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Antidepressant Treatments Regulate Matrix Metalloproteinases-2 and -9 (MMP-2/MMP-9) and Tissue Inhibitors of the Metalloproteinases (TIMPs 1–4) in the Adult Rat Hippocampus

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KEY WORDS depression; dentate gyrus; fluoxetine; tranylcypromine; desipramine; electroconvulsive seizure; zymography; *in situ* hybridization

ABSTRACT Antidepressants induce structural remodeling in the adult hippocampus, including changes in dendritic arbors, axonal sprouting, neurogenesis, and endothelial cell proliferation. Such forms of structural plasticity take place in the context of the extracellular matrix environment and are known to be regulated by matrix metalloproteinases (MMPs), in particular MMP-2/9, and their endogenous regulators, the tissue inhibitors of the metalloproteinases (TIMPs 1–4). Given the hippocampal structural remodeling associated with antidepressant treatments, we hypothesized that antidepressants may regulate the expression and activity of MMP-2/9 and TIMPs 1–4. The influence of distinct classes of antidepressants, namely, electroconvulsive seizure, fluoxetine, tranylcypromine, and desipramine, on the gene expression of MMP-2, MMP-9, and TIMPs 1–4 in the hippocampus was determined using radioactive *in situ* hybridization. In addition, zymography studies addressed the regulation of the gelatinase activity of MMP-2/9 following acute and chronic antidepressant administration. We observed that acute and chronic ECS differentially regulate the transcript levels of MMP-2/9 and TIMPs 1–4 and also increase gelatinase activity in the hippocampus. Acute and chronic pharmacological antidepressants on the other hand differentially alter the expression of the TIMPs without any observed effect on hippocampal MMP-2/9 expression or activity. These findings raise the possibility that extracellular matrix modifying enzymes and their endogenous regulators may serve as targets for antidepressant treatments and suggests the possibility that they may contribute to antidepressant-mediated structural plasticity in the hippocampus. **Synapse 62:590–600, 2008.**

INTRODUCTION

Antidepressants induce cellular remodeling in the hippocampus, a key limbic brain region involved in cognitive and mood-related behaviors (Duman et al., 1999). Chronic antidepressant treatments enhance hippocampal neurogenesis (Malberg et al., 2000), a process implicated in the behavioral effects of antidepressants (Airan et al., 2007; Santarelli et al., 2003). Electroconvulsive seizures (ECS), the most robust antidepressant treatment, besides its neurogenic effects, also produce axonal sprouting of the hippocampal mossy fiber pathway (Vaidya et al., 1999). In addition, the proliferation of endothelial cells (Hellsten et al., 2004) and neuroglial precursors (Wennstrom et al., 2003) in the hippocampus is also modulated by ECS. Not only do antidepressants promote structural reorganization, they ameliorate the

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damaging effects of depression models on hippocampal cytoarchitecture (Malberg and Duman, 2003). Building evidence suggests that such adaptive changes in hippocampal structural plasticity may contribute to the therapeutic effects of antidepressant treatments (Nestler et al., 2002).

Though hippocampal structural plasticity has been implicated in the behavioral effects of antidepressants, the pathways that underlie antidepressant mediated hippocampal remodeling remain poorly understood (Vaidya and Duman, 2001). However, plastic changes in structure occur within the milieu of the extracellular environment and in this context, enzymes that modify the extracellular matrix, cleave key growth factors like brain-derived neurotrophic factor (BDNF) to their active forms and modulate both axonal guidance and cell adhesion molecules are of particular relevance (Ethell and Ethell, 2007). The matrix metalloproteinases (MMPs) are one such group of proteases that are postulated to play important roles in synaptic and structural plasticity in the adult brain (Bozdagi et al., 2007). MMPs are a family of endopeptidases with 24 identified members, belonging to distinct subgroups: gelatinases, collagenases, stromelysins, and membrane-type MMPs (MT-MMPs) (Nagase and Woessner, 1999). Thus far, their major substrates in the brain include the proneurotrophins like pro-BDNF (Lee et al., 2001), axonal guidance molecules like the ephrins (Hattori et al., 2000), cytokines like tumor necrosis factor alpha (Black et al., 1997), and cell adhesion molecules such as tenascin-R, laminin, cadherins, and integrins (Dzwonek et al., 2004). MMPs synthesized as prepro-enzymes and secreted as inactive pro-MMPs are enzymatically cleaved and thus activated. Activated MMPs are subject to inhibition by the tissue inhibitors of metalloproteinases (TIMPs) that bind MMPs to form tight noncovalent 1:1 complexes with them. There are four TIMPs identified so far: TIMP-1, -2, -3, and -4 (Sternlicht and Werb, 2001).

MMP-2 and MMP-9, also referred to as the gelatinases, are among the best studied of the MMPs in the brain. The gelatinases have been implicated in dendritic remodeling (Szklarczyk et al., 2002), axonal sprouting (Reeves et al., 2003), synaptic plasticity, and learning in the hippocampus (Bozdagi et al., 2007; Meighan et al., 2006). We hypothesized that antidepressant treatments may influence the expression and activity of the gelatinases, MMP-2 and -9, and their endogenous inhibitors, the TIMPs.

