*Neuroscience* 169 (2010) 229–235

# RECEPTOR-GENES CROSS-TALK: EFFECT OF CHRONIC 5-HT$_{1A}$ AGONIST 8-HYDROXY-2-(DI-N-PROPYLAMINO) TETRALIN TREATMENT ON THE EXPRESSION OF KEY GENES IN BRAIN SEROTONIN SYSTEM AND ON BEHAVIOR

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**Abstract—Dysfunction in brain serotonin (5-HT) system has been implicated in the psychopathology of anxiety, depression, drug addiction, and schizophrenia. The 5-HT$_{1A}$ receptors play a central role in the control of 5-HTergic neurotransmission. There are some scarce data showing cross-regulation between 5-HT receptors. Here, we investigated whether interaction exists between 5-HT$_{1A}$ receptor and genes encoding key members in brain 5-HT system. Chronic treatment with selective agonist of 5-HT$_{1A}$ receptor 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) (1.0 mg/kg i.p., 14 days) produced considerable decrease in hypothermic response to acute administration of 8-OH-DPAT in CBA/Lac mice indicating desensitization of 5-HT$_{1A}$ receptors. The decrease in 5-HT$_{1A}$ gene expression as well as decrease in the expression of gene encoding key enzyme in 5-HT synthesis, tryptophan hydroxylase-2 (TPH-2) in the midbrain, and the expression of the gene encoding 5-HT$_{2A}$ receptor in the frontal cortex was shown. There were no significant changes in 5-HT transporter mRNA level in the midbrain. Despite considerable decrease in the expression of the genes encoding tryptophan hydroxylase-2, 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors, chronic 8-OH-DPAT treatment failed to produce significant changes in 5-HT$_{1A}$-linked behavior (intermale aggression, open-field behavior, light-dark box, and pinch-induced catalepsy), suggesting compensatory and adaptive effect of genes suppression. The obtained data on the effect of 8-OH-DPAT-induced desensitization of 5-HT$_{1A}$ receptors on 5-HT$_{1A}$, 5-HT$_{2A}$ and TPH-2 gene expression demonstrated the role of 5-HT$_{1A}$ receptor as indirect regulator of gene expression. The results provide the first evidence of receptor-key genes interaction in brain 5-HT system and may have profound implications in understanding the functioning of the brain neurotransmitter systems.**


**Key words: chronic 8-OH-DPAT treatment, 5-HT$_{1A}$, 5-HT$_{2A}$ receptors, 5-HT transporter, tryptophan hydroxylase-2 genes expression, behavior.**

Brain serotonin (5-HT) system has been implicated in psychopathology of anxiety, depression, drug addiction, obsessive-compulsive disorder, and schizophrenia. Among large family of related receptors mediating 5-HT neurotransmittion in the brain particular attention has focused on the 5-HT$_{1A}$ receptor. In contrast to other 5-HT receptors, 5-HT$_{1A}$ receptors are localized predominantly in the brain (Saudou and Hen, 1994), and play central role in the control of 5-HT system functional activity due to unique presynaptic (on the soma and dendrites in the 5-HT neurons) and postsynaptic localization. In the midbrain raphe neurons, 5-HT$_{1A}$ presynaptic receptor regulates 5-HT neurons firing rate modulating the neurotransmitter release (Barnes and Sharp, 1999), and is thought to play a role in the feedback regulation of the 5-HT system. 5-HT$_{1A}$ receptor is implicated in the mechanisms of stress–response (Resstel et al., 2009; Vianna and Carrive, 2009), thermoregulation (Hjorth, 1985; Goodwin et al., 1987), depression, anxiety (Ramboz et al., 1998; Overstreet et al., 2003), depressive psychosis (Maes and Meltzer, 1995), aggressive behavior (de Boer et al., 1999; Pruus et al., 2000), catalepsy (Kulikov et al., 1993; Neal-Beliveau et al., 1993). Agonists of 5-HT$_{1A}$ receptor have been shown to be clinically effective anxiolytics (Rakel, 1990; Handley, 1995), and are used as antidepressants (Robinson et al., 1989; Blier and de Montigny, 1994).

