Contractions mediated by α_1 -adrenoceptors and P_2 -purinoceptors in a cat colon circular muscle

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1 The postjunctional excitatory and inhibitory effects of adrenoceptor and purinoceptor agonists and antagonists were studied in circular smooth muscle strips of cat colon.

2 In the presence of tetrodotoxin (0.5 μ M), noradrenaline caused contraction or relaxation of circular smooth muscle at resting tension or with raised tone, respectively. The noradrenaline-evoked contractions were potentiated and the noradrenaline-evoked relaxations were antagonized by propranolol (1 μ M), suggesting β -adrenoceptor involvement.

3 At resting tension, noradrenaline, adrenaline and the selective α_1 -adrenoceptor agonist, phenylephrine, caused concentration-dependent contractile responses, with EC_{50} values of 1.8 ± 0.2 μ M, 1.9 ± 0.4 μ M and 4.3 ± 1.7 μ M, respectively. The EC_{50} values and the amplitude of maximal responses were not significantly different from one another. Clonidine (0.1–500 μ M), a selective α_2 -adrenoceptor agonist, was not effective.

4 Prazosin (0.1–9 μ M), competitively antagonized the contractile effects of noradrenaline with an estimated pA_2 value of 6.93 and a slope of 1.07 ± 0.03 . The K_b values, estimated from a single shift (0.1 μ M prazosin) of the concentration-response curves to noradrenaline, adrenaline and phenylephrine were 92.8 ± 9.3 nM, 108.7 ± 6.4 nM and 18.4 ± 3.1 nM, respectively.

5 At resting tension, adenosine 5' triphosphate (ATP, 5–1000 μ M), α,β -methylene adenosine 5'-triphosphate (α,β -MeATP, 0.05–50 μ M), β,γ -methylene adenosine 5'-triphosphate (β,γ -MeATP, 0.5–100 μ M), and 2-methylthioadenosine 5'-triphosphate (2-MeSATP, 1–500 μ M) caused concentration-dependent contractions with EC_{50} values of 60.5 ± 15.9 μ M, 0.7 ± 0.1 μ M, 7.6 ± 0.1 μ M and 25.3 ± 12.8 μ M, respectively. The maximal responses to α,β -MeATP and β,γ -MeATP were greater than maximal responses to 2-MeSATP and ATP.

6 Suramin (50–500 μ M), competitively antagonized the contractile responses of α,β -MeATP with an estimated pA_2 value of 4.92 and a slope of 1.08 ± 0.04 . The K_b values, estimated from a single shift (100 μ M suramin) of the concentration-response curves to ATP, α,β -MeATP, β,γ -MeATP and 2MeSATP were 52.3 ± 20.2 μ M, 25.2 ± 4.5 μ M, 21.7 ± 11.0 μ M and 11.6 ± 2.7 μ M, respectively.

7 At resting tension, reactive blue 2 (100 μ M), a selective antagonist of the P_{2Y} -purinoceptor, and 8-(*p*-sulphophenyl)-theophylline (8-SPT) (1 μ M), a selective antagonist of the P_1 -purinoceptor, did not antagonize the contractile responses to α,β -MeATP (0.5–5 μ M). Contractile responses to ATP (50–500 μ M) were not altered by 8-SPT (1 μ M) but were potentiated by reactive blue 2 (100 μ M).

8 With raised tone, ATP and 2-MeSATP caused a relaxant effect. This effect of ATP was not altered by either tetrodotoxin (TTX) (0.5 μ M) or suramin (100 μ M), but was antagonized by reactive blue 2 (100 μ M) and 8-SPT (1 μ M), suggesting that inhibitory P_{2Y} - and P_1 -purinoceptors are involved. In contrast, α,β -MeATP and β,γ -MeATP caused only contractions. This contractile effect of α,β -MeATP was resistant to TTX (0.5 μ M) and antagonized by suramin (100 μ M).

9 In summary, cat colon circular muscle contains postjunctional α_1 -adrenoceptors and P_{2X} -purinoceptors which mediate contractions and P_{2Y} - and P_1 -purinoceptors which mediate relaxation. Postjunctional α_2 -adrenoceptors appear not to be present.

Keywords: α_1 -Adrenoceptors; P_{2X} -purinoceptors; contractile responses; cat colon

Introduction

Electrical stimulation of lumbar sympathetic fibres contracts circular muscle of sphincter regions of the colon via postjunctional α -adrenoceptors (Pahlin & Kewenter, 1976; Bouvier & Gonella, 1981) and inhibits neurogenic contractions in non-sphincter regions of colon circular and longitudinal muscle via prejunctional α -adrenoceptors (Gillespie & McKenna, 1961; de Groat & Krier, 1976; Gillespie & Khoyi, 1977) and pre- and postjunctional β -adrenoceptors (Ek, 1985).

