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RESEARCH ARTICLE

Gonadotropin Releasing Hormone antagonist attenuates antidepressant-like effect of fluoxetine in mice against CRF-induced depression.

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Abstract

The present study was designed to study the influence of gonadotropin releasing hormone (GnRH) receptor antagonist (antide) on antidepressant-like effect of fluoxetine in mice in which depression like effect was induced by i.c.v. administered corticotrophin releasing factor (CRF), and assessed by using forced swim test. CRF (0.1 and 0.3 nmol/mouse) administration increased immobility time indicating depression-like effect, and 15 min prior treatment with fluoxetine (10 and 15 mg/kg, s.c.) was found to attenuate the same. However, this attenuation by fluoxetine pre treatment was not evident in a group which received GnRH antagonist – antide (10 nmol/mouse, i.c.v.) 10 min prior to fluoxetine. These observations point towards the possible role of GnRH in antidepressant –like effect of fluoxetine.

1. Introduction

Fluoxetine, a selective serotonin reuptake inhibitor (SSRI) is clinically used for the treatment of major depressant, obsessive–compulsive and panic disorder. In vitro studies have shown that, serotonin stimulates the release of Gonadotropin Releasing Hormone (GnRH) (Meyer *et al.*, 1992; Vitale *et al.*, 1986). Similarly, treatment with amitriptyline — a non-selective monoamine reuptake inhibitor is reported to increase the immuno-reactivity of GnRH neurons in rat brain (Jain and Subhedar, 1993). Cell line studies demonstrated the release of GnRH from GT-1 cells on exposure to 5-hydroxytryptamine-1A/7 (5-$HT_{1A/7}$) receptors agonist [8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT)] (Hery *et al.*, 1997). Earlier studies have shown that GnRH receptor agonist attenuates depressant and anxiogenic-like effect of CRF (Umathe *et al.*, 2008).

Electrophysiologically, GnRH has been shown to alter the firing rate in different sites; neurones in the hippocampus, septum, arcuate nucleus, hypophysiotrophic, cortical and cortico-spinal regions are either stimulated or inhibited by GnRH (Herbison *et al.*, 1984; Palovcik and Phillips, 1986; Phillis and Kirkpatrick, 1980). Incidentally, GnRH receptors have been identified in various regions of the brain that relates with anxiety, depression, obsessive-compulsion and schizophrenia like disorders (Jennes *et al.*, 1988; Rance *et al.*, 1994; Reubi *et al.*, 1987; Reubi and Maurer 1984; Shin and Liberzon, 2010; Aouizerate *et al.*, 2004). The limbic system is known to regulate the mood and behavior and also serves as a site for the action of antidepressant drugs (Contreras *et al.*, 1989, 1990; Horovitz 1965; Huang 1979).

Literature documents that anxiety, depression and immunosuppression are the charactorestic features of stress, and also evident on CRF administration (Irwin, 1993; Todorovic *et al.*, 2005). Therefore, CRF is considered to play a pivotal role in stress manifestation. Further, GnRH is known to positively regulate CRF binding protein (Westphal and Seasholtz, 2005). CRF binds with this protein with equal or greater affinity than its receptors, and therefore this protein is considered as an important modulator of CRF activity (Sutton *et al.*, 1995).

Incidentally, fluoxetine is shown to inhibit corticotropin-releasing factor (CRF)-induced behavioural responses in rats (Lowry *et al.*, 2009).

These evidences indicate an interplay between serotonergic system, GnRH and CRF in the expression and control of depressant disorder. It is possible that fluoxetine induced synaptic rise in serotonin content might cause the release of GnRH which oppose the behavioural effects of CRF by enhancing the expression of CRF binding protein. To support such possibility we tested the effect of GnRH antagonist on earlier reported attenuation of CRF induced depression by fluoxetine. This study was conducted in mice and depression was assessed using forced swimming test.

