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Characterization of adenosine receptors evoking excitation of mesenteric afferents in the rat

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1 We examined the effects of adenosine receptor agonists and antagonists on the discharge of mesenteric afferent nerves supplying the jejunum in pentobarbitone sodium-anaesthetized rats.

2 Adenosine (0.03–10 mg kg^{-1}, i.v.), NECA (0.3–300 μg kg^{-1}, i.v.) and the A_1 receptor agonist, GR79236 (0.3–1000 μg kg^{-1}, i.v.), each induced dose-dependent increases in afferent nerve activity and intrajejunal pressure, hypotension and bradycardia. The A_1 receptor antagonist, DPCPX (3 mg kg^{-1}, i.v.), antagonized all the effects of GR79236 but only the haemodynamic effects of adenosine and NECA. The A_{2A} receptor antagonist, ZM241385 (3 mg kg^{-1}, i.v.), antagonized the hypotensive effect of NECA but none of the effects of GR79236.

3 The A_{2A} receptor agonist, CGS21680 (0.3–300 μg kg^{-1}, i.v.), and the A_3 receptor agonist, IB-MECA (0.3–300 μg kg^{-1}, i.v.), each induced only a dose-dependent hypotension. Subsequent administration of adenosine (3 mg kg^{-1}, i.v.) induced increases in afferent nerve activity and intrajejunal pressure and bradycardia. ZM241385 (3 mg kg^{-1}, i.v.) antagonized the hypotensive effect of CGS21680 but not the effects of adenosine.

4 Bethanechol (300 μg kg^{-1}, i.v.) evoked increases in afferent nerve activity and intrajejunal pressure, hypotension and bradycardia. However, adenosine (3 mg kg^{-1}, i.v.) evoked greater increases in afferent nerve activity than bethanechol despite inducing smaller increases in intrajejunal pressure.

5 In summary, A_1 and A_{2B} and/or A_{2B}-like receptors evoke adenosine-induced increases in mesenteric afferent nerve activity and intrajejunal pressure in the anaesthetized rat. Furthermore, elevations in intrajejunal pressure do not wholly account for adenosine-evoked excitation of mesenteric afferent nerves.

Keywords: Adenosine receptors; jejunum; afferent nerves; *in vivo*; NECA; CGS21680; GR79236; IB-MECA; DPCPX; ZM241385

Introduction

The physiological actions of adenosine are mediated by at least four distinct receptor types; A_1, A_{2A}, A_{2B} and A_3, for which several selective agonists and antagonists exist (see Collis & Hourani, 1993; Fredholm *et al*., 1994; Olah & Stiles, 1995). The A_1 adenosine receptor is selectively activated by N-[(1S, *trans*)-2-hydroxycyclopentyl] adenosine (GR79236; Gurden *et al*., 1993) and selectively inhibited by 1,3-dipropyl-8-cyclopentylxanthine (DPCPX; Bruns *et al*., 1987). A_{2A} adenosine receptors are preferentially activated by 2-[[2-[4-(2-carboxyethyl) phenyl] ethyl] amino]-N-ethyl-carboxamidoadenosine (CGS21680; Jarvis *et al*., 1989; Gurden *et al*., 1993). Furthermore, selective A_{2A} receptor antagonists such as 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*] pyrimidine (SCH-58261; Baraldi *et al*., 1994) and 4-(2-[7-amino-2-(2-furyl)[1,2,4] triazolo[2,3-*a*][1,3,5]triazin-5-ylamino]ethyl)phenol (ZM241385; Poucher *et al*., 1995) have been recently developed. N-[(2-methylphenyl)methyl] adenosine (metrifudil) exhibits modest selectivity for the A_{2B} over the A_{2A} receptor (Gurden *et al*., 1993) although it is also active at the A_3 receptor (Patel *et al*., 1994). Finally, the A_3 receptor (Zhou *et al*., 1992; Fozard & Carruthers, 1993), is selectively activated by aminobenzyl-5′-N-methylcarboxamidoadenosine, (AB-MECA; Olah *et al*., 1994) and N-6-(3-iodobenzyl)-5′-N-methylcarboxamidoadenosine (IB-MECA; Jacobson *et al*., 1993) and is characterized further, at least in the rat, by its resistance to xanthine and non-xanthine receptor antagonists such as DPCPX and 9-fluoro-2-(2-furyl)-5,6-dihydro [1,2,4] triazolo{1,5-*c*}-quinazin-5-imine (CGS15943A; Fozard & Carruthers, 1993; Carruthers & Fozard, 1994; Patel *et al*., 1994; van Galen *et al*., 1994), respectively.

Adenosine has widespread actions in the gastrointestinal tract. In addition to classical inhibitory actions such as attenuation of intestinal secretion in the rat (Hancock & Coupar, 1995a) and relaxation of rat intestinal smooth muscle (Bailey & Hourani, 1990; 1992; Hancock & Coupar 1995b; Nicholls *et al*., 1992; Nicholls & Hourani, 1997), adenosine exhibits several excitatory effects. For example, adenosine can evoke contraction of the *muscularis mucosae* in the rat colon, ileum and duodenum (Bailey & Hourani, 1990; Bailey *et al*., 1992; Nicholls *et al*., 1996; Nicholls & Hourani, 1997). Furthermore, adenosine elicits depolarization and action potential discharge in guinea-pig myenteric (Katayama & Morita, 1989) and submucosal neurones (Barajas-López *et al*., 1991). Although adenosine stimulates cutaneous nociceptive afferents (Bleehen *et al*., 1976), it is not known what effect(s) adenosine has on intestinal afferent nerve endings. Since extracellular levels of adenosine are likely to be elevated in inflamed or ischaemic segments of intestine (Bleehen *et al*., 1976; Edlund *et al*., 1983), it was of interest to investigate whether adenosine could modulate mesenteric afferent nerve activity and, if so, to characterize the receptor subtype involved. Thus, we have examined the actions of adenosine and synthetic analogues on the afferent innervation of the

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jejunum in the anaesthetized rat. A preliminary account of some of these observations has been published (Kirkup *et al*., 1997).

Methods

Animals

Experiments were conducted using 79 Sheffield-strain male or female Wistar rats (300–450 g) allowed free access to food and water. General anaesthesia was established with a single intraperitoneal injection of pentobarbitone sodium (60 mg kg^{-1}) and maintained by intravenous (i.v.) infusion (0.5–1 mg kg^{-1} min^{-1}). Animals were killed by anaesthetic overdose followed by exsanguination.

