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ACTIONS OF SEVERAL SUBSTITUTED SHORT ANALOGUES OF PORCINE GALANIN ON ISOLATED RAT FUNDUS STRIPS: A STRUCTURE-ACTIVITY RELATIONSHIP

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The activity of porcine galanin (Gal) fragments and analogues were tested *in vitro* using rat gastric fundus strips. The peptides contracted longitudinal smooth muscle in a concentration-dependent manner with the following order of potency: [Nle^4]Gal(1-15), Gal(1-15), [Cle^4]Gal(1-15), [Hse^6]Gal(1-15), [Val^4]Gal(1-15), [Ile^4]Gal(1-15), [endoTrip^{2a}, Cle^4]Gal(1-15), [desThr^3, Cle^4]Gal(1-15), [D-Leu^4]Gal(1-15), [desLeu^4]Gal(1-15). On the contrary [desTrp^2, Val^4]Gal(1-15) remained inactive up to 10 μM. The values of Hill's coefficients estimated from the appropriate concentration-contraction curves for all analogues except for [Val^4]Gal(1-15), [Hse^6]Gal(1-15), [endoTrp^{2a}, Cle^4]Gal(1-15), [desLeu^4]Gal(1-15) and [D-Leu^4]Gal(1-15) did not significantly differ from unity. Our results indicate that the integrity of the first four N-terminal amino acids of Gal molecule is essential for the full excitatory myogenic action of the peptide in rat gastric fundus. Similarly, substitution, addition or deletion of amino acid residues in positions two, three, four and six can considerably influence the ability of Gal analogues to interact with Gal receptors. The data acquired in the course of our structure-activity study suggest that both N- and C-terminals of Gal molecule contribute towards the affinity and activity of Gal in rat gastric smooth muscle cell receptors.

Key words: *galanin, gastric fundus, smooth muscle, rat.*

INTRODUCTION

Porcine Gal is a 29 amino acid peptide isolated from the extracts of the upper intestine by the method based on detecting peptide's C-terminal amidated structure (1). Gal-like immunoreactivity is widely distributed in the central, peripheral nervous systems, endocrine system, genitourinary tract and gut of several mammalian species including man (2—3). All Gal molecules except for the tuna fish Gal share a conserved 14 amino acid N-terminal and

a variant COOH-terminal region. Gal is a ubiquitous neuropeptide transmitter activating at least three types of G-protein coupled receptors in order to regulate a variety of physiologic processes such as feeding, lactation, growth, gastrointestinal (GI) motility, gastric acid secretion and memory (2, 4—7). There is abundant evidence that Gal modulates gut motility by acting at specific receptors, but due to the lack of specific antagonists in the Gl tract the actual level of Gal involvement in gut motility remains largely unknown (8—11).

Both N- and C-terminal Gal sequences are important for specific types of biological responses and different Gal fragments might be recognised as ligands by different receptors in a species and locus-specific manner. Currectly, we have performed structure-activity studies of the substituted short analogues of porcine Gal, investigating their contractile action on rat isolated gastric fundus strips, employed as *in vitro* assay of peptides activity (8). We aimed at a closer characterisation of the identity of the molecular domains responsible for binding and activation of Gal receptors in rat gastric fundus. We have chosen this particular group of substituted 15 amino acid analogues of Gal for our studies, because of the interesting properties of [Lys^{14}]Gal(1-15)-NH_2, which is a partial agonist at Gal receptors in rat stomach (12). Consequently it seemed clear that data obtained in the course of such studies might be helpful in a purposeful search for specific Gal receptor antagonists in the GI tract.

MATERIALS AND METHODS

Animals and tissue preparation

Albino-Wistar rats of either gender (180—250 g) were kept in normal laboratory conditions, with standard chow pellets and tap water available *ad libitum*. Animals were fasted overnight, sacrificed by cervical dislocation and longitudinal muscle strips of gastric fundus were prepared and suspended at a resting tension of 2.0 g (13). Organ baths contained Tyrode solution (pH 7.4; 37°C, gassed with carbogen) and the mechanical activity of the tissues were recorded isotonically. Composition of Tyrode buffer was as follows (mM): NaCl 136.9, KCl 3.35, $CaCl_2$ 1.46, $MgCl_2$ 1.03, $NaHCO_3$ 11.9, NaH_2PO_4 0.48, glucose 5.0. All studies were carried out in the presence of atropine sulphate (1 μM), guanethidine (3 μM), amastatin (10 μM) and phosphoramidon (1 μM). Tissues were allowed to equilibrate for 90 min before the beginning of experiment. The buffer was changed every 5 min, except for the contact time of the test peptides with tissues.

