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Pharmacological characterization of alpha adrenoceptor-mediated motor responses in the rat colon

Sara Traserra[1] | Marc Grao[1] | Sonia Trujillo[1] | Francesc Jiménez-Altayó[2,3] | Patri Vergara[1,4] | Marcel Jimenez[1,4]

[1]Department of Cell Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, Barcelona, Spain

[2]Department of Pharmacology, Universitat Autònoma de Barcelona, Barcelona, Spain

[3]Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares (CIBERCV), Instituto de Salud Carlos III, Madrid, Spain

[4]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Barcelona, Spain

Correspondence
Marcel Jimenez, Department of Cell Biology, Physiology and Immunology, Veterinary Faculty, Neurosciences Institute, Universitat Autònoma de Barcelona, Bellaterra 08173, Barcelona, Spain.
Email: marcel.jimenez@uab.cat

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Abstract

Background: Inhibitory neuromuscular transmission in the gastrointestinal tract is mediated by intrinsic nitrergic and purinergic neurons. Purines activate G protein-coupled receptor $P2Y_1$ receptors, increasing intracellular Ca^{2+} that activates small conductance calcium-activated potassium (SK_{Ca}) channels. Little is known about the effect of adrenergic receptor activation on intestinal smooth muscle. In vascular tissue, stimulation of α-adrenoceptors causes smooth muscle contraction, while their effect on intestinal tissue is poorly understood. This study aimed to pharmacologically characterize the effect of α-adrenoceptor activation in the rat colon, which shares similar inhibitory pathways to the human colon.

Methods: Muscle bath experiments were performed with the rat proximal, mid, and distal colon oriented both circularly and longitudinally.

Results: The α_1-adrenoceptor agonist phenylephrine (PE) (10^{-8}–10^{-5} M) evoked concentration-dependent relaxations of the intestinal smooth muscle from all regions and orientations. However, in the mid-circular colon at low PE concentrations, a contraction sensitive to 10^{-5} M phentolamine (non-selective α-adrenoceptor blocker), the neural blocker tetrodotoxin (TTX; 10^{-6} M), and atropine (10^{-6} M) was recorded. PE-induced relaxations were insensitive to TTX (10^{-6} M) and the nonselective β-adrenoceptor blocker propranolol (10^{-6} M). In contrast, PE-induced relaxations were blocked by phentolamine (10^{-5} M), prazosin (10^{-6} M) (α_1-adrenoceptor blocker), and RS17053 (10^{-6} M) (α_{1A}-blocker), but not by yohimbine (10^{-6} M) (α_2-adrenoceptor blocker). Apamin (10^{-6} M), a SK_{Ca} channel blocker, abolished PE-induced relaxations.

Conclusions: Contractile responses in the circular muscle of the mid colon could be attributed to α-adrenoceptors located on enteric cholinergic neurons. Stimulation of α_{1A}-adrenoreceptors activates SK_{Ca} channels to cause smooth muscle relaxation, which constitutes a signaling pathway that shares similarities with $P2Y_1$ receptors.

KEYWORDS
alpha adrenoceptors, apamin, colon, phenylephrine, relaxation

1 | INTRODUCTION

Inhibitory neuromuscular transmission in the gastrointestinal (GI) tract is mediated by nitric oxide (NO) and ATP, or a related purine. This mechanism has been described in several species including humans,[1,2] rodents,[3,4] pigs,[5] and horses.[6] NO binds to cytosolic guanylyl cyclase (Gc), while purines bind on post-junctional $P2Y_1$ receptors. $P2Y_1$ receptors are G protein-coupled receptors that upon activation induce an increase in cytosolic Ca^{2+} in post-junctional cells. This, in turn, activates apamin-sensitive SK_{Ca} channels (sK3), resulting in smooth muscle hyperpolarization.[7,8] Experimental data suggest that NO may play a role in prolonged tonic relaxation, while the activation of $P2Y_1$ receptors likely induces phasic relaxation.[9] Accordingly, the electrophysiological basis of this process consists of a biphasic inhibitory junction potential (IJP), composed of a fast and transient purinergic hyperpolarization (IJPf) followed by a slow, long-lasting but sustained nitrergic response (IJPs).[10] Moreover, it has been proposed that PDGFRα+ cells, participate in purinergic inhibitory neuromuscular transmission.[11,12]

Rodents serve as a valuable model for studying neuromuscular transmission in the GI tract due to the presence of a composed IJP.[3] Besides, in mice, distinctions in innervation have been observed not only between the circular and longitudinal layers of the colon[13] but also among the proximal, mid, and distal colon.[4,13] In the circular muscle of the colon, an inverse gradient in both inhibitory pathways has been described.[4,14] Notably, the proximal and mid colon exhibit predominantly nitrergic inhibitory neuromuscular transmission, whereas in distal areas, the primary inhibitory pathway is purinergic.[4] In contrast, in the longitudinal muscle, neuromuscular innervation was mainly nitrergic.[13]

