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Biochemical properties in membrane of rat astrocytes under oxidative stress

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ABSTRACT

Oxidative stress induced by the treatment with 100 μM hydrogen peroxide (H_2O_2) for 10 min enhances release of cytosolic proteins along with fibroblast growth factor 1 (FGF-1) from rat astrocytes without inducing apoptosis. FGF-1 promotes the generation of apolipoprotein E-containing high-density lipoprotein-like particles (apoE/HDL) in astrocytes, which contributes to cholesterol homeostasis in the brain. In this work, we studied various effects of oxidative stress on rat astrocyte's membrane to understand the mechanism underlying release of cytosolic proteins and FGF-1. The oxidative stress using 100 μM H_2O_2 enhanced lipid release from rat astrocytes in addition to suppression of lipid synthesis. The lipid synthesis, however, was hardly suppressed by H_2O_2 in the cell lines such as bovine endothelial cells and HepG2 cells from which the release of cytosolic proteins is not increased by H_2O_2 unlike rat astrocytes. The treatment of rat astrocytes with H_2O_2 changed the distribution of lipids and proteins in the caveolin-1-rich domain of membrane to the non-raft domain, which was canceled by the pretreatment of cells with low-density lipoproteins (LDL). These findings suggest that oxidative stress induced by H_2O_2 changes lipid level of the plasma membrane to make the membrane structure fragile in rat astrocytes. The direct treatment with H_2O_2 of membrane fraction prepared from rat astrocytes did not enhance lipid release from the membrane. The lipid release, however, was enhanced from the isolated membrane fraction, after the cells were treated with H_2O_2 and incubated in H_2O_2-free DPBS. Hydrogen peroxide enhanced phosphorylation of protein kinases such as Akt, MEK, and ERK in intact astrocytes without injury and stress. A MEK inhibitor, U0126, suppressed not only the H_2O_2-induced ERK phosphorylation but also cytosolic protein release from rat astrocytes. These findings suggest that the H_2O_2-induced

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; BBB, blood–brain barrier; apo E, apolipoprotein E; DPBS, Dulbecco's phosphate buffered saline; FCS, fetal calf serum; DMEM, Dulbecco's modified Eagle medium; BSA, bovine serum albumin; TLC, thin layer chromatography; SDS-PAGE, 0.5% SDS/10% polyacrylamide gel electrophoresis; TCA, trichloroacetic acid; CNS, central nervous system; FGF-1, fibroblast growth factor 1

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release of cytosolic proteins depends on imbalance of lipid level in the membrane through suppressing lipid synthesis and increasing lipid release induced by the intracellular biochemical reaction such as signal transduction generated in intact rat astrocytes treated with H_2O_2.

1. Introduction

Astrocytes are known to have many functions to support structure, function, and distribution of neurons in the brain, to regulate molecular transport between the inside and outside of brain through the blood–brain barrier (BBB), and to preserve homeostasis for extracellular environment of neural cells (Abbott, 2002; Sofroniew and Vinters, 2010; Wegrzynowicz et al., 2011). Accordingly, astrocytes are generally evaluated as supporting actors for the organization of neuronal signal networks in the central nervous system (CNS). Role of astrocytes in protection of neuronal cells against stress and injury in the brain, furthermore, has been recently taken into notice. An increasing extracellular glutamate level induces neuronal apoptosis in the brain. Astrocytes uptake excess glutamate to reduce extracellular glutamate level and protect neuronal survival (Lehre et al., 1995). In order to protect neurons against oxidative stress in the brain, astrocytes produce and release glutathione (GSH) and metallothionein, and enhance ATP release to up-regulate expression of thioredoxin reductase through ATP receptor (P2Y1) (Butt, 2011; Dringen and Hirrlinger, 2003).