Concentration-response curves for Gal and its short analogues

Experiments were started when reproducible response to carbachol was obtained (30 nM). No more than two, non-cumulative concentration-response curves were constructed using one

analogue on each strip: a control one for Gal and another for its analogues (applied alternately). The contact time of the peptide with muscle strips ranged from 1 to 3 min (12; 14). The tissues were washed out until the length of the strip returned to basal level. In order to avoid tachyphylaxis, Gal was applied at 20—30 min intervals. Viability and reproducible contractility of each strip were examined at the end of each experiment by a contractile response to carbachol.

Drugs

Carbachol was obtained from Sigma (St. Louis, MO, USA). Other chemicals were purchased from P.P.H. Polskie Odczynniki Chemiczne (Gliwice, Poland). Gal peptides (for detailed structure see *Table 1*) were synthesized by Rekowski, Ruczyński and Szyk, as described elsewhere (12, 14).

Table 1. Detailed structure of the investigated peptides.

Peptide	1 5 10 15 20 25 29
Gal(1-29)	G-W-T-L-N-S-A-G-Y-L-L-G-P-H-A-I-D-N-H-R-S-F-H-D-K-Y-G-L-A-NH_2
Gal(1-15)	G-W-T-L-N-S-A-G-Y-L-L-G-P-H-A-NH_2
[desTrp^2, Val^4]Gal(1-15)	G----T-X_1-N-S-A-G-Y-L-L-G-P-H-A-NH_2
[Val^4]Gal(1-15)	G-W-T-X_1-N-S-A-G-Y-L-L-G-P-H-A-NH_2
[Cle^4]Gal(1-15)	G-W-T-X_2-N-S-A-G-Y-L-L-G-P-H-A-NH_2
[desThr^3, Cle^4]Gal(1-15)	G-W—X_2-N-S-A-G-Y-L-L-G-P-H-A-NH_2
[Nle^4]Gal(1-15)	G-W-T-X_3-N-S-A-G-Y-L-L-G-P-H-A-NH_2
[Ile^4]Gal(1-15)	G-W-T-X_4-N-S-A-G-Y-L-L-G-P-H-A-NH_2
[D-Leu^4]Gal(1-15)	G-W-T-X_5-N-S-A-G-Y-L-L-G-P-H-A-NH_2
[desLeu^4]Gal(1-15)	G-W-T----N-S-A-G-Y-L-L-G-P-H-A-NH_2
[Hse^6]Gal(1-15)	G-W-T-L-N-X_6-A-G-Y-L-L-G-P-H-A-NH_2
[endoTrp^{2a}, Cle^4]Gal(1-15)	G-W-W-T-X_2-N-S-A-G-Y-L-L-G-P-H-A-NH_2

Abbreviations: A-Ala; D-Asp; F-Phe; G-Gly; H-His; I-Ile; K-Lys; L-Leu; M-Met; N-Asn; P-Pro; Q-Gln; R-Arg; T-Thr; S-Ser; Y-Tyr; W-Trp; X_1-Val; X_2-Cle; X_3-Nleu; X_4-Ile; X_5-D-Leu; X_6-Hse; [endoTrp^{2a}]Gal(1-15) — contains two Trp residues, Gal-galanin.

Statistical analysis of the results

Results are expressed as a percentage of the maximum response induced by each peptide. Efficacy, potency (EC_{50}) and the slope of the concentration-response curves are expressed as means with 95% confidence limits. Efficacy is expressed as a percentage of the maximum contractile effect of Gal. EC_{50}, the slopes of the dose response curves, relative potencies of Gal analogues and their statistical significance were determined using version 4 of the Pharm/PCS computer programme (15). Efficacy, EC_{50} were compared using non-parametric Mann-Whithey, Wilcoxon signed-rank test for pairs or one-way analysis of variance (ANOVA) plus Bonferroni post-ANOVA tests, where required. Hill's coefficient was calculated using a program based on *Biodata handling with microcomputers* (16). Hill's coefficient is expressed as a mean ± standard error of mean (SEM). To test whether the Hill's coefficient is different from unity a non-parametric Mann-Whitney test was used. Two-tailed p values of less than 0.05 were taken to indicate a significant difference.

