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Amyloid-beta neurotoxicity and clearance are both regulated by glial group II metabotropic glutamate receptors

Daniela Durand, Lila Carniglia, Juan Turati, Delia Ramírez, Julieta Saba, Carla Caruso, Mercedes Lasaga*

INBIOMED Instituto de Investigaciones Biomédicas UBA-CONICET, Facultad de Medicina, UBA, Ciudad Autónoma de Buenos Aires, 1121 ABG, Argentina

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ABSTRACT

Astrocytes are now fully endorsed as key players in CNS functionality and plasticity. We recently showed that metabotropic glutamate receptor 3 (mGlu3R) activation by LY379268 promotes non-amyloidogenic cleavage of amyloid precursor protein (APP) in cultured astrocytes, leading to increased release of neuroprotective sAPPα. Furthermore, mGlu3R expression is reduced in hippocampal astrocytes from PDAPP-J20 mice, suggesting a role for these receptors in Alzheimer's disease. The present study enquires into the role of astroglial-derived neurotrophins induced by mGlu3R activation in neurotoxicity triggered by amyloid β (Aβ). Conditioned medium from LY379268-treated astrocytes protected hippocampal neurons from Aβ-induced cell death. Immunodepletion of sAPPα from the conditioned medium prevented its protective effect. LY379268 induced brain-derived neurotrophic factor (BDNF) expression in astrocytes, and neutralizing BDNF from conditioned medium also prevented its neuroprotective effect on Aβ neurotoxicity. LY379268 was also able to decrease Aβ-induced neuron death by acting directly on neuronal mGlu3R.

On the other hand, LY379268 increased Aβ uptake in astrocytes and microglia. Indeed, and more importantly, a reduction in Aβ-induced neuron death was observed when co-cultured with LY379268-pretreated astrocytes, suggesting a link between neuroprotection and increased glial phagocytic activity.

Altogether, these results indicate a double function for glial mGlu3R activation against Aβ neurotoxicity: (i) it increases the release of protective neurotrophins such as sAPPα and BDNF, and (ii) it induces amyloid removal from extracellular space by glia-mediated phagocytosis.

1. Introduction

Currently, Alzheimer's disease (AD) is the most common type of age-associated dementia, and there are no disease-modifying treatments (Habib et al., 2017). Aβ accumulation has been largely linked to the pathogenesis of AD and, although this notion has often been questioned after each failure of Aβ immunization-based clinical trials (commented on Belluk (2016), it remains still in vogue. The proteolytic cleavage of amyloid precursor protein (APP) by β-secretase (a.k.a. BACE1) combined with the subsequent γ-secretase cleavage, leads to $A\beta_{40/42}$ peptide generation (Kang et al., 1987). On the other hand, in the non-amyloidogenic pathway, APP is cleaved at the plasma membrane by α-secretase, which precludes Aβ formation but produces a soluble peptide: sAPPα (Haass and Selkoe, 1993). Whereas the neurotoxic profile of Aβ is broadly accepted, several authors have demonstrated beneficial effects of sAPPα in terms of protecting hippocampal neurons from excitotoxicity, glucose deprivation, Aβ toxicity, ischemia, and traumatic

Abbreviations: mGluR, metabotropic glutamate receptor; AD, Alzheimer's disease; Aβ, amyloid β; sAPPα, soluble amyloid precursor protein α; BDNF, brain derived neurotrophic factor; NAM, negative allosteric modulator; PAM, positive allosteric modulator; LY379268, (1*R*,4*R*,5*S*,6*R*)-4-Amino-2-oxabicyclo [3.1.0] hexane-4,6-dicarboxylic acid; LY2389575, (3*S*)-*N*-(2,4-Dichlorobenzyl)-1-(5-bromopyrimidin-2-yl) pyrrolidinyl-3-amine; GCM, glial conditioned medium; AG1, arginase 1; SR, scavenger receptor.

* Corresponding author. INBIOMED Instituto de Investigaciones Biomédicas UBA-CONICET, Facultad de Medicina, UBA, Ciudad Autónoma de Buenos Aires, 1121 ABG, Argentina.

E-mail addresses: ddurand@fmed.uba.ar (D. Durand), lcarniglia@fmed.uba.ar (L. Carniglia), jturati@fmed.uba.ar (J. Turati), dramirez@fmed.uba.ar (D. Ramírez), juli_saba@hotmail.com (J. Saba), ccaruso@fmed.uba.ar (C. Caruso), mlasaga@fmed.uba.ar (M. Lasaga).

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brain injury (Barger and Harmon, 1997; Corrigan et al., 2011; Furukawa et al., 1996; Goodman and Mattson, 1994; Mattson et al., 1993; Smith-Swintosky et al., 1994). sAPPα also supports axonal and neurite outgrowth and synaptic plasticity (Chasseigneaux et al., 2011; Hick et al., 2015; Qiu et al., 1995; Ring et al., 2007; Taylor et al., 2008), promotes neural differentiation of human embryonic stem cells (Freude et al., 2011), and attenuates Aβ pathology by inhibiting β-secretase (Obregon et al., 2012; Peters-Libeu et al., 2015), glycogen synthase kinase (GSK)-3β activity, and tau hyperphosphorylation (Deng et al., 2015). Indeed, sAPPα improves cognition in animal models of AD (Corrigan et al., 2012; Meziane et al., 1998; Ring et al., 2007) and aging (Bour et al., 2004; Roch et al., 1994). However, it has not yet been considered a relevant target for AD in clinical trials.

Metabotropic glutamate receptors (mGluR) belong to the superfamily of class III G-protein coupled receptors. Unlike fast responses triggered by ionotropic receptors, mGluR mediate slower responses by coupling to second messenger-mediated reactions. mGluR have been classified into three groups based on molecular structure, sequence homology, pharmacological profile, and associated second messengers (Table 1). Group I includes mGlu1R and mGlu5R subtypes whose activation leads to phospholipase C activity, inositol-1,4,5-triphosphate and diacylglycerol production, calcium mobilization, and protein kinase C activation (Cartmell and Schoepp, 2000). Group II (mGlu2R and mGlu3R subtypes) and group III (mGlu4/6/7/8 subtypes) receptors are coupled to G_i/G_0 proteins which inhibit adenylate cyclase and reduce cyclic AMP levels (Cartmell and Schoepp, 2000). The mGlu2R subtype is mainly located in the presynaptic terminals of glutamatergic neurons where it inhibits glutamate release, maintaining glutamatergic transmission within physiological range (Schoepp, 2001), whereas the mGlu3R subtype is preferentially present in postsynaptic membranes and in glial cells (Riedel et al., 2003; Schoepp, 2001).

Neuron-glia interaction has been broadly shown to yield enhanced neuroprotection, in part through mechanisms involving group II mGluR activation. mGlu3R activation prevents glucose-induced oxidative injury in dorsal root ganglion neurons co-cultured with Schwann cells by increasing free radical scavenging and antioxidant defense (Berent-Spillson and Russell, 2007). Astrocytic mGlu3R cause neuroprotection against NMDA toxicity (Corti et al., 2007). Orthosteric mGlu2/3R agonists (such as LY379268) exert neuroprotective functions via mGlu3R signaling, which stimulates production of transforming growth factor-β (TGF-β) and glial cell line-derived neurotrophic factor (GDNF) (Battaglia et al., 2009, 2015; Bruno et al., 1998; Bruno et al., 1997; Caraci et al., 2011; Corti et al., 2007; Di Liberto et al., 2010). However, the influence of mGlu2R and mGlu3R activation on neurodegeneration is not univocal (reviewed in (Bruno et al., 2017)). Selective activation of mGlu2R enhances neuron death (Caraci et al., 2011; Corti et al., 2007; Motolese et al., 2015). Group II mGluR activation induces the secretion of $Aβ_{1-42}$ from nerve endings, and antagonizing group II mGluR enhances hippocampal neurogenesis and improves learning in AD mice (Higgins et al., 2004; Kim et al., 2010, 2014). Indeed, activation of mGlu2R in microglia promotes acquisition of a pro-inflammatory phenotype with ensuing release of neurotoxic cytokines (Taylor et al., 2002, 2005). Concordantly, Bruno et al. (2017) suggested that a combination of mGlu2R blockade and mGlu3R activation might be an optimal strategy in the treatment of AD.

A currently well-established concept in neuroscience is that both increased Aβ production and impaired Aβ clearance are responsible for amyloidosis in AD. According to the Aβ-cascade hypothesis, therapeutic intervention in any of these opposite processes is expected to be successful. Regarding Aβ production, we previously demonstrated that mGlu3R activation in astrocytes promotes *non-amyloidogenic* or *alpha*-cleavage of APP, leading to increased release of sAPPα, and simultaneously inhibits β-secretase expression (Durand et al., 2014). The present investigation enquired into neuroprotective actions that astroglial-derived sAPPα exerts against Aβ toxicity, and explored the possibility that mGlu3R might modulate Aβ clearance by glial cells.

