5HT\(_{1A}\) and 5HT\(_{1B}\) receptors of medial prefrontal cortex modulate anxiogenic-like behaviors in rats

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**A B S T R A C T**

Medial prefrontal cortex (MPFC) is one of the brain regions which play an important role in emotional behaviors. The purpose of the present study was to evaluate the role of 5HT\(_{1A}\) and 5HT\(_{1B}\) receptors of the MPFC in modulation of anxiety behaviors in rats. The elevated plus maze (EPM) which is a useful test to investigate the effects of anxiogenic or anxiolytic drugs in rodents, was used. Bilateral intra-MPFC administration of 5HT\(_{1A}\) receptor agonist, 8-OH-DPAT (5, 10, and 50 ng/rat) decreased the percentages of open arm time (OAT\%) and open arm entries (OAE\%), indicating an anxiogenic response. Moreover, administration of 5HT\(_{1A}\) receptor antagonist, NAN-190 (0.25, 0.5, and 1 µg/rat) significantly increased OAT\% and OAE\%. Pre-treatment administration of NAN-190 (0.5 µg/rat), which was injected into the MPFC, reversed the anxiogenic effects of 8-OH-DPAT (5, 10, and 50 ng/rat). Intra-MPFC microinjection of 5HT\(_{1B}\) receptor agonist, CGS-12066A (0.25, 0.5, and 1 µg/rat) significantly decreased OAT\% and OAE\%, without any change in locomotor activity, indicating an anxiogenic effect. However, injection of 5HT\(_{1B}\) receptor antagonist, SB-224289 (0.5, 1, and 2 µg/rat) into the MPFC showed no significant effect. In conclusion, these findings suggest that 5HT\(_{1A}\) and 5HT\(_{1B}\) receptors of the MPFC region modulate anxiogenic-like behaviors in rats.

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Several lines of evidence show that the prefrontal cortex (PFC) is involved in the regulation of stress and anxiety [8], and most of the behavioral studies investigating the function of the PFC in anxiety behaviors have focused mainly on the medial prefrontal cortex (MPFC) [22].

The expression of anxiety in human has similar characteristics as in animals and serotonergic agents as a first-line treatment option for human anxiety disorders have highlighted the key role which is likely played by serotonin in anxiety. Serotonin receptors have been classified into several distinct families: 5HT1-7 [18]. The distinct involving mechanisms in the etiology of anxiety are unclear, however one of the likely hypothesized mechanism is that increased 5HT neurotransmission is associated with anxiogenic responses, while decreased 5HT function is associated with anxiolytic effects [6,20]. In support of this hypothesis, a number of clinical studies relate functioning of 5HT\(_{1A}\) Receptors (5HT\(_{1A}\)-Rs) to anxiety disorders, resulting in successful use of this receptor protein as a molecular target for anxiolytic drugs [1]. In addition, findings of limited clinical and animal studies indicated that stimulation of 5-HT\(_{1B}\)-Rs produces anxiolytic or anxiogenic effects [9,25].

Relationship between the 5HT-R of PFC and emotionality has been repeatedly shown by several experiments, imaging techniques, and also rodent models with inactivated 5HT gene searching for mechanisms underlying different regions of brain leading to the anxiety behaviors [15,23,24,27]. Several serotonin receptors have been localized to the MPFC, including members of the 5HT1 families such as 5HT\(_{1A}\) and 5HT\(_{1B}\) [11,26]. Several studies reported that conditioned fear stress increases serotonin metabolism in the rat MPFC, an observation that suggests that the 5HT system in the PFC is involved in emotional behavior related to anxiety or fear [19,34]. These results support the hypothesis that aspects of human anxiety are mediated through prefrontal cortical mechanisms. Thus, the present study aimed to understand the links between anxiety behaviors and 5HT\(_{1A}\)- and 5HT\(_{1B}\)-Rs of MPFC.

