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Adrenoceptors and regulation of intestinal tone in the isolated colon of the mouse

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1 Adrenaline, noradrenaline, phenylephrine, dopamine, clonidine and apomorphine at low concentrations (from 10^{-9} M to 10^{-6} M) contracted the longitudinal muscle of the isolated distal colon of the mouse. Phentolamine, tetrodotoxin and indomethacin antagonized these contractile responses. Yohimbine antagonized them at lower concentrations than prazosin. Dopamine and clonidine had the same contractile activity on preparations from mice pretreated with 6-hydroxydopamine (6-OHDA). Isoprenaline (10^{-9} to 3×10^{-7} M) induced relaxations of the colon which were antagonized by propranolol.

2 At higher concentrations, adrenaline and noradrenaline (from 3×10^{-7} M), dopamine (from 3×10^{-5} M), phenylephrine (from 3×10^{-6} M) and apomorphine (from 10^{-4} M) relaxed the colon. Clonidine (10^{-6} to 3×10^{-5} M) inhibited the spontaneous activity of the colon but never induced relaxations. At 10^{-4} and 10^{-3} M clonidine elicited contractions. Prazosin antagonized the inhibitory effect of phenylephrine and clonidine, a mixture of propranolol and prazosin antagonized the relaxations to adrenaline, noradrenaline and dopamine and unmasked contractions that were sensitive to yohimbine and tetrodotoxin. The relaxations induced by apomorphine and the contractions induced by clonidine ($> 10^{-6}$ M) were resistant to all these antagonists.

3 Electrical field stimulation (1 ms, 2 Hz, 2–20 V) of the mouse colon induced contractile responses which increased with the frequency of the stimulus. After cessation of stimulation at 4 Hz a rebound contraction was generally observed, followed by a progressive decline in tone. In the presence of atropine, the contractile response to field stimulation was abolished and transformed into a rapid and sustained relaxation. A rebound contraction was always observed after cessation of stimulation. The responses to electrical stimulation (in the presence or absence of atropine) were abolished by tetrodotoxin. The rebound contractions were abolished by indomethacin. The relaxations induced in the presence of atropine were not modified by phentolamine, propranolol, guanethidine, methysergide, mepyramine, cimetidine or naloxone.

4 Tetrodotoxin (from 3×10^{-8} M) caused a sustained contraction of the colon with increased spontaneous activity. This contraction was not modified by atropine, phentolamine, propranolol, guanethidine, methysergide, mepyramine, cimetidine, naloxone, but was abolished by preincubation of the preparation with indomethacin.

5 These results indicate that, at low concentrations, various sympathomimetics contracted the mouse distal colon by stimulating α_2 presynaptic adrenoceptors. The responses appeared provided intramural prostaglandin synthesis was unaffected. Higher concentrations of sympathomimetics induced relaxations by stimulation of postjunctional α_1- and β-adrenoceptors. Electrical field stimulation of the mouse colon produced cholinergically mediated contractions or, in the presence of atropine, non-adrenergic non-cholinergic (NANC) relaxations followed by rebound contractions, provided prostaglandin synthesis was unaffected.

6 These data suggest that in the mouse isolated colon, muscle tone and contractility are regulated by 2 opposing mechanisms: (1) a neurogenic cholinergic activity and a local prostaglandin synthesis leading to an increase in muscle tone; (2) a neurogenic NANC inhibitory control the nature of which remains to be elucidated. α_2-Presynaptic receptors, when activated inhibit the neurogenic inhibitory control and liberate the mechanism by which muscle tone is increased, causing a contraction.

Introduction

The effects of catecholamines on the gastrointestinal tract of several mammalian species are mediated by α- and β-adrenoceptors. Stimulation of these receptors produces mainly inhibitory responses (Ahlquist & Levy, 1959; Furchgott, 1960; Furness & Burnstock, 1974). The relative importance of the two receptor populations varies in different parts of the alimentary tract; β-adrenoceptors on the muscle are nearly always inhibitory and α-adrenoceptors may mediate excitation (Furness & Costa, 1974). For example contractions of the upper terminal portion of the guinea-pig ileum induced by sympathomimetic agents (Munro, 1951; Lands, 1952; Innes & Kohli, 1969; Minker, *et al.*, 1977; Bauer, 1981), and of the rat isolated colon induced by adrenaline following β-blockade (Regoli & Vane, 1964; Gagnon, 1970) have been reported. No information concerning the adrenoceptors of the mouse colon has been found though segments of mouse ileum exhibit spontaneous movements that are inhibited by sympathomimetic amines (Large & Wright, 1974). The purpose of the present investigation was, therefore, to investigate the mechanism of action and the effects of a number of sympathomimetics on the mouse colon, as well as the responses of this preparation to electrical field stimulation.

Methods

Swiss Webster mice (20–30 g) were stunned and killed by exsanguination; the terminal colon was dissected out and placed under an initial load of 1 g in an organ bath containing Krebs solution of the following composition (mM): KCl 4.7, NaCl 118.1, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2 and glucose 5. The bath (50 ml) was maintained at 36 ± 1°C and bubbled continuously with a mixture of 95% O_2 and 5% CO_2. The mechanical activity of the longitudinal muscle was measured, isometrically, with a Grass force displacement transducer. Preparations were allowed to equilibrate in Krebs solution for at least 60 min before agonists were added.

Agonists were left in contact with the tissue for periods of 5–6 min at intervals of 15 min. Antagonists were added at least 10 min before agonists were examined or electrical stimulation applied.

IC_{50} values for antagonists were determined graphically by testing the effect of three different concentrations ($n = 4$ to 7 for each concentration) on submaximal responses to each agonist. Dose-response curves were constructed for each agonist ($n = 4$ to 6), the pD_2 ($-\log ED_{50}$) was determined graphically from the mean ED_{50} in these experiments.

Chemical sympathectomy

In some mice, 6-hydroxydopamine (6-OHDA) 200 mg kg^{-1} was injected intraperitoneally. The animals were killed 24 h after treatment and each terminal colon dissected out. In these conditions, a total degeneration of adrenergic nerve fibres in Auerbach's plexus of the guinea-pig ileum has been described (Quayym, 1976). Acetylcholine (ACh 10^{-6} M) was always added to the bath at the beginning of the experiment to test the reactivity of the colon. The contractions to sympathomimetics have been expressed as percentages of the maximal ACh-induced response in the same preparation.

Electrical stimulation

Field stimulation of the mouse colon was carried out by square-wave pulses of 2–20 V, 1 ms duration, 2 or 4 Hz for 30–60 s at 10 min intervals.

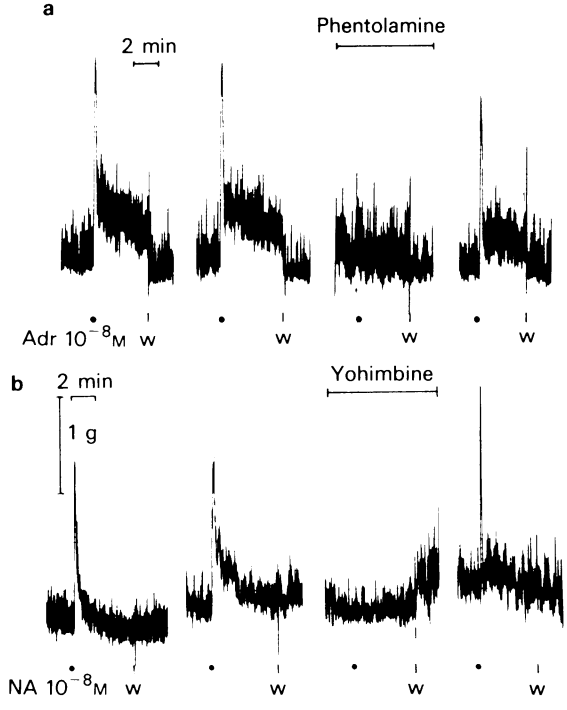

Figure 1 Contractile activity of adrenaline (Adr) and noradrenaline (NA) each 10^{-8} M on the mouse isolated colon. The responses were reproducible at 15 min intervals and abolished by phentolamine (3×10^{-8} M) (a) or yohimbine (3×10^{-8} M) (b). The contractile responses were restored 15 min after washing out the antagonist. W = washout.

