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Postjunctional α_1- and β-adrenoceptor effects of noradrenaline on electrical slow waves and phasic contractions of cat colon circular muscle

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1 The postjunctional excitatory and inhibitory effects of noradrenaline and selective α_1- and β-adrenoceptor agonists on electrical and mechanical activity of cat colon muscle strips were studied by microelectrode recordings and isometric force measurements. Experiments were performed in the presence of tetrodotoxin (0.5 μM) or atropine (0.5 μM).

2 Circular muscle cells near the submucosal border had a mean resting membrane potential of -76.1 ± 1.2 mV and exhibited electrical slow waves at frequencies of 4-6 cycles min^{-1}. The mean values of electrical slow wave components were: upstroke potential, -40.7 ± 1.2 mV; plateau potential, -43.7 ± 0.8 mV; and duration, 4.9 ± 0.4 s. Electrical slow waves were in phase with rhythmic contractions of the circular muscle layer. Muscle cells near the myenteric border had a mean resting membrane potential of -51.1 ± 5.5 mV and did not exhibit electrical slow waves.

3 Noradrenaline (1 μM) increased the duration of electrical slow waves. This effect was inhibited by prazosin (1 μM) and potentiated by propranolol (5 μM), indicating activation of α_1- and β-adrenoceptors. Also, when α_1-adrenoceptors were irreversibly blocked by phenoxybenzamine (1 μM), noradrenaline decreased the duration of electrical slow waves. Phenylephrine (1 μM), a selective α_1-adrenoceptor agonist, and isoprenaline (1 μM), a β-adrenoceptor agonist, increased or decreased the duration of electrical slow waves, respectively.

4 Phenylephrine (0.01-5 μM) caused a linear increase in the area of electrical slow waves and phasic contractions but did not affect resting membrane potential or resting muscle tension. Higher concentrations of phenylephrine (5-50 μM) depolarized the resting membrane potential (2-6 mV) and increased muscle tone.

5 Nitrendipine or verapamil (each at 5 μM) reduced the amplitude of the upstroke potential and nearly abolished the plateau phase of the electrical slow waves. In the presence of L-type Ca^{2+} antagonists, noradrenaline (1-10 μM) or phenylephrine (1-100 μM) had no effect on electrical slow waves and phasic contractions. This indicates that the effects of noradrenaline and phenylephrine involve the influx of extracellular Ca^{2+} through voltage-dependent L-type Ca^{2+} channels.

6 Ryanodine, an alkaloid that depletes intracellular Ca^{2+} stores nearly abolished phasic contractions. In muscle strips, pretreated with ryanodine (10 μM for 30 min), phenylephrine (1 μM) increased and isoprenaline (1 μM) decreased the duration of electrical slow waves but neither was able to reverse the ryanodine-suppressed phasic contractions. This suggests that adrenoceptor effects on electrical slow waves are coupled to contractions via Ca^{2+} release from ryanodine-sensitive intracellular stores.

7 In summary, noradrenaline activates postjunctional α_1- and β-adrenoceptors. Activation of α_1-adrenoceptors increases the magnitude of electrical slow waves and phasic contractions, whereas activation of β-adrenoceptors decreases them. The α_1-adrenoceptor mediated effects on electrical slow waves and phasic contractions require the influx of Ca^{2+} through voltage-gated L-type Ca^{2+} channels. Phasic contractions also involve Ca^{2+} release from ryanodine-sensitive intracellular stores.

Keywords: Noradrenaline; α_1-adrenoceptors; β-adrenoceptors; nitrendipine; verapamil; ryanodine; electrical slow waves; contractions; cat colon

Introduction

In non-sphincteric regions of the colon, stimulation of lumbar sympathetic fibres reduces neurogenic contractions via prejunctional adrenoceptors (Gillespie & McKenna, 1961; de Groat & Krier, 1976; Gillespie & Khoyi, 1977). These effects are mediated by adrenergic fibres, which surround neurones in the myenteric plexus (Gabella, 1987). In addition, lumbar sympathetic nerves contract circular muscle of non-sphincteric regions of the colon via postjunctional excitatory α_1-adrenoceptors (Venkova & Krier, 1993). These effects could be mediated by adrenergic fibres located in colon circular muscle (Furness *et al.*, 1990). Also, excitatory α- and inhibitory β-adrenoceptors have been characterized in circular muscle of cat (Anuras & Christensen, 1981; Ek *et al.*, 1987; Venkova *et al.*, 1994), dog (Zang *et al.*, 1992; Smith *et al.*, 1993) and human colon (Gangon *et al.*, 1972). Therefore, sympathetic regulation of colonic motility by the release of noradrenaline could involve simultaneous interactions with prejunctional and different postjunctional adrenoceptor types (see review by Bylund, 1994).

Noradrenaline action on circular muscle via postjunctional excitatory α- and inhibitory β-adrenoceptors involves regulation of electrical activity. However, the excitatory effects of noradrenaline on the electrical activity of muscle cells at the submucosal border are not yet completely characterized (Smith & Sanders, 1985) nor are the mechanisms that couple noradrenaline effect on electrical activity to contractions. Cells near

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the submucosal border show rhythmic depolarizations and repolarizations of the resting membrane potential, termed electrical slow waves that are in phase with rhythmic contractions (for review see Sanders & Smith, 1989).

Our aim is to characterize the postjunctional effect(s) of noradrenaline on electrical slow waves and phasic contractions in colon circular muscle cells. Experiments will test the hypothesis that the effects of noradrenaline involve simultaneous interactions with postjunctional excitatory α_1- and inhibitory β-adrenoceptors. The role of extracellular Ca^{2+} in α_1-adrenoceptor effect was estimated using nitrendipine and verapamil to block L-type voltage-gated Ca^{2+} channels. A role of intracellular Ca^{2+} was estimated using ryanodine, an alkaloid that depletes intracellular Ca^{2+} stores by blocking intracellular calcium channels in their open state (Hwang & van Breemen, 1987). Ryanodine depletes the intracellular Ca^{2+} pool that is activated by the influx of extracellular calcium (Ca^{2+}-induced Ca^{2+} release mechanism) (Iino *et al.*, 1988). A preliminary account of some of the data has been presented in abstract form (Venkova & Krier, 1994).

