Mechanism of galanin-induced contraction of longitudinal smooth muscle of the rat jejunum

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Galanin is a 29-amino acid peptide that was first isolated from the myenteric plexus of pig ileum (38). On the basis of immunoreactivity studies, galanin appears to be widely distributed in most mammalian species. Immunoreactivity to galanin is present in the central and peripheral nervous system (3, 34) as well as the gastrointestinal tract (9, 15). In contrast, the physiological function of galanin is quite diverse and extremely species and tissue specific. Galanin dilates arterioles from guinea pig intestine (24), whereas widespread vasoconstriction in response to galanin is seen in the opossum (7) and little to no response is seen in the vasculature of the cat (7).

The smooth muscle of the gastrointestinal tract also demonstrates species- and location-specific responses to galanin. Ileal smooth muscle of the pig, rat, rabbit, and guinea pig contracts, whereas canine ileum relaxes in response to galanin (5, 6). Galanin relaxes gastric smooth muscle cells of the guinea pig (17, 18) but is a potent contractile agonist of rat jejunum (20).

The contractile actions of galanin are believed to be a direct myogenic action due to the lack of inhibitory effect of tetrodotoxin (8). Specific galanin receptors have been identified on several smooth muscle cells that either contract or relax in response to galanin (17, 18). Moreover, previous studies have shown that at least in pig ileum, galanin induces contraction by activation of a pertussis toxin-sensitive G protein and influx of extracellular Ca2+ (5). However, very little else is known about how the peptide initiates contraction of smooth muscle at the intracellular level.

The goal of this study was to determine the mechanism(s) by which galanin contracts rat jejunal smooth muscle. Specifically, we were interested in determining if galanin-induced contractions are a direct myogenic response, the source of the activator Ca2+ for contraction (intracellular release or extracellular influx), and the relationship between galanin-induced force and levels of myosin light chain (MLC) phosphorylation. In addition, we were interested in determining if galanin stimulation enhances the myofilament Ca2+ sensitivity of the jejenum and if so, the response is coupled to a pertussis toxin-sensitive G protein.

MATERIALS AND METHODS

Tissue preparation. All experimental protocols were approved by the Animal Care and Use Committee of the Allegheny University Hospitals, Graduate. Male Sprague-Dawley rats, weighing 250–300 g, were provided standard rat chow and water ad libitum. The rats were killed by inhalation overdose using 100% CO2. The abdominal cavity was opened, and the jejenum was quickly excised and placed in ice-cold physiological salt solution (PSS) composed of (in mM) 140.1 NaCl, 4.7 KCl, 1.2 Na2HPO4, 1.2 MgSO4, 1.6 CaCl2, 2 3-(N-morpholino)propanesulfonic acid (pH 7.4), 0.02 Na2EDTA, and 5 d-glucose. The jejenum was cut along the long axis of the tissue, cleaned of all intestinal contents, and cut into longitudinal strips 1 × 0.2 cm (length × width), averaging 0.5 mm in thickness for experiments measuring isometric force and MLC phosphorylation levels of intact tissue, and 0.5 × 0.08 cm (length × width), averaging 0.2 mm in thickness for experiments measuring isometric force and MLC phosphorylation levels of α-toxin-permeabilized tissue.

