

Adenosine A_{2A} receptors in portal hypertension: their role in the abnormal response to adenosine of the cranial mesenteric artery in rabbits

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1 Adenosine is a regulator of mesenteric vasodilation involved in auto-regulation and post-prandial hyperemia, but the adenosine receptor subtype involved in this relaxant effect is poorly characterized. We have now pharmacologically characterized this receptor in rabbit mesenteric arteries and investigated how this adenosine receptor response changes in portal hypertensive animals since the adenosine response is decreased.

2 The closest non-metabolisable adenosine analogue, 2-chloroadenosine (CADO), the mixed A₁/A₂ receptor agonist, 5'-ethylcarboxamidoadenosine (NECA), and the selective A_{2A} receptor agonist, 2-[4-(2-p-carboxyethyl)phenylamino]-5'-N-ethylcarboxamidoadenosine (CGS 21680) (1 pM–1 mM) relaxed noradrenaline pre-contracted arteries with a rank order of potency of CGS 21680 (EC₅₀ = 20 nM) ≥ NECA (60 nM) >> CADO (640 nM).

3 The selective A_{2A} receptor antagonist, 4-(2-[7-amino-2-(2-furyl)-[1,2,4]-triazolo[2,3-a][1,3,5]-triazin-5-ylamino]ethyl)phenol (ZM 241385, 100 nM), shifted to the right the CADO concentration-response curve.

4 In portal hypertensive animals, there was mainly a decreased potency but also a decreased efficacy of all tested adenosine agonists compared to normal animals. Concomitantly, there was a decreased adenosine plasma level and a decreased binding density of [³H]-CGS 21680 and [³H]-ZM 241385 to mesenteric artery membranes from portal hypertensive compared to normal rabbits.

5 These results indicate that A_{2A} receptor activation is required for the adenosine-induced mesenteric relaxation and that the decreased density of A_{2A} receptors may contribute to the decreased relaxation induced by adenosine of mesenteric arteries in portal hypertensive animals.

British Journal of Pharmacology (2002) **135**, 1324–1330

Keywords: Portal hypertension; adenosine; rabbit; mesenteric artery; A_{2A} receptors

Abbreviations: CADO, 2-chloroadenosine; CGS 21680, 2-p-(2-carboxyl-ethyl)phenylethylamino-5'-N-ethylcarboxamidoadenosine; NECA, 5'-ethylcarboxamido adenosine; ZM 241385, 4-(2-[7-amino-2-(2-furyl)-[1,2,4]-triazolo[2,3-a][1,3,5]-triazin-5-ylamino]ethyl)phenol

Introduction

Adenosine is a potent vasodilator that mediates several physiological phenomena in the splanchnic circulation, including autoregulation of the cranial mesenteric artery (Lautt, 1986), the hepatic buffer response (Lautt, 1985; Lautt & Greenway, 1987), and post-prandial hyperemia (Sawmiller & Chou, 1988). There has been some controversy about the adenosine receptor involved in mesenteric artery vasodilatation, with claims of both A_{2A} (Hiley *et al.*, 1995) or A_{2B} receptor involvement (Rubino *et al.*, 1995; Prentice *et al.*, 1997), although it is clear that adenosine-mediated mesenteric vasodilation is dependent on the presence of a functional endothelium (Hiley *et al.*, 1995; Tabrizchi & Lupichuk, 1995; Villa de Brito *et al.*, 1999). In different vascular beds, adenosine vasodilation mostly involves A_{2A} receptor activation, although in some instances A_{2B} receptors may also play

a role (reviewed by Olsson & Pearson, 1990; Belardinelli *et al.*, 1998; Feoktistov & Biaggioni, 1997). The importance of this adenosine-induced vasodilation is best exemplified by the observation that chronic blockade of adenosine receptors leads to hypertension (Guimarães & Albino-Teixeira, 1996).

The rabbit model of partial portal vein ligation is an increasingly used means of study for portal hypertension characterized by the development of portal-systemic shunting (Chojkier & Groszmann, 1981; Atucha *et al.*, 1998). The vascular responses to several vasoactive agents are modified in portal hypertension (Lee *et al.*, 1992; Clària *et al.*, 1994; Gadano *et al.*, 1997). In a previous study, we showed that the vasodilation of the cranial mesenteric artery induced by adenosine is decreased in rabbits with portal hypertension both *in vitro* (Villa de Brito *et al.*, 1998) and *in vivo* (Marques *et al.*, 1999).

