Inhibitory effects of galanin on evoked \([\text{Ca}^{2+}]_i\) responses in cultured myenteric neurons

Giovanni Sarnelli, Pieter Vanden Berghe, Petra Raeymaekers, Jozef Janssens, and Jan Tack

Center for Gastroenterological Research, Katholieke Universiteit Leuven, 3000 Leuven, Belgium

Submitted 5 June 2003; accepted in final form 13 January 2004

Sarnelli, Giovanni, Pieter Vanden Berghe, Petra Raeymaekers, Jozef Janssens, and Jan Tack. Inhibitory effects of galanin on evoked \([\text{Ca}^{2+}]_i\) responses in cultured myenteric neurons. Am J Physiol Gastrointest Liver Physiol 286: G1009–G1014, 2004. First published January 22, 2004; 10.1152/ajpgi.00255.2003.—Galanin modulates gastrointestinal motility by inhibiting the release of ACh from enteric neurons. It is, however, not known whether galanin also inhibits neuronal cholinergic transmission postsynaptically and whether galanin also reduces the action of other excitatory neurotransmitters. The aim of the present study was thus to investigate the effect of galanin on the evoked intracellular \([\text{Ca}^{2+}]_i\) concentration (\([\text{Ca}^{2+}]_i\)) responses in myenteric neurons. Cultured myenteric neurons from small intestine of adult guinea pigs were loaded with the \([\text{Ca}^{2+}]_i\) indicator fluo-3 AM, and the \([\text{Ca}^{2+}]_i\) responses following the application of different stimuli were quantified by confocal microscopy and expressed as a percentage of the response to high-K\(^+\) solution (75 mM). Trains of electrical pulses (2 s, 10 Hz) were applied to stimulate the neuronal fibers before and after a 30-s superfusion with galanin (10\(^{-6}\) M). Substance P (SP), 5-HT, 1,1-dimethyl-4-phenyl-piperazinium iodide (DMPP), and carbachol were used as direct postsynaptic stimuli (10\(^{-5}\) M, 30 s) and were applied alone or after galanin perfusion. Galanin significantly reduced the responses induced by electrical fiber stimulation (43 ± 2 to 35 ± 3\%, \(P = 0.01\)), SP (15.4 ± 1 to 8.0 ± 0.3\%, \(P < 0.05\)), and 5-HT (26 ± 2 to 21.4 ± 1.5\%, \(P < 0.05\)). On the contrary, galanin did not affect the responses induced by local application of DMPP and carbachol. We conclude that in cultured myenteric neurons, galanin inhibits the excitatory responses induced by electrical stimulation, SP, and 5-HT. Finally, the inhibitory effect of galanin on electrical stimulation, but not on DMPP- and carbachol-induced responses, suggests that, at least for the cholinergic component, galanin acts at the presynaptic level.

galanin; myenteric neurons; cholinergic transmission; substance P; serotonin

GALANIN IS A 29-AMINO ACID peptide that is widely distributed in several tissues including the gastrointestinal tract of different mammalian species (2, 14, 15, 19, 29). In the myenteric plexus, galanin-expressing neurons have been found with fibers projecting either to the circular muscle layer and mucosa (9, 19) or terminating in myenteric ganglia, suggesting a modulatory role for galanin at different levels (7). This was supported by several studies in which galanin indeed altered gastrointestinal motility, either via a direct contractile action on the intestinal smooth muscle (5) or through an inhibitory effect on cholinergic myenteric neurons (7, 15, 35). The latter was also confirmed by Mulholland et al. (21), who showed that electrically induced ACh release in cultured myenteric neurons was reduced by galanin. Further evidence for this inhibitory role was recently presented by Sternini et al. (26) who showed inhibition of the contractile responses using longitudinal, muscle-myenteric plexus preparations (LMMP) from the guinea pig ileum. Finally, it has been reported that the inhibitory action of galanin on myenteric neurons leads to the suppression of cellular excitability through a hyperpolarization of the somatic membrane (22, 23, 28). Thus although there is evidence that galanin inhibits cholinergic transmission at the presynaptic level, it remains to be elucidated whether this holds true for other neurotransmitters and whether galanin also has a direct effect on the postsynaptic action of other neurotransmitters.