The autonomic nervous system regulates GI motility through the sympathetic, parasympathetic, and enteric nervous systems (ENS). The sympathetic nervous system exerts its effects through epinephrine and norepinephrine released by the adrenal gland (hormonal effect), as well as norepinephrine released by postganglionic sympathetic neurons (neural effect). These catecholamines act on α- and β-adrenoceptors located in various structures, such as neurons of the ENS and intestinal smooth muscle cells, thereby regulating GI motility. Adrenoceptors belong to the superfamily of G protein-coupled receptors (GPCR) and are activated by catecholamines. α-adrenoceptors are divided into α_1 and α_2-adrenoceptors.[15] The three α_1-adrenoceptor subtypes α_{1A}, α_{1B}, and α_{1D} are activated by the endogenous agonists, adrenaline and noradrenaline. Phenylephrine (PE) is a selective α_1–adrenoceptor agonist. α_1-adrenoreceptors are coupled to stimulatory Gq proteins, activate the enzyme phospholipase C, and are mainly found in the smooth muscle cells of blood vessels and the urinary tract, where they induce constriction. β-adrenoceptor are subdivided into three subtypes β_1, β_2, and β_3. All three β-adrenoceptor subtypes dilate blood vessels,[16] bronchioles, and relax the muscles of the uterus, bladder, and GI tract (see for review[17]). In contrast, very little is known about the activation of α-adrenoreceptors in the GI tract. Two recent papers have reported that α_{1A}-adrenoceptors activate SK_{Ca} channels, leading to smooth muscle hyperpolarization and relaxation. This postulates a non-nitrergic, non-purinergic mechanism of relaxation in the human and murine colon.[18,19] In another study, region-specific parasympathetic innervation has been described in the mouse colon affecting both myenteric neurons and post-junctional cells including PDGFRα+ cells and ICC.[20] Despite these previous studies, the contribution of α-adrenergic signaling throughout the intestine remains unknown.

The present study aimed to pharmacologically characterize the mechanism of smooth muscle relaxation induced by α-adrenoceptor activation in the rat colon and to properly assess this mechanism in the proximal, mid, and distal colon from the circular and longitudinal layers.

Key Points

- α-Adrenoceptors cause vasoconstriction, however, its effect in intestinal tissue is poorly characterized.
- In contrast to what occurs in vascular tissue, activation of α_{1A}-adrenoceptors cause colonic relaxation attributable to the opening of SK_{Ca} channels.
- The pathway is different from relaxation associated to ß-adrenoceptor activation.

2 | MATERIALS AND METHODS

2.1 | Ethical approval

Experimental procedures were approved by the Ethics Committee of the Universitat Autònoma de Barcelona with the approval code MJF-eut/01 and followed the European Community Council Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes.

2.1.1 | Animals and tissue samples

Thirty-five Sprague–Dawley rats (20 females and 15 males) of 7- to 10-week old were housed under controlled conditions: temperature (22 ± 2°C) and humidity (55 ± 10%), 12-h light/dark cycle, and ad libitum access to water and food. Rats were euthanized by decapitation without prior anesthesia.

For functional experiments, the colon was quickly removed and immersed in a cold carbogenated (95% O_2 and 5% CO_2) Krebs solution. The mesenteric fat was removed, and the colon was carefully opened along the mesenteric border and pinned to a Sylgard base with the mucosa facing upward. Subsequently, the colon was partitioned into three segments: proximal, mid, and distal colon. The

mucosal and submucosal layers were removed, and 4 × 10 mm muscle strips were cut in circular and longitudinal directions.

2.1.2 | Mechanical studies

Muscle strips were set up in a 10-mL organ bath filled with Krebs solution and maintained at a temperature of 37 ± 1°C and carbogenated (95% O_2 and 5% CO_2). A tension of 1 g was applied, and tissues were equilibrated for 1 h. After this period, strips displayed spontaneous phasic contractions. Mechanical activity was measured using an isometric force transducer (UF-1 Harvard Apparatus) connected to a computer through an amplifier. Data were digitally recorded at a rate of 25 Hz using Data 2001 software (Panlab) coupled with an A/D converter integrated into the computer.

2.1.3 | Solutions and drugs

The composition of the Krebs solution was (in mmol/L): glucose 10.10, NaCl 115.48, $NaHCO_3$ 21.90, KCl 4.61, NaH_2PO_4 1.14, $CaCl_2$ 2.50, and $MgSO_4$ 1.16 bubbled with a mixture of 5% CO_2–95% O_2 (pH 7.4).

The following drugs were used: L-phenylephrine (PE; CAS number: 59–42-7; Merck), isoprenaline hydrochloride (CAS number: 51-30-9; Merck), tetrodotoxin (TTX; CAS number: 1078; Tocris), Nω-Nitro-L-arginine (L-NNA; CAS number: 2149-70-4; Merck), apamin (CAS number: 24345-16-2; Merck), (N-[2-(2-cyclopropylmethoxyphenoxy) ethyl]-5-chloro-α, α-dimethyl-1H-indole-3-ethanamine) hydrochloride (RS17053, CAS number: 0985; Tocris), yohimbine hydrochloride (CAS number: 65-19-0; Merck), propranolol hydrochloride (CAS number: 318-98-9; Tocris), phentolamine hydrochloride (CAS number: 73-05-2; Merck), atropine (CAS number: 51-55-8; Merck), Carbachol (CAS number: 51-83-2, Merck), and MRS 2500 tetraammonium salt (CAS number: 630103-23-0, Tocris).

Stock solutions were prepared by dissolving drugs in distilled water except for RS17053, which was dissolved in DMSO; and L-NNA, which was dissolved in a physiological saline solution by sonication.

2.1.4 | Experimental procedure, data analysis, and statistics

Concentration–response curves were obtained at increasing concentrations of PE (10^{-8}–10^{-5} M). Each concentration was incubated for 5–10 min until a stable response was obtained. Experiments were also performed with 30 min incubation with different blockers. Isoprenaline was rested at 10^{-8} M in the presence of different blockers. Each strip was used for one experiment.

The area under the curve (AUC; g × min) of contractions calculated from the baseline was measured to estimate the response to drugs, which was expressed as a percentage of the basal AUC of contractions, being 100 the basal activity before drug addition. Concentration–response curves of phenylephrine under different pharmacological conditions were performed using the following formula $Y = 100/ (1 + 10^{(X\text{-}LogIC50)})$. Responses to drugs were compared using one-way and two-way ANOVA tests. Data are expressed as mean ± SEM. Data were considered significant when $p < 0.05$. *n* values represent strips from different animals. Statistical analysis was performed with GraphPad Prism software version 6.01 (GraphPad Software, San Diego, CA, USA).