To investigate the reasons of this decreased responsiveness to adenosine in hypertensive animals, we now decided to characterize the adenosine receptor involved in adenosine-

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induced mesenteric vasodilation and to investigate if this receptor activity was changed in portal hypertensive animals.

Methods

Animals and surgical procedures

Adult male New Zealand White rabbits (2.7–3.3 kg) were used for all studies. Rabbits were housed in a controlled environment and allowed free access to food and water. All experiments were performed according to the EU guidelines of care and use of laboratory animals.

Portal hypertension was produced by partial portal vein ligation (Chojkier & Groszmann, 1981). Briefly, a laparotomy was performed under ketamine (25 mg kg⁻¹, i.m.) and medetomidine (0.5 mg kg⁻¹, s.c.) anaesthesia. A ligature (3/0 silk) was placed, distal to the confluence of the right and left portal vein branches. An 18-gauge blunt needle was placed beside the portal vein, the ligature was tightened around both needle and vein, and the needle was removed, producing a standard, calibrated mechanical resistance. The abdomen was closed and the animal allowed recovery for 6 weeks. In sham-operated rabbits, the same procedure was carried out, with the exception that after the portal vein was isolated no ligature was placed.

Organ bath experiments

The rabbits were killed by an i.v. bolus of pentobarbitone (2 ml of a saturated solution), after which the cranial mesenteric arteries were removed, cleaned of fat and connective tissue, and cut into rings of approximately 3 mm length. The rings were suspended on tungsten wires under 2.0 g resting tension and allowed to equilibrate for 60 min in Krebs-Henseleit (mM): NaCl 118.3, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.2; KH₂PO₄ 1.2, NaHCO₃ 25.0 and glucose 11.1. Organ baths were maintained at 38°C and oxygenated with 95% O₂ and 5% CO₂. Tension was recorded with a Lectromed UF1 isometric transducer connected to a Lectromed polygraph 5041.

The mesenteric rings were sub-maximally contracted with noradrenaline (10 μM) and the integrity of the endothelium was confirmed by the response to acetylcholine (10 nM–1 mM). The rings that did not relax to acetylcholine were discarded. Cumulative concentration-response curves to the tested adenosine receptor agonists (CGS 21680, NECA and CADO) were constructed by the addition of increasing concentrations of each drug (1 pM–1 mM) to the organ bath. The responses to increasing concentrations of CADO were also evaluated, in normal rings, before and after incubation with ZM 241385 (100 nM). Since all these adenosine receptor ligands are hydrophobic, they were all dissolved in concentrated solutions of dimethylsulphoxide and we verified that dimethylsulphoxide, in the maximal concentration applied to the preparations (2%), was devoid of effects. The E_{max} and the EC₅₀ values were calculated using a sigmoidal dose response curve with variable slope and no weighting with the logistic equation of $Y = E_{\min} + (E_{\max} - E_{\min}) / (1 + 10^{-(\log EC_{50} - X) \cdot \text{Hill slope}})$, where X is the log of the agonist concentration, Y is the response measured and E_{min} is fixed at 0, using the GraphPad Prism software (GraphPad Software, San Diego, U.S.A.).

Adenosine plasma levels quantification

Normal rabbits and rabbits with portal hypertension were anaesthetized with sodium pentobarbitone (40 mg kg⁻¹, i.v.) and blood was obtained from the caudal vena cava near the liver, at the end of the suprahepatic vein, by venipuncture after laparotomy. Each blood sample (1.8 ml) was rapidly collected into a syringe containing 82 μl of a stopping solution consisting of 1 mM dilazep (to inhibit adenosine uptake into and release from red blood cells), 10 μM erythro-9-(2-hydroxy-3-nonyl)adenine (to block adenosine deaminase activity) and 525 μM α,β-methylene adenosine-5'-diphosphate (to inhibit ecto-5'-nucleotidase) (Slowiaczek & Tattersall, 1982; McCann & Katholi, 1990; Miura *et al.*, 1991; Zhang *et al.*, 1991). Only the samples free of observable haemolysis were considered. The blood samples were immediately centrifuged at 14,000 × g for 1 min. Aliquots (100 μl) of plasma were collected and deproteinated with 30 μl of 7% perchloric acid. Samples were centrifuged again for 5 min at 14,000 r.p.m., and 75 μl of clear supernatant were immediately neutralized with a 0.7 M KOH and 50 mM Tris solution. The samples were kept at -70°C until HPLC analysis.

