ORIGINAL RESEARCH

Excessive Glutamate Stimulation Impairs ACE2 Activity Through ADAM17-Mediated Shedding in Cultured Cortical Neurons

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Abstract

The excitotoxicity of glutamate plays an important role in the progression of various neurological disorders via participating in inflammation and neuronal damage. In this study, we identified the role of excessive glutamate stimulation in the modulation of angiotensin-converting enzyme type 2 (ACE2), a critical component in the compensatory axis of the renin–angiotensin system (RAS). In primary cultured cortical neurons, high concentration of glutamate (100 μM) significantly reduced the enzymatic activity of ACE2. The elevated activity of ADAM17, a member of the 'A Disintegrin And Metalloprotease' (ADAM) family, was found to contribute to this glutamate-induced ACE2 down-regulation. The decrease of ACE2 activity could be prevented by pre-treatment with antagonists targeting ionotropic glutamate receptors. In addition, the glutamate-induced decrease in ACE2 activity was significantly attenuated when the neurons were co-treated with MitoTEMPOL or blockers that target oxidative stress-mediated signaling pathway. In summary, our study reveals a strong relationship between excessive glutamate stimulation and ADAM17-mediated impairment in ACE2 activity, suggesting a possible cross-talk between glutamate-induced excitotoxicity and dysregulated RAS.

Keywords Glutamate · Excitotoxicity · ACE2 · ADAM17

Introduction

Within the renin–angiotensin system (RAS), angiotensin-converting enzyme type 2 (ACE2) has been established as a major component of the compensatory axis for its role in the metabolism of angiotensin II (Ang II). ACE2 transforms Ang II into Ang-(1–7), thus turning the vasoconstrictor into a vasodilator peptide, promoting nitric oxide (NO) release, reducing sympathetic outflow, increasing baroreflex sensitivity, and ultimately reducing high blood pressure (BP). In addition to cardiovascular diseases, the brain RAS also participates in the pathological process of various neurological diseases, including stroke, traumatic brain injury, and neurodegenerative diseases, such as Alzheimer's disease, as evidenced by both the protective and therapeutic effects of RAS blockers (Villapol et al. 2015; Kusaka et al. 2004; Ongali et al. 2014). The ACE2/Ang-(1–7) pathway also exhibits neuroprotective effects by reducing oxidative stress and suppressing inflammation. For example, overexpression of ACE2 exhibited a protective role in brain ischemic damage by decreasing the detrimental effects of Ang II-induced reactive oxygen species (ROS) production (Zheng et al. 2014). And the anti-inflammatory action of ACE2/Ang-(1–7), including the inhibition of microglial activation and inflammatory cytokine expression (Liu et al. 2016), was found to contribute to the attenuation in chronic heart failure-induced cognitive impairment (Hay et al. 2017). Despite those protective actions, down-regulated activity of ACE2-Ang-(1–7)-Mas axis in the brain is observed in patients or experimental models of Alzheimer's disease and ischemic stroke (Jiang et al. 2013a; Kehoe et al. 2016), suggesting

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that impaired ACE2/Ang-(1–7) compensatory activity might play a role in the neurodegeneration and its related process.

During brain injury and inflammation, the neurons can be damaged by excessive stimulation of glutamate, which is released from the activated glial cells surrounding those neurons. The pathologically high concentration of glutamate can cause excitotoxicity through massive Ca^{2+} influx, which will damage the function of mitochondria, increase oxidative stress, and go on to damage cell structures and DNA through various downstream signals, including Ca^{2+}/calmodulin-dependent protein kinase II, protein kinase C (PKC), nitric oxide synthase, calpain, and mitogen-activated protein kinase (MAPK) (Bal-Price and Brown 2001; Minger et al. 1998; Chaparro-Huerta et al. 2005). It has been shown that Ang II can inhibit the function of glutamate transporter (GLT1) on astrocytes, which is the major source of glutamate release, increasing the extracellular glutamate levels and extra-synaptic glutamate excitatory tone (Stern et al. 2016). In this case, if the compensatory activity of ACE2 is also impaired by glutamate-induced excitotoxicity, in turn, the inflammatory process and glial activation could be exacerbated via the over-activated RAS, further increasing the level of local glutamate.

