Effect of Galanin on the Release of Growth Hormone in Perifused Bovine Pituitary and Hypothalamus

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Abstract The effect of galanin was studied on the release of growth hormone (GH) from perifused medial basal hypothalami (MBH) and/or adenohypophyses of steers in a sequential double chamber perifusion system. Galanin stimulated significant GH release at the doses of $10^{-7}$, $10^{-8}$ ($P<0.01$) and $10^{-9}M$ ($P<0.05$) in perifusion groups containing pituitary fragments alone compared with control perifusion group. The elevation induced by galanin in perifusion groups containing pituitary fragment alone tended to be in a dose dependent manner. In contrast to these results, galanin stimulated significant GH release only at the dose of $10^{-7}M$ in perifusion groups containing both the hypothalamus and pituitary fragments in tandem ($P<0.01$). The present results suggest that galanin stimulates GH release by acting directly on the adenohypophysis in cattle.

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Galanin, a 29 amino acid peptide originally discovered in the porcine intestine$^{20}$, has been found to be widely distributed in the mammalian central nervous system$^{4,15,18,19}$. The peptide is known to stimulate pituitary growth hormone (GH) release in rats$^{6,11,13,14,16,17}$ and humans$^{1,5,10}$. The hypothalamic site of galanin action to release GH has been suggested, since intraventricular injections of galanin stimulated GH release, but failed to stimulate GH release from the adenohypophyses in vitro$^{16,17}$. On the contrary to this report, galanin acts directly on rat anterior pituitary cells stimulating GH release$^{6}$. Considering these controversial results, we examined to explore whether galanin stimulates the release of GH indirectly through the hypothalamus or directly on the adenohypophysis. Moreover, the effect of galanin on GH secretion in domestic animals has not been elucidated.

In the present study, the effect of galanin on the secretion of GH from the adenohypophysis of steers was examined using an in vitro superfusion system of the hypothalamus and anterior pituitary fragments.

Materials and Methods

Preparation of anterior pituitary fragments and medial basal hypothalamus (MBH) : The MBH and anterior pituitary glands were removed from 17 Holstein steers (age in 17-18 months) at a local slaughter house and were transported to the laboratory in Krebs-Ringer bicarbonate solution to which was added 1.8 mg glucose/ml, 0.5 mg bacitracin/ml, 1.0 mg bovine serum albumin/ml, 0.1 mg streptomycin/ml and 100 IU penicillin/ml (modified KRB solution) at 5°C within 2 h. The anterior pituitary glands were sagittally sectioned prior to immersion into the modified KRB solution.
The medial part of the sagittal halves of the pituitary glands was sectioned into small cubes of 1.5 mm dimensions. The medial basal hypothalamic tissue prepared for perifusion was limited dorsally 3 mm deep, frontally by the caudal border of the optic chiasm, laterally by 1 mm lateral to the tuber cinereum, and caudally by the rostral border of the mamillary body. The MBH included the pituitary stalk.

**Anterior pituitary perifusion**: The perifusion system was a modified method of MIYAKE and YEN\(^1\). Five cubes of the anterior pituitary fragments were loaded into a small plastic chamber of 0.2 ml size and were perifused at 38°C, in a modified KRB medium saturated with 95% O\(_2\) and 5% CO\(_2\). After equilibration for 3 h, the effluent was collected in 3 ml fractions at 10 min intervals at a flow rate of 18 ml/h. The validity of the system used in the experiment was confirmed in our previous study\(^7\). Six samples of the effluent were collected first over a period of 1 h for determinations of basal control values. Solutions containing various concentrations (10\(^{-7}\), 10\(^{-8}\) and 10\(^{-9}\)M) of galanin (Sigma Chemical Co., St. Louis, MO, U.S.A.) or the modified KRB solution alone (control) were then perifused for the next 1.0 h. After these period of treatments, the modified KRB solution was perifused again for the next 1.0 h. At the termination of the experiment, modified KRB solution containing high potassium ion (123.2 mM KCl in KRB solution) was perifused to test the viability of the tissues used.

