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HALOPERIDOL DISRUPTS LIPID RAFTS AND IMPAIRS INSULIN SIGNALING IN SH-SY5Y CELLS

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Abstract—Haloperidol exerts its therapeutic effects basically by acting on dopamine receptors. We previously reported that haloperidol inhibits cholesterol biosynthesis in cultured cells. In the present work we investigated its effects on lipid-raft composition and functionality. In both neuroblastoma SH-SY5Y and promyelocytic HL-60 human cell lines, haloperidol inhibited cholesterol biosynthesis resulting in a decrease of the cell cholesterol content and the accumulation of different sterol intermediates (7-dehydrocholesterol, zymostenol and cholesta-8,14-dien-3β-ol) depending on the dose of the drug. As a consequence, the cholesterol content in lipid rafts was greatly reduced, and several pre-cholesterol sterols, particularly cholesta-8,14-dien-3β-ol, were incorporated into the cell membrane. This was accompanied by the disruption of lipid rafts, with redistribution of flotillin-1 and Fyn and the impairment of insulin-Akt signaling. Supplementing the medium with free cholesterol abrogated the effects of haloperidol on lipid-raft composition and functionality. LDL (low-density lipoprotein), a physiological vehicle of cholesterol in plasma, was much less effective in preventing the effects of haloperidol, which is attributed to the drug's inhibition of intracellular vesicular trafficking. These effects on cellular cholesterol homeostasis that ultimately result in the alteration of lipid-raft-dependent insulin signaling action may underlie some of the metabolic effects of this widely used antipsychotic.

Key words: lipid rafts, haloperidol, antipsychotic drug, insulin, cholesterol biosynthesis, signal transduction.

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Abbreviations: BCA, bicinchoninic acid; CtxB, cholera toxin B; DiI, 1,1′-dioctadecyl-3,3,3,3′-tetramethylindocarbocyanineperchlorate; FBS, fetal bovine serum; GC/MS, gas chromatography/mass spectrometry; IR, insulin receptor; LDL, low-density lipoprotein; LPDS, lipoprotein-deficient serum; PI3K, phosphatidylinositol-3-kinase; pTyr, phosphotyrosine; TfR, transferrin receptor; 7DHC, 7-dehydrocholesterol.

Haloperidol was introduced in therapeutics over 40 years ago and is still widely used for the treatment of acute and chronic psychosis. Its antipsychotic activity is thought to be mediated, at least in part, by its dopamine D2 receptor antagonist properties (Miyamoto et al., 2005). Haloperidol is also a σ1 and σ2 receptors antagonist (Hashimoto and Ishiwata, 2006) and acts, albeit with lower activity, on dopamine D1, D3 or D4, serotonin 2A, and α1 adrenergic receptors (Miyamoto et al., 2005). Haloperidol exerts its therapeutic effects by acting preferentially in the brain and, in fact, it accumulates in brain tissue (Korpi et al., 1984; Kornhuber et al., 1999), however, it has been found also to produce some metabolic disturbances, including weight gain (Bobes et al., 2003; Zipursky et al., 2005), impaired glycemic control (Wirshing et al., 2002; Perez-Iglesias et al., 2009), insulin resistance (Perez-Iglesias et al., 2009), and a worsening of the lipid profile (Perez-Iglesias et al., 2009).

In membranes, cholesterol is concentrated in lipid rafts, which are also enriched in sphingolipids (Pike, 2005). Lipid rafts serve as major platforms for the initiation, propagation, and maintenance of signal transduction events (Pike, 2005). Thus, it is not surprising that dysfunctions in cholesterol synthesis, storage, transport, and removal may lead to important cell physiology disturbances (Vance et al., 2005). Many key signaling molecules have been shown to localize to lipid rafts, including the insulin receptor (IR) (Bickel, 2002). The IR is expressed at high levels in many brain areas and in several different cell types (Havrankova et al., 1978). Stimulation of the IR triggers the phosphorylation of tyrosine receptor kinases and the activation of a downstream signal transduction pathway coupled to phosphatidylinositol-3-kinase (PI3K) and Akt. In brain, insulin regulates key processes such as energy homeostasis, reproductive endocrinology, and neural survival, and has been implicated in neurodegenerative diseases (Plum et al., 2005). IR and functional tyrosine kinases have been shown to concentrate in lipid rafts from neuronal plasma membranes (Wu et al., 1997). However, whether localization of IR in the different membrane domains modulates IR function is controversial (Vainio et al., 2002; Taghibiglou et al., 2009). Recently, cavin/polymerase I and transcript release factor-knockout mice, which lack morphologically detectable caveolae and have markedly diminished expression of the three caveolin isoforms, have been shown to develop glucose intolerance and hyperinsulinemia (Liu et al., 2008), underscoring the role of caveolae/lipid rafts in insulin action.

