Differential gene expression in acute and chronic seizure models in the rat brain

Summary of Ph.D. Thesis

Andrea Bagosi, M.D.

Department of Anatomy
Faculty of Medicine
University of Szeged

Doctoral School of Clinical Medicine

Supervisor: Prof. Mihály András, M.D., Ph.D., D.Sc.

2008

CONTENTS

1. INTRODUCTION

Acute and chronic application of a great variety of stimuli to the brain have been shown to lead to differential responses reflecting short-term and long-term neural plasticity. Thus, the cellular consequences of repeated seizure activity characteristic of epilepsy differ from that of a single, acute seizure episode. This fact is demonstrable by the expression of transcription factors (TFs), proteins that control gene expression in response to receptor activation.

Fos-family TFs exert their effects via heterodimeric complexes that constitute the activator protein -1 (AP-1) complex and activate or repress the transcription of many neurobiologically important target genes (Herdegen and Leah, 1998). While c-fos and full-length FosB are induced rapidly and transiently in specific brain areas after different types of stimuli, the truncated form of FosB gene, named ΔFosB, codes for a highly stable protein with a half-life of approximately 1 week and functions as a type of 'molecular switch' that gradually converts acute responses into relatively stable adaptations that underlie long-term neural and behavioural plasticity to repeated stimuli (Nestler et al., 1999).

The epileptogenic effects of the potassium channel blocker 4-aminopyridine (4-AP) has been extensively studied in acute experiments. The c-fos expression, that can be regarded as a tool to study neuronal activation and seizure spread (Morgan and Curran, 1991), has a characteristic pattern and is maximal at 1–3 h following systemic 4-AP administration in the hippocampus and neocortex (Miháy et al., 2001, 2005). However, repeatedly elicited 4-AP seizures are expected to cause qualitatively different cellular modifications as compared to a single seizure episode.

The importance of gamma aminobutyric acid (GABA) systems in the control of neural activity and the suppression of epileptiform discharge is well established. It is less clear whether long term changes in the function of inhibitory systems play a role in the development of epilepsy. Though, literature data show, that GABA systems can be modified by seizure activity (Morimoto et al., 2004). Accordingly, a large number of studies focused on a specific group of fast spiking interneurons, which use mainly GABA and express the Ca^{++}- binding protein parvalbumin (PV). Postseizure modification of the PV content could be both indicative of, and a reason for the alterations in their physiological function.

2. AIMS

The present study comprises investigations directed towards revealing some features of the mechanisms and consequences of seizure activity in acute and chronic conditions.
The K^+ channel blocker 4-AP was used in order to initiate brief generalized seizures in rats. The differential response after varying number of convulsive episodes was investigated at behavioural and molecular level.
The specific aims of the experiments were as follows:

- Investigation of the relevance of transcriptional regulation in the mechanism of seizure induction and seizure spread by detection of time-dependent c-fos expression.
- Characterization of a chronic seizure model in terms of transcriptional regulation after repeatedly induced seizures that imply long-term alterations in neural plasticity.
- Investigation of the effects of repeatedly induced seizures related to the inhibitory neuronal circuit by examination of changes of a subset of parvalbumin-containing neurons.

3. MATERIALS AND METHODS

3.1. Experimental animals and their treatments

Adult rats were housed in groups under standard conditions (temperature 23 °C, lights on from 06:00 to 18:00 h) and with free access to water and food. The animals were injected intraperitoneally with 0.067% (w/v) solution of the convulsant 4-AP (Sigma; St. Louis, MO, USA) dissolved in physiological saline (0.9% NaCl in distilled water). The applied dose was 5 mg/kg body weight, as this concentration proved to be convulsive in previous studies (Mihály et al., 2001, 2005). Control animals received appropriate volumes of physiological saline. All procedures of the animal experiments were licensed by the Committee for Animals in Experimental Research, University of Szeged, according to the directive of the European Council (86/609/EEC) and to the Hungarian Animal Act.
The investigations were conducted with the following experimental groups:

- 15 Wistar rats were used for the immunodetection of c-fos 30 min, 1 h, 3 h, 5 h, and 8 h after a single injection of 4-AP.
- Male Wistar rats (150–180 g) were anaesthetized with Calypsol (100 mg/kg) plus atropine (0.01 mg/kg) given intraperitoneally (i.p.). The head of the animal was fixed in a stereotaxic frame, and following a vertical skin incision, the soft tissues and the temporalis muscle were cut in order to expose the temporal squama on the left side. The bone was cut with a dental drill, removed and the rhinal sulcus was identified. The cortical area inferior to the rhinal sulcus was electrocoagulated and suctioned (lateral entorhinal cortex ablation, LECA). The lesion of the LEC extended to all cortical layers and also the subcortical white matter, including the temporoammonic pathway. At the end of the procedure, the bone defect was covered with the temporalis muscle and skin was closed with clamps. In sham-operated control (SOC) animals the same procedure was performed except for coagulation – the meninges and the brain were not injured. 40 days following the surgical destruction of the lateral entorhinal cortex (LECA) one group of Wistar rats (3 animals) received a single injection of 4-AP. Three SOC rats received the same treatment. These rats were sacrificed 3 h after the treatment and their brains were processed for c-fos immunohistochemistry.
- Three groups of Wistar rats received daily 4-AP injections for 1, 4, 8 and 12 days and were sacrificed 24 h after the last injection to be investigated by FosB and PV immunodetection.
- Three groups of Sprague-Dawley rats were treated by daily injections of 4-AP for 12 days and sacrificed 3 h after the last injection to be investigated by PV immunohistochemistry, Western blotting and in situ hybrization, respectively.

For IHC, animals were deeply anesthetized with diethylether and perfused transcardially with 200 ml of 0.1 M phosphate buffered saline (PBS, pH 7.4), followed by 300 ml of 4% phosphate buffered paraformaldehyde (pH 7.4). The brains were removed and postfixed in 4% phosphate buffered paraformaldehyde (pH 7.4) for 1h. After postfixation, the brains were cryoprotected overnight in 30% sucrose in 0.1 M phosphate buffer (pH 7.4).

3.2. Immunohistochemistry

3.2.1. FosB immunolabeling
Sections were incubated in 0.2% Triton X-100/PBS for 20 min, followed by washing in three changes of PBS. Then 20% normal pig serum (NPS; Sigma) was applied for 1 h to block nonspecific labeling. Sections from rats injected for 1, 4 and 8 days were used for immunohistochemical analysis of FosB protein, while sections from the rats treated for 12 days were used for simple FosB and double FosB+parvalbumin immunolabeling. Since antibodies selective for ΔFosB are not available, we used a rabbit polyclonal antiserum raised against an internal region of FosB (sc-48; Santa Cruz Biotechnology), that has been shown to recognize both FosB and ΔFosB. The antibody was used diluted 1:4000 in 0.05M PBS containing 10% NPS. The sections were incubated in the primary antibody overnight at room temperature.
The biotinylated secondary antibody (goat antirabbit IgG; Vector Laboratories, CA) was diluted 1:40 in PBS, and sections were incubated for 1 h. After additional washing, sections were transferred to streptavidin-peroxidase (1:1000; Jackson ImmunoResearch), and incubated for 1 h. Then the sections were washed and processed using 0.05% 3,3-diaminobenzidine tetrahydrochloride (DAB; Sigma) containing 0.3% nickel sulphate and 0.01% hydrogen peroxide. After 20 min of incubation, the chromogen yielded a black reaction product.
The sections were dehydrated with an ascending series of ethanol (40%, 70% and 100%), cleared in xylene and mounted with Entellan (Fluka).