**MBH and anterior pituitary perifusion**: One half of the MBH was loaded into the first plastic chamber of 0.8 ml size, and five fragments of the anterior pituitary glands into the second chamber (0.2 ml size), and perifused at 38°C and 18 ml/h flow rate with modified KRB solution, saturated with 95% O\(_2\) and 5% CO\(_2\). The same perifusion procedure described above for the perifusion containing the pituitary fragments alone was applied to the serial double chambers. Effluent fractions were stored at -20°C until assayed for GH.

**Radioimmunoassay (RIA)**: GH in effluent fractions was determined by RIA as described previously\(^9\). The GH standard preparation used and hormone for iodination were bGH-B1. The hormone was supplied by the USDA Reproduction Laboratory, Beltsville MD. Bovine growth hormone (bGH) antiserum to bGH prepared in monkeys was supplied by Dr. JOHKE, T., National Institute of Animal Industry, Japan, and goat anti-monkey IgG serum (2nd antibody) was supplied by Dr. WAKABAYASHI, K, Institute of Endocrinology, Gunma University, Japan. In this assay, the intra- and inter-assay coefficients of variation were 1.7 and 16.7%, respectively. The least detectable value of assay was 2.54 ng/ml.

**Statistical analysis**: Values during the experiment were expressed as percent changes from means of 6 determinations during the 1 h basal control period. Data were presented as means±S.E.. For statistical analysis the levels of each effluent fraction after the treatment were compared with respective control values. The mean GH concentrations during and after infusion of galanin from each 6 fractions were also compared with the mean value of their respective controls. Statistical comparisons were made by using the Student’s t test after the BARTLETT’S test for uniformity of the variances\(^8\).

**Results**

The effects of the various molar concentrations of galanin for the release of GH from the perifusion containing pituitary fragments alone are shown in Figs. 1 and 2. As shown in Fig. 1, perifusion with the media containing 10\(^{-7}\) and 10\(^{-8}\)M galanin for 60 min significantly stimulated GH release from the pituitary fragments alone when compared with respective control values (P<0.05, P<0.01). The increasing rates of mean GH concentrations during and after infusion of galanin from each 6 fractions were also compared with the mean value of their respective controls. Statistical comparisons were made by using the Student’s t test after the BARTLETT’s test for uniformity of the variances\(^8\).
Fig. 1. Effects of galanin (10^-7 - 10^-9 M) on GH release from perifusion containing only the pituitary fragments. The mean GH concentrations of the initial 6 fractions during 1 h perifusion was expressed as basal control values. All values in the controls and groups perifused galanin were calculated as percent changes from the each basal control value. Each value represents the mean of 4 or 5 experiments ± S.E.. The letter of galanin indicate the period of galanin perifused. K+ indicates high potassium ion perifused. *, **: Significantly different from respective controls (* P<0.05, ** P<0.01).

The elevated levels of GH induced by 10^-7M galanin were maintained up to at least 60 min after the termination of the perifusion of the peptide (Fig. 2). The 10^-9M galanin also significantly (P<0.05) increased the mean GH concentrations during the treatments compared with the mean GH concentrations of 60-min control perifusion period, but the increasing rates (14%) of the 10^-9M galanin was significantly (P<0.05) lower than that (37%) of the 10^-7M galanin (Fig. 2). The elevation induced by galanin in perifusion groups containing pituitary fragment alone tended to be in a dose dependent manner.

The effects of the various molar concentrations of galanin for the release of GH from the perifusion containing pituitary fragments in series with the hypothalamus are shown in Figs. 3 and 4. As shown in Fig. 3, perifusion with the medium containing 10^-7M galanin for 60 min significantly stimulated GH release from the pituitary fragments in series with MBH compared with respective control values (P<0.05, P<0.01). The increasing rates of mean GH concentrations during the treatments in the 10^-7M group was 32% (P<0.01), and
Fig. 2. Secretory response of in vitro GH release from perifusion containing only pituitary fragments to different galanin concentrations. Values represent the mean GH levels and SE from 6 fractions during the perifusion of galanin (Left) and from the next 6 fractions after the perifusion of galanin (Right). Numbers at the bottom of bars represent number of experiments.

*, **: Significantly different from controls (*P<0.05, **P<0.01). a, b: a was significantly different from b (P<0.05).

this increase in GH concentrations was maintained up to at least 60 min after the termination of the perifusion of the peptide (Fig. 4). There were no significant difference at other galanin doses between the control groups and the perifusion groups containing pituitary fragments in series with the hypothalamus.