Surgical procedures

A mid-line incision in the neck was performed and the trachea was intubated with a short length of Portex tubing (PP40) to facilitate spontaneous respiration and to permit artificial ventilation if necessary. The right external jugular vein was bi-cannulated with two saline-filled Luer-mounted Portex cannulae; one long cannula (approximately 32 cm) to facilitate maintenance anaesthesia and one short cannula (approximately 8 cm) for the systemic administration of drugs. The left common carotid artery was cannulated with a heparinized Portex catheter (200 U ml^{-1} heparin in 0.9% *w*/*v* NaCl solution; PP30) to record blood pressure (Elcomatic EM760). The heart rate was electronically-derived from the arterial pressure record. Body temperature was monitored with a rectal probe and maintained at around 37°C by means of a thermostatically-controlled heating blanket.

A mid-line laparotomy was performed and the caecum was ligated and excised. A 10–15 cm loop of proximal jejunum was located (typically 1–5 cm from the ligament of Trietz). The loop was isolated with ligatures around the oral and aboral ends and incisions were made on the anterior mesenteric border at each end. The loop was cannulated with Portex tubing (PP100) passed through the right abdominal wall *via* two small incisions. The oral cannula was inserted first and isothermic saline was flushed through the loop to remove the luminal contents. The aboral cannula contained two smaller bore saline-filled Portex tubes (PP30), one for the local administration of saline to produce distension (applications were drained *via* the oral cannula) and the other was connected to a pressure transducer (Elcomatic EM760) to measure intrajejunal pressure. The aboral and oral cannulae were closed to provide isovolumic recording conditions and the intestinal loop was filled with saline up to a baseline pressure of around 5 cm H_2O. This basal pressure was maintained by perfusion of the loop with saline (0.4–1.0 ml h^{-1}) through the aboral cannula connected to the pressure transducer. The small incisions through which the intestinal cannulae passed were sewn up and the muscle and skin comprising the perimeter of the abdominal incision were sutured to a 5 cm steel ring to form a well and the peritoneal cavity was filled with pre-warmed (37°C) light liquid paraffin.

Nerve preparation and afferent recording

Two paravascular nerve bundles run alongside the major blood vessels located in the mesenteric arcades supplying the jejunum. A single arcade was placed on a black Perspex platform in order to steady the preparation. With the aid of a dissection microscope (12–60 × magnification, Kyowa, Model SZM) and illumination by a fibre optic source (Schott, KL 1500-T) a single nerve bundle was isolated from the surrounding adipose and connective tissue. A mesenteric nerve was exposed, severed distally from the wall of the jejunum (approximately 1–1.5 cm) in order to eliminate efferent nerve activity and cleaned until satisfactory for recording. It was then attached to one arm of a bipolar platinum recording electrode, with a length of connective tissue wrapped around the other to act as a reference. The electrodes were connected to a Neurolog head stage (NL100, Digitimer, Welwyn Garden City, U.K.) and the signal from them was differentially pre-amplified (NL103), amplified (NL105) and then filtered with a bandwidth of 100–1000 Hz (NL125). This filtered nerve signal was displayed on a storage oscilloscope (Tektronix 5113), digitized (PCM-2 A/D VCR adaptor, Medical Systems Corp., Greenvale, N.Y.11548, U.S.A.) and recorded on video tape (Saisho, VR3300X) for subsequent post-experimental analysis. The filtered nerve signal was also relayed to a spike processor (Digitimer D130) that counted over a 1.25 s period each spike crossing a threshold (which was not altered for the duration of an experiment) set above the noise level. Threshold settings were confirmed at the end of an experiment by dripping 5% *w*/*v* lignocaine solution onto the nerve bundle to abolish all neural activity. For on-line visualization and data-capture the outputs from the spike processor (essentially a measure of whole nerve discharge in terms of a frequency histogram), together with the signals from the arterial and intrajejunal pressure transducers, were relayed to a PC (Viglen 4DX266) running Spike 2 software (Cambridge Electronic Design (CED), Cambridge, U.K.) *via* a 1401 plus interface board (CED).

Experimental protocols

After a 45–60 min stabilization period, the viability of the preparation was assessed with separate intravenous (i.v.) bolus doses (5–10 min apart) of 2-methyl-5-hydroxytryptamine (2-methyl-5-HT, 10 μg) and cholecystokinin (CCK, 50 or 100 ng) and, after a further 5–10 min, the intestinal loop was distended for 15 s with the rapid introduction (<1 s) of 0.5 and/or 1.0 ml of isothermic saline, which was subsequently drained away. Nerve preparations which failed to respond to all three stimuli were discarded. After a further 15–20 min stabilization period, dose-response curves to agonists were produced. In the case of adenosine, a sequential i.v. administration protocol was utilized, with the interval between each successive dose being sufficient to permit recovery of variables to their basal values. As a result of the long duration of action of the synthetic agonists, a cumulative i.v. administration protocol was employed, the time between doses being sufficient to allow a plateau response to develop. Animals were treated with either DPCPX (3 mg kg^{-1}), ZM241385 (3 mg kg^{-1}) or vehicle i.v. 15 min prior to construction of dose-response curves to one of the agonists. Only one dose response curve to an agonist was generated per vehicle or antagonist-treated animal and therefore comparisons were made between vehicle- and antagonist-treated groups. In some cases, after completion of the dose-response curve, the preparations were then challenged with adenosine (3 mg kg^{-1}, i.v.) and/or the three initial test stimuli. The studies examining the actions of adenosine and the muscarinic agonist, bethanechol (300 μg kg^{-1}), were conducted in a separate group of preparations. In each animal, adenosine and bethanechol were administered intravenously, in a randomized order, and the parameters were allowed to return to basal values before exposure to the second drug treatment.

All drugs were administered in a 0.1–0.15 ml dose volume followed by a 0.25 ml saline flush.

