

# Galanin inhibits insulin secretion by direct interference with exocytosis

Susanne Ullrich and Claes B. Wollheim

*Division de Biochimie Clinique, Centre Médical Universitaire, 9, avenue de Champel, CH-1211 Genève 4, Switzerland*

Received 23 February 1989

Electrically permeabilized RINm5F cells were used to study whether galanin inhibits insulin secretion distally to the generation of soluble second messengers.  $\text{Ca}^{2+}$ -induced insulin secretion was inhibited by the neuropeptide in a dose-dependent manner. Galanin appears to act via a G-protein as pertussis toxin treatment abolished the effect. GTP (100  $\mu\text{M}$ ), GDP (100  $\mu\text{M}$ ) and a low dose of  $\text{GTP}\gamma\text{S}$  (10  $\mu\text{M}$ ) did not affect galanin-mediated inhibition of secretion. In contrast, at 100  $\mu\text{M}$ ,  $\text{GTP}\gamma\text{S}$  attenuated and  $\text{GDP}\beta\text{S}$  abolished the effect of the peptide. We conclude that galanin inhibits exocytosis directly by a mechanism involving a G-protein.

Galanin; Insulin secretion; Permeabilization; Pertussis toxin; Guanine nucleotide; (RINm5F cell)

## 1. INTRODUCTION

Galanin, a 29 amino acid neuropeptide, has been found to inhibit basal and stimulated insulin secretion in a variety of systems [1,2]. Immunocytochemical staining revealed that galanin is present in intrapancreatic nerves [3]. The peptide may therefore play an important role in the regulation of hormone secretion from pancreatic islets. Galanin receptors have been characterized in brain and Syrian hamster insulinoma tissue [4,5]. In the insulin-secreting cell line RINm5F, galanin inhibits insulin release and adenylate cyclase activity, both effects being attenuated by pertussis toxin pretreatment [6]. These properties are also shared by other inhibitory hormones, somatostatin and  $\alpha_2$ -adrenergic agonists, e.g. epinephrine [7]. Since cyclic AMP levels do not correlate with the rate of insulin secretion, it is unlikely that lowering of cyclic AMP is the sole factor mediating the inhibition of secretion [8]. It has been found that galanin hyperpolarizes RINm5F cells [9,10] and attenuates glucose-in-

duced depolarization in islet cells [11,12]. The changes in membrane potential are due to activation of ATP-sensitive  $\text{K}^+$  channels [9] via a pertussis toxin-sensitive G-protein [10]. However, neither the action on membrane potential nor the lowering of cytosolic  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_i$ ) [11,12] offers sufficient explanation for the potent reduction of insulin release. To elucidate the action of galanin we used permeabilized RINm5F cells, a system in which the regulation of cyclic AMP levels and  $[\text{Ca}^{2+}]_i$  can be bypassed [13]. We demonstrate that galanin inhibits  $\text{Ca}^{2+}$ -induced secretion in a dose-dependent manner and that pertussis toxin abolishes the effect. These findings suggest that galanin inhibits exocytosis at a site distal to and independent of the gating of  $\text{K}^+$  channels and the inhibition of adenylate cyclase activity.

## 2. MATERIALS AND METHODS

RINm5F cells were cultured and permeabilized as in [13]. In brief, before use, the cells were kept for 3 h in spinner culture. Permeabilization by high-voltage discharge (10 exposures to an electrical field of 3 kV/cm each of 30  $\mu\text{s}$  duration) was carried out in mannitol buffer (pH 7.0), containing 270 mM mannitol, 10 mM potassium glutamate, 5 mM MgATP, 0.4 mM EGTA, 20 mM Hepes. Permeabilized cells were incubated in potassium

*Correspondence address:* S. Ullrich, Division de Biochimie Clinique, Centre Médical Universitaire, 9, avenue de Champel, CH-1211 Genève 4, Switzerland

glutamate buffer (pH 7.0), containing 140 mM potassium glutamate, 5 mM NaCl, 7 mM MgSO<sub>4</sub>, 5 mM Na<sub>2</sub>ATP, 10.2 mM EGTA and Ca<sup>2+</sup> and other test substances at appropriate concentrations for 15 min at 4°C and subsequently for 5 min at 37°C. Incubations were stopped by chilling to 4°C followed by centrifugation. The nucleotide triphosphate-regenerating system consisting of 21 U/ml of creatine kinase and 2 mM phosphocreatine was only added when GTP was present in the incubation medium. Immunoreactive insulin was measured in the supernatant as described [13]. Pertussis toxin (100 ng/ml) treatment of the cells was carried out during the 3 h spinner culture prior to permeabilization.

