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Pre- and postjunctional actions of purine and xanthine compounds in the guinea-pig caecum circular muscle

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1 The sucrose-gap technique was used to study pre- and postjunctional actions of P_1-purinoceptor and P_2-purinoceptor agonists and a range of xanthine derivatives in the guinea-pig caecum circular muscle.

2 Adenosine, 2-chloroadenosine (2-ClAd), ATP and α,β-methylene ATP all caused concentration-dependent hyperpolarization of the smooth muscle membrane with a rank order of potency of 2-ClAd > α,β-methylene ATP > adenosine.

3 The xanthine derivatives caffeine, theophylline, 8-phenyltheophylline and 1,3-dipropyl-8-(2-amino-4-chlorophenyl) xanthine (PACPX) at submicromolar concentrations evoked depolarization of the smooth muscle membrane. At higher concentrations, all these compounds and enprofylline caused concentration-dependent hyperpolarization.

4 All the purine compounds tested caused a reduction in the amplitude of the non-adrenergic, non-cholinergic inhibitory junction potential (i.j.p.). For the P_1-purinoceptor agonists adenosine and 2-ClAd this was almost entirely a prejunctional effect. For the P_2-purinoceptor agonists this was mostly a postjunctional effect because both ATP and α,β-methylene ATP caused significantly greater increases in the conductance of the smooth muscle membrane than did adenosine or 2-ClAd.

5 All the xanthine compounds tested (up to 100 μM), except enprofylline, were capable of increasing the amplitude of the i.j.p. At millimolar concentrations both caffeine and theophylline could reduce the i.j.p. amplitude.

6. It is concluded that there are inhibitory prejunctional P_1-purinoceptors on the i.j.p.-producing neurones in the guinea-pig caecum circular muscle and that, of the xanthine derivatives tested, none of them would be suitable to use as a P_1-purinoceptor antagonist in this preparation because of their own direct effects.

Introduction

Adenosine and adenosine triphosphate (ATP) elicit relaxant responses in many gastrointestinal smooth muscles, following hyperpolarization of the smooth muscle membrane, resulting from activation of extracellular purinoceptors (Axelsson & Holmberg, 1969; Baer & Müller, 1983; Ferrero & Frischknecht, 1983; Burnstock *et al.*, 1984). Adenosine and ATP preferentially activate P_1- and P_2-purinoceptors, respectively (Burnstock, 1978).

Considerable evidence has accumulated, showing that neuromuscular transmission in many tissues can be modulated by activation of prejunctional purinoceptors (Paton, 1981; Baer & Müller, 1983). For example, prejunctional P_1-purinoceptors have been described which mediate inhibition of noradrenaline release from sympathetic nerves supplying the canine saphenous vein (De Mey *et al.*, 1979), cholinergic transmission in the guinea-pig ileum (Moody & Burnstock, 1982) and at the neuromuscular junction on frog skeletal muscle (Ribeiro & Sebastião, 1985), and inhibition of parasympathetic transmission in the urinary bladder (Gustaffson *et al.*, 1978) and of sympathetic transmission in the vas deferens (Sneddon *et al.*, 1984).

Alkylxanthines in general are known to act as P_1-purinoceptor antagonists in a variety of tissues

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(see Burnstock, 1978; Rall, 1980; Daly et al., 1981; Berne et al., 1983) but some arylxanthines, notably 8-phenyltheophylline (8-PT) and 1,3-dipropyl-8-(2-amino-4-chlorophenyl) xanthine (PACPX) are more potent (Smellie et al., 1979; Bruns, 1981; Griffith et al., 1981; Bruns et al., 1983; Burnstock & Hoyle, 1985). However, 3-propylxanthine (enprofylline) is almost devoid of antagonistic activity at P_1-purinoceptors (Lunell et al., 1982; Fredholm & Persson, 1982; Persson, 1982; 1984; Vinge et al., 1984).

The aim of this study was to investigate the post-junctional actions of P_1-purinoceptor agonists (adenosine and stable analogue 2-chloroadenosine) and P_2-purinoceptor agonists (ATP and stable analogue α,β-methylene ATP) in the guinea-pig caecum circular muscle, and to ascertain whether there is a purinoceptor-mediated modulation of non-adrenergic, non-cholinergic inhibitory neuromuscular transmission in the tissue (Ito & Kuriyama, 1973). The actions of a selection of xanthine compounds: caffeine, theophylline, 8-PT, PACPX and enprofylline were also investigated. This selection includes potent and impotent P_1-purinoceptor antagonists.

