Several chimeric peptides consisting of the N-terminal fragment of galanin (GAL) and C-terminal fragments of other bioactive peptides (e.g. substance P, bradykinin, neuropeptide Y, mastoparan) have been synthesized and reported as high-affinity galanin receptor antagonists. Recently we have synthesized a new chimeric peptide, GAL(1-13)-[Ala\textsuperscript{10,11}]-ET-1(6-21)-NH\textsubscript{2}, consisting of the N-terminal fragment of GAL and the C-terminal fragment of endothelin-1 (ET-1) analogue. This chimera was previously shown to be a moderate-affinity ligand to hypothalamic galanin receptors with a $K_D$ value of 205 nM. However, its biological action has been unknown so far. In our studies we characterized the biological properties of this new chimeric analogue, investigating its action on rat isolated gastric smooth muscles and influence on insulin secretion from rat isolated islets of Langerhans. Data acquired in the course of our studies suggest that analogue GAL(1-13)-[Ala\textsuperscript{10,11}]-ET-1(6-21)-NH\textsubscript{2} does not seem to be a potent galanin receptor antagonist in the gastrointestinal tract.

Key words: galanin, chimeric analogues, insulin secretion, gastric smooth muscles contraction
INTRODUCTION

Galanin (GAL), a 29-amino-acid-residue neuropeptide, displays many types of interesting physiological and behavioural activity, which are mediated through its interaction with distinct G-protein-coupled membrane receptor subtypes (GALR1, GALR2 and GALR3), subsequently activating several signalling pathways (1-22). GAL has the ability to modulate pituitary hormone release, inhibits insulin secretion, affects memory, learning, feeding, pain threshold control and sexual behaviour. In the gastrointestinal (GI) tract GAL modulates gastric smooth muscles activity (12-16). However, due to the lack of specific antagonists in the GI tract the actual level of GAL involvement in GI motility remains largely unknown. Moreover, different GAL fragments might be recognized as ligands by different receptors in a species and locus-specific manner (3,22).

In recent years several chimeric peptides consisting of the N-terminal fragment 1-13 of galanin and C-terminal fragments of other bioactive peptides (e.g. substance P, bradykinin, neuropeptide Y, mastoparan) have been synthesized and reported as high-affinity GAL receptor antagonists in feeding, learning and pain paradigms (*Table 1*) (18-33). Some of them have been employed as valuable tools in studying the mechanisms responsible for the diverse biochemical actions of GAL. However, it is still unclear why some of these peptides act as antagonists whereas others act as agonists. The first hybrid obtained was 20-amino-acid-residue peptide of the composition GAL(1-13)-SP(5-11)-NH₂ (28). This peptide, named galantide or M15, was found to be potent antagonist for the inhibitory effect of GAL on glucose-induced insulin secretion from pancreatic islets of Langerhans (29). M15 blocks also the neuronal action of GAL in the central nervous system (23). However, in the gastrointestinal smooth muscles galantide acts as full agonist of galanin (25).

In present studies we have synthesized a new chimeric GAL analogue, GAL(1-13)-[Ala₁₀,₁₁]ET-1(6-21)-NH₂, consisting of the N-terminal fragment 1-13 of galanin and the C-terminal fragment 6-21 of endothelin-1 (ET-1) analogue, in which the Cys residues, involved in disulphide bridges, were replaced by L-Ala residues (*Table 2*). This chimera was previously shown to be a moderate-affinity ligand to hypothalamic GAL receptors with a Kᵥ value of 205 nM (32). However, its biological action has been unknown so far. In our studies we aimed to characterize the biological properties of this new chimeric analogue, investigating its action on rat isolated gastric smooth muscles and influence on glucose-induced insulin secretion from rat isolated islets of Langerhans. We have also synthesized porcine galanin, its N-terminal fragment GAL(1-15)-NH₂ and the C-terminal fragment 6-21 of endothelin-1 analogue, Ac-[Ala₁₀,₁₁]ET-1(6-21)-NH₂, as a control. We expected that the results obtained in the course of such studies may provide more information about molecular mechanism of GAL action and may be helpful in a purposeful search for specific GAL receptor antagonist in the gastrointestinal tract.
MATERIALS AND METHODS

Synthesis of peptides.