3.2.2. C-fos immunolabeling
Frozen, coronal plane sections were cut at 20 μm thickness with cryostat. Polyclonal c-fos antibody (raised in rabbit; Santa Cruz Biotechnology, CA, USA) and the peroxidase-antiperoxidase (PAP) method was used for immunohistochemistry. The sections were incubated in 20% normal pig serum, next in primary c-fos antibody (1:1000 in 20% normal pig serum), and then in donkey antirabbit IgG (1:40; Jackson Immuno-Research, PA, USA). The secondary antibody was detected by PAP complex diluted to 1:1000 (Jackson). The peroxidase reaction was localised with nickel chloride-containing diaminobenzidine tetrahydrochloride (Sigma), yielding a black reaction product.

3.2.3. PV immunolabeling
Coronal, frozen brain sections (24 μm thin) were reacted with monoclonal mouse anti-PV antibody (Sigma PA-235, dilution 1:15,000). For the detection of the primary antibody, biotinylated goat anti-mouse IgG (Vector) and streptavidin-HRP (Vector) were employed. The immunoperoxidase reaction was developed by using 3'3'-diaminobenzidine tetrahydrochloride (DAB; Sigma) as a chromogen. The sections were mounted on microscope slides, dehydrated and coverslipped with Entellan (Fluka).

3.2.4. PV and FosB double immunolabeling
Double immunolabeling was performed using primary antibody cocktails: mouse anti-PV (Sigma), diluted 1:100,000 and rabbit anti-FosB (Santa Cruz Biotechnology), diluted 1:500. The sections were incubated in the primary antibody cocktail overnight at room temperature,

then transferred to the secondary antibody cocktail: biotinylated anti-mouse IgG (Sigma), diluted 1:600, and unlabeled goat anti-rabbit IgG (Vector Laboratories) diluted 1:40. The sections were incubated for 1 h in the secondary antibody cocktail and then transferred to streptavidin-peroxidase (1:1000; Jackson ImmunoResearch). The streptavidin-peroxidase was developed by using 0.05% DAB (Sigma) with 0.01% hydrogen peroxidase. Following a wash in TBS, the sections were transferred to peroxidase-anti-peroxidase (PAP; Jackson ImmunoResearch), diluted 1:1000, and incubated for 1 h. The PAP was developed using 0.05% DAB (Sigma) with 0.3% nickel sulphate and 0.01% hydrogen peroxide, as described previously.

3.3. Evaluation of immunolabeling and statistical analysis

The quantitative analysis of each immunolabeling was performed on five histological sections per animal. The immunoreactive cells were counted using a Nikon Eclipse 600 microscope equipped with a Spot RT Slider digital camera (1600x1200 dpi in 8 bits), and the Image Pro Plus 4 morphometry software (Media Cybernetics, Silver Spring, MD).

Areas of interest were selected on the basis of the same stereotaxic coordinates (Paxinos and Watson, 1998): from regions CA1, CA2, CA3 of the Ammon's horn, the granule cell layer of the dentate gyrus, the hilum of the dentate gyrus, and from the neocortex.

In the neocortex, the area of interest was the rectangular image-capturing field of the camera which included all neocortical layers (I–VI) from the pia mater to the subcortical white matter. Frozen sections stained with cresyl violet were used as reference for the thickness of the neocortical layers. The counting was done by using a 10x objective. Following background subtraction, the threshold was adjusted so that all positively labeled cells could be recognized. Cell counts were then normalized to 1 mm^2.

In the hippocampus, the area of interest was the rectangular image-capturing field of the camera. The hilum of the dentate gyrus was outlined manually, according to Amaral (1978), and used as area of interest. The counting was done by using a 40x objective. Following background subtraction, the threshold was adjusted so that all positively labeled cells could be recognized. Cell counts were then normalized to 1 mm^2.