Analyses of data

Mean baseline values for the parameters of blood pressure, heart rate, intrajejunal pressure and mesenteric afferent nerve activity were calculated using an averaging facility available in the Spike2 software (CED) from a 30 s period prior to commencement of drug administration. Hypotension was measured as the peak change compared with the average basal mean arterial pressure in mmHg. Bradycardia was measured as the peak change compared with the average basal heart rate in beats min^{-1}. Effects on intrajejunal pressure were measured as the peak change compared with baseline and expressed in units of cm H_2O. Effects on afferent nerve discharge, expressed in spikes per second (spikes s^{-1}), were calculated as the difference between mean basal discharge and the peak increase in the primary burst of discharge produced an agent. In vehicle-pre-treated animals the effective doses of agonist required to produce 25% or 50% of the maximal effect (ED_{25} and ED_{50} values, respectively) on blood pressure, heart rate, intrajejunal pressure and afferent nerve discharge were determined, where appropriate, by non-linear curve-fitting using a 3 parameter logistical equation in Prism 2 (Graphpad Software). In other instances, data were fitted by point-to point plotting and the values determined by linear interpolation. As a result of the variability in the magnitude of maximal responses, agonist dose ratios in individual antagonist pre-treated animals, were determined by comparison of the ED_{25} or ED_{50} (that is the doses which produced 25% or 50% of the mean maximal effect) with the mean ED_{25} or ED_{50} in vehicle-pre-treated rats. Data are presented as the arithmetic mean $\pm$ s.e.mean or geometric mean with 95% confidence limits as appropriate, from four or more animals per vehicle- or antagonist-treated or untreated group. Where *n* values are given they refer to the number of animals. Where ED_{50} values are given, they represent the $-\log_{10}$ of the dose administered in g kg^{-1}. Significant differences between data points were determined by using parametric or non-parametric analysis of variance followed by the Student's paired or unpaired *t*-test (with Bonferroni corrections) or Dunn's analysis, respectively. A probability of $P<0.05$ was considered to be indicative of a statistically significant difference.

Drugs

Adenosine, bethanechol, CCK (in the form of sulphated CCK_8) and lignocaine were obtained from Sigma Chemical Co., Poole, Dorset, U.K. IB-MECA, 2-methyl-5-HT and 5′-N-ethylcarboxamido-adenosine (NECA) were purchased from Research Biochemicals Inc., SEMAT, St Albans, U.K. CGS21680, DPCPX and ZM241385 were obtained from Research Biochemicals Inc. and Tocris Cookson Ltd, Bristol, U.K. GR79236 was a gift from Dr M. Sheehan and was synthesized in the Chemistry Division of Glaxo Wellcome Research and Development, Stevenage, U.K.

Adenosine, bethanechol, GR79236, lignocaine and 2-methyl-5-HT were each dissolved in 0.9% *w*/*v* NaCl solution (saline). CCK was dissolved in 2% *w*/*v* bovine serum albumin (Sigma) in saline. NECA was dissolved in 50 μl 1 M HCl and made up to 1 ml total volume in saline giving a stock concentration of 3 mg ml^{-1}. CGS21680 was dissolved in 50 μl 1 M NaOH and made up to 1 ml total volume in saline giving a stock concentration of 3 mg ml^{-1}. IB-MECA was dissolved in 50% *v*/*v* dimethylsulphoxide (DMSO) in saline giving a stock concentration of 1 mg ml^{-1}. Serial dilutions of these stock concentrations of agonists were made in saline. In control experiments, cumulative administration of corresponding vehicle dilutions produced no effect on mesenteric afferent nerve activity or intrajejunal pressure. Transient perturbations in the blood pressure and heart rate traces occurred on administration of the vehicle but these were no greater than those produced by administration of similar volumes of saline. DPCPX and ZM241385 were each dissolved in 5% *v*/*v* DMSO/5% *v*/*v* 1 M NaOH in saline to give a stock concentration of 3 mg ml^{-1}. Administration of antagonist or vehicle did not affect mesenteric afferent nerve activity or intrajejunal pressure but produced transient effects on blood pressure and heart rate which recovered to baseline within 5 min.

Results

Effects of adenosine and NECA

In vehicle-treated animals, sequential administration of adenosine (0.03–10 mg kg^{-1}) elicited a dose-dependent increase in mesenteric afferent nerve discharge ($ED_{50}=3.32\pm0.12$, $n=7$), intrajejunal pressure ($ED_{50}=3.20\pm0.19$), hypotension ($ED_{50}=3.68\pm0.07$) and bradycardia ($ED_{50}=3.17\pm0.11$; Figure 1). The effects of adenosine on mesenteric afferent nerve discharge and intrajejunal pressure were rapid, occurring within 5–15 s of intravenous administration (Figure 2). Up to doses of 1 mg kg^{-1} the effects of adenosine on mesenteric afferent nerve discharge comprised a rapidly-developing early phase of activation which decayed over a period of <1 min. However, in 100% of preparations, at doses of 3 and 10 mg kg^{-1} a late (secondary) burst of mesenteric afferent nerve activity followed and was superimposed upon the early (primary) burst (Figure 2). For example, after injection of 3 mg kg^{-1} adenosine the primary burst of mesenteric afferent nerve discharge attained a peak increase of 77 ± 10 spikes s^{-1} and the time period between the initiation of the primary and commencement of the secondary burst was 20 ± 1 s. The secondary burst of mesenteric afferent nerve discharge lasted for 28 ± 3 s and during this period achieved a peak elevation of 142 ± 19 spikes s^{-1} ($P<0.05$ compared with the peak primary burst) before decaying to a similar level of activity as the primary burst and then to basal levels of discharge over a further period of 55 ± 10 s (Figure 2). A similar time course of mesenteric afferent nerve activation was produced by adenosine at the dose of 10 mg kg^{-1}, although the decay of the response was prolonged in comparison. The intrajejunal pressure elevations evoked by the higher doses of adenosine sometimes comprised early and late increases followed by a decay to basal but this pattern of activity occurred in only 81% of preparations.