Methods

Preparation of material

Guinea-pigs of either sex (400–850 g) were killed by stunning and bleeding. A ventral mid-line incision was made, the caecum was exposed, freed from mesentery, and a segment approximately 5 cm long, from the middle, was removed. The segment was opened up along the mesenteric taenia, washed out very briefly under a cold tap and then rinsed several times in cold Krebs solution of the following composition (mM): NaCl 133, KCl 4.7, $MgSO_4$ 0.6, NaH_2PO_4 1.3, $NaHCO_3$ 16.3, $CaCl_2$ 2.5 and glucose 7.7 (Bülbring, 1953); atropine (3×10^{-7} M) and guanethidine (2×10^{-6} M) were also included in order to inhibit expression of cholinergic and adrenergic neurotransmission. The caecum wall was pinned flat, mucosal surface down, onto a wax tray and kept immersed in Krebs solution. Strips of circular muscle 200–500 μm wide were dissected free from the mucosa, parallel to the direction of the muscle fibres, from between the ventral and medial taenia, and were 10–15 mm long. The prepared muscle strips were allowed to equilibrate in Krebs solution at room temperature for at least 2 h before being mounted in the sucrose gap apparatus. The Krebs solution was gassed continually with 95% O_2/5% CO_2, bringing the pH to 7.35 ± 0.05.

Sucrose-gap apparatus

The apparatus used was a rubber-membrane type of single sucrose-gap (Artemenko et al., 1982; Hoyle, 1987). A multiport tap allowed the superfusing solution to be changed rapidly from Krebs to a drug-containing solution. Superfusates were gassed continuously, and the superfusate temperature around the preparation was 35 ± 1°C although temperature fluctuation during any one experiment was ± 0.5°C. Preparations were allowed to stabilize before drugs were applied, usually for about 15 min. In the presence of atropine (0.3 μM) and guanethidine (2 μM) single pulse field stimulation (0.5 ms, 14 V) evoked a non-adrenergic, non-cholinergic neurally-mediated transient membrane hyperpolarization, i.e. an inhibitory junction potential (i.j.p.). Stimulus strength was adjusted to give i.j.ps of about 80–90% maximum amplitude. Preparations in which the i.j.p. did not initially develop to 7 mV were discarded.

Drug-containing superfusates were applied until a maximum response had been reached (generally about 2 min) and applications were followed by washout with Krebs solution until control levels had been regained or, in some experiments, by replacement with a different drug-containing solution.

Analysis of results

Because the i.j.p. amplitude is linearly dependent upon membrane potential, within the range of membrane potentials encountered in these experiments, it is possible to use regression analysis to separate drug-induced modulatory effects on i.j.p. amplitude from changes in i.j.p. amplitude due to changes in membrane potential (Hoyle, 1987; Reilly et al., 1987). For inhibitory responses, the maximum hyperpolarization was measured, along with the time taken to reach half maximum hyperpolarization ($t_{1/2}$) and the rate of hyperpolarization. The rate constant of hyperpolarization was calculated from $1/2t_{1/2}$ or from rate of hyperpolarization/amplitude of hyperpolarization. This derived value is therefore normalized and states the number of maximum hyperpolarizations which could occur in a unit of time.

The calculated regressions of i.j.p. amplitude on change in membrane potential were tested for significance and the constant of the derived linear equation was evaluated. The value of this constant is effectively an extrapolation of the regression line to a zero change in membrane potential, i.e. it is a theoretical value for the amplitude of an i.j.p. evoked at the resting membrane potential but in the presence of an applied drug. Using this method of analysis, the i.j.p. amplitude becomes emancipated from its dependency on membrane potential.

Changes in membrane resistance were measured as the percentage change in amplitude between control and test analectrotonic potentials, and are expressed relative to the change in membrane potential at which the test potential was evoked.

Drugs and solvents

Adenosine, ATP (Na salt), α,β-methylene ATP (α,β-Me ATP; Li salt), 2-chloroadenosine (2-ClAd), caffeine (1,3,7-trimethylxanthine) and theophylline (1,3-dimethylxanthine) were obtained from Sigma. 8-Phenyltheophylline (8-PT) was obtained from Calbiochem. 1,3 - Dipropyl - 8 - (2 - amino - 4 - chlorophenyl) xanthine(PACPX) was generously donated by Warner-Lambert Co., USA. Enprofylline (3-propylxanthine) was generously donated by courtesy of Dr C.G.A. Persson from AB-Draco, Sweden. Atropine sulphate was obtained from Antigen and guanethidine monosulphate (Ismelin) from Boehringer Ingelheim.

8-PT and PACPX were dissolved in 80% methanol/20% molar NaOH (v/v) to produce stocks of 10^{-2} M and were subsequently diluted with distilled water or fresh Krebs solution. The other xanthines were dissolved in distilled water to produce stock solutions that were subsequently diluted in fresh Krebs solution. Solvent control experiments were performed with methanolic NaOH and no part of the response to 8-PT or PACPX was due to the solvent.

Both 8-PT and PACPX precipitate out of Krebs solution above 10^{-5} M but PACPX at 10^{-4} M will stay in solution for a short period of time at high pH. Therefore, in the case of PACPX alone (10^{-4} M) the Krebs solution was titrated to pH 8.0 with NaOH.

Results

Effects of purine compounds on the membrane potential

ATP (10–1000 μM), α,β-Me ATP (10–100 μM), adenosine (10–1000 μM) and 2-ClAd (0.05–100 μM) all produced concentration-dependent hyperpolarization of the circular smooth muscle of the guinea-pig caecum (Table 1, Figure 1). The rate constant for induced hyperpolarizations is not dependent on absolute values of hyperpolarization (unlike the rate of hyperpolarization) and is therefore more meaningful when comparing the actions of different drugs (Table 1).