The myenteric plexus consists of several morphological and electrophysiological classes, in which not only ACh but also other excitatory neurotransmitters such as substance P (SP) and 5-HT are endogenously present (11, 12, 20, 27). Myenteric neurons in primary culture reliably reflect this diversity and are suitable models for the study of intracellular \([\text{Ca}^{2+}]_i\) concentrations ([Ca\(^{2+}\)]_i) signaling properties (30). In earlier studies, it was shown that electrical fiber strand stimulation (EFSS) (32) and the exogenous application of excitatory neurotransmitters such as SP, 5-HT, and selective neuropeptides such as 1,1-dimethyl-4-phenyl-piperazinium iodide (DMPP) induced a \([\text{Ca}^{2+}]_i\) rise via different mechanisms, indicating that the expression of functional receptors is preserved in culture (17, 25, 31). Recently, galanin has been shown to suppress calcium conductances in cultured myenteric neurons, but it is unknown whether its hyperpolarizing action affects the \([\text{Ca}^{2+}]_i\) (23).

The aim of the present study was 1) to investigate the effect of galanin and of cell hyperpolarization on neuronal \([\text{Ca}^{2+}]_i\), in cultured myenteric neurons, 2) to evaluate the influence of galanin on the \([\text{Ca}^{2+}]_i\), rise induced by electrical or cholinergic stimulation, and 3) to assess whether galanin also modulates the neuronal activation induced by SP and 5-HT. Finally, we also wanted to investigate whether a hyperpolarization (induced by low K\(^+\)) affected the \([\text{Ca}^{2+}]_i\) responses to electrical and chemical stimuli in a way similar to galanin.

MATERIALS AND METHODS

Myenteric neuron cultures. Primary cultures of myenteric neurons were prepared from adult guinea pig small intestine according to previously described methods (12, 13, 31). All procedures were approved by the animal ethics committee of Katholieke Universiteit Leuven.

Briefly, the longitudinal muscle and the adherent myenteric plexus were dissected from the circular muscle and mucosa. LMMP was digested in an enzymatic solution containing protease (1 mg/ml) and collagenase (1.25 mg/ml). After a 30-min incubation (37°C), the
G1010 EFFECTS OF GALANIN ON MYENTERIC NEURONAL Ca2+ RESPONSES

induced responses induced before and after galanin in low-K+ perfusion, were compared by unpaired Student

Medium 199 enriched with 10% fetal bovine serum and 50 ng/ml of 7-s nerve growth factor (7sNGF) was used. Antibiotics (100 μg/ml streptomycin, 100 U/ml penicillin, and 50 μg/ml gentamicin) were added to the medium, and glucose concentration was elevated to 30 mM. Cultures were kept in an incubator at 37°C and continuously gassed with 95% O2-5% CO2. Arabinose-C-furanoside (10 μM) was added to prevent proliferation of dividing cells (glial cells, fibroblasts).

[Ca2+]i measurements. Neurons were loaded for 30–45 min at 37°C in Krebs solution containing 10 μM fluo-3 AM and 2.5 μM Pluronic F-127 (25% wt/wt). Fluo-3 AM is taken up by the cells and entrapped intracellularly after hydrolysis by cytoplasmic esterases. After being incubated in fluo-3 loading medium, the neurons were washed four times in fresh modified Krebs and transferred to a coverslip chamber mounted on an inverted confocal laser-scanning microscope (Naroe OZ). Fluo-3 was excited by the 488-nm line of an Argon laser, and Ca2+-bound fluo-3 emission was recorded in the 500- to 560-nm range. The number of neurons (n) within one microscopic field ranged from 2 to 8. Changes in [Ca2+]i, are reflected in the changes in fluorescence of the fluo-3 indicator that was calculated for selected regions using InterVision software.

Fluo-3 fluorescence values were exported to a personal computer, and relative fluorescence changes were calculated by using Microsoft Excel; relative fluorescence was considered as the fluorescence of a certain region minus the fluorescence of the background divided by the fluorescence of that region at time 0 minus the background fluorescence at time 0 (32).

Study protocols. 5-HT, SP, DMPP, and carbachol (all at a 10−5 M concentration) were used as direct postsynaptic stimuli, and all were perfused for 30 s. A platinum electrode (diameter, 50 μm) was placed on the interconnecting strands 0.3–0.7 mm away from neurons of interest. Trains (2 s, 10 Hz) of electrical pulses (Grass Instruments, Quincy, MA) were used to stimulate the neuronal fibers. All stimuli were applied immediately after a 30-s superfusion with normal Krebs solution or galanin (10−6 M). To avoid desensitization, all neurons were exposed to galanin only once.

