β-DYSTROGLYCAN AS A TARGET FOR MMP-9, IN RESPONSE TO ENHANCED NEURONAL ACTIVITY*

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Running Title: β-Dystroglycan as a Synaptic Target for MMP-9

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Matrix Metalloproteinase-9 has recently emerged as an important molecule in control of extracellular proteolysis in the synaptic plasticity. However, no synaptic targets for its enzymatic activity had been identified before. In this report, we show that β-dystroglycan comprises such a neuronal activity-driven target for Matrix Metalloproteinase-9. This notion is based on the following observations: (i) recombinant, autoactivating Matrix Metalloproteinase-9 produces limited proteolytic cleavage of β-dystroglycan; (ii) in neuronal cultures β-dystroglycan proteolysis occurs in response to stimulation with either glutamate or bicuculline and is blocked by Tissue Inhibitor of Metalloproteinases-1, a metalloproteinase inhibitor; (iii) β-dystroglycan degradation is also observed in the hippocampus in vivo in response to seizures but not in the Matrix Metalloproteinase-9 knockout mice; (iv) β-dystroglycan cleavage correlates in time with increased Matrix Metalloproteinase-9 activity and (v) β-dystroglycan and Matrix Metalloproteinase-9 co-localize in postsynaptic elements in the hippocampus. In conclusion, our data identify the β-dystroglycan as a first Matrix Metalloproteinase-9 substrate digested in response to enhanced synaptic activity. This demonstration may help to understand possible role of both proteins in neuronal functions, especially in synaptic plasticity, learning and memory.

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases acting outside the cells and therefore attributed with digesting extracellular matrix components. These enzymes are produced in a latent form and after release to extracellular space are activated by cleavage off the propeptide (1,2). MMPs are involved in a number of physiological and pathological conditions, including development, tissue remodelling, inflammation and tumour metastasis (1-4). Specifically, multiple data show increased expression and activity of MMPs after the brain injury and in certain diseases of the central nervous system (5). On the other hand, physiological roles of MMPs in the adult brain have only recently been appreciated (4). In particular, MMP-9 (also known as gelatinase B) has been implicated in synaptic plasticity, learning and memory (6,7). Furthermore, a marked increase in MMP-9 mRNA, protein and its enzymatic activity in the hippocampal dentate gyrus after kainate-evoked seizures have been shown (8). Kainate, a glutamate analog, produces excitotoxicity in the CA subfields of the hippocampus, sparing...
the dentate gyrus’ granule neurons that, however, undergo aberrant plastic changes (9).

Despite data implicating MMP-9 in neuronal/synaptic plasticity, no synaptic targets for its enzymatic activity have as yet been identified in neurons. However, recent studies have suggested that this enzyme may digest the 43 kDa β-dystroglycan (β-DG) to release a 30 kDa product from the full length subunit. First, Yamada et al. (10) have shown that un-identified MMPs digest β-DG to reveal the 30 kDa product in the peripheral tissues. Second, Kaczmarek and co-workers (11) demonstrated that the appearance of the 30 kDa β-DG degradation product in the hippocampus following kainate treatment coincides with the increased levels of MMP-9. Recently, it was reported that β-DG expressed at the astrocyte endfeet can be specifically cleaved by macrophage-derived gelatinases (MMP-2 and MMP-9) during leukocyte penetration of the basement membrane in experimental autoimmune encephalomyelitis (12). Finally, selective cleavage of β-DG by MMP-2 and MMP-9 was also observed in schwannoma cell line RT4 by Zhong and co-workers (13). Although, none of those studies has directly addressed β-DG proteolysis in neurons or, more specifically, the synapses, it is of note that β-DG can be expressed in the postsynaptic membranes in the brain, as previously shown by Zaccaria et al. (14).