MATERIALS AND METHODS

Animal treatment paradigms

Male Sprague-Dawley rats (200–250-g) bred in our animal-breeding colony were used in all experiments. Animals were group housed and maintained on a controlled 12 h light–dark cycle with access to food and water *ad libitum*. All animal procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the TIFR Institutional Animal Ethics Committee. Animals received bilateral ECS treatment via ear clip electrodes (UGO Basile, Comerio, Italy) or sham treatment (ear clip electrode application without electrical stimulation). For the acute ECS study, animals (n = 5/group) received a single sham/ECS treatment, while for the chronic ECS paradigm, animals (n = 5/group) received sham/ECS treatment once daily for 10 consecutive days and were sacrificed 2 h after the last ECS treatment. In the acute antidepressant study, animals received a single injection of fluoxetine (5 mg/kg, Sigma, St. Louis) (n = 4/group), tranylcypromine (7.5 mg/kg, Sigma) (n = 4/group), desipramine (15 mg/kg, Sigma) (n = 4/group), or vehicle (0.9% saline) by i.p. (i.p.) injection. For the chronic antidepressant treatments, animals received fluoxetine (n = 4 /group), tranylcypromine (7.5 mg/kg for 7 days and then 10 mg/kg for 14 days) (n = 4/group), desipramine (n = 6/group), or vehicle treatment once daily for 21 days. For both the ECS and pharmacological antidepressant experiments, we used the 2-h time point after last treatment for sacrifice based on previous results that chronic treatment experiments induce changes in neurogenesis at this time point and acute treatments modulate several target genes after 2 h (Dias et al., 2003; Malberg et al., 2000; Newton et al., 2003). Rats were sacrificed 2 h after the last injection and the brains were rapidly removed, frozen on dry ice, and stored at −70°C before cryostat sectioning. For zymography, separate experiments of acute (n = 6/group) and chronic ECS (n = 9/group) treatment, and acute (n = 9/group) and chronic (n = 6/group) fluoxetine, tranylcypromine, and desipramine treatments were performed as described earlier.

In situ hybridization

In situ hybridization for MMP-2, MMP-9, and TIMPs 1–4 was performed on cryostat cut, fixed sections. In brief, 14-μm thick coronal sections mounted on ribonuclease free Probe-on plus slides (Electron Microscopy Services) were fixed, acetylated, and dehydrated before storage at −70°C. Rat MMP-2, MMP-9, and TIMPs 1–4 riboprobes were transcribed using ^{35}S-labeled UTP (Amersham, Buckinghamshire, UK) from transcription competent plasmids provided by Dr. Thomas Curry, Dr. Robert Nuttall, and Dr. Diane M. Jaworski. *In situ* hybridization was performed as described previously (Dias et al., 2003). In brief, slides were incubated with ^{35}S-UTP labeled riboprobes (1×10^6 cpm/150 μL) in hybridization buffer for 20–24 h at 60°C. After hybridization, the slides were

subjected to stringent washes in decreasing concentrations of SSC and air dried prior to exposure (3–6 weeks) to Hyperfilm β-max (Amersham, UK). Sense riboprobes for the MMP-2, MMP-9, and the TIMPs 1–4, or a ribonuclease (40 μg/mL at 37°C for 30 min) prehybridization wash did not yield significant hybridization (Supplementary Fig. 1) confirming the specificity of the signal.

In situ zymography

In situ zymography was carried out on unfixed cryostat cut hippocampal sections (14 μm) to localize gelatinase activity as described previously (Jourquin et al., 2003). Slides were incubated with the fluorescent substrate gelatin Oregon green 488 conjugate (50 μg/ml; Molecular Probes) in reaction buffer (100 mm NaCl, 100 mM Tris–HCl, pH 7.5, 10 mM CaCl2, 20 mM ZnCl2, 0.05% Triton X-100) for 24 h at 37°C. Gelatinolytic cleavage of the substrate was detected as increased fluorescence intensity. Some slides were preincubated with the MMP inhibitor GM6001 (50 μg/ml in DMSO; Chemicon) for 2 h before adding gelatin-Alexa 488 conjugate. Slides were fixed and coverslipped with Vectashield (Vector) as the mountant.

In-gel zymography

The hippocampal MMP enriched fraction was prepared as previously described (Szklarczyk et al., 2002). Dissected hippocampi were weighed, dounce homogenized in a buffer containing 10 mM $CaCl_2$ and 0.25% Triton X-100 and homogenates were centrifuged at 6,000*g* for 30 min. The pellet was resuspended (50 mM Tris and 0.1 M $CaCl_2$), heated for 15 min at 60°C followed by centrifugation at 10,000*g* for 30 min at 4°C. The supernatant was precipitated using 60% ethanol, solubilized in sample buffer, and used for in gel zymography.

Samples, molecular weight markers, and gelatinase standards (human MMP-2 and MMP-9, Chemicon) were subjected to electrophoresis in native PAGE gels (7.5% acrylamide, 0.5% gelatin). Gels were incubated in 2.5% Triton-X-100 overnight followed by incubation in 50 mM Tris, 10 mM $CaCl_2$, 1 μM $ZnCl_2$, 1% Triton X-100, and 0.02% sodium azide at 37°C for 2 days. Gels were stained with 0.1% Coomassie blue, followed by destaining until clear bands of gelatinolysis appeared on a dark background.

Quantitation and data analysis

MMP-2/9 and TIMPs 1–4 mRNA levels were analyzed with Scion Image software (Scion) using ^{14}C standards to calibrate for nonlinearity. All hippocampal subfields including the dentate gyrus (DG), CA3, CA1, and CA4 were outlined and optical density measurements from both sides of 3–4 individual sections/animal were analyzed. After in gel zymography, the bands corresponding to MMP-2 (70 kDa) and MMP-9 (97 kDa) were outlined, and densitometric measurements were normalized to total protein loaded using Image J (NIH). Results were subjected to statistical analysis using Prism software (Graphpad) with Student's *t* test for experiments with two groups and an analysis of variance (ANOVA) followed by a Tukey-Kramer posthoc test for experiments with three or more groups. Statistically significant differences were determined at p values <0.05.