From these results it is apparent that ATP hyper-

Table 1 Effects of purine nucleosides and nucleotides on membrane potential in guinea-pig caecum circular muscle

Agent	*Concentration* (μM)	*Hyperpolarization* (mV)	*Rate of hyperpolarization* (mV s^{-1})	*Rate constant of hyperpolarization* (K s^{-1})
ATP	10	2.3 ± 0.20 (5)	0.20 ± 0.031 (5)	37 ± 5.2 (5)
	50	5.5 ± 0.69 (13)	0.48 ± 0.056 (4)	69 ± 13.1 (4)
	100	6.4 ± 0.56 (16)	0.44 ± 0.081 (9)	72 ± 10.5 (9)
	500	9.3 ± 0.60 (13)	0.68 ± 0.128 (9)	75 ± 10.6 (9)
	1000	13.2 ± 1.59 (5)	1.13 ± 0.193 (5)	86 ± 13.1 (5)
α,β-Me ATP	1	1.8 ± 0.21 (16)		
	5	3.2 ± 0.36 (11)	0.15 ± 0.021 (9)	32 ± 4.5 (9)
	10	8.8 ± 0.71 (15)	0.44 ± 0.245 (3)	40 ± 18.6 (3)
	50	8.4 ± 0.47 (20)	0.36 ± 0.062 (7)	45 ± 9.4 (7)
	100	8.6 ± 0.61 (9)	0.32 ± 0.022 (9)	33 ± 3.4 (9)
Adenosine	10	3.5 ± 0.49 (9)	0.08 ± 0.030 (6)	18 ± 3.5 (6)
	50	6.1 ± 1.05 (8)	0.09 ± 0.019 (6)	25 ± 4.5 (6)
	100	8.3 ± 0.37 (22)	0.36 ± 0.048 (17)	44 ± 5.2 (17)
	500	7.8 ± 0.92 (4)	–	–
	1000	10.9 ± 0.40 (22)	0.41 ± 0.021 (17)	38 ± 1.6 (17)
2-ClAd	0.5	2.8 ± 1.25 (3)		
	1	2.9 ± 1.42 (3)		
	5	6.1 ± 0.72 (4)		
	10	8.7 ± 1.45 (5)	0.27 ± 0.060 (5)	29 ± 3.6 (5)
	100	8.8 ± 0.96 (7)	0.32 ± 0.035 (6)	43 ± 4.9 (6)

All values are mean ± s.e.mean (n).
α,β-Me ATP = α,β-methylene ATP; 2 ClAd = 2 chloradenosine.

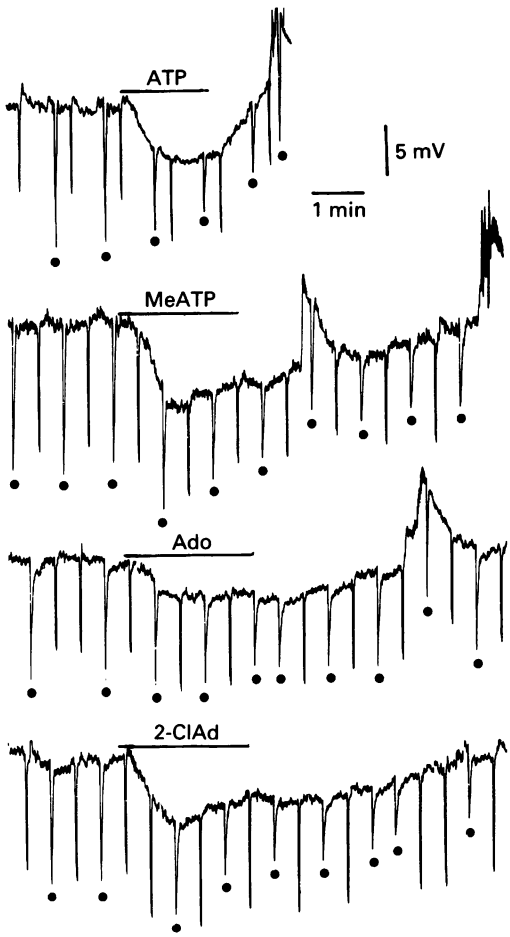

Figure 1 Guinea-pig caecum circular muscle: effects of purine compounds on membrane potential. Inhibitory junction potentials (i.j.ps) were evoked by single pulses of field stimulation 0.5 ms, submaximal voltage (●). Between junction potentials, analectrotonic potentials were evoked by passing pulses of constant current (1 s, 15–25 μA). In all panels, drug-containing Krebs solution was applied as indicated and was washed out following the maximum level of response. ATP, 100 μM, caused a rapid hyperpolarization during which the i.j.p. amplitude was reduced more than can be accounted for by the change in membrane potential. α,β-Methylene ATP, 100 μM, (MeATP), caused hyperpolarization and the i.j.p. amplitude decreased. Note the change in membrane resistance, monitored by the analectrotonic potentials and also note that the first i.j.p. evoked in the presence of MeATP is large. Adenosine, (Ado, 100 μM), caused hyperpolarization and markedly reduced the i.j.p. amplitude but with no change in membrane resistance. 2-Chloroadenosine (2Cl-Ad, 100 μM) was more potent than adenosine at inducing a membrane hyperpolarization, and at this concentration had a large effect on i.j.p. amplitude yet little effect on membrane resistance.

polarizes the smooth muscle membrane at a faster rate and with a higher rate constant than any of the other agents tested and that the synthetic analogues are more potent than their natural counterparts, although they have slower rates and smaller rate constants at equimolar concentrations (Table 1). For any single preparation the rank order of agonist potency at inducing hyperpolarization was 2 ClAd > α,β-Me ATP > ATP > adenosine.

