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Scavenger receptor control of chromogranin A-induced microglial stress and neurotoxic cascades

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ABSTRACT

Chromogranin A (CgA), a neuroactive glycoprotein, is associated with microglial activation cascades implicated in neurodegeneration. Here we show that CgA-dependent inducible nitric oxide synthase (iNOS) expression and stress responses in microglia involved signalling via scavenger receptors (SR), since SR class-A (SR-A) ligands blocked iNOS expression, mitochondrial depolarisation, apoptosis and glutamate release. Furthermore, block of SR-A ameliorated CgA-induced microglial neurotoxicity. In contrast, block of CD36, or the receptor for advanced glycation end products (RAGE) did not prevent CgA-induced microglial activation and neurotoxicity. Thus, manipulation of specific scavenger receptor-coupled signalling pathways may provide avenues for therapeutic intervention in neurodegenerative diseases implicating microglial activation with chromogranin peptides.

1. Introduction

The glycoprotein CgA is implicated in neurodegeneration in Alzheimer's (AD), stroke, Parkinson's, Pick's and prion disease [1–4]. Chromogranins can mediate secretion of mutant super oxide dismutase proteins linked to amyotrophic lateral sclerosis [5]. Microglial activation is involved in the degenerative processes of many neurological conditions [6] and suppression of the microglial response may decrease the development and impact of neuroinflammation and neurodegeneration [6]. CgA is a potent activator of microglia [7,8], inducing their production of potential neurotoxins [7,9] and activating microglial stress pathways [7,9,10].

Here, we show that CgA can signal in microglia via scavenger receptors (SR), and the neurotoxicity of CgA-exposed microglia can be prevented through the inhibition of SR-A. SR are upregulated in microglia around senile plaques in AD tissue [11] and, in vitro, amyloid-beta peptides act as SR ligands [12–15]. Thus processes which modulate the interaction of CgA with SR may have therapeutic potential in neurodegenerative diseases where microglial activation is implicated.

2. Materials and methods

2.1. Animals and materials

Wistar rats were bred and reared in house from stock animals originally purchased from Charles River UK Ltd. (Kent, UK). Foetal calf serum (FCS), and Dulbecco's modified Eagle's medium (D-MEM) were obtained from Invitrogen (Paisley, UK). Synthetic CgA was purchased from Scientific Marketing Associates, Hertfordshire, UK (from the Peptide Institute, Japan) and was verified endotoxin-free. Neutralising antibody against RAGE was a kind gift from Dr. Larry Denner (Texas Biotechnology Corporation, TX, USA). Neutralising antibody against SR-A (clone 2F8) was from Serotec (Kidlington, Oxford, UK). Mouse anti-CD36/SMø neutralising monoclonal antibody and fluorescein isothiocyanate-annexin V (annexin V) was from Autogen Bioclear (Calne, Wiltshire, UK). 5,5′,6,6′-Tetraethylbenzimidazole carbo-cyanine iodide (JC-1) was from Molecular Probes (Leiden, The Netherlands). Poly-inosine, fucoidan and all other reagents were from Sigma (Dorset, UK).

2.2. Preparation and treatment of cell cultures

Primary cultures of rat microglia and cerebellar granule neurons (CGC) were prepared and maintained as previously described [7,16]. The BV-2 microglia cell line, kindly provided by Dr. S.F. Tzeng (Department of Life Sciences, National Cheng Kung University, Taiwan), was cultured in D-MEM plus 10% FCS, 2 mM

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glutamine, 44 mM $NaHCO_3$, 100 U/ml penicillin, 100 μg/ml streptomycin and 125 μg/l amphotericin B. Microglia were pre-incubated with poly-inosine (0.1 or 0.5 μg/ml), or fucoidan (50 or 200 μg/ml; a complex sulphated polysaccharide found in brown seaweed), both of which are inhibitory ligands of SR-A [15] for 1 h before the addition of CgA (100 nM). Alternatively, microglia were pre-incubated with neutralising antibodies against RAGE (200 or 400 μg/ml) [17], SR-A (10–100 μg/ml) [18], or CD36 (SMø) (1 or 10 μg/ml) [13], for 1 h before the addition of CgA (100 nM). Following activation, microglial-conditioned medium (MGCM) was collected, added to CGC cultures at a 1:1 ratio with CGC medium and incubated for a further 24 h before analysis.