There is accumulating evidence of cross-regulation between different kinds of receptors and profound impact of receptor interplay in neurotransmitter functions. Two types of interaction between effector signaling pathways were elucidated (Berg et al., 1998): (1) homologous interactions within the same receptor system producing loss (desensitization) or increase (sensitization) of responsiveness, and (2) heterologous interactions that occur between different receptor systems, that is cross-talk. It was suggested that some kinds of interactions between neurotransmitter receptors has important implications for rapid modulation of receptor function and the integration of multiple signals at a synapse (Brandon and Moss, 2000).

It was shown that chronic administration of 5-HT$_{1A}$ receptor agonist produced desensitization of 5-HT$_{1A}$ receptors (De Souza et al., 1986; Martin et al., 1992; Manahan-Vaughan et al., 1994) and the reduction of [$^{35}$S]-GTP$\gamma$S binding (Hensler and Durgam, 2001). The data on the effect of chronic 5-HT$_{1A}$ agonist treatment on 5-HT$_{1A}$ receptor density are scarce and contradictory. Some authors have found the considerable reduction of brain 5-HT$_{1A}$ receptor density (Fanelli and McMonagle-Strucko, 1992; Le Poul et al., 1999), whereas others did not reveal

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*Abbreviations:* DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; rPol II, DNA-dependent RNA-polymarese II; TPH-2, tryptophan hydroxylase-2; 5-HTT, 5-HT transporter; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)tetralin.

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doi:10.1016/j.neuroscience.2010.04.044

any effect of 5-$HT_{1A}$ agonist long-term treatment on 5-$HT_{1A}$ receptor density (Larsson et al., 1990; Schechter et al., 1990). Chronic treatment with high doses of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) (5.0 mg/kg s.c.) (De Souza et al., 1986) produced modest enhancement of 5-$HT_{2A}$ receptor mediated head-twitch and decreased 5-$HT_{2A}$ receptor number in frontal cortex. There were no association between head-twitch response and 5-$HT_{2A}$ receptor density in the frontal cortex. Acute exposure to 5-$HT_{1A}$ receptor agonist 8-OH-DPAT produced prolong desensitization of 5-$HT_{2A}$ receptors (Carrasco et al., 2007), while activated by 5-$HT_{2A}$ receptor protein kinase C produces desensitization of the 5-$HT_{1A}$ receptor in human cell culture (Raymond, 1991) suggesting heterologous interactions between 5-$HT_{1A}$ and 5-$HT_{2A}$ receptors.

There were no data on the impact of chronic 5-$HT_{1A}$ agonist treatment on expression of genes encoding key members of brain serotonin system.

To elucidate the cross-talk between 5-$HT_{1A}$ receptor and genes encoding key members in 5-HT brain system we investigated functional activity of 5-$HT_{1A}$ receptor, the expression of 5-$HT_{1A}$ receptor, 5-$HT_{2A}$ receptor, 5-HT transporter (5-HTT) and tryptophan hydroxylase-2 (TPH-2) genes, as well as 5-$HT_{1A}$ receptor-related behavior in mice chronically treated with selective 5-$HT_{1A}$ agonist 8-OH-DPAT.

## EXPERIMENTAL PROCEDURES

### Animals

Experiments were carried out on adult (6 weeks) male mice of CBA/Lac inbred strain. The mice were housed under standard laboratory conditions in a natural light–dark cycle (16 h light and 8 h dark) with free access to water and food. All experimental procedures were in compliance with Guidelines for the Use of Animals in Neuroscience Research, 1992. For 14 days the animals weighted about 25 g were treated with selective agonist of 5-$HT_{1A}$ receptor—8-OH-DPAT (Research Biochemicals Inc. USA). The drug was dissolved in saline in the concentration of 1 mg/kg of body weight and administered i.p. (10 mice in the group). The control group (10 mice) obtained saline injections instead of the drug. Two days before experiments the mice were isolated into individual cages to remove the group effect. The treatment with 8-OH-DPAT was continued until animals were decapitated for gene expression estimation; the injection of the drug was carried out after experimental manipulations. The behavioral tests were separated with one-day span and performed using EthoStudio software described in details elsewhere (Kulikov et al., 2008).