Methods

Isolated muscle strips

Cats of either sex, weighing 2.5–4 kg, were anaesthetized with sodium pentobarbitone (50 mg kg^{-1} i.p.). A segment of mid colon (approximately 3 cm long) was excised at the level of the inferior mesenteric artery. The segment was cut along the mesenteric border and rinsed in Krebs solution. The tissue was pinned flat with the mucosal surfact facing the Sylgard bottom of the dissecting dish. Using a double-blade scalpel (1.2 mm fixed distance between the blades), two types of muscle strips were excised: strips (approximately 1.2 mm wide and 6 mm long) cut in the direction of the longitudinal muscle layer and strips (approximately 1.2 mm wide and 12 mm long) cut in the direction of the circular muscle layer. The muscle strips were transferred into a recording chamber, turned on their side and pinned to the Sylgard base using very fine pins and with the aid of a microscope. This exposed a cross-sectional view of the bowel wall, showing the longitudinal muscle, the circular muscle, the submucosa and the mucosa. Small regions, adjacent to the submucosal or to the myenteric border, were surrounded by pins to prevent, as much as possible, the movement of the muscle strip due to spontaneous contractions. The procedure of immobilizing the muscle without stretching it has been described for cross-sectional preparations isolated from the canine colon (Smith *et al.*, 1987). After dissection of the mucosal layer, the preparation consisted of the entire muscularis and an attached submucosal segment. The organ bath was continuously superfused with Krebs solution of the following composition (mM): NaCl 117, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11. The Krebs solution was gassed with 95% O_2–5% CO_2 (pH 7.2–7.4) and preheated to 36.5°C at the recording site. Muscle strips were equilibrated for 2 h before experiments. Adrenoceptor effects on electrical and contractile activity were recorded in the presence of tetrodotoxin (TTX, 0.5 μM) or atropine (0.5 μM).

Microelectrode recordings

Microelectrodes were filled with 3 M KCl. Tip resistances of the microelectrodes ranged between 30–60 MΩ. Membrane potential was measured by a high-impedance electrometer and displayed on an oscilloscope and a chart recorder. Electrical signals were recorded on magnetic tape. Microelectrodes were positioned to impale muscle cells near the submucosal or myenteric border. Microelectrode recordings were made from cells within 0.1–0.3 mm of the submucosal or myenteric edge of the immobilized regions of the muscle strip. Cells near the submucosal border showed spontaneous oscillations of the membrane potential, termed electrical slow waves. The rates of depolarization and repolarization were recorded simultaneously as the first derivative of voltage (dV/dT). The magnitude of an electrical slow wave was measured as the area (mm^2) under the slow wave. There were no significant differences between the resting membrane potential and the parameters of electrical slow waves (rate of depolarization, amplitude, duration and frequency) recorded in cat colon muscle strips cut parallel or perpendicular to the circular muscle layer. This allowed us to summarize data obtained in both kinds of strips.

Mechanical activity

When strips were cut parallel to the circular muscle layer, one end of the strip was tied with a silk thread to a force transducer. During equilibration the strips were repeatedly stretched in small increments (equal to a load of 50 mg) until the level of resting tension and the amplitude of spontaneous phasic contractions remained constant. Spontaneous or drug-evoked isometric contractions were displayed and recorded simultaneously with the electrical activity. The area (mm^2) of the phasic contraction was measured and expressed as the percentage of control. Changes in tone were measured as shifts in the level of resting muscle tension and expressed in mN.

Statistics

Resting membrane potential, parameters of electrical slow waves (rates of depolarization and repolarization, potentials of the upstroke and the plateau phase, duration and frequency), as well as the areas under electrical slow waves and phasic contractions were measured for time periods of 1 min before treatment and during the maximal effect for each of the drug concentrations tested. Data are mean ± s.e.mean. Significance of differences was assessed by Student's *t* test for paired or unpaired observations and *P* values of less than 0.05 were

Table 1 Electrical activity recorded in cat mid colon from circular muscle cells at the submucosal border

Parameters	*Control* ($n=33$)	*NA* ($n=9$)	*PE* ($n=13$)	*Iso* ($n=11$)
Resting membrane potential (mV)	−76.1 ± 1.2	−74.6 ± 2.6	−74.3 ± 2.0	−77.0 ± 1.1
Upstroke potential (mV)	−40.7 ± 1.2	−38.5 ± 2.3	−39.2 ± 1.9	−41.2 ± 1.8
Plateau potential (mV)	−43.7 ± 0.8	−40.9 ± 1.6	−42.4 ± 1.2	−46.6 ± 1.2
Rate of depolarization (Vs^{-1})	0.65 ± 0.03	0.71 ± 0.06	0.68 ± 0.05	0.61 ± 0.01
Rate of repolarization (Vs^{-1})	0.16 ± 0.01	0.13 ± 0.02	0.14 ± 0.01	0.19 ± 0.02
ESW duration (s)	4.9 ± 0.4	6.8 ± 0.9*	7.9 ± 0.6*	2.3 ± 0.4*
ESW frequency (cycles min^{-1})	4.2 ± 0.4	4.0 ± 0.4*	3.8 ± 0.3*	4.6 ± 0.2*

Values are means ± s.e. n = number of muscle strips, *$P<0.05$.
Resting membrane potential and parameters of electrical slow waves (ESW) in the absence and presence of noradrenaline (NA, 1 μM), phenylephrine (PE, 1 μM) or isoprenaline (Iso, 1 μM).

considered significant. Number of observations (n) refers to the number of muscle strips.

Drugs

Drugs used were: atropine sulphate, isoprenaline, nitrendipine, (−)-noradrenaline bitartrate, phenoxybenzamine, (−)-phenylephrine hydrochloride, prazosin hydrochloride, ryanodine (AgriSystems International, PA) and verapamil hydrochloride. Drugs were obtained from Sigma Chemical Co., unless specified. All drugs, except nitrendipine and ryanodine were dissolved in distilled water and diluted to final concentrations in Krebs solution. Nitrendipine and ryanodine were dissolved in dimethyl sulphoxide (DMSO), diluted in distilled water to a concentration of 1 mM and stored as stock solution at −20°C. The final concentration of DMSO in Krebs for the bath solution was less than 0.001% (v/v) and had no effect on electrical and mechanical activity. Drug concentrations presented refer to final bath values.

Results

Electrical slow waves and phasic contractions

The electrical and contractile activity of cat colon circular muscle was studied in the presence of tetrodotoxin (0.5 μM) or atropine (0.5 μM). Muscle cells near the submucosal border had a mean resting membrane potential of -76.1 ± 1.2 mV ($n = 33$). They exhibited electrical slow waves at a frequency of 4–6 cycles min^{-1} that were in phase with rhythmic changes in the isometric force of the circular muscle, termed phasic contractions. Each electrical slow wave consisted of an upstroke depolarization, a sustained plateau that occurred for several seconds and a repolarization. The mean values of resting membrane potential and the components of electrical slow waves are shown in Table 1 (control values). These values are similar to those in dog colon circular muscle submucosal region (Smith *et al.*, 1987; Barajas-Lopeza & Huizinga, 1989).

