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Immunohistochemical evidence for the involvement of gonadotropin releasing hormone in neuroleptic and cataleptic effects of haloperidol in mice

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ABSTRACT

Blockade of dopamine D2 receptor by haloperidol is attributed for neuroleptic and cataleptic effects; and also for the release of gonadotropin releasing hormone (GnRH) from the hypothalamus. GnRH agonist is reported to exhibit similar behavioural effects as that of haloperidol, and pre-treatment with GnRH antagonist is shown to attenuate the effects of haloperidol, suggesting a possibility that GnRH might mediate the effects of haloperidol. To substantiate such possibility, the influence of haloperidol on GnRH immunoreactivity (GnRH-ir) in the brain was studied in vehicle/antide pre-treated mice by peroxidase–antiperoxidase method. Initially, an earlier reported antide–haloperidol interaction in rat was confirmed in mice, wherein haloperidol (250 μg/kg, i.p.) exhibited suppression of conditioned avoidance response (CAR) on two-way shuttle box, and induced catalepsy in bar test; and pre-treatment with antide (50 μg/kg, s.c., GnRH antagonist) attenuated both effects of haloperidol. Immunohistochemical study was carried out to identify GnRH-ir in the brain, isolated 1 h after haloperidol treatment to mice pre-treated with vehicle/antide. The morphometric analysis of microphotographs of brain sections revealed that haloperidol treatment increased integrated density units of GnRH-ir in various regions of the limbic system. Considering basal GnRH-ir in vehicle treated group as 100%, the increase in GnRH-ir after haloperidol treatment was by 100.98% in the medial septum; 54.26% in the bed nucleus of the stria terminalis; 1152.85% in the anteroventral periventricular nucleus; 120.79% in the preoptic area–organum vasculosum of the lamina terminalis and 138.82% in the arcuate nucleus. Antide did not influence basal and haloperidol induced increase in GnRH-ir in any of the regions.

As significant increase in GnRH-ir after haloperidol treatment was observed in such regions of the brain which are reported to directly or indirectly communicate with the hippocampus and basal ganglia, the regions respectively responsible for neuroleptic and cataleptic effects; and as GnRH antagonist eliminated the effects of haloperidol without affecting GnRH-ir, it appears that GnRH released by haloperidol mediates its neuroleptic and cataleptic effects.

1. Introduction

Haloperidol (HAL) is a clinically well proven antipsychotic agent and its prolong use is associated with extra pyramidal side effects (Moleman et al., 1982; Baldessarini et al., 1988; Schlosser et al., 1997). These effects of HAL are consequent to dopamine D2 receptor blockade. These receptors have inhibitory control over the release of prolactin from the pituitary and GnRH from the hypothalamic nuclei, and hence HAL is clinically known to produce hyperprolactinemia (Ben-Jonathan and Hnasko, 2001; David et al., 2000), and experimentally shown to produce lordosis in ovariectomised animals (Pednekar and Mascarenhas, 1993).

Our earlier studies in rat demonstrated that GnRH antagonist attenuated neuroleptic and cataleptic effects of HAL (Umathe et al., 2009). Direct infusion of GnRH into the lateral ventricles, hippocampus, NAc is reported to impair acquisition of conditioned avoidance performance in 15 min, and its infusion in the striatum is shown to produce similar inhibition of CAR immediately after administration (Mora et al., 1991). Similarly i.c.v. administration of GnRH is reported to induce catalepsy in rats (Kádár et al., 1990, 1992; Umathe et al., 2009). Moreover, GnRH has been shown to antagonise amphetamine-induced improvement in acquisition of CAR (Mora and Diaz-Veliz, 1983), and pre-treatment with L-DOPA is shown to reverse inhibitory effect of GnRH on CAR (Nasello et al., 1990). Parkinson's like syndrome is often evident in postmenopausal state (Alexander et al., 2007) and this state is characteristically having higher release of GnRH and low levels of oestrogens in the blood (Kim et al., 2009; Erlik et al., 1982). In addition, in vitro studies demonstrated

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the inhibitory action of GnRH on the synthesis and release of dopamine in the corpus striatum (Wang et al., 1982a, 1982b; Mora et al., 1987).

Autoradiographic studies demonstrated rich population of GnRH receptors in the hippocampus and various components of the basal ganglia (Jennes et al., 1988), the areas which respectively relate with conditioned avoidance response and coordination of motor activities (Gralewicz, 1976; Lipska et al., 1995). GnRH has been shown to alter neuronal firing rate (Herbison et al., 1984; Palovcik and Phillips, 1986; Phillis and Kirkpatrick, 1980). In view of these reports it is speculated that the behavioural effects of HAL can also be attributed to GnRH, released by it. To substantiate such possibility, it was proposed to investigate GnRH immunoreactivity after HAL treatment to vehicle/antide, a GnRH antagonist, pre-treated mice. To correlate the changes in GnRH immunoreactivity with neuroleptic and cataleptic effects of HAL, these behavioural parameters were also studied by using two-way shuttle box and bar test.

2. Materials and methods

2.1. Subjects

Adult male Swiss albino mice (22–25 g) were purchased from the National Centre for Laboratory Animal Sciences, Hyderabad, India, and acclimatised to our animal house for at least 10 days prior to the experiments. The animals were housed ($n = 6$) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25 ± 2 °C, 55–65%). Mice received standard rodent chow (Gold Mohar brand, Lipton India Ltd.) and water ad libitum. All the experiments were carried out in a noise-free room between 9.00 and 13.00 h. The study was carried out only in male mice as females have fluctuating hormonal levels due to their oestrus cycle. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals under the Ministry of Environment and Forests, Government of India, New Delhi, India.