Adenosine was separated by HPLC using a 5 μm reverse-phase C18 column and a 100 mM KH₂PO₄ with 15% (v v⁻¹) methanol eluent (pH 6.5) at a flow rate of 1.75 ml min⁻¹ and quantified by area integration of the peak detected spectrophotometrically at 254 nm (Cunha *et al.*, 1989; Cunha & Sebastião, 1993).

Adenosine A_{2A} receptor binding assays

Membranes were prepared basically as previously described (Cunha *et al.*, 1999) from frozen arteries. Briefly, the tissue was mechanically ground and sonicated to achieve homogenization in 10 volumes of sucrose solution (0.32 M), containing (mM): Tris-HCl 50, EGTA 2 and dithiothreitol 1, pH 7.6. The homogenates were centrifuged at 1000 × g for 10 min at 4°C. The supernatants were collected and centrifuged at 21,000 × g for 20 min at 4°C. The pellets were then resuspended in a solution containing (mM): Tris-HCl 50, EGTA 2, EDTA 1 (pH 7.4 at 23°C) with 5 u ml⁻¹ adenosine deaminase and incubated for 30 min at 37°C to remove endogenous adenosine. The mixture was then centrifuged at 21,000 × g for 10 min at 4°C, and the pellets resuspended in the incubation solution containing 50 mM Tris and 10 mM MgCl₂, pH 7.4, with 5 u ml⁻¹ adenosine deaminase.

[³H]-ZM 241385 (0.1–6 nM) and [³H]-CGS 21680 (30 nM) binding studies were performed as previously described (Cunha *et al.*, 1999). Briefly, binding reactions were carried out for 30 min (for [³H]-ZM 241385 binding) or 2 h (for [³H]-CGS 21680 binding) at room temperature (23–25°C) with 82–124 μg of membrane protein in a final volume of 300 μl of the incubation solution. The binding reactions were stopped by vacuum filtration through Whatman GF/C glass fibre filters, followed by washing of the filters and reaction tubes with 10 ml of the incubation solution, kept at 4°C. The filters were then placed in scintillation vials, and 5 ml of scintillation liquid (Scintran Cocktail T, Wallac) were added. Radioactivity bound to the filters was determined after 12 h with an efficiency of 50%. Results are expressed as specific binding, determined by subtraction of the non-specific binding, which was measured in the

presence of 2 μM 8-[4-[(2-aminoethyl)amino]carbonylmethyl-oxyphenyl]xanthine, and normalised per amount of protein, determined following the method of Peterson (1977). All binding assays were performed in duplicate. To calculate the binding parameters of [³H]-ZM 241385 binding, the saturation isotherm was adjusted by a rectangular hyperbola to estimate the K_D and B_{max} values using the GraphPad Prism software.

Drugs

Noradrenaline-L-hydrogen tartrate, acetylcholine chloride, NECA (5'-ethylcarboxamido adenosine), CADO (2-chloroadenosine), adenosine deaminase (type VI, 1803 u ml⁻¹, EC 3.5.4.4.), dilazep, α,β -methylene adenosine-5'-diphosphate and erythro-9-(2-hydroxy-3-nonyl)adenine were from Sigma, CGS 21680 (2-p-(2-carbonylethyl)phenylethylamino-5'-N-ethylcarboxamidoadenosine) and 8-[4-[(2-aminoethyl)amino] carbonylmethyl-oxyphenyl]xanthine were from Research Biochemicals Inc., 4-(2-[7-amino-2-(2-furyl)-[1,2,4]-triazolol[2,3-a][1,3,5]-triazin-5-ylamino]ethyl)phenol (ZM 241385) and [³H]-ZM 241385 (specific activity of 17 Ci mmol⁻¹) were from Tocris and [³H]-CGS 21680 (specific activity 37.5 Ci mmol⁻¹) was from DuPont-New England Nuclear.

The solutions of all adenosine receptor ligands were prepared in dimethylsulphoxide and diluted in Krebs buffer on the day of use.

