Available online at www.sciencedirect.com

SCIENCE DIRECT®

Molecular Brain Research xx (2004) xxx–xxx

MOLECULAR BRAIN RESEARCH

www.elsevier.com/locate/molbrainres

Research report

Dendritic BC1 RNA: modulation by kindling-induced afterdischarges

Volker Nimmrich[a,b,1], Eric L. Hargreaves[a,2], Ilham A. Muslimov[a], Riccardo Bianchi[a], Henri Tiedge[a,b,c,*]

[a]*Department of Physiology and Pharmacology, State University of New York, Health Science Center at Brooklyn, 450 Clarkson Avenue, Brooklyn, NY 11203, USA*

[b]*Program in Neural and Behavioral Sciences, State University of New York, Health Science Center at Brooklyn, Brooklyn, NY 11203, USA*

[c]*Department of Neurology, State University of New York, Health Science Center at Brooklyn, Brooklyn, NY 11203, USA*

Abstract

Local protein synthesis in dendrites is thought to provide a mechanism for long-lasting modifications of synapses in response to physiological activity and behavioral experience. New synthesis of dendritic proteins may be triggered by various paradigms, including induction of epileptiform activity. Prerequisite for such modulated synthesis is a mechanism that limits translation of synaptodendritic mRNAs to times of demand. Recently identified as a translational repressor that is localized to dendrites, small untranslated BC1 RNA has been implicated in the regulation of postsynaptic protein synthesis. Here we show that translational repressor BC1 RNA is itself undergoing modulation as a result of neuronal stimulation. Induction of hippocampal epileptiform activity resulted in a significant decrease of BC1 RNA in the CA3 region over several hours after excitation. The observed decrease was cell-wide, thus indicating reduced expression rather than intracellular redistribution. We suggest that a downregulation of the translational repressor BC1 RNA serves to modulate postsynaptic protein complements in response to the induction of epileptiform activity. Such increased protein synthesis in dendrites may be required for the consolidation of enduring epileptogenic mechanisms.

Theme: Disorders of the nervous system
Topic: Epilepsy: basic mechanisms

Keywords: Local protein synthesis; Kindling; Epilepsy

1. Introduction

A prominent property of nerve cells lies in their ability to modify synaptic connections, and thus to modulate communication between them in response to functional stimulation [4,24]. Long-lasting changes in synaptic strength require the de novo synthesis of proteins [8,15,47]. Increasing evidence is now suggesting that local translation in synaptodendritic microdomains serves as a mechanism to generate synaptic proteins rapidly and in a manner that is both activity-dependent and input-specific (reviewed by Refs. [15,47]).

The discovery in dendrites of polyribosomal [32] and of other components of the translation machinery [11,38,43] provided initial evidence that proteins can be synthesized in postsynaptic microdomains. This notion received further support by the identification in dendrites of various types of RNAs, including mRNAs as well as untranslated functional RNAs (for review, see Refs. [15,16,18,33,40,47]). Subsequently, local translation in neurons has been demonstrated in a variety of experimental systems (for review, see Refs. [9,13,15,30,33,40,47]), lending further support to the notion that on-site protein synthetic mechanisms contribute to long-term plastic modulations of synapses. BC1 RNA is an untranslated neuronal polymerase III transcript that is

* Corresponding author. Tel.: +1 718 270 1370; fax: +1 718 270 2223.
E-mail address: htiedge@downstate.edu (H. Tiedge).
[1] Present address: Abbott GmbH & Co. KG, Department of Pharmacology, Knollstrasse 50, 67061 Ludwigshafen, Germany.
[2] Present address: Center for Neural Science, New York University, 4 Washington Place, New York, NY 10003, USA.

0169-328X/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.molbrainres.2004.10.008

expressed at high levels in synaptodendritic domains [6,39]. BC1 RNA has recently been identified as a repressor of translation initiation that specifically targets recruitment of the small ribosomal subunit to the mRNA [45]. Translational repression may be the default state for dendritically localized mRNAs [45]: in this model, translational activation (i.e. de-repression) is triggered during times of demand, a notion in line with previous observations that protein synthesis in neurons is increased after synaptic excitation (reviewed by Refs. [15,47]).

This concept raises an important question: is expression of BC1 RNA itself subject to modulation by synaptic activity? In other words, is the modulator itself being modulated, thus providing long-term feedback control? To address this question, we examined BC1 expression after kindling-induced epileptiform activity, an event associated with increased protein synthesis. We report that in adult rat brain in vivo, somatodendritic levels of BC1 RNA in hippocampal neurons were significantly decreased after induction of epileptiform activity. We suggest that a downregulation of BC1 expression levels results in the facilitated translation of somatodendritic mRNAs after induction of seizures.

2. Materials and methods

2.1. Surgery and electrophysiology

Male Sprague–Dawley rats (22 total, 350–600 g) were anaesthetized with urethane (1 g/kg administered i.p.). Animals were implanted with monopolar stimulating and recording electrodes composed of single Teflon coated stainless steel wires, cut flush at the tips (diameter 65 μm). Both stimulating and recording electrodes were referenced to stainless steel screws implanted in the skull.

Animals were implanted unilaterally on the right side with stimulating and recording electrodes in the stratum radiatum of CA3 and CA1, respectively. CA3 stimulating coordinates were −3.5 mm posterior and −3.5 mm lateral to the bregma suture intersection, while CA1 recording coordinates were −3.8 mm posterior and 2.5 mm lateral to the bregma suture intersection.

Final depth positioning of all electrodes was done under physiological control, and set to optimize the response from the appropriate implanted pathways. Electroencephalogram (EEG) was similarly amplified but band-pass filtered from 1 to 200 Hz (A-M Systems Model No. 1700 differential AC amplifier, Carlsborg, WA) and digitized at 400 Hz.

