RNA (no template control).

Quantification of the RT-PCR results was performed using the comparative Ct method. Fluorescence signal intensities were plotted against the number of PCR cycles on a semilogarithmic scale using the ABI Prism 7000 SDS software. A threshold cycle (CT) was designated as the amplification cycle at which the first significant increase in fluorescence occurred. The C_T value of each sample was compared with that of β-actin as internal standard: $\Delta C_t = C_t(\text{target gene}) - C_t(\beta\text{-actin})$. In a second step, samples of GAERS were compared to those of NEC: $\Delta\Delta C_t = \Delta C_t(\text{GAERS}) - \Delta C_t(\text{NEC})$. The change of expression of the target gene in GAERS was calculated by the term $2^{-\Delta\Delta Ct}$.

Statistics

Results were subjected to statistical analysis using the Student's t-test. Differences were considered to be statistically significant at p-values <0.05. Experiments were performed with a total of 10 NEC and GAERS each and repeated 3 times.

Results

BDNF protein levels were measured using Western blot analyses of brain homogenates followed by quantification of the pixel intensities. In cortex, BDNF levels in juvenile GAERS are significantly decreased (72 %; p<0.05) as compared to NEC **(figure 1A, left)**. In contrast, cortices from adult GAERS show significantly elevated BDNF levels up to 121 % (p<0.05); **figure 1A**, right). In hippocampus, elevated BDNF levels are detected in both, juvenile (123 %; p<0.05; **figure 1B, left**) as well as adult (119 %; p<0.05; **figure 1B, right**) GAERS as compared to age-matched NEC. No differences in BDNF protein levels were observed in thalamus of neither juvenile nor adult GAERS (data not shown). Of particular interest is the finding that a significant elevation of BDNF protein levels in hippocampus already occurs before the onset of SWDs.

Changes in mRNA expression were measured using real-time PCR with a fluorescent dye. In juvenile GAERS, BDNF mRNA expression is highly elevated in cortex (160 %; p<0.05; **figure 2A**) and in hippocampus (212 %; p<0.05; **figure 3A**) as compared to age-matched NEC. However, in adult GAERS differences in BDNF mRNA expression are less pronounced displaying a significant elevation up to 140 % (p<0.05) only in cortex **(figure 2B)**. Surprisingly, differences in BDNF mRNA expression were found neither in hippocampus of adult animals (**figure 3B**) nor in thalamus of both, juvenile and adult GAERS (data not shown).

NPY mRNA expression clearly is altered in GAERS versus NEC, but no significant age-dependent differences were found in juvenile and adult animals (**figures 2, 3**). In cortex, NPY mRNA levels are elevated to 161 % (p<0.05) in juvenile and 175 % (p<0.05; **figures 2A, B**) in adult GAERS. Similarly, in hippocampus NPY mRNA ex-

