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Neural modulation of the cyclic electrical and mechanical activity in the rat colonic circular muscle: putative role of ATP and NO

[1]Lídia Plujà, [1]Ester Fernández & *,[1]Marcel Jiménez

[1]Department of Cell Biology and Physiology, Universitat Autònoma de Barcelona, 08193 Bellaterra Barcelona, Catalunya Spain

1 The rat colonic circular muscle displays cyclic episodes of myenteric potential oscillations (MPOs), each of them associated with a spontaneous contraction. Nifedipine 1 μM abolished both MPOs and their associated contractions. TTX (1 μM) increased the amplitude and frequency of spontaneous contractions.

2 Electrical field stimulation (EFS) induced a non-adrenergic non-cholinergic (NANC) inhibitory junction potential (IJP), with two phases: an initial fast hyperpolarization (characterized by IJP amplitude) and a sustained hyperpolarization (characterized by IJP duration).

3 Sodium nitroprusside (10 μM) hyperpolarized and abolished spontaneous contractions even in presence of TTX or 1 μM apamin. ATP (100 μM) also hyperpolarized and abolished spontaneous contractions but its effects were decreased by TTX and abolished by apamin.

4 Suramin (100 μM) or apamin reduced the amplitude of the IJPs, but did not affect their duration. Incubation with L-NOARG (1 mM) reduced the duration but not the amplitude of the IJPs. In presence of L-NOARG plus suramin or L-NOARG plus apamin, both duration and amplitude of the IJPs were reduced but a residual IJP could still be recorded.

5 We conclude that the mechanical and electrical cyclic activity of the rat colonic circular muscle is modulated but not originated by the enteric nervous system and involves L-type calcium channel activity. EFS induces release of NANC inhibitory neurotransmitters which hyperpolarize and relax smooth muscle cells. Both ATP and NO are involved in IJP generation: ATP is responsible for the first phase of the IJPs involving activation of apamin-sensitive potassium channels, whereas NO initiates the second phase which is independent of the activation of such channels.

Keywords: Myenteric potential oscillations (MPOs); non-adrenergic non-cholinergic (NANC); inhibitory junction potential (IJP); ATP; NO; apamin; rat colon

Introduction

Intracellular recordings from colonic smooth muscle cells have revealed a spontaneous cyclic electrical activity. In several species, such as human and cat, rapid oscillations (20–40 cycles min^{-1}) that might trigger action potentials at their peaks, have been described. This electrical activity has been designated as myenteric potential oscillations (MPOs) (Duthie & Kirk, 1978; Sanders & Smith, 1989). In the mouse colon, successive depolarizations take place and might allow action potentials during them. These 'myoelectric complexes' alternate with quiescence periods (Lyster *et al*., 1993; 1995). Thus, MPOs are very similar to depolarizations recorded in the myoelectric complexes.

Electrical field stimulation (EFS) of isolated gastrointestinal preparations causes a neurogenic transient hyperpolarization, known as the inhibitory junction potential (IJP), which results in relaxation of the muscle. IJPs are mediated by inhibitory non-adrenergic non-cholinergic (NANC) neurotransmitters (Bywater & Taylor, 1986). The identity of these neurotransmitters has been extensively investigated. First ATP and then VIP, were regarded as putative neurotransmitters in the gut (Burnstock *et al*., 1978; Manzini *et al*., 1986; Grider & Makhlouf, 1988). More recently, nitric oxide (NO) has been viewed as the main NANC inhibitory neurotransmitter in the gastrointestinal tract of many species (Boeckxstaens *et al*., 1991; Burleigh, 1992; Suthamnatpong *et al*., 1994; Serio *et al*., 1995; Takeuchi *et al*., 1996). However, it has become evident that IJPs may often show two phases, a fast and a slow hyperpolarization. Thus, this profile has been described for the IJP elicited by EFS of mouse colon (Shuttleworth *et al*., 1997), mouse ileum (Ward *et al*., 1994), guinea-pig taenia caeci (Bridgewater *et al*., 1995), guinea-pig ileum (Crist *et al*., 1992), human colon (Keef *et al*., 1993) and human jejunum (Stark *et al*., 1993). This biphasic shape of the IJP may suggest the release of more than one neurotransmitter. In other species such as dog and pig, IJPs are monophasic with a triggered slow wave afterwards (Stark *et al*., 1993; Borderies *et al*., 1997).

In the rat colon, co-localization of ATP and NO has been described in neurons of the myenteric plexus using the quinacrine fluorescence technique to identify purinergic nerves and NADPH-diaphorase histochemistry to localize nitrergic neurons. This co-localization suggests a putative role for both substances in NANC neurotransmission (Belai & Burnstock, 1994). In fact, a role for NO in the IJP has been proposed from results obtained with the sucrose-gap technique (Serio *et al*., 1995). However, NO is not fully responsible for the hyperpolarization induced by EFS since, in the presence of NO synthase (NOS) inhibitors, EFS does still cause a residual IJP. This may suggest a co-transmission in this tissue (Serio *et al*., 1995). In fact, in the rabbit stomach, NO, ATP and VIP have all been described as NANC mediators (Baccari *et al*., 1994).

Accordingly, the aims of this study have been: (1) to investigate the spontaneous electrical and mechanical activity of the isolated circular muscle of the rat colon; (2) to study the putative neurotransmitter(s) that mediate the inhibitory NANC response to EFS.

*Author for correspondence; E-mail: marcel.jimenez@blues.uab.es

Methods

Tissue preparation

Male Sprague-Dawley rats (300–350 g), 8–10-weeks-old, were kept at a constant temperature (21–23°C), with lighting cycle of 12 h light/12 h dark, fasted overnight (18 h) but allowed *ad libitum* access to water. Rats were killed by decapitation and bled. This procedure was approved by the Ethics Committee of the Universitat Autònoma de Barcelona. The colon was quickly removed, placed in Krebs solution on a dissection dish, and the mucosal and submucosal layers removed. Circular muscle strips were cut 1 cm long and 0.3 cm wide. The composition of the Krebs solution was (in mM) 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).

Recordings of spontaneous mechanical activity

Muscle strips were attached with silk threads to a stable mount in the bottom of a 10 ml organ bath filled with carbogenated Krebs solution at 37±1°C. The upper end was tied to an isometric force transducer (Harvard VF-1) connected to an amplifier and then to a computer. Data were digitalized (25 Hz) and simultaneously displayed and collected using Datawin1 software (Panlab-Barcelona) coupled to an ISC-16 A/D card installed in a PC Pentium computer. A tension of 1 g was applied and the tissue was allowed to equilibrate for 1 h. After this period, strips displayed spontaneous phasic activity. To estimate the responses to drugs, the amplitude, duration and frequency of the contractions were measured before and after drug addition.

Simultaneous recordings of electrical and mechanical activities

Muscle strips were placed (circular muscle side up) in a Sylgard coated chamber and continuously perfused with carbogenated Krebs solution at 37±1°C. One end was pinned for intracellular recordings and the opposite end was attached to an isometric transducer and preloaded with 1 g. Preparations were allowed to equilibrate for approximately 1 h before experiments started. Circular muscle cells were impaled with glass microelectrodes (R = 40–60 MΩ) filled with 3 M KCl. Membrane potential was measured using standard electrometer Duo773 (WPI Inc., FL, U.S.A.). Both electrical and mechanical activities were displayed on a digital storage oscilloscope 4026 (Racal-Dana Ltd., England), and simultaneously digitalized (100 Hz) and collected using EGAA software coupled to an ISC-16 A/D card (RC Electronics Inc., CA, U.S.A.) installed in a 486 PC computer.

