Elevated DNA-binding activity of AP-1,1 a dimeric transcription factor composed of Fos and Jun proteins, as well as increased expression of its components have been observed repeatedly in a variety of neuronal activation phenomena (for reviews, see Refs. 1–4). The use of c-Fos labeling of neurons has repeatedly in a variety of neuronal activation phenomena (for reviews, see Refs. 1–4). The use of c-Fos labeling of neurons has proven to be especially useful in delineating functional pathways in the brain (for review, see Refs. 2 and 3). Furthermore, it became clear that AP-1 could serve as a prototypical excitatory-response transcription factor (1–3).

However, despite the accumulated knowledge about expression patterns of c-Fos, AP-1, etc., in the brain in response to various stimuli, very little is known about physiological (and possibly pathological) function(s) of this transcription factor. An obvious obstacle in elucidating this function is the lack of reliable interventional strategies to affect AP-1 within neuronal tissue in vivo. An alternative approach is the identification of AP-1-driven genes. However, only very few such targets in the brain have so far been identified, and moreover, none of them apparently unequivocally (see Ref. 4, for discussion). This situation stems from the fact that this task is very complex and requires a multitude of approaches, correlative in the brain and functional in the culture dish.

Recently, several pieces of information have pointed to the gene timp-1, encoding the tissue inhibitor of metalloproteinases (TIMP-1) as a possible AP-1 target in the central nervous system. Increased expression of this gene has been reported in rat brain following treatment with kainate, a glutamate receptor agonist as well as pro-convulsive and neurotoxic drug that is also well known to activate AP-1 (1, 5–7). At the same time, Bugno et al. (8), Logan et al. (9) as well as Botelho et al. (10) identified three major promoter elements located in the vicinity of the transcription initiation site, including the one interacting with AP-1 transcription factor, to be of critical importance in control of inducible timp-1 expression in non-neuronal cells in vitro.

The present studies were designed to test the hypothesis that AP-1 may control timp-1 gene expression in rodent hippocampus in response to enhanced neuronal excitation. Such a hypothesis appears to be very attractive, since if proven, could reveal an important biological function of AP-1.

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**EXPERIMENTAL PROCEDURES**

**Animals and Their Treatment**

Rats—Wistar male rats weighing 250–300 g from the Nencki Institute animal facility were used for the studies. In the experiments with animals, the rules established by the Ethical Committee on Animals Research of the Nencki Institute and based on national laws were strictly followed.

Intraperitoneal administration of either kainate (10 mg/kg) or pentylenetetrazole (PTZ, 50 mg/kg) was performed as described previously (11–13). Briefly, to exclude effects of the injection itself, the animals were handled and injected with physiological saline daily for 3–4 days before the experimental treatment. The rats were then given either sodium kainate (Sigma) or PTZ (BB) by intraperitoneal injection and observed for up to 90 min to confirm the occurrence or absence of convulsions. Only the animals displaying clear seizures (that occur a few minutes after PTZ treatment and are initiated at 30–60 min following kainate administration) were used for the experiments (that is, typically in our hands, more than 80% of rats). For collection of the material, rats were decapitated at different times after the drug administration (4–6 animals for each time point), the brains were removed and processed as described in details below.

Transgenic Mice—TIMP-LacZ transgenic mice were obtained from Dr. B. R. Williams (14). The animals carry the fragment of timp-1 gene extending from −1373 to +727 (see Fig. 1) fused to LacZ gene encoding...
reporter enzyme β-galactosidase. Fidelity of the gene construct expression has been documented during development, when patterns of β-galactosidase activity were found to mimic expression of the endogenous timp-1 gene (14).

Northern Blot RNA Analysis

Isolated brains were rapidly dissected on ice and the hippocampi were snap-frozen on dry ice. RNA was isolated from frozen tissue according to the procedure of Chomczynski and Sacchi (15) and electrophoresed through a 1% agarose gel as described (12). After blotting onto nylon membranes (Hybond N, Amersham) the filters were prehybridized for 2 h and then hybridized overnight with random primer-labeled probes in Church buffer (12, 16).