In addition to internalization (Deshotels et al. 2014), cell membrane-bound ACE2 can be impaired through ectodomain shedding. ADAM17, a member of the 'A Disintegrin And Metalloprotease' (ADAM) family, is known to cleave a variety of membrane-anchored proteins, including ACE2 (Xu et al. 2016; Lambert et al. 2005). We previously demonstrated the opposite relationship between ADAM17 and the compensatory activity of ACE2 in the mouse brain, especially in neurons (Xu et al. 2017; Xia et al. 2013). Upon brain RAS over-activation, ADAM17 is up-regulated through increased ROS and activated MAPK pathway, leading to an increased ectodomain shedding of ACE2.

Glutamate contributes to neuronal injury not only through the ionotropic glutamate receptors (iGluR)-mediated excitotoxic pathway, but also via oxidative stress, including the ROS/MAPK pathway (Bossy-Wetzel et al. 2004; Molz et al. 2008; Stanciu et al. 2000). Several pro-inflammatory cytokines can be up-regulated during glutamate-induced excitotoxicity. Among them, tumor necrosis factor α (TNFα) production in hippocampus is significantly increased by glutamate in neonatal rats, but inhibited by a p38 MAPK blocker, which has no effect on the glutamate-induced increase in TNFα gene expression (Chaparro-Huerta et al. 2005), indicating that the shedding activity of ADAM17 (also known as TNFα converting enzyme) could be increased during glutamate stimulation. In addition, stress-induced increase in ADAM17 activity in the cortex of adult male rats can be blocked by treatment with MK-801, an antagonist of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor (Madrigal et al. 2002). Altogether, these studies

suggest that the shedding activity of ADAM17 can be elevated during excessive glutamate stimulation. This increase in ADAM17 activity might compromise the compensatory effects of ACE2/Ang-(1–7) mediated signaling, in favor of inflammatory progression and glial activation, which can cause further neuronal damage through this vicious cycle.

In this study, we investigated the relationship between excessive glutamate stimulation and ADAM17-mediated impairment of ACE2 activity. In primary cultured cortical neurons, high dose of glutamate treatment significantly decreased the enzymatic activity of ACE2, while blockade of iGluR with specific antagonists restored the ACE2 activity completely. Via specific ADAM17 inhibitor, it was demonstrated later that glutamate-induced ADAM17 activation contributed to this reduction in ACE2 activity. Although blocking iGluR had limited effect on ADAM17 activation, signals like mitochondrial ROS, p38 MAPK, and inducible nitric oxide synthase (iNOS), which are known to be involved in glutamate-induced neuronal damage, seemed to contribute to the ADAM17-mediated ACE2 shedding during excessive glutamate stimulation.

Materials and Methods

All procedures conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Louisiana State University Health Sciences Center Animal Care and Use Committee (#3418). Dams (C57BL/6j) were housed under standard 12-h light/dark cycle with ad libitum access to food and water.

Cortical Primary Neurons Culture

Cortical primary neurons were isolated from neonatal or 1-day-old wild type or pups lacking AT_{1a} receptors (AT_1R) specifically on neurons (AT1N) (Xu et al. 2017). Brains were dissected immediately and the cortex was collected in ice-cold Hank's balanced salt solution (HBSS) (Gibco 14175-079). Following brief dissociation, the cortices were washed and dispersed in HBSS containing 1% trypsin (Sigma-Aldrich T1426) and 1.5 kU/mL DNaseI (Sigma-Aldrich D5025), and then digested for 10 min at 37 °C. The digested samples were then dissociated by gentle pipetting in HBSS containing 1.5 KU/mL DNase I. After brief centrifugation, the cell pellets were re-suspended in complete Neurobasal culture medium, supplemented with 2% B27 and 0.5 mM GlutaMax (Gibco), and plated at a density of 10^6 cells/ml onto poly-L-lysine-coated plates. Cytosine arabinofuranoside (Ara-C, 2 μM, Sigma-Aldrich C1768) was added to the cultures 48 h after plating to arrest the growth of non-neuronal cells. Cultured cortical neurons were validated via immunofluorescence labeling with MAP2

(microtubule-associated protein 2), as shown in Fig. 1a. Cortical primary neurons were cultured for at least 10 days and then treated with specific agonist or blockers. All blockers, except for MitoTEMPOL and 1400W, were given 1 h before L-glutamic acid treatment (100 μM): NBQX (AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, receptor antagonist, 100 μM), D-AP5 (NMDA receptor, 50 μM), and DL-AP4 (non-selective ionotropic receptors antagonist, 50 μM), SB203580 (p38 MAPK blocker, 10 μM). MitoTEMPOL (mitochondria-targeted antioxidant, 10 μM) and 1400W (iNOS inhibitor, 5 μM) were given 30 min before L-glutamic acid treatment.