Electrophysiological response to electrical field stimulation (EFS)

Tissue samples were placed in a silgard coated chamber in the same conditions described above. Electrical field stimulation (EFS) was applied using two silver chloride plates placed perpendicular to the longitudinal axis of the preparation and

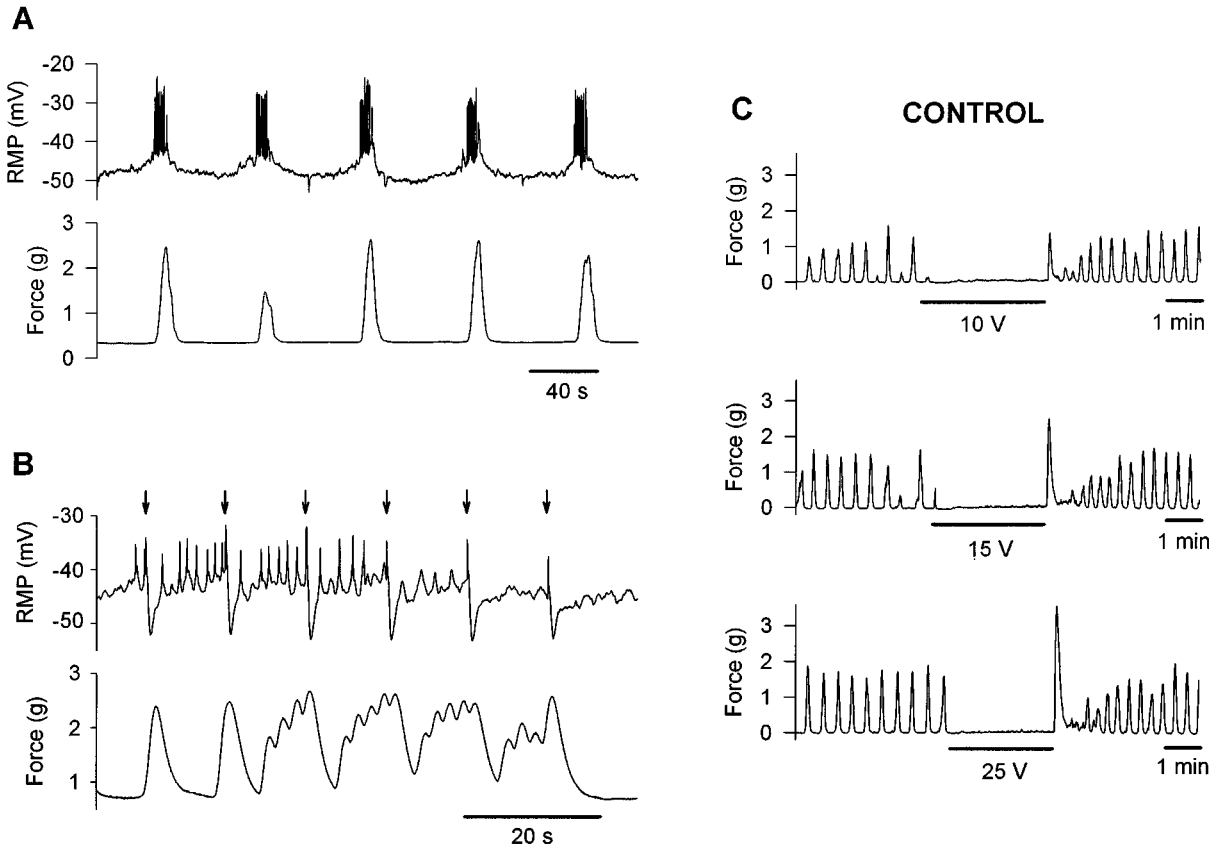

Figure 1 (A) Simultaneous intracellular microelectrode (top) and mechanical (bottom) recordings from the circular muscle layer of the rat colon showing cyclic episodes of MPOs and associated contractions. (B) Intracellular recording showing IJPs (top) evoked by EFS (12 V, 20 Hz, train duration 100 ms and pulse duration 0.3 ms) and the simultaneous associated relaxations on the mechanical activity (bottom). Arrows indicate the start of the stimuli. (C) Mechanical recordings showing the response to EFS elicited during 3 min (1 Hz, train duration 100 ms, frequency 20 Hz, pulse duration 0.3 ms) and increasing amplitude strengths (10, 15 and 25 V).

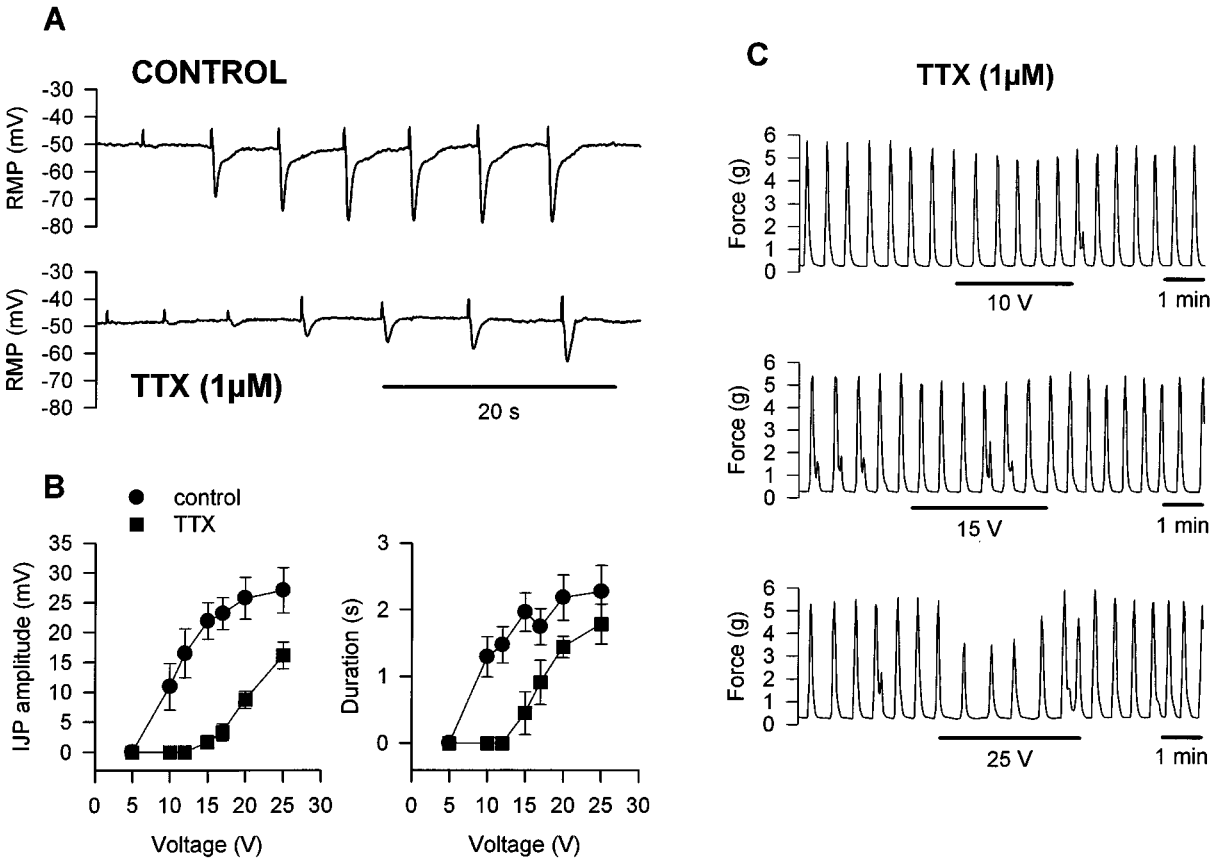

Figure 2 (A) Intracellular recordings showing IJPs elicited by EFS increasing the voltage of stimulation (5, 10, 12, 15, 17, 20 and 25 V) in control conditions (top), and in the presence of TTX (1 μM) (bottom). Both recordings were obtained in the presence of nifedipine (1 μM) to abolish mechanical activity and to increase the duration of the impalement. (B) Effect of TTX (1 μM) on the amplitude (left) and duration (right) of the IJPs. (C) Mechanical recording, in the presence of TTX (1 μM), showing the response to EFS elicited during 3 min at 1 Hz (train duration 100 ms, frequency 20 Hz, pulse duration 0.3 ms) and increasing amplitude strengths (10, 15 and 25 V).