3 | RESULTS

3.1 | Effect of PE on the circular and longitudinal layers from the proximal, mid, and distal colon

The α_1-adrenoceptor agonist, PE (10^{-8}–10^{-5} M) evoked concentration-dependent relaxations of the colon smooth muscle from all regions and orientations with an IC_{50} in the range of 10^{-7} M in all regions and orientations (Table 1). In the longitudinal muscle, no differences were observed between colonic zones ($p = 0.9286$). In contrast, in the circular muscle, PE elicited differences in the response across colonic zones ($p < 0.0001$), being more potent in the distal colon with an IC_{50} of 2.8×10^{-7} M, followed by the proximal colon with an IC_{50} of 5.7×10^{-7} M and lastly the mid colon with an IC_{50} of 6.5×10^{-7} M. Moreover, at concentrations from 10^{-8} to 10^{-7} M of PE, an increase in the AUC was also observed in the circular muscle from the mid colon (Figure 1).

3.2 | Effect of PE on the circular in control, in the presence of atropine, phentolamine, and propranolol

The response observed with PE in the mid colon, circular muscle was pharmacologically characterized. Tissues were incubated with 10^{-6} M atropine, 10^{-5} M phentolamine, 10^{-6} M propranolol, and in control conditions. The increase in the amplitude of the contractile response (measured as increase in AUC) observed in the middle circular colon, at low PE concentrations, was sensitive to 10^{-5} M phentolamine and 10^{-6} M atropine. Consistent with the activation of α-adrenoceptors, PE-induced relaxation was phentolamine-sensitive and propranolol- and atropine-insensitive (Figure 2). The contractile response but not the relaxation induced by PE was 10^{-6} M TTX-sensitive (Figures 2 and 3). It is important to note that the increase in AUC was mimicked by muscarinic stimulation with low concentration of carbachol (see Figure S1).

3.3 | Effect of PE on the circular and longitudinal muscle in control conditions, in the presence of TTX, phentolamine, and apamin

Responses induced by PE were assessed in the presence of 10^{-6} M TTX, 10^{-5} M phentolamine, and 10^{-6} M apamin. PE-induced

TABLE 1 Outcome parameters from PE concentration–response curve evaluation in the proximal, mid, and distal colon from the circular and longitudinal muscle.

	Circular muscle			Longitudinal muscle		
	Proximal	Mid	Distal	Proximal	Mid	Distal
IC_{50} (*M*)	5.7×10^{-7}	6.5×10^{-7}	2.8×10^{-7}	8.8×10^{-7}	8.0×10^{-7}	8.6×10^{-7}
Log $IC_{50}\pm$SE	-6.2 ± 0.03	-6.2 ± 0.06	-6.5 ± 0.04	-6.1 ± 0.03	-6.1 ± 0.03	-6.1 ± 0.02