Hyperpolarization of the smooth muscle membrane induced by ATP and α,β-Me ATP was accompanied by a decrease in membrane conductance. At 100 μM, ATP and α,β-Me ATP caused a significant decrease in amplitude of applied analectrotonic potentials of 6.5 ± 1.45 ($n = 16$) and 5.3 ± 1.28 ($n = 17$) % per mV of hyperpolarization, respectively (both values $P < 0.001$). In contrast, adenosine and 2-ClAd, both at 100 μM, caused reductions in amplitude of analectrotonic potentials of 2.6 ± 1.9 ($n = 18$) and 3.0 ± 1.38 ($n = 12$) % per mV of hyperpolarization, respectively.

Effects of xanthine derivatives on membrane potential

Caffeine (1–1000 μM), theophylline (1–1000 μM), 8-PT (0.01–10 μM), PACPX (0.1–100 μM) and enprofylline (0.01–100 μM) all induced concentration-dependent hyperpolarizations of the circular muscle of the guinea-pig caecum (Figure 2; Table 2). At their lower concentrations, all the compounds except enprofylline induced small transient depolarizations which preceded either a return to the resting membrane potential or a sustained hyperpolarization. At higher concentrations there were no depolarizations. The rates of hyperpolarization for all the compounds were slow and concentration-dependent, and the rate constants were low (Table 2). For equi-effective concentrations of all the compounds, there were no significant differences between either the rates of hyperpolarization or rate constants of hyperpolarization. The order of potency for inducing hyperpolarization was 8-PT = enprofylline > theophylline > caffeine > PACPX. Because of the limited solubility of these compounds, especially 8-PT, PACPX and enprofylline, this rank order of potency was based on a concentration range of 1–10 μM rather than a comparison between given levels of effective concentrations. Time courses for responses, as is indicated by the rates and rate constants of hyperpolarization, were long and maximum responses took up to 5 min to develop.

None of the xanthines at any concentration, except for caffeine at 1000 μM, had any effect on membrane resistance. However, at 1000 μM, caffeine caused a decrease in amplitude of applied analectrotonic potential of 6.0 ± 1.18 ($n = 8$) % per mV hyperpolarization.