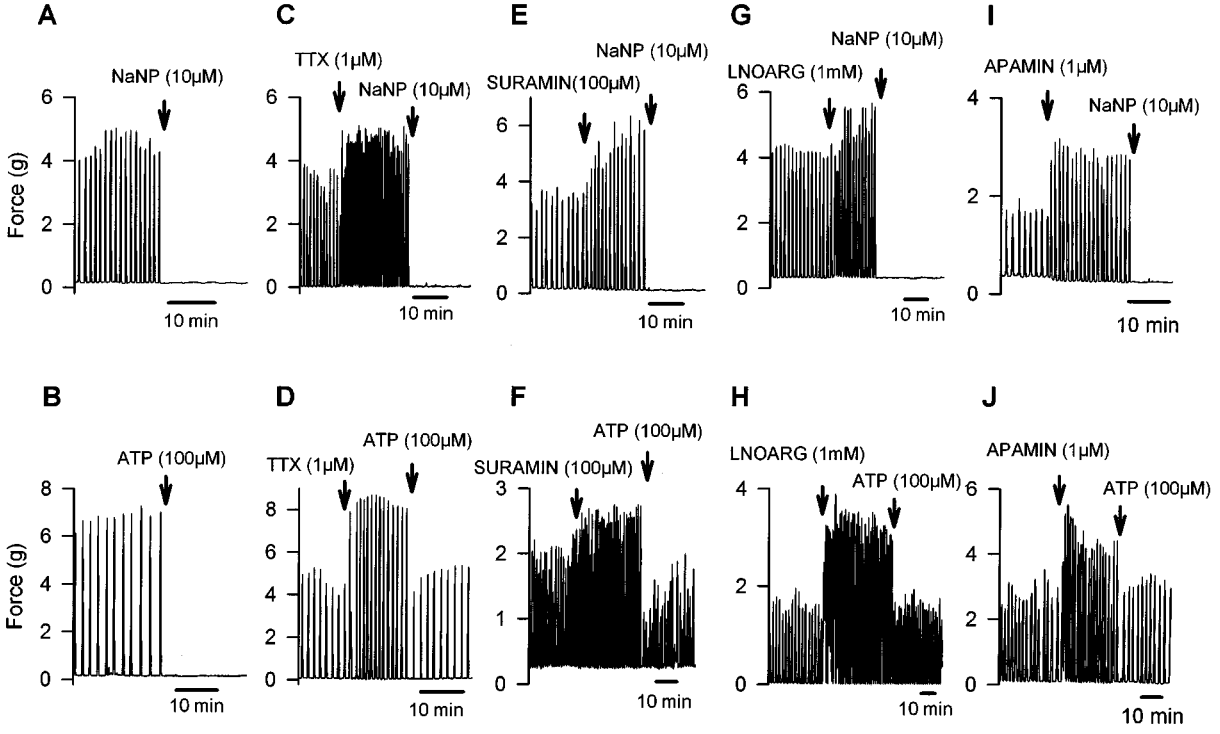

Figure 3 Recording showing the effects of NaNP (10 μM) and ATP (100 μM) on the spontaneous mechanical activity under control conditions (A and B); in the presence of TTX (1 μM) (C and D); in the presence of suramin (100 μM) (E and F); in the presence of L-NOARG (1 mM) (G and H); and in the presence of apamin (1 μM) (I and J, respectively).

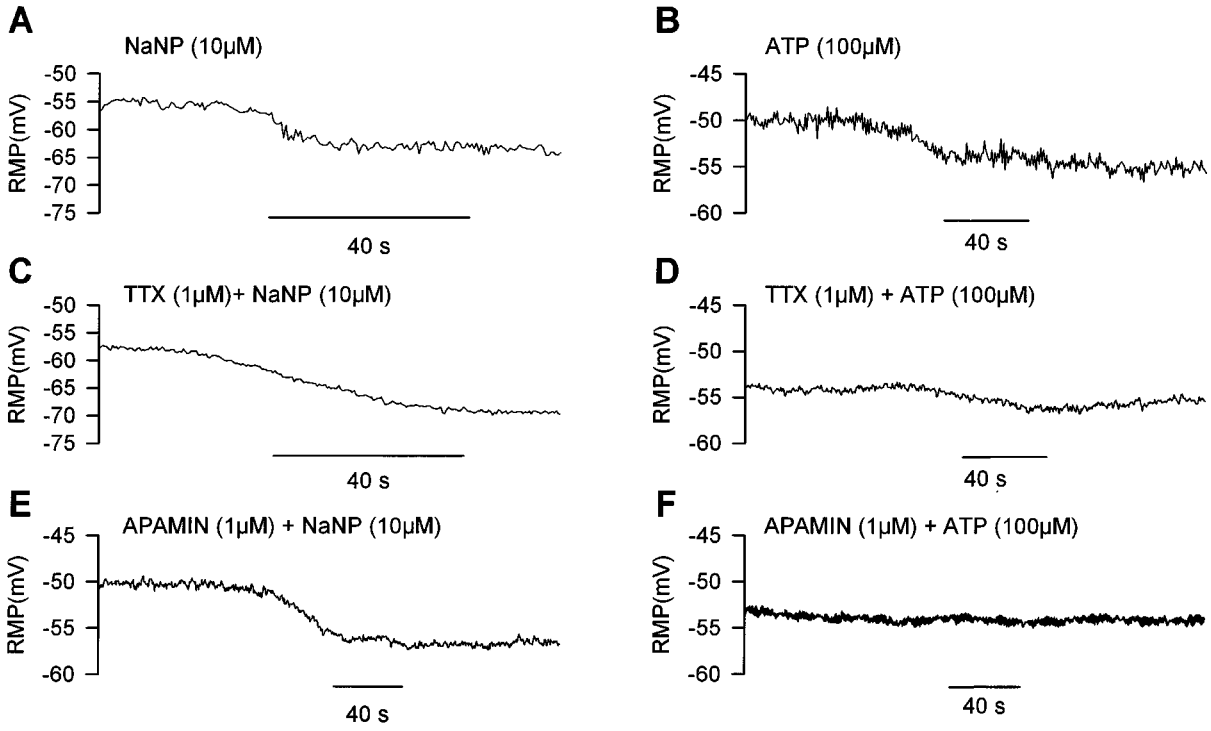

Figure 4 Intracellular microelectrode recordings showing the effects of NaNP (10 μM) (A) and ATP (100 μM) (B) on the resting membrane potential under control conditions. The same subtances were tested in the presence of TTX 1 μM (C and D, respectively) and in the presence of apamin (1 μM) (E and F, respectively). All intracellular recordings were obtained in the presence of nifedipine (1 μM).

