Activation of NMDA Receptors Upregulates A Disintegrin and Metalloproteinase 10 via a Wnt/MAPK Signaling Pathway

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A disintegrin and metalloproteinase 10 (ADAM10) is the constitutive α -secretase that governs the nonamyloidogenic pathway of β -amyloid precursor protein processing and is an attractive drug target for treating Alzheimer's disease. To date, little is known about the mechanism by which ADAM10 is regulated in neurons. Using mouse primary cortical neurons, we show here that NMDA receptor (NMDAR) activation led to upregulation of the genes encoding ADAM10 and β -catenin proteins. Interestingly, the ADAM10 upregulation was abolished by inhibitors of Wnt/ β -catenin signaling. Conversely, activation of the Wnt/ β -catenin signaling pathway by recombinant Wnt3a stimulated ADAM10 expression. We further showed that both the NMDAR- and Wnt3a-induced ADAM10 upregulation was blocked by ERK inhibitors. We suggest that the NMDARs control ADAM10 expression via a Wnt/MAPK signaling pathway.

Introduction

Amyloid- β (A β) peptides (A β_{1-40} and A β_{1-42}) are the proteolytic products of β -amyloid precursor protein (APP), and they likely play a central role in the pathogenesis of Alzheimer's disease (AD). A β accumulation in the brain has been shown to impair neurotransmitter release, long-term potentiation, synapse formation (Hu et al., 2009), and learning and other cognitive functions (Johnson-Wood et al., 1997). Although APP processing can undergo the amyloidogenic pathway to generate A β , it may also go through the nonamyloidogenic pathway to block A β production and generate soluble APP α (sAPP α). sAPP α is known to play neuroprotective and neurotrophic roles (Cheng et al., 2002; Stein et al., 2004). Thus, effective therapeutic approaches could be involved in switching APP processing to the nonamyloidogenic pathway (Dewachter and Van Leuven, 2002).

While the amyloidogenic pathway is mediated by the β - and γ -secretases (BACE1 and presenilin1/2, respectively), the nonamyloidogenic pathway is regulated by the α - and γ -secretases (Lichtenthaler, 2011). α -Secretase is the key proteinase that controls APP

processing in the nonamyloidogenic pathway. A disintegrin and metalloproteinase (ADAM) family, including ADAM9, ADAM10, and ADAM17, constitutes the main α -secretase candidates (Lichtenthaler, 2011). Among them, ADAM10 is the primary endogenous α -secretase (Jorissen et al., 2010; Kuhn et al., 2010). Evidence from both AD mouse models and AD human patients suggests the involvement of ADAM10 in the regulation of AD pathogenesis (Epis et al., 2010; Kim et al., 2010). In addition to APP processing, ADAM10 is involved in the shedding of many other biologically important proteins (e.g., Notch, N-cadherin, and Nectin-1) from cell membranes (Hartmann et al., 2002; Uemura et al., 2007; Kim et al., 2010).

Our understanding of the mechanisms that regulate ADAM10 activity in neurons is limited. ADAM10 may be regulated at multiple levels, including mRNA translation (Lammich et al., 2010) and processing of the pro-ADAM10 (Anders et al., 2001). Retinoic acid receptors can increase the expression of ADAM10, leading to a decrease in A β peptides (Tippmann et al., 2009; Donmez et al., 2010). The activity of ADAM10 is regulated by NMDA receptors (NMDARs), which modulate synaptic trafficking of ADAM10 via synapse-associated protein-97 (SAP97) (Marcello et al., 2007). Interference of the interaction between ADAM10 and SAP97 causes sporadic AD (Epis et al., 2010).

In the current study, we found that NMDAR activation increased ADAM10 protein expression in primary cortical cultures. Furthermore, we also revealed that this NMDAR-regulated ADAM10 protein expression was mediated by Wnt and MAPK/ERK signaling. Our findings uncover novel molecular mechanisms that control synaptic activity-induced synthesis of ADAM10.

