Purinergic and nitrergic junction potential in the human colon

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Gallego D, Gil V, Aleu J, Auli M, Clavé P, Jiménez M. Purinergic and nitrergic junction potential in the human colon. Am J Physiol Gastrointest Liver Physiol 295: G522–G533, 2008. First published July 3, 2008; doi:10.1152/ajpgi.00510.2007.—The aim of the present work is to investigate a putative junction transmission [nitric oxide (NO) and ATP] in the human colon and to characterize the electrophysiological and mechanical responses that might explain different functions from both neurotransmitters. Muscle bath and microelectrode techniques were performed on human colonic circular muscle strips. The NO donor sodium nitroprusside (10 μM), but not the P2Y receptor agonist adenosine 5′-O-2-thiodiphosphate (10 μM), was able to cause a sustained relaxation. N6-nitro-l-arginine (l-NNA) (1 mM), a NO synthase inhibitor, but not 2′-deoxy-N6-methyl adenosine 3′,5′-diphosphate tetraammonium salt (MRS 2179) (10 μM), a P2Y antagonist, increased spontaneous motility. Electrical field stimulation (EFS) at 1 Hz caused fast inhibitory junction potentials (IJPs) and a relaxation sensitive to MRS 2179 (10 μM). EFS at higher frequencies (5 Hz) showed biphasic IJP with fast hyperpolarization sensitive to MRS 2179 followed by sustained hyperpolarization sensitive to l-NNA; both drugs were needed to fully block the EFS relaxation at 2 and 5 Hz. Two consecutive single pulses induced MRS 2179-sensitive fIJPs that showed a rundown. The rundown mechanism was not dependent on the degree of hyperpolarization and was present after incubation with l-NNA (1 mM), hexamethonium (100 μM), MRS 2179 (1 μM), and NFO23 (10 μM). We concluded that single pulses elicit ATP release from enteric motor neurons that cause a fIJP and a transient relaxation that is difficult to maintain over time; also, NO is released at higher frequencies causing a sustained hyperpolarization and relaxation. These differences might be responsible for complementary mechanisms of relaxation being phasic (ATP) and tonic (NO).

ATP; IJP; smooth muscle; rundown; purinergic receptors

The identity of the inhibitory neurotransmitter(s) involved in nonadrenergic, noncholinergic (NANC) inhibitory neurotransmission is still being debated, but ATP and nitric oxide (NO) are presently recognized as two of the major inhibitory mediators in the gastrointestinal (GI) tract. Nitrergic neurons mediate lower esophageal sphincter relaxation after swallowing (34), and vagally induced gastric (11) and pyloric (2) relaxation regulate colonic transit time (26) and mediate the rectoanal inhibitory reflex (28). Fewer studies have been published on the putative role of ATP as a neurotransmitter mediating these physiological functions. ATP and NO might participate in the regulation of gastroduodenal motility in rats, (17) and ATP might participate in vagally induced gastric relaxation (4) and intrinsic gastric adaptive relaxation in mice (12). These (and other) data suggest that these neurotransmitters have important physiological functions; however, smooth muscle relaxation might have different properties (i.e., tonic vs. phasic) that should be better characterized. The release of inhibitory neurotransmitter(s) at the neuromuscular junction is the final step for smooth muscle relaxation. Inhibitory junction potentials (IJPs) have been well characterized in animal samples, and two components, i.e., fast and slow IJPs, have been reported (9, 18), suggesting the release of at least two transmitters that might have different functions. However, few studies have been published that examine these results on the human GI tract.

NO is a potent inhibitory neurotransmitter in the GI tract (7). Nitrergic neurons have the apparatus of NO synthesis (24, 30), and NO released from inhibitory motor neurons causes the slow component of the IJPs and smooth muscle relaxation (6, 21). Inhibitors of NO synthase, such as N6-nitro-l-arginine (l-NNA) or N6-nitro-l-arginine methyl ester (l-NAME), increase muscular contractions (21), showing that NO might be tonically released from inhibitory motor neurons. However, several results are inconsistent with the hypothesis that NO is the sole NANC inhibitory neurotransmitter in the human GI tract: the fast component of the IJP is l-NNA insensitive (15, 21). In the presence of NO synthase inhibitors, an important NANC-nonnitrergic relaxation is present (6, 29, 31). These results are consistent with the presence of at least one complementary inhibitory neurotransmitter, which might have complementary or redundant functions.

