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Effects of taurine on glutamate uptake and degradation in Müller cells under diabetic conditions via antioxidant mechanism

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ABSTRACT

Glutamate is the excitatory neurotransmitter in the retina, but it is neurotoxic in excessive amounts. A decrease in the ability of Müller cells to remove glutamate from the extracellular space may play a crucial role in the disruption of glutamate homeostasis that occurs in the diabetic retina. Previously we have shown that taurine has protective effects against diabetes-induced glutamate dysmetabolism in retinal Müller cells. The aim of this study is to examine the effects and underlying mechanism of taurine on high glucose-induced alterations of Müller cells glutamate uptake and degradation. Müller cells cultures were prepared from 5- to 7-day-old Sprague–Dawley rats. Glutamate uptake was measured as ^{3}H-glutamate content of the lysates. Glutamine synthetase (GS) activity was assessed by a spectrophotometric assay. The expressions of glutamate transporters (GLAST) and GS were examined by RT-PCR and western-blot. In 25 mmol/l high glucose-treated cultures, Müller cells glutamate uptake, GS activity and GLAST, GS expressions were decreased significantly compared with 5 mmol/l normal glucose cultures ($p<0.05$). Taurine (1 and 10 mmol/l) significantly inhibited the high glucose-induced decreases in glutamate uptake, GS activity and GLAST, GS expressions ($p<0.05$). The generation of TBARS, ROS and NO in Müller cells increased significantly after treatment with high glucose compared with normal glucose. However, treatment of 1 and 10 mmol/l taurine resulted in a significant decrease in TBARS, ROS and NO levels ($p<0.05$). The high glucose treatment decreased antioxidant enzyme (catalase, SOD and GSH-px) activities compared with normal glucose. Taurine treatment increased the catalase, SOD and GSH-px activity in a dose-dependent manner. These findings suggest that taurine may regulate Müller cells' glutamate uptake and degradation under diabetic conditions via its antioxidant mechanism.

Introduction

Glutamate is the main excitatory neurotransmitter in the mammalian brain and retina, but it is neurotoxic when present in excessive amounts. In the retina, glutamate is the primary excitatory transmitter in the vertical pathway from photoreceptors to ganglion cells (Lucas and Newhouse, 1957; Massey and Miller, 1987; Choi, 1988; Ng et al., 2004). Several recent studies have shown that a significant increase in glutamate in the retina is associated with the development of diabetic retinopathy (DR), a disease characterized by neurodegeneration and vasculopathy (Li and Puro, 2002; Diederen et al., 2006). Although the detailed mechanism remains unknown, it may be of critical importance for neuroprotection to remove excess glutamate from the extracellular space in the retina.

Müller cells, the main glial cells within the retina, are vital for maintaining the normal health of the retina and have been implicated in many retinal diseases, including DR (Hicks and Courtois, 1990; Takei et al., 2000). A major physiological function of these cells is to regulate the ionic and molecular composition of the retinal microenvironment. An intensively studied function of Müller cells is their uptake of synaptically released glutamate (Newman and Reichenbach, 1996). Müller cells surround glutamatergic synapses, and express glutamate transporters (GLAST) and the glutamatemetabolizing enzyme, glutamine synthetase (GS). Extracellular glutamate is transported into Müller cells by GLAST and amidated by GS to the non-toxic amino acid glutamine. Glutamine is then released by the Müller cells and taken up by neurons, where it is hydrolyzed by glutaminase to form glutamate again. Thus, the cells not only play a substantial role in transmitter clearance, but also in the retinal glutamate/glutamine cycle. Therefore, increased function in retinal Müller cells may prevent excessive accumulation of glutamate and therefore protect neurons against excitotoxicity in the retina (Kirk and Moscona, 1963; Rowe et al., 1970; Riepe and Norenburg, 1977; Sarthy and Lam, 1978; Moscona and Moscona, 1979; Puro, 2002).

