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Brain Research 1051 (2005) 81 – 89
BRAIN RESEARCH
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Research Report

Prepulse inhibition and fear-potentiated startle are altered in tissue inhibitor of metalloproteinase-2 (TIMP-2) knockout mice

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Accepted 27 May 2005
Available online 24 June 2005

Abstract

The ability to discriminate between potential dangers and recall those stimuli is essential for survival. This emotional learning requires the involvement of higher brain structures, including the amygdala, hippocampus and related cortical structures. Long-term changes in synaptic transmission and structure are important for the establishment and consolidation of fear memory. The structural changes associated with this synaptic plasticity likely require alterations in the composition of the extracellular matrix (ECM). ECM integrity is maintained by the opposing action of matrix metalloproteinases (MMPs) and their specific inhibitors, tissue inhibitors of metalloproteinases (TIMPs). To date, no studies have examined the role of MMPs or TIMPs in conditioned fear. Here, we show that neither male nor female mice deficient in TIMP-2 (knockout) exhibit prepulse inhibition of the startle reflex, suggesting deficits in pre-attentional sensorimotor gating. In addition, knockout mice and mice expressing a mutant truncated TIMP-2 (knock-down) show deficits in fear-potentiated startle. This is the first report of a phenotype for the TIMP-$2^{-/-}$ mice and suggests that TIMP-2 may play a role in the synaptic plasticity underlying learning and memory.

Theme: Neural basis of behavior
Topic: Neural plasticity

Keywords: Extracellular matrix; Fear conditioning; Learning; MMP; Synaptic plasticity; TIMP

1. Introduction

The ability to learn and form memories depends on specific patterns of synaptic activity that produces rapid and long lasting modifications of synaptic structure. Extracellular matrix (ECM) molecules are thought to participate in this activity-dependent plasticity by regulating the extracellular milieu (reviewed in [11]). In particular, proteases play a role in structural rearrangements in the synapse/spine complex associated with neural plasticity [39,41]. Matrix metalloproteinases (MMPs) are proteases involved in the turnover of a broad-spectrum of ECM proteins and are the main physiological mediators of matrix degradation (reviewed in [42]). Tissue inhibitors of metalloproteinases (TIMPs) inhibit MMP activity by forming tight, but relatively low selectivity complexes with the active forms of MMPs. Although it has been reported that MMP-9 [40] and TIMP-1 [24] expressions are up-regulated in response to kainic acid-induced seizures, the role of MMPs or TIMPs in normal physiological activity-dependent synaptic plasticity has not been investigated. The spatial distribution and temporal regulation of TIMP-2 expression in brain regions known to be involved in learning and memory, including the cerebral cortex, hippocampus and amygdala, suggested a role in synaptic plasticity.

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doi:10.1016/j.brainres.2005.05.057

To test whether TIMP-2 plays a role in synaptic plasticity, mice deficient for TIMP-2 were examined. Two laboratories independently generated mice with altered TIMP-2 expression (TIMP-$2^{-/-}$). Soloway and colleagues developed mice carrying a targeted mutation in the TIMP-2 gene in which no TIMP-2 mRNA was detected in mutant mice, indicating a null mutation [44]. These mice will be referred to as TIMP-2 knockout. In contrast, the homologous recombination targeting strategy used by Birkedal-Hansen and colleagues resulted in the creation of a mutant locus that gives rise to a mRNA with an in-frame deletion that results in a mutant 14 kDa protein with residual MMP-inhibitory activity [6]. These hypomorphs will be referred to as TIMP-2 knock-down mice. Thus far, the only phenotype reported for both TIMP-$2^{-/-}$ mice is the impairment of proMMP-2 activation [6,44]. This reflects the unique ability of TIMP-2 to regulate both the activation of latent proMMPs to the proteolytically active form and the inhibition of MMP activity.