Statistical analysis

Results are expressed as mean \pm s.e.mean, except the K_D values, which are presented as mean (95% confidence interval). Statistical analysis was performed using ANOVA (parameters derived from the concentration response curves and adenosine plasma levels) and unpaired Student's *t*-test (binding data). A *P* value of less than 0.05 was considered statistically significant.

Results

Pharmacological characterization of the adenosine-induced mesenteric relaxation

All adenosine receptor agonists tested produced arterial vasodilation in a concentration dependent manner (Figure 1a) with similar maximal response (Table 1). However, the potency of the tested adenosine receptor agonists differed. The selective A_{2A} receptor agonist, CGS 21680, was the most potent, closely followed by the mixed A₁/A₂ receptor agonist, NECA, both of them markedly (*P* < 0.05) more potent than

the closest chemical analogue of adenosine, CADO (Figure 1a and Table 1). This observed rank order of potency is indicative of the involvement of an A_{2A} receptor in the adenosine-induced mesenteric relaxation.

To further confirm the main involvement of the A_{2A} receptor subtype in mesenteric artery response to adenosine,

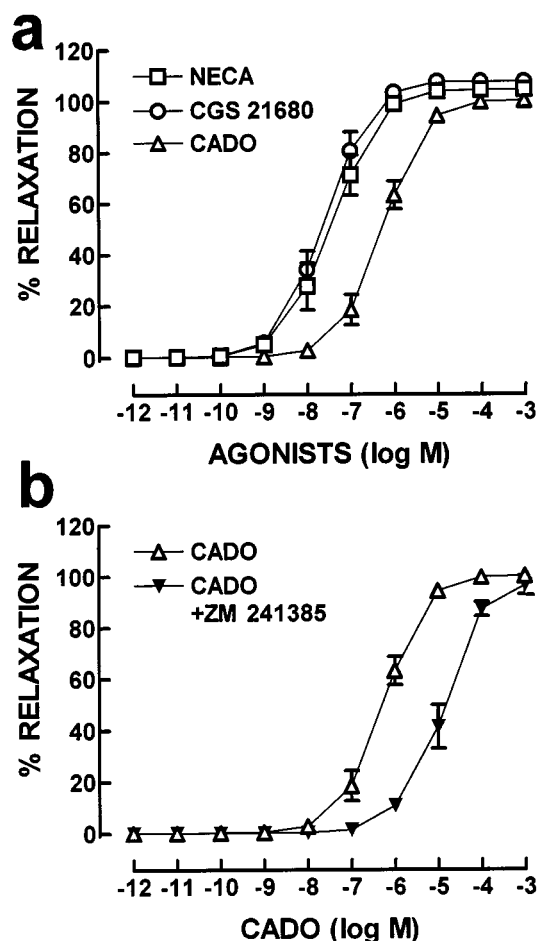


Figure 1 Relative potency of adenosine receptor agonists to relax rabbit cranial mesenteric arteries. The mesenteric artery rings were pre-contracted with noradrenaline (10 μM) and the functionality of the endothelium was verified by testing the response to acetylcholine. Then, cumulative concentration-response curves with increasing concentrations of the selective A_{2A} receptor agonist, CGS 21680, of the mixed A₁/A₂ receptor agonist, NECA, or with the closest non-metabolizable adenosine analogue, CADO, were carried out in (a). In (b), cumulative concentration-response curves with increasing concentrations of CADO were performed either in the absence or in the presence of the selective A_{2A} receptor antagonist, ZM 241385 (100 nM). The data are mean \pm s.e.mean of 5–6 experiments.

Table 1 Different potency and efficacy of the adenosine receptor agonist (NECA, CGS 21680 and CADO) to relax noradrenaline-precontracted cranial mesenteric artery rings from control and portal hypertensive rabbits

	NECA	Control CGS 21680	CADO	NECA	Portal hypertensive CGS 21680	CADO
EC ₅₀ (μM)	0.06 \pm 0.02	0.02 \pm 0.01	0.64 \pm 0.11	6.6 \pm 1.6*	8.8 \pm 2.7*	4.16 \pm 1.1*
E _{max} (%)	104.3 \pm 1.6	107.9 \pm 2.7	100.2 \pm 1.9	91.3 \pm 1.2*	90.2 \pm 3.4*	78.0 \pm 5.8*

The maximal relaxation (E_{max}) as well as the concentration leading to 50% of maximal relaxation (EC₅₀) are the mean \pm s.e.mean of 5–6 experiments. **P* < 0.05 (ANOVA) compared to control.