Dystroglycan (DG) is a central protein in the dystrophin-glycoprotein complex (DGC) that links dystrophin and the intracellular cytoskeleton with extracellular matrix and anchors the whole complex at the membrane. DG is translated from a single mRNA as a precursor peptide, which is subsequently cleaved into two, non-covalently associated subunits: extracellular (α) and transmembrane (β) (15,16). α-DG is highly glycosylated peripheral membrane protein that binds via its carbohydrate side chains to many extracellular matrix ligands such as laminins, agrin, perlecan (17) and to the presynaptic neurexins in the brain (18). β-DG in turn, through its proline-rich C-terminus binds dystrophin or urotrophin, and this interaction links DGC to F-actin cytoskeleton (17,19). Even though the role of β-DG in the brain, especially in neurons is not known, it is worth mentioning that the specific gene ablation of dystroglycan in the brain produces deficits in neuronal plasticity, similar to those caused by inhibition of MMP-9 (7,20).

Taken together, the aforementioned data lead us to hypothesize that β-DG is a synaptic target for MMP-9. In this report we provide several lines of evidence supporting such a notion.

Experimental procedures

Animals. In these studies we used 12 Wistar adult rats and 30 newborns. We also used 15 MMP-9 knockout mice described previously (21) and 15 wild-type mice. Mice were kindly provided by Dr Zena Werb. Prior to the experiment, the animals were kept in the laboratory animal facility with free access to food and water, with a 12 hour light/dark cycle. All the procedures with animals were carried out according to guidelines of 1st Warsaw Ethical Committee on animal research and based on permissions nos.: 480/2005 and 523/2005.

Recombinant autoactivating MMP-9 (aaMMP-9). Expression of previously described (22) auto-activating mutant of MMP-9 was performed according to Bac-to-Bac Baculovirus Expression System manual (Invitrogen). Briefly, G100L MMP-9 mutant (generous gift from Katherine Fisher, Pfizer) was cloned to pFastBac1 and resulting recombinant plasmid was used to transform DH10Bac competent cells. Colonies, which performed transposition were identified by blue-white selection and recombinant bacmid was isolated and verified by PCR. The S21 insect cells were transfected with recombinant bacmid using Cellfectin® reagent (Invitrogen) to obtain recombinant baculovirus. After amplification and titration of the recombinant baculovirus, S21 cells were infected and incubated in SF-900ISFM serum free medium (Gibco). Forty eight hours post-infection the culture medium was collected and recombinant aaMMP-9 was purified on Gelatin Sepharose™ 4B (GE Healthcare) as previously described (23).

Adenoviral vectors. Viral stocks were prepared according to the procedure described before (24). The recombinant Ad-β-Galactosidase (Ad-β-Gal) has also been described previously (25). The recombinant Ad-TIMP-1 has been described by Okulski et al. (26).
Pentylenetetrazole (PTZ) stimulation. Pharmacological stimulation was done by the intra-peritoneal PTZ injection (50 mg/kg) and animals were sacrificed five minutes after the seizure onset. Hippocampi were isolated and homogenized in ice-cold homogenization buffer (50 mM Tris-HCl pH 7.5; 150 mM NaCl; 5 mM CaCl$_2$ and 10 μl/ml Protease Inhibitor Cocktail, Sigma). Homogenates were then analyzed by the Western blot approach.

Hippocampal extracts and their treatment with αMMP-9. Wistar rats were sacrificed and hippocampi were isolated, and homogenized in homogenization buffer (20 mM Tris-Cl pH 7.4 at 4°C; 137 mM NaCl; 25 mM β-glycerophosphate; 2 mM NaPPi; 1 mM Na$_3$VO$_4$; 1% Triton X-100; 10% glycerol; 2 mM benzamidine; 0.5 mM DTT; 1 mM PMSF). One hundred micrograms of hippocampal extracts were incubated at 37°C for an hour with 100 ng of recombinant MMP-9 in reaction buffer (50 mM Tris-Cl pH 7.5; 10 mM CaCl$_2$; 1 μM ZnCl$_2$), also in the presence of 10 mM 1, 10 phenathroline. Homogenates were then analysed by the Western blot.