ACE2 and ADAM17 Activity Assay

Primary cultured neurons collected from drug- or vehicle-treated groups were processed for ACE2 and ADAM17 activities, as reported previously (Pedersen et al. 2015, 2011). Data are normalized according to the activity of vehicle-treated group in each experiment.

RNA Isolation and Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

Primary cultured neurons were extracted with TRIzol, phase separated with 1-bromo-3-chloropropane (BCP, MRC BP151) and spun at $13,000 \times g$ for 15 min at 4 °C. For primary neurons, the supernatant was added to an equal volume of ethanol and then processed using an RNA Mini-Prep kit from ZYMO Research (R2070). Real-time RT-PCR amplification reactions were performed with Power SYBR Green RNA-to-CT one-step kit (Thermo Fisher 4389986). Data were normalized to β-actin expression by the $^{\Delta\Delta}$CT comparative method and expressed as fold change compared to sham.

Results

To test the hypothesis that stimuli (e.g., glutamate and inflammation), other than Ang II, can reduce the compensatory activity of ACE2 in the central nervous system, cultured primary cortical neurons were treated with L-glutamic acid (glutamate, 100 μM) and lipopolysaccharide (LPS, 100 ng/ml), respectively. Glutamate significantly reduced the enzymatic activity of ACE2 (Fig. 1b) in cultured cortical neurons, after 18 h of treatment. Similarly, an inflammatory stimulus, such as LPS, also compromised the activity of ACE2. Since the treatment with glutamate can also promote neuronal inflammation, evidenced by up-regulated expression of interleukin 6 (IL-6) and cyclooxygenase-2 (COX-2, Fig. 1d, e) (Sriramula et al. 2015), in the following

experiments we investigated the contribution of glutamate to reduced ACE2 activity in neurons.

We previously found that neuronal AT_1R plays a pivotal role in DOCA-salt-induced reduction of ACE2 compensatory activity in the mouse hypothalamus (Xu et al. 2017). To further investigate the involvement of neuronal AT_1R, we treated cortical neurons, isolated from mice lacking AT_1R selectively from neurons, with glutamate. As shown in Fig. 1c, the enzymatic activity of ACE2 was still significantly decreased in the glutamate-treated neurons, compared to vehicle-treated controls, while the effect of Ang II was completely abolished. This result indicates that glutamate can decrease ACE2 activity through a neuronal AT_1R-independent mechanism.

To determine whether ionotropic glutamatergic receptors are involved in the glutamate-induced impairment of ACE2, the cultured primary neurons were pre-treated with NBQX (an AMPA receptor antagonist), D-AP5 (a NMDA receptor blocker), and DL-AP4 (a non-selective ionotropic receptors antagonist), before neurons exposure to glutamate (100 μM) for 18 h. As previously, glutamate reduced ACE2 activity in cortical neurons and this effect was completely prevented by all three ionotropic antagonists (Fig. 2a). Interestingly, NMDA blockade resulted in significant increase in ACE2 activity above baseline levels, suggesting that this receptor is involved in some intrinsic inhibition of ACE2, while the others appear to be involved in the excitotoxic inhibition of the carboxypeptidase.

We previously demonstrated the opposite relationship between ACE2 and ADAM17, a protease responsible for ACE2 ectodomain shedding, in mouse hypothalamus during deoxycorticosterone acetate (DOCA)-salt hypertension (Xia et al. 2013). Here, we hypothesized that glutamate-induced inhibition of ACE2 activity could also be the consequence of ADAM17-mediated shedding. Interestingly, although we did observe a significant increase in ADAM17 activity in glutamate-treated neurons, none of the three ionotropic antagonists, NBQX, D-AP5, or DL-AP4, could block this effect (Fig. 2b).

To confirm the involvement of ADAM17 in the glutamate-induced reduction of ACE2 activity, neurons were pre-treated with DPC333, a specific ADAM17 inhibitor (Fig. 3a) (Le Gall et al. 2010). Fig. 3b shows that blockade of ADAM17 fully restored the glutamate-impaired ACE2 activity, implicating the role of this sheddase in the decrease of ACE2 activity in the glutamate-treated neurons.