RESULTS

The influence of acute and chronic ECS and pharmacological antidepressants on MMP-2/9 and TIMPs 1–4 mRNA expression, as well as on the gelatinase activity of MMP-2/9 was quantified using *in situ* hybridization and gelatinase zymography, respectively. The baseline MMP-2 and MMP-9 mRNA expression profiles were in agreement with earlier reports (Szklarczyk et al., 2002) with low expression of MMP-2 and higher levels of MMP-9 seen in all hippocampal subfields. In the case of the TIMPs, subfield specific differential patterns of expression were observed. TIMP-1 and -4 were found to be expressed in all hippocampal subfields. TIMP-2, on the other hand, was expressed at higher levels in DG and CA1 in comparison with the other hippocampal subfields. TIMP-3 mRNA had a characteristic profile of a low level diffuse expression in the hippocampal subfields and hilus. Although all hippocampal subfields were quantitated for the following experiments, significant changes were observed in the DG subfield, and figures contain graphical representations of the levels of MMP-2/9 and TIMPs 1–4 mRNAs only for the DG, where the effects were observed.

Influence of acute and chronic electroconvulsive seizures on the hippocampal mRNA expression of MMP-2 and MMP-9

To address the regulation of MMP-2 and -9 by electroconvulsive seizure (ECS), rats were administered ECS either once (acute ECS) or repeatedly for 10 days (chronic ECS), and the levels of MMP-2 and -9 transcripts were quantitated using *in situ* hybridization. Acute ECS did not significantly alter the mRNA expression of MMP-2 or MMP-9 within the DG (Fig. 1), although a trend toward a small increase in MMP-2 expression ($P = 0.06$) was observed. In striking contrast, chronic ECS resulted in a region-specific, robust increase (47%) in the expression of MMP-9 mRNA observed in the DG (Fig. 1). Chronic ECS did not change the levels of MMP-2 mRNA in the hippocampus.

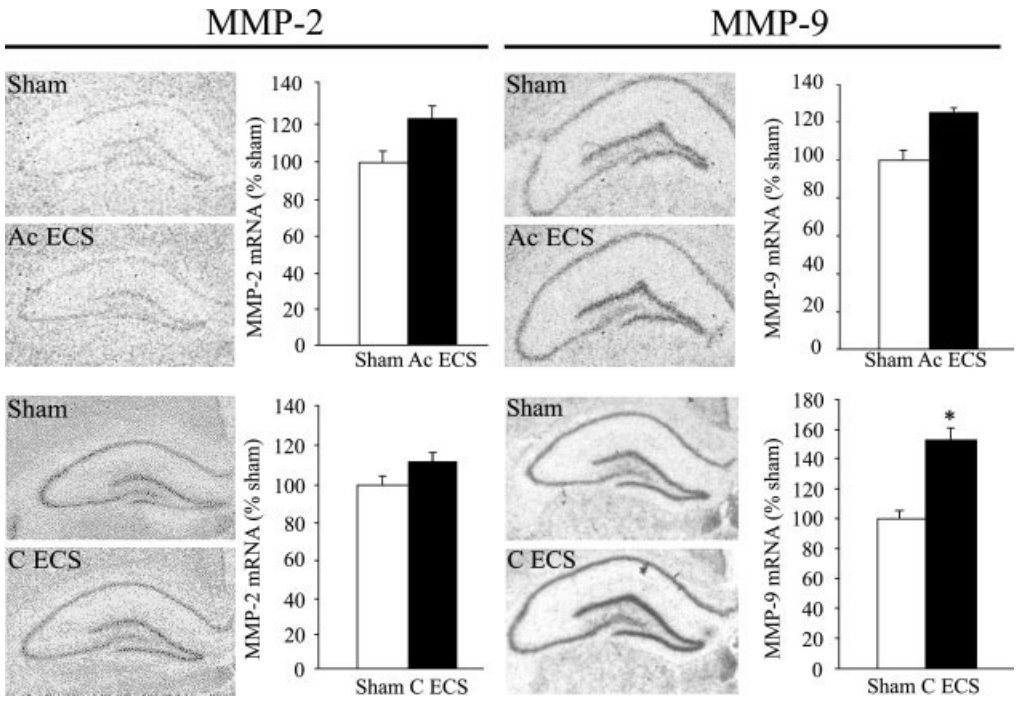

Fig. 1. Regulation of matrix metalloproteinase-2 and -9 (MMP-2 and -9) mRNA levels in the hippocampus by acute and chronic electroconvulsive seizures (ECS). Animals received sham or ECS either as a single treatment (Ac ECS) or once daily for 10 days (C ECS) as described in Materials and methods section, and levels of MMP-2 and MMP-9 mRNA were determined by *in situ* hybridization and quantitated densitometrically. Representative autoradiograms of MMP-2 and MMP-9 mRNA from sham and ECS-treated animals are shown. Levels of MMP-2 and MMP-9 mRNA in the dentate gyrus subfield are graphically represented as the mean ± SEM percent of sham ($n = 5$ animals /group); $^*P < 0.05$ when compared with sham (Student's t test).

EFfect of acute and chronic electroconvulsive seizures on the hippocampal expression of TIMPs 1–4 mRNA

Given the importance of TIMPs in modulating MMP activity, we examined the influence of electroconvulsive seizures (ECS) on the hippocampal mRNA expression of TIMPs 1–4 by *in situ* hybridization. Acute ECS resulted in a differential regulation of TIMPs 1–4 within the DG, with an increase in TIMP-1 (40%), TIMP-2 (25%), and TIMP-4 (61%) mRNA levels. In contrast, no change was seen in the expression of TIMP-3 (Fig. 2). As opposed to the changes following acute ECS, chronic ECS administration did not regulate TIMP-1, -2, and -3 but caused a small, significant increase (18%) in TIMP-4 mRNA expression in the DG (Fig. 2). The mRNA regulation of the TIMPs following a single seizure was found to be robustly attenuated following repeated administration of seizures for 10 days.