In Situ Hybridization

For in situ mRNA analysis the procedure described by Konopka et al. (17) was followed. Isolated brains were immediately frozen on dry ice. Twenty micrometer cryostat sections were fixed in 4% cold paraformaldehyde in PBS, dehydrated, and prehybridized for 2 h at 37 °C in buffer containing: 50% formamide, 2 × SSC (0.3 M sodium chloride, 0.03 M sodium citrate, pH 7.0), 1 × Denhardt’s solution (0.02% Ficoll 400, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), 200 μg/ml single-stranded DNA, and 20 μM dithiorthreitol. Next, the sections were hybridized overnight in the above solution containing additionally 10% dextran sulfate and 32P-labeled cDNA (random primers) probe at 37 °C. The probe was kindly provided by Dr. Y. Citri (5). The sections were washed for 15 min and then for 60 min in 50% formamide, 2 × SSC at room temperature, and then exposed against β-Max Hyperfilm (Amersham). The autoradiograms were analyzed with an aid of PC-based computer program Provision.

c-Fos Immunocytochemistry

The expression of c-Fos protein was assessed essentially as described before (18). Following the appropriate treatments, as indicated under “Results,” the rats were anesthetized with chloral hydrate and perfused with saline followed by 2% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The brains were removed and stored in the same fixative for 24 h at 4°C and then in 30% sucrose with 0.02% sodium azide at 4°C until needed. The brains were slowly and gradually frozen in a heptane/dry ice bath and sectioned at 20 μm on a cryostat. The sections were washed three times in PBS, pH 7.4, incubated 10 min in 0.3% H2O2 in PBS, washed twice in PBS, then incubated with a polyclonal antibody (anti-c-Fos, 1:1000, Santa Cruz number sc-52) for 48 h at 4°C in PBS with azide (0.01%) and normal goat serum (3%). After that the sections were washed three times in PBS containing Triton X-100 (0.3%, Sigma), incubated with goat anti-rabbit biotinylated secondary antibody (1:1000, Vector) in PBS/Triton and normal goat serum (3%) for 2 h, washed three times in PBS/Triton, incubated with avidin-biotin complex (1:1000, Vector, in PBS/Triton) for 1 h and washed three times in PBS. The immunostaining reaction was developed using the glucose oxidase-3,3'-diaminobenzidine tetra-chloride-nickel method. The sections were incubated in PBS containing 3,3'-diaminobenzidine tetrachloride (0.05%), glucose (0.2%), ammonium chloride (0.04%), ammonium nickel sulfate (0.1%) (all from Sigma) for 5 min, then 10% (v/v) glucose oxidase (Sigma, 10 units/ml in H2O) was added. The staining reaction was stopped by two to three washes with PBS. The sections were mounted on gelatin-covered slides, air-dried, dehydrated in ethanol solutions and xylene, and embedded in Entellan (Merck).

Electrophoretic Mobility Shift Assay (EMSA)

Preparation of Protein Extracts and Analysis of DNA Binding Activities—Brain tissue from the rat hippocampi was extracted and immediately processed on ice for nuclear protein extraction (12, 18). The tissue was manually pulverized with a Teflon pestle and suspended in 0.5 ml of buffer A (10 mM Hepes, pH 7.9, 1.5 mM MgCl2, 10 mM KCl, 1 mM dithiothreitol, and protease inhibitors: 1 mg/ml phenylmethylsulfonyl fluoride, 10 mg/ml aprotinin, 10 mg/ml leupeptin, 1 mg/ml pepstatin A) (all products from Sigma). After incubation for 15 min on ice, Nonidet P-40 was added to final concentration of 1% before centrifugation at 12,000 rpm for 10 min at 4°C. The supernatant buffer was removed and the pellet was resuspended in buffer B (20 mM Hepes, 0.84 mM NaCl, 1.5 mM MgCl2, 0.4 mM EDTA, 1 mM dithiothreitol, 25% (v/v) glycerol, and protease inhibitors as above) and incubated for 15 min at 4°C. After centrifugation for 15 min at 12,000 rpm, the supernatant was frozen at −70°C. The protein content was estimated by the Bradford method and verified by Coomassie staining of SDS-polyacrylamide gel electrophoresis 12% Tris glycine gels. We applied the EMSA technique to assess the DNA binding activities of the extracted nuclear proteins from the different experimental conditions. Fifteen micrograms of nuclear proteins were preincubated for 10 min at room temperature in binding buffer (10 mM Hepes, 25 mM KCl, 0.5 mM EDTA, 0.25 μg/ml bovine serum albumin, 1 mM dithiothreitol, 20 μg/ml poly(dI-dC) and subsequently incubated with 40 fmol (3 × 104-40,000 Cerenkov’s cpm) of end-labeled probe for 20 min at room temperature.

