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

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Abstract The neuropeptide galanin mediates a diverse array of physiological functions through activation of specific receptors. Roles of the three recently cloned galanin receptors (GalRs) in rat intestinal contraction and food intake were examined using GalR-selective ligands and the results were compared with the pharmacological profiles of defined GalRs. The action profile of these ligands in jejunal contraction resembled only that of GalR2 and only a high level of GalR2 mRNA was detected in the tissue, supporting GalR2 as the receptor mediating jejunal contraction. The action profile for food intake in rats excluded GalR2, GalR3 and the putative pituitary galanin receptor as the 'feeding receptor', suggesting that either GalR1 or an unidentified GalR is responsible for mediating this function.

Key words: Galanin; Galanin receptor; Ligand binding; Intestinal contraction; Food intake

1. Introduction

Galanin is a neuropeptide with 29–30 amino acids originally isolated from porcine small intestine [1]. Galanin contains an amino acid sequence unique to any known family of biologically active peptides. It is widely distributed in the central and peripheral nervous systems and is expressed abundantly in various regions of the brain. Numerous studies have revealed roles of galanin in modulation of functions in the gastrointestinal system, including motility of the digestive system [1–6], and in inhibition of release of gastrin, somatostatin, insulin and gastric acid [7–10]. Several functions associated with the central nervous system were subsequently shown to be mediated by galanin, including neurotransmitter and hormone release, spinal reflexes and nociception [11–15]. Centrally administered galanin potently stimulates food intake in animals [16]. The actions of galanin are thought to be mediated through specific galanin receptors. Pharmacological studies using galanin fragments and chimerical galanin peptides have indicated the existence of more than one galanin receptor in different tissues and cell lines, and have revealed pharmacological differences between the brain and gut galanin receptors [17,18].

Recent molecular cloning of galanin receptor subtypes has provided further understanding of the molecular and pharmacological characteristics of galanin receptors. The first galanin receptor (GalR1) has been cloned from several species [19–25], followed by cloning of two more subtypes of galanin receptor (GalR2 and GalR3) using homology- and function-based approaches [26–31]. All three receptor subtypes consist of seven putative transmembrane domains and belong to the G-protein coupled receptor (GPCR) superfamily. These receptors are distinguished by their pharmacological profiles in membrane binding assays and by their tissue distributions. Galanin-(2–29), which lacks Gly[1] of galanin, retains high affinity for GalR2 and GalR3 but possesses reduced affinity for GalR1, whereas galanin(1–16) displays low affinity for GalR3 and high affinity for GalR1 and GalR2 [28,30]. [D-Trp[2]]-Galanin(1–29) also has high affinity for rat GalR2 but low affinity for rat GalR1 [29]. The expression of GalR1 appears to be restricted to the central nervous system, only detectable in the brain and spinal cord [19,23,32]. In contrast, the expression of GalR2 is more widespread and can be readily detected in both central and peripheral tissues [26–29]. Unlike GalR1 and GalR2, the expression of GalR3 is more restricted to peripheral tissues [30]. These results suggest a possibility that different galanin receptor subtypes mediate separate central and peripheral functions of galanin. However, despite the cloning of three galanin receptors, the roles of the cloned GalRs have not been determined. In this report, we describe studies examining the roles of the GalR1, GalR2 and GalR3 galanin receptors in modulation of small intestine contraction and galanin-stimulated food intake and the results reveal that the small intestinal contraction is mediated by GalR2 whereas the feeding behavior is not regulated by GalR2/GalR3 activation.

2. Materials and methods

2.1. Materials

[^{125}I]Porcine-galanin (2200 Ci/mmol) and α-[^{32}P]dATP (5000 Ci/mmol) were purchased from DuPont-NEN (Boston, MA). Rat galanin and rat galanin(1–16) were purchased from Peninsula Laboratories (Belmont, CA). Rat galanin(2–29) and rat galanin(3–29) were synthesized by Bio-synthesis, Inc. (Lewisville, TX).

