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Neuroprotective effects of mGluR II and III activators against staurosporine- and doxorubicin-induced cellular injury in SH-SY5Y cells: New evidence for a mechanism involving inhibition of AIF translocation

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ABSTRACT

There are several experimental data sets demonstrating the neuroprotective effects of activation of group II and III metabotropic glutamate receptors (mGluR II/III), however, their effect on neuronal apoptotic processes has yet to be fully recognized. Thus, the comparison of the neuroprotective potency of the mGluR II agonist LY354740, mGluR III agonist ACPT-I, mGluR4 PAM VU0361737, mGluR8 PAM AZ12216052 and allosteric mGluR7 agonist AMN082 against staurosporine (St-) and doxorubicin (Dox)-induced cell death has been performed in undifferentiated (UN-) and retinoic acid differentiated (RA-) human neuroblastoma SH-SY5Y cells. The highest neuroprotection in UN-SH-SY5Y cells was noted for AZ12216052 (0.01–1 μM) and VU0361737 (1–10 μM), with both agents partially attenuating the St- and Dox-evoked cell death. LY354740 (0.01–10 μM) and ACPT-I (10 μM) were protective only against the St-evoked cell damage, whereas AMN082 (0.001–0.01 μM) attenuated only the Dox-induced cell death. In RA-SH-SY5Y, a moderate neuroprotective response of mGluR II/III activators was observed for LY354740 (10 μM) and AZ12216052 (0.01 and 10 μM), which afforded protection only against the St-induced cell damage. The protection mediated by mGluR II/III activators against the St- and Dox-evoked cell death in UN-SH-SY5Y cells was not related to attenuation of caspase-3 activity, however, a decrease in the number of TUNEL-positive nuclei was found. Moreover, mGluR II/III activators attenuated the cytosolic level of the apoptosis inducing factor (AIF), which was increased after St and Dox exposure. Our data point to differential neuroprotective efficacy of various mGluR II/III activators in attenuating St- and Dox-evoked cell damage in SH-SY5Y cells, and dependence of the effects on the cellular differentiation state, as well on the type of the pro-apoptotic agent that is employed. Moreover, the neuroprotection mediated by mGluR II/III activators is accompanied by inhibition of caspase-3-independent DNA fragmentation evoked by AIF translocation.

1. Introduction

In spite of many years of investigations the causes as well the mechanisms of neuronal cell damage in various neurodegenerative diseases (e.g. Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and Amyotrophic lateral sclerosis (ALS)) have yet to be fully recognized (Agostini et al., 2011; Dauer and Przedborski, 2003; Rothstein, 2009). Despite the fact that many putative neuroprotective agents were beneficial in experimental models of neurodegenerative diseases, there is still a lack of clinically efficacious neuroprotective drugs (Faden and Stoica, 2007; Kabadi and Faden, 2014; Majid, 2014; Schapira et al., 2014). Many factors may contribute to neuronal cell demise among which overproduction of reactive oxygen species (ROS), depletion of endogenous antioxidant system, mitochondrial dysfunction, aggregates of misfolded

Abbreviations: AIF, apoptosis inducing factor; Dox, doxorubicin; FBS, fetal bovine serum; GABA, γ-aminobutyric acid; GPCRs, G-protein-coupled receptors; iGluRs, ionotropic glutamate receptors; LDH, lactate dehydrogenase; mGluRs, metabotropic glutamate receptors; MPP(+), 1-methyl-4-phenylpyridinium ion; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PAMs, positive allosteric modulators; PCD, programmed cell death; pHi, intracellular pH; PI, propidium iodide; RA-SH-SY5Y, retinoic acid-differentiated SH-SY5Y cells; St, staurosporine; TUNEL, the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling; UN-SH-SY5Y, undifferentiated SH-SY5Y cells.

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proteins, neuroinflammation, excitotoxicity and apoptotic-like programmed cell death (PCD) are the most common (Agostinho et al., 2010; Agostini et al., 2011; Navarro and Boveris, 2009; Swerdlow, 2009; Yong-Kee et al., 2012). The participation of apoptotic-like PCD in progression of various chronic neurodegenerative conditions (AD, PD, ALS, HD) is well documented and the regulation of this type of cell demise by various putative neuroprotectants has been demonstrated in many experimental models (Bredesen et al., 2006; Cavallucci and D'Amelio, 2011; Mattson, 2006; Nagley et al., 2009).