Electrical stimulation of lumbar sympathetic fibres (lumbar colonic nerves) also cause contractions of circular muscle, in non-sphincter regions of cat colon. The contractions are partially reduced by either prazosin or by desensitization with α,β -methylene ATP, but are almost abolished by application of both compounds. This suggests that the

neurogenic contractions are mediated by the co-release of noradrenaline and ATP or a related nucleotide through postjunctional α_1 -adrenoceptors and P_{2X} -purinoceptors (Venkova & Krier, 1993).

The circular muscle layer of cat, dog, rat and human colon contains excitatory α -adrenoceptors (Gagnon & Belisle, 1970; Gagnon *et al.*, 1972; Anurus & Christensen, 1981; Zang *et al.*, 1992; Venkova & Krier, 1993) and P_2 -purinoceptors (Hedlung *et al.*, 1986; Venkova & Krier, 1993). In cat colon circular muscle, postjunctional adrenoceptors and purinoceptors have not been pharmacologically characterized. Our aim was to study the excitatory effects of adrenoceptor agonists and purine nucleotides in circular muscle strips of cat colon *in vitro*. Agonist potency and the effects of selective antagonists were used to characterize the α -adrenoceptors and purinoceptors involved. Prazosin, a selective antagonist of the α_1 -adrenoceptors was used to study agonist responses

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mediated by α_1 -adrenoceptors. Suramin, an antagonist of the P_{2X} -purinoceptor (Dunn & Blackley, 1988; Hoyle *et al.*, 1990; Leff *et al.*, 1990; Kennedy, 1990) and reactive blue, an antagonist of the P_{2Y} -purinoceptor (Kerr & Krantis, 1979) were used to study agonist responses mediated by P_{2X} - and P_{2Y} -purinoceptors. 8-(*p*-sulphophenyl)-theophylline (8-SPT), a putative selective antagonist of the P_1 -purinoceptor (Collis *et al.*, 1987), was used to distinguish agonist responses mediated by P_1 - and P_2 -purinoceptors.

Methods

Male or female cats, weighing 2.5–4.5 kg were anaesthetized with sodium pentobarbitone (50–70 mg kg⁻¹, i.p.). After exsanguination, a segment (4–5 cm) of the mid colon, 4 cm caudad to the ileocecal sphincter, was excised and placed in ice-cold Krebs solution. It was cut along the antimesenteric border and the mucosa removed. Muscle strips (about 10 mm long and 3 mm wide) were cut in the long axis of circular muscle fibres. They contained both muscle layers and the myenteric plexus. Isolated preparations were mounted vertically in the circular axis in 10 ml organ baths containing a modified Krebs solution [(mm): NaCl 117, KCl 4.7, CaCl₂ 2.5, MgCl₂ 1.2, NaH₂PO₄ 1.2, NaHCO₃ 25 and glucose 11] maintained at 37°C and continuously aerated with 95% O₂ and 5% CO₂.

Spontaneous isometric contractions of the circular smooth muscle layer were recorded. Muscle strips were equilibrated for 60–90 min under a load of 5 g. Optimal tension was determined previously by repeatedly stretching the muscle strips and adding acetylcholine (10 μ M). When contractions to acetylcholine ceased to increase with increasing stretch, that tension was considered optimal. This corresponded to a load of approximately 5 g. Muscle strips which changed their tone after equilibration were discarded.

Experimental protocol

The mechanical responses of cat colon circular muscle strips to exogenous adrenoceptor agonists were studied at resting tension and with raised tone. At resting tension, concentration-response curves to noradrenaline, adrenaline and phenylephrine were constructed. Peak contractions to adrenoceptor agonists occurred within 3–4 min. Increasing concentrations of adrenoceptor agonists were added cumulatively during the plateau phase of each agonist contraction. After completion of each concentration-response curve, drugs were 'washed out' and tension returned to the resting level. Also, concentration-response curves to noradrenaline were constructed in the absence and presence of either prazosin or propranolol. Muscle strips were pretreated with antagonists for 20–25 min before construction of a concentration-response curve. Each muscle strip served as its own control. Only one agonist and one antagonist was applied per muscle strip.

The relaxation responses to noradrenaline were studied at raised tone. To study relaxation, carbamylcholine (carbachol, 1 μ M) was added to the Krebs solution. This concentration of carbachol caused a submaximal, sustained rise in tone. Tetrodotoxin (TTX) (0.5 μ M) was used to abolish neuronal input to smooth muscle. The effects of noradrenaline (10 μ M) were tested during the 'plateau phase' of the evoked contraction. The involvement of β -adrenoceptors was studied by examining relaxant responses to noradrenaline in the absence and presence of propranolol (1 μ M).

