Effects of galanin on growth hormone release in isolated cultured rat somatotrophs

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Several studies suggest that galanin stimulates growth hormone release through effects on the hypothalamus. It is not known if galanin has acute effects directly on the somatotrophs. We now find that 0.5 μmol/l galanin stimulates growth hormone release within the first minute of exposure in isolated, purified, cultured male rat somatotrophs. The effect persisted for 15 min and was reversible when galanin was omitted. Galanin reduced the effect of 1 nmol/l growth hormone-releasing hormone (hGHRH(1–29)) on growth hormone release. Galanin stimulated 45Ca efflux from prelabelled cells but had no effect on 86Rb efflux (tracer for potassium). The findings support the fact that galanin can stimulate growth hormone release directly at the level of the somatotrophs. The cellular mechanisms for the effect of galanin probably differ from those of growth hormone-releasing hormone.

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Galanin is found in the central nervous system, especially in the hypothalamus, and may function as a neurotransmitter (1, 2). Galanin stimulated growth hormone (GH) release when injected intravenously in humans (3) and hypothalamic galanin could be a modulator of GH secretion. The effect may be through inhibition of somatostatin release (4) but a stimulation of growth hormone-releasing hormone (GHRH) secretion also has been demonstrated (5).

Galanin is colocalized with GHRH in nerve endings in the median eminence (6, 7) and, moreover, also found in anterior pituitary endocrine cells (8, 9). We do not know if direct effects of galanin on the somatotrophs can play a role in the regulation of GH release. Galanin had no effect on basal GH release in isolated rat anterior pituitary cells in studies by Ottlécz et al. (10) and Meister and Hulting (11), but others have found stimulatory effects in rat (12–14) and chicken (15). When an effect of galanin has been observed, GH release was measured either over several hours or was slow in onset.

We now want to know if galanin can stimulate somatotroph hormone secretion within a time period suggesting a role in the moment-to-moment regulation of GH release, and if so, what can be the underlying cellular mechanisms. We have tested the effect of galanin in a population of purified and highly responsive cultured rat somatotrophs (16) and find that galanin stimulates basal GH release within 1 min of exposure and reduces the effect of GHRH. Galanin stimulated somatotroph calcium efflux but had no effect on rubidium (tracer for potassium) flux.

Materials and methods

Animals

Adult male Sprague-Dawley rats (200–250 g) were obtained from ALAB, Stockholm, Sweden. The rats were housed at 55–65% humidity and 22–24°C, with illumination from 06.00 to 18.00 h and water and pelleted food ad libitum. The local animal ethics committee has given consent for these studies.

Preparation and culture of anterior pituitary cells

The methods have been described in detail elsewhere (16) and will only be summarized here. Dispersion of anterior pituitary glands was done by incubation at 37°C and shaking (200 strokes per min) with trypsin (1 g/l) and deoxyribonuclease (0.25 g/l). A cell fraction known to contain consistently more than 95% somatotrophs with 98–99.5% viability (16) was isolated by centrifugation on a preformed, self-generated Percoll density gradient and cultured for 3 days at 37°C in Dulbecco’s modified Eagle’s medium (Flow Laboratories Ltd., Irvine, UK) with 10% fetal calf serum (Flow Labs.) to enhance GHRH responsiveness (16).

Labelling and perfusion of cultured somatotrophs

Cultured cells were harvested and resuspended in 1.0 ml of perfusion medium. A 100-μl aliquot was taken for protein determination (17) and the remaining 900 μl (~1.3 × 10⁶ cells; haemocytometer) was incubated in a
70° tilted conical glass vial (14 x 110 mm) for 90 min at 37°C (air/CO₂, 95:5) with 31 μmol/l ⁴⁰RbCl (6-13 TBq/mol) and 1850 TBq of ⁴⁰Ca (Amersham International plc, Bucks., UK). The cells were washed by centrifugation in non-radioactive medium and put on top of 2-mm pre-swollen Sephadex G-10 in the perfusion chamber (a Nucleopore 13-mm Pop-Top holder; Nucleopore Corporation, Pleasanton, CA). The perfusion system was as described previously (16) and enclosed entirely in an incubator maintained at 37°C. Basal perfusion medium contained 115 mmol/l NaCl, 4.7 mmol/l KCl, 1.2 mmol/l KH₂PO₄, 1.2 mmol/l MgSO₄, 2.56 mmol/l CaCl₂, 20 mmol/l NaHCO₃, 20 mmol/l HEPES, 1 mmol/l d-glucose and 10 g/l bovine serum albumin, pH 7.40.