In another group of animals, the effects of adenosine (3 mg kg^{-1}) and the muscarinic agonist, bethanechol (300 μg kg^{-1}) were assessed. In these preparations, bethanechol and adenosine each induced rapid increases in mesenteric afferent nerve discharge and intrajejunal pressure with mean latencies to onset, after administration, of 7 ± 1 s and 6 ± 1 s, and 6 ± 1 s and 5 ± 1 s, respectively ($n=16$; Figure 3). Both agents exhibited a similar time-course to peak increases in intrajejunal pressure (bethanechol $=43\pm8$ s; adenosine $=47\pm3$ s), although bethanechol induced a greater overall peak increase in intrajejunal pressure than adenosine (bethanechol $=11.1\pm0.6$ cm H_2O; adenosine $=6.6\pm0.7$ cm H_2O, $P<0.0001$). However, the time-course to the peak increase in mesenteric afferent nerve discharge was much greater for bethanechol (49 ± 5 s) than that to the peak increase in the

primary burst of discharge induced by adenosine (16±1 s, $P<0.0001$; Figure 3). Moreover, bethanechol evoked a smaller peak activation of mesenteric afferent nerve discharge than adenosine (bethanechol = 112±15 spikes s^{-1}, adenosine = 143±12 spikes s^{-1}, $P<0.05$) despite the elevation in intrajejunal pressure at these peak levels of activation being greater for the muscarinic agonist (bethanechol = 7.4±0.8 cm H_2O; adenosine = 1.8±0.3 cm H_2O, $P<0.0001$). Bethanechol and adenosine each evoked similar peak falls in blood pressure of 91±5 mmHg and 89±5 mmHg and similar peak falls in heart rate of 241±19 beats min^{-1} and 261±5 beats min^{-1}, respectively.

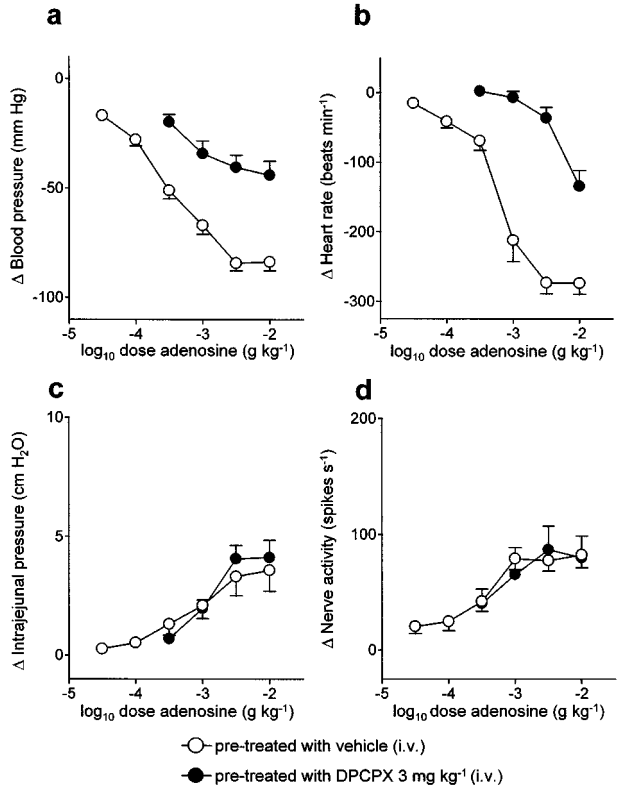

Figure 1 Effect of pre-treatment with DPCPX (3 mg kg^{-1}, i.v.) on the effects of adenosine (i.v.) on (a) blood pressure, (b) heart rate, (c) intrajejunal pressure and (d) mesenteric afferent nerve activity in the anaesthetized rat. Points represent the mean±s.e.mean of $n=6-7$.

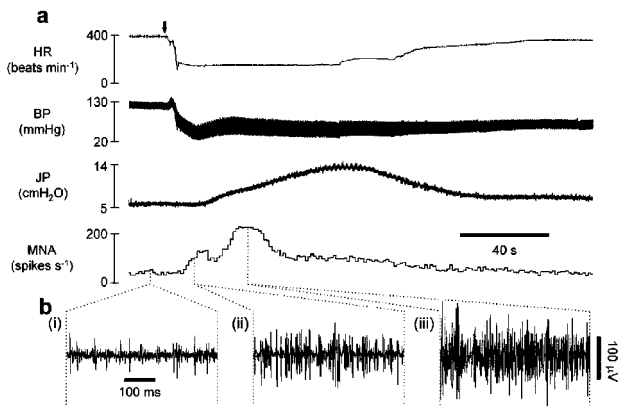

Figure 2 (a) Representative traces of simultaneous recordings of the effects of 3 mg kg^{-1} (i.v.) adenosine on heart rate (HR), blood pressure (BP), intrajejunal pressure (JP) and mesenteric afferent nerve activity (MNA) in the anaesthetized rat. The point of injection of the agonist is indicated by the arrow. (b) Extracellular recordings of afferent nerve discharge obtained from the same animal. Each section of trace is of the first 500 ms of the 1.25 s bin denoted by the vertical dashed line in the trace of nerve discharge frequency of (i) basal activity and during the (ii) primary and (iii) secondary phases of discharge induced by adenosine.

Pre-treatment with the selective A_1 receptor antagonist, DPCPX (3 mg kg^{-1}), antagonized the bradycardia and the hypotension evoked by adenosine (0.3–10 mg kg^{-1}, Figure 1, Table 1). However, the effects of adenosine on mesenteric afferent nerve discharge and intrajejunal pressure were unaffected by treatment with DPCPX (Figure 1, Table 1). Furthermore, DPCPX did not affect increases in mesenteric afferent nerve discharge induced by CCK ($n=6$; data not shown) or by 2-methyl-5-HT ($n=6$; data not shown) or by a distension with 0.5 ml saline ($n=6$; data not shown).

Cumulative intravenous administration of NECA (0.01–1000 μg kg^{-1}), induced dose-dependent increases in mesenteric afferent nerve discharge ($ED_{50}=5.33\pm0.19$, $n=5$), intrajejunal pressure ($ED_{50}=5.07\pm0.22$), hypotension ($ED_{50}=5.95\pm0.07$) and bradycardia ($ED_{50}=5.04\pm0.09$) in vehicle-treated animals (Figure 4). The patterns of increased mesenteric afferent nerve activity and intrajejunal pressure evoked by NECA mirrored those of adenosine with early and late bursts of mesenteric afferent nerve activity (with quantitatively similar temporal characteristics to those obtained with adenosine) occurring at doses >3 μg kg^{-1} (not shown).

Pre-treatment with the A_1 receptor-selective antagonist DPCPX (3 mg kg^{-1}) antagonized only the bradycardia induced by NECA (0.01–3000 μg kg^{-1}; Figure 4, Table 1). Treatment with the antagonist did not modify the peak effects of NECA on mesenteric afferent nerve discharge, intrajejunal pressure or blood pressure (Figure 4, Table 1). Pre-treatment with the selective A_{2A} receptor antagonist, ZM241385, caused a significant increase in the threshold dose for the hypotensive action of NECA (Figure 4a) but did not affect the ED_{50} value for the effect of the agonist on this parameter (Table 1). ZM241385 did not antagonize the peak effects of NECA on mesenteric afferent nerve activity, intrajejunal pressure and heart rate (Figure 4, Table 1).