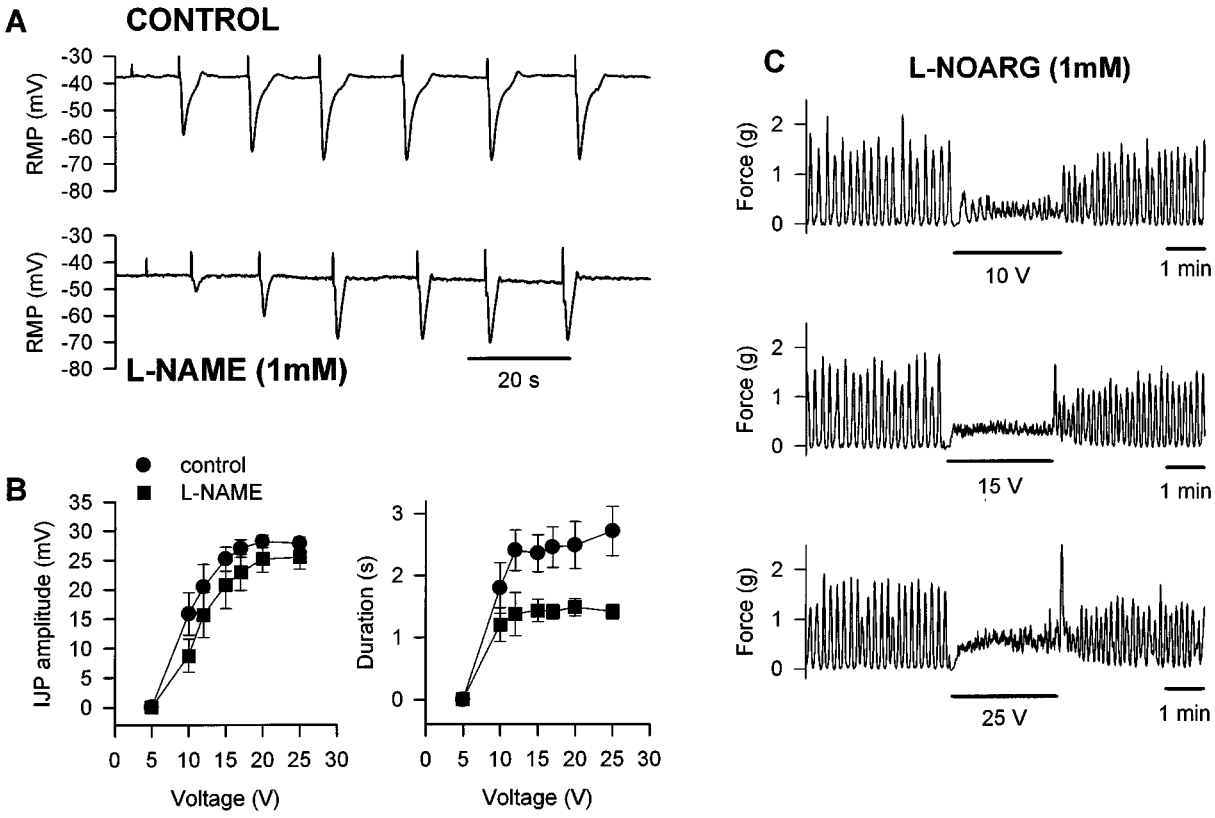

Figure 5 (A) Intracellular recording showing the effect of L-NAME (1 mM) on the IJPs obtained by EFS (same procedure as in Figure 2). All recordings were obtained in the presence of 1 μM nifedipine (B) Effect of L-NAME (1 mM) on the amplitude (left) and duration (right) of the IJPs. (C) Mechanical recording, in the presence of L-NOARG (1 mM), showing the response to EFS elicited during 3 min at 1 Hz (train duration 100 ms, frequency 20 Hz, pulse duration 0.3 ms) and increasing amplitude strengths (10, 15 and 25 V).

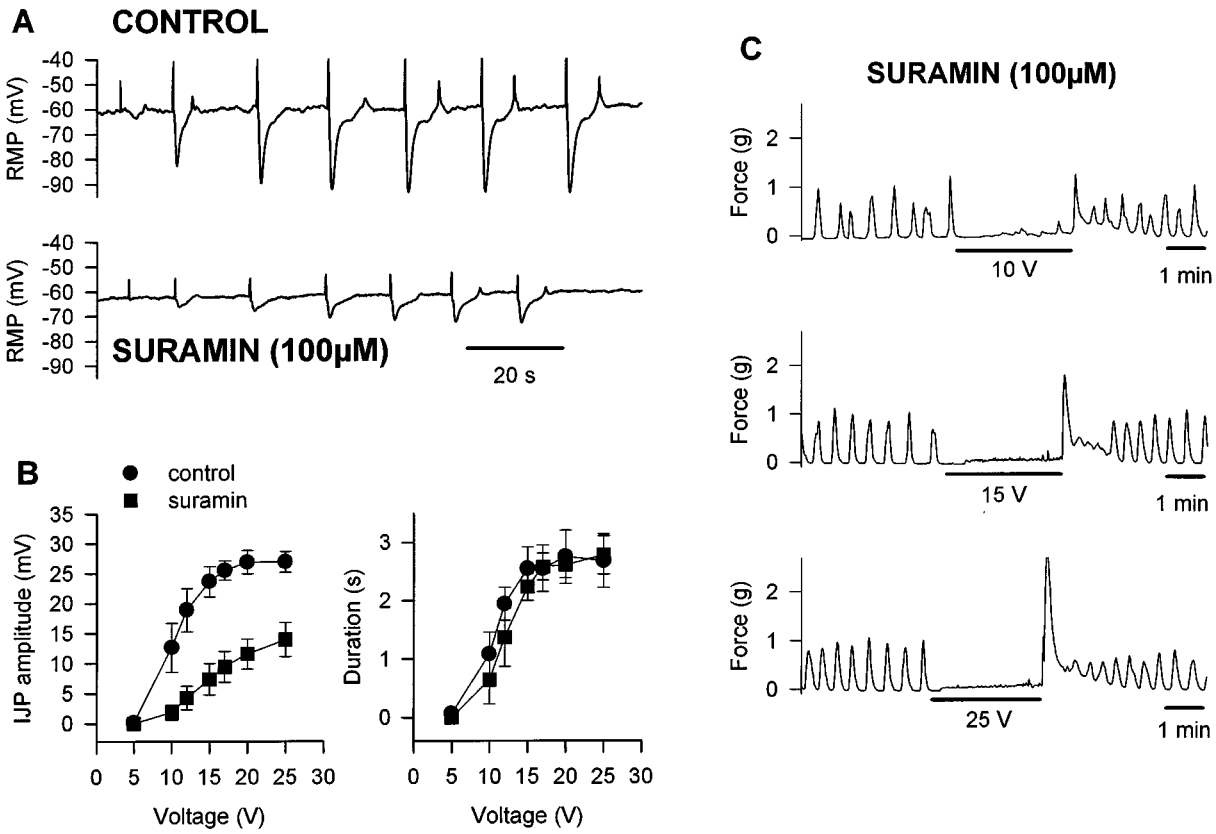

Figure 6 (A) Microelectrode recording showing the IJPs obtained by EFS in control conditions (top) and in the presence of suramin (100 μM), (same procedure as in Figure 2). All recordings were obtained in the presence of nifedipine (1 μM). (B) Effect of suramin (100 μM) on the amplitude (left) and duration (right) of the IJPs obtained at different stimulus strength. (C) Mechanical recording, in the presence of suramin (100 μM), showing the response to EFS elicited during 3 min at 1 Hz (train duration 100 ms, frequency 20 Hz, pulse duration 0.3 ms) and increasing amplitude strengths (10, 15 and 25 V).