2. Materials and Methods

2.1 Animals

The studies were carried out in adult male albino Swiss mice (22–25 g), group housed (n=6), under a standard 12 h light/dark cycle and controlled conditions of temperature (25±2°C) and humidity (55±2%) maintained in animal house facility of Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur from a stock originally purchased from National Institute of Nutrition, Hyderabad, India. They received standard rodent chow (Goldmohar brand, Lipton India Ltd.) and water *ad libitum*. The experiments were carried between 9.00 to 14.00 h in a noise-free room. Separate groups (n=6) of mice were used for each set of experiments and each animal was used only once. The experimental procedures were in strict accordance with the guidelines approved by the Institutional Animal Ethics Committee constituted for the purpose of control and supervision of experimental animals under Ministry of Environment and Forests, Government of India, New Delhi, India.

2.2 Drugs

Fluoxetine was received as a gift from Reliance Laboratories Ltd., India, for research purpose. CRF and antide were purchased from Sigma-Aldrich Ltd., USA. Fluoxetine was dissolved in 0.9% saline, whereas CRF and antide were dissolved in artificial cerebrospinal fluid aCSF having composition 0.2 M NaCl, 0.02 M NaH2CO3, 2 mM KCl, 0.5 mM KH2PO4, 1.2 mM CaCl2, 1.8 mM MgCl2, 0.5 mM Na2SO4, and 5.8 mM D-glucose. The selection of the doses was based on our preliminary observations, and the previous reports (Matsumoto *et al.*, 1997; Pelleymounter *et al.*, 2004; Umathe *et al.*, 2008; Uday *et al.*, 2007).

2.3 Intracerebroventricular (i.c.v.) injection

The *i.c.v.* cannulation was carried out as described earlier (Umathe *et al.*, 2008). In brief, mice were anesthetized with ketamine (100 mg/kg, i.m.) and xylazine (5 mg/kg, i.m.) combination. A guide cannula (24 gauge) was stereotaxically implanted with the stereotaxic coordinates from Paxinos and Franklin (AP -0.82 mm; ML +1.5 mm and DV +2.0 mm; related to bregma). The guide cannula was secured to the skull using mounting screws and dental cement (Dental Products of India, Mumbai, India). A stainless steel dummy cannula was used to occlude the guide cannula when not in use. The animals were then allowed to recover for a week under the cover of cefotaxim (50 mg/kg, *s.c.*), during which they were habituated to the experimental protocols to minimize nonspecific stress. Injections were made using a Hamilton microliter syringe (Hamilton, Nevada, USA) connected to internal cannula (24 gauge) by polyethylene tubing and a volume of 2.0 µl was administered over a period of 1 min into the right lateral ventricle. The injection cannula was left in place for further 1 min before being slowly withdrawn to avoid back flow. At the end of all *i.c.v.* experiments, dilute India ink was injected (2 µl, *i.c.v.*) and animals were killed immediately. Only 5–10% of the mice were eliminated because of inaccurate cannula placement or injection leakage.

2.4 Forced swim test(FST)

Forced swim test was carried out by a method described earlier (Porsolt *et al.*, 1977). Mice were placed for 6 min in a glass cylinder (height: 35 cm; diameter: 17 cm) filled with water (25 ± 1°C) to a depth of 25 cm. The water depth was adjusted so that the animals must swim or float without their hind limbs or tail touching the bottom. During testing, individual mouse was placed in the cylinder for 6 min, and the duration of immobility was scored. As suggested by Porsolt, only the data scored during the last 4 min were analyzed and presented.

2.5 Treatments

aCSF (2µl/mouse) or antide (10 nmol / mouse) was administered by intracerebroventric-

