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Short communication

A possible participation of gonadotropin-releasing hormone in the neuroleptic and cataleptic effect of haloperidol

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ABSTRACT

Haloperidol, an antipsychotic agent, stimulates the release of gonadotropin-releasing hormone (GnRH), and this hormone is known to mimic some of the behavioral effects of haloperidol. Hence, the present study was carried out to find out the contribution of GnRH in the behavioral effects of haloperidol. The studies revealed that haloperidol (0.15, 0.25 and 0.5 mg/kg, i.p.) and leuprolide (GnRH agonist; 50, 100, 200 and 400 μg/kg, s.c.) dose-dependently inhibited conditioned avoidance response (CAR) in male Sprague–Dawley rats. In higher doses, haloperidol (0.5, 1 mg/kg, i.p.) and leuprolide (200, 400 μg/kg, s.c.) produced catalepsy in rats. Co-administration of sub-effective dose of leuprolide (50 or 100 μg/kg, s.c.) and haloperidol (0.15 or 0.5 mg/kg, i.p.) similarly inhibited CAR and induced catalepsy. Pre-treatment of rats with antide (GnRH antagonist; 10 μg/rat, s.c.), attenuated the inhibitory effect of both the agents on CAR; blocked leuprolide-induced catalepsy; and attenuated the intensity and reduced the duration of haloperidol-induced catalepsy. In conclusion, the studies suggest a possible role of GnRH in the neuroleptic and cataleptic effect of haloperidol.

1. Introduction

Haloperidol is a widely used antipsychotic agent. The neuroleptic effect and extrapyramidal symptoms of haloperidol are attributed to its ability to block dopamine D2 receptors (Baldessarini et al., 1988; Schlösser et al., 1997). Its interaction with D2 receptor and antagonism with dopamine is well demonstrated in several experimental studies (Arnt, 1982; Yurek and Randall, 1985).

Literature documents that D2 receptors situated proximal to hypothalamus/pituitary exert inhibitory influence on the release of some tropic hormones. Therefore, haloperidol is known to stimulate the release of prolactin from anterior pituitary and gonadotropin-releasing hormone (GnRH) from hypothalamus (Tasaka et al., 1985; Lacau-Mengido et al., 1993; Inoue et al., 1998; Mohan Kumar et al., 1998; Spitzer et al., 1998). While haloperidol-induced increased release of prolactin is known to cause hyperprolactemia, the consequences of increased release of GnRH are not known.

The early studies established a key role of GnRH in endocrine system, while later studies indicated its direct influence on central nervous system. Immunocytochemical studies demonstrated the presence of GnRH and its receptors in various regions of rat brain (Reubi and Maurer, 1984; Leblanc et al., 1988). Moreover, arcuate nucleus projects its neurons to regions such as limbic system, ventral tegmental area, anterior cingulated gyrus, frontal cortex, caudate, putamen and thalamus (Merchenthaler et al., 1984; Jennes et al., 1988).

GnRH as well as GnRH agonists is reported to inhibit conditioned avoidance response (CAR) (Mora et al., 1991, 1998) and produce catalepsy in experimental animals (Kádár et al., 1990, 1992). Pre-treatment with L-DOPA is known to reverse the inhibitory effect of GnRH on CAR (Nasello et al., 1990). Moreover, GnRH has been shown to antagonize amphetamine-induced improvement in acquisition of CAR (Mora and Díaz-Véliz, 1983). Further, GnRH is also reported to inhibit the synthesis and release of dopamine from rat striatal tissues (Wang et al., 1982; Mora et al., 1987). Incidentally, Parkinson's like syndrome is often evident in postmenopausal state (Alexander et al., 2007) that is characterized by low levels of estrogen (Erlik and Judd, 1982) and higher levels of GnRH released from hypothalamus (Kim et al., 2009). These evidences point towards the behavioral effects of GnRH that are probably mediated by its anti-dopaminergic action.

Thus, it was speculated that GnRH released by haloperidol from hypothalamus, may be contributing to its neuroleptic and cataleptic effect. We therefore, tested this possibility by studying the influence of GnRH antagonist pre-treatment on haloperidol-induced inhibition of conditioned avoidance response and catalepsy. The studies were carried out only in male Sprague–Dawley rats to

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avoid inconsistent effects of GnRH arising out of the changes in sex hormone levels (Mora et al., 1983, 1998).

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats (220–250 g) were purchased from National Centre for Laboratory Animal Sciences, Hyderabad, India, and acclimatized to our animal house for at least 10 days prior to the experiments. The animals were housed (n = 2–3) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25 ± 2 °C, 55–65%). Rats received standard rodent chow (Goldmohar brand, Lipton India Ltd.) and water *ad libitum*. The animals were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 8.00 and 15.00 h. Separate group (n = 6 or 10) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), 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 and administration

Leuprolide acetate and antide (GnRH antagonist) were purchased from Sigma–Aldrich Ltd., USA. Haloperidol (Searle, India) was received as a gift. All above drugs were dissolved in 0.9% saline. Drug solutions were freshly prepared, and the selection of the doses was based on the previous reports (Nasello et al., 1990; Kádár et al., 1992; Mora et al., 1998).