1.5 cm apart. Train stimulation had the following parameters: total duration 100 ms, frequency 20 Hz, pulse duration 0.3 ms and increasing amplitude strengths (5, 10, 12, 15, 17, 20 and 25 V). The amplitude and duration of IJPs were measured under control conditions and after infusion of each drug. In order to obtain stable impalements, nifedipine (1 μM) was perfused to abolish mechanical activity.

Mechanical response to electrical field stimulation (EFS)

In separate experiments the mechanical responses to electrical field stimulation were recorded using the same set up in the absence of nifedipine. EFS was applied during 3 min, 1 train s^{-1} (train duration 100 ms, frequency 20 Hz, pulse duration 0.3 ms) and increasing amplitude strengths (10, 15 and 25 V). To estimate the responses to EFS, the amplitude, duration and frequency of the contractions were measured before and during EFS. When a drug qualitatively modified the response, a description of the effect is reported.

Solutions and drugs

The following drugs were used: nifedipine, N-nitro-L-arginine (L-NOARG), N-methyl-L-arginine ester (L-NAME), adenosine 5′-triphosphate (ATP), phentolamine (Sigma Chemicals, St. Louis, U.S.A.); tetrodotoxin (TTX), atropine sulphate, propranolol, suramin, apamin, sodium nitroprusside (NaNP) (Research Biochemicals International, Natick, U.S.A.). Stock solutions were made by dissolving drugs in distilled water except for nifedipine which was dissolved in ethanol.

Data analysis and statistics

Data are expressed as mean $\pm$ s.e.mean. Paired Student's *t*-test was used to compare mechanical activity in the absence and in the presence of drugs or in the absence and presence of EFS. The differences between amplitude or duration of the IJPs before and after drug infusion were compared by analysis of variance for repeated measurements followed by pairwise comparisons using Student-Newman-Keuls method. A P value <0.05 was considered as statistically significant.

Results

Spontaneous electrical and mechanical activity

The resting membrane potential of circular muscle cells from the rat colon was -50.2 ± 1.3 mV ($n=41$). This membrane potential was spontaneously unstable showing cyclic episodes of depolarization (5–10 mV) that trigger action potentials which were considered myenteric potential oscillations (MPOs) (Figure 1A). This cyclic activity occurred at a frequency of 0.78 ± 0.17 episodes min^{-1}. Each episode of MPOs is associated with a spontaneous muscle contraction which averaged 3.3 ± 0.1 g amplitude and 22.6 ± 1.4 s duration ($n=29$). The cyclic electrical activity (depolarizations and

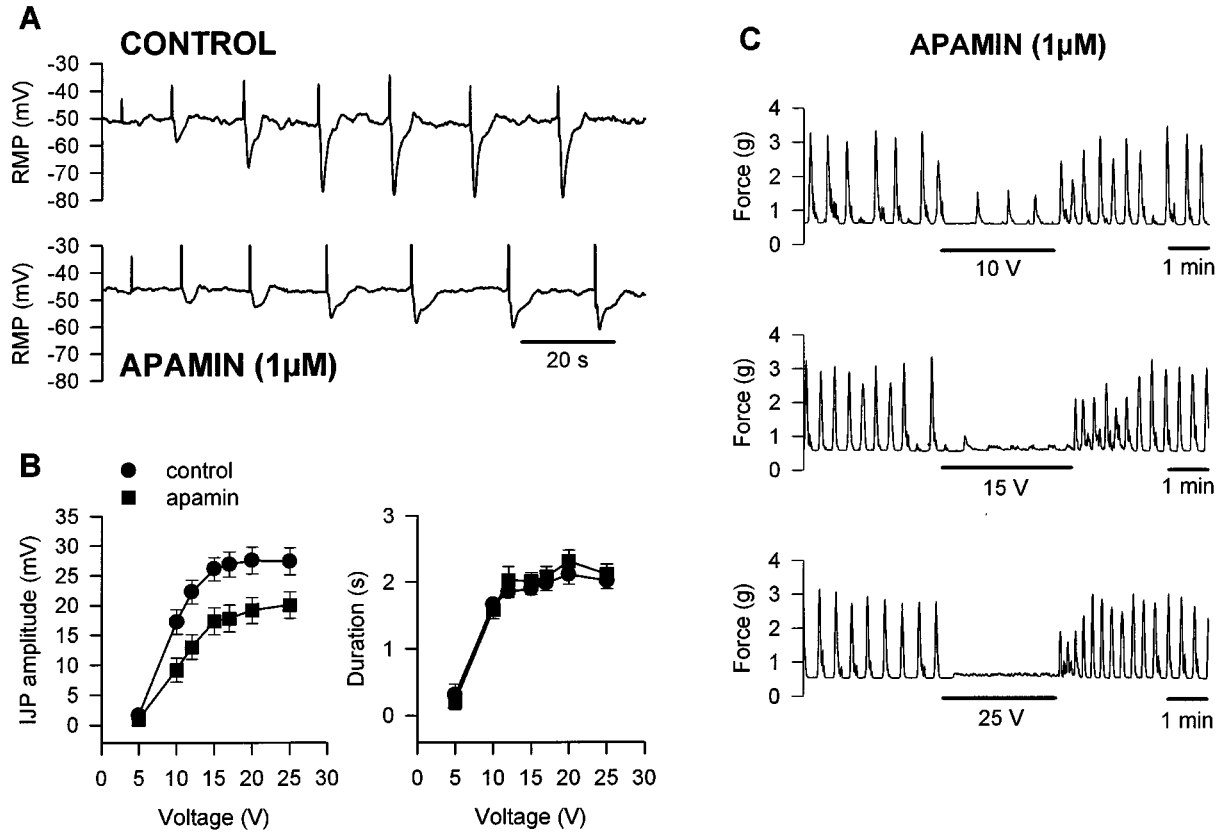

Figure 7 (A) Effect of apamin (1 μM) on the IJPs obtained at different stimulus strengths (same procedure as in Figure 2). All recordings were performed in the presence of nifedipine (1 μM). (B) Effect of apamin (1 μM) on the amplitude (left) and duration (right) of the IJP. (C) Mechanical recording, in the presence of apamin (1 μM), showing the response to EFS elicited during 3 min at 1 Hz (train duration 100 ms, frequency 20 Hz, pulse duration 0.3 ms) and increasing amplitude strengths (10, 15 and 25 V).

action potentials) and associated contractions were abolished by 1 μM nifedipine ($n=5$).

Relationship between electrical field stimulation and the mechanical activity

EFS induced a membrane hyperpolarization considered as an inhibitory junction potential (IJP) (see next paragraph). IJPs could be elicited both during the resting state and during the episode of depolarization. When an IJP was triggered during the resting period the mechanical activity was not modified. However, when the IJP was elicited during the spontaneous contraction, a transient relaxation was immediately recorded (Figure 1B). Moreover, when repetitive IJPs (1 Hz) were elicited (10, 15, 25 V) for 3 min, spontaneous contractions were abolished. An off-response was observed at the end of the stimulation (Figure 1C).

Characterization of the inhibitory junction potential

EFS evoked a transient membrane hyperpolarization which showed two phases: a fast hyperpolarization followed by a slow one (Figure 2A). These phases were characterized by the amplitude and the duration of the transient hyperpolarization respectively. Both parameters increased when increasing voltages were applied (Figure 2B). The maximum response (-26.6 ± 0.6 mV in amplitude and 2.4 ± 0.1 s in duration) was reached at EFS of 25 V ($n=60$).