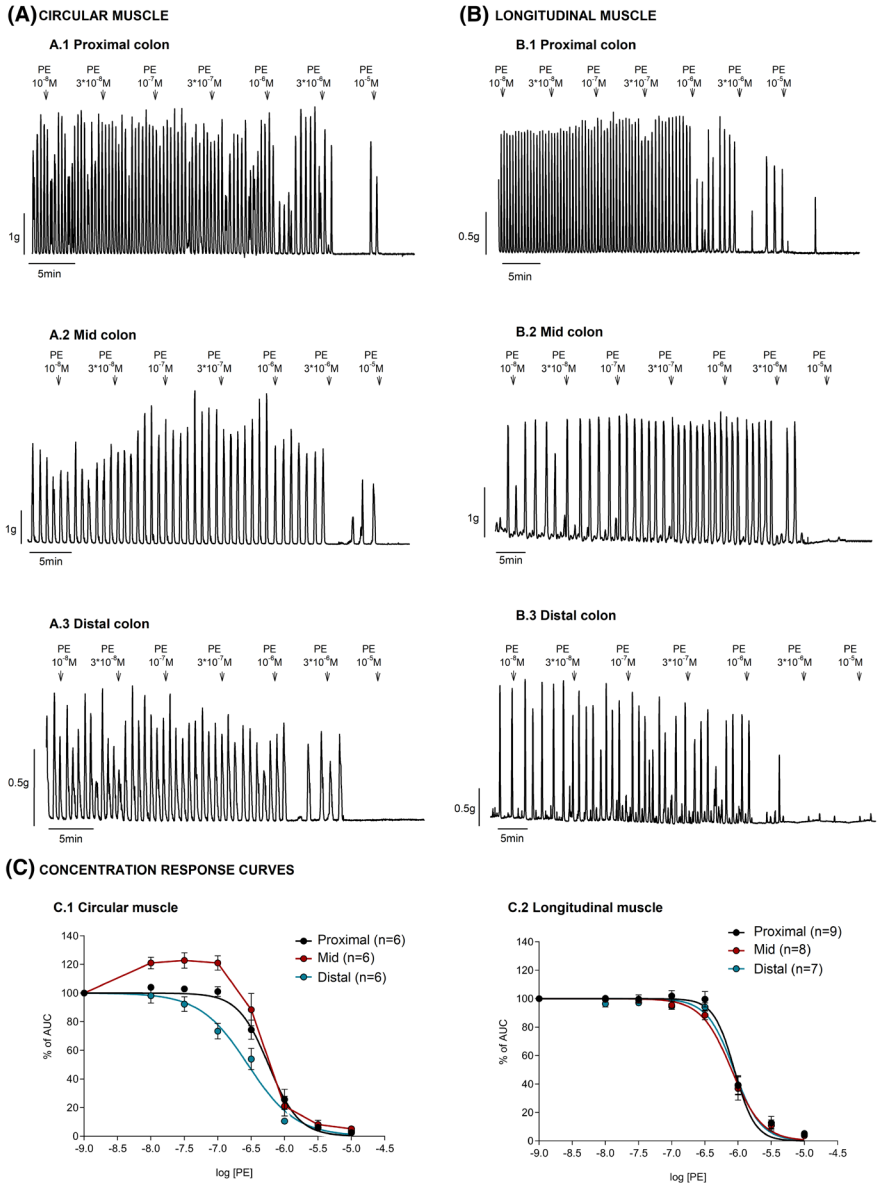

FIGURE 1 Effect of phenylephrine (PE) on the circular and longitudinal layers from the proximal, mid, and distal colon. Top: Representative mechanical recording showing the effect of PE (10^{-8}–10^{-5} M) on the circular (A.1–A.3) and longitudinal (B.1–B.3) layers from the proximal, mid, and distal colon. Bottom: Concentration–response curves in each segment and muscle orientation (C.1–C.2). Data were normalized (i.e., 100%) to the basal AUC before drug addition. Data are expressed as mean ± SEM.

relaxations were insensitive to the neural blocker, TTX 10^{-6} M. In contrast, 10^{-5} M phentolamine and 10^{-6} M apamin, an SK_{Ca} channel blocker, completely abolished PE-induced relaxations in both circular and longitudinal muscle strips from the mid colon. To assess whether PE-induced relaxation was associated with the neural release of inhibitory neurotransmitters, tissue was incubated with a cocktail of blockers including L-NNA 10^{-3} M + MRS2500 10^{-6} M + propranolol 10^{-6} M and atropine 10^{-6} M. Under these conditions, PE-induced relaxations were still observed in both muscle layers. The addition of 10^{-6} M TTX to the cocktail did not modify PE-induced relaxations. However, incubation with 10^{-6} M apamin and 10^{-5} M phentolamine abolished PE-induced relaxations (Figure 3). It is important to note that all experiments were performed on different strips. However, in a subset of experiments, the concentration–response curves were performed twice with PE. A washout was performed between the two curves.

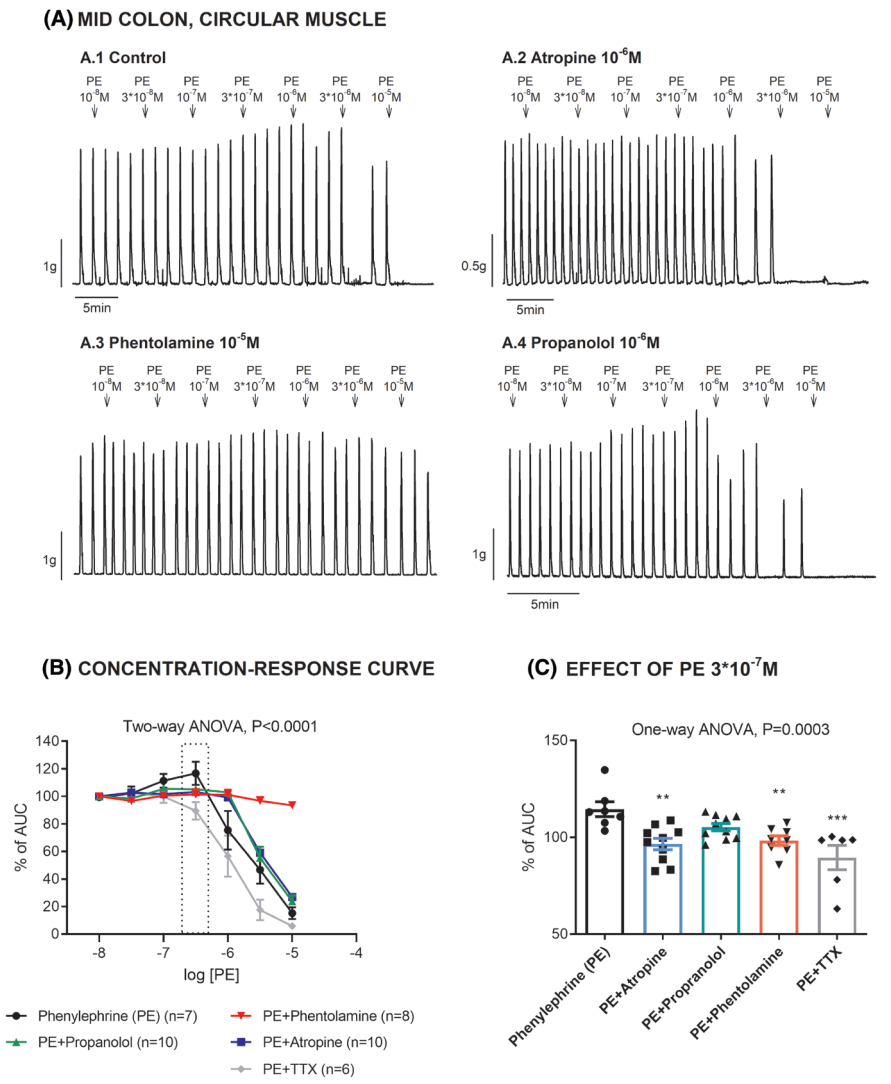

FIGURE 2 Effect of phenylephrine (PE) on the circular middle colon in control conditions and in the presence of atropine, phentolamine, propranolol, and TTX. (A) Representative mechanical recordings showing the effect of PE in control conditions (A.1), in the presence of atropine (A.2), phentolamine (A.3), and propranolol (A.4). (B) Graph showing the effects of PE in each experimental condition. C. Histogram showing the effect of PE 3×10^{-7} M in each experimental condition. Data were normalized (i.e., 100%) to the basal AUC before drug addition. $^{**}p<0.01$, $^{***}p<0.001$, compared with PE alone by one-way ANOVA with Dunnett's post-test. Data are expressed as mean ± SEM.

These experiments correspond to circularly oriented strips and were performed in the presence of TTX ($n=6$). Both responses were coincident.