Materials and Methods

Primary cortical cultures. Primary cortical cultures from mouse embryos of either sex were prepared as described previously (Hoey et al., 2009). Briefly, cortices were dissected from C57BL/6J mouse embryos (E18) and

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Figure 1. NMDAR activation increases ADAM10 protein in cortical cultures. **A**, NMDA toxicity. Cultures were treated with NMDA for 90 min, and cell viability was measured by MTT 24 h later. **B**, NMDA-induced ADAM10 upregulation. Cortical cultures (10DIV) were stimulated with 50 μ M NMDA, and cell lysates were analyzed by immunoblotting with BACE1 or ADAM10 antibody ($n = 5$, *** $p < 0.001$ vs 0 min). **C**, NMDA-induced α -secretase activity revealed by immunoblotting of APP-CTFs (C83 and C99) (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs 0 min). **D**, D-APV blocks NMDA-induced ADAM10. Cultures preincubated with 100 μ M D-APV for 30 min were treated with 50 μ M NMDA for 15 min ($n = 4$). **E**, Effect of actinomycin D on NMDA-induced ADAM10. Cultures were treated with 25 μ M actinomycin D for 30 min before NMDA administration (15 min); both actinomycin D and NMDA were dissolved in 0.1% DMSO, and cultures treated with 0.1% DMSO were used as controls ($n = 3$). The signal intensity of the bands on immunoblots was normalized to GAPDH. **F**, Effect of α -amanitin on NMDA-induced ADAM10. **G**, NMDA-regulated ADAM10 transcription. Cultures were treated with 50 μ M NMDA. The mRNA level of ADAM10 was measured by quantitative PCR and normalized to β -actin mRNA ($n = 4$, * $p < 0.05$, *** $p < 0.001$ vs 0 min).

washed in HBSS (Invitrogen). Meninges and excess white matter were removed. Cortical slices (~ 0.5 mm²) were prepared with microscopic pinches and transferred to a sterile tube containing HBSS. Cortical slices were treated with 0.25% trypsin (Sigma) for 8 min at 37° and were dissociated to single-cell suspension, followed by centrifugation at $120 \times g$ for 5 min. The cell pellets were resuspended in DMEM (Invitrogen) containing 10% FBS and 1% penicillin-streptomycin (Weijia). Cells were planted on culture plates coated with 20 μ g/ml poly-D-lysine (Sigma) in 0.1 M borate buffer, pH 8.5 (50 mM H₃BO₃, 12.5 mM Na₂B₄O₇ (10H₂O)). Two hours later, DMEM was replaced with Neurobasal Medium (Invitrogen) supplemented with 2% B27 (Invitrogen), 1% penicillin-streptomycin, and 0.5 mM L-glutamine (Invitrogen). Cells were cultured at 37° in a humidified incubator of 95% air and 5% CO₂. One-third of the medium was replenished every 3 d until 10 d *in vitro* (DIV). Under our culturing conditions, 90% of the cells were neurons, as revealed by MAP2 staining.

MTT assay. Cortical cultures in 96-well plates at 9DIV were treated with NMDA for 90', followed by continued culturing in fresh medium for 24 h. For MTT assays, 20 μ l of 5 mg/ml MTT (Sigma) was added to individual wells and incubated for 4 h. The water-insoluble formazan was

dissolved in dimethyl sulfoxide (DMSO), and the optical absorbance was measured at 490 nm using Multiskan Ascent.

Antibodies. Millipore; mouse monoclonal β -catenin antibody (1:1000, Cell Signaling Technology); rabbit APP C-terminal antibody (1:8000, Sigma); mouse monoclonal BACE1 antibody (1:1000, R&D Systems); mouse monoclonal *p*-ERK1/2 antibody (1:500, Santa Cruz Biotechnology); rabbit ERK1 antibody (1:500, Santa Cruz Biotechnology); rabbit ERK2 antibody (1:500, Santa Cruz Biotechnology); mouse monoclonal GAPDH antibody (1:1000, Santa Cruz Biotechnology); HRP-conjugated mouse IgG antibody (1:10,000, Pierce); and HRP-conjugated rabbit IgG antibody (1:5000, Pierce).