2.2. Rat jejunal contraction

Male Sprague-Dawley rats (250–300 g, Charles River, Kingston, NY) were killed by carbon dioxide inhalation. The jejunal segment was removed quickly from each animal, and dissected free of vascular and connective tissues. After washing out the luminal contents, the jejunum was cut into 15 mm segments and suspended longitudinally in 20 ml organ chambers containing Krebs solution. The Krebs composition was (mM) NaCl 118, $CaCl_2$ 2.55, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 24.9, glucose 11.1. The organ chamber was kept at 37°C and continuously gassed with 95% O_2/5% CO_2 to maintain the pH at 7.4. Tension changes were measured with a Grass force-displacement transducer (model FT03) and recorded on a Gould

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(TA4000) recorder. The optimal resting tension, determined by length-tension relationship with repeated KCl (40 mM) challenge, was 0.5 g. After equilibration for 60 min at optimal tension, the tissues were challenged with acetylcholine (ACh) (5 μM). Tissues were washed several times with Krebs solution and equilibrated for another 45 min. Each jejunal segment was tested with a single concentration of a galanin analog. The contractile response was expressed as percentage of the ACh (5 μM) response. EC_{50} was calculated as log concentration causing a half-maximal effect of each galanin analog. Data are expressed as mean ± S.E.M.

2.3. Northern blot analysis

Jejuna from five rats were removed and transferred immediately to 100 ml of Tri-reagent solution (Molecular Research Center, Cincinnati, OH). 1 mg of the total RNA (2.8–3.7 mg) was used for poly(A)$^+$ RNA isolation using an oligo(dT) affinity protocol (Fast Track, Invitrogen). 5 μg of the poly(A)$^+$ RNA was loaded on a 1% denaturing agarose gel containing 2.2 M formamide. The gel was electrophoresed at 5 V/cm for approximately 2 h. The RNA was then transferred to a positively charged Nylon membrane (BrightStar-plus, Ambion, Austin, TX) by the capillary transfer method [44]. After UV-cross linking, the blot was hybridized for 15 h at 55°C in an ExpressHyb solution (Clontech) using ^{32}P-labeled rat GalR1 (full length cDNA cut with restriction enzymes *Hin*dIII and *Xba*I from plasmid pcDNA3-rGalR1 and gel purified), rat GalR2 cDNA (near full length of cDNA (1.3 kb insert) cut with restriction enzymes *Hin*dIII and *Xba*I from clone pcDNA3-B45-16-11 [27] and gel purified), or a GalR3 0.7 kb cDNA [30] as a probe. After hybridization, the blot was washed with solution I (2×SSC, 0.05% SDS) for 30 min at room temperature then with wash solution II (0.1×SSC, 0.1%SDS) for 30 min at room temperature, 1 h at 48°C, and 1 h at 55°C. The blot was then wrapped with Saran Wrap and exposed to Kodak BioMax films for 5 h at −80°C. The same blot was stripped and similarly hybridized with ^{32}P-labeled actin cDNA to ensure loading of poly(A)$^+$ mRNA from the tissues onto the blot.

2.4. Feeding behavior

Male Sprague-Dawley rats (Charles River) were chronically implanted with a single 22G stainless steel guide cannula (Plastics One, Roanoke, VA) in the lateral ventricle of the brain under ketamine:xylazine (100:10 mg/kg i.p.) anesthesia with the following coordinates: −1.0 mm relative to bregma, 1.5 mm lateral of midline, and 3.6 mm below the surface of the skull. The cannula was secured on the surface of the skull with jeweler's screws and dental cement, and a 28 gauge wire was inserted into the cannula to maintain patency. Animals were allowed to recover for 2 weeks, and then acclimated to consume a milk-mash diet (500 g powdered #5001 rat chow, 400 g sugar, and 12 oz of condensed milk) for 3 days prior to study. Sterile saline or galanin(1–29, 1–16, 2–29, or 3–29) in saline was infused i.c.v. in a total volume of 5 μl over the course of 1 min via a 28G internal cannula 4.6 mm below the surface of the skull (Plastics One) attached to a BAS Bee Syringe Pump (Bioanalytical Systems, West Lafayette, IN). The infusion cannula was left in place for an additional minute following the infusion.