Cell culture stimulation. The cultures were stimulated as described (27) with slight modifications. Cells were treated with either 50 μM glutamate (in BME medium) on 7 DIV, or 10 μM bicuculline (cultured in Neurobasal medium) on 14 DIV. For the cells treated with glutamate, CNQX (40 μM) was added to all plates the night before the stimulation, to reduce endogenous synaptic activity, and 5 μM nimodipine was added 30 minutes before the stimulation.

Western blotting. After the stimulation the cells were lysed in the lysis buffer (20 mM Tris-Cl pH 7.4 at 4°C; 137 mM NaCl; 25 mM β-glycerophosphate; 2 mM NaPPi; 1 mM EDTA; 1 mM Na$_3$VO$_4$; 1% Triton X-100; 10% glycerol; 2 mM benzamidine; 0.5 mM DTT; 1 mM PMSF and 10 μl/ml Protease Inhibitor Cocktail, Sigma), the protein concentration in each sample was measured using Bradford method (Sigma) and samples were brought to equal protein concentration by H$_2$O dilution. Lysates were mixed with 5 × SDS sample buffer, denatured and 20 μg of total protein samples were loaded on 12% SDS-polyacrylamide gels. The samples were electrotransferred onto polyvinylidene difluoride membranes (Immobilon-P, Millipore), which were blocked 2 hours at RT with 10% nonfat milk in Tris-buffered saline with 0.1% Tween-20 (TBS-T). After blocking, the membranes were incubated at 4°C overnight with following antibodies: anti-β-dystroglycan (NCL-b-DG, Novocastra, 1:500), anti- Glyceraldehyde-3-phosphate dehydrogenase – GAPDH (MAB374, Chemicon, 1:2000) and anti-β-actin (A5441, Sigma, 1:6000), all diluted in 5% nonfat milk in TBS-T. Membranes were than incubated 2 hours at RT with peroxidase-labelled secondary antibody diluted 1:10,000 in 5% nonfat milk in TBS-T. After washing, peroxidase activity were visualized with ECLplus reagent (GE Healthcare).

Gel zymography. Samples of the culture medium were mixed with 3 × SDS sample buffer without DTT and loaded on 8% SDS-polyacrylamide gels containing 2 mg/ml gelatine. After electrophoresis, the gels were washed twice with 2.5% Triton X-100 for 30 minutes at RT and incubated overnight in developing buffer (50 mM Tris-Cl pH 7.5; 10 mM CaCl$_2$; 1 μM ZnCl$_2$; 1% Triton X-100; 0.05% Triton X-100). The gel was stained with Coomassie Blue and destained with 10% acetic acid. The gel was then soaked in a solution of 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 50 mM Tris HCl (pH 7.4) for 15 minutes, washed once with water and incubated overnight in developing buffer (50 mM Tris-Cl pH 7.5; 10 mM CaCl$_2$; 1 μM ZnCl$_2$; 1% Triton X-100; 0.05% Triton X-100) at 37°C, then washed with 2.5% Triton X-100 for 15 minutes and stained.
Gels were stained with Coomassie Brilliant Blue and shortly destained. Electron-microscopic immunocytochemistry. This was performed according the previously described procedures (28,29). In brief, normal Wistar rats were perfused transcardially with a fixative consisting of 4% paraformaldehyde plus 0.1% glutaraldehyde. The brains were postfixed in the same fixative, cut into 0.5- to 1.0-mm slices, cryoprotected, snap-frozen in liquid propane (-170°C), and subjected to freeze substitution (in Leica EM AFS apparatus). Specimens were then embedded in Lowicryl HM20 resin, polymerized by UV light at -45°C to 0°C, and cut into ultrathin sections. The immunoreactions consisted of sequential incubations with a mixture of primary antibodies: (i) a mouse anti-β-dystroglycan (NCL-b-DG, Novocastra) (undiluted) and (ii) rabbit anti-MMP-9 (Torrey Pines Labs) diluted 1:50, followed by species-specific goat secondary antibodies coupled to 6nm or 10 nm gold particles (GE Healthcare). The specimens were examined with a Philips CM 10 electron microscope at 60 kV.