The jejunal strips to be used for intact muscle experiments were mounted vertically between a micrometer for control of muscle length and a Grass FT.03 force transducer attached to a Grass model 7 polygraph for recording of all force responses. The mounted strips were maintained in water-jacketed, glass muscle chambers warmed to 37°C and aerated with 100% O2. All muscle chambers were siliconized with Prosil-28 (PCR, Gainesville, FL). Initial experiments demonstrated that maximal active force development in response to 30 µM carbachol was produced at a resting force of 400–500 mg. Therefore, all subsequent experiments were performed at this resting force. The strips were equilibrated in the warmed, aerated PSS for 30–40 min, then contracted by the addition of 30 µM carbachol. The strips were rinsed several times and allowed to equilibrate for another 30–40 min, after which they were contracted by the addition of 30 nM galanin. The strips were then washed and equilibrated for another 30–40 min before stimulation with carbachol to determine their initial force. Each strip was then stimulated with a variety of substances, including 30 nM galanin, as well as 10 µM carbachol, 0.1 µM saxitoxin, and 10 nM tetrodotoxin. The vehicle for these substances was a solution of 0.02% Evans blue and 0.1% bovine serum albumin in 140.1 mM NaCl, 3.5 mg/l glutamate, 4.7 mM KCl, 1.2 mM Na2HPO4, 1.2 mM MgSO4, 1.6 mM CaCl2, 2 3-(N-morpholino)propanesulfonic acid (pH 7.4), 0.02 mM Na2EDTA, and 5 d-glucose. The tissue was rinsed several times and allowed to equilibrate for another 30–40 min before stimulation with carbachol to determine their initial force. Each strip was then stimulated with a variety of substances, including 30 nM galanin, as well as 10 µM carbachol, 0.1 µM saxitoxin, and 10 nM tetrodotoxin. The vehicle for these substances was a solution of 0.02% Evans blue and 0.1% bovine serum albumin in 140.1 mM NaCl, 3.5 mg/l glutamate, 4.7 mM KCl, 1.2 mM Na2HPO4, 1.2 mM MgSO4, 1.6 mM CaCl2, 2 3-(N-morpholino)propanesulfonic acid (pH 7.4), 0.02 mM Na2EDTA, and 5 d-glucose. The tissue was rinsed several times and allowed to equilibrate for another 30–40 min before stimulation with carbachol to determine their initial force.
allowed a third 30- to 40-min equilibration period before they were used in any experimental protocol.

α-Toxin permeabilization. The jejunal strips were depleted of Ca²⁺ by the addition of 30 µM carbachol in a Ca²⁺-free PSS containing 1 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N’-tetraacetic acid (EGTA). The strips were then exposed to a solution containing (in mM) 10 EGTA, 1 Mg²⁺, 25 creatine phosphate (CP), 4 MgATP, 20 imidazole (pH 6.8), 50 K-acetate, 1 dithiothreitol (DTT), and 2,500 U/ml creatine phosphokinase (CP), 4 MgATP, 20 imidazole (pH 6.8), 1 DTT, sufficient K-acetate to maintain ionic strength constant at 120, 1 phenylmethylsulfonyl fluoride (PMSF), and 1 µM ionomycin. The composition of all Ca²⁺-contracting solutions was determined by a computer program that calculates the exact amounts of CaCl₂, MgCl₂, and MgATP at a specific pH, ionic strength, and temperature (27, 36).

MLC phosphorylation measurement. Intact or α-toxin-permeabilized strips were frozen at various times before and during a contraction by immersion in a dry ice-acetone slurry containing 6% trichloroacetic acid. The strips were slowly thawed, weighed, and then homogenized in a solution composed of 10% glycerol, 20 mM DTT, and 2% sodium dodecyl sulfate, using glass-glass homogenizers. The homogenized strips were subjected to two-dimensional electrophoresis as previously described (28). Quantitation of MLC phosphorylation levels was performed using a Molecular Dynamics personal laser densitometer. Phosphorylation levels were calculated by volume (area × density of each pixel in area) analysis of the electrophoretic spot corresponding to the phosphorylated MLC as a percentage of the total volume of both the phosphorylated and unphosphorylated MLC. Satellite spots were not included in the analysis or quantitation.

Experimental procedures. All tissues to be contracted by the addition of galanin, intact or permeabilized, were incubated first in an appropriate solution containing 1 mM PMSF, 10 µM amastatin, 10 µM leupeptin, and 1 µM phosphoramidon for 20 min. Carbachol-induced contractions of the longitudinal smooth muscle were unaffected by the presence of these protease and peptidase inhibitors. All subsequent solutions contained these protease and peptidase inhibitors.

Cumulative galanin concentration-response curves were performed on intact strips. The active, stimulated force produced by the intact strips in response to each addition of galanin was taken as the peak of the phasic activity minus the peak of the active, unstimulated phasic activity. The experimental solutions also contained 1 µM atropine, 10 µM hexamethonium, 10 µM guanethidine, and in selected experiments 1 µM tetrodotoxin to block neuronal input, thus ensuring that all contractions in response to galanin were due to a direct myogenic effect.