RESULTS

Effects of Gal and Gal(1-15) on rat gastric fundus strips

Gal and Gal(1-15) evoked concentration-dependent contractions of rat fundus strips, each yielding a typically shaped response curve. Gal gave reproducible effects at 1 nM, a maximum at 1000 nM and a fall-down effect at the supramaximal concentrations. EC_{50} of Gal was 13.39 nM (6.17—29.05). Hill's coefficient equalled 0.96 (*Table 2*). The concentration-contraction curve of Gal(1-15) was to the right of that of Gal, with detectable contractions occurring at 10 nM and a maximum at 6 μM; EC_{50} reached 174 nM (105—288) and Hill's coefficient amounted to 0.99. The efficacy of Gal(1-15) was remarkably lower than that of Gal (*Table 3*).

Table 2. A comparison of some pharmacological variables as obtained from their respective Gal and Gal(1-15) concentration-contraction curves.

Peptide	Efficacy [%]	EC_{50} (nM)	Relative potency	Slopes of concentration-response curves	Hill's coefficient	Number of experiments
Gal	100	13.39 (6.17—29.05)	1	34.63 (23.72—45.54)	0.96 ± 0.05	10
Gal(1-15)	59.64 * (53.39—65.32)	174 * (105—288)	0.08 (0.04—0.16)	35.57 (27.59—43.54)	0.99 ± 0.08	6

Data are expressed as means with 95% confidence limits (ranges given in parentheses). Hill's coefficient is given as a mean ± standard error of the mean (SEM). Efficacy refers to the maximum response produced by the test peptide and is expressed as a percentage of the maximum contraction to Gal. The potency of each test peptides (EC_{50}) was calculated from the appropriate concentration-response curves. Relative potency was described as the ratio of the equieffective concentrations of each peptide from their respective concentration-effect relations. * $P < 0.05$ Gal(1-15) vs. Gal.

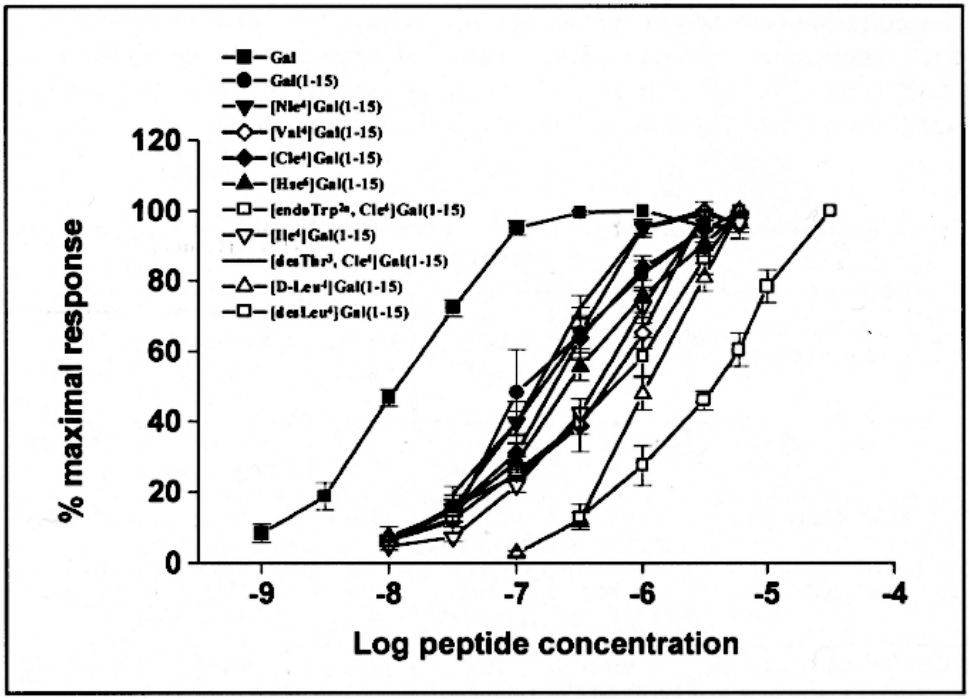

Fig. 1. Non-cumulative concentration-response curves of gastric fundus smooth muscle exposed to Gal, Gal(1-15) and its analogues. Data were normalised as percentage of the maximal response to peptide and plotted against log peptide concentration. Data were as means ± SEM for at least 6—10 different tissue strips.