Mitochondrial function is impaired by excitotoxicity upon excessive glutamate stimulation (Pereira and Oliveira 2000). Meanwhile, oxidative stress and the p38 MAPK pathway activation are also found to be critical to ADAM17 activation (Patel et al. 2014; Xu and Derynck 2010; Xu et al. 2017). Accordingly, we further investigated the participation of mitochondrial ROS, iNOS, and p38 MAPK in the
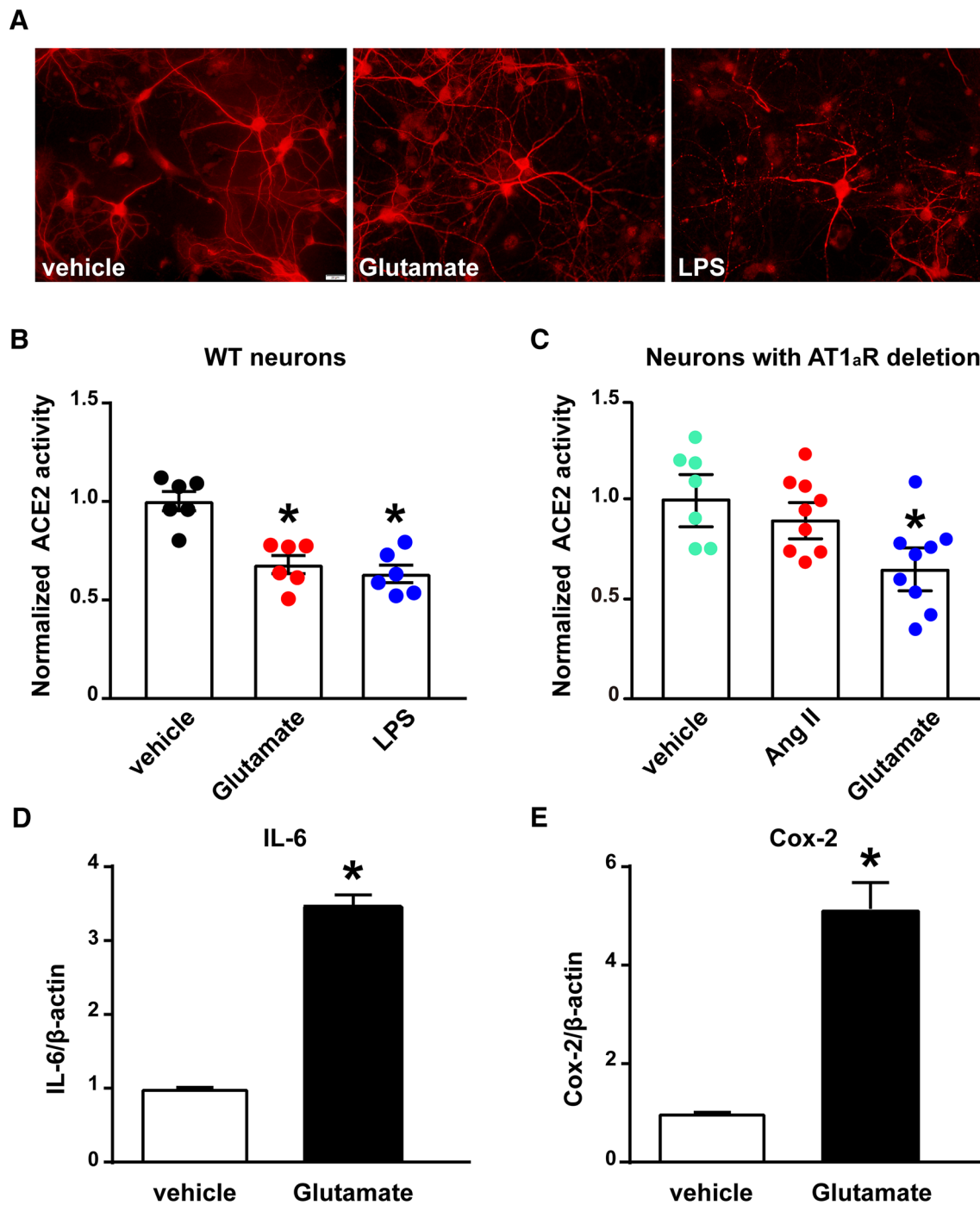
Fig. 1 The compensatory activity of ACE2 is impaired by glutamate and inflammatory stimuli in cultured cortical neurons. **a** Immunofluorescence labeling of primary cultured cortical neurons for visualization of MAP2 (red). The scale bar represents 20 μm. **b** ACE2 activity assay was performed in primary cultured cortical neurons isolated from wild-type neonates. The cultured neurons were treated with ʟ-glutamic acid (glutamate, 100 μM) or lipopolysaccharide (LPS, 100 ng/ml) for 18 h (*n*=3 independent cultures/group). **c** ACE2 activity assay was performed in cultured cortical neurons isolated from neonates with neuronal $AT_{1a}R$ deficiency. The cultured neurons were treated with glutamate (100 μM) or Angiotensin II (Ang II, 300 nM) for 