concentration-dependent vasodilation caused by CADO, the closest chemical analogue of adenosine that is non-metabolizable, was examined in the absence and presence of a selective A_{2A} adenosine receptor antagonist, ZM 241385. As illustrated in Figure 1b, ZM 241385 (100 nM) shifted to right ($P < 0.05$) the concentration-response curve to CADO (EC_{50} increased to $8.2 \pm 2.1 \mu\text{M}$, $n = 6$) with no change ($P > 0.05$) in efficacy ($E_{\text{max}} = 98.4 \pm 3.9\%$, $n = 6$). By itself, ZM 241385 (100 nM) caused a slight non-significant ($P > 0.05$) relaxation of noradrenaline pre-contracted arteries ($n = 6$).

Changes in adenosine responsiveness in portal hypertension

As illustrated for CGS 21680 in Figure 2, we observed that the relaxant response to all tested adenosine receptor agonists was decreased in cranial mesenteric arteries of rabbits subjected to partial portal vein ligation. There was a significant ($P < 0.05$) reduction mainly of the potency of all tested agonists, as well as a decrease in their maximal response (see Table 1). It should be noted that the artery rings from the two groups of animals displayed a similar ($P > 0.05$) contractile response to $10 \mu\text{M}$ noradrenaline ($0.96 \pm 0.06 \text{ g}$ in control and $1.00 \pm 0.04 \text{ g}$ in hypertensive animals, $n = 6$).

The decreased relaxant responsiveness to adenosine analogues of mesenteric arteries from portal hypertensive animals is not due to a general decrease of its relaxation properties. Thus, the acetylcholine-induced relaxation was observed not to be statistically ($P > 0.05$) different in mesenteric arteries from control and portal hypertensive rabbits. Thus, acetylcholine displayed an EC_{50} of $0.57 \pm 0.26 \mu\text{M}$ and of $0.80 \pm 0.18 \mu\text{M}$ and a E_{max} of $107.7 \pm 2.7\%$ and of $100.8 \pm 2.1\%$ in control and portal hypertensive animals ($n = 12$). This is in accordance with the previous observation of an increased nitric oxide synthase-mediated responsiveness in mesenteric arteries of portal hypertensive animals (Heinemann & Stauber, 1996).

To further investigate the reasons underlying this diminished relaxant response to adenosine in mesenteric arteries from portal hypertensive animals, we investigated if this could either be due to an increase in the levels of endogenous extracellular adenosine or to a decrease in the density of the receptors involved in this effect of adenosine, i.e. A_{2A} receptors.

Adenosine plasma levels, quantified in the vena cava near the liver, were significantly reduced ($P < 0.001$) in portal hypertensive rabbits, when compared to those obtained in normal rabbits. Thus, the measured concentrations of adenosine were $244 \pm 13 \text{ nM}$ in control and $78 \pm 5 \text{ nM}$ in portal hypertensive rabbits ($n = 8$). This makes it unlikely that the lower potency of adenosine receptor agonists might be due to an increased tonic activation of adenosine receptors by higher levels of endogenous extracellular adenosine.

In relation to the density of adenosine A_{2A} receptors, there was a significant ($P < 0.05$) decrease in the binding density of the selective A_{2A} receptor antagonist, [³H]-ZM 241385, to membranes of mesenteric arteries of portal hypertensive ($B_{\text{max}} = 384 \pm 29 \text{ fmol mg}^{-1} \text{ protein}$, $n = 4$) when compared to control rabbits ($B_{\text{max}} = 522 \pm 28 \text{ fmol mg}^{-1} \text{ protein}$, $n = 5$). The saturation curves of [³H]-ZM 241385 binding (Figure 3) show that the tested A_{2A} receptor antagonist