2. Materials and methods

2.1. Materials

LY379268 was purchased from TOCRIS Biosciences. Fluorescent microspheres (Molecular Probes, F8823) were kindly provided by Dr. Candolfi (INBIOMED, UBA-CONICET). The negative allosteric modulator (NAM) LY2389575 was kindly provided by Stephan Schann (Domain Therapeutics). $Aβ_{25-35}$ was purchased from Sigma-Aldrich Argentina. $Aβ_{25-35}$ HiLyte Fluor™-488 labeled was purchased from Anaspec Inc. Cell culture inserts (Becton-Dickinson) were kindly provided by Dr. Theas (INBIOMED, UBA-CONICET).

2.2. Cell cultures

Neuron cultures were obtained from gestation day-18 fetal hippocampi. Pregnant Wistar rats were decapitated and fetuses immediately and aseptically removed. Cerebral hemispheres were freed from meninges and hippocampi dissected under a microscope. Tissue was placed in 3 ml of 0.25% trypsin (Invitrogen Life Technologies) in Hank's Balanced Salt Solution (HBSS) for 10 min at 37 °C. After washing with HBSS, cells were centrifuged, resuspended in 1–2 ml DMEM (Invitrogen Life Technologies, supplemented with 2 mM L-glutamine, 10% horse serum, Streptomycin 50 μg/ml, and Penicillin 50 U/ml), and dissociated by repeated passage through a series of three sequentially smaller-bore, fire-polished Pasteur pipettes until a single-cell suspension was obtained. Cells were plated on poly-L-lysine-coated culture dishes and maintained with DMEM plus 10% horse serum (Gibco, Invitrogen Life Technologies) for 2 h. Then, the medium was entirely replaced by serum-free medium DMEM-N2 (supplemented with N2 and B27 (Invitrogen Life Technologies)), 2 mM L-glutamine, and 1 mg/ml chicken egg albumin (Sigma-Aldrich Co.). Cell culture was kept in a humidified incubator at 37 °C with 5% CO_2 until 8–9 DIV. Purity of cultures routinely ranged between 90 and 98% as determined by immunofluorescence detection of neurons with anti-MAP2 monoclonal antibody (Santa Cruz Biotechnology, Inc.).

Astrocytes and microglia were prepared from cerebral hemispheres of 1- to 2-day-old postnatal Wistar rat pups as previously described (Carniglia et al., 2013). Microglial cultures were stained with a microglial marker (anti-rat CD11b monoclonal antibody, OX-42, Millipore) to assess their purity, which was routinely close to 98%. Astrocyte cultures were routinely close to 95% purity, as assessed by immunostaining with anti-GFAP monoclonal antibody (Millipore, MA, USA).

Experimental procedures were carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals, with approval by the Committee on Ethics of the University of Buenos Aires Medical School (Res. 2068/2015).

2.3. Treatments

A summary of cell treatments is shown in Fig. 1.

2.3.1. Glial conditioned medium (GCM)

Astrocytes were incubated with DMEM-N2 with or without LY379268 (0.1 μM) for 3 h. Conditioned medium was collected and centrifuged to eliminate debris. Supernatants were either used as

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Table 1
Metabotropic glutamate receptor classification.

Group	Subtype	Expression[a]	Agonists	Antagonists	Signaling[b]
I	mGlu1R	Neurons	Quisqualate	MPEP	$G_{q/11}$/PLC stimulation/MAPK activation
	mGlu5R	Neurons and astrocytes	3,5-DHPG CHPG	LY367385 AIDA CPCCOEt YM298198	
II	mGlu2R	Neurons	mGlu2R PAMs (BINA, LY487379, LY566332)	EGLU LY341495	$G_{i/0}$/Adenylate Cyclase inhibition
	mGlu3R	Neurons and astrocytes	DCG-IV L-CCG-I 2R,4R-APDC LY354740 LY379268	mGlu3R NAM (LY2389575)	Predominantly neuroprotective through astrocytes[c]
III	mGlu4R	Neurons	L-AP4	CPPG	$G_{i/0}$/Adenylate Cyclase inhibition
	mGlu6R	Expressed only in retina	3,4-DCPG	MSOP	
	mGlu7R	Neurons	(RS)PPG	UBP1112	
	mGlu8R	Low expression	VU0155041		

[a] (Niswender and Conn, 2010).
[b] (Cartmell and Schoepp, 2000).
[c] (Durand et al., 2013).

complete GCM or subjected to immunoprecipitation (IP) for sAPPα. For IP, GCM was incubated with 2 μg monoclonal anti-sAPPα antibody (Immuno-Biological Laboratories Co., Ltd.) overnight at 4 °C on a rotator, then protein A/G agarose beads (Santa Cruz Biotechnology) were added for 30 min. Immune complexes were pelleted by centrifugation at 4000 rpm; supernatants were used as *sAPPα-immunodepleted GCM*, whereas pellets were run in a western blot for sAPPα detection (10% SDS-PAGE; 1 μg/ml anti-sAPPα antibody, 130 KDa band). A negative control of IP was performed in absence of antibody. For BDNF neutralization, a neutralizing anti-BDNF polyclonal antibody (1:50, Millipore Co.) was added to complete-LY-GCM just before addition to neuron cultures.

2.3.2. Neuron treatments

For GCM assays, neuron culture medium was half-replaced by DMEM-N2, complete-control-GCM, complete-LY-GCM, sAPPα-immunodepleted-control-GCM, sAPPα-immunodepleted-LY-GCM, or BDNF-neutralized-LY-GCM and incubated for 1 h before adding $A\beta_{25\text{-}35}$ (25 μM, pre-aggregated for 4 days at 37 °C) for 24 h.

For other assays, neuron culture medium was half-replaced by DMEM-N2 or the mGlu3R-selective NAM LY2389575 (0.1 μM) and incubated for 1 h before adding DMEM-N2 or LY379268 (0.1 μM) for an additional 1 h, and then adding $A\beta_{25\text{-}35}$ (25 μM, pre-aggregated for 4 days at 37 °C) for 24 h.

2.3.3. Astrocyte treatments

For real-time PCR experiments, cultured astrocytes were incubated with 0.1 μM LY379268 in serum-free Minimum Essential Medium Eagle (MEM, Sigma-Aldrich) for 3 h (for BDNF determinations) or 24 h (for scavenger receptor determinations).

2.3.4. Astrocyte-neuron co-cultures

Astrocytes were plated on cell culture inserts (transwells, 0.4 μm pore size, PET track-etched membrane) and treated with LY379268 (0.1 μM in serum-free MEM) for 24 h. After two washes in phosphate-buffered saline (PBS), inserts were transfered to a 24-well dish containing cultured neurons. $A\beta_{25\text{-}35}$ HiLyte Fluor™ 488-labeled (5 μM) was added to the neuronal compartment for 24 h, whereas inserts were filled with DMEM-N2.

2.3.5. Microglia treatments

Cultured microglia were incubated with vehicle (DMEM with 2% fetal bovine serum (FBS, Natocor, Argentina) and 2 mM L-glutamine) or 0.1 μM NAM LY2389575 for 1 h prior to incubation with vehicle or 0.1 μM LY379268 for 24 h. Fluorescent microspheres (in a 100:1 bead to cell ratio) or 5 μM $A\beta_{25\text{-}35}$ HiLyte Fluor™ 488-labeled (fluor-Aβ) were added 2 or 1 h before ending the experiment, respectively. When needed, after LY379268 treatment, microglia were incubated with anti-CD11b antibody (Millipore, 5 μg/mL) for 15 min prior to replacing the medium with fluor-Aβ.

2.4. Determination of neuron death

Neuron death was determined by Trypan blue exclusion and by the TUNEL technique (Roche Diagnostics GmbH) as described before (Caruso et al., 2004). For Trypan blue exclusion assays, viable and dead cells were counted on a haemocytometer, ranging typically between 20 and 50 total cells per replicate. Three technical replicates were performed for each experiment. Dead/viable cells were counted under blind conditions.

2.5. Reverse transcription (RT) and real time polymerase chain reaction (qPCR)

Total RNA was extracted using TRIZOL reagent (Invitrogen Life Technologies, CA, USA) according to the manufacturer's instructions. 2 μg of total RNA were treated with 1 U RQ1 RNAse free-DNAse (Promega Corporation, WI, USA) at 37 °C for 10 min. RT reaction was carried out using 0.4 μg oligo-dT primers (Biodynamics) plus 1 μL ImProm-II™ Reverse Transcriptase (Promega) for 1 h at 42 °C with 3 mM $MgCl_2$. Rat BDNF (NM_012513.3), SRB1 (NM_031541.1) and CD36 (NM_031561.2) genes were analyzed using the following PCR primer sets: BDNF forward 5′-GATGAGGACCAGAAGGTTCG-3', BDNF reverse 5′-TCCAGCAGAAAGAGCAGAGG-3', SRB1 forward 5′-GCATTCGGAACAGTGCAACA-3', SRB1 reverse 5′-TCATGAATGGTGCCCACATC-3', CD36 forward 5′-CATGATTAATGGCACAGATGCA-3', CD36 reverse 5′-ACCTCAGTGTTCGAGACTTCTCAA-3'. HPRT was used as endogenous control (HPRT forward 5′-CTCATGGACTGATTATGGACAGGAC-3′, HPRT reverse 5′-GCAGGTCAGCAAAGAACTTATAGCC-3′). PCR amplifications were done in a StepOne_ Real-Time PCR System (Applied Biosystems) and PCR reactions were set up to a final volume of 16 μL containing 4 μL of 1:2 or 1:6 or 1:10 diluted cDNA (for CD36, SRB1, and BDNF, respectively), 900 nM primers (for CD36) or 150 nM primers (for BDNF, SRB1, and HPRT), and SYBR Select Master Mix (Applied Biosystem, Life Technologies Co). PCR conditions were UDG activation at 50 °C for 2 min and denaturation at 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. PCR product