The selection of an appropriate model system to investigate synaptic plasticity in TIMP-$2^{-/-}$ mice required special consideration. In the Morris water maze, a classical behavioral test to assess learning and memory, the experimental subject is required to navigate a swimming pool to find a hidden platform [31]. Animals locate the platform and acquire a spatial memory based on extramaze information [30]. Both the hippocampus [32] and the cerebellum [36] participate in this spatial learning task. Here, we show that both TIMP-2 and TIMP-1 are expressed in the hippocampal formation. Thus, TIMP-1 could compensate for the loss of TIMP-2 and no phenotype would be detectable. Moreover, TIMP-2 knockout mice display motor defects, including decreased time on a motorized rotating rod (RotaRod) and decreased cerebellar neurite outgrowth [25]. In contrast to the Morris water maze, where motor deficits could confound interpretation of learning, fear conditioning does not involve an appreciable motor component. Here, we show that TIMP-2, but not other TIMPs, is expressed in the basolateral nucleus of the amygdala, which has been implicated in the synaptic plasticity associated with conditioned fear. Thus, if TIMP-2 plays a role in synaptic plasticity associated with fear conditioning, TIMP-2 knockout mice may show alterations in either the acquisition or maintenance of conditioned fear. Furthermore, TIMP-2 knockout mice exhibit high levels of exploratory behavior, a subjective measure of anxiety often associated with amygdalar defects. Therefore, fear conditioning appears to be the most appropriate behavioral test to study the role of TIMP-2 in synaptic plasticity.

Pavlovian fear conditioning is an ideal model for examining activity-dependent plasticity associated with learning and memory. In Pavlovian fear conditioning, a neutral conditioned stimulus (e.g., tone) is paired with an aversive stimulus (e.g., foot shock). After a few of these parings, the tone comes to elicit a variety of responses that are indicative of conditioned fear. The startle reflex can show several forms of plasticity, including habituation [7] and potentiation [10]. Fear-potentiated startle (FPS) is defined as an increase in the acoustically elicited startle reflex following tone-shock pairing. The neural circuitry underlying fear conditioning has been well characterized. Plasticity regarding contextual aspects of fear conditioning is thought to be mediated in part by the hippocampus, while cued fear conditioning plasticity is dependent upon the amygdala, particularly the basolateral nuclear complex [8,9,14,16,26]. Defects in fear conditioning in humans are thought to underlie the development of pathological fears, phobias and anxiety.

Here, we report that neither male nor female TIMP-2 knockout mice exhibit prepulse inhibition (PPI) of the startle reflex (a measure of pre-attentional sensorimotor gating) and that FPS is impaired in both male TIMP-$2^{-/-}$ genotypes. This represents the first report of a phenotype for the TIMP-$2^{-/-}$ mice and substantiates examining mice with targeted gene disruptions for phenotypic alterations using behavioral methodology.

2. Experimental procedures

2.1. Animals

Procedures that involved animals were in accordance with the institutional guidelines of the Animal Care and Use Committee at the University of Vermont. Mice bearing a targeted disruption of the TIMP-2 gene have been described elsewhere [6,44]. Several strains of mice, including C57BL/6, display age-dependent high frequency hearing loss [45]. To diminish the likelihood that our findings were influenced by basal differences in hearing, young (postnatal day 90) mice were utilized for the studies. Mice are maintained on a C57BL/6 background after 10 backcrosses. To obtain the three genotypes required for behavioral testing, heterozygous breedings were performed. TIMP-2 knock-down mice are maintained as homozygous recessive. Offspring carrying the TIMP-2 mutant gene were identified by PCR analysis. Genomic DNA was prepared using the DNA extraction kit (Stratagene) following manufacturer's instructions. Products were amplified using HotStartTaq master mix (Qiagen) and either a forward primer for neomycin (5′-GCCCAGTCA-TAGCCGAA-TAGCC-3′) or a forward primer specific for TIMP-2 (PT2: 5′-CCCCTCCACCGTTCCTCTCT-TTTC-3′) in conjunction with a reverse primer specific for TIMP-2 (PT1: 5′-TCACCCAGCCA-GCACCTCACC-3′). PCR products (600 bp for the mutant gene and 250 bp for wild type) were separated on a 1.8% agarose gel.

2.2. Histology

In situ hybridization was performed as previously described [22]. The generation of cDNA probes for the four TIMPs was described in [21]. Immunohistochemistry