Because of the lack of a specific antagonist, the identity of the receptor involved in purinergic relaxation has been difficult to establish. (5, 31). However, 2′-deoxy-N6-methyl adenosine 3′,5′-diphosphate tetraammonium salt (MRS 2179), which is the N6-methyl modification of 2′-deoxyadenosine 3′,5′-bisphosphate, is a potent P2Y1 receptor antagonist (8) and is presently considered competitive and specific (1, 19). P2Y1 receptors have several physiological functions in the GI tract (33). They are present both in enteric neurons (16) and smooth muscle cells (15) and might participate in smooth muscle relaxation (10), synaptic transmission (19), and neurogenic secretion (13). We have recently shown that P2Y1 receptors are localized in smooth muscle cells and are responsible for the fast component of the IJP and the NANC nonnitrergic relaxation (15). In addition, we have found that both l-NNA and MRS 2179 are required to fully block mechanical relaxation induced by stimulation of inhibitory motor neurons by electric field stimulation (EFS) or through nicotinic receptors in the...
human colon (3). All these data are consistent with a putative role of purinergic inhibitory neurons causing the release of ATP or a related purine, acting through P2Y₁ receptors and causing smooth muscle hyperpolarization and relaxation (15).

At present, the relative contribution of purinergic and nitric neurotransmission mediating smooth muscle hyperpolarization and relaxation is unknown. The aim of the present paper is to investigate the electrophysiological basis of inhibitory transmission in the human colon and to examine the role of the two inhibitory neurotransmitters to find out whether either is redundant or whether they are complementary in smooth muscle relaxation.

MATERIALS AND METHODS

Tissue preparation. Specimens of distal and sigmoid colon (N = 31) were obtained from patients aged 47 to 80 yr during colon resections for neoplasm. Colon segments from macroscopically normal regions were collected and transported to the laboratory in cold saline buffer. The tissue was placed in Krebs solution in a dissection dish, and the mucosal layer was gently removed. Circular muscle strips, 10 × 4 mm, were cut. The patients provided written, informed consent, and the experimental procedure was approved by the Ethics Committee of the Hospital of Mataró (Barcelona, Spain).

Mechanical experiments. Muscle strips were examined in a 10-ml organ bath filled with Krebs solution at 37 ± 1°C containing phentolamine, atropine, and propranolol (each 1 μM) to block adrenergic and muscarinic receptors. An isometric force transducer (Harvard VF-1) connected to an amplifier was used to record the mechanical activity. Data were digitalized (25 Hz) using Datawin1 software (Panlab, Barcelona, Spain) coupled to an ISC-16 analog-to-digital card installed in a PC. A tension of 4 g was applied, and the tissue was allowed to equilibrate for 1 h. After this period, strips displayed spontaneous phasic activity. EFS was applied for 2 min (pulse duration 0.4 ms, frequency 1, 2, and 5 Hz, and amplitude 50 V).

Stimulation protocols. To study the putative nitric and purinergic neurotransmission, the following protocols were performed for each frequency of stimulation: 1) study of the nonnitric inhibitory component: EFS was applied in the presence of l-NNA (1 mM) (20 min); 2) study of the nonpurinergic inhibitory component: EFS was applied in the presence of MRS 2179 (10 μM) (20 min); and 3) study of the response during EFS of simultaneous blockade of both nitric and purinergic components by l-NNA and MRS 2179. Protocol 3 was performed by adding MRS 2179 to strips previously incubated with l-NNA and by adding l-NNA to strips previously incubated with MRS 2179 because the addition of l-NNA and MRS 2179 produces a total reversion of the EFS-induced relaxation independently of the sequence of the addition. To check that the response was not time dependent, longer incubations with the antagonists alone (40 min) were performed.

Data analyses and statistics. To estimate the responses to drugs, the area under the curve (AUC) of spontaneous contractions from the baseline was measured before and after drug addition (each 2 min) or before and during EFS (each 30 s). To normalize data, the value of

Fig. 1. A: mechanical recordings from human colonic circular muscle strips showing the inhibition of the spontaneous motility caused by ATP (1 mM), adenosine 5′-O-2-thiodiphosphate (ADPβS) (10 μM), and sodium nitroprusside (NaNP) (10 μM). B: histograms showing the time course of the inhibition during drug incubation. Data are expressed as mean ± SE. *P < 0.05; ***P < 0.001; ns, not significant.
AUC obtained before the treatment was considered to be 100, and the percentage of inhibition of the spontaneous motility was estimated with the AUC obtained after the treatment. One-way ANOVA followed by a Bonferroni post hoc test was performed to compare the response measured in each interval of time and control. N values represent the number of strips from different patients.