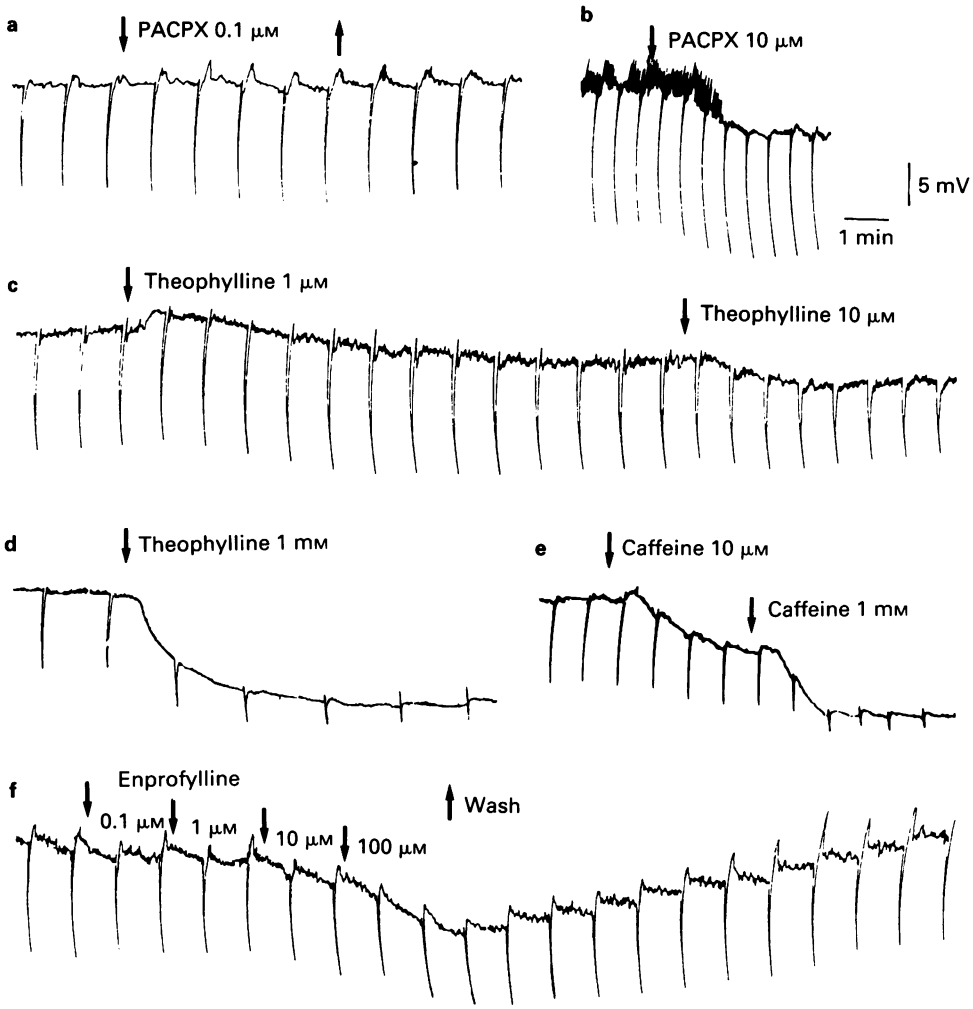

Figure 2 Guinea-pig caecum circular muscle: effects of xanthine compounds on membrane potential and i.j.ps which were evoked at regular intervals (0.5 ms, submaximal voltage). (a) 1,3-Dipropyl-8-(2-amino-4-chlorophenyl) xanthine (PACPX) at 0.1 μM had little effect on membrane potential in this preparation but enhanced the amplitude of the i.j.p. (b) At a higher concentration of 10 μM, PACPX caused hyperpolarization accompanied by abolition of the spontaneous excitatory activity. (c, d) Theophylline, at a low concentration of 1 μM, evoked a transient depolarization while, at higher concentrations, it evoked hyperpolarization. Note the appearance of a small excitatory response which precedes the final i.j.ps in (d). Enhancement of i.j.p. amplitude is particularly evident at the lowest concentration shown here. (e) Caffeine caused concentration-dependent hyperpolarization and marked reduction of i.j.p. amplitude at 1 μM. (f) Enprofylline caused concentration-dependent hyperpolarization without marked change in i.j.p. amplitude.

Effects of purine compounds on the inhibitory junction potential

During a hyperpolarization induced by ATP, adenosine, α,β-Me ATP or 2-ClAd, the amplitudes of evoked i.j.ps decreased (Figure 1).

In the presence of adenosine above 50 μM, ATP and α,β-Me ATP at or above 50 μM, or 2-ClAd above 10 μM, the i.j.p. constant was significantly less than 1 (Figure 3; Table 3). This implies that all these purine compounds could reduce the amplitude of the i.j.p. in a manner not directly related to the extent of induced hyperpolarization. However, for α,β-Me ATP at 1 μM or above, the first i.j.p. evoked in its

Table 2 Effects of xanthine derivatives on membrane potential in guinea-pig caecum circular muscle

Xanthine	*Concentration* (μM)	*Hyperpolarization* (mV)	*Rate of hyperpolarization* ($\mu V s^{-1}$)	*Rate constant of hyperpolarization* ($K s^{-1}$)
Caffeine	1	0.4 ± 0.20 (7)	–	–
	10	3.8 ± 0.72 (4)	19.4 ± 6.69 (4)	6.6 ± 0.60 (4)
	100	5.4 ± 2.68 (4)	65.7 ± 12.39 (4)	12.4 ± 2.45 (4)
	1000	9.5 ± 1.49 (8)	83.9 ± 12.74 (5)	11.1 ± 1.52 (5)
Theophylline	1	0 (7)	–	–
	10	2.0 ± 0.86 (5)	–	–
	100	5.8 ± 0.66 (5)	27.8 ± 4.39 (5)	4.8 ± 0.40 (5)
	1000	9.5 ± 0.69 (12)	121.8 ± 14.70 (11)	13.2 ± 0.43 (11)
8-Phenyl-theophylline	0.01	0 (7)	–	–
	0.1	2.0 ± 0.65 (9)	24.4 ± 3.26 (6)	7.5 ± 1.16 (6)
	1	3.8 ± 0.52 (7)	33.4 ± 3.65 (5)	9.4 ± 1.59 (5)
	10	7.3 ± 0.52 (7)	53.0 ± 8.99 (6)	7.2 ± 0.86 (6)
PACPX	0.1	0 (7)	–	–
	1	0.8 ± 0.28 (6)	–	–
	10	4.3 ± 0.45 (7)	37.3 ± 5.79 (6)	9.2 ± 0.68 (6)
	100	6.3 ± 0.59 (7)	57.2 ± 9.10 (5)	10.2 ± 1.90 (5)
Enprofylline	0.01	0.7 ± 0.25 (9)	–	–
	0.1	1.7 ± 0.55 (9)	18.3 ± 0.68 (4)	9.9 ± 2.30 (4)
	1	3.0 ± 0.54 (10)	36.8 ± 4.43 (5)	13.9 ± 1.16 (5)
	10	4.2 ± 0.61 (13)	47.4 ± 14.00 (5)	10.4 ± 1.82 (5)
	100	6.7 ± 0.59 (18)	63.2 ± 9.83 (11)	9.6 ± 0.84 (11)

All values are mean ± s.e.mean (n).
PACPX = 1,3-dipropyl-8-(2-amino-4-chlorophenyl)xanthine.

presence was often enlarged, despite a decrease in membrane resistance (e.g. Figure 1).