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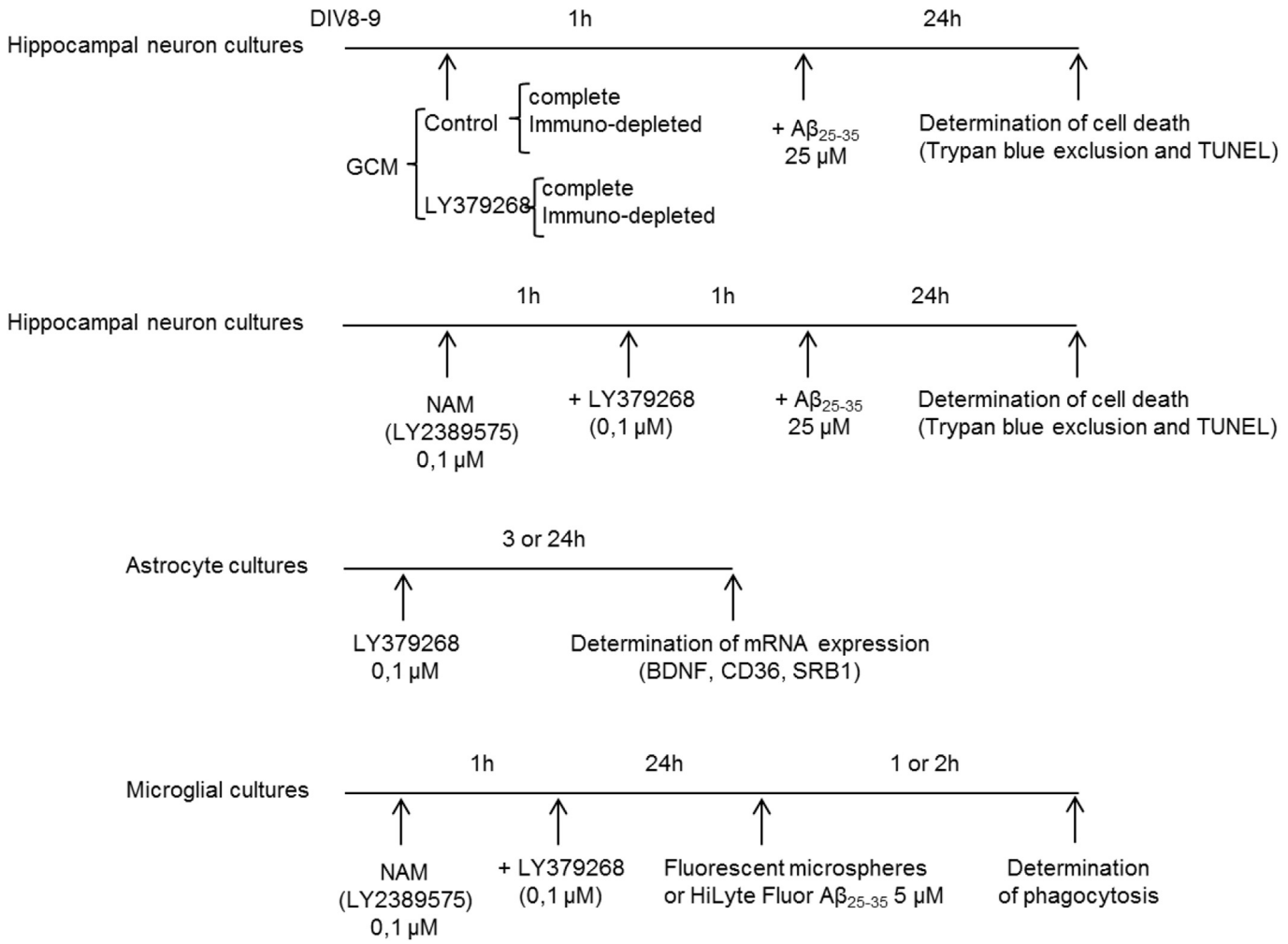

Fig. 1. Cell treatments.

specificity was verified by a melting curve analysis. No-RT controls omitted the reverse transcriptase enzyme, and no-template controls were performed by addition of nuclease-free water instead of cDNA. Levels of BDNF, SRB1, and CD36 expression were normalized to the endogenous control gene HPRT and analyzed with Step-One Software (Applied Biosystems) using the comparative ΔΔCt method (Livak and Schmittgen, 2001). Data were reported as RQ mean of 3–4 biological replicates (with 3 technical replicates each) ± SE.

2.6. Immunocytochemistry

2.6.1. MAP2/mGluR

Cells were fixed with 4% paraformaldehyde, incubated in Citrate Buffer pH 6.0 under microwave irradiation, and blocked with PBS+10% donkey serum+10% goat serum. Primary antibodies anti-mGlu2/3R (10 μg/mL, Upstate Millipore) or anti-mGlu3R (16 μg/mL, Alomone Labs) combined with anti-MAP2 (2 μg/mL) were diluted in PBS-BSA 1% (for mGlu3R) or PBS (for mGlu2/3R) plus 1% donkey serum and 1% goat serum. Secondary antibodies were anti-mouse-Cy3 (1:400, Chemicon) and anti-rabbit-FITC (1:200, Vector Laboratories). Cells were mounted with DAPI and visualized under a fluorescence microscope (Axiophot, Carl Zeiss, Jena, Germany). Quantification of mGlu3R expression was done with ImageJ software and reported as total green fluorescence (Integrated OD) per field normalized to the number of cells present in each field.

2.6.2. GFAP

Cells were fixed with 4% paraformaldehyde, permeabilized with PBS-triton (PBS-T) 0.2%, and blocked with PBS-T 0.1% + 10% donkey serum. Primary antibody (anti-GFAP, 1:500, Millipore) was diluted in PBS-T 0.1% + 1% donkey serum. As a secondary antibody we used anti-mouse-Cy3 (1:400). Cells were mounted with DAPI.

2.6.3. CD11b

Cells were fixed with 4% paraformaldehyde and blocked with 10% donkey serum in PBS. Anti-CD11b (5 μg/mL in PBS plus 1% donkey serum) was incubated overnight at 4 °C, followed by incubation with the secondary antibody (anti-mouse Cy3 1:200 diluted in PBS-T 0.2% plus 1% donkey serum). Cells were mounted with DAPI.

2.6.4. Arginase 1 (AG1)

Microglia were fixed with 4% paraformaldehyde and blocked with 10% goat serum in PBS-T 0.2%. Anti-AG1 polyclonal antibody (4 μg/ml, Santa Cruz Biotechnology) was diluted in PBS-T 0.2% plus 1% goat serum and incubated overnight at 4 °C, followed by incubation with the secondary antibody (anti-rabbit-FITC 1:300 in PBS-T 0.2% plus 1% goat serum). Cells were mounted with DAPI.

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2.7. Microsphere phagocytosis and Aβ uptake analysis

After CD11b or GFAP staining, cells were visualized under a fluorescence microscope (Axiophot, Carl Zeiss, Jena, Germany). Percentage of bead-containing cells or Aβ-positive cells over total cells was recorded, whereas phagocytic index was calculated as the percentage of cells containing more than 10 beads over total cells, as published elsewhere (Karlstetter et al., 2014).

2.8. Statistical analysis

Data were expressed as mean ± SEM and analyzed by one-way analysis of variance (ANOVA) or repeated measures ANOVA, followed by the Bonferroni or Dunnett post-test, or by one- or two-sample Student's *t*-test, when appropriate. Differences with a $p < 0.05$ were considered statistically significant.

3. Results

3.1. sAPPα and BDNF involvement in the neuroprotective action of GCM from LY379268-treated astrocytes

Based on evidence suggesting an antiapoptotic effect of GCM derived from LY379268-treated astrocytes on Aβ-induced neuron death (Caraci et al., 2011), and considering previous results from our group showing that mGlu3R activation in astrocytes induces sAPPα release (Durand et al., 2014), we sought to determine whether sAPPα could be responsible for the neuroprotective action of GCM. Primary hippocampal neuron cultures were incubated with complete-control-GCM, complete-LY-GCM, sAPPα-immunodepleted-control-GCM, or sAPPα-immunodepleted-LY-GCM for 1 h prior to exposure to 25 μM $Aβ_{25-35}$ (pre-aggregated for 4 days at 37 °C) for 24 h. Then, neuron viability was assessed by Trypan Blue exclusion (Fig. 2a) and the TUNEL method (Fig. 2b). As expected, both complete-control-GCM and complete-LY-GCM reduced the percentage of dead neurons after Aβ challenge, and LY-GCM exhibited a higher neuroprotective effect than control-GCM (Fig. 2a and b). In agreement with our hypothesis, sAPPα depletion from LY-GCM reversed the effect of LY-GCM, restoring neuron death levels to those observed in Aβ+complete-control-GCM treated neurons (Fig. 2a and b). Moreover, sAPPα depletion from control-GCM had no effect compared to complete-control-GCM (Fig. 2a). Therefore, we propose that once released from astrocytes after mGlu3R activation, sAPPα mediates the neuroprotective action of GCM against Aβ toxicity. None of the GCM alone had any significant effect on basal neuron death (Fig. 2a).