Table 1 The activity of various agonists and antagonists on the isolated colon of the mouse

	Dose-response curves (n)		*Antagonists*	
			Yohimbine	*Prazosin*
	pD_2	*Maximal contractile response* (g)	IC_{50}	IC_{50}
Agonists	($-\log ED_{50}$)	± s.e. of mean	($\times 10^{-8}$ M)	($\times 10^{-8}$ M)
Adrenaline	8.2 (6)	+2.5±0.2	1	60
Noradrenaline	8.4 (4)	+1.3±0.3	1	100
Dopamine	7.6 (5)	+2.2±0.4	0.6	100
Clonidine	8.3 (4)	+2.4±0.5	1	1500
Phenylephrine	7.5 (4)	+2.6±0.3	2	200
Isoprenaline	8.1 (5)	−1.2±0.2	>1000	>1000
Apomorphine	7.3 (4)	+2.6±0.4	1	86

Figure in parentheses denotes number of observations.

Drugs

The following were used: acetylcholine hydrochloride (Roche), (−)-adrenaline bitartrate (Fluka), atropine sulphate (Fluka), apomorphine hydrochloride (Hoffman-La Roche), cimetidine (SKF), clonidine hydrochloride (Boehringer), domperidone (Janssen), (±)-dopamine hydrochloride (Koch Light), guanethidine sulphate (Ciba), 6-hydroxydopamine hydrobromide (Aldrich), indomethacin (Merck Sharp & Dohme), (−)-isoprenaline hydrochloride (Winthrop), mepyramine maleate (Rhône-Poulenc), methysergide hydrogenmaleate (Sandoz), naloxone hydrochloride (Endo), (−)-noradrenaline bitartrate (Winthrop), phentolamine methanesulphonate (Ciba), phenylephrine hydrochloride (Winthrop), prazosin hydrochloride (Pfizer), (±)-propranolol hydrochloride (ICI), sulpiride (Delagrange), tetrodotoxin (Calbiochem), yohimbine hydrochloride (Aldrich). Indomethacin (31 mg) was dissolved in ethanol (4 ml) and diluted with distilled water to give 0.31 mg ml^{-1}; 0.05 or 0.1 ml of this solution was added to the organ bath. Doses refer to molar concentrations (M) of salts in the bath.

Results

Effects of adrenaline and noradrenaline

Adrenaline ($10^{-9}-3\times10^{-8}$ M) in 86% of cases ($n=45$) and noradrenaline in 80% of cases ($n=25$) induced reproducible contractions of the terminal colon. In the other preparations no responses were observed. The contractions induced by adrenaline

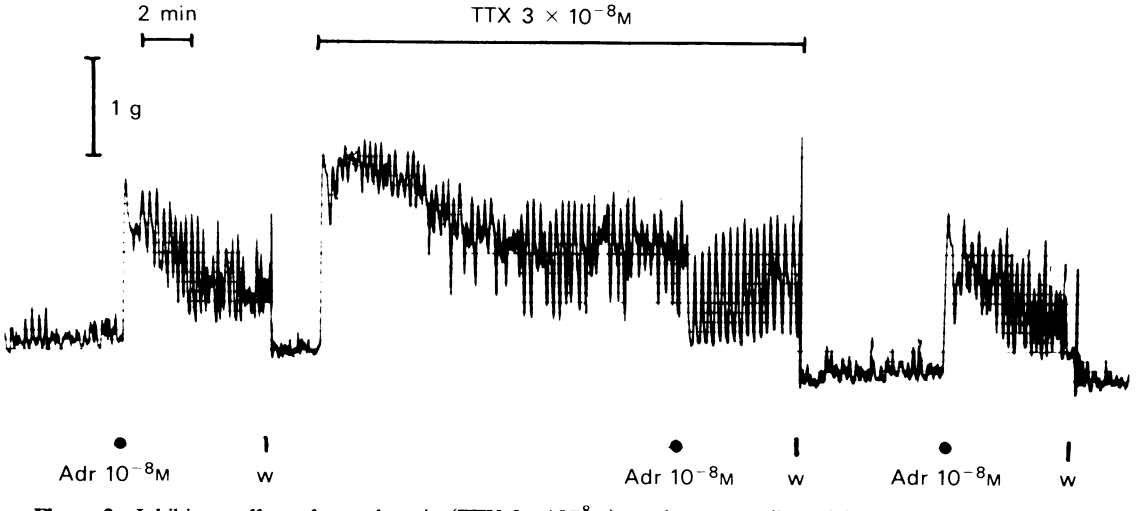

Figure 2 Inhibitory effect of tetrodotoxin (TTX 3×10^{-8} M) on the contractile activity of a low concentration (10^{-8} M) of adrenaline (Adr). Tetrodotoxin induced a sustained contraction and increased spontaneous activity. The response to adrenaline was restored 20 min after washing out tetrodotoxin. W = washout.

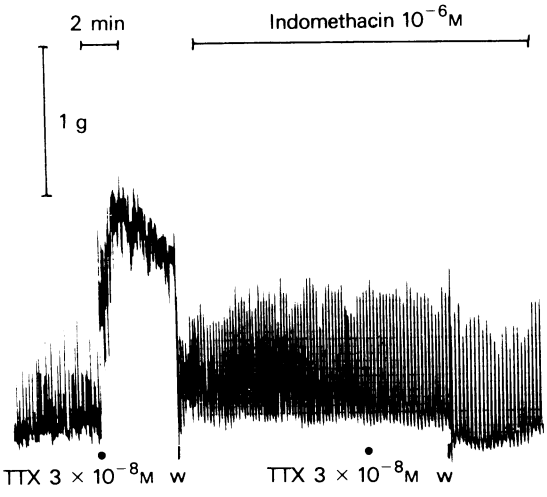

Figure 3 Inhibitory effect of indomethacin 10^{-6} M on the contractile activity of tetrodotoxin (TTX) 3×10^{-8} M. W = washout.