Hippocampal afterdischarges (ADs) were evoked using 1 ms biphasic pulses delivered in a 60 Hz train for an initial duration of 1 s [5]. If an AD was not elicited, the duration of the train was increased, or the intensity was increased and the train delivered again after several minutes. Tetanization was repeated in this manner until an AD of at least 10 s duration was elicited and recorded from the EEG. Using this approach, we produced either a single AD of at least 10 s duration or, typically, two ADs in which case only the second one was of at least 10 s duration. The duration of recorded hippocampal ADs was typically between 10 and 30 s. Animals were perfusion-fixed 2–3 h after induction of an AD. Weight- and gender-matched control animals were anaesthetized and processed in parallel.

For LTP experiments, animals were implanted unilaterally with stimulation and recording electrodes, and LTP was induced by tetanization of the perforant path using standard paradigms [34].

2.2. Preparation of specimens

Cardiac perfusion was performed with 150 ml freshly prepared 4% formaldehyde (made from paraformaldehyde) in phosphate-buffered saline (PBS; 13.7 mM NaCl, 0.27 mM KCl, 0.43 mM Na_2HPO_4, 0.14 mM KH_2PO_4, pH 7.4). Brains were placed in ice-cold formaldehyde solution overnight, transferred successively to 12%, 16% and 20% sucrose solution, and embedded in Tissue-Tek (Sakura Finetek USA, Torrance, CA). Specimens were then cryosectioned onto microscope slides (Fisher, Pittsburgh, PA) [21]. All tissue sections used for this work were from equivalent caudo-rostral positions, corresponding to plate number 34–36 in the atlas of Paxinos and Watson [29]. In particular, to ensure comparability, sections from stimulated brains were chosen from a narrow area in the immediate vicinity of the stimulating electrode.

2.3. In situ hybridization and immunocytochemistry

RNA probes against BC1 RNA were generated from plasmid pMK1 [37,39]. Probes specific for Arc mRNA were generated from a clone containing a 3.032 kb cDNA insert [23]. This plasmid contains the coding region, the 3′ UTR and part of the 5′ UTR. Arc mRNA was used as a positive control in all experiments. ^{35}S-labeled RNA probes were transcribed from linearized templates, using T3 or T7 RNA polymerase as recommended by the manufacturer (Roche Diagnostics, Indianapolis, IN). Prehybridization and hybridization steps were carried out as described [37]. High stringency washes were performed at 50 °C.

For immunocytochemistry, sections were re-fixed in 4% formaldehyde/PBS directly after thawing, and then washed in PBS for 15 min. Unspecific binding was blocked with 5% BSA in PBS for 15 min. Sections were incubated with anti-synaptophysin monoclonal antibody C7.2 (courtesy of R. Jahn, Göttingen, Germany) for 24 h at 4 °C (1:200 dilution in PBS). A biotinylated secondary antibody (anti-mouse IgG; Amersham Biosciences, Piscataway, NJ) was applied for 2 h (1:100 dilution) and decorated with a streptavidine–rhodamine conjugate (Molecular Probes, Eugene, OR). Between all steps, sections were washed in PBS for 30 min. Sections were mounted in glycerol and immediately examined by fluorescence microscopy. To prevent drying

out of tissue sections, all procedures were performed in a humid-atmosphere box. Control sections were processed the same way except that the primary antibody mixture was replaced by PBS.

2.4. Emulsion autoradiography

Emulsion autoradiography was performed as previously described [37]. In brief, dried sections were dipped in NTB2 emulsion (Eastman Kodak, Rochester, NY) diluted 1:1 with HPLC-grade water, air dried, and exposed at 4 °C for 3 days (BC1 RNA) or 7 days (Arc mRNA). After photographic development (D-19 developer, 50% strength, and Rapid-Fix; Eastman Kodak), sections were stained with cresyl violet, dehydrated, and mounted in DPX (Fluka, Ronkonkoma, NY).

2.5. Quantitative analysis

Sections were analyzed and photographed on a Nikon Microphot-FXA microscope (Nikon, Melville, NY), using dark field or epifluorescence optics. X-ray autoradiograms were either analyzed with the Nikon Microphot or with a Nikon Diaphot 300 inverted microscope. Films were exposed to labelled sections for various times (usually ranging from 1 h to over night) and autoradiograms of medial intensities were analyzed. Images were acquired with a SONY DKC-5000 3CCD camera. Photoshop software (Adobe Systems, San Jose, CA) was used to measure expression levels as described [19,20]. For quantitative analysis of autoradiograms, regions of interest (ROIs) were selected in CA3 (stratum radiatum), CA1 (stratum radiatum and pyramidale), and dentate gyrus (stratum moleculare). Optical densities in ROIs were calculated from measured luminosity values using Lambert–Beer's law. To identify activity-dependent changes in RNA expression, ipsi- and contralateral sides were measured separately for all three ROIs and were plotted as ratios of signal intensities (ipsi/contra). Because of animal-to-animal variation of the hybridization signal (and, to a lesser degree, section-to-section variation within the same animal), we restricted all quantitative analyses to comparisons within the same section. For quantitative analysis of autoradiographic silver grains, ROIs in each of stratum radiatum and stratum pyramidale were selected, and signal intensities in ROIs were calculated by subtracting background luminosity over glass from luminosity over ROIs. To test for activity-dependent changes in subcellular RNA distribution, the values were plotted as ratios of radiatum/pyramidale for both stimulated and unstimulated hippocampi. Three to six coronal sections of the area of the mid-dorsal hippocampus were selected from each animal. Results were statistically evaluated by analysis of variance (one-way ANOVA) or by Student's *t*-test, using InStat software (http://www.rdg.ac.uk/ssc/instat/instat.html; University of Reading, UK). In either case, level of significance was set at $p<0.05$.

3. Results

It was the overall objective of this work to establish whether expression of the translational modulator BC1 RNA is itself subject to activity-dependent modulation. To address this question, we examined BC1 expression patterns after induction of epileptiform activity. To assure that seizure activity was restricted to the ipsilateral hemisphere, Arc mRNA was probed as a positive control in the same respective animal as BC1 RNA.