Western blotting and quantification. Cortical cultures (10DIV; 0.5×10^6 cells/well on 12-well plates) were lysed with 100 μ l of 2 $\times$ SDS sample buffer (62.5 mM Tris-HCl buffer, pH 6.8, 10% glycerol, 2% SDS, 5% β -mercaptoethanol, and 0.01% bromophenol blue). Lysates were transferred to 1.5 ml Eppendorf tubes, boiled for 10 min, and centrifuged at $13,400 \times g$ at 4° for 5 min. Total protein (40 μ g) was loaded on 10% SDS-PAGE. After electrophoresis, the protein was transferred to a 0.2 μ m polyvinylidene fluoride membrane (Millipore). The membrane with transferred protein was blocked with 5% skim milk in TBS-T (20 mM

Figure 2. Blockage of Wnt/ β -catenin signaling inhibits NMDAR-dependent ADAM10 expression. **A**, NMDA stimulation caused β -catenin increase. Cortical cultures were stimulated with 50 μ M NMDA before immunoblotting ($n = 5$; $*p < 0.05$ vs 0 min). **B**, DKK1 impaired NMDA-induced ADAM10 increase. Cultures were treated with or without DKK1 and/or NMDA. When cotreated with both, cultures were pretreated with 100 ng/ml DKK1 for 60 min before NMDA administration (50 μ M; 15 min) ($n = 4$). **C**, Anti-Wnt3a antibody diminished NMDA-induced ADAM10 increase. Wnt3a antibody was used to neutralize Wnt3a in the medium. ($n = 3$). **D**, Indo (50 μ M, 30 min) and XAV939 (1 μ M, 30 min) abolish NMDA-induced β -catenin increase. ($n = 3$). **E**, Indo (50 μ M, 30 min) and XAV939 (1 μ M, 30 min) abolish NMDA-induced ADAM10 upregulation ($n = 3$). **F**, **G**, Effects of IWR-1 (10 μ M, 30 min) on NMDA-induced β -catenin and ADAM10, respectively.

Tris-HCl, pH 7.4, 0.15 M NaCl, 0.1% Tween 20) for 2 h at room temperature (RT), incubated with primary antibodies in blocking solution overnight at 4°C, and washed with TBST (3×10 min). After incubating with HRP-conjugated anti-mouse or rabbit secondary antibodies (1 h at RT) and washing, protein bands were detected with an ECL kit (Pierce), according to manufacturer's instructions. The optical density of each band was quantified using Quantity One 4.6.2 (Bio-Rad).

Quantitative RT-PCR. Cortical cultures (10DIV) were stimulated with 50 μ M NMDA. Total RNA was purified from the cultures with TRIZOL (Invitrogen) according to the manufacturer's instructions. Total RNA (0.5 μ g) was used for reverse transcription, followed by quantitative real-time PCR using PrimeScript RT reagent kit (TaKaRa). Real-time PCRs [25 μ l mixture, which contains 12.5 μ l of 2 $\times$ SYBR Premix Ex Taq, 0.5 μ l of PCR forward primer (10 μ M), 0.5 μ l of PCR reverse primer (10 μ M), 9.5 μ l of H₂O, and 2 μ l of cDNA] were performed with the iQ5 system (Bio-Rad); cycling protocol: preincubation at 95°C for 30 s followed by 40 cycles of 95°C/5 s and 60°C/20 s. Primer sequences were as follows: ADAM10 reverse primer: 5'-ACCACTGAAGTCTTGCTC-CAC-3'; ADAM10 forward primer: 5'-TTATGCCATGTTTGCTG-CATGA-3'; β -actin reverse primer: 5'-GCAATGCCTGGGTACATG-

GTGG-3'; β -actin forward primer: 5'-ACGCGTCGACCTCCTT-GCAGTCCATTTT-3'.

Statistical analysis. Data in summary graphs (mean $\pm$ SEM) were from at least three independent experiments. One-way ANOVA was performed with SPSS 16.0.

Results

NMDAR activation increases ADAM10 protein

NMDAR activation is known to stimulate α -secretase activity, which is responsible for shedding APP and other membrane proteins (Uemura et al., 2007; Hoey et al., 2009). Because ADAM10 is the primary candidate of α -secretase (Jorissen et al., 2010; Kuhn et al., 2010), we tested the hypothesis that ADAM10 synthesis is regulated by NMDARs. Cultures were stimulated with different concentrations of NMDA. Stimulation with up to 50 μ M NMDA did not change cell viability (Fig. 1A). After stimulation with 50 μ M NMDA, ADAM10 and APP protein levels were measured by Western blotting analysis. NMDA caused a rapid transient increase of ADAM10 protein, which peaked with a 1.7-fold increase at 15 min