From almost 20 years the metabotropic glutamate receptors (mGluRs) have been studied as promising new targets for neuroprotection, and several ligands for these receptors have been shown to attenuate neuronal cell injury induced by various factors either via direct or indirect means of evoking overactivation of glutamatergic signaling (Byrnes et al., 2009; Caraci et al., 2012; Maiese et al., 2008; Nicoletti et al., 2011). However, the role of particular subtypes of mGluRs (mGluR group I: mGluR1 and mGluR5; mGluR group II: mGluR 2 and mGluR3; mGluR group III: mGluR4, mGluR7 and mGluR8) in models of neuronal apoptotic-like PCD has been only partially recognized. Earlier pertinent studies revealed the neuroprotective effects of non-specific mGluR agonists (e.g. 1,S,3R-ACPD, DCG-IV, L-CCG-I), as well as group specific ones (e.g. mGluR I agonist – DHPG, mGluR II agonist – LY354740, mGluR III agonists: L-AP4, L-SOP), in primary neuronal cell cultures where cell damage was induced by various pro-apoptotic stimuli (e.g. low potassium; β-amyloid, NMDA, NO, high glucose, staurosporine, etoposide) (Allen et al., 1999, 2000; Berent-Spillson et al., 2004; Borodezt and D'Mello, 1998; Copani et al.,1995; Kingston et al., 1999; Vincent et al., 1997, 1999a; Vincent and Maiese, 2000). Unlike in neuronal cells, the anti-apoptotic action of mGluRs activators was previously described for other cells e.g. in glia and epithelial cells (Lin and Maiese, 2001; Luyt et al., 2006; Moldrich et al., 2002; Pinteaux-Jones et al., 2008; Taylor et al., 2003; Zhou et al., 2006), suggesting that regulation of apoptotic-like processes may be a part of neuroprotective mechanism induced by mGluR ligands. When considering the influence of mGluR I specific ligands in models of neuronal apoptotic-like PCD, there are data showing that an agonist of this receptor, DHPG attenuates neuronal cell damage induced by some pro-apoptotic factors, such as staurosporine, etoposide or NMDA (Allen et al., 2000; Blaabjerg et al., 2003). However, a cautionary note here is the linkage of mGluR I to IP3-sensitive calcium pools which could under some conditions exaggerate the neuronal cell death (Nicoletti et al., 1999; Pshenichkin et al., 2008; Werner et al., 2007) as it has been shown for example for DHPG (20 μM) in a model of neuronal cell death induced by oxygen–glucose deprivation (Allen et al., 2000). Moreover, it has been shown that higher concentration of DHPG (5 mM) could induce cell death in striatal neurons, which is executed by apoptotic- and necrotic-like mechanisms (Diwakarla et al., 2009). Accordingly, the II and III groups of mGluRs could be considered as a safer target for neuroprotection, especially in light of the fact that subtype-specific positive allosteric modulators (PAMs) with improved potency, solubility, and pharmacokinetic properties have been recently developed (Acher et al., 1997; Conn et al., 2009; Flor and Acher, 2012; Nickols and Conn, 2014; Nicoletti et al., 2011; Niswender et al., 2008; Robichaud et al., 2011). In our recent study we compared the efficiency of LY354740 (an orthosteric mGluR II agonist), ACPT-I (an orthosteric mGluR III agonist), VU0361737 (a mGluR4 PAM), (S)-3,4-DCPG (an orthosteric mGluR8 agonist), AZ12216052 (a mGluR8 PAM) and AMN082 (an allosteric mGluR7 agonist) against MPP(+)-evoked cell damage in undifferentiated (UN-) and retinoic acid (RA-) differentiated human neuroblastoma SH-SY5Y cells. In the above study we proposed for the first time SH-SY5Y cells as a useful model to study the neuroprotective potential of mGluR II/III ligands (Jantas et al., 2014b). MPP(+) (1-methyl-4-phenylpyridinium ion) is a dopaminergic toxin used for cellular and animal modeling of PD and has been shown to induce cell death in SH-SY5Y cells via apoptotic-like and necrotic mechanisms (Gómez et al., 2001; Sun et al., 2011; Wang and Xu, 2005; Zhang et al., 2013). In the MPP(+) model we demonstrated the highest neuroprotection mediated by mGluR8-specific agents in UN- and RA-SHSY5Y cells. Moreover, the protection mediated by mGluR II/III activators against the MPP(+)-evoked cell death was not accompanied by the modulation of caspase-3 activity; however, a decrease in the number of apoptotic and necrotic nuclei was found. In order to broaden the understanding of the role of mGluR II/III in the modulation of apoptotic processes, herein we tested and compared the neuroprotective potency of LY354740, ACPT-I, VU0361737, AZ12216052 and AMN082 against the cell damage of UN- and RA-SH-SY5Y cells induced by staurosporine (St) and doxorubicin (Dox), inducers of intracellular (mitochondrial) and extracellular (death receptor mediated) apoptotic pathways, respectively (Jantas et al., 2008).