After 30 min of preperifusion in basal medium, synthetic human GHRH (1-29) (kindly provided by Kabi Vitrum, Stockholm, Sweden) was added for 3 min. This was followed by a second similar preperifusion and test period. Previous experiments have shown that the effect of GHRH does not differ appreciably between the first and second of two such periods (16). Samples from each fraction of effluent were saved (−20°C) for GH radioimmunoassay (16). Cells and fractions of effluent were then analysed for radioactivity by liquid scintillation spectrometry using Aquasol (NEN Research Products, Boston, MA) as the scintillation liquid. The lag period for a new medium to reach the chamber was 43 s and has been corrected for in the figures. Cell viability (Evans Blue exclusion) was consistently more than 99% after the perifusions.

Expression of results

Efflux patterns for ⁴⁰Ca and ⁶⁸Rb were expressed in terms of "fractional outflow rates". Herein, the efflux of radioactivity at each point is expressed as a function of the residual radioactivity within the cells, as estimated by back-calculation from the preceding collections. This derivation is designed to compensate for differences in the persistent pool of label.

Statistical analyses have been made using Student’s t-test for paired or independent observations, as indicated in the Results section and the figures.

Results

We wanted to find out if galanin has acute effects on GH release in isolated rat somatotrophs. Figure 1 shows that 0.5 μmol/l galanin stimulated GH release during the first minute of exposure. The level of GH release reached after the first few minutes of stimulation persisted during the 15-min period with galanin (Fig. 1). Basal values were obtained 5–10 min after omission of galanin from the perfusion medium. Basal GH release during the 5-min period preceding galanin stimulation was 129.7 ± 23.5 (mean ± SEM, N = 12) ng GH·mg protein⁻¹·min⁻¹. Mean GH release during stimulation with galanin was 201.1 ± 26.0 ng GH·mg protein⁻¹·min⁻¹. This is comparable to the effect observed in similar types of perifusions with 0.1 nmol/l GHRH, where there was a rise from 89.8 ± 3.5 to 217.4 ± 16.8 ng GH·mg protein⁻¹·min⁻¹ (N = 4). Thus, effects of GHRH are observed at concentrations several orders of magnitude lower than for galanin.

Growth hormone-releasing hormone is the most important physiological stimulus for GH release. Because galanin is present in the median eminence and may be released to the portal vessels, we wanted to know if galanin can affect the GH response to GHRH. We found that GHRH stimulated GH release also in cells that had been pretreated with 0.5 μmol/l galanin for 10 min (Fig. 2). However, when calculated as a percentage of the release during the last 5 min with basal medium, the effect of GHRH during the first 5 min was less in cells perifused with galanin (288.6 ± 45.0%) than in controls (63.6 ± 84.8%, p < 0.05).

To investigate the possible mechanisms underlying the effects of galanin on GH secretion, we studied calcium and potassium flux. Galanin (0.5 μmol/l) stimulated somatroph ⁴⁰Ca flux (Fig. 3) but had no effect on ⁶⁸Rb outflow (tracer for K⁺): GHRH (0.1 nmol/l) affected both calcium and rubidium fluxes (Figs. 3 and
4). This concentration of GHRH was chosen because it produces about the same stimulation of GH release as 0.5 μmol/l galanin (see above).

Discussion

A number of studies support the hypothesis that galanin stimulates GH secretion through effects on the hypothalamus (3–5, 10, 11, 18). However, galanin has been localized in nerve endings in the median eminence (6, 7), with the majority of corresponding nerve cell bodies in the arcuate nucleus (19). Galanin is found also in anterior pituitary cells (8, 9). The concentration of galanin in the hypophysial portal circulation is higher than in peripheral blood (20). A role for galanin in the regulation of GH release at the level of the pituitary is therefore possible. In humans, galanin seems to be present predominantly in adrenocorticotrophic cells (9). In male rats, galanin is present in somatotroph secretory granules (8). However, thyrotropin-releasing hormone, but not GHRH, stimulated galanin release in isolated male rat pituitary cells (21).

No strong support has been presented for the role of galanin in the control of GH release at the level of the pituitary. Effects of galanin have been observed either
this. In other cell types, galanin can affect cAMP levels (26), phospholipid inositol breakdown (27), potassium flux (28), cytoplasmic calcium levels (29) and late unidentified steps in the exocytosis (30).

In conclusion, our findings suggest that galanin, along with its hypothalamic effects, can modulate somatotroph GH secretion directly at the pituitary level.

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References


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