In one series of experiments the concentrations of agonists were balanced so that they were equi-effective at hyperpolarizing the smooth muscle (Figure 4), and in another series of experiments equi-effective concentrations of α,β-Me ATP and adenosine were mixed in various ratios (Figure 5). These experiments enabled the effects of agents on i.j.ps to be compared while the membrane potential remained unaltered. For equi-effective hyperpolarizing concentrations the rank order of potency for reducing i.j.p. amplitude was adenosine > 2-ClAd > ATP > α,β-Me ATP.

Effects of xanthine derivatives on the inhibitory junction potential

Except for caffeine at 1000 μM, the presence of any of the xanthine compounds did not cause a reduction in i.j.p. constant. In fact the i.j.p. constant became either greater than 1 or was equal to 1 (Figure 3; Table 4). At 1000 μM, caffeine caused a significant reduction in the i.j.p. constant (Table 4).

Discussion

Effects of ATP, α,β-methylene ATP, adenosine and 2-chloroadenosine

All the P_1- and P_2-purinoceptor agonists used caused concentration-dependent hyperpolarizations of the smooth muscle membrane. For the P_2-purinoceptor agonists, although the rates of hyperpolarization and the related rate constants induced by ATP were faster than those induced by α,β-Me ATP, the analogue of ATP was more potent at inducing hyperpolarization of the membrane. The same picture was true for the P_1-purinoceptor agonists. The P_1-purinoceptor agonists at equi-effective hyperpolarizing concentrations reduced the i.j.p. amplitude by the same extent, whereas for the P_2-purinoceptor agonists, α,β-Me ATP was more potent than ATP at increasing the membrane potential, but was much less potent than an equi-effective ATP concentration at reducing the i.j.p. This may be because ATP can be broken down to adenosine before having a prejunctional action while α,β-Me ATP would be resistant to such degradation (Moody & Burnstock, 1982).

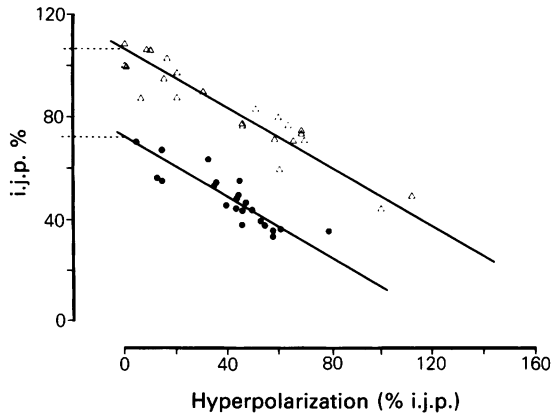

Figure 3 Guinea-pig caecum circular muscle: effects of adenosine and 8-phenyltheophylline on i.j.p. amplitude. Regression of i.j.p. amplitude on hyperpolarization induced by adenosine (100 μM, ●) and 8-phenyltheophylline (8PT) (10 μM, △). Values are expressed as a percentage of control i.j.p. amplitude evoked at resting membrane potential. For 8PT, the extrapolation to a zero change in membrane potential yields an i.j.p. constant of 106% which was significantly greater than 100 ($P < 0.05$), while the correlation coefficient of 0.930 was also significant ($P < 0.001$). For adenosine the i.j.p. constant was much reduced at 72% ($P < 0.001$) while the correlation coefficient was significant, 0.616 ($P < 0.001$).

The extents and rates of hyperpolarization due to adenosine and 2-ClAd in the circular muscle preparation were very similar to those reported in the longitudinal muscle preparation (taenia coli), also recorded by the sucrose-gap technique (Burnstock *et al.*, 1984), and the magnitude of ATP-induced hyperpolarization was also similar to values recorded from the taenia coli (Tomita & Watanabe, 1973).

All the purine compounds caused a reduction of the i.j.p. amplitude during a hyperpolarization. Analysis of the regression of i.j.p. amplitude on change in membrane potential revealed that, for higher agonist concentrations, the i.j.p. amplitude was reduced more than could be accounted for by the reduction in K^+ potential. Furthermore, the reduction in i.j.p. amplitude appeared to be dependent on the concentration of the agonist. The reduction in i.j.p. amplitude could be mediated via a junctional receptor on the inhibitory neurones responsible for the i.j.p. Since adenosine and 2-ClAd reduced the i.j.p., this receptor would be a type of P_1-purinoceptor.

It is apparent that the P_1-purinoceptor agonists and P_2-purinoceptor agonists mediate their postjunctional actions in different ways. ATP and α,β-Me ATP both significantly increased their membrane conductance during hyperpolarization while adenosine and 2-ClAd did not, which is similar for the taenia coli (Jager, 1979). The action of ATP is dependent on the concentration of extracellular potassium ions, but not chloride ions (Tomita & Watanabe, 1973; Ferrero & Frischknecht, 1983), whereas the action of adenosine is dependent on both extracellular potassium and chloride ions, although less dependent on potassium ions than ATP; also adenosine is less dependent on extracellular calcium ions than ATP (Banks *et al.*, 1979; Maas *et al.*, 1980; Ferrero & Frischknecht, 1983; Burnstock *et al.*, 1984).