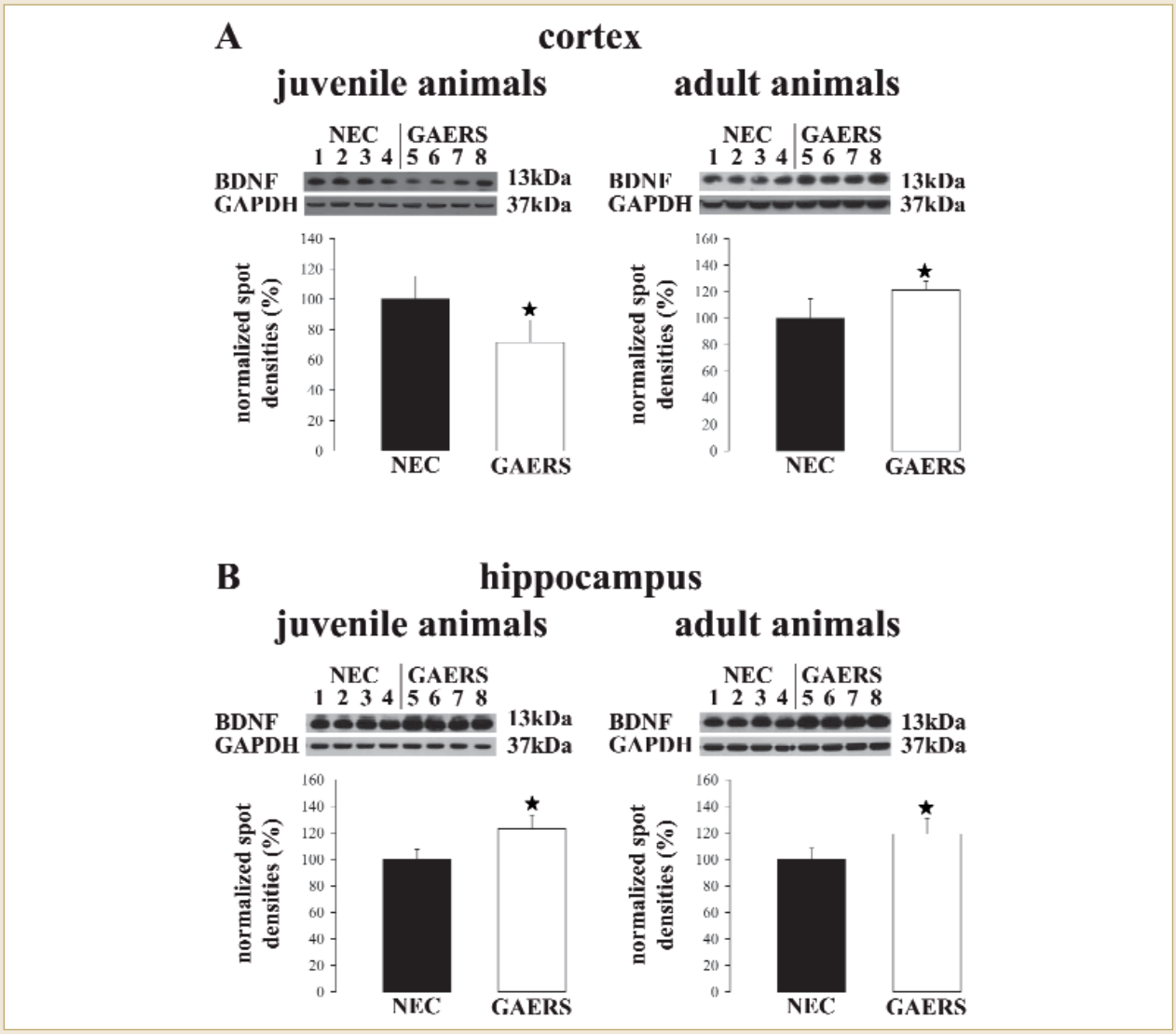

Figure 1: Western blot analysis of cortex (A) and hippocampus (B) homogenates derived from juvenile (left) and adult (right) non-epileptic control rats (NEC; 1-4) and GAERS (5-8) immunoassayed with BDNF (N-20)-antibody. Quantitative evaluation of BDNF levels is shown below (n=10). GAPDH (6C5) immunoreactivity was used for normalisation. *p<0.05 compared to age-matched NEC (Student's t-test).

pression is increased to 227 % ($p<0.05$) and 189 % ($p<0.05$) in juvenile and adult GAERS **(figures 3A, B)**. No changes were found in the thalamus of juvenile and adult animals (data not shown).

Significant differences between juvenile and adult animals were found in TrkB mRNA expression. In all three examined brain regions of juvenile GAERS no significant changes in TrkB expression were detected as compared to age-matched NEC (**figures 2A, 3A**; data for thalamus not shown). However, in adult GAERS TrkB mRNA expression is significantly reduced in both, cortex (83 %; $p<0.05$; **figure 2B**) and hippocampus (69 %; $p<0.05$; **figure 3B**). No changes in TrkB mRNA expression levels were found in the thalamus of adult animals (data not shown). **Table 1** summarizes all obtained results.