All peptides apart from [desTrp^2, Val^4]Gal(1-15) contracted longitudinal rat gastric fundus strips in a concentration-dependent manner. [desTrp^2, Vat^4]Gal(1-15) was inactive up to 10 μM. However this inactive analogue did not act as an antagonist of Gal receptors in the gastric fundus even at the highest concentration studied. Potency of Gal(1-15) analogues declined in the following order: [Nle^4]Gal(1-15), Gal(1-15), [Cle^4]Gal(1-15), [Hse^6]Gal(1-15), [Val^4]Gal(1-15), [Ile^4]Gal(1-15), [endoTrip^{2a}, Cle^4]Gal(1-15), [desThr^3, Cle^4]Gal(1-15), [D-Leu^4]Gal(1-15), [desLeu^4]Gal(1-15). EC_{50}s of [desThr^3, Cle^4]Gal(1-15), [D-Leu^4]Gal(1-15), [desLeu^4]Gal(1-15) and [endoTrp^{2a}, Cle^4]Gal(1-15) were significantly higher than that of Gal(1-15). However, only the efficacy of [desLeu^4]Gal(1-15) was notably lower than that of Gal(1-15).

[Nle^4]Gal(1-15), [Cle^4]Gal(1-15), [Val^4]Gal(1-15), [Hse^6]Gal(1-15) and [endoTrp^{2a}, Cle^4]Gal(1-15) evoked reproducible contractions at 10 nM reaching the maximum effect at 3 or 6 μM. Their EC_{50}s equalled 143, 196, 296, 235 and 351 nM, respectively. Hill's coefficients obtained from the

concentration-contraction curves of [Nle^4]Gal(1-15), [Cle^4]Gal(1-15) were not significantly different from unity, whereas Hill's coefficients for [Val^4]Gal(1-15), [Hse^6]Gal(1-15) and [endoTrp^{2a}, Cle^4]Gal(1-15), were significantly lower than unity, namely 0.67, 0.78 and 0.56.

Table 3. A comparison of some pharmacological variables of Gal(1-15)-NH_2 and some substituted analogues.

Test peptide	Efficacy [%]	EC_{50} [nM]	Relative potency	Slopes of the concentration--response curves	Hill's coefficient
Gal(1-15)	100	174 (105—288)	1	35.57 (27.59—43.54)	0.99±0.08
[Cle^4]Gal(1-15)	108 (62.28—146)	196 (129—298)	0.91	37.22 (30.21—44.22)	0.99±0.04
[Val^4]Gal(1-15)	93.84 (72.03—135)	296 (159—553)	0.59	35.85 (25.68—46.01)	0.67±0.05*
[desThr^3, Cle^4]Gal(1-15)	103 (69.81—133)	365* (243—547)	0.45	43.39 (34.05—52.74)	0.88±0.04
[Nle^4]Gal(1-15)	111 (80.92—140)	143 (90.44—226)	1.13	41.80 (32.08—51.52)	0.90±0.02
[Ile^4]Gal(1-15)	104 (61.43—169)	302 (175—520)	0.49	38.32 (28.84—47.80)	0.86±0.04
[D-Leu^2]Gal(1-15)	102 (72.73—154)	1001* (399—2510)	0.19	54.21 (18.88—89.54)	1.53±0.04*
[desLeu^4]Gal(1-15)	43.97* (15.43—65.06)	2640* (1730—4010)	0.07	44.98 (33.41—56.55)	0.72±0.01*
[Hse^6]Gal(1-15)	65.48 (51.14—105)	235 (168—328)	0.74	35.65 (30.28—41.03)	0.78±0.03*
[endoTrp^{2a}, Cle^4]Gal(1-16)	101 (72.11—139)	351* (210—585)	0.49	34.42 (26.44—42.39)	0.56±0.02*

