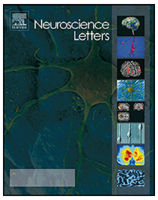
Neuroscience Letters 638 (2017) 51–54

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet



Research article

5-HT1A receptor (HTR1A) 5′ region haplotypes significantly affect protein expression in vitro

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HIGHLIGHTS

- A fragment spanning nucleotides −551 to +672 of the HTR1A (Transcription start site +1) 5′ promoter region was used to construct three haplotype plasmids (1-1 to 3-1).
- The relative fluorescence intensity of plasmids 1-1 and 3-1 was compared to the wild type plasmid 2-1 after transfection of HEK-293 and SK-N-SH cells.
- In the HEK-293 cells, there was a significant difference between plasmid 1-1(GC) and plasmid 2-1(GG).
- A significant difference between plasmid 1-1 and plasmid 2-1 was also observed in the SK-N-SH cells.

ARTICLE INFO

Article history:
Received 18 October 2016
Received in revised form 2 December 2016
Accepted 6 December 2016
Available online 7 December 2016

Keywords:
HTR1A
Polymorphism
Promoter
Transcription
Schizophrenia

ABSTRACT

The aim of this study was to explore the role of two SNPs (rs6295 & rs113195492) in the HTR1A gene promoter region that regulates the expression of the 5-HT1A receptor. A fragment spanning from −551 to +672 of HTR1A (Transcription start site +1) was cloned into the pGL-3 Basic Vector and three haplotype plasmids composed of two SNPs were constructed. HEK-293 cells and the SK-N-SH cells were transfected with the three plasmids, and the relative fluorescence intensity was measured. In HEK-293 cells, there was a significant difference when the relative fluorescence intensity of plasmid 1-1 was compared to that of plasmid 2-1. However, no significant difference was observed when the luciferase expression of plasmid 2-1 and plasmid 3-1 was analyzed. We also found that the expression trend of the SK-N-SH cells was similar to the HEK-293 cells, but the overall relative fluorescence intensity of the SK-N-SH cells was lower than that of the HEK-293 cells. Our finding showed that the rs6295 SNP, as a suspected variant that indicates susceptibility to schizophrenia, exhibited a higher transcriptional activity. The influence of the rs113195492 locus on schizophrenia needs to be explored further.

1. Introduction

Schizophrenia is a serious, chronic disease that may cause brain disorders. The worldwide prevalence reaches twenty-one million, with a lifetime rate of 1% of the population [1,2]. As a complex mental disorder influenced by the interaction between the environment and genetic factors, the etiology of schizophrenia is not clear. The current hypotheses to explain the pathological mechanisms of schizophrenia involve the neurotransmitters dopamine, serotonin, γ-aminobutyric acid and others. It is noted that 5-HT, which is an important neurotransmitter affecting a variety of physiological functions, including the nervous system, gastrointestinal system and cardiovascular system, is well-studied in schizophrenia research.

The serotonin receptor is an important part of the serotonin system in the brain. The 5-HT1A receptor represents the most abundant 5-HT receptor in each region of the brain [3]. As the presynaptic autoreceptor, the 5-HT1A receptor can inhibit the release of 5-HT. A great number of studies have shown that the reduction of 5-HT was linked with multiple mood disorders, including schizophrenia [4], manic depressive psychosis [5], severe depression [6,7] and suicide [8]. However, the function of the 5-HT1A postsynaptic receptor is exactly the opposite. It will lead to an increase in the concentration of serotonin. The gene HTR1A, encoding the 5-HT1A receptor, is located on human chromosome 5. A large number of SNPs in the control area of this gene, comprising different haplotypes, directly or indirectly affect the expression of the mRNA and, thus, the 5-HT1A receptor protein. Therefore, the different haplotypes will further influence the function of sero-

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http://dx.doi.org/10.1016/j.neulet.2016.12.011
0304-3940/

tonin. Many researchers have reported the rs6295 SNP. It is within the promoter region −1019 (regard ATG as +1) of the HTR1A gene, but its role in the expression of the 5-HT1A receptor is still controversial. Katharina Pernhorsta reported that the rs6295 (C/G) mutation had an effect on the basal activity of the HTR1A promoter. However, there was no significant difference between the C allele and the G allele [9]. However, Albert and Czesak believed that the rs6295G allele increased the expression of 5-HT1A autoreceptors, and reduced the 5-HT1A postsynaptic receptors, which led to a decrease in the total levels of 5-HT [10]. We also examined another polymorphism, rs113195492, which is located in the −1068 promoter region of the HTR1A gene (this number refers to the ATG as +1). Our previous study confirmed that this polymorphism might be one of the risk factors of schizophrenia [11].

The effect of the HTR1A gene in schizophrenia is still unknown, therefore, in this study we confirmed the role of the two SNPs in the regulatory area of the HTR1A gene.

2. Materials and methods

2.1. Sample

DNA samples were chosen in accordance with the three haplotypes that were intended for follow-up based our previous analysis of the haplotypes of the different samples.