The insulin signaling pathway appears to be important for neuronal growth, synaptic development and neuronal

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survival/apoptosis during brain development (Girgis et al., 2008). Alterations of this pathway may have profound implications for pathogenesis, pathophysiology and treatment of neurodevelopmental disorders of the brain, including schizophrenia (Girgis et al., 2008). Using post-mortem material, Zhao et al. (2006) showed a functional decrease in IR-mediated signal transduction in the dorsolateral prefrontal cortex of medicated schizophrenics relative to control patients.

There are few data about the effects of antipsychotics on cholesterol biosynthesis. Summerly and Yardley (1965) were the first to demonstrate that haloperidol inhibits cholesterol biosynthesis in rat skin. In humans, Simpson et al. (1967) found no effect on plasma cholesterol levels in schizophrenic patients receiving up to 30 mg haloperidol/day, whereas Kelley and Hennekam (2000) reported increased levels of 7-dehydrocholesterol (7DHC)—an immediate sterol precursor of cholesterol in the cholesterol biosynthesis pathway—in plasma from patients treated with haloperidol. We recently reported that SH-SY5Y cells treated with haloperidol accumulated cholesta-8,14-dien-3β-ol and decreased cholesterol content, suggesting the inhibition of Δ^{14}-reductase enzyme efficiency (Sanchez-Wandelmer et al., 2009b). This effect resulted in the alteration of somatostatin signaling (Sanchez-Wandelmer et al., 2009b).

In the present work, we examined the effects of haloperidol on intracellular cholesterol homeostasis and its repercussion in lipid raft -dependent insulin signaling. Our results show that, by inhibiting cholesterol biosynthesis, haloperidol causes a change in total sterol content and in the composition of lipid raft and non-raft membrane domains. This is accompanied by disruption of lipid rafts, redistribution of flotillin-1, and impairment of insulin signaling. The ability of the antipsychotic haloperidol to deplete cholesterol in lipid rafts alters signal processing in these domains.

EXPERIMENTAL PROCEDURES

All chemicals, unless otherwise stated, were purchased from Sigma (Sigma-Aldrich Química, S.A., Tres Cantos, Madrid, Spain).

Cell cultures

SH-SY5Y neuroblastoma cells (ATCC CRL-2266) were cultured in RPMI 1640 medium containing L-glutamine and supplemented with MEM non-essential amino acids, 10% fetal bovine serum (FBS), and antibiotics (Gibco BRL, Invitrogen S.A., Barcelona, Spain) at 37 °C in a 5% CO_2 atmosphere. HL-60 cells (ECACC 98070106) were cultured in cholesterol-free medium containing ITS+supplements: RPMI 1640 (Gibco BRL) supplemented with 625 μg transferrin (Roche, Basel, Switzerland)/ml, 625 μg insulin/ml, 535 μg linoleic acid-BSA/ml, 625 ng sodium selenite (Sigma)/ml, 125 mg human serum albumin (Grifols 20%, Barcelona, Spain)/ml, and antibiotics. 3T3 cells (ECACC 93061524) were cultured in DMEM (Gibco BRL) supplemented with 10% FBS and antibiotics. Lipoprotein-deficient serum (LPDS) was prepared from FBS by ultracentrifugation at a density of 1.21 kg/L. For experiments, cells were cultured in medium with 10% LPDS and treated with haloperidol (Sigma) for 24 h. Cholesterol (Steraloids Inc., Newport, RI, USA) was dissolved in ethanol; haloperidol was dissolved in DMSO. The final concentrations of DMSO and ethanol in the medium were 0.044% and 0.44%, respectively. Human low-density lipoproteins (LDLs) were isolated as described elsewhere (Martinez-Botas et al., 2001).