**Electrophysiological experiments.** Muscle strips were dissected parallel to the circular muscle and placed in a Sylgard-coated chamber continuously perfused with NANC Krebs solution at 37 ± 1°C. Strips were meticulously pinned in a cross-sectioned slab allowing microelectrode recordings from both circular and longitudinal muscles. This procedure was previously reported with canine ileum (20). Preparations were allowed to equilibrate for ~1 h before experiments started. Circular and longitudinal muscle cells were impaled with glass microelectrodes (40–60 MΩ) filled with 3 M KCl. Membrane potential was measured using standard electrometer Duo773 (WPI, Sarasota, FL). Tracings were displayed on an oscilloscope 4026 (Racal-Dana, UK), and simultaneously digitalized (100 Hz) using EGAA software coupled to an ISC-16 A/D card (RC Electronics, Thousand Oaks, CA) installed

![Fig. 2. Mechanical recordings from human colonic circular muscle strips showing the inhibition of the spontaneous motility caused by electrical field stimulation (EFS) (1 Hz, 50 V) in control conditions (A and B, top), after N⁵-nitro-L-arginine (L-NNA) (1 mM) incubation (A, middle), 2'-deoxy-N⁶-methyl adenosine 3',5'-diphosphate tetraammonium salt (MRS 2179) (10 μM) incubation (B, middle), and incubation with both L-NNA and MRS 2179 (A and B, bottom). C: histograms showing the percentage of inhibition measured at 30-s intervals during the stimulation period and in each of the above experimental conditions. Data are expressed as mean ± SE. **p < 0.01; ***p < 0.001.](http://ajpgi.physiology.org/10.1152/ajpgi.00001.2008)
in a computer. EFS was applied using two silver chloride plates placed perpendicular to the longitudinal axis of the preparation and 1.5 cm apart. Preparations were perfused with nifedipine (1 μM) to abolish mechanical activity and obtain stable impalements.

**Stimulation protocols and data analysis.** Trains of 5 s (supramaximal voltage 50 V, 0.3 ms) were performed at 1 Hz (5 pulses: P1 to P5) and 5 Hz (25 pulses). The amplitude of the 5 IJP was calculated when 1 Hz (5 pulses) was applied. At 5 Hz, the response consisted of a fast component followed by a slow one (see **RESULTS**), and in this case the fast component was estimated with the maximum amplitude and the slow component was estimated at 2.5 and 3.75 s after the beginning of the stimulus. A two-way ANOVA test was performed to compare data before and after drug addition. N values represent the number of strips from different patients.

Two pulses (pulse duration: 0.3 ms) were performed at the same time from 1 to 20 s. Both pulses had the same voltage, 20 V, 30 V, or

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**Fig. 3.** Mechanical recordings from human colonic circular muscle strips showing the inhibition of the spontaneous motility caused by EFS (5 Hz, 50 V) in control conditions (A and B, top), after L-NNA (1 mM) incubation (A, middle), MRS 2179 (10 μM) incubation (B, middle), and combination of both L-NNA and MRS 2179 (A and B, bottom). C: histograms showing the percentage of inhibition measured at 30-s intervals during the stimulation period and in each of the above experimental conditions. Data are expressed as mean ± SE. *P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant.
50 V depending on the protocol. The response consisted of a fast IJP1 elicited by the first pulse followed by a second IJP2 elicited by the second pulse. The ratio $Y/IJP2/IJP1$ was plotted vs. $X = \text{time interval between pulses}$. Data were fitted in a curve with an initial plateau at 0 (IF: $X < X_0 Y = 0$) followed by an exponential curve [IF: $X > X_0 Y = 100^\% (1 - \exp(-k^\% (X - X_0)))$, $X_0$, $K$, and $R^2$ were calculated using a nonlinear regression with GraphPad Prism software version 4.00 (GraphPad Software, San Diego, CA). $N$ values represent the number of strips from different patients, and $n$ values the number of pair data analyzed.