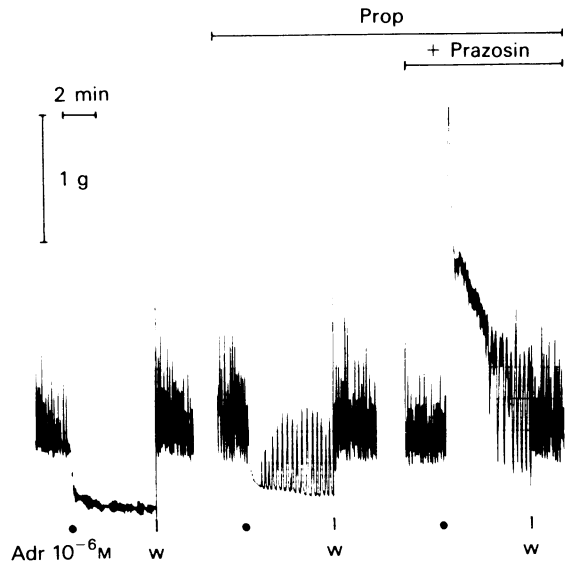

Figure 5 Inhibitory effect of adrenaline (Adr) at a high concentration (10^{-6} M was antagonized partially by propranolol 10^{-6} M (Prop) and converted to a contraction by the additional presence of prazosin 10^{-6} M (+ Prazosin). W = washout.

were rapid in onset, an initial peak being followed by a progressively declining response (Figure 1a). Noradrenaline caused contractions which were very short lasting (the initial peak fading after a maximum of 60 s) (Figure 1b). The responses were reproduci-

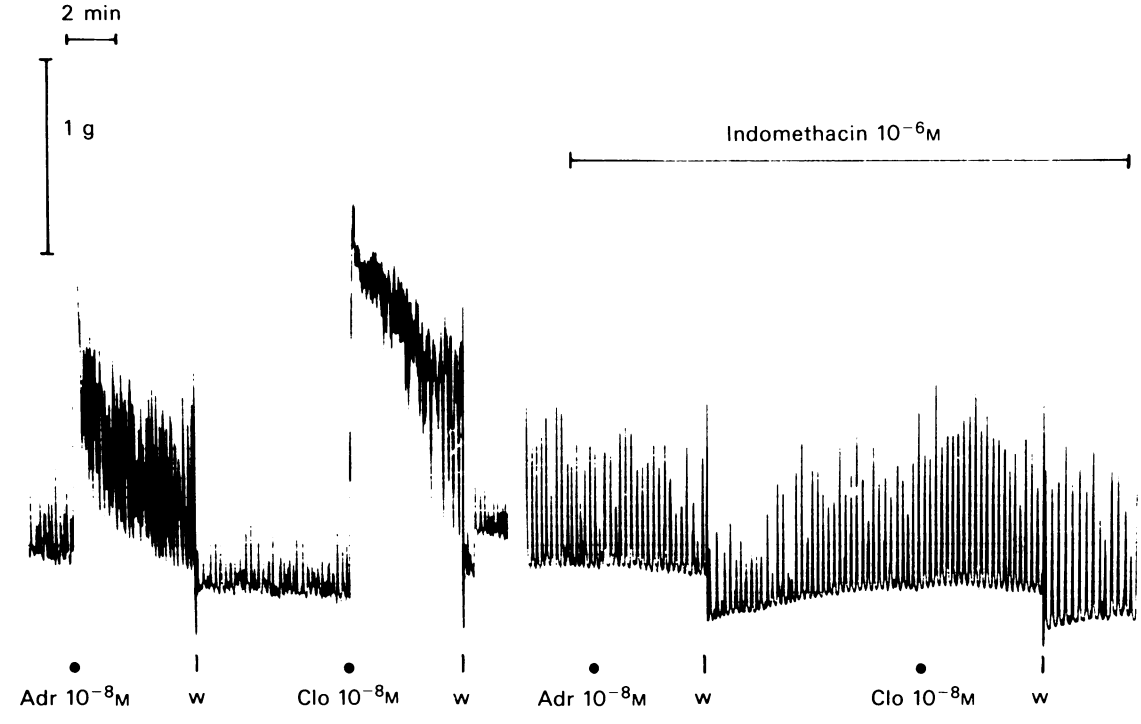

Figure 4 Inhibitory effect of indomethacin 10^{-6} M on the contractile activity of adrenaline (Adr) 10^{-8} M and clonidine (Clo) 10^{-8} M. W = washout.

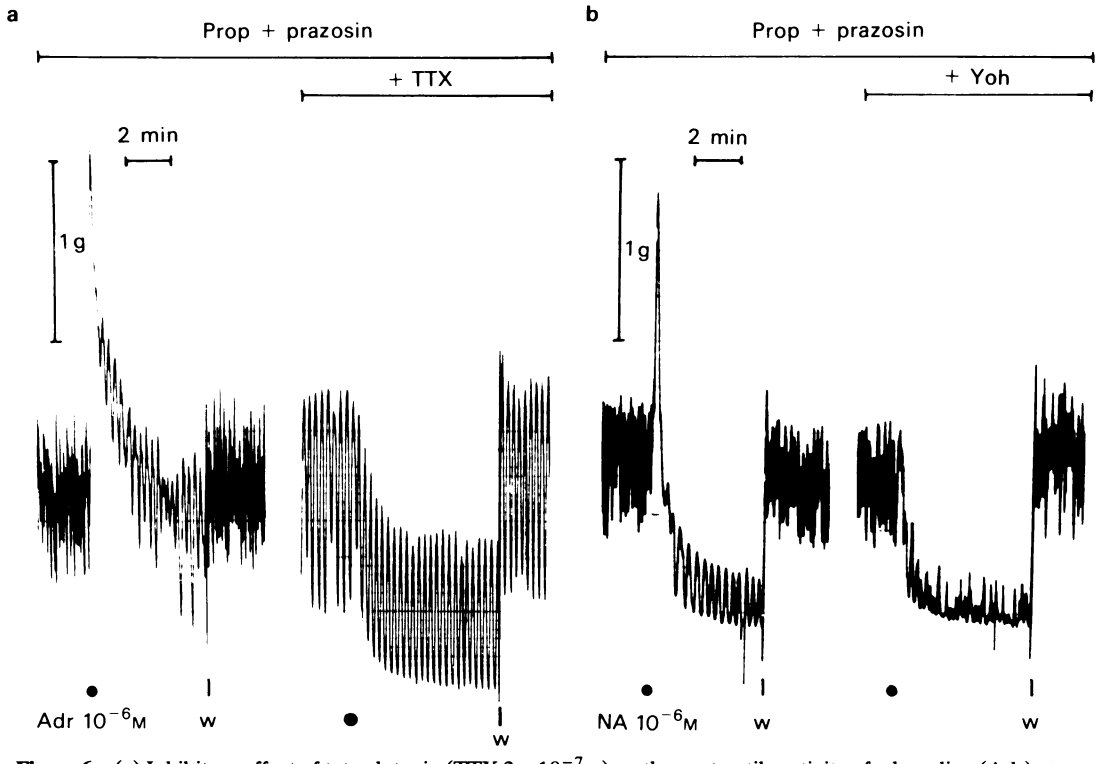

Figure 6 (a) Inhibitory effect of tetrodotoxin (TTX 2×10^{-7} M) on the contractile activity of adrenaline (Adr) at a high concentration (10^{-6} M) in the combined presence of propranolol and prazosin, each 10^{-6} M (Prop + prazosin). (b) Contraction and relaxation induced by noradrenaline (NA) at a high concentration (10^{-6} M) in the combined presence of propranolol and prazosin, each 10^{-6} M (Prop + prazosin) and the inhibitory effects of yohimbine (+ Yoh) 2.5×10^{-6} M on the contractile response. W = washout.

ble at 15 min intervals (Figure 1). The pD_2 values for these amines (Table 1) were relatively high (between 8 and 9) but the maximum tension (in g) induced by noradrenaline was lower than that induced by adrenaline (Table 1). The contractions were not modified by atropine (3×10^{-7} M), mepyramine (2.5×10^{-7} M), methysergide (5×10^{-7} M), propranolol (10^{-6} M), guanethidine (5×10^{-6} M), cimetidine (10^{-5} M) or naloxone (4×10^{-7} M). They were abolished by low concentrations of phentolamine (3×10^{-8} M) (Figure 1a) and by yohimbine (3×10^{-8} M) (Figure 1b) but not significantly modified by the same concentration of prazosin. The IC_{50} of yohimbine and prazosin are indicated in Table 1.