Acute and chronic electroconvulsive seizures (ECS) enhance hippocampal gelatinase activity

To address whether acute and chronic electroconvulsive seizures (ECS) regulated the proteolytic activity of MMP-2 and MMP-9, we measured their gelatinolytic activity using *in situ* and in-gel zymographic analysis. The *in situ* zymography assay indicated very low levels of gelatinase activity in sham-treated sections with a robust increase observed in the neuropil of the DG and hilus in sections from acute ECS animals (Fig. 3A). The enhanced gelatinase activity in ECS-treated sections could be blocked by preincubation with the MMP inhibitor GM6001 (Fig. 3A). Acute ECS significantly increased the gelatinase activity in the hippocampus with two gelatinolytic bands observed corresponding to gelatinase A (MMP-2; 70 kDa) and gelatinase B (MMP-9; 97 kDa) (Fig. 3B). The increase in gelatinolytic activity was observed for both MMP-2 and MMP-9 (Fig. 3B) following acute ECS. Chronic ECS treatment selectively increased the gelatinolytic activity of MMP-9, but not MMP-2, in the hippocampus (Fig. 3B).

EFfect of pharmacological antidepressant treatments on MMP-2 and MMP-9 mRNA levels in the hippocampus

We next addressed the effects of acute and chronic treatment with three distinct classes of pharmacological antidepressants, the serotonin selective reuptake inhibitor (SSRI) fluoxetine, the monoamine oxidase inhibitor (MAOI) tranylcypromine, and the norepinephrine selective reuptake inhibitor (NARI) desipramine, on mRNA levels of MMP-2/9 in the hippocampus. In

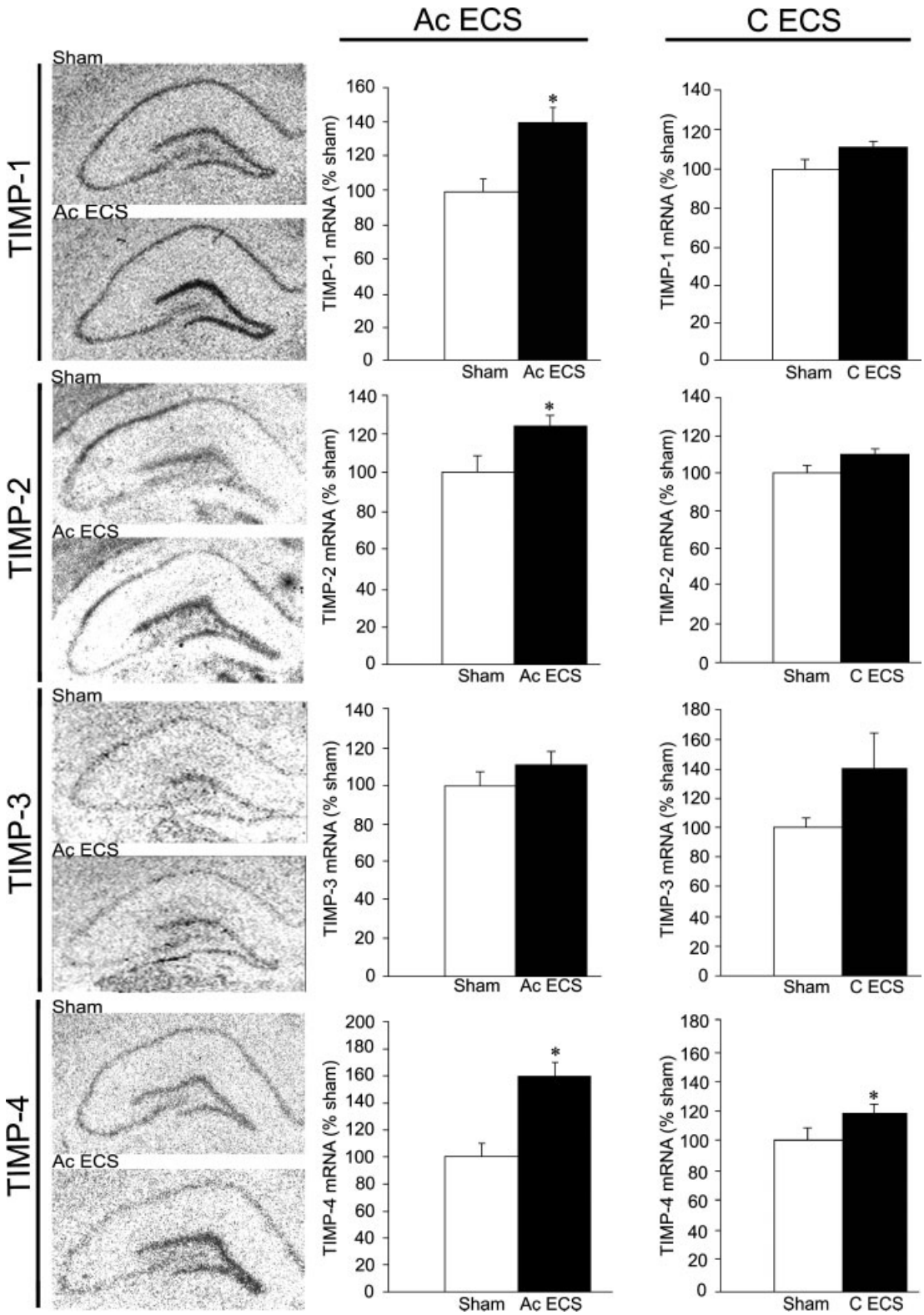

Fig. 2. Differential regulation of the tissue inhibitors of metalloproteinases 1–4 (TIMPs 1–4) mRNA expression in the hippocampal dentate gyrus subfield by acute and chronic electroconvulsive seizures (ECS). Animals received sham or ECS either as a single treatment (Ac ECS) or once daily for 10 days (C ECS) as described in Materials and methods section, and levels of TIMPs 1–4 mRNA were determined by *in situ* hybridization and quantitated densitometrically. Representative autoradiograms of TIMPs 1–4 mRNA from sham and acute ECS-treated animals are shown. The levels of TIMPs 1–4 mRNA are represented in the graphs as the mean ± SEM percent of sham ($n = 5$ animals /group); $^*P < 0.05$ when compared with sham (Student's t test).