We previously showed (31, 32) that the [Ca2+]i responses elicited by repetitive electrical stimuli or by repetitive application of agonists are not significantly different from each other. Thus to mimic membrane hyperpolarization, commonly thought to be the mechanism of action of galanin, we performed another set of experiments in which all the stimuli (electrical stimulation and agonists) were applied after a 30-s superfusion with low-K+ medium (4 mM).

Also, to further explore the possibility that inhibition of SP- and 5-HT-mediated responses by galanin can be due to a pre- or a postsynaptic site of action, we conducted additional experiments in which galanin was perfused during a 5-min pretreatment with TTX (10−6 M).

[Ca2+]i responses were expressed as a percentage of the response to high-K+ solution (75 mM) and expressed as means ± SE. The agonist-induced [Ca2+]i rises, elicited after normal Krebs or galanin perfusion, were compared by unpaired Student’s t-tests. Excitatory-induced responses induced before and after galanin in low-K+ medium were analyzed with paired Student’s t-tests. Differences were considered to be significant if P < 0.05.

Experiments were performed in a modified Krebs solution containing (in mM) 150 NaCl, 6 KCl, 1.5 CaCl2, 1 MgCl2, 10 glucose, and 10 HEPES (pH 7.38). The high-K+ medium contained 75 mM K+ . Osmolarity was adjusted by lowering the Na+ concentration to 81 mM. The low-K+ medium contained 4 mM K+, and the osmolarity was adjusted by increasing the Na+ concentration to 152 mM.

RESULTS

Effect of galanin and hyperpolarization on [Ca2+]i signaling. Similar to previous reports (31, 32), cultured myenteric neurons were first identified by a brief depolarization step (75 mM K+), which opened the voltage-operated Ca2+ channels (VOCC) leading to a rise in [Ca2+]i. The perfusion of galanin (≤10−6 M for 30 s) did not induce any detectable changes in [Ca2+]i (n = 10).

Galanin has been shown to hyperpolarize some cells (23). We therefore mimicked a hyperpolarization by switching the K+ concentration from 5.9 to 4 mM. In 30 neurons, the perfusion with 4 mM K+ solution (30 s) did not induce any changes in [Ca2+]i.

Influence of galanin on electrically induced stimulation. The [Ca2+]i response to EFSS was studied in 40 neurons with such a response. The relative amplitude of the response to electrical stimulation was 42.7 ± 1.8% of the K+-induced Ca2+ response. However, after a 30-s perfusion with galanin (10−6 M), the Ca2+-evoked responses were significantly reduced (34.9 ± 2.6%, n = 38, P = 0.01) (Fig. 1A). A similar decrease in the EFSS-induced [Ca2+]i response was observed in 17 neurons in which the electrical stimulation was applied after the perfusion with low-K+ solution (30 s, 4 mM) (38.4 ± 3.8 to 33.3 ± 3.4%, P < 0.05) (Fig. 1B).

Effect of galanin on cholinergic [Ca2+]i signaling induced by selective agonists. To test the hypothesis that galanin would affect the cholinergic transmission pathway, we used DMPP (10−5 M) and carbachol (10−5 M) to activate nicotinic and muscarinic receptors. Galanin did not significantly affect the responses induced by DMPP (26.5 ± 0.8 to 24.6 ± 1.2%, n = 44 and 37, respectively, P = NS), and, also, the carbachol-induced [Ca2+]i rise was unchanged after galanin (17.0 ± 1.8 to 16.9 ± 1.1, n = 38 and 40, respectively, P = NS) (Fig. 2).

Similarly, the excitatory responses induced by DMPP (25.6 ± 1.7 vs. 24.8 ± 1.5%, n = 20) and carbachol (27.1 ± 4.0 vs. 24.8 ± 3.6%, n = 20, P = NS) were not significantly changed after perfusion of low K+ (Fig. 3).

Influence of galanin on SP- and 5-HT-induced [Ca2+]i signaling. ACh is not the only neurotransmitter in the myenteric plexus of the ileum. We therefore also tested the effect of galanin on two transmitters believed to be involved in the slow synaptic transmission between myenteric neurons (6, 16, 33).