For the experiments designed to examine the source of activator Ca²⁺ during a galanin-induced contraction, several protocols were performed. First, to determine if intracellular Ca²⁺ was the source of activator Ca²⁺, the tissues were incubated in a Ca²⁺-free PSS containing 2 mM EGTA for 10 min, at which time 30 µM carbachol was added, producing a rapid but transient contraction. After the strips fell to baseline force, the strips were rinsed twice with Ca²⁺-free PSS containing 2 mM EGTA following again by the addition of 30 µM carbachol. In no strips did the second challenge with carbachol elicit a contraction. The strips were rinsed three times with Ca²⁺-free PSS (without EGTA), then exposed to normal CaCl₂-containing PSS plus 30 nM galanin and the cocktail of protease and peptidase inhibitors and neuronal blockers.

EGTA has been suggested to increase membrane permeability, and as such the use of EGTA may alter the responsiveness of the strip. Therefore a second protocol was used to examine the role of intracellular Ca²⁺ release in a galanin-induced contraction. The strips were incubated in Ca²⁺-free PSS containing 1 µM thapsigargin, an inhibitor of the sarcoplasmic reticulum (SR) Ca²⁺-adenosinetriphosphatase (39) for 30 min. The strips were then challenged with 30 µM carbachol to ensure that all SR Ca²⁺ was depleted from the cell. If a contraction was observed, the strips were again incubated in Ca²⁺-free PSS containing 1 µM thapsigargin for 30 min, at which time they were challenged with carbachol. In no strips did the addition of the second challenge of carbachol elicit a contraction. The strips were then exposed to normal CaCl₂-containing PSS plus 30 nM galanin and the cocktail of protease and peptidase inhibitors and neuronal blockers.

To determine the role of extracellular Ca²⁺ in a galanin-induced contraction, we performed two protocols. First, the strips were rinsed three times, for 2 min each, with a Ca²⁺-free PSS containing 0.1 mM EGTA to remove extracellularly bound Ca²⁺. The strips were then rinsed twice, for 2 min each, with a Ca²⁺-free PSS to remove any EGTA and then challenged with 30 nM galanin and the cocktail of protease and peptidase inhibitors and neuronal blockers. The second protocol was to simply add 0.1 µM nifedipine to the normal CaCl₂-containing PSS for 30 min and then challenge the strips with 30 nM galanin and the cocktail of protease and peptidase inhibitors and neuronal blockers.

Chemicals. Galanin was obtained from Bachem Biosciences (Philadelphia, PA). ATP, amastatin, atropine, carbachol, guanethidine, GTP, hexamethonium, leupeptin, nifedipine, PMSF, phosphoramidon, pertussis toxin, and tetrodotoxin were purchased from Sigma Chemical (St. Louis, MO). Ionomycin was purchased from Calbiochem. Staphylococcus aureus α-toxin was purchased from Gibco-BRL (Gaithersburg, MD).

Data analysis and statistics. Data are presented as means ± SE. Statistical differences between means were determined using the Student's t-test for unpaired data. P < 0.05 was taken as significant.

RESULTS

The cumulative addition of the 29-amino acid peptide galanin to an intact strip of rat jejunal smooth muscle induced a concentration-dependent contraction. Figure 1 shows a representative tracing of the galanin-induced response of the jejunum strip. Galanin increased both the phasic and tonic components of the contraction. The response to galanin was not affected by 1 µM atropine, 10 µM hexamethonium, or 10 µM guanethidine. The addition of 1 µM tetrodotoxin did not affect the galanin-
induced response either, supporting the conclusion that the increase in force was a direct myogenic effect of galanin and not the indirect effect of stimulation of excitatory neurons or depression of inhibitory neurons. The addition of a cocktail of peptidase and protease inhibitors was, however, required for the reliable demonstration of galanin-induced contraction, demonstrating the presence of active proteolytic enzymes in the jejunum preparation. The inclusion of 1 mM PMSF, 10 µM amastatin, 10 µM leupeptin, and 1 µM phosphoramidon produced very reproducible contractions in response to the cumulative addition of galanin as shown in Fig. 2. This figure shows the results of several of the experiments represented by the tracing in Fig. 1. All forces were normalized to the maximal contraction in response to galanin. Galanin contracted the jejunum strip with a threshold concentration of 1 nM, a half-maximum effective concentration (EC50) of 9.2 ± 0.1 nM, and a maximal contraction at 100 nM. The contractile response to galanin was 87 ± 6% of the response to carbachol.

