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Research report

5-HT$_{1A}$ receptor gene silencers Freud-1 and Freud-2 are differently expressed in the brain of rats with genetically determined high level of fear-induced aggression or its absence

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HIGHLIGHTS

- Aggressive and tame rats differ in 5-HT$_{1A}$ protein but not 5-HT$_{1A}$ mRNA level.
- Investigated rat strains differ in 5-HT$_{1A}$ receptor silencers Freud-1 and Freud-2.
- Freud-1 protein level was decreased in hippocampus of aggressive rats.
- Freud-2 protein level was increased in frontal cortex of highly aggressive rats.
- Freud-1 and Freud-2 are involved in mechanisms of genetically defined aggression.

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ABSTRACT

Serotonin 5-HT$_{1A}$ receptor is known to play a crucial role in the mechanisms of genetically defined aggression. In its turn, 5-HT$_{1A}$ receptor functional state is under control of multiple factors. Among others, transcriptional factors Freud-1 and Freud-2 are known to be involved in the repression of 5-HT$_{1A}$ receptor gene expression. However, implication of these factors in the regulation of behavior is unclear. Here, we investigated the expression of 5-HT$_{1A}$ receptor and silencers Freud-1 and Freud-2 in the brain of rats selectively bred for 85 generations for either high level of fear-induced aggression or its absence. It was shown that Freud-1 and Freud-2 levels were different in aggressive and nonaggressive animals. Freud-1 protein level was decreased in the hippocampus, whereas Freud-2 protein level was increased in the frontal cortex of highly aggressive rats. There no differences in 5-HT$_{1A}$ receptor gene expression were found in the brains of highly aggressive and nonaggressive rats. However, 5-HT$_{1A}$ receptor protein level was decreased in the midbrain and increased in the hippocampus of highly aggressive rats.

These data showed the involvement of Freud-1 and Freud-2 in the regulation of genetically defined fear-induced aggression. However, these silencers do not affect transcription of the 5-HT$_{1A}$ receptor gene in the investigated rats. Our data indicate the implication of posttranscriptional rather than transcriptional regulation of 5-HT$_{1A}$ receptor functional state in the mechanisms of genetically determined aggressive behavior. On the other hand, the implication of other transcriptional regulators for 5-HT$_{1A}$ receptor gene in the mechanisms of genetically defined aggression could be suggested.

1. Introduction

Brain serotonin (5-HT) system is known to play an important role in the regulation of aggressive behavior [1,2]. Among numerous receptors mediating 5-HT action, 5-HT$_{1A}$ receptor attracts particular attention due to its key role in the autoregulation of the brain 5-HT system functioning [3,4] and a plenty of data on the

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involvement of 5-HT_{1A} receptor in the control of different kinds of aggressive behavior [5–9].

Recent studies provided new evidences that being a key regulator of the brain 5-HT system 5-HT_{1A} receptor is under control of multiple factors [10,11] including transcriptional factors [12,13]. Freud-1 is one of the most important transcriptional factors for 5-HT_{1A} receptor gene. Freud-1 binds to a specific element in the promoter of the 5-HT_{1A} receptor gene and mediates strong repression of the 5-HT_{1A} receptor gene expression. The deletion of this binding site from 5-HT_{1A} receptor gene promoter leads to intense increase of 5-HT_{1A} receptor gene expression. In its turn, overexpression of the gene encoding Freud-1 causes inhibition of 5-HT_{1A} receptor gene expression and reduction of 5-HT_{1A} receptor protein level [12]. Freud-1 was initially identified as a strong repressor of 5-HT_{1A} autoreceptors on raphe neurons, but now it is also known to represses the receptor in non-serotonergic neuron. Freud-1 is strongly expressed in raphe, PFC and hippocampus and co-localized with 5-HT_{1A} receptors. There are some data indicating the participation of Freud-1 in the development of psychopathology [14,15]. Among others, Freud-1 was shown to be involved in the mechanisms of major depression. Freud-1 protein level was significantly decreased in the prefrontal cortex of the subjects with major depressive disorder [16]. Freud-1 is also known to play role in the mechanisms of prenatal stress response [17], social defeat stress [18] and chronic restraint stress [19]. In our previous studies we showed that Freud-1 is implicated in the autoregulation of the brain 5-HT system [20].

Recently novel silencer for 5-HT_{1A} receptor Freud-2 was revealed [13,21]. Freud-2 is a homologue of Freud-1 and binds to a site adjacent to Freud-1 to repress 5-HT_{1A} receptor expression [13,21]. Unlike Freud-1, Freud-2 sparsely expressed in the raphe, but is strongly expressed in prefrontal cortex where it is co-localized with 5-HT_{1A} receptors and a trend for reduced Freud-2 in the prefrontal cortex of depressed patients was observed [13]. Down-regulation of Freud-2 may be beneficial in depression to increase 5-HT_{1A} expression in pyramidal neurons and enhance their firing activity.