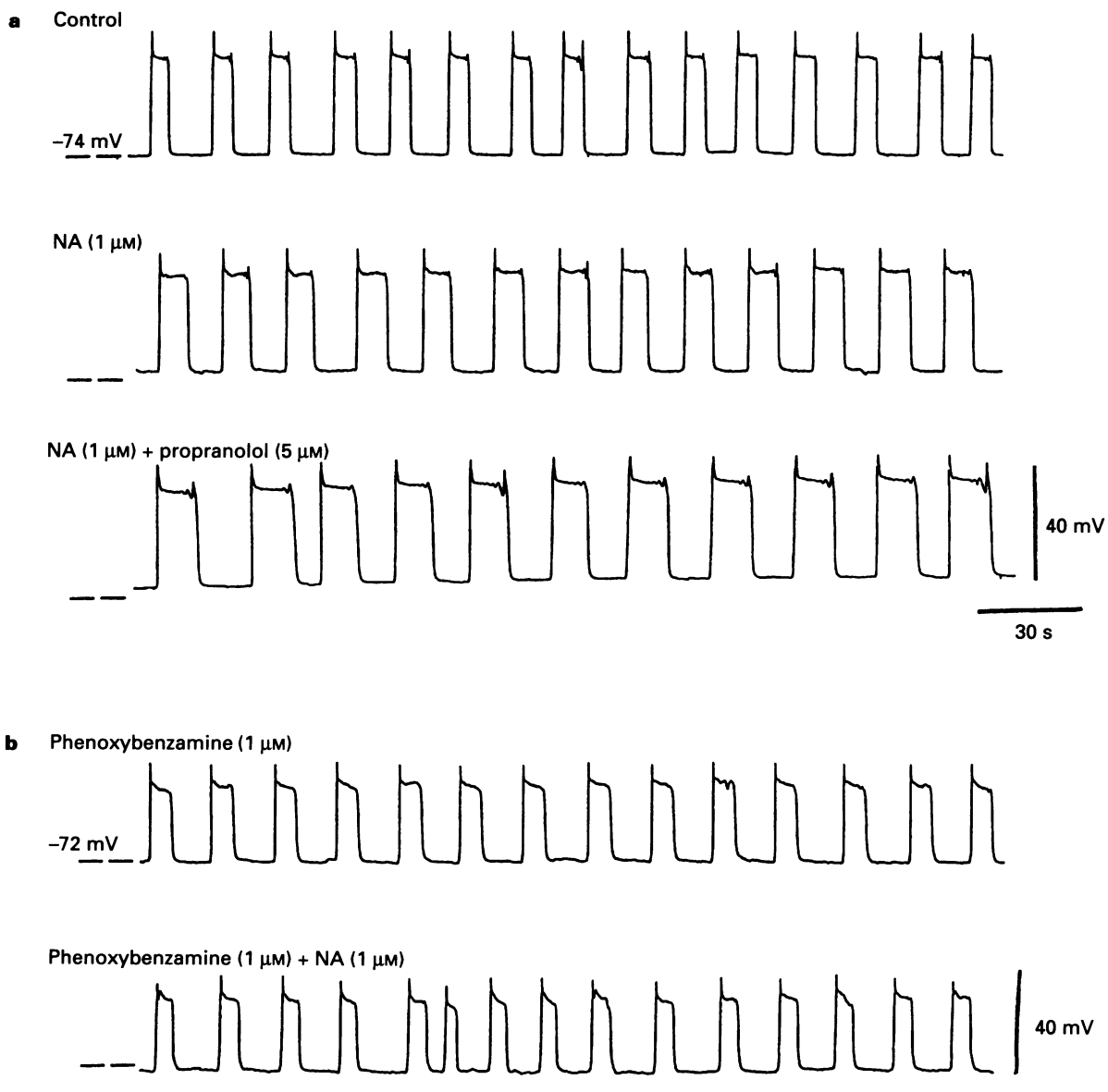

Figure 1 Electrical slow waves of cat colon circular muscle in the presence of atropine (0.5 μM). (a) Slow waves before treatment (upper trace), in the presence of noradrenaline (NA, 1 μM for 3 min) (middle trace) and after superfusion of propranolol (5 μM for 5 min) in the presence of noradrenaline (lower trace). Traces are sections of continuous recording from same cell near the submucosal border. Resting membrane potential (−74 mV) is indicated by dotted lines. (b) Electrical slow waves in the presence of phenoxybenzamine (1 μM for 25 min) (upper trace) and after superfusion of noradrenaline (NA, 1 μM for 3 min) in the presence of phenoxybenzamine (lower trace). Traces are sections of continuous recording from same cell near the submucosal border. Resting membrane potential (−72 mV) is indicated by dotted lines.

Effects of noradrenaline

Superfusion of muscle strips with noradrenaline (1 μM for 5 min) did not significantly alter the resting membrane potential of muscle cells near the submucosal border, but significantly increased the duration of electrical slow waves and decreased their frequency (Table 1 and Figure 1a). In addition noradrenaline slightly increased the amplitude and the rate of depolarization of the upstroke and depolarization of the plateau potential. The increase in the magnitude of electrical slow waves, was coupled to an increase in the magnitude of phasic contractions. The effect reached maximum within the first 2–3 min of noradrenaline superfusion and remained stable until noradrenaline was washed out. Pretreatment of the muscle strips ($n=3$) with prazosin (1 μM for 10 min) abolished the effects of noradrenaline on electrical slow waves and phasic contractions. In contrast, propranolol (1 μM) significantly potentiated the effects of noradrenaline on electrical slow waves by 32±8% ($P<0.05$, paired observations in 4 cells) (Figure 1a). The effect of noradrenaline on phasic contractions was potentiated by 42.9±9% in the presence of propranolol. Superfusion of propranolol (5 μM for 5 min) alone showed no effect on either electrical or mechanical activity.

The effects of noradrenaline were also tested in muscle strips ($n=3$), pretreated with phenoxybenzamine (1 μM for 25 min), which acts as an irreversible antagonist of α_1-adrenoceptors. In these strips noradrenaline significantly ($P<0.05$, paired observations in 5 cells) reduced the magnitude of electrical slow waves by 29±9% (Figure 1b) and inhibited phasic contractions by 52±11%. The inhibitory effect of noradrenaline on both electrical slow waves and contractions was abolished by propranolol (1 μM).

