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THE σ1 RECEPTOR REGULATES ACCUMULATION OF GM1 GANGLIOSIDE-ENRICHED AUTOPHAGOSOMES IN ASTROCYTES

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Abstract—GM1 gangliosides (GM1) are acidic glycosphingolipids that are present in cell membranes and lipid raft domains, being particularly abundant in central nervous systems. GM1 participate in modulating cell membrane properties, intercellular recognition, cell regulation, and signaling. We previously demonstrated that GM1 are expressed inside astrocytes but not on the cell surface. We investigated whether the antipsychotic drug haloperidol induces GM1 expression in astrocytes, and found that the expression of GM1 was significantly upregulated by haloperidol in the intracellular vesicles of cultured astrocytes. The effects of haloperidol on GM1 expression acted through the σ1 receptor (σ1R), but not the dopamine-2 receptor. Inhibition of the ERK pathway blocked the induction of GM1 through the σ1R by haloperidol. Interestingly, this increase in GM1 expression induced the accumulation of autophagosomes in astrocytes. Moreover, the effect of haloperidol on the σ1R induced a decrease in GM1 in the cellular membrane of astrocytes. These findings suggested that the effects of haloperidol on the σ1R induced GM1 accumulation in the autophagosomes of astrocytes through activating the ERK pathway and a decrease in GM1 expression on the cell surface.

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Abbreviations: σ1R, sigma (σ)-1 receptor; Aβ, amyloid β-protein; AD, alzheimer disease; APP, amyloid precursor protein; CTX-HRP, Horseradish peroxidase-cholera toxin B subunit; DMEM, Dulbecco's modified Eagle's medium; DR, dopamine receptor; DRMs, detergent-resistant membrane microdomains; ERK, extracellular signal-regulated kinase; FBS, fetal bovine serum; GM1, GM1 ganglioside; PBS, phosphate-buffered saline; PDMP, (±)-threo-1-phenyl-2-decanoyla mine-3-morpholino-1-propanol hydrochloride; PFA, paraformaldehyde; siRNA, stealth small interfering RNA; SKF10,047, (+)-SK&F 10,047 hydrochloride.

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INTRODUCTION

GM1 gangliosides (GM1), sialic acid-containing glycosphingolipids, are composed of a ceramide moiety linked to an oligosaccharide chain. GM1 are expressed primarily, but not exclusively, on the outer leaflet of the plasma membrane of cells in all vertebrates. They are particularly abundant in nervous tissues. Disturbance in ganglioside metabolism has been correlated with the development of neurodegenerative diseases, such as Alzheimer's disease (AD) (Yanagisawa, 2011). AD is a progressive neurodegenerative disease of the elderly characterized by the deposition of amyloid β-protein (Aβ) in the brain as amyloid plaques. Aβ is physiologically produced by the proteolytic cleavage of amyloid precursor protein (APP) by β- and γ-secretase and becomes cytotoxic upon aggregation and accumulation (reviewed by Selkoe et al.) (Selkoe, 2001). In addition, it has been suggested that the early pathological changes that are associated with AD are caused by the formation in the brain of a complex of GM1 gangliosides (GM1) and Aβ, which is termed GAβ (Yanagisawa et al., 1995). The unique molecular characteristics of GAβ allow it to act as a seed for Aβ fibrillogenesis in the brain of AD patients (Yanagisawa, 2011). The assembly of Aβ is initiated by GM1 accumulation and clustering at presynaptic neuron terminals in the brains of these patients (Bugiani et al., 1990; Probst et al., 1991). We recently confirmed that the expression of GM1 is distributed around nuclei of astrocytes (Yamamoto et al., 2007). Haloperidol has been reported to induce a decrease in APP protein levels, inhibit Aβ production, and attenuate the neurotoxicity of Aβ (Higaki et al., 1997; Palotás et al., 2003; Wei et al., 2003).

Sigma receptors (σRs) are unique in having two transmembrane segments and differ in this respect from all other mammalian G protein-coupled receptors (Bowen, 2000). These receptors are expressed in various areas of the brain and in peripheral organs including liver, testis, kidney, spleen, and intestine. σRs have been classified into at least two subtypes, termed σ1 (σ1R) and σ2 (σ2R), which were distinguished by means of biochemical and pharmacological studies (Quirion et al., 1992). σRs are high-affinity binding sites for several important classes of psychotic drugs, such as haloperidol (Musacchio et al., 1989; Itzhak and Stein, 1990; Walker et al., 1990, 1993; Bowen et al., 1993; Debonnel, 1993). σ1R is strongly

expressed in neurons and glia, and has been implicated in cognition. The occupancy of σ1Rs by agonists causes translocation of the receptor protein from the endoplasmic reticulum to the cell membrane where the receptor can regulate ion channels and neurotransmitter release. σ1R has been implicated in cellular differentiation, neuroplasticity, neuroprotection, and cognitive functioning of the brain (van Waarde et al., 2011). σ2R agonists produce transient and sustained increases in the intracellular calcium ion concentration $[Ca^{2+}]i$, and have been implicated in the induction of apoptosis (Wei et al., 2003).