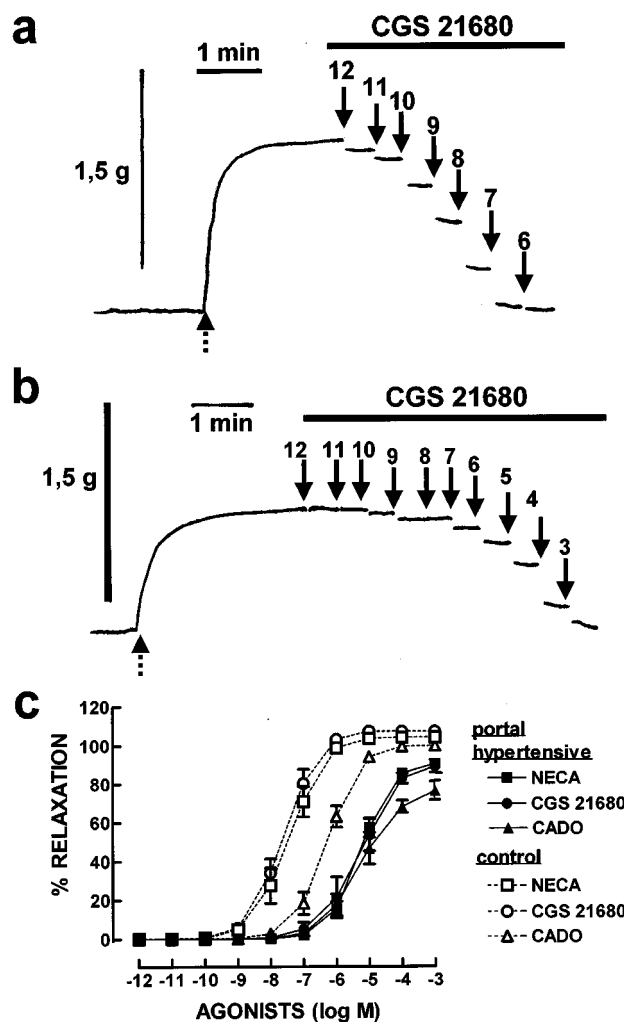


Figure 2 Modification of the potency of adenosine receptor ligands in cranial mesenteric arteries from portal hypertensive compared to control rabbits. In (a) and (b) are shown contractile recordings illustrating a cumulative concentration-response curve of the effect of CGS 21680 in cranial mesenteric arteries from control (a) and portal hypertensive rabbits (b). The mesenteric artery rings were pre-contracted with noradrenaline ($10 \mu\text{M}$, as shown by the dashed arrows in a and b). Then, a cumulative concentration-relaxant response to increasing concentration of CGS 21680 was carried out, as indicated by the upper bar in (a) and (b), and the recordings correspond to the last 30 s of recording out of each of the 5 min of recording after starting the superfusion of each concentration of CGS 21680, whose concentration is presented (as $-\log$ concentration) above each arrow. In (c) are shown the average results in preparations of control (open symbols, dashed lines) and portal hypertensive (filled symbols, filled lines) rabbits of the cumulative concentration-response curves with increasing concentrations of the selective A_{2A} receptor agonist, CGS 21680, with the mixed A₁/A₂ receptor agonist, NECA, and with the closest non-metabolizable adenosine analogue, CADO. The data are mean \pm s.e.mean of 5–6 experiments.

bound to mesenteric artery membranes from control rabbits to a single binding site with a K_D of 0.29 nM (95% confidence interval: 0.22 – 0.37 nM , $n = 5$) that was unchanged ($P > 0.05$) in portal hypertensive rabbits ($K_D = 0.26 \text{ nM}$; 95% confidence interval: 0.18 – 0.33 nM , $n = 4$). We also found that the single concentration of the selective A_{2A} receptor agonist used, [³H]-CGS 21680 (30 nM), showed a reduced ($P < 0.05$) amount of