The timp-1 promoter-derived sequences as well as their mutated variants were used in the experiments (Fig. 1). The probes were labeled with [α-32P]dCTP (Amersham) and purified on Sephadex G-50 spin columns. Following incubation, 2 μl of loading buffer containing 0.3% bromophenol blue (5% glycerol was added to the samples and electrophoresed at 130 V for 2 h in a nondenaturing 4% polyacrylamide gel. Electrophoresis was performed in a low ionic strength buffer (6.7 mM Tris-HCl, pH 7.5, 1 mM EDTA, and 3.3 mM sodium acetate). Gels were dried and exposed to phosphor screens (Molecular Dynamics) overnight. Gel images were obtained with a PhosphorImager (Molecular Dynamics). To facilitate comparison among the different conditions, the autoradiograms were scanned densitometrically and average gray/pixel level was measured in the area of the band.

Supershift Analysis—To identify the components of the AP-1 complex, supershift analysis was applied (12, 13). Commercially available (Santa Cruz) polyclonal antibodies against the following members of AP-1 family were used: c-Fos (sc-52X), FosB (sc-48X), Fra-1 (sc-183X), Fra-2 (sc-246X), c-Jun (sc-82XX), and JunD (sc-189X). Antibodies (1 mg/1 ml) were affinity purified by the manufacturer and had no detectable cross-reactivity with other members of the Fos and Jun families. This was confirmed by Western blot analysis (see Refs. 12 and 18).

One microliter of each antibody was added to 10 μl of reaction volume containing the nuclear protein extract (10 μg) and incubated for 1 h at 4°C. Afterward, the labeled oligonucleotide was added to the reaction mixture and the EMSA protocol was followed as described, above. The samples were then electrophoresed at 110 V for 5 h with recirculation of the electrophoresis buffer. Gels were dried and exposed to phosphor screens and analyzed with a PhosphorImager as described above.

In Vitro Culture of Hippocampal Dentate Gyrus

Primary cultures of dentate gyrus cells were obtained from 5-day-old rat pups using a modification of procedure described previously (19). Briefly, hippocampal tissue was sliced mechanically and placed in Krebs-Ringer bicarbonate medium supplemented with 1.0 mg/ml bovine serum albumin and 1.2 mM MgSO4 (solution A). The area dentata was then dissected from each slice and transferred to a tube containing the same solution. After a short centrifugation at 150 × g, the tissue was resuspended in 5 ml of the solution A containing 0.05 mg/ml trypsin (Life Technologies, Inc.), and left in a rotary bath at 37°C (200 rpm) for 20 min. Next, 5 ml of solution A, containing 12.8 μg/ml DNase I (Sigma) and 0.1 mg/ml soybean trypsin inhibitor was added, which was immediately centrifuged. The pellet was resuspended in 3 ml of the same solution, containing in addition 80 μg/ml DNase 1, 0.52 μg/ml trypsin inhibitor, and 2.7 mM MgSO4. The tissue was dissociated with a Pasteur pipette, sedimented for 15 min, and the pellet was resuspended as above in 2 ml. The two supernatants were collected and supplemented with 3.5 ml of Krebs-Ringer bicarbonate medium containing 1 mg/ml bovine serum albumin, 2.4 mg/ml MgSO4, and 0.1 μM CaCl2. After centrifugation for 10 min at 150 × g the pellet was resuspended in the culture medium. Cells were plated on poly-L-lysine coated glass coverslips at a density of 150,000 per coverslip. Cultures were maintained for 24 h in Dulbecco’s modified Eagle’s (DMEM, Life Technologies, Inc.) medium containing 10% fetal calf serum (with 25 mM KCl, 2 μM glutamine and penicillin (50 units/ml)/streptomycin (50 μg/ml)). Twenty-four h later the cultures were transferred into the chemically defined medium consisting of DMEM supplemented with 1 × N-2 Supplement (Life Technologies, Inc.), 25 mM KCl, 2 mM glutamine, and 100 units/ml/streptomycin (50 μg/ml). Cells grown for 4 days in vitro were transfected using the calcium-phosphate procedure. A day later, the cells were exposed to glutamate (0.1 mM).