3.1. Kindling

To establish whether expression of translational repressor BC1 RNA is modulated after strong neural excitation, we induced limbic seizure events in brains of live animals. Animals were implanted with electrodes to the right hippocampus for Schaffer collateral stimulation and recording of the hippocampal EEG. A 60 Hz kindling protocol was used to generate single hippocampal afterdischarges (ADs) of 10–30 s rhythmic network activity (see Materials and methods).

Fig. 1 shows the hippocampal EEG during a kindling-induced AD. Spike-wave discharges occurred shortly after stimulation and usually lasted for more than 10 s.

3.2. RNA expression in kindled animals

Arc mRNA is an activity-regulated dendritic mRNA [22,23], which was used in our work for reference as a molecular positive control. Kindling strongly induced expression of Arc mRNA (Fig. 2C). The result indicates that induction of an epileptic discharge was sufficient to modulate expression of a dendritic RNA. Autoradiograms in Fig. 2A,B show the distribution of BC1 RNA after seizure induction, compared to that in an unstimulated control animal. In unstimulated animals (Fig. 2B), we consistently observed higher expression of BC1 RNA in the right hippocampus than in the left one. Such asymmetric expression may be due to differences in morphology, preferred usage of one hemisphere, or other left-right functional brain asymmetries that have previously been reported in various animal systems [7,12,14,41]. Induction of epileptiform activity in the right, i.e. the higher-expressing hippocampus caused a marked decrease of the BC1 RNA signal, resulting in now virtually identical expression levels in the CA3 regions of both hemispheres (Fig. 2A). Downregulation of BC1 expression appeared throughout the stimulated hippocampus, with a significant decrease (18%, $p=0.032$) of BC1 expression levels in the CA3 field (Fig. 3). Over the entire hippocampus (i.e. dentate gyrus, CA3, CA1), BC1 expression levels were reduced by an average of 14% ($p=0.002$). Image analysis of CA3 revealed no relative change in the laminar (dendritic vs. cell body) distribution of BC1 RNA (Figs. 3D and 4), suggesting that the decrease was cell-wide. In summary, induction of

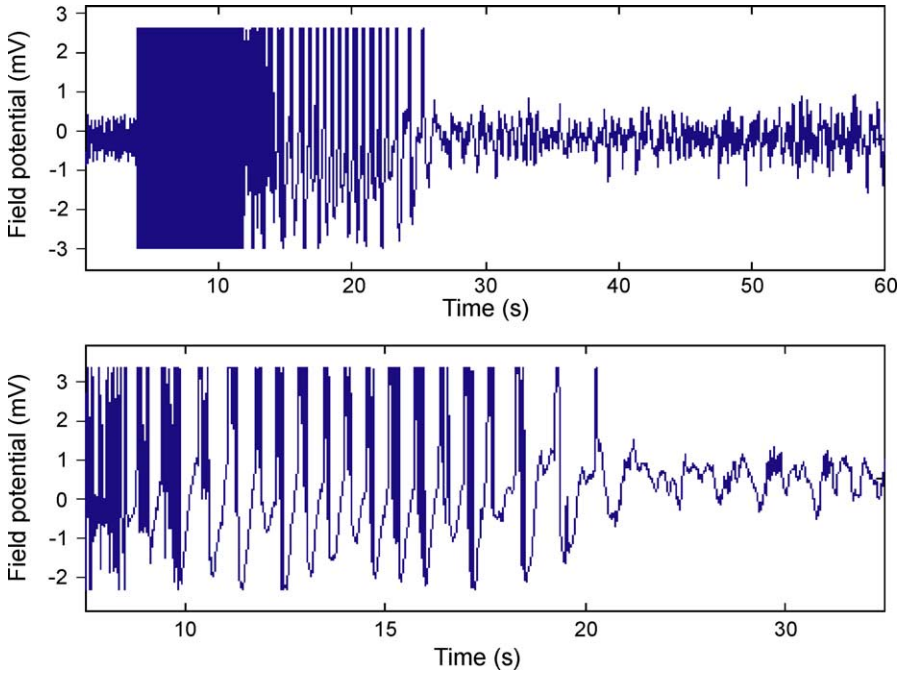

Fig. 1. Representative electrophysiological recording of a kindling experiment. Shown is a hippocampal EEG that includes induction and development of an epileptic AD. Animals were implanted unilaterally with stimulating and recording electrodes in the stratum radiatum of CA3 and CA1, respectively. A 60 Hz train is followed by a 10-s AD. The lower panel shows the AD at a higher temporal resolution. The typical appearance of hippocampal epileptiform activity is evidenced by spikes displayed on depolarizing waves (spikes are clipped in this illustration).

epileptiform activity resulted in a marked downregulation of somatodendritic BC1 RNA in the stimulated hippocampus, whereas—in the same area of the same animals—a control RNA (Arc mRNA) was upregulated.

3.3. RNA expression and LTP

We also analyzed BC1 RNA expression in all hippocampal areas after LTP induction. In none of these regions did high-frequency stimulation-induced LTP result in a significant alteration of BC1 RNA levels, although Arc mRNA expression was strongly and specifically upregulated (n=7; data not shown). The time range examined here was similar to the seizure experiments (2–3 h).

3.4. Control experiments

To ascertain tissue integrity after induction of afterdischarges, we probed for proteins as well as a control RNA in the CA3 region of the kindled animals. First—using an antibody specific for synaptophysin—we observed that the density of mossy fiber terminals in CA3 was comparable in both hemispheres of unilaterally kindled animals (Fig. 5A,B). The result confirms that innervation of CA3 pyramidal cells was not negatively affected by afterdischarges during the time frame of our experiments. Cresyl violet staining also failed to reveal any signs of tissue deterioration (data not shown). Second, we examined all animals for expression of Arc mRNA. In all cases, Arc mRNA was significantly upregulated in the seizured hippocampus (e.g. Fig. 2C), thus confirming that gene expression mechanisms were not compromised in hippocampal neurons. When autoradiograms were exposed longer, upregulation of Arc mRNA was also observed in CA3 (Fig. 5C), showing higher levels in the stimulated side. These data clearly show that cell viability and functionality were not adversely affected by AD induction, and that the observed downregulation of BC1 levels was specific and not the result of a general downregulation of gene expression.