### Functional activity of 5-$HT_{1A}$ and 5-$HT_{2A}$ receptors

For estimation of functional activity (sensitivity) of 5-$HT_{1A}$ receptors the intensity of hypothermic reaction produced by administration of 5-$HT_{1A}$ receptor agonist 8-OH-DPAT was evaluated (Hjorth, 1985; O'Connell and Curzon, 1996; Popova et al., 2005). Although 8-OH-DPAT has moderate affinity for the 5-$HT_7$ receptor, its hypothermic effect is mediated by 5-$HT_{1A}$ receptors. In our preliminary experiments, 8-OH-DPAT-induced hypothermia was abolished by pretreatment with selective 5-$HT_{1A}$ receptor antagonist WAY 100635 (delta t°C was 2.3±0.2 in 8-OH-DPAT group and 0.3±0.3 in WAY 100635-pretreated mice (1.0 mg/kg), $F_{1,19}=34.1$; $P<0.001$). The body temperature was measured before and in 20 min after the drug administration (1.0 mg/kg i.p.) by means of a KJT thermocouple thermometer (Hanna Instruments, Singapore) with a copper-constantan rectal probe for mice (Physitemp Instruments, USA).

After all behavioral procedures the functional activity of 5-$HT_{2A}$ receptors was investigated by evaluation of the number of head-twitches induced by selective agonist of 5-$HT_{2A}$ receptors—DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane, Sigma, USA; Green and Heal, 1985). DOI is 5-$HT_{2A/2C}$ agonist with principal effect on 5-$HT_{2A}$ receptor. Importantly, pharmacological analysis showed that the head-twitches were mediated primarily by 5-$HT_{2A}$ receptors (Sanchez and Arnt, 2000). DOI was dissolved in saline in the dose of 1 mg per kg of body weight and administered i.p. The head-twitches were measured during 20 min in 5 min after drug administration.

### Open field

Open field was performed using open chamber (40×40×25 $cm^3$), made from opaque plastic. The floor of the device was made of mat and semitransparent plastic and placed on the mount of 40 cm above two halogen lamps of 12 W each (Kulikov et al., 2008). Each animal was placed near the wall of the chamber equally spaced from the corners and tested during 5 min. The total path length, time spent in center, number of groomings, number of rearings and number of defecations were measured.

### Pinch-induced catalepsy

Catalepsy was tested on the same day, after the open-field test. It was performed according to the early described procedure (Kulikov et al., 1993). Animals were firmly pinched between two fingers for 5 s at the scruff of a neck, placed on parallel bars, with the forepaws at 5 cm above the hind legs and then were released gently. The catalepsy duration was recorded in seconds from the instant when an animal was released to the instant when the animal shifted its front paws from the initial position on the upper bar or made gross body or head movements. The trial ended either when a mouse started to move or after 120 s of immobility. Every animal was submitted to 10 successive trials with 2-min intervals. Mice were kept in the home cages between the trials. The mouse was considered as cataleptic if the time of immobility was above 20 s in no less than three of 10 trials. The percent of cataleptics in group and the time of total freezing were measured.

### Light–dark box

Light–dark box test was performed using plastic white-colored device (20×20×27 $cm^3$) divided by opaque partition with the 7×7 $cm^2$ aperture into two compartments—bright, with semitransparent plastic flour alighted from below by two halogen lamps of 12 W each, and dark (closed). The animal was placed in the light part of the device faced towards aperture. The number of entering in dark compartment, latent time of entering in dark compartment, duration of staying in dark compartment and the number of peeks were measured during 5 min of the test.

### Intermale aggression test

Intermale aggression was measured in encounters between pairs of male and characterized by the number and duration of fights (Popova and Kulikov, 1986). A random-bred male albino mouse (intruder) of the same age and weight as the tested male (resident) was introduced into the home cage of the resident. Trials were limited to 10 min. To exclude the influence of previous fighting experience and familiarity with intruder, all residents were tested once only.

### Tail suspension test

For Tail Suspension Test (TST; Trullas et al., 1989) the animals were fixed suspended by the tail using adhesive tape on the horizontal crossbar positioned at the 30 cm height. The time of complete immobility, number of immobility episodes and latent time of immobility were evaluated during 6 min of the test.