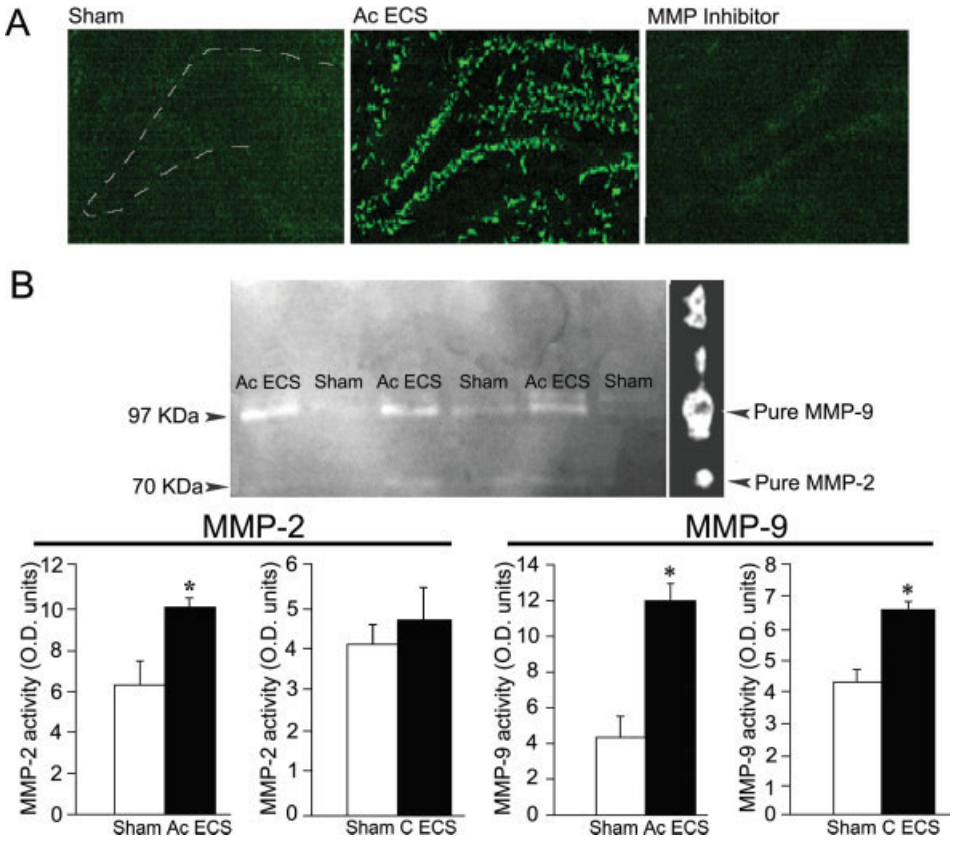

Fig. 3. Regulation of gelatinolytic activity of matrix metalloproteinase-2 and -9 (MMP-2 and MMP-9) in the hippocampus in response to acute and chronic electroconvulsive seizures (ECS). Animals received sham or ECS either as a single treatment (Ac ECS) or once daily for 10 days (C ECS) as described in Materials and methods section, and activity levels of MMP-2 and MMP-9 were analyzed using *in situ* zymography for acute ECS (**A**) and in gel zymography for both acute and chronic ECS (**B**). Representative images of *in situ* gelatinase zymograms are shown from sham and acute ECS-treated animals. The specificity of the gelatinase activity was confirmed using the MMP inhibitor GM 6001 which quenched the increased gelatinolytic activity observed in acute ECS sections (A). In gel zymography was performed for acute and chronic ECS treatments and the extent of gelatinolytic activity was quantitated by densitometry using Image J (NIH, USA). A representative gel from the acute ECS experiment is shown (B) and the densitometric results are expressed as optical density (O.D.) units and are the mean ± SEM (n = 4-9 animals /group); *$P < 0.05$ when compared with sham (Student's t test).

contrast to the effects observed with acute and chronic ECS, neither acute nor chronic treatment with the pharmacological antidepressants resulted in any change to the levels of MMP-2 and MMP-9 in the DG (Table I). In addition, no changes in MMP-2/9 mRNA levels were seen in other hippocampal subfields (data not shown).

TABLE I. Regulation of MMP-2 and MMP-9 mRNA levels by acute and chronic treatment with pharmacological antidepressants

Treatment	MMP-2 mRNA	MMP-9 mRNA
Ac ADT		
VEH	100.00 ± 7.14	100.00 ± 9.69
FLX	85.53 ± 7.56	83.27 ± 4.29
TCP	83.66 ± 7.05	86.14 ± 16.16
VEH	100.00 ± 5.56	100.00 ± 9.04
DMI	97.63 ± 4.44	103.94 ± 8.49
C ADT		
VEH	100.00 ± 25.60	100.00 ± 4.27
FLX	66.53 ± 22.25	83.50 ± 3.76
TCP	109.14 ± 9.7	104.45 ± 5.8
VEH	100.00 ± 10.07	100.00 ± 6.09
DMI	91.86 ± 33.33	78.06 ± 15.95

Animals were administered vehicle (VEH). Desipramine (DMI), Fluoxetine (FLX), or Tranylcypromine (TCP) as acute (Ac ADT) or chronic (C ADT) treatments. mRNA levels of MMP-2 & MMP-9 in the hippocampal dentate gyrus were detected by in situ hybridization followed by densitometric analysis. Both acute and chronic FLX & TCP treatments have the same vehicle group. Data are expressed as mean ± S.E.M. percent vehicle.