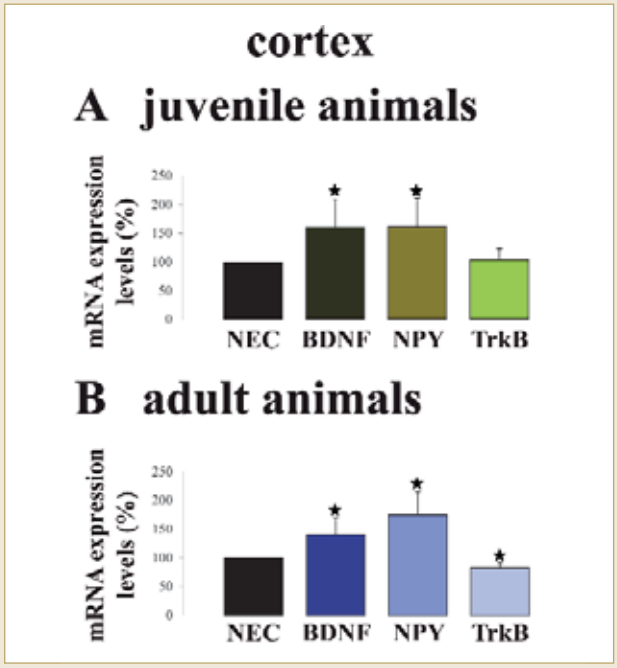

Figure 2: Detection of changes in BDNF-, NPY- and TrkB mRNA expression in cortex of juvenile GAERS (A) and adult GAERS (B) as compared to NEC using RT-PCR analyses. *p<0.05 compared to age-matched NEC (Student's t-test).

Discussion

The original working hypothesis was to elaborate a direct correlation between BDNF levels and the existence of SWDs. Preliminary data revealed clear differences in BDNF protein as well as mRNA expression in GAERS compared to NEC, supporting the hypothesis that BDNF is indeed an important regulator in the pathophysiology of absence epilepsy. However, the most surprising observation was that there were no explicit differences in BDNF levels between juvenile and adult animals. The obtained data indicate that BDNF levels are already elevated in juvenile GAERS although SWDs are not yet present at this age.

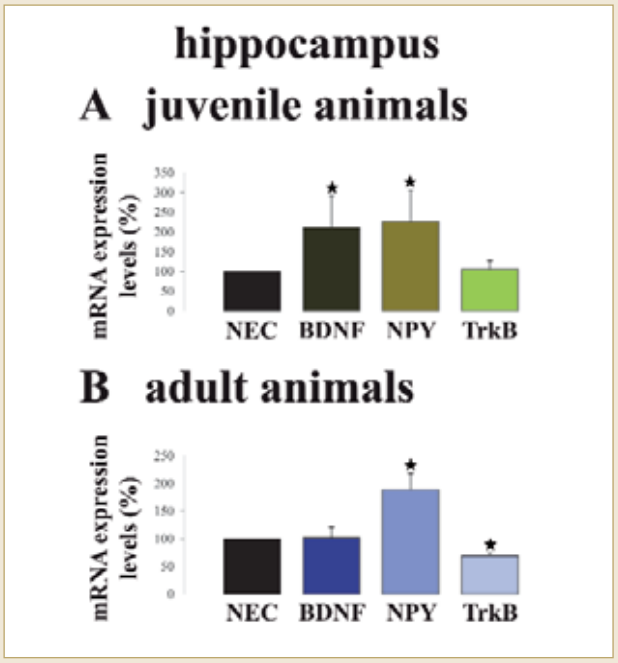

Figure 3: Detection of changes in BDNF-, NPY- and TrkB mRNA expression in hippocampus of juvenile GAERS (A) and adult GAERS (B) as compared to NEC using RT-PCR analyses. *p<0.05 compared to age-matched NEC (Student's t-test).