Tetrodotoxin (3×10^{-8} M) reversibly antagonized the contractions induced by adrenaline and noradrenaline. Figure 2 shows the reversibility of the adrenaline-induced contraction 20 min after washing out tetrodotoxin (4 intermediate washings of the preparation at 5 min intervals).

Tetrodotoxin itself caused a sustained contraction of the colon and increased spontaneous activity (Figure 2). This contraction was reproducible and atropine-resistant (3×10^{-7} M). It was not modified by phentolamine (3×10^{-6} M), propranolol (10^{-6} M), guanethidine (5×10^{-6} M), methysergide (5×10^{-7} M), mepyramine (2.5×10^{-7} M), cimetidine (10^{-5} M), naloxone (4×10^{-7} M) but abolished by preincubation with indomethacin 10^{-6} M (Figure 3). Indomethacin (10^{-6} M) left in contact with the tissue for 20 min also abolished contractions induced by adrenaline (10^{-8} M) (Figure 4) and noradrenaline (10^{-8} M). In the same experimental conditions, the solvent for indomethacin (40% ethanol in water) had no effect. The inhibitory effects of indomethacin were slowly reversible. A near restoration of the responses to adrenaline and noradrenaline was usually observed 60–90 min after washing out indomethacin.

The effect of indomethacin itself on the amplitude of the spontaneous activity varied from one preparation to another; in some experiments the tone was lowered by this drug.

In contrast to their effects at lower concentrations, adrenaline (Figure 5) and noradrenaline (3×10^{-7} M to 10^{-5} M) relaxed the preparations and abolished

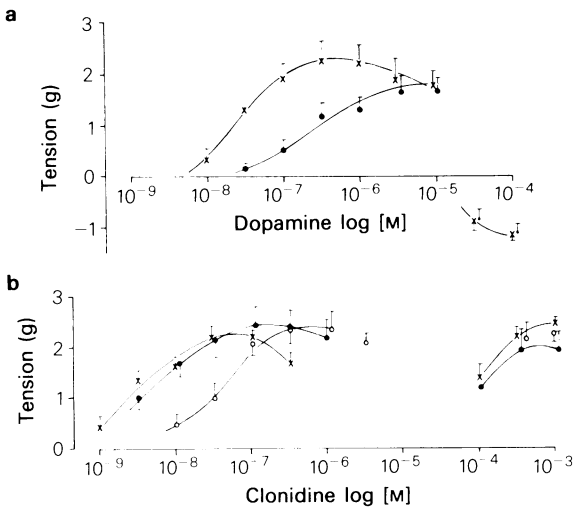

Figure 7 (a) Dopamine-induced concentration-effect curves in the absence (X) and presence (●) of yohimbine 10^{-8} M. Mean values are shown, vertical lines indicate s.e.mean; $n = 4$ or 5. (b) Clonidine-induced concentration-effect curves in the absence (X) and presence of yohimbine 10^{-9} M (●), 10^{-8} M (○). Mean values are shown, vertical lines indicate s.e.mean; $n = 4$.

their spontaneous activity. Indomethacin 10^{-6} M had no effect on these relaxations. Propranolol (10^{-6} M) partially reversed the effect of high concentrations of adrenaline and noradrenaline on the phasic activity, while in the presence of a mixture of propranolol and prazosin (each 10^{-6} M), the relaxations produced by adrenaline were changed into contractions (Figures 5, 6a) or in the case of noradrenaline into contractions followed by relaxations (Figure 6b).

The contractions were inhibited by yohimbine (2.5×10^{-6} M) and by tetrodotoxin (2×10^{-7} M) after the contraction produced by the latter had subsided (Figure 6a, b).

Effects of dopamine

Dopamine (10^{-8} to 10^{-6} M) induced a contraction of the mouse terminal colon in a dose-dependent manner (Figure 7a). The pD_2 values and maximal tensions (in g) developed are shown in Table 1. The response was characterized by a peak followed by a sustained contraction and increased phasic activity (Figure 8a). Dopamine had the same contractile activity on preparations from mice pretreated with 6-OHDA. In order to avoid any interference by

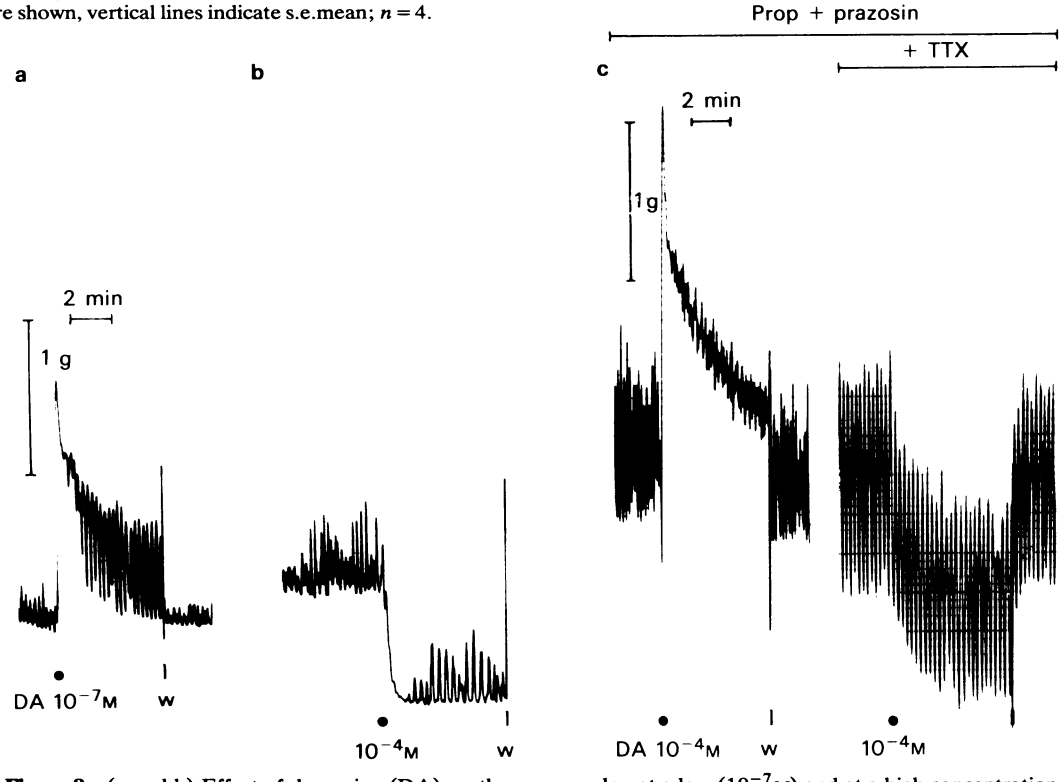

Figure 8 (a and b) Effect of dopamine (DA) on the mouse colon at a low (10^{-7} M) and at a high concentration (10^{-4} M). (c) Contractile activity of dopamine (DA) at a high (10^{-4} M) concentration, in the presence of propranolol plus prazosin each 10^{-6} M (Prop + prazosin) and inhibitory effect of tetrodotoxin (TTX) 2×10^{-7} M. W = washout.

