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Gonadotropin-releasing hormone agonist blocks anxiogenic-like and depressant-like effect of corticotrophin-releasing hormone in mice

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Abstract

Corticotrophin-releasing factor (CRF) is reported to inhibit the release of gonadotropin-releasing hormone (GnRH). In addition to the endocrine effects, GnRH is reported to influence the behavior via its neuronal interactions. We therefore, hypothesized that anxiety and depression produced by CRF could be also subsequent to the decrease in GnRH. To support such possibility, we investigated the influence of GnRH agonists on CRF or CRF antagonist induced changes in social interaction time in social interaction test, and immobility time in forced swim test in mice, as the indices for anxiety and depression, respectively. Results indicated that GnRH agonists [leuprolide (20–80 ng/mouse, i.c.v.), or D-Trp-6-LHRH (40–160 ng/mouse, i.c.v.)] dose dependently increased social interaction time and decreased immobility time indicating anxiolytic- and antidepressant-like effect, respectively. Such effects of GnRH agonists were even evident in castrated mice, which suggest that these effects were unrelated to their endocrine influence. Administration of CRF (0.1 and 0.3 nmol/mouse, i.c.v.) produced just opposite effects as that of GnRH agonist on these parameters. Further, it was seen that pretreatment with leuprolide (10 or 20 ng/mouse, i.c.v.) or D-Trp-6-LHRH (20 or 40 ng/mouse, i.c.v.) dose dependently antagonized the effects of CRF (0.3 nmol/mouse, i.c.v.) in social interaction and forced swim test. CRF antagonist [α-Helical CRF (9–41), (1 or 10 nmol/mouse, i.c.v.)] was found to exhibit anxiolytic- and antidepressant-like effect, and its sub-effective dose (0.1 nmol/mouse, i.c.v.) when administered along with sub-threshold dose of leuprolide (10 ng/mouse, i.c.v.), or D-Trp-6-LHRH (20 ng/mouse, i.c.v.) also produced significant anxiolytic- and antidepressant-like effect. These observations suggest reciprocating role of GnRH in modulating the CRF induced anxiogenic- and depressant-like effects.

Keywords: Luteinizing hormone-releasing hormone; Corticotrophin-releasing factor; HPA axis; HPG axis; Stress; Anxiety; Depression; Leuprolide; D-Trp-6-LHRH; Castration

1. Introduction

Gonadotropin-releasing hormone (GnRH) is mostly known for its endocrine influence on reproductive system. However, some studies in the recent past suggested its additional role in modulating the behavior. In humans, GnRH or its receptor agonist increases alertness, reduces anxiety and fatigue (McAdoo et al., 1978; Soysal et al., 2001), and therefore such agents are clinically used in anxiety related disorders such as obsessive–compulsive disorder, premenstrual dysphoria and postmenopausal depression (West and Hillier, 1994; Eriksson, 2000; Bixo et al., 2001; Breckwoldt and Keck, 2002; Studd, 2006). These evidences point some role of GnRH in the modulation of anxiety.

Studies conducted in human subjects using various psychological tests reported an antidepressant-like effect

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of GnRH (German and Stampfer, 1979; Soulairac et al., 1983). Similar effect has been demonstrated in rats using conditioning and forced swim test (Massol et al., 1989; Jain and Subhedar, 1993). Moreover, GnRH is reported to increase the binding sites of imipramine—a clinically used antidepressant (Bernardi et al., 1990). It is also shown that treatment with antidepressant such as amitriptyline increases the number of GnRH neurons (Jain and Subhedar, 1993). These evidences indicate some ill-defined role of GnRH in depression.

Recent studies demonstrated that GnRH agonist attenuates anxiety related marble-burying behavior (Uday et al., 2007; Umathe et al., 2008b), ethanol-withdrawal induced hyperexcitability (Umathe et al., 2008a), and stress-induced immunosuppression (Bobyntsev and Sever'yanova, 2002; Umathe et al., 2008c). Anxiety, depression, and immunosuppression are some of the imperative characteristic features of stress response to fear provoking stimuli, and corticotrophin-releasing factor (CRF) administration produces similar effects (Irwin, 1993; Todorovic et al., 2005). Therefore, CRF is considered to be the key molecule in processing the stress-induced responses.

Hypothalamic–pituitary–gonadal (HPG) axis plays an interactive role in controlling stress-activated central fear mechanisms (Rubinow et al., 2005; Van Honk et al., 2005; Hermans et al., 2007). Castrated animals exhibit higher levels of anxiety and depression (Bernardi et al., 1990; Maayan et al., 2006), and low levels of GnRH/GnRH mRNA (Rudenstein et al., 1979; Kalra, 1985; Rothfeld et al., 1987; Park et al., 1988; Emanuele et al., 1996). In addition, GnRH positively regulates CRF binding protein (Westphal and Seasholtz, 2005), which binds to CRF with an equal or greater affinity than the CRF receptors, and hence suggested to be an important modulator of CRF activity (Sutton et al., 1995). Moreover, CRF is known to inhibit GnRH release, mRNA expression and transcription (Petraglia et al., 1987; Williams et al., 1990; Chen et al., 1992; Tellam et al., 1998; Kinsey-Jones et al., 2006), and glucocorticoids inhibit the release of luteinizing hormone from pituitary, and secretion of estrogen and progesterone from ovary. These effects are responsible for 'hypothalamic amenorrhea' of stress, which is observed in anxiety and depression (Chrousos et al., 1998).

In view of these evidences, it was contemplated that stress-induced behavioral changes could be also related to inhibition of GnRH by CRF. To support this hypothesis, we studied the influence of GnRH agonists, such as leuprolide and D-Trp-6-LHRH, on CRF induced anxiety and depression in mice by using social interaction and forced swim test, respectively. In addition, the role of peripheral sex hormones in the *per se* behavioral effects of GnRH agonists was studied in castrated mice.

2. Materials and methods

2.1. Animals

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. Subjects were young healthy male Swiss mice (24–28 g), born and reared in the animal house of Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur from a stock originally purchased from National Institute of Nutrition, Hyderabad, India. Mice were group housed (7–9 per cage) in opaque polypropylene cages and maintained at 25 ± 2 °C under 12:12 h light/dark cycle (07:00–19:00 h) with rodent chow (Trimurti Feeds, Nagpur, India) and water *ad libitum*. Animals were naive to drug treatment and experimentation at the beginning of all studies. Each experimental group had a separate set of 7–9 animals.

2.2. Drugs and solutions

Leuprolide acetate, D-Trp-6-LHRH, CRF (human/rat) and CRF antagonist [α-Helical CRF (9–41)] (Sigma Aldrich, St. Louis, USA) was dissolved in artificial cerebrospinal fluid (aCSF) having composition 0.2 M NaCl, 0.02 M NaH_2CO_3, 2 mM KCl, 0.5 mM KH_2PO_4, 1.2 mM $CaCl_2$, 1.8 mM $MgCl_2$, 0.5 mM Na_2SO_4, 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., 2008a).

2.3. Intracerebroventricular (i.c.v.) injection

The i.c.v. cannulation was carried out as described earlier (Umathe et al., 2008a,c). 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 (Plastics One Inc., USA) 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