In several experiments, the magnitude of developed force in response to galanin was not maintained (e.g., as shown in Fig. 3) and fell to a lower yet still suprabasal value. We determined whether this was the typical physiological response of the tissue to galanin or simply the result of proteolytic degradation of galanin. A large volume of PSS containing 30 nM galanin was divided in half. One half was used to contract several tissues, whereas the other half was maintained aerated at 37°C. After the contracted tissues achieved a steady-
state level of force, the bathing solution was replaced with the aerated, warmed PSS containing 30 nM galanin; no change in the level of force was noted in any of the tissues. If the fall in force was the result of degradation of galanin, one would expect then an increase in the level of force on the addition of fresh solution. Conversely, we contracted several tissues with PSS containing 30 nM galanin, allowed the tissues to reach a steady-state level of force, and then removed the bathing PSS and applied it to a set of uncontracted, resting tissues; the second set of tissues rapidly developed force with a similar temporal profile and magnitude as the first set of tissues. This demonstrates that a rapid increase in force followed by a slow decline to a suprabasal value is not the result of proteolytic degradation of galanin.

The next series of experiments was performed to determine the source of the activator Ca\(^{2+}\) for the galanin-induced contraction. The results of these experiments are shown in Table 1. Galanin-induced force development was not significantly affected by either depletion of intracellular Ca\(^{2+}\) stores or by the addition of thapsigargin. Galanin-induced force development was, however, completely abolished by either blockade of dihydropyridine Ca\(^{2+}\) channels or removal of extracellular Ca\(^{2+}\). The lack of a galanin-induced contraction in the absence of extracellular Ca\(^{2+}\) was not due to tissue damage resulting from either protocol, as the addition of carbachol produced a maximal contraction. Therefore, although galanin-induced contractions are apparently receptor mediated (6, 19), they are dependent on influx of extracellular Ca\(^{2+}\) and not on release from intracellular stores.

The time course of MLC phosphorylation and force development during a galanin-induced contraction in normal CaCl\(_2\)-containing PSS is shown in Fig. 3. MLC phosphorylation levels were quantified from two-dimensional electrophoretic gels as described in MATERIALS AND METHODS. The location of the MLC, both unphosphorylated and phosphorylated, on the electrophoretic gel was initially determined by transfer of the proteins to nitrocellulose paper by standard techniques (2, 29), then visualized using a rabbit polyclonal antibody against chicken gizzard MLC and an anti-rabbit goat secondary antibody conjugated with alkaline phosphatase. After several experiments were performed in which Coomassie blue-stained electrophoretic gels and immunoblotting techniques used simultaneously provided similar quantitative values, only Coomassie blue-stained electrophoretic gels were utilized. The addition of 30 nM galanin produced a rapid increase in both MLC phosphorylation levels and force. Interestingly, MLC phosphorylation levels were maintained while force fell to suprabasal values. These results are consistent with previously published reports on smooth muscle (13, 14) in which high levels of MLC phosphorylation were measured although force had significantly declined during carbachol stimulation.

The results shown in Fig. 3 suggest that galanin stimulation of the rat jejunum may induce alterations in myofilament Ca\(^{2+}\) sensitivity. To examine this possibility, we performed experiments using the \(\alpha\)-toxin-permeabilized jejunum. This preparation allows the precise control of the intracellular environment while retaining receptor- and G protein-dependent transduction pathways intact. Figure 4 contains a representative tracing of an experiment in which the force response of an \(\alpha\)-toxin-permeabilized strip was measured during the cumulative addition of Ca\(^{2+}\) in the absence and presence of 30 nM galanin plus 10 \(\mu\)M GTP. The \(\alpha\)-toxin-permeabilized strip contracted rapidly and in a concentration-dependent manner in response to an increase in the Ca\(^{2+}\) concentration ([Ca\(^{2+}\)])). The addition of galanin plus GTP enhanced the level of force developed in response to each [Ca\(^{2+}\)]. Galanin did not increase force in the absence of exogenously added GTP, and GTP alone had no effect on force. Additionally, the galanin plus GTP-dependent increase in force was reversed by the addition of guanosine 5'-O-(2-thiodiphosphate), demonstrating that the increase is G protein dependent.

Galanin plus GTP induced a significant increase in the Ca\(^{2+}\) sensitivity of force as shown by the leftward shift of the [Ca\(^{2+}\)]-force response curve (Fig. 5). The EC\(_{50}\) for a Ca\(^{2+}\)-dependent contraction of the \(\alpha\)-toxin-permeabilized jejunum strip was 2.32 ± 0.08 \(\mu\)M, whereas in the presence of galanin plus GTP it decreased to 1.03 ± 0.05 \(\mu\)M Ca\(^{2+}\) (P < 0.05). In addition to increasing the myofilament Ca\(^{2+}\) sensitivity, galanin plus GTP augmented the maximal level of force developed.