Peptides (Table 2) were synthesized by the solid phase peptide synthesis with the use of a Labortec AG model SP 650 peptide synthesizer and 9-fluorenylmethoxycarbonyl (Fmoc) strategy (34). TentaGel S RAM resin for peptide amides (capacity 0.25 mmol/g) was used as the starting material. All amino acids were coupled as active derivatives with the use of the O-(benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate with addition of N-hydroxybenzotriazole (1:1) coupling method. Deprotection of the Fmoc group was carried out with 20% piperidine in N,N-dimethylformamide. After synthesis had been completed the peptides were cleaved from resin with

Table 1. The structure of some chimeric galanin analogues and its affinity to rat hypothalamic galanin receptors (18-33).

<table>
<thead>
<tr>
<th>Ligand</th>
<th>K&lt;sub&gt;D&lt;/sub&gt; [nM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAL (porcine)</td>
<td></td>
</tr>
<tr>
<td>GWTLNSAGYLLGP HAIDNHSFHDKYGLA-NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.8</td>
</tr>
<tr>
<td>GAL(1-13)-NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>GWTLNSAGYLLGP-NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>150</td>
</tr>
<tr>
<td>M15; galantide; GAL(1-13)-SP(5-11)-NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>GWTLNSAGYLLGP QQFFGLM-NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.1</td>
</tr>
<tr>
<td>M32; GAL(1-13)-NPY(25-36)-NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>GWTLNSAGYLLGP RHYINLITRQRY-NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.1</td>
</tr>
<tr>
<td>M35; GAL(1-13)-BK(2-9)-NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>GWTLNSAGYLLGP PPGFSPFR-NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.3</td>
</tr>
<tr>
<td>M40; GAL(1-13)-PPALALA-NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>GWTLNSAGYLLGP PPALALA-NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>10</td>
</tr>
<tr>
<td>C7; GAL(1-13)-spantyd-NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>GWTLNSAGYLLGP (D-R)PKPQQ(D-W)F(D-W)LL -NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.2</td>
</tr>
<tr>
<td>Galparan; GAL(1-13)-Mas-NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>GWTLNSAGYLLGP INLKALAALAKKIL-NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>5.8</td>
</tr>
</tbody>
</table>

BK - bradykinin, GAL - galanin, Mas - mastoparan, NPY - neuropeptide Y, SP - substance P

Table 2. The primary structures of the synthesized peptides.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Amino acid sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAL (porcine)</td>
<td>GWTLNSAGYLLGP HAIDNHSFHDKYGLA-NH&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td>GAL(1-15)-NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>GWTLNSAGYLLGPHA-NH&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td>GAL(1-13)-[Ala&lt;sup&gt;11,15&lt;/sup&gt;]ET-1(6-21)-NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>GWTLNSAGYLLG[PLMDKEAVYFAHLDIIW]-NH&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td>Ac-[Ala&lt;sup&gt;11,15&lt;/sup&gt;]ET-1(6-21)-NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Ac-LMDKEAVYFAHLDIIW-NH&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

Ac - acetyl, ET-1 - endothelin-1, GAL - galanin
trifluoroacetic acid/phenol/triisopropylsilane/water (88:5:2:5 v/v/v/v) mixture. Crude peptides thus obtained were purified by Reverse Phase High Performance Liquid Chromatography (RP-HPLC) on preparative Vydac C-18 column (32 x 240 mm, 15-20 µm particle size) with several isocratic systems and linear gradients of acetonitrile (ACN) in 0.1% trifluoroacetic acid (TFA). Eluates were fractioned and analyzed by the analytical RP-HPLC. Purity of the peptides (greater than 98%) was checked by an analytical Beckman "System Gold" chromatograph with a Vydac C-18 column (4.6 x 250 mm, 5µm particle size) with several isocratic systems and linear gradients of ACN in 0.1% TFA. Identities of peptides were confirmed by amino acid analysis and Electrospray Ionization Mass Spectrometry (ESI-MS).