Nifedipine (1 μM, $n=5$) abolished MPOs and spontaneous contractions but did not modify the resting membrane potential. The amplitude and duration of IJPs remained unchanged (not shown). After this fact was evidenced, all intracellular recordings were made in the presence of nifedipine, as the absence of spontaneous contractions increased significantly the duration of impalements. TTX (1 μM, $n=6$) abolished the hyperpolarizations evoked by EFS at stimuli ranging from 5–12 V. At higher voltages, a residual hyperpolarization was still present (Figures 2A and B) which could be due to a direct stimulation of nerve endings. Thus, transient hyperpolarizations evoked by train stimuli were (IJPs). Atropine, phentolamine and propranolol (each at 1 μM, $n=6$) did not change IJP characteristics regardless of the strength of the stimulus used (not shown). The spontaneous mechanical activity displayed by the tissue in control conditions was abolished when EFS was applied (Figure 1C). TTX 1 μM ($n=4$) decreased but did not block the inhibitory effects of stimuli of higher voltages (Figure 2C).

Effect of NaNP and ATP on the mechanical activity

Sodium nitroprusside (NaNP) (10 μM, $n=4$) and ATP (100 μM, $n=4$) abolished spontaneous contractions (Figures 3A and B). TTX (1 μM, $n=8$) increased the amplitude (4.0 ± 0.6 vs 7.5 ± 1.1 g) and the frequency (0.66 ± 0.10 vs 0.83 ± 0.13 c.p.m.) of phasic contractions ($P<0.01$ and $P<0.05$, respectively) (Figures 3C and D). The duration of the contractions was not affected. NaNP (10 μM, $n=4$) caused a TTX resistant abolition of the spontaneous mechanical activity (Figure 3C). In the presence of TTX (1 μM, $n=4$), ATP reduced the amplitude (9.4 ± 1.3 vs 5.3 ± 0.7 g; $P<0.05$)

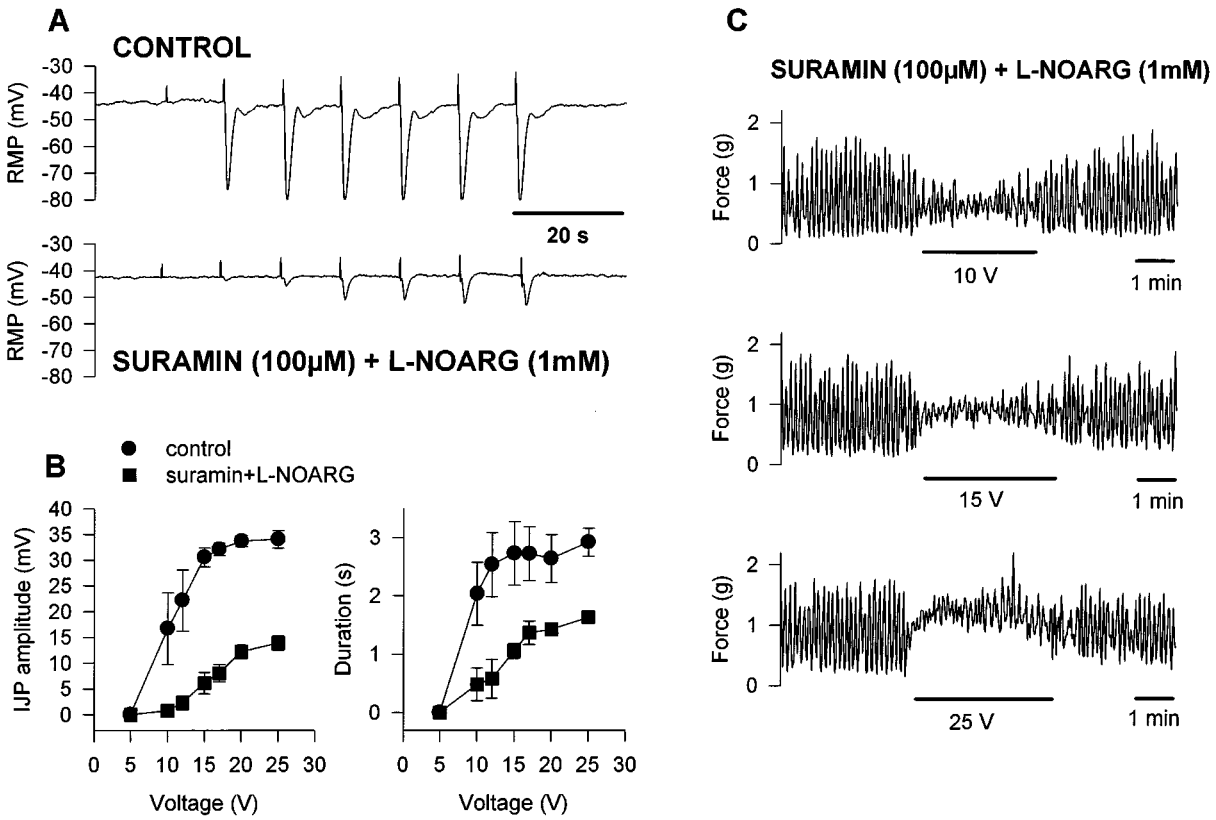

Figure 8 (A) Microelectrode recording showing the IJPs obtained by EFS (same procedure as in Figure 2), in control conditions (top) and in the presence of suramin (100 μM) plus L-NOARG (1 mM). All recordings were obtained in the presence of nifedipine (1 μM). (B) Effect of suramin (100 μM) plus L-NOARG (1 mM) on the amplitude (left) and duration (right) of the IJP. (C) Mechanical recording, in the presence of suramin (100 μM) plus L-NOARG (1 mM), showing the response to EFS elicited during 3 min at 1 Hz (train duration 100 ms, frequency 20 Hz, pulse duration 0.3 ms) and increasing amplitude strengths (10, 15 and 25 V).

and frequency of the contractions (0.88 ± 0.10 vs 0.51 ± 0.12 c.p.m.; $P<0.01$), but a complete blockade of the mechanical activity was not achieved (Figure 3D).

In the presence of suramin (100 μM, $n=8$), the amplitude (3.4 ± 0.6 vs 4.5 ± 0.5 g; $P<0.01$) and frequency (0.55 ± 0.06 vs 0.74 ± 0.09 c.p.m.; $P<0.05$) of the contractions increased (Figures 3E and F) whereas the duration of the contractions was not affected. NaNP (10 μM, $n=4$) abolished the cyclic mechanical activity in the presence of suramin (100 μM) (Figure 3E). In contrast, ATP (100 μM, $n=4$) reduced but did not abolish the amplitude of spontaneous mechanical activity (4.3 ± 0.9 vs 1.9 ± 0.9; $P<0.05$) (Figure 3F).

L-NOARG (1 mM, $n=5$) increased the amplitude (3.1 ± 1.0 vs 5.0 ± 1.4 g; $P<0.01$), duration (22.0 ± 1.1 vs 33.2 ± 1.7 s; $P<0.01$) and frequency (0.55 ± 0.08 vs 0.80 ± 0.03 c.p.m.; $P<0.05$) of the contractions (Figures 3G and H). L-Arg (10 mM, $n=6$) partly reversed this effect (not shown). In the presence of L-NOARG (1 mM), NaNP (10 μM, $n=4$) abolished the mechanical activity (Figure 3G), whereas ATP (100 μM, $n=6$) reduced the amplitude (4.6 ± 1.2 vs 2.3 ± 0.7; $P<0.01$) and frequency (0.98 ± 0.18 vs 0.67 ± 0.14; $P<0.01$) of cyclic contractions (Figure 3H).