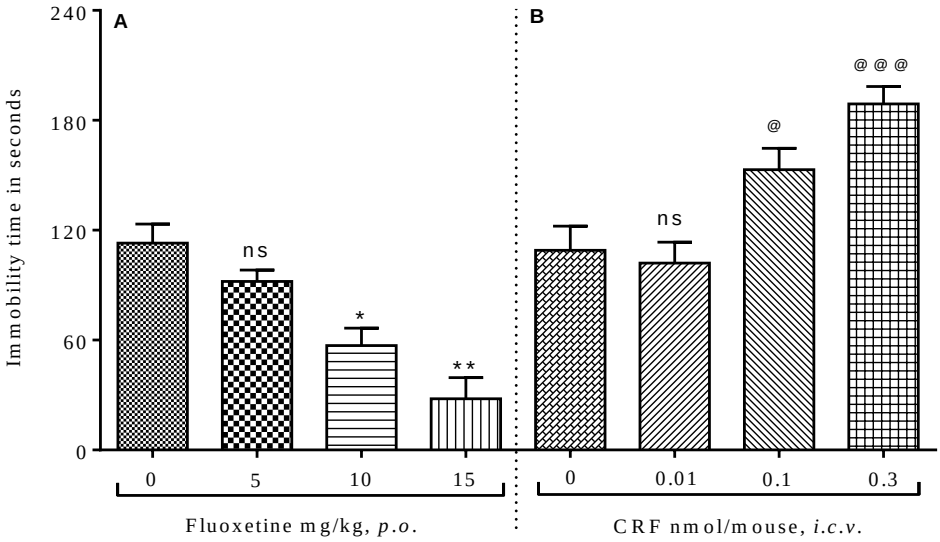

Fig.1: Dose response of fluoxetine and CRF in forced swim test- A: immobility time after *p.o.* administration of fluoxetine (5, 10, 15 mg/kg, *p.o.*) and B: immobility time after *i.c.v.* administration of CRF (0.01, 0.1, or 0.3 nmol/mouse). Each bar represents Mean ± S.E.M. of 6 observations. * $P < 0.01$, ** $P < 0.001$, $^{@}$ $P < 0.05$, $^{@@@}$ $P < 0.001$vs respective vehicle treatments.

-ular route 10 min prior to the subcutaneous administration of 0.9 % saline (10ml/kg) or fluoxetine (5, 10, 15 mg/kg, *s.c.*), 15 min followed by aCSF (2µl/mouse) or CRF (0.01, 0.1, or 0.3 nmol/mouse) intracerebroventricular administration. 5 min after last treatment mice were subjected for FST.

3. Results

Fluoxetine has shown its obvious antidepressant-like effect (Fig.1A), whereas CRF has shown depressant-like effect (Fig.1B) in forced swim test. One-way ANOVA followed by dunnett's multiple comparison test revealed that acute administration of fluoxetine (5, 10, 15 mg/kg, *s.c.*) dose dependently decreases the immobility time as compared to vehicle control where effects of fluoxetine 10 and 15 mg/kg, *s.c.* were statistically significant [$F (3, 20) = 15.27$, $P < 0.0001$]. On the other hand acute administration of CRF (0.01, 0.1, or 0.3 nmol/mouse, *i.c.v.*) significantly increased immobility time [$F (3, 20) = 12.40$, $P < 0.0001$]. Lower doses of fluoxetine (5 mg/kg, *s.c.*) and CRF (0.01 nmol/mouse, *i.c.v.*) were devoid of any effect ($P > 0.05$).

Fluoxetine has attenuated the CRF induced depressant-like effect in forced swim test (Fig.2). One-way ANOVA followed by dunnett's multiple comparison test revealed that CRF (0.3 nmol/mouse, *i.c.v.*) has significantly increased the immobility time as compared to vehicle treated group, whereas fluoxetine (10, 15 mg/kg, *s.c.*) significantly reduced the immobility time in CRF treated group when compared to the respective vehicle treated group [$F (3, 20) = 6.248$, $P < 0.05$].

Treatment of antide-GnRH receptor antagonist (10 nmol/mouse, *i.c.v.*) blocked the antidepressant-like effect of fluoxetine in CRF induced depressant-like effect in forced swim test (Fig.2). One-way ANOVA followed by dunnett's multiple comparison test revealed that fluoxetine (10, 15 mg/kg, *s.c.*) was unable to reduce immobility time in all the tested doses [$F (3, 20) = 0.4037$, $P > 0.05$].