Taken together, these data establish that BC1 expression is specifically and significantly reduced following induction of epileptiform activity. We conclude that BC1 RNA, itself a translational repressor, is subject to modulation by strong synaptic activation in vivo.

4. Discussion

Transsynaptic activity may result in long-lasting changes of synaptic form and function. On-site translation in dendrites is thought to provide, in an input-specific manner, some of the proteins that are required for long-term modifications of functional synaptic architecture that occur in response to neural activity (for review, see Refs. [13,15,30,40,47]). Prerequisite for this scenario are local regulatory mechanisms to promote translation at stimulated synapses, and to repress translation at those synapses that remain quiescent [15,47]. Dendritic BC1 RNA, a small untranslated RNA that has previously been shown to depend

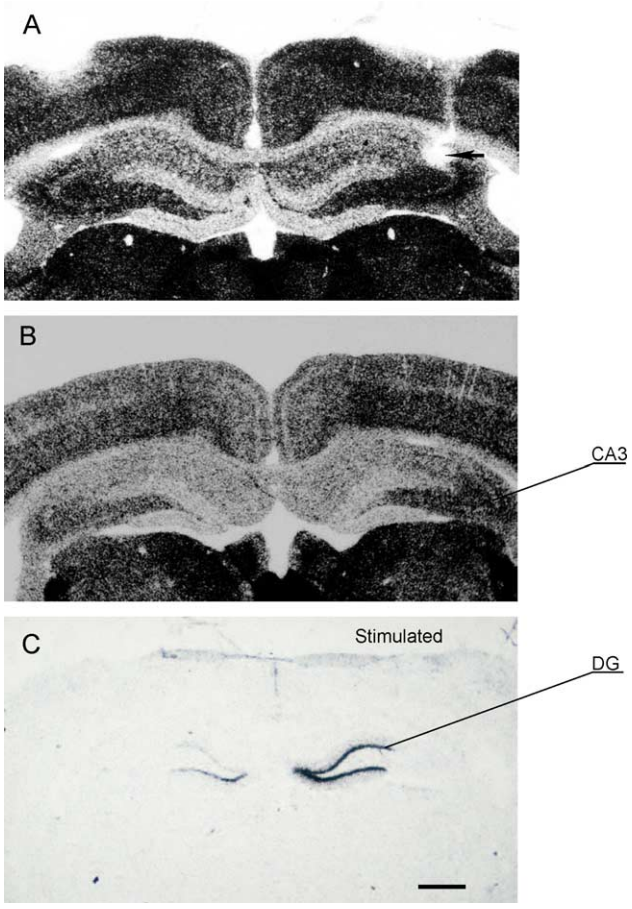

Fig. 2. Expression and localization of BC1 RNA and Arc mRNA after AD induction. Labeling intensities are indicated by darkness of the autoradiographic signal. Brain areas shown include the mid-dorsal hippocampus. The right side was stimulated in all experiments. (A) Expression of BC1 RNA after AD induction; (B) expression of BC1 RNA in a control animal; (C) expression of Arc mRNA after AD induction. Arc mRNA expression is strongly upregulated in the stimulated dentate gyrus (right hemisphere) but also shows some induction contralaterally (C). No significant expression of Arc mRNA was observed in unstimulated animals (not shown). In A, the puncture introduced by the stimulating electrode is indicated by an arrow. The line of reduced signal above the puncture in CA3 is produced by the physical insertion of the electrode through the neocortex. In the control animal (B), BC1 expression is higher in the stimulated (right) hemisphere than in the left hemisphere. After AD induction, BC1 expression levels in the stimulated (right) hippocampus are similar to levels in the unstimulated (left) side, indicating a downregulation of the RNA (A). Note that absolute expression levels between animals may vary. Scale bar, 800 μm.

on electrical activity for long-term stable expression [28], has recently been identified as a specific modulator of translation initiation [45].

A key question that is prompted by these previous findings is whether expression of the translational repressor BC1 RNA is itself subject to regulation by synaptic activity. We therefore decided to examine if BC1 expression is subject to modulation in response to altered physiological activity in brains of live animals. It has previously been reported that induction of epileptiform activity is accompanied by new synthesis of various proteins, including such that are localized to dendrites [1,10,17,23,44,46]. We therefore examined BC1 expression after induction of hippocampal ADs in vivo. The combined experimental results demonstrate that somatodendritic levels of BC1 RNA were significantly downregulated in hippocampus subsequent to kindling-induced epileptiform activity.

Seizure activity is accompanied by increased synthesis of various neuronal proteins [1,10,17,23,44,46]. These observations invite the speculation that a translational repressor is inhibited or downregulated under such conditions. We now show that in response to single kindling-induced ADs in vivo, levels of BC1 RNA are significantly reduced ipsilaterally in CA3. While the mechanistic basis for this reduction remains to be examined (e.g. decreased transcription and/or increased degradation), we suggest that such downregulation of the translational repressor BC1 RNA in hippocampal CA3 may be a mechanism that promotes somatodendritic protein synthesis.

To generate hippocampal ADs in a controlled fashion, we decided to use the kindling-model of seizure induction. Seizures were restricted to the ipsilateral hemisphere, as indicated by the Arc mRNA control. In previous work that has used similar kindling paradigms, neuronal or terminal degeneration has not been observed in CA3 [35,36]. We performed additional controls to ascertain that no histomorphological alterations had occurred in neurons or neuronal terminals secondary to epileptic discharges. Further corroborating that neurons around the seizure focus were functionally intact, Arc mRNA was found robustly upregulated in stimulated hippocampi. We therefore conclude that the observed downregulation of BC1 RNA after AD induction occurred specifically as a result of neuronal stimulation.