We observed that the production and release of fibroblast growth factor 1 (FGF-1) are increased in rat astrocytes during stressful long-term culture accompanied with oxidative stress (Nagayasu et al., 2008; Nagayasu et al., 2014). Furthermore, we found that cryoinjury stimulates production of FGF-1 and apoE in the astrocytes around the injury in mouse brain and the FGF-1 production is ahead of apoE production, and that the wound healing of cryoinjury is delayed in apoE-deficient mouse brain in compared with wild type mouse brain (Tada et al., 2004). FGF-1 enhances syntheses and release of apoE and cholesterol in stress-less astrocytes to up-regulate apoE/HDL generation through at least three kinds of signal transductions in vitro (Ito et al., 2005; Ito et al., 2007; Lu et al., 2009). FGF-1 enhances cholesterol synthesis through the activation of MAP kinase cascade to phosphorylate MEK and ERK, increases apoE expression to activate LXR, and accelerates intracellular apoE transport through the activation of PI3-kinase/Akt pathway, respectively, in rat astrocytes. These findings suggest that astrocytes contribute to protect the brain against stress and injury through the production and release of FGF-1 and apoE/HDL. The brain HDL is predominantly composed of an apoE produced mainly by astrocytes as an apolipoprotein (Boyles et al., 1985; Fujita et al., 1999; Ito et al., 1999). Generally apoE/HDL contributes to the intercellular cholesterol transport for cholesterol homeostasis in the CNS (Barres and Smith, 2001; Ito and Yokoyama, 2004; Popko et al., 1993). As the phenotype of apoE in the CNS of recipient remains unchanged even after liver transplantation (Linton et al., 1991), it is seemingly un-exchangeable with the plasma apoE. In culture cell system, suppression of cholesterol synthesis decreases viability of neurons and addition of cholesterol as apoE/HDL resumes and develops synapse formation and neurite outgrowth (de Chaves et al., 1997; Ko et al., 2005; Michikawa and Yanagisawa, 1999). It has been observed that biosynthesis of apoE is increased after injury of rat sciatic and optic nerves (Skene and Shooter, 1983), suggesting close relation between the production of apoE and apoE/HDL and nerve injury.

As FGF-1 has no N-terminal signal peptide like FGF-2, FGF-1 is considered secreted through the mechanism different from the classical secretory pathway dependent on endoplasmic reticulum and Golgi apparatus (Mason, 1994; Mohan et al., 2010; Wiedlocha et al., 2005). FGF-1 is known to be released in response to various stress conditions such as heat shock, hypoxia, serum starvation, and oxidative stress (Ito et al., 2013; Jackson et al., 1992; Mouta et al., 2001; Shin et al., 1996). We reported in previous paper that the treatment of rat astrocytes with 100 μM hydrogen peroxide (H_2O_2) for 10 min enhances the release of FGF-1 along with cytosolic proteins such as HSP70, HSP90, and HSP110 without inducing apoptosis. Oxidative stress induced by H_2O_2 facilitates inflow of exogenous molecules into rat astrocytes also. We considered that the release of FGF-1 seemingly relates to the change of lipid metabolism in H_2O_2-treated astrocytes, as the enhancement of FGF-1 release was suppressed by pretreatment with lipoproteins such as HDL and LDL. The oxidative stress-induced FGF-1 release may relate to the change of plasma membrane structure. Therefore, we studied changes of membrane structure and lipid metabolism of rat astrocytes treated with H_2O_2 to understand the mechanism underlying FGF-1 release.

2. Results

The stability of membrane lipids in rat astrocytes treated with H_2O_2 was first studied by analysis of cellular lipid release from the cells. The membrane lipids such as cholesterol (Cho), sphingomyelin (SM), and phosphatidylcholine (PC) biosynthesized using [^{14}C]-acetate as a precursor were very greatly released to the conditioned medium within 3 h after treatment of the cells with 100 μM H_2O_2 for 10 min (Fig. 1A). The lipid release was decreased at 24 h after the end of treatment with H_2O_2. The release of both SM and PC biosynthesized using [^{14}C]-choline as a precursor was also enhanced during 3–6 h after H_2O_2 treatment, although the lipid release was slightly suppressed during the treatment with H_2O_2 for 10 min (0 h) (Fig. 1B). The treatment of rat astrocytes with catalase neutralized H_2O_2-induced lipid release (Fig. 2A) and weakened partly H_2O_2-induced inhibition of lipid synthesis (Fig. 2B). We previously observed that the treatment with 100 μM H_2O_2 for 10 min does not enhance the release of cytosolic proteins from HepG2 cells and bovine endothelial cells. In this study, it was shown that the syntheses of Cho and SM in HepG2 cells and bovine endothelial cells are not so much suppressed by H_2O_2 or tBHP unlike rat