The last set of experiments performed were designed to determine if the galanin-induced contraction involved a pertussis toxin-sensitive G protein (Fig. 6). The \(\alpha\)-toxin-permeabilized strips were contracted with Ca\(^{2+}\), and the contraction was augmented by the addition of galanin plus GTP, then relaxed by the addition of a high-EGTA relaxing solution. The strip was then perfused with relaxing solution containing 5 \(\mu\)g/ml pertussis toxin for 60 min. The strips were then recontracted with the same Ca\(^{2+}\) solution followed again by the addition of galanin plus GTP. Control tissues were

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Table 1. Source of activator Ca\(^{2+}\) for galanin-induced contraction of intact rat jejunum

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Force Development, mg</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>453 ± 39.5</td>
<td>20</td>
</tr>
<tr>
<td>Thapsigargin (1 µM)</td>
<td>470 ± 66</td>
<td>7</td>
</tr>
<tr>
<td>Ca(^{2+}) depleted</td>
<td>380 ± 94</td>
<td>3</td>
</tr>
<tr>
<td>Nifedipine (0.1 µM)</td>
<td>0*</td>
<td>7</td>
</tr>
<tr>
<td>0 CaCl(_2) PSS</td>
<td>0*</td>
<td>3</td>
</tr>
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</table>

Values are means ± SE; n, no. of determinations. Strips of intact rat jejunum were contracted with 30 nM galanin (control) and then treated with the appropriate agent and exposed a second time to 30 nM galanin. To inhibit influx of extracellular Ca\(^{2+}\), strips were incubated with the dihydropyridine Ca\(^{2+}\) channel blocker nifedipine (0.1 µM) before the addition of galanin or were exposed to a 0 CaCl\(_2\) physiological saline solution (PSS) containing 0.1 mM EGTA as described in MATERIALS AND METHODS. To inhibit release of intracellular Ca\(^{2+}\) stores, tissues were either incubated with the inhibitor of sarcoplasmic reticular Ca\(^{2+}\)-ATPase activity, thapsigargin (1 µM), or depleted of intracellular Ca\(^{2+}\) as described in MATERIALS AND METHODS. * Significantly different from control value (P < 0.05).
contracted in response to galanin, allowed to relax for 60 min, and then restimulated with galanin; the two contractions were essentially identical. As shown in the tracing in Fig. 6, pertussis toxin almost completely abolished the galanin-induced increase in myofilament Ca\(^{2+}\) sensitivity.

DISCUSSION

Galanin is a naturally occurring peptide to which all smooth muscles studied to date are responsive. Whether galanin-induced stimulation of smooth muscle results in a contraction or relaxation depends on both the species and tissue. Consistent with previous reports (20), we have shown in this study that galanin is a contractile agonist in the longitudinal smooth muscle of rat jejunum. Based on the finding that neither atropine, hexamethonium, guanethidine, nor tetrodotoxin affected the galanin-induced contraction, our results confirm the finding (20) that galanin contracts the longitudinal smooth muscle from rat jejunum by a direct myogenic effect. We now extend these findings to demonstrate that galanin stimulation of rat jejunum activates a pertussis toxin-sensitive G protein coupled to the influx of extracellular Ca\(^{2+}\). The results of our study present, to the best of our knowledge, the first information concerning the relationship between force and MLC phosphorylation levels during a galanin-induced contraction of any smooth muscle tissue. Moreover, our results are also the first to demonstrate that galanin stimulates the biochemical pathway(s) responsible for enhancement of myofilament Ca\(^{2+}\) sensitivity as well as that responsible for desensitization of the myofilaments by uncoupling MLC phosphorylation from steady-state force. Of particular interest is the novel finding that galanin stimulation is apparently associated with a receptor-G protein complex that initiates the pathway(s) that increases myofilament Ca\(^{2+}\) sensitivity, but does not apparently produce the second messengers that release intracellular Ca\(^{2+}\). The importance of this finding lies in the fact that all smooth muscle agonists tested to date that increase myofilament Ca\(^{2+}\) sensitivity also result in inositol 1,4,5-trisphosphate (IP\(_3\))-induced Ca\(^{2+}\) release. Carbachol stimulation of most gastrointestinal smooth muscles, for example, is tightly coupled to G protein-dependent increases in IP\(_3\) and the subsequent release of intracellular Ca\(^{2+}\) (23). This is not to suggest that IP\(_3\) is involved in the pathway(s) that modulates myofilament Ca\(^{2+}\) sensitivity, but it has been implicitly assumed that the same initiating events result in both IP\(_3\) and sensitization. Our results now clearly demonstrate that the two pathways are not coupled.