Discrepancies between BDNF protein and mRNA expression were found in both, cortex of juvenile and hippocampus of adult GAERS which is in good correlation to published data suggesting that a tight correlation between the localization of BDNF mRNA and protein may not necessarily to be expected. In the literature, several possible explanations are discussed: (i) The amount of protein may be too low to be detected by a specific antibody. Alternatively, the protein may be in a precursor form or in an unfolded state, which prevents recognition by the antibody. (ii) The protein may be localized to a subcellular region distinct from the mRNA. (iii) BDNF synthesis could be regulated at a post-transcriptional level and not directly coupled to mRNA transcription [10,11]. (iv) The protein may be very rapidly transported or secreted after processing. The presence of BDNF protein in axon terminals throughout the brain suggests that anterograde transport might be a common feature of neurotrophin trafficking in the central nervous system [12]. (v) BDNF mRNA transcripts are translocated to the dendritic compartment after neuronal activation which conceivably could affect BDNF mRNA translation rate. (vi) BDNF may have a neurotransmitter-like role in the brain. Increased BDNF turnover could reduce total BDNF protein, if BDNF synthesis was not activated accordingly [13].

As it was not possible to demonstrate a direct correlation between BDNF protein expression and the existence of SWDs, we extended our analyses on the expression of TrkB the main receptor for BDNF. Whereas no differences in TrkB mRNA expression were found in all brain regions of juvenile animals examined, adult

Table 1:
Summary of changes in protein and mRNA expression of different members of the BDNF-system in juvenile and adult GAERS compared to age-matched NEC. ↑: significant increase; ↓: significant decrease; ↔ no significant changes.

			cortex	hippocampus
adult GAERS	BDNF	protein	↑	↑
		mRNA	↑	↔
	TrkB	mRNA	↓	↓
	NPY	mRNA	↑	↑
juvenile GAERS	BDNF	protein	↓	↑
		mRNA	↑	↑
	TrkB	mRNA	↔	↔
	NPY	mRNA	↑	↑

GAERS display significant decreases in TrkB expression both in cortex as well as in hippocampus. These observations speak in favour that the BDNF-system is critically involved in the generation of absence epilepsy. Long-time exposure to high levels of BDNF might lead to internalization or diminished synthesis of TrkB receptors in order to protect neuronal cells from NT overstimulation. This protective mechanism might need time to establish and therefore not yet be present in juvenile animals. These considerations were in line with recent results obtained from cell culture experiments. A three-day chronic BDNF treatment of cortical cultures led to a significant down-regulation of full-length TrkB, but not truncated TrkB receptor protein [14].

Our data on reduced TrkB expression suggest that the BDNF signalling-pathway appears to be disturbed in adult GAERS. It was shown that BDNF knock-out mice exhibited only a modest impairment of limbic epileptogenesis as evidenced by the increased number of stimulations required to elicit behavioural seizures. Moreover, in the BDNF knock-out mice an increased expression of hippocampal NT-3 protein was found which may be a compensatory response to the lack of BDNF. However, increased NT-3 expression may contribute to TrkB activation and limbic epileptogenesis. In distinct contrast, it was not possible to induce limbic epileptogenesis in TrkB knock-out mice. No behavioural seizures could be evoked even after a maximum of 50 stimulations [15].

As it is well documented that BDNF induces NPY gene expression, this neuropeptide was also included in the analyses. In cortex as well as in hippocampus of juvenile and adult GAERS, NPY mRNA expression is significantly elevated as compared to age-matched NEC. Although there might be a strong correlation between NPY expression and the pathophysiology of absence seizures, NPY overexpression does not seem to be the cause for the generation of SWDs. A so far unknown mechanism distinct from BDNF signalling might additionally be involved in the upregulation of NPY mRNA expression in adult GAERS, since NPY expression is significantly elevated, even though TrkB receptor – and thus BDNF-signalling – is diminished. In a recent publication it was shown that a 7-day continuous infusion of NPY in the hippocampus delayed the progression of hippocampal kindling in the rat, whereas administration of anti-NPY antibody had a rather aggravating effect [16]. These results support the hypothesis that BDNF delays kindling at least in part through upregulation of NPY expression. This might be an endogenous mechanism that counteracts excessive hippocampal activity.