3.4 | Effect of PE on the circular and longitudinal muscle in the presence of propranolol, yohimbine, prazosin, and RS17053 in tissue incubated with TTX

Lastly, different α-adrenoceptor blockers were tested to assess the response induced by PE. Phenylephrine-induced relaxation in the mid colon was blocked by 10^{-6} M prazosin (α_1-adrenoceptor blocker) and 10^{-6} M RS17053 (α_{1A} preferential blocker), but not by 10^{-6} M yohimbine (α_2-adrenoceptor blocker) in both muscle layers (Figure 4).

3.5 | Effect of isoprenaline on the circular muscle

To investigate the involvement of β-adrenoceptors on colonic inhibitory responses, isoprenaline (β-agonist) was tested at 10^{-8} M. Isoprenaline strongly decreased spontaneous phasic contractions. Th effect was reversed by propranolol 10^{-6} M, and further incubation with Phe at 10^{-5} M completely abolished spontaneous contractions ($n=6$). Incubation with propranolol at 10^{-6} M prevented the isoprenaline response ($n=7$). In contrast, incubation with phentolamine at 10^{-6} M did not modify the isoprenaline response ($n=7$). Tracings and data are shown in Figure 5. These findings are consistent with β-adrenoceptor activation that is totally different from the previously described response to Phe. Differences are detailed in Table 2.

4 | DISCUSSION

In the present study, we found that PE causes a TTX-insensitive relaxation in both muscle layers and regions of the colon, whereas a TTX-sensitive contractile response was observed in the circular muscle of the mid colon.

The inhibitory response induced by PE was similar to the one described by Kurahashi et al. in murine distal colon.[19] In this study,

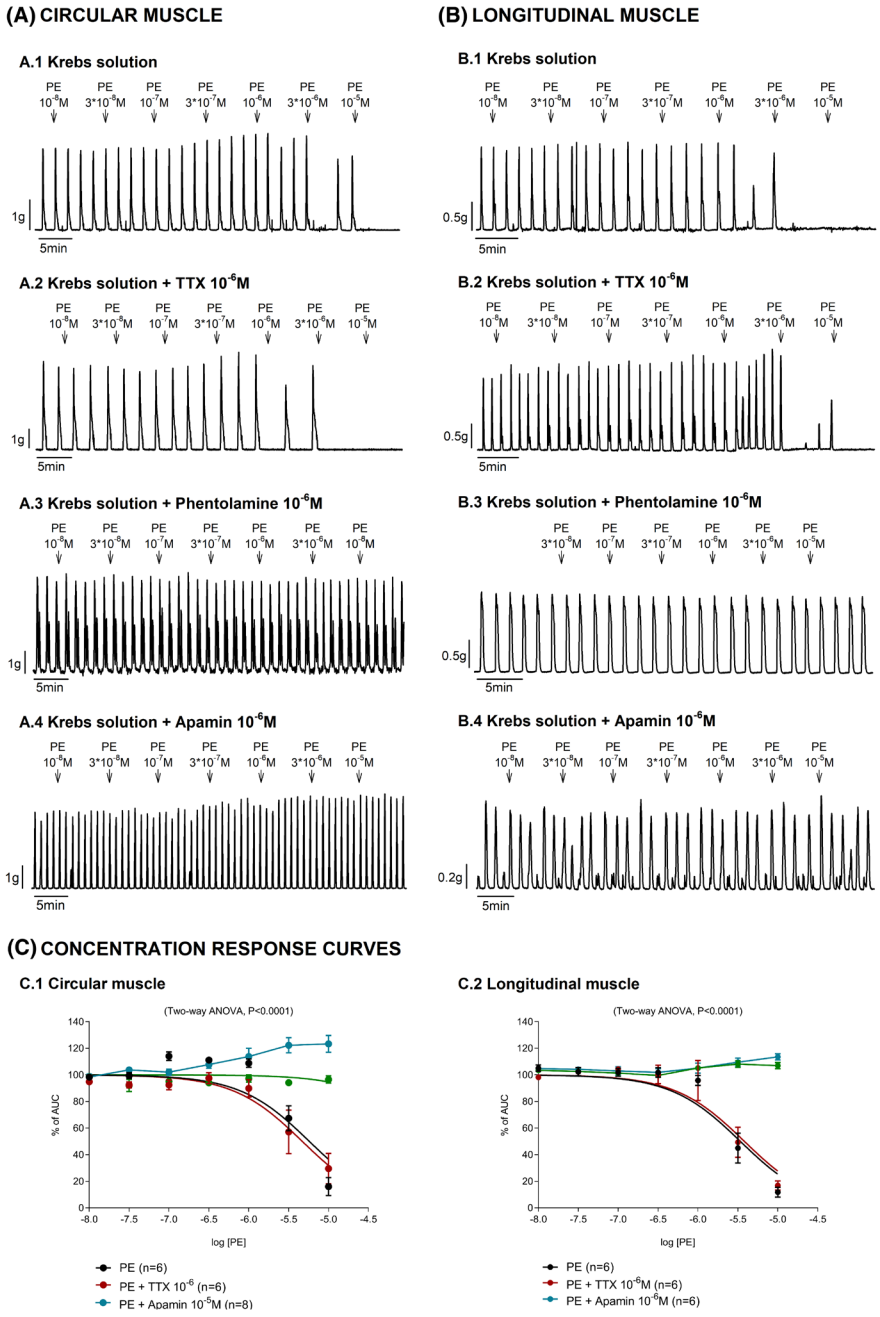

FIGURE 3 Effect of phenylephrine (PE) on the circular and longitudinal muscle from the mid colon in the presence of TTX, phentolamine, and apamin. Representative mechanical recordings showing the effect of PE in the circular (A: left panel) and longitudinal (B: right panel) muscle in control conditions (A.1, B.1), in the presence of TTX (A.2, B.2), phentolamine (A.3, B.3), and apamin (A.4, B.4). Concentration–response curves assessed in each muscle layer (C). Data were normalized (i.e., 100%) to the basal AUC before drug addition. Data are expressed as mean ± SEM.