GTP, GTP $\gamma$ S, GDP, GDP $\beta$ S, Na<sub>2</sub>ATP and MgATP were from Sigma (St. Louis, MO). Galanin was purchased from Bachem Bioscience (Bubendorf, Switzerland) and pertussis toxin from List Biological Labs (Campbell, CA).

### 3. RESULTS AND DISCUSSION

Galanin decreases Ca<sup>2+</sup>-induced insulin secretion in electrically permeabilized RINm5F cells in a dose-dependent manner (fig.1). 10 nM galanin attenuates insulin secretion by 23%, while the peptide at 0.1 and 1  $\mu$ M inhibits secretion by 32 and 43%, respectively. These galanin concentrations are one order of magnitude higher than those reported for inhibition of insulin secretion in intact isolated islet cells [11] and in RINm5F cells [6].

When RINm5F cells were treated with pertussis toxin prior to permeabilization, the inhibitory effect of galanin on Ca<sup>2+</sup>-induced secretion was abolished (fig.2). Thus, galanin interference with exocytosis may be mediated by a pertussis toxin-sensitive G-protein. A previous study suggested that pertussis toxin causes ADP-ribosylation of at least two protein bands in RINm5F cell membranes [13], which may represent G<sub>i</sub> and G<sub>o</sub> [14]. It is of interest to compare the effects of galanin to those of  $\alpha_2$ -adrenergic agonists. The latter compounds also inhibit stimulated insulin secretion from permeabilized islets [15,16] and RINm5F cells [17]. In our previous study [17] several lines of evidence, including the abrogation of the inhibition by pertussis toxin, suggest that the inhibition is secondary to the coupling of  $\alpha_2$ -adrenoceptors to a G-protein. Galanin action, however, is not mediated by adrenergic receptors since adrenergic antagonists did not attenuate its effects [18]. Such receptors have indeed been characterized by <sup>125</sup>I-galanin binding in rat brain [4] and hamster pancreatic tumor cells [5].

To substantiate further the involvement of a G-protein we examined the dependence of the galanin

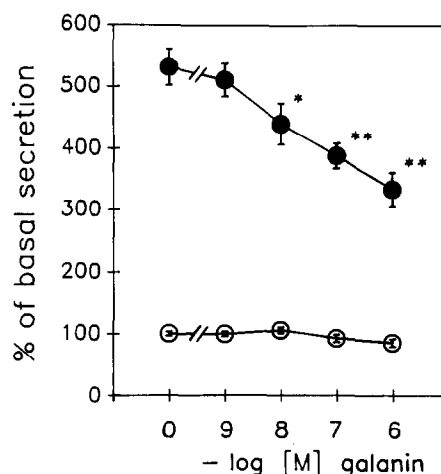


Fig.1. Concentration dependency of galanin-induced inhibition of basal and Ca<sup>2+</sup>-stimulated insulin secretion in permeabilized RINm5F cells. Cells were incubated for 5 min in the presence of 0.1  $\mu$ M Ca<sup>2+</sup> (open circles) or 10  $\mu$ M Ca<sup>2+</sup> (filled circles) and increasing amounts of galanin as indicated. 100  $\mu$ M GTP and a nucleotide-regenerating system were present throughout; for details see section 2. Secretion at 0.1  $\mu$ M Ca<sup>2+</sup> in the absence of galanin was set to 100%. Results are means  $\pm$  SE of 20 observations from 4 independent experiments. Statistical analysis was by Student's *t*-test for unpaired data: \* *P* < 0.05, \*\* *P* < 0.001.

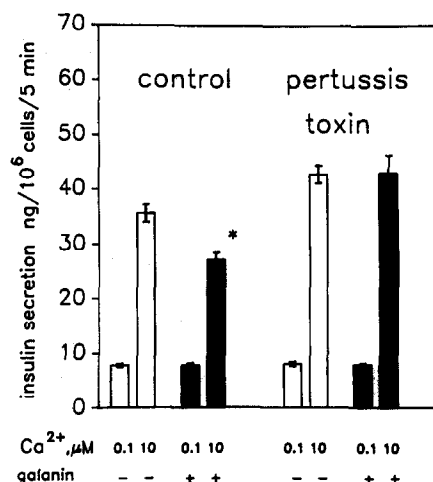


Fig.2. Effect of pertussis toxin treatment on galanin-induced inhibition of insulin secretion in permeabilized RINm5F cells. Cells were kept 3 h in spinner culture without (control) or with pertussis toxin prior to permeabilization as described in section 2. Incubations were carried out in the presence of 100  $\mu$ M GTP at 0.1 or 10  $\mu$ M Ca<sup>2+</sup>. Galanin was used at 0.1  $\mu$ M (filled bars). Results are means  $\pm$  SE of 15 observations from 3 independent experiments. \* *P* < 0.05.

inhibition upon exogenously added guanine nucleotides. The potency of the peptide to inhibit  $\text{Ca}^{2+}$ -induced secretion does not change irrespective of whether GTP (100  $\mu\text{M}$ ) is added to the incubation medium (table 1). This finding differs from the inhibitory effect of epinephrine on  $\text{Ca}^{2+}$ -induced insulin secretion which was augmented by the addition of GTP up to 500  $\mu\text{M}$  [17].