Haloperidol is a typical antipsychotic used worldwide in the treatment of many mental health conditions, particularly psychosis and to a lesser extent major agitation in the elderly, owing to its calming effects. Patients with AD often develop psychotic features, such as delusions and hallucinations, as well as disruptive behavior, such as psychomotor agitation and physical aggression (Devanand et al., 1997). Atypical antipsychotics improve the psychobehavioral disturbances in patients with AD (Street et al., 2000; Brodaty et al., 2003) and are tolerated better by these patients than the typical form of antipsychotic haloperidol, which produces significantly more extrapyramidal symptoms (Devanand et al., 1998). Because many reports have indicated that AD neuropathology is not common in demented schizophrenia patients (Casanova et al., 1993; Arnold et al., 1994; Purohit et al., 1998), it has been proposed that medications used to treat schizophrenia may actually have protective effects against the development of AD neuropathology, which would account for the low frequency of these changes in elderly schizophrenics (Arnold et al., 1994). The mechanism by which antipsychotic drugs may exert this effect is unknown. Haloperidol is a dopamine-2 receptor (D2R), σ1R antagonist, and σ2R agonist with apoptotic activity (Giambalvo, 1988; Walker et al., 1990; Bowen, 2000), and is associated with neurotoxicity and has extrapyramidal side effects (Morimoto et al., 2002).

In this study, we investigated the effects of haloperidol (σ1R antagonist) on GM1 expression in primary cultured astrocytes. We also investigated the involvement of σ1R, σ2R, and D2R, which are the molecular targets of haloperidol. Our results suggested that the effects of haloperidol on σ1R might impact GM1 expression and distribution both in the intracellular spaces and cell membranes of astrocytes. In addition, these effects appear to be regulated by activation of the extracellular signal-regulated kinase (ERK) signaling pathway.

EXPERIMENTAL PROCEDURES

Materials

Bromocriptine mesylate, cycloheximide, Dulbecco's modified Eagle's medium (DMEM), DMEM-F-12, haloperidol, ifenprodil, PD98059, (±)-threo-1-phenyl-2-decanoylamine-3-morpholino-1-propanol hydrochloride (PDMP), and (±)-sulpirido were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Fetal bovine serum (FBS) was obtained from Hyclone Laboratories, Inc. (Logan, UT, USA). N2 supplements and a 0.1% bovine serum albumin fraction solution were purchased from Life Technologies (Grand Island, NY, USA). Horseradish peroxidase-conjugated cholera toxin B subunit (CTX-HRP) was obtained from Sigma–Aldrich Co. (St. Louis, MO, USA). SCH23390 hydrochloride and L745-870 trihydrochloride were purchased from Tocris Bioscience (Ellisville, MO, USA). Cycloheximide and Akt inhibitor V Triciribine (Triciribine) were obtained from Calbiochem (San Diego, CA, USA). Antibodies for phospho-ERK1/2, ERK1/2, and phospho-Akt (Ser473, D9E), Alix (a phylogenetically conserved cytosolic scaffold protein, 3A9), and LC3 (Microtubule-associated protein light chain 3) were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). Anti-Akt antibody, anti-Flotillin-1 antibody, and anti-Nucleoporin p62 antibody were obtained from BD Biosciences (San Jose, CA, USA). L-745, 890, SCH23390 hydrochloride (SCH23390), and (+)-SK&F 10,047 hydrochloride (SKF10,047) were purchased from Tocris Bioscience (Bristol, UK). Amyloid β-protein (human, 1-40) (Aβ40) was obtained from the Peptide Institute (Osaka, Japan).

Animals and cell culture

Sprague–Dawley rats (SLC, Shizuoka, Japan) were housed in a room maintained at 24 ± 1 °C and illuminated daily for 12 h (08:00–20:00). Free access was granted to food and water. All animal procedures were performed in accordance with a protocol approved by the Institutional Animal Care and Use Committee of Ritsumeikan University (Kyoto, Japan) and Hokuriku University (Ishikawa, Japan).

Cultures of cortical astrocytes were prepared from Sprague–Dawley rats at embryonic day 20 according to a previously described method (Kato et al., 1979). Trypsinized and dissociated cortical cells were cultured in 25-cm^2 culture flasks (Corning Inc., Corning, NY, USA) containing DMEM supplemented with 10% FBS in humidified 5% CO_2 at 37 °C. After incubation for 7 days, the cells were trypsinized and subcultured in 60- or 90-mm-diameter culture dishes (Corning Inc.). These cells were pharmacologically treated with haloperidol, other reagents, or vehicle (control).

SDS–PAGE and western blotting

Cells were lysed in Triton X-100-containing Tris buffer [5 mM Tris–HCl (pH 7.4), 2 mM EDTA, 1% Triton X-100, Complete protease inhibitor cocktail (Roche Molecular Biochemicals, Penzberg, Germany)]. The protein concentration of each sample was determined using a BCA protein assay kit (Thermo Fisher Scientific Inc., Rockford, IL, USA). The samples were separated on a 10/20% polyacrylamide gel (Wako Pure Chemical Industries Ltd.) and then electrotransferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). The blots were probed with CTX-HRP that was diluted 1:15,000, and anti-phospho-ERK1/2 antibody, anti-ERK1/2 antibody, anti-phospho-Akt antibody, or anti-Akt antibody. All antibodies were diluted 1:2,000. The bands were visualized by reaction with HRP-linked secondary antibodies (Cell Signaling