Figure 3. Wnt3a induces ADAM10 expression. **A**, Time course of Wnt3a-induced ADAM10 expression. Immunoblots of β -catenin, BACE1, and ADAM10 from cultures treated with recombinant Wnt3a (100 ng/ml) for various time ($n = 4$, * $p < 0.05$ vs 0 min (β -catenin); * $p < 0.05$ vs 0 min (ADAM10)). **B**, Dose effect of Wnt3a on ADAM10 expression. Immunoblots of β -catenin, BACE1, and ADAM10 from cultures treated with different amounts of recombinant Wnt3a [$n = 4$, * $p < 0.05$, ** $p < 0.01$ vs 0 min (β -catenin); ## $p < 0.01$ vs 0 min (ADAM10)]].

after NMDA administration and returned to basal level by 60 min (Fig. 1B). On the other hand, NMDA stimulation did not change BACE1 (Fig. 1B), suggesting that NMDA stimulation specifically upregulated α -secretase ADAM10. Because ADAM10 is a major α -secretase in APP processing, we sought to determine whether NMDA stimulation also elevated the α -cleavage of APP processing. To this end, immunoblotting was performed to measure the levels of C-terminal fragments (CTFs) of APP. The results showed that the ratio between C83-CTF (an AAP processing product from α -cleavage mediated by α -secretase) and C99 (an AAP processing product from β -cleavage) was elevated following NMDA stimulation (Fig. 1C). This result suggested that NMDAR activation facilitated α -cleavage, which was consistent with the findings reported earlier (Hoey et al., 2009). Interestingly, although NMDA stimulation promoted the APP α -cleavage, we did not observe significant concomitant decrease of full-length APP (Fig. 1C), probably because only a small fraction of APP was processed.

To confirm that the observed effect was mediated by NMDARs, we determined the effect of NMDA in the presence of D-APV (100 μ M, pretreated for 30 min), a specific antagonist of NMDARs. D-APV abolished NMDA-induced ADAM10 increase (Fig. 1D).

To determine whether NMDA stimulates ADAM10 protein upregulation by transcription activation, we tested the effect of actinomycin D, a transcription inhibitor. As shown in Fig. 1E, 25 μ M actinomycin D abolished NMDA-induced ADAM10 increase. Another transcription inhibitor, α -amanitin, showed a similar effect (Fig. 1F). These results suggest that NMDAR-activation elicited ADAM10 protein synthesis by activating ADAM10 gene transcription. To investigate this possibility further, we measured the level of ADAM10 mRNA by quantitative real-time RT-PCR, and observed that ADAM10 mRNA was upregulated by NMDA in a time-dependent manner (Fig. 1G). It appeared that the significant increase of ADAM10 protein (Fig. 1B) preceded that in mRNA (Fig. 1G). This discrepancy is probably due to NMDA-stimulated rapid translation from pre-existing mRNA, as suggested by previous studies (Ouyang et al., 1999; Gong et al., 2006; Li et al., 2012). Also, the ADAM10 protein increase is more transient than the increase of ADAM10 mRNA (Fig. 1B, G), indicating that there probably is a negative feedback mechanism that tightly controls ADAM10 protein level.

Blockage of Wnt/ β -catenin signaling inhibits NMDAR-dependent ADAM10 upregulation

Previous studies indicate that NMDAR activation leads to Wnt release and nuclear accumulation of β -catenin in hippocampal slices (Chen et al., 2006). Because both NMDAR activation (Hoey et al., 2009; Tampellini et al., 2009) and Wnt/ β -catenin signaling (De Ferrari et al., 2003) have protective effects on AD pathologies, we tested whether NMDAR activation stimulated ADAM10 synthesis via Wnt/ β -catenin signaling. To this end, we first determined the effect of NMDA stimulation on β -catenin in cortical cultures. As shown in Fig. 2A, β -catenin protein started to elevate at 5 min and last until 30 min after 50 μ M NMDA application, indicating that NMDAR activation resulted in upregulation of β -catenin signaling in cortical cultures.

