Regulatory role of galanin in control of hypothalamic–anterior pituitary function
(stimulation of growth hormone and prolactin/inhibition of thyroid-stimulating hormone/third-ventricular injection/galanin antiserum/ dispersed pituitary cell incubation)

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ABSTRACT The role of the neuropeptide galanin in the regulation of anterior pituitary function was studied in vivo in conscious male rats and in vitro with cultured anterior pituitary cells. Galanin (50–200 ng; 15–60 pmol) injected into the third cerebral ventricle of rats produced highly significant, dose-related increases of plasma growth hormone (GH) concentrations, whereas galanin increased prolactin (PRL) and decreased thyroid-stimulating hormone (TSH) levels only at the highest dose (60 pmol) tested. Intravenous galanin failed to alter PRL and TSH levels in these rats. In contrast with the results with intraventricular injection of the peptide, intravenous injection of 30 or 300 pmol of galanin produced small, brief, dose-related increases in plasma GH. The response to the 300-pmol dose was less than that induced by a factor of 20–lower intraventricular dose, which establishes a central action of galanin. Galanin in concentrations ranging from 1 nM to 1 μM failed to alter significantly GH, PRL, or TSH release from dispersed anterior pituitary cells. It also failed to alter GH secretion in response to 100 nM GH-releasing hormone; however, at this dose galanin did potentiate the effect of 100 nM TSH-releasing hormone on TSH and PRL release. Thus, the effects of third-ventricular injection of the peptide are mediated by the hypothalamus. To determine the physiological significance of galanin in control of pituitary hormone release, highly specific antisera against galanin was injected intraventricularly. Third-ventricular injection of 3 μl of galanin antiserum resulted in a dramatic decrease in plasma GH values as compared with those of normal rabbit serum-injected controls within 15 min, which persisted until the end of the experiment (5 hr postinjection). Galanin antiserum did not decrease plasma PRL or TSH levels at any time period after its third-ventricular injection; however, a transient increase of plasma TSH levels occurred after 30 and 60 min in comparison with TSH levels in normal rabbit serum-injected controls. Since there was no effect of the antiserum on plasma PRL and only a transient elevation in TSH, galanin may not be physiologically significant enough during resting conditions to alter PRL and TSH release in the male rat. The results of the experiments with galanin antiserum indicate that endogenous galanin has a tonic action within the hypothalamus to stimulate GH release. The rapidity of onset of the effects of galanin and the antiserum directed against it suggest that it acts to stimulate release of GH-releasing hormone from periventricular structures, which then stimulates the release of GH.

The gastrointestinal peptide galanin has been isolated from porcine intestinal extracts (1). Galanin has been shown to cause contraction of smooth muscle preparations (1, 2) and to inhibit both substance P- and acetylcholine-induced smooth muscle contraction (2). Furthermore, galanin causes hyperglycemia in dogs via inhibition of insulin release (1, 3).

Galanin is widely distributed in the rat brain and intestine (4–8). In the central nervous system, the hypothalamus is rich in fibers and cell bodies containing galanin-like immunoreactivity, with the highest concentration of galanin being found in the median eminence (6, 8). Recently, receptors for galanin have been shown throughout the rat central nervous system (9, 10). The presence of galanin-like immunoreactivity was established in the cholinergic neurons of the basal forebrain (9). Coexistence of galanin-like immunoreactivity with catecholamines, 5-hydroxytryptamine, γ-aminobutyric acid, and vasopressin has been reported in neurons (11, 12).

The high-density network of galanin-like immunoreactive fibers in the hypothalamus is suggestive that there may be a neuroendocrine role for this peptide. We reported previously that injections of minute doses of galanin into the third ventricle (3V) caused an increase of plasma growth hormone (GH) levels in conscious rats (13). Subsequently, much higher doses were reported to increase plasma prolactin (PRL) following lateral ventricular injection (14).

In the present study, we have further examined the GH-stimulating activity of galanin in vivo by evaluating the effects of intravenous and 3V injections of the peptide on anterior pituitary hormone release in conscious, freely moving male rats. We have also studied the effect of galanin on the release of pituitary hormones by dispersed pituitary cells in vitro. To examine the physiological significance of galanin in control of pituitary hormone secretion, we have evaluated the effects of 3V injections of highly specific antisera directed against galanin on the release of GH, PRL, and thyroid-stimulating hormone (TSH).