In the mammillary bodies, the areas of interest (AOI) for cell counts were selected from the medial mammillary nucleus. Following background subtraction, the threshold was adjusted so that the counting program could equally recognize pale- and deep-stained cells. Cell counts were done using a 40x objective. The AOI was the rectangular image-capturing field of the camera (222 μm x 296 μm).

The statistical analysis was performed with the SPSS 9.0 computer program.

Differences of FosB, parvalbumin and double immunolabeling between control and 4-AP treated groups were analysed by a paired t-test in case of each time-course experiment. A significance criterion of $p<0.05$ was used.

Differences in the number of c-fos IR cells in the control and convulsing animals were analysed with one-way analysis of variance (ANOVA), followed by the Bonferroni post hoc test.

3.4. Western blotting

Rats in diethyl-ether anesthesia were decapitated 3 h after the last 4-AP or saline injection. The heads were immersed in liquid nitrogen, and the brains were dissected on ice. The mammillary area was cut out, chilled in liquid nitrogen and stored at -80 °C until use. The number of animals was 16: 8 treated with 4-AP for 12 days, and 8 controls (injected daily with saline for 12 days).

The tissues were homogenized in 50 mM Tris–HCl (pH 7.5) containing 2 mM phenylmethanesulfonyl fluoride (PMSF), 150 mM NaCl, 0.1% Nonidet-P-40, 2 μg/ml leupeptin, 1 μg/ml pepstatin and 2mM EDTA.

Thirty-five micrograms protein was separated on a 10% SDS-polyacrylamide gel and transferred onto nitrocellulose membrane (Schleicher and Schuell, Dassel, Germany). Membranes were blocked for 1 h in 5% nonfat dry milk in Tris–HCl (pH 7.5) containing 0.9% NaCl (TBS) and 0.05% Tween 20, and incubated overnight with mouse anti-PV antibody (Sigma PA-235; dilution 1:3000). After 5x5 min washes in TBS-Tween 20 on room temperature, the membranes were incubated for 1 h with biotinylated goat anti-mouse IgG (Vector Laboratories; Burlingame, CA, USA; dilution 1:1000), and washed three times as before.

The enhanced chemiluminescence method (ECL Plus Western blotting detection reagents; Amersham Biosciences, Little Chalfont, Buckinghamshire, UK) was then used to reveal immunoreactive bands, according to the manufacturer's protocol. The other chemicals were from Sigma. The intensity of the bands was quantified by densitometry and expressed as pixel volume (pixel intensity x area) using the Gel-Doc system (Biorad). For quantitative comparisons, each value was calculated based on at least seven independent determinations.

An analysis of variance (ANOVA) followed by post hoc analysis (Tukey's multiple comparison) was used to evaluate the significance.

3.5. cRNA probes

The only PV gene in the rat contains five exons and codes for two mRNA species by means of alternative polyadenylation. A template for a gene-specific cRNA probe was created by isolating the 133 bp long third exon of the rat PV gene by genomic polymerase chain reaction (PCR) using the primers 5' CCCCCCAACAGCTGCAGACTCC- 3' and 5'-TTTACCCCAGCTCATCCTCCTCAATG- 3'. The PCR product was inserted into the pcDNA3 vector (Invitrogene, Carlsbad, CA) between BamHI and EcoRI restriction sites and cloned using standard methods. Identity of the insert was confirmed by sequencing (AB 373 DNA Sequencer; PE Applied Biosystems, Foster City, CA). For the production of antisense and sense PV cRNA probes, the vector construct was linearized either with BamHI or EcoRI (Promega, Madison, WI, USA), respectively. Then, the linearized plasmid was transcribed in vitro by using Riboprobe System-T7 and Riboprobe System-SP6 (Promega) according to the manufacturer's instructions. For radiolabeling, [^{35}S]UTPα´ S (ICN Biomedicals; Costa Mesa, CA) was incorporated during the syntheses of the cRNA probes. The antisense and sense PV [^{35}S]cRNA probes were purified by size exclusion chromatography using ProbeQuant G-50 Sephadex microcolumns (Pharmacia Biotech; Uppsala, Sweden) and their specific activities were determined to be 3.1x107–6.4x10^{7} cpm/ pmol by liquid scintillation counting.

