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Research Report

Riluzole-induced glial cell line-derived neurotrophic factor production is regulated through fibroblast growth factor receptor signaling in rat C6 glioma cells

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ABSTRACT

Riluzole is approved for the treatment of amyotrophic lateral sclerosis (ALS); however, recent accumulating evidence suggests that riluzole is also effective for the treatment of psychiatric disorders, such as mood disorders. Plastic change in the brain induced by neurotrophic factors/growth factors is thought to be involved in the mechanism of antidepressants. This study investigated the mechanism of riluzole-induced glial cell line-derived neurotrophic factor (GDNF) production in rat C6 glioma cells (C6 cells), a model of astrocytes. The study investigated the phosphorylation of cAMP response element binding protein (CREB), an important transcriptional factor of the *gdnf* gene, and found that riluzole increased CREB phosphorylation in a time-dependent manner, peaking at 40 min after treatment. The riluzole-induced CREB phosphorylation was completely blocked by a mitogen-activated protein kinase kinase (MEK) inhibitor (U0126). Riluzole increased extracellular signal-regulated kinase (ERK) activation prior to CREB phosphorylation. These results suggest that riluzole rapidly activates the MEK/ERK/CREB pathway. Furthermore, two types of fibroblast growth factor receptor (FGFR) tyrosine kinase inhibitors (SU5402 and PD173074) completely blocked riluzole-induced CREB phosphorylation. In addition, riluzole rapidly phosphorylated FGFR substrate 2α (FRS2α), a major adaptor protein of FGFR. These findings suggest that riluzole induces CREB

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Abbreviations: 5-HT, 5-hydroxytriptamine (serotonin); 5-HTR, 5-HT receptor; ALS, amyotrophic lateral sclerosis; BCA, bicinchoninate; BSA, bovine serum albumin; BDNF, brain-derived neurotrophic factor; C6 cells, rat C6 glioma cells; CREB, cAMP response element binding protein; DMEM, Dulbecco's modified Eagle's medium; EAAC1, excitatory amino acid carrier 1; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; FGFR, fibroblast growth factor receptor; FRS2α, FGFR substrate 2α; GAPDH, glyceraldehydes-3-phosphate dehydrogenase; GDNF, glial cell line-derived neurotrophic factor; GLAST, glutamate–aspartate transporter; Glu, glutamate; GLT-1, Glu transporter-1; GluT, glutamate transporter; MEK, mitogen-activated protein kinase kinase; MMP, matrix metalloproteinase; NGF, nerve growth factor; PBS, phosphate buffered saline; SDS, sodium dodecyl sulfate; TK, tyrosine kinase; t-PDC, L-trans-pyrrolidine-2, 4-dicarboxylate; Trk, tropomyosin-related kinase; Tukey's HSD test, Tukey's honestly significant difference test

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phosphorylation through FGFR. In addition, PD173074 inhibited riluzole-induced GDNF production. In contrast, L-glutamate and a glutamate transporter inhibitor (t-PDC) did not yield any effects in either CREB phosphorylation or GDNF production. These findings suggest that riluzole rapidly activates a MEK/ERK/CREB pathway through FGFR in a glutamate transporter-independent manner, followed by GDNF expression in C6 cells.

1. Introduction

Although riluzole, 2-amino-6-trifluoromethoxybenzothiazole, is approved for the treatment of amyotrophic lateral sclerosis (ALS), recent accumulating evidence suggests that riluzole is also effective for the treatment of psychiatric disorders, such as mood disorders (Zarate and Manji, 2008). Riluzole is known as a modulator of glutamatergic neurotransmission. It blocks the excess release of glutamate from presynaptic terminals by a blockade of voltage-dependent sodium channels, as well as voltage-activated calcium channels. On the other hand, riluzole promotes glutamate uptake through the glutamate transporter (GluT) in glia (Fumagalli et al., 2008; Pittenger et al., 2008). These mechanisms allow riluzole to regulate glutamate homeostasis in the synaptic cleft and protect the brain from glutamatergic neurotoxicity. The neuroprotective effects of riluzole are thought to be dependent on not only glutamatergic modulating effects but also the production of neurotrophic factors (Mizuta et al., 2001; Zarate and Manji, 2008). Therefore, multiple mechanisms are possibly involved in the pharmacological benefit of riluzole on the treatment for neurodegenerative disorders as well as psychiatric disorders.