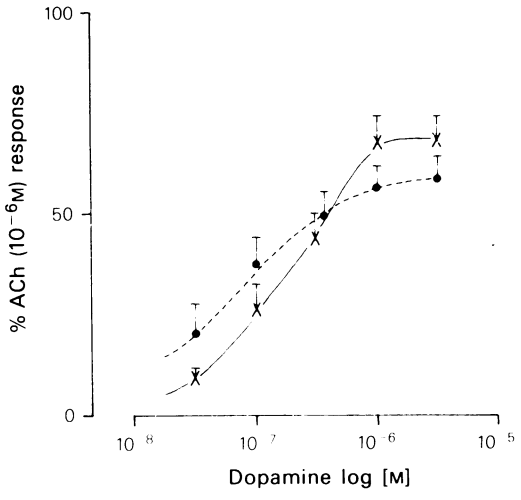

Figure 9 Concentration-effect curves for dopamine expressed as a percentage of the acetylcholine (ACh)-induced maximal response in the same preparations. Mean values are shown, vertical lines indicate s.e.mean. (X) Controls ($n = 4$); (●) preparations from mice pretreated with 6-hydroxydopamine (200 mg kg^{-1}) ($n = 7$).

chemical sympathectomy with the general reactivity of the smooth muscle to contractile agonists, dopamine-stimulated concentration effect curves on preparations from control and pretreated mice were expressed as percentages of the acetylcholine-induced maximal response in the same preparation (Figure 9). In both pretreated and control colons the maximal response induced by dopamine was about 50% of that induced by acetylcholine; there was no significant difference (Student's t test) between the responses of control and pretreated tissues.

Like those to adrenaline and noradrenaline, dopamine-induced contractions were not modified by atropine (3×10^{-7}M), mepyramine (2.5×10^{-7}M), methysergide (5×10^{-7}M), propranolol (10^{-6}M), guanethidine (5×10^{-6}M), cimetidine (10^{-5}M) or naloxone (4×10^{-7}M). However, they were abolished by phentolamine (3×10^{-8}M), tetrodotoxin (3×10^{-8}M), indomethacin (10^{-6}M) and competitively antagonized by yohimbine 10^{-8}M) (Figure 7a).

The IC_{50} of prazosin (10^{-6}M) was much higher than that of yohimbine (6×10^{-9}M) (Table 1), indicating the weaker effect of the α_1-antagonist.

By increasing the doses of dopamine, the contractions decreased and the responses became biphasic, the initial peak being followed by a relaxation.

Dopamine (3×10^{-5}M to 10^{-4}M) elicited a dose-dependent relaxation of the preparation (Figures 7a, 8b) and partially inhibited the spontaneous phasic activity. The relaxations induced by the higher doses of dopamine (3×10^{-5}–10^{-4}M) were abolished by a mixture of propranolol (10^{-6}M) and prazosin (10^{-6}M) and converted to contractions (Figure 8c).

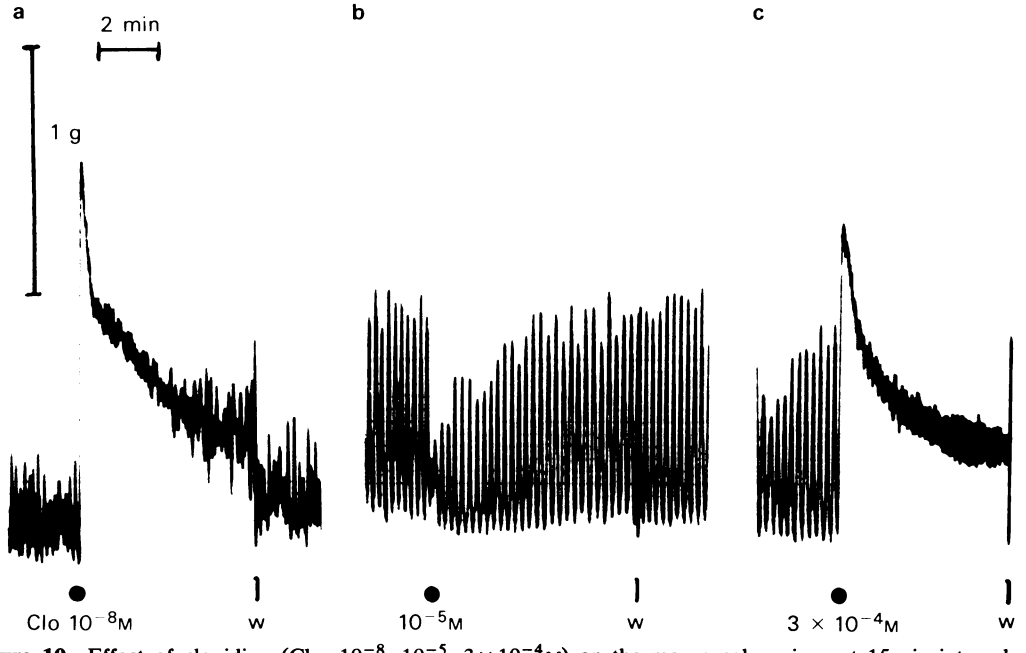

Figure 10 Effect of clonidine (Clo, 10^{-8}, 10^{-5}, 3×10^{-4}M) on the mouse colon given at 15 min intervals. W = washout.

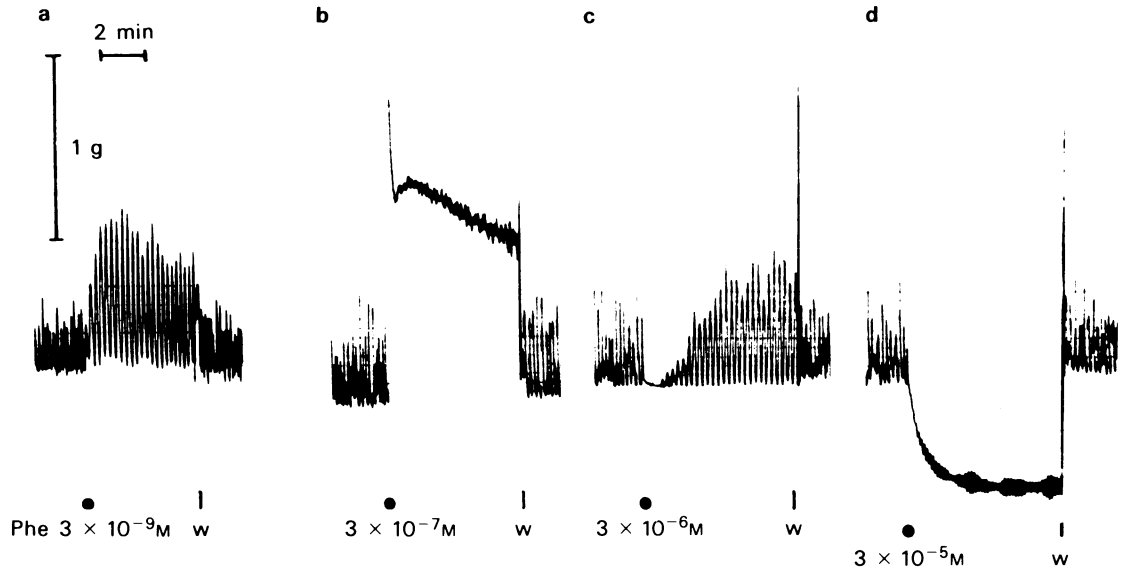

Figure 11 Effects of phenylephrine (Phe, 3×10^{-9}, 3×10^{-7}, 3×10^{-6} and 3×10^{-5} M) given at 15 min intervals on the mouse colon. W = washout.