EXPERIMENTAL PROCEDURES

Male rats (200–240 g) of the Sprague–Dawley strain were purchased from Sasco (Omaha, NE). Animals were maintained under controlled conditions (23–25°C; lights on at time 0500 to 1900 and provided Rat Chow (British Petroleum, Saint Louis) and water ad libitum.

In Vivo Studies. Intravenous testing. Indwelling, right atrial cannulae were implanted via the external jugular vein; ether was the anesthetic (15). Twenty-four hours later, individually caged, cannulated animals were moved to a quiet laboratory, and extension tubing (30.5-cm, PE-50 tubing, Clay Adams) was attached to the jugular cannula at its exit in the dorsal of the neck. Animals were then left undisturbed for 1 hr. After removal of the zero-time blood sample (0.6 ml), 0.2 ml of isotonic saline (0.9% NaCl) alone or saline containing 30 or

Abbreviations: PRL, prolactin; TSH, thyroid-stimulating hormone; GH, growth hormone; TRH, TSH-releasing hormone; GRH, GH-releasing hormone; NRS, normal rabbit serum; 3V, third ventricle.

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300 pmol of synthetic porcine galanin (Peninsula Laboratories, San Carlos, CA) was injected via the atrial cannula, followed by 0.4 ml of isotonic saline to replace the volume of blood withdrawn. Subsequent samplings (0.6 ml) at 5, 15, 30, and 60 min postinjection were followed by infusion of 0.6 ml of heparinized saline (20 units of heparin per ml). Blood samples were centrifuged at 4°C, and plasma was stored frozen until hormone assays were performed. All experiments were carried out between times 0900 and 1200.

Intraventricular testing. A chronic, indwelling cannula was implanted in the 3V of male rats as described with slight modification (16, 17). One week later a jugular-atrial cannula was implanted (15). Again, 24 hr later, extension cannulae were attached, and the rats were left undisturbed for 1 hr. After removal of the zero-time blood sample (0.6 ml), 2 µl of saline alone or an equal volume of saline containing 15, 30, or 60 pmol of synthetic galanin was injected into the 3V.

In another group of rats, 3 µl of rabbit galanin antiserum (RAS-7153N, Peninsula Laboratories) or normal rabbit serum (NRS) was microinjected into the 3V. Blood samples (0.6 ml) were removed at 5, 15, 30, and 60 min or 1, 2, 3, and 4 hr after the 3V injections. The galanin antiserum had no cross-reactivity to secretin, PHM-27, or vasoactive intestinal polypeptide in RIA (personal communication, G. Chang of Peninsula Laboratories).

Cell Dispersion. Anterior pituitaries removed from male rats after decapitation were minced into small pieces with a razor blade and then dispersed enzymatically by using a solution of 0.1% trypsin (1:250; Difco, Detroit) in minimal essential medium without calcium (GIBCO) containing 0.1% bovine serum albumin (18).

After 2 hr, the cells were collected by centrifugation and resuspended in medium 199 containing 10% (vol/vol) horse serum and 20 mM HEPES (GIBCO). The medium was adjusted to pH 7.2–7.4 with 0.1 M NaOH. The medium also contained 100 units of penicillin and 100 µg of streptomycin per ml. Cells were counted, placed in 12 × 75 mm culture tubes at 5 × 10⁵ cells per tube and cultured overnight at 37°C under an air atmosphere.

The following day, the overnight culture medium was removed, and the cells were resuspended in 1 ml of medium 199 containing 0.1% bovine serum albumin for preincubation for 30 min (37°C) in a Dubnoff metabolic shaker. After removal of preincubation medium, the cells were resuspended in 1 ml of medium 199 plus 0.1% bovine serum albumin alone or in 1 ml of a similar medium containing synthetic porcine galanin at final concentrations of 1 nM to 1 µM. Cells were incubated alone or in combination with various concentrations of galanin with or without 2.5 nM GH-releasing hormone (GRH), 30 nM somatostatin [somatotropin (growth hormone) release-inhibiting hormone (GRIH in Fig. 5), or 70 or 140 nM TSH-releasing hormone (TRH). All peptides were purchased from Peninsula Laboratories. After incubation for 1 hr at 37°C, the cells were pelleted by centrifugation at 25°C for 10 min at 500 × g. The media were stored frozen at −20°C until measurement of hormone content.