3.6. In situ hybridization

Coronal rat brain sections were fixed for 5 min in 2x SSC containing 4% formaldehyde at room temperature (RT) and washed twice in 2xSSC for 1 min at RT. Then, the sections were rinsed in 0.1 M triethanolamine containing 0.25% acetic anhydride and 0.9% NaCl, pH 8.0 for 5 min at RT, dehydrated, and air-dried. Next, the sections were hybridized in 50 μl hybridization solution (50% formamide, 6x SSPE, 5x Denhardt's reagent, 10% dextran sulfate, 50 mM DTT, 100 μg/ml salmon sperm DNA, and 50 μg/ml yeast tRNA) containing a [35S]cRNA probe. The concentrations of the PV antisense and sense hybridization solutions ranged from 247 to 288 fmol/ml. Hybridization was performed under Parafilm coverslips in a humid chamber at 55 °C for 24±0.5 h. The sections were rinsed once in 2x SSC/50% formamide at RT for 5 min, twice in 2x SSC/50% formamide at 50 °C for 10 min, and then in 2x SSC at RT for 5 min. The sections were next incubated in 1x TE containing 0.5 M NaCl and $1.32x10^{-3}$ Kunitz U (~25 mg protein)/ml RNase A at 37 °C for 30 min, and rinsed in 2x SSC/50% formamide at 50 °C for 10 min and in 2x SSC at 50 °C for 10 min. Sections were dehydrated, air-dried and processed for phosphorimaging.

4. RESULTS

4.1. 4-AP-precipitated seizure behaviour

1.1. Acute seizure model

Following the injection of the convulsive dose of 4-AP, the animals displayed behavioural seizures which lasted for 60–80 min. The characteristic symptoms occured within approximately 10 min of injection and developed gradually, as described previously (Mihály et al., 1990). Initially, animals exhibited increased exploratory activity which was followed by tremors of the vibrissal muscles, shivering, forelimb clonus with increasing frequency conducting to the development of generalized tonic-clonic seizure with loss of postural control. After a postictal period of 10–20 min, some of the animals displayed generalized seizure again.

The seizure symptoms were registered according to a scale established by Racine: mouth and facial movements (stage 1), head nodding and muscle tremor (stage 2), forelimb clonus (stage 3), rearing (stage 4) and generalized tonic-clonic seizure (GTCS, stage 5), with complete loss of postural control. The average latency of the first GTCS was 30.3 min (±1.4 min).

4.1.2. Chronic seizure model

During the 12 days' treatment the above-mentioned seizure symptoms were detected every day, although the severity and the stages showed slight variations.

The analysis of the daily elicited seizure symptoms according to the Racine scale proved that the convulsion pattern changing slightly towards the end of the experiment: decreases of the seizure intensity were observed, i.e. after 4-AP administration the animals developed a single full motor seizure (stage 5) instead of two seizures, or displayed less severe, stage 4, symptoms.

4.1.3. Lateral entorhinal cortex ablation

In the LECA group (n = 7) only three animals responded to 4-AP injection with a single seizure and the remaining four animals produced no seizure at all. In the SOC group the first seizure lasted for 55.5 ± 4.2 s, and developed during the first 30 min following the 4-AP injection with an average latency of 20.8 ± 6.4 min. These events were followed by a second seizure within the first hour following the 4-AP treatment. The mean delay between the two seizures was 16.2 ± 4.9 min. The second seizure was longer, lasted for 73.4 ± 13.9 s. In the LECA group the latency of first seizure increased to 31.8 ± 4.3 min, and only one rat presented a second epileptic event.