Effects of the A_1-selective agonist, GR79236

Cumulative intravenous administration of GR79236 (0.3–1000 μg kg^{-1}) elicited a dose-dependent increase in mesenteric

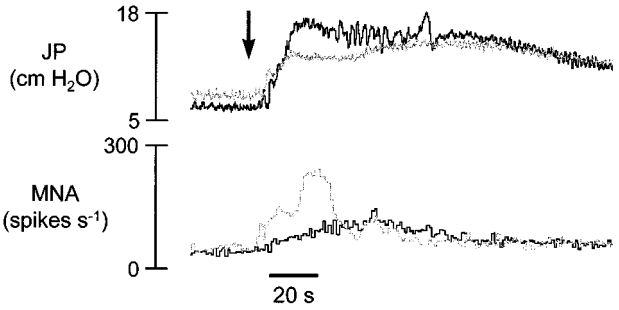

Figure 3 Superimposed traces of the effects of 3 mg kg^{-1} (i.v.) adenosine (grey) and 300 μg kg^{-1} (i.v.) bethanechol (black) on intrajejunal pressure (JP) and mesenteric afferent nerve activity (MNA) in the anaesthetized rat. Note that adenosine induced a more rapidly developing and larger change in mesenteric afferent nerve activity than bethanechol whereas the muscarinic agonist evoked a larger increase in intrajejunal pressure. These traces were obtained from the same animal and the point of injection of the two agents is indicated by the arrow.

Table 1 Antagonism of the effects of adenosine and analogues on blood pressure (BP), heart rate (HR), intrajejunal pressure (IP) and afferent discharge (AD) by DPCPX and ZM241385

	DPCPX Dose ratio					*ZM241385* Dose ratio				
	BP	*HR*	*IP*	*AD*	n	*BP*	*HR*	*IP*	*AD*	n
Adenosine	6 (2, 16)	29 (15, 58)	1.3 (0.8, 2.1)	1.9 (0.7, 5)	6	–	–	–	–	–
NECA	1.5 (0.9, 2.4)	208 (126, 345)	1.2 (0.6, 2.3)	1.6 (1.0, 2.7)	6	5.9 (0.4, 86.1)	1.4 (0.4, 4.5)	2.1 (0.3, 14.3)	1.9 (0.7, 5.5)	4
GR79236	16 (11, 27)	88 (56, 139)	12 (6, 25)	72 (10, 499)	6	2.0 (0.8, 5.1)	1.4 (1.0, 1.9)	1.4 (0.3, 5.7)	1.0 (0.4, 2.4)	5
CGS21680	–	–	–	–	–	106 (57, 199)	–	–	–	6

Values are the geometric mean of the dose ratio with the 95% confidence intervals in parentheses.

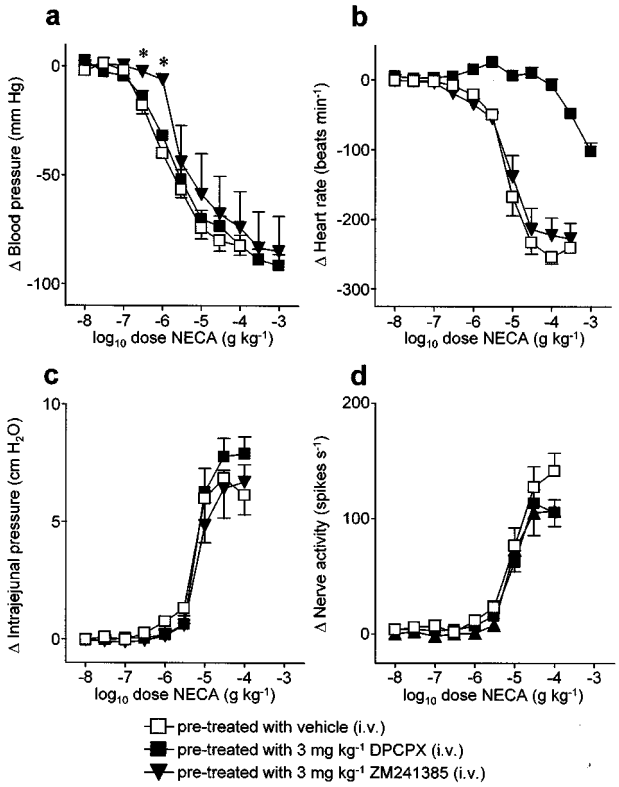

Figure 4 Effect of pre-treatment with DPCPX (3 mg kg^{-1}, i.v.) or ZM241385 (3 mg kg^{-1}, i.v.) on the effects of NECA (i.v.) on (a) blood pressure, (b) heart rate, (c) intrajejunal pressure and (d) mesenteric afferent nerve activity in the anaesthetized rat. *Indicates a significant difference ($P<0.05$) from the vehicle-treated group. Points represent the mean ± s.e.mean of $n=4-6$.

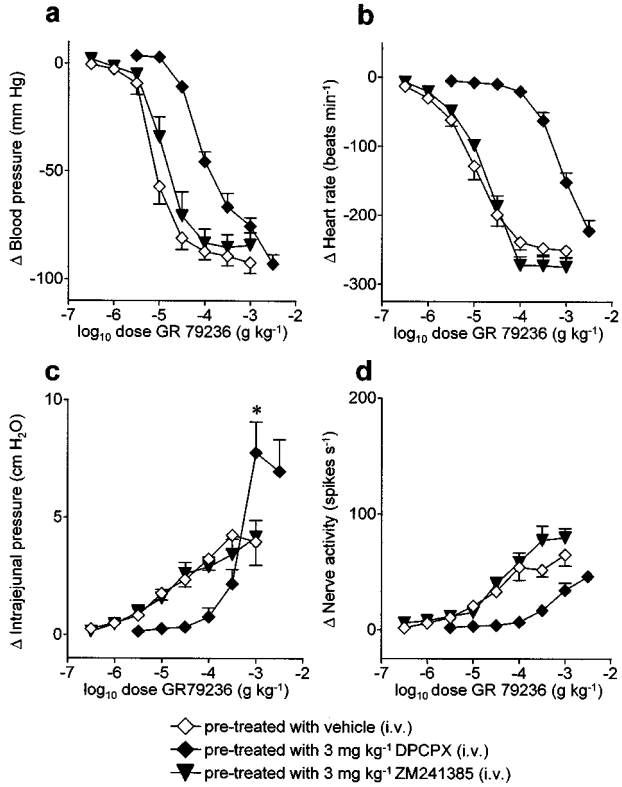

Figure 5 Effect of pre-treatment with DPCPX (3 mg kg^{-1}, i.v.) or ZM241385 (3 mg kg^{-1}, i.v.) on the effects of GR79236 (i.v.) on (a) blood pressure, (b) heart rate, (c) intrajejunal pressure and (d) mesenteric afferent nerve activity in the anaesthetized rat. *Indicates a significant difference ($P<0.05$) from the vehicle-treated group. Points represent the mean ± s.e.mean of $n=5-6$.

