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Galanin-induced inhibition of insulin release from cultured rat islets

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ABSTRACT To determine the effect of galanin on insulin secretion from islets of Langerhans *in vitro*, isolated rat islets were cultured in the medium containing various concentrations of galanin and glucose. Insulin secretion was inhibited markedly and dose-dependently by galanin, when the glucose concentration in the culture medium was 5.5 mmol/L. Galanin 0.3 μmol/L had a more pronounced inhibitory effect on insulin secretion in culture medium with a glucose concentration of 5.5 mmol/L than in medium with 2.7 or 20 mmol/L of glucose. Without glucose in the medium, galanin had a negligible effect on insulin secretion from the cultured islets, Galanin did not affect glucagon release.

In conclusion, galanin inhibits effectively insulin release from isolated islets, thus suggesting that it may act as a modulator in controlling insulin secretion from pancreatic beta cells under normal physiological conditions.

KEY WORDS galanin; islands of Langerhans; insulin; glucagon; rats; cultured cells

Galanin, a 29 amino acid peptide, was recently isolated from the porcine upper small intestine[1]. An immunoreactive substance similar to galanin has been detected in the central nervous systems of rats[2] and in neural structures throughout the gastrointestinal tracts of rats[3] and pig[4]. Recently, galanin immunoreactive nerves were demonstrated also in the pancreas, in particular associated with the islets[5]. It has been reported that galanin resulted in pronounced hyperglycemia and a decrease in plasma insulin when administered intravenously to conscious dogs[6] and inhibited insulin secretion by perfusing rat pancreas [7]. These findings suggest that galanin may be an important neuropeptide in the gut[5].

The objectives of our current study are to determine the effect of galanin on the insulin secretion from islets of Langerhans, an isolated and denervated cell system, and to examine the mechanisms responsible for such effects.

MATERIALS AND METHODS

Islet isolation and culture Male wistar rats (Charles River, Canada), weighing 253 ± SD 21 g, were anesthetized by ip sodium pentobarbital (55 mg/kg, MTC Pharmaceuticals). Islets were isolated from the pancreas of the rats by a collagenase (Type V, Sigma) digesting technique[8] and hand picked with the aid of a dissecting microscope. The isolated islets (200/flask) were then cultured for 24 h in 10 ml of CMRL-1969 medium[9] (Connaught Laboratories

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Ltd) supplemented with glucose 5.5 mmol/L, 7.5% (vol/vol) fetal bovine serum, and gentamicin 50 μg/ml in a humidified atmosphere containing 5% CO_2 at 37℃.

After 24 h of pre-incubation, the islets were rinsed 3 times with CMRL-1969 and placed in a series of 12×75 mm culture tubes (10 islets/tube). Added to each tube were 1 ml CMRL-1969, various concentrations of glucose (2.7, 5.5 and 20 mmol/L) and galanin (Sigma: 3,15,30,300 nmol/L), and bovine serum albumin 1 mg/ml (Sigma). The islets were incubated for 60 min at 37℃ in a Dubnoff shaker (60 strokes/min) with air. Incubated samples were then taken for insulin and glucagon determinations. In some experiments islets were cultured in CMRL-1969 containing galanin 0.3 μmol/L without glucose, or neither glucose nor galanin. After the first hour of incubation the medium was withdrawn from the tubes and replaced by fresh medium. After another hour of incubation samples were taken for testing.

Hormone assays The insulin content of the culture medium was determined by radioimmunoassay with solid-phase ^{125}I-insulin supplied by Diagnostic Products Corp, USA. Following the Coat-A-Count insulin procedure, in brief, ^{125}I-insulin competes with insulin in the sample for sites on insulin-specific antibody immobilized to the wall of a polypropylene tube. After incubation, isolation of the antibody-bound fraction is achieved simply by decanting the supernatant[10].

Glucagon in the medium was also measured by radioimmunoassay, using ^{125}I-porcine glucagon (Novo Research Institute, Denmark). The glucagon standard and antiserum used for determining glucagon of medium were purified pork glucagon standard and glucagon rabbit serum K 5563, respectively.

Statistical analysis The statistical significance of the results was determined by a Student's two-tailed t test. All values are expressed as a $\bar{x}\pm$SD of the number of observations indicated.

RESULTS

In culture medium with a glucose 5.5 mmol/L, the degree to which galanin inhibited insulin secretion from isolated islets varied with the amount of galanin used (Fig 1). With the addition of galanin 3 nmol/L to the medium, insulin secretion was decreased only slightly. However, in medium containing 15,30,300 nmol/L of galanin, insulin secretion was decreased significantly and dose-dependently.