Gene Constructs

The gene constructs used in this study to assess the timp-1 AP-1 activity in cultured dentate gyrus neurons are schematically depicted in the Fig. 1. The genes were constructed from fragments of wild type timp-1 promoter or promoter mutant in AP-1 site that were obtained by polymerase chain reaction with prT-61CAT and prTmut(AP1)CAT plasmids (8) used as templates for reactions. For all reactions the same pair of polymerase chain reaction primers were used: 5′-gagcttagctactggagctgagggtg-3′ and 5′-ggagtagctacgtttaataaatctcgccaaagtctgtcgag-3′.
Both, p61PTimp1(1AP1)GFP and p61PTimp1(1AP1mut)GFP, were generated by subcloning the aforementioned polymerase chain reaction products digested with XhoI-BamHI into the XhoI-BamHI sites of pEGFP-1 plasmid (CLONTECH). All constructs were verified by sequencing. prT-61CAT and prTmut(1AP1)CAT plasmids were kindly provided by Dr. T. Kordula (8).

**Transfection of Cultured Hippocampal Neurons**

For each transfection 3 μg of total plasmid DNA per coverslip were used. Usually a 2:1 ratio of timp-1 promoter containing vector p61PTimp1(1AP1)GFP or p61PTimp1(1AP1mut)GFP to pSVβgal (used for assessing the transfection efficiency during the normalization steps of evaluation of the results) was used. All plasmids were purified using EndoFree Plasmid Maxi Kit (Qiagen).

Neurons from dentate gyrus of hippocampus were transfected by the calcium phosphate method described earlier (20) with modifications. Cells growing for 4–5 days were used for transfection. The culture medium was removed and saved. The cells growing on coverslips were moved into a 24-well dish (one coverslip per each well) and incubated for another 24 h.

**RESULTS**

**Seizure-evoked timp-1 mRNA Accumulation**—In the first experiment we analyzed, with Northern blot technique, the time course of timp-1 mRNA accumulation in the hippocampus after treating rats with kainate. We found significant increases in timp-1 mRNA at 6–24 h after the treatment (Fig. 2A). Notably, using exactly the same conditions as previously reported, the mRNA level of c-fos as well as other AP-1 components peak at 1–6 h (12). The kainate-evoked increase in timp-1 mRNA accumulation at 6 h was greatly suppressed by pretreatment of the rats with the protein synthesis inhibitor, cycloheximide (Fig. 2B), suggesting that a significant component of timp-1 mRNA accumulation requires preceding de novo protein synthesis.

To extend the observation of seizure-driven timp-1 mRNA accumulation, and to exclude the possibility that our observations are solely linked to kainate neurotoxicity, we employed another seizure model that involves an application of PTZ, known to produce massive neuronal excitation in the hippocampus without, however, any adverse effects on neuronal survival. Again, a significant increase in timp-1 mRNA levels was observed after the PTZ-evoked seizures, with peak values of timp-1 mRNA levels at 2 h following treatment. Importantly, this elevation was slightly delayed in comparison with c-fos mRNA, that peaked at 45 min after the PTZ injection (Fig. 2C).