In the presence of the non-hydrolyzable GTP-analog  $\text{GTP}\gamma\text{S}$  (10  $\mu\text{M}$ ) galanin is still able to exert its inhibitory effect on secretion. In contrast, a higher concentration of  $\text{GTP}\gamma\text{S}$  (100  $\mu\text{M}$ ) reduces the peptide-mediated inhibition (table 1). As in our previous studies [13,17] insulin secretion stimulated with 10  $\mu\text{M}$   $\text{Ca}^{2+}$  in the absence of galanin was not affected significantly by GTP or  $\text{GTP}\gamma\text{S}$  (not shown). These results indicate that galanin, in contrast to epinephrine, does not require the presence of hydrolyzable GTP, to cause the inhibition of insulin secretion.

In the next series of experiments the importance of endogenous GTP was further tested by the addition of GDP and its stable analog  $\text{GDP}\beta\text{S}$ . These nucleotides displace GTP from its protein-binding sites. GDP (100  $\mu\text{M}$ ) affects neither  $\text{Ca}^{2+}$ -induced secretion nor the inhibitory effect of galanin (fig.3).  $\text{GDP}\beta\text{S}$  (100  $\mu\text{M}$ ), in agreement with our earlier studies [13,17], attenuates  $\text{Ca}^{2+}$ -stimulated insulin secretion and abolishes the effect of galanin (fig.3). These results demonstrate that the effects of  $\text{GDP}\beta\text{S}$  and galanin on secretion are not additive, perhaps indicating a common site of action

Table 1

Effects of guanine nucleotides on galanin-induced inhibition of insulin secretion in permeabilized RINm5F cells

Addition	Inhibition (%)
A no guanine nucleotide	29.6 $\pm$ 6.2 (7)
B 100 $\mu\text{M}$ GTP	33.9 $\pm$ 2.7 (7)
C 10 $\mu\text{M}$ $\text{GTP}\gamma\text{S}$	35.4 $\pm$ 4.6 (7)
D 100 $\mu\text{M}$ $\text{GTP}\gamma\text{S}$	15.0 $\pm$ 2.5 (3)

RINm5F cells were permeabilized and incubated as described in section 2. Galanin was tested at 0.1  $\mu\text{M}$  in the absence or presence of GTP plus a regenerating system or  $\text{GTP}\gamma\text{S}$  as indicated. Results are expressed as % inhibition of insulin secretion due to increasing  $\text{Ca}^{2+}$  from 0.1 to 10  $\mu\text{M}$  in the absence or presence of the respective guanine nucleotide. Data are means  $\pm$  SE from the given number of independent experiments each performed in quintuplet

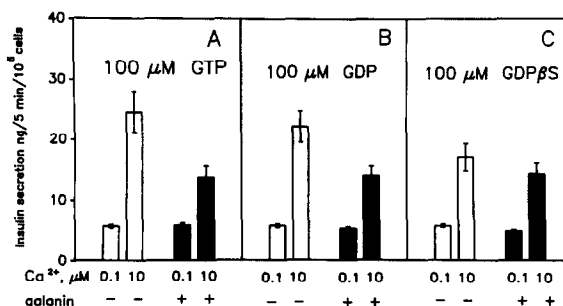


Fig.3. Effect of galanin on  $\text{Ca}^{2+}$ -induced secretion in the presence of GTP, GDP and  $\text{GDP}\beta\text{S}$ . Permeabilized RINm5F cells were incubated at 0.1 or 10  $\mu\text{M}$   $\text{Ca}^{2+}$  in the presence of 100  $\mu\text{M}$  GTP and a regenerating system (A), 100  $\mu\text{M}$  GDP (B) or 100  $\mu\text{M}$   $\text{GDP}\beta\text{S}$  (C) without (open bars) and with 0.1  $\mu\text{M}$  galanin (filled bars). Results are means  $\pm$  SE of 10 observations from 2 separate experiments.