The mechanical responses of cat colon circular muscle strips to ATP, 2-MeSATP, α,β -MeATP and β,γ -MeATP were studied at resting tension. At resting tension, purinoceptor agonists caused contractions of circular smooth muscle. Concentration-response curves to ATP, 2-MeSATP, α,β -MeATP and β,γ -MeATP were constructed. Purine nucleotides were consecutively applied at intervals of 20 min to

avoid desensitization. Concentration-response curves to ATP, 2-MeSATP, α,β -MeATP and β,γ -MeATP were constructed in the absence and presence of suramin, a P_{2X} -purinoceptor antagonist. Contractile responses to ATP and α,β -MeATP were studied in the absence and presence of reactive blue 2, a P_{2Y} -purinoceptor antagonist and 8-(*p*-sulphophenyl)-theophylline (8-SPT), a P_1 -purinoceptor antagonist. Each muscle strip served as its own control. Only one agonist was applied per muscle strip.

Some experiments examined the effects of ATP (10 μ M), α,β -MeATP (5 μ M), 2-MeSATP (5 μ M) and β,γ -MeATP (10 μ M) in muscle strips with raised tone. Noradrenaline (10–50 μ M) was added to the bathing solution to raise tone. The involvement of P_{2Y} -purinoceptors and P_1 -purinoceptors in relaxation was studied by examining responses to ATP (10 μ M) in the absence and presence of reactive blue 2 (100 μ M) and 8-SPT (50 μ M).

Data analysis

The amplitude of contractile responses to agonists for each muscle strip was measured and expressed either as a percentage of the maximal response or as percentage of the carbachol (10 μ M)-evoked contractile response. The concentration of agonists required to cause 50% of the maximal effect (EC₅₀ values) was calculated by linear regression of the relation between negative log₁₀ of the concentration of agonist and the percentage maximum response. Data obtained from responses between 20% and 80% of the maximum were used to fit linear regression lines for each concentration-response curve. Linear equations were used to calculate the slope and EC₅₀ value for each concentration-response curve. The correlation coefficients of the linear regression lines ranged between 0.91 and 0.99.

The antagonistic potency and the type of antagonism of prazosin (0.1–9 μ M) against noradrenaline and suramin (50–500 μ M) against α,β -MeATP were defined by Schild plot analysis (Arunlakshana & Schild, 1959). Concentration-response curves were constructed in the absence and in the presence of multiple concentrations of antagonists. The pA₂ values and slopes were calculated from regression lines of the relation between logarithmic concentration of antagonist and the logarithmic concentration ratio (DR-1).

The antagonistic actions of single concentrations of prazosin (0.1 μ M) against noradrenaline, adrenaline and phenylephrine and suramin (100 μ M) against α,β -MeATP, β,γ -MeATP, 2-MeSATP and ATP were also studied. Antagonist potency was expressed as the dissociation constant K_b , which was estimated from a single shift in the agonist concentration-response curves. The K_b values were calculated by the equation:

$$K_b = a/(DR-1)$$

where a is the antagonist concentration and DR is the ratio of the equieffective agonist concentration (at the EC₅₀ level) determined in the absence and presence of the antagonist (Kenakin, 1993).

The EC₅₀ slopes and K_b values were calculated for each muscle strip and data are presented as mean $\pm$ s.e. mean. The significance of differences was assessed by a Student's *t* test for paired and unpaired observations and *P* values of less than 0.05 were considered significant. The number of observations (*n*) are the number of muscle strips.

Drugs

Drugs used were: tetrodotoxin, acetylcholine chloride, carbamylcholine chloride (carbachol), adenosine hemisulphate, ($\pm$)-propranolol hydrochloride, prazosin hydrochloride, suramin hexasodium salt, (–)-noradrenaline bitartrate, (–)-phenylephrine hydrochloride, adenosine 5'-triphosphate disodium salt (ATP), α,β -methylene adenosine 5'-triphosphate lithium salt (α,β -methylene ATP), 2-methylthioadenosine 5'-triphosphate tetrasodium salt (2-MeSATP), β,γ -methylene

adenosine 5'-triphosphate sodium salt (β,γ -methylene ATP), adenosine hemisulphate salt and reactive blue 2 (cibacron blue 3GA). All drugs except 8-(*p*-sulphophenyl)-theophylline (Research Biochem. Inc.), 2-methylthioadenosine 5' triphosphate tetrasodium salt (Research Biochem. Inc.) and suramin hexasodium salt (a gift from Center for Disease Control; Atlanta, Georgia, U.S.A.) were obtained from the Sigma Chemical Co. Drugs were dissolved in distilled water and diluted to final concentrations in Krebs solution. Drug concentrations reported refer to final bath values.