**Solutions and drugs.** The composition of the Krebs solution was (in mM) 10.10 glucose, 115.48 NaCl, 21.90 NaHCO₃, 4.61 KCl, 1.14 NaH₂PO₄, 2.50 CaCl₂, and 1.16 MgSO₄ bubbled with a mixture of 5% CO₂-95% O₂ (pH 7.4). The following drugs were used: nifedipine, l-NNA, ATP, adenosine 5’-O-2-thiodiphosphate (ADP₂S), apamin, phentolamine, tetrodotoxin (TTX), atropine sulphate, propranolol, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), adenosine, hexamethonium chloride (Sigma Chemicals, St. Louis, MO), sodium nitroprusside (NaNP) (Research Biochemicals International, Natick, MA), MRS 2179, and NF023 (Tocris, Bristol UK). Stock solutions were made by dissolving drugs in distilled water except for nifedipine [ethanol (96%)], and DPCPX (DMSO) (0.01% final concentration).

**RESULTS**

**Mechanical responses in the human colon.** Circular muscle strips showed cyclic spontaneous mechanical activity as previously described by Gallego et al. (15). In the presence of the neural blocker TTX, the NO donor NaNP (10 μM) abolished the spontaneous motility. The inhibitory effect was prominent for more than 10 min ($n = 5$) (Fig. 1). In contrast, ATP (1 mM, $n = 4$) and ADP₂S (10 μM, $n = 6$), a preferential and stable P₂Y agonist, transiently inhibited the motility, but subsequent spontaneous contractions were recorded during the incubation of the purinergic agonists, starting about 5 to 6 min after drug addition (Fig. 1). After incubation with l-NNA (1 mM), the AUC or basal tension was increased from 57.4 ± 7.5 g/min to 73.9 ± 9.2 g/min. ($P < 0.05$). Incubation with MRS 2179 (10 μM) slightly decreased the AUC in a nonsignificant manner.

EFS (1, 2, 5 Hz) for 2 min caused almost complete cessation of spontaneous motility (Figs. 2 and 3). In all cases, during EFS, spontaneous motility was <20% of the basal values. To study the putative nitricergic and purinergic neurotransmission,
strips were incubated with l-NNA (1 mM, 20 min), with MRS 2179 (10 μM, 20 min), and with both l-NNA (1 mM) and MRS 2179 (10 μM). The latter protocol was performed adding MRS 2179 in strips previously incubated with l-NNA and adding l-NNA in strips previously incubated with MRS 2179. To check that the response was not time dependent, longer incubations with l-NNA or MRS 2179 alone (40 min) were performed, and no differences were observed compared with the results obtained after 20-min incubation.

At 1 Hz (Fig. 2), the EFS inhibition was still observed in the presence of l-NNA (1 mM) (ANOVA, \( P \leq 0.001 \) vs. control), but MRS 2179 (10 μM) partially antagonized EFS relaxation [ANOVA: not significant (ns) vs. basal values]. Simultaneous addition of both MRS 2179 and l-NNA fully blocked the inhibition of spontaneous motility induced by EFS (ANOVA: ns vs. control). These results suggest that, at this frequency of stimulation, the response was mainly MRS 2179 sensitive (10 μM) and was therefore considered mainly purinergic with a minor nitrergic component.

At 2 Hz (not shown) and 5 Hz (Fig. 3) a strong inhibition was observed in the presence of MRS 2179 (10 μM) and in the presence of l-NNA (1 mM). In the presence of both MRS 2179 and l-NNA, the inhibition of spontaneous motility during EFS was not observed (ns). In some recordings a noncholinergic contractile response was observed. These results suggest that, at this range of frequencies, the response was partially MRS 2179 and l-NNA sensitive and therefore was considered both purinergic and nitrergic.

It is important to notice that at 2 Hz (not shown) and 5 Hz when the purinergic component was blocked with MRS 2179 (10 μM), the remaining nitrergic component could abolish the mechanical activity during all the EFS. In contrast, when the nitrergic component was inhibited, the time course of the inhibitory response was different, being a greater inhibition at the beginning of the EFS and partially recovering with time (Fig. 3).