RESULTS

Previous studies localized dystroglycan to various cell compartments in the brain, including dendritic spines (14,30). Similarly, we have shown that MMP-9 is expressed postsynaptically in asymmetric synapses (31). To confirm that the two molecules are colocalized, we performed a high-resolution, double-labelling immunogold analysis. Gold particles representing β-dystroglycan (6 nm) were found in close vicinity to gold particles signaling MMP-9 (10 nm) (Fig. 1). The subcellular colocalization of β-dystroglycan and MMP-9 is consistent with the idea that MMP-9 could be secreted to the extracellular space, allowing for a focal, MMP-9 mediated proteolysis of membrane-bound β-dystroglycan.

To examine whether β-DG is cleaved upon neuronal stimulation, we employed cortical primary neuronal cultures that include minimal component of glia (around 10%) (32). The cultures were stimulated in vitro with 50 μM glutamate and the whole cell lysates were analyzed by the Western blotting. As shown in Figure 2, glutamate exposure led to a significant increase in the level of the cleaved, 30 kDa form of β-DG. This increase was notable at 10 min of stimulation and was followed by a decrease over the next 10 min period.

To resolve whether cleavage of β-DG after neuronal stimulation is MMP-dependent we infected the cortical neuronal cultures with the adenoviral vector (Ad-TIMP-1), carrying cDNA encoding tissue inhibitor of metalloproteinases -1 (TIMP-1). TIMP-1 is known to bind MMP-9 with high affinity and to block its enzymatic function (33). Twenty four hours after the infection, the cultures were stimulated with 50 μM glutamate and the cell lysates were analyzed by the Western blotting. As shown in the Figure 3, the glutamate-stimulation-dependent increase in β-dystroglycan cleavage was abolished following infection with Ad-TIMP-1 but not following infection with Ad-β-Galactosidase, used as a control (Fig. 3A). In the absence of glutamate exposure, there was no difference between TIMP-1 infected and β-Galactosidase infected cells with respect to the level of the β-DG degradation product.

Next, we investigated whether exogenous, extracellular MMP-9 can cleave β-DG on the cultured. Indeed, treatment of the neuronal cultures for 30 min with recombinant autoactivating mutant protein of MMP-9 (aaMMP-9) caused β-DG cleavage that could not be significantly increased by subsequent glutamate stimulation (Fig. 3B). Furthermore, when hippocampal homogenates were incubated with recombinant aaMMP-9 (Figure 3C), there was an accumulation of the degradation (30 kDa) product of β-DG that was completely abolished in the presence of 1, 10-phenathroline, the Zn²⁺ chelator, known to inhibit MMP activity.

We have also investigated whether rapid activity-induced proteolysis of β-DG can occur in vivo and if so, whether this cleavage is MMP-9 dependent. MMP-9 knockout and wild-type mice were injected intraperitoneally with pentylenetetrazole, PTZ, a proconvulsant and GABA_A receptor antagonist that is known in order to provoke increased synaptic stimulation in vivo (34). Five minutes after the seizure onset, the hippocampi were isolated and subjected to the Western blot analysis. Figure 3D shows a significant increase in the level of 30 kDa β-DG form in the PTZ treated.
Finally, we recorded cleavage of β-DG in neuronal cultures treated with bicuculline. At the concentration used (10 μM) this GABA<sub>A</sub> receptor blocker induces an increased glutamate receptor-dependent activity in a functional neuronal network (35). Figure 4A shows that the MMP-9 (but not MMP-2) activity is strongly increased at five minutes of stimulation and returns to basal level within the next five minutes. An accumulation of the 30-kDa β-DG form was observed to follow directly enhanced MMP-9 activity (Fig. 4B). Expression of TIMP-1 effectively prevented β-DG cleavage after bicuculline administration (Fig. 4C).