The role of Müller cells in maintaining a low extracellular concentration of glutamate may be particularly critical in diabetes. As in the normal retina, synaptically released glutamate must be removed. However, in addition, neurons in the diabetic retina must be protected from glutamate leaking into the retina because the blood-retinal barrier is compromised

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early in diabetes. Since plasma contains 100 to 300 μM of glutamate (Castillo et al., 1997) and as little as 5 μM glutamate can be lethal to neurons, (Lipton and Rosenberg, 1994) it seems apparent that a breakdown in the blood-retinal barrier could have dire consequences for retinal function and neuronal survival (Cunha-Vaz et al., 1975; Do Carmo et al., 1998). Thus, the transport of glutamate into Müller cells may be essential in order to prevent toxic levels of this amino acid from reaching neurons located near defects in the blood-retinal barrier. Several studies have suggested that the function of Müller cells is abnormal in streptozotocin model of diabetes or in high glucose cultures for diabetes studies (Ishikawa et al., 1996; Ward et al., 2005; Xi et al., 2005; Kusner et al., 2004). We have shown that Müller cells undergo diabetes-induced glial reactivity and glutamate dysmetabolism in vivo (Zeng et al., 2009).

The underlying mechanism for the regulation of glutamate uptake at the transporter level results from an interaction of redox agents with the transporter sulfhydryl groups. GLAST, contains functional cysteine residues that are sensitive to oxidative formation of cystine bridges and cause inhibition of glutamate flux through the transporters (Trotti et al., 1998). Hydrogen peroxide, nitric oxide, superoxide anion and peroxynitrite anion can all inhibit glutamate uptake through GLAST. Overexpression of the superoxide anion scavenger superoxide dismutase-1 protected astrocyte glutamate transporters from inhibition by reactive oxygen species (Pogun et al., 1994; Volterra et al., 1994; Trotti et al., 1996). Oxidative stress is believed to play a key role in DR progression, although the molecular mechanisms involved are not clear. Numerous evidences on alterations in Müller cells function during diabetes have been related to changes in redox homeostasis. These observations provide links between diabetes-induced oxidative stress and glutamate excitotoxicity.

Taurine, a ß-aminosulfonic acid, is the most abundant retinal amino acid and is essential for sustain of retinal structure and function. Several studies have recently indicated that taurine exhibites beneficial effects in adults with diabetes. Taurine influences various biological and physiological functions, including brain and retinal development, cell membrane stabilization, antioxidation, detoxification, osmoregulation, hypoglycemic action and neuromodulation. In addition, taurine has been shown to have neuroprotective effects. Neuroprotective effects of taurine in the present study are consistent with many observations reported before (Thurston et al., 1980; Bernardi, 1985; Pasantes-Morales et al., 1985; El Idrissi and Trenkner, 2004). O'Byrne and Tipton (2000) have shown a protective effect of taurine against 1-methyl-4-phenylpyridinium-induced neurotoxicity in coronal slices of rat brain. Louzada et al. (2004) have also reported in an in vitro study that taurine protects chick retinal neurons in culture against the neurotoxicity of β-amyloid and glutamate receptor agonists. Our previous experiments showed that dietary taurine supplementation could decrease the level of glutamate, inhibit glial reactivity and glutamate dysmetabolism in diabetic retina, then exhibits neuroprotective effects (Zeng et al., 2009).

In this study, we address the possible effects and underlying mechanism of taurine on glutamate uptake, GS activity and GLAST, GS expression in high glucose-treated retinal Müller cells.

Results

Immunocytochemical characterization of cultured retinal Müller cells

We characterized cultured rats Müller cells by their expression of GS, vimentin and GLAST, as judged by immunocytochemical staining. Nuclei were stained with hoechst. Double labeling was performed with rabbit anti-GS antibodies in combination with mouse anti-vimentin antibodies. Cells in this culture system showed positive labeling for GS, vimentin and GLAST (Fig. 1), molecular markers for Müller cells in the retina. By this immunocytochemical labeling, the cultured cells were thought to be Müller cells.