18 h (*n*=4 independent cultures/group). Statistical significance: One-way ANOVA: *$P<0.05$ versus vehicle. **d, e** Glutamate significantly upregulates the gene expression of pro-inflammatory cytokines in cultured cortical neurons: qPCR was performed to assess the mRNA levels of IL-6 (**d**), and Cox-2 (**e**) in neurons treated with glutamate (100 μM) or vehicle. Statistical significance: Student *t* test: *$P<0.05$ versus vehicle
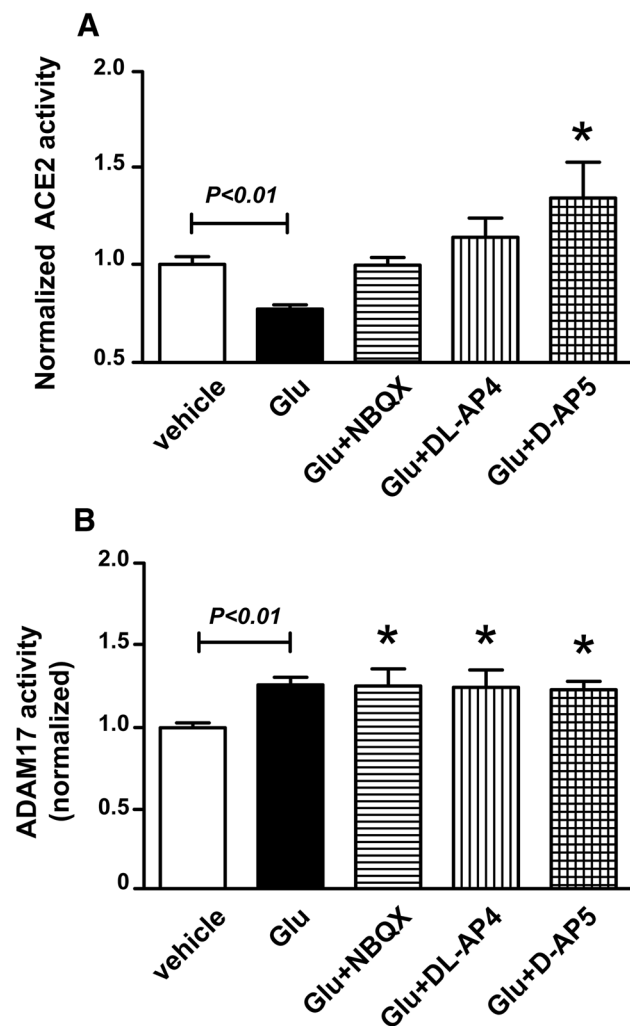
Fig. 2 The glutamate-induced reduction of ACE2 activity, but not the ADAM17 activation, is mediated via the ionotropic glutamate receptors. ACE2 (**a**) and ADAM17 (**b**) activity assay were performed in cultured cortical neurons isolated from wild-type neonates. After 1 h of pre-treatment with NBQX (an AMPA receptor antagonist, 100 μM), D-2-amino-5-phosphonopentanoate (D-AP5, a NMDA receptor antagonist, 50 μM), or D,L-2-amino-4-phosphonobutyric acid (DL-AP4, a non-selective ionotropic glutamate receptors antagonist, 50 μM), the cultured neurons were then treated with glutamate (100 μM) for 18 h ($n = 6$ independent cultures/group). Statistical significance: One-way ANOVA: *$P < 0.05$ versus vehicle