**Smooth muscles preparation.**

Longitudinal muscle strips of Albino-Wistar rats (weighing 180-250 g) were prepared according to Vane's procedure (35) and mounted vertically in organ baths bubbled with carbogen (O2/CO2 95/5). The organ baths were filled with Tyrode solution (37°C). The composition of Tyrode solution (pH 7.2-7.4) was (mM): NaCl 136.9, KCl 3.35, CaCl2 1.46, MgCl2 1.03, NaHCO3 11.9, NaH2PO4 0.48, glucose 5.0. The tissues were kept at a resting tension of 2.0 g. One end of each strip was attached to a fixed support and the free one to a lever connected via a spring to force displacement transducers for the isotonic registration of mechanical activity. Tissues were allowed to equilibrate for 90 min before the start of the experiment. The buffer was changed every 5 min, except for the contact time of the tested peptides with the tissues. To eliminate the probable involvement of cholinergic and adrenergic components in the responses investigated, the studies were carried out in the presence of atropine (1µM), hexamethonium (10 µM) and guanethidine (10 µM). To prevent excessive degradation of peptides the experimental buffer contained amastatin (10 µM) and phosphoramidon (1 µM). Viability and contractility of each strip were examined by the addition of acetylcholine chloride (10 µM/l).

**Pancreatic islets preparation.**

Male Wistar rats (weighting 180-220 g) were kept under standard laboratory conditions (a natural light-dark cycle) with unrestricted access to food and tap water. Pancreatic rat islets of Langerhans were isolated by collagenase digestion using an intraductal injection technique (36). After the decapitation, a midline laparotomy was performed and 7-10 ml of the collagenase type XI solution (1mg/ml) was injected into the duct system of the pancreas. The inflated pancreas was removed from the animal and placed in a water bath at 37°C. Digestion of the pancreas was completed after 10 min and the tissue was rinsed three times with ice-cold Krebs buffer to remove exocrine cells and collagenase. The composition of Krebs buffer (pH 7.38) was (mM): NaCl 120, KCl 4.8, CaCl2 2.5, MgCl2 1.2, NaHCO3 24, bovine serum albumin (0.1%). The islets of comparable size were hand picked under a stereomicroscope and preincubated in Krebs buffer containing 15 mM of glucose. The buffer was bubbled with carbogen (O2/CO2 95/5). Selected islets were allowed to equilibrate for 60 min and then were rinsed with Krebs buffer. Next, they were divided into groups of 3, and each was transferred to separate test tube containing Krebs buffer and placed in a water bath at 37°C.

**Determination of smooth muscles contraction.**

Experiments were started when reproducible contractile responses to acetylcholine chloride (10 µM/l) were obtained. Conventional concentration-contraction curves were constructed in a non-cumulative manner, by adding the increasing concentrations of peptides directly into the organ baths, until the maximum muscular effect occurred (when the contraction could not be further increased by
a higher concentration of the peptide). When the maximum contraction had developed, the tissues were washed out for 2-3 min until the length of the strip returned to basal level. In order to avoid tachyphylaxis in isolated gastric fundus strips, the peptides were applied at 30 min intervals. No more than two complete concentration-response curves were plotted for each strip. Viability and reproducible contractility of each strip were examined at the end of each experimental session by a submaximal response to acetylcholine chloride, at the same concentration as at the start.

**Determination of insulin secretion**

*In vitro* insulin secretion from the isolated rat pancreatic islets was studied during static incubation. All peptides were tested at two concentrations: 100 nM and 1 µM. Each test tube contained Krebs buffer supplemented with glucose at a concentration of 3, 10 and 20 mM (as control) or 10 mM of glucose with the addition of one peptide tested. However, the antagonistic/agonistic activities of GAL analogues were checked by the simultaneous application of GAL and one of the analogues, used at the same concentration. The isolated islets were incubated for 60 min in a water bath at 37°C and an atmosphere of carbogen. After 1 h, samples of the incubation medium were collected and immediately frozen at -20°C for subsequent analysis of insulin. The concentration of insulin in the incubation medium was determined radioimmunologically (37) using anti-rat insulin antibody and 125I-labelled rat insulin (DRG, USA).

**Statistical analysis of the acquired data.**

Efficacy and potency (EC50) are expressed as mean values with 95% confidence limits. EC50, relative potencies of galanin analogues and their statistical significance were determined using the Pharmacological Calculation System version 4 computer program. Efficacy and EC50 were compared using the non-parametric Mann-Whitney, Wilcoxon signed-rank test for pairs or one-way analysis of variance (ANOVA) plus Bonferroni post-ANOVA tests. Hill's coefficients are expressed as mean±SEM (standard error of mean). To examine whether the value of Hill's coefficients are different from unity a non-parametric Mann-Whitney test was used. Two-tailed P values of less than 0.05 were interpreted as indicating a significant difference. Values of insulin level [ng/60min/islet] are presented as means±SEM (standard error of mean). Student's t-test and one-way ANOVA were used to test the degree of significance.