Although variability is often expected when comparing results from two different viability indicators, the present differences between cell death magnitudes obtained by Trypan blue exclusion and the TUNEL technique may respond to the different end-points being measured by each technique, as reported elsewhere (Gain et al., 2002; Gilbert and Boutros, 2016; Hanly et al., 2015). Whereas the Trypan blue exclusion assay detects cells with impaired plasma membrane integrity, the TUNEL assay detects DNA fragmentation, which occurs much earlier than membrane disintegration. Therefore, at the 24 h time-point it is likely that most of the TUNEL-positive cells are in the early stages of death but cannot yet be detected by Trypan blue exclusion.

As a control of immunoprecipitations we performed a western blot for immunoprecipitated sAPPα (Fig. 2c). Blots showed positive immunoreactivity for sAPPα in the pellet fraction of IP (lane 3) as well as in the positive control (astrocyte lysates, lane 2), but no reactivity in the pellet of IP reaction incubated without antibody (lane 1). Concordantly, LY-GCM subjected to IP in absence of anti-sAPPα antibody did not modify the effect of complete-LY-GCM (data not shown).

To the best of our knowledge there are no precedents linking mGlu3R activation to BDNF production in astrocytes. Therefore, we studied BDNF mRNA levels after treating astrocytes with LY379268 and found that BDNF expression was significantly increased by 40% after mGlu3R activation (Fig. 3a). Concordantly, neutralization of BDNF from LY-GCM increased basal neuron death (Fig. 3b) and voided the protective effect of LY-GCM against Aβ neurotoxicity (Fig. 3b and c). Hence, BDNF may also be implicated in the pro-survival effect of LY-GCM on hippocampal neurons, not only upon Aβ challenge but also in resting conditions.

3.2. Effect of LY379268 through neuronal mGlu3R

Next, we investigated the effect of LY379268 on Aβ-induced cell death through binding at neuronal mGlu3R or mGlu2R. As shown in Fig. 4a, primary cultured hippocampal neurons were stained by both an anti-mGlu2/3R antibody as well as a selective anti-mGlu3R antibody, thereby confirming the expression of mGlu3R in cultured hippocampal neurons. Interestingly, LY379268 directly added to neuron cultures partially prevented Aβ-induced neuron death (Fig. 4b). This result was also observed by the TUNEL technique (data not shown). The aforementioned effect was due to activation of mGlu3R subtype since the mGlu3R-selective NAM (LY2389575) reverted the anti-apoptotic action of LY379268 (Fig. 4b). In order to test whether the observed changes in neuron viability responded to variations in mGlu3R expression, mGlu3R immunoreactivity was quantified in hippocampal neurons treated with Aβ, LY379268, or their combination. No significant differences in mGlu3R expression were found between groups (Fig. 4c).

3.3. LY379268 stimulates glial Aβ uptake

Aside from producing soluble neurotrophins, another major neuroprotective function of glial cells in the context of amyloidosis is amyloid clearance, which is achieved by endocytic/phagocytic mechanisms followed by intracellular degradation (Ries and Sastre, 2016). We consequently decided to ascertain the effect of astroglial mGlu3R activation on Aβ uptake and its impact on neuron survival. The repercussion of Aβ uptake by astrocytes on neuron survival was studied by using a non-contact co-culture approach, as shown in Fig. 5a. For this purpose we did not pre-aggregate Aβ in order to favor its passage through the transwell porous membrane.

In this co-culture system we observed a significant increase in astroglial Aβ uptake after treatment with LY379268 (Fig. 5c, brown bars). In parallel, analysis of neuron death by TUNEL revealed that, whereas fluor-Aβ significantly increased neuron death (as did unlabeled Aβ), neurons co-cultured with LY379268-pretreated astrocytes exhibited reduced cell death, comparable to that of the control group (Fig. 5c, pink bars). However, co-culturing neurons with control astrocytes did not modify cell death induced by Aβ (Fig. 5c, pink bars). Hence, an association between increased Aβ uptake and reduced neuron death after astroglial mGlu3R activation is suggested.

It is noteworthy that the percentage of neuron death observed after pre-aggregated, unlabeled Aβ treatment (Fig. 2b) was comparable to that obtained with non-pre-aggregated fluor-Aβ (Fig. 5c), suggesting that fibrillization might not be essential for the neurotoxic effect of $Aβ_{25-35}$.

Next, we tested two scavenger receptors possibly linked to amyloid phagocytosis by astrocytes: CD36 and SRB1. We found that LY379268 induced a weak but significant increase in CD36 mRNA levels in astrocytes, but did not modify SRB1 expression (Fig. 5d).

Next, we analyzed microglial phagocytic activity in response to LY379268. Since microglia express mGlu2R as well as mGlu3R

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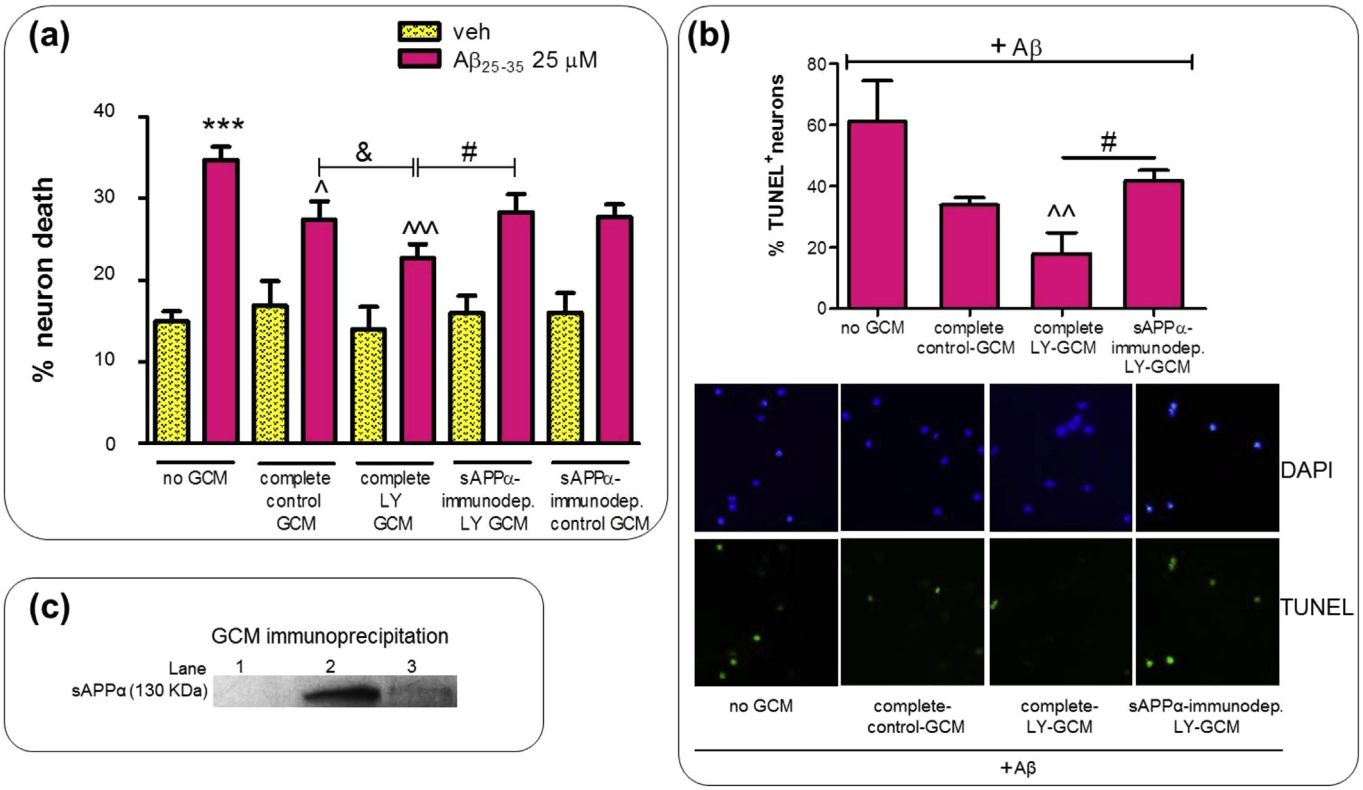