Results

Responses of circular muscle to adrenoceptor agonists

Effects of noradrenaline at different tone levels At resting tension, noradrenaline (10–100 μM) caused concentration-dependent contractions of circular muscle (Figure 1b). The noradrenaline-evoked contractions were potentiated by tetrodotoxin (0.5 μM) and blocked by the α_1 -adrenoceptor agonist, prazosin (Figure 1a). Propranolol (3 μM) significantly ($P < 0.05$) potentiated the amplitude of noradrenaline-evoked con-

Figure 1 Cat mid-colon circular muscle strip *in vitro*. (a) Mechanical responses to noradrenaline (NA, 10 μM), at resting tension. Responses of one muscle strip in the absence and presence of tetrodotoxin (TTX, 0.5 μM) or TTX and prazosin (Praz, 1 μM). (b) Effect of propranolol on the contractile responses to noradrenaline, at resting tension. Abscissa scale, concentration of noradrenaline (μM). Ordinate scale, contractile responses expressed as a percentage relative to the contraction caused by carbachol (10 μM). Contractile responses to noradrenaline in the absence (●, $n = 10$) and presence of propranolol (1 μM , ○, $n = 6$). Noradrenaline was applied in cumulatively increasing concentrations. Effects of propranolol were significant (* $P < 0.05$, paired test). Mean values with s.e. mean are shown.

Figure 2 Cat mid-colon circular muscle strip *in vitro*. Mechanical responses to noradrenaline (NA, 10 μM) when tone was raised by carbachol (CCh, 10 μM). Responses of single muscle strip in the absence and in the presence of tetrodotoxin (TTX, 0.5 μM) or TTX and propranolol (Prop, 1 μM).

tractile responses (Figure 1b). When tone was raised by carbachol (1 μM), noradrenaline (0.1–10 μM) caused relaxation (Figure 2, for 10 μM noradrenaline). The relaxant responses to noradrenaline occurred in muscle strips pretreated with tetrodotoxin (0.5 μM) and were reduced by the β -adrenoceptor antagonist, propranolol (1 μM , $n = 6$). At resting tension, the inhibitory effect of noradrenaline is masked by the contraction caused by excitatory α_1 -adrenoceptors.

Potency of α -adrenoceptor agonists At resting tension, phenylephrine, noradrenaline and adrenaline (each at 0.1–1000 μM) caused concentration-dependent contractions in colon circular muscle strips whereas clonidine (1–1000 μM) did not. The amplitude of each agonist-evoked contraction was expressed as the percentage of the response to carbachol (10 μM). With low concentrations of agonists (0.1–10 μM), the amplitude of contractile responses to adrenaline were greater than those to noradrenaline and phenylephrine. This resulted in significant differences ($P < 0.05$) in the slopes of the linear regression lines between adrenaline and noradrenaline and between adrenaline and phenylephrine but not between noradrenaline and phenylephrine (Table 1). In contrast, EC_{50} values for phenylephrine, noradrenaline and adrenaline and the amplitude of the maximal responses were not significantly different (Table 1). This suggests that each adrenoceptor agonist has a similar potency for contracting circular smooth muscle.

Antagonist potency of prazosin The contractile responses to noradrenaline, adrenaline and phenylephrine were antagonized by prazosin. Increasing concentrations of prazosin (0.1, 1, 3, 9 μM) caused parallel shifts to the right of the concentration-response curves to noradrenaline (Figure 3a). The Schild plot analysis of the rightward shifts yielded a pA_2 value of 6.93 and a slope of 1.07 ± 0.03 (Figure 3b). The K_b values for prazosin, estimated from a single shift (0.1 μM prazosin) of the concentration-response curves to noradrenaline, adrenaline and phenylephrine were $92.8 \pm 9.3 \text{ nM}$ ($n = 8$), $108.7 \pm 6.4 \text{ nM}$ ($n = 6$) and $18.4 \pm 3.1 \text{ nM}$ ($n = 5$), respectively. The K_b value against phenylephrine was significantly lower than the K_b values against the other two adrenoceptor agonists. Also, there was no difference between the K_b values estimated against noradrenaline by Schild plot analysis and by the single shift of the concentration-response curves.