Discussion

The present study demonstrates that galanin induces GH release directly from the anterior pituitary gland in vitro in cattle. Heretofore, effects of galanin on the release of pituitary GH have never been reported in domestic animals in vivo as well as in vitro. To our knowledge, the present study is the first demonstration of the effect of galanin in stimulating pituitary GH release in ruminant animals. O'TTLECZ et al. reported that the intraventricular or intravenous injection of galanin stimulated GH release in rats. They failed, however, to induce release of GH from cultured anterior pituitary cells in vitro. Hence they suggested that the peptide had no direct action on the adenohypophysis, but acts on the level of the hypothalamus. But GABRIEL et al. observed the direct galanin stimulation of GH release from rat anterior pituitary cells. The results of the present investigation are consistent with the observations of GABRIEL et al.

In contrast to the results that galanin induced a significant release of GH only in the 10^{-7}M in the pituitary fragments in series with the MBH group, the GH releasing effects of galanin in the group of pituitary fragments alone could be seen at a dose as low as 10^{-9}M. A concomitant somatostatin infusion inhibits GH release induced by galanin in man. And somatostatin was isolated from the hypothalamus on the basis of its ability to inhibit GH release from the anterior pituitary cells in...
Galanin Release Bovine Growth Hormone

Fig. 3. Effects of galanin (10^{-7} \sim 10^{-9} M) on GH release from the perifused bovine pituitary fragments and hypothalamus in series. Each value represents the mean of 5 experiments±S.E.. *, **: Significantly different from controls (*P<0.05, **P<0.01). Other explanations are described in Fig. 1.

Therefore, the inhibitory responses observed in perifusions containing both the pituitary fragments and hypothalamus in series could be attributed to the action of somatotropin release inhibiting factor (SRIF), such as somatostatin. Further studies such as perifusion of MBH extracts or somatostatin instead of MBH tissues would be necessary in order to clarify the inhibitory responses appeared in the present study.

The GH releasing effect of galanin seems to be weaker than that of GRF since the GH releasing effect of GRF was seen at concentrations as low as 10^{-11}M GRF in the same perifusion system\(^7\). The physiological importance of galanin-stimulated GH secretion is uncertain. However, galanin was reported to be a physiological regulator of spontaneous pulsatile secretion of GH in rats\(^1\). Also the combined effect of galanin and GRF produces considerably greater GH release than that produced by GRF alone\(^5,6\). Binding sites of galanin receptor and galanin mRNA have been found in the hypothalamus of humans and rats\(^7\). It is not clear, at present, whether the specific binding sites for galanin exist in the bovine adenohypophysis or not. The present results, however, suggest that galanin may be involved in the regulation of GH secretion by a direct action on the adenohypophysis in cattle.
These findings suggest that galanin may be added to the list of factors that act to modify GRF and somatostatin effects on GH secretion in the cattle.

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References

6) GABRIEL, S.M., C.M. MILBURY, J.A. NATHANSON and J.B. MARTIN, Galanin stimulates rat pituitary growth hormone
Galanin Release Bovine Growth Hormone


ガラニンが牛の成長ホルモン（GH）の放出に及ぼす影響について検討した。去勢雄牛から得た視床下部-下垂体組織を in vitro 灌流系に置き、10^{-2} ～ 10^{-9} M のガラニンを灌流し、灌流液中の GH 濃度をラジオイムノアッセイで測定した。灌流は、下垂体組織のみを灌流する単独灌流と第一室に視床下部の内側底部組織を入れ、そこを流れた液が下垂体前葉組織を入れた第二室に流れるようにした連続灌流を行った。灌流液には38℃に保持した修正クレブス液を用いた。ガラニンは単独灌流時には 10^{-7} ～ 10^{-8} M 濃度で GH 濃度を有意（P<0.05, P<0.01）に増加させたが、連続灌流時には 10^{-7} M 濃度で有意な （P<0.01）増加反応が認められたに過ぎなかった。また単独灌流時に見られた反応は用量依存性であった。これらの結果は、ガラニンは GH の放出に対して下垂体に直接作用することを示唆している。