**RESULTS**

**Effects of peptides on rat gastric smooth muscles.**

Isotonic contractions of the isolated gastric fundus strips were measured. Results are presented as a comparison of some pharmacological variables obtained from the respective concentration-response curves. Results were expressed as the maximum response (efficacy) produced by the peptide tested and as the effective concentration required to produce 50% of the maximum contraction (EC50). Efficacies were expressed as a percentage of the maximum contractile effect of GAL control. Moreover, Hill's coefficients were calculated to examine whether ligand-receptor interactions followed the classical receptor theory, according to which the biological effect is proportional to the concentration of the hormone-receptor complex in which one hormone molecule
binds to one receptor. In this case, Hill's coefficient is equal to unity. Otherwise (when Hill's coefficient is significantly lower or greater than unity) the action of the hormone is not consistent with the classical receptor theory and may either affect secondary activity carriers, such as cyclic nucleotides, or bind to several agonist molecules.

All peptides contracted longitudinal rat gastric fundus strips in a concentration-dependent manner (Table 3). Porcine GAL evoking reproducible contraction at 3 nmol/l and a maximum contraction at 1 mmol/l. The EC50 value estimated from the concentration-contraction curves equaled 13.39 nM (Table 3). Hill's coefficient for GAL was not different from unity indicating an interaction of one molecule with one receptor, thus fulfilling the criteria of the classical receptor theory. The GAL(1-15)-NH2 fragment was also active, exhibiting about 8% of the native GAL potency, with a significantly lower maximum response of 59.6% and a higher EC50 of 174 nM than GAL. Hill's coefficient for GAL(1-15)NH2 was not different from unity.

GAL(1-13)-[Ala11,15]ET-1(6-21)-NH2, the new chimeric galanin analogue (designed as GAL antagonist), has shown activity similar to that of GAL(1-15)-NH2. This new analogue displayed about 7% of GAL potency, with a significantly lower maximum response of 59.61% and a higher EC50 of 193.5 nM than GAL. Hill's coefficient for GAL(1-13)-[Ala11,15]ET-1(6-21)-NH2 was significantly lower than unity, suggesting that peptide molecule activates of more than one receptor type, prompting negative ligand-receptor cooperativity or a multi-step ligand-receptor interactions. However, peptide Ac-[Ala11,15]ET-1(6-21)-NH2 (used as a control) has shown about 3.59-fold higher potency than GAL. This analogue has shown a significantly lower EC50 of 3.69 nM and a lower maximum response of 71.57% than GAL. Hill's coefficient for Ac-[Ala11,15]ET-1(6-21)-NH2 was also significantly lower than unity, indicating that the rules of the classical receptor theory may not apply.


<table>
<thead>
<tr>
<th>Peptide</th>
<th>Efficacy [%]</th>
<th>EC50 (nM)</th>
<th>Relative potency</th>
<th>Hill's coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAL (porcine)</td>
<td>100</td>
<td>13.39 (6.17-29.05)</td>
<td>1</td>
<td>0.96±0.05</td>
</tr>
<tr>
<td>GAL(1-15)-NH2</td>
<td>59.64* (53.39-65.32)</td>
<td>174* (105-288)</td>
<td>0.08</td>
<td>0.99±0.08</td>
</tr>
<tr>
<td>GAL(1-13)-[Ala11,15]ET-1(6-21)-NH2</td>
<td>59.61* (33.58-79.67)</td>
<td>193.54* (99.17-373.82)</td>
<td>0.07</td>
<td>0.53±0.02 b</td>
</tr>
<tr>
<td>Ac-[Ala11,15]ET-1(6-21)-NH2</td>
<td>71.57 (53.76-77.80)</td>
<td>3.69* (1.36-10.10)</td>
<td>3.59</td>
<td>0.65±0.09 b</td>
</tr>
</tbody>
</table>

*P < 0.05 vs. GAL, b value of Hill's coefficient significantly different from 1.0 at P < 0.05
Effects of peptides on insulin secretion from rat isolated islets of Langerhans.