Fig. 2. sAPPα mediates neuroprotection exerted by glial conditioned medium (GCM) from LY379268-treated astrocytes on Aβ-challenged hippocampal neurons. Primary cultured hippocampal neurons were treated with 25 μM pre-aggregated $A\beta_{25-35}$ for 24 h (pink bars) in the presence of DMEM-N2 (no GCM); complete-control-GCM (GCM derived from astrocytes treated with DMEM-N2 for 3 h); complete-LY-GCM (GCM derived from astrocytes treated with 0.1 μM LY379268 for 3 h); sAPPα-immunodepleted LY- or control-GCM (GCM derived from astrocytes treated or not with LY379268, which was depleted of sAPPα using an anti-sAPPα monoclonal antibody). veh: respective vehicle for each treatment in absence of Aβ. Neuron death was determined by Trypan Blue exclusion (a, N = 6) or TUNEL (b, N = 3). ***$p < 0.001$ versus veh-no GCM; ^$p < 0.05$/^^$p < 0.01$/^^^$p < 0.001$ versus Aβ-no GCM; &$p < 0.05$ versus control-GCM; #$p < 0.05$ versus complete LY-GCM. (c) Western blot for sAPPα showing specificity of immunoprecipitation (IP). Lane 1= pellet from IP with water instead of antibody; Lane 2= positive control (astrocyte lysate); Lane 3= pellet from IP with anti-sAPPα antibody. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

subtypes (Taylor et al., 2002), the agonist LY379268 may activate both receptors in this cell type. Primary cultured microglia were incubated with LY379268 for 24 h and their phagocytic activity was determined by using latex beads and/or fluor-Aβ. LY379268 significantly increased the percentage of phagocytic microglial cells (bead-containing microglia), whereas in the presence of the mGlu3R-selective NAM (LY2389575) this effect was blocked (Fig. 6a and b), evidencing involvement of the mGlu3R subtype in the induction of phagocytosis. Furthermore, microglial mGlu3R activation also increased the phagocytic burden, expressed as the *phagocytic index* (Fig. 6a and c). LY379268-mediated increase in the phagocytic index was also impaired by LY2389575 (Fig. 6c). Since these results may or may not be representative of what microglial mGlu3R activation does regarding specific Aβ phagocytosis, we studied Aβ uptake in these cells. LY379268 was able to induce fluor-Aβ uptake (Fig. 6e); however, this effect does not seem to be mediated by mGlu3R subtype since LY2389575 treatment did not modify LY379268-induced Aβ uptake (Fig. 6e), suggesting involvement of mGlu2R rather than mGlu3R in microglial Aβ uptake. We also found a strong inhibitory effect of CD11b neutralization on LY379268-induced Aβ uptake (Fig. 6f), evidencing a role of this surface molecule in microglial-mediated Aβ phagocytosis.

While analyzing the phagocytic activity of microglia, we were surprised to notice a strong increment in the number of microglial cells following LY379268 treatment, which was also reversed by LY2389575 (Fig. 6g). This finding may be of particular relevance considering that this proliferating population is actively phagocytic. In order to investigate whether these microglia exhibited a more anti-inflammatory/neuroprotective profile (M2-like), we stained microglia for arginase-1 (AG1), a classical marker of M2 phenotype (Fig. 6h). No significant difference in the percentage of AG1-positive cells was found after 24 h-treatment with LY379268 (data not shown). Instead, as shown in Fig. 6h, the AG1-positive population in the control group was higher than 95%.

4. Discussion

In line with the arduous task of searching for novel and efficacious therapies for AD, our present results, combined with previous results from our group, postulate glial mGlu3R as promising therapeutic targets for treating this disease based on their demonstrated ability to:

i) Promote the non-amyloidogenic cleavage of APP in astrocytes, in detriment of the β-site cleavage, leading to increased sAPPα secretion (Durand et al., 2014);
ii) Prevent Aβ-induced neuron death through neuroprotective actions of astroglial sAPPα and BDNF;
iii) Rescue hippocampal neurons from Aβ-induced cell death, even in absence of glial cells;
iv) Stimulate Aβ uptake by astrocytes and microglia, which may translate into reduced neuron death in the context of amyloidosis.

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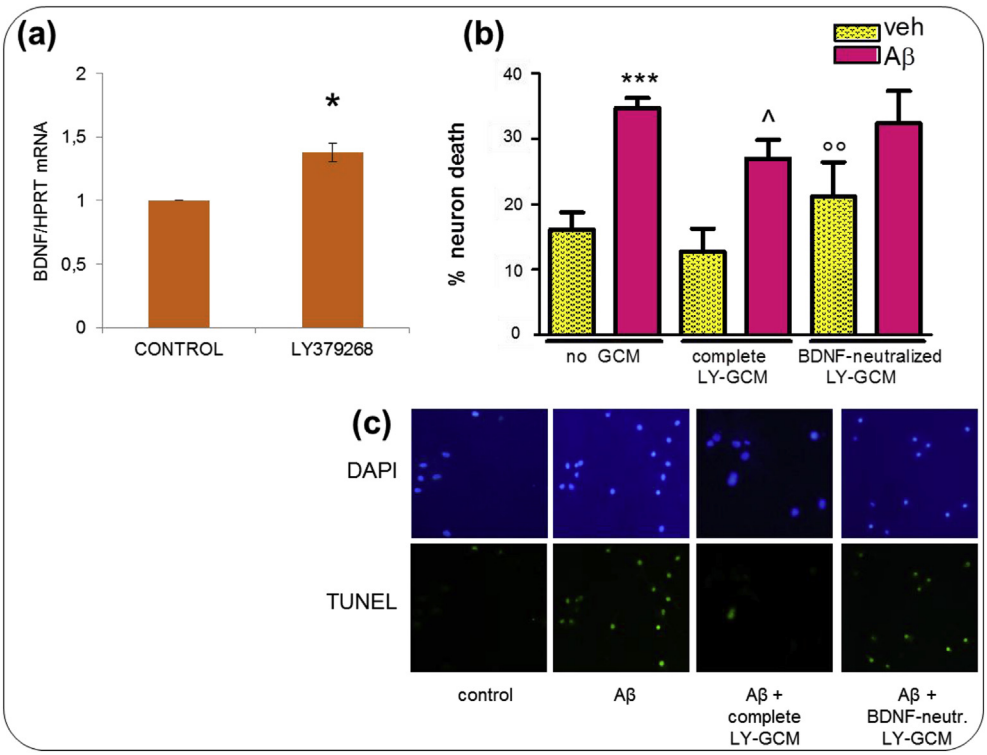

Fig. 3. BDNF is involved in the neuroprotective effect of LY-GCM on Aβ-challenged hippocampal neurons. (a) Primary cultured astrocytes were incubated with 0.1 μM LY379268 for 3 h, and BDNF mRNA levels were quantified by RT-qPCR. *$p < 0.05$ versus control, N = 4. (b and c) Primary cultured hippocampal neurons were treated with 25 μM pre-aggregated $Aβ_{25-35}$ for 24 h in the presence of DMEM-N2 (no GCM); complete-LY-GCM (GCM derived from astrocytes treated with 0.1 μM LY379268 for 3 h); or BDNF-neutralized LY-GCM (LY-GCM, in which BDNF activity was inhibited using a neutralizing anti-BDNF antibody). veh: respective vehicle for each treatment in absence of Aβ. Neuron death was determined by Trypan Blue exclusion (b) or TUNEL (c). ***$p < 0.001$ versus veh-no GCM; ^$p < 0.05$ versus Aβ-no GCM; °°$p < 0.01$ versus veh-complete-LY-GCM, N = 3.

Our results using GCM complement those from Caraci et al. (2011), who showed that GCM from control astrocytes is neuroprotective against $Aβ_{25-35}$ toxicity, and that GCM from LY379268-treated astrocytes exerts further neuroprotection in cortical primary cultured neurons. Here, we reproduced those results using hippocampal cultured neurons but, although Caraci et al. (2011) completely blocked GCM protective effect through TFGβ neutralization, we have clearly demonstrated here that sAPPα and BDNF are two additional key players in neuroprotection exerted by mGlu3R-stimulated astrocytes on Aβ-challenged neurons. Contrary to the well-known properties of BDNF as a neurotrophin, sAPPα has been studied much less. sAPPα is composed of up to 6 domains, domains D1 and D6a being those which likely carry intrinsic neuroprotective activity, since they present beneficial actions *per se in vitro* (Jin et al., 1994; Ohsawa et al., 1997; Qiu et al., 1995) and improve motor and cognitive outcome in a rat model of traumatic brain injury (Corrigan et al., 2011). sAPPα plays a role in modulation of neuron excitability, synaptic plasticity, neurite outgrowth, synaptogenesis, and cell survival after Aβ challenge (Habib et al., 2017; Stein and Johnson, 2003), and has memory-enhancing effects in certain behavioral paradigms (Bour et al., 2004; Meziane et al., 1998; Xiong et al., 2017). It is noteworthy that sAPPα is able to directly associate and modulate β-secretase activity (Obregon et al., 2012), and can also inhibit tau phosphorylation through repression of the GSK3β signaling pathway, which thereby define a central role for sAPPα in APP autoregulation and AD pathogenesis (Deng et al., 2015). Interestingly, astrocyte-derived exosomes from plasma of patients with AD or matched controls present much higher levels of sAPPα than neuron-derived exosomes (Goetzl et al., 2016). The fact that sAPPα immunodepletion in our *in vitro* system had a suppressive effect only on LY-GCM (but not on control-GCM)-mediated neuroprotection highlights the particular neuroprotective profile of mGlu3R-induced astroglial sAPPα compared to the basal condition. On its own, BDNF present in LY-GCM seems to be a key player for basal neuron survival, as its neutralization from GCM increased hippocampal neuron death even in absence of Aβ. To the best of our knowledge, there is no previous evidence of changes in astrocytic BDNF expression after mGlu3R activation. Jean et al. (2008) showed an increase in BDNF levels and release from basal forebrain astrocytes in culture after group I mGluR activation. Di Liberto et al. (2010) showed that LY379268 upregulates BDNF mRNA and protein levels in the cerebral cortex and in the hippocampal formation, but this effect was restricted to neurons. On the other hand, Venero et al. (2002) injected the group II mGluR agonist DCG-IV into the striatum and found high levels of BDNF mRNA only in microglial cells. Other authors found no changes in BDNF levels after injection of LY379268 (Bond et al., 2000; Gewirtz et al., 2002). Nevertheless, here we describe an upregulation of BDNF mRNA levels in cultured astrocytes exposed to LY372968.