Forty-two i.c.v. cannulated Sprague-Dawley rats were divided into groups of six and administered saline vehicle or galanin(1–29) (1 nmol, 3 nmol, 10 nmol), galanin(1–16), galanin(2–29), or galanin(3–29) (3 nmol, 10 nmol, 30 nmol) i.c.v. in a crossover design. The animals were allowed a 48 h washout period between treatments. Food consumption (milk-mash diet) was monitored at 1 h post-dosing, which was 3 h into the light cycle in the satiated rats. All studies were conducted in an AAALAC accredited facility following protocols approved by the Schering-Plough Research Institute's Animal Care and Use Committee. The procedures were performed in accordance with the principles and guidelines established by the NIH for the care and use of laboratory animals.

2.5. Radioligand binding assay

Chinese hamster ovary (CHO) cell lines expressing rat GalR1 and rat GalR2 were obtained as previously described [33,34]. Binding of [^{125}I]porcine galanin to intact CHO cells expressing rat GalR1 or rat GalR2 was performed in a buffer containing PBS (without Ca^{2+}/Mg^{2+}), 0.1% bovine serum albumin (w/v), 0.1% bacitracin, 2 μg/ml leupeptin, 0.1 mM phenylmethylsulfonyl fluoride (PMSF) and 10 mM $MgCl_2$. Peptide competition studies were performed in 200 μl of the binding buffer containing 5×10^5 cells and 0.3 nM [^{125}I]porcine galanin. After 1 h incubations at room temperature, the assays were terminated by rapid vacuum filtration through 0.3% polyethyleneimine-pretreated Multiscreen FB Filter Plates (cat# MAFB NOB50, Millipore, Bedford, MA). The filters were then washed three times with 100 μl of phosphate buffered saline (pH 7.4) and counted with a TopCount counter (Hewlett Packard). All data were analyzed using non-linear regression software (Prism, GraphPad, San Diego, CA) and the K_i calculated according to the method of Cheng and Prusoff [35].

3. Results

3.1. Effects of galanin analogs on contractility of jejunal longitudinal muscle

We first tested the effects of galanin and its analogs in a bioassay of jejunal contraction, a tissue in which galanin was originally discovered [1]. Rat galanin(1–29) elicited a concentration-dependent contraction of rat jejunal longitudinal muscle over the dose range 10 nM to 10 μM (EC_{50} = 76 ± 26 nM, Fig. 1). Galanin(2–29) also stimulated the contraction with a potency (EC_{50} = 269 ± 57 nM) 3-fold less than that of galanin(1–29) (Fig. 1). Galanin(1–16), a ligand with low affinity for GalR3, produced an EC_{50} value similar to that of galanin(2–29) (370 ± 195 nM, Fig. 1), indicating that the action is mediated by a receptor with high affinity for galanin(1–29), galanin(1–16) and galanin(2–29). In contrast, elimination of Trp at position 2 of rat galanin resulted in an inactive peptide (galanin(3–29)), which did not induce any contraction in rat jejunal muscle (Fig. 1). The maximum effects of the contraction for all the three active galanin peptides were comparable, ranging from 50 to 60% of that elicited by ACh at 5 μM (Fig. 1).

3.2. Expression of the galanin receptor subtypes in rat jejunum

Since the levels of expression of rat GalR2 and GalR3 mRNA in jejunum have not been reported, we measured the mRNA levels of the rat galanin receptor subtypes in rat jejunum by Northern blot analysis. Poly(A)$^+$ RNA was isolated from rat jejunum, separated on an agarose gel, blotted onto