**Junction potential in the human colon.** EFS at 1 Hz and 50 V for 5 s generated five consecutive pulses (P1 to P5). The response consisted of a first fast IJP of about 15 to 20 mV recorded after the first pulse (P1), followed by a very small response after the second pulse (P2) and fast IJPs of about 5 to 10 mV after the other three pulses (P3, P4, and P5) (Fig. 5). The response was almost abolished by MRS 2179 (10 μM).
and a minor difference was observed in the presence of L-NNA 1 mM (N = 5) (Fig. 4). The response obtained when the tissue was incubated with both MRS 2179 (10 μM) and L-NNA (1 mM) (n = 10) was similar to the response obtained with MRS 2179 (10 μM) alone. Accordingly, the response at 1 Hz was considered mainly purinergic through P2Y1 receptors.

EFS at 5 Hz for 5 s generated 25 consecutive pulses. At this frequency the response consisted of an IJP with a fast component followed by a sustained one (Fig. 5). The amplitude of the fast component was about 20 to 25 mV, whereas the slow component, measured at 2.5 s and 3.75 s after the beginning of the stimulus, was about 10 to 15 mV. L-NNA (1 mM) did not modify the fast component but significantly reduced the slow component. In contrast, addition of MRS 2179 (10 μM) decreased the fast component, and no major effect on the slow component was observed (Fig. 6). Addition of both L-NNA (1 mM) and MRS 2179 (10 μM) completely inhibited both the slow and fast component.

To illustrate the mechanism of neurotransmission, responses obtained in the presence of L-NNA, MRS 2179 and both antagonists together were subtracted from the response under control conditions (Fig. 5). The subtraction of the response obtained in control minus the response obtained in the presence of L-NNA consisted mainly of a slow component, corresponding to the response attributable to NO release. In contrast, the subtraction of the response obtained in control-MRS 2179 consisted of a fast component corresponding to the response attributable to a purine acting on P2Y1 receptors. Notice that, when the subtraction of the response in control from the response obtained after incubation with L-NNA and MRS 2179, both the fast and slow components were present (Fig. 6).

Characterization of the rundown of the fast component of the IJP in the human colon. The fast component of the IJP (fIJP) that is attributable to activation of P2Y1 receptors shows a rundown, i.e., a decrease in the response observed after 10.220.33.6 on April 25, 2017 http://ajpgi.physiology.org/ Downloaded from

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Fig. 6. To illustrate the neurotransmission mechanism, a subtraction (right) from the electrophysiological response obtained in control (left) and the response obtained after incubation with L-NNA (1 mM; A), MRS 2179 (10 μM; B), or both drugs (C) (middle) was performed. Notice that the L-NNA-sensitive response, probably attributable to NO release, is mainly the sustained IJP causing a sustained hyperpolarization, and the MRS 2179-sensitive response, probably attributable to activation of P2Y1 receptors, is mainly the fast component of the IJP.
when two stimuli are close together (Fig. 7). The mathematical model proposed in the present paper shows high correlation values (Figs. 7, 8, and 9). About 2.5 s after the first pulse, the amplitude of the response obtained after the second pulse was about 50% of the IJP elicited by the first pulse. 80–90% of recovery was achieved with intervals from 5–8 s after the first pulse. This rundown mechanism is present both in the circular and longitudinal muscle layers (Fig. 7). To characterize the fIJP rundown, the rundown mechanism might be attributable to the percentage of receptor occupation. Accordingly, we have performed the same analysis in the presence of graded responses, i.e., supra-maximal fIJPs (about 30 mV hyperpolarization), intermediate fIJPs (about 20 mV hyperpolarization), and small fIJPs (about 10 mV hyperpolarization) (Fig. 9). In each case, the voltage of stimulation was selected to obtain an appropriate response. All these responses are MRS 2179 sensitive (15). The rundown mechanism was equally present independently of the amplitude of the first hyperpolarization (Fig. 8). The rundown mechanism was present after incubation of the tissue with L-NNA (1 mM), MRS 2179 (1 μM), NF2173 (10 μM), or hexamethonium (100 μM) (Fig. 9). Neither adenosine (0.5 nM-1 μM) nor DPCPX (10 μM) had any effect on the IJP amplitude and rundown (data not shown). It is

Fig. 7. Rundown response obtained after 2 consecutive stimuli obtained from the circular and longitudinal layer (A). Percentage of the first response (Y-axis) obtained at different time intervals (X-axis) (B). Experimental data (dots) were fitted with an exponential curve (see MATERIALS AND METHODS), and R² was estimated.
important to notice that MRS 2179 (1 μM) reduced the amplitude of the fIJP by about 50% (15) without modifying the rundown mechanism.