Responses of circular muscle to purinoceptor agonists

Effects of purinoceptor agonists at different tone levels The effects of ATP and ATP analogues were tested on the mechanical activity of colon circular muscle at resting tension and when tone was raised. At resting tension, the purinoceptor agonists evoked contractions that were resistant to tetrodotoxin (0.5 μM). They were reduced by the $\text{P}_{2\text{U}}$ -purinoceptor antagonist, suramin (100 μM , $n = 3$ for each agonist) (Figure 4a,b, for ATP and α,β -MeATP).

Table 1 Effects of adrenoceptor agonists on cat colon circular smooth muscle

	Maximal contraction (% of carbachol)	EC_{50} (μM)	Slope	r
Noradrenaline ($n = 14$)	66.0 ± 3.0	1.8 ± 0.2	46.0 ± 2.4	0.92–0.99
Adrenaline ($n = 10$)	72.2 ± 10.5	1.9 ± 0.4	$33.0 \pm 2.3^*$	0.91–0.92
Phenylephrine ($n = 12$)	56.1 ± 8.3	4.3 ± 1.7	46.1 ± 3.3	0.92–0.99

Values are means $\pm$ s.e., n = number of preparations.
 r = correlation coefficient of regression line.

*Significantly different from all others at $P < 0.05$.

Contractile responses to ATP (50–500 μM) and α,β -MeATP (0.5–5 μM) were also studied in the absence and presence of reactive blue 2 (100 μM) or 8-(*p*-sulphophenyl)-theophylline (8-SPT) (50 μM). In other visceral and vascular smooth muscle, similar concentrations of reactive blue 2 and 8-SPT antagonized the effect of ATP at P_{2Y} -purinoceptors (Burnstock & Warland, 1987; Hopwood & Burnstock, 1987; Reilly *et al.*, 1987) and the effect of adenosine at P_1 -purinoceptors (Kennedy, 1990; Matharu & Hollingsworth, 1992), respectively. In muscle strips, where tone was raised with noradrenaline, 8-SPT antagonized relaxations evoked by adenosine (1 μM , $n = 5$) (data not shown) but had no significant effect on contractions evoked by ATP (Figure 4c) or α,β -MeATP (Figure 4d). Reactive blue 2 significantly ($P < 0.05$) potentiated the responses to ATP but did not alter contractions to α,β -MeATP (Figure 4c,d).

When tone was raised by noradrenaline, ATP and 2-MeSATP caused relaxation. In the presence of TTX (0.5 μM), the ATP-evoked relaxation was reduced by reactive blue 2 (100 μM) and abolished by both reactive blue and 8-SPT (50 μM) (Figure 5a). Also, the ATP-evoked relaxant response

was resistant to suramin (100 μM , $n = 4$, data not shown). In contrast, α,β -MeATP and β,γ -MeATP induced low-amplitude contractile responses that were superimposed on the plateau of the noradrenaline-evoked contraction. The contractile responses to α,β -MeATP were resistant to TTX but were blocked by suramin (100 μM , $n = 5$) (Figure 5b).

Potency of P_2 -purinoceptor agonists At resting tension ATP (1–1000 μM), α,β -MeATP (0.05–10 μM), β,γ -MeATP (0.1–

Figure 3 Cat mid-colon circular muscle strip *in vitro*. (a) Effects of prazosin on the contractile responses to noradrenaline, at resting tension. Ordinate scale, responses expressed as a percentage relative to the maximum contraction. Abscissa scale, concentration of noradrenaline (μM). Contractile responses to noradrenaline in the absence ($\bullet$, $n = 22$) and presence of prazosin (0.1 μM , $\circ$, $n = 7$), (1 μM , ∇ , $n = 6$), (3 μM , $\square$, $n = 6$), (9 μM , Δ , $n = 5$). (b) Schild plot of the relation between $\log$ (agonist dose ratio – 1) and $\log$ molar concentration of the antagonist, prazosin. Noradrenaline was applied in cumulatively increasing concentrations. Mean values with s.e. mean are shown. The values of pA_2 and slope of the Schild plot are 6.9 and 1.07 ± 0.03 , respectively. The correlation coefficient of the regression line is 0.97.