Male Wistar rats from the Pasteur Institute of Iran, weighing 220–250g at the time of surgery, were used. Animals were housed 4 per cage in a room with a 12:12 h light/dark cycle (lights on at 7:00 A.M.) and controlled temperature (23 ± 1 °C). Rats had access to food and water ad libitum and were allowed to adapt to the laboratory conditions for at least 1 week before surgery. All experiments were performed according to the protocols approved by institutional animal ethics committee of Islamic Azad University, and each rat was tested only once. Seven animals were used in each group of experiments.
Animals were anesthetized intraperitoneally with ketamine hydrochloride (50 mg/kg) plus xylazine (4 mg/kg) and placed in a Stoelting stereotaxic instrument. The stainless steel guide cannula (21-gauge) was implanted in the right and left MPFC regions according to Paxinos and Watson [28]. Stereotaxic coordinates for the MPFC were AP: +3.5 mm from bregma, L: ±0.8 mm from midline and V: −3.3 mm from the skull surface. The cannula was fixed to the skull with acrylic dental cement. Rats were handled about 4 min each day and were allowed 7 days before the test to recover from surgery. The left and right MPFC were infused by means of an internal cannula (27-gauge), terminating 1 mm below the tip of the guides, connected by polyethylene tubing to a 1-μl Hamilton syringe. On each side 0.5 μl solution was injected. The inner cannula was left in place for an additional 60 s to allow diffusion of the solution and to reduce the possibility of reflux [35].

The elevated plus-maze test was performed as previously described [35]. Five minutes following their respective drug treatment, rats were placed individually at the center of the EPM, facing one of the open arms and allowed 5 min of free exploration. The percentage of open arm time (OAT%) and open arm entries (OAE%) as the standard anxiety indices were calculated as follows: (1) OAT%; time in open arm/time in open + closed arm × 100; (2) OAE%; number of open arm entries/number of open arm + closed arm entries × 100; (3) total arm entries used as an index of locomotor activity (LMA).

The following drugs were used in the experiments: (±)-8-hydroxy-2-(di-n-propyl-amino) tetralinhydrobromide (8-OH-DPAT; Sigma, USA), NAN-190 hydrobromide (Sigma Chemical Co., USA), CGS-12066A maleat salt (Sigma Chemical Co., USA), and SB-224289 hydrochloride (Tocris, Bristol, UK). 8-OH-DPAT and NAN-190 were dissolved in 0.9% saline; CGS-12066A was dissolved in a minimal volume of diluted acetic acid (1 drop; 5 μl by Hamilton micro-syringe 10 μl) and made up to a volume of 5 ml with saline and was then diluted to the required volume with vehicle and SB-224289 was dissolved in dimethylsulphoxide (DMSO; up to 10%, v/v) and sterile saline. Control animals received either saline or vehicle.

Four groups of rats received bilateral MPFC injection of 5HT1A receptor agonist, 8-OH-DPAT (5, 10, and 50 ng/rat) [10]. The other three groups received 5HT1A receptor antagonist, NAN-190 (0.25, 0.5, and 1 μg/rat) and then were compared with the saline group. The test session was performed 5 min after intra-MPFC injections.

Three groups of rats received bilateral MPFC injection of 5HT1B receptor agonist, CGS-12066A (0.25, 0.5, and 1 μg/rat). The other three groups received 5HT1B receptor antagonist, SB-224289 (0.5, 1, and 2 μg/rat) and subsequently were compared with the vehicle control group.

Four groups of rats received saline (1 μl/rat, intra-MPFC). Three minutes after the administration, one group received saline (1 μl/rat) and the other three groups received 3 different doses of 8-OH-DPAT (5, 10, and 50 ng/rat). Other four groups of rats received an intra-MPFC injection of NAN-190 (0.5 μg/rat) at 3 min before injection of saline (1 μl/rat) or 8-OH-DPAT (5, 10, and 50 ng/rat).

At the end of the behavioral tests, each rat was sacrificed by chloroform overdose and then 0.5 μl/side of a 1% Methylene-Blue solution was bilaterally injected into the MPFC as a marker of the injection sites. Brains were removed after decapitation and fixed in a 10% formalin solution at least for 10 days. The brains were sliced and the sites of injection were verified according to the atlas of Paxinos and Watson [28]. Data from animals with injection sites located outside the MPFC region were not used in the analysis (Fig. 1).

All values were expressed as mean ± SEM. Analysis of results was performed using one-way and two-way analyses of variance (ANOVA). Following a significant F-value, post-hoc analysis (Tukey test) was performed for assessing specific group comparisons. P < 0.05 was considered statistically significant.