The latter were abolished by tetrodotoxin (2×10^{-7} M) (Figure 8c) and by yohimbine (10^{-5} M).

Effects of clonidine

Clonidine (10^{-9} M to 10^{-7} M) contracted the preparation in a dose-dependent manner (Figure 7b). The responses were similar to those obtained with dopamine (Figures 4 and 10a). The pD_2 and maximal tensions developed are shown in Table 1.

As with dopamine, the contractions were competitively antagonized by yohimbine (Figure 7b, left part) and by prazosin, the IC_{50} for which was 1500 higher than that of yohimbine (Table 1). The contractions were also abolished by tetrodotoxin (3×10^{-8} M) and by indomethacin (10^{-6} M) (Figure 4) but unaffected by atropine (3×10^{-7} M), mepyramine (2.5×10^{-7} M), methysergide (5×10^{-7} M), propranolol (10^{-6} M), guanethidine (5×10^{-6} M), cimetidine (10^{-5} M) or naloxone (4×10^{-7} M). At increasing doses of clonidine (10^{-6} to 3×10^{-5} M) the contractions disappeared and a dose-dependent (up to 3×10^{-5} M) decrease in the spontaneous activity of the preparation was obtained (Figure 10b). This inhibitory effect was abolished in the presence of prazosin (10^{-6} M).

By increasing the doses of clonidine further (10^{-4} to 10^{-3} M) and, in contrast with adrenaline, noradrenaline and dopamine, no relaxations were observed but dose-related, transient contractions were elicited (Figure 7b, right part; Figure 10c). These latter were not easily reproducible and showed tachyphylaxis, they were not inhibited by yohimbine (10^{-8} M) (Figure 7b, right part) or by large concentrations of tetrodotoxin (3×10^{-7} M) phentolamine or propranolol (each 3×10^{-6} M).

Clonidine had the same contractile activity on preparations from mice pretreated with 6-OHDA ($n = 6$).

Effects of phenylephrine

In most cases (68%, $n = 19$), phenylephrine stimulated the preparation. Lower concentrations (3×10^{-9} M) increased the spontaneous phasic activity of the colon (Figure 11a), while higher concentrations (10^{-8} M to 10^{-6} M) contracted the preparations in a dose-dependent manner (Figure 11 and Table 1). Yohimbine was much more effective than prazosin in antagonizing the contractions induced by phenylephrine (Table 1) while they were abolished by tetrodotoxin (3×10^{-8} M) and indomethacin (10^{-6} M).

At concentrations above 10^{-6} M the contractions induced were smaller and at 3×10^{-6} M phenylephrine inhibited spontaneous activity (Figure 11c). From 3×10^{-5} to 3×10^{-3} M, phenylephrine relaxed the preparation (Figure 11d). The inhibitory effects of phenylephrine were resistant to tetrodotoxin (3×10^{-7} M) and to high doses of yohimbine (10^{-5} M) but susceptible to prazosin (10^{-7} M). The relaxations induced by phenylephrine 10^{-4} M were inhibited by

prazosin (10^{-6} M) and changed to a short lasting contraction which was sensitive to yohimbine (10^{-8} M) and tetrodotoxin (3×10^{-8} M).

In other preparations (32%, $n = 19$) no contractions or stimulatory effects were induced by phenylephrine. Only inhibition of the spontaneous activity of the preparation was observed at concentrations of 10^{-6} M, and dose-related relaxations from 3×10^{-6} M to 3×10^{-4} M. These effects were antagonized only by prazosin.

Effect of isoprenaline

Isoprenaline, 10^{-9} M–3×10^{-7} M, relaxed the mouse colon in a dose-dependent manner. These relaxations were competitively antagonized by propranolol (10^{-6} M) and resistant to tetrodotoxin.

Effect of apomorphine

From 10^{-8} M to 10^{-6} M, apomorphine contracted the preparation in a similar way to dopamine (Figure 12). The pD_2 and maximal responses (Table 1) were similar to those induced by dopamine. A competitive antagonism with yohimbine could be demonstrated (Figure 12) and the contractions produced by apomorphine were abolished by tetrodotoxin and indomethacin.

As with dopamine, apomorphine at high concentrations (10^{-4} M to 10^{-3} M) elicited a dose-dependent relaxation. However, and in contrast to dopamine, adrenaline and noradrenaline, these relaxations were not modified by any of the antagonists tested: they were resistant to both neuronal and α- and β-adrenoceptor blockade. They were not modified by the dopamine antagonists, sulpiride or domperidone (10^{-5} M) (Laduron & Leysen, 1979; O'Connor & Brown, 1982).

Response to electrical stimulation

Electrically-induced responses of the mouse colon could be elicited by field stimulation of 1 ms, 8 V, 2 Hz (Figure 13a). The contractile response had a short delay in onset (10–15 s) while spontaneous activity was inhibited, and progressively reached its maximum in 1 min. After cessation of stimulation there was a progressive decline of the tone (2–3 min). An increase in voltage of the stimulus (16 V, Figure 13a) did not modify significantly the pattern or the amplitude of the response. However, when the frequency of the stimulus was increased (4 Hz, Figure 13b) the delay in onset of the contraction was shortened and the amplitude increased, although it never reached more than 50% of the maximal response induced by acetylcholine (Figure 13).

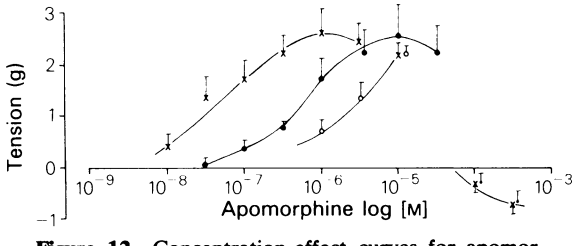

Figure 12 Concentration-effect curves for apomorphine in the absence (X) and presence of yohimbine 10^{-8} M (●) and 10^{-7} M (○). Mean values are shown, vertical lines indicate s.e.mean; $n = 4$.

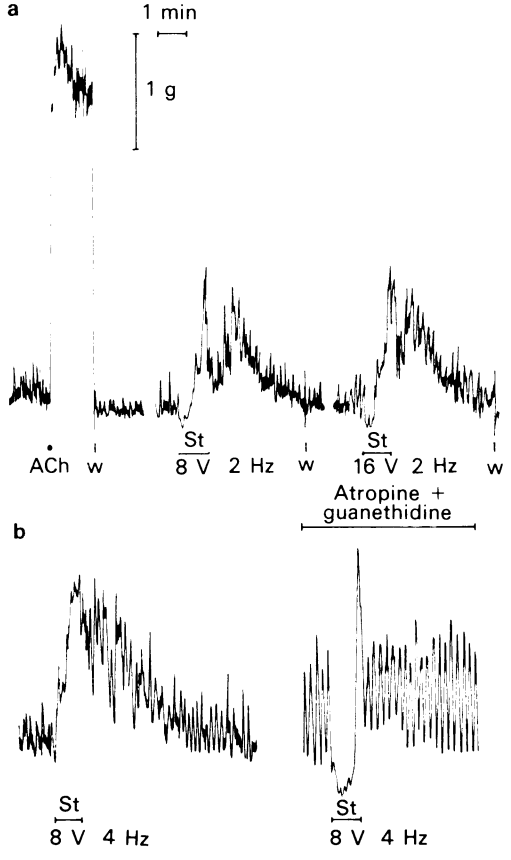

Figure 13 (a) Responses of the mouse colon to acetylcholine (ACh) 10^{-6} M and field stimulation, 1 ms, 2 Hz, 8 and 16 V (St). The contractile response develops after a short delay; after cessation of stimulation there is a progressive decline in tone. W = washout. (b) Responses to electrical field stimulation 1 ms 4 Hz, 8 V (same preparation as in a). There is no delay for the contractile response. In the presence of atropine (2×10^{-7} M) and guanethidine (5×10^{-6} M) the same electrical stimulus elicits a relaxation and at the end of St, a rebound contraction.