for the two substances. In contrast to epinephrine inhibition of  $\text{Ca}^{2+}$ -induced insulin secretion [17], galanin action is thus not sensitive to blockade of GTP-binding sites by GDP. The present findings that galanin inhibits  $\text{Ca}^{2+}$ -stimulated insulin secretion in permeabilized cells indicate an action exerted at a point distal to the generation of soluble messenger molecules. Thus, in permeabilized cells (i) ion channels are short-circuited and the opening of  $\text{K}^+$  channels can be disregarded [9,10]; (ii) interference with  $\text{Ca}^{2+}$  handling can be excluded, since the  $\text{Ca}^{2+}$  concentration is clamped with EGTA; (iii) cyclic AMP levels are very low under the experimental conditions used [17] and alterations in adenylate cyclase activity [6] cannot explain the inhibition of insulin secretion. We describe here that the inhibition of  $\text{Ca}^{2+}$ -stimulated insulin release is overcome by pertussis toxin, as is the case for the other known actions of galanin, i.e. the opening of  $\text{K}^+$  channels [10], the lowering of  $[\text{Ca}^{2+}]_i$  [12] and the inhibition of adenylate cyclase [6] in intact cells.

The identity of the pertussis toxin-sensitive G-protein mediating the galanin effect, as well as the mechanism beyond G-protein activation, remain unknown. The putative site of action may be a fusion pore [19,20] formed by oligomerization of a specific pore-forming protein in a manner suggested for exocytosis of synaptic vesicles [21]. It is conceivable that complete inhibition of insulin secretion is a result of the concerted action of galanin on ion channels, adenylate cyclase and the direct interference with exocytosis.

**Acknowledgements:** We wish to thank A.-S. Annen and D. Nappay for technical assistance. This work was supported by the Swiss National Science Foundation (grant no. 3.214-0.85).

## REFERENCES

- [1] Ahrén, B., Rorsman, P. and Berggren, P.-O. (1988) *FEBS Lett.* 229, 233-237.
- [2] Dunning, B.E. and Taborsky, G.J. jr (1988) *Diabetes* 37, 1157-1162.
- [3] Dunning, B.E., Ahrén, B., Veith, R.C., Böttcher, G., Sundler, F. and Taborsky, G.J. jr (1986) *Am. J. Physiol.* 251, E127-E133.
- [4] Servin, A.L., Amiranoff, B., Rouyer-Fessard, C., Tatemoto, K. and Laburthe, M. (1987) *Biochem. Biophys. Res. Commun.* 144, 298-306.
- [5] Amiranoff, B., Servin, A.L., Rouyer-Fessard, C., Couvineau, A., Tatemoto, K. and Laburthe, M. (1987) *Endocrinology* 121, 284-289.
- [6] Amiranoff, B., Lorinet, A.-M., Lagny-Pourmir, I. and Laburthe, M. (1988) *Eur. J. Biochem.* 177, 147-152.
- [7] Wollheim, C.B. and Sharp, G.W.G. (1981) *Physiol. Rev.* 61, 914-973.
- [8] Ullrich, S. and Wollheim, C.B. (1984) *J. Biol. Chem.* 259, 4111-4115.
- [9] De Wille, J., Schmid-Antomarchi, H., Fosset, M. and Lazdunski, M. (1988) *Proc. Natl. Acad. Sci. USA* 85, 1312-1316.
- [10] Dunne, M.J., Bullett, M.J., Li, G., Wollheim, C.B. and Petersen, O.H. (1989) *EMBO J.* 8, 413-420.
- [11] Ahrén, B., Arkhammar, P., Berggren, P.-O. and Nilsson, T. (1986) *Biochem. Biophys. Res. Commun.* 140, 1059-1063.
- [12] Nilsson, T., Arkhammar, P., Rorsman, P. and Berggren, P.-O. (1989) *J. Biol. Chem.* 264, 973-980.
- [13] Vallar, L., Biden, T.J. and Wollheim, C.B. (1987) *J. Biol. Chem.* 262, 5049-5056.
- [14] Terashima, T., Katada, T., Oinuma, M., Inoue, Y and Ui, M. (1987) *Brain Res.* 417, 190-194.
- [15] Tamagawa, T., Niki, I., Niki, H. and Niki, A. (1985) *Biomed. Res.* 6, 429-432.
- [16] Jones, P.M., Fyles, J.M., Persaud, S.J. and Howell, S.L. (1987) *FEBS Lett.* 219, 139-144.
- [17] Ullrich, S. and Wollheim, C.B. (1988) *J. Biol. Chem.* 263, 8615-8620.
- [18] Hermansen, K. (1988) *Acta Endocrinol.* 119, 91-98.
- [19] Breckenridge, L.J. and Almers, W. (1987) *Proc. Natl. Acad. Sci. USA* 84, 1945-1949.
- [20] Breckenridge, L.J. and Almers, W. (1987) *Nature* 328, 814-817.
- [21] Thomas, L., Hartung, K., Langosch, D., Rehm, H., Bamberg, E., Franke, W.W. and Betz, H. (1988) *Science* 242, 1050-1053.