Efficacy, EC_{50} and the slopes are expressed as means with 95% confidence limits (ranges given in parentheses). Hill's coefficient is presented as a mean value arithmetic mean±SEM. Efficacy refers to the maximum response produced by the investigated peptide and is expressed as a percentage of the maximum contraction to Gal(1-15). Potency of each peptide (EC_{50}) was calculated from the appropriate concentration-response curve. Relative potency was described as the ratio of the equieffective concentrations of each peptide obtained from their respective concentration-effect relations. * $P < 0.05$ vs. respective Gal(1-15) values, *significancy different from 1.0.

[Ile^4]Gal(1-15) and [desThr^3,Cle^4]Gal(1-15) contracted gastric fundus strips at 10 or 30 nM with a maximal tissue responses at 3 or 6 μM. EC_{50}s of both peptides were 302 and 365 nM. Hill's coefficients were not significantly different from unity.

[D-Leu^4]Gal(1-15) and [desLeu^4]Gal(1-15) showed significantly lower potency than Gal(1-15) when contraction of gastric smooth muscle preparations is concerned. They induced relable myogenic effects at 300 nM, attaining maximum at 6 or 30 μM respectively. EC_{50}s of both analogues were 1.00 and 2.64 μM and their Hill's coefficients differed from unity.

DISCUSSION

In order to perform the structure-activity relationship analysis of Gal action in gastric motility we utilised synthetic Gal fragments and analogues, although using porcine rather than rat Gal might have influenced the range of biological actions observed. Consistent with the observations of Kuwahara *et al.* [17] we have noticed that partial sequences of Gal do not satisfy the structural requirements for the full potency and efficacy of the peptide using rat stomach or guinea-pig ileum as targets for the muscular or neuronal Gal receptors. This seems to be in general agreement with results of studies performed in several other organs, such as rat pancreas, guinea-pig taenia coli, canine small intenstine and rabbit iris sphincter [17—20].

Although Gal (1-15) showed significant myogenic activity in gastric fundus strips, the peptide was over 10-fold weaker and 40% less efficacious than the intacz Gal molecule. On the other hand the C-terminal fragments of Gal were not active in rat gastric or guinea pig smooth muscle strips by themselves [8, 14, 17]. The results of our experiments and previously published studies hint that the active site of Gal molecule causing myogenic activity in rat fundus, ileum or suppressing neurally evoked guinea-pig ileum contractions resides mainly in the N-terminal half part of the peptide moiety. The same outcome seems to apply to the inhibition of insulin, gastrin or pentagastrin-stimulated gastric acid release or a decrease in the C-fibre response in rat isolated pancreas, stomach or spinal cord [8, 14, 17, 21—25]. However the C-terminal end of the molecule is of considerable importance for maintaining the complete potency and efficacy of Gal, at least in the first two preparations mentioned above. The role of the C-terminal in gut motility appears to be further substantiated by the observations showing that Gal(1-20) showed residual activity in the dog small intestine, whereas Gal(1-10) was completely inactive in guinea-pig taenia coli [19, 20]. Besides Fox *et al.* found out that the C-terminal fragments of Gal such as Gal(15-29) and Gal(21-29) inhibit neurally evoked ileal contractions in the dogs [23]. Moreover

neuromodulatory effects of Gal in the guinea-pig taenia coli and rabbit iris sphincter required the presence of the whole molecule, since Gal(1-10) proved to be ineffective (20).

Our findings on the function of the N- and C-terminals in gastric motility seem to differ from those of Katsoulis (8), as in the light of our experiments both ends play an important role when affinity and internal activity of Gal in gastric fundus is concerned. The reasons for the discrepancies remain largely unknown at the moment.