4. Discussion

The present investigations demonstrated that *i.c.v.* administration of GnRH antagonist attenuated the antidepressant-like effect of fluoxetine against CRF-induced depression-like behaviour in mice. In accordance with the earlier reports, *i.c.v.* administration of CRF exhibited depressant-like behaviour (Swiergiel *et al.*, 2007) and fluoxetine attenuated this effect of CRF (Lowry *et al.*, 2009). However, the ability of fluoxetine to attenuate CRF induced increase in immobility time was not evident in GnRH antagonist pre-treated group. Further it was evident that antide had no per se effect on immobility time. This indicates that GnRH is probably involved in the antidepressant effect of fluoxetine. These observations corroborate well

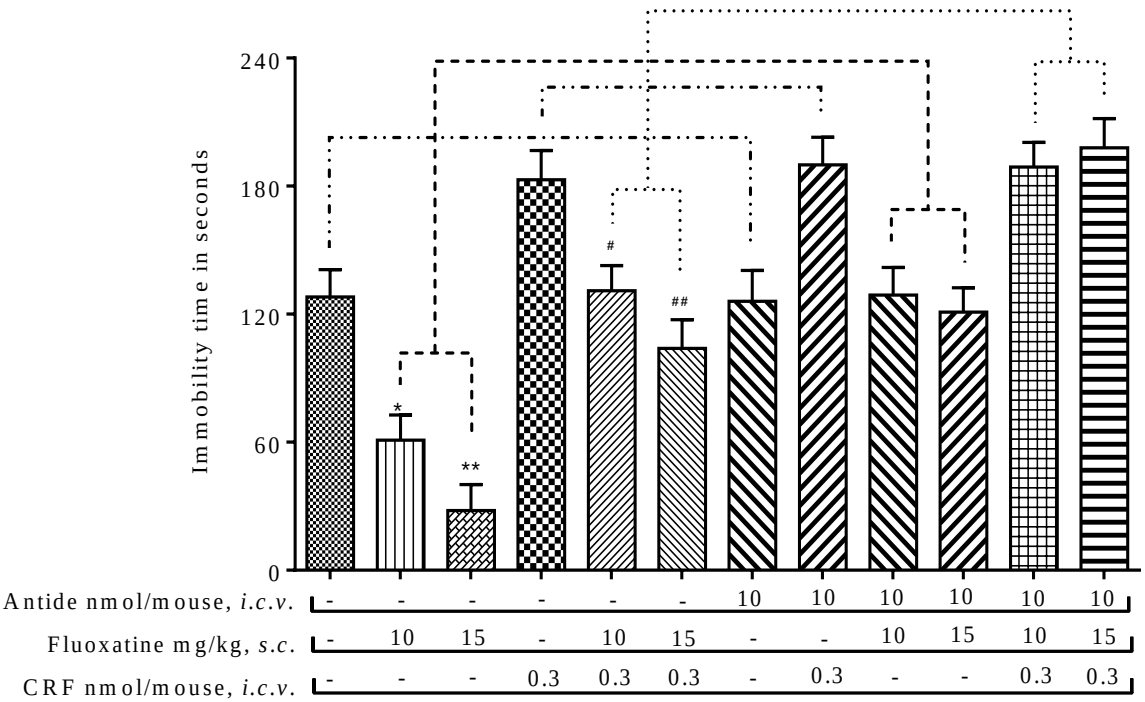

Fig.2 Influence of GnRH antagonist (antide) on antidepressant-like effect of fluoxetine against CRF induced dipression-like effect in forced swim test. Each bar represents Mean ± S.E.M. of 6 observations. * $P < 0.01$, ** $P < 0.001$ vs vehicle control; $^{\#}P < 0.01$, $^{\#\#}P < 0.001$; $^{\#}P < 0.001$vs vs CRF (0.3 nmol/mouse, *i.c.v.*) treatment. (------): fluoxetine (10 and 15 mg/kg, s.c.) vs antide (10 nmol/mouse, *i.c.v.*) + fluoxetine (10 and 15 mg/kg, s.c.), (.....): fluoxetine (10 and 15 mg/kg, s.c.) + CRF (0.3nmol/mouse, *i.c.v.*) vs antide (10 nmol/mouse, *i.c.v.*) + fluoxetine (10 and 15 mg/kg, s.c.) + CRF (0.3nmol/mouse, *i.c.v.*), (-..-..-): no *per se* effect of antide

with the earlier report that pre-treatment with GnRH antagonist attenyates fluoxetine induced decrease in marble burying behaviour in mouse (Uday *et al.*, 2007).