Effects of isoprenaline

Activation of β-adrenoceptors inhibits electrical slow waves and phasic contractions in dog colon circular muscle (Smith *et al.*, 1993; Du *et al.*, 1993). In cat colon circular muscle, isoprenaline (1 μM for 5 min) significantly decreased the duration of electrical slow waves and increased their frequency without altering the resting membrane potential of muscle cells near the submucosal border (Table 1). In addition to the decrease in duration, isoprenaline reduced the amplitude and the rate of depolarization of the upstroke and slightly hyperpolarized the plateau potential of electrical slow waves. The reduction of the magnitude of electrical slow waves was coupled to a decrease in the magnitude of phasic contractions. The effect of isoprenaline was maximum within 2–3 min and remained constant in the presence of isoprenaline. A period of 20–30 min was required for isoprenaline to be washed out and for control activity to be restored.

Superfusion of the muscle strips ($n=9$) with increasing concentrations of isoprenaline (0.01 μM–5 μM) caused a concentration-dependent decrease in magnitude of electrical slow waves and phasic contractions. Higher concentrations of isoprenaline (>1 μM) hyperpolarized the resting membrane potential (1–5 mV) and decreased resting tension (Figure 2a).

Effects of phenylephrine

Phenylephrine (1 μM), a selective α_1-adrenoceptor agonist, showed no significant effect on resting membrane potential of muscle cells near the submucosal border, but significantly increased the duration of electrical slow waves and reduced their frequency (Table 1). Similar to noradrenaline, phenylephrine also increased the amplitude and rate of depolarization of the upstroke and depolarized the plateau phase of electrical slow waves in some cells.

Superfusion of muscle strips with increasing concentrations of phenylephrine caused a concentration-dependent increase in the magnitude of electrical slow waves and phasic contractions (Figure 2b). Phenylephrine (0.01–5 μM) increased the area of electrical slow waves (Figure 3a) and phasic contractions (Figure 3b), but did not effect resting membrane potential or resting tension. Within this concentration range, there was a linear relation (correlation coefficient is 0.98) between the effects of phenylephrine on electrical slow waves and phasic contractions (Figure 4). Higher concentrations of phenylephrine (5–50 μM) depolarized the resting membrane potential and increased muscle tone (Figure 3a,b).

Effects of Ca^{2+} channel antagonists on responses to noradrenaline, phenylephrine and isoprenaline

The organic Ca^{2+} channel antagonists, nitrendipine or verapamil (each at 5 μM for 15 min), did not change resting membrane potential but decreased the amplitude of the up-

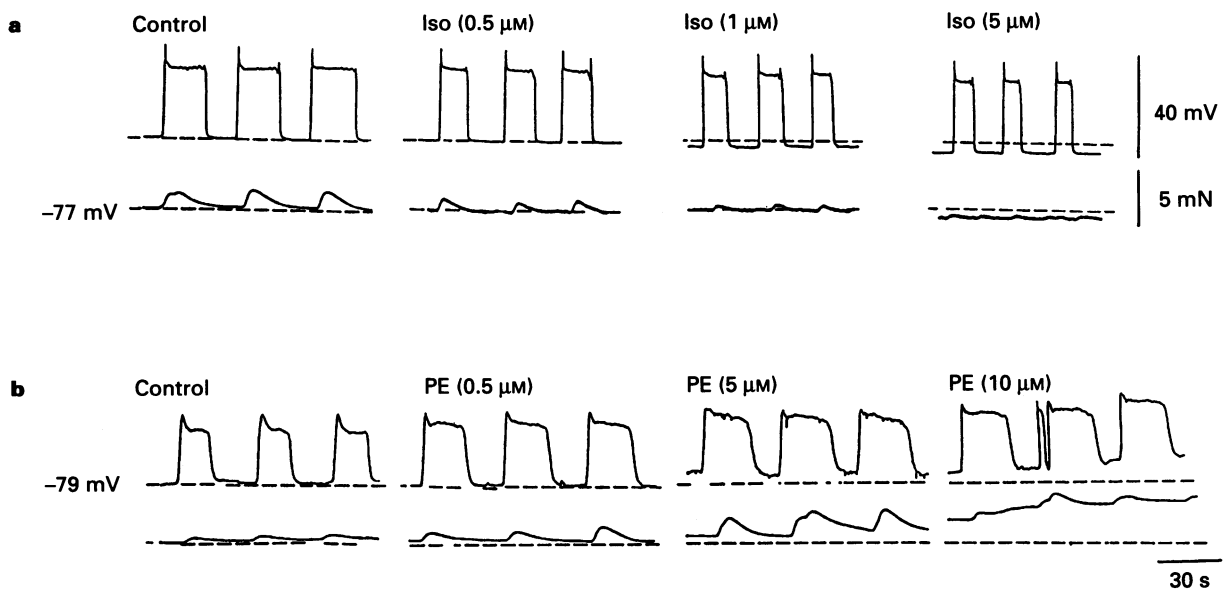

Figure 2 Electrical (upper traces) and mechanical (lower traces) activity of cat colon circular muscle in the presence of atropine (0.5 μM) in (a) and (b). (a) Cumulative effects of isoprenaline (Iso) superfused for 3 min at concentrations increasing from 0.01 μM to 5 μM. (b) Cumulative effects of phenylephrine (PE), superfused for 3 min at concentrations increasing from 0.05 μM to 10 μM. In (a) and (b) traces are a continuous recording from the same cell near the submucosal border. Resting membrane potential (−79 mV) and level of resting tension is indicated by the dashed lines.