following experiments. While both iNOS and p38 MAPK inhibitors blocked the glutamate-induced increase in ADAM17 activity, MitoTEMPOL (MT), a mitochondrial ROS scavenger, potentiated the sheddase activity (Fig. 4a). This is most likely due to the conversion of ROS into H_2O_2 after restoration of manganese-dependent superoxide dismutase (MnSOD) activity, as H_2O_2 was previously shown to increase ADAM17 activity in monocytes (Scott et al. 2011). Although MitoTEMPOL failed to prevent ACE2 impairment, it partially restored the ACE2 activity in cortical neurons after glutamate stimulation (Fig. 4b). Unlike

MitoTEMPOL, the p38 MAPK blocker and iNOS inhibitor completely prevented the increase in ADAM17 activity (Fig. 4a) and the inhibition of ACE2 activity (Fig. 4b) suggesting that these signaling components are part of the same pathway.

Discussion

The inhibition of ACE2 enzymatic activity has been observed in the central nervous system and peripheral organs, such as heart and kidney, during cardiovascular diseases and diabetes (Cherney et al. 2014; Epelman et al. 2008; Úri et al. 2014). In the brain, through its conversion of Ang II into the vasodilator peptide Ang-(1–7), ACE2 promotes the reduction of oxidative stress and attenuates neuronal apoptosis, in addition to its protective role in the maintenance of blood–brain barrier function (Wu et al. 2015; Jiang et al. 2013b). Therefore, reduced ACE2 activity favors the progression of pathological processes. Our group previously identified the deleterious effects of an over-activated RAS in blunting ACE2 activity (Xia et al. 2013). By activating AT_1R, Ang II induces both internalization and ectodomain shedding of ACE2, inhibiting both ACE2 expression and enzymatic activity (Deshotels et al. 2014; Xia et al. 2013). In this study, we demonstrate that an excitotoxic concentration of glutamate can markedly reduce ACE2 activity in cultured primary cortical neurons. Excessive stimulation by glutamate can induce excitotoxicity and damage the nerve cells. The excitotoxicity of glutamate is known to be involved in stroke, traumatic brain injury, and neurodegenerative diseases, such as Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's disease (Doble 1999). Therefore, loss of ACE2 compensatory activity might also contribute to the development of those neurological disorders or to the progression of their pathological mechanisms, such as inflammation and apoptosis.

We previously discovered that ACE2 is undergoing ectodomain shedding, mediated by ADAM17 and possibly resulting in compromised ACE2 efficiency in transforming Ang II to Ang-(1–7). Interestingly, unlike the previous studies in hypertensive models, the role of glutamate in ACE2 inhibition is independent of neuronal AT_1R, suggesting that glutamate signaling might have a direct effect on ACE2. To further identify the mechanism of the glutamate-induced inhibition of ACE2 activity, we used DPC333, a specific ADAM17 inhibitor, and showed that blockade of the sheddase activity completely abolished the reduction of ACE2 activity in glutamate-treated neurons, indicating that excessive glutamate stimulation decreases ACE2 activity though an ADAM17-mediated mechanism. Moreover, our data showing that none of the ionotropic inhibitors was able to reduce the glutamate-mediated increase in ADAM17
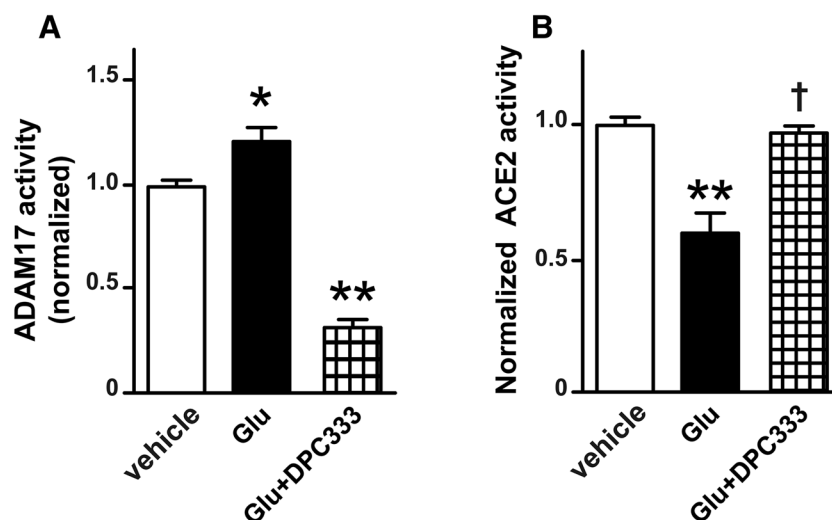
A

B

Fig. 3 ADAM17-mediated shedding is responsible for the glutamate-induced impairment of ACE2 activity in cortical neurons. ADAM17 (**a**) and ACE2 (**b**) activity assay were performed, respectively, in cultured cortical neurons isolated from wild-type neonates. The cultured neurons were treated with glutamate (100 μM) or glutamate with 1 h of pre-treatment with DPC333 (a specific ADAM17 inhibitor, 10 μM) for 18 h ($n = 3$–6 independent cultures/group). Statistical significance: One-way ANOVA: *$P < 0.01$ and **$P < 0.001$ versus vehicle; †$P < 0.001$ versus glutamate