2.2. Construction of the PGL-3 vectors

With the following primers, the target fragments were amplified using the PrimerSTAR® kit (Takara, Dalian, China).

Forwards: 5′-CTAGCTAGCTTGTCGTCGTTGTTCGTTTG-3′

Reverse: 5′-CCCAAGCTTGTCGGAGATACCAGTAGTGTTGC-3′

Cleavage sites for the restriction enzymes Nhe I and Hind III were introduced into the 5′ end of the primers. The purified PCR products were then cloned into the pGM-T vectors using the pGM-T Ligation® Kit (TIANGEN, Beijing, China). The recombinant pGM-T vectors were transformed into JM109 competent cells, and the plasmids extracted with SanPrep® Column Enodotoxin-Free Plasmid Mini-Preps Kit (Sangon Biotech, Shanghai, China) was sequenced to confirm the correct inserted fragments.

Finally, the correct pGM-T vectors with the three haplotypes were subcloned into the pGL-3 Basic Vector (Promega, Madison, Wisconsin, USA) and then transfected into the eukaryotic cell lines.

2.3. Cell culture

The two cell lines were cultured in a stable environment of 5% CO_2 + 95% mixed air, 37°, and 100% humidity. The HKE-293 cells were cultured in HyClone® DMEM high glucose medium containing 10% fetal bovine serum (Thermo Fisher Scientific, Massachusetts, USA), while the SK-N-SH cells were cultured in KeyGEN BioTECH ® DMEM high glucose medium with 0.011 g/L sodium pyruvate containing 15% fetal bovine serum. When the density reached 90% or more, the cells were inoculated in 24-well plates (2×10^5 cells per well). Then, using Lipofectamine® 2000 reagent (Invitrogen, California, USA), the recombinant plasmids of the three haplotypes were co-transfected into the two cell lines with Renilla luciferase-expressing vector pRL-TK (Promega) as a control. After 24 h of culture, the cells were harvested. Finally, firefly luciferase activity (LUC value) and renilla luciferase activity (TK value) were measured. Each assay was performed in triplicate in two cell lines.

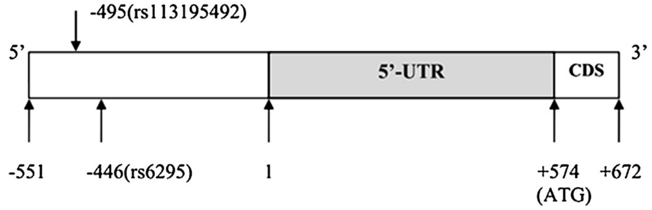

Fig. 1. The cloned fragment spans from −551 to +672 of the HTR1A gene including two SNPs.

Table 1
The base composition of the two polymorphisms distributed in the three haplotypes.

SNP	rs113195492	rs6295
PLASMID1-1	G	C
PLASMID2-1	G	G
PLASMID3-1	A	G

2.4. Statistical

LUC/TK value was the relative fluorescence intensity. We used the LSD-T test to compare the means of the relative fluorescence intensity among the three different haplotypes.

3. Results

3.1. Construction of the luciferase reporter gene vector in the HTR1A 5′ regulatory region

In this study, a 1223 bp target gene was cloned into the PGL-3 Basic vector by the PCR amplification technique, and the length of the inserted fragments were detected by double enzyme digestion and agarose gel electrophoresis. The length of the target fragments containing the two SNPs was consistent with the designed length (Fig. 1). Sequencing was then used to screen the recombinant plasmids of the three haplotypes (Table 1).

3.2. Analysis of the relative fluorescence intensity of the three haplotypes

The two cell lines, HEK-293 and SK-N-SH, were used to carry out the luciferase reporter experiments, which further proved that the two SNPs in the HTR1A regulatory region were involved in the expression of the 5-HT1A receptor. By comparing the LUC/TK values of the three haplotypes with the pGL-3 Basic vector and the pGL-Control vector, all three haplotypes were shown to express the luciferase. As the wild-type, the relative fluorescence intensity of plasmid 2-1 (GG) was the highest. In HEK-293 cell lines, the LUC/TK values of the two other plasmids (1-1 and 3-1) were lower than that of plasmid 2-1, and the fluorescence intensity of plasmid 1-1 was lowest. However, the only significant difference found was between the fluorescence intensity of plasmid 1-1 and plasmid 2-1 ($P = 0.001$). A significant difference between plasmid 3-1 and plasmid 2-1 was not observed (Fig. 2). Analysis of the SK-N-SH cells showed a trend similar to that seen with the HEK-293 cells with respect to the expression of the relative fluorescence intensity. However, overall luciferase activity was relatively higher in the HEK-293 cells (Fig. 3).

4. Discussion

In this study, a fragment of the HTR1A gene containing 551 bp of the 5′ flanking region and 672 bp of the 5′-UTR was cloned into the pGL-3 Basic Vector. To examine the regulatory activity of the promoter region, HEK-293 cells and SK-N-SH cells were transfected