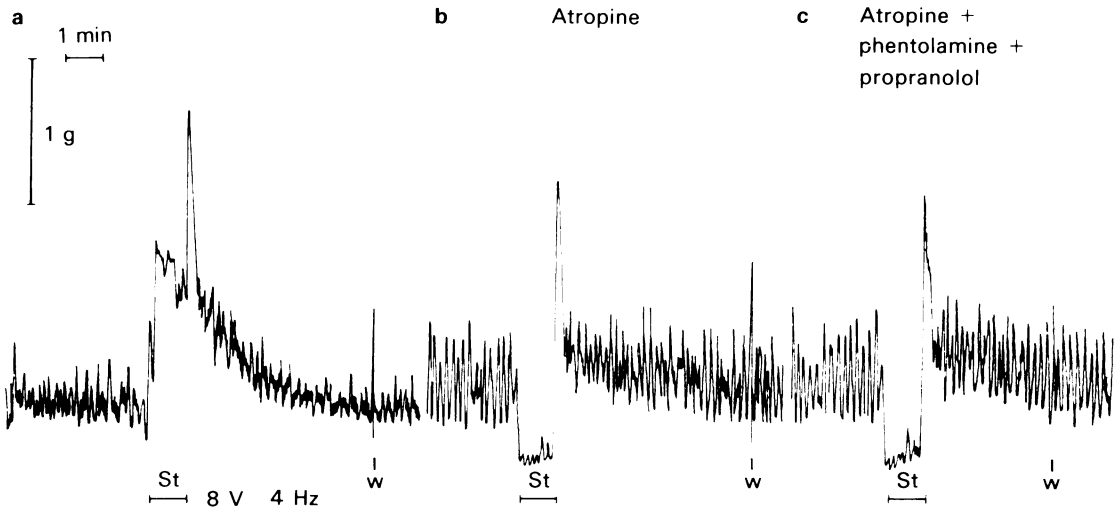

Figure 14 Responses of the mouse colon to electrical field stimulation 1 ms, 4 Hz, 8 V (St). Note the rebound contraction after cessation of St (a). In the presence of atropine (2×10^{-7} M) the same electrical stimulus elicits a relaxation and a rebound-contraction (b). This relaxation was not modified by the additional presence of phentolamine (3×10^{-6} M) and propranolol (10^{-6} M) (c). W = washout.

After cessation of stimulation at 4 Hz, a rebound contraction was generally but not always observed (Figures 14a and 13b) and the tone declined more progressively than after cessation of stimulation at 2 Hz.

In the presence of atropine (2×10^{-7} M) the contractile response to field stimulation was abolished and transformed into a rapid and sustained relaxation (Figure 14b). Immediately after cessation of stimulation, a rebound contraction (peak) was always observed (Figures 13b and 14b). Phentolamine (3×10^{-6} M), propranolol (10^{-6} M), (Figure 14c), guanethidine (5×10^{-6} M) (Figure 13b), methysergide (5×10^{-7} M), mepyramine (2.5×10^{-7} M); cimetidine (10^{-5} M) and naloxone (4×10^{-7} M) failed to modify either the relaxation or the rebound contraction.

The responses to electrical stimulation in the absence or the presence of atropine were abolished by low concentrations (3×10^{-8} M) of tetrodotoxin. This effect was reversed by washing (Figure 15a). After preincubation with indomethacin (2×10^{-6} M) for 30 min the rebound contraction, always observed after cessation of the stimulation in the presence of atropine and guanethidine (Figure 15b) was inhibited. This inhibitory effect was slowly reversible (60–90 min).

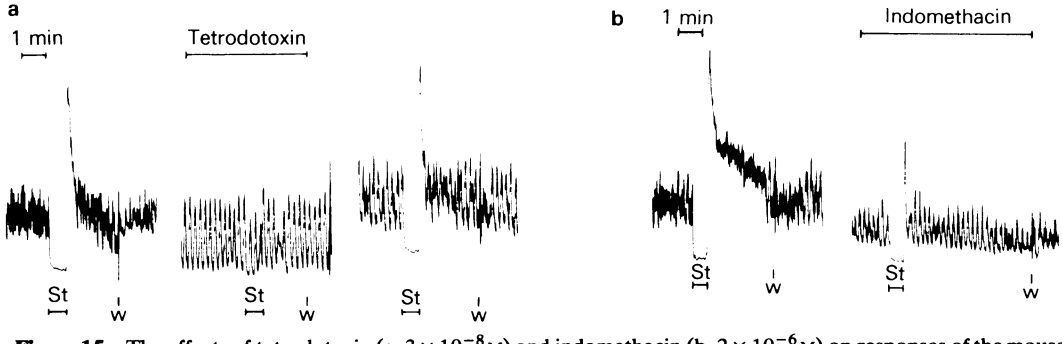

Figure 15 The effects of tetrodotoxin (a, 3×10^{-8} M) and indomethacin (b, 2×10^{-6} M) on responses of the mouse colon to electrical field stimulation (St) 2 Hz, 0.5 ms, 20 V in the presence of atropine (2×10^{-7} M) and guanethidine (5×10^{-6} M). W = washout. Tetrodotoxin (a) abolished the response and increased spontaneous activity. The inhibition was reversed 20 min after washing out tetrodotoxin. After preincubation (30 min) with indomethacin 2×10^{-6} M (b) the rebound contraction of the muscle was inhibited and the tone decreased in the presence of drug. W = washout.

Discussion

Presynaptic adrenoceptors and the regulation of muscle tone in the mouse colon

Adrenaline, noradrenaline, phenylephrine and dopamine, at low concentrations ($pD_2 = 7$ to 8), contracted the mouse distal colon by stimulating α-adrenoceptors; the responses were abolished by low concentrations of phentolamine. Clonidine, an α-adrenoceptor agonist (see Isaac, 1980) and apomorphine, a dopamine agonist (Ernst, 1967; Willems & Bogaert, 1975) had similar effects. The contractions were reproducible and competitively antagonized by much lower concentrations of yohimbine than of prazosin, suggesting that they were produced by stimulation of α_2-adrenoceptors (Langer, 1974; Starke, 1977). The contractions were also abolished by tetrodotoxin, which blocks neuronal activity (Gershon, 1967) and by indomethacin which inhibits prostaglandin synthesis (Vane, 1971).

On the other hand, the contractions were not modified by atropine, mepyramine, cimetidine, methysergide, propranolol or naloxone. Pretreatment with 6-OHDA (Quayyum, 1976) did not modify the responses to dopamine or clonidine. The integrity of adrenergic nerves is clearly not required for the contractile responses. Field stimulation induced cholinergically-mediated contractions which, in the presence of atropine were transformed into relaxations followed by a rebound contraction after cessation of stimulation. While phentolamine, propranolol and guanethidine did not modify these responses tetrodotoxin was effective, indicating their neural origin. These results demonstrate the existence of both cholinergic and of non-adrenergic, non-cholinergic neurones (NANC) in the mouse colon which can be activated by electrical stimulation (see review by Gillespie, 1982).