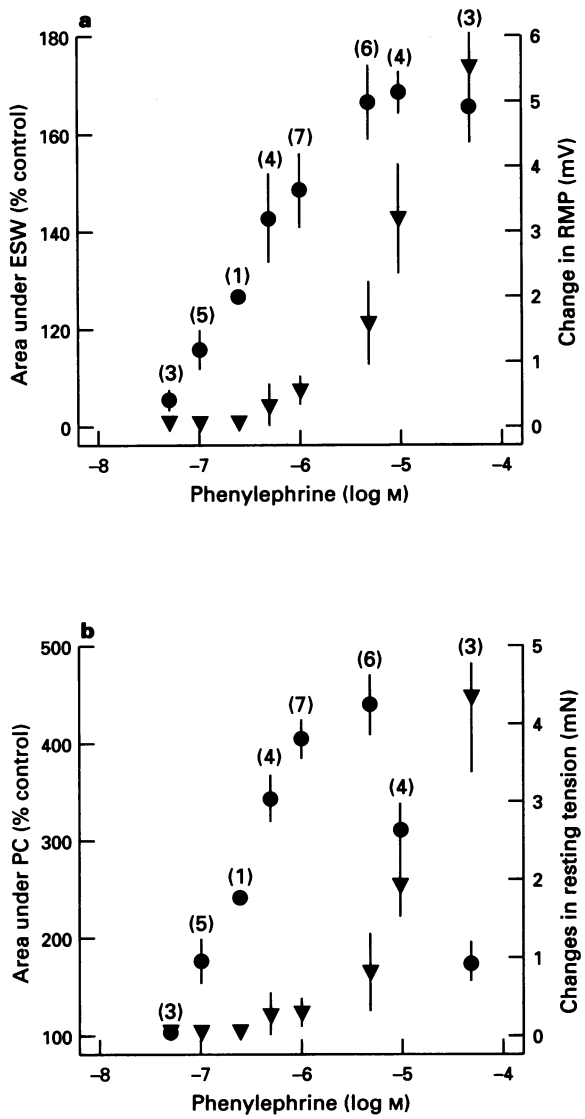

Figure 3 Effects of phenylephrine on the electrical (a) and mechanical (b) activity of cat colon circular muscle in the presence of atropine (0.5 μM). (a) Electrical slow waves (●), and changes in resting membrane potential (▼), plotted against phenylephrine concentration. Left ordinate scale, area under electrical slow waves (ESW) expressed as % of control. Right ordinate scale, changes in resting membrane potential (RMP) measured in mV. Numbers above symbols indicate the number of muscle cells tested at each concentration of phenylephrine. (b) Phasic contractions (●) and changes in resting tension (▼) plotted against phenylephrine concentration. Left ordinate scale, area under phasic contractions (PC) expressed as % of control. Right ordinate scale, changes in resting tension measured in mN. Numbers above symbols indicate number of muscle strips tested at each concentration of phenylephrine. Muscle strips were isolated from 8 experimental animals.

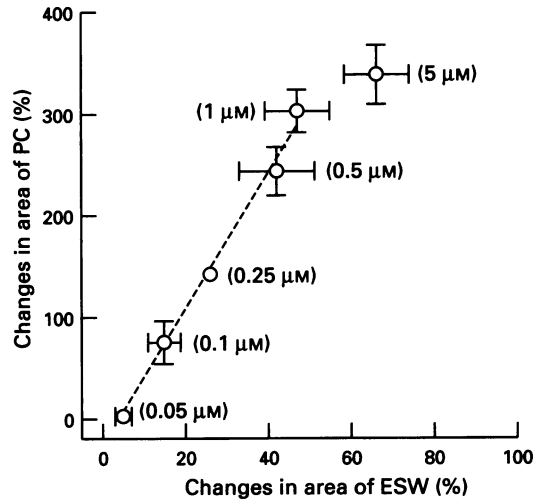

Figure 4 Relationship between phenylephrine-mediated changes in the area of phasic contractions (PC) and electrical slow waves (ESW), expressed as % of controls before phenylephrine. Concentrations of phenylephrine are shown in parentheses. Regression line illustrates linear relation ($r=0.98$) between changes in electrical slow waves and phasic contractions. Vertical and horizontal bars are s.e.mean for changes in area of phasic contractions and electrical slow waves, respectively. Data are means from muscle strips isolated from 8 experimental animals.

stroke and abolished the plateau phase of electrical slow waves. Phasic contractions were also inhibited (Figure 5 a,c). In muscle strips ($n=3$) pretreated with nitrendipine, noradrenaline (1–10 μM) had no effect on resting membrane potential, and on the nitrendipine-resistant components of electrical slow waves and phasic contractions (Figure 5 b,d).

The effects of phenylephrine (0.5–100 μM) on electrical slow waves and phasic contractions were also antagonized by nitrendipine. Figure 6 shows the effects of increasing concentrations of phenylephrine on the magnitude of electrical slow waves and phasic contractions in the absence and presence of nitrendipine (5 μM). A similar antagonism occurred in muscle strips ($n=4$) pretreated with verapamil (5 μM for 15 min).

In the presence of nitrendipine ($n=2$ muscle strips) or verapamil ($n=2$ muscle strips), isoprenaline (0.1–50 μM) did not cause a further reduction of the nitrendipine-resistant components of the electrical slow waves and phasic contractions and did not change resting membrane potential.

Effects of ryanodine on responses to noradranaline, phenylephrine and isoprenaline

The effects of adrenoceptor agonists were studied when intracellular calcium stores were depleted by ryanodine (Hwang & van Breemen, 1987). Ryanodine (10 μM for 30 min) did not abolish electrical slow waves but reduced phasic contractions by 80–95%. When muscle strips were pretreated with ryanodine, phenylephrine (0.1–10 μM) increased the magnitude of electrical slow waves but had no effect on contractile activity. The summarized results in Figure 7 show that in the presence of ryanodine the effect of phenylephrine on electrical slow waves is uncoupled from the contractile effect. This indicates the involvement of ryanodine-sensitive intracellular Ca^{2+} stores in the coupling between electrical slow waves and phasic contractions during α_1-adrenoceptor activation.

In muscle strips ($n=3$) pretreated with ryanodine noradrenaline increased while isoprenaline reduced the magnitude of electrical slow waves. However, neither noradrenaline nor isoprenaline had any apparent effect on the magnitude of the ryanodine-resistant components of contractile activity.

Effects of noradrenaline, phenylephrine and isoprenaline in circular muscle cells near the myenteric border

Experiments were carried out to study the effects of adrenoceptor activation on the electrical activity of muscle cells in the myenteric border region. Intracellular recordings were made from cells in close proximity (0.1–0.2 mm) to the myenteric border. Circular muscle cells in the myenteric border region have a resting membrane potential of -51.1 ± 5.5 mV ($n=22$ cells) and exhibit myenteric potential oscillations (amplitude 2–11 mV; frequency 18–32 cycles min^{-1}). Values of resting membrane potential and the amplitude and frequency of myenteric potential oscillations are similar to those obtained in dog colon circular muscle myenteric region (Smith *et al.*, 1987; Barajas-Lopez & Huizinga, 1989).