Glial cell line-derived neurotrophic factor (GDNF) is a member of the GDNF family and a distant member of the transforming growth factor-β superfamily. GDNF has trophic effects on several neuronal populations and glia (Airaksinen and Saarma, 2002; Lin et al., 1993). GDNF plays an important role in cognition and acquisition processes (Gerlai et al., 2001; Messer et al., 2000) and has the potential to regulate neuronal and/or glial plasticity as well as higher order brain functions such as mood alteration. In addition to brain-derived neurotrophic factor (BDNF), accumulating evidence indicates that the alterations in the GDNF levels are observed in the peripheral blood as well as post-mortem brain tissue with mood disorders (Michel et al., 2008; Takebayashi et al., 2006). Furthermore, the serum levels of GDNF increase following antidepressive treatment (Zhang et al., 2009). On the other hand, antidepressants and serotonin (5-hydoroxytriptamine: 5-HT), both of which are relevant agents for the treatment of mood disorders, increase GDNF production in glia (Hisaoka et al., 2001, 2004). These findings suggest that GDNF production in glia may be relevant to the mechanism of the antidepressive effects. Caumont et al. (2006) recently reported that riluzole also induced GDNF production in rat C6 glioma cells (C6 cells), a model of astrocytes. However, the precise mechanism is still not understood. This study examined the mechanism of riluzole-induced GDNF production in order to reveal the effects of the riluzole in glia. This study first examined how riluzole induced cAMP response element binding protein (CREB) phosphorylation because CREB is an important transcriptional factor of the *gdnf* gene. Further investigations were thus conducted to reveal the consequences of the riluzole-induced CREB phosphorylation pathway which is involved in GDNF production.

2. Results

2.1. *Riluzole rapidly activates an MEK/ERK/CREB pathway in a GluT-independent manner*

We investigated the effect of riluzole on the phosphorylation of CREB. Riluzole increased CREB phosphorylation in a time-dependent manner, where phosphorylation gradually increased after 10 min and peaked at 40 min (Fig. 1A). The effect of riluzole on the phosphorylation level of CREB (40 min treatment) was dependent on the concentration of riluzole. A statistically significant increase was observed above 25 μM (Fig. 1B). A cell viability assay with trypan blue staining revealed a significant induction of cell death above 500 μM for 48 h. However, it was not observed at concentrations less than 200 μM riluzole (Fig. 1C). Therefore, riluzole was used at 25 μM in the subsequent experiments.

It is possible that riluzole induces CREB phosphorylation via GluT since riluzole is a modulator of glutamatergic neurotransmission. Then, the effect of two types of transportable GluT related agents on CREB phosphorylation were investigated. As shown in Table 1, L-glutamate and L-*trans*-pyrrolidine-2, 4-dicarboxylate (t-PDC), a transportable glutamate uptake inhibitor, did not increase CREB phosphorylation even at high concentrations (Table 1). These findings suggest that there might not be a regulatory pathway from GluT to CREB phosphorylation in C6 cells. Because mitogen-activated protein kinase kinase (MEK) is a major modulator of CREB phosphorylation and rapid MEK/ERK/CREB activation is critically involved in a GDNF production pathway in C6 cells (Hisaoka et al., 2007; Tsuchioka et al., 2008), the involvement of MEK was investigated subsequently. The riluzole-induced CREB phosphorylation was completely blocked by U0126, a MEK inhibitor (Fig. 2A). Elk-1 is a downstream substrate of ERK. The amounts of phospho-Elk-1 indirectly show ERK activity. As shown in Fig. 2B, riluzole indeed increased extracellular signal-regulated kinase (ERK) activation. ERK activation was first detected within 2 min and then peaked at 5 min, which was therefore earlier than CREB phosphorylation. The phosphorylation level was maintained for 60 min and then returned to the basal level by 3 h after treatment (Fig. 2B). These findings suggest that riluzole rapidly activates the MEK/ERK/CREB pathway independent of GluT in C6 cells.

2.2. *Riluzole induces CREB phosphorylation via FGFR*

Tyrosine kinases (TK) are involved in the regulation of rapid MEK/ERK/CREB activation in GDNF production by antidepressants and