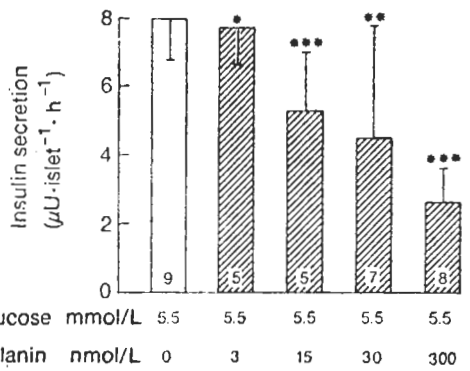

Fig 1. Inhibitory effects of galanin on insulin secretion from rat islets after 1 h incubation in CMRL-1969 medium. In this and subsequent illustrations, numbers within the bars represent the number of tubes (10 islets/tube) per group. Open bars: control group, 5.5 mmol/L glucose in CMRL-1969 alone. Shaded bars: test group, 5.5 mmol/L glucose in CMRL-1969 with various concentrations of galanin. *p>0.05, **p<0.05 ***p<0.01 *vs* glucose alone.

The inhibitory effect of galanin 0.3 μmol/L on insulin secretion varied in response to graded concentrations of glucose in the culture medium (Fig 2A, 2B). While insulin secretion was increased when glucose concentration was raised from 2.7 to 20 mmol/L, the most pronounced inhibitory effect on secretion was observed when the

glucose concentration in the culture medium was 5.5 mmol/L (2.3±0.7 *vs* 8.3±1.5; $\mu U \cdot islet^{-1} \cdot h^{-1}$; $p<0.01$). To emphasize the differences between the amounts of insulin secreted with or without the influence of galanin, Fig 2B shows the galanin-inhibited secretion levels as % of the levels secreted without galanin in the culture medium. Galanin had a more pronounced inhibitory effect on insulin secretion in culture medium with glucose 5.5 mmol/L than in medium with glucose 2.7 or 20 mmol/L (28±7% *vs* 74±10% and 75±11%, respectively, $p<0.01$).

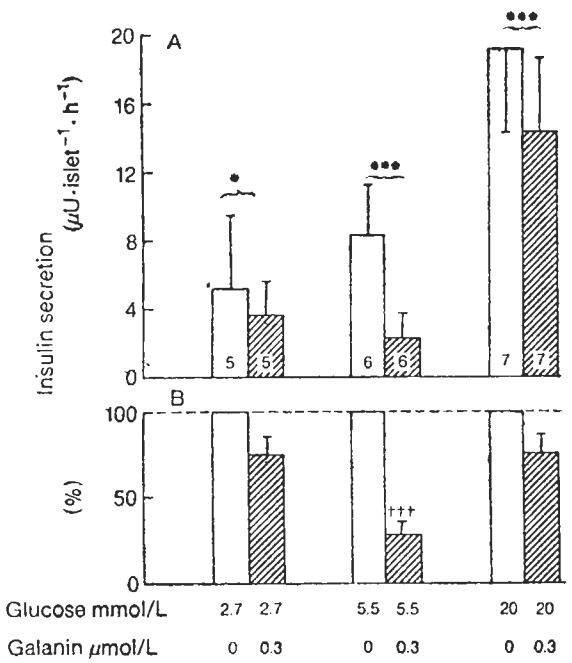

Fig 2 Inhibitory effect of galanin on glucose-induced insulin secretion from isolated islets, expressed in microunits (2A) and as % of insulin released in medium containing various concentrations of glucose without the addition of galanin (2B) †††$p<0.01$ *vs* insulin secretion from islets incubated in medium containing either 2.7 or 20 mmol/L glucose. **$p<0.05$, ***$p<0.01$

Other tests showed that the inhibitory effect of galanin on insulin secretion was determined by the presence of glucose. Fig 3 illustrates that: 1) galanin reduced the quantity of insulin secreted by isolated islet cells to about the basal level; 2) galanin had no significant effect on insulin secretion in the absence of glucose in the culture medium; and 3) islet cells that had been incubated 1 h without exposure to glucose responded to glucose challenge during incubation for an additional hour.

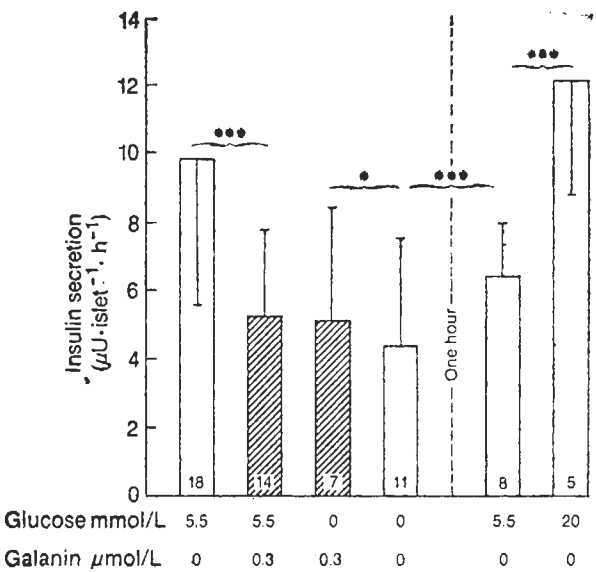

Fig 3. Comparison between effects of galanin on insulin secretion from islets incubated 1 h in medium with and without glucose. Medium without glucose and galanin was replaced by fresh medium containing glucose. 5.5 or 20 mmol/L Islets were then incubated an additional hour in medium. The results were shown in the right side of the dashed line. *$p>0.05$, **$p<0.05$, ***$p<0.01$

Although glucagon secretion decreased as glucose levels were raised from 2.7 to 20 mmol/L, it was not affected significantly by the addition of galanin 0.3 μmol/L to the culture medium (Fig 4).

