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Research Report

The role of Homer 1a in increasing locomotor activity and non-selective attention, and impairing learning and memory abilities

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ABSTRACT

The current study aimed to investigate the possible role of Homer 1a in the etiology and pathogenesis of attention deficit hyperactivity disorder (ADHD). We divided 32 rats into four groups. The rats in the RNAi-MPH group were given the lentiviral vector containing Homer 1a-specific miRNA (Homer 1a-RNAi-LV) by intracerebroventricular injection, and 7 days later they were given three daily doses of methylphenidate (MPH) by intragastric gavage. The RNAi-SAL group was given Homer 1a-RNAi-LV and saline later. The NC-MPH group was given the negative control lentiviral vector (NC-LV) and MPH later. The NC-SAL group was given NC-LV and saline later. Rats that were given Homer 1a RNAi exhibited increased locomotor activity and non-selective attention, and impaired learning and memory abilities, which is in line with the behavioral findings of animal models of ADHD. However, MPH ameliorated these abnormal behaviors. All findings indicated that Homer 1a may play an important role in the etiology and pathogenesis of ADHD.

1. Introduction

Attention deficit hyperactivity disorder (ADHD) is a neuropsychiatric disorder characterized by inattentiveness, impulsivity and hyperactivity (Komatsu et al., 2012). It is associated with co-morbidities such as learning disorders, tics, anxiety, oppositional defiant disorder and conduct disorder (Hanwella et al., 2011). This disorder occurs in approximately 3–7% of the childhood population and approximately 2–5% of the adult population (Lyon et al., 2011). There is increasing evidence to indicate that genetic and neurobiological factors are the main likely causes, greatly reducing the role of purely social factors (Siqueira and Gurgel-Giannetti, 2011).

Researchers therefore focus on studying ADHD using modern molecular technology. Studies have indicated that changes in the dopamine (DA), norepinephrine (NE) and 5-hydroxytryptamine (5-HT) signal pathways may be associated with the clinical symptoms of ADHD. For instance, suboptimal levels of DA and NE transmission can lead to prefrontal cortex (PFC) dysfunction and symptoms resembling ADHD (Russell, 2002). In addition, drugs that increase brain DA neurotransmission (e.g., amphetamine and methylphenidate) produce

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divergent effects on impulsive behavior, generally acting to improve stopping performance on stop-signal reaction time tasks and reducing delay-discounting impulsivity in ADHD participants (Hammerness et al., 2009; Prehn-Kristensen et al., 2011). However the exact etiology of ADHD is still unknown.

In our previous studies, we found that the Homer 1a neuronal protein was significantly decreased in the PFC or the hippocampus of the spontaneous hypertensive rat (SHR) (Hong et al., 2009, 2011; Qiu et al., 2010). (SHR is the most widely used animal model of ADHD.) Homer 1a is one of the most important proteins of the Homer family of scaffolding proteins, which is localized to the postsynaptic density (PSD) of glutamatergic excitatory synapses (Qiu et al., 2010). It has been found to be involved in the dopamine, norepinephrine and glutamate signal pathways (de Bartolomeis and Tomasetti, 2012; Iasevoli et al., 2009). Homer 1a competes with the long-form Homer proteins to combine with some PSD-related components, such as metabotropic glutamate receptors (mGluR1, mGluR5) (Ghasemzadeh et al., 2009; Zhang et al., 2007), inositol trisphosphate receptors (IP3Rs) and Shank (de Bartolomeis and Iasevoli, 2003). These proteins are closely related with the transmitting of neurotransmitters between neurons, and behave as a dominant negative in PSD remodeling, which may represent a mechanism of synaptic plasticity (Roselli et al., 2009). Meanwhile, Homer 1a is one of the targeted molecules in behavior. Homer 1 knockout mice exhibit pronounced learning deficits during acquisition in both the Morris water maze (MWM) and radial arm maze tests, indicating poor reference and impaired working memory (Jaubert et al., 2007; Szumlinski et al., 2005). Meanwhile, Homer 1a overexpression can improve cognitive function in rats (Lominac et al., 2005). From such findings we infer that Homer 1a plays an important role in the etiology and pathogenesis of ADHD, however, further evidence is needed.