Interestingly, BC1 expression in unstimulated brain was consistently higher in the right hemisphere. Such left–right asymmetry could reflect differential usage of hemispheres, or laterally asymmetric neuroanatomical or functional features. Laterally asymmetric gene expression in brain has been reported in various animal systems (reviewed by Refs. [7,12,14,41]). The question of whether the observed lateral asymmetry of BC1 expression is reflected in functional corollaries, although important and fascinating, was outside the scope of this work. Here we show that higher expression levels of BC1 RNA in the right hippocampus are significantly downregulated by kindling-induced ADs in this hemisphere. While we cannot formally rule out that BC1 expression increased on the non-stimulated side, rather than decreased on the stimulated side, we note that all seizured animals showed a predominant increase of Arc mRNA in the stimulated hemisphere and only minor expression contralaterally, indicating that the contralateral side was unaffected by seizures.

A reduction of BC1 expression levels was also observed after chronic block of action potential propagation in cultured neurons [28]. It thus appears that BC1 expression can be downregulated by either very high or very low levels of neuronal activity. A robust increase in protein synthetic

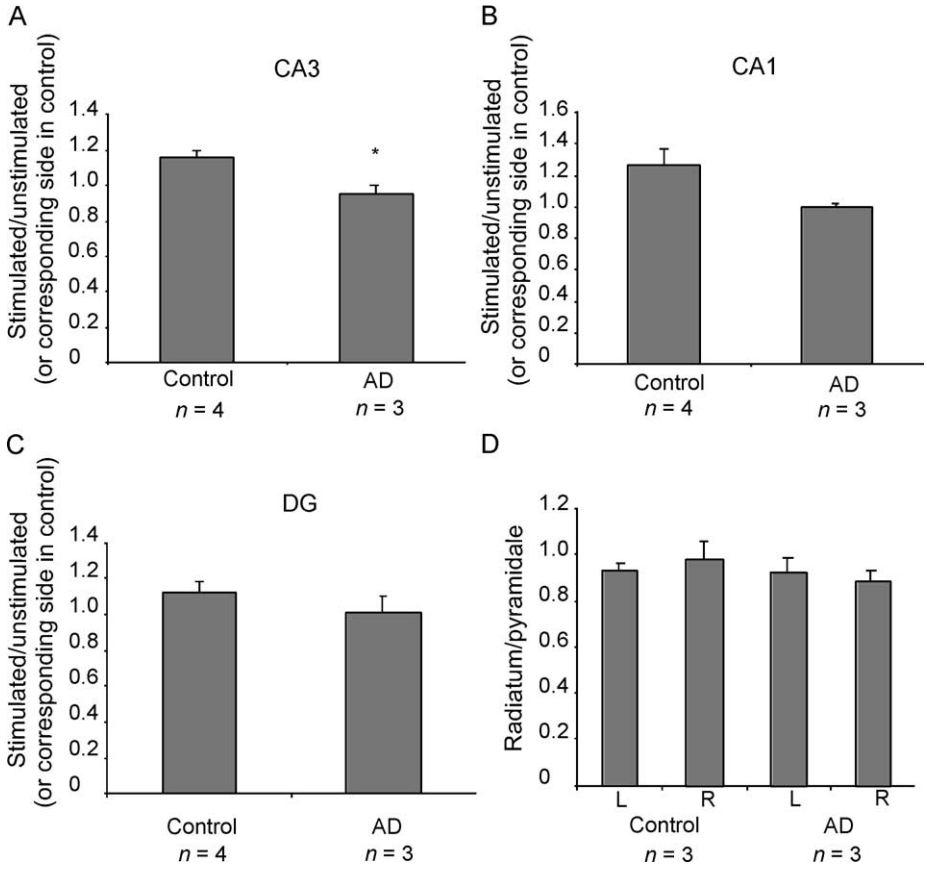

Fig. 3. Levels of BC1 RNA after induction of AD. AD induction results in a significant reduction of somatodendritic BC1 levels in the CA3 region of the hippocampus. Shown are histograms of BC1 expression and distribution in control and stimulated animals. A–C show columns reflecting the signal ratios of stimulated to unstimulated hippocampus, or the corresponding sides (right to left) in control groups. Note that control animals express higher levels of BC1 RNA in the right hippocampus. While BC1 expression levels appear reduced ipsilaterally throughout the hippocampus in stimulated animals, such decrease was found statistically significant in stratum radiatum of CA3 (A). D shows signal ratios of CA3 stratum radiatum to CA3 stratum pyramidale for the unstimulated (left, L) and the stimulated (right, R) hippocampal side. Numbers of animals analyzed are indicated (n). Autoradiograms of 4–6 sections (A–C) were examined. Silver grain densities of 3–4 sections (D) of each animal were also analyzed for subcellular quantification. (A) CA3; (B) CA1; (C) dentate gyrus. Student's t-test was performed for dentate gyrus, CA3, and CA1. A significant difference (decrease by 18%) was revealed for CA3 (A, p=0.032) but not for CA1 (B, p=0.08) or dentate gyrus (C, p=0.178). A significant decrease (14%) was also revealed when the combined data (i.e. dentate gyrus, CA3, and CA1) were analyzed by t-test (p=0.002). Analysis of variance (one-way ANOVA) was performed for data in D (p=0.534). Significance (p<0.05) is indicated by an asterisk.

activity may therefore be required in either scenario, in a situation analogous to dendritic spines where both very low and very high synaptic activity results in the same response, i.e. shortening and loss [31].