Table 3 Effects of purine nucleosides and nucleotides on i.j.p. constant in guinea-pig caecum circular muscle

Purine compound	*Concentration* (μM)	*i.j.p. constant* ± 95% c.l. (n)	*Probability** (P)
ATP	50	0.81 ± 0.103 (14)	<0.005
	100	0.81 ± 0.181 (10)	<0.05
	500	0.69 ± 0.153 (22)	<0.001
α,β-Me ATP	10	1.08 ± 0.145 (16)	NS
	50	0.75 ± 0.163 (14)	<0.01
	100	0.74 ± 0.091 (9)	<0.001
Adenosine	10	1.01 ± 0.155 (3)	NS
	50	0.87 ± 0.295 (6)	NS
	100	0.72 ± 0.096 (25)	<0.001
	1000	0.71 ± 0.114 (30)	<0.001
2-ClAd	0.5	0.99 ± 0.125 (5)	NS
	1	0.98 ± 0.120 (4)	NS
	10	0.81 ± 0.131 (7)	<0.01
	100	0.63 ± 0.108 (12)	<0.001

c.l. = confidence limits.

* Probability that the i.j.p. constant is not unity. NS = not significantly different ($P > 0.05$). Abbreviations as Table 1.

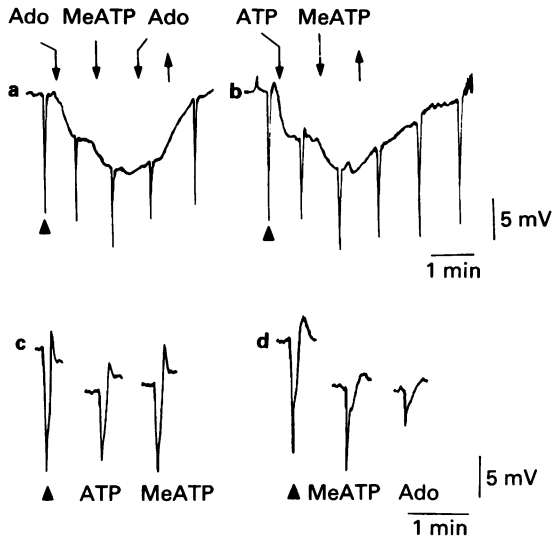

Figure 4 Guinea-pig caecum circular muscle: effects of ATP, α,β-methylene ATP and adenosine on i.j.p. amplitude. (a) control i.j.p. (▲) was evoked in normal Krebs solution. Adenosine (Ado, 50 μM) induced hyperpolarization and reduction of i.j.p. amplitude. An equimolar concentration of α,β-methylene ATP (MeATP) caused additional hyperpolarization but without further reduction in the i.j.p. Adenosine then caused further reduction in the i.j.p. (b) Control i.j.p. (▲) was evoked in normal Krebs solution. ATP (50 μM) caused hyperpolarization and reduction in i.j.p. amplitude, which remained unchanged despite further hyperpolarization in the presence of equimolar MeATP. (c) Control i.j.p. (11.0 mV) evoked at resting membrane potential (▲). The next i.j.p. (6.3 mV) was evoked after 2 min in the presence of ATP (50 μM) which had hyperpolarized the membrane by 2.6 mV and the final i.j.p. (8.0 mV) was evoked after 2 min in the presence of 10 μM MeATP which had maintained a hyperpolarization of 2.6 mV. (d) Control i.j.p. (10.5 mV) was evoked at the resting membrane potential (▲). The next i.j.p. (7.8 mV) was evoked after 1 min in the presence of 50 μM MeATP which had caused a hyperpolarization of 4.6 mV. The third i.j.p. (2.8 mV) was evoked after 70 s in 100 μM Ado while the membrane potential was still hyperpolarized by 4.6 mV.

It is unlikely that the effect of adenosine and 2-ClAd on the i.j.p. amplitude is a function of the change in conductance of the postjunctional membrane rather than a purinoceptor-mediated prejunctional action. This is because neither adenosine nor 2-ClAd had significant action on membrane resistance in contrast to ATP and α,β-Me ATP, yet, like ATP and α,β-Me ATP, adenosine could cause a decrease in i.j.p. amplitude independent of change in membrane potential.