Noradrenaline (1 μM) and phenylephrine (1 mM) depolarized the resting membrane potential by 16.8 ± 5.2 mV ($n=4$) and by 14.7 ± 4.1 mV ($n=5$), respectively. In the majority of

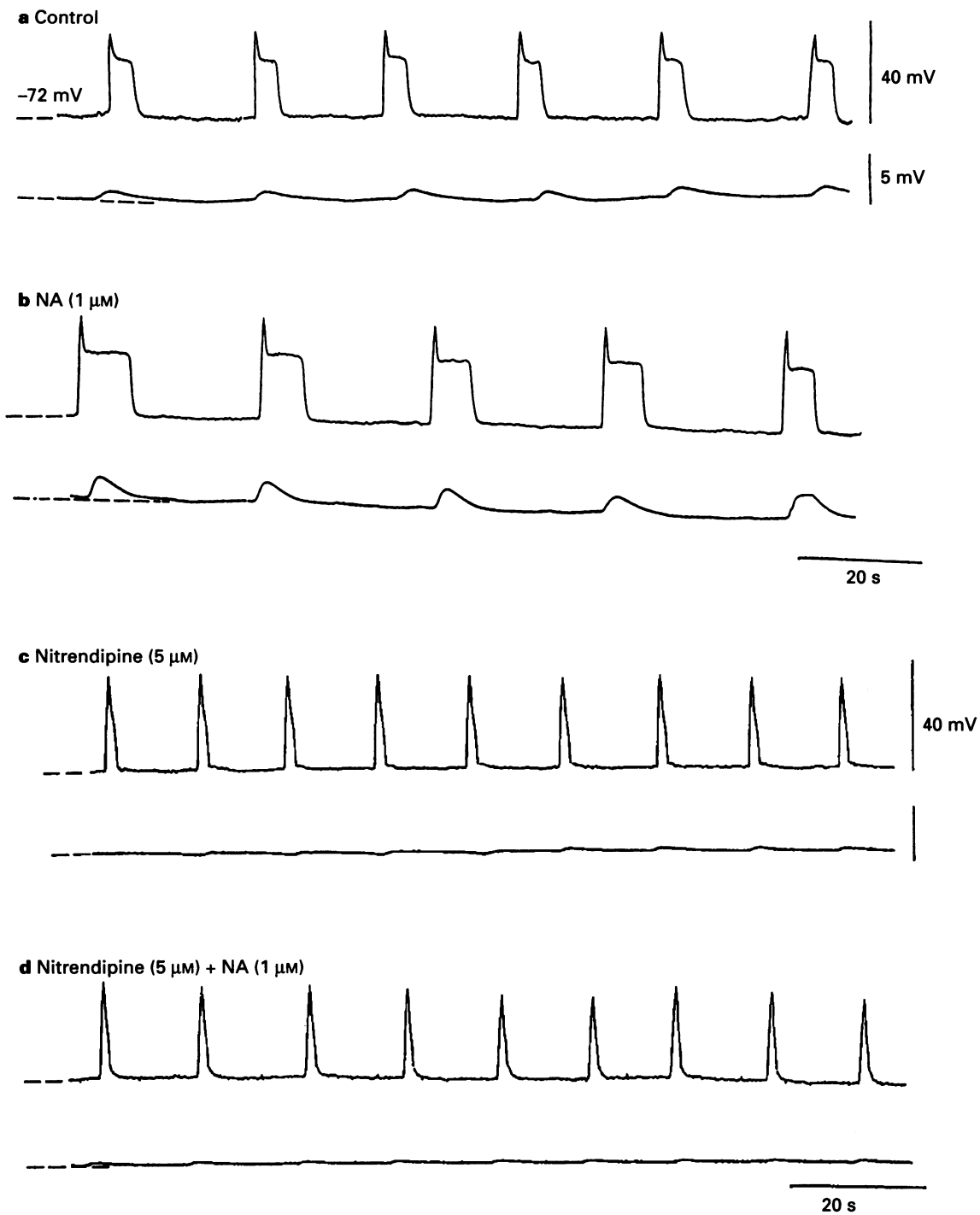

Figure 5 Electrical (upper traces) and mechanical (lower traces) activity of cat colon circular muscle in the presence of atropine (0.5 μM). (a) Control activity. (b) Effects of noradrenaline (NA, 1 μM for 3 min). (c) Effects of nitrendipine (5 μM for 15 min). (d) Effects of noradrenaline (NA, 1 μM for 3 min) superfused in the presence of nitrendipine. (a–d) Traces are sections of continuous recording from same cell near the submucosal border. Resting membrane potential (−72 mV) is indicated by dotted lines. Dotted lines at lower traces indicate level of resting tension.

muscle strips there was no significant effect on the membrane potential oscillation (Figure 8a). Only in two of the strips noradrenaline and phenylephrine slightly increased the amplitude of myenteric potential oscillations and evoked an irregular appearance of spike-like activity (amplitude 12–16 mV). The effects of noradrenaline and phenylephrine were inhibited by prazosin (1–5 μM). In contrast, isoprenaline (1 μM) hyperpolarized the resting membrane potential by 8.7 ± 3.3 mV ($n=3$) (Figure 8b) and the effect was antagonized by propranolol (1 μM).

In order to define the role of α_1-adrenoceptor-evoked potentiation of electrical slow waves at the submucosal border and depolarization of the membrane potential at the myenteric border for the development of the contractile response, experiments were performed on muscle strips in which the submucosal region (about 25% of the thickness of the circular layer) was removed by dissection. These muscle strips, comprising about 75% of the circular muscle layer, lack rhythmic spontaneous contractile activity, but respond with high-amplitude contractions to acetylcholine (0.1–1 μM). Microelec-

trode recordings from muscle cells in the bulk of the circular layer (about 50% of the distance between the myenteric and submucosal border) showed a pattern of low-amplitude membrane potential oscillations and lacked a slow-wave component, which was characteristic for the bulk of the circular muscle in intact strips. In muscle strips with the submucosal region removed ($n=3$), noradrenaline (1 μM) and phenylephrine (1 μM) depolarized the membrane potential and caused a sustained contraction (Figure 9). The effects were blocked by prazosin (1 μM).

Discussion

Our data show that noradrenaline simultaneously activates postjunctional excitatory α_1- and inhibitory β-adrenoceptors in cat colon circular muscle. At the myenteric border, noradrenaline and phenylephrine depolarized the resting membrane potential via α_1-adrenoceptors and isoprenaline hyperpolarized the resting membrane potential via β-adrenoceptors. At the submucosal border, α_1-adrenoceptor activation caused an increase in the magnitude of electrical slow waves, due to sig-

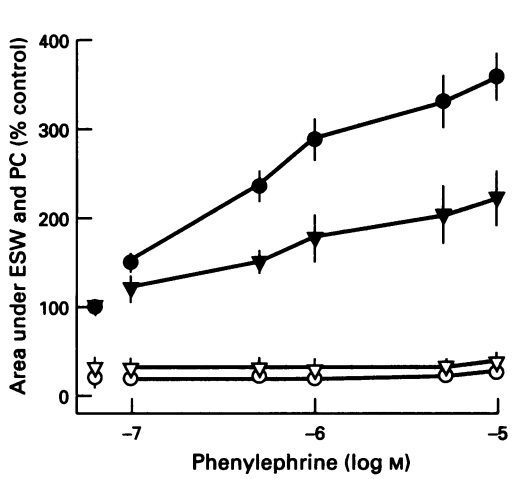

Figure 6 Effects of nitrendipine (5 μM for 15 min) on the concentration-response curves for phenylephrine (0.1–10 μM) in cat colon circular muscle in the presence of atropine (0.5 μM). Magnitude of electrical slow waves (▼, ▽) and phasic contractions (●, ○) are expressed as % of controls, measured in the absence of nitrendipine. Filled and open symbols represent data in the absence and presence of nitrendipine, respectively. Symbols, left to the abscissa scale, represent the magnitudes of electrical slow waves and phasic contractions before application of phenylephrine. Ordinate scale, area under electrical slow waves (ESW) and phasic contractions (PC) expressed as % of control. Data are means±s.e.mean from muscle strips, isolated from 3 experimental animals.