contractile responses were evaluated in the circular muscle from the rat distal colon and a reduction in the contractility was observed after PE addition. Our study shows similar results in the different regions of the rat colon analyzed, showing the same mechanism involved in both muscle layers. PE is slightly more potent in rats compared to mice,[19] as the IC_{50} is between 10^{-6} and 10^{-7}M. To check a possible involvement of the NO/Purine pathway, we incubated the tissue with the NOS inhibitor L-NNA and the $P2Y_1$ blocker MRS2500. We also added TTX and atropine to the cocktail to abolish the influence of both neural excitatory and inhibitory pathways. Under these experimental conditions, PE was still able to inhibit spontaneous phasic contractions (SPC), suggesting that inhibitory motor neurons are not involved in PE response (see below).

The receptor involved in the smooth muscle relaxation is probably an α_{1A}-adrenoceptor. This is consistent with the blockade with prazosin (α_1-adrenoceptor blocker) and RS17053 (α_{1A} preferential blocker), but not with yohimbine (α_2-adrenoceptor blocker). Similar results were obtained with RS100329 (α_{1A} preferential blocker) in the mouse distal colon.[19] Moreover, the reduction in contractility induced by PE was absent in α_{1A}-receptor KO mice. A similar mechanism has been described in human tissue where at 10^{-5} M norepinephrine reduced SPC and this mechanism was blocked by prazosin and RS100329.[18] Similar to what we found in our study, the authors observed that the mechanism was present in different regions of the human colon. The pathway described in the present study and in these previous studies[18,19] is distinct

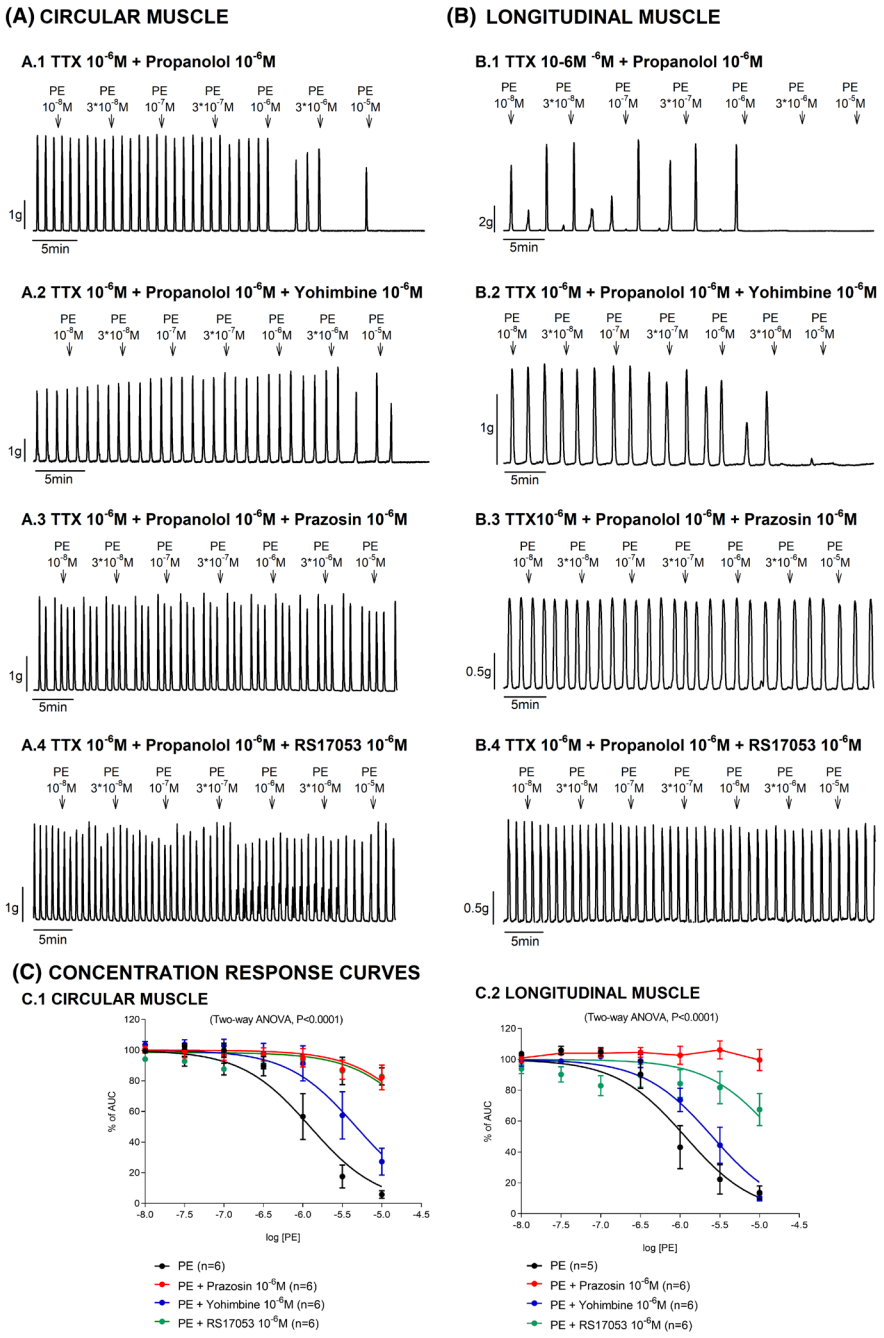

FIGURE 4 Effect of phenylephrine (PE) on the circular and longitudinal muscle in the presence of propranolol, yohimbine, prazosin, and RS17053 under TTX conditions. Mechanical recordings from the circular (A: left panel) and longitudinal (B: right panel) muscle showing the effect of PE in the presence of propranolol (A.1, B.1), yohimbine (A.2, B.2), prazosin (A.3, B.3), and RS17053 (A.4, B.4). Concentration–response curves assessed in each muscle layer (C). Data were normalized (i.e., 100%) to the basal AUC before drug addition. Data are expressed as mean ± SEM.