This NMDA-stimulated β -catenin increase was blocked by pretreatment with 100 ng/ml recombinant DKK1 protein (a specific endogenous antagonist for the Wnt/ β -catenin signaling) at 60 min before NMDA administration (Fig. 2B), indicating that NMDAR stimulation activated this pathway. Next, we investigated whether NMDA-stimulated ADAM10 expression was the result of the NMDA-induced upregulation of Wnt/ β -catenin signaling. DKK1 diminished the ADAM10 increase induced by NMDA (Fig. 2B). Since NMDAR activation causes the secretion of Wnt3a (Chen et al., 2006), which is a prototypic Wnt ligand for the Wnt/ β -catenin pathway, we specifically tested the involvement of Wnt3a in NMDA-induced ADAM10 upregulation. In this experiment, we used Wnt3a antibody to neutralize Wnt3a. As shown in Fig. 2C, the Wnt3a antibody completely abolished the ADAM10 increase. The inhibitory activity of the antibody on Wnt/ β -catenin signaling was verified by its blockage of NMDA-induced β -catenin upregulation (Fig. 2C).

Next, we performed experiments to test the role of β -catenin, using pharmacological approaches. Indomethacin (Indo) and XAV939, two different inhibitors of β -catenin expression (Goessling et al., 2009; Huang et al., 2009), were used in these experiments. The cultures were pretreated with Indo (50 μ M) or XAV939 (1 μ M) for 30 min, followed by NMDA (50 μ M) stimulation for 15 min before harvesting for immunoblotting. The results showed that treatments of cortical cultures with either inhibitor completely blocked NMDA-induced β -catenin increase (Fig. 2D). Importantly, these inhibitors also abolished the NMDA-induced ADAM10 increase (Fig. 2E). These observations were further confirmed with another recently developed small molecule (IWR-1) that promotes β -catenin degradation by abrogating Axin2 turnover (Chen et al., 2009) (Fig. 2F, G). Collectively, these results suggest that the Wnt/ β -catenin signaling is critical for NMDA-stimulated ADAM10 synthesis.

Wnt3a stimulates ADAM10 increase

The results described above indicate that secretion of Wnt3a following NMDAR activation is required for NMDAR-elicited ADAM10 increase. Next, we sought to investigate whether Wnt3a is able to stimulate ADAM10 expression. To this end, we determined the time course and dose effects of Wnt3a on ADAM10 protein level. As

Figure 4. MAPK/ERK pathway is involved in ADAM10 regulation. **A**, PD98059 blocked NMDA-induced ADAM10 increase. When cotreated with PD98059 and NMDA, PD98059 (20 μ M) was applied 30 min before NMDA (50 μ M), followed by an additional 15 min of incubation before cell harvest for immunoblotting. Both PD98059 and NMDA were dissolved in 0.05% DMSO, which was used in vehicle controls ($n = 4$; both p -ERK1 and p -ERK2 were included in quantification). **B**, **C**, Time course (**B**) and dose effect (**C**) of Wnt3a on ERK phosphorylation ($n = 4$; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ vs 0 min or 0 ng/ml). **D**, PD98059 inhibited Wnt3a-induced ADAM10 increase. When cotreated with both PD98059 and Wnt3a, cultures were preincubated with PD98059 (20 μ M) for 30 min before adding Wnt3a (100 ng/ml), and incubation was continued for 60 min before cell harvest for immunoblotting. DMSO (0.05%) was used as vehicle for both PD98059 and Wnt3a and in control treatment ($n = 3$). **E**, A model of NMDAR/Wnt/MAPK signaling in the regulation of ADAM10 expression. NMDAR activation stimulates the secretion of Wnt3a, which then activates ERKs to elicit ADAM10 expression, likely via β -catenin.

shown in Fig. 3A, addition of recombinant Wnt3a to cultures caused a time-dependent increase of β -catenin, demonstrating the activity of the purified Wnt3a in activating the Wnt/ β -catenin pathway. Importantly, ADAM10 protein was also elevated by Wnt3a; the ADAM10 increase peaked at 30 min after Wnt3a application (Fig. 3A). In dosage experiments, we measured ADAM10 protein levels in cortical cultures treated with various concentrations (0, 25, 50, or 100 ng/ml) of recombinant Wnt3a for 60 min. As expected, Wnt3a caused a dose-dependent increase of β -catenin (Fig. 3B). Importantly, ADAM10 protein also displayed a similar profile, with a 1.7-fold increase at 100 ng/ml Wnt3a (Fig. 3B). In contrast, Wnt3a treatment did not change BACE1 (Fig. 3A,B). Together, these results show that Wnt3a is sufficient to specifically induce the upregulation of ADAM10 protein.