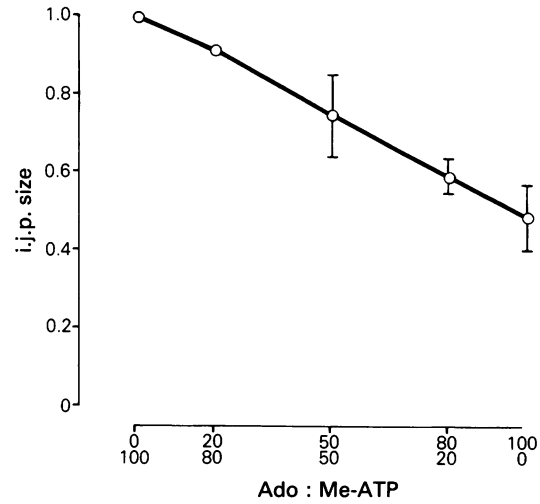

Figure 5 Guinea-pig caecum circular muscle: effects of α,β-methylene ATP and adenosine on i.j.p. amplitude. In the preparations used for these experiments α,β-methylene ATP at 10 μM produced the same level of hyperpolarization as did adenosine at 100 μM. These concentrations were mixed in different ratios and such solutions were applied to the tissue in random order and, although the membrane potential remained consistently hyperpolarized (6 ± 1 mV), the i.j.p. amplitude varied according to the composition of the superfusing solution. Ordinate scale is the i.j.p. amplitude expressed relative to the i.j.p. size in the presence of 100% α,β-methylene ATP (10 μM). Abscissa scale is the ratio, v/v, of adenosine (Ado, 100 μM, upper row), to α,β-methylene ATP (Me-ATP, 10 μM, lower row). Each point represents the mean of three observations and the bars show their standard errors. The graph shows that the i.j.p. amplitude was inversely proportional to the partial volume of adenosine in the superfusing solution.

Effects of xanthine derivatives

All the xanthine derivatives tested here were capable of producing large membrane hyperpolarizations and, at 100 μM, caffeine, theophylline, PACPX and enprofylline were equi-effective with 8-PT at 10 μM. However, the rates of hyperpolarization induced by these xanthines and the time courses as expressed by the rate constants are all much lower than those seen with the purine compounds. The potency order for inducing hyperpolarization (8-PT = enprofylline > theophylline > caffeine > PACPX) is consistent with that observed for enprofylline, theophylline and caffeine in causing tracheal relaxation (Karlsson *et al.*, 1982; Persson *et al.*, 1982).

The xanthine compounds had mixed effects on the i.j.p. The four relatively potent adenosine antagonists could all, at some concentration, enhance the i.j.p.

Table 4 Effects of xanthine derivatives on i.j.p. constant in guinea-pig caecum circular muscle

Xanthine	*Concentration* (μM)	*i.j.p. constant* ± 95% c.l. (n)	*Probability** (*P*)
Caffeine	100	1.05 ± 0.045 (7)	<0.05
	1000	0.84 ± 0.079 (20)	<0.001
Theophylline	0.1	0.99 ± 0.221 (13)	NS
	1	1.07 ± 0.030 (9)	<0.001
	100	1.03 ± 0.069 (20)	NS
	1000	0.77 ± 0.263 (11)	NS
8-Phenytheophylline	0.1	1.14 ± 0.043 (28)	<0.001
	1	1.02 ± 0.039 (17)	NS
	10	1.06 ± 0.054 (22)	<0.05
PACPX	1	1.13 ± 0.075 (9)	<0.005
	10	1.03 ± 0.058 (9)	NS
	100	1.09 ± 0.208 (9)	NS
Enprofylline	10	0.98 ± 0.094 (20)	NS
	100	0.97 ± 0.082 (30)	NS

c.l. = confidence limits.
* Probability that the i.j.p. constant is not unity. NS = not significantly different ($P > 0.05$).

amplitude, while enprofylline had no such action. The increase in i.j.p. amplitude was not due to an increase in membrane resistance because no change in membrane resistance was observed. Possibly the increase in i.j.p. amplitude was due to an inhibition of an adenosine-tone which acts prejunctionally to depress transmitter release, in a manner similar to the 'purinergic tone' of the central nervous system (Harms *et al.*, 1978) or the endogenous adenosine release in adipose tissue (Fredholm & Lindgren, 1984) or at the neuromuscular junction (Ribeiro & Sebastião, 1987). Other possible explanations for the raised i.j.p. constant are that there may be a non-receptor-mediated facilitation of transmitter release from the non-adrenergic, non-cholinergic inhibitory neurones, due to calcium mobilization (Huddart *et al.*, 1983) or that there may be an allosteric reaction with the postjunctional receptor leading to an increase in the affinity for the inhibitory transmitter. However, there is no evidence presently available to support these possibilities; nevertheless, the facilitatory actions of the xanthine derivatives are in marked contrast to the inhibitory action of purine compounds on the i.j.p. in the same type of preparation.

Caffeine at 1000 μM reduced the i.j.p. amplitude in a manner unrelated to dependency on membrane potential. This was possibly due to the decrease in membrane resistance but could also have been due to other effects such as phosphodiesterase inhibition, induced Ca^{2+} chelation or K^+-channel blockade (Ito *et al.*, 1984). Any of these actions, either pre- or postjunctionally, could be responsible for a diminished amplitude of the i.j.p. However, it is interesting that the reduction in i.j.p. amplitude due to caffeine (1000 μM), ATP and α,β-Me ATP (both 100 μM) were all similar, as were the changes in membrane resistance induced by these compounds.

These results show that P_1-purinoceptor agonists act prejunctionally to inhibit non-adrenergic, non-cholinergic inhibitory neuromuscular transmission in the circular muscle coat of the guinea-pig caecum. The results also show that none of the xanthine compounds tested would be suitable to use as an antagonist directly against the prejunctional effects of adenosine or ATP in the intestine in view of the relative increase in i.j.p. amplitude associated with a membrane hyperpolarization.

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