Apamin (1 μM, $n=6$) increased the amplitude (3.3 ± 0.4 vs 4.7 ± 0.6 g; $P<0.01$) and frequency (0.66 ± 0.16 vs 0.92 ± 0.13 c.p.m.; $P<0.05$) of contractions, but their duration was unchanged (Figure 3I and J). In the presence of apamin (1μM), NaNP (10 μM, $n=4$) was able to abolish the spontaneous mechanical activity (Figure 3I) whereas ATP (100 μM, $n=4$) did not abolish but reduced the amplitude of contractions (5.5 ± 0.6 vs 3.6 ± 0.7 g; $P<0.05$) (Figure 3J).

Effects of NaNP and ATP on the membrane potential

The NO donor, sodium nitroprusside (10 μM, $n=5$; $P<0.001$), caused a hyperpolarization of 13 ± 1 mV of the muscle cells (Figure 4A). This hyperpolarization was resistant to TTX (1 μM) (9 ± 2 mV, $n=4$, $P<0.01$) (Figure 4C), and apamin (1 μM) (10 ± 2 mV, $n=4$, $P<0.01$) (Figure 4E).

Perfusion of ATP (100 μM, $n=6$; $P<0.001$) caused a hyperpolarization of 12 ± 2 mV of the muscle cells (Figure 4B). In the presence of TTX (1 μM, $n=5$), the ATP-induced hyperpolarization was also observed (7 ± 2 mV; $P<0.05$) (Figure 4D). In the presence of apamin (1 μM), ATP (100 μM) did not change the resting membrane potential ($n=4$) (Figure 4F).

Effect of NO synthase inhibitors, suramin and apamin on the IJP and spontaneous contractions

NO synthase inhibitors, L-NOARG ($n=5$) and L-NAME ($n=6$) (1 mM), reduced the duration of the IJPs ($P<0.001$) but did not affect their amplitude (Figures 5A and B). In the presence of L-NOARG (1 mM, $n=4$) EFS still abolished the cyclic mechanical activity but a sustained tone was reached (Figure 5C).

The purinoceptor blocker suramin (100 μM, $n=6$; $P<0.001$) reduced the amplitude of IJPs at all stimulus

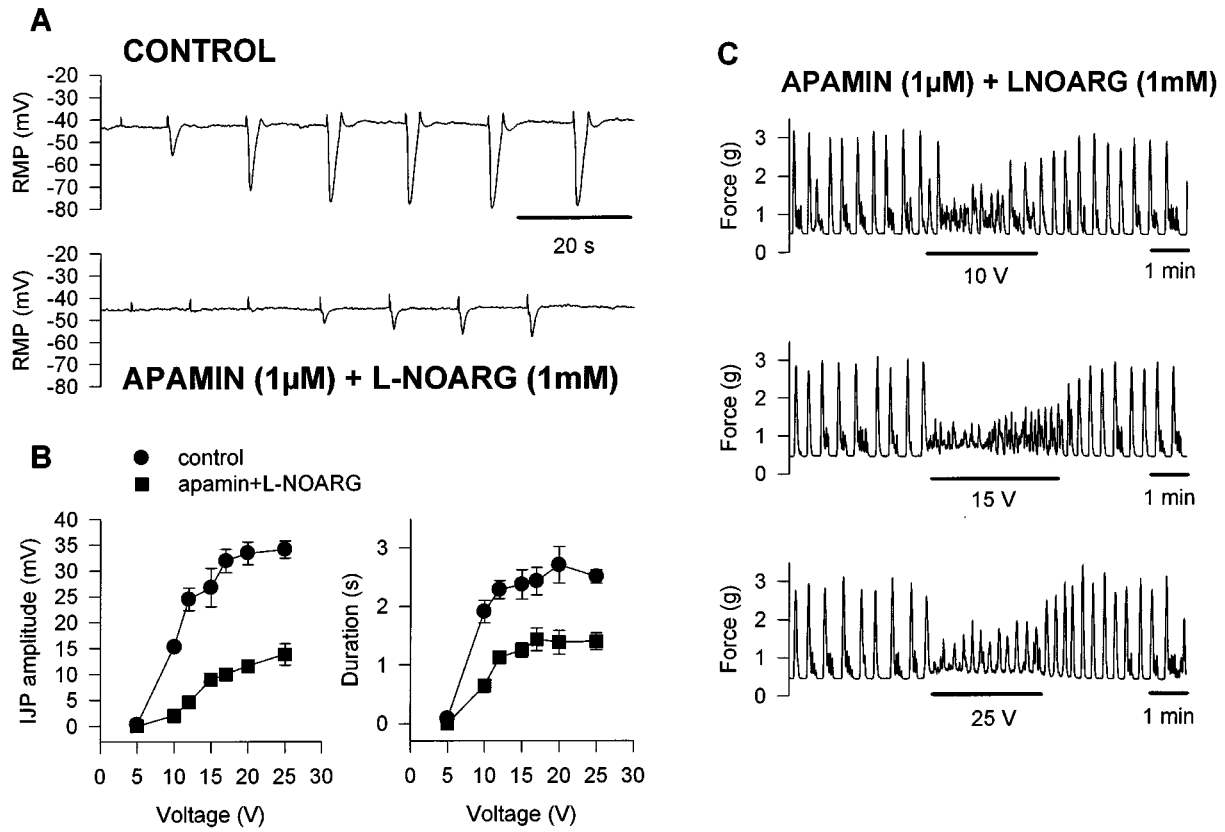

Figure 9 (A) Intracellular recordings showing the effect of apamin (1 μM) plus L-NOARG (1 mM) on the IJPs obtained by EFS (same procedure as in Figure 2). All recordings were obtained in the presence of nifedipine (1 μM). (B) Effect of apamin (1 μM) plus L-NOARG (1 mM) on the amplitude (left) and duration (right) of the IJP. (C) Mechanical recording, in the presence of apamin (1 μM) plus L-NOARG (1 mM), showing the response to EFS elicited during 3 min at 1 Hz (train duration 100 ms, frequency 20 Hz, pulse duration 0.3 ms) and increasing amplitude strengths (10, 15 and 25 V).

strengths. The duration of the IJPs was not affected (Figures 6A and B). In the presence of suramin (100 μM, $n=4$), repetitive EFS (1 Hz) still caused an abolition of the spontaneous mechanical activity (Figure 6C).

Apamin (1 μM, $n=12$) reduced the amplitude but not the duration of the IJPs ($P<0.001$; Figures 7A and B). However, in the presence of apamin (1μM, $n=4$) repetitive EFS (1 Hz) was still able to abolish the spontaneous mechanical activity at 15 and 25 V. (Figure 7C)

In the presence of L-NOARG (1 mM) plus suramin (100 μM) ($n=4$) the amplitude and duration of IJPs were reduced ($P<0.01$) (Figures 8A and B). However, a residual IJP was still recorded (Figure 8A). In the presence of both drugs, EFS was still able to decrease phasic contractions (Figure 8C).

In the presence of apamin (1 μM) plus L-NOARG (1 mM) ($n=4$), the amplitude and duration of IJPs were decreased ($P<0.01$) (Figures 9A and B) but a residual IJP could be recorded. In the presence of both drugs, EFS (1 Hz) was still able to reduce the spontaneous mechanical activity but phasic contractions were observed during EFS.