Figure 4 Cat mid-colon circular muscle strip *in vitro*. Contractile responses to ATP and α,β -MeATP at resting tension. (a,b) Responses to ATP (10 μM) and α,β -MeATP (5 μM) were obtained in two different muscle strips. Contractile responses in the absence and presence of tetrodotoxin (TTX, 0.5 μM) or suramin (Sur, 100 μM). (c,d) Effects of 8-sulphophenyl-theophylline (8-SPT, 50 μM) and reactive blue 2 (100 μM) on the contractile responses to ATP and α,β -MeATP. Abscissa scale, agonist concentration, (μM). Ordinate scale, contractile responses expressed as a percentage of the contraction caused by carbachol (10 μM). (c) Responses to ATP in the absence ($\bullet$, $n = 8$) and presence of 8-SPT (∇ , $n = 4$) or reactive blue 2 ($\circ$, $n = 4$). (d) Responses to α,β -MeATP in the absence ($\bullet$, $n = 8$) and presence of 8-SPT (∇ , $n = 4$) or reactive blue 2 ($\circ$, $n = 4$). Mean values with s.e. mean are shown. Effects of reactive blue 2 were significant ($*P < 0.05$, paired test).

100 μM) and 2-MeSATP (1–500 μM) caused concentration-dependent contractions of circular muscle. The concentration-response curves for each agonist were sigmoidal with different maxima. The maximal responses to α, β -MeATP and

Figure 5 Cat mid-colon circular muscle strip *in vitro*. Mechanical responses to ATP (10 μM) and α, β -MeATP (MeATP, 5 μM) when tone was raised by noradrenaline (NA, 10 μM). Responses to ATP (a) and α, β -MeATP (b) were recorded in two different muscle strips in the absence and in the presence of tetrodotoxin (TTX, 0.5 μM), suramin (Sur, 100 μM), reactive blue 2 (RB, 100 μM) or reactive blue 2 and 8-sulphophenyl-theophylline (SPT, 50 μM).

Figure 6 Cat mid-colon circular muscle strip *in vitro*. (a) Effect of suramin on the contractile responses to α, β -MeATP. (a) Abscissa scale, concentration of α, β -MeATP (μM). Ordinate scale, responses expressed as a percentage relative to the maximum contraction. Contractile responses in the absence of suramin ($\bullet$, $n = 16$), and in the presence of suramin (50 μM , $\circ$, $n = 5$), (100 μM , ∇ , $n = 6$), (500 μM , $\square$, $n = 5$). (b) Schild plot of the relation between $\log$ (agonist dose ratio - 1) and $\log$ molar concentration of the antagonist, suramin. α, β -MeATP was applied non-cumulatively. The values of pA_2 and slope of the Schild plot are 4.52 and 1.06 ± 0.34 , respectively. The correlation coefficient of the regression line is 0.95. Mean values with s.e. mean are shown.

β, γ -MeATP did not differ from one another but were significantly greater than maximal responses evoked by ATP and 2-MeSATP (Table 2). The order of potency for contractile responses was α, β -MeATP $>$ β, γ -MeATP $>$ 2-MeSATP $>$ ATP. There were no significant differences in the slopes of the linear regression lines (Table 2).

Antagonist potency of suramin The contractile responses to ATP and the ATP analogues were antagonized by suramin (100 μM). At this concentration, suramin is a selective P_{2X} -purinoceptor antagonist in guinea-pig vas deferens (Dunn & Blakely, 1988), submucosal arterioles (Evan & Surprenant, 1992) and urinary bladder (Hoyle *et al.*, 1990). The concentration-effect curves to α, β -MeATP were shifted to the right by increasing concentrations of suramin (50–500 μM) (Figure 6a). The Schild plot analysis of the rightward shifts yielded a slope of 1.06 ± 0.3 and a pA_2 value of 4.45 ($K_b = 30.6 \mu\text{M}$) (Figure 6b). The K_b values for suramin, estimated from single shifts (100 μM suramin) of the concentration-effect curves for α, β -MeATP, β, γ -MeATP, 2-MeSATP and ATP, were $25.2 \pm 4.5 \mu\text{M}$ ($n = 5$), $21.7 \pm 9.9 \mu\text{M}$ ($n = 6$), $11.5 \pm 2.7 \mu\text{M}$ ($n = 3$) and $52.3 \pm 10.2 \mu\text{M}$ ($n = 5$), respectively. The K_b value against ATP was significantly ($P < 0.05$) higher when compared to the K_b values for suramin against the ATP analogues. There were no significant differences between K_b values for suramin calculated against α, β -MeATP and β, γ -MeATP. Also, there were no significant differences between K_b values for suramin against α, β -MeATP calculated by either Schild plot analysis or by a single-shift of the concentration-response curves.

Discussion

The data show that the mechanical responses of cat colon circular muscle to noradrenaline and ATP involve postjunctional α_1 -adrenoceptors, β -adrenoceptors, P_{2X} -purinoceptors and P_{2Y} -purinoceptors. Postjunctional adrenoceptors and purinoceptors in cat colon circular muscle were characterized by agonist potency and the effects of antagonists.