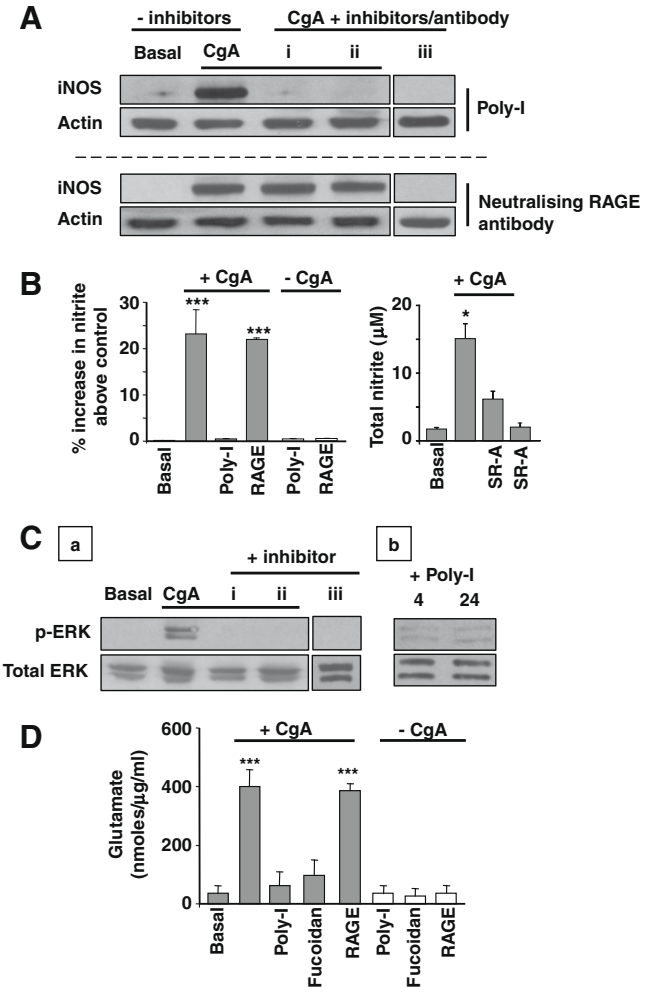

Fig. 1. Block of SR but not RAGE inhibits CgA-induced microglial reactivity. (A) Poly-inosine blocks CgA-induced iNOS expression. Western blot of iNOS and β-actin in microglia treated with 100 nM CgA ± poly-inosine (i: 0.1 μg/ml; ii: 0.5 μg/ml), neutralising RAGE antibody (i: 200 μg/ml; ii: 400 μg/ml) or with inhibitors alone (iii: 0.5 μg/ml poly-inosine or 400 μg/ml neutralising RAGE antibody) for 24 h. "Basal" is untreated microglia at 24 h. (B) Poly-inosine blocks CgA-induced nitrite production. Total nitrite levels in culture medium from microglia treated as in A or with 100 μg/ml anti-SR-A antibody, measured with Griess reagent. $^{***}P < 0.001$ or $^{*}P < 0.05$ versus basal. (C) Poly-inosine blocks CgA-induced ERK phosphorylation. Western blot of anti-phospho-ERK or total-ERK in (a) primary microglia 3 h after treatment with 100 nM CgA or together with poly-inosine (i: 0.1 μg/ml; ii: 0.5 μg/ml), with poly-inosine alone (0.5 μg/ml) (iii) or in untreated cells (Basal). (b) Control lysates from microglia treated for 4 or 24 h with poly-inosine (0.5 μg/ml). (D) Poly-inosine blocks CgA-induced glutamate release. Glutamate in MGCM from primary microglia treated with 100 nM CgA ± 0.5 μg/ml poly-inosine, 200 μg/ml fucoidan or 400 μg/ml neutralising RAGE antibody for 24 h. "Basal" indicated MGCM from microglia untreated for 24 h. Units describe nmoles of glutamate per μg of microglial protein per ml of medium. $^{***}P < 0.001$ versus basal.

