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Galanin receptors in the rat gastrointestinal tract

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Abstract

Galanin functions are mediated by three distinct G-protein-coupled receptors, galanin receptor 1 (GalR1), GalR2 and GalR3, which activate different intracellular signaling pathways. Here, we quantified mRNA levels of GalR1, GalR2 and GalR3 in the gastrointestinal tract using real time RT-PCR. GalR1 and GalR2 mRNAs were detected in all segments with the highest levels in the large intestine and stomach, respectively. GalR3 mRNA levels were quite low and mostly confined to the colon. We also investigated the effect of galanin 1–16, which has high affinity for GalR1 and GalR2 and low affinity for GalR3 on depolarization-evoked Ca^{2+} increases in rat cultured myenteric neurons using Ca^{2+} -imaging. Intracellular Ca^{2+} changes in myenteric neurons were monitored using the Ca^{2+} sensitive dye, fluo-4, and confocal microscopy. Galanin 1–16 (1 μM) markedly inhibited the K^{+} -evoked Ca^{2+} increases in myenteric neurons. In summary, the differential distribution of GalRs supports the hypothesis that the complex effects of galanin in the gastrointestinal tract result from the activation of multiple receptor subtypes. Furthermore, this study confirms the presence of functional GalRs and suggests that galanin modulates transmitter release from myenteric neurons through inhibition of voltage-dependent calcium channels involving a $\text{G}_{i/o}$ -coupled GalR.

Keywords

Real time RT-PCR; Voltage-dependent calcium channel; Enteric nervous system

1. Introduction

In the gastrointestinal tract, galanin modulates numerous biological activities, including regulation of transmitter release, secretion and motility. The effects of galanin are mediated by three distinct G-protein-coupled receptors that involve different signaling pathways:

galanin receptor 1 (GalR1) ($G_{i/o}$), GalR2 ($G_{q/11}$) and GalR3 ($G_{i/o}$) (Smith et al., 1997; Branchek et al., 1998, 2000; Wang et al., 1998). The effect of galanin on gastrointestinal motility varies according to the species and the segment investigated, suggesting activation of multiple receptors (Tamura et al., 1987; Muramatsu and Yanaihara, 1988; Fontaine and Lebrun, 1989; Katsoulis et al., 1990). To better understand which receptor mediates a particular galanin effect, it is critical to determine the tissue specific distribution of individual GalRs. Moreover, galanin has been shown to modulate transmitter release by altering Ca^{2+} influx through voltage-dependent Ca^{2+} channels (Sarnelli et al., 2004). GalR1 is expressed by enteric neurons, and it mediates galanin-induced inhibition of cholinergic transmission in the small intestine (Sternini et al., 2004). It is likely that galanin reduces acetylcholine release from myenteric neurons by inhibiting voltage-gated Ca^{2+} channels. The aims of this study were to: (1) quantify the tissue distribution of rat GalR1, GalR2 and GalR3 mRNAs in different regions of the rat gastrointestinal tract using quantitative real time RT-PCR; and (2) investigate the effect of galanin 1–16, which has high affinity for GAL-R1 and GAL-R2 (K_i 4.8 ± 1.5 and 5.66 ± 3.7 , respectively) and low affinity for GAL-R3 (K_i 49.6 ± 15.3) (Wang et al., 1997) on K^+ -evoked intracellular Ca^{2+} responses in cultured myenteric neurons.

2. Real time quantitative RT-PCR

Adult (200–250 g) Sprague–Dawley rats were used. Total RNA was extracted from stomach, small intestine and large intestine, followed by first strand cDNA synthesis. Specific primers for the three GalR genes were designed for this study (Anselmi et al., in press). The levels of mRNA for the three GalRs in the different regions of the gastrointestinal tract were analyzed by real time quantitative RT-PCR. The data acquired from each sample were normalized to the housekeeping gene β -actin. Results were analyzed using the comparative Ct method to derive relative quantities (User Bulletin #2, ABI Prism 7700 Sequence Detection System, December 11, 1997).

GalR1 mRNA was detected in all regions of the gastrointestinal tract, with the highest levels in the large intestine (Fig. 1(a)). The highest relative expression of GalR2 was in the stomach, and high levels were found in the large intestine (Fig. 1(b)). Unlike GalR1 and GalR2, GalR3 mRNA was expressed at low levels in all tissues examined (Fig. 1(c)).