afferent nerve discharge ($ED_{50}=4.50\pm0.12$, $n=7$), intrajejunal pressure ($ED_{50}=4.80\pm0.18$), hypotension ($ED_{50}=5.03\pm0.08$) and bradycardia ($ED_{50}=5.00\pm0.10$; Figure 5). As observed with adenosine and NECA, the GR79236-induced increases in mesenteric afferent nerve discharge and intrajejunal pressure were rapid in onset (10–15 s; not shown). The profiles of the effects of GR79236 on these parameters were similar to those elicited by adenosine and NECA with early and late bursts of mesenteric afferent nerve activity (with quantitatively similar temporal characteristics to those observed with adenosine and NECA) occurring at doses >100 μg kg^{-1} (not shown).

In contrast to its effects on responses to adenosine and NECA, pre-treatment with DPCPX (3 mg kg^{-1}) antagonized the action of GR79236 (3–3000 μg kg^{-1}) on mesenteric afferent nerve discharge, intrajejunal pressure, blood pressure and heart rate (Figure 5, Table 1). Interestingly, the peak increase in intrajejunal pressure was significantly augmented in DPCPX-treated preparations (Figure 5).

Pre-treatment with the A_{2A}-selective antagonist, ZM241385, did not antagonize the actions of GR79236 on mesenteric afferent nerve discharge, intrajejunal pressure, blood pressure and heart rate (Figure 5, Table 1).

Effects of the A_{2A}-selective agonist, CGS 21680

Cumulative systemic administration of CGS21680 (0.3–300 μg kg^{-1}) induced a dose-dependent hypotension ($ED_{50}=5.31\pm0.08$, $n=7$) and a (presumably reflex) tachycar-

dia (Hutchison *et al.*, 1989). CGS21680 did not significantly affect either afferent nerve discharge or intrajejunal pressure (Figure 6). Subsequent administration of adenosine (3 mg kg^{-1}) elicited a significant increase in mesenteric afferent nerve discharge, an increase in intrajejunal pressure and a bradycardia (Figure 6). These effects of adenosine were similar to those previously observed with this dose (see Figure 1). Pre-treatment with the A_{2A}-selective antagonist, ZM241385 (3 mg kg^{-1}) antagonized the hypotensive action of CGS21680 (0.3–1000 μg kg^{-1}, Table 1) and, furthermore, there was no tachycardia over the dose-range of agonist tested (Figure 6). Moreover, there was still no apparent effect of CGS21680 on mesenteric afferent nerve discharge and intrajejunal pressure in antagonist-treated animals (Figure 6). The effects of adenosine (3 mg kg^{-1}) on heart rate were not altered by treatment with ZM241385, although its effects on mesenteric afferent nerve activity and intrajejunal pressure were significantly augmented (Figure 6). Nevertheless, treatment with the A_{2A}-selective antagonist did not significantly affect the peak mesenteric afferent nerve discharge evoked by CCK ($n=6$; data not shown) or by 2-methyl-5-HT ($n=6$; data not shown) or by a distension with 0.5 ml saline ($n=6$; data not shown).

Effects of the A_3-selective agonist, IB-MECA

Cumulative intravenous administration of IB-MECA (0.3–300 μg kg^{-1}) induced only a dose-dependent hypotension ($ED_{50}=4.75\pm0.07$, $n=5$) but did not significantly affect mesenteric afferent nerve activity, intrajejunal pressure of heart rate (data not shown). Nonetheless, subsequent administration of adenosine (3 mg kg^{-1}) evoked increases in mesenteric afferent nerve discharge (peak increase of 99 ± 16 spikes s^{-1}) and intrajejunal pressure (peak increase of 3.8 ± 1.1 cm H_2O) and a bradycardia (peak decrease of 277 ± 13 beats min^{-1}).

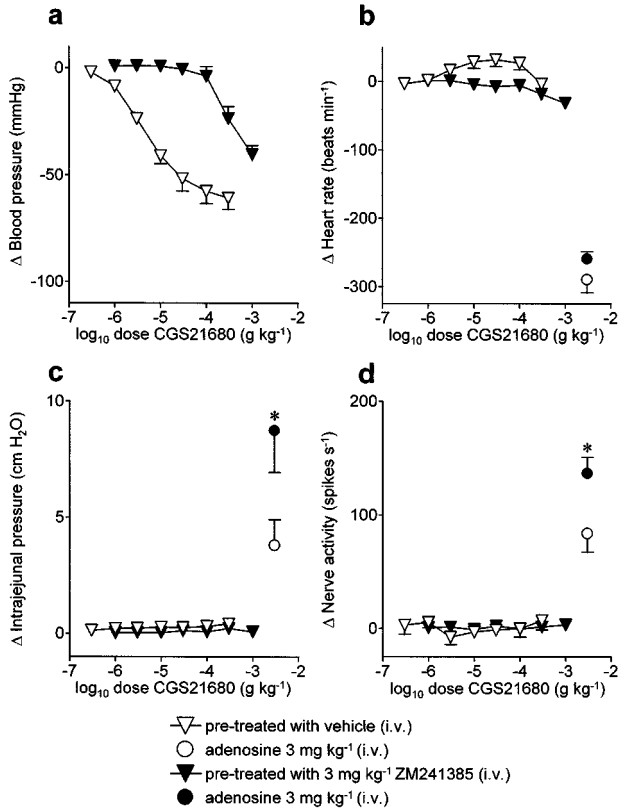

Figure 6 Effect of pre-treatment with ZM241385 (3 mg kg^{-1}, i.v.) on the effects of CGS21680 (i.v.) on (a) blood pressure, (b) heart rate, (c) intrajejunal pressure and (d) mesenteric afferent nerve activity in the anaesthetized rat. Note that only subsequent intravenous administration of adenosine (3 mg kg^{-1}, i.v.) produced a bradycardia and stimulated increases in intrajejunal pressure and afferent nerve activity. *Indicates a significant difference ($P<0.05$) from the vehicle-treated group. Points represent the mean$\pm$s.e.mean of $n=6-7$.