Effects of noradrenaline at different tone levels

The responses of colon circular smooth muscle to noradrenaline were dependent on tone. At resting tension, noradrenaline caused contractions that were potentiated by propranolol, a selective β -adrenoceptor antagonist and blocked by prazosin, a selective α_1 -adrenoceptor antagonist whereas with raised tone, noradrenaline caused relaxation blocked by propranolol. The α - and β -adrenoceptors were considered postjunctional, because contractions or relaxations evoked by noradrenaline occurred in the presence of tetrodotoxin. The

Table 2 Effects of purinoceptor agonists on cat colon circular smooth muscle

	Maximal contraction (% of carbachol)	EC_{50} (μM)	Slope	r
α, β -MeATP ($n = 9$)	94.3 ± 8.0	$0.7 \pm 0.1^*$	$39.5 \pm 0.9^*$	0.91–0.99
β, γ -MeATP ($n = 9$)	98.5 ± 9.5	$7.6 \pm 0.1^*$	46.2 ± 8.4	0.93–0.99
2-MeSATP ($n = 6$)	65.3 ± 10.8	$25.3 \pm 12.8^*$	50.4 ± 2.4	0.94–0.99
ATP ($n = 14$)	70.8 ± 8.6	$60.5 \pm 15.9^*$	48.6 ± 3.9	0.93–0.99

Values are means $\pm$ s.e., n = number of preparations.

r = correlation coefficient of regression lines.

*Significantly different from all others at $P < 0.05$.

inhibitory β -adrenoceptors present on cat colon circular muscle are the β_2 -subtype (Ek & Lundgren, 1982; Ek *et al.*, 1987). In rat and human isolated colon smooth muscle strips, β -adrenoceptor blocking agents inhibit relaxations to adrenaline and unmask contractile responses (Gagnon & Belisle, 1970; Gagnon *et al.*, 1972).

Characteristics of postjunctional α_1 -adrenoceptors

At resting tension, postjunctional α_1 -adrenoceptors that mediate contractions were characterized in cat colon circular muscle by the potency of adrenoceptor agonists and the antagonist effects of prazosin. There were no significant differences among the EC_{50} values for the contractile effects of noradrenaline, adrenaline and the selective α_1 -adrenoceptor agonist phenylephrine. In guinea-pig terminal ileum (Bauer, 1981), the EC_{50} values, calculated for the contractile effects of noradrenaline (7.32 μ M; 5.24–10.47 μ M), adrenaline (2.56 μ M; 1.91–3.40 μ M) and phenylephrine (5.23 μ M; 3.02–9.13 μ M), were similar to the EC_{50} values we obtained in the present study. Also, there were no differences between the slopes of the regression lines for noradrenaline and phenylephrine, while the slope of the regression line for adrenaline was significantly different. At low concentrations, adrenaline evoked contractile responses which are greater than those evoked by either phenylephrine or noradrenaline. This higher sensitivity to low concentrations of adrenaline may represent its interaction with adrenoceptors that do not have an innervation (extrajunctional location) and are activated by circulating catecholamines (Illes, 1983).

Prazosin antagonized the contractile effects of adrenoceptor agonists. The Schild analysis of the rightward shifts of the concentration-effect curves to noradrenaline yielded a slope that was not different from unity and a pA_2 value of 6.93. This pA_2 value is similar to that obtained against noradrenaline in human saphenous vein (pA_2 value = 6.45, Connaughton & Docherty, 1989). Also, the K_b value, against phenylephrine, was significantly lower than the K_b values against noradrenaline and adrenaline. This suggests that noradrenaline and adrenaline activate adrenoceptors less sensitive to prazosin. In rat tail artery, two subtypes of smooth muscle α_1 -adrenoceptors have been proposed. One type was neurally innervated and exhibited a high affinity (low K_b value) for prazosin while the other was not neurally innervated (extrajunctional location) and exhibited a lower affinity (high K_b value) for prazosin (Medgett & Langer, 1984).

Effects of ATP and analogues of ATP at different tone levels

At resting tension, all purinoceptor agonists caused only contractions of circular smooth muscle, mediated by suramin-sensitive P_{2X} -purinoceptors. At raised tone ATP and 2-MeSATP caused only relaxations whereas α, β -MeATP and β, γ -MeATP, specific P_{2X} -purinoceptor agonists (Kennedy, 1990), caused only contractions. Both the contractions and relaxations to exogenous ATP are due to activation of postjunctional purinoceptors, since they were resistant to tetrodotoxin.