3. Cultured myenteric plexus neurons

The longitudinal muscle and myenteric plexus preparation (LMMP) used for this study was obtained from two week old Sprague–Dawley rats and digested first with collagenase and then treated with trypsin (0.025%). The LMMP was carefully triturated and plated at a cell density of 2.5×10^5 cells/ml onto poly-L-lysine coated coverslips with Dulbecco's Modified Eagle Medium containing 10% fetal bovine serum (FBS), penicillin-streptomycin-glutamine and DNase. After 3 h, the coverslips were flipped over (cell side down) and placed in new plates containing the defined media without FBS (Anselmi et al., unpublished). Cultured rat myenteric neurons were loaded with $0.5 \mu M$ fluo-4 (calcium sensitive dye). To activate voltage-dependent Ca^{2+} channels, cells were depolarized by increasing $[K^+]_o$ from 5 to 72 mM. The change produced by elevated $[K^+]_o$ applied in the presence of the test solution was compared to the changed produced in control conditions. Images were collected with a Zeiss 510 META Laser Scanning Microscope. Statistical analysis was performed using paired and unpaired Student's *t*-test, significance was chosen at $p < 0.05$, and variability is reported as $\pm SEM$.

Fig. 2(a) shows a plot of the $[Ca^{2+}]_i$ response measured in a myenteric neuron before, during and after the application of galanin 1–16 ($1 \mu M$). Galanin 1–16 produced a reversible

inhibition of the depolarization-evoked $[Ca^{2+}]_i$ increase (Fig. 2(b)). On average, galanin 1–16 (1 μ M) caused a $54.9 \pm 7.2\%$ ($n = 7$; $p = 0.0078$) reduction in the K^+ -evoked fluorescence change in cultured myenteric neurons.

4. Discussion

These studies show that all three GalR mRNAs are expressed throughout the gastrointestinal tract with different levels of expression. Both GalR1 and GalR2 mRNAs were more abundant in the large than in the small intestine. GalR2 mRNA was the most abundant in the stomach. The levels of GalR3 mRNA were the lowest throughout the entire length of the gut with the highest levels in the large intestine. Galanin exerts both direct and indirect effects via regulation of transmitter release. For instance, galanin inhibition of the excitatory neuroneuronal and neuromuscular transmission is partly mediated by GalR1 that is predominantly $G_{i/o}$ -coupled, which is consistent with our finding of GalR1 mRNA in the intestine (Fig. 1(a)). Our data also match the immunohistochemical distribution of GalR1 immunoreactivity in the gut (Pham et al., 2002). GalR3, which is also $G_{i/o}$ -coupled, might participate to GalR-mediated neurogenic response inhibition, which is in agreement with the presence of small levels of GalR3 mRNA in the intestine. GalR2, the only GalR that signals predominantly through a stimulatory $G_{q/11}$ pathway, is likely to mediate the direct effect of galanin on gastrointestinal motility. This is consistent with our finding of high levels of GalR2 in the stomach and intestine.

Our study also shows that galanin 1–16 inhibits voltage-dependent Ca^{2+} influx in cultured myenteric neurons, which is likely to be mediated by GalR1, which is coupled to $G_{i/o}$ inhibitory proteins thus mediates galanin inhibitory effects. GalR3 is also coupled to $G_{i/o}$ proteins, but the galanin fragment used in this study, galanin 1–16 has low affinity for GAL-R3 and high affinity for GAL-R1 and GAL-R2. However, GalR2 is predominantly coupled to $G_{q/11}$ excitatory proteins, thus its activation would more likely result in a direct stimulation of Ca^{2+} instead of an inhibition of evoked- Ca^{2+} .

In summary, the differential distribution of mRNA transcripts for GalRs, together with the distinct pharmacological profiles and signal transduction pathways of GalRs, supports the concept that the different actions of galanin in the gastrointestinal tract are dependent upon the interaction of galanin with multiple GalRs. Moreover, the Ca^{2+} imaging study supports our hypothesis that GalR activation alters transmitter release from myenteric neurons through inhibition of voltage-dependent calcium channel and provides additional support for the presence of functional GalRs in the myenteric plexus.