Aside from the protective effect exerted by the mGlu3R agonist on Aβ-challenged neurons through astrocyte-derived neurotrophins, we show here that neuronal mGlu3R activation is also neuroprotective against Aβ toxicity. This result disagrees with previous reports showing no effect of mGlu3R agonists on neuron viability affected by Aβ (Caraci et al., 2011), staurosporine, glutamate (Jantas et al., 2016), or myelin (Pinteaux-Jones et al., 2008). Indeed, it has been shown that neuronal mGlu2R activation enhances neuron death (Caraci et al., 2011; Corti et al., 2007). Such differences may well be due to the developmental stage of the cells in culture and the brain region from which neurons have been isolated, as previously demonstrated (Copani et al., 1995; Jantas et al., 2016). However, another set of papers have demonstrated

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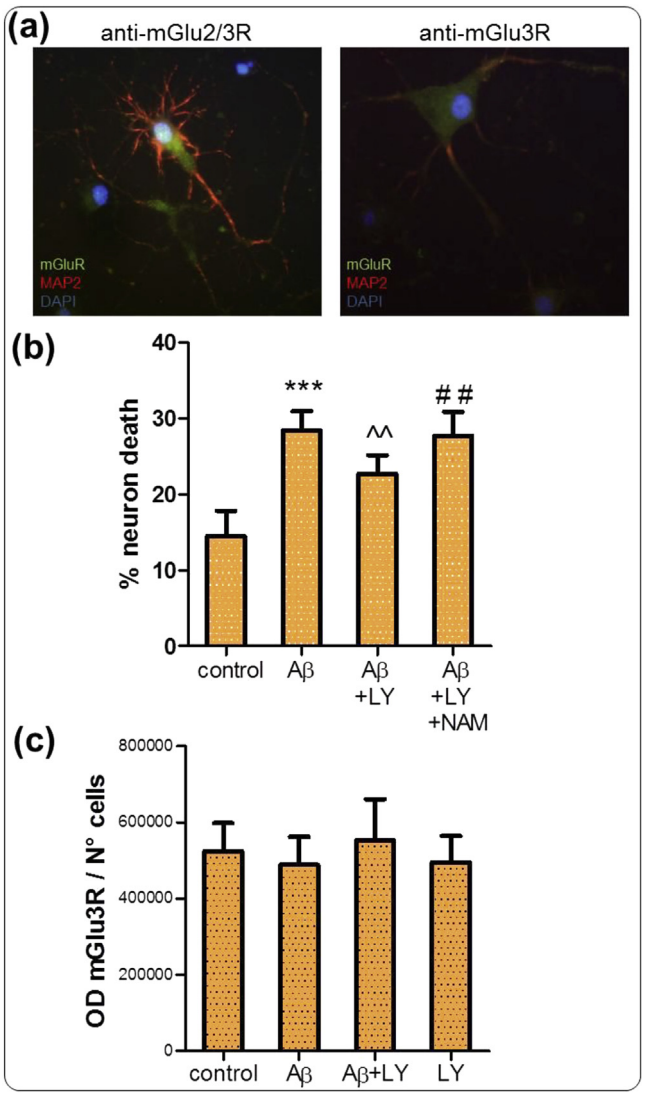

Fig. 4. LY379268 prevents Aβ-induced neuron death by acting directly at neuronal mGlu3R. (a) Neuron immunostaining for group II mGluR using a non-selective anti-mGlu2/3R antibody (left, green) or an mGlu3R-selective antibody (right, green). Neurons were co-stained for MAP2 (red) and DAPI (blue). (b) Primary cultured hippocampal neurons were treated with DMEM-N2 (control) or 25 μM pre-aggregated $A\beta_{25-35}$ in the presence or absence of 0.1 μM LY379268 ± the mGlu3R-selective NAM LY2389575 (0.1 μM) for 24 h. Neuron death was determined by Trypan blue exclusion. ***$p < 0.001$ versus control, ^^$p < 0.01$ versus Aβ, ##$p < 0.01$ versus Aβ+LY, $N = 4$. (c) Immunofluorescence for mGlu3R subtype was expressed as Integrated Density per field normalized to the number of cells present in each field. The mean of 10–14 fields per group analyzed from 1 representative experiment is shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

neuroprotective properties of these receptors in primary neuron cell cultures (Berent-Spillson et al., 2004; Kingston et al., 1999; Vincent and Maiese, 2000; Zhou et al., 2006) even against Aβ toxicity (Copani et al., 1995). Nevertheless, we cannot absolutely rule out the possibility that the effect we observed on neuron survival may be mediated by mGlu3R from contaminating glial cells present in our neuron cultures, as neuron purity ranged between 90 and 98%.

Although it is clear that mGlu3R, but not mGlu2R, is expressed in astrocytes throughout the brain, the precise localization of mGlu3R in neuronal elements remained elusive (Tamaru et al., 2001). The typical pattern of mGlu3R expression is: high levels in the cerebral cortex, striatum, and reticular thalamic nucleus, and low levels in the hippocampus (Mudo et al., 2007). Here we show the presence of mGlu3R in cultured hippocampal neurons by immunostaining with a selective-anti-mGlu3R antibody. Janssens and Lesage (2001) detected both mGlu2R and mGlu3R mRNA in primary cultured embryonic hippocampal neurons. Jantas et al. (2016) showed mRNA expression of mGlu2R and mGlu3R, the former being 10/20-fold lower than mGlu3R mRNA levels in 7-DIV hippocampal neurons, whereas mGlu3R gene expression was inhibited in 12-DIV neuron cultures, suggesting strong dependence between mGlu3R levels and neuronal developmental stage. In our different experimental groups, however, mGlu3R expression remained stable.

In order to achieve the goal of reducing Aβ accumulation in the CNS in AD, we must consider balance between Aβ genesis (through amyloidogenic APP processing) and Aβ removal and degradation. In addition, the cerebral impact of Aβ clearance dysregulation is strengthened considering collateral Aβ accumulation in blood vessel walls, or *cerebral amyloid angiopathy*. As a major phagocytic cell in the CNS, microglial participation in Aβ clearance has been thoroughly studied. On the contrary, Aβ removal by astrocytes has been explored far less, despite evidence indicating that microglial ablation has no effect on Aβ accumulation in transgenic models of AD, at least during the specific time period studied (Grathwohl et al., 2009), and that transplanted astrocytes successfully clear Aβ deposits in AD mice through upregulation of specific proteases (Pihlaja et al., 2008, 2011). The presence of Aβ-containing-astrocytes near amyloid plaques suggests that astrocytes participate in the clearance of Aβ in AD, or rather that dysregulation of astrocyte-mediated Aβ clearance may underlie Aβ accumulation in this disease (Alarcón et al., 2005). Our present results show that mGlu3R subtype increases Aβ uptake by cultured astrocytes. Moreover, underscoring this result, we found that the increase in Aβ uptake after astroglial mGlu3R activation was accompanied by reduced neuron death, as demonstrated by the neuron-astrocyte non-contact co-culture system, suggesting a relevant association between mGlu3R-mediated Aβ clearance and neuroprotection. In microglia, mGlu3R subtype activation increases phagocytosis of fluorescent microspheres as well as the phagocytic burden. However, specific phagocytosis of Aβ apparently responded to mGlu2R subtype activation, indicating differential uptake mechanisms depending on the *cargo* molecule to be phagocytized, as suggested by Jones et al. (2013).