On the other hand, we observed no significant modulation of BC1 levels during the protein-synthesis dependent maintenance phase of LTP. These results indicate that strong neuronal activation per se is not sufficient to trigger a

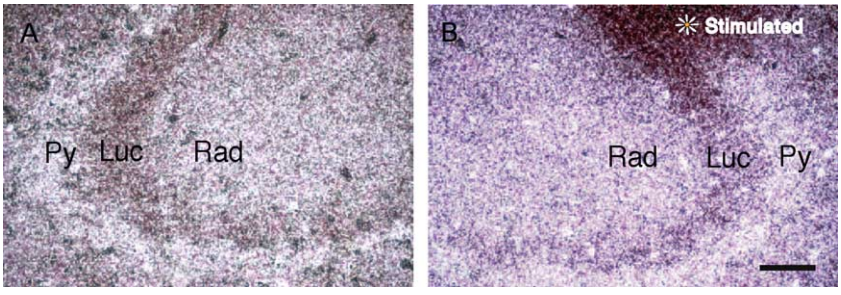

Fig. 4. Microscopic distribution of BC1 RNA after AD induction. Shown are the hippocampal CA3 regions of the left and right hemispheres. Labeling intensities are indicated by white silver grains. Asterisk indicates the area that was punctured by electrode implantation on the stimulated side. No redistribution of the RNA through neuronal processes was observed following AD induction. Luc, stratum lucidum; Py, stratum pyramidale; Rad, stratum radiatum. Scale bar, 200 μm.

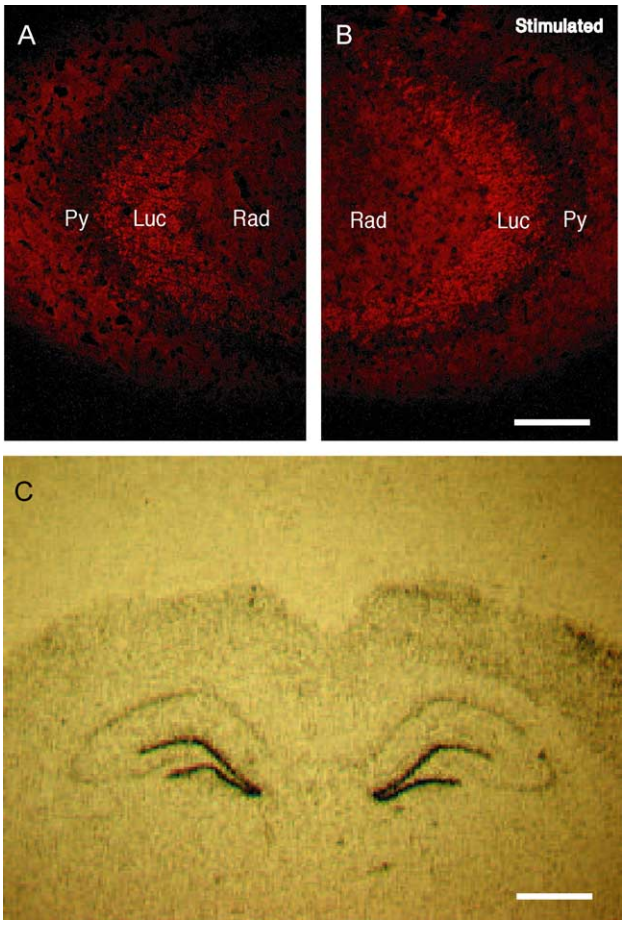

Fig. 5. Control experiments to ascertain that induction of ADs did not result in tissue damage. (A, B) Presynaptic specializations were visualized in the CA3 region of a seizured animal by immunocytochemistry. B shows fluorescence signal (red) for synaptophysin in the stimulated hippocampus, A in the control hemisphere. Mossy fiber terminals are abundant in both stimulated and unstimulated hippocampus. (C) Expression of Arc mRNA after kindling of the right hemisphere. Stimulation paradigms were similar to other kindling experiments used in this work but yielded in a more generalized and bilateral RNA induction in this case. This animal was used as a control showing that RNA expression was intact also in the immediate vicinity of the electrode puncture. Luc, stratum lucidum; Py, stratum pyramidale; Rad, stratum radiatum. Scale bar, 250 μm (A, B), 1000 μm (C).

downregulation of BC1. It is certainly conceivable that protein synthetic requirements during the LTP maintenance phase in hippocampus can be met without changes in BC1 expression levels. This notion is also fully compatible with the functional role of BC1 RNA as a translational repressor that directly modulates postsynaptic protein repertoires, presumably in an activity-dependent fashion, by targeting translation initiation [45]. Such interaction of BC1 RNA with the translational initiation machinery would be sufficient to effectuate translational modulation in LTP, with no need for further, i.e. indirect, modulation at the level of BC1 expression.

As LTP and epileptogenesis do not share the same cellular pathways [5], reduction of BC1 expression appears to be a consequence of kindling-induced downstream processes. RNA regulation in dendrites after epileptogenic stimuli, but not after high-frequency stimulation, has also been observed for BDNF mRNA. After such epileptogenic stimuli (including kindling), this mRNA is increasingly targeted to and translated in dendrites [42]. Seizure activity triggers a pathological pathway that is associated with significantly elevated levels of protein synthesis [1,10,17,23,44,46]. Such robust increases in translational activity may become manifest only if translational repression is alleviated. We thus submit the hypothesis, to be tested in future experiments, that downregulation of the translational repressor BC1 RNA is causally correlated with 'deregulated' protein synthesis in epileptogenic paradigms. We envision that the dendritic translational machinery operates such that a moderate offset of a translational modulator during seizures may result in a large change in protein synthetic output.

We do not know whether increased protein synthesis during epilepsy is in fact required to maintain prolonged epileptic manifestation. However, it has previously been shown that application of translational inhibitors forestalls expression of sustained epileptiform activity in vitro [25]. These data suggest that protein synthesis is an obligatory step in the pathway from induction to long-term consolidation of the epileptogenic process. It is therefore tempting to suggest that reduced BC1 expression, by way of downregulation of translational repression in dendrites, may be linked to the expression of prolonged epileptic activity.