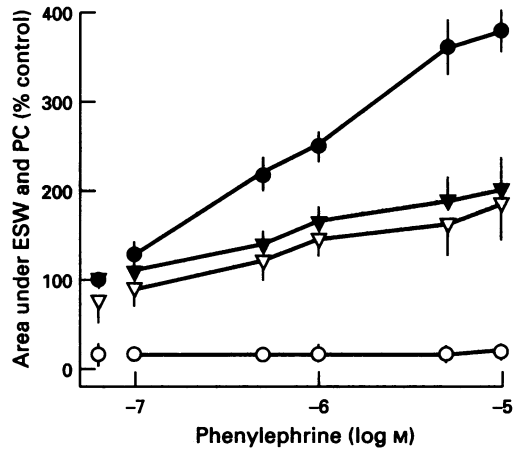

Figure 7 Effects of ryanodine (10 μM for 30 min) on the concentration-response curves for phenylephrine (0.1–10 μM) in cat colon circular muscle in the presence of atropine (0.5 μM). Magnitude of electrical slow waves (▼, ▽) and phasic contractions (●, ○) are expressed as % of controls, measured in the absence of ryanodine. Filled and open symbols represent data in the absence and presence of nitrendipine, respectively. Symbols, left to the abscissa scale, represent the magnitude of electrical slow waves and phasic contractions before application of phenylephrine. Ordinate scale, area under electrical slow waves (ESW) and phasic contractions (PC) expressed as % of control. Data are means±s.e.mean from muscle strips, isolated from 6 experimental animals.

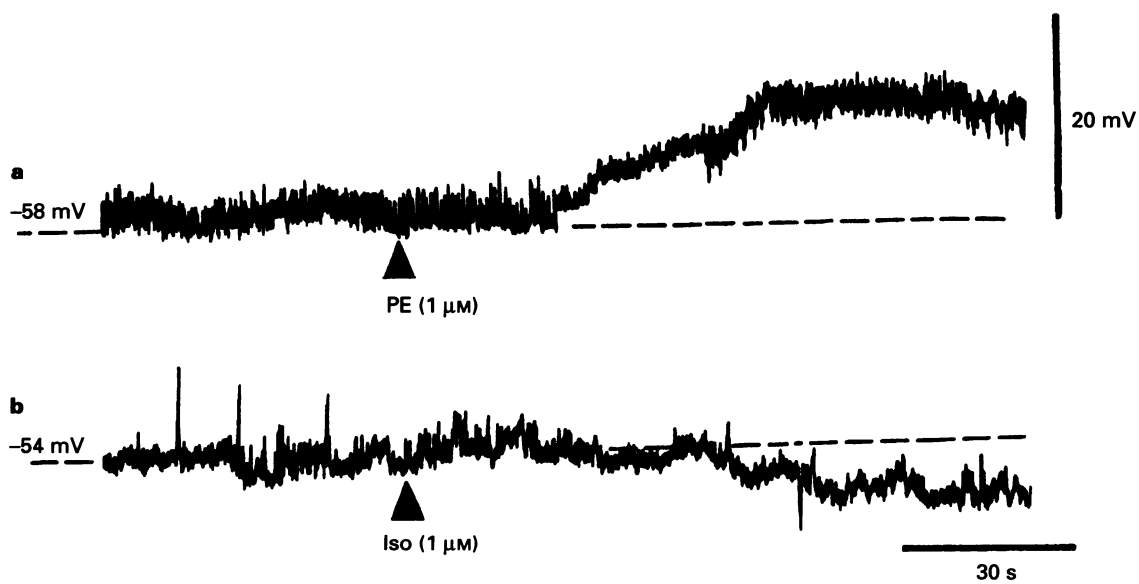

Figure 8 Effects of phenylephrine (PE, 1 μM) and isoprenaline (Iso, 1 μM) on electrical activity recorded from cells in the myenteric border region of cat colon circular muscle in the presence of atropine (0.5 μM). Resting membrane potential is depolarized compared to cells in the submucosal region. The membrane activity of cells at the myenteric border lacks electrical slow waves and shows low-amplitude myenteric potential oscilations. (a) Membrane activity before treatment and in the presence of phenylephrine (PE, 1 μM). Resting membrane potential (−58 mV) is indicated by dotted lines. (b) Membrane activity before treatment and in the presence of isoprenaline (Iso, 1 μM). Resting membrane potential (−54 mV) is indicated by dotted lines. (a,b) Traces are sections of continuous recordings from two different cells near the myenteric border.

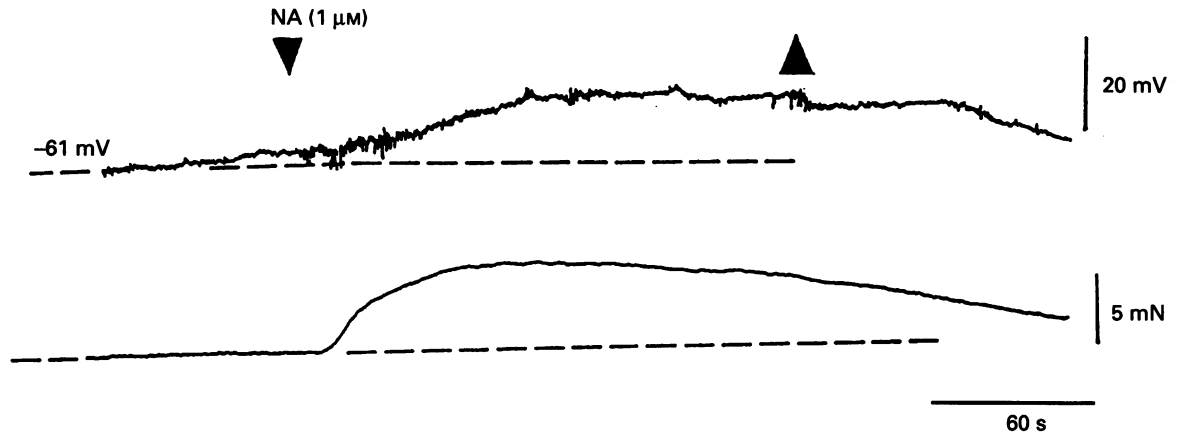

Figure 9 Effect of noradrenaline (NA, 1 μM) on the electrical activity recorded from a cell in the bulk of the circular layer (upper trace) and on the mechanical activity of cat colon circular muscle (lower trace) in the presence of atropine (0.5 μM). The muscle strip is devoid of submucosal pacemaker region. The membrane activity in the presence and absence of noradrenaline lacks electrical slow wave components. Membrane potential is -61 mV, as indicated by dotted line. Dotted line at lower trace indicates the level of resting tension.