activity, pointing to a contribution of the metabotropic receptors. This is in agreement with previous observations by Cho et al. that stimulation of mGluR1/5, the Group I metabotropic glutamate receptors (mGluR), can activate ADAM17, further promoting the shedding of neuronal pentraxin receptor and endosome-dependent removal of AMPA receptors from the synapses (Cho et al. 2008). Stimulation of Group I mGluR leads to activation of a wide variety of signaling pathways. mGluR couple to $G\alpha_{q/11}$ proteins, activating phospholipase C and resulting in both diacylglycerol and inositol-1,4,5-triphosphate formation followed by the activation of PKC. It is well known that activation on PKC-mediated signaling can increase ADAM17 enzymatic activity, evidenced by the prominent effect of phorbol myristate acetate (PMA), a potent PKC activator, on ADAM17 activation (Zhang et al. 2000; Kveiborg et al. 2011). Moreover, a study by Horiuchi et al. pointed that the activation of ADAM17 did not rely on the existence of calcium (Ca^{2+}), unlike other member of its family (Horiuchi et al. 2007). This might explain why pre-incubating the neurons with ionotropic antagonists has no effect in diminishing glutamate-induced ADAM17 activity. Interestingly, unlike their role in ADAM17 modulation, those iGluR antagonists successfully prevented the inhibition of ACE2 activity in glutamate-treated neurons. This result seemingly goes against the previous findings on the opposite relationship between ADAM17 and ACE2. A previous study from the group that originally reported ADAM17-mediated ACE2 shedding indicated that the interaction between ACE2 and calmodulin (CaM), a ubiquitous Ca^{2+} sensing protein, could

inhibit the shedding of its ectodomain (Lambert et al. 2008). Furthermore, our group found that this ACE2-CaM interaction was significantly down-regulated in the brain when local Ang II stimulation was increased by DOCA-salt treatment (Xia et al. 2013). Ang II is known to have direct effects on multiple Ca^{2+} channels and can rapidly increase the level of intracellular Ca^{2+} (Zimmerman et al. 2005). In the event of a transient rise in $[Ca^{2+}]_i$, the Ca^{2+} ion will interact with each Ca^{2+}-binding loop on CaM (Chin and Means 2000) and the structural rearrangements in CaM might result in decreased binding with ACE2. In this case, it is conceivable that glutamate activates ADAM17, while ACE2 activity remains intact due to the inadequate intracellular Ca^{2+} level, considering that the major Ca^{2+} influx is blocked by the treatment of iGluR antagonists.

The neurotoxicity of glutamate is triggered primarily by a massive Ca^{2+} influx arising from over-stimulation of the iGluR (Szydlowska and Tymianski 2010). The elevation of intracellular $[Ca^{2+}]$ can primarily cause mitochondrial dysfunction and increase ROS accumulation (Pereira and Oliveira 2000; Almeida et al. 1998), which plays a positive role on ADAM17 via increasing its translocation and activation (Patel et al. 2014; Dreymueller et al. 2012). However, we did not see any significant inhibition but a small elevation in ADAM17 activity when the neurons were co-treated with MitoTEMPOL; this is possibly because MitoTEMPOL restores MnSOD activity, which causes H_2O_2 accumulation, and H_2O_2 was previously shown to increase ADAM17 activity in monocytes (Scott et al. 2011). Upon mitochondrial ROS blockade, ACE2 activity was partially restored in the
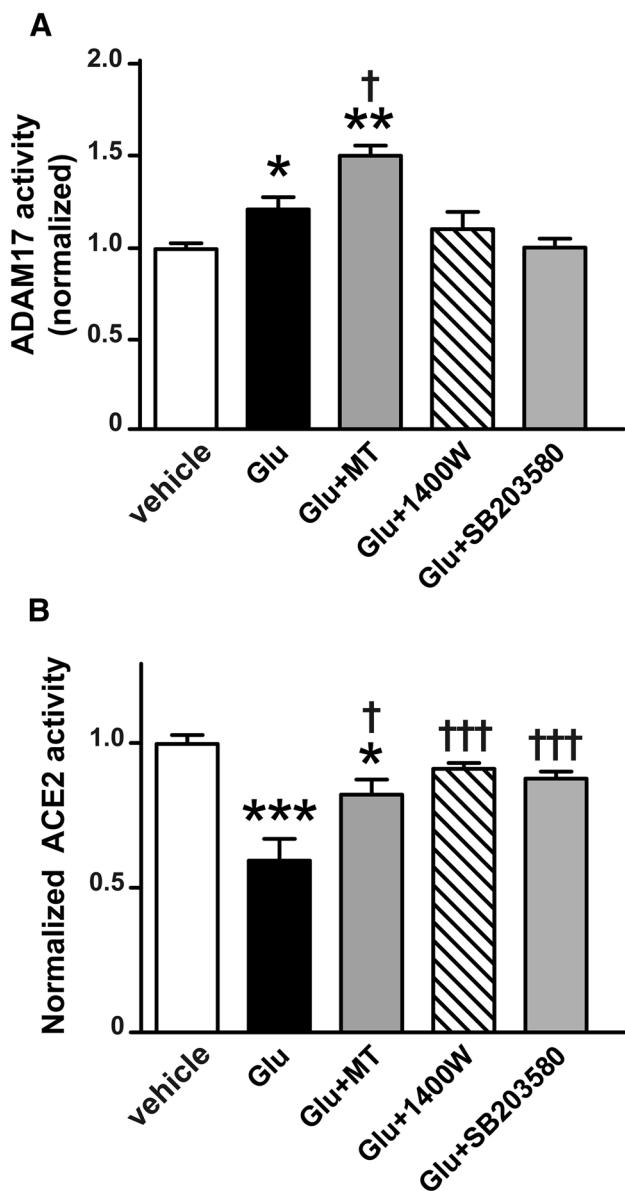
A