Pharmacological antidepressant treatments differentially regulate the hippocampal levels of TIMP1–4 mRNA

Given the dynamic effects of TIMP expression on the MMPs, we addressed whether the pharmacological antidepressants regulated the mRNA expression of TIMPs 1–4. Our studies revealed differential regulation of TIMP mRNA levels in response to both acute and chronic antidepressant treatment. Acute fluoxetine and tranylcypromine both resulted in a region-specific downregulation (~50%) of TIMP-2 mRNA in the DG (Fig. 4). These effects of acute fluoxetine and tranylcypromine were restricted to TIMP-2 with no changes observed in the mRNA levels of TIMP-1, -3,

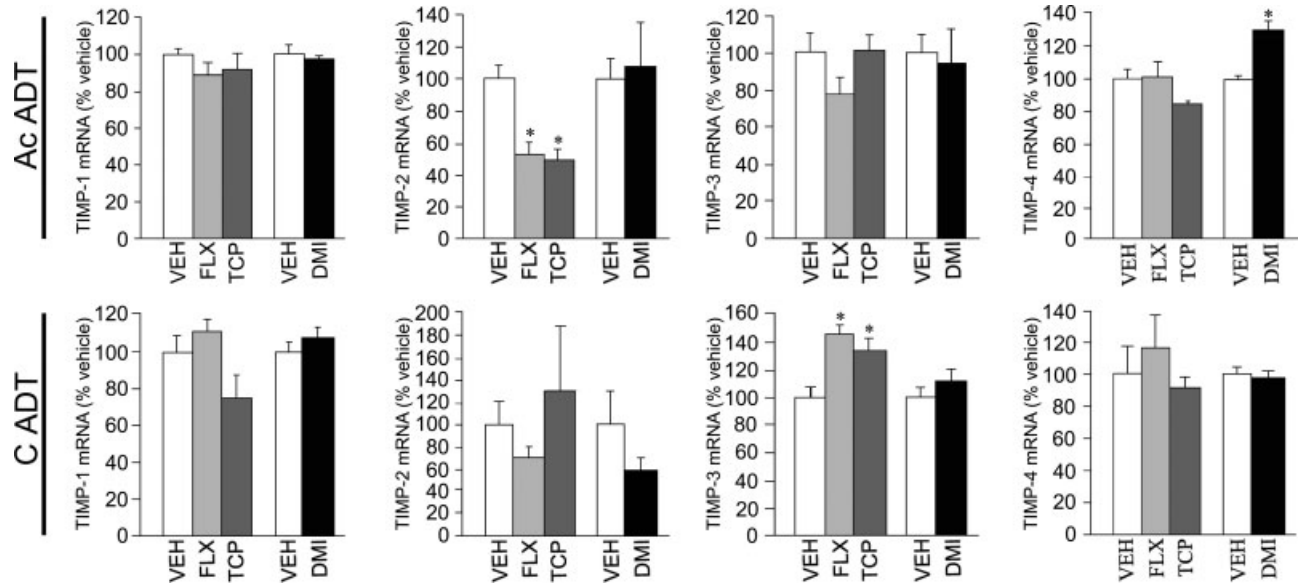

Fig. 4. Influence of acute and chronic antidepressant treatment on tissue inhibitors of metalloproteinases 1-4 (TIMPs 1-4) mRNA levels in the hippocampal dentate gyrus subfield. Rats received either acute (Ac ADT) or chronic treatment (C ADT) with vehicle (VEH), fluoxetine (FLX), tranylcypromine (TCP), or desipramine (DMI) as described in Materials and methods section, and levels of tissue inhibitors of metalloproteinases 1–4 (TIMPs 1–4) mRNA were determined with *in situ* hybridization analysis and quantitated densitometrically. The FLX and TCP experiment had a common vehicle group. The levels of TIMPs 1–4 mRNA are graphically represented as percent of vehicle and are the mean ± SEM (n = 4–6 animals/group); *$P < 0.05$ when compared with vehicle (Student's t test for the DMI experiment; ANOVA with Tukey-Kramer posthoc test for the FLX and TCP experiment).

and -4 in any of the hippocampal subfields (Fig. 4). Interestingly, acute treatment with the NARI, desipramine, led to a selective and significant increase (30%) in TIMP-4 mRNA levels in the DG with no changes observed for the others TIMPs (Fig. 4).

In striking contrast to the effects of acute fluoxetine and tranylcypromine, chronic fluoxetine, and tranylcypromine, treatments failed to regulate TIMP-2 but resulted in a significant increase (45%-fluoxetine, 35%-tranylcypromine) in TIMP-3 mRNA levels in the DG (Fig. 4). The mRNA levels of TIMP-1 and -4 continue to remain unchanged after chronic fluoxetine and tranylcypromine. Chronic treatment with desipramine did not regulate the hippocampal expression of any of the TIMPs and sustained administration of desipramine resulted in a loss of the acute desipramine mediated upregulation of TIMP-4 in the DG (Fig. 4).

Effect of pharmacological antidepressant treatments on the gelatinase activity in the hippocampus

Given the effects of pharmacological antidepressants on TIMP mRNA expression, we next sought to address whether treatment with the pharmacological antidepressants regulates the gelatinolytic activity of MMP-2 and MMP-9 in the hippocampus. In-gel zymography assays were performed to test the effects of acute and chronic fluoxetine, tranylcypromine, and desipramine treatment on MMP-2 and MMP-9 activity. The results indicated that acute and chronic fluoxetine, tranylcypromine, and desipramine treatments did not alter gelatinolytic activity of either MMP-2 or MMP-9 (Fig. 5).