Discussion

The electrical activity pattern of rat colonic circular smooth muscle consists of cyclic episodes of slow depolarizations that trigger smooth muscle action potentials which induce a contraction. Accordingly, cyclic depolarizations are related to cyclic contractions of smooth muscle. In the mouse colon, neurogenic cyclic depolarizations have been reported and designated as 'myoelectric complexes' (Lyster *et al*., 1993; 1995). Smooth muscle action potentials are similar to those described in the human colon (Duthie & Kirk, 1978). In our study TTX increased the amplitude and frequency of cyclic contractions which could be taken as evidence against the concept that the cyclic activity is dependent on a neural input. These results suggest that the rat colonic circular muscle is neurally inhibited. NO and ATP are putative neurotransmitters involved in this tonic neural inhibition because NO synthase inhibitors and suramin increased the frequency and amplitude of contractions. Accordingly, our data suggest that although the cycle is neurally modulated, myogenic mechanisms may originate this cyclic activity. Our results do also suggest that in the rat colon, the cyclic electrical and mechanical activities are mainly due to calcium influx through L-type calcium channels because in the presence of nifedipine, slow depolarizations, action potentials and the corresponding mechanical activity are abolished.

Inhibitory junction potentials (IJPs) could be elicited during the whole cycle. Between contractions when the muscle was relaxed, IJPs caused hyperpolarization without any change in mechanical activity. When a contraction occurred, IJPs induced a relaxation. The fact that repetitive IJPs inhibited cyclic activity shows that inhibitory mediators could be released during the whole cycle. IJPs and relaxations elicited by EFS were sensitive to TTX when a low voltage was applied. However, at higher voltages a residual IJP and relaxation was still observed. TTX-resistant IJPs and relaxations might be due to a direct stimulation of nerve endings. This has also been described in the canine ileum (Cayabyab *et al*., 1996) and

(using the same set-up) in the porcine ileum (Borderies *et al*., 1997). In this last case, N-type calcium channels allow the calcium influx that triggers the release of inhibitory transmitters. However, in the rat colon, N-type calcium channels are not fully responsible for neurotransmitter release from nerve endings (Borderies *et al*., 1996) and further investigations are needed to characterize the voltage gated calcium channels involved.

IJPs did not change under NANC conditions, which shows that they are due to the release of NANC inhibitory mediator(s). IJPs recorded from the rat colon showed two phases: a sharp decrease of the membrane potential (fast component) followed by a sustained hyperpolarization (slow component). This biphasic IJP is similar to those described in other preparations such as mouse colon (Shuttleworth *et al*., 1997), mouse ileum (Ward *et al*., 1994), guinea-pig taenia caeci (Bridgewater *et al*., 1995), guinea-pig ileum (Crist *et al*., 1992), human colon (Keef *et al*., 1993) and human jejunum (Stark *et al*., 1993), whereas a monophasic IJP has been recorded from the pig (Borderies *et al*., 1997) and dog (Stark *et al*., 1993) ileum. The presence of two components in the IJP may suggest a cotransmission. The amplitude of the IJP was used to define the fast component and the duration as a feature of the second one.

We tested the ability of several putative inhibitory transmitters to hyperpolarize the smooth muscle cell and to decrease the mechanical activity. Exogenous addition of both ATP and the NO donor NaNP, hyperpolarized and relaxed the smooth muscle. Since the effects of NaNP were unchanged in the presence of TTX, NO may be considered as a putative final inhibitory NANC transmitter in this preparation. The inhibitory effect of ATP on the mechanical activity was partially decreased by TTX, suggesting that both muscle cells and neurons may have purinoceptors. In the presence of suramin, NaNP but not ATP abolished the mechanical activity. The same result was found when apamin was added to the bath. Moreover, apamin blocked the hyperpolarization induced by ATP but did not modify the hyperpolarization induced by NaNP. Altogether, these results show that both suramin and apamin might be pharmacological tools to discriminate between ATP and NO effects in this tissue. An interesting finding was that although NaNP was able to abolish cyclic contractions in the presence of L-NOARG, ATP did not abolish the cyclic mechanical activity when the preparation was incubated with a NO synthase inhibitor. This result is consistent with the fact that suramin and TTX only partially blocked the effects of ATP and suggests that ATP might also be releasing NO from neural and/or non neural sources.

When EFS was applied, L-NOARG or L-NAME reduced the slow component and suramin reduced the fast component of the IJP. These effects were selective for each phase suggesting that ATP might be involved in the sharp hyperpolarization and NO might be related to the sustained phase. Morphological studies have shown that ATP and NO colocalize in neurons of the myenteric plexus of the rat colon (Belai & Burnstock, 1994), providing structural support to a pressumptive cotransmission. A mechanism of cotransmission between NO and another neurotransmitter, perhaps ATP, has been proposed in the circular muscle of human (Boeckxstaens *et al*., 1993) and guinea-pig (Zagorodnyuk & Maggi 1994; Selemedis *et al*., 1997) colon.

In the presence of L-NOARG or suramin, a residual IJP could be recorded. This is consistent with results related to spontaneous mechanical activity where an inhibitory effect was still present when L-NOARG or suramin were perfused. However, the increase in tone recorded in the presence of L-NOARG might be explained in terms of reduction of the IJP duration. In this case EFS at 1 Hz is not able to keep the smooth muscle relaxed because the sustained hyperpolarization is clearly reduced (total duration of the IJP 1s). Apamin selectively reduced the amplitude of the IJP suggesting that the fast component, probably ATP mediated, involves small conductance calcium activated potassium channels. As we found in the case of suramin, a residual IJP (mainly the slow component) is recorded in the presence of apamin and this residual IJP might keep the smooth muscle relaxed when EFS was applied. Apamin-sensitive IJPs elicited in the rat colon have been reported (Suthamnatpong *et al*., 1994). As we found in our study the fast hyperpolarization is not NO-mediated (Suthamnatpong *et al*., 1994). In this study, the slow component of the IJP could not be recorded probably due to differences in EFS parameters or location of stimulating electrodes. In contrast to what we found in the rat, in the human colon apamin seems to affect both the fast and slow components of the IJP (Keef *et al*., 1993). In the canine small intestine, NO has been reported to be the only neurotransmitter responsible for the IJPs by acting partially through apamin-sensitive potassium channels (Christinck *et al*., 1991; Stark *et al*., 1993). On the other hand, ATP-mediated IJPs, are apamin-sensitive in the porcine intestine (Fernandez *et al*., 1998). These discrepancies might be explained in terms of different mechanism of action of inhibitory mediators in different species.

When both suramin and L-NOARG were perfused together into the bath, the amplitude and duration of the IJP were reduced, showing an additive effect. The same result was found when apamin plus L-NOARG were perfused. However, a residual IJP was still present in both situations. Two hypothesis might explain these results: (1) Concentrations of antagonists used in this study are not sufficient to completely abolish the effects of neural release of ATP and NO. (2) Other neurotransmitters might also be involved in the inhibitory response. In relation to this hypothesis, our results do not rule out the possibility that VIP and PACAP might act as putative inhibitory neurotransmitters too.

In conclusion, the present findings show that the electrical activity of the circular muscle of the rat colon displays cyclic episodes of depolarizations that trigger MPOs which in turn cause contractions. This cyclic activity is not of neural origin but is neurally modulated. Both electrical and mechanical activities are dependent on calcium influx through L-type calcium channels. EFS induces the release of inhibitory NANC neurotransmitters which hyperpolarize and relax the smooth muscle cell. In the rat colon the hyperpolarization shows two components: a fast hyperpolarization followed by a sustained one. Our results suggest that the neurotransmitter responsible for the first phase may be ATP, acting through purinoceptors sensitive to suramin and probably located on the muscle cell. The fast hyperpolarization is due to the activation of apamin-sensitive potassium channels. Finally, the release of NO may initiate the sustained hyperpolarization. The ionic basis of such sustained hyperpolarization does not involve apamin-sensitive potassium channels and awaits further investigation. In contrast to what has been found in other species (where IJPs are monophasic and due to NO release), the circular muscle of the rat colon provides a model of contransmission very similar to that found in the human colon.

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