Transcriptional regulation of the 5-HT_{1A} receptors could play an considerable role in the psychopathology of different kinds of mental diseases [22]. Changes in the 5-HT_{1A} gene expression could differentially affect pre- and postsynsptic 5-HT_{1A} receptors and, hence, the functional activity of the brain 5-HT system. These data taken together with a crucial role of 5-HT_{1A} receptor in the autoregulation of the brain 5-HT system function [4] suggest transcriptional factors of 5-HT_{1A} receptor gene as potential regulators of 5-HT-dependent behavior. However, the relationships between Freud-1 and Freud-2 transcriptional factors and their roles in the regulation of behavior are still remaining unclear.

Earlier, using rats of the 59th generation of selective breeding for either high level or for the lack of fear-induced aggression we revealed significant differences in the expression of 5-HT_{1A} receptor. Considerable reduction of 5-HT_{1A} receptor density was shown in the hypothalamus, frontal cortex and amygdala of rats with genetic predisposition to aggressive behavior compared with tame animals. These changes were accompanied by significant decrease of 5-HT_{1A} receptor gene expression in the midbrain, the brain area of serotonergic cell bodies localization [7]. Taking into account the important role of Freud-1 and Freud-2 in the regulation of 5-HT_{1A} receptor gene transcription the involvement of these silencers in the regulation of 5-HT_{1A} receptor expression alterations in the brain of rats with genetically defined aggression and its absence was suggested.

So, the aim of the current study was to investigate the expression of the 5-HT_{1A} receptor and its transcriptional factors Freud-1 and Freud-2 in the brain structures of rats selectively bred for 85 generation for either high level or for the lack of aggression towards man.

2. Materials and methods

2.1. Animals

The experiments were carried out on adult male Norway rats (Rattus norvegicus) selectively bred for 85 generation for either high level or for the lack of fear-induced aggression at the Institute of Cytology and Genetics, Novosibirsk [23,24]. The animals were housed in metallic cages (50 × 33 × 20 cm) under standard laboratory conditions in a natural light-dark cycle (12 h light and 12 h dark) with free access to water and food in groups of four individuals. Two days before experiments 6 month old rats weighted 300–350 g (20 animals of each strain) were isolated into individual cages to remove the group effect. All experimental procedures were in compliance with Guidelines for the Use of Animals in Neuroscience Research, 2010. All efforts were made to minimize the number of animals used and their sufferings. The study was carried out on the base of ICG SD RAS vivarium (RFMEFI61914 × 0005 and RFMEFI61914 × 0010).

2.2. Handling test

The response to handling by gloved hand was used for estimation of aggressiveness [8]. The intensity of response was evaluated using following five-score system: 0 – rat permits to handle and does not make any attempts of avoiding; 1– permits to handle and makes evasive movements in the hand; 2 – moves away from the hand and while being picked up breaks loose from the grip; 3– actively escapes handling and while being picked up, rat can emit loud screaming noises, opens mouth or bites; 4 – rat does not permit to handle, attacks the hand and emits loud screaming noises.

2.3. RT-PCR

Total RNA was extracted using TRIzol Reagent ("Lifetechnologies", USA) according to the manufacturer's instructions, treated with RNA-free DNAse (Promega, USA), and diluted to 0.125 μg/μl with DEPC-treated water. One microgram of total RNA was taken for cDNA synthesis with a random hexanucleotide mixture [25]. The concentration of genomic DNA in the cDNA samples did not exceed 30 copies/μl. The number of copies of DNA-dependent RNA polymerase II (rPol II), 5-HT_{1A} receptor, Freud-1 and Freud-2 cDNA were evaluated by real-time quantitative PCR using selective primers (Table 1), SYBR Green I fluorescence detection (R-414 Master mix, Syntol, Moscow, Russia), and genomic DNA extracted from the livers of male Wistar rats as the external standard (200 copies/ng of genomic DNA). We used 50, 100, 200, 400, 800, 1600, 3200 and 6400 copies of genomic DNA as external standards for all studied genes. Reagent controls were carried out under the same conditions but without the template. Gene expression was evaluated as the number of cDNA copies with respect to 100 copies of rPol II cDNA [25–27]. Melting curve analysis was performed at the end of each run for each primer pair, allowing us to control amplification specificity.

2.4. Western blot analysis

For assessment of 5-HT_{1A} receptor levels, total protein was isolated from frontal cortex, hippocampus and midbrain, which were homogenized in HB buffer (10 mM Tris-HCl, pH = 7.2, 1 mM EDTA, 5 mM β-mercaptoethanol), and protease inhibitors (GE Healthcare, USA). Homogenates were centrifuged on 2000*g* during 15 min on 4 °C, supernatant was transferred to clean 1.5 ml tube and