Conclusion

A number of questions arise from the results obtained in my work.

The most straightforward work to do is the performance of kindling experiments in juvenile GAERS. Two different mechanisms could contribute to the protection against kindling. (i) Assuming that young animals are already protected it appears that NPY is the key player with its antiepileptic property. (ii) If only adult animals are protected against kindling, the BDNF receptor TrkB seems to be the key player by impairing the BDNF-signalling pathway. (iii) A further possibility would be that a different, yet unknown molecule or mechanism is involved in the protection against kindling. This might be a downstream molecule in the BDNF-signalling pathway such as cyclic AMP response element-binding protein (CREB; see **figure 4**).

Activation of TrkB by BDNF-binding leads to autophosphorylation at two different tyrosine residues. Thus, two distinct signalling pathways can be activated resulting in phosphorylation of CREB. One pathway leads to CREB phosphorylation by ribosomal S6 kinase

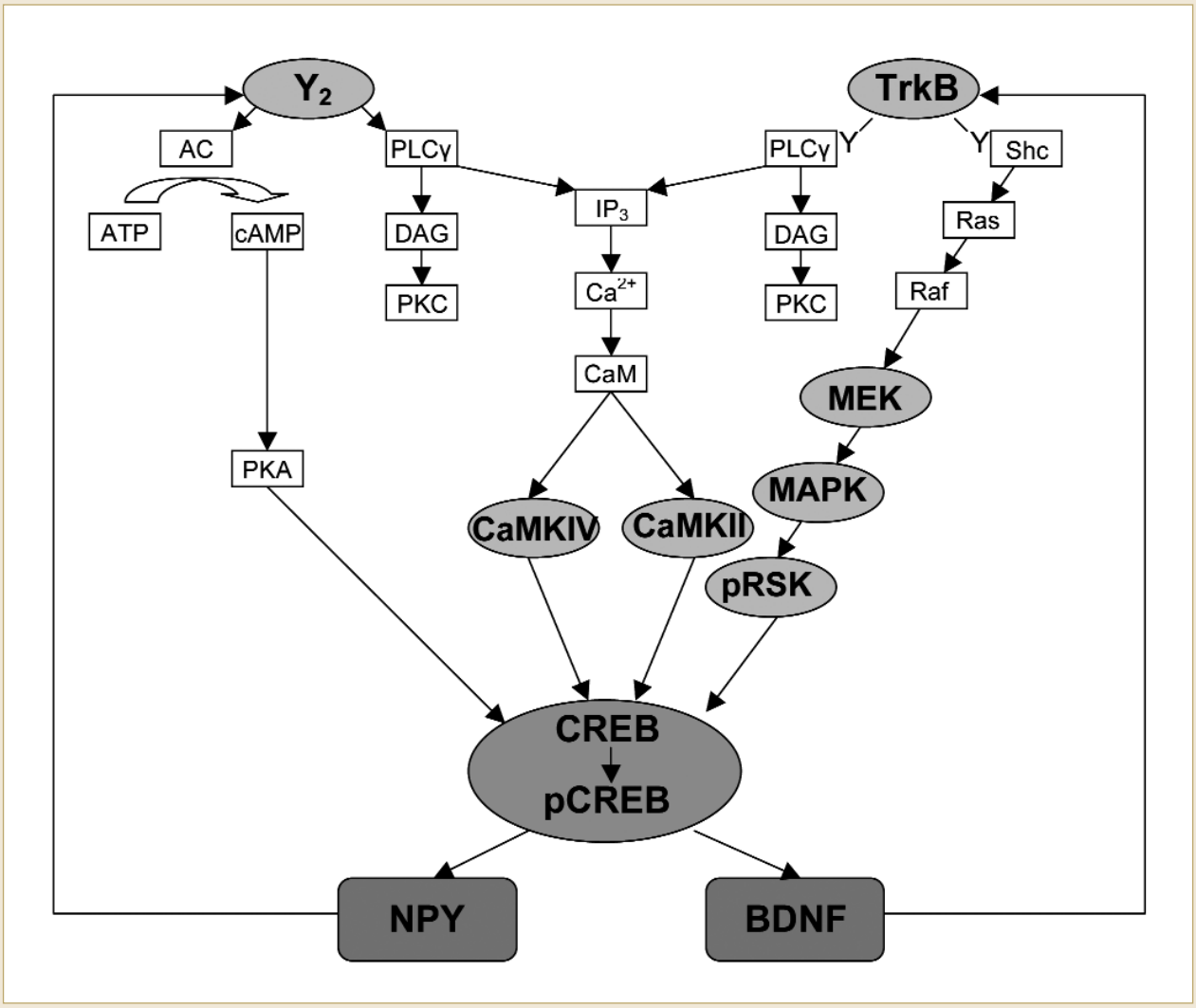

Figure 4: Schematic model of CREB as a molecular switch in the coordinate regulation of BDNF and NPY expression. BDNF binding to TrkB stimulates receptor phosphorylation, resulting in the recruitment of a series of signalling proteins to docking sites on the receptor, finally leading to CREB phosphorylation. This transcription factor in turn regulates the expression of NPY and BDNF and their specific receptors. In addition, NPY via stimulation of NPY-Y2 receptors is also competent to activate CREB by phosphorylation.

(RSK) via mitogen-activated protein kinase (MAPK). The other one involves release of intracellular calcium thus activating Calcium-calmodulin-dependent protein kinases (CaMKIV) and finally leading to CREB phosphorylation.