The direct myogenic effect of galanin is apparently due to binding at specific galanin receptors on the smooth muscle membrane. Recent work has suggested that there are at least three galanin receptor subtypes...
Botella et al. (6), using two galanin-receptor antagonists, M15 and M35, demonstrated that M15 was more potent in inhibiting contractile responses to galanin, whereas M35 was more potent in inhibiting galanin-induced relaxation. Therefore, our present results taken in the context of the work by Botella et al. (5, 6) suggest that galanin contracts rat jejunum by binding to an M15-sensitive receptor coupled to a pertussis toxin-sensitive G protein.

Most G protein-coupled receptors in smooth muscle that initiate contraction activate phospholipase C with the resultant liberation of IP3 and release of intracellular stores of Ca2+(23). This cascade of events has been shown for vascular, airway, and gastrointestinal smooth muscles (1). Murthy et al. (30, 31) have previously demonstrated that receptor-G protein mediated events in circular smooth muscle are coupled to IP3-induced release of intracellular Ca2+, whereas those in longitudinal smooth muscle of the gastrointestinal tract are coupled to influx of intracellular Ca2+. Our results are consistent, in part, with these studies. The galanin-induced contraction of rat jejunum was not affected by either depletion of SR Ca2+ or by the addition of an inhibitor of SR function, thapsigargin. In contrast, the galanin-induced contractions were completely abolished by removal of extracellular Ca2+ or addition of the dihydropyridine Ca2+ channel blocker nifedipine. This suggests that the galanin-receptor-G protein complex on rat jejunal smooth muscle membranes may be coupled to phospholipase D, which increases only diacylglycerol and not IP3, or is directly coupled to a Ca2+ channel. On the other hand, carbachol stimulation of longitudinal jejunal smooth muscle was unaffected by removal of extracellular Ca2+ and abolished by depletion of intracellular Ca2+ stores. This suggests that, at least in the jejunum of the rat, the agonist-induced contraction of longitudinal smooth muscle can be coupled to either influx or intracellular release, depending on the receptor(s) involved.

Based on data from a vascular smooth muscle cell line (10, 21), protein kinase C activated by diacylglycerol directly increases Ca2+ conductance through a dihydropyridine-sensitive Ca2+ channel. On the other hand, it has also been shown that protein kinase C inhibits Ca2+ channel function in smooth muscle (12). Because receptor-activated Ca2+ channels as described by Bolton and Pacaud (4) are not dihydropyridine sensitive, these entities are probably not important in the galanin-induced contraction. Whether a diacylglycerol-activated Ca2+ channel could be involved in the galanin-induced contraction is unknown.

![Fig. 5](http://ajpgi.physiology.org/) Concentration-response curves of α-toxin-permeabilized jejunal smooth muscle to Ca2+ in the presence and absence of galanin. The results of several experiments of the type shown in Fig. 4 were normalized to the maximal force developed in response to 10 μM Ca2+ alone, averaged, and plotted against the free Ca2+ concentration. GTP significantly enhanced the development of force at every [Ca2+] tested. Galanin also increased the maximal level of force attained. Force in response to Ca2+ alone; 4, force in response to Ca2+ and galanin + GTP. Values shown are means ± SE for at least 5 determinations.

![Fig. 6](http://ajpgi.physiology.org/) Galanin enhances myofilament Ca2+ sensitivity of rat jejunal smooth muscle through a pertussis toxin-sensitive pathway. Addition of 0.7 μM Ca2+ caused the rapid but transient development of force, which reached a stable value within several minutes. The addition of 30 nM galanin + 10 μM GTP significantly increased steady-state force (left). After incubation of the tissue in 5 μg/ml pertussis toxin, the sensitizing action of galanin was almost completely abolished (right). Tracings shown are representative of the results of 4 experiments.
erol/protein kinase C-dependent pathway or direct G protein activation of nifedipine-sensitive Ca²⁺ channels is involved is not known. It is clear from several lines of evidence that galanin-induced contractions are supported by influx of extracellular Ca²⁺ (5, 11).