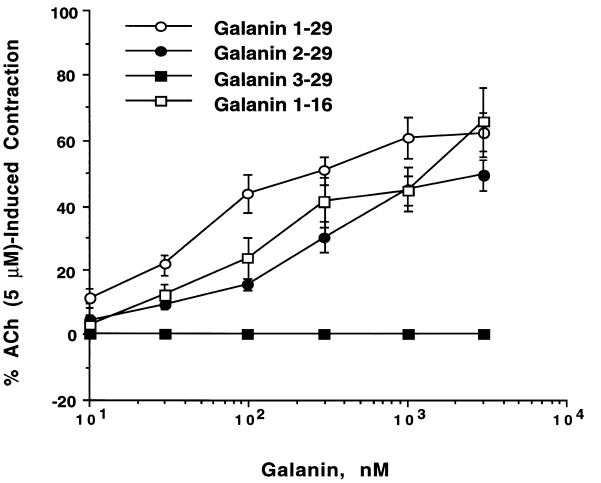

Fig. 1. Concentration-response curves showing the contractile effects of galanin(1–29) and galanin fragments on longitudinal smooth muscle of rat jejunum. The contractile responses are expressed as a percentage of the contraction induced by ACh (5 μM). Each value is the mean of 5–8 experiments. Vertical bars represent the standard error of means.

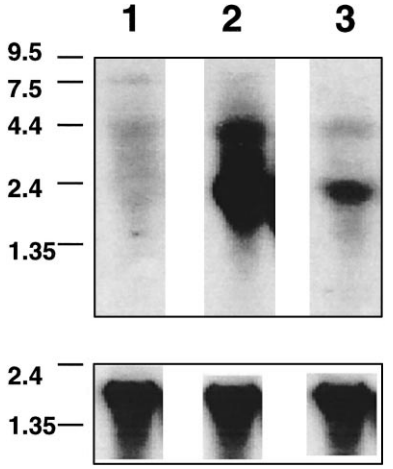

Fig. 2. Expression of rat GalR1, GalR2 and GalR3 in jejunum. 5 μg poly(A)$^+$ RNA was loaded on each lane of an agarose gel and after transfer of the RNA from the gel to Nylon filter, the blot was cut into three parts and then hybridized with ^{32}P-labeled GalR1 (lane 1), GalR2 (lane 2) and GalR3 (lane 3) probes (labeled with a random priming kit, BRL, specific activity 2×10^9, 0.44×10^9 and 1×10^9 cpm/μg for rat GalR1, GalR2 and GalR3 cDNA probes, respectively). The hybridized blots were then washed and exposed to a film identically. The blot was subsequently stripped and hybridized with ^{32}P-labeled β-actin cDNA (bottom panel). The numbers on the left indicate standard molecular weights (kb).

Nylon membranes and hybridized with ^{32}P-labeled rat GalR1, GalR2 or GalR3 cDNA. Shown in Fig. 2 is a representative blot of two independent Northern blot analyses. A band with a strong signal at ~2.4 kb was detected when hybridized with radiolabeled rat GalR2 cDNA (Fig. 2, lane 2). The molecular weight was similar to that of rat GalR2 transcripts found in other tissues (~2–2.4 kb, [26,27,29]. In contrast, when the hybridization was performed with the rat GalR1 or GalR3 cDNA as probe, a very faint band at the expected molecular weight of 7–9 kb for GalR1, or of ~4 kb for GalR3 mRNA was observed (Fig. 2, lanes 1 and 3, respectively), indicating that GalR1 and GalR3 were expressed at very low level in this tissue. The faint bands seen at 2.4 kb on the blot arose from cross-hybridization of the rat GalR1 and GalR3 cDNA probes to the large amount of GalR2 transcript migrated at this molecular weight (Fig. 2, lanes 1 and 3).