2.2. Drugs, groups and treatments

Haloperidol (Searle, India) was received as a gift and antide (GnRH antagonist) was purchased from Sigma-Aldrich Ltd., USA. Drugs were dissolved in 0.9% saline. Drug solutions were freshly prepared. For dose dependent study, HAL (62.5, 125, 250 μg/kg)/vehicle (10 ml/kg) was administered by intraperitoneal (i.p.) route. Another group of animal received antide (50 μg/kg)/vehicle (10 ml/kg) subcutaneously (s.c.) 30 min prior to HAL/vehicle treatment. They were divided into three groups; one group each ($n = 6$) was used for respectively studying CAR, catalepsy and GnRH-ir by immunohistochemistry. Selection of doses was based on the previous reports (Britton et al., 1992; Kobayashi et al., 1997; Umathe et al., 2009).

2.3. Conditioned avoidance response (CAR)

The conditioned avoidance response was performed according to the procedure described earlier (Bianchi and Marazzi-Uberti, 1969) with few modifications. The conditioning experiments were carried out in a two-way shuttle box made of opaque Plexiglas ($40 \times 10 \times 20$ cm) having a partition with a small opening at the floor level and a transparent ceiling with two 10-Watt lamps. The floor is made of 2 mm stainless steel rods and 10 mm apart connected to a master shock supply, and was free to tilt on its short axis. The experiments were carried out in a sound-attenuated chamber with low, indirect incandescent lighting (about 20 lx). Mice were trained individually to move from one compartment of a shuttle box to the other upon presentation of conditioned stimulus (lighting the lamps located on the ceiling) for 10 s, and during the last 3 s of the conditioned stimulus the grid floor was electrified with 88 V and 0.16 mA current (unconditioned stimulus). The mouse escaping in the second compartment of the cage could interrupt the illumination of the cage and prevent the foot shock, as the tilting floor was connected to a micro-switch governing the electric supply of the cage. Crossings made during the conditioned stimulus period were recorded as avoidance response and those made during unconditioned stimulus were recorded as escape response. Each animal was subjected to a daily session of 20 trials separated by 20 s inter-trial interval. All animals were trained for a week. Only those animals characterised by a high level of avoidance responding (>90%) were used for further experiments. The results are expressed as percent of trials avoided and percent of escape failure. The observations were made by a trained experimenter who was unaware of the treatments given.

2.4. Catalepsy

Catalepsy is defined as the long-term maintenance of an externally imposed abnormal posture in experimental animals. The severity of catalepsy in individual mouse was assessed using the bar test. The apparatus consists of a wooden bar of 0.8 cm in diameter placed at a height of 3.0 cm above the tabletop. It was determined by gently placing the forepaws of the mouse over the wooden bar, and fixed horizontally at a height of 3.0 cm above the tabletop. The time required in seconds (s) to bring both forepaws down by a mouse to the tabletop was recorded, with maximum cutoff time of 300 s. The catalepsy was recorded at 15, 30, 60, 90, 120, 150 and 180 min after administration of drugs. Test was performed in a sound-attenuated chamber lit with low, indirect incandescent lighting (about 20 lx). In order to normalise the data, duration of catalepsy obtained in seconds was converted into natural logarithms (ln). A trained person who conducted the experiment was blind to the given treatments.

2.5. Immunohistochemistry (IHC)

After 1 h of the last treatment, animals were anesthetised with subcutaneous administration of ketamine 80 mg/kg–xylazine 10 mg/kg cocktail, and slowly perfused transcardially with heparinised phosphate-buffered saline (PBS; pH 7.4) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 8–10 min. Following the completion of the perfusion, the brain was carefully isolated and stored in the perfusion fixative at 4 °C for a minimum of 24 h followed by cryoprotection at 4 °C in 30% sucrose solution till the brain sinks to the bottom. The brains were then embedded in 15% polyvinylpyrrolidone (PVP) at −28 °C and the serial sections were collected on a cryostat at 30 μm thickness in coronal plane.

The sections from all the groups were processed at the same time to ensure uniformity of immunostaining. Brain sections were washed three times in 0.1 M PBS (pH 7.4), and then treated with 0.1% Triton X 100 for 20 min. Further, sections were incubated in blocking solution (5% bovine serum albumin, Sigma-Aldrich, USA., in 0.1 M PBS, pH 7.4) for 30 min and were then incubated with rabbit anti-GnRH antibodies (G8294-Sigma-Aldrich Ltd., USA) at 1:750 dilutions at 4 °C overnight. The following morning, the sections were rinsed with 0.1 M PBS (pH 7.4) and then treated with a secondary antibody consisting of diluted (1:200) biotinylated mouse anti-rabbit IgG at room temperature for 1 h, followed by ExtrAvidin®-Peroxidase, 1:100 for 1 h (EXTRA3-1KT, ExtrAvidin® Peroxidase Staining Kit for rabbit antibodies, Sigma-Aldrich Ltd., USA). After incubation the sections were stained with AEC staining kit (AEC101-1KT, Sigma-Aldrich Ltd., USA). Each section was then washed in double distilled water and mounted on slides with glycerol–gelatin mounting medium. All the sections in series were studied under the microscope and the sections that displayed GnRH immunoreactive cells/fibres were photographed using Leica DM2500 microscope. GnRH-ir positive regions were identified by using Paxinos and Franklin's mouse brain atlas (Paxinos and Franklin, 2001).