DISCUSSION

This study examines the regulation of the hippocampal mRNA expression of MMP-2/9 and TIMPs 1–4, as well as the gelatinolytic activity of MMP-2/9 following acute and chronic antidepressant treatments. Although acute and chronic ECS treatments robustly enhanced gelatinase activity in the hippocampus and also increased MMP-9 mRNA expression in the DG subfield following chronic administration, the pharmacological antidepressants did not influence either MMP-2/9 expression or activity in the hippocampus. However, both ECS treatments as well as pharmacological antidepressant administration resulted in significant changes in the expression of the TIMPs 1–4. Following acute ECS, a robust increase in TIMP-1, -2, and -4 mRNA levels was seen in the DG, and a selective small increase was retained in TIMP-4 mRNA expression following repeated ECS exposure. Pharmacological antidepressants induced a differential regulation of the TIMPs with striking parallels in the effects of fluoxetine and tranylcypromine (decrease TIMP-2: acute, increase TIMP-3: chronic) as opposed to desipramine (increase TIMP-4: acute). Our

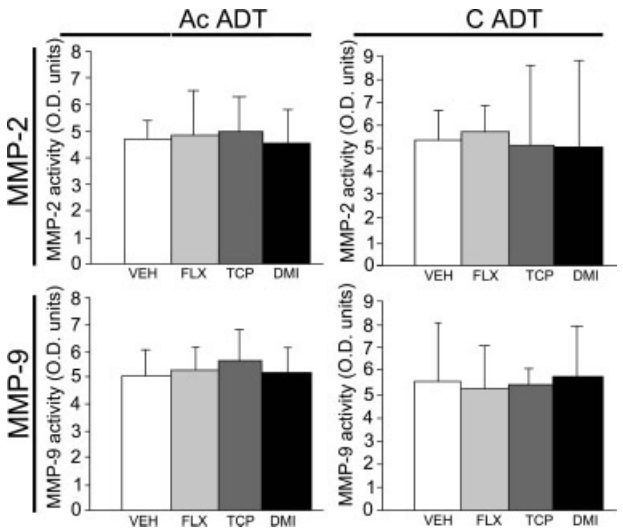

Fig. 5. Influence of acute and chronic antidepressant treatment on gelatinase activity of matrix metalloproteinase-2 and -9 (MMP-2 and -9) in the hippocampus. Rats received either acute (Ac ADT) or chronic treatment (C ADT) with vehicle (VEH), fluoxetine (FLX), tranylcypromine (TCP) or desipramine (DMI), and gelatinase activity was measured by in-gel zymography as described in Materials and methods section. The extent of gelatinolysis was quantitated by densitometry using Image J and graphical results are expressed as mean ± SEM optical density (O.D.) units (n = 6–9 animals/group).

results provide novel evidence that distinct classes of antidepressants regulate the MMP-2/9 and TIMP systems in the hippocampus, in particular within the DG subfield, a region known to undergo structural changes in response to antidepressant treatments.

Previous studies indicate that neuronal activity, both physiological—stimuli that induce long-term potentiation (LTP) (Bozdagi et al., 2007) and pathophysiological—epileptic kainate seizure models (Jourquin et al., 2003), induce changes in hippocampal MMP-2/9 expression and activity. Interestingly, our study points to the possibility that the MMP/TIMP system may also be regulated by clinically relevant antidepressant treatments. Acute ECS induces a rapid (2 h) upregulation of gelatinase activity in the hippocampus, with a similar temporal profile to synaptic plasticity inducing stimuli (1 h), (Nagy et al., 2006) and distinct from excitotoxic kainate treatment (12–72 h) (Jourquin et al., 2003; Szklarczyk et al., 2002). Unlike stimuli that induce LTP, which selectively enhance MMP-9 activity (Nagy et al., 2006), acute ECS upregulates both MMP-2 and -9 activity. However, chronic ECS, which is the therapeutic model, strongly resembles the effects of LTP as it selectively upregulates MMP-9 expression and activity with no regulation of MMP-2. Also the induction of MMP-9 activity by ECS (threefold-acute, 1.5-fold-chronic) is similar in magnitude to the increases seen in MMP-9 activity by LTP (2.5-fold), (Nagy et al., 2006) and is far less than that observed following kainate seizures (sixfold), (Jourquin et al., 2003). Given that ECS causes robust increases in neuronal activity but differs from pathophysiological seizure models in that there is no excitotoxic cell death (Dam and Dam, 1986; Vaidya et al., 1999), it is particularly intriguing to observe the parallels in the manner in which chronic ECS and synaptic plasticity inducing stimuli enhance MMP-9 activity and expression. This raises the possibility that the extent of MMP activation may contribute to a shift from a physiological to pathological outcome in response to heightened neuronal activity.

Acute ECS results in a rapid, robust, and region-specific upregulation of the mRNA expression of TIMP-1, -2, and -4 in the DG. Although previous microarray studies have identified TIMP-1 as a transcriptional target of ECS (Altar et al., 2004; Newton et al., 2003), our study identifies novel effects of acute ECS on TIMP-2 and -4 mRNA expression. Although our results for TIMP-1 regulation agree with the two previous reports (Altar et al., 2004; Newton et al., 2003), indicating an increase in TIMP-1 expression with acute ECS, the extent of regulation we see is smaller in magnitude which may be due to differences in ECS paradigms, time points assessed as well as methodology. Although TIMP-1 is a known activity responsive gene (Nedivi et al., 1993), thus far, no reports indicate an activity-dependent regulation for the other TIMPs. In striking contrast to the TIMP regulation seen with a single seizure, repeated ECS administration results in the loss of TIMP regulation with the exception of a small increase still seen in TIMP-4 mRNA levels. This suggests the possibility that acute versus chronic ECS differentially modulate the MMP/TIMP ratio and thus could influence consequent changes in proteolytic functions.