4.2. Immunohistochemical results

2.1. Seizure induced c-fos

The c-fos IR cell nuclei were present in every layer of the somatosensory neocortex following 4-AP administration. The increase of the number of c-fosIR cell nuclei was significant already at 30 min in layers II, III, IV, V and VI. The highest values were detected at 1 h in each layer. The increase was still significant at 3 h following 4-AP injection. Cell counts decreased to control levels at 5 and 8 h following 4-AP administration. The highest elevation in the number of c-fosIR cells at 30 min was seen in layer IV: other layers

contained less c-fosIR cells. At 1 h, every layer (except layer I) stained strongly; only layer V stood out with a lower amount of c-fos IR cells. At 3 h, the number of c-fos IR cells decreased to control levels, and remained so at 8 h. The values in convulsing animals displayed highly significant differences ($P \leq 0.01$) compared to the controls. In controls, scattered c-fosIR cells were seen in layer IV.

4.2.2. Seizure induced c-fos after lateral entorhinal cortex

Convulsions induced by 4-AP 40 days after the surgery caused the expression of c-fos protein in every part of the hippocampus and the subiculum. The overall pattern of c-fosIR in SOC and LECA animals 3 h after the 4-AP injection was similar to the previous findings obtained with intact animals (Mihály et al. 2001, 2005; Szakács et al. 2003): strong c-fosIR in the dentate granule cell layer, medium c-fosIR in the stratum pyramidale of CA1–3, and scattered c-fosIR nuclei in the hilum of the dentate gyrus, and in the infra- and suprapyramidal layers of CA1–3. Quantitative analysis of immunostained cell nuclei revealed that the convulsions in LECA animals induced significantly less c-fos immunopositive cells in the pyramidal cell layer of the Ammon's horn, and in the granule cell layer and in the hilum of the dentate gyrus, compared with the SOC group. Because the number of the haematoxylin–eosin-stained cell bodies did not change in LECA brains, the significant reduction in the number of c-fos immunopositive cells indicates the decrease of neuronal activity, and not the loss of neurons in the investigated hippocampal regions.

4.2.3. FosB

The FosB immunohistochemistry revealed a very weak labeling in the Ammon's horn of both the control and 4-AP treated rats in every time-course group. A very small number of weakly labeled cell nuclei were found in this hippocampal region and it was therefore not evaluated by statistical analysis. The dentate gyrus was characterized by more striking FosB immunolabeling. There was a significant increase in FosB-immunopositivity in the 4-AP treated animals compared with controls after 4, 8 and 12 days of treatment. Increase in immunopositivity after a single 4-AP treatment did not reach statisitical significance in comparison to the corresponding control level. Neocortical FosB immunoreactivity increased significantly after 4-AP elicited convulsions at every time point compared to controls. Sections stained with cresyl violet showed no gross cell loss.

4.2.4. Parvalbumin in the medial mammillary nucleus

The PV immunoreactivity in the MM was similar to that described (Celio, 1990). Medium-sized neurons with few dendritic processes contained PV-like staining. Regional densities of immunolabeled cell bodies were determined for each experimental group. Values from 4-AP treated groups were compared to those from corresponding control groups (independent samples Student's ttest, $p<0.05$). The number of PV-immunoreactive neurons did not change in 4-AP-treated animals, compared to the controls, although the intensity of the staining did show a visible decrease. No pathological cell forms were detected in the 12 days 4-AP samples.

4.2.5. PV and FosB in the cortex

In the brain sections from animals treated for 12 days from both 4-AP injected and control animals, parvalbumin-positive neurons were distributed in every layer of the frontal cortex. In the hippocampus, parvalbumin-positive neurons were present mainly in the pyramidal layer of the Ammon's horn and in the hilar region, while the granule cell layer comprised

scattered parvalbumin-immunopositive cells. No significant changes were revealed in the overall number of parvalbumin-immunopositive cells in any of the investigated brain regions after 4-AP treatment. There was, however, a significant increase in double-labeled neurons after 4-AP treatment in comparison to control levels in the neocortex, hilum of the dentate gyrus and region CA.