from the activation of β-adrenoceptors with isoprenaline, which induces propranolol-sensitive but phentolamine-insensitive inhibitory responses. β-Adrenoceptors couple to Gs-proteins to activate adenylyl cyclase. Stimulation of adenylyl cyclase leads to the conversion of ATP into cAMP, which subsequently activates protein kinase A, that in turn phosphorylates several substrates. This pathway is responsible for colonic relaxation and differs from α_{1A}-receptor activation. Understanding these distinct signaling pathways is crucial for elucidating the complex regulation of gastrointestinal motility and may offer insights into potential therapeutic targets for gastrointestinal disorders characterized by abnormal smooth muscle contractility. One important question is where α_{1A}-adrenoceptors are located in the GI tract. According to cell-specific transcriptome data, α_{1A}-adrenoceptors are located in PDGFRα$^+$ cells from mouse colon.[19] These cells are interstitial cells that are c-kit-negative and constitute another population of interstitial cells different from c-kit-positive interstitial cells of Cajal (ICC).[21] Smooth muscle cells are electrically coupled to interstitial cells of Cajal (ICC) and PDGFRα+ cells forming an integrated motor unit, known as SIP syncytium.[21,22] In the mouse colon, PDGFRα + cells are located in both muscle layers and are more numerous compared with cKit + cells, which both form a heterologous cellular network.[14] Based on these results it is reasonable to speculate that the inhibitory effect observed in our study is due to activation of α_{1A}-adrenoceptors in PDGFRα + cells. Consistent with this hypothesis, norepinephrine increased Ca^{2+} transients in PDGFRα