Leuprolide and antide were injected by subcutaneous (s.c.) route while haloperidol was intraperitoneally (i.p.) administered. At the most, two injections were given to each rat in both paradigms of CAR and catalepsy. Respective controls were maintained simultaneously by vehicle treatment at specific time intervals to study the effect of multiple injections or repeated handling stress for each experiment.

2.3. Treatments

Leuprolide (50, 100, 200, or 400 μg/kg, s.c.) or haloperidol (0.15, 0.25, 0.5, or 1 mg/kg, i.p.) or leuprolide (50 or 100 μg/kg, s.c.) plus haloperidol (0.15 or 0.5 mg/kg, i.p.) were administered 30 min prior to the assessment of either conditioned avoidance response or catalepsy.

In another set of experiment, GnRH antagonist antide (10 μg/rat, s.c.) was given 30 min prior to the administration of leuprolide (400 μg/kg, s.c.) or haloperidol (0.25, 0.5, or 1 mg/kg, i.p.). Thirty minutes thereafter, rats were subjected to the both the behavioral tests. Alone, antide (10, 20, or 40 μg/rat, s.c.) was administered 30 min prior to the assessment of either conditioned avoidance response or catalepsy.

For each of the above treatment, there was a separate control group, which received vehicle (0.9% saline, 10 ml/kg) by respective routes (s.c. or i.p.) at corresponding time intervals. Separate groups (n = 6–10) of rats were used for each set of experiments.

To avoid the biphasic effect of leuprolide, it was administered only once, and immediately after 30 min the animals were subjected to behavioral tests.

2.4. Conditioned avoidance response (CAR)

The conditioned avoidance response was performed according to the procedure described earlier (Ugale et al., 2004). The conditioning experiments were carried out in a two-way shuttle box (Jumping box, Techno Labs, Lucknow), composed of two stainless steel modular testing units equipped with an 18 bar insulated shock grid floor and buzzer. Electric shocks were provided to the grid floor by a master shock supply. The experiments were carried out in a sound-attenuated chamber with low, indirect incandescent lighting (about 20 lx). Rats were trained individually to move from one compartment of a shuttle box into other upon presentation of the 10 s buzzer tone (conditioned stimulus). If the rat failed to respond, the tone was further conditioned with an unconditioned stimulus in the form of an electric shock (0.5 mA), delivered to the grid floor of the chamber for a period of 10 s. Each animal was subjected to a daily session of 10 trials separated by 20 s inter-trial interval. The trial terminated once the rat has moved into the other compartment during the conditioned stimulus and unconditioned stimulus period. Crossings made during the conditioned stimulus period were recorded as avoidance response and those made during unconditioned stimulus were recorded as escape response. All animals were trained for a week. Only those animals characterized by a high level of avoidance responding (>90%) were used for further experiments. Separate group of trained rats (n = 10 per group) were employed for individual dose effect of the drugs. After treatment, rats were placed individually in the shuttle box for the standard 10 trial session of CAR. The results were expressed as number of trials avoided. The observations were made by a trained experimenter who was unaware to the treatments given.

2.5. Catalepsy

Catalepsy is defined as the long-term maintenance of an animal in an externally imposed abnormal posture. The severity of catalepsy in individual rat was assessed using the bar test. The apparatus consist of a wooden bar of 0.8 cm in diameter placed at a height of 9.0 cm above the tabletop. It was determined by gently placing the forepaws of the rat over a 0.8 cm diameter wooden bar, fixed horizontally at a height of 9.0 cm above the tabletop. The time required in seconds (sec) to bring both the forepaws down by rat to the tabletop was recorded, with maximum cut off time of 300 sec. The catalepsy was recorded at 30, 60, 90, 120, 150, and 180 min or 0.5, 1, 2, 3, 6, and 24 h after administration of drug. The catalepsy test was performed in a sound-attenuated chamber lit with low, indirect incandescent lighting (about 20 lx). In order to normalize the data, duration of catalepsy obtained in seconds was converted into natural logarithms (ln). The behavior of the rat was video recorded by a camera placed 2 m parallel to the horizontal table. A trained experimenter analyzed the video recordings blind to the given treatments.

2.6. Statistical analysis

The results of CAR were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test or Newman–Keuls test. The data of catalepsy were analyzed using two-way ANOVA followed by Bonferroni test for multiple comparisons. The results are expressed as mean ± SEM of 6–10 animals. $P < 0.05$ was considered to be statistically significant in all the cases.

3. Results

3.1. Effect of haloperidol and GnRH agonist on CAR

As shown in Fig. 1A, acute administration of haloperidol (0.15, 0.25 and 0.5 mg/kg, i.p.), dose-dependently produced suppression of conditioned avoidance response. One-way ANOVA followed by