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Stress-induced upregulation of VLDL receptor alters Wnt-signaling in neurons

Kai Kysenius, Henri J. Huttunen *

Neuroscience Center, University of Helsinki, Finland

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ABSTRACT

Lipoprotein receptor family members hold multiple roles in the brain, and alterations in lipoprotein receptor expression and function are implicated in neuronal stress, developmental disorders and neurodegenerative diseases, such as Alzheimer's disease. Berberine (BBR), a nutraceutical shown to have both neuroprotective and neurotoxic properties, is suggested to regulate lipoprotein receptor expression. We show that subtoxic concentration of BBR regulates neuronal lipoprotein receptor expression in a receptor- and time-dependent fashion in cerebellar granule neurons (CGN). Similarly to BBR, subtoxic concentrations of neuronal stressors cobalt chloride, thapsigargin and rotenone increased very-low-density lipoprotein receptor (VLDLR) mRNA and protein expression in CGN suggesting a conserved pathway for stress-induced upregulation of VLDLR in neurons. We also show that VLDLR upregulation is accompanied by transiently increased stabilization of hypoxia-induced factor 1 alpha (HIF-1α) and decreased β-catenin levels affecting the Wnt pathway through GSK3β phosphorylation, a crucial player in neurodegenerative processes. Our results indicate that neuronal stress differentially regulates lipoprotein receptor expression in neurons, with VLDLR upregulation as a common element as a modulator of neuronal Wnt signaling.

1. Introduction

Lipoprotein receptor family members have both physiological and pathophysiological functions in the brain [1]. Apolipoprotein E receptor 2 (ApoER2/LRP8) and very-low-density lipoprotein receptor (VLDLR) function as canonical Reelin receptors and are associated with brain development [2], synaptic plasticity [3], neuronal survival [4,5] and Alzheimer's disease (AD) pathogenesis [6]. Low-density lipoprotein receptor (LDLR) and low-density lipoprotein receptor-related protein 1 (LRP1) have central roles in lipid metabolism and clearance pathways within the brain [7,8], especially through their interaction with apolipoprotein E (ApoE), an important lipid- and amyloid-β-binding protein associated with AD [9,10]. VLDLR, LRP4, LRP5 and LRP6 are involved in Wnt signaling and are implicated in synaptic plasticity, adult neurogenesis and AD pathogenesis [11-13].

Neurons are subject to various forms of stress during aging and in neurodegenerative diseases (NDDs), such as AD [14]. Reduced neuroprotective signaling, disturbed proteostasis and altered mitochondrial function and ion homeostasis increase susceptibility to neuronal cell death [15]. Additionally, metabolic disorders, such as hypercholesterolemia, are associated with elevated risk of late-life dementia and increased neuronal susceptibility to stress [16]. Excessive dietary cholesterol intake, increased de novo cholesterol production and mutations in the LDLR, apolipoprotein B and proprotein convertase subtilisin kexin/type 9 (PCSK9) genes can increase circulating low-density lipoprotein (LDL) levels to cause hypercholesterolemia [17,18]. Statins, the most commonly used anti-hypercholesterolemic drugs, decrease de novo cholesterol production, but their cholesterol lowering effect is counteracted by an increase in PCSK9 expression, which downregulates hepatic LDLR levels and increases circulating LDL levels [19]. Recently, multiple clinical studies with PCSK9 antibodies have demonstrated high efficacy in cholesterol reduction with a good safety profile [20]. Interestingly, PCSK9 can also bind and degrade lipoprotein receptor family members ApoER2, VLDLR and LRP1, potentially affecting systemic and CNS functions beyond cholesterol metabolism [5,21–23].