Phosphorylated CREB is the transcription factor regulating the expression of cyclic AMP-inducible genes. Interestingly, it was shown that both, NPY via its specific receptors and BDNF using the TrkB signalling pathway have been identified as CREB-target genes. CREB might therefore be a hot spot in the whole signalling cascade and act as a common molecular switch between BDNF and NPY **(**see **figure 4)** [17,18]. Moreover, NPY in turn by stimulation of NPY-Y2 receptors is also competent to activate CREB phosphorylation via cyclic AMP dependent protein kinase or via Calcium-calmodulin-dependent protein kinases. Thus, it is obvious that a detailed investigation of CREB phosphorylation and expression is needed.

Outlook

Current experiments are concentrated on the protein expression and phosphorylation of CREB in the different brain regions in order to identify age-related differences of GAERS versus NEC in juvenile and adult animals. In addition, ELISA for detection of NGF and NT-3 protein expression as well as Western blot analyses for detection of $p75^{NTR}$ expression will be performed to demonstrate the exact and unique role of the BDNF system.

Moreover, immunohistochemical studies will be performed to reveal the region-specific expression pattern of NTs and neuropeptides. Special attention will be directed to the CA1 region of hippocampus and thalamus, in particular the thalamic reuniens nucleus (RN) and the centromedian nucleus as previous studies have

provided evidence that the RN is involved in the promotion of limbic hippocampal seizures [19].

Moreover, kindling-experiments will be performed to investigate whether there exists a correlation between resistance to propagation of amygdaloid kindling seizures and appearance of SWDs.

To characterize the role of BDNF in preventing the kindling response, functional blockade of BDNF activity should be performed using antibodies against BDNF receptors. In addition, BDNF and proBDNF proteins could be applied exogenously and their effects studied.