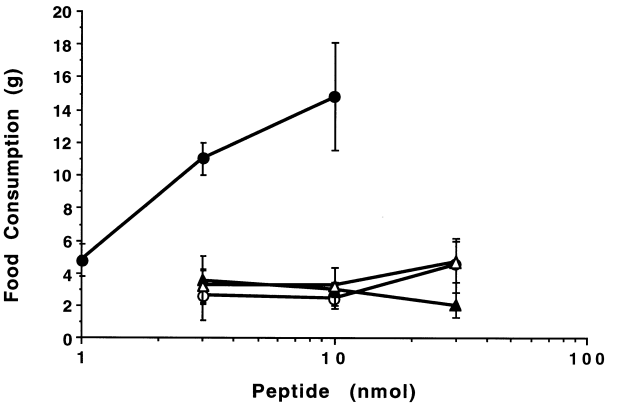

Fig. 3. Effects of galanin and galanin fragments on food intake in rats. Galanin peptides were infused i.c.v. into rats and food consumption was monitored 1 h after the dosing. Values are presented as mean ± S.E.M. (n = 6–24 animals/group). Statistical significance between feeding responses to the galanin peptides was compared to the saline vehicle treatment (1.31 ± 0.2 g food) by the paired Student's t-test. Symbols denote galanin(1–29) (●), galanin(1–16) (△), galanin(2–29) (○) and galanin(3–29) (▲).

3.3. *Effects of the galanin analogs on food intake in rats*

Because of the well-established orexigenic activity of galanin itself [16], we compared the activity of galanin(1–29), galanin(1–16), galanin(2–29) and galanin(3–29) in a rat feeding model. Galanin(1–29) potently stimulated food consumption as evidenced by a dose-dependent increase in 1 h food intake

Table 1
Relative affinities and potencies of galanin and galanin fragments in GalR1, GalR2 and GalR3 receptor binding, jejunal contraction and feeding assays

Ligand		Receptor						Jejunum bioassay	Feeding bioassay
		GalR1		GalR2		GalR3			
		memb[a]	cells[b]	memb	cells	memb	cells		
Galanin(1–29)	K_i (nM)	1.0	0.9 ± 0.1	1.5	5.6 ± 0.3	1.5	nd (~3.5)[c]	76 ± 26	+++++[d]
	K_i/K_{igal}	1	1	1	1	1		1	
Galanin(2–29)	K_i (nM)	85	1100 ± 225	1.9	56 ± 5	12.6	nd (~264)	269 ± 57	±
	K_i/K_{igal}	85	1222	1.3	10	8.6		3.5	
Galanin(1–16)	K_i (nM)	4.8	6 ± 1	5.7	13 ± 2	50	nd (~88)	370 ± 195	±
	K_i/K_{igal}	4.8	6.6	3.9	2.3	34		4.8	
Galanin	K_i (nM)	> 1000	> 2500	> 1000	> 3125	> 1000	nd (> 3125)	> 10 000	−
	K_i/K_{igal}	> 1000	> 2778	> 650	> 558	> 650		> 132	

In the radioligand binding assays, CHO cells were incubated with 0.3 nM [^{125}I]porcine galanin in the presence of various concentrations of galanin and its fragments (Fig. 4). Values are mean ± S.D. from two independent experiments performed in duplicate. Receptor affinities of the peptides are K_i (nM) calculated from EC_{50} values obtained by non-linear regression analysis of the binding data. Data of binding assays using membranes are from [30]. In the jejunal contraction assays, jejunal contraction was measured in the presence of various concentrations of galanin and its fragments and compared to that with 5 μM ACh. EC_{50} was determined from 5–8 measurements and represents mean ± S.E.M. (Fig. 1). Food intake by rats in the feeding bioassay was measured after i.c.v. infusion of the peptide (Fig. 3.)

[a]'memb' indicates membrane binding assays.

[b]'cells' denotes the whole-cell binding assays. K_i/K_{igal} represents affinity or potency of a ligand relative to that of galanin(1–29).

[c]Values in parentheses are estimated using the ratios of K_{icell}/K_{imemb} of GalR1 and GalR2 obtained with the same peptides. Note that the K_{icell}/K_{imemb} ratio of each ligand is within 4-fold of variability, e.g. the K_{icell}/K_{imemb} values of galanin(1–29) are 0.9(0.9/1) for GalR1 and 37.5(5.6/1.5) for GalR2, those of galanin(2–29) are 12.9(1100/85) for GalR1 and 29.4(56/1.9) for GalR2, and those of galanin(1–16) are 1.3(6/4.8) for GalR1 and 2.3(13/5.7) for GalR2.