DISCUSSION

In this report we provide direct evidence that β-DG is a target of neuronal MMP-9 in the brain in vivo as well as in neuronal cultures in vitro. The following lines of evidence support this notion: (i) recombinant, auto-active MMP-9 produces limited proteolytic cleavage of β-DG in brain neurons cultured in vitro as well as in the hippocampal ex vivo extracts. (ii) in neuronal cultures β-DG proteolysis occurs in response to synaptic activation with either glutamate or bicuculline. The β-DG proteolysis is blocked by TIMP-1, a metalloproteinase inhibitor; (iii) the same phenomenon of β-DG degradation is also observed in the hippocampus in vivo in response to seizures and it is absent in the MMP-9 knockout mice; (iv) both in the cultured brain neurons and in the hippocampus in vivo, β-DG cleavage correlates in time with increased MMP-9 activity and β-DG and MMP-9 co-localize in postsynaptic elements in the hippocampus.

The fact that β-DG was found by electron-microscopic immunocytochemistry to be membrane-bound is consistent with its previously identified localization in various cell types, including neurons (17,36). Our data also support the notion that the protein can be present in asymmetric postsynaptic specializations, which belong to the excitatory neurons (14). In the close vicinity of the β-DG we have also observed MMP-9. However, it is known that the enzyme is released in the latent form to acquire its enzymatic activity outside the cells (1,2) and thus it is not very probable that MMP-9 could digest β-DG intracellularly. Notably, we have not observed colocalization of MMP-9 with β-DG within the synaptic cleft, however, this result can be explained by our data obtained with neuronal cultures (see Fig. 4A), as well as literature indicating that MMP-9 is available outside the cells only very transiently (37). Thus our colocalization results indicated only a potential role of MMP-9 in β-DG proteolysis. The extensive evidence for such a role has been obtained in subsequent functional studies, carried out mostly in vitro.

We demonstrated limited β-DG proteolysis in the neuronal cultures as well as in the hippocampus in vivo. This phenomenon was found to be a very rapid process, since already within a few minutes after the neuronal stimulation either in vitro or in vivo we observed appearance of the truncated form of the protein. Furthermore, of special note is the finding that β-DG processing is also very transient phenomenon, as already within 20 minutes after the stimulation a decrease in the level of the 30 kDa-form has been observed. This result could be explained by a rapid turnover of the molecule.

We have also demonstrated that activity-dependent processing of β-DG occurs via MMP activity as it is blocked by TIMP-1 and furthermore this processing is critically dependent on MMP-9 and not other MMPs, since it does not take place in MMP-9 knockout mice. Moreover, incubation of both neuronal culture and hippocampal homogenate with recombinant active MMP-9 significantly increased the level of 30 kDa form of β-DG. Altogether, these results suggest that MMP-9 is responsible for activation-dependent cleavage of β-DG. Interestingly, however, in non-stimulated neurons in culture, as well as in the hippocampi ex vivo and in vivo we observed noticeable levels of truncated form of β-DG. These may be due to basal activity of either MMP-2 or MMP-9 as both proteases can cleave β-DG (12,13). However, in MMP-9 knockout mice the level of cleaved β-DG is not increased in response to enhanced neuronal activity, indicating that MMP-9 is critical for stimulation-driven β-DG cleavage. Furthermore, treatment of the neuronal cultures with 10 μM bicuculline shows that after the stimulation only MMP-9 but not MMP-2 levels
are increased in the cell medium. This result also indicates that activation of synaptic receptors is sufficient for releasing MMP-9 to extracellular environment as quickly as 5 minutes after the stimulus (see also: 37) and is immediately followed by limited proteolysis of β-DG. Importantly, this time-frame of the accumulation of 30 kDa β-DG cleavage product after the synaptic activation, is the same as following glutamate stimulation of the cultured neurons.