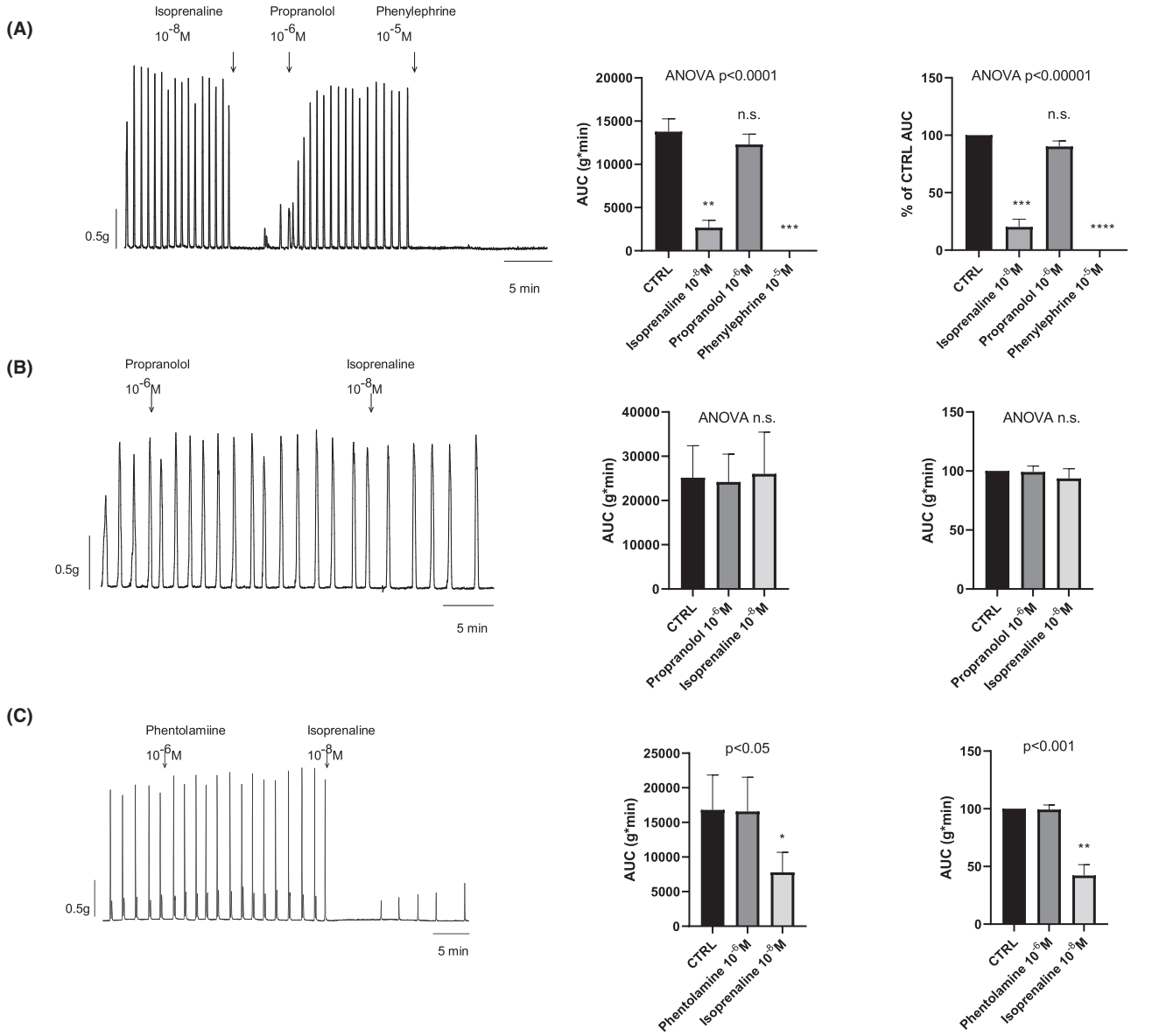

FIGURE 5 Effect of isoprenaline on the circular muscle from the mid colon. Isoprenaline 10^{-8} M strongly reduced spontaneous contractions. The effect was reversed by propranolol 10^{-6} M and further addition of Phe 10^{-5} M abolished spontaneous contractions ($n=6$) (Panel A). Incubation with propranolol 10^{-6} M prevented isoprenaline 10^{-8} M responses ($n=8$; Panel B). Incubation with phentolamine 10^{-6} M did not prevent isoprenaline 10^{-8} M responses ($n=8$; Panel C). Data in histograms are shown in AUC and normalized (i.e., control = 100%) for each experimental protocol. Dunnet's post hoc test was performed after paired ANOVA test to check differences from control (CTRL) conditions. To avoid neural-mediated responses, TTX 10^{-6} M was added 30 min before the start of the experiments.

TABLE 2 Pharmacological profile of α- and β-agonists on inhibitory motor responses in the rat colon.

Agonists	Antagonists that block the inhibitory response	Antagonists that do not block the inhibitory response
Phenylephrine (α-adrenoceptor agonist)	Phentolamine: α-adrenoceptor blocker Apamin: sK_{Ca} blocker Prazosin: α_1-adrenoceptor blocker RS17053: α_{1A}-adrenoceptor blocker[a]	Propranolol: β-adrenoceptor blocker Yohimbine: α2-adrenoceptor blocker
Isoprenaline (β-adrenoceptor agonist)	Propranolol: β-adrenoceptor blocker	Phentolamine: α-adrenoceptor blocker

[a]Preferential α_{1A} as it might also block α_{1D}-adrenoceptor.

+ cells and this response was blocked by the preferential α_{1A}-adrenoceptors blocker RS100329.[19]

Alpha$_{1A}$-adrenoceptors belong to the family of G protein-coupled receptors (GPCR). They activate the Gq/$_{11}$ and phospholipase Cβ, thus increasing the production of inositol 1,4,5-triphosphate (IP3) and initiating Ca^{2+} release from intracellular stores via IP3 receptors.[23] This is probably the basis of contraction in many smooth muscle cells including vascular tissue. However, the opposite result was found in the colon. It is possible that the Ca^{2+}-dependent increase in PDGFRα + cells activates SK_{Ca} channels, and this hyperpolarization is transmitted to smooth muscle cells through the SIP syncytium. Consistent with this hypothesis, PE caused a smooth muscle hyperpolarization (results not shown) that probably reduced open probability of L-type Ca^{2+} channels, leading to a decrease in SPC. In this study, we found that the effect of PE was blocked by apamin. Similar results were reported both in mice and human colon.[18,19] Therefore, our data support previous evidence in these two species. In addition, previous data from our laboratory showed that the fast component of the IJP is sensitive to apamin and blocked with the $P2Y_1$ blocker MRS2500.[24,25] The fast component of the IJP is absent in $P2Y_1$ KO mice,[26,27] demonstrating that the IJPfast is due to ATP or a related purine acting on $P2Y_1$ receptors. The IJPf is usually recorded in non-adrenergic non-cholinergic conditions and therefore activation of α_{1A}-adrenoceptors is unlikely to mediate the IJPf and the corresponding relaxation. However, it is possible that $P2Y_1$ receptors in PDGFRalfa+ cells also mediate the purinergic relaxation[26] and consequently α_{1A}-adrenoceptors share the same inhibitory pathway as $P2Y_1$ receptors.

The physiological significance of our results is associated with an effect of the sympathetic nervous system on colonic motility, which could be overactivated in several pathophysiological conditions such as stress and hypertension. The sympathetic nervous system may exert its effects through epinephrine and norepinephrine released by the adrenal gland (hormonal effect) and norepinephrine released by postganglionic sympathetic neurons (neuronal effect). A recent study in mice showed that postganglionic sympathetic neurons are closely associated with myenteric neurons, as well as with c-KIT and PDGFRα+ cells. Both electrical and optogenetic stimulation of postganglionic sympathetic neurons inhibit spontaneous contractions.[20] In this study, a decrease in Ca^{2+} transients were observed in ICC-MP and this effect was sensitive to prazosin, suggesting the involvement of α-adrenoceptors.[20] In mice, this effect was most prominent in the proximal colon but according to our results in the rat, the mechanism is present in all regions of the colon and affects both muscle layers.

Sympathetic innervation can also inhibit and activate different populations of enteric neurons.[20] In our study, a contraction was also observed at low concentrations of PE in the circular muscle from the mid colon. This response was found to be sensitive to phentolamine and, therefore, mediated by α-adrenoreceptors. The contractile response induced by PE was possibly associated with the presence of α-adrenoceptors located on enteric cholinergic neurons, as the response was TTX and atropine-sensitive. Consistently, in the human colon, the contractile response observed to norepinephrine was associated to α-adrenoceptors (possibly α_{1D} adrenoceptors) located in smooth muscle.[18]

5 | CONCLUSION

We conclude that the contractile responses in the circular muscle of the mid colon could be attributed to α-adrenoceptors located on enteric cholinergic neurons. Post-junctional stimulation of α_{1A}-adrenoreceptors possibly located in PDGFRα+ cells activates SKCa channels to cause smooth muscle hyperpolarization and relaxation. This pathway is coincident with that previously described for $P2Y_1$ receptors in the colon of different species, including humans. Activation of α_{1A}-adrenoceptors is therefore a new pathway of smooth muscle relaxation through sympathetic regulation of colonic motility.

AUTHOR CONTRIBUTIONS

Sa.T. wrote the paper, prepared the figures, and did some of the experiments. M.G. and So.T. performed part of the experiments. F. J-A. and P.V. contributed to the design of the study and participated in its funding and M.J. supervised the study.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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