We also analyzed the spatial pattern of timp-1 mRNA expression at either 6 h after kainate administration or 2 h following PTZ treatment. The in situ hybridization autoradiograms were compared with c-Fos immunochemistry performed on parallel sections. Fig. 3 presents the striking resemblance between timp-1 mRNA and c-Fos protein distribution patterns. In the hippocampus of PTZ-treated animal, both
senger stability, we employed transgenic mice harboring a gene accumulation might be solely derived from the increased mes-
vated following seizures and thus to exclude that
PTZ-exposed animal the most intense signal of both c-Fos and
timp-1 results. rRNA ethidium bromide staining of the blots before hybridization,
strongly labeled.
whereas in KA-treated rats also the cornu ammonis (DG)
detection or
and their brains isolated and snap-frozen on dry ice. Twenty microme-
hybridization procedures. Please note that in
timp-1 in situ
FIG. 2 . Seizure-evoked timp-1 mRNA accumulation in rat hippocampus. The animals were treated with kainate (10 mg/kg, intraperitoneal) either alone (A) or following pre-administration of 3 mg/kg subcutaneously of protein synthesis inhibitor cycloheximide (B) or PTZ (50 mg/kg, intraperitoneal) (C). At the times indicated at the top, the rats were killed, their hippocampi dissected and processed in pairs from individual animals for RNA extraction and then Northern blot hybridization. Duplicate lanes in A are shown to indicate reproducibility of the results. rRNA ethidium bromide staining of the blots before hybridization, is presented to show similar amounts of total cellular RNA (15–20 μg) in each lane. Panel C presents, in addition to timp-1, also c-fos mRNA to indicate a time delay in accumulation of both messages. Each experiment was performed at least twice, representative results are shown.
timp-1 mRNA and c-Fos protein are most abundant in the dentate gyrus, whereas in the kainate-treated rat expression of both timp-1 and c-fos occurs throughout the neuronal cell body layer of all hippocampal subfields. The animals were treated to either physiological saline (Control), kainate (KA), or pentylenetetrazole (PTZ) and then at the times indicated to the left, the rats were killed and their brains isolated and snap-frozen on dry ice. Twenty micrometer sections were cut and processed in parallel to either c-Fos immunode-
tion or timp-1 in situ hybridization procedures. Please note that in
PKZ-exposed animal the most intense signal of both c-Fos and timp-1 expression is limited to the dentate gyrus neuronal cell layer (DG), whereas in KA-treated rats also the cornu ammonis (CA) subfields are strongly labeled.

Activation of timp-1 Promoter in TIMP-LacZ Transgenic Mice—To investigate whether timp-1 promoter can be activated following seizures and thus to exclude that timp-1 mRNA accumulation might be solely derived from the increased messenger stability, we employed transgenic mice harboring a gene containing the timp-1 regulatory region fused to β-galactosidase coding region (see Fig. 1). The mice were treated with kainate (20 mg/kg) and 24 h later their brains were analyzed for β-galactosidase activity. Fig. 4 shows that kainate administration resulted in a marked stimulation of the timp-1 promoter, most notably in the dentate gyrus. A similar, albeit less pronounced, effect was also observed in animals treated with PTZ (50 mg/kg, not shown).

Transcription Factor Binding to timp-1 Promoter Regulatory Elements—In the next series of experiments, we used synthetic oligonucleotides carrying timp-1 gene regulatory sequences spanning the promoter region from −66 to −35 (see Fig. 1), to investigate transcription factors binding before and after either kainate or PTZ treatment. In the first experiment, we investiga-
ted the pattern of DNA binding at different times after treatment with kainate. Three major bands of DNA binding could be recognized. Fig. 5A shows the strong binding that was observed in the band designated as I and that was markedly induced at 6 h after kainate administration, i.e. when the timp-1 mRNA starts to peak and thus when one could expect the gene transcription to be active. The intensity of the binding decreased at 24 h following treatment (not shown).

In our previous study using a consensus AP-1 sequence as a probe for DNA binding experiments, we reported that 2–6 h after kainate exposure the AP-1 complex is composed predominantly of c-Fos, FosB, JunB, and JunD proteins (12). Using the AP-1/Stat/Ets sequence derived from the timp-1 promoter, we found similar AP-1 DNA-binding proteins, including phospho-
ylated c-Jun (Fig. 5B). Next, we used wild type (wt) as well as variants of the timp-1 regulatory region, mutated specifically at either AP-1 (mutAP-1) or Stat/Ets (mutStat/Ets) element, to identify its binding proteins present in rat hippocampus 6 h after KA administration. Fig. 5C shows that a mutation in the AP-1 element completely prevented the kainate-evoked band I protein binding, whereas mutations in Stat/Ets region did not affect any of the bands. Furthermore, we carried out competition experiments in which the hippocampal extracts, obtained from animals at 6 h after kainate, were preincubated with an excess of unlabeled timp-1 regulatory region (either wt or mut) before adding the wild type 32P-labeled probe. Fig. 5C docu-
ted that competition with the wild type probe abolishes DNA binding, most notably in bands I and II. Similar effects were also observed in the case of a probe mutated within the Stat/Ets element. In contrast, oligonucleotides mutated in the AP-1 site only weakly affected binding to the wild type probe (Fig. 5C).