Thirty-seven neurons with an SP-induced [Ca2+]i rise were studied. The relative amplitude of the response (15.4 ± 1.0%) was markedly reduced (8.0 ± 0.3, n = 59, P < 0.01) by the perfusion with galanin (Fig. 4A). In 35 neurons, the application of 5-HT (10−5 M) induced a [Ca2+]i rise of 25.8 ± 1.7%. A small but significant decrease in the 5-HT-induced [Ca2+]i increase was observed when 5-HT was superfused after galanin (21.4 ± 1.5%, n = 27, P < 0.05) (Fig. 4B).

Similarly, the perfusion of low K+ significantly reduced the [Ca2+]i rise induced by SP in 15 neurons (22.9 ± 2.1 to 17.3 ± 1.9%, P < 0.01) and by 5-HT in 32 cells (27.3 ± 1.3 to 23.7 ± 1.1%, P < 0.05) (Fig. 5).
Pretreatment with TTX did not significantly affect the SP- and 5-HT-induced responses (17.8 ± 1 and 29 ± 1.5%, n = 52 and 38, respectively). However, SP- and 5-HT-induced \([\text{Ca}^{2+}]_\text{i}\) rises were significantly reduced when galanin was added to TTX (11.8 ± 1.2 and 20.6 ± 1.7%, n = 33 and 42, respectively, \(P < 0.01\)).

**DISCUSSION**

In this study, we showed that galanin causes inhibition of the \([\text{Ca}^{2+}]_\text{i}\) responses induced by several excitatory stimuli in myenteric neurons. We used primary cultures of myenteric neurons, shown to be a good model for the study of the myenteric plexus, because the neurons in culture express functional receptors for various neurotransmitters and neuroactive compounds (11, 17, 24, 25, 31, 32). As previously reported, both the electrical stimulation of neuronal processes and the exogenous administration of excitatory neuroligands induced a \([\text{Ca}^{2+}]_\text{i}\) rise in a subset of cultured neurons (31, 32).

Application of galanin did not induce any significant changes in the \([\text{Ca}^{2+}]_\text{i}\) of myenteric neurons. This finding is consistent with previous electrophysiological observations stating that the action of galanin in the guinea pig small intestine was inhibitory and brought about a membrane hyperpolarization (22, 28).

---

**Fig. 1.**

A: effect of galanin on \([\text{Ca}^{2+}]_\text{i}\) signaling evoked by electrical stimulation in cultured myenteric neurons. Fluo-3-emitted fluorescence is plotted against time. Arrow represents the addition of 75 mM K\(^+\) (10 s). Electrical stimulation of neuronal fiber strands (EFSS; 2 s, 10 Hz, gray arrow) induce an intracellular \([\text{Ca}^{2+}]_\text{i}\) concentration (\([\text{Ca}^{2+}]_\text{i}\)) rise in 40 neurons (black line). After perfusion with galanin \(10^{-6}\) M (gray line), a significant decrease of the \([\text{Ca}^{2+}]_\text{i}\) increase was observed (\(n = 38\) cells, \(P = 0.01\)).

B: effect of hyperpolarization on \([\text{Ca}^{2+}]_\text{i}\) signaling induced by electrical stimulation. Electrically induced \([\text{Ca}^{2+}]_\text{i}\) rises (2 s, 10 Hz, gray arrow) were significantly reduced after perfusion with 4 mM K\(^+\) solution (\(n = 17\), \(P < 0.05\)).

---

**Fig. 2.**

A: increase of \([\text{Ca}^{2+}]_\text{i}\) induced by perfusion with 10 \(\mu\)M 1,1-dimethyl-4-phenyl-piperazine (DMPP; \(n = 37\), black trace) was unaffected by galanin (\(n = 44\), gray trace). B: increase of \([\text{Ca}^{2+}]_\text{i}\) induced by perfusion with 10 \(\mu\)M carbachol (\(n = 38\), black trace) was similarly unchanged by galanin (\(n = 40\), gray trace). Arrows indicate addition of 75 mM K\(^+\) (10 s); horizontal bars represent the application of agonists.