Oligomeric and fibrillar Aβ are primarily internalized through receptor-mediated endocytosis (reviewed in (Ries and Sastre, 2016)). Scavenger receptors (SR), Toll-like receptors, receptor for advanced glycation end products (RAGE), Fc receptors, and lipoprotein receptor-related proteins (LRP), among others, account for this function (Ries and Sastre, 2016). As for SR, SR type-A, type B1 (SR-B1), SR-MARCO, CD36, or CD40 can be expressed by astrocytes and microglia where they bind to and mediate endocytosis of Aβ (Alarcón et al., 2005; Husemann et al., 2002; Ries and Sastre, 2016). SR-B1 plays a role in C1q-facilitated $A\beta_{1-42}$ uptake by astrocytes, whereas both SR-B1 expression and $A\beta_{1-42}$ uptake are impaired in old 5xFAD astrocytes (Iram et al., 2016). In addition, individual neutralization of CD36, CD47, or RAGE in astrocytes partially inhibits their phagocytic capability (Jones et al., 2013). Among the mechanisms underlying glia-mediated Aβ phagocytosis, we hereby propose involvement of CD11b in the process of recognition/endocytosis of Aβ by microglia. CD11b (a.k.a. complement receptor 3) is an integrin implicated in intercellular adhesion. Phagocytosis induced via CD11b may or may not be dependent on the opsonin C3b (Choucair et al., 2006); thus CD11b is considered a professional phagocytic receptor (Le Cabec et al., 2002). Microglial expression of CD11b is enhanced in the brain of AD patients (Akiyama and

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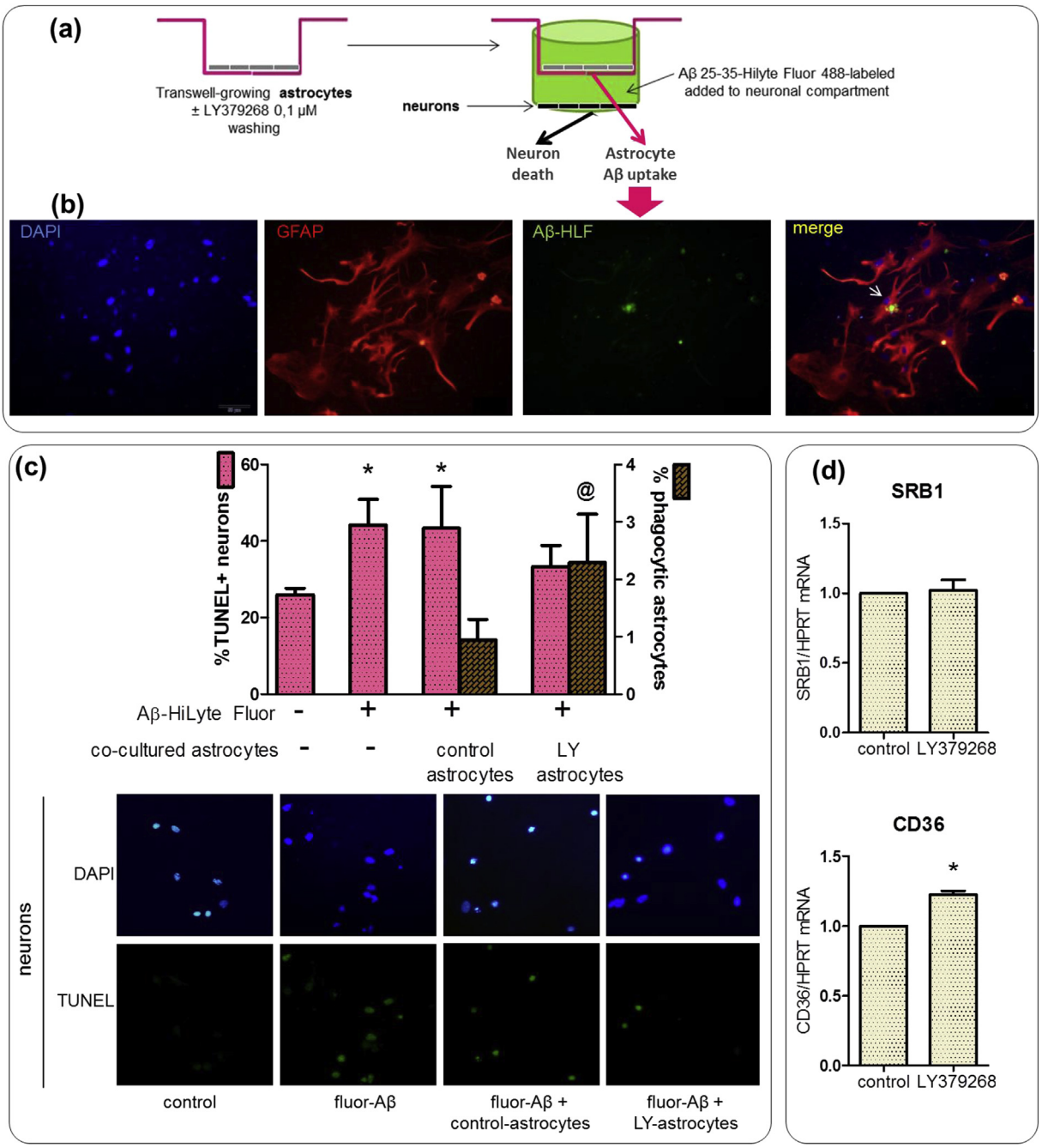

Fig. 5. Aβ neurotoxicity is reduced in neurons co-cultured with LY379268-pretreated astrocytes. (a) Schematic diagram of co-culture experiments: Transwell-growing astrocytes were treated with MEM (control astrocytes) or with 0.1 µM LY379268 (LY astrocytes) for 24 h. After washing with PBS, transwells were transferred to 24-well dishes containing cultured hippocampal neurons. $A\beta_{25-35}$-HiLyte Fluor™ 488-labeled was added to neuronal compartments and inserts were filled with DMEM-N2. 24 h later, astrocytes and neurons were fixed and stained for GFAP or TUNEL, respectively. (b) Representative images of Aβ uptake by astrocytes. (c) The percentage of astrocytes positive for Aβ was quantified (% phagocytic cells, brown bars, right Y-axis, @$p < 0.05$ versus control astrocytes), whereas neuron death was determined by TUNEL (pink bars, left Y-axis, *$p < 0.05$ versus control neurons). $N = 5$. (d) Astroglial mRNA expression of scavenger receptors SRB1 and CD36 after 24 h treatment with 0.1 µM LY379268 was assessed by RT-qPCR. *$p < 0.05$ versus control, $N = 3$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

McGeer, 1990; Strohmeyer et al., 2002) and CD11b is implicated in the elimination of $A\beta_{1-42}$ and $A\beta_{1-40}$-coated yeast particles (Choucair-Jaafar et al., 2011). SR-A can act in conjunction with CD11b to promote Aβ uptake in microglia (Fu et al., 2012). Here, we showed that CD11b neutralization robustly inhibited both basal and LY379268-induced $A\beta_{25-35}$ uptake by microglia. As for astrocytes, we detected a significant change in expression of CD36 mRNA after mGlu3R activation but no change in SRB1, suggesting possible involvement of CD36 in astroglial phagocytic activity. However, to ascribe changes in phagocytic function to a specific receptor is at least an ambitious task given the complexity of these processes. For example, a mechanism of redundancy among SRs has been suggested, since individuals genetically deficient for some SR exhibit no evident brain pathology (Trigatti et al., 1999; Yamamoto et al., 1994). On the other hand, an interesting association between sAPPα and Aβ clearance has been demonstrated. sAPPα interacts

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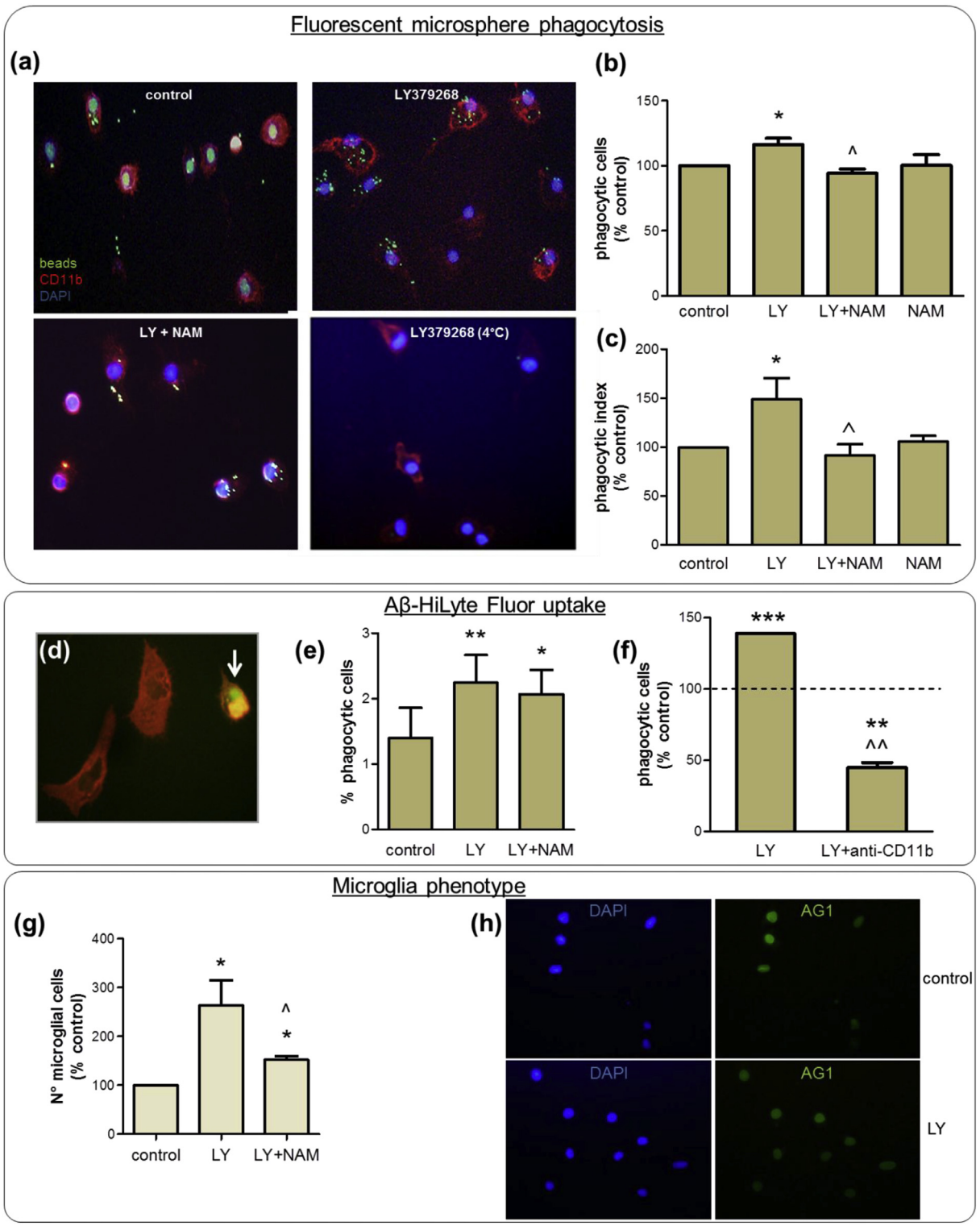