Glutamate uptake in cultured retinal Müller cells

We investigated the effect of taurine on glutamate uptake activity in retinal Müller cells cultured with high glucose. Fig. 2 shows the changes in the high glucose-induced glutamate uptake in rats retinal Müller cells with or without taurine treatment. Treatment with 25 mmol/l D-glucose for 72 h significantly decreased L-[2,3-^{3}H] glutamate uptake activity from 726 ± 12 cpm/min/mg protein in control cultures to 352 ± 10 cpm/min/mg protein in high glucose-treated cultures ($p<0.05$). When the cells were incubation with high glucose and taurine at 1 and 10 mmol/l, the L-[2,3-^{3}H] glutamate uptake activity significantly

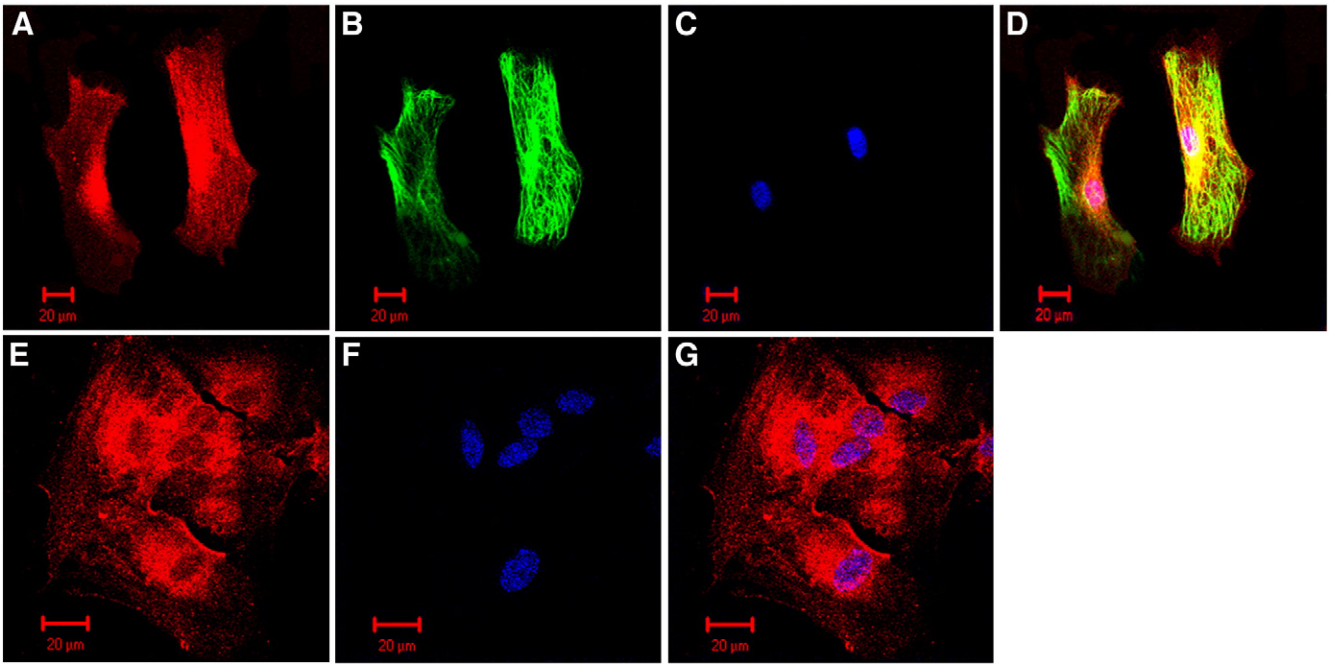

Fig. 1. Retinal Müller cells were identified by their expression of glutamine synthetase (GS) (A), vimentin(B) and glutamate transporter (GLAST) (E), as judged by immunocytochemical staining. Nuclei were stained with Hoechst (C, F). D: Merged labeling of GS, vimentin and hoechst. g: Merged labeling of GLAST and hoechst.