---

**Fig. 3.**

A: effect of hyperpolarization on \([\text{Ca}^{2+}]_\text{i}\) signaling induced by cholinergic agonists. Relative amplitudes of \([\text{Ca}^{2+}]_\text{i}\) increases induced DMPP (A; \(n = 20\)) and carbachol (B; \(n = 20\)) were, respectively, unchanged before and after perfusion with low-K\(^+\) solution (30 s, 4 mM) (\(P = \text{all not significant}\)). Fluo-3-emitted fluorescence is plotted against time, and black arrow represents the addition of 75 mM K\(^+\) (10 s).
Moreover, both in hippocampal and AH/type II myenteric neurons, galanin has been shown to have a direct inhibitory effect via a hyperpolarizing effect, which involves both the activation of an inwardly rectifying K\(^+\) current and the blockade of voltage-gated Ca\(^{2+}\) channels (1, 15, 23, 28). In the present study, we used a perfusion step with low-K\(^+\) solution to verify whether the effect of hyperpolarization is affecting the \([\text{Ca}^{2+}]_{\text{i}}\) and also to mimic the hyperpolarizing effect of galanin on myenteric neurons. Similar to galanin, low-K\(^+\) perfusion did not induce any detectable \([\text{Ca}^{2+}]_{\text{i}}\) changes, suggesting that the pattern of \([\text{Ca}^{2+}]_{\text{i}}\) recording during galanin perfusion is similar to that observed during hyperpolarization of the neuronal membrane.

Galanin is well known to have multiple effects on the gastrointestinal tract (3, 10, 29), probably acting via different receptors (4) and at different sites. Galanin-immunoreactive fibers have been widely found in the long, inhibitory, descending neural pathways, and they colocalize with other inhibitory neurotransmitters, such as vasoactive intestinal peptide or nitric oxide fibers (8). In view of their location, it is likely that galanin-immunoreactive fibers mainly act to inhibit the release of, or the activity of, cholinergic and noncholinergic neurotransmitters (7). In the LMMP, galanin was shown to inhibit the induced release of ACh (35), which was confirmed by Mulholland et al. (21), who showed that galanin inhibits the induced \(^{3}H\)ACh release in cultured guinea pig myenteric plexus neurons. Data from the present study corroborate these observations. The \([\text{Ca}^{2+}]_{\text{i}}\) rise induced by electrical fiber tract stimulation, used to induce a general synaptic activation, was decreased by galanin application.

### Figures

**Fig. 4.** Involvement of galanin in the substance P (SP)- and 5-HT-induced \([\text{Ca}^{2+}]_{\text{i}}\) increase. A: perfusion with 10^{-5} M SP (horizontal bar) induced an increase of \([\text{Ca}^{2+}]_{\text{i}}\), in 37 neurons (black trace), which was markedly reduced \((P < 0.01)\) in 59 neurons after superfusion with galanin 10^{-6} M (gray trace). B: perfusion with 10^{-5} M 5-HT (horizontal bar) induced an increase of \([\text{Ca}^{2+}]_{\text{i}}\), in 35 neurons (black trace), which was significantly reduced \((P < 0.05)\) in 27 neurons after superfusion with galanin 10^{-6} M (gray trace). Arrows represent addition of 75 mM K\(^+\) (10 s).

**Fig. 5.** Effect of hyperpolarization on \([\text{Ca}^{2+}]_{\text{i}}\) signaling induced by noncholinergic agonists. A: perfusion with 10^{-5} M SP (black horizontal bar) induced an increase of \([\text{Ca}^{2+}]_{\text{i}}\), that was reduced after a 30-s perfusion with 4 mM K\(^+\) solution (gray horizontal bar). B: 5-HT-induced increase of \([\text{Ca}^{2+}]_{\text{i}}\), (black horizontal bar) was reduced after superfusion with 4 mM K\(^+\) (gray horizontal bar). Arrows represent the addition of 75 mM K\(^+\) (10 s). B and D: relative amplitudes of \([\text{Ca}^{2+}]_{\text{i}}\), increases induced by SP (22.9 ± 2.1%, \(n = 15\)) and 5-HT (27.3 ± 1.3%, \(n = 32\)) were significantly reduced after a 30-s perfusion with 4 mM K\(^+\) solution (17.3 ± 1.9 and 23.7 ± 1.1%, respectively). \(*P < 0.01, \quad \**P < 0.05.\)
significantly reduced by galanin, suggesting that the reduction of the electrically induced responses after galanin may be due to a reduced release of ACh. This was confirmed by the results of the direct postsynaptic cholinergic stimulation.

