Galanin regulation of LH release in male rats

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The present study examines the role of cerebroventricular administered (IIIrd ventricle) galanin on LHRH and LH release in adult and immature male rats. In both age groups, galanin stimulated LHRH synthesis and release from the hypothalamus, leading to a higher release of pituitary LH which in turn increased plasma LH levels. Galantide, a galanin receptor blocker, on the other hand, drastically reduced hypothalamic LHRH and plasma LH while increasing pituitary LH. In vitro incubation of anterior pituitary cells with galanin followed by LHRH resulted in increased release of pituitary LH but not by galanin alone. Galantide exhibited no such effect either alone or with LHRH. These results indicate that galanin is an important regulator for both hypothalamic LHRH and hypophysial LH and its role is independent of age in the case of male rats.

Keywords: Galanin, Galantide, LHRH, LH, Neuropeptide

Galanin, a 29/30 amino acid c-terminally amidated neuropeptide was discovered in the year 1983 by Tatemoto et al.1 from porcine intestine and is now known to be widely distributed in the central and peripheral nervous systems of many mammalian species. In the central nervous system (CNS), it is found in the cerebral cortex, nucleus accumbens, striatum, hippocampus, dentate gyrus, hypothalamus, cerebellum, medulla and the dorsal horn of the spinal cord2-4. The highest levels of its synthesis and storage is found within the hypothalamus and the median eminence respectively5. Its presence is also reported in the rat anterior pituitary6,7.

Galanin is usually found coexpressed with some of the brain neurotransmitters and neuropeptides. Among the neurotransmitters to which it is commonly associated are acetylcholine8, serotonin9 and noradrenaline9,10. Some of the neuropeptides with which it is co-localized are growth hormone releasing hormone (GHRH)11, neurotensin, vasopressin12,13 and gonadotropin releasing hormone (GnRH)14.

Luteinizing hormone releasing hormone (LHRH), produced by a network of neurons in the hypothalamus is the primary signal that stimulates the release of the pituitary LH. A pulse generator within the hypothalamus has been shown to be the controlling center responsible for both its basal and cyclic patterns of release15. Of the many excitatory components controlling LHRH secretion via the pulse generator, galanin has been demonstrated to be an important factor because (a) it induces the full expression of the LH surge and the administration of its antiserum or receptor antagonist, galantide significantly blunts the surge16,17, (b) the pulsatile secretion of galanin and LHRH in the portal circulation is nearly coincident18, (c) galanin neurons innervate LHRH cells in the hypothalamus and also co-localise as a subset of LHRH neurons in the preoptic area14, (d) both peptides are seen in the same secretory vesicles in the axonal terminals in the median eminence, and (e) under in vitro conditions also, galanin can stimulate LHRH release from arcuate nucleus-median eminence fragments19.

The above observations suggest that galanin is one of the important hypothalamic factors that control LHRH release from the hypothalamus. The present work, involves a detailed study on changes in hypothalamic LHRH levels by directly injecting galanin in the brain (III ventricle) and evaluating LHRH in the hypothalamic extract. Pituitary and plasma LH levels have also been estimated in order to understand the pathway and mechanism of action of galanin. Both adult and immature male rats have been used to understand at what age galanin or galantide is effective in modulating the hypothalamic LHRH. In vitro studies were also carried out to ascertain its
Materials and Methods

Laboratory bred adult (60–80 days) and immature (21-30 days) male rats of Holtzman strain were used. They were kept in groups of 3-4 individuals per cage under standard conditions of photoperiod (14 h light: 10 h darkness) and temperature (23° ± 2°C) with ad libitum provision of laboratory chow and water. The following in vivo and in vitro experiments were conducted.