Essentially the same pattern, indicating requirement for intact AP-1 site of DNA binding of the timp-1 promoter region, was observed at 2 h after treating rats with pentylenetetrazole, i.e. at the time coinciding with timp-1 mRNA accumulation in this seizure model (Fig. 5D).

timp-1 Promoter Activity following Transfection of Dentate Gyrus Granule Cells Cultured in Vitro—In the previous experiments we found the most robust timp-1 mRNA accumulation.
in hippocampal dentate gyrus granule cell neuronal layer, in response to markedly elevated neuronal excitation. To further investigate the role of timp-1 AP-1 regulatory sequence in excitatory amino acid-driven gene expression we turned to glutamate-stimulated dentate gyrus cells in culture (19). These cultures derived from 5-day-old rat pups are composed of neuronal and glial elements.

The cultures were transfected with a modified calcium phosphate technique (see "Experimental Procedures" for details). Fig. 6 shows that L-glutamate may activate a short timp-1 promoter region fused to GFP coding region. Morphology of successfully transfected cells indicated their neuronal character (Fig. 6A). Fig. 6B presents results of the experiment in which the cultures were transfected with either promoter-less GFP gene or the same reporter sequence driven by timp-1 short promoter region (see Fig. 1 for details of the gene constructs). The presence of the timp-1 regulatory region allowed the reporter gene to be expressed, and this expression was significantly potentiated (p < 0.01) by treating the cultures with 0.1 mM glutamate. In the next experiment we analyzed whether the intact wild type (wt) AP-1 site, contained within the timp-1 promoter, was indispensable for glutamate-driven gene expres-
The dentate gyrus cultures were transfected with gene constructs carrying GFP under either wt or AP-1-mutated sequences. Fig. 6 shows that wt AP-1 produced much stronger gene activation than the mutant (p < 0.01).

**DISCUSSION**

**Importance, Novelty, and Accuracy of the Findings—**The combined results of this study strongly implicate AP-1 transcription factor in the regulation of timp-1 gene transcription in the brain in response to enhanced neuronal excitation. This notion is supported by the following multiple evidence. timp-1 mRNA accumulation, both after KA and PTZ, occurs in the hippocampus with a delayed, in comparison with AP-1 components time course, as well as with a spatial overlap with c-Fos protein expression. Furthermore, AP-1-like sequence derived from timp-1 promoter is specifically bound by AP-1 proteins, after either KA or PTZ treatment. Finally, timp-1 promoter responds to excitatory activation both in vivo and in vitro. In the dentate gyrus cultures, this activation critically depends on an intact timp-1 AP-1 site.

Despite the fact that the present study represents probably the most advanced analysis of AP-1-dependent neuronal gene expression in the brain, it is important to note that each of the individual tests does not provide fully conclusive results. For instance, EMSA is done on protein extracts isolated post-mortem, that do not necessarily reflect the exact nature of the protein complexes present within the living brain. Next, correlation of consecutive c-fos and timp-1 expression phenomena as well as spatial overlap between c-Fos protein and timp-1 mRNA distribution can be only taken as a suggestion of a functional link between these events. However, it is important to note that, whereas kainate administration produces a variety of effects, including neuronal excitation, plasticity, and cell death (7, 21) our results showing that PTZ, known to be devoid of neurodegenerative action, may also activate timp-1 expression point to increased neuronal activity as the major trigger of timp-1 expression. Furthermore, the fact that timp-1 expression is strongly induced by KA within the dentate gyrus, known to survive the insult, implies that it is a neuronal excitation, rather than neurotoxicity-related phenomenon.