Discussion

Type of the receptor mediating the excitatory effect of adenosine

In the present study, conducted in the anaesthetized rat, we have observed that adenosine stimulates mesenteric afferent nerves and increases pressure in the jejunum, in addition to its haemodynamic effects. Adenosine has been reported also to stimulate carotid chemoreceptors (McQueen & Ribeiro, 1986), enteric neurones (Katayama & Morita, 1989; Barajas-Lopez *et al.*, 1991) and isolated vagus nerve trunks (Trezise *et al.*, 1993), as well as cutaneous nociceptive (Bleehen *et al.*, 1976), pulmonary (Cherniak *et al*, 1987) and renal afferent nerves (Katholi *et al.*, 1985). However, this is, to our knowledge, the first demonstration of an excitatory effect of adenosine on afferent nerves supplying the intestine.

The stimulatory effect of adenosine on afferent nerve activity and intrajejunal pressure was mimicked by the stable, non-selective analogue, NECA, but not by CGS21680 or IB-MECA, agonists selective for A_{2A} and A_3 receptors (see Collis & Hourani, 1993; Olah & Stiles, 1995), respectively, which only produced a hypotension as observed previously (Hutchison *et al.*, 1989; Patel *et al.*, 1994; Patel & Ramage, 1997). However, intravenous administration of adenosine initiated increases in mesenteric afferent nerve activity and intrajejunal pressure in the animals treated with the latter two agonists, thus suggesting that A_{2A} or A_3 receptors are not responsible for either of these stimulatory effects of adenosine. Our observations contrast with these of Barajas-Lòpez *et al.* (1991) in which A_{2A} receptors are implicated in the excitation induced by adenosine on intestinal submucosal neurones. Nevertheless, the inability of the A_{2A}-selective antagonist ZM241385 (at a dose which produced a 100 fold shift in the hypotension dose-response curve for the A_{2A}-selective agonist, CGS21680) to antagonize the excitatory effects of adenosine and NECA on intestinal afferent nerve activity and intrajejunal pressure is consistent with the lack of involvement of A_{2A} receptors in mediating these increases. Interestingly, an augmentation of the increased mesenteric afferent nerve activity and intrajejunal pressure in response to adenosine occurred in animals pre-treated with ZM241385. This observation suggests a possible role of A_{2A} receptors in regulating the magnitude of increases in mesenteric afferent nerve activity and intrajejunal pressure produced by adenosine. However, in the ZM241385-treated group, administration of the dose regimen of CGS21680 did not produce as great a fall in blood pressure as observed in those treated with vehicle alone (see Figure 5). Thus, it cannot be excluded that differences in blood flow between these groups account for this phenomenon.

As expected, the actions of the two non-selective agonists on heart rate were sensitive to the A_1-selective antagonist, DPCPX. Although the bradycardia induced by adenosine appeared to be less sensitive to DPCPX than that of NECA it is likely that the discrepancy is pharmacokinetic in origin because of the differing administration protocols used for the

two agonists (sequential for adenosine *versus* cumulative for NECA). The hypotensive action of adenosine but not NECA was antagonized by DPCPX. The reasons for this anomaly are not immediately clear. However, it may result from the stimulation of A_3 receptors by NECA which are known to lower blood pressure and are insensitive to DPCPX in the rat (Fozard & Carruthers, 1993; Carruthers & Fozard, 1994; Patel *et al*., 1994). Furthermore, it is interesting that the maximal responses to NECA are approximately twice those seen with adenosine. This could possibly reflect differences in their disposition since adenosine is more susceptible to breakdown and/or uptake than NECA. Nevertheless, the effects of adenosine and NECA on afferent nerve discharge and intrajejunal pressure were not inhibited by the A_1-selective antagonist, DPCPX, suggesting that A_1 receptors are not involved. Thus, collectively, the observations with the non-selective agonists suggest, 'by default', that an A_{2B} or an A_{2B}-like receptor evokes these excitatory effects in the rat. However, the A_1-selective agonist, GR79236 (Gurden *et al*., 1993; Patel *et al*., 1994), induced increases in mesenteric afferent nerve discharge and intrajejunal pressure and, furthermore, these effects were sensitive to the A_1-selective antagonist, DPCPX, but not the A_{2A}-selective antagonist, ZM241385. Thus, these data suggest that A_1 receptors are indeed functional in this preparation and adenosine-induced increases in mesenteric afferent nerve activity and pressure in the jejunum are not mediated exclusively by an A_{2B} or an A_{2B}-like receptor. To explain the lack of sensitivity of these effects of adenosine and NECA to DPCPX it could be possible that both A_1 and A_{2B} and/or A_{2B}-like receptors are present on the effector(s) and that blockade of the A_1 population does not prevent non-selective agents from evoking mesenteric afferent nerve discharge and increases in intrajejunal pressure.

The peak effect of higher doses of GR79236 on intrajejunal pressure was significantly augmented in DPCPX-treated animals. This observation may reflect the unmasking of an inhibitory action of this receptor on increases in intrajejunal pressure possibly in combination with a loss of selectivity of GR79236 at the A_1 subtype. The former mechanism may explain why GR79236 did not produce maximal responses of similar magnitude to NECA on this parameter in vehicle- and ZM241385-treated animals. However, compromised blood flow in these latter two treatment groups compared with the DPCPX-treated group may have contributed to this discrepancy. Interestingly, the mesenteric afferent nerve activity evoked by GR79236 was not augmented in DPCPX-treated animals. We have no explanation for this apparent anomaly. However, it suggests that the elevation in intrajejunal pressure and mesenteric afferent nerve activity evoked by adenosine receptors may not be closely related, a posit that is discussed in further detail below. Nevertheless, these data suggest that a mixed population of adenosine receptors (A_1 and A_{2B} and/or A_{2B}-like) evoke the excitation of afferent nerves and increases in intrajejunal pressure.