4.3. Western blotting of the mammillary area

PV immunoreactive bands were sharp. The samples from control and 4-AP-treated rats displayed a visible density difference. Densitometry of the membranes revealed a 20.1±5.5% decrease of PV signal intensity in rat MM treated with 4-AP for 12 days, compared to the controls (data from eight samples of convulsing brains and seven samples from controls). The difference between control and 4-AP-treated values was significant ($p<0.05$).

4.4. Quantification of regional PV mRNA contents in the rat brain following acute and chronic 4-AP treatment

Regional quantities of PV mRNAs in the brains of rats subjected to acute or chronic 4-AP treatment were assessed by ISH employing PV [^{35}S]cRNA probes. Autoradiograms of sections hybridized with antisense PV [^{35}S]cRNA probes displayed a characteristic labeling pattern specific for PV mRNAs. Images of sections hybridized with sense PV [^{35}S]cRNAs had very low level of labeling and did not exhibit brain structure specific signals. Normal distribution of experimental data was confirmed for each data set except that of group 12 days 4-AP medial mammillary nucleus. In this group, several data points with very similar values compromised the normal distribution of data (skewness) even when the number of measurements was doubled by quantifying contralateral as well. The subnuclei of the medial mammillary nucleus (MM) could not be separated on the ISH images. The PV mRNA content of the forebrain regions of the 4- AP treated rats were compared to that of the corresponding control rats by using two-tailed Student's t-test ($p<0.01$). None of the investigated neocortical and allocortical areas displayed changes in PV mRNA levels in our experiments. Daily convulsions induced by 4-AP through 12 days did not have any impact on the transcription of the PV gene in the cerebral cortex. In the diencephalon, PV mRNA levels displayed a slight increase in group 3 h in the thalamic reticular nucleus (nRt) (116%), while in group 12 days significant decreases (77%, $n=20$, $p<0.001$) were observed in the MM of the hypothalamus. Data sets were also compared with ANOVA followed by post hoc Bonferroni test ($p<0.05$). When regional PV mRNA abundances in 4-AP groups were compared to those in the corresponding controls groups, ANOVA and Bonferroni tests indicated that changes of the nRt of the thalamus are not significant. Data sets of MM were further analysed with the nonparametric Median test, which reinforced that the decrease of PV mRNA level in group 12 days 4-AP in the MM was highly significant, when compared to 12 days control value, confirming the results for this brain region obtained with the above parametric tests. The decreased level of PV mRNA in the MM was visible on the in situ hybridization images, too.

5. CONCLUSIONS

- The acute 4-AP seizure model provided a tool for the study of the development of generalized seizure activity in the rat brain. Time-dependent expression of neocortical c-fos correlated with electrophysiological analysis supported the importance of the thalamo-corticothalamic circuit in the activation and maintanace of the neocortical seizure, and suggested a proconvulsant role of the transcriptional activity of c-fos. The ablation of the entorhinal cortex is followed by the suppression of seizure activity and c-fos expression, suggesting the importance of the limbic structures for seizure generation after systemically applied K^+ channel blocker.
- Daily injections of the convusing dose of 4-AP demonstrate changes in excitability and long-lasting expression of FosB. The symptoms of the first 4-AP treatment were usually characterized by the development of generalized tonic-clonic seizures. Then, after an interval of tolerance to seizure occurence of sensitization was observed followed by a further desensitization period towards the 12th day. This is in accordance with literature data concerning the differential regulation of gene expression by FosB and its isoforms depending on the duration and level of expression. Therefore, the expression of FosB appeared to have protective effect against seizure and cell damage in an initial period of repeated brief generalized seizure sequence, and the cortical expression involved mainly the neocortex and dentate gyrus and proved to be protective also for the hippocampus proper where no cell death was observed.
- A large number of parvalbumin containing cells expressed FosB in the neocortex and regions of the hippocampal formation, suggesting plasticity changes of inhibitory neurons after chronic seizure activity. In our experiments, the only brain region presenting alteration of parvalbumin expression was the medial mammillary nucleus, where the preserved number of PV positive cells had significantly decreased PV content.