It is well demonstrated that pre-treatment with CRF antagonist decreases immobility time in forced swimming test, the immobility is attributed to CRF, which is a stress hormone. Therefore, in the present study we administered CRF *i.c.v.* to induce depression which was probably in addition to the depression caused by endogenous CRF. It is known that CRF inhibits the release of GnRH and vice-versa. Probably therefore qualitatively opposite behavioural effects of GnRH and CRF are well demonstrated in experimental animals. In the present study we did not observe any per se effect of antide, which suggested that endogenous GnRH was unavailable as probably inhibited by CRF released due to forced swimming. Therefore, to decrease the influence of CRF in stressful condition in experimental animals either GnRH itself is to be administered *i.c.v.* or a treatment that can cause the release of endogenous GnRH is indicated. Earlier it has been shown that fluoxetine treatment increases immobility time. In the present study we categorically observed that fluoxetine pre-treatment not only shown antidepressant effect in control animal but also attenuated CRF-induced increase in immobility time. Incidentally, fluoxetine is shown to inhibit corticotropin-releasing factor (CRF)-induced behavioural responses in rats (Lowry *et al.*, 2009).

Incidentally, GnRH receptors have been identified in anterior cingulated cortex, thalamus, amygdala, caudate, putamen and hippocampus (Jennes *et al.*, 1988; Rance *et al.*, 1994; Reubi *et al.*, 1987; Reubi and Maurer 1984), and these areas relate with anxiety, depression, obsessive-compulsion and schizophrenia like disorders (Shin and Liberzon, 2010; Aouizerate *et al.*, 2004). Electrophysiologically, GnRH has been shown to alter the firing rate in different sites; neurones in the hippocampus, septum, arcuate nucleus, hypophysiotrophic, cortical and cortico-spinal regions are either stimulated or inhibited by GnRH (Herbison *et al.*, 1984; Palovcik and Phillips, 1986; Phillis and Kirkpatrick, 1980). Neurones in the preoptic area, organum vasculosum of lamina terminalis (OVLT), and mediobasal hypothalamic regions are inhibited

(Felix and Phillips, 1979; Renaud, 1977). The limbic system is known to regulate the mood and behavior and also serves as a site for the action of antidepressant drugs (Contreras *et al.*, 1989, 1990; Horovitz 1965; Huang 1979). Further, studies conducted largely in human subjects using various psychological tests reported an antidepressant-like effect of GnRH (German and Stampfer, 1979; McAdoo *et al.*, 1978; Soulairac *et al.*, 1983)

Corticotrophin-releasing factor (CRF) is known to produce some imperative characteristic features of stress response like anxiety, depression and immunosuppression (Irwin, 1993; Todorovic *et al.*, 2005). Therefore, CRF is well thought-out to be the key molecule in dealing out the stress-induced responses. Further, GnRH is known to positively regulate CRF binding protein (Westphal and Seasholtz, 2005). CRF binds with this protein with equal or greater affinity than its receptors, and therefore this protein is considered as an important modulator of CRF activity (Sutton *et al.*, 1995). Serotonin is reported to induce the release of GnRH from median eminence (Vitale *et al.*, 1986).

These evidences indicate an interplay between serotonergic system, GnRH and CRF in the expression and control of depressant disorder. Our observations that pretreatment with antide eliminated fluoxetine induced attenuation of CRF mediated depression like behaviour in forced swimming test points towards the possibility that fluoxetine induced synaptic rise in serotonin, and consequent increase in GnRH release and physiological antagonism with CRF could be the possible reason for antidepressant effect of fluoxetine. Further studies that would demonstrate the release of GnRH by fluoxetine treatment in various brain regions that contributes to depression like behaviour may further clear the role of GnRH in the effect of fluoxetine.

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