Hormone Assays. PRL, TSH, and GH levels in plasma samples and incubation media were determined by RIA as recommended by the National Institute of Diabetes and

![Figure 1](image-url) **Fig. 1.** Effect of 3V injection of galanin (GAL) on plasma GH and PRL concentrations in freely moving male rats. In this and subsequent figures, injection took place just after removal of the initial blood sample. Time is on a nonlinear scale, and values are means ± SEM. *, P < 0.05; **, P < 0.01 (versus normal saline (2 µl)-injected controls). ivtr, Intraventricular.

Table 1. The effect of 3V injection (in 2 µl of 0.9% NaCl) of synthetic porcine galanin on plasma TSH in freely moving male rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose, ng</th>
<th>TSH levels (ng/ml) at indicated minutes postinjection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>275.9 ± 8.9</td>
</tr>
<tr>
<td>Galanin</td>
<td>50</td>
<td>263.5 ± 9.6</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>311.1 ± 8.9</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>292.4 ± 11.1</td>
</tr>
</tbody>
</table>

Values presented are means ± SEM.

*P < 0.05 (versus initial values).

†P < 0.02 (versus initial values).

‡P < 0.05 (versus saline-injected controls).
Digestive and Kidney Diseases and expressed in terms of the respective RP2 reference standards. Analysis of variance with repeated measures followed by the Student–Newman–Keul multiple comparison test was used for assessment of significance.

RESULTS

In Vivo. Low doses of galanin (15, 30, or 60 pmol) corresponding to 50, 100, and 200 μg, respectively, were injected into the 3V of freely moving male rats, and plasma GH, PRL, and TSH levels were determined. All doses of galanin increased plasma GH levels significantly at 5, 15, and 30 min after injection. The increases were dose-related, with the two higher doses (30 and 60 pmol) being equally effective at both 5 (P < 0.01) and 15 (P < 0.001) min after injection (Fig. 1). Plasma PRL levels increased slightly after the injection of 30 and 60 pmol of galanin. However, only the 60-pmol dose of galanin increased plasma PRL levels significantly (Fig. 1). Statistically, galanin decreased plasma TSH levels only at the 60-pmol dose at 30 and 60 min (Table 1).

In a second experiment 30 (100 ng) or 300 (1000 ng) pmol of galanin were injected i.v. Galanin (30 pmol) increased plasma GH levels significantly (P < 0.05) by 5 min as compared with values in saline-injected controls (Fig. 2). Plasma levels of GH after injection of 300 pmol of the peptide were highly significantly increased at 5 min postinjection (P < 0.01) when compared with plasma GH levels in either saline-injected controls or the preinjection levels (Fig. 2). The response to this dose given i.v. was less than that to a dose lower by a factor of 20 administered intraventricularly. At this concentration of galanin (300 pmol)-injected i.v., no alterations of the plasma levels of PRL and TSH were measured at any sampling time.

Passive immunoneutralization of endogenous galanin by 3V injection of 3 μl of rabbit antiserum to galanin resulted in a rapid 80% decrease of plasma GH levels (Fig. 3). Plasma levels of GH in the galanin antiserum-injected group decreased abruptly by 15 min postinjection (P < 0.01), while plasma levels of GH in the NRS (3 μl)-injected groups remained stable. The low plasma GH levels persisted for the 5-hr duration of the experiment (P < 0.001).

After 3V injection of galanin antiserum, an increase of plasma levels of TSH was found at 30 and 60 min (P < 0.05 and P < 0.02, respectively (Fig. 4). By 2 hr, however, TSH values were similar to control TSH levels. Thereafter, TSH levels from anti-galanin antiserum-injected rats showed no significant alteration in comparison with those of NRS-injected controls.

In Vitro. In five separate cell preparations, 1 nM to 1 μM galanin failed to alter significantly GH, PRL, or TSH release (Figs. 5–7). However, cells from each preparation did respond to GRH or TRH by releasing significantly greater amounts of GH (Fig. 5) or PRL and TSH than did cells incubated only in control medium (Figs. 6 and 7). When 100 nM galanin was combined with 70 nM TRH in the incubation medium, TRH-stimulated TSH release was potentiated (P < 0.02). Galanin did not alter significantly the effect of the higher concentration of 140 nM TRH on TSH release (Fig. 6). The same dose of galanin also potentiated TRH-stimulated PRL release at both TRH concentrations (P < 0.05 and P < 0.02, respectively) (Fig. 7). In contrast, galanin failed to alter the stimulatory effect of GRH on GH release or the inhibitory effect of somatostatin (Fig. 5).