The insulin level for 10 mM of glucose (4.23 ng/60min/islet) was a reference for all experiments. Studies have shown that all peptides decrease glucose-induced insulin secretion from rat pancreatic islets in a concentration-dependent manner (Figure 1 and 2). Porcine GAL used at a concentration of 1 µM caused a

![Graph showing effects of peptides on insulin secretion from rat pancreatic islets induced by 10 mM glucose. Results are presented as means±SEM of insulin level induced by the tested peptide. Asterisks indicate the probability level of random difference vs. 10 mM of glucose (*p<0.01).]
decrease in the insulin secretion by about 40% (2.54 ng/60min/islet) and a concentration of 100 nM lowered it by about 38% (2.59 ng/60min/islet) as compared to the control tissue incubated 10 mM of glucose. The N-terminal fragment of GAL inhibited insulin secretion with lower potency in comparison to

Figure 2. Effects of peptides on insulin secretion from the rat pancreatic islets induced by 10 mM glucose in the presence of appropriate concentration of GAL. Results are presented as means±SEM of insulin level induced by the tested peptide. Asterisks indicate the probability level of random difference vs. 10 mM of glucose (*p<0.01).
native GAL. This 15-amino-acid fragment of GAL decreased the insulin level by about 16% (3.56 ng/60min/islet) at a concentration of 1 µM and by about 11% (3.78 ng/60min/islet) at a concentration of 100 nM. The simultaneous application of GAL and GAL(1-15)-NH₂, used at the same concentration, caused an increase in the inhibitory action of GAL by about 30% and 37% (at a lower and higher concentration, respectively).

GAL(1-13)-[Ala¹¹,¹⁵]ET-1(6-21)-NH₂, new chimeric galanin analogue (designed as GAL antagonist), has also shown the inhibitory activity (stronger than GAL) on glucose-induced insulin secretion as it lowered the insulin level by about 45% (2.33 ng/60min/islet) at a concentration of 100 nM and about 58% (1.76 ng/60min/islet) at a concentration of 1 µM. The simultaneous application of GAL and GAL(1-13)-[Ala¹¹,¹⁵]ET-1(6-21)-NH₂, used at the same concentration, caused an increase in the inhibitory action of GAL by about 41% and 66% (at a lower and higher concentration, respectively). However, the C-terminal fragment of endothelin-1 analogue Ac-[Ala¹¹,¹⁵]ET-1(6-21)-NH₂ (used as a control) lowered the insulin level by about 40% (2.54 ng/60min/islet) at a concentration of 100 nM and about 41% (2.48 ng/60min/islet) at a concentration of 1 µM. Moreover, this analogue increased the inhibitory effect of GAL by about 65% and 53% (at a lower and higher concentration, respectively).

DISCUSSION

To determine structural requirements for the biological action of GAL numerous galanin fragments and analogues have been synthesized and studied (18-33,38-42). Structure-activity studies showed that the activity of GAL is connected with the N-terminal 1-15 fragment, needed for full activation of GAL receptors and the biological action (22,27,32,38-40). Trp² in the amino acid sequence of GAL is crucial for high-affinity binding to its receptors. Substitution of this residue with L-Ala, D-Trp, L-Phe or L-Tyr markedly decreased or abolished GAL action. This observation suggests the crucial role of the aromatic ring of the amino acid residue in position 2 for the effect of GAL. Other important residues are Leu⁴, Ser⁶, Gly⁸ and Tyr⁹, which also contribute significantly to the activation of GAL receptors (22,27,32,38-40).

So far several chimeric galanin analogues have been also synthesized and their activities tested. M15 (galantide), the first hybrid obtained, antagonized the GAL mediated insulin secretion from pancreatic rat islets (28,29). Moreover, M15 blocked the neuronal action of GAL in the central nervous system (23), but in gastrointestinal smooth muscles galantide acted as a full agonist of GAL (25). Another chimeric peptide, M35, was found to be a high-affinity galanin receptor antagonist in rat spinal cord, rat hippocampus and isolated mouse pancreatic islets (24,25,33). In RINm5F cells M35 had a dual effect on the GAL-mediated inhibition of insulin secretion (34). At low concentrations M35 antagonized the
effect of GAL, whereas at higher concentrations M35 acted as a GAL receptor agonist. Thus, M35 has shown mixed agonist/antagonist properties, which may result from the chimeric nature of M35 (39).