References

- Branchek TA, Smith KE, Walker MW. Molecular biology and pharmacology of galanin receptors. *Ann. N.Y. Acad. Sci.* 1998; 863:94–107. [PubMed: 9928163]
- Branchek TA, Smith KE, Gerald C, Walker MW. Galanin receptor subtypes. *Trends Pharmacol. Sci.* 2000; 21:109–117. [PubMed: 10689365]
- Fontaine J, Lebrun P. Galanin: Ca^{2+} -dependent contractile effects on the isolated mouse distal colon. *Eur. J. Pharmacol.* 1989; 164:583–586. [PubMed: 2475349]
- Katsoulis S, Schmidt WE, Schworer H, Creutzfeldt W. Effects of galanin, its analogues and fragments on rat isolated fundus strips. *Brit. J. Pharmacol.* 1990; 101:297–300. [PubMed: 1701674]
- Muramatsu I, Yanaihara N. Contribution of galanin to noncholinergic, non-adrenergic transmission in rat ileum. *Brit. J. Pharmacol.* 1988; 94:1241–1249. [PubMed: 2463026]
- Pham T, Guerrini S, Wong H, Reeve J Jr, Sternini C. Distribution of galanin receptor 1 immunoreactivity in the rat stomach and small intestine. *J. Comp. Neurol.* 2002; 450:292–302. [PubMed: 12209857]

- Sarnelli G, Vanden Berghe P, Raeymaekers P, Janssens J, Tack J. Inhibitory effects of galanin on evoked $[Ca^{2+}]_i$ responses in cultured myenteric neurons. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2004; 286:G1009–G1014. [PubMed: 14739140]
- Smith KE, Forray C, Walker MW, Jones KA, Tamm JA, Bard J, Branchek TA, Linemeyer DL, Gerald C. Expression cloning of a rat hypothalamic galanin receptor coupled to phosphoinositide turnover. *J. Biol. Chem.* 1997; 272:24612–24616. [PubMed: 9305929]
- Sternini C, Anselmi L, Guerrini S, Cervio E, Pham T, Balestra B, Vicini R, Baiardi P, D'Agostino GL, Tonini M. Role of galanin receptor 1 in peristaltic activity in the guinea pig ileum. *Neuroscience.* 2004; 125:103–112. [PubMed: 15051149]
- Tamura K, Palmer JM, Wood JD. Galanin suppresses nicotinic synaptic transmission in the myenteric plexus of guineapig small intestine. *Eur. J. Pharmacol.* 1987; 136:445–446. [PubMed: 2440701]
- Wang S, He C, Hashemi T, Bayne M. Cloning and expressional characterization of a novel galanin receptor. Identification of different pharmacophores within galanin for the three galanin receptor subtypes. *J. Biol. Chem.* 1997; 272:31949–31952. [PubMed: 9405385]
- Wang S, Hashemi T, Fried S, Clemmons AL, Hawes BE. Differential intracellular signaling of the GalR1 and GalR2 galanin receptor subtypes. *Biochemistry.* 1998; 37:6711–6717. [PubMed: 9578554]

Fig. 1.

Expression of GalR mRNAs in different regions of the rat gastrointestinal tract: stomach, small intestine and large intestine. Plot of GalR1 mRNA levels is shown in (a), of GalR2 mRNA in (b) and of GalR3 mRNA in (c). The levels of GalR mRNAs were measured by real time RT-PCR. The data acquired from each sample were normalized to those of β -actin. Relative quantities (RQ) of mRNA were analyzed using the comparative Ct method. Each cDNA sample was amplified in triplicate and all data are expressed as the means $\pm$ SEM.

Fig. 2.

Galanin 1–16 inhibits voltage dependent Ca^{2+} increases in myenteric neurons. (a) Effects of galanin 1–16 on depolarization evoked Ca^{2+} increases in the soma of a myenteric neuron in culture produced by 1 min applications of elevated $[\text{K}^+]_o$. Example of somatic $[\text{Ca}^{2+}]_i$ recorded from a cultured myenteric neuron. (b) Bar graph of galanin 1–16 (1 μM) mediated inhibition of the K^+ -evoked Ca^{2+} increase in cultured myenteric neurons.