In vivo

Effect of galanin and its receptor antagonist, galantide on hypothalamic LHRH, pituitary and plasma LH—Adult and immature male rats were divided in the following 4 groups (4-6 animals per group) and injected with galanin and galantide through cerebroventricular (III ventricle) route using the methods and coordinates of Noble et al.\(^\text{20}\) and Saxena et al.\(^\text{21}\) with slight modifications:

- Gr. I-Control: received only 2 µl saline.
- Gr. II-Galanin: 0.31 nM (in 2 µl saline) in adults and 0.62 nM in immature rats.
- Gr. III-Galantide: 2.5 nM (in 6 µl saline) in both age groups.
- Gr. IV-Galantide and galanin: In this group, the rats were first injected with galantide then 1 h later with galanin. The doses were the same as in separate injections.

The doses of galanin and galantide were same as in the earlier work of Sahu et al.\(^\text{17}\).

Animals were autopsied (by decapitation) after 1 hr for:

1. Blood plasma: Blood was collected in heparinized (0.1% heparine) tubes and centrifuged at 750 rpm for 15 min. Plasma was decanted and stored frozen (at −20°C) in sodium azide rinsed vials for later estimation of LH and
2. Pituitary and hypothalamus: After decapitation, the brain and pituitary gland were dissected out immediately under cold conditions. The pituitaries were acetone dried and stored at −20°C for estimation of pituitary LH. The hypothalamus was also quickly dissected out, acetone dried and stored separately at −20°C for LHRH estimation.

In vitro

Effect of galanin and galantide on pituitary cell response to LHRH—The pituitary cells were isolated as per Weiner et al.\(^\text{22}\) and Saxena and Misra\(^\text{23}\). Cells equivalent to half pituitary (in 0.5 ml suspension) were incubated with galanin (100 nM), galantide (100 nM) and LHRH (2.5 ng) either alone or in combinations. In the combination studies, the anterior pituitary cells were first first incubated with galantide or galanin for an hour, washed twice with HBSS, centrifuged at 750 rpm for 15 min after which the supernatant was discarded and the cells were finally incubated with LHRH for 3 h. All the incubations were done in 2.0 ml of HBSS and incubated at 37°C in a metabolic shaker with 25 cycles/ min. After 3 h of incubation, the contents were centrifuged at 750 rpm for 5 min at 4°C and the supernatant was used for estimation of LH released.

Hormone estimation—Pituitary and plasma LH levels were determined by RIA following the double antibody method described by Niswender et al.\(^\text{24}\). To estimate hypothalamic LHRH, acetone dried hypothalami were homogenized in chilled 0.1N HCl and centrifuged at 12000 rpm for 1 hr at 4°C. The residue was discarded and the pH of the supernatant was adjusted to 7.3 with 5.6% sodium bicarbonate solution. This was used as a semi purified hypothalamic extract. Isolated anterior pituitary cells from adult male rats were incubated in the extract and the LH released into the medium was estimated and plotted on a standard curve drawn by use of different doses of LHRH. The value of LHRH on the standard curve corresponding to the LH release gave the hypothalamic LHRH content.

Statistical analysis—Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test and values of \(P < 0.05\) were taken as significant.

Results

In vivo

Effect of galanin and its receptor antagonist, galantide on hypothalamic LHRH, pituitary and plasma LH—

Hypothalamic LHRH: In adult and immature rats, administration of galanin significantly increased \((P < 0.05)\) hypothalamic LHRH while galantide \((P < 0.05)\) decreased it even lower than the control level (Fig. 1A). In combined treatment (galantide+galanin), the level was not significantly different from galantide treatment.
Pituitary LH: Corresponding to hypothalamic LHRH, the pituitary LH level in both ages decreased significantly ($P < 0.001$) after galanin administration but elevated significantly following galantide treatment (Fig. 1B). In combined treatment, it also increased as that in case of galantide alone.

Plasma LH: In both age groups, galanin administration caused a significant rise ($P < 0.05$) in plasma LH (Fig. 1C). However a drastic decline was seen after galantide treatment which was even lower than in control and galanin treated groups. In combined treatment also it was same as that in case of galantide alone.