Immunofluorescence microscopy

Human LDL was labelled with the fluorescent probe DiI (1,1′-dioctadecyl-3,3,3,3′-tetramethylindocarbocyanineperchlorate) as described elsewhere (Calvo et al., 1998). SH-SY5Y and 3T3 cells were cultured on glass coverslips and fixed on the coverslips with 4% paraformaldehyde/PBS for 5 min. Next, the cells were permeabilized in 0.1% Triton X-100/PBS for 5 min and incubated with 2% BSA in PBS for 45 min. Free cholesterol was stained by exposing the cells to Filipin (50 mg/L in PBS, Sigma) for 45 min. For LAMP2 staining, cells were incubated with a 1:200 dilution of specific antibody (Abcam, Cambridge, UK) for 2 h and then with a 1:500 dilution of Alexa fluor 488-conjugated anti-mouse IgG (Molecular Probes, Invitrogen S.A.) in PBS for 45 min. For ganglioside GM1 staining, cells were incubated with a 1:100 dilution of cholera toxin B-FITC (CtxB-FITC, Sigma) for 30 min before fixation. Cells were mounted for microscopy and examined on an Olympus BX51 reflected fluorescence microscope. Images of Filipin staining were pseudocolored in red.

Sucrose gradient fractionation of membranes

Membrane fractions were separated according to a modified version of a previously described detergent-free method (Song et al., 1996). SH-SY5Y cells cultured in a 175-mm flask were scraped into 2 ml of 500 mM sodium carbonate, pH 11, sonicated for two bursts of 30 s each, and centrifuged for 30 min at 14 000$\times g$ to obtain a Golgi-free supernatant. The Golgi-free supernatant proteins were measured using a bicinchoninic acid (BCA) protein assay (Pierce Biotechnology, Inc., Rockford, IL, USA). Equal amounts of membrane proteins were then adjusted to 42.5% sucrose by the addition of 2 ml of 85% sucrose prepared in MBS (25 mM Mes, pH 6.5, 0.15 M NaCl) and placed at the bottom of an ultracentrifuge tube. A 5%–35% discontinuous sucrose gradient was formed above (2 ml of 5% sucrose, 5 ml of 30% sucrose, 1 ml of 35% sucrose) and the tube was centrifuged at 190,000 g for 18–20 h in an SW41 rotor (Beckman Instruments, Palo Alto, CA, USA). Twelve 1-ml fractions were collected from the top of the gradient and processed for Flotillin-1 (BD Bioscience) and anti-transferrin receptor (TfR) (Zymed, Invitrogen S.A.) analysis by Western blotting and for analysis of sterol by HPLC. The fractions were concentrated by 10% trichloroacetic acid precipitation prior to electrophoretic analysis for phosphotyrosine (p-Tyr), IR β subunit, and Fyn (Santa Cruz Biotechnology, Santa Cruz, CA, USA) by Western blotting. The amount of protein in the samples was measured using the BCA protein assay.

Western blotting

Whole-cell extracts were obtained as described elsewhere (Sanchez-Wandelmer et al., 2009a). Gradient fractions from each experiment (control, haloperidol, haloperidol+cholesterol and haloperidol+LDL conditions) were processed simultaneously. Equal volumes of each gradient fraction or whole-cell lysate (50 μg) were subjected to 8%–12% SDS-PAGE and transferred to nitrocelullose membranes (Bio Rad Laboratories S.A., Barcelona, Spain). After blocking, the blots were probed with specific antibodies followed by incubation with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology). The immunoblots were incubated with the enhanced chemiluminescence reagent (Bio-Rad) and exposed for equal times on a VersaDoc MP 4000 System (Bio-Rad) and images were obtained. Densitometric analysis was performed by using the Quantity One 4.5.2 version program (Bio-Rad). Phospho-Akt-S473 and Akt antibodies were