[d]Feeding data are estimated based on the potencies of the peptides. +++++ denotes the highest effect and − denotes no effect (Fig. 3). nd, not determined.

when infused i.c.v. at 1–10 nmol (Fig. 3). At all doses the increase of food intake at 1 h was significantly higher than that when saline was infused as control ($P < 0.05$, paired t-test). In contrast, galanin(3–29) did not stimulate food intake even at doses as high as 30 nmol, i.c.v. (Fig. 3). Galanin(2–29) was also inactive in stimulation of food intake at 3 and 10 nmol. Only a small increase was observed when 30 nmol galanin(2–29) was infused as compared to the animal's saline response (Fig. 3). Galanin(1–16) did not increase food intake consistently over the three doses. The 10 nmol dose of galanin(1–16) was significantly increased over the animals' saline infused baseline ($P < 0.05$, paired t-test), but the 30 nmol dose was not significantly increased. These results demonstrate that the galanin-stimulated food intake was mediated by a galanin receptor that is activated by galanin(1–29), slightly activated by galanin(1–16) and galanin(2–29), and incapable of being activated by galanin(3–29).

3.4. Effects of galanin analogs in [^{125}I]galanin binding assay

To correlate the results of jejunal contraction and food intake with the pharmacological profiles of the defined GalR subtypes, we compared these results with pharmacological profiles of the three cloned GalRs (Table 1) determined in previous membrane binding assays [30] and binding assays with intact CHO cells expressing GalR1 or GalR2 (Fig. 4). In the whole-cell binding assays, galanin(1–29) competitively inhibited the binding of [^{125}I]galanin to both rat GalR1 and GalR2 receptors with K_i values of 0.9 and 5.6 nM, respectively, whereas galanin(3–29) did not displace the radioligand from either GalR1 or GalR2 (Fig. 4). Galanin(1–16) bound both GalR1 and GalR2 with high affinity ($K_i = 6$–13 nM). However, galanin(2–29) potently competed the radioligand from GalR2 but not GalR1 (K_i of 1100 nM and 56 nM for GalR1 and GalR2, respectively) (Fig. 4). Comparison of the membrane and whole-cell binding data indicates that the two types of assays produced consistent profiles for the GalRs (Table 1). The pharmacological profiles of GalR1 and GalR2 obtained with radioligand binding assays with whole-cells (Fig. 4 and Table 1) and membranes (Table 1 and [30]) are consistent with those obtained with functional assays, such as the inhibitory effects of galanin and the fragments galanin(2–29) and galanin(3–29) on forskolin-stimulated cAMP production [33]. Although the low expression level of GalR3 in mammalian cells [30] did not allow generation of a CHO cell line expressing enough GalR3 for the whole-cell binding assay, the use of the GalR3 pharmacological profile obtained with membrane assay most likely represents the profile of GalR3 in the whole-cell assay (Table 1). Taken together, these ligand binding studies have defined distinct pharmacological profiles for the three GalR subtypes: galanin(1–29) and galanin(1–16) possess high and galanin(2–29) and galanin(3–39) possess low relative binding affinities (K_{ilig}/K_{igal}) for GalR1 (Table 1). All the ligands, except galanin(3–29), possess high affinities for GalR2. For GalR3, the relative affinities of galanin(1–29) and galanin(2–29) are higher than those of galanin(1–16) and galanin(3–29) (Table 1).