In vitro

**Effect of galanin and galantide on pituitary cell response to LHRH**

Isolated anterior pituitary cells (equivalent to half pituitary) taken from adult and immature males were incubated with LHRH, galanin and galantide either singly or in combinations. The LH release into the medium was measured by RIA. In each case duplicate sets of incubation were used.

In both, adult and immature male rats (Fig. 2), incubation of the anterior pituitary cells with galanin or galantide failed to induce any release of LH while incubation with LHRH for 3 h caused a significant release of LH into the medium. Preincubation with galanin for an hour followed by LHRH resulted in a significantly higher ($P < 0.05$) LH release as compared to LHRH alone but prior treatment with galantide followed by LHRH showed no significant change in comparison to LHRH alone.

**Discussion**

Neuropeptides play an important role in the regulation of reproductive events at all levels of the hypothalamic-pituitary-gonadal axis. Their key center for controlling reproduction is the hypothalamus, where a number of peptidergic signals interact to control the release of gonadotropin releasing hormone, GnRH. Galanin has now been shown to have a positive effect on gonadotropin secretion and is considered to be an important neuropeptide for regulation of gonadotropin secretion.

In the present study, cerebroventricular (IIIv) administration of galanin caused a significant elevation in hypothalamic LHRH, a decrease in pituitary LH and a rise in plasma LH levels in both immature and adult male rats. The increased hypothalamic LHRH points towards the possibility
that galanin enhances the rate of synthesis of LHRH far more than its release into the hypophysial circulation. In the preoptic region of the male brain approximately 15-20% of galanin immunoreactive neurons are also immunopositive for LHRH and in the medial preoptic area (MPOA) and diagonal band of broca (DBB), the LHRH cells are surrounded by gal-IR nerve terminals, suggesting the presence of synaptic contacts between LHRH perikarya and gal-IR terminals. These studies show the presence of a link between galanin and LHRH neurons which could be getting further activated on exogenous administration of galanin leading to increased synthesis and release of LHRH. The effective difference in the dose of galanin for significant increase in plasma LH between adult and immature rats is indicative of the possibility that in the pre-pubertal males either the number of functional receptors is lower than the adults or their binding potency is not fully developed. The number and / or sensitivity of the galanin receptors probably increases with the onset of puberty and galanin becomes more effective in stimulating LHRH release as seen in the case of the adults.

On the other hand, galantide has been shown to bind with GalR1 receptor subtypes present in the brain and gut. The present study reveals that its central injection suppressed both LHRH and LH release, indicating that GalR1 could be mediating the galanin induced responses. This is also similar to the work of Sahu et al. on female rats which demonstrated that galantide suppressed both basal and cyclic LH release in female rats by competitively antagonizing galanin`s binding to GalR1 receptors in the brain.

Galanin mRNA in the rat pituitary is far less abundant than in the hypothalamus and its expression is strongly dependent on the circulating levels of estrogens. Apart from increasing LH release by increase in the hypothalamic LHRH, the presence of galanin in the anterior pituitary raises the possibility that it could also be having a local stimulatory effect whereby it enhances greater binding and / or action of LHRH on the gonadotrophs. This possibility was confirmed by the in vitro experiments where a greater LH release by LHRH was noted from the galanin primed pituitary cells in both the age groups (adult and immature) of rats. Therefore the effect of galanin at the level of the pituitary is probably to potentiate the influence of LHRH by enhancing the binding potency of its functional receptors. Galantide, on the other hand failed to suppress the stimulatory action of galanin at the pituitary level. It is therefore possible that another receptor subtype apart from GalR1 may be modulating the stimulation of LH release from the pituitary.

Based on the present results it can be stated that, both immature and adult male rats respond to galanin in a similar manner. The results are substantive of the assumption that prior to sexual maturity also galanin receptors are present and are functional as exhibited by its (galanin) direct influence at the level of the pituitary and the hypothalamus in male rats. At the level of the hypothalamus, it enhances the synthesis and release of LHRH whereas in pituitary it induces increased release of luteinizing hormone and thereby an overall increase in plasma LH is noted.

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References