Interestingly in the present study deletions of the second or the third N-terminal amino acids in [desTrp^2, Val^4]Gal(1-15) and [desThr^3, Cle^4] Gal(1-15) rendered the former agent completely inactive and markedly reduced the potency of the latter peptide indicating that Trp and Thr play a crucial role in the recognition and/or stimulation of Gal receptors in rat stomach. Similarly the addition of a second Trp residue in [endoTrp^{2a}, Cle^4]Gal 1-15), the substitution of Leu in position 4 with D-Leu in [D-Leu^4]Gal(1-15) or its deletion in [desLeu^4]Gal(1-15) conspicuously attenuated the strength of those peptides. These findings are in concert with other papers emphasizing that the replacement of Trp in position two with Tyr, Phe, D-Trp or deletions of the first two or three N-terminal amino acids led to a dramatic loss of activity in many tissues such as guinea-pig ileum, rat stomach, intestine or pancreas [14, 17—18, 20, 22—25]. Contrastingly, the change of Ser to Hse in position six in [Hse^6]Gal(1-15) did not influence the potency of the active peptide, but affected the value of Hill's coefficient so that it was significantly lower than unity, that is 0.78. Much alike, the addition of an extra Trp residue in [endoTrp^{2a}, Cle^4]Gal(1-15), substitution of Leu with Val in [Val^4]Gal(1-15) or amino acid deletion in [desLeu^4]Gal(1-15) led to a decrease in the values of Hill's coefficients calculated from the appropriate concentration-response curves, equalling 0.56, 0.67 or 0.72, respectively. According to classical receptor theory Hill's slopes of less than unity may indicate either a heterogenity of binding sites or negative cooperativity. On the other hand the substitution of Leu with D-Leu led to an increase in the value of Hill's coefficient well above unity, namely 1.53, suggesting positive cooperativity (26). However the exact molecular nature of the observed phenomena could not be determined based on our experiments alone. In conclusion, our experiments indicate that Trp, Thr and Leu in positions 2, 3 and 4 of the N-terminal play a vital role in the biologic activity of Gal(1-15) molecule in rat fundus. Conversely, Ser in position six seems to be less important for maintaining the peptide's stength. However structural changes of amino acids in all of these positions can influence their interactions with Gal receptors in gastric smooth muscle. Both N- and C-terminals of Gal molecule seem to be necessary for the peptide molecule to be able to exert a full excitatory action on the receptors located in gastric smooth muscle cell membranes.