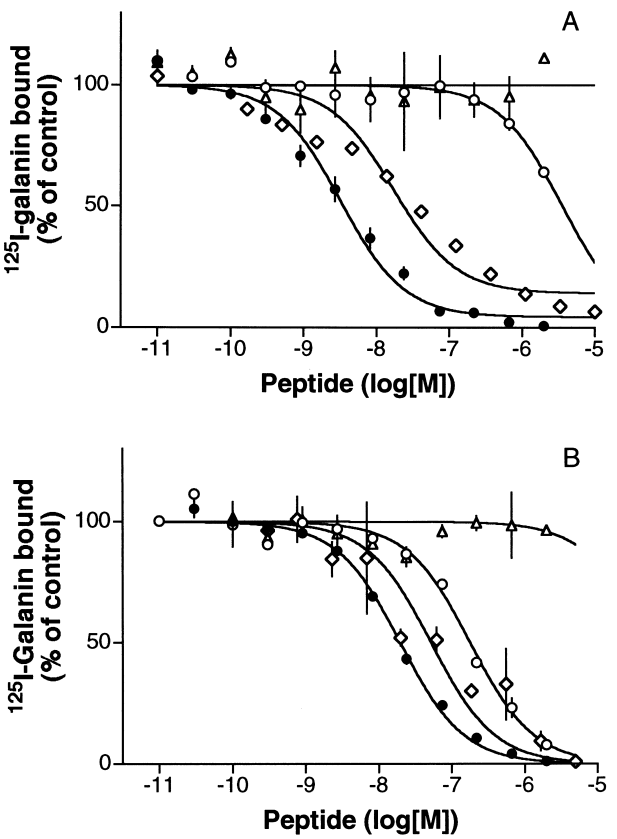

Fig. 4. Ligand binding profiles of cloned GalR1 and GalR2 receptors. [^{125}I]Porcine galanin at 0.3 nM was incubated with CHO cells expressing GalR1 (A) or GalR2 (B) in the presence of rat galanin(1–29) (●), galanin(1–16) (◇), galanin(2–29) (○) and galanin(3–29) (△). The data are expressed as percent of control (total binding minus non-specific binding). The total binding was 6354 ± 320 dpm ($n = 6$) and 16 790 ± 1122 dpm ($n = 6$) for GalR1 and GalR2, respectively; and the non-specific binding, defined by including 5 μM rat galanin in the assays, was 647 ± 63 dpm ($n = 4$) and 2178 ± 163 dpm ($n = 4$) for GalR1 and GalR2, respectively.

4. Discussion

Galanin is an unique gut-brain neuropeptide that regulates a variety of physiological functions via activation of specific G protein-coupled receptors. Analysis of the structure-activity relationship of galanin peptides with the three cloned GalR subtypes demonstrated that the ligand binding is independent of the C-terminus of galanin [20,26,30]. In other studies, a putative galanin receptor that may require the C-terminus of galanin has been suggested [8,18,36]. GalR1, GalR2 and GalR3 may mediate different physiological functions because the three cloned galanin receptors (1) are differentially expressed among central and peripheral tissues; (2) activate different signal transduction pathways (G_i for GalR1, and G_q, G_o and G_i for GalR2); and (3) are pharmacologically distinct [19,20,23,24,26–28,30]. The cloning of the three receptor subtypes and identification of receptor subtype-selective ligands galanin(2–29) and galanin(1–16) have enabled us to perform experiments to directly differentiate the roles of these receptor subtypes in intestinal contraction and feeding paradigms (Table 1). We have demonstrated that the two gut/brain functions are mediated by different galanin receptors, as suggested by earlier pharmacological studies [17].

Two lines of evidence support GalR2 as the galanin receptor that mediates intestinal contraction. The pharmacological profile in jejunal contractions (Fig. 1 and Table 1), in which high affinity ligands galanin(1–29), galanin(2–29) and galanin(1–16) are all active in stimulating jejunal contraction

whereas galanin(3–29) is ineffective, resembles only the pharmacological profile of the GalR2 galanin receptor (Fig. 4 and Table 1). The ligand profile of the contraction is markedly different from those of GalR1 and GalR3, suggesting that these GalRs do not play a role in this function. In addition, abundant expression of GalR2 and minimum expression of GalR1 and GalR3 detected in jejunum, as revealed by Northern blot analyses (Fig. 2), correlates with the observed physiological activity of GalR2 (Fig. 1). The present finding that galanin(1–29), galanin(1–16) and galanin(2–29) are active but galanin(3–29) is inactive in stimulation of the contraction is consistent with previous results utilizing galanin fragments in jejunal smooth muscle contraction models [37,38]. However, in other studies, galanin(9–29) [8] and residues 25–29 of galanin [18] were found to bind to jejunal membrane preparations. Binding of ligands to jejunal membranes may reflect binding to more than one receptor subtype, obscuring the true pharmacological profile of each receptor. Thus a ligand such as galanin(3–29), although binding the membrane preparations, may not activate the appropriate receptor subtype to cause jejunal contraction. The approach taken in the present study, in which jejunal contraction can be compared to the pharmacological profile of each individual receptor subtype expressed in CHO and COS-7 cells (Table 1), allows a clearer correlation to emerge. Human GalR1 receptor has been isolated from mucosal cells lining the human gastrointestinal tract by RT-PCR using human GalR1-specific primers [21], suggesting a role for galanin in regulation of intestinal epithelial cell absorption [39]. In the present study, we extended to measure the relative amounts of all the three GalR subtypes in rat jejunum by Northern blot analysis. While the dominant expression of GalR2 mRNA (Fig. 2) suggests a regulatory role for this receptor in intestine, the detection of low levels of GalR1 and GalR3 (Fig. 2) is consistent with the existence of GalR1 in the mucosal cells, which can be readily amplified by PCR using GalR1-specific primers [21].