Finally, it should be emphasized that this demonstration of the first specific MMP-9 target in response to enhanced synaptic activity, at the central nervous system synapses, reported herein, may help to understand possible role of both MMP-9 and β-DG proteins in neuronal functions, especially in synaptic plasticity, learning and memory. Recent studies have shown that MMP-9 is activated during LTP and learning, and its inhibition by either genetic or pharmacological means prevents formation of long-lasting plastic changes as well as long-term memory (6,7). Also in humans increased MMP-9 activity, resulting from the specific gene polymorphism, was found to ameliorate dementia symptoms (38). In turn, Moore and co-workers (20) have shown that mutant mice selectively deficient in the brain dystroglycan suffer from L-LTP deficits in the hippocampus. Furthermore, functional disruption of the DGC is observed in several forms of inherited muscular dystrophies such as muscle–eye–brain disease, Walker-Warburg syndrome, Fukuyama congenital muscular dystrophy, which are all associated with cognitive deficits (39-41). In addition, mutations in the genes encoding dystroglycan-binding proteins such as laminin (its extracellular ligand) as well as dystrophin (linking it with the F-actin) are associated with mental retardation (congenital and Duchenne muscular dystrophy, respectively). Notably, we have previously demonstrated specific changes in the hippocampal expression patterns of transcripts encoding dystrophin and neurexins (the pre-synaptic interacting partners of DG) following kainate and PTZ treatment in vivo (42,43). This suggests a functional role for the entire DAG complex at central synapses and in their plasticity. Moreover, it was shown that β-DG can either directly or indirectly interact with extracellularly regulated kinases (ERK) as well as with focal adhesion kinase (FAK) (30,44) and both those kinases are important for neuronal plasticity including the induction of LTP (45). It was also shown recently in Schwann cells that the 30 kDa-form of β-DG has a greater affinity fore the short isoform of utrophin (Up71) than for the dystrophin isoform (Dp116), which in turn has greater affinity to full length β-DG (46). Although these interactions have been found in glia, it is probable that similar change in affinity may influence actin cytoskeleton in neurons and allow for changes in, for example, dendritic spine morphology.

REFERENCES


FOOTNOTES
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The abbreviations used are: MMP, Matrix metalloproteinase; MMP-2, Matrix metalloproteinase-2, EC 3.4.24.24; MMP-9, Matrix metalloproteinase-9, EC 3.4.24.35; TIMP, tissue inhibitors of matrix metalloproteinases; LTP, long-term potentiation; β-DG, β-dystroglycan; DGC, dystrophin-glycoprotein complex; DG, Dystroglycan; aaMMP-9, autoactivating MMP-9; Ad-β-Gal, Adenovector with β-Galactosidase; Ad-TIMP-1, Adenovector with Tissue Inhibitor of Metalloproteinase-1; PTZ, pentylenetetrazole, BME, Basal Medium Eagle; AraC, Cytosine-β-D-arabinofuranoside; TBS-T, Tris-buffered saline supplemented with 0.1% Tween-20; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; ERK, extracellularly regulated kinases; FAK, focal adhesion kinase

FIGURE LEGENDS
Figure 1. Fine structural immuno-colocalization of β-dystroglycan and Matrix Metalloproteinase-9 in the rat hippocampus at the electron microscopic level. Ultrathin sections of rat hippocampus were sequentially incubated with a mixture of primary antibodies: mouse anti-β-dystroglycan and rabbit anti-MMP-9, followed by species-specific goat secondary antibodies coupled to 6 nm or 10 nm gold particles. The specimens were examined with an electron microscope. Immunogold electron-microscopic visualization of β-dystroglycan (6 nm particles, arrows) and MMP-9 (10 nm particles,
arrowheads) immunoreactivities in dendritic spines. Note strict colocalization of the two proteins at some sites, including postsynaptic density.