At present, the mechanisms that underlie the regulation of MMP-2/9 expression and activity and TIMP-1, -2, and -4 mRNA levels in response to ECS are unknown. MMP-9 (Sato and Seiki, 1993) and TIMP-1 (Jaworski et al., 1999) have been suggested to be regulated by the AP-1 transcriptional complex, and TIMP-2 has reported AP-1-binding sites (Hammani et al., 1996). Dynamic changes in the composition of the AP-1 complex known to occur in acute versus chronic ECS (Hope et al., 1994) may contribute to the differential effects of acute ECS on TIMP-1 and -2 and of chronic ECS treatment on MMP-9 transcription. In addition, growth factors like BDNF and basic fibroblast growth factor (bFGF) that are known to be enhanced by ECS (Dias et al., 2003; Gwinna et al., 2002) have also been implicated in the modulation of MMP-9 expression (Im et al., 2007; Miyagi et al., 1998; Sartor et al., 2002). At the level of MMP-2/9 proteolytic activity, multiple pathways may contribute to the effects of ECS. The tissue plasminogen activator, an immediate early gene target of neuronal depolarization (Qian et al., 1993), through changes in the

proteolytic actions of plasmin, could influence MMP-2/9 activity (Werb, 1997). Alternatively, the cAMP cascade, a known target for ECS (Nomikos et al., 2000) and a modulator of MMP-9 activation (Nagy et al., 2006) may also contribute to the effects of acute ECS.

In contrast to chronic ECS, the most robust form of antidepressant therapy, we find selective effects of pharmacological antidepressants on the mRNA expression of specific TIMPs with no accompanying change in either the expression or activity of MMP-2/9. Although we cannot rule out the possibility that we may have missed localized changes in gelatinolytic activity with the zymographic analysis used or that changes in MMP-2/9 mRNA expression may have occurred at time points not tested in this study, our results suggest that unlike ECS, pharmacological antidepressants do not appear to target MMP-2/9 expression or activity. Our findings indicate that the SSRI fluoxetine and the MAOI tranylcypromine similarly modulate TIMP-2 (decrease- acute) and TIMP-3 (increase-chronic) mRNA expression suggesting the recruitment of common regulatory mechanisms. In contrast, the NARI desipramine selectively modulates TIMP-4 mRNA levels. Recent reports in nonneuronal cells suggest a role for serotonin and norepinephrine in the modulation of MMP-2/9 (Yang et al., 2006) and MMP-13 expression (Shum et al., 2002), respectively. However, the influence of monoaminergic neurotransmission on the MMP/TIMP system has not been well addressed in the brain. In this context, our studies point to the intriguing possibility that serotonin and norepinephrine may differentially influence hippocampal TIMP-2, -3, and -4 expression. Collectively, our findings indicate that distinct TIMPs are differentially modulated by various classes of antidepressants. Although our focus in this study has been to address influences on the MMP-2/9 system, TIMPs are known to be inhibitory modulators of the entire MMP family (Kaczmarek et al., 2002). Hence an influence of antidepressants on TIMP expression could bear ramifications to the regulation of other MMP members within the hippocampal DG subfield. Given recent reports of a role for MMP-3 and -7 in hippocampal synaptogenesis (Falo et al., 2006) and dendritic spine plasticity (Bilousova et al., 2006), future studies are required to address whether antidepressants modulate the activity of other key MMPs through an influence on the MMP/TIMP ratio.

Given the recent evidence implicating extracellular proteolysis in the modulation of structural and synaptic plasticity (Dzwonek et al., 2004), the physiological consequences of a regulation of the MMP/TIMP system in the hippocampus by clinically relevant agents like antidepressants is interesting. Proteolytic substrates of the MMPs that may be important to antidepressant-mediated effects include the growth factors BDNF and vascular endothelial growth factor (VEGF) (Chun-Yan et al., 2006; Wary et al., 2003). BDNF and VEGF are thought to play a key role in the effects of antidepressants on hippocampal neurogenesis, endothelial cell proliferation, axonal sprouting, synaptogenesis as well as hippocampal-dependent behavior (Scharfman et al., 2002; Shirayama et al., 2002; Vaidya et al., 1999; Warner-Schmidt and Duman, 2007). In addition, BDNF and VEGF have been reported to exert antidepressant-like effects themselves (Sairanen et al., 2005; Warner-Schmidt and Duman, 2007). Via a modulation of the activity of BDNF and VEGF, the MMP/TIMP system may play a critical role in contributing to the effects of antidepressants on both structural reorganization and behavior. In addition, effects on other MMP substrates such as the cell adhesion molecules brevican, laminin, integrins, and tenascin (Sternlicht and Werb, 2001), as well as guidance molecules like ephrins, could facilitate the effects of antidepressants like ECS on mossy fiber sprouting, synaptogenesis, and angiogenesis in the hippocampus (Eliceiri and Cheresh, 1999; Xu et al., 2003). MMP-2 and -9 have also been implicated in neuronal progenitor migration (Wang et al., 2006) and through a modulation of the microenvironment in the neurogenic niche may contribute to antidepressant effects on hippocampal neurogenesis. Given a recent report that the MMP/TIMP system may regulate dopaminergic neurotransmission (Mizoguchi et al., 2007), it is possible to speculate that antidepressant effects on the MMP/TIMP system in the hippocampus may also influence monoaminergic release and receptor signaling. Possible MMP independent, growth promoting effects of TIMPs have also been suggested and may be relevant to the effects of antidepressants on TIMP expression (Sternlicht and Werb, 2001). Given that the DG is the major site for antidepressant-induced hippocampal remodeling (Warner-Schmidt and Duman, 2006), it is particularly interesting that the effects of antidepressants on MMP-2/9 and TIMPs 1–4 expression in the hippocampus are predominantly observed in this subfield.

In conclusion, our results identify MMP-2/9 and specific TIMPs in the hippocampus as targets of antidepressant treatments. Further, they raise the possibility that ECS and pharmacological antidepressants through effects on MMP-2/9 expression and activity, or changes in MMP/TIMP ratios, could regulate the microenvironment in the hippocampal DG and thus influence structural and synaptic plasticity. Future studies are required to address the functional consequences of an antidepressant regulation of the MMP/TIMP system.

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