In further experiments, various concentrations of galanin were added to the medium containing glucose, but no significant changes in glucagon release were observed (Tab 1).

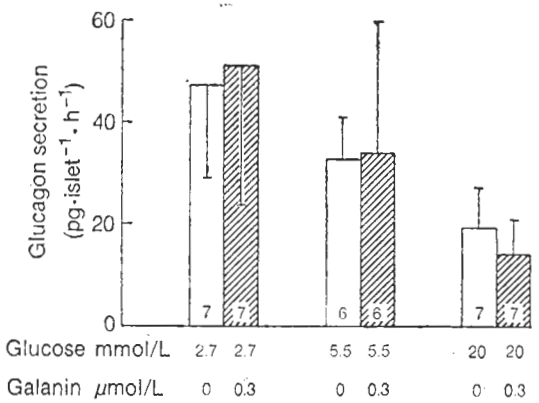

Fig 4. After islets were incubated 1 h, glucagon secretion was decreased with increases in glucose concentration but was not affected significantly by galanin.

Tab 1. Insignificant effect of galanin on glucagon secretion of isolated rat islets.

Medium contents			
Glucose (mmol/L)	Galanin (nmol/L)	Tubes	Glucagon secretion (pg·islet⁻¹·h⁻¹)
5.5	0	10	28±10
5.5	15	7	28±14
5.5	30	6	30±29
5.5	300	6	34±27

DISCUSSION

Our findings indicate that galanin inhibits insulin secretion by islets *in vitro*. This is consistent with experiments performed on canine pancreas[6,11], and also in good agreement with the report of Silvestre *et al*[7] which indicated that insulin release was depressed by galanin in perfused rat pancreas. The fact that insulin secretion was inhibited most prominently in a glucose concentration of 5.5 mmol/L suggests that galanin may play a role in the regulation of beta cell function under normal physiological conditions.

Our study also shows that galanin does not significantly inhibit the secretion of insulin in the absence of glucose, indicating that galanin inhibition acts on the glucose-stimulated insulin release. Glucose stimulates the overall rate of islet RNA synthesis, thereby affecting the formation of messenger RNA for proinsulin production. Based on the evidence available to date, it appears that the specific action of glucose on insulin release involves a change in the permeability of the beta cell membrane to calcium, resulting in the transport of beta granules associated with microtubular and microfilamentary systems to the cell surface, where the granules are liberated by emiocytosis[12]. Recently, Ahren *et al*[13] reported that galanin inhibition of glucose-stimulated insulin release in mice involves hyperpolarization of beta cell with a subsequent decrease in cytoplasmic free Ca^{2+}. It does suggest that galanin may act as a physiological modulator in glucose homeostasis.

In addition, our study demonstrates that galanin does not significantly affect the release of glucagon from isolated islets, confirming several previous studies which indicated that galanin neither stimulated nor depressed pancreatic glucagon release[6,7,11]. However, in other studies galanin stimulates *in vivo* glucagon release in dogs[5] and mice[14], but inhibited *in vitro* glucagon release in dog[15]. This discrepancy between their data and ours may be due to the different experimental preparations in different animal species. Manabe *et al* also reported that levels of epinephine, norepinephrine, cortisol and growth hormone in plasma were not affected significantly by galanin[8].

It has been suggested that galanin, being a pancreatic neuropeptide[5], may directly affect the release of pancreatic hormones without being mediated by the sympathetic nervous system[11]. while our *in vitro* study is consistent with that observation, the mechanism of galanin action has to be elucidated in further investigation.

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Galanin抑制离体大鼠胰岛释放胰岛素

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提要 离体大鼠胰岛孵育在含有不同浓度的葡萄糖和galanin的培养液中1 h(37℃, 空气). 用放射免疫法测定培养液中的胰岛素和胰高血糖素含量. Galanin抑制离体大鼠胰岛释放胰岛素呈剂量-效应关系. Galanin 0.3 μmol/L对胰岛素释放的最大抑制效应仅出现在培养液中葡萄糖浓度为5.5 mmol/L时(相当于血浆生理浓度). 在无葡萄糖的培养液中, galanin抑制胰岛素释放的作用消失. Galanin不影响胰高血糖素的释放.

关键词 galanin; 胰岛; 胰岛素; 高血糖素; 大鼠; 培养的细胞

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