DISCUSSION

The present results confirm our earlier finding (13) that intraventricular injection of galanin has a powerful, concentration-dependent stimulatory effect on GH release at very low doses (<15 pmol). Furthermore, galanin is more effective when administered via the intraventricular rather than the i.v. route. Since galanin had no effect on basal, stimulated, or inhibited GH release from anterior pituitary cells in vitro, it is apparent that its primary action is on the hypothalamus.
The action of galanin to stimulate GH release appears to be of physiological significance because intraventricular injection of highly specific antiserum directed against the peptide produced a dramatic lowering of plasma GH that was apparent within 15 min after the injection of the antiserum. The mechanism by which galanin stimulates GH release has not been determined but is most likely the result of either a stimulation of GRH discharge and/or an inhibition of somatostatin release. The rapidity of the action suggests that the GH response was mediated by GRH neurons near the 3V wall. Whether the action of galanin is directly on the hypophysiotropic neurons or is mediated by interneurons remains to be established.

In our previous report (13), we did not observe any effect of galanin on the release of other pituitary hormones; however, Koshiyama et al. (14) reported recently that lateral ventricular injection of galanin increased plasma PRL levels in urethane-anesthetized or conscious rats. In our present work, a clear stimulation of PRL release was observed only with the highest dose (60 pmol) of galanin that was injected into the 3V. This may explain the failure to observe any effect of galanin on PRL release at the lower doses used in our earlier experiments. It is apparent that the dose of galanin required to stimulate PRL release is at least 4 times greater than that needed to stimulate GH release. In view of the fact that the stimulation of PRL release by TRH in vitro was augmented by galanin, the effect of galanin to stimulate PRL release may be mediated by direct action on the pituitary. It may be that at the higher doses of galanin, there is sufficient transport of galanin from the hypothalamus to the anterior pituitary via the hypophysial portal vessels to cause the observed increase in PRL secretion.

However, it also is likely that at least part of galanin's effect is due to changes in the hypothalamic balance of PRL-releasing and -inhibiting factors. The inhibitory effect of

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**Fig. 5.** Effect of coincubation of porcine galanin (GAL), GRH, and somatostatin [GH release-inhibiting hormone (GRH)] on GH release from dispersed, male rat anterior pituitary cells in vitro. The data represent the mean ± SEM of five samples from a representative example of five separate experiments. NS, not significant.

**Fig. 6.** Effect of coincubation of porcine galanin (GAL) and TRH on TSH release from dispersed, male rat anterior pituitary cells in vitro. The data represent the mean ± SEM of five samples from a representative example of five separate experiments. NS, not significant.

**Fig. 7.** Effect of coincubation of porcine galanin (GAL) and TRH on PRL release from dispersed, male rat anterior pituitary cells in vitro. The data represent the mean ± SEM of five samples from a representative experiment. The other experiments gave similar results.
galanin on dopamine release may be one of these intrahypothalamic mechanisms. Nordstrom et al. (19) have reported that galanin can inhibit [3H]dopamine release from the median eminence, a site at which galanin and dopamine have been found to coexist in nerve endings. Another possible mechanism by which galanin may affect PRL release is via stimulation of vasoactive intestinal polypeptide release from the hypothalamus. This peptide can stimulate PRL release, and Koshiyama et al. (14) reported that passive immunization with specific anti-vasoactive intestinal peptide rabbit serum suppressed the plasma PRL response to galanin (500 pmol; administered to lateral ventricle) in anesthetized rats. In unanesthetized animals, intraventricular antiserum galanin suppressed TRH-stimulated TSH release but had no effect on the in vivo release of PRL. In contrast to these findings, we observed that galanin antiserum failed to alter plasma PRL levels in unanesthetized rats. However, it should be noted that galanin was injected into the ventricle rather than into the hypothalamus, a site at which galanin is known to coexist with other hypothalamic neuropeptides.

Recently, estrogen has been shown to stimulate dramatically galanin mRNA and galanin synthesis in the anterior pituitary of female rats and to increase circulating galanin (21). Therefore, it should be important to evaluate the actions of galanin and their physiological significance in normal female animals and those treated with estrogen.

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