Characteristics of postjunctional P_{2X} -purinoceptors

The data show that cat colon circular muscle has P_{2X} -purinoceptors which mediate contractions. At resting tension, these receptors were characterized by the potency of purinoceptor agonists and the antagonist effects of suramin. The potency order was: α, β -methylene ATP > β, γ -methylene ATP > 2-methylthio ATP > ATP. This suggests the presence of P_{2X} -purinoceptors. A similar potency order of purinoceptor agonists to contract smooth muscle via P_{2X} -purinoceptors occurs in urinary bladder (Hoyle *et al.*, 1990) and in a

number of blood vessels (see Burnstock, 1991). However, in the present study, receptor classification based solely on agonist potency may be limiting. For example, the differences in potency as well as the maximal responses could occur because α, β -MeATP and β, γ -MeATP caused only contractions at resting tension and at raised tone whereas 2-MeSATP and ATP causes contractions and relaxation at resting tension and raised tone, respectively. Also differences in potency we report could reflect different rates of the metabolism of ATP and its analogues. Similar data were reported in the rat mesenteric artery (Burnstock & Warland, 1987).

Suramin antagonized the contractile responses to all the purinoceptor agonists. The Schild analysis of the rightward shifts of the concentration-effect curves to α, β -MeATP indicated that suramin is a competitive antagonist of α, β -MeATP-evoked contractile responses in colon circular muscle. Suramin is also a competitive antagonist of contractions to α, β -methylene ATP and ATP in mouse vas deferens (von Kugelgen *et al.*, 1990) and in rabbit ear artery (Leff *et al.*, 1990).

The P_2 -purinoceptor agonists showed different sensitivities to suramin. ATP had a lower sensitivity (high K_b value) to suramin than did the analogues of ATP, suggesting that ATP activates a suramin-insensitive component of the P_{2X} -purinoceptor (von Kugelgen *et al.*, 1990) and/or another nucleotide receptor (O'Connor *et al.*, 1991). The higher sensitivity of 2-MeSATP evoked contractions to suramin may reflect a predominant effect of 2-MeSATP on the P_{2Y} -purinoceptor. P_2 -purinoceptors mediating biphasic muscle effects that depend on smooth muscle tone occur in rat gastric fundus (Lefebvre & Burnstock, 1990). In contrast, in cat colon, ATP and 2-MeSATP evoked either contraction at resting tension or relaxation at high tone.

Postjunctional P_{2Y} - and P_1 -purinoceptors

The relaxation responses to ATP were mediated by reactive blue-sensitive P_{2Y} -purinoceptors since they were resistant to suramin and reduced approximately 80–90 percent by reactive blue 2 (100 μ M), a selective inhibitor of relaxation responses mediated by P_{2Y} -purinoceptors. Also, we surmise that a small component of the ATP-evoked relaxation is due to activation of P_1 -purinoceptors by the hydrolytic breakdown of ATP to adenosine because it was inhibited by the addition of 8-SPT. A similar antagonism of ATP-evoked inhibitory responses by reactive blue 2 (100 μ M) occurs in rat gastric fundus (Lefebvre & Burnstock, 1991), duodenum (Manzini *et al.*, 1985) and caecum (Manzini *et al.*, 1986) and in rabbit mesenteric and ear arteries (Kennedy & Burnstock, 1985; Burnstock & Warland, 1987). Furthermore, the antagonism of ATP-evoked relaxations by reactive blue 2 and 8-SPT in the cat colon appears specific, because neither reactive blue 2 nor 8-SPT altered contractile responses to α, β -MeATP at resting tension, whereas the contractile responses to ATP were significantly potentiated by reactive blue 2 and slightly increased by 8-SPT. These results suggest that the contractile responses to α, β -MeATP do not involve the P_{2Y} -purinoceptor whereas ATP causes contractile and relaxant responses via P_{2X} - and P_{2Y} -purinoceptors, respectively. In mouse urinary bladder (Boland *et al.*, 1993) and guinea-pig coronary vessels (Hopwood & Burnstock, 1987), ATP causes contractile and relaxant responses mediated by P_{2X} - and P_{2Y} -purinoceptors, respectively.

In summary, we have characterized postjunctional α_1 -adrenoceptors and P_{2X} -purinoceptors that mediate colonic contractions. In addition, postjunctional β -adrenoceptors and P_{2Y} -purinoceptors mediate muscle relaxation. It is likely that all the post-junctional receptors are activated by the co-release of ATP and noradrenaline from lumbar sympathetic nerves (Venkova & Krier, 1993). We speculate that the lumbar sympathetic nerves regulate colonic tone and phasic contractions *in vivo* by activating both postjunctional receptors

and the prejunctional receptors (Vizi *et al.*, 1991) which modulate cholinergic and noradrenergic, noncholinergic neurotransmission.

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