Fig. 6. LY379268 induces phagocytic activity and Aβ uptake by microglia. Primary cultured microglial cells were incubated with 0.1 μM LY379268 in the presence or absence of mGlu3R-selective NAM (LY2389575, 0.1 μM) for 24 h. The last 1–2 h of each experiment, either fluor-Aβ or fluorescent microspheres, respectively, was added. After fixation, cells were stained for CD11b. (a) Representative images for microsphere phagocytosis. A negative control incubated at 4 °C was included. (b) Percentage of bead-containing cells (phagocytic cells, N = 5). (c) Percentage of cells containing more than 10 beads (phagocytic index, N = 6). *p < 0.05 versus control; ^p < 0.05 versus LY. (d) A representative image shows a microglial cell positive for Aβ (arrow). (e) Percentage of cells positive for Aβ. *p < 0.05, **p < 0.01 versus control, N = 4. (f) Microglial cells were incubated with LY379268 for 24 h, then washed with PBS and incubated with anti-CD11b antibody for 15 min prior to replacing the medium for fluor-Aβ. After 1 h, Aβ uptake was quantified. **p < 0.01/ ***p < 0.001 versus control; ^^p < 0.01 versus LY, N = 3. (g) Total number of microglial cells per experimental group was estimated by counting cells present in vertical plus horizontal diametrical lines of each cover glass. *p < 0.05 versus control; ^p < 0.05 versus LY, N = 7. In b, c, f, and g figures, control values were set as 100%. (h) AG1 staining for microglia treated with LY379268 for 24 h. A representative experiment from 3 independent experiments is shown.

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with several partners involved in Aβ clearance such as SR-A and LRP1 in microglia, neurons, peripheral monocytes, and endothelial cells from the blood-brain barrier (reviewed in (Habib et al., 2017), thereby adding new components to the Aβ clearance machinery.

Despite the fact that we and others have demonstrated protective actions of astrocytes in favor of neuron survival, a variety of papers are focused on the deleterious effects that Aβ-activated glial cells have on neuronal function. In fact, the classical notion in AD research sustains that pro-inflammatory mediators derived from activated glia accompany or even cause AD pathological features. For example, using glia-neuron co-cultures, Jana and Pahan (2010) showed that Aβ1-42 + IL1β-activated astrocytes release nitric oxide, which may stimulate neuronal ceramide production, which in turn causes neuron death. Also, other authors showed that exposure of hippocampal glia-neuron co-cultures to Aβ decreased nicotinamide adenine dinucleotide and mitochondrial membrane potential in astrocytes and increased neuron death (Abeti et al., 2011). Moreover, reactive astrocytes can accumulate and release Aβ (Nagele et al., 2003, 2004), and the amount of Aβ-positive material in activated astrocytes correlates with the extent of local AD pathology (Nagele et al., 2004). It has been suggested that astrocytes accumulate Aβ through internalization of debris from Aβ-containing neurons (Nagele et al., 2003), and that Aβ-burdened astrocytes can later undergo lysis to form astrocyte-derived amyloid plaques (Nagele et al., 2004). Furthermore, astrocytes can produce and secrete Aβ after challenging with cytokines or Aβ (Blasko et al., 2000; Ourdev et al., 2015; Prà et al., 2011; Zhao et al., 2011) or even under basal conditions. By using a novel method to detect single-cell-derived analytes, Liao et al. (2016) showed that astrocytes from induced pluripotent stem cells (iPSC) secrete high levels of both Aβ and sAPPα. The authors also showed that 8.8% of purified human astrocytes secrete detectable levels of sAPPα, whereas only 1.6% of these cells secrete detectable levels of Aβ, and that levels of Aβ secreted by iPSC-derived astrocytes represent 1/3 of that secreted by iPSC-derived neurons. Concordantly, β-secretase expression is higher in neurons than in glia (Armato et al., 2013). On the contrary, Busciglio et al. (1993) had previously shown that human astrocytes generate higher levels of Aβ than neurons or than rat astrocytes. Also, Goetzl et al. (2016) isolated astrocyte-derived exosomes (ADEs) and neuron-derived exosomes (NDEs) from healthy or AD patients and found that ADEs contain significantly higher levels of Aβ, β- and γ-secretases, sAPPα, glial-derived neurotrophic factor, and tau than NDEs. Evidence suggests that astrocytes may significantly contribute to Aβ burden in AD. However and in spite of all this evidence, deleterious actions of astroglia in neuron viability cannot be assumed in our experimental conditions due to the fact that no additional neuron death was observed when neurons were co-cultured with untreated astrocytes in the presence of Aβ. Furthermore, LY379268-pretreated astrocytes protected co-cultured neurons from Aβ toxicity. It should also be pointed out that, since internalization of fluorescent-labeled Aβ is visualized, our results reflect exogenous amyloid uptake and not endogenous production of Aβ.

Aβ removal from the brain parenchyma has been associated with an M2 or alternatively activated microglial phenotype (Tang and Le, 2016). Also, Cherry et al. (2015) demonstrated that AG1-positive microglia are involved in Aβ-plaque reduction during sustained, IL-1β-dependent neuroinflammation. On the contrary, inflammatory microglia are poor phagocytes of Aβ, perhaps because they cannot properly degrade it (Cherry et al., 2015). Although we found no differences in the percentage of AG1-positive microglial cells after LY379268 treatment, almost all microglia present in our cultures were AG1-positive. Taking into account that LY379268 strongly incremented the number of microglial cells, enrichment in the AG1-positive microglia population may ultimately be occurring. In turn, these microglia could improve Aβ clearance and promote an anti-inflammatory scenario. This topic requires much further investigation in order to fully characterize this microglial population, highly relevant to the modulation of amyloidosis, and to better understand the complexity of microglial response.

Controversial results have been reported regarding mGluR functions in microglia. Activation of microglial mGlu2R exacerbates myelin-induced neurotoxicity in cerebellar granule neurons, whereas the mGlu3R agonist NAAG exerts a neuroprotective effect (Pinteaux-Jones et al., 2008). However, induction of microglial apoptosis by group II mGluR activation was reported (Taylor et al., 2002). Soluble toxins released by microglia after group II mGluR activation cause neuron death, but do not mediate Aβ-induced neuron death (Taylor et al., 2002). These effects were attributed to the mGlu2R subtype (Taylor et al., 2005). Glucose deprivation-challenged neurons induce microglial activation through stimulation of group II mGluR, and these activated microglia are neurotoxic to naïve neurons (Kaushal and Schlichter, 2008). Our results, however, showed that mGlu2R mediates Aβ clearance by microglia, thereby underscoring the need for a more comprehensive study of the dynamics of microglial group II mGluR and neurotoxicity in the brain.

In summary, our results indicate that group II mGluR exhibit neuroprotective properties in all three cell types studied: astrocytes, microglia, and neurons, and that astroglial mGlu3R-mediated neuroprotection rests on its capacity to induce the release of neurotrophic factors as well as to promote Aβ clearance.

5. Conclusion

Dysregulation of glial function can contribute to the development or progression of AD and other neurodegenerative diseases. Considering our previously published results showing that mGlu3R expression in astrocytes is reduced in the hippocampus of AD mice (Durand et al., 2014) and our present findings describing a clear neuroprotective effect of mGlu3R activation in astrocytes, we propose that deficient astroglial mGlu3R signaling may underlie some pathogenic mechanisms of AD. As for microglia, specific effects of mGlu2R and mGlu3R subtypes require further study in order to improve understanding of the net effect of group II mGluR activation on this cell type.

Disclosure statement

There are no current or potential conflicts of interest for any author or any of the authors' institutions.

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