The second finding in present study is that feeding behavior is not mediated by either GalR2 or GalR3. GalR1 and GalR2 mRNAs have been found wildly distributed in rat brain by in situ hybridization studies [23,28,32]. In these studies, the patterns of the distributions were distinct but overlapping in several regions of the brain, including hypothalamus and amygdala, suggesting that one or both of the receptor subtypes may mediate galanin-induced food intake. Chimeric galanin antagonists, M40 and C7, have been found to block galanin-induced feeding in rats [40] but these ligands are generally not GalR subtype-selective [30]. In the present study, we directly employed feeding behavioral model and of GalR2/GalR3-selective agonist galanin(2–29) (Fig. 2 and Table 1) to test the possible roles of the three GalRs in feeding. The inability of galanin(2–29) to stimulate food intake indicates that galanin-stimulated food intake is mediated by a galanin receptor other than GalR2 and GalR3 (Fig. 3 and Table 1).

The results obtained from the present study, however, cannot define GalR1 as the feeding receptor because the low activity of galanin(1–16) in the food intake assay (Fig. 3) is not consistent with the high affinity of the ligand for GalR1 (Table 1). Two possibilities exist to explain the data. (1) The inactivity of galanin(1–16) may be resulted from endogenous protease degradation. Galanin(1–16), lacking the C-terminal 13 amino acids of galanin, has been found to be markedly more susceptible to proteolytic degradation in hypothalamus membranes ($t_{1/2} = \sim 28$ min) than galanin ($t_{1/2} = \sim 100$ min) [41]. Similarly, a higher degradation rate of galanin(1–16) in cerebrospinal fluid, spinal cord membranes and plasma has been observed [42,43]. It is therefore possible that the reduced 1 h food intake-stimulating activity of galanin(1–16) resulted from i.c.v. degradation (Table 1). (2) An unidentified galanin receptor (other than the three cloned GalRs [30] and the putative pituitary GalR [36]) that possesses a pharmacological profile matching that demonstrated in the feeding behavior of rats (Fig. 3) may be responsible for mediating this function of galanin. The putative pituitary galanin receptor, which requires the C-terminus of galanin for high affinity binding [36], is unlikely to mediate either food intake or intestinal contraction, since galanin(3–29), a ligand that binds to this receptor with high affinity and is active in stimulating prolactin release [36], does not bind either GalR1, GalR2, or GalR3 (Table 1) and is inactive in both food intake and intestinal contractions (Figs. 1 and 3).

In summary, we have studied roles of the three cloned galanin receptors in two important central and peripheral functions mediated by galanin. GalR2/GalR3-selective ligand galanin(2–29) and GalR1/GalR2-selective ligand galanin(1–16) were used in these studies to implicate the GalR2 galanin receptor in the excitatory mechanism for small intestinal smooth muscle contraction. In addition, our data suggest that neither GalR2 nor GalR3 mediates galanin-stimulated food intake. Positive identification of the 'feeding receptor' will require identification of potent GalR1-selective antagonists or generation of animals in which GalR1 is specifically disrupted by molecular approaches, and/or require isolation of a new GalR subtype with an unique pharmacological profile identical to that of the feeding behavior.

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