It has been known for several years that receptor and G protein stimulation increases the myofilament Ca²⁺ sensitivity of all the smooth muscles so far examined (23, 29). Galanin stimulation of the rat jejunum is no exception because our results (Figs. 4–6) clearly demonstrate an increase in the magnitude of force development at any given [Ca²⁺] in the presence of galanin plus GTP. The exact mechanism responsible for the increase in Ca²⁺ sensitivity is not known. However, two prevailing hypotheses have been proposed. The first is that G protein activation results in inhibition of MLC phosphatase activity such that for any level of MLC kinase activation, MLC phosphorylation levels, and therefore force, will be enhanced (37). The mechanisms responsible for inhibition of MLC phosphatase have been proposed to be arachidonic acid (16) or protein kinase C (26). It has also been suggested that protein kinase C-catalyzed phosphorylation of the thin filament protein calponin may be involved in the enhanced Ca²⁺ sensitivity (35). Our present results do not address potential mechanisms involved in myofilament Ca²⁺ sensitivity. However, our results do demonstrate that G protein-dependent enhancement of Ca²⁺ sensitivity is an important aspect of the mechanism of a galanin-induced contraction.

Galanin stimulation of the rat jejunum also initiates events that desensitize the myofilaments to Ca²⁺. This phenomenon, originally described extensively by Gerthoffer (13, 14), is shown by the fall in force while MLC phosphorylation levels remain elevated in the intact tissue and by the transient contractile response in the α-toxin-permeabilized tissue. Because stimulation of the α-toxin-permeabilized tissue with Ca²⁺ alone results in Ca²⁺ desensitization, it is probable that the increase in intracellular [Ca²⁺] per se and not explicitly galanin stimulation is responsible. This interesting yet surprising result is well documented, as most studies that have utilized α-toxin-permeabilized preparations of gastrointestinal smooth muscle have demonstrated transient contractions during conditions of constant [Ca²⁺] (22). The mechanism(s) responsible for the fall in force during maintained levels of MLC phosphorylation in the intact tissue and maintained levels of both Ca²⁺ and MLC phosphorylation in the permeabilized preparation is unknown. Although only speculation at this point, one potential mechanism for this phenomenon may involve the thin filament protein caldesmon (33). If one assumes that desinhibition of caldesmon is required for full activation of the contractile machinery, then a time-dependent return of caldesmon to the inhibitory state could account for a fall in force during maintained levels of MLC phosphorylation. Further studies on this interesting phenomenon are required to verify or dispel this speculation.

The potential physiological importance of galanin-induced changes in myofilament Ca²⁺ sensitivity is also of interest. Because galanin has both secretory and contractile actions (25), it would be beneficial to be able to modulate the contractile response to promote both mixing and transport of intestinal materials. An increase in myofilament Ca²⁺ sensitivity would increase both mixing and transport, whereas desensitization would increase transit time and thus reabsorption of nutrients. Based on the tissue-specific nature of the galanin response and the widespread distribution of galanin receptors, we suggest that the secretory and contractile effects of this peptide are tightly coupled and temporally fine tuned to provide efficient gastrointestinal function.

In summary, we have presented information that describes the intracellular events involved in a contraction of rat jejunal smooth muscle in response to galanin stimulation. Based on our current results and those previously published, galanin stimulates jejunal smooth muscle by activation of a specific galanin receptor coupled to a pertussis toxin-sensitive G protein, which in turn increases the influx of extracellular Ca²⁺, and not release from intracellular stores. The increase in cellular Ca²⁺ stimulates the MLC kinase to increase levels of MLC phosphorylation and force. Galanin stimulation of the receptor-G protein complex also initiates a cascade of events that increase the myofilament Ca²⁺ sensitivity such that greater force is developed at any given steady-state level of MLC phosphorylation and [Ca²⁺]. Simultaneously or most likely after a short time delay, the galanin-induced increase in cellular Ca²⁺ induces a state of myofilament Ca²⁺ desensitization and produces a fall in force to suprabasal values. The delicate balance between Ca²⁺-dependent activation and alterations in myofilament Ca²⁺ sensitivity is an important part of smooth muscle regulation and must be taken into account when examining contractile mechanisms.

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