B

Fig. 4 Downstream signals of mitochondrial ROS, MAPK, and iNOS are involved in glutamate-induced ADAM17 activation and the reduction of ACE2 activity in cortical neurons. ADAM17 (**a**) and ACE2 (**b**) activity assay were performed, respectively, in cultured cortical neurons isolated from wild-type neonates. The cultured neurons were treated with glutamate (100 μM), glutamate plus co-treatment with MitoTEMPOL (MT, a mitochondrial ROS scavenger, 10 μM), or glutamate plus 0.5–1 h of pre-treatment with 1400W (an iNOS inhibitor, 5 μM), SB203580 (a p38MAPK blocker, 10 μM), for 18 h ($n = 3$–6 independent cultures/group). Statistical significance: One-way ANOVA: $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ versus vehicle; $^{\dagger}P < 0.05$ and $^{\dagger\dagger\dagger}P < 0.001$ versus glutamate

neurons subjected to high dose of glutamate. Again, these results confirm that activation of iGluR through excessive glutamate stimulation does not significantly contribute to ADAM17 activation, but it is critical to the glutamate-induced process of ACE2 shedding.

The excitotoxicity induced by excessive glutamate stimulation contributes to the development of inflammatory neurodegeneration through oxidative stress and/or iNOS-mediated mechanisms (Chang et al. 2008; Ward et al. 2009). It has been shown that chronic NMDA receptor activation increases inflammatory markers, including iNOS expression, in rat frontal cortex (Chang et al. 2008). In addition, the production of iNOS is also found to be downstream of PKC-mediated signaling in various cell systems (McKenna et al. 1995; Ginnan et al. 2006; Salonen et al. 2006). Increased iNOS can promote the release of glutamate from neighboring astrocytes and furtherly worsen the neuro-inflammation (Brown and Bal-Price 2003). Interestingly, iNOS leads to the translocation and activation of ADAM17 through its NO/cGMP/protein kinase G (PKG) signaling in hepatocytes (Chanthaphavong et al. 2012). Here, we show that iNOS-mediated signaling is also involved in the glutamate-induced ACE2 impairment through an increase in ADAM17 shedding activity, supported by an attenuated reduction in ACE2 activity after excessive glutamate stimulation.

Although ADAM17-mediated ACE2 shedding could contribute to the neurotoxicity of glutamate because of the impaired compensatory role of ACE2/Ang-(1–7), blocking ADAM17 activation may not improve neuronal survival. Indeed, it was shown in rat cortical cultures that inhibiting ADAM17 worsens neuronal apoptosis after glutamate exposure (Hurtado et al. 2002). ADAM17 promotes the shedding of multiple substrates, some involved in cell survival while others are pro-inflammatory and pro-apoptotic (Dreymueller et al. 2012). For example, by shedding TNFα from activated glial cells, ADAM17 is pro-inflammatory, but by cleaving its receptor from the plasma membrane, the sheddase disconnects this receptor from its downstream signaling and thus regulate the inflammatory process and help to reduce the neuronal damage induced by TNFα (Marchetti et al. 2004; Tellier et al. 2006)

Although the current study identifies a new stimulus and potential receptors leading to ADAM17-mediated inhibition of ACE2 activity in the brain, further investigation is needed to elucidate the mechanisms leading to ADAM17 substrate selectivity and uncover effective new therapeutic targets for treatment of related neurological diseases.

Compliance with Ethical Standards

Conflict of interest The authors report no conflict of interest.

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