2.3. Measurement of mitochondrial depolarisation, apoptosis and cell death

Mitochondrial depolarisation was assessed using JC-1 [9,10]. Apoptosis and cell death were assessed in unfixed cells using 2′[epoxyphenyl]-5-[4-methyl-1-piperazinyl]-2,5′-bi-1H-benzimidazol Hoechst 33342 (Hoechst 33342) for total cell number and pyknotic nuclei, in conjunction with propidium iodide (PI) [19] for dead cells or annexin V [9] for exterior staining of phosphatidylserine [20]. Cell counts were performed on at least five fields per coverslip, three coverslips per treatment from three independent experiments.

2.4. Immuno-blotting

Immuno-blotting was performed using standard procedures with 1:5000 rabbit anti-iNOS (BD Biosciences Pharmingen, Oxford, UK) or 1:750 mouse anti-phospho-extracellular signal regulated kinase (p-ERK; Autogen Bioclear, Wiltshire, UK) and ECL (Amersham Pharmacia, Buckinghamshire, UK). Protein loading controls used were 1:1000 mouse anti-β-actin (Sigma, UK) or 1:500 rabbit anti-total ERK (Autogen Bioclear, Wiltshire, UK), respectively. Immunoblots are representative of three independent experiments.

2.5. Measurement of nitrite and glutamate production

Total nitrite levels in MGCM were determined using Griess reagent [7]. Glutamate was measured using fluorometric quantification of NADPH, produced through the reaction of glutamate with $NADP^+$ in the presence of glutamate dehydrogenase [9,21].

2.6. Statistics

To compare two or more treatments with a control group (or other treatment) a one-way analysis of variance (ANOVA) was used followed by a Tukey post test. P values < 0.05 were considered statistically significant, and $P < 0.05^*$, $<0.005^{**}$, $<0.001^{***}$.

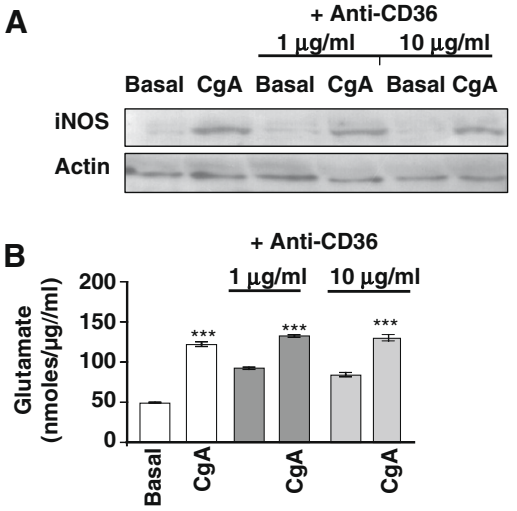

Fig. 2. Block of CD36 does not prevent CgA-induced iNOS expression or glutamate release. (A) Anti-CD36/SMø does not block CgA- induced iNOS expression. Western blot of iNOS and β-actin in primary microglia incubated with 1 or 10 μg/ml anti-CD36/SMø in the absence (Basal) or presence of 100 nM CgA for 24 h. (B) Anti-CD36/SMø does not block CgA-induced glutamate release. Glutamate in MGCM from BV-2 microglia treated with 100 nM CgA ± 10 μg/ml anti-CD36/SMø after 24 h. Units describe nmoles of glutamate per μg of microglial protein per ml of medium. $^{***}P < 0.001$ versus basal condition.