REFERENCES

1. Tatemoto K, Rökaeus A, Jörnvall H, McDonald TJ, Mutt V. Galanin-a novel biologically active peptide from porcine intestine. *FEBS Lett* 1983; 164: 124—128.
2. Bartfai T. Galanin: a neuropeptide with important central nervous system actions. In: Psychopharmacology: The fourth generation of progress. Bloom FE, Kupfer DJ (eds). New York, Raven Press Ltd, 1995, pp. 563—571.
3. Lorimer DD, Benya RV. Cloning and quantification of galanin-1 receptor expression by mucosal cells lining the human gastrointestinal tract. *Biochem Biophys Res Common* 1996; 222: 379—385.
4. Bennet A, Stamford IF, Sanger GJ, Bloom SR. The effects of various peptides on human isolated gut muscle. *J Pharm Pharmocol* 1992; 44: 960—967.
5. Soldami G, Mengozzi G, Della-Longa A, Intorre L, Martelli F, Brown DR. An analysis of the effects of galanin on gastric acid secretion and plasma levels of gastrin in the dog. *Eur J Pharmacol* 1988, 154: 313—318.
6. Wang S and Parker EM. Galanin receptor subtypes as potential therapeutic targets. *Exp Opin Ther Patents* 1998; 8: 1225—1235.
7. Chan-Palay V. Galanin hyperinnervates surviving neurons of the human basal nucleus of Meynert in dementias of Alzheimer's and Parkinson's disease: a hypothesis for the role of galanin in accentuating cholinergic dysfunction in dementia. *J Comp Neurol* 1988; 273: 543—57.
8. Katsoulis S, Schmidt WE, Schwörer H, Creutzefeld W. Effects of galanin, its analogues and fragments on rat isolated fundus strips. *Br J Pharmacol* 1900; 101: 297—300.
9. Katsoulis S, Clemens A, Mory-Wortmann S, Schwörer H, Schaube H, Klomp H-J, Fölsch UR, Schmidt WE. Human galanin modulates human colonic motility in vitro. Characterization of structural requirements. *Scand J Gastroenterol* 1995; 31: 446—451.
10. Wang S, Ghibaudi L, Hashemi T, He C, Strader C, Bayne M, Davis H, Hwa JJ. The GalR2 galanin receptor mediates galanin-induced jejunal contraction, but not feeding behavior, in the rat: differentiation of central and peripheral effects of receptor subtype activation. *FEBS Lett* 1998; 434: 277—282.
11. Rossowski WJ, Rossowski TM, Zacharia S, Ertan A, Coy DH. Galanin binding sites in rat gastric and jejunal smooth muscle membrane preparations. *Peptides* 1990; 11: 333—338.
12. Korolkiewicz R, Śliwiński W, Rekowski P, Szyk A, Mucha P, Konstański Z, Korolkiewicz KZ. Lysine^{14}galanin(1-15)-NH_2: a partial agonist at galanin receptors in rat isolated gastric fundus. *Pharmacology* 1997; 55: 179—184.
13. Vane JR. A sensitive method for assay of 5-hydroxytryptamine. *Br J Pharmacol* 1957; 12: 344—349.
14. Korolkiewicz R, Śliwiński W, Rekowski P, Halama-Borowiec A, Mucha P, Szczurowicz A and Korolkiewicz KZ. Galanin, galantide and galanin(1-14)-[α-aminobutyric acid^{8}]-scyliorhinin-1: structure dependent effects on the rat isolated gastric fundus. *Pharmacol Res* 1997; 35: 7—16.
15. Tallarida RJ, Murray RB. Manual of pharmacologic calculations with computer programs, 2nd edition, New York, Springer Verlag, 1986.
16. Barlow RB. Biodata handling with microcomputers. Programs written in BASIC for handling biological, biochemical, pharmacological and physiochemical results-with a commentary on the calculations involved. Amsterdam, Elsevier Science Publishers BV, 1983.
17. Kuwahara A, Ozaki T and Yanaihara N. Structural requirements for galanin action in the guinea-pig ileum. *Regul Pept* 1990; 29: 23—29.
18. Amiranoff B, Lorimer A-M, Yanaihara N and Laburthe M. Structural requirements for galanin action in the pancreatic beta cell line Rin m5F. *Eur J Pharmacol* 1989; 163: 205—207.

19. Fox JET, Brooks B, McDonald TJ, Barnett W, Kostolanska F, Yanaihara C, Yanaihara N and Röhaeus A. Actions of galanin fragments on rats, guinea-pig and canine intestinal motility. *Peptides* 1988; 9: 1183—1189.
20. Ekblad E, Håkanson R, Sundler F and Wahlstedt C. Galanin: neuromodulatory and direct contractile effects on smooth muscle preparations. *Br J Pharmacol* 1985; 86: 241—246.
21. Yanaihara N, Mochizuki T, Takatsuka N, Iguchi K, Sato K, Kakayuma H, Li M and Yanaihara C. Galanin analogues: agonist and antagonist. *Reg Pept* 1993; 46: 93—101.
22. Lagny-Pourmir I, Lorinet A-M, Yanaihara N and Laburthe M. Structural requirements for galanin interaction with receptors from pancreatic beta cells and from brain tissue of the rat. *Peptides* 1980; 10: 757—761.
23. Nagashima T, Takatsuka N, Mochizuki T, Hoshino M, Yanaihara C, Greeley Jr, GH and Yanaihara N. Effects of galanin-related peptides on gastrin and somatostatin release from the isolated perfused rat stomach. *Biomed Res* 1992; 13 (Suppl. 2): 329—336.
24. Mungan Z, Ozmen V, Ertan A, Coy DH, Baylor LM, Rice JC and Rossowski WJ. Structural requirements for galanin inhibition of pentagastrin-stimulated gastric acid secretion in conscious rats. *Eur J Pharmacol* 1992; 214: 53—57.
25. Rossowski WJ, Zacharia S Jiang NY, Mungan Z, Mills M, Ertan A, Coy DH. Galanin: structure-dependent effect on pancreatic amylase secretion and jejunal strip contraction. *Eur J Pharmacol* 1993; 240: 259—267.
26. Laurence D, Carpenter J. A dictionary of pharmacology and clinical drug evaluation. 2nd London, UCL Press Ltd, 1994; pp. 102.

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