Recently we studied the effects of galantide (M15) and six new chimeric peptides on the glucose-induced insulin secretion from isolated rat pancreatic islets of Langerhans and on contraction of rat gastric smooth muscles (41-43). All chimeric peptides were composed of the N-terminal galanin 1-13 fragment or its analogues linked to the C-terminal portions of substance P analogues or scylloinrin I (SCY-I) analogues. Our studies have shown that the inhibitory effect of GAL on insulin secretion was antagonized (in a dose-dependent manner) by galantide, which almost completely abolished the action of galanin. GAL(1-13)-[Nle\textsuperscript{10}]SCY-I(3-10)NH\textsubscript{2} and [Cle\textsuperscript{4}]galantide were stronger antagonists of GAL than M15, completely abolished the inhibitory effect of GAL and caused a considerable increase in the insulin secretion. Other chimeric peptides: GAL(1-13)-[Abu\textsuperscript{8},Nle\textsuperscript{10}]SCY-I(3-10)-NH\textsubscript{2}, GAL(1-14)-[Abu\textsuperscript{8}]SCY-I, [Hse\textsuperscript{6}]galantide and [Phe(4F)\textsuperscript{17}]galantide were very weak antagonists of GAL. However, in rat gastric smooth muscles these chimeric ligands were found to be full GAL receptor agonists (25,42,43). They contracted rat gastric smooth muscles in a concentration-dependent manner with significantly increased activity as compared to GAL(1-15)-NH\textsubscript{2}.

Some authors suggest that the effects of some of the chimeric GAL ligands are mediated through other receptors that are still not characterized (22). Another explanation might be that the chimeric ligands are degraded and subsequently bind as shorter fragments to the galanin receptors, mediating the same agonistic effect as short fragments of GAL (22). However, our latest studies suggest that the action of some of the chimeric peptides (e.g. M15) on smooth muscles of rat gastric fundus depended not only on the myogenic interaction of this peptide with galanin binding sites, but also on activation of tachykinin receptors or release of endogenous mediators from presynaptic terminals (43). This might explain the lack of antagonistic action of chimeric ligands in gastric smooth muscles.

In the present study we have researched biological properties of a new chimeric GAL analogue, GAL(1-13)-[Ala\textsuperscript{10,11}]ET-1(6-21)-NH\textsubscript{2}, investigating its action on rat isolated gastric smooth muscles and the influence on glucose-induced insulin secretion from rat isolated islets of Langerhans. This chimera, consisting of the C-terminal fragment 6-21 of endothelin-1 analogue, BQ-3020 (ET-B receptor agonist), in which the Cys residues were replaced by L-Ala residues, has been previously shown to be a moderate-affinity ligand to hypothalamic GAL receptors (32). However, its biological action had been unknown so far.

Our studies confirmed the earlier observations that porcine GAL is a full GAL receptor agonists in isolated rat gastric smooth muscles and pancreatic islets of Langerhans. Its N-terminal fragment GAL(1-15)-NH\textsubscript{2}, which plays a key role in high-affinity binding of GAL to its receptors, was also active, exhibiting about
8% and 35% of the native GAL potency (in gastric smooth muscles and pancreatic islets, respectively). This suggests that the C-terminal fragment GAL(16-29) is required for full activation of GAL receptors in the GI tract. The analogue GAL(1-13)-[Ala$^{11,15}$]ET-1(6-21)-NH$_2$ (designed as GAL antagonist), in contrast to other chimeric GAL analogues such as M15, M35 or galparan (reported as high-affinity galanin receptor antagonists), has shown agonistic activity both in rat pancreatic islets of Langerhans and rat gastric smooth muscle cells. In gastric smooth muscles this chimera has shown activity similar to that of GAL(1-15)-NH$_2$, however, in pancreatic islets of Langerhans GAL(1-13)-[Ala$^{11,15}$]ET-1(6-21)-NH$_2$ has shown inhibitory activity stronger than GAL.

Our studies suggest that the analogue GAL(1-13)-[Ala$^{10,11}$]ET-1(6-21)-NH$_2$ does not seem to be a potent galanin receptor antagonist in the gastrointestinal tract. We conclude that the analogue GAL(1-13)-[Ala$^{11,15}$]ET-1(6-21)-NH$_2$ may activate different receptor subtypes and/or use different mechanism(s) (activate the release of endogenous mediators from presynaptic terminals) to regulate its action in rat gastric smooth muscles and rat pancreatic islets of Langerhans. However, further studies are required to fully explain the activity of this analogue. We expect that results obtained in the course of such studies may provide more information about molecular mechanism(s) of galanin (and its chimeric analogues) action and may be helpful in a purposeful search for specific GAL receptor antagonist in the gastrointestinal tract.

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