References

1. *Danober L, Deransart C, Depaulis A et al. Pathophysiological mechanisms of genetic absence epilepsy in the rat. Progress in Neurobiology 1998; 55: 27-57*
2. *Dechant G. Molecular interactions between neurotrophin receptors. Cell Tissue Res 2001; 305: 229-238*
3. *Eskazan E, Onat FY, Aker R, Oner G. Resistance to propagation of amygdaloid kindling seizures in rats with genetic absence epilepsy. Epilepsia 2002; 43: 1115-1119*
4. *Xu B, Michalski B, Racine RJ, Fahnestock M. The effects of brain-derived neurotrophic factor (BDNF) administration on kindling induction, Trk expression and seizure-related morphological changes. Neuroscience 2004; 126: 521-531*
5. *Blum R, Kafitz KW, Konnerth A. Neurotrophin-evoked depolarization requires the sodium channel Nav1.9. Nature 2002; 419: 687-693*
6. *Reibel S, Larmet Y, Lê BT et al. Brain-derived neurotrophic factor delays hippocampal kindling in the rat. Neuroscience 2000; 100: 777-788*
7. *Reibel S, Larmet Y, Carnahan J et al. Endogenous control of hippocampal epileptogenesis: a molecular cascade involving brain-derived neurotrophic factor and neuropeptide Y. Epilepsia 2000; 41(Suppl 6): 127-133*
8. *Nanobashvili Z, Chachua T, Nanobashvili A et al. Suppression of limbic motor seizures by electrical stimulation in thalamic reticular nucleus. Exp Neurol 2003; 181: 224-230*
9. *Vergnes M, Marescaux C, Depaulis A et al. Ontogeny of spontaneous petit mal-like seizures in Wistar rats. Brain Research 1986; 395: 85-87*
10. *Dugich-Djordjevic MM, Peterson C, Isono F et al. Immunohistochemical visualization of brain-derived neurotrophic factor in the rat brain. Eur J Neurosci 1995; 7: 1831-1839*
11. *Yan Q, Rosenfeld RD, Matheson CR et al. Expression of brain-derived neurotrophic factor protein in the adult rat central nervous system. Neuroscience 1997; 78: 431-448*
12. *Altar CA, DiStefano PS. Neurotrophin trafficking by anterograde transport. Trends in Neurosciences 1998; 21: 433-437*
13. *Jacobsen JP, Mork A. The effect of escitalopram, desipramine, electroconvulsive seizures and lithium on brain-derived neurotrophic factor mRNA and protein expression in the rat brain and the correlation to 5-HT and 5-HIAA levels. Brain Research 2004; 1024: 183-192*
14. *Knusel B, Gao H, Okazaki T et al. Ligand-induced down-regulation of Trk messenger RNA, protein and tyrosine phosphorylation in rat cortical neurons. Neuroscience 1997; 78: 851-862*
15. *He XP, Kotloski R, Nef S et al. Conditional deletion of TrkB but not BDNF prevents epileptogenesis in the kindling model. Neuron 2004; 43: 31-42*
16. *Reibel S, Benmaamar R, Lê BT et al. Neuropeptide Y delays hippocampal kindling in the rat. Hippocampus 2003; 13: 557-560*
17. *Kaplan DR, Miller FD. Neurotrophin signal transduction in the nervous system. Curr Opin Neurobiol 2000; 10: 381-391*
18. *Williams AG, Hargreaves AC, Gunn-Moore FJ, Tavaré JM. Stimulation of neuropeptide γ gene expression by brain-derived neurotrophic factor requires both the phospholipase Cγ and Shc binding sites on its receptor, TrkB. Biochem J 1998; 333: 505-509*
19. *Bertram EH, Zhang DX, Mangan P et al. Functional anatomy of limbic epilepsy: a proposal for central synchronization of a diffusely hyperexcitable network. Epilepsy Research 1998; 32: 194-205*

Korrespondenzadresse:
Svenja Landweer, PhD student
Molecular Neurobiology Research Group
Institute of Physiology
Department of Biomedicine
University of Basel
c/o Institute of Anatomy
Pestalozzistr. 20
CH 4056 Basel
Tel. 0041 61 267 3545
Fax 0041 61 267 3582
s.landweer@unibas.ch