Both NMDAR- and Wnt3a-dependent ADAM10 increases are mediated by the MAPK/ERK pathway

We sought to further understand the potential mechanism by which NMDARs and Wnt3a regulate ADAM10. Because the MAPK pathway is critically involved in NMDAR-regulated protein synthesis (Kelleher et al., 2004; Gong and Tang, 2006; Wayman et al., 2006) and major components in the MAPK signaling cascades (including $p38$, JNKs and ERKs) are regulated by Wnt

signaling (Yun et al., 2005; Bikkavilli and Malbon, 2009), we investigated the role of MAPK signaling in NMDAR- and Wnt3a-regulated ADAM10 expression. As shown in Fig. 4A, NMDA stimulation activated the MAPK/ERK pathway, as indicated by the increase of phosphorylated ERK1/2. Importantly, pretreatment of the cultures with a specific ERK inhibitor, PD98059 (20 μ M), blocked NMDAR-induced ADAM10 increase (Fig. 4A). These results indicate that ERK is critical for the upregulation of ADAM10 by NMDARs. Next, we investigated whether MAPK/ERK signaling was also involved in Wnt3a-stimulated ADAM10 upregulation. We observed that Wnt3a administration also stimulated ERK1/2 phosphorylation in a time- and dose-dependent manner (Fig. 4B,C). Furthermore, PD98059 abolished Wnt3a-induced ADAM10 upregulation (Fig. 4D). Thus, these observations suggest that NMDA and Wnt3a stimulate the ADAM10 increase via the MAPK/ERK pathway.

Discussion

We show here that NMDAR activation stimulates ADAM10 protein expression and that this NMDAR-dependent ADAM10 expression is elicited via a Wnt/MAPK/ERK signaling pathway.

Previous studies revealed that synaptic activity stimulated α -secretase, and thereby reduced $A\beta$ generation and release

(Hoey et al., 2009; Tampellini et al., 2009; Kim et al., 2010). How synaptic activity regulates α -secretase remains unclear. Here, we reveal a novel pathway by which NMDARs control ADAM10 activity. Our results revealed that ADAM10 expression was increased by NMDA stimulation and abolished by D-APV (Fig. 1*B,C*). These observations suggest that synaptic activity may stimulate α -secretase activity by upregulation of ADAM10 protein as a result of NMDAR activation.

Our studies further uncover a novel mechanism by which NMDARs regulate ADAM10 expression. Chen et al. (2006) previously reported that NMDAR activation in response to tetanus elicited rapid Wnt3a secretion in hippocampal slices. In support of this notion, stimulation of hippocampal and cortical neurons in cultures by glutamate or NMDA also leads to Wnt3a secretion (unpublished observations). We observed that either DKK1 or Wnt3a antibody blocks NMDAR-induced ADAM10 upregulation (Fig. 2*B,C*) and that inhibition of β -catenin with two different inhibitors abolished the upregulation. Thus, we conclude that the canonical Wnt signaling activated by Wnt3a is critical for NMDAR activation to lead to upregulation of ADAM10.

Furthermore, we showed that exogenous Wnt3a induced ADAM10 expression (Fig. 3). Because NMDAR activation is known to elicit Wnt3a secretion (Chen et al., 2006), this finding suggests that NMDAR activation causes ADAM10 expression by stimulating Wnt3a secretion. Previous work indicates that the canonical Wnt signaling has a protective effect on AD development in animal models (De Ferrari et al., 2003). Our findings of Wnt3a as a positive regulator of the expression of the α -secretase ADAM10 suggest that Wnt3a may downregulate A β production and thus A β toxicity.

We observed that Wnt3a stimulated ERK activity (Fig. 4*B*). Consistent with our findings, prior studies revealed that Wnt3a induced a rapid and β -catenin-independent activation of ERKs in non-neuronal cells (Yun et al., 2005; Caverzasio and Manen, 2007). Interestingly, ERK inhibitors suppressed not only NMDAR-dependent but also Wnt3a-induced ADAM10 increase (Fig. 4*A,D*). These findings suggest that the MAPK/ERK signaling is essential for NMDAR and Wnt3a to stimulate ADAM10 expression. Together, we propose that an NMDAR/Wnt/MAPK signaling pathway (Fig. 4*E*) controls synaptic activity-regulated expression of ADAM10.

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