Fig. 2 (left panel) shows the effects of intra-MPFC injections of 8-OH-DPAT (5, 10, 50, and 100 ng/rat). A one-way ANOVA and post hoc analysis revealed that the effects of 8-OH-DPAT were significant in all cases, except for highest dose used. 8-OH-DPAT decreased OAT% [F (4,30) = 5.71, P < 0.05] and OAE% [F (4,30) = 5.96, P < 0.05], but no significant change in the LMA [F (4,30) = 1.389, P > 0.05] was observed. The data indicates the induction of anxiogenic response following the administration of 8-OH-DPAT.

However, rats infused intra-MPFC of NAN-190 (0.25, 0.5, and 1 μg/rat) showed significant increase in OAT% [F (3,24) = 5.354, P > 0.05] and OAE% [F (3,24) = 4.481, P < 0.05] at all doses, but no significant change in the LMA [F (3,24) = 1.011, P > 0.05] was observed. The data indicates the induction of anxiolytic response following the injection of NAN-190 (Fig. 2, right panel).

Fig. 3 (left panel) shows the effects of intra-MPFC injections of CGS-12066A (0.25, 0.5, and 1 μg/rat). A one-way ANOVA and post hoc analysis revealed that CGS-12066A decreased OAT% [F (3,24) = 7.121, P < 0.05] and OAE% [F (3,24) = 6.199, P < 0.05] at the
Fig. 2. Effects of intra-MPFC injection of 5HT1A receptor agonist or antagonist on anxiety. Rats were treated with saline (1 μl/rat); 8-OH-DPAT (5, 10, 50, and 100 ng/rat) or NAN-190 (0.25, 0.5, and 1 μg/rat) in left and right panels. Each bar represents mean±S.E.M. (N=7) of OAT% (A), OAEX (B) or locomotor activity (C). Significant differences: *P<0.05, and **P<0.01 compared to the saline rats.

Fig. 3. Effects of intra-MPFC injection of 5HT1B receptor agonist or antagonist on anxiety. Rats were injected with saline (1 μl/rat); CGS-12066A (0.25, 0.5, and 1 μg/rat) or SB-224289 (0.5, 1, and 2 μg/rat) in left and right panels. Each bar represents mean±S.E.M. (N=7) of OAT% (A), OAEX (B) or locomotor activity (C). Significant differences: *P<0.05, and **P<0.01 compared to the vehicle group.

doses of 0.5 and 1 μg/rat, but no significant change in the LMA [F (3,24) = 1.246, P>0.05] was observed. The data indicates the induction of anxiogenic response following the injection of CGS-12066A.

However, intra-MPFC infusions of SB-224289 (0.5, 1, and 2 μg/rat) has not significant effects on OAT% [F (3,24) = 1.646, P>0.05], OAEX [F (3,24) = 2.243, P>0.05], and LMA [F (3,24) = 1.54, P>0.05] at all doses (Fig. 3, right panel).

Fig. 4 indicates the effects of intra-MPFC administration of 8-OH-DPAT (5, 10, and 50 ng/rat) alone or in combination with NAN-190 (0.5 μg/rat) on anxiety parameters in the EPM. Two-way ANOVA indicated an interaction between most effective dose of NAN-190 (Factor B) and 8-OH-DPAT (Factor A) on OAT% [Factor A; F (3,48) = 4.82, P<0.05, Factor B; F (1,48) = 6.16, P<0.05, Factor (A x B); F (3,48) = 4.25, P<0.05], OAEX [Factor A; F (3,48) = 5.261, P<0.05, Factor B; F (1,60) = 6.161, P<0.05, Factor (A x B); F (4,60) = 5.696, P<0.05]. No significant interaction indicated on LMA [Factor A; F (3,48) = 0.765, P>0.05, Factor B; F (1,48) = 1.226, P>0.05, Factor (A x B); F (3,48) = 0.774, P>0.05].