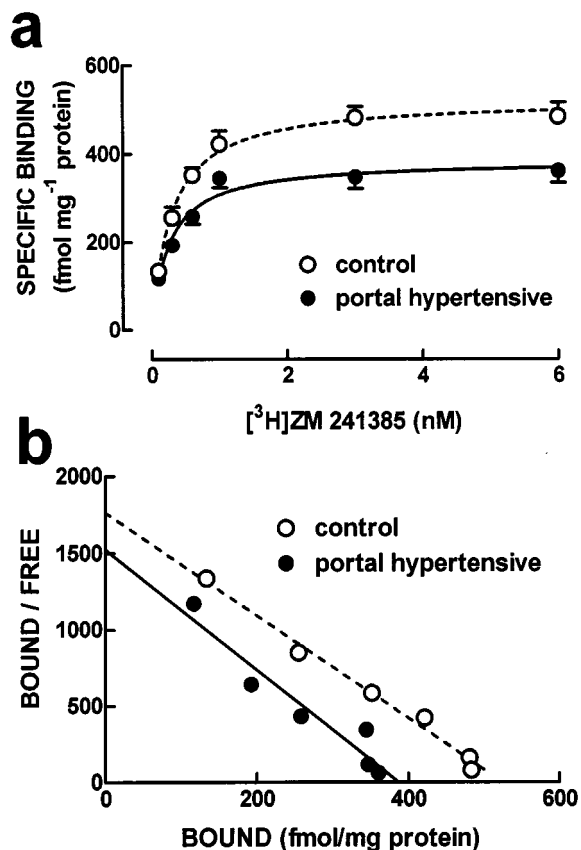


Figure 3 Average saturation curves (a) and corresponding Scatchard plots (b) of the binding of the A_{2A} receptor antagonist, ZM241385, to whole membranes of mesenteric arteries from control (open symbols and dashed lines) and portal hypertensive rabbits (filled symbols and filled lines). The ordinates in (a) represent the specific binding of [³H]-ZM 241385 on subtraction of non-specific binding, determined in the presence of 2 μM 8-[4-[(2-aminoethyl)amino]carbonylmethoxyphenyl] xanthine, from total binding. The curves in (a) were generated from the average binding parameters obtained on fitting by non-linear regression assuming a single binding site, whereas the curves in (b) correspond to linearizations of the data points. Results are mean ± s.e.mean of 4–5 experiments. The s.e.mean values are not presented in the Scatchard plots for the sake of simplicity.

binding in mesenteric artery membranes prepared from portal hypertensive (233 ± 29 fmol mg⁻¹ protein, *n* = 6) when compared to control rabbits (344 ± 12 fmol mg⁻¹ protein, *n* = 6).

Discussion

The present results indicate the involvement of adenosine A_{2A} receptors in the adenosine-induced vasodilation in rabbit mesenteric arteries. Furthermore, the previously observed decreased ability of adenosine to cause vasodilation in mesenteric arteries of portal hypertensive rabbits (Villa de Brito *et al.*, 1998) was now confirmed to also occur for all tested adenosine receptor agonists and likely involves a decreased density of adenosine A_{2A} receptors in rabbit mesenteric artery membranes.

The conclusion of a main involvement of adenosine A_{2A} receptors in adenosine-mediated vasodilation of mesenteric arteries stems from the observation that the prototypical A_{2A}

receptor agonist, CGS 21680, has a potency in the low nanomolar range and is equipotent to NECA. Furthermore, the selective A_{2A} receptor antagonist, ZM 241385, antagonized the relaxant effect caused by CADO, the closest non-metabolizable adenosine analogue. Finally, the parallel between the decrease in the binding density of A_{2A} receptors and the decreased potency of the tested adenosine receptor agonists in portal hypertensive animals is another argument favouring our conclusion that A_{2A} receptors mediate adenosine-induced vasodilation in cranial mesenteric arteries. This conclusion is in good agreement with previous observations in other studies performed in mesenteric arteries but using different experimental conditions and different animal species (Balwierczak *et al.*, 1991; Hiley *et al.*, 1995). However, some other studies in mesenteric artery preparations could not obtain evidence for the involvement of A_{2A} receptors, and concluded that adenosine-induced mesenteric vasodilation was mediated mostly via A_{2B} receptor (Rubino *et al.*, 1995; Prentice *et al.*, 1997) with the contribution of an yet undefined intracellular site (Prentice *et al.*, 1997). The studies that concluded on the involvement of A_{2B} rather than A_{2A} receptors in the vasodilation induced by adenosine in the mesenteric artery were carried out in a different species, i.e. the rat (Rubino *et al.*, 1995; Prentice *et al.*, 1997). However, another study, also carried out in the rat, clearly shows relaxant responses caused by the selective A_{2A} receptor agonist, CGS 21680 (Hiley *et al.*, 1995). There are experimental differences between these three studies that may explain the opposite conclusion reached in regard to the adenosine receptor responsible for the relaxant response in mesenteric arteries of the rat. Two of the studies were carried out with bolus injections of different doses of CGS 21680 in perfused mesenteric beds (Hiley *et al.*, 1995; Rubino *et al.*, 1995). The study that observed relaxant effects of CGS 21680 used a lower perfusion rate (Hiley *et al.*, 1995) and it is known from other systems that CGS 21680 equilibrates slowly with rat preparations (e.g. Cunha *et al.*, 1997). Also, the concentration range where CGS 21680 is selective for A_{2A} receptors is in the low nanomolar range and one of the studies, which failed to detect CGS 21680-induced relaxation, started to superfuse CGS 21680 at a concentration of 10 μM (Prentice *et al.*, 1997). In different rat preparations, it has been observed that CGS 21680-induced responses, pharmacologically identified as being mediated by A_{2A} receptors, cannot be revealed by the use of high (micromolar) concentrations of CGS 21680, possibly due to A_{2A} receptor desensitization (reviewed in Cunha, 2001). Therefore, it can be concluded that, when the necessary precautions are taken to safely use CGS 21680 as a selective A_{2A} receptor agonist, an A_{2A} receptor-mediated relaxation of mesenteric arteries can be observed in the rat, as it is now described to occur in the rabbit. The possible involvement of 'atypical' A_{2A} binding sites, which have been described in the CNS (Johansson & Fredholm, 1995; Cunha *et al.*, 1996), cannot be ruled out from the present set of data. However, it is important to note that these 'atypical' A_{2A} binding sites, which do not yet have a molecular correlate, appear to function mostly as 'typical' A_{2A} receptors although coupling to different transducing systems (e.g. Cunha & Ribeiro, 2000). Thus, irrespective of whether they are 'typical' or 'atypical', the present result extend to the mesenteric bed the major role played by A_{2A} receptors in the adenosine-induced vasodilation that has been