6. CITED REFERENCES

Celio MR. Calbindin D-28 k and parvalbumin in the rat nervous system. Neuroscience 1990;35:375–475.

Herdegen T, Leah JD. Inducible and constitutive transcription factors in the mammalian nervous system: control of gene expression by Jun, Fos and Krox, and CREB/ATF proteins. Brain Res Brain Res Rev 1998;28:370–490.

Nestler EJ, Kelz MB, Chen J. DeltaFosB: a molecular mediator of long-term neural and behavioral plasticity. Brain Res 1999;835:10–7.

Mihály A, Bencsik K, Solymosi T. Naltrexone potentiates 4-aminopyridine seizures in the rat. J Neural Transm (GenSect) 1990;79:59–67.

Mihály A, Szakács R, Bohata C, Dobó E, Krisztin-Péva B. Time-dependent distribution and neuronal localization of c-fos protein in the rat hippocampus following 4-aminopyridine seizures. Epilepsy Res 2001;44:97–108.

Mihály A, Borbély S, Világi I, Détári L, Weiczner R, Zádor Z, et al. Neocortical c-fos mRNA transcription in repeated, brief, acute seizures: is c-fos a coincidence detector? Int J Mol Med 2005;15:481–6.

Morimoto K, Fahnestock M, Racine RJ. Kindling and status epilepticus models of epilepsy: rewiring the brain. Progress in Neurobiology 2004;73:1–60.

Racine RJ. Modification of seizure activity by electrical stimulation: II. Motor seizure. Electroencephalogr Clin Neurophysiol 1972;32:281–94.

7. LIST OF PUBLICATIONS

Journal of Neurochemistry, 2005, 95, 111-124
Lateral entorhinal cortex lesions rearrange afferents, glutamate receptors, increase seizure latency and supress seizure-induced c-fos expression in the hippocampus of adult rat
Zsolt Kopniczky, Endre Dobó, Sándor Borbély, Ildikó Világi, László Détári, Beáta Krisztin-Péva, Andrea Bagosi, Elek Molnár, András Mihály

Molecular Brain Research 131 (2004) 110-118
Repeated 4-aminopyridine seizures reduce parvalbumin content in the medial mammillary nucleus of the rat brain
Sándor Vizi, Andrea Bagosi, Beáta Krisztin-Péva, Károly Gulya, András Mihály

Int J Mol Med. 2005 Mar; 15(3):481-6
Neocortical c-fos mRNA transcription in repeated, brief , acute seizures:
Is c-fos a coincidence detector?
Mihály A, Borbély S, Világi I, Détári L, Weiczner R, Zádor Z, Krisztin-Péva B, Bagosi A, Kopniczky Z, Zádor E
Acta Biologica Szegediensis, 51 (Suppl. 1) 2007
Repeated brief seizures induce long-lasting rearrangement of ionotropic glutamate receptor subunits in the rat hippocampus
A Mihály, E Dobó, E Molnár, A Bagosi, M Bakos, B Szűcs, A Vincze, N Károly

Acta Histochemica 110 (2008) 418-426
Late expression of FosB transcription factor in 4-aminopyridine-induced seizures in the rat cerebral cortex
Andrea Bagosi, Moónika Bakos, Beáta Krisztin-Péva, András Mihály

8. ACKNOWLEDGEMENT

I express my gratitude to those who contributed to the present work: Professor András Mihály, Sándor Vizi, Mónika Bakos, Beáta Krisztin-Péva, Zsolt Kopniczky, Márta Dukai, Katalin Lakatos.