Mechanism of the afferent activation induced by adenosine

The excitatory effect of adenosine on intestinal afferents occurs in conjunction with increases in intrajejunal pressure raising the possibility that the observed afferent nerve discharge may reflect the stimulation of mechano-sensitive fibres. However, in the present study, bethanechol (300 μg kg^{-1}), produced a greater peak increase in intrajejunal pressure than the maximal dose of adenosine (3 mg kg^{-1}). Nevertheless, adenosine evoked a greater peak increase in mesenteric afferent nerve discharge. Furthermore, at the peak bursts of afferent nerve activity evoked by bethanechol and adenosine, the elevation in intrajejunal pressure was greater for the former compared with the latter. Moreover, distension of the jejunum with saline elicited smaller peak increases in mesenteric afferent nerve activity than the maximal dose of adenosine despite inducing significantly larger increases in intrajejunal pressure (Kirkup *et al*., 1997). Thus collectively, these observations suggest that increases in pressure in the jejunum do not alone account for adenosine-mediated increases in afferent nerve discharge.

The rapid increases in intrajejunal pressure provoked by adenosine are likely to be the result of the contraction of intestinal smooth muscle. Adenosine analogues are known to cause contraction, *via* either A_1 or A_{2B} receptors, when acting on the *muscularis mucosae* along the length of the gastrointestinal tract of the rat (duodenum, Nicholls *et al*., 1996; ileum, Nicholls & Hourani, 1997; colon, Bailey & Hourani, 1990; Bailey *et al*., 1992). Contraction of this muscle layer may contribute to the observed increases in intrajejunal pressure although it must be stated that the effects of adenosine analogues on the *muscularis mucosae* in the jejunum have not been determined. However, it is unlikely that contraction of this muscle layer alone, which is one or two smooth muscle cells in thickness, could generate the observed increases in intrajejunal pressure. Furthermore, adenosine also relaxes the longitudinal muscle in these preparations and it is the response from this layer that predominates in the response of the intact tissue (Bailey *et al*., 1992; Nicholls *et al*., 1996; Nicholls & Hourani, 1997). It would therefore seem likely that several indirect mechanisms contribute to the observed rises in intrajejunal pressure. Stimulation of enteric motorneurones could be involved since adenosine depolarizes 'S-type' neurones in the myenteric plexus (Katayama & Morita, 1989) and, furthermore, adenosine-induced intrajejunal pressure increases are sensitive to the N-type calcium channel inhibitor, ω-conotoxin GVIA (Kirkup & Grundy, unpublished observations). Interestingly, adenosine-induced increases in mesenteric afferent nerve activity were unaffected in these toxin-treated animals, thus supporting our postulate that increases in intrajejunal pressure do not completely account for the elevation in mesenteric afferent nerve discharge.

The actions of adenosine also occur in conjunction with significant falls in blood pressure and we have previously compared the effects of adenosine with the hypotensive agent, sodium nitroprusside, on mesenteric afferent nerve discharge (Kirkup *et al*., 1997). Both of these agents produced rapid and similar peak falls in blood pressure. However, only adenosine induced a rapid and intense mesenteric afferent nerve activation suggesting that transient decreases in blood pressure do not influence the ongoing activity of intestinal afferent nerves. Furthermore, this observation is supported by findings of the present study since the increases in intrajejunal pressure and mesenteric afferent nerve activity in response to adenosine were unaffected by pre-treatment with DPCPX which otherwise attenuated the adenosine-induced decrease in blood pressure.

It is thus apparent that additional mechanisms contribute to the increases in afferent nerve discharge provoked by administration of adenosine. Adenosine is known to sensitize afferent nerves in a number of preparations (Taiwo & Levine, 1990; see Burnstock & Wood, 1996) and this mechanism may contribute to the stimulation of mesenteric afferent fibres. Indeed, sensitization could explain why adenosine and its analogues produce pronounced increases in mesenteric afferent nerve activity despite inducing only small increases in intrajejunal pressure compared with bethanechol and disten-

sion. A direct action on the mesenteric afferent nerve terminals cannot, as yet, be ruled out and previous studies have described excitatory actions of adenosine on cutaneous nociceptive (Bleehen *et al*., 1976), pulmonary (Cherniak *et al*., 1987) and renal afferent fibres (Katholi *et al*., 1985). Furthermore, adenosine can weakly depolarize the rat isolated vagus nerve, although it is not certain whether this is an effect on the population of afferent and/or efferent nerve fibres present in this preparation (Trezise *et al*., 1993).

A component of the excitation of mesenteric afferent nerves evoked by adenosine may be secondary to the release of substances from cells present in the tissues of the jejunum. Mast cells are one possible source of mediators. Indeed, they are found in close apposition to the terminations of afferent nerves in the intestinal mucosa (Williams *et al*., 1995) and, furthermore, the mast cell-degranulator, compound 48/80, stimulates mesenteric afferent nerves in the anaesthetized rat (Eastwood & Grundy, 1996). Moreover, depending on the mast cell-like cell line utilized, adenosine analogues either augment the release of mediators *via* A_3 (rat mucosal mast cell-like cell line, RBL-2H3; see Olah & Stiles, 1995) or induce interleukin-8 release and calcium mobilization *via* A_{2B} adenosine receptors (human mast cell line, HMC-1; Feoktistov & Biaggioni, 1995; 1998). Nevertheless, the mediators released from mast cells could produce direct and indirect actions on intestinal afferent nerves. 5-HT, a major constituent of secretory granules in mast cells of the rat, directly stimulates vagal afferent nerves (Hillsley *et al*., 1998) and other mediators such as histamine and prostaglandins could have indirect actions by causing contraction of intestinal smooth muscle or sensitizing intestinal afferent nerves (see Hill, 1990; Stebbins *et al*., 1985; Brunsden *et al*., 1998).

Conclusions

In conclusion, adenosine evokes the excitation of mesenteric afferent nerves and elevates intrajejunal pressure in the anaesthetized rat. These actions may be involved directly and/or indirectly, *via* the activation of reflex pathways, in the pathophysiology of inflammatory and ischaemic bowel disorders. Our experiments with adenosine analogues indicate that these effects on mesenteric afferent nerves and intrajejunal pressure are mediated *via* activation of A_1 and A_{2B} and/or A_{2B}-like receptors. Furthermore, the increases in intrajejunal pressure elicited by adenosine but not its haemodynamic effects are likely to contribute to some of the excitation of mesenteric afferent nerves.

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