2. Materials and methods

2.1. Chemicals

LY354740 (*1S,2S,5R,6S-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid*), ACPT-I *(1S,3R,4S)-1-aminocyclo-pentane-1,3,4-tricarboxylic acid*), VU0361737 (*N-(4-chloro-3-methoxyphenyl)-2-pyridinecarboxamide*), AMN082 (*dihydrochloride (N,N′-dibenzhydrylethane-1, 2-diamine dihydrochloride)*), UBP1112 (*α-methyl-3-methyl-4-phosphonophenylglycine*), LY341495 ((*2S)-2-Amino-2-[(1S,2S)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid)* and MMPIP (*6-(4-methoxyphenyl)-5-methyl-3-pyridin-4-ylisoxazolo[4,5-c]pyridin-4(5H)-one*) were from Tocris Bioscence (Bristol, UK). AZ12216052 was purchased from Axon Medchem BV (Groningen, The Netherlands).

Dulbecco's modified Eagle medium (DMEM) and fetal bovine serum (FBS) were from Gibco (Invitrogen, Poisley, UK). The Cytotoxicity Detection Kit, *In Situ* Cell Death Detection Kit Fluorescein and BM Chemiluminescence Western Blotting Kit were from Roche Diagnostic (Mannheim, Germany). Primary polyclonal rabbit antibodies: anti-AIF (sc-5586) and anti-GAPDH (sc-25778), MW standards (sc-2035) and goat anti-rabbit secondary antibodies (sc-2004 and sc-2030) were purchased from Santa Cruz Biotechnology Inc. (CA, USA). pHrodo™ Red AM Intracellular pH Indicator and Intracellular pH Calibration Buffer Kit were from Molecular Probes (Eugene, OR, USA). All other reagents were from Sigma (Sigma-Aldrich Chemie GmbH, Germany).

2.2. Neuroblastoma SH-SY5Y cell culture

Human neuroblastoma SH-SY5Y cells (ATCC, passages 5–25) were grown in DMEM supplemented with a 10% heat-inactivated FBS and 0.1% penicillin/streptomycin mixture. Cells were maintained at 37 °C in a saturated humidity atmosphere containing 95% air and 5% CO_2. The cells, after having reached 80% confluence were trypsinized and seeded at a density of 3×10^5 cells per ml into multi-well plates. In order to obtain differentiated cells (RA-SH-SY5Y), they were cultured with retinoic acid (RA, 10 μM) for 6 days and the culture medium was changed every two days during that period. One day before cell treatment the culture medium in UN- and RA-SH-SY5Y cells was replaced by DMEM containing antibiotics and 1% FBS.

2.3. Cell treatment

The UN- and RA -SH-SY5Y cells were treated with LY354740 (0.001–100 μM); ACPT-I (0.001–100 μM); VU0361737 (0.001–10 μM); AZ12216052 (0.001–10 μM) and AMN082 (0.001–1 μM) and staurosporine (St: 0.15 and 0.5 μM for UN- and RA-SH-SY5Y cells, respectively) or doxorubicin (Dox: 0.25 and 1 μM for UN- and