DISCUSSION

In the human colon, single pulses or short trains induce fIJs that are insensitive to L-NNA (15, 21), and consequently fIJs are not mediated by NO. In contrast, in other species, short pulses cause a biphasic IJP with a fast component followed by a sustained one (9, 18, 27). Usually, the fast component is L-NNA insensitive, and the sustained component is L-NNA sensitive, showing that NO and a second neurotransmitter are involved in the inhibitory response. In the guinea pig, small intestine fIJs are sensitive to MRS 2179, and sustained IJP are mainly sensitive to L-NNA (32), showing that purinergic components through P2Y₁ receptors and NO mediate inhibitory neurotransmission in that species. In the human colon, this response is much more difficult to record because long pulses

Fig. 8. Effect of the amplitude of the first response (IJP1) on the rundown mechanism. Rundown recorded with 30-mV IJP1 amplitude (A), 20-mV IJP1 amplitude (B), and 10-mV IJP1 amplitude (C). Experimental data (dots) were fitted with an exponential curve (see MATERIALS AND METHODS), and $R^2$ was estimated (right).
are needed to demonstrate the presence of these two components (21). In a previous paper, we demonstrated that the fast component of the IJP was sensitive to MRS 2179, and, therefore, it might be attributable to a purinergic mediator acting at P2Y₁ receptors (15). In the present work, we have studied the transmission between NO and ATP using this pharmacological approach, and we have tried to evaluate whether both neurotransmitters are complementary or redundant, causing smooth muscle inhibition in the human colon.

**Characterization of the purinergic component: the IJP rundown.** Single or short train pulses elicit fIJP that show a rundown when a second pulse (test pulse) is applied at different time intervals after the first conditioning pulse. This mechanism has been previously denominated as IJP rundown in animal studies (22, 25). In contrast, in other tissues, such as the mouse cecum, this mechanism is absent (35). In our study, the amplitude of the IJP elicited by the test pulse was very small at short time intervals and recovered when time intervals were increased. The time to recover 50% of the first IJP was around 2.5 s both in circular and longitudinal muscle layers, which is quite similar to those previously described in animal studies (22, 25). Our data show that this mechanism is largely independent of the amplitude of the conditioning IJP. The rundown mechanism can be attributable to a prejunctional mechanism, i.e., prejunctional receptors causing the inhibition of the release of the inhibitory mediator or alternatively to a postjunctional mechanism including receptor or intracellular pathways. Regarding the prejunctional hypothesis, a previous study on hamster proximal colon demonstrated that NO might be responsible for the IJP rundown (25). In this species, short pulses elicited a fast component followed by a sustained nitrergic one, and the IJP rundown was inhibited with L-NNA incubation. Unfortunately, we could not demonstrate this mechanism in the human colon because single pulses elicit fIJP that are L-NNA insensitive, and incubation with L-NNA did not inhibit the rundown mechanism. Moreover when pulses of 1 Hz were applied (see below) the response was not modified in the presence of L-NNA. Another putative mechanism of prejunctional inhibition might be through adenosine receptors. Adenosine might cause a prejunctional inhibition of neurotransmitter release as has previously been demonstrated for excitatory neurotransmitters in animal models (23). Moreover, adenosine

![Fig. 9. Rundown recorded after incubation with L-NNA (1 mM) (A), MRS 2179 (1 μM) (B), NF023 (10 μM) (C), or hexamethonium (100 μM) (D). Closed circles are experimental data obtained in control, and open circles are experimental data after drug incubation.](http://ajpgi.physiology.org/)

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inhibited, the sustained component is reduced and the mechanical activity is transiently inhibited, but it cannot be maintained over time. This response appears at both 2 and 5 Hz of stimulation. It is possible that continuous release of ATP or a related purine causes a rundown mechanism that is unable to cause hyperpolarization and relaxation of smooth muscle cells. According to this result, incubation of the tissue with ADPβS or ATP causes transient relaxation that partially recovers with time. It is important to notice that incubation with NO inhibitors causes an increase in motility. In contrast, no effect or even a slight decrease in spontaneous motility is observed after MRS 2179 incubation. These results show that NO but not ATP might contribute to the inhibitory neural tone.