It is of note in this context that the kindling-induced reduction of somatodendritic BC1 levels was most pronounced in the CA3 field, an area that has been suggested to be a generator of epileptiform activity [2,27,48,49]. We assume that CA3 neurons were stimulated antidromically in our experimental setup, resulting in the predominant decrease of BC1 RNA levels and initiation of the epileptic cascade in CA3. Schaffer collateral stimulation has previously been shown to activate CA3 pyramidal neurons antidromically through recurrent glutamatergic synapses, thereby producing epileptiform activity in this region [26]. This mechanism would thus account for the fact that BC1 levels decreased predominantly in CA3, as compared with other hippocampal areas.

Synthesis of brain-derived neurotrophic factor (BDNF) has previously been shown to be markedly increased after kindling-induced single seizures, and such increased BDNF synthesis was also most prominent, or even exclusive, in CA3 [10,42]. Upregulated BDNF synthesis has in turn been implicated in the generation and spread of seizures [3,10]. Increased protein synthesis has also been shown to be necessary for the persistent manifestation of epileptogenic mechanisms in vitro [25]. These observations may suggest a scenario in which reduced BC1 levels in CA3 facilitate increased somatodendritic translation of BDNF mRNA [42] (and possibly other neuronal mRNAs [17]). In this model,

'inappropriate' modulation of BC1 levels is an upstream event in the pathway that results in the deregulated translation of select somatodendritic mRNAs and thus triggers the cascade that ultimately results in the development of enduring epileptic discharges. While these questions remain conjectural at this time, they nevertheless introduce a testable hypothesis that will be open to future experimental investigation.

Acknowledgements

We thank Dr. Robert U. Muller for use of his recording equipment, Dr. Matt Stead for writing parts of the data acquisition software, Dr. Paul Worley for the Arc plasmid, and Dr. Jeremy Weedon for advice on statistical procedures. This work was supported in part by a German Academic Exchange Service (DAAD) Fellowship (V.N.), by a New York City Council Speaker's Fund for Biomedical Research Grant (I.A.M.), and by National Institutes of Health Grants NS34158 and 46769 (H.T.).

References

[1] W.C. Abraham, S.E. Mason, J. Demmer, J.M. Williams, C.L. Richardson, W.P. Tate, P.A. Lawlor, M. Dragunow, Correlations between immediate early gene induction and the persistence of long-term potentiation, Neuroscience 10 (1993) 717–727.
[2] R. Bianchi, R.K. Wong, Excitatory synaptic potentials dependent on metabotropic glutamate receptor activation in guinea-pig hippocampal pyramidal cells, J. Physiol. 487 (1995) 663–676.
[3] D.K. Binder, S.D. Croll, C.M. Gall, H.E. Scharfman, BDNF and epilepsy: too much of a good thing, Trends Neurosci. 24 (2001) 47–53.
[4] T.W. Bliss, T. Lømo, Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path, J. Physiol. 232 (1973) 331–356.
[5] D.P. Cain, F. Boon, E.L. Hargreaves, Evidence for different neurochemical contributions to long-term potentiation and to kindling and kindling-induced potentiation: role of NMDA and urethane-sensitive mechanisms, Exp. Neurol. 116 (1992) 330–338.
[6] M.E. Chicurel, D.M. Terrian, H. Potter, mRNA at the synapse: analysis of a synaptosomal preparation enriched in hippocampal dendritic spines, J. Neurosci. 13 (1993) 4054–4063.
[7] R.J. Davidson, K. Hugdahl, Brain Asymmetry, MIT Press, Cambridge, MA, 1994.
[8] H.P. Davis, L.R. Squire, Protein synthesis and memory: a review, Psychol. Bull. 96 (1984) 518–559.
[9] J. Eberwine, K. Miyashiro, J.E. Kacharmina, C. Job, Local translation of classes of mRNAs that are targeted to neuronal dendrites, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 7080–7085.
[10] M. Elmér, Z. Kokaia, M. Kokaia, J. Carnahan, H. Nawa, O. Lindvall, Dynamic changes of brain-derived neurotrophic factor protein levels in the rat forebrain after single and recurring kindling-induced seizures, Neuroscience 83 (1998) 351–362.
[11] A. Gardiol, C. Racca, A. Triller, Dendritic and postsynaptic protein synthetic machinery, J. Neurosci. 19 (1999) 168–179.
[12] S.D. Glick, D.A. Ross, Lateralization of function in the rat brain, Trends Neurosci. 4 (1981) 196–199.
[13] W.T. Greenough, A.Y. Klintsova, S.A. Irwin, R. Galvez, K.E. Bates, I.J. Weiler, Synaptic regulation of protein synthesis and the fragile X protein, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 7101–7106.
[14] O. Hobert, R.J. Johnston, S. Chang, Left–right asymmetry in the nervous system: the *Caenorhabditis elegans* model, Nat. Rev., Neurosci. 3 (2002) 629–640.
[15] C. Job, J. Eberwine, Localization and translation of mRNA in dendrites and axons, Nat. Rev., Neurosci. 2 (2001) 889–898.
[16] S. Kindler, E. Mohr, D. Richter, Quo vadis: extrasomatic targeting of neuronal mRNAs in mammals, Mol. Cell. Endocrinol. 128 (1997) 7–10.
[17] D. Koubi, A. Gharib, J. Gagnon, J.P. Andrieu, P. Bobillier, N. Sarda, Early and prolonged widespread increase in brain protein synthesis following a single electroconvulsive shock in free-moving rats, Brain Res. 821 (1999) 111–116.
[18] D. Kuhl, P. Skehel, Dendritic localization of mRNAs, Curr. Opin. Neurobiol. 8 (1998) 600–606.
[19] H.A. Lehr, D.A. Mankoff, D. Corwin, G. Santeusanio, A.M. Gown, Application of photoshop-based image analysis to quantification of hormone receptor expression in breast cancer, J. Histochem. Cytochem. 45 (1997) 1559–1565.
[20] H.A. Lehr, C.M. van der Loos, P. Teeling, A.M. Gown, Complete chromogen separation and analysis in double immunohistochemical stains using Photoshop-based image analysis, J. Histochem. Cytochem. 47 (1999) 119–126.
[21] Y. Lin, J. Brosius, H. Tiedge, Neuronal BC1 RNA: co-expression with growth-associated protein-43 messenger RNA, Neuroscience 103 (2001) 465–479.
[22] W. Link, U. Konietzko, G. Kauselmann, M. Krug, B. Schwanke, U. Frey, D. Kuhl, Somatodendritic expression of an immediate early gene is regulated by synaptic activity, Proc. Natl. Acad. Sci. 92 (1995) 5734–5738.
[23] G.L. Lyford, K. Yamagata, W.E. Kaufmann, C.A. Barnes, L.K. Sanders, N.G. Copeland, D.J. Gilbert, N.A. Jenkins, A.A. Lanahan, P.F. Worley, Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites, Neuron 14 (1995) 345–433.
[24] R.C. Malenka, R.A. Nicoll, Long-term potentiation—a decade of progress? Science 285 (1999) 1870–1874.
[25] L.R. Merlin, P.J. Bergold, R.K. Wong, Requirement of protein synthesis for group I mGluR-mediated induction of epileptiform discharges, J. Neurophysiol. 80 (1998) 989–993.
[26] R. Miles, R.K. Wong, Excitatory synaptic interactions between CA3 neurons in the guinea-pig hippocampus, J. Physiol. 373 (1986) 397–418.
[27] R. Miles, R.K. Wong, R.D. Traub RD, Synchronized afterdischarges in the hippocampus: contribution of local synaptic interactions, Neuroscience 12 (1984) 1179–1189.
[28] I.A. Muslimov, G. Banker, J. Brosius, H. Tiedge, Activity-dependent regulation of dendritic BC1 RNA in hippocampal neurons in culture, J. Cell. Biol. 141 (1998) 1601–1611.
[29] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, Academic press, San Diego, 1998.
[30] D. Richter, Cell Polarity and Subcellular RNA Localization, Springer, Berlin, 2001.
[31] I. Segal, I. Korkotian, D.D. Murphy, Dendritic spine formation and pruning: common cellular mechansisms? Trends Neurosci. 23 (2000) 53–57.
[32] O. Steward, W.B. Levy, Preferential localization of polyribosomes under the base of dendritic spines in granule cells of the dentate gyrus, J. Neurosci. 2 (1982) 284–291.
[33] O. Steward, E.M. Schuman, Protein synthesis at synaptic sites on dendrites, Annu. Rev. Neurosci. 24 (2001) 299–325.
[34] O. Steward, C.S. Wallace, G.L. Lyford, P.F. Worley, Synaptic activation causes the mRNA for the IEG Arc to localize selectively near activated postsynaptic sites on dendrites, Neuron 21 (1998) 741–751.