Our results demonstrate that 5HT1A- and 5HT1B-Rs of the MPFC play an active role in modulating anxiety behaviors. 5HT1A- and 5HT1B-Rs are localized both pre- and post-synaptically in Rat brain. Presynaptic receptors are mostly located in dorsal and medial raphe nuclei on serotonergic neurons, whereas postsynaptic 5HT1A-Rs are mainly found on the glutamatergic and γ-aminobutyric acid (GABA)-ergic pyramidal neurons. Furthermore, animal studies have proven the modulatory effects of postsynaptic receptors on glutamatergic, GABAergic, and dopaminergic neurons, especially in the PFC and limbic areas [3,12,29,31].
One of the possible explanations of these data is that activation of postsynaptic 5HT1A-Rs will induce inhibition in neurons of different neurotransmitter systems, including GABAergic interneurons [5]. 5HT1A-Rs were localized to pyramidal neurons in the MPFC [12]. Activation of 5HT1A-Rs in MPFC inhibits cortical glutamatergic activity and 5HT1A-R agonist 8-OH-DPAT mimics the inhibitory effects of serotonin on the pyramidal cells [4]. Moreover, studies conducted on the 5HT1A receptor null mouse showed an increased c-Fos expression and also more excitation activity in the MPFC. Therefore, serotonin and its analogues can inhibit excitatory signal through modifying the function of 5HT1A-Rs located on MPFC pyramidal neurons [7,33].

Another possible explanation of our findings is that there are different communications between various regions of the brain involving in anxiety, the hypothesis which previous studies proposed it. For instance, connections between amygdala and MPFC play an important role in regulating anxiety behaviors and since amygdala is a downstream structure of MPFC which is involved in emotional regulation [2], it is reasonable to suppose that the connections between these regions may act as a bridge, through which the MPFC functions can actively regulate the different functions of amygdala. Therefore, across the whole brain, anxiety levels exclusively predict the functional connectivity between the amygdala and MPFC [2,16]. Different studies clearly demonstrated that there are anatomical pathways between the MPFC and the raphe nuclei and the dorsal raphe nucleus [16,21], for example glutamatergic pathway originates in the MPFC and projects to the raphe nuclei [32]. Since previous studies showed that 5HT1A-Rs are involved in GABA neurotransmission in the MPFC, the GABAergic pathways originate in the MPFC may be involved in the control of anxiety [7].

In the terminal areas of serotonergic neurons, 5HT1B-Rs were identified as inhibitory auto-receptors [5], as postsynaptically located 5HT1B-Rs inhibit the release of some neurotransmitters involving in the modulation of anxiety, such as 5HT, acetylcholine, glutamate and GABA in the cortex [17,30]. Together with serotonergic transporters, 5HT1A- and 5HT1B-Rs regulate the fine-tuning of the electrical activity of the 5HT neurons and the release of 5HT [14]. Therefore, 5HT1B auto-receptors constitute a negative feedback system regulating the 5HT neurotransmission based upon the local conditions at the site of release. Previous studies have reported that 5HT1B-R agonist exerts an anxiolytic-like effect as measured with separation-induced ultrasonic vocalization test [13], and also an anxiogenic effect in the EPM [26]. A few studies have described anxiolytic effects of 5HT1B-R antagonists. However, our results indicate that the injection of 5HT1B antagonist into the MPFC region produces only partial and non-significant effects on anxiety, while 5HT1B agonist clearly affected anxiety behaviors. Since the application of 5HT1B-R antagonist was ineffective, and its agonist effectively showed anxiogenic response, it is reasonable to suggest that there is no active anxiety endogenous 5HT1B-R activity in MPFC.

The EPM test is based on the propensity of rats to show their avoidance of heights and open spaces. Thus, rats tend to avoid the open arms and stay more on the enclosed arms, while in the present study the control rats spent approximately equal amounts of time in both the open and closed arms of the maze or presented quite the same number of entries into the open and closed arms. A possible reason for this discrepancy could be attributed to the experimental conditions used in our study such as the placement of the EPM in the center of a quiet and dimly lit room and placement of the rats at the center of the EPM facing one open arm, while usually the animals are placed facing one of the closed arms. Considering that, all experiments were performed in a similar condition for the control and drug groups. Therefore, the results of this study show a moderate level of anxiety so that the observed drug-induced effects could not occur if the aversive state or open/closed arms conflict generated by the experimental conditions of the study were more intense.

In conclusion, our data showed that stimulation of 5HT1A- and 5HT1B-Rs of the MPFC modulates anxiogenic-like behaviors in the EPM test in rats.

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References