Taken together, our results demonstrate that the two inhibitory neurotransmitters have different functions in inhibiting colonic motility. NO is 1) responsible for sustained hyperpolarization, 2) can cause sustained relaxation, and 3) can be tonically released from inhibitory motor neurons. In contrast, the purinergic mediator acting on P2Y₁ receptors is 1) responsible for fast hyperpolarization (which usually has bigger amplitude than the sustained component), 2) might cause a transient relaxation that is difficult to maintain over time due to the rundown mechanism, and 3) is probably not tonically released from enteric neurons.

It is conceivable that both neurotransmitters are involved in inhibiting the motility of other areas of the gastrointestinal tract such as the small intestine (31) or LES (14). When a tonic relaxation should occur such as in gastric accommodation, NO can probably accomplish the function without a purinergic input. In contrast, when a sudden transient relaxation is needed, ATP through P2Y₁ receptors can probably accomplish this function. More studies are needed on other areas of the human GI tract to demonstrate this hypothesis and to investigate the putative role of neuromuscular transmission impairment in human neuropathologies that affect the regulation of gut motility.

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inhibits IJP in the guinea pig ileum, but prejunctional P1 purinoceptors are not responsible for the IJP rundown (22). In the human colon, we did not find an inhibitory effect of adenosine on the IJP, and inhibitors of adenosine receptors such as DPCPX did not inhibit the IJP rundown. Other antagonists such as low concentration of MRS 2179 (1 μM) that inhibits the IJP by about 50%, or NF023, a putative P2X antagonist, do not inhibit the IJP rundown, suggesting that these receptors are not involved in the prejunctional inhibition of the inhibitory transmitter. Hexamethonium did not affect the IJP rundown. Although we do not have a final explanation of the mechanism in the human colon, a postjunctional mechanism might be responsible for the IJP rundown. Incubation of P2Y agonist causes transient hyperpolarization, and a partial inhibition of the IJP occurs when the membrane potential is recovered (15). Moreover, stable agonists have been widely used as “desensitizers” of purinergic receptors.

Characterization of the purinergic component: pulses at 1 Hz. Pulses elicited at 1 Hz for 5 s (5 pulses) elicited five fIJP of variable amplitude. The first IJP was of high amplitude compared with the others probably due to the rundown mechanism. A similar result has been reported in the guinea pig ileum where trains of 1 Hz caused IJPs of smaller amplitude compared with the first response (22). In this case, the recovery of rundown may coincide with the release of new inhibitory neurotransmitters with the next pulse, and a compromise between both mechanisms exists. The mechanical response of this EFS protocol consists of a sustained inhibition of spontaneous motility, probably based on the successive fIJP. Both the electrophysiological and the mechanical responses were mainly inhibited by MRS 2179, showing the involvement of ATP or a related purine acting at P2Y₁ receptors.

Characterization of the purinergic and nitrergic component: pulses at 5 Hz. EFS at 5 Hz for 5 s (25 pulses) clearly demonstrates that two neurotransmitters are released by inhibitory neurons. The electrophysiological response shows a fast component followed by a sustained one. It is important to notice that the fast component is mainly MRS 2179 sensitive, whereas the second component is mainly L-NNA sensitive, showing a neurotransmission of ATP or a related purine, acting on P2Y₁ receptors, and NO. This pharmacological approach has been recently used in the guinea pig ileum where the majority of IJPs with a fast and sustained component were MRS 2179 and L-NNA sensitive, respectively (32). The mechanical response of this protocol of EFS consisted of a complete inhibition of spontaneous motility. Incubation of the tissue with both MRS 2179 and L-NNA blocked the inhibitory effect induced by EFS.

It is important to notice that electrical and mechanical responses are correlated when a single inhibitor is infused. In the presence of MRS 2179, the fast component is inhibited and the sustained component is present. This sustained hyperpolarization is probably responsible for the sustained relaxation observed in the presence of the P2Y₁ antagonist. This is an interesting result because it demonstrates that nitrergic neurotransmission can cause sustained hyperpolarization causing sustained relaxation in the absence of purinergic inhibitory neurotransmission. This result fits with the effect of NO donors on spontaneous motility because NO causes sustained hyperpolarization (15) and causes long-lasting inhibition of spontaneous motility. In contrast, when nitrergic neurotransmission is