observed in different vascular beds (reviewed by Olsson & Pearson, 1990; Belardinelli *et al.*, 1998; Feoktistov & Biaggioni, 1997).

The present results also confirm our previous observation that adenosine-induced vasodilation of cranial mesenteric artery is decreased in rabbits with portal hypertension both *in vitro* (Villa de Brito *et al.*, 1998) and *in vivo* (Marques *et al.*, 1999). In fact, we now have found that the relaxant response to all adenosine receptor agonists tested was also decreased in mesenteric arteries from portal hypertensive rabbits. Portal hypertension is characterized by pronounced arterial vasodilation and increased systemic and mesenteric artery blood flow. This vasodilation leads to systemic hypotension that occurs despite an increased blood volume and cardiac output (Bosch *et al.*, 1989; Groszmann, 1994). According to the liver artery buffer response (Lautt, 1985), when portal blood flow decreases, adenosine accumulates in the space of Mall and induces hepatic artery vasodilation. Therefore, it would be expected that the concentration of adenosine in the caudal vena cava would be increased or at least maintained in our study. Thus, we hypothesize that hypertension-induced changes in the uptake and/or metabolism of plasmatic adenosine may have contributed for the significant decrease in adenosine plasma levels observed in our study. The most likely explanation for the observed decrease in the potency and efficacy of adenosine receptor agonists to induce mesenteric relaxation in portal hypertensive rabbits is based on the observed decreased binding density of adenosine A_{2A} receptors in mesenteric artery membranes of portal hypertensive rabbits. Indeed, theoretical studies (Kenakin, 1993)

predict that in systems where the receptor is significantly distributed between two different affinity states for the agonists, as is the case for the adenosine A_{2A} receptor (e.g. Cunha *et al.*, 1999), a decrease in receptor number may produce a dextral displacement of the concentration-response curve together with a decrease of the maximal response. It is important to note that the overall response to A_{2A} receptor agonists results from the number of A_{2A} receptors as well as from the efficiency of the transduction systems operated by the receptor and of the effector pathways targeted. Thus, one would not expect that the percentage change in receptor number would match the percentage change in the efficiency of the tested adenosine agonists and, although the decreased number of A_{2A} receptors is the prime explanation for the decreased efficiency of A_{2A} receptor agonists in portal hypertensive animals, it cannot be excluded that other factors may help contributing for the decreased relaxant effect of adenosine receptor agonists in the mesenteric artery of portal hypertensive animals.

In conclusion, the present results indicate that the adenosine receptor responsible for the adenosine-induced relaxation of mesenteric arteries is of the A_{2A} subtype and suggest that the reduced density of these A_{2A} receptors may contribute for the reduced relaxant response to adenosine in the mesenteric arteries of portal hypertensive animals.

This study was supported by grants from CIISA, Faculty of Veterinary Medicine and by FCT.

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(Received October 3, 2001

Revised December 20, 2001

Accepted January 3, 2002)