Figure 2. β-dystroglycan is cleaved shortly after neuronal stimulation. After stimulation of cortical neuronal culture with 50 μM glutamate, cells were lysed and β-dystroglycan cleavage was checked by the Western blot. Activity-dependent proteolysis of the full length β-dystroglycan (β-DG43) is transient since 20 minutes after stimulation the levels of the cleaved form (β-DG30) are significantly lower in comparison to 10 minutes after stimulation. Glu 5’, Glu 10’, Glu 20’ – samples (all sets in triplicate) stimulated with glutamate for 5, 10 and 20 minutes respectively; as a control serves non-stimulated cells; GAPDH – Glyceraldehyde-3-phosphate dehydrogenase – loading control. The representative out of 4 experiments is shown.

Figure 3. Cleavage of β-dystroglycan after stimulation is Matrix Metalloproteinase-9 dependent. A) Cortical neuronal culture was infected with adenoviral vector caring cDNA of TIMP-1 (Ad-TIMP-1) and a control adenovector caring cDNA of β-Galactosidase (Ad-β-Gal). Forty eight hours post infection the cultures were stimulated for 10 minutes with 50 μM glutamate (Glu 10’) and the results of β-DG cleavage was checked by the Western blot. Increase in the amount of cleaved β-dystroglycan (β-DG30) after the stimulation with glutamate is reduced in samples derived from the cultures infected with Ad-TIMP-1, when compared to infection with a control adenovector. B) Thirty minutes incubation of cortical culture with recombinant autoactivating mutant of Matrix Metalloproteinase-9 (MMP-9) causes limited proteolysis of β-dystroglycan and this effect is not additive to glutamate-dependent cleavage of β-dystroglycan (Glu 10’). As a control, the cultures were treated with vehiculum (Vehic.) C) One hour incubation of hippocampal homogenates with αMMP-9 causes cleavage of β-dystroglycan and this effect is completely eliminated by Zn²⁺ chelator, phenanthroline. β-DG30, truncated form of β-DG. D) Seizures induced by intraperitoneal injection of pentylenetetrazole (PTZ) causes β-dystroglycan cleavage shortly after the onset of seizures in wild type (MMP-9 WT) mice, but not in MMP-9 knockout mice (MMP-9 KO). GAPDH– Glyceraldehyde-3-phosphate dehydrogenase and β-actin – loading controls. All sets of samples are derived from triplicate experiments, i.e, either three animals or three cultures were employed. In addition, the experiments were reproduced at least once.

Figure 4. Limited proteolysis of β-dystroglycan is synaptic stimulation-dependent and follows the release of MMP-9. Bicuculline stimulation blocks GABAₐ receptors and reduces threshold of the neuronal activation. A) Cortical cultures (in triplicate) were stimulated with 10 μM bicuculline and conditioned media were then collected and analysed with gelatine zymography. Gelatinolytic activity (degradation of gelatine presented in the SDS gel) of MMP-9 demonstrates rapid release of MMP-9 to the culture media already within 5 minutes after the bicuculline administration (Bic. 5’). This phenomenon is transient as 10 minutes after the stimulation, the level of MMP-9 is not different from the control. act-MMP-9 – MMP-9 without propeptide (activated), pro-MMP-9 – latent form of MMP-9. B) The Western blot showing β-dystroglycan cleavage after the bicuculline stimulation. As soon as 10 minutes after the drug administration there is a significant increase in the level of cleaved, 30 kDa form (β-DG30). C) Infection of the cortical culture with adenoviral vector carrying cDNA of TIMP-1 (Ad-TIMP-1) abolishes cleavage of β-dystroglycan after bicuculline stimulation in comparison to infection with a control adenovector (Ad-β-Gal). GAPDH – Glyceraldehyde-3-phosphate dehydrogenase – loading control.
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Figure 2, Michaluk et al.
Figure 3, Michaluk et al.
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