[35] T. Sutula, A. Pitkänen, Do Seizures Damage the Brain? Progress in Brain Research, Elsevier, Amsterdam, 2002.
[36] T. Sutula, X.X. He, J. Cavazos, G. Scott, Synaptic reorganization in the hippocampus induced by abnormal functional activity, Science 239 (1988) 1147–1150.
[37] H. Tiedge, The use of UV light as a cross-linking agent for cells and tissue sections in in situ hybridization, DNA Cell Biol. 10 (1991) 143–147.
[38] H. Tiedge, J. Brosius, Translational machinery in dendrites of hippocampal neurons in culture, J. Neurosci. 16 (1996) 7171–7181.
[39] H. Tiedge, R.T. Fremeau Jr., P.H. Weinstock, O. Arancio, J. Brosius, Dendritic location of neural BC1 RNA, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 2093–2097.
[40] H. Tiedge, F.E. Bloom, D. Richter, RNA, whither goest thou? Science 283 (1999) 186–187.
[41] A.W. Toga, P.M. Thompson, Mapping brain asymmetry, Nat. Rev., Neurosci. 4 (2003) 37–48.
[42] E. Tongiorgi, M. Armellin, P.G. Giulianini, G. Bregola, S. Zucchini, B. Paradiso, O. Steward, A. Cattaneo, M. Simonato, Brain-derived neurotrophic factor mRNA and protein are targeted to discrete dendrtitic laminas by events that trigger epileptogenesis, J. Neurosci. 24 (2004) 6842–6852.
[43] E.R. Torre, O. Steward, Protein synthesis within dendrites: glycosylation of newly synthesized proteins in dendrites of hippocampal neurons in culture, J. Neurosci. 16 (1996) 5967–5978.
[44] C.S. Wallace, G.L. Lyford, P.F. Worley, O. Steward, Differential intracellular sorting of immediate early gene mRNAs depends on signals in the mRNA sequence, J. Neurosci. 18 (1998) 26–35.
[45] H. Wang, A. Iacoangeli, S. Popp, I.A. Muslimov, H. Imataka, N. Sonnenberg, I.B. Lomakin, H. Tiedge, Dendritic BC1 RNA: functional role in regulation of translation initiation, J. Neurosci. 22 (2002) 10232–10241.
[46] C.J. Watkins, Q. Pei, N.R. Newberry, Differential effects of electroconvulsive shock on the glutamate receptor mRNAs for NR2A, NR2B and mGluR5b, Mol. Brain. Res. 61 (1998) 108–113.
[47] D.G. Wells, J.D. Richter, J.R. Fallon, Molecular mechanisms for activity-regulated protein synthesis in the synapto-dendritic compartment, Curr. Opin. Neurobiol. 10 (2000) 132–137.
[48] R.K. Wong, R.D. Traub, Synchronized burst discharge in disinhibited hippocampal slice: I. Initiation in CA2–CA3 region, J. Neurophysiol. 49 (1983) 442–458.
[49] R.K. Wong, R.D. Traub, R. Miles, Cellular basis of neuronal synchrony in epilepsy, Adv. Neurol. 44 (1986) 583–592.