nificant increase in the duration of the plateau and to slight depolarization of the upstroke and plateau potentials. A secondary effect of α_1-adrenoceptor activation was depolarization of the resting membrane potential. This occurred when the concentration of phenylephrine exceeded 5 μM. In contrast to α_1-adrenoceptor activation, the predominant effect of β-adrenoceptor activation was a decrease in the magnitude of electrical slow waves, due primarily to reduction of the plateau phase. Similar effects of isoprenaline have been obtained in dog colon (Smith *et al.*, 1993).

The effects of noradrenaline on ion currents underlying electrical slow waves were not studied. However, in cat isolated colon myocytes action potentials involve membrane currents through voltage-gated L-type Ca^{2+} channels (Bielefeld & Krier, 1991). The adrenoceptor effects on electrical slow wave duration (primarily plateau phase) are due to the influx of Ca^{2+} through L-type voltage-gated Ca^{2+} channels, since the effects of noradrenaline and the selective α_1- and β-adrenoceptor agonists on electrical slow waves were abolished by nitrendipine or verapamil. It is possible that the action of adrenoceptor agonists on the plateau phase is due to direct effects on voltage-gated inward L-type Ca^{2+} currents, and/or to the effects on voltage- and Ca^{2+}-dependent outward K^+ currents (Cole & Sanders, 1989; Carl & Sanders, 1989; Carl *et al.*, 1990). For example, α_1-adrenoceptor-mediated increase in the magnitude of electrical slow waves may occur by increasing the inward voltage-dependent L-type Ca^{2+} current and/or inhibiting a voltage- or Ca^{2+}-dependent outward K^+ current in cat colon myocytes (Bielefeld *et al.*, 1990). In dog colon myocytes muscarinic cholinoceptor agonists increase the magnitude of electrical slow waves and phasic contractions by suppressing the Ca^{2+} activated outward K^+ current (Cole *et al.*, 1989). In contrast, β-adrenoceptor-mediated decrease in electrical slow wave duration may occur by decreasing the voltage-dependent L-type Ca^{2+} current and/or by inhibiting a voltage- or Ca^{2+}-dependent K^+ current. In dog colon myocytes activation of adenylate cyclase by forskolin or adenosine 3′:5′-cyclic monophosphate (cyclic AMP) decreases the plateau phase of electrical slow waves. It has been suggested that the inhibitory effect of cyclic AMP is due to an increase of the open probability of Ca^{2+} activated K^+ channels (Smith *et al.*, 1993; Du *et al.*, 1993).

In cat colon circular muscle, electrical slow waves recorded at the submucosal border are coupled to phasic contractions (Christensen *et al.*, 1969). Our data show a linear relationship between the changes in magnitude of electrical slow waves and phasic contractions evoked by α_1-adrenoceptor activation. However, the linear relation between electrical slow waves and phasic contractions was limited to a certain concentration range of the α_1-agonist (0.01 – 5 μM phenylephrine). Other agonists show a similar relationship between electrical slow waves and phasic contractions of circular muscle in other regions of the gastrointestinal tract (Szurszewski, 1987). In addition, higher concentrations of phenylephrine depolarized the resting membrane potential and caused tonic contractions. This also occurred in the absence of the submucosal pacemaker region and in muscle cells near the myenteric border, suggesting that noradrenaline-evoked tonic contractions are independent of electrical slow wave mechanisms. Thus, the α_1-adrenoceptor-mediated effect involves an increase in the magnitude of electrical slow waves that is coupled to an increase in magnitude of phasic contractions and a depolarization of resting membrane potential, associated with an increase in muscle tone. In dog colon circular muscle cholinoceptor agonists cause similar changes in phasic contractions and muscle tone. These changes may be due to effects on electrical slow waves in the submucosal region and on resting membrane potential in the myenteric region of the circular muscle layer (Barajas-Lopez & Huizinga, 1989; Keef *et al.*, 1992).

Data from the present study suggest that the increase in phasic contractions that is mediated by α_1-adrenoceptors requires extracellular Ca^{2+} entry through dihydropyridine-sensitive Ca^{2+} channels, that induces the release of Ca^{2+} from a ryanodine-sensitive intracellular Ca^{2+} pool, since contractions were abolished by either organic Ca^{2+} channel antagonists or by ryanodine. Similar dihydropyridine- and ryanodine-sensitive mechanisms are involved in α_1-adrenoceptor-mediated contractions of rat small mesenteric arteries (Gustafsson & Nilsson, 1993) and guinea-pig vas deferens (Drescher *et al.*, 1993).

In summary, the present results indicate that the postjunctional effect of noradrenaline on electrical slow waves and phasic contractions represents a balance between α_1-adrenoceptor and β-adrenoceptor-mediated components that can be of physiological significance. Based upon previous studies (Venkova & Krier, 1993; Venkova *et al.*, 1994) we can suggest that the prevalence of an α_1- or a β-adrenoceptor-mediated component during the postjunctional action of noradrenaline depends upon the background activity of the circular muscle. For instance, in muscle strips pretreated with tetrodotoxin or atropine, noradenaline or stimulation of the lumbar colonic nerve caused a contractile response mediated by α_1-adrenoceptors. When muscle strips were precontracted with carbachol the same stimuli caused relaxing responses, mediated by β-adrenoceptors. In addition, the responses to lumbar sympathetic nerve stimulation were regulated by prejunctional α_2-adrenoceptors.

Altogether, our data provide the concept that adrenocep-

tors located on both sites of the neuromuscular junction are involved in sympathetic nerve regulation of colonic motility. Noradrenaline, released from sympathetic nerve terminals regulates muscle contractions by prejunctional modulation of neurogenic activity (see Vizi *et al.*, 1991) and by direct interaction with α_1- and β-adrenoceptors on circular muscle cells.

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