Tetrodotoxin itself induced a contraction that was inhibited by preincubation with indomethacin but was not abolished by atropine, yohimbine, prazosin, propranolol, methysergide, mepyramine, cimetidine or naloxone.

These results suggest that in the mouse colon, muscle tone and contractility may be regulated by two opposing mechanisms (see Figure 16): (i) a neural cholinergic component and a local synthesis and release of prostaglandin synthesis which lead to an increase in muscle tone; (ii) a neural non-adrenergic non-cholinergic (NANC) inhibitory control. Adrenergic nerves terminals have not been represented in Figure 16 since in our experimental conditions there is no evidence of their being stimulated. However, the presence of α_2-presynaptic receptors on noradrenergic terminals in the mouse colon is not excluded.

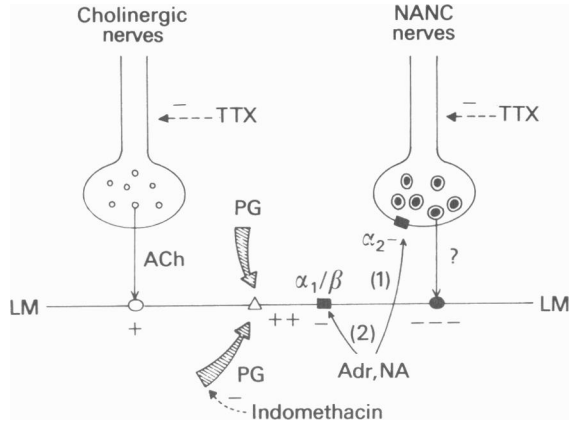

Figure 16 Schematic representation of the regulatory mechanisms of tone in longitudinal muscle (LM) of the mouse isolated colon. Non-adrenergic non-cholinergic (NANC) nerves inhibit tone (---). Cholinergic nerves and local production of prostaglandins (PG) postsynaptically are involved in the increase of muscle tone (+++). Noradrenaline (NA) and adrenaline (Adr) at low concentrations each stimulate α_2-presynaptic adrenoceptors (1) on NANC nerve terminals. This leads to a contraction, by suppressing the release of the inhibitory NANC nerve transmitter, and liberating the mechanism which increases muscle tone. Indomethacin inhibits (−) the catecholamine-induced contractions by inhibition of the intestinal prostaglandin synthesis. Tetrodotoxin (TTX) also inhibits (−) the inhibitory control exerted by NANC nerves; this leads to a contraction. After TTX the response to α_2-adrenoceptor agonists is also inhibited. Adr or NA at high concentrations stimulate postjunctional α_1- and/or β-adrenoceptors (2) which leads to a relaxation of the longitudinal muscle.

Our hypothesis is supported by the following observations: (a) Tetrodotoxin itself induced a contraction. This drug is known for its neuronal blocking activity (Gershon, 1967) and suppression of the NANC inhibitory component, (ii), so the first mechanism (i) mediated by prostaglandin synthesis and cholinergic nerve becomes dominant. Indomethacin which inhibits prostaglandin synthesis (Vane, 1971) also inhibits tetrodotoxin-induced contractions. (b) In the presence of atropine and guanethidine, nerve stimulation, presumably of NANC nerves led to a relaxation followed by rebound contraction. (c) When the neurogenic relaxation is inhibited by tetrodotoxin, there is no rebound contraction at the end of the stimulation period. On the other hand, indomethacin was found to inhibit the rebound contraction, not the relaxation.

α_2-Presynaptic receptors, when activated, are thus presumed to inhibit a neurogenic inhibitory control which is mediated by NANC nerves.

α_2-Adrenoceptor agonists by diminishing or sup-

pressing the release of the inhibitory NANC transmitter, allow prostaglandins to predominate and increase muscle tone and cause a contraction.

Electrical stimulation liberates the inhibitory transmitter from intrinsic nerves, opposing the effects of prostaglandins and preventing the increase in tone. The rebound contraction at the end of the stimulation period may arise from unopposed prostaglandins.

Inhibition by indomethacin of the contraction elicited by sympathomimetics and of the rebound contraction after field stimulation supports the existence of a prostaglandin-dependent mechanism that increases longitudinal smooth muscle tone in the mouse colon.

The absence of inhibition of either of these contractions by atropine, mepyramine or methysergide suggests that acetylcholine, histamine or 5-hydroxytryptamine are not involved. Prostaglandins are released from gastrointestinal smooth muscle (Bennet, 1972) and may help to maintain intrinsic tone in various isolated preparations (Ferreira *et al.*, 1972; Eckenfels & Vane, 1972) including the guinea-pig colon (Bennet *et al.*, 1975). Prostaglandins are not neurogenic, and are therefore insensitive to tetrodotoxin. Tetrodotoxin causes a contraction, and is not an inhibitor of the direct effect of prostaglandins on smooth muscle.

As the response to tetrodotoxin is inhibited by indomethacin, one may consider that tetrodotoxin, by inhibiting the neurogenic inhibitory mechanism, 'reveals' the prostaglandin producing mechanism that elevates tone.

Postsynaptic adrenoceptors

Catecholamines and the other drugs tested have additional effects at high concentrations. Adrenaline, noradrenaline (from 3×10^{-7} M) and dopamine (from 3×10^{-5} M) relaxed the longitudinal muscle of the mouse colon, as a result of α_1- and β-adrenoceptor stimulation. Inhibition of these responses by a mixture of prazosin and propranolol unmasked the α_2-receptor stimulation already observed at lower concentrations of these amines.

Above 3×10^{-6} M or from 3×10^{-8} M in 40% of the preparations, phenylephrine inhibited the spontaneous phasic activity of the colon and, above 10^{-4} M, relaxed the preparation. These inhibitory effects were abolished by prazosin and were presumably mediated by stimulation of α_1-adrenoceptors. Like adrenaline, noradrenaline and dopamine, the α_2 stimulatory effects of phenylephrine at high concentrations were unmasked after blockade of its inhibitory effects.

Clonidine, from 3×10^{-7} M to 3×10^{-5} M exerted the same type of inhibition as phenylephrine on the spontaneous activity of the colon but never induced relaxations. At very high concentrations (10^{-4} M, 10^{-3} M) clonidine elicited non-sustained contractions by a still undefined mechanism.

Finally, the relaxations induced by high concentrations of apomorphine (from 10^{-4} M) were unrelated either to α_1, β-adrenoceptor or dopamine receptor stimulation but may have arisen from a direct action on smooth muscle. Isoprenaline at all concentrations tested (3×10^{-9} M–3×10^{-7} M) induced dose-related relaxations by β-adrenoceptor stimulation. These results demonstrate the presence of postsynaptic inhibitory α and β-adrenoceptors in the longitudinal muscle of the mouse colon. These receptors have been described in various gastrointestinal preparations including the guinea-pig isolated ileum (Wikberg, 1977; Fagbemi & Salako, 1980), the rabbit intestine (Bowman & Hall, 1970; Wikberg, 1977), the mouse ileum (Large & Wright, 1974). In conclusion, in the mouse colon, the activation of prejunctional α_2-adrenoceptors leads to a contraction. This appears to be related to modifications of the local control of intestinal tone exerted by inhibitory NANC nerves, and by excitatory prostaglandins. The nature of the inhibitory transmitter (whose release should be antagonized by catecholamines acting presynaptically on α_2-adrenoceptors) remains unknown. Moreover, the activation of postjunctional α_1 and/or β-adrenoceptors leads to a relaxation of the longitudinal muscle. There is no evidence for the presence of dopamine receptors in the mouse colon.

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