In cultured neurons, both postsynaptic nicotinic and muscarinic receptors are involved in the neuronal ACh-induced \([\text{Ca}^{2+}]\) signaling (25, 31); thus we used specific ligands to activate nicotinic and muscarinic receptors. Galanin failed to reduce the \([\text{Ca}^{2+}]\), signaling evoked either by DMPP or carbachol, suggesting that its inhibitory effect on cholinergic transmission does not involve an action on postsynaptic receptors. In the same line, we showed that hyperpolarization of neurons with low K\(^+\) perfusion inhibits the electrically induced responses, whereas postsynaptic stimulation by cholinergic agonists was not affected. The fact that galanin blocks VOCC (23, 28) and intracellular calcium influx plays a central role in the initiation of neurotransmitter release might explain how galanin inhibits the cholinergic transmission at the postsynaptic terminals.

However, several other excitatory neurotransmitters are endogenously present in the myenteric neuronal network where they contribute to excitatory transmission. Therefore, we used exogenous administration of SP and 5-HT to investigate the ability of galanin to inhibit noncholinergic excitatory responses. In cultured myenteric neurons, SP and 5-HT signaling is mediated via neurokinin-1 and 5-HT3 receptors, respectively (6, 24, 31). We showed that galanin reduced the \([\text{Ca}^{2+}]\), increase induced by local application of SP and 5-HT, suggesting that, in contrast to the cholinergic transmission, galanin inhibited noncholinergic neurotransmission, at least in part, at the postsynaptic level. In this context, our findings that SP and 5-HT-induced responses were not affected by TTX, whereas they were significantly lowered by the addition of galanin, are likely to confirm a postsynaptic site of action of galanin.

The mechanism by which galanin also inhibits noncholinergic transmission seems most likely to be attributable to its ability to hyperpolarize the neuronal membrane (23). Indeed, it was shown that both SP- and 5-HT-induced \([\text{Ca}^{2+}]\); responses were reduced after hyperpolarization of neurons with low K\(^+\) perfusion. Alternatively, when exogenous neurostimulants are applied to myenteric neurons, a secondary release of ACh may occur, which, in turn, may induce a \([\text{Ca}^{2+}]\), rise in neighboring cells (34). Therefore, we cannot completely rule out that the inhibition of the SP- and 5-HT-induced \([\text{Ca}^{2+}]\), rise is secondary to the reduced release of ACh (35, 36).

Also, different mechanisms underlying intracellular calcium increase, rather than receptor location, may represent an alternative hypothesis to explain the different inhibitory effects of galanin. However, it seems unlikely that galanin would similarly act on different receptors that belong to the same family class. In cultured myenteric neurons, DMPP- and 5-HT-induced responses are, respectively, mediated by nicotinic and 5-HT\(_3\) receptors, which are both considered ion channels. In keeping with this, if galanin inhibits calcium entry by inhibiting the calcium entry through this type of receptor, it would result in a significant inhibition of both nicotinic- and 5-HT-induced responses, whereas we only observed a significant inhibition of 5-HT-induced \([\text{Ca}^{2+}]\), increase.

Further studies are thus needed to clarify whether galanin selectively inhibits postsynaptic SP- and 5-HT-induced responses or whether it also reduces the cholinergic presynaptic responses. Taken together, our results confirm once again the wide inhibitory effect of galanin on excitatory responses in myenteric neurons.

In summary, we studied the inhibition of galanin on \([\text{Ca}^{2+}]\), rise induced by major excitatory stimuli on cultured myenteric neurons of guinea pig small intestine. We conclude that galanin inhibits the excitatory responses induced by electrical stimulation and reduces the \([\text{Ca}^{2+}]\), increase induced by SP and 5-HT. Conversely, galanin does not affect the increase in \([\text{Ca}^{2+}]\), induced by DMPP and carbachol. This suggests that the inhibitory effect of galanin is due to a predominant presynaptic effect for the cholinergic transmission and/or a postsynaptic effect on noncholinergic excitatory neurotransmission. Finally, our data support the hypothesis that galanin is a modulating neuropeptide that exerts an inhibitory action on myenteric neurons through the suppression of cholinergic transmission.

**ACKNOWLEDGMENTS**

Present address of G. Sarnelli: Division of Hepatogastroenterology, Department of Clinical and Experimental Medicine, University of Naples “Federico II,” Via Pansini 5, 80131, Naples, Italy.

**GRANTS**

P. Vanden Berge is a postdoctoral fellow of the Fonds voor Wetenschappelijk Onderzoek, Vlaanderen, Belgium.

**DISCLOSURE**

This work was presented, in part, at the Digestive Disease Week, Atlanta, GA, May 20–23, 2001, and it was published as an abstract in *Gastroenterology* 120: A1046, 2001.

**REFERENCES**