Our recent study showed that endogenous PCSK9 downregulation is neuroprotective in cerebellar granule neurons (CGN), an effect dependent on ApoER2 expression [5]. Although VLDLR levels were not affected by PCSK9 downregulation in this study [5], VLDLR are implicated in inflammatory signaling and upregulated during hypoxia and ER stress [24,25]. Interestingly, berberine (BBR), a plant-derived alkaloid used as a dietary supplement in a number of indications including diabetes, hypercholesterolemia and dysentery [26], is shown to increase LDLR and decrease PCSK9 transcriptional expression in hepatocytes through the transcriptional factors sterol-responsive element binding protein 2 (SREBP2) and hepatocyte nuclear factor-1 (HNF-1) [27]. BBR is largely known for its neuroprotective properties [28–30], although we recently showed that BBR can act as a neuronal

* Correspondence to: Neuroscience Center,University of Helsinki, P.O. Box 56 (Viikinkaari 4), FIN-00014, Finland.
E-mail address: henri.huttunen@helsinki.fi (H.J. Huttunen).

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stressor in CGN at micromolar concentrations (30). In this study, we utilized BBR as a neuronal stressor to elucidate the effects of neuronal stress on the expression of lipoprotein receptors and PCSK9 in neurons.

Our results show that BBR differentially regulates the expression of neuronal lipoprotein receptors in CGN. Most notably, in concentrations tolerated by neurons in vitro [30], BBR increased the transcriptional and protein expression of VLDLR to a similar extent with subtoxic concentrations of hypoxia-mimetic cobalt chloride ($CoCl_2$), ER Ca^{2+}-ATPase inhibitor thapsigargin and mitochondrial complex I inhibitor rotenone. Additionally, hypoxia-induced factor 1α (HIF-1α) and β-catenin levels showed a transient biphasic response to BBR. At 6 h, HIF-1α levels were stabilized and β-catenin levels diminished, whereas at 24 h, HIF-1α levels and β-catenin levels returned to control levels. The effect of BBR on β-catenin levels at 6 h coincided with decreased serine-9 phosphorylation of GSK3β, a multifunctional kinase inactivated by serine-9 phosphorylation and implicated as a key target of Wnt signaling [31]. Interestingly, while increased expression of VLDLR, previously associated with antagonism of Wnt signaling [13,32], was observed at 6 h, a delayed increase in expression of Wnt signaling co-receptors LRP5/6 occurred at the 24 h timepoint. Taken together, our results indicate that stress acutely modulates lipoprotein receptor expression in neurons, particularly increasing VLDLR expression, coinciding with transient alterations in the Wnt-signaling pathway.

2. Results

2.1. Differential transcriptional regulation of lipoprotein receptors and PCSK9 by berberine

Lipoprotein receptor expression and associated pathways are altered in conditions with a significant neuronal stress component, such as AD [33]. Subtoxic concentrations of compounds affecting neuronal metabolism, such as BBR [30], are proposed to precondition neurons to withstand injuries such as hypoxia or oxidative stress [28], and are hence considered as potential therapeutic options against neurodegenerative diseases. However, the involvement and transcriptional regulation of lipoprotein receptors under neuronal stress is currently poorly understood.

We assessed the relative mRNA levels of lipoprotein receptor family members LDLR, VLDLR, ApoER2, LRP1, LRP4, LRP5 and LRP6 and a modulator of lipoprotein receptors, PCSK9, in CGN at concentrations of BBR, 0.1 and 1 μM. These concentrations do not acutely affect neuronal viability as determined in an earlier study [5] and shown by the Alamar blue cytotoxicity assay (Fig. 1a). Treatment with 0.1 μM BBR did not significantly affect lipoprotein receptor or PCSK9 mRNA levels at either 6 or 24 h (data not shown). However, 1 μM BBR treatment differentially regulated the expression of PCSK9 and lipoprotein receptors at the transcriptional level at 6 and 24 h (Fig. 1b). After 6 h of BBR treatment, only VLDLR mRNA levels showed a significant increase (2.16-fold, $p < 0.001$), whereas LRP1 showed a trend of increase (1.57-fold). After 24 h of 1 μM BBR treatment, a trend of PCSK9 (0.60-fold) and significant LDLR (0.42-fold, $p < 0.05$) reduction were observed in their respective mRNA levels; ApoER2 and LRP4 remained unaffected; and the transcriptional expression of VLDLR (2.64-fold; $p < 0.001$), LRP1 (1.94-fold; $p < 0.05$), LRP5 (2.26-fold; $p < 0.001$) and LRP6 (1.99-fold; $p < 0.05$) were upregulated (Fig. 1b). A Western blot analysis confirmed the upregulation of VLDLR also at the protein level in 1 μM BBR-treated CGN (1.45-fold, $p < 0.05$)(Fig. 1c and d).

2.2. Berberine-induced VLDLR upregulation is associated with a transient HIF-1α activation

Berberine affects the stability and expression of several

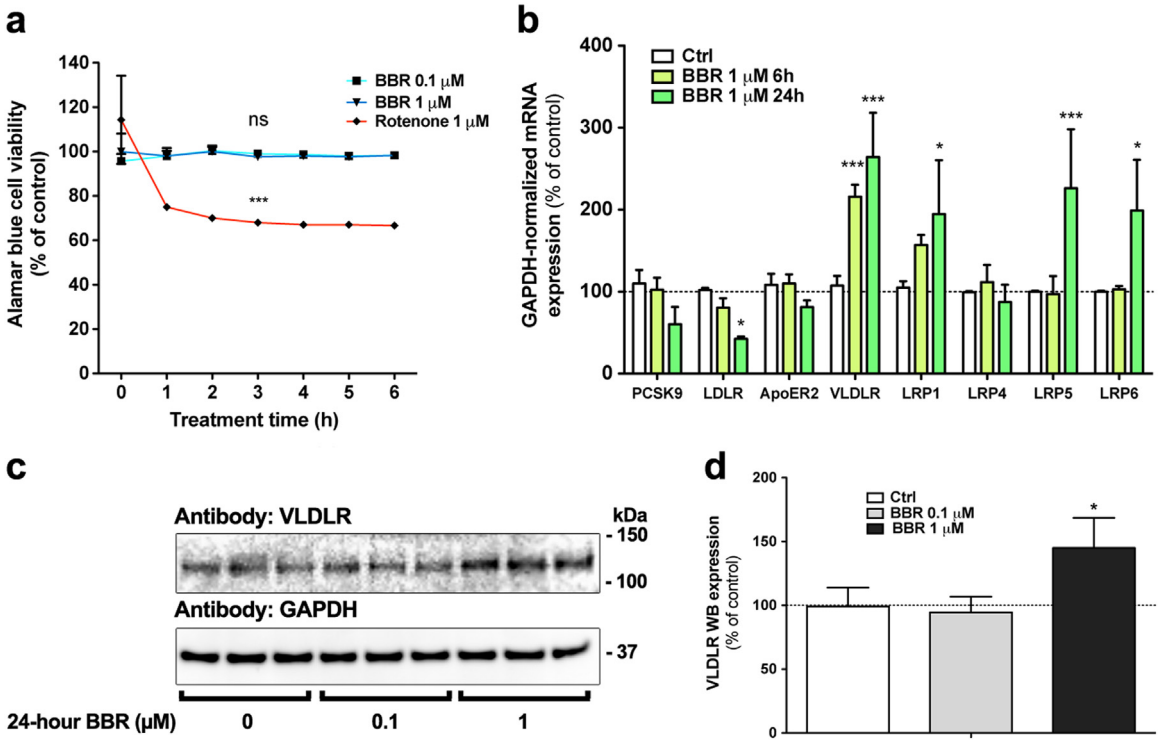

Fig. 1. Differential regulation of PCSK9 and lipoprotein receptor transcriptional expression by BBR. (a) Cell viability of BBR-treated CGN as assessed by Alamar blue assay with rotenone as a positive control. (b) The levels of PCSK9 and lipoprotein receptors mRNA normalized to GAPDH mRNA as determined by qPCR. (c) Representative Western blot of CGN treated with 0.1 and 1 μM BBR for 24 h. (d) Protein levels of VLDLR normalized to relative GAPDH levels (c). For a, b and d, $n=4$. Significance levels: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ between control and treatments groups.

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