The observation that timp-1 could be activated both by kainate and PTZ is not trivial, as there are many genes, whose expression is stimulated by kainate, but evidence for their PTZ responsive-
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ness is either missing or non-existent. For instance, in our studies, out of five genes cloned by Nedivi et al. (5) only timp-1 has proven to be PTZ-responsive (see Refs. 17 and 22). Such results greatly complicate the understanding of AP-1 function in the brain. AP-1 is activated to a roughly similar degree by various physiological and pathological stimuli, including both kainate and PTZ and, still, genomically responses to both proconvulsants appear to be clearly different (see also Ref. 23).

Finally, it is important to bear in mind that results obtained with transgenic animals can depend on a position effect of the gene construct within the chromatin milieu. We should also explain that these experiments were done on a different species (mouse versus rat) because of the availability of appropriate transgenic mice. On the other hand, rat is a model system routinely used in our laboratory and we have collected a multitude of information about AP-1 activation in the rat brain (see Ref. 7, for review). Importantly, the patterns of c-Fos and AP-1 expression in response to either KA or PTZ have been found to be essentially the same for rat and mouse (1, 2).

AP-1 Involvement Does Not Explain all Phenomena of timp-1 mRNA Accumulation—Whereas our study was focused on AP-1 involvement in timp-1 promoter inducibility, it is of note that neither our EMSA nor in vitro culture experiments provided any evidence implying Stat/Ets regulatory region in timp-1 control in hippocampus. These factors were previously characterized to be pivotal for timp-1 expression in non-neuronal cells in vitro (8–10). Clearly we have not provided any proof for lack of their involvement in timp-1 gene inducibility in the brain in vivo. However, our results do not help to resolve a puzzle reported by Rivera et al. (6) that protein synthesis inhibition did not affect timp-1 expression in dentate gyrus at 90 min after KA treatment. Interestingly, we have also observed a low, albeit clear, timp-1 mRNA accumulation after treating rats with both cycloheximide and KA, under conditions that have proven to be effective in blocking other KA-driven genes (17). An involvement of some pre-existing transcription factors (e.g. STAT 3), activated via post-translational modifications could provide an explanation for the phenomenon of early phase of timp-1 mRNA accumulation in response to KA. However, results obtained by Rivera et al. (6) could also be interpreted in a context of previous observation that timp-1 mRNA accumulation may result from enhanced messenger stabilization as opposed to an increased rate of transcription (24). Despite the complex picture emerging from various reports on timp-1 gene regulation in response to extracellular stimuli, both in previous experiments carried out on cell lines (8–10) as well as in our study on the brain, AP-1 appears to be of critical importance for the timp-1 promoter activation.

Functional Consequences of Elevated timp-1 Gene Expression—Recent advances in the biology of the extracellular matrix (ECM) strongly suggest that the ECM may play an important role in brain organization and functioning not only by providing the mechanical frame for the nerve tissue but also by regulating its cellular organization (25–27). Several pieces of evidence imply ECM components in regulating neural plasticity and cell death (28–30). It has also lately been shown that extracellular matrix proteases such as the tissue plasminogen activator/plasmin system might be associated with nerve tissue remodeling and neuronal plasticity (29, 31, 32). Matrix metalloproteinases (MMPs) and their natural inhibitors TIMPs are known to be specifically active in ECM reorganization during histogenesis as well as in some pathological conditions (33). Recent data show that increased activity of MMPs as well as elevated TIMP-1 expression are associated with such brain pathologies as Alzheimer disease, stroke, ischemia, and epilepsy (for review, see Refs. 34 and 35). However, the physiologic-ical functions of the brain MMPs/TIMPs system remain largely unknown. Recent in vitro experiments have shown that MMP-dependent selective cleavage of ECM proteins may provide a signal for regulated repositioning and migration of neuronal processes (36). It is thus tempting to hypothesize that MMP-dependent ECM remodeling in the brain could be responsible for neuronal circuitry formation and synaptic plasticity.

Our finding that neuronal activity dependent timp-1 expression could be regulated in the brain by AP-1 suggests that this transcription factor may exert its role by affecting the ECM. It is then conspicuous that several other components of the MMP-TIMP system have also been found to be regulated by AP-1 in non-neuronal cells (see Refs. 37 and 38). It remains to be elucidated whether this could also be the case in the central nervous system in response to enhanced neuronal excitation that may result in plastic changes within the brain.

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