RNA interference (RNAi) can be used as a tool to silence expression of target genes by triggering post-transcriptional degradation of homologous transcripts through a multi-step mechanism involving double-stranded, small silencing RNA. As ADHD is a behavior disorder related to brain development, the primary technique used in this study was in vivo delivery of an RNAi vector to neurons. The psychostimulant methylphenidate (MPH) is the most prescribed drug to treat children, adolescents and adults with ADHD (Findling, 1996). The clinical effect of MPH made it a useful tool to study the pathogenesis.

In the current study, we constructed a lentiviral vector containing an artificial Homer 1a-specific miRNA. This was transduced into primary neuronal cells obtained from Sprague Dawley (SD) rat cortices. The effectiveness of the RNAi was validated by detecting the mRNA and protein expressions of Homer 1a. An in vivo experiment was then carried out by using 32 SD rats, which were divided into four groups. The RNAi-MPH group was given the lentiviral vector containing Homer 1a-specific miRNA (Homer 1a-RNAi-LV) by intracerebroventricular injection, and 7 days later these rats were given three daily doses of MPH by intragastric gavage. The RNAi-SAL group was given Homer 1a-RNAi-LV and then saline. The NC-MPH group was given the negative control lentiviral vector (NC-LV) and then MPH. Finally, the NC-SAL group was given NC-LV and then saline (Bello and Hajnal, 2006). After that, we initially examined the effects of Homer 1a RNAi on behavior, and then examined MPH effects on the behavior of rats that were given RNAi, using the Làt maze and the MWM. The mRNA and protein expression levels of Homer 1a were detected by qPCR and Western blotting, respectively. The findings revealed increased locomotor activity and non-selective attention, and impaired learning and memory abilities, which are characteristics of ADHD. However, MPH ameliorated these abnormalities. All findings indicated that Homer 1a may be related to the etiology and pathogenesis of ADHD.

2. Results

2.1. *Homer 1a-RNAi-LV down-regulates expression of Homer 1a mRNA and proteins in the primary neural cells*

After transduction with Homer 1a-RNAi-LV, the primary neural cells showed a significant reduction in the level of Homer 1a mRNA expression (vs. control groups, $F=5.93$, $P<0.05$), and protein expression (vs. control groups, $F=6.40$, $P<0.05$; Fig. 1).

2.2. *The changes in behavior and Homer 1a expression in rats after RNAi*

In the Làt maze (Fig. 2), the RNAi-SAL group exhibited significantly higher levels of both horizontal and vertical activities than the NC-SAL group (among groups, horizontal: $F=4.18$, $P<0.05$; vertical: $F=3.22$, $P<0.05$). In the MWM (Fig. 3), the RNAi-SAL group had longer latency times before locating the platform and longer total swim distances than the NC-SAL group during the first 2 days (among groups, Day 1 latency: $F=3.15$, $P<0.05$; distance: $F=3.95$, $P<0.05$. Day 2 latency: $F=3.30$, $P<0.05$; distance: $F=4.04$, $P<0.05$).

In the striatum and hippocampus, Homer 1a mRNA and protein expression levels were significantly decreased for the RNAi-SAL group compared with the NC-SAL group (among groups, striatum mRNA: $F=6.84$, $P<0.05$, striatum protein: $F=9.66$, $P<0.01$; hippocampus mRNA: $F=5.22$, $P<0.05$, hippocampus protein: $F=5.46$, $P<0.05$), but there was no difference in the PFC (Fig. 4).

2.3. *The changes in behavior and Homer 1a expression in rats after MPH treatment*

In the Làt maze, there were no significant differences between the RNAi-MPH group and the NC-MPH group for either horizontal or vertical activities. However, the RNAi-MPH group showed a decreased trend in locomotor activity compared with the RNAi-SAL group, without statistical significance (Fig. 2). In the MWM, the RNAi-MPH group had a longer total swim distance and latency time than the NC-MPH group, but with no statistical significance. The RNAi-MPH group showed a trend for increased learning and memory abilities compared with the RNAi-SAL group, without statistical significance (Fig. 3).

Homer 1a mRNA and protein expression levels were significantly decreased in the striatum for the RNAi-